Dear Colleagues

We would like to welcome you to - as far as we know - the first Congress of our Society to be held in an oceanic island. We think this geographic characteristic is nowadays no longer a limitation.

In fact, we now know from the quantity and quality of presentations, that we will continue the excellent standards of our previous meetings. We hope to have staged the conditions for a fruitful exchange of information, so highly required in our profession.

We take the opportunity to thank our sponsors and to call your attention to the exhibitors booths that will present the latest commercially available products and prototypes. They also need our feedback as not-no-usual consumers.

We also hope that you can spare some time to enjoy the beautiful landscape of the Azorean archipelago.

The Executive Organising Committee,

António Amorim

Francisco Corte-Real

21st. Congress

International Society for Forensic Genetics

13-17 September 2005

Ponta Delgada

S. Miguel Island, Azores

Portugal

PROGRAMME

General information

Location

Teatro Micaelense / Centro Cultural e de Congressos
Largo de S. João
9500 Ponta Delgada, S. Miguel, Azores, Portugal
tel:(+351) 296 308 340
fax:(+351) 296 308 344
http://www.teatromicaelense.pt/

ISFG Board and Scientific Committee

Peter M. Schneider (Germany)
Angel Carracedo (Spain)
Mechthild Prinz (USA)
Niels Morling (Denmark)
Wolfgang R. Mayr (Austria)

Executive Organisers

António Amorim
Francisco Corte-Real

Language

English (no simultaneous translation provided)

Social programme

Friday, Sept. 16, 20h30
Gala Dinner - Clube Naval, Avenida Infante D. Henrique
Saturday, Sept. 17
Island Tour: visit to Furnas and lunch

Satellite meeting

Monday, Sept. 12, 14h00
to Tuesday, Sept. 13, 18h00
10th. Annual Meeting of GEP-ISFG (Spanish and Portuguese Working Group)

Travel Operator

Top Atlântico, Viagens e Turismo S.A.
Contact Person: Adélia Nunes
E-mail: group-dept@topatlantico.com
Phone: +351213108810
Fax: +351213108896

Presentations technical requirements

Speakers are required to submit their presentations in a PC compatible format (preferably PowerPoint) at least one hour before their scheduled time in a storage medium such as CD-ROM or USB flash memory.

Posters are displayed throughout the Congress duration and are required to be removed before Friday, Sept. 16, 19h.

Tuesday, Sept. 13

19h00 Reception and Registration

Wednesday, Sept. 14

09h00 Opening ceremony

09h30 – Oral presentations – Session 1; chairpersons: *Peter Schneider, Walther Parson*

Setting Standards and Developing Technology to Aid the Human Identity Testing Community - *John M. Butler*

10h15

O-01: Validation of a 21-locus Autosomal SNP Multiplex for Forensic Identification - *Dixon LA, Murray CM, Archer EJ, Dobbins AE, Koumi P, Gill P*

10h30 Coffee break

11h00 Oral presentations – Session 1 (ctd.); chairpersons: *Peter Schneider, Walther Parson*

O-02: Multiplex genotyping of 22 autosomal SNPs and its application in forensic field - *Turchi C, Onofri V, Alessandrini F, Buscemi L, Pesaresi M, Presciuttini S, Tagliabracci A*

O-03: Development of a multiplex PCR assay with 52 autosomal SNPs - *Sanchez JJ, Phillips C, Børsting C, Bogus M, Carracedo A, Court DS, Fondevila M, Harrison CD, Morling N, Balogh K, Schneider P*

O-04: Application of Nanogen Microarray Technology for Forensic SNP Analysis - *Balogh MK, Bender K, Schneider PM and the SNPforID Consortium*

O-05: Fluorescence labelling and isolation of male cells - *Anslinger K, Mack B, Bayer B*

O-06: Low Volume PCR (LV-PCR) for STR typing of forensic casework samples - *Proff C, Rothschild MA, Schneider PM*

O-07: Forensic Response Vehicle: Rapid analysis of evidence at the scene of a crime - *Hopwood A, Fox R, Round C, Tsang C, Watson S, Rowlands E, Titmus A, Lee-Edghill J, Cursiter L, Proudlock J, McTernan C, Grigg K, Kimpton C*

12h30 Lunch break

14h00 Poster Session I (posters 1, 4, 7, 10, …)

14h45-15h45 Sponsor Presentation

(room: sala Congro)

Improved Results from Integrating DNA Quantitation with AmpFlSTR Yfiler in a Sexual Assault investigation - *Yogesh Prasad (Applied Biosystems)*

15h30 Coffee break

16h00 Oral presentations – Session 2; chairpersons: *Mechthild Prinz; Christian Doutremepuich*

Evolution of microsatellite sequences - *Christian Schlötterer*

16h45

O-08: The evolution of European national DNA databases – conventional STRs, mini-STRs or SNPs?- *Gill P, Curran J, Elliot K*

O-09: Characterization and Performance of New MiniSTR Loci for Typing Degraded Samples – *Coble MD, Hill CR, Vallone PM, Butler JM*

O-10: Development of a new multiplex assay for STR typing of telogen hair roots - *Bender K, Schneider PM*

O-11: Evaluation of methodology for the isolation and analysis of LCN-DNA before and after dactyloscopic enhancement of fingerprints - *Leemans P, Vanderheyden N, Cassiman J-J, Decorte R*

O-12: Characterization of parameters influencing autosomal STR mutations - *Hohoff C, Fimmers R, Baur MP, Brinkmann B*

O-13: Highly efficient semi-quantitative genotyping of single nucleotide polymorphisms in mitochondrial DNA mixtures by liquid chromatography electrospray ionization time-of-flight mass spectrometry - *Niederstätter H, Oberacher H, Parson W*

18h00 ISFG Working parties meetings

Thursday, Sept. 15

09h00 Oral presentations – Session 3; chairpersons: *Angel Carracedo; Leonor Gusmão*

Forensic Interpretation of Haploid DNA Mixtures -*Michael Krawczak*

09h45

O-14: A Problem in Paradise: the Development and Forensic Interpretation of the Y Chromosome in New Zealand - *Harbison S, Brash K, Fris B, McGovern C*

O-15: Relative Y-STR mutation rates estimated from the variance inside SNP defined lineages - *Soares PA, Pereira F, Brion M, Alves C, Carracedo A, Amorim A, Gusmão L*

O-16: Relaunch of the Y-STR haplotype frequency surveying method based on metapopulations - *Willuweit S, Krawczak M, Roewer L*

10h30 Coffee break

11h00 Oral presentations – Session 3(ctd.); chairpersons: *Angel Carracedo; Leonor Gusmão*

O-17: Mitochondrial DNA pseudogenes in the nuclear genome as possible sources of contamination - *Goios A, Amorim A, Pereira L*

O-18: Genotyping coding region mtDNA SNPs for Asian and Native American haplogroup assignation - *Álvarez-Iglesias V, Salas A, Cerezo M, Ramos-Luis E, Jaime JC, Lareu MV, Carracedo A*

O-19: Haplogroup-level coding region SNP analysis and subhaplogroup-level control region sequence analysis for East Asian mtDNA haplogroup determination in Koreans - *Lee H-Y, Yoo J-E, Park MJ, Chung U, Shin K-J, Kim C-Y*

O-20: Dissection of mitochondrial haplogroup H using coding region SNPs - *Brandstätter A, Salas A, Gassner C, Carracedo A, Parson W*

O-21: Analysis of mtDNA mixtures from different fluids: an inter-laboratory study - *Montesino M, Salas A, Crespillo M, Albarrán C, Alonso A, Alvarez-Iglesias V, Cano JA, Carvalho M, Corach D, Cruz C, Di Lonardo AM, Espinheira R, Farfán MJ, Filippini S, Garcia-Hirchfeld J, Hernández A, Lima G, López-Cubría CM, López-Soto M, Pagano S, Paredes M, Pinheiro MF, Sala A, Sóñora S, Sumita DR, Vide MC, Whittle MR, Zurita A, Prieto L*

12h00 ISFG General Assembly

13h00 Lunch break

14h00 Poster Session II (posters 2, 5, 8, 11, …)

14h45-15h45 Sponsor Presentation (room: sala Congro)

Seeger/Aslinger- (Molecular Machines & Industries)

15h30 Coffee break

16h00 Oral presentations – Session 4; chairpersons: *Niels Morling; Bernd Brinkmann*

Forensic molecular pathology and pharmacogenetics – *Antti Sajantila*

16h45

O-22: Real-time PCR assays for the detection of tissue and body fluid specific mRNAs - *Fang R, Manohar C, Shulse C, Brevnov M, Wong A, Petrauskene OV, Brzoska P, Furtado MR*

O-23: Determination of forensically relevant SNPs in MC1R gene - *Branicki W, Kupiec T, Wolańska-Nowak P, Brudnik U*

O-24: Hair colour in Danish families: Genetic screening of 15 SNPs in the MC1R gene by analysis of a multiplexed SBE reaction using capillary electrophoresis or MALDI-TOF MS - *Mengel-Jørgensen J, Eiberg H, Børsting C, Morling N*

O-25: Initial Study of Candidate Genes on Chromosome 2 for Relative Hand Skill - *Phillips C, Barbaro A, Lareu MV, Salas A, Carracedo A*

O-26: Analysis of inter-specific mitochondrial DNA diversity for accurate species identification - *Pereira F, Meirinhos J, Amorim A, Pereira L*

O-27: The Development of a DNA Analysis System for Pollen - *Eliet J, Harbison S*

Public report on the activities of the EDNAP & ENFSI Groups

18h00 Review of EDNAP activities and update on current activities

Niels Morling (Summary of EDNAP activities); *Walther Parson* (Establishment of the forensic mtDNA population database EMPOP); *Peter Gill* (A collaborative study of the EDNAP group to compare SNPs, miniSTRs and conventional STRs to analyse degraded samples).

18h30 Review of ENFSI activities and update on current activities

ENFSI DNA WG Delegates

Friday, Sept. 16

09h00 Oral presentations – Session 5; chairpersons: *Wolfgang Mayr; Walter Bär.*

Representing and solving complex DNA identification cases using Bayesian networks - *Philip Dawid*

09h45

O-28: Characterizing Population Structure - *Weir BS*

O-29: Autosomal Markers for Human Population Identification from Whole Genome SNP Analyses - *Kayser M, Lao O, van Duijn JK, Kersbergen P, de Knijff P*

O-30: A Compact Population Analysis Test Using 25 SNPs With Highly Diverse Allele Frequency Distributions - *Phillips C, Sanchez J, Fontadevila M, Gómez-Tato A, Alvarez-Dios J, Calaza M, Casares de Cal M, Salas A, Ballard D, Carracedo A, The SNPforID Consortium*

10h30 Coffee break

11h00 Oral presentations – Session 5 (ctd.); chairpersons: *Wolfgang Mayr; Walter Bär.*

O-31: A Bayes net solution that simulates the entire DNA process associated with analysis of short tandem repeat loci - *Gill P, Curran J, Elliot K*

O-32: Maximisation of STR DNA typing success for touched objects - *Prinz M, Schiffner L, Sebestyen J, Bajda E, Tamariz J, Shaler R, Baum H, Caragine T*

O-33: Multi-substrata analysis on Siberian mummies: A different way for validation in ancient DNA studies? - *Amory S, Keyser-Tracqui C, Crubézy E, Ludes B*

11h30 Round Table: Interpretation of forensic mixtures; chairperson: *Peter Gill* *Panel Members: Bruce Weir, Charles Brenner, Michael Krawczak, Philip Dawid*

12h30 Lunch break

14h00 Poster Session III (posters 3, 6, 9, 12, …)

14h45-15h45 Sponsor Presentation

(room: sala Congro)

Chargeswitch® technology - a novel highly sensitive DNA purification technology, optimised for forensic applications - *Richard Watts (Invitrogen)*

15h30 Coffee break

16h00 Round Table: Lessons from the Tsunami experience

Chairperson: *Mechthild Prinz*

Speakers:

Ruediger Lessig - The first days after the Tsunami - a report about the situation. *Gunilla Holmlund* - Where can we find reference DNA when several generations of families are missing?

Martin Steinlechner - Sri Lanka victim samples typed using a streamlined PM sample processing strategy

Antti Sajantila - The Finnish contribution and issues regarding non DNA identification methods for children

Charles Brenner - Simultaneous versus serial DNA identification of related Tsunami victims

Hermann Schmitter - The need for international DVI standards

Bertil Lindblom - Data management and profile matching at the IMC in Phuket

17h00 Quality Control reports

Chairperson: *Niels Morling*

Speakers:

Gjertson DW (for the Parentage Standards Program Unit of the AABB) - Accredited Relationship Testing and Current Practices in the United States

Simonsen BT, Hallenberg C, Morling N - Results of the 2005 Paternity Testing Workshop of the English Speaking Working Group

García-Hirschfeld J, Alonso A, García O, Amorim A, Gómez J - 2004-2005 GEP proficiency testing programs: special emphasis on the interlaboratory analysis of mixed stains

Hohoff C, Schürenkamp, M, Brinkmann B - The GEDNAP Proficiency tests. Recent trends and developments

18h30 closing remarks

20h30 gala dinner

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Krawczak M	Forensic Interpretation of Haploid DNA Mixtures
Sajantila A	Forensic molecular pathology and pharmacogenetics
Dawid P	Representing and solving complex DNA identification cases using Bayesian networks

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INVITED

PRESENTATIONS

ABSTRACTS

Setting Standards and Developing Technology to Aid the Human Identity Testing Community

John M. Butler,

P.M. Vallone, M.D. Coble, A.E. Decker, C.R. Hill, J.W. Redman, D.L. Duewer, and M.C. Kline

National Institute of Standards and Technology, 100 Bureau Drive, Mail Stop 8311, Gaithersburg, MD 20899 USA

Our project team at the U.S. National Institute of Standards and Technology (NIST) is funded by National Institute of Justice (NIJ) to conduct research that benefits the human identity testing community and to create tools that enable forensic DNA laboratories to be more effective in analyzing DNA. We conduct interlaboratory studies, produce new assays to enable improved recovery of information from degraded DNA, evaluate new loci for potential future use in human identity applications, and generate standard information and training materials that are made available on the NIST STRBase website: http://www.cstl.nist.gov/biotech/strbase/. New genetic markers and assays involving STR and SNP loci are examined in a U.S. reference population data set involving approximately 650 samples that are of Caucasian, Hispanic, and African American origin. A portion of this presentation will also be devoted to discussing the results from the mixture interpretation interlaboratory study (MIX05) conducted in early 2005 where over 50 different laboratories returned interpretation results on the same DNA samples. Our efforts to improve STR and SNP typing resources and assays for the community will also be described.

John M. Butler, National Institute of Standards and Technology, 100 Bureau Drive MS 8311, Building 227, Room B250, Gaithersburg, MD 20899 USA; Tel: 301-975-4049; Fax: 301-975-8505; email: john.butler@nist.gov

Evolution of microsatellite sequences

Christian Schlötterer

Forensic Interpretation of Haploid DNA Mixtures

Michael Krawczak

Institut für Medizinische Informatik und Statistik
Christian-Albrechts-Universität Kiel
Brunswiker Strasse 10
24105 Kiel

The mathematical concept previously introduced for the forensic interpretation of DNA mixtures using non-associated genetic markers has been adapted to the assessment of haplotypes. Such calculus is required, for example, when mitochondrial or Y-chromosomal markers are used in forensics. In addition to outlining the general mathematical framework, we devise two approaches to its practical computational implementation, involving either the inclusion-exclusion principle of probability theory or a recursion in the number of unknown contributors invoked. The two approaches scale differently, depending upon the complexity of the case and the diversity of the markers used. The performance of Y-chromosomal microsatellites (Y-STRs) as a means of trace donor discrimination has been assessed, using the derived formulas. Dased upon data from the Y-chromosomal Haplotype Reference Database (YHRD), the exclusion chance of a non-contributor is shown to vary between 95% in the case of two contributors to the trace, and 70% for five contributors. It must be emphasised that these estimates are likely to be conservative since the calculations involved only haplotypes known to occur in YHRD. Along the same line, the correct and unbiased interpretation of haploid DNA mixtures may still be hampered by the fact that the respective evidence is impossible to quantify if haplotypes necessary to explain the trace have not been observed before.

Contact:krawczak@medinfo.uni-kiel.de

Forensic molecular pathology and pharmacogenetics

Antti Sajantila

Representing and solving complex DNA identification cases using Bayesian networks

Philip Dawid
Department of Statistical Science, University College London, Gower Street, London WC1E 6BT, United Kingdom.
with Julia Mortera and Paola Vicard, Universita Roma Tre

Problems of forensic identification from DNA profile evidence can become extremely challenging, both logically and computationally, in the presence of such complicating features as missing data on individuals, mixed trace evidence, mutation, silent alleles, laboratory and handling errors, etc. etc. In recent years it has been shown how Bayesian networks can be used to represent and solve such problems.

"Object-oriented" Bayesian network systems, such as Hugin version 6, allow a network to contain repeated instances of other networks. This architecture proves particularly natural and useful for genetic problems, where there is repetition of such basic structures as Mendelian inheritance or mutation processes.

I will describe a "construction set" of fundamental networks, that can be pieced together, as required, to represent and solve a wide variety of problems arising in forensic genetics. Some examples of their use will be provided.

Contact: dawid@stats.ucl.ac.uk

ORAL

PRESENTATIONS

ABSTRACTS

O-01

Validation of a 21-locus Autosomal SNP Multiplex for Forensic Identification Purposes

Dixon LA, Murray CM, Archer EJ, Dobbins AE, Koumi P, Gill P

The Forensic Science Service, 2960 Trident Court,, Solihull Parkway, Birmingham, UK

A single nucleotide polymorphism (SNP) multiplex has been developed to analyse highly degraded and low copy number (LCN) DNA template, i.e. <100pg, for scenarios including mass disaster identification. The multiplex consists of 20 autosomal non-coding loci plus Amelogenin for sex determination, amplified in a single tube PCR reaction and visualised on the Applied Biosystems 3100 capillary electrophoresis system. Allele-specific primers tailed with shared universal tag sequences were designed to speed multiplex design and to balance the amplification efficiencies of all loci through the use of a single reverse and two differentially labelled allele denoting forward universal primers. As the multiplex is intended for use with samples too degraded for conventional profiling, a computer program was specifically developed aid interpretation. Critical factors taken into account by the software include empirically determined extremes of heterozygote imbalance (*Hb*) and the drop-out threshold (*Ht*) defined as the maximum peak height of a surviving heterozygote allele, where its partner may have dropped out. The discrimination power of the system was estimated at 1 in 4.5 million, using a White Caucasian population database. Comparisons using artificially degraded samples profiled with both the SNP multiplex and AMPFlSTR SGM plus (Applied Biosystems) demonstrated a greater likelihood of obtaining a profile using SNPs for certain sample types. Saliva stains degraded for 147 days generated an 81% complete SNP profile whilst STRs were only 18% complete; similarly blood degraded for 243 days produced full SNP profiles compared to only 9% with STRs. Reproducibility studies showed concordance between SNP profiles for different sample types, such as blood, saliva, semen and hairs, for the same individual, both within and between different DNA extracts.

Contact: DNAPGill@compuserve.com

O-02

Multiplex genotyping of 22 autosomal SNPs and its application in forensic field

Turchi C[1], Onofri V[1], Alessandrini F[1], Buscemi L[1], Pesaresi M[1], Presciuttini S[2], Tagliabracci A[1]

[1]*Institute of Legal Medicine, Università Politecnica delle Marche, Ancona, Italy*
[2]*Center of Statistical Genetics, University of Pisa, Italy*

The sequence of the human genome, within the framework of the Genome Project, has revealed the existence of a new class of DNA polymorphisms involving one single base-pair, called SNPs (single nucleotide polymorphisms), constituting the most abundant form of genetic variation. This new class of markers offers interesting prospects in the forensic field, given their abundance and low mutation rates. Moreover, SNPs can be analyzed using throughput technologies and, most importantly, they can be used when DNA is highly degraded because they can be tested in short amplicons. In such instances, they could be used with proficiency in association with classical markers. On the other hand, the number of SNPs required to achieve a significant discrimination power is higher than the number of STRs commonly used. It has been estimated that nearly 60 SNPs are needed to match the power of the CODIS STRs set.
The aims of this study were to set up multiplex PCRs of 22 autosomal SNPs suitable for forensic purposes to assay their discrimination power in a population sample, and to compare it with the already known power of STRs commonly used in forensic work
22 binary polymorphisms, with an allele frequency of 0.5 in at least two different Caucasian population studies, were extrapolated from the "SNP Consortium" database (http://snp.cshl.org). One SNP was chosen for each autosomal chromosome. Three multiplex PCRs were constructed with primer pairs designed to produce amplicons in a range between 56 and 151 bp. 1 nanogram of DNA template, extracted from 50 healthy Italian subjects, was submitted to amplification reactions. SNPs typing was performed by fluorescently labelled dideoxy single-base extension of unlabelled oligonucleotide primers using the ABI PRISM SNaPshot™ Multiplex Kit (Applied Biosystems). The extension products were electrophoresed in an automated ABI 310 5-colour sequencer (Applied Biosystems).

contact: a.tagliabracci@univpm.it

O-03

Development of a multiplex PCR assay with 52 autosomal SNPs

Sanchez JJ[1], Phillips C[2], Børsting C[1], Bogus M[3], Carracedo A[2], Court DS[4], Fondevila M[2], Harrison CD[4], Morling N[1], Balogh K[3] and Schneider P[3]

1 Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark
2 Institute of Legal Medicine, University of Santiago de Compostela, Spain
3 Institut für Rechtsmedizin, Johannes Gutenberg University, Mainz, Germany
4 Department of Haematology, ICMS, Queen Mary's School of Medicine and Dentistry, London, UK

An efficient method that can be used to simultaneously amplify a set of genetic loci across the genome with high reliability can provide a valuable tool for single nucleotide polymorphism (SNP) forensic genotyping. A crucial element is the number of individual biochemical reactions that must be performed. The SNPforID consortium (www.snpforid.org) was established in 2003 with the principal goal of developing a SNP-based system of DNA analysis that would have comparable discrimination power and ease of use to existing short tandem repeat (STR) based techniques. Here, we describe a strategy for amplifying 52 genomic DNA fragments, each containing one SNP, in a single tube, and accurately genotyping the PCR product mixture using two single base extension reactions. This multiplex approach reduces the cost of SNP genotyping and requires as little as 0.5 ng of genomic DNA to detect 52 SNPs. We used a multiple injection approach for sequencers that can effectively detect all the SNPs amplified in a single electrophoretic run. We present SNP data for 709 unrelated individuals from 9 populations. Statistical interpretation of the results and comparisons between the 52 SNP multiplex and commercial STR kits are discussed.

contact: juan.sanchez@forensic.ku.dk

O-04

Application of Nanogen Microarray Technology for Forensic SNP Analysis

Balogh MK, Bender K, Schneider PM and the SNP*for*ID Consortium

Institute of Legal Medicine, Johannes Gutenberg University of Mainz, Germany
www.snpforid.org

The NanoChip® Molecular Biology Workstation (developed by Nanogen Inc.) using electronic microarrays is a particularly attractive microarray approach for rapid and high throughput analysis of SNPs. This instrument is fully automated and uses a proprietary semiconductor microchip for electronic addressing of capture probes to specific array sites followed by electronic hybridisation of the single stranded PCR products, and passive hybridisation of fluorescently labelled reporter oligos. Discrimination is achieved by applying thermal stringency to denature the mismatched reporters. Allele calling is carried out immediately using a built-in laser-activated fluorescence detection system.

The main purpose of the performance assessment was to evaluate the sensitivity, the accuracy and the multiplex capability of the platform by using 24 autosomal SNPs chosen from 52 non-coding SNPs, previously selected for the SNP*for*ID project. In the initial phase of the project, the amplicon down assay was used for addressing the biotinylated amplicons directly to the surface of the microarray, followed by hybridisation of the labelled reporter probes, separately for all the 24 SNPs. However, forensic typing requires rapid multiplex analysis from limited samples under high throughput conditions. Therefore, the capture down assay is more suitable, since fragment specific capture probes are bound to the array and the PCR amplicons are captured simultaneously by electronic hybridisation, followed by passive hybridisation of all labelled reporter probes in a single reaction.

24 SNP assays have already been designed using a modification of the capture down assay which applies a “touch down” strategy to obtain the best reporter probe discrimination. Overall the Nanochip platform appears to be suitable for SNP multiplex typing and presently, an additional 24 SNPs are under evaluation to be combined into a 48-plex.

Balogh MK, Institute of Legal Medicine, Johannes Gutenberg University of Mainz,
Am Pulverturm 3, 55131 Mainz, Germany
contact: balok000@students.uni-mainz.de

O-05

Fluorescence labelling and isolation of male cells

Anslinger K[1], Mack B[2], Bayer B[1]

[1] *Institute of Legal Medicine, Ludwig-Maximilians-University, Munich*

[2] *Department of Head and Neck Surgery, Ludwig-Maximilians-University, Munich*

Laser capture microdissection (LMD) is a relatively new technique for the isolation of single cells. In forensic science for example, LMD is used to select spermatozoa out of Haematoxylin / Eosin stained vaginal smears. In particular in cases with low numbers of sperm and in cases with aspermatozooic perpetrator or men, which have undergone a vasectomy, it could be profitable to isolate male cells in general, instead of focussing on the sperm only. Similarly, the specific detection of male cells in a female/male epithelial cell mixture, for example male saliva on female skin, could be of real advantage. The aim of this study was to find a staining method, which allows the identification of male cells from different origins for isolation via LMD. Therefore, we used a fluorescence-in-situ–hybridisation kit from Vysis (Downers Grove, IL), which includes probes for the X- and the Y-chromosome. The X-specific probe hybridises to multicopy alphoid DNA located at the centromere and was labelled with a red fluorescence dye. The Y-specific probe hybridises to Satellite-III-DNA located on Yq12 and was labelled with a green fluorescence dye. The simultaneous detection of the X- and the Y-chromosome could be seen as an internal positive control for the success of the hybridisation. Different mixtures of male and female cell samples were stained, and the male cells were isolated via SL µCut LMD system from Molecular Machines & Industries AG (MMI, Glattburg, Zurich, Switzerland). In comparison with other LMD systems, the SL µCut doesn't works with glass slides. The samples are spread on a membrane, which is placed on a special metal holder. The laser cuts the membrane around the cells of interest and they are securely removed with an adhesive film technology.

DNA was isolated from the LMD separated cells and a STR profiling was performed using different multiplex PCRs. In parallel we determined the overall content of male DNA of the different mixtures using the Quantifiler Human and Quantifiler Human Male DNA Quantification Kits (AB, PE Corporation, Forster City, CA). Taken together, the results of our study revealed that the staining method in combination with LMD seems to be a real advantage when dealing with unfavourable male/female cell mixtures. In cases where every single cell is important for a successful STR profiling of the male component, this technique can definitely increase the amount of male material that could be extracted via LMD. Moreover it's suitable to select male cells out of male/female mixtures with identical cell types.

Contact: Katja.Anslinger@med.uni-muenchen.de

O-06

Low Volume PCR (LV-PCR) for STR typing of forensic casework samples.

Proff C, Rothschild MA, Schneider PM

Institute of Legal Medicine, University Clinic Cologne, Germany

Commercial multiplex STR typing kits are often used with reduced PCR volumes. A volume reduction of 30-50% normally does not result in a significant loss of quality regarding signal intensity, allele balance etc. This is especially true for reference samples extracted from blood or buccal swabs where sufficient DNA of good quality is available. But even in low copy number (LCN) amplifications reproducible results can be obtained. This may be due to the assumption that DNA in a lower PCR volume could get into better contact with primer or polymerase molecules because the overall amount of DNA is less diluted than in a higher volume. Otherwise the volume of extracted DNA that can be used for the PCR assay is limited.

In the days of nanotechnology everything is getting smaller. In this case commercial PCR chips (*Ampli* Grid® A60, Alopex, Kulmbach, Germany; originally designed for diagnostic single cell PCR) have been developed where multiplex PCR can be performed in a 1 µL-PCR volume on a 60 well glass chip in microscopic slide format. Circular hydrophilic wells are separated by hydrophobic regions to ensure that the liquid PCR components do not get into contact with each other and stay in a drop form comparable to the 'lotus effect'. The fluids are then covered by mineral oil to prevent evaporation before the slide is put on a suitable in-situ PCR adapter that fits into a common 96-well thermocycler. Using this technology, it is possible to obtain a full 16-locus DNA profile in a 1 µl volume consisting of 0.5 µl DNA sample and 0.5 µl PCR reaction master mix.

We have tested LV-PCR with DNA from typical forensic casework samples using different commercial STR typing kits with a variable number of STRs from different manufacturers. The following criteria have been considered for this study: sensitivity, reproducibility, contamination risk, total DNA amount and relative DNA concentration, PCR cycling protocol, Taq polymerase, LCN amplification, allele balance, allelic dropout, and signal intensities.

Address for correspondence:
Dr. Carsten Proff, Institute of Legal Medicine,
Melatenguertel 60-62, 50823 Cologne, Germany;
phone +49 221 47886623, fax +49 221 4783496,
contact: carsten.proff@uk-koeln.de

O-07

Forensic Response Vehicle: Rapid analysis of evidence at the scene of a crime.

Hopwood A, Fox R, Round C, Tsang C, Watson S, Rowlands E, Titmus A, Lee-Edghill J, Cursiter L, Proudlock J, McTernan C, Grigg K, Kimpton C.

Forensic Science Service, Trident Court, Solihull Parkway, Birmingham Bus. Park, B'ham B37 7YN

The first hours of a criminal investigation can be the most important. A suspect arrested soon after a crime has less time to remove evidence from their person, possibly allowing stronger forensic ties between the individual and the crime scene.
We have developed a mobile laboratory with designated work areas for the searching of small items and pre and post PCR work.
An SGM+ profile can be produced and compared to the National DNA Database in approximately 5 hours, potentially providing the police with valuable intelligence early in the investigation of a crime.
The DNA analysis process utilises off the shelf equipment and consumables for the most part but a novel instrument for the separation and detection of fluorescently labelled STR amplicons has been developed from which data is directly imported to I^3 expert system software to provide an automated solution to profile designation.
The vehicle also carries the capability for the interrogation of electronic items such as mobile phones, and satellite communication systems allow direct connection with the FSS computer network allowing images of fingermarks and footwear marks to be searched against the appropriate databases.

Contact:
Andy.Hopwood@fss.pnn.police.uk

O-08

The evolution of European national DNA databases – conventional STRs, mini-STRs or SNPs?

Peter Gill and Lindsey Dixon

Forensic Science Service, Trident Court, 2960 Solihull Parkway, Birmingham, UK

Recently, an EDNAP exercise was instigated to compare the efficacy of conventional high molecular weight STR systems compared to low molecular weight (mini-STR) loci and SNP-plex of 21 loci, to analyse highly degraded stain material. We also assessed the relative statistical attributes of SNPs v. STRs and present a computer model that simulates DNA degradation. We concluded that the evidence suggests that substantial improvements can be expected by moving to multiplex systems that analyse smaller fragments of DNA than those in commercial kits that are currently in common use. To improve existing STR multiplexes, we propose that loci are re-engineered to produce smaller amplicons. In addition, 3 new European loci that have specific low molecular weight characteristics have been suggested for universal adoption – namely D10S1248, D14S1434, D22S1045.
The substantial difficulties associated with preparing large multiplexed reactions suggests that for routine stain work where the size of the sample is very limited, mini-STRs are the best option, whereas SNPs are an option when there is effectively unlimited sample, such as bone, that is available for analysis because several different multiplexes can be successfully utilised. If new loci are introduced into routine casework use it will be important to coordinate throughout Europe. To encourage this change, it will be essential to facilitate the process via international collaborative groups such as EDNAP and ENFSI.

Contact: DNAPGill@compuserve.com

O-09

Characterization and Performance of New MiniSTR Loci for Typing Degraded Samples

Coble MD[1], Hill CR[1], Vallone PM[1], Butler JM[1]
[1]*Biotechnology Division, National Institute of Standards and Technlogy, Gaithersburg, Maryland*

A number of studies have demonstrated that successful analysis of degraded DNA specimens from mass disasters or forensic evidence improves with smaller sized polymerase chain reaction (PCR) products (1). Forensic DNA analysts often perform short tandem repeat (STR) typing on highly degraded biological material and then turn to mitochondrial DNA testing, which is less variable but more likely to obtain a result due to higher copy numbers in cells, if many or all of the STRs fail. The commercially available kits for multiplex amplification of the 13 CODIS (FBI's COmbined DNA Index System) STR loci usually exhibit allele or locus-dropout for larger sized loci with degraded DNA or samples containing PCR inhibitors.

By moving PCR primers closer to the STR repeat region, we have demonstrated that it is possible to obtain fully concordant results to the commercial kits while improving successful analysis of degraded DNA with smaller PCR products or "miniSTRs" (1). However, many of the CODIS core loci have large allele ranges (e.g., D21S11 and FGA) that make it impossible to create small PCR products. We have examined a battery of new potential STR loci that can be made less than 100 bp in size (in most cases) and would therefore be helpful in testing highly degraded DNA samples. These new STR loci are being put together into novel DNA testing assays and evaluated across more than 600 samples representing the three largest populations in the U.S.: Caucasian, African American, and Hispanic. A set of six non-CODIS markers have been characterized and published (2). More markers that have been recently developed will be presented.

We have shown that the selection of STR loci that have a narrow allele range (e.g., less than 50 bp) and can be made smaller than 100 bp works well with degraded DNA samples, such as shed hairs and old bones. The successful typing of even a small number of nuclear loci from shed hairs can greatly increase the forensic discrimination of the sample compared to mtDNA testing alone, especially where a significant number of common types are present in the population.

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contact: michael.coble@nist.gov

O-10

Development of a new multiplex assay for STR typing of telogen hair roots

Bender K[1] and Schneider PM[2]

[1] *Institute of Legal Medicine, Johannes Gutenberg University Mainz, Germany*
[2] *Institute of Legal Medicine, University Clinic of Cologne, Germany*

We have developed a new strategy in which 10 STR systems plus amelogenin were simultaneously amplified with maximal fragment sizes smaller than 270 base pairs. This method can be used for the amplification of DNA from casework samples from which only limited amounts or highly degraded DNA can be isolated, for instance when DNA is isolated from telogen hair roots.

The multiplex amplification reaction includes six STR loci from the European standard set of loci (ESS) for DNA databases (D3S1358, D8S1179, D21S11, THO1, FGA and VWA) as well as four additional STR systems selected for their robustness and short amplicon sizes (D2S1338, D12S391, TPOX and D5S818) together with the sex-specific locus amelogenin. After PCR amplification, the multiplex reaction is splitted into two sets of STR multiplexes.Using streptavidin-coated Sepharose beads five STR systems are separated from the other six systems prior to being analyzed in two different runs on a capillary gel electrophoresis instrument.

To verify the specificity of the new STR multiplex undegraded human DNA samples from blood were amplified at least twice, separated and analysed by capillary gel electrophoresis. The results were compared to the typings with the SGM Plus™ (Applied Biosystems) or PowerPlex® 16 (Promega) kits and the single amplification of the D12S391 STR system. Furthermore DNA samples from artificially degraded DNA and from real case work were analyzed.

Dr. Klaus Bender, Institut Institute of Legal Medicine, Johannes Gutenberg University of Mainz, Am Pulverturm 3, D-55131 Mainz, Germany. Tel. 00 49 (0)6131 3932733; Fax: 00 49 (0)6131 393183
Contact: kbender@mail.uni-mainz.de

O-11

Evaluation of methodology for the isolation and analysis of LCN-DNA before and after dactyloscopic enhancement of fingerprints.

Peter Leemans, Nancy Vanderheyden, Jean-Jacques Cassiman and Ronny Decorte*

Laboratory for Forensic Genetics and Molecular Archaeology, Department of Human Genetics, K.U.Leuven, Belgium

Several studies have demonstrated the feasibility of using latent fingerprints for forensic DNA analysis. The analysis of these LCN-DNA samples is however not trivial and leads frequently to no results, partial results or the recovery of mixed DNA profiles. As this kind of evidence material is increasingly be submitted by the police for DNA analysis, we wanted to evaluate if current methodologies of sampling by the police and DNA methods (mainly DNA extraction) in the laboratory are optimal for DNA analysis of latent fingerprints. Fingerprints were applied by 6 different donors onto clean microscope glass slides and the fingerprints were recovered by using either cellotape, cotton swabs wetted with physiologic water, cotton swabs wetted with ATL-lysis buffer (Qiagen) or cotton tissue wetted with physiologic water. Four different methods were used for DNA extraction: QIAamp DNA Mini kit (Qiagen), QIAamp PCR Purification kit (Qiagen), a combination of both kits were the flow-through of the QIAamp DNA Mini kit columns was applied to the QIAamp PCR Purification kit columns, and the CST Forensic DNA Purification kit (Invitrogen). Four different methods for the enhancement of fingerprints were applied: white powder, black powder, cyanoacrylate fuming and enhancement of cyanoacrylate with basic yellow. The DNA extracts were evaluated quantitatively and qualitatively, respectively with the Quantifiler Human DNA Quantification kit, and AmpFlSTR SGM Plus and Profiler (Applied Biosystems). In addition, a multiplex of Y-SNPs (De Maesschalck et al., in preparation) was applied in order to evaluate the analysis of SNPs in LCN-DNA. The following conclusions could be drawn from the results of these experiments: (1)Cotton swabs showed to be the preferable method for the collection of latent fingerprints. The amount of DNA recovered with the cellotape was significantly (4 times) lower than with the other methods. (2)No difference was observed between the use of physiologic water and ATL-buffer for collecting the fingerprints. Only when the swabs were left at room temperature for at least 6 weeks, slightly more results were obtained with the STR-kits when ATL-buffer was used. However, we cannot exclude the possibility that this difference was due to differences in the amount of fingerprints present on the glass slide. (3)The amount of DNA recovered when the swabs were left at room temperature for at least 1 week until 8 weeks was slightly lower than when DNA extraction was done immediately. However, there was no decrease in the amount of DNA recovered between the different time periods neither in the possibility to type the STRs suggesting that degradation and loss of DNA is a slow process after sampling fingerprints when swabs remain at room temperature. (4)From the 6 donors, only one person was a good "shedder". This was reproducible and the amount of DNA recovered was sufficient for STR analysis and SNP amplification. For the other donors, either no DNA or low quantity DNA was obtained and the STR-profiles showed evidence for allele-drop-out, locus-drop-out and the presence of additional alleles. The presence of mixed profiles indicates the presence of additional DNA on the glass slide. We cannot exclude the possibility of secondary transfer. Further experiments should clarify this. (5)No significant difference was seen between the different methods used for DNA extraction. (6)We were able to obtain DNA after enhancement with different methods of the glass slides. The amount of DNA recovered was slightly less than without enhancement and no inhibition was observed in the PCR-reactions.

* Presenting and corresponding author: ronny.decorte@med.kuleuven.be

O-12

Characterization of parameters influencing autosomal STR mutations

Hohoff C[1], Fimmers R[2], Baur, MP[2] und Brinkmann B[1]

[1] Institut für Rechtsmedizin, Universitätsklinikum Münster, Röntgenstr. 23, 48149 Münster
[2] Institut für Medizinische Biometrie, Informatik und Epidemiologie, Medizinische Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn

In our routine parentage testing and additional meioses studies in Caucasoid populations we have observed 189 *de novo* mutations in more 100,000 meiotic transfers at autosomal STR loci.

The following criteria were chosen to call a Non-Mendelian transfer a mutation: isolated mismatch(es) (1 - 2), sequencing of all involved alleles and inclusion of the mutation in the biostatistical calculation with a resulting paternity value W > 99,97%.

By application of 'maximum likelihood' estimates we have been able to evaluate system-specific parameters such as gender, gain or loss of repeat units, parental age at conception and the sequence structure of the particular repeat.

This work is an important step to increase our understanding of the basic principles of STR mutations to estimate allele-related mutation rates in the future.

Address for correspondence
Prof. Dr. med. Bernd Brinkmann, Institut für Rechtsmedizin, Universitätsklinikum Münster, Röntgenstrasse 23, D-48149 Münster, Germany, Fax: 00 49 (0) 251 8355158,
eMail: remed@uni-muenster.de

O-13

Highly efficient semi-quantitative genotyping of single nucleotide polymorphisms in mitochondrial DNA mixtures by liquid chromatography electrospray ionization time-of-flight mass spectrometry

Niederstätter H, Oberacher H, Parson W

Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria

Sanger sequencing represents the "golden standard" for the typing of mtDNA. Accordingly, sequencing is commonly used for the detection and the semi-quantification of heteroplasmic mixtures. Typically, a minimum contribution of 20% of the minority allele is required to unequivocally reveal the presence of point-heteroplasmy, which clearly restricts the use of this method for the quantification of allelic contents. A number of different technologies have been introduced for the determination of allelic frequencies in DNA mixtures including allele-specific oligonucleotide hybridization, minisequencing, denaturing gradient gel electrophoresis, real-time PCR, and denaturing high-performance liquid chromatography.
Here, the combination of ion-pair reversed phase high-performance liquid chromatography and electrospray ionization quadrupole time-of-flight mass spectrometry is presented as an efficient method for the semi-quantitative genotyping of single nucleotide polymorphisms (SNPs). Artificially prepared and naturally occurring mitochondrial DNA mixtures showing different levels of heteroplasmy at nucleotide position 16519 served as reference samples. Allelic frequency determinations were based on the comparisons of allele-specific peak intensities in the obtained deconvoluted mass spectra. Deviations between measured and observed allelic frequencies were caused by differential PCR amplification and ionization of single alleles. Biased estimates were corrected by measuring the allele-specific signal intensities of equimolar allelic mixtures. Afterwards, measured and expected allelic frequencies correlated well (R^2 = 0.9983). An average error of 1.2% and a maximum error of 2.2% demonstrated the accuracy of the method. An average standard deviation of 2.45% and a maximum deviation of 5.35% proved the reproducibility of the assay. Due to the sensitivity of the applied mass spectrometric detection system alleles occurring at a frequency of 1.0% were unequivocally detected. The limit of quantification was found in the range of 5% minority component. The observed assay performance suggests that the described mass spectrometric technique represents one of the most powerful semi-quantitative genotyping assays available today.
Contact: harald.niederstaetter@uibk.ac.at

O-14

A Problem in Paradise: the Development and Forensic Interpretation of the Y Chromosome in New Zealand

Harbison S[1], Brash K[1], Fris B[2], McGovern C[1]

[1]*Forensic Biology, Institute of Environmental Science and Research Ltd, Auckland, New Zealand*
[2]*Forensic Science Programme, Department of Chemistry, University of Auckland, New Zealand*

The Y chromosome offers much for the analysis of forensic samples in both a traditional crime solving capacity and also in matters of mass disaster such as experienced recently in Asia. At ESR we are interested in developing Y chromosome systems for use in forensic casework to complement the SGM Plus™ system currently in place. A difficulty we face is the unique, multicultural nature of New Zealand, comprised as the population is of individuals of European, Asian and Polynesian descent and mixtures thereof. This complexity was demonstrated by the discovery of significant amounts of linkage disequilibrium in our STR population data and consequent adoption of relatively high Fst (θ) values of up to 5% in calculations of match probabilities.
In this paper we have evaluated both Y chromosome STRs and Y chromosome SNPs as potential systems for development. We have used both the Y plex 12™ system from Reliagene and the Powerplex-Y system from Promega to investigate the distribution of Y chromosome haplotypes in our population. We have found differences between population groups, including haplotypes common to some population groups and not others. We offer suggestions as to how these differences could be utilized in a forensic investigation.
We also describe the development of Y-chromosome based SNP marker systems, designed with the requirements of a forensic laboratory in mind. These multiplex systems comprise 5 or 6 SNP loci and have been built using mini-sequencing technology from Applied Biosystems, the SNAPSHOT™ SNP system. Loci were specifically selected for typing Y-chromosome lineages within Polynesian populations. These systems stand as a stepping-stone to the development of larger broad-based Y-chromosome and autosomal SNP marker systems that could complement or replace the STR systems currently in use.
We illustrate our work with case examples that demonstrate the usefulness of these techniques.

Contact: SallyAnn.Harbison@esr.cri.nz

O-15

Relative Y-STR mutation rates estimated from the variance inside SNP defined lineages

Soares P[1], Pereira F[1,2], Brion M[3], Alves C[1], Carracedo A[3], Amorim A[1,2], Gusmão L[1]

[1]IPATIMUP, Instituto de Patologia e Imunologia da Universidade do Porto, Portugal; [2]Faculdade de Ciências da Universidade do Porto, Portugal; [3]Unidad de Genética Forense, Inst. Medicina Legal, Univ. Santiago de Compostela, Spain.

Apart from the important role in general population genetics and in discerning male counterpart of demographic history, Y-chromosome has major forensic applications. Y specific microsatellites (STRs) have been widely used in forensic and population genetics in age estimates of human male lineages. Previously estimates of mutation rates from father-son pairs have shown quite variable results in different studies, essentially due to the rare nature of the mutational phenomenon. We propose an indirect approach for the determination of relative mutation rate of Y-chromosome microsatellites based on STR allele size intra-lineages variance. Indeed, the present distribution of STR alleles offers us an insight into the mechanisms that have generated that diversity, not a direct observation of a mutation occurrence but the observation of past mutations. We performed simulations using a Stepwise Mutation Model which showed that the variance of allele size distribution in Y-chromosome presented a linear relation with elapsed time that could be expressed as $V=d\times t$ (where V is the variance of the allele size distribution, d is the slope of the curve and t is the number of generations). From this equation: $t=V/d$. Therefore, if we compare the variances inside the same SNP defined lineage for two microsatellites (e.g. A and B), as time lapse is the same for both, we will have $V_A/d_A=V_B/d_B$ and therefore $d_A=(V_A/V_B)\times d_B$. Since the slope of the curve and mutation rates also presented a linear relation in the simulations, the equation can be changed to $\mu_A=(V_A/V_B)\times\mu_B$ (or $\mu_A=R_{AB}\times\mu_B$ if we considered R as the relative mutation rate of A to B). Using the calculated relative mutation rates, a linear relation shows up between the variance of each microsatellite inside a lineage (V) and $1/R$. For new STRs, or to those where few data concerning mutation studies have been accumulated, it would be thus possible to establish a relative mutation rate using lineage's microsatellite variance, according to the equation $V=m\times(1/R)$ (where m is the lineage specific slope). The relative mutation rate (R) between two microsatellites will be the same in the distinct SNP defined lineages (p and q) so we will have $V_p/m_p=V_q/m_q$ and, since $V=d\times t$ and $d_p=d_q$ for the same microsatellite, we could modify the equation to $t_p\times m_q=t_q\times m_p$, and therefore, $t_p=(m_p/m_q)\times t_q$. This means that the relative age of the lineage could be obtained through the relation of the lineages' specific slopes (m). In a sample of 950 unrelated Iberian individuals belonging to different Y-lineages, we selected those which presented n>30 to avoid too large confidence intervals and spurious results. Among those we selected those with well defined unimodal distributions and not too dispersed allele distributions in order to decrease inaccuracies due to random demographic factors (genetic drift, bottlenecks and founder effects) and to avoid significant departures from the theoretical model due to different mutational behaviour of the extreme size alleles in a STR allele distribution. Applying these criteria, we obtained three lineages (defined by SNPs M172, M201 and M269) and we calculated the relative mutation rates for all pairs of seven commonly used Y-microsatellites (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393). Contact: pedros@ipatimup.pt

O-16

Relaunch of the Y-STR haplotype frequency surveying method based on metapopulations

Willuweit S[1], Krawczak M[2], Roewer L[1]

[1]Inst. of Legal Medicine, Charité-University Medicine Berlin, Germany; [2]Institute of Medical Informatics and Statistics, Christian-Albrechts-University, Kiel, Germany

The successful implementation of Y-STR analysis in forensic practice led to the establishment of large web-based population databases which facilitate the assessment of match probabilities for haplotypic profiles. Thanks to international collaboration the current release 15 of the Y-STR Haplotype Reference Database (YHRD) consists of more than 22,000 different haplotypes from 249 population samples. YHRD provides frequencies for haplotypes found in geographically or linguistically defined metapopulations. Metapopulations are here defined as pools of population samples with an (assumed) high degree of relatedness. To obtain the observed haplotype frequency, it is sufficient to search a profile against the database or metapopulation and count the number of matches. The frequency surveying approach instead [1] takes the genetic similarities of a searched haplotype profile to its closely related "neighbours" into account and thus allows the frequency estimation even of those haplotypes which are rare and not observed in the database. In order to ensure that the extrapolated frequencies retain their high evidential power, it is important to perform the analysis for specified homogeneous data sets which can be identified by population genetic analysis. Using AMOVA and MDS analysis such pools with a high degree of genetic relatedness of Y-STR haplotypes have been identified for Europe based on a representative dataset 12.700 haplotypes from 91 populations [2]. Starting with release 16 all European haplotypes of the YHRD were assigned to these genetically defined metapopulations. Now we present the re-programmed web-based frequency surveying method adapted to metapopulation pools. To define such data pools we introduce a test for the assessment of homogeneity of population pools used for the frequency extrapolations.

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Contact: sascha.willuweit@charite.de

O-17

Mitochondrial DNA pseudogenes in the nuclear genome as possible sources of contamination

Goios A[1,2], Amorim A[1,2], Pereira L[1]

[1]IPATIMUP (Instituto de Patologia e Imunologia Molecular da Universidade do Porto), Porto, Portugal; [2]Faculdade de Ciências Univ. Porto, Porto, Portugal

Since shortly after the discovery of the mitochondrial genome, mitochondrial DNA-like sequences have been identified in the nuclear genome. The presence of these nuclear mitochondrial insertions (NUMTs) may lead to accidental amplifications of nuclear fragments with primers specifically designed for mitochondrial DNA (mtDNA). Depending on the homology of each NUMT to the mtDNA, this problem may be more or less relevant. In this work, we focused on the NUMTs that may be a cause of contamination in forensic analyses. Following the report of the complete human genome sequence, various studies have been published describing and listing all mitochondrial pseudogenes that exist in the different chromosomes. Of the 247 NUMTs reported in one of these studies (Mishmar et al, 2004), we analysed 19 that encompass the fragments of the D-loop that are usually used for forensic purposes, and identified the homologies to the primer annealing zones. We observed that none of the primers used for amplifying the Hypervariable Regions (HVRs) I and II in forensic studies (Wilson et al, 1995) anneals completely in any NUMT. The highest homology was observed in three NUMTs (chromosomes 4 and 17), where the annealing sites of both forward and reverse HVRI primers present one point substitution. Therefore, we concluded that an accidental amplification of one of these NUMTs with the HVRI and HVRII primers is very unlikely to occur. However, forensic and anthoropological studies have been focusing more and more on the coding region, and much information is now obtained by using SNaPshot multiplexes or sequencing of various fragments outside the D-loop. The longest and most similar NUMT from Mishmar's study, with 97% homology to the region between 3914-9755np of the Cambridge Reference Sequence (CRS), encompasses a target region for several analyses performed by forensic researchers. This high homology enables 11 primers used in a SNaPshot multiplex for mtDNA typing (Quintáns et al, 2004) to anneal perfectly to this NUMT. In order to establish whether this is an issue that forensic investigators must take into account when studying mtDNA, we will perform PCR with primers specifically designed for the NUMT sequences that may be a source of accidental amplifications and compare the results with what is obtained for mtDNA-targeted primers. We will present results of this analysis made on samples with different mtDNA content, such as blood, hair and buccal swabs.

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Contact: aalmeida@ipatimup.pt

O-18

Genotyping coding region mtDNA SNPs for Asian and Native American haplogroup assignation

Álvarez-Iglesias V, Salas A, Cerezo M, Ramos-Luis E, Jaime JC, Lareu MV, Carracedo A

Unidad de Genética Forense, Instituto de Medicina Legal, Universidade de Santiago de Compostela, Galicia, Spain

Based on phylogenetic criteria we selected 34 mitochondrial DNA (mtDNA) coding region SNPs that allow to distinguish Asian and Native American mtDNA haplogroups. SNP genotyping is carried out in a single multiplex reaction that involves a 20-amplicons PCR amplification, followed by a single minisequencing reaction using SNaPshot (Applied Biosystems, Foster City, CA, USA). The polymorphisms selected increase the discrimination power of the mtDNA hypervariable regions (HVS-I/II) in populations. Consequently, these combined SNPs are of particular interest in forensic casework, clinical and population genetics research. Here we show preliminary results using a sample from south-east Asia (Taiwan) and Native Americans from Argentina.

Contact: apimlase@usc.es

O-19

Haplogroup-level coding region SNP analysis and subhaplogroup-level control region sequence analysis for East Asian mtDNA haplogroup determination in Koreans

Hwan Young Lee[1], Ji-Eun Yoo[1], Myung Jin Park[1], Ukhee Chung[1], Kyoung-Jin Shin[1,2], Chong-Youl Kim[1,2]
[1]Department of Forensic Medicine, College of Medicine, Yonsei University, Seoul, Korea; [2]Human Identification Research Institute, Yonsei University, Seoul, Korea

We have established a high quality mtDNA control region sequence database for 593 Koreans. Based on previously reported patterns of shared haplogroup-specific or haplogroup-associated polymorphisms in control region sequence, we also classified 592 Korean mtDNAs (99.8%) into various East Asian haplogroups or subhaplogroups using the program mtDNAmanager (K-J Shin, Yonsei University, unpublished). These haplogroup-directed database comparisons and posteriori phylogenetic analysis confirmed the absence of major systematic errors in our data. We collated the basic informative control region SNPs and suggested the important mutation motifs for the assignment of East Asian haplogroups. However, quite a few haplogroup-diagnostic SNPs are located in mtDNA coding region, and in some haplogroups, scoring of coding region SNPs is required for exact haplogroup determination due to the lack of informativity in their control region sequences. Accordingly, we have selected 21 coding region SNP markers and designed the 3 multiplex systems applying single base extension methods. Using 2 multiplex systems, we allocated all 593 Korean mtDNAs into 15 haplogroups: M, D, G, D4, D5, M7, M8, M9, M10, M11, R, F, B, A and N9. Using the other multiplex, we further determined D4 subhaplogroups; D4a, D4b D4e, D4g and D4j, since D4 haplotypes occurred most frequently in Koreans. In this way, we could complement coding region information to control region mutation motifs and also confirm our control region mutagenic motifs for the assignment of East Asian haplogroups. Moreover, these 3 multiplex systems are expected to work well in degraded samples, since they have been designed to contain small PCR products (101~163 bp) for SNaPshot reactions. Therefore, we performed SNP scoring in 98 old skeletal remains using 3 multiplex and proved the utility of these multiplex in degraded samples. The targeting and preferential amplification of mtDNA control region using small amplicons and the selective scoring of highly informative SNPs in coding region using the 3 multiplex systems in this study is expected to represent a promising means for most application involving East Asian mtDNA haplogroup determination and haplogroup-directed stringent quality control even in degraded samples.
Contact: hylee192@yumc.yonsei.ac.kr

O-20

Dissection of mitochondrial haplogroup H using coding region SNPs

Brandstätter A[1], Salas A[2], Gassner C[3], Carracedo A[2], Parson W[1]

[1]Institute of Legal Medicine, Innsbruck Medical University, 6020 Innsbruck, Austria
[2]Instituto de Medicina Legal, Facultad de Medicina, 15705 Santiago de Compostela, Spain
[3]Central Institute for Blood Transfusion, General Hospital and University Clinics, Innsbruck, Austria

Analysis of single nucleotide polymorphisms (SNPs) is a promising application in forensic human identification. We selected 45 SNPs from the coding region of the human mitochondrial DNA in order to ascribe samples belonging to mitochondrial haplogroup H (hg-H) to one of the previously described sub-lineages of hg-H. SNP selection was carried out using the available literature on population and forensic genetics and extended by means of phylogenetic analysis of complete genomes (>400) and control region profiles. The selected SNPs are amplified in two PCR-multiplex reactions and subsequently targeted in three multiplex-systems via the application of the SNaPshot™ kit. Samples belonging to haplogroup H (approximately 40% of West-Eurasians) can in most cases not be distinguished from each other based on control region polymorphisms. By screening the selected coding region SNPs after sequencing of the control region however, we would be able to rapidly differentiate between stains or hairs in high volume case work or to eliminate multiple suspects from an inquiry. The presented hg-H screening strategy was conceived as a high-throughput method and the distribution of the selected SNPs and targeted haplogroups was inferred from a huge population sample.

contact: Walther.Parson@uibk.ac.at

O-21

Analysis of mtDNA mixtures from different fluids: an inter-laboratory study

Montesino M[1], Salas A[2], Crespillo M[3], Albarrán C[4], Alonso A[4], Alvarez-Iglesias V[2], Cano JA[5], Carvalho M[6], Corach D[7], Cruz C[8], Di Lonardo[9], Espinheira R[8], Farfán MJ[10], Filippini S[9], Garcia-Hirchfeld J[4] , Hernández A[11], Lima G[12], López-Cubría CM[5], López-Soto M[10], Pagano S[13], Paredes M[3] , Pinheiro MF[12], Sala A[7], Sóñora S[13], Sumita DR[14], Vide MC[6], Whittle MR[14], Zurita A[11], Prieto L[1]

[1]Comisaría General de Policía Científica, Spain [2]Inst. Medicina Legal Santiago de Compostela, Spain [3]Instituto de Toxicología y Ciencias Forenses de Barcelona, Spain [4]Instituto de Toxicología y Ciencias Forenses de Madrid, Spain. [5]Dirección General de la Guardia Civil, Spain. [6]Delegação de Coimbra do Inst. Nacional de Medicina Legal, Portugal [7]Servicio de Huellas Digitales Genéticas, U. Buenos Aires, Argentina [8]Delegação de Lisboa do Instituto Nacional de Medicina Legal, Portugal [9]Banco Nacional de Datos Genéticos, Buenos Aires, Argentina [10]Inst. de Toxicología y Ciencias Forenses de Sevilla, Spain [11]Inst. Toxicología y Ciencias Forenses de Canarias, Spain [12]Delegação do Porto do Instituto Nacional de Medicina Legal, Portugal. [13]Dirección Nacional de Policía Técnica, Uruguay [14]Genomic Engenharia Molecular Ltda, São Paulo, Brasil.

The analysis of mixed stains is a routine practice in forensic casework, mainly related to sexual assault cases. These analyses are commonly performed using differential lysis that allows the separation of epithelial cells DNA from that at of spermatozoa, followed by nuclear STR typing. In a number of cases, however, it could be interesting to know the mitochondrial DNA (mtDNA) haplotypes that contributed to the mixture (e.g. degraded or low-copy number reference samples, exclusion of a maternal relationship between victim and suspect in rape cases, etc). In the last GEP-ISFG mtDNA proficiency exercise (2003'04), the mtDNA analysis of a mixture stain (saliva from a female plus 1:20 diluted semen) yielded an unexpected consensus result: only the mtDNA hypervariable I and II saliva haplotype was detected, in contrast to the predominant presence of the male autosomal STR profile. Hence, the use of only mtDNA typing for this mixture sample could in this case lead to a false exclusion. Several additional experiments carried out by some laboratories pointed to the existence of different relative amounts of nuclear and mtDNA in saliva and semen (Crespillo et al. 2005, in press). In order to disentangle this puzzle, the mtDNA GEP-ISFG working group decided to carry out an inter-laboratory study.

We have studied mixtures from three semen donors and three saliva/blood female donors. Three semen dilutions (pure, 1:10 and 1:20) from each donor were mixed with saliva or alternatively, blood taken from each female donor (see Table 1). No *a priori* information was provided to the participating laboratories concerning either the mitochondrial haplotypes of contributors or the dilutions of semen. Each laboratory used their routine methodologies in order to carry out differential lysis, cell count, nuclear or mtDNA quantification, PCR and sequencing. There was a high consensus between labs for the epithelial fractions. In contrast, results concerning the seminal fractions were more ambiguous. In addition, some laboratories reported contamination problems in the male fraction. The most plausible explanation to this finding is that, after differential lysis, female and male mitochondria remain in the epithelial fraction and, theoretically, no mtDNA should be found in the male fraction (assuming effective differential lysis). Nevertheless, the first lysis is not always completely effective, so that mtDNA is also detected in the seminal fraction. The detection level of the male component decreased in accordance with the degree of semen dilutions, although the loss of signal was not uniform throughout all the nucleotide positions. There were clear differences between the mixtures prepared from different donors and body fluids. In some cases the male component was not detected. This may indicate that there are differences in the number of mitochondria (or cellular content) contributed by different donors and body fluids.

In conclusion, we can tentatively say that special care should be taken when analysing mtDNA in mixtures. There are several variables that we should bear in mind: the types of body fluids involved in the mixture, the possibility of contamination mainly in male fractions, the loss of signal in some nucleotide positions (but not in others), and the fact that differences in cellular content between donors are also possible. In addition, unlike the autosomal STR mixtures, the interpretation of mtDNA mixtures can be supported by using a phylogenetic approach.

contact: lourditasmt@ya.com

Female/male pair number	**Haplogroups**	Female saliva / semen mixtures	**Female blood / semen mixtures**
1	Female T2	50 µl of saliva + 50 µl of pure semen	50 µl of blood + 50 µl of pure semen
	Male H	50 µl of saliva + 50 µl of semen 1:10	50 µl of blood + 50 µl of semen 1:10
		50 µl of saliva + 50 µl of semen 1:20	50 µl of blood + 50 µl of semen 1:20
2	Female K	50 µl of saliva + 50 µl of pure semen	50 µl of blood + 50 µl of pure semen
	Male H	50 µl of saliva + 50 µl of semen 1:10	50 µl of blood + 50 µl of semen 1:10
		50 µl of saliva + 50 µl of semen 1:20	50 µl of blood + 50 µl of semen 1:20
3	Female H	50 µl of saliva + 50 µl of pure semen	50 µl of blood + 50 µl of pure semen
	Male J2	50 µl of saliva + 50 µl of semen 1:10	50 µl of blood + 50 µl of semen 1:10
		50 µl of saliva + 50 µl of semen 1:20	50 µl of blood + 50 µl of semen 1:20

Table 1. Composition of the mixture stains analysed in the inter-laboratory study.

O-22

Real-time PCR assays for the detection of tissue and body fluid specific mRNAs

Fang R[1], Manohar C[2], Shulse C[1], Brevnov M[1],Wong A[1], Petrauskene OV[1], Brzoska P[1], Furtado MR[1]

[1]*Applied Biosystems, Foster City CA*
[2]*Lawrence Livermore National Laboratories, Livermore CA.*

Identification of tissue parts and body fluids is frequently required in crime scene investigations. Conventional methods are often labor-intensive, not confirmatory and employ a diverse range of methodologies. Several forensic laboratories have pioneered the selection of specific protein or mRNA markers for identification of tissues and body fluids. Applied Biosystems has designed and tested real-time PCR based Taqman® assays that target the detection of over 20,000 mRNAs encoded by the human genome. We have employed proprietary methods to design assays specific to a target transcript avoiding amplification of related gene transcripts. We have developed methods for extraction of both RNA and DNA from samples. We have also developed methods for pre-amplification of hundreds of targets present in a single sample preserving relative quantification information. These methods will be useful when dealing with heterogeneous mixtures.
In this study we have tested the performance of assays targeting saliva specific markers, Statherin, Histatin, PRB1, PRB2, PRB3; menstrual blood markers like mettaloproteinases; and semen specific markers like protamines. Data will be presented to demonstrate the capability to pre-amplify small amounts of RNA enabling testing for the presence of multiple mRNA species when the amount of RNA is limiting. Capability to multiplex these assays will also be presented.

Contact: furtadmr@appliedbiosystems.com

O-23

Determination of forensically relevant SNPs in MC1R gene

Branicki W[1], Kupiec T[1], Wolańska-Nowak P[1], Brudnik U[2]
[1] *Institute of Forensic Research, Cracow, Poland*
[2] *Collegium Medicum of the Jagiellonian University, Cracow, Poland*

High variation present among humans in pigmentation causes that genetic prediction of this physical trait seems attractive for forensic investigations. Genetic typing of biological traces collected at scenes of crime could be a source of valuable information about the donor's characteristics. More than 60 genes are expected to be involved in the process of pigmentation in humans, but until present the only gene which influence for physiological variation on human pigmentation has been proved is the melanocortin 1 receptor gene (MC1R). The MC1R plays a key role in eumelanin/pheomelanin ratio in humans and hence its influence on hair and skin colour is crucial. Some allelic variants of the MC1R are significantly associated with the overproduction of pheomelanin, which is manifested with such phenotypic features as red hair or light skin. It has been suggested that analysis of MC1R variation could serve as a good indicator of the red hair phenotype. However, the postulated dosage effect of the MC1R variants on pigment phenotype disables the simple inference in the red/ non red mode. The influence of other genes on ultimate hair or skin colour makes the analysis even more complicated. Our goal was to check the variation within MC1R gene characteristic for Polish population and evaluate the usefulness of its analysis in forensic studies. A complete sequence data determined for the MC1R gene revealed, that in our population, red hair colour is mainly associated with the following variants: R151C, R160W and D294H what remains in good concordance with data for other European population samples. In our region blond-red hair phenotype is relatively common and seems mostly associated with heterozygotes or compound heterozygotes for the above alleles. Pure red hair colour can be, however, associated with homozygotes and compound heterozygotes. Hence, using simple sequence analysis more certain conclusions predicting the pure red hair colour can be drawn only for homozygous individuals, who are poorly represented in the studied population sample. Individual cases suggesting actions of other genes that could mask the influence of the MC1R variants on pigmentary status or determine a similar pigmentary effect has also been noted. Additionally a SNaPshot based assay has been developed, providing a selective analysis of the variable sites within MC1R gene, which have a significant correlation with red hair. Performed validation confirmed that the developed test enables reliable analysis of forensic specimens. We can conclude that at present the forensic usefulness of MC1R SNPs is of rather low value, but the growing data on association of particular gene variants with different phenotypic characteristics allow us to optimistically look ahead.
Contact: tkupiec@ies.krakow.pl

O-24

Hair colour in Danish families: Genetic screening of 15 SNPs in the *MC1R* gene by analysis of a multiplexed SBE reaction using capillary electrophoresis or MALDI-TOF MS

Mengel-Jørgensen J[1], Eiberg H[2], Børsting C[1], Morling N[1]

[1]Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark.
[2]Institute of Medical Biochemistry and Genetics, University of Copenhagen, Denmark

Hair, eye and skin pigmentation in humans is a result of the synthesis and the deposition of melanin. The Melanocortin 1 Receptor (MC1R) is an important regulator of melanin synthesis and numerous mutations in the single-exon *MC1R* gene encoding MC1R have been reported. Some of these mutations affect the function of MC1R and they have been found in high frequencies in individuals with red and blond hair. A total of 15 SNPs from the *MC1R* gene were selected: Eight missence mutations (V60L, D84C, V92M, R142H, R151C, R160W, R163Q, D294H), two insertion mutations (179InsC, 29InsA), two silent mutations (P300P, T314T) and three SNPs near the important regulatory element, SP-1, in the *MC1R* promoter (rs3212359, rs3212360, rs3212361). Two PCR strategies were applied. Five short fragments covering 793 bp of the *MC1R* gene were amplified in a multiplex PCR to allow amplification of DNA purified from decomposed samples. Alternatively, a 1,648 bp fragment covering the entire coding region, 626 bp of the promoter and 59 bp downstream of the coding region was amplified with the purpose of determining the haplotypes of selected samples. The 15 SNPs were typed with a multiplexed single base extension reaction and detected by either capillary electrophoresis or MALDI-TOF MS. Examples of *MC1R* SNPs in Danish families with red haired members will be presented. Red haired individuals were typically homozygous for the mutant allele at one locus or compound heterozygous for two of the selected loci.

Contact: Jonas.Mengel@forensic.ku.dk

O-25

Initial Study of Candidate Genes on Chromosome 2 for Relative Hand Skill

Phillips C[1], Barbaro A[1,2], Lareu MV[1], Salas A[1] and Carracedo A[1]

1. Institute of Legal Medicine, University of Santiago de Compostela, Galicia, Spain
2. SIMEF, 4, Via Nicolò da Reggio, Reggio Calabria, Italy

Relative hand skill or handedness (HSR, OMIM: 139900) is a physical characteristic trait that divides people into two groups: one comprising 89-91% of individuals with a preference to use the right hand for complex manipulative tasks (typically handwriting) and the other comprising 9-11% with a left hand preference. Until recently the trait was thought to have a significant environmental component and a low heritability (1) due principally to the repeated observation of discordance for hand skill in half of left handed monozygotic twins studied, together with, often, inadequate measurement of subjects in hand skill studies. However, following the development of the random recessive, non-determinate theory for the genetic control of hand skill and other laterality traits, a robust and predictive model now exists that is consistent with the simple, mendelian inheritance of a single locus. This model implies a recessive allele frequency of ~0.48 based on an observed total of 18% discordant individuals amongst all monozygotic twin groups tested to date (2).
We have used the results of two STR based linkage analysis studies that measured hand skill as a quantitative trait (3, 4) to focus on a 3.5Mb peri-centromeric region of chromosome 2 to search for candidate genes. We aim to refine the linkage signal, initially genotyping a reduced subset of the coding SNPs in 42 genes found in the region defined by the strongest signal previously reported from a limited STR marker set. The SNP genotyping efforts required to scan such a large group of loci are considerable. This workload may be reduced by selecting candidates for study on the basis of probable gene function, SNP allele frequencies, haplotype block distribution and current studies of human: Chimpanzee gene orthology.

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contact: c.phillips@mac.com

O-26

Analysis of inter-specific mitochondrial DNA diversity for accurate species identification

Pereira F[1,2], Meirinhos J[1], Amorim A[1,2], Pereira L[1]
[1] Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Portugal; [2] Faculdade de Ciências, Universidade do Porto, Portugal

Elucidation of several forensic casework studies relies on the precise identification of the species of origin for a variety of biological materials. With the advent of DNA based techniques, this correct identification has become of primary importance in different fields, such as in criminal investigations, food industry, protection of endangered species, etc. Nevertheless, the correct assignment of the biological samples sent to forensic laboratories has frequently proven to be a difficult task due to the high level of degradation and low quality DNA present in many samples as in the case of ancient materials (bone or teeth remains), stomach contents, hair, processed food (dairy products, roasted meat), etc. Several studies demonstrated that the information enclosed in the mitochondrial DNA (mtDNA) is the most useful and reliable tool for species identification, when compared with nuclear DNA based approaches, especially due to the large number of copies in each cell (raising the sensitivity of the analysis) and to the increasing number of sequences available in different databases. Commonly used mtDNA typing systems are based on the PCR amplification of a particular region of this molecule (usually the cytochrome b gene) followed by an RFLP or sequence analysis. However, attention should be paid to some points when using these techniques: (i) RFLP analysis are prone to false results due to undigested PCR fragments; (ii) the use of only one informative region may be not sensitive enough for the correct assignment; (iii) and the very difficult PCR amplification of larger fragments (> 300 bp) in old and/or degraded samples. In this work we attempt to develop a strategy to avoid some of these drawbacks and to produce more sensitive and reliable results for species identification. The first step was the construction of a large database, for different mtDNA regions, using all the available reference sequences for the class *Mammalia* (123 records; http://www.ncbi.nlm.nih.gov/genomes/ORGANELLES/40674.html). The alignment of these sequences allowed the correct identification of the diversity patterns found across the different mtDNA regions. We calculated these patterns of diversity splitting the aligned sequences in consecutive short windows of 100 bp overlapped by 50bp, using a home developed software. This characterisation will be useful for two main objectives: determination of the minimum fragment size suitable for typing highly degraded samples and the identification of regions for primer design. Conserved regions found in a wide range of species will be used for the design of primers that amplify segments containing species-specific information for species identification purpose. On the other hand, inter-specific variable regions are ideal for the design of primers for specific amplification in cases were mixed samples in very different proportions are suspected (a PCR with universal primers would lead to the identification of the most represented species only). Therefore, this information will be particularly useful in the development of a multiplex-PCR of short amplicons (≈100 – 130 bp) in different mtDNA regions for post-sequencing analysis, more informative and suitable for samples with degraded DNA.
Contact: fpereira@ipatimup.pt

O-27

The Development of a DNA Analysis System for Pollen

Eliet J[1] and Harbison S[2]
[1]*Forensic Science Programme, Dep. Chemistry, Univ. Auckland, NZ;* [2]*Forensic Biology, Inst. Environmental Science and Research Ltd, Auckland, New Zealand* (SallyAnn.Harbison@esr.cri.nz)

Pollen is commonly identified as the yellow powdery substance that is found in flowers or released in large amounts from trees such as pines. The use of pollen grains for forensic evidence was established in 1959 and has since been used successfully in many cases. The traditional method of pollen analysis is microscopy where the pollen grains are identified by the distinct patterns of the pollen wall. This is time consuming and requires a trained and experienced palynologist, of which there are few, and an extensive reference collection. This thesis aimed to develop a DNA analysis system for pollen grains extracted from soil that could be applied to forensic samples in casework. The focus on pollen grains recovered from soil was because a number of casework pollen samples are in the form of a soil sample, for example, mud from a shoe. DNA analysis techniques could provide advantages because it is quick and simple. The DNA analysis investigated does not require in depth knowledge of the pollen types and reduces subjectivity associated with human judgment. The DNA analysis technique, terminal restriction fragment length polymorphism (tRFLP), was tested using the plant material from eight different species. Plant material was chosen to test the technique as plant material contains the same genes as pollen and the DNA extraction is relatively simple and effective. The DNA was extracted using a commercially available kit, the DNeasy® Plant Minikit (Qiagen). The tRFLP technique involved amplifying the extracted DNA using primers for the *Adh1* gene, the forward primer labeled with FAM fluorescent dye. The amplification products were precipitated with ethanol prior to digestion with the *Msp1* restriction enzyme. The restricted amplified product was analyzed by capillary electrophoresis on the ABI Prism® 3100 Genetic Analyzer (Applied Biosystems). As only the forward primer was labeled, the 5' terminal restriction fragment was detected by the analyzer and this should be a different size for each different species. The results were interpreted using Genescan 3.7 software. The output values of peak area and fragment length were analyzed using a Euclidean distance measure to compare samples. The analysis showed that the tRFLP technique had good reproducibility as 93% of comparisons between replicates from the same species provided very strong support for them being the same species. The results also showed high discrimination between different species as the electropherogram profiles could be distinguished visually and the statistical analyses showed very high variation values for comparisons between samples from different species. Classification of an unknown as a particular species could be done correctly 97% of the time. A technique using glass bead maceration was found to be suitable for extracting amplifiable DNA from pollen grains. The pollen grain has a very tough wall made from a substance called sporopollenin. The force required to disrupt this wall, such as grinding in liquid nitrogen with mortar and pestle, is often too severe for the DNA to remain intact and results in damaged DNA, unable to be amplified. The glass bead maceration involved the addition of 1mm diameter glass beads to the sample with a sodium buffer and vortexing. This was sufficient to disrupt the pollen wall and release the DNA but did not result in the DNA being damaged. The same tRFLP technique was applied to the pollen DNA extract, as detailed above for the plant material. Analysis of the DNA from five pollen species also indicated good reproducibility of the technique and discrimination between species. Pollen was seeded into soil samples to determine if soil had an effect on the extraction and amplification of DNA from pollen grains present in soil. Many chemicals used to remove pollen from soil for microscopic analysis are very harsh and remove everything inside the pollen grain including the DNA. Therefore, these methods are not suitable when DNA is to be extracted. The method of specific gravity separation with zinc bromide (ZnBr) was used to remove pollen grains from soil, as this was the least invasive method currently used. However, it was found to be unsuitable as the DNA extracted from the pollen grains that had been removed from the soil using ZnBr was degraded and non-amplifiable. An alternative method using sucrose was suggested, as it is less dangerous and should have no harmful effects on the DNA. In summary, the tRFLP technique was a reliable and reproducible technique that provides considerable discriminating power between samples. It will be suitable for application to forensic casework pollen samples after further work to improve the recovery of pollen from soil.

O-28

Characterizing Population Structure

Weir BS

Program in Statistical Genetics, Department of Statistics North Carolina State University, Raleigh NC 27695-7566, USA

The population structure parameter theta, or Fst, is used in forensic match probability equations and also in results for parentage determination and remains identification. Although it describes the relationship among alleles within a population, it does so only with reference to alleles in different populations so that estimation requires data from more than one population. Standard methods for estimating theta provide an average over several populations and do not pay much attention to the sampling distributions of these estimates. A method for estimating population-specific values of theta will be described and illustrated with forensic STR data and for very dense SNP datasets.

Contact: weir@stat.ncsu.edu

O-29

Autosomal Markers for Human Population Identification from Whole Genome SNP Analyses

Kayser M[1], Lao O[1,2], van Duijn JK[1,2], Kersbergen P[2,3], de Knijff P[3]

[1]*Department of Forensic Molecular Biology, Erasmus University Medical Centre Rotterdam, NL*
[2]*Department of Biology, Netherlands Forensic Institute, The Hague, NL*
[3]*Department of Human and Clinical Genetics, Leiden University Medical Centre, Leiden, NL*

Identifying the population of origin (="ethnic origin") of a perpetrator by DNA-analysis of a biological sample found at a crime scene would be highly useful for the police in order to concentrate their investigation on a specific group of individuals for finding an unknown suspect. For this purpose the use of sex-specific inherited Y-chromosomal and mitochondrial DNA markers is suitable due to their high degree of population affinity but the degree of confidence is limited by potential sex-biased genetic admixture. Therefore, autosomal markers are needed in addition to Y and mtDNA markers to identify the population / geographic region of genetic origin of an unknown individual with high degree of certainty.
We will present an approach for identifying informative autosomal markers for human population identification from whole genome SNP analysis. We have applied a whole genome scan including more than 10.000 single nucleotide polymorphisms (SNPs) in a set of globally dispersed human individuals and have used different statistical means to identify markers with maximal performance in population differentiation. Based on this dataset we have identified a small set of autosomal SNPs than can identify major human population groups. In order to test the capacity of those markers in other datasets we have typed them in a different set of human population samples including >50 regions from all over the world.
We want to emphasise that for those human populations showing a strong association with certain physical traits the genetic identification of the population of origin indirectly allows the prediction of externally visible characteristics (e.g. identification of African genetic origin predicts dark skin / hair /eye pigmentation). Thus, genetic markers for population identification, as presented here, will be the first attempt for predicting externally visible characteristics of an unknown individual by means of DNA-analysis, before a direct approach using markers that are functionally responsible for those phenotypic traits might be available in the future.
contact: m.kayser@erasmusmc.nl

O-30

A Compact Population Analysis Test Using 25 SNPs With Highly Diverse Allele Frequency Distributions

Phillips C[1], Sanchez J[2], Fontadevila M[1], Gómez-Tato A[3], Alvarez-Dios[3] J, Calaza M[3], Casares de Cal, M[3], Salas A[1], Ballard D[4], Carracedo A[1] and The SNPforID Consortium[5]

[1]Institute of Legal Medicine, University of Santiago de Compostela, Galicia, Spain; [2]Department of Forensic Genetics, University of Copenhagen, Denmark; [3] Faculty of Mathematics, University of Santiago de Compostela; [4] Department of Haematology, Queen Mary's School of Medicine, London, UK; [5]www.snpforid.org

By selecting a total of 25 SNP loci that exhibit marked contrasts in allele frequency distributions (median highest frequency differential, $\delta = 0.525$ for 21/25 SNPs) in three major population groups: African, European and East Asian, we have developed a multiplex PCR assay and web based analysis tool that provides a predicted population of origin for a sample of unknown source. The genotyping assay was designed to use a single tube PCR and primer extension reaction and to be sensitive enough for routine forensic analysis. Appropriate markers were chosen from previously collected groups of population specific SNPs and non-binary SNPs (1, 2), from published ancestry informative marker sets, and from scrutiny of genes known to have been subject to diversifying selection in the recent evolutionary history of the population groups under study; e.g. FY in Africans and LCT in Europeans (3). The 25 SNPs comprising the final set were carefully selected to ensure as wide a distribution in autosomes as possible, maximising the potential for segregation of each marker. This is an important aspect of any population analysis test examining urban populations, since it can be expected that a large proportion of individuals from highly admixed populations or of immediate mixed descent (i.e. parental or grandparental), if undetected, would be incorrectly assigned to one of the contributing population groups.

SNP profiles generated from the genotyping assay can be submitted and analyzed with an open access web portal that uses a probability ratio approach based on the assumption of random variable independence for all markers. Three samples of 90 individuals each from Mozambique, Spain and Taiwan were used as training sets for the classification algorithm used. The error rate for a three population group classification was been estimated to be 2% from modelling (cross validation and bootstrapping) and below 1% from analysis of new profiles obtained from a different sample population in each group (60 Somali, Danish and Chinese samples).

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contact: c.phillips@mac.com

O-31

A Bayes net solution that simulates the entire DNA process associated with analysis of short tandem repeat loci

Peter Gill[1], James Curran[2], Keith Elliot[1]

[1]Forensic Science Service, Trident Court, 2960 Solihull Parkway, Birmingham, UK
[2]Department of Statistics, University of Waikato, New Zealand.

The use of expert systems to interpret short tandem repeat (STR) DNA profiles in forensic, medical and ancient DNA applications is becoming increasingly prevalent as high-throughput analytical systems generate large amounts of data that are time-consuming to process. With special reference to low copy number (LCN) applications we use a graphical model to simulate stochastic variation associated with the entire DNA process starting with extraction of sample, followed by the processing associated with preparation of a PCR reaction mix, and PCR itself. Each part of the process is modelled with input efficiency parameters (π). Then, the key output parameters that define the characteristics of a DNA profile are derived - namely heterozygous balance (*Hb)* and allele dropout *p(D)*. The model can be used to estimate unknown efficiency parameters such as $\pi_{Extraction}$. 'What-if' scenarios can be used to improve and optimise the entire process - e.g. by increasing the aliquot forwarded to PCR the improvement expected to a given DNA profile can be reliably predicted. We demonstrate that heterozygote balance and dropout are mainly a function of stochastic effect of pre-PCR molecular selection and can be predicted relative to the quantity of DNA analysed. For mixture analysis, we show that the method is much more powerful than others suggested, since we simulate at the molecular level, without having to make assumptions based on a collection of output data (which may be unrepresentative). We also show that whole genome amplification is unlikely to give any benefit over conventional PCR for LCN as there is no theoretical basis.

Contact: DNAPGill@compuserve.com

O-32

Maximisation of STR DNA typing success for touched objects

Prinz M, Schiffner L, Sebestyen J, Bajda E, Tamariz J, Shaler R, Baum H, Caragine T

Department of Forensic Biology, Office of Chief Medical Examiner, New York, NY

In order to produce database eligible DNA profiles from touched objects each individual step leading up to a DNA type was evaluated and optimized. The procedures were tested on fingerprints deposited on a variety of substrates, touched objects such as pens and credit cards, purified human embryonic kidney (HEK) cells with defined cell counts and diluted DNA from buccal swabs and other body fluids. For the initial swabbing several types of swabs and solutions were compared. DNA recovery was better for cotton fabric than for the conventional twisted thread cotton or Dacron swabs, while a 0.01% SDS solution performed better than water or other buffers. For the extraction, it was found that simple procedures with fewer steps were superior to commercial kits, such as DNA IQ™ (Promega, Madison, WI) and QiaAmp (Qiagen, Valencia, CA), and other protocols with many manipulations. The optimized protocol included a thirty-minute incubation with 0.01% SDS and proteinase K at 56°C, followed by an incubation at 100°C for 10 minutes. Concentration of the extract and removal of the SDS was accomplished through centrifugation with a Microcon 100 (Millipore, Bedford, MA) column. The addition of 1ng Poly A RNA to the Microcon significantly improved DNA recovery. Samples were quantitated on a Rotorgene 3000 (Biotage) using an ALU repeat based real time DNA quantitation procedure as described by Nicklas and Buel (1). Based on work presented by Whitaker et al (2) samples were amplified in triplicate, with a minimum of 6.2 pg of DNA per amplification. Database compatible commercial megaplex kits were used for the amplification. For the Identifiler kit (Applied Biosystems, Foster City, CA) the annealing time was increased from 1 to 2 minutes and the cycle number was raised to 31 cycles. Initial experiments also involved Profiler Plus kits (Applied Biosystems, Foster City, CA) and the Poweplex 16 kit (Promega, Madison, WI). 6µL of amplified product were mixed with 15µLHiDi Formamide and 0.375µL LIZ size standard and analyzed on the 3100 Genetic Analzyer (all Applied Biosystems, Foster City, CA). Injection conditions were adjusted based on DNA input and three different conditions are being used routinely: 1kV 22 seconds, 3kV 20 seconds and 6 kV 30 seconds. Samples around 100pg gave the best results with 1kV 22 seconds, while 50 and 25pg samples were optimal at 3kV 20 seconds. The high injection conditions of 6kV, 30 seconds do result in broader peak shapes and but can be useful for the identification of low peaks. Peak intensities were maximised by not using variable binning and by setting the baseline window to 251. Data were analyzed empoying a minimum threshold of 75RFU. Alleles are only included in the interpretation if the allele is present in at least two of the three amplifications (2). The high injection conditions distort the expected peak intensities for low DNA amounts, therefore stochastic effects and allelic drop out events have to be newly characterized. Overall the increased cycle number and higher injection conditions allow reproducible DNA testing down to 20pg of DNA. For DNA dilutions, 25 pg routinely resulted in full profiles. For the touched objects, 78% of the 20pg to 100pg samples yielded database eligible profiles; the other samples were either mixtures or contained an insufficient number of allele calls. Here, the three amplification approach was crucial and yielded more complete profiles with more confidence in the allele calls. DNA amounts below 20pg did show partial profiles with correct allele calls that could have been compared in a specific case but were too incomplete for database entry.

Nicklas JA., and Buel E (2003) J. of Forensic Science 48: 282-291.
Whitaker JP, Cotton EA, and Gill P (2001) Forensic Sci.Int. 123: 215-223.

Contact: mprinz@nyc.rr.com

O-33

Multi-substrata analysis on Siberian mummies: A different way for validation in ancient DNA studies?

Amory S [1,2], Keyser-Tracqui C [1,2], Crubézy E [2], Ludes B [1,2].

[1] Laboratoire d'Anthropologie Moléculaire, Institut de Médecine Légale, Strasbourg Cedex, France; [2] Laboratoire d'Anthropobiologie, Université Paul Sabatier, CNRS, UMR 8555, Toulouse, France 31000.

Ancient DNA results are always submitted to caution due to the technical difficulties induced by the minute amounts, the degraded nature of the template and the high risk of contamination. A list of criteria of validation has been published as a guideline for ancient DNA researchers[1], including a dedicated and separated work area, controlled amplification, reproducibility of the results, etc… In addition to these criteria, the analysis of different substrates: bones, teeth and hairs of the same individual could be another way to ensure the reliability of the results.

This study presents the first results obtained on bones, molar teeth and hairs of two Siberian samples dated from the 18th Century. Thus, the grave of Munur Urek, a burial site of an important clan chief and the multiple grave of the "Chamanic tree" site, gave us the opportunity to sample these different type of substrates. These two subjects excavated from frozen graves, were mummified. This exceptional state of preservation allowed us to test the amplification of autosomal and Y chromosome STRs and the sequencing of the HVI region on the three types of substrates. All experiments were done in a dedicated laboratory and negative controls were run for each step. The persons in contact with the samples were typed for the same markers in order to determine exogenous contamination.

This method permitted the identification of artefacts on STRs profiles, common when working with Low Copy Number amounts of DNA. Indeed, the comparison of the profiles obtained for bones and teeth highlights allelic dropouts and spurious alleles for the bone samples.

The possibility to compare results from different substrates, in spite of the limited numbers of possible cases, represent another, and interesting, criterion to confirm the authenticity of ancient DNA results.

[1]*Cooper A, Poinar HN. Ancient DNA: Do It Right or Not at All. 2000. Science, Vol 289, Issue 5482, 1139.*

Contact: Sylvain.Amory@iml-ulp.u-strasbg.fr

POSTER PRESENTATIONS

ABSTRACTS

P-001

Complex Paternity investigations: The need for more genetical information

Abrantes D[1], Pontes ML[1], Lima G[1], Cainé L[1], Pereira MJ[1], Matos P[1], Pinheiro MF[1,2]

[1]*Instituto Nacional de Medicina Legal – Delegação do Porto*
[2]*Faculdade de Ciências da Saúde – Universidade Fernando Pessoa*

In the past few years our laboratory has been registering a raising demand of difficult parentage investigations, namely those performed in the absence of the putative father's genetical data. Due to the also increasing complexity of the aforementioned investigation cases themselves, the results provided by the routinely used commercial kits AmpFℓSTR® Identifiler™ (Applied Biosystems) and Powerplex® 16 (Promega), in our laboratory, even in conjunction with the complementary commercial kit Powerplex® ES Monoplex System (SE33), are no longer always satisfactory. Therefore, there is an urgent need for more (preferably) easily- and rapidly-analysable markers. In this sense, our laboratory resorted to the long-time commercialized kit Gene Print® Fluorescent STR Systems FFFL Multiplex (Promega), which allows for the co-amplification of four more STR-*loci* (F13A01, FES/FPS, F13B e LPL) and, thus, for the acquisition of the required supplementary genetical data. In this work, we describe several cases to whose solution the FFFL data were crucial. We also report the allelic frequencies and some parameters of forensic interest, relative to FFFL *loci*, for the Northern Portuguese population.

Contact: Biologia@dpinml.mj.pt

P-002

Accurate mtDNA mixture quantification using the Pyrosequencing technology

Allen M, Nilsson M, Andréasson H
Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Sweden

Analysis of mtDNA variation using Sanger sequencing does not allow accurate quantification of mtDNA mixtures. Thus, a method to determine the specific mixture ratios in samples displaying heteroplasmy, consisting of DNA contribution from several individuals or containing contamination would be valuable. In this study, a novel quantification system for mtDNA mixture analysis is described. The assay is based on pyrosequencing technology, in which the linear relationship between incorporated nucleotides and released light allows accurate quantification. The routinely applied Sanger sequence analysis of mtDNA is robust and in most cases successful due to the high copy number of mtDNA per cell. However, occasionally samples show a DNA mixture as a consequence of multiple contributors, heteroplasmy or contamination. In contrast to STR analysis, quantification of mixed samples based on mtDNA sequence analysis is not feasible using the current sequencing methodology. The ABI PRISM® BigDye™ Primer Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) is commonly used for mtDNA analysis. Although this chemistry easily and effectively determines the sequence in single source samples, the uneven peak heights and sequence-dependent variation in dideoxynucleotide incorporation efficiency prevent quantification of mixtures. Thus mixtures can be detected and visualised using Sanger sequencing, but the information cannot be used to determine the exact quantities of the different mitochondrial types and in most cases the results are called as inconclusive. Resolution of mtDNA mixtures has been demonstrated previously using alternative technologies such as cloning and denaturing high-performance liquid chromatography. However, in order to resolve and accurately quantify multiple individuals contributing to a sample in equal or unequal ratios, an easy to use quantitative assay would be useful. Pyrosequencing is a technology based on the release of pyrophosphate during strand elongation, producing light. The light signal is proportional to incorporated nucleotides, allowing allele quantification utilising PSQ™96MA SNP Software (Version 2.02, Biotage, Uppsala, Sweden). The allele quantification capability has been previously used in a number of studies, including allele frequency measurements in pooled DNA samples and quantitative analysis of methylation status at CpG islands. Other studies involve gene copy number measurements and determination of allele-specific transcript expression. In this study, a subset of PCR fragments previously developed for a fast and simple pyrosequencing analysis of variation in the mtDNA control and coding region, were used for pyrosequencing based quantification. Seven polymorphic sites, three in the control region and four in the coding region, within five PCR fragments, were selected and successfully used for mtDNA mixture quantification. For all SNPs quantified in this study, a linear relationship was observed between measured and expected mixture ratios. The average standard deviations of each of the seven SNPs fell within the expected 1-2% (for 10 replicate reactions). In conclusion, this mtDNA mixture quantification system is an alternative application of the pyrosequencing technology and is useful in forensic DNA analysis. Pyrosequencing has been shown to provide a very rapid, accurate and easy to use quantification system that can be used in forensic casework investigations to resolve and interpret major and minor mtDNA contribution from multiple individuals, determine heteroplasmy ratios and monitor contamination.marie.allen@genpat.uu.se

P-003

Reducing mtDNA sequencing efforts by half in forensic casework

Allen M, Nilsson M, Calloway C, Divne A-M

Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Sweden

Mitochondrial DNA (mtDNA) sequencing can be time-consuming and laborious, limitations that can be minimized using a faster typing assay. The LINEAR ARRAY mtDNA HVI/HVII Region-Sequence Typing Kit (Roche Applied Science) uses sequence-specific probes immobilized in 31 lines. Linear array typing of mtDNA polymorphisms is a simple and fast pre-screening method with potential to substantially reduce sequencing efforts due to exclusion of samples. The analysis is performed in less than 3 hours without the need for expensive equipment.

Over a five-year period more than 300 forensic samples have been successfully typed for mtDNA polymorphisms using these linear arrays in our laboratory. A majority of the specimens that were analyzed using the combined HVI/HVII linear array were shed hairs. However, previous use of the HVII linear array has been successful on samples obtained from a variety of items, such as epithelial cells collected from areas in close contact with the skin. Furthermore, successful linear HVI/HVII array typing results have been obtained in a cold case investigation of 15-years old hair samples, one shed head hair (3 cm long) and two reference hairs.

A high sensitivity in the assay was shown by typing of TaqMan quantified DNA samples with limited amounts. Successful and reliable results were obtained from three centimetre pieces of distal shaft parts of shed and plucked hairs. Moreover, strong and easily interpretable array signals were obtained from control samples containing 100 -10 000 mtDNA copies, equivalent to 0.6 pg to 60 pg of genomic DNA (333 genome equivalents/ng DNA) indicating a highly sensitive typing system.

The exclusion capacity has been evaluated by a retrospective study of 90 previously HVI/HVII sequenced samples (57 evidence samples and 33 reference samples) from 16 forensic cases. Using the HVI/HVII mtDNA linear array, 56% of the samples were excluded and thus less than half of the samples require further sequencing due to a match or inconclusive results. Of all the samples that were excluded by sequence analysis, 79% could be excluded using the HVI/HVII linear array alone.

The use of the mtDNA linear arrays in our laboratory has served as a valuable pre-screening method and demonstrates the potential to reduce the required sequencing efforts by more than half. Thus, this rapid and user-friendly linear array typing system provides a convenient and efficient pre-screening method for selection of the samples of most interest for further investigation.

contact: marie.allen@genpat.uu.se

P-004

The Amelogenin locus displays a high frequency of X homologue failures in São Tomé island (West Africa)

Alves C[1], Coelho M[1], Rocha J[1,2], Amorim A[1,2]

[1] *IPATIMUP, R. Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal*

[2] *Faculdade de Ciências da Universidade do Porto, Pr. Gomes Teixeira, 4009-002 Porto, Portugal*

A multiplex STR study using the Powerplex 16 System (Promega) in 503 unrelated individuals from the island of São Tomé (Gulf of Guinea, West Africa) revealed 10 male individuals presenting only the Y homologue of the Amelogenin locus (~2%). These individuals were further typed with other commercial kits which also amplify the Amelogenin locus, namely the AmpFLSTR Identifler (Applied Biosystems) and Y-Plex™12 (Reliagene) kits, and an X/Y genotype was only obtained with the primers used in Y-Plex™12.

Although this X Amelogenin drop-out was only detected in males, this does not rule out the fact that females may also carry it. Since women have two X chromosomes, in some instances it could be suspected that an X failure was also present in females, by simple observation of differences in electrophoregram peak heights, in comparison with XY profiles. Although we cannot objectively consider these apparent nulls in females for frequency estimate purposes, there is no doubt of its magnitude in this population. With a 2% frequency in males, it is expected that the frequency of female carriers and homozygotes will be 3.92% and 0.04%, respectively. It is also noteworthy that the previously reported frequency for X Amelogenin null (which was estimated in Caucasians) was much lower (0.3%).

Failure to amplify alleles in the Amelogenin locus has been described before, mainly in cases where the Y homologue fails, which can have critical consequences in forensic casework. Cases where the X counterpart fails to amplify, as described here, are not of fundamental significance in forensic genetics, since there is no danger of a male individual being mistaken for a female one. However, this can have a different impact in other fields, such as in prenatal diagnosis of certain XY chromosome abnormalities, like XXY, using quantitative assays.

The high frequency of amplification failures already detected for either the X or Y chromosome Amelogenin locus, only draws our attention more to the need for caution when applying solely the amelogenin test for sex determination.

Sequencing of the X Amelogenin allele responsible for the amplification failure in the 10 male individuals is undergoing.

Contact: calves@ipatimup.pt

P-005

Making the most of Y-STR haplotypes. The HapYDive

Alves C[1], Gusmão L[1], Meirinhos J[1], Amorim A[1,2]
[1] IPATIMUP, R. Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal; [2] Faculdade de Ciências da Universidade do Porto, Pr. Gomes Teixeira, 4009-002 Porto, Portugal

Since the informative power of a Y-STR marker can only be recognised in a haplotype context, a software was devised to evaluate the increase of haplotype diversity (HD) by the addition of any combination of markers to a fixed number of markers. The first version of the program was quite limited and not very user-friendly. The HapYDive is the latest version, created in Excel format (available at www.ipatimup.pt/app/). It´s not only a software for Y-STR HD calculation but, more importantly, it allows the determination of which combination of Y-STRs is the most informative in a certain population sample. With the HapYDive it is possible to analyse any set of Y markers up to a maximum of 20, with a minimum number of 4 markers fixed for calculations.

As an example, let's consider the fixed set of Y-STRs currently used in the "YHRD - Y Chromosome Haplotype Reference Database" (http://www.yhrd.org), comprising the "minimal haplotype" markers (9 loci) plus DYS438 and DYS439. Depending on the population sample, these 11 loci together will have a certain HD value. Which set and what number of the other available Y-STRs will increase more rapidly the HD value?

Applying the HapYDive program to a population sample from Portugal (N=657) with haplotypes containing the 11 Y-STRs plus DYS437, DYS460, DYS461, DYS635, GATA A10 and GATA H4, the best order of markers is shown in Table 1. In this sample, all the other Y-STRs contribute to a certain degree to an increment of HD, but DYS460 contributes the most and DYS635 the least.

However, in other samples, particularly in those from different population groups, one or more Y-STRs may not contribute in any way, and the order in which they´ll contribute more may be quite different. For example, by applying the HapYDive to a population sample from Mozambique (N=112) using the same markers, the best order is shown in Table 2. In this case, the order is different and there is one marker, DYS437, that does not contribute in any way to a higher HD.

Apart from applying this program to different sample origins and to different sets of Y-STR markers (namely from the recent commercial kit Yfiler from Applied Biosystems), it is also worth studying the effect of sample size. This study is still undergoing and a discussion of the results will be shown. Contact: calves@ipatimup.pt

Table 1
(Portugal)

Y-STR sets	HD
11 Y-STRs	0.99771
11 Y-STRs + DYS460	0.99866
12 Y-STRs + GATA H4	0.99915
13 Y-STRs + GATA A10	0.99936
14 Y-STRs + DYS461	0.99950
15 Y-STRs + DYS437	0.99960
16 Y-STRs + DYS635	0.99966

Table 2
(Mozambique)

Y-STR sets	HD
11 Y-STRs	0.99212
11 Y-STRs + GATA A10	0.99437
12 Y-STRs + DYS460	0.99582
13 Y-STRs + DYS635	0.99646
14 Y-STRs + DYS461	0.99695
15 Y-STRs + GATA H4	0.99727
16 Y-STRs + DYS437	0.99727

P-006

Estimating the post-mortem interval (I) The use of genetic markers to aid in identification of Dipteran species and subpopulations

Ames C, Turner B, Daniel B

Department of Forensic Science and Drug Monitoring, King's College London, UK

Insect evidence can be utilised in a forensic investigation in a variety of ways. For instance, insects are most commonly used to help in the estimation of time since death of a discovered corpse. With a knowledge of recent environmental conditions of the scene of crime, an entomologist can predict how long it has taken for any insects present to have reached their particular developmental stage and hence the minimum time since death. The insect species present on a corpse will also indicate the time since death as insect species colonise a carcass in a distinct succession. Some insects have distinct geographical distributions, their presence outside of their normal habitat could indicate post mortem movement of a corpse or link a suspect to a scene of crime. Their lifecycles are seasonal and presence of insects can therefore indicate the time of year a crime occurred. The presence of drugs or poisons within feeding insects may give an indication of cause of death of a body.

All forensic entomology techniques depend upon accurate identification of insect species. At present this is mainly based upon morphological differences between species. This can be difficult as the early lifecycle stages of many forensically important Dipteran species are very hard to distinguish.

One aim of this work was to use DNA molecular markers to help in identification of forensically important fly species and ultimately populations within the UK.

Wild populations of *Calliphora vicina* and *Calliphora vomitoria* (Diptera: Calliphoridae) caught from various locations around the UK were raised and maintained in the laboratory. Both these species are early corpse invaders in the United Kingdom. To ensure the identity of both species before experimental work began, they were characterised morphologically using a key (Smith 1986). DNA was extracted from adults and larval forms. Regions of both the nuclear (xanthine dehydrogenase exon 2) and mitochondrial (cytochrome oxidase I and the control region) genomes were amplified using PCR and then sequenced or digested using restriction enzymes.

These molecular markers have been shown to contain both interspecific and intra specific variation and thus can be used to distinguish between the two species and also between English populations of both species.

Contact: carole.ames@kcl.ac.uk

P-007

Estimating the post-mortem interval (II) The use of differential temporal gene expression to determine the age of blowfly pupae

Ames C, Turner B, Daniel B
Department of Forensic Science and Drug Monitoring, King's College London, UK

Insect evidence can be utilised in a forensic investigation in a variety of ways. For instance, insects are most commonly used to help in the estimation of time since death of a discovered corpse. With a knowledge of recent environmental conditions of the scene of crime, an entomologist can predict how long it has taken for any insects present to have reached their particular developmental stage and hence the minimum time since death. The insect species present on a corpse will also indicate the time since death as insect species colonise a carcass in a distinct succession. Some insects have distinct geographical distributions, their presence outside of their normal habitat could indicate post mortem movement of a corpse or link a suspect to a scene of crime. Their lifecycles are seasonal and presence of insects can therefore indicate the time of year a crime occurred. The presence of drugs or poisons within feeding insects may give an indication of cause of death of a body.
To establish time since death, an entomologist requires accurate assessment of the age of insects discovered associated with a corpse. At present this is done using morphological features or biometric characteristics such as length or weight. The aim of this work was to use molecular techniques to determine the age of immature forms of forensically important fly species. Throughout the developmental lifecycle of insects different genes will be expressed at specific time points. Once identified these temporally expressed genes could provide markers as to the age of an insect.

Wild populations of *Calliphora vicina* (Diptera: Calliphoridae) were maintained in the laboratory. This species is an early corpse invader in the United Kingdom. Initially the pupal stage would be focussed upon. Adult females were encouraged to lay eggs and this was taken as 'time zero'. Eggs were placed at a constant 20□C until the pupal stage. At specific timepoints total RNA was extracted from pupal samples. The extracted mRNA was reverse transcribed to cDNA. Potential markers were located either from the use of differential display techniques (DD) or from the literature. DD is a method that detects changes in gene expression between samples by the random amplification of cDNA. Fragments are visualised on a gel and differences in banding pattern can be focussed upon.
Once potential markers were located their expression in differently aged pupal samples was quantified using Real-time PCR. The results indicated that this is a viable method for age determination of Dipteran immature stages.
Contact: carole.ames@kcl.ac.uk

P-008

Extended Northern Portuguese database on 21 autosomal STRs used in genetic identification

Amorim A[1,2], Alves C[1], Gusmão L[1], Pereira L[1]
[1]IPATIMUP, R. Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal;[2]Faculdade de Ciências da Universidade do Porto, Pr. Gomes Teixeira, 4009-002 Porto, Portugal

Routine casework in our genetic identification laboratory is carried out with two commercially available autosomal STR kits, namely Identifiler (Applied Biosystems) and Powerplex 16 (Promega), which together amplify a total of 17 STR loci. In some instances, namely in deficient paternity cases, we can also count on an in-house multiplex system which amplifies 4 more loci, totalling 21 STRs. Along the years, we have accumulated population frequency data in our database, namely from individuals residing in Northern Portugal, which we are now presenting extensively, together with parameters of forensic interest. This is also the first time we are presenting data on both D2S1338 and D19S433. The following table summarises our results:

	CD4 (N=382)	CSF1PO (N=1825)	D2S1338 (N=760)	D3S1358 (N=1816)	D5S818 (N=1816)	D7S820 (N=1809)	D8S1179 (N=1822)
Ho	0.696	0.731	0.833	0.784	0.702	0.794	0.808
He	0.705	0.724	0.877	0.787	0.702	0.810	0.811
PD	0.855	0.872	0.973	0.921	0.859	0.937	0.941
CE	0.422	0.452	0.741	0.556	0.436	0.601	0.616
P	0.929	0.496	0.798	0.754	0.597	0.112	0.034

	D13S317 (N=1818)	D16S539 (N=1283)	D18S51 (N=1810)	D19S433 (N=761)	D21S11 (N=1817)	F13A01 (N=484)	FES (N=487)
Ho	0.773	0.760	0.891	0.806	0.843	0.711	0.700
He	0.782	0.776	0.876	0.794	0.843	0.754	0.700
PD	0.922	0.915	0.972	0.930	0.957	0.899	0.852
CE	0.566	0.546	0.733	0.587	0.673	0.509	0.417
P	0.495	0.760	0.919	0.820	0.552	0.098	0.818

	FGA (N=1833)	MBPB (N=371)	Penta D (N=1280)	Penta E (N=1281)	TH01 (N=2403)	TPO (N=2402)	VWA (N=2303)
Ho	0.857	0.741	0.837	0.899	0.783	0.639	0.804
He	0.866	0.728	0.839	0.885	0.796	0.648	0.810
PD	0.967	0.879	0.953	0.976	0.927	0.823	0.937
CE	0.712	0.469	0.657	0.755	0.570	0.386	0.602
P	0.316	0.360	0.015	0.786	0.658	0.691	0.915

N: nº individuals; Ho: observed heterozygosity; He: expected heterozygosity according to Nei; PD: power of discrimination; CE: a priori chance of exclusion; P: Hardy-Weinberg equilibrium, exact test based on more than 2000 shufflings, for standard error <0.01.

Deviations from Hardy-Weinberg equilibrium were detected in D8S1179 and Penta D loci, but applying the Bonferroni correction for the number of loci analysed, the departure in both loci was not significant (0.05/21=0.0024).
Both commercial STR kits share 13 loci but use different primer pairs, and so genotype inconsistencies may occur. For individuals genotyped as homozygotes with one kit and as heterozygotes with the other, the latter genotype was the one considered.
The overall matching probability for the 21 STRs in our population sample is of 1 in 1.56 x 10^{24} individuals, and combined power of exclusion of 0.9999999914.
aamorim@ipatimup.pt

P-009

Evaluation of the 11 Y-STR loci in the PowerPlex® Y-system;
Experience from analyses of single male samples and simple male: male mixtures.

Andreassen R, Heitman IK

Institute of Forensic Medicine, University of Oslo, Norway

Interpretation of sample mixtures requires knowledge about the efficiency of the system. In this study we have measured the amount of stutter and pull-up in 100 male samples analysed at 11 Y-STR loci (DYS 391, DYS389I/II, DYS 439, DYS 393, DYS 390, DYS 385, DYS 438, DYS 437, DYS 19, DYS 392) using the PowerPlex® Y-system as described by the manufacturer. The proportion of stutter differed among the loci in the multiplex-kit and was associated with allele length (number of repeats) and repeat size (3-5 basepairs). The best performing locus was DYS 438 while largest proportion of stutter was observed in locus DYS 389II. Area of stutter was less than 0.2 for all loci tested. Both stutter in position N-2 and N+1 was observed at certain loci. No pull up larger than 0.1 was observed in the loci analysed. Locus DYS 393 was amplified less efficient than other loci in the multiplex mix giving alleles with low peak heights compared to the others. Efficiency for each Y-STR locus will be presented in detail. Fourteen samples from males with known Y-haplotypes were used to compose male:male mixtures. Mixture ratios varied within the interval 1:3 to 1:1. A total of sixty-five samples were analysed. In each sample the peak areas of alleles were used to type a "minor" and a "major" Y-haplotype consisting of all minor alleles or major alleles, respectively, at loci with two alleles. The results from this exercise were compared with the two known Y-haplotypes in each sample. Disregarding locus DYS 393 and DYS 385 a/b, the two Y-haplotypes in a sample was correctly typed in all samples with a relative peak area difference (average of peak areas of minor alleles / peak area of major alleles) less than 0.6. The results from this simple test indicate that peak area of alleles in PowerPlex® Y-system provides quantitative information that might be used to interpret the most likely Y-haplotypes in a simple male:male mixture.

E-mail: r.j.andreassen@medisin.uio.no

P-010

Icelandic population data for the 10 autosomal STR loci in the AMP*Fl*STR®SGM Plus™ system and the 11 Y-STR loci in the PowerPlex® Y-system

Andreassen R, Heitman IK, Hansen L, Mevaag B

Institute of Forensic Medicine, University of Oslo, Norway

Autosomal STR polymorphisms at 10 loci (D3S1358, vWA, D16S539, D21S1338, D8S1179, D21S11, D18S51, D19S433, TH01 and FGA) and Y-STR polymorphisms at 11 loci (DYS 391, DYS389I/II, DYS 439, DYS 393, DYS 390, DYS 385, DYS 438, DYS 437, DYS 19, DYS 392) are presented. Samples from a population material of unrelated individuals from Iceland were analysed using the AMP*Fl*STR®SGM Plus™ system (n=110) and the PowerPlex® Y-system (n=76) as described by the manufacturer. For the autosomal polymorphisms the observed heterozygosities ranged from 0,764 (vWA) to 0,891 (FGA). No significant deviation from Hardy-Weinberg equilibrium was observed. For the Y-chromosome polymorphisms 62 different haplotypes were observed in the 76 male samples analysed. No haplotype was observed more than three times in the population sample.

Locus diversity, allele distributions and other relevant forensic genetic parameters will be presented in detail.

E-mail: r.j.andreassen@medisin.uio.no

P-011

Low Copy Number: interpretation of evidence results

Anjos MJ[1], Andrade L[1], Carvalho M[1], Lopes V[1], Serra A[1], Oliveira C[1], Balsa F[1], Brito P[1], Corte-Real F[2], Vide MC[1]

[1] Forensic Genetic Service. National Institute of Legal Medicine. Largo da Sé Nova, 300 Coimbra. Portugal
[2] National Institute of Legal Medicine. Largo da Sé Nova, 300 Coimbra. Portugal
geneforense@dcinml.mj.pt

Evidence of crime scene and samples from decomposed or skeletal remains have in many cases, low amounts or degraded DNA.

The choice of extraction protocols as well as sensitive and robust STR's is crucial to obtain good results but in many situations is not enough; it is necessary to make protocol adaptations of amplification instructions even when are used commercial kits.

One of the most common modifications is changing the number of cycles in the amplification protocol, with an increment of 4, 6 or more cycles. However in some cases there are difficulties in interpreting results because extra peaks appear or an imbalance between them seems to have no sense.

In this work we show some cases where changes of technical approaches produced better results and others where we had problems in interpreting evidence results.

Contact: geneforense@dcinml.mj.pt

P-012

Amelogenin as a Target for Real Time PCR Quantitation of Forensic Templates

Anwar N, Goodman M, Hulme P, Elsmore P, Greenhalgh M and McKeown B

Orchid BioSciences (Europe) Ltd, Abingdon, UK

PCR is the ubiquitous method of forensic DNA analysis, but prior to amplification, two other processes are crucial to obtaining a satisfactory result: template DNA extraction and template quantitation. Here we will presume the extraction process has been performed, and concentrate on the quantitation step: a focus of recent advances. Real time quantitative PCR (RT-QPCR) is an advantageous alternative to probe hybridisation or fluorescent dye association, which are (respectively) laborious and less accurate procedures. We reviewed the available commercial methods of RT-QPCR and concluded that for our requirements, a more attractive solution was the in-house development and validation of an ultra-rapid, small batch size solution. Our solution is real time detection using the Roche LightCycler 2.0 and the amplification of a 106/112bp amelogenin amplicon. Melt-curve analysis and back extrapolation to the starting template-dependant crossing point generates results from 32 samples in ~30 minutes (post PCR assembly) and this approach has advantages in that a positive quantitation result implies that in the SGM Plus™ amplification that follows, at the very least, an amelogenin product should be generated. The use of the amelogenin target also provides an indication of the possibility of PCR product travelling from the separate PCR product room backwards into the clean PCR set-up environment, something that the use of telomerase or β-globin amplicons cannot provide. We have validated the use of our LightCycler-amelogenin based quantitation system and have seen significant improvements in the reliability of quantitation measurements in our forensic laboratories.

email: nanwar@orchid.co.uk , bmckeown@orchid.co.uk

P-013

Population study of small-sized short tandem repeat in Japan and its application to analysis of degraded samples

Asamura H, Tsukada K, Ota M, Sakai H, Takayanagi K, Kobayashi K, Saito S, Fukushima H

Department of Legal Medicine, Shinshu University School of Medicine

Analysis of short tandem repeat (STR) is the most valuable tool for the clarification of personal identity. Recently, several commercial multiplex STR kits are regularly used in forensic practice. However, for some highly degraded samples, analysis of STR by means of the commercial STR kits is practically impossible due to DNA fragmentation and occurrence of PCR inhibitors. Some papers reported that smaller–sized PCR products were effective in analyzing such highly degraded samples. We previously performed multiplex PCR for the TH01, TPOX, CSF1PO, and vWA loci using a newly designed pair of primers that yield smaller fragment, and reported several successful analyses of the degraded samples. In this study the six mini-STR loci (D1S1677, D2S441, D4S2364, D10S1248, D14S1434, and D22S1045), which Coble et al. reported, were investigated in Japanese population. As the result, analysis with use of the six loci demonstrated the moderate degree of polymorphisms in Japanese population. Moreover, it was confirmed that these six loci assays for typing degraded samples were more successful than those of commercial STR kits. Consequently, it was considered that combination analyses of the six mini-STR loci and the four loci, which we previously reported, are highly beneficial in the context of Japanese forensic practice from degraded DNA samples.

Contact: asamura@sch.md.shinshu-u.ac.jp

P-014

Allele distribution of two X chromosomal STR loci in a population of Sicily (Southern Italy)

Asmundo A., Perri F., Sapienza D.

Dipartimento di medicina sociale del territorio, Sezione di medicina legale, Università di Messina, Azienda Ospedaliera Universitaria "Policlinico G. Martino", Via Consolare Valeria 98125 Messina, Italy

Population genetic data for two X-chromosomal STR loci (DXS7423 and DXS9902) were obtained by analysing a population sample (n= 60 males and 60 females) and 43 family trios (showing a probability of paternity and maternity > 99.9%) from Sicily (Southern Italy) by using PCR and PAGE followed by silver staining. Five and four different alleles of DXS7423 and DXS9902 loci were detected, respectively. The allele frequencies of both ChrX markers were in good agreement with Hardy-Weinberg equilibrium. The analysis of the family trios, based on the investigated meiotic events, showed no mutation. The observed eterozigosity of DXS7423 and DXS9902, together with other forensic parameters were determined, so confirmating that these markers are useful tools for parentage testing, mainly in deficiency paternity cases when the disputed son is female.

Alessio.Asmundo@unime.it

P-015

Forensic evaluation of three closely linked STR markers in a 13 kb region at Xp11.23

Augustin C[1], Cichy R[1], Hering S[2], Edelmann J[3], Kuhlisch E[4], Szibor R[5]

[1]*Institute of Legal Medicine, University of Hamburg, Germany*
[2]*Institute of Legal Medicine, Technical University of Dresden, Germany*
[3]*Institute of Legal Medicine, University of Leipzig, Germany*
[4] *Institut für Medizinische Informatik und Biometrie, Technical University of Dresden, Germany*
[5]*Institute of Legal Medicine, University of Magdeburg, Germany*

Searching for markers located in the Xp11 region the sequence of the clone AF196972 was checked for its content of microsatellites. Two tetranucleotide STRs and one trinucleodide STR were tested in respect of their forensic efficiency and registered in the GDB as DXS10076, DXS10077 and DXS10078.
DXS10076 is located 48 065.564 - 48 065.759 kb from the Xptel. DXS10077 and DXS10078 are located further 7.701 kb and 12.879 kb downstream, respectively.
The three STRs differ clearly in their individualization capacity as could be shown in a population sample of 201 male and 151 female Germans. Whereas at Locus DXS10076 10 alleles and at Locus DXS10078 13 alleles could be detected resulting in PIC and HET values of 0.767 and 0.747 (DXS10076) and 0.811 and 0.861 (DXS10078), respectively, the trinucleotide STR DXS10077 consists of 5 alleles leading to much lower PIC (0.492) and HET (0.507) values.
Two paternal mutations were detected at DXS10078 in 150 families with confirmed paternity while no mutations could be found until now at the other two loci.
Theoretically, this cluster could give rise to 650 different haplotypes in the German population. In fact, genotyping of 201 males revealed the presence of 72 haplotypes. Due to their closely linked location the three STRs form a cluster free of recombination. The stability of haplotypes was tested in 90 three-generation families. Hence, the Xp11.23 STR cluster reported here can contribute to solving complex kinship cases. Special aspects such as linkage disequilibrium etc. will be discussed in detail.

Contact: augustin@uke.uni-hamburg.de

P-016

Danger of false inclusion among deficient paternity case

Babol-Pokora K, Jacewicz R, Szram S

Department of Forensic Medicine, Medical University of Lodz, Lodz, Poland

Deficient cases, such as motherless cases, are more difficult then standard ones, but a conclusion can still be derived, based on the types of the child and alleged father. More complicated are cases in which the alleged father is unavailable for testing – these are difficult to calculate. But the real problem is when both mother and father are unavailable. Such cases can be burdened with danger of false inclusion.

In our practice we had a deficiency case, in which the alleged father was unavailable, so we had to test his parents. We used Identifiler™ system and there was no exclusion in that case, when we typed the child and his grandparents only. The probability of paternity that we obtained was 99,9 percent. Mother's typing, however, revealed exclusions in 3 STR loci among the Identifiler™ system. Further researches showed exclusions in 4 STR loci out of 21 tested STR loci.

contact: katarzynababol@wp.pl

P-017

IDENTIFILER™ system as an inadequate tool for judging deficient paternity cases

Babol-Pokora K, Jacewicz R, Szram S

Department of Forensic Medicine, Medical University of Lodz, Lodz, Poland

Identifiler™ is known to be one of the most useful multiplex systems for standard paternity testing. But in some cases the mother is unavailable and there can be a problem with obtaining sufficient value of probability of paternity. We typed nine hundred unrelated individuals from Central Poland population (Lodz region), in order to check the usefulness of Identifiler™ for analysis of motherless cases. The most important thing was to compare the evidence value between standard cases (trios) and deficient ones (duos). One hundred and fifty excluding cases and one hundred and fifty including ones were analysed and the results were estimated for trios and duos. Power of exclusion and paternity index were analysed for each locus as well as for the entire set of the fifteen STR markers. Our researches confirmed the usefulness of Identifiler™ system for standard paternity testing, and showed that the minimal probability of paternity that can be obtained, is 99,999 percent. In motherless cases however, the average value of probability of paternity was as low as 99,9 percent. The minimal number of excluding *loci* among trio cases was four, whilst among duo ones there were events of exclusion in one *locus* only. That is why Identifiler™ is proper for standard paternity cases, however motherless cases need to be examined more widely.

contact: katarzynababol@wp.pl

P-018

Is SGM Plus™ the sufficient system for paternity testing?

Babol-Pokora K, Jacewicz R, Szram S

Department of Forensic Medicine, Medical University of Lodz, Lodz, Poland

SGM Plus™ is one of the multiplex systems commonly used in forensic genetics. It consists of 10 STR loci which can be useful for parentage testing. We present results of typing in nine hundred unrelated individuals from Central Poland population (Lodz region), in order to check the usefulness of SGM Plus™ system for paternity testing. One hundred and fifty excluding cases and one hundred and fifty including ones were analysed in the range of 10 STR *loci* of SGM Plus™ system and the results were estimated for standard and motherless cases. Power of exclusion and paternity index were analysed for each of ten *loci* as well as for entire SGM Plus™ set. Our researches showed that the SGM Plus™ is not sufficient for parentage testing. The minimal number of excluding *loci* for SGM Plus™ analyses was one among duo cases and two among trio ones and there was an event of false inclusion, which was revealed after Identifiler™ analysis. Additionally the number of excluding loci among twenty seven percent of duo cases was less than four. The probability of paternity in almost sixty percent of trio cases was 99,99 percent and lower. Additionally the average value of probability of paternity among duo cases was as low as 99,0 percent. That is why we consider SGM Plus™ not to be sufficient for paternity testing among deficient cases as well as standard ones.

contact: katarzynababol@wp.pl

P-019

The Beneficial Effect of Extending the Y Chromosome STR Haplotype

Ballard DJ, Khan R, Thacker CR, Harrison C, Musgrave-Brown E, Syndercombe Court D

Centre for Haematology, ICMS, Barts & The London, Queen Mary's School of Medicine & Dentistry, UK

Y chromosome testing is becoming a more frequently used technique both in criminal and relationship cases. One drawback with this method however, is the relatively low haplotype diversity when compared with autosomal DNA profiles. The consequence of this lower diversity is that individuals can present with the same Y-STR haplotype even if they are not closely related.

During the development of Y chromosome testing there was a scarcity of known Y-STR loci and it is only recently that larger numbers of polymorphic markers have been discovered. Haplotype diversity is therefore partially compromised in standard forensic Y chromosome testing protocols by the need to use the established markers, not all of which are highly polymorphic. We have taken a number of the recently discovered highly polymorphic markers and analysed them in addition to the standard set of Y chromosome loci. Presented here are the allele frequencies for these loci in the British population along with the resulting increase in haplotype diversity associated with their incorporation. Also detailed is a relationship case that demonstrates the advantages that additional informative Y chromosome loci can confer when used in forensic casework.

Email : d.j.ballard@qmul.ac.uk

P-020

Application of Whole Genome Amplification for Forensic Analysis

Balogh MK[1], Børsting C[2], Sánchez Diz P[3], Thacker C[4], Syndercombe-Court D[4], Carracedo A[3], Morling N[2], Schneider PM[1], and the SNP*for*ID Consortium

[1] *Institute of Legal Medicine, Johannes Gutenberg University of Mainz, Germany*
[2] *Institute of Legal Medicine, University of Santiago de Compostela, Spain*
[3] *Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark*
[4] *Centre for Haematology, ICMS, Barts and The London, Queen Mary's School of Medicine and Dentistry, UK*
www.snpforid.org

Fundamental to most forensic analysis is the availability of genomic DNA of adequate quality and quantity. To perform a multitude of genetic analysis and assays requires sufficiently large amount of template. However, DNA yield from forensic samples is frequently limiting. Whole Genome Amplification appears to be a promising tool to obtain sufficient DNA amounts from samples of limited quantity. The WGA method is based upon the "Strand-Displacement Amplification" approach used in rolling circle amplification. The exponential amplification process theoretically enables the amplification of DNA from one single cell up to a million-fold. Therefore the main purpose of our study was to systematically investigate its sensitivity, accuracy and suitability for DNA diluted with quantities of 50, 100, 150, 250 and 500pg. We have performed the study using diluted DNA from two cell lines, HepG2 and K562. The WGA reactions were repeated five times, followed by STR PCR carried out twice for each cell line and dilution. To generate sufficient data, to assess the sensitivity, accuracy and suitability of the Whole Genome Amplification four laboratories were included in this study.

WGA was found to be very efficient, all sample dilutions amplified well, and the amplification yield does not relate to the amount of input DNA. In general ~500ng/µl were obtained, independently of the amount of target DNA. However, reliable STR amplification was dependent on the DNA quantity used for WGA. Consistent and reliable STR typing was only obtained using 500pg genomic DNA. Dropouts and allelic imbalance started to occur at 250pg and more dramatically at 100 and 50pg.

Therefore the usefulness of WGA in forensic casework is limited, however the method may be very useful for saving rare samples provided that the DNA is of adequate quality.

Email: balok000@students.uni-mainz.de

P-021

DNA typing from 15 years old bloodstains

Barbaro A.[1], Cormaci P[1] and Barbaro A.[2]

[1] *Department of Forensic Genetics,* [2]*Director of SIMEF*
SIMEF - Reggio Calabria-ITALY
www.simef.com - e-mail: simef_dna@tiscali.it

The aim of this study is to compare the efficiency of different validated methods for DNA extraction on old bloodstains. The study has been performed on bloodstains placed on a cotton surface, stored at room temperature for 15 years. As reference were used liquid blood samples,stored at –20°C, belonging to the same donors above. DNA has been extracted from all samples using different procedures (chelex, paramagnetic silica particles, silica membrane column, desalting procedure), then quantified in Real-Time PCR by the Quantifiler Human DNA Quantification kit (Applied Biosystems) and amplified by AmpFlSTR Identifiler kit (Applied Biosystems).

We've evaluated the ability of each method to extract DNA, the quantity of human DNA extracted with each procedure, the ability to perform multiplex STRs amplification and the reproducibility of results obtained

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Contact: anniebar@tiscali.es

P-022

Multiplex STRs amplification from hair shaft validation study

Barbaro A.[1], Cormaci P[1] and Barbaro A.[2]

[1] *Department of Forensic Genetics,* [2]*Director of SIMEF*
SIMEF - Reggio Calabria-ITALY
www.simef.com - e-mail: simef_dna@tiscali.it

Mt-DNA analysis, that is widely used in forensic genetics in case where the amount of DNA is very small or degraded, is unfortunately a complex and time-consuming procedure, so, since several years in other our previous papers, we've showed the possibility to amplify in single-plex DNA extracted from hair shaft. Now in the present study we've evaluated the ability to perform multiplex STRs amplification and the reproducibility of results obtained.

In particular we analysed 20 hair shafts beloging to known donors (2 male and 2 female) using different DNA extraction procedures (fenol-clorophorm, paramagnetic silica particles, silica membrane column,chelex).

Extracted DNA has been quantified by Quantifiler Human DNA Quantification kit (Applied Biosystems) using a 7300 Real-Time PCR System and amplified by AmpFlSTR Identifiler and AmpFlSTR Y-Filer kits (Applied Biosystems).

Amplified samples have been analyzed on an ABI PRISM 3130 multicapillary sequencer.

As reference were used saliva samples coming from the same hairs donors.

We verified that in some cases where there's a sufficient quantity and a good quality of medulla cells inside the hair stem a multiplex amplification can be performed and this is very useful for obtaining in a single step the typing of many loci avoiding the loss of DNA.

The ability to identify STRs markers in difficult samples as hair shafts gives a great opportunity to obtain DNA profiles useful for any further comparison or searching in DNA database.

Contact: anniebar@tiscali.es

P-023

LCN DNA typing from touched objects

Barbaro A.[1], Cormaci P[1] and Barbaro A.[2]

[1] *Department of Forensic Genetics,* [2]*Director of SIMEF*
SIMEF - Reggio Calabria-ITALY
www.simef.com - e-mail: simef_dna@tiscali.it

A married beautiful woman received to her home, in different slots, 2 envelops containing pornographic photos and indecent proposals from an anonymous persistent admirer.
The woman sent the material above to our laboratory for latent prints development and for searching biological traces for DNA typing.
While latent prints research gave negative results, we were able to found on the stamps some saliva traces useful for DNA analysis. STRs typing showed that both stamps were licked by the same male individual.
Since the husband of the woman suspected a colleague, after some weeks from the analysis above, he brought us two marking pens (one red and the other one black), that the man was used to utilize at the workplace, for performing DNA typing from any eventual sweat/skin residual found on them, with the aim to compare DNA profiles obtained with the one from stamps.
We were able to obtain from biological traces on the red marking pen a mixed DNA profile, while from the black pen we had a partial DNA profile: all profiles found matched with the one from the stamps.So DNA analysis confirmed the hypothesis: the husband colleague was the bother perpetrator.
This casework is a further confirmation that it's possible to type LCN DNA with very good results if an appropriate collection and analysis of biological material is performed.

Contact: anniebar@tiscali.es

P-024

X-STRs typing for an identification casework.

Barbaro A.[1], Cormaci P[1] and Barbaro A.[2]

[1] *Department of Forensic Genetics,* [2]*Director of SIMEF*
SIMEF - Reggio Calabria-ITALY
www.simef.com - e-mail: simef_dna@tiscali.it

X-STRs have been proven to be useful in case of deficiency paternity testing and in effective mother-son kinship and father-daughter testing. Male individuals inherit their one X-Chr from their mother, while female individuals receive one X from the mother and the other one from the father. So, female individuals fathered by the same man share their paternal Chromosome X.
Hence in case of deficiency paternity in which the mother is available for typing, the possible X alleles of the putative father can be determined and the paternal profile can be reconstructed.
In the present casework we used X-STRs for the identification of a biological material supposed to be belonging to a girl disappeared from several years. In fact in the house of a man (suspected to be the author of another woman murder) was found a headscarf similar to a one obelonging to the girl and inside it some hairs. In absence of any biological sample belonging to the disappeared girl we verified the relationship between hairs above and the mother and the sister of the disappeared girl.
In particular we used Mentype® Argus X-UL that is a new kit commercialized by Biotype for fast and reliable profiling of the following 5 unlinked X chromosomal STRs markers DXS8378, DXS7132, HPRTB, DXS7423 and Amelogenin.
Additionally we investigated in triplex DXS101, DX6789, HumSTRX1 and in duplex GATA1872D05, DX7133 using MWG-Biotech primers and our own amplification protocols.
By comparison between DNA profiles it was possible to identify in the woman, that was surely daughter of the not available father, the paternal possible X alleles and then to verify the presence in the questioned samples of maternal and paternal X-STRs.
The present case demonstrates the impact of additional X-STRs markers in special reverse paternity case that cannot be solved using autosomal markers.

Contact: anniebar@tiscali.es

P-025

Study of 16 Y-STRs in the population of Calabria using AmpFlSTR Y-filer kit

Barbaro A.[1], Cormaci P[1] , Falcone G. and Barbaro A.[2]

[1] *Department of Forensic Genetics,* [2]*Director of SIMEF*
SIMEF - Reggio Calabria-ITALY
www.simef.com - e-mail: simef_dna@tiscali.it

Y-STRs are very useful for forensic laboratories to identify and analyse male DNA from evidence-containing mixtures of male and female DNA (for example in case of sexual assault), in difficult paternity analysis or for reconstruction of male lineage or application in kinship analysis.
AmpFLSTR® Yfiler™ PCR Amplification kit is the last commercial kit for Y-STRs analysis produced by Applied Biosystems. It uses the 5-dyes chemistry for co-amplification, in a single PCR reaction, of 16 Y-chromosome STRs (DYS456, DYS389I, DYS390, DYS389II, DYS458 DYS19 DYS385 DYS393 DYS391 DYS439 DYS635 DYS392 YGATAH4 DYS437 DYS438 DYS635 DYS448), including the European Minimal Haplotype loci, the loci recommended by the Scientific Working Group on DNA Analysis Methods (SWGDAM) and 6 additional highly polymorphic loci.
In the present study we analysed the distribution of the Y-STRs above in 3 populations from a Southern Italy region: Calabria.
In particular DNA was extracted, by Instant Gene Matrix (Biorad) treatment, from blood/saliva samples of male unrelated healthy donors (100 per each area), since 3 generations, at least, belonging to the populations of Reggio Calabria, Catanzaro and Cosenza.
All samples were quantified by the Quantifiler™ Human DNA Quantification Kit using a 7300 Real Time System and then amplified according to the Yfiler™ kit protocol using GeneAmp PCR Systems 9600,9700,2400,2720 thermal cyclers (Applied Biosystems). Female and Male Positive controls and negative controls were used during all amplification steps.
Amplified products were analyzed by capillary electrophoresis on ABI PRISM 310 and ABI PRISM 3130 Genetic Analyzers (Applied Biosystems) employing Genotyper and GeneMapper 3.2 softwares.

Contact: anniebar@tiscali.es

P-026

Male contribution in the constitution of the Brazilian Centro-Oeste populations estimated by Y-chromosome binary markers

Barcelos RSS[1,2,3], Ribeiro GGBL[1], Silva Jr. WA[4], Abe-Sandes K[4,5], Godinho NMO[1,2], Marinho-Neto F[7], Gigonzac MAD[7], Klautau-Guimarães MN[1], Oliveira SF[1].
[1]*Departamento de Genética e Morfologia, Universidade de Brasília, Distrito Federal, Brazil;* [2]*Superintendência de Criminalística, Secretaria de Segurança Pública do Estado de Goiás, Brazil;* [3]*Departamento de Biomedicina, Universidade Católica de Goiás, Brazil;* [4] *Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil;* [5]*Departamento de Ciências da Vida, Universidade do Estado da Bahia, Campus I - Salvador, Bahia, Brazil.*

Due to Brazil's large dimension, this country is divided in five geo-political regions: South, Southeast, Center-West, North and Northeast. The Center-West region is the subject of our study and is composed by three states - *Goiás*, *Mato Grosso* and *Mato Grosso do Sul* - and the Federal District. The settlement of this territory, which was a result of the miscegenation among different ethnic groups, especially Europeans, Africans and Amerindians, did not happen in a homogeneous way, which reflects in the current genetic population composition. Meanwhile the Brazilian colonization was initiated in XVI century, the Center-West region settlement took place only after XVII century and the Federal District, where is placed the Federal capital (Brasília), was founded in the late 1950s. Differently from the others Brazilian's regions, the colonization of Center-West region was derived from internal migrations of already mixed individuals from all others Brazilian regions. Another Brazilian peculiarity is the directional mating between European males and Amerindian or African females. Therefore, after consider these characteristics, could this region be considered as the best representative population group of the Brazilian population? How is the male constitution in the Brazilian Center-West? Seeking answer these questions, we studied eleven unique-event polymorphism (UEPs), located in the non-recombinant region of the Y chromosome, in 200 unrelated men from *Goiás* state and Federal District. The results showed that the last population presented a greater genetic diversity than the first one, which reflects in a low divergence between these two populations. The greater genetic diversity of Federal District corroborated the historic data of migrations from all regions of the country and indicated this population as the most representative group of the Brazilian population genetic constitution. The most common haplogroup in this survey, P92R7, presents a wide geographic distribution. However, due to Brazilian settlement history, its presence may reflect a European contribution. The contribution estimated using European haplogroups was similar in both population and is greater than the African one. It was also observed a little male contribution from Amerindian to the constitution of both populations and from Japanese only to Federal District constitution. These results demonstrated a greater male contribution of Europeans than Africans or Amerindians to the formation of both populations, which corroborated the historic data of this region settlement.

contact: silviene@unb.br

P-027

Evaluation of seven autosomal STR loci in a German population

Becker D, Vogelsang D, Brabetz W

Biotype AG, Moritzburger Weg 67, 01109 Dresden, Germany

Population data for the seven autosomal STR loci D4S2366, D6S474, D14S608, D19S246, D20S480, D21S226 and D22S689 were analysed in a German population of unrelated individuals (n =189) by capillary gel electrophoresis. For this purpose two screening multiplex polymerase chain reactions (triplex and quadruplex) were developed with fluorescently labelled primers.

Different alleles for all loci were sequenced and an allelic nomenclature consistent with the ISFG recommendations was defined. Simple, compound and complex repeats could be distinguished. Allele frequencies and further population statistical data for all loci were described and will be discussed with regard to forensic applications.

E-Mail: d.becker@biotype.de

P-028

The Comparison of mtDNA and Y chromosome

Diversity in Malay Populations

Bekaert B, Hadi S, Goodwin W

Department of Forensic& Investigative Sciences, University of Central Lancashire, Preston, UK

Analysis of the mtDNA hypervariable region I of 106 modern Malay samples and 59 Orang Asli samples had previously found that the mtDNA diversity in the modern Malay was comparable to other Caucasian and Asian populations while, unsurprisingly the diversity in the isolated Orang Asli was very low, with only 14 different haplotypes being observed.

A follow up study was carried out to assess the effect of isolation on the levels of diversity of the Y chromosome in the Orang Asli polulation. Thrity three samples from the Orang Asli and thirty eight from the modern Malay population were analysed using the Promega Y-Plex kit. The Orang Asli population consisted of two subpopulations: the Jahai population and the Kensiu population. Fifteen samples were profiled from the Jahai population with the most common haplotype occuring in 3 individuals, over all the gene diversity value was 0.9536. Eighteen Kensiu samples were profiled and the most common haplotype again occurred 3 times, the gene diversity was 0.961. In 38 modern Malay samples no common haplotype was found and the gene diversity value was calculated as 0.9999. In both the Orang Asli and Malay population the diversity of the Y chromosome was higher than had been detected in the mtDNA genome.

The different frequency of haplotypes in isolated populations is an important consideration when applying lineage markers to casework.

contact: WHGoodwin@uclan.ac.uk

P-029

A Multiplex SNP Typing Approach for the DNA Pyrosequencing Technology

Bender K[1], Nehlich C[1], Harrison C[2], Musgrave-Brown E[2], Syndercombe-Court D[2], Schneider PM[1]
and the SNP*for*ID Consortium

[1] *Institute of Legal Medicine, Johannes Gutenberg University Mainz, Germany*
[2] *Centre for Haematology, ICMS, Barts and The London, Queen Mary's School of Medicine and Dentistry, UK*
www.snpforid.org

Multiplex Pyrosequencing enables simultaneous analyses of multiple target DNA. Single and multiplex PCR was employed to amplify target DNA templates each containing one of 23 single nucleotide polymorphisms (SNPs) from genomic DNA selected by the SNP*for*ID Consortium. In our investigations we have looked for the multiplex capacity of the PSQ™ 96MA instrument (Biotage AB). To test the reliability of the SNP typing by Pyrosequencing we have analysed each of the SNPs by using the SNaPshot minisequencing technique in parallel as reference method.
For developing a multiplex assay, in the first step 23 PCR products were divided into 8 aliquots of equal volume and each aliquot was typed in parallel with a set of three different SNPs. In the next step the same set of SNPs was typed by using one duplex and seven 3plex PCR reactions side by side. Because the amount of DNA is limited in the majority of casework samples it is necessary to amplify all relevant SNPs in one or only a few PCR reactions. Therefore we have addressed the questions, whether it is possible to perform a successive typing of two 3plex SNP typing reactions out of a 6plex PCR reaction and how often this SNP typing reaction can be repeated. Finally we could show that the typing of 23 SNPs out of a 23plex PCR reaction seems to be possible under optimized conditions. Due to the lack of an adequate instrument software for our strategy the dispensation orders for the nucleotides used in the pyrosequencing had to be designed manually in a time-consuming step. To improve the method different purification steps and the use of single strand binding protein (SSB) were tested.

Contact: kbender@mail.uni-mainz.de

P-030

Y Chromosome variation in Gabon

Gemma Berniell-Lee, Elena Bosch, Jaume Bertranpetit, David Comas

Unitat de Biologia Evolutiva, Departament de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, Doctor Aiguader 80, 08003 Barcelona, Spain

Of the 6,900 known living languages worldwide, one third are spoken in Africa. African languages are divided into four main language families or phyla, the largest of these being the Niger – Congo family (both in terms of geographical area, the number of speakers, and the number of different languages). In turn, one third of the Niger-Congo phyla are Bantu languages. Bantu languages are spoken throughout Sub-Saharan Africa (i.e. the Congo Basin, Angola, Mozambique etc...) and are thought to have obtained this distribution through one of the major cultural expansions in Africa; the Bantu Expansion. This expansion is thought to have taken place around 5,000 years ago, and to have originated in southern Nigeria and/or northwestern Cameroon. Although its linguistic side has been widely studied, little is known about the demographic processes associated to it. Our aim is to provide insights into the origin and diffusion of Bantu and Bantu-speaking populations by means of genetic data. Since the human Y chromosome is uniparentally inherited, and its phylogeny has been exhaustively described, it is possible to reconstruct a phylogeography of the human male lineages in sub-Saharan Africa. We have typed 18 STRs in over 1,100 samples from multiple ethnic groups (i.e. Galoa, Benga, Nzebi etc...) from the area of Gabon, located in the Guinea Gulf, which together with SNP data, should enable us to identify admixture, possible migration routes, and to study correlations between languages, cultures and genes.

contact: gemma.berniell@upf.edu

P-031

Diversity of maternal and paternal lineages in the geographic extremes of the Azores (Santa Maria and Flores Islands): insights from mtDNA, Y-Chromosome and Surname data

Bettencourt C[1], Montiel R[1], Santos C[2,3], Prata MJ[4], Aluja MP[2], Lima M[1]

[1]Center of Research in Natural Resources (CIRN), University of the Azores, Ponta Delgada, Portugal
[2]Unity of Anthropology, Department BABVE, Autonomous University of Barcelona, Barcelona, Spain
[3]Department of Anthropology, University of Coimbra, Coimbra, Portugal
[4]Institute of Pathology and Molecular Immunology of the University of Porto (IPATIMUP), Porto, Portugal

The Azores Islands were discovered, uninhabited, by Portuguese navigators in the early 15th century. The 9 islands that form this Archipelago are clustered in three geographic groups (Eastern, Central and Western). The peopling process was initiated in 1439 by the Eastern group (S. Miguel and Santa Maria, proceeding slowly to the remaining islands. Santa Maria (population of 5 490 inhabitants; area of 96.9 km2) and Flores (population of 3 949 inhabitants; area of 141 km2) occupy respectively the eastern and western limits of the Archipelago. These two small islands represent not only geographic extremes but also are chronologically distant in terms of settlement history, since Santa Maria was the first to be peopled whereas Flores was the last to be occupied. With the purpose of analysing the impact that the effective population size, geographic distance and chronology of settlement had on the genetic structure of these islands, we characterized the maternal and paternal lineages of both populations by:

a) determining the sequence of HVRI region and specific polymorphic positions of the non-coding region of mitochondrial DNA (mtDNA);

b) analysing 20 binary polymorphisms located in the non-recombining portion of the Y-chromosome (NRY); and c) studying patterns of surname composition.

Contact: mcbettencourt@notes.uac.pt

P-032

Validation of a single expert system to automate the interpretation of STR data, including mixtures

Bill M[1], Gill P[1], Young R[1], Maguire C[1], Healy M[1], Thornton L[1], Curran J[2]

[1]Forensic Science Service, Trident Court, 2960 Solihull Parkway, Solihull B620LS UK
[2]Department of Statistics, University of Waikato, New Zealand.

The Forensic Science Service® (FSS) has utilised computer software to automate the interpretation of STR data since 1998. The introduction of these systems has resulted in dramatic improvements in the quality, speed and efficiency of the analytical stage of the DNA profiling process. The FSS have developed an expert system suite called FSS-i³ (FSS i-cubed) which brings together the technical knowledge and experience acquired .The suite uses complex heuristic rule-sets developed with the Forensic Science

Service's most experienced reporting officers (ROs) and analysts, and is designed for use with any STR multiplex and any PCR cycle number. The software is used to completely automate the designation of alleles so that genotypes are now down-loaded to the UK national DNA database without the need of an operator interface. In addition to the 'core' interpretative processes, the software has alternate algorithmic solutions using least squared approach and geometric means to interpret mixtures. Apart from completely de-skilling the interpretative process, the net outcome is a significant reduction in unit's costs and an increase in the success rate of crime-stain data by circa 20%. The FSS-i³ software has proven to be so robust in its ability to correctly interpret data that its usage as a single expert system has been approved.

Martin.Bill@fss.pnn.police.uk

P-033

CYP2C9 Polymorphism in Iranian population with three different ethnicity

Biramijamal F[2] ,Tanhaei s[1],Sanati M.H[2],Sheidai M[1]

[1]Shahid Beheshti University,Tehran-Iran
[2]National Institute for Genetic Engineering and Biotechnology(NIGEB),Tehran-Iran

The Cytochrome P450 2C9 gene has a function in detoxification of carcinogenic compounds. Recently, described the polymorphism at codon 144 of the CYP2C9gene (Cys/Arg) and susceptibility of several types of cancer. Also it is reported that CYP2C9 polymorphism is involved in drug resistance.
To investigate the CYP2C9 codon 144 polymorphism among different ethnicity, we collected samples from healthy population from three different ethnicity groups. The CYP2C9 Cys144Arg genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and direct DNA sequencing analysis in 120 healthy controls.
Among the healthy subject with Mazandarani, Turkaman and Kord ethnicity, the allelic frequency of CYP2C9 Cys144Arg were 16%,9% and 14% for Cys allele,84%,91% and 86% for Arg allele.
No significant difference in CYP2C9 allele distribution was observed between Mazandarani, Turkaman and Kord healthy individuals.In each group distribution of genotypes fits the Hardy-Weinberg equilibrium. Our initial study of 120 Iranian healthy individuals calls for future work in Iranian population genetics, also finding the CYP2C9 genotypes between Iranian cancer patient and comparing it with healthy controls. Indeed pharmacogenetics studies can be done according our data.
This work was supported by NIGEB project number 197.

contact: stanhaei@yahoo.com

P-034

Analysis of Y chromosome lineages in native South American population

Blanco-Verea A, Brion M, Sanchez-Diz P, Jaime JC, Lareu MV, Carracedo A

Institute of Legal Medicine. University of Santiago de Compostela. Spain

The object of this work is to try and identify both the evolutionary footprints and the origin of native populations of Argentina, and to compare them with those of other populations from South America. We analyzed 32 SNPs and 11 STRs of the Y chromosome in 126 samples from three different native populations from Argentina (Kollas, Mapuches and Diaguitas). The STR markers were amplified by means of the commercial kit PowerPlex Y system (Promega Corporation), the SNPs were amplified by means of four multiplex reactions and genotyped using the SNaPshot minisequencing Multiplex Kit (Applied Biosystems), and the products were analyzed with an ABI Prism 3100 Genetic Analyzer. Our results reveal that haplogroups R1b, Q3, G, I are the main haplogroups present in these populations, indicating the introduction of European Y chromosome lineages during the colonization of the American continent.

Contact: brioniml@usc.es

P-035

Rapid Microarray-based Typing of Forensic SNPs.

Bogus M,[1], Sobrino B[2], Bender K[1], Carracedo A[2], Schneider PM[1]
and the SNP*for*ID Consortium

[1] *Institute of Legal Medicine, Johannes Gutenberg University Mainz, Germany*
[2] *Institute of Legal Medicine, CEGEN, University of Santiago de Compostela, Spain*
www.snpforid.org

The Single Base Extention-Tag Array (SBE-Tag Array) method is carried out on glass slides and combines the specificity of minisequencing for SNP typing with the high troughput capacity of microarrays. Following multiplex PCR, a single tube SBE reaction is carried out, and the labelled extension products are hybridized to an array for locus-specific analysis. The aim is to prove and optimise the conventional microarray reaction on accuracy and efficiency for forensic applications.
From a list of non-cross-reacting sequences, 29 tag sequences were chosen and the complementary sequences were spotted as capture probes in duplicates on glass slides. Each slide contains four to ten arrays (MWG/CodeLink), which can provide results for the same number of individuals, using a design called “array of arrays” (Pastinen et al., Genome Res. 2000, 10:1031). In a minisequencing reaction containing fluorophore (Cy5, Cy3, Rox) labelled ddNTPs and 5´-tagged SBE primers, the extention reaction is performed and finally demultiplexed by hybridization to the arrays. Genotyping is carried out using an Affymetrix 428 scanner. Detection is carried out at three wavelengths, therefore the assays have been designed to avoid A/C polymorphisms, as these bases had to be labelled with the same dye. Alternatively, if a two-wavelength scanner is used, minisequencing can be performed using only a single dye label in four separate reactions. Then the reaction products have to be hybridized to four separate arrays on the same slide, and analyzed individually for each base. At present, 23 SNPs are combined into a single reaction.
The SBE-Tag array on glass slides is a promising and cost-efficient genotyping technology, which can be further extended in respect of the number of simultaneously anlysed individuals and the size of the multiplex PCR reaction.

bogus@uni-mainz.de

P-036

Internal Validation of AmpFlSTR Identifiler PCR Amplification Kit with detection on ABI Prism 3100 Genetic Analyzer for Use in Forensic Casework at the Department of Chemistry, Malaysia.

Lim Kong Boon

Department of Chemistry Malaysia

According to the guidelines of quality assurance standards for forensic DNA testing laboratories, prior to implementing a new DNA analysis procedure or an existing DNA analysis procedure developed by another laboratory, the forensic laboratory must first demonstrate reliability of the procedure by carrying out internal validation. Seven elements were design by the forensic laboratory at the Department of Chemistry, Malaysia to validate the use of the AmpFlSTR(r) Identifiler PCRTM Amplification Kit with detection on ABI Prism(r) 3100 Genetic Analyzer using POP-4TM polymer. The presentation summarizes the results obtained for each of the seven elements of the validation studies, which include the following evaluation: sensitivity, precision, reproducibility, non-probative casework, stutter, heterozygous ratio and mixtures. With these data, guidelines for the interpretation of STR DNA profiles based on the AmpFlSTR(r) Identifiler genetic loci were documented for use by the DNA laboratory.

Contact: kblim@kimia.gov.my

P-037

Comparison of calculated paternity indices based on the typing of 15 STRs, 7 VNTRs, and 52 SNPs in 50 Danish mother-child-father trios

Børsting C, Sanchez JJ, Birk AH, Bruun HQ, Hallenberg C, Hansen AJ, Hansen HE, Simonsen BT, Morling N

Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark

Fifty Danish paternity cases from the year 2004 were selected based on the results obtained with the AmpF*l*STR® Identifiler® PCR amplification kit (Applied Biosystems). In all cases, the calculated paternity index (PI) was higher than 10,000, and there was not observed any genetic inconsistensies between mother and child, or between father and child. DNA from the selected trios was used to type 7 VNTRs (D2S44, D5S43, D5S110, D7S21, D7S22, D12S11, and D16S309) using the RFLP technique, and 52 SNPs using a PCR multiplex with 52 PCR primer pairs and two SBE multiplexes with 23 and 29 SBE primers, respectively (for details of the 52-SNP-plex, see SNPforID presention). PIs were calculated based on each set of loci (STRs, VNTRs and SNPs) and the results were compared.

Contact: Claus.Boersting@forensic.ku.dk

P-038

Whole genome amplification of blood and saliva samples placed on FTA® cards

Børsting C[1], Thacker C[2], Syndercombe Court D[2], Morling N[1]

[1]*Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark*
[2]*Centre for Haematology, ICMS, Barts and The London, Queen Mary's School of Medicine and Dentistry, UK*

Cells that come in contact with FTA® cards (Whatman Bioscience) lyse. The DNA is released and irreversibly bound to the filter matrix, from where the DNA can be assayed directly. The GenomiPhi™ DNA amplification kit (Amersham Biosciences) utilizes Phi29 DNA polymerase and random hexamer primers to exponentially amplify DNA by strand displacement amplification (SDA). We tested the GenomiPhi DNA amplification kit on 50 blood and 50 saliva samples placed on FTA cards. A 1.2 mm disk was punched out of the FTA cards using the BSD600-duet (BSD Robotics). The disk was washed three times with 150 □l Milli-Q water using the THEONYX robotic system (MWG) and left overnight in 150 □l Milli-Q water to remove all inhibitors of Phi29 polymerase. The disk was dried and used as target for the GenomiPhi DNA amplification kit. On average, the Phi29 polymerase produced 2 µg DNA (100 ng/□l) with DNA fragment sizes ranging from a few hundred bp to 12 kbp. A total of 1-2 ng Phi29 amplified DNA was typed using the AmpF*l*STR® SGM Plus™ amplification kit (Applied Biosystems) and the resulting STR profiles analysed according to the guidelines of each of the two forensic laboratories.

Contact: Claus.Boersting@forensic.ku.dk

P-039

Autosomal microsatellite analysis of the Azorean population

Branco CC[1,2], Pacheco PR[1,2], Cabral R[1,2], de Fez L[1,2], Peixoto BR[1,2], Mota-Vieira L[1,2]

[1] *Molecular Genetic and Pathology Unit, Hospital of Divino Espírito Santo, São Miguel, Azores, Portugal*
[2] *Instituto Gulbenkian de Ciência, Oeiras, Portugal*

The knowledge of population history, demography and genetic structure has proven to be fundamental to address research in human genetics. Here, we describe the genetic diversity of Azorean population and its affinity with other populations by the analysis of 13 microsatellite loci (TPOX, D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, TH01, vWA, D13S317, D16S539, D18S51 and D21S11) in 222 unrelated blood donors. These short tandem repeat (STR) markers were typed by Polymerase Chain Reaction (PCR) with fluorescently labelled primers. Statistical analysis was performed using Arlequin v.2.0, and Nei's genetic distance was calculated with DISPAN software and trees were constructed by Neighbor-Joining (NJ) using PHYLIP 3.63. To quantify the genetic contribution of Portuguese, African and European populations we calculated the admixture coefficient (mY) using Admix v. 2.0.
The analysis of microsatellite loci shows that the Azorean population presents an average gene diversity of 0.776. For each marker, gene diversity range between 0.661 for TPOX and 0.8812 for D18S51. Heterozigosity values calculated for each STR varies from 63.9% for TPOX to 89.2% for D18S51, although the majority of markers show values superior to 80%. In addition, the admixture coefficient reveals North Portuguese as the major contributors to the genetic background of the Azoreans. These results are corroborated by the dendrogram, in which Azores is closer to Belgians, Portuguese and Spanish, apart from Moroccans and Cabo Verdeans.
Taken together, these data indicate that the gene pool of the Azorean population is very diverse and are consistent with our previous results on Y-chromosome (Pacheco et al., Ann Hum Genet 69: 145-156, 2005). Moreover, no genetic differentiation between Azores and Portugal is observed.

contact: claudiacbranco@hdes.pt
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P-040

Simultaneous versus serial DNA identification of related tsunami victims

Brenner CH

School of Public Health, UC Berkeley, California, USA

DNA has proven to be a major and essential tool for identification in recent mass fatality incidents including wars, bombings, airplane crashes, and the World Trade Center attack. It will surely prove to be so in dealing with the hundreds of thousands of victims of the 2004 Indian Ocean tsunami. Among the many mathematical complications characteristic of this sort of mass fatality is the prevalence of related victims. When several bodies are found that are suspected of being members of the same family and are to be identified through DNA profile comparison with to other, living, family members, the right method of analysis is to consider all the identities at once. Only a *simultaneous* approach takes full account of the power of the evidence, takes into account the extent to which each dead body's identity is supported by its DNA similarity to the other dead bodies. By contrast, the *serial* method, which assigns the identities one at a time, thus letting each victim identity once established participate in the identification of the subsequent bodies, is superficially attractive but unfortunately it often understates the true value of the evidence. As an extreme example, imagine a father and daughter as the only two related victims of a small airplane crash. The two of them can probably be picked out and therefore identified from the DNA similarity even if no reference relatives are available, so simultaneous consideration of their types is almost infinitely better in this case. I will illustrate the "simultaneous method" with a realistic example and show how the logic and confidence of identification is stronger than using a serial identification approach.

Contact: cbrenner@berkeley.edu

P-041

Analysis of 29 Y-chromosome SNPs in a single multiplex useful to predict the geographic origin of male lineages

Brión M[1], Sanchez JJ[2], Balogh K[3], Thacker C[4], Blanco-Verea A[1], Børsting C[2], Stradmann-Bellinghausen B[3], Bogus M[3], Syndercombe-Court D[4], Schneider PM[3], Carracedo A[1], Morling N[2], and the SNP*for*ID Consortium

[1] *Institute of Legal Medicine, CEGEN, University of Santiago de Compostela, Spain*
[2] *Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark*
[3] *Institute of Legal Medicine, University of Mainz, Germany*
[4] *Barts and the London, Queen Mary's School of Medicine and Dentistry, London, UK*
www.snpforid.org

The European Consortium "High throughput analysis of single nucleotide polymorphisms for the forensic identification of persons – SNPforID", has performed a selection of candidate Y-chromosome SNPs (single nucleotide polymorphisms) for making inferences on the geographic origin of an unknown sample. From more than 200 SNPs compiled in the phylogenetic tree published by the Y Chromosome Consortium, and looking at the population studies previously published, a package of 29 SNPs has been selected for the identification of major population haplogroups.
A "Major Y chromosome haplogroup typing kit" has been developed, which allows the multiplex amplification of all 29 SNPs in a single reaction followed by a single base extension (SBE) reaction (minisequencing) and separation of the resulting extension products by capillary electrophoresis.
Validation of the kit was performed, firstly to check the accuracy and reproducibility of the 29-plex in different laboratories, and secondly to obtain haplogroup frequencies in samples from the major population groups. To compile the sample collections each of the participating groups reported the samples they had available in their labs. Among all the populations reported, a set of 1126 unrelated male samples distributed in 12 populations was selected. This selection was performed to obtain the best possible representation of the general worldwide distribution of populations. Selected population samples were distributed equally among the participating laboratories to perform the validation as a collaborative exercise.
The approach takes advantage of the specific geographic distribution of the Y-chromosome haplogroups and demonstrates the utility of binary polymorphisms to infer the origin of a male lineage.
Contact: brioniml@usc.es

P-042

Y-chromosomal and mitochondrial markers: a comparison between four population groups of Italy

Brisighelli F[1], Capelli C[13], Álvarez-Iglesias V[2], Arredi B[1], Baldassarri L[1], Boschi I[1], Dobosz M[1], Scarnicci F[1], Salas A[2], Carracedo A[2], Pascali VL[1]

[1]*Forensic Genetics Laboratory, Istituto di Medicina Legale, Università Cattolica del S. Cuore, Roma Italy*
[2] *Instituto de Medicina Legal, Facultad de Medicina, Universidad de Santiago de Compostela, Galicia, Spain*
[3]*PromegaGenetic Identity Europe, Promega Corporation , Madison WI, USA*

The investigation on the genetic diversity of humans has become fundamental to the complete understanding of the pre-history and history of populations, and it is presently addressing crucial issues of the human evolution that intersect with demographic, cultural and linguistic events. Numerous studies have been recently focused on the Italian Peninsula, and the current set of data regarding this country can now fit into a general frame in which local differences seems to emerge and be interpreted in the context of other cultural and historical knowledge. However, a comprehensive study based on multiple genetic systems and on extensive sampling is still missing. Here we report new data on the Y chromosome and mitochondrial DNA (mtDNA) over a significant larger Italian sample. In particular we address four geographic sites that in the past have been the theatre of significant events in the framework of Italy's peopling: Latium (central-west), Piceno (central-east), Calabria (south-west) and Messapia (south-east). Concerning the Y haplotype, we based our study on STRs and SNPs polymorphisms in order to tackle populational events positioned at various stages of the evolutionary history of Italy, and to account of local differences. Much to the same purpose, mtDNA has been characterized for the complete sequence of the two hypervariable segments (HVS-I and HVS-II) and to a selection of informative mtDNA coding region SNPs. The availability of both sets of loci including slow- and fast-evolving markers has enabled us to undertake multiple-level comparisons. We paid special interest to the distribution of genetic variability across our populations and we aimed to compare the mainframe emerging from the haploid male and female inherited loci. Preliminary results provided us with some intriguing inference regarding the prehistory and history of Italy will be discussed.

Contact: francesca.brisighelli@rm.unicatt.it

P-043

A comparative study between Brazilian, Iberian and African populations in an evolutionary perspective

Brito P[1], Carvalho M[1], Lopes V[1], Andrade L[1], Anjos MJ[1], Serra A[1], Balsa F[1], Oliveira AC[1], Oliveira C[1], Batista L[1], Gamero JJ[2], Romero JL[2], Corte-Real F[3], Vieira DN[3], Vide MC[1]

[1]Forensic Genetic Service. National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal
[2]Departament of Legal Medicine, Faculty of Medicine, University of Cádiz, Spain
[3]National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal

The STR's study of the Y chromosome has a great importance on Forensic Genetics, namely, in parentage investigations and biological crime evidence investigations. These markers pass from father to son without suffering recombination and with a very low occurrence of mutations. These characteristics combined with the high level of Y chromosome polymorphisms, made it in one of the main elements of study in forensic genetics, as like as in population genetics, allowing the study of lineages and the origin of a certain population.

Basing on the minimum haplotype of the Y chromosome STR's (DYS19, DYS385, DYS389 I, DYS389 II, DYS390, DYS391, DYS392, DYS393), the haplotype diversity was calculated according to Nei (1973), in Brazilian, African and Iberian populations. The analysis of Molecular Variance (AMOVA) was determinate by Markov test using the Arlequin Software (ver. 2.000). The distance matrix between populations was obtained by the genetic differences between haplotypes.

Contact: geneforense@dcinml.mj.pt

P-044

Analysis of 16 Y-chromosomal STRs in a Valle (Colombia) population sample

Builes JJ[1,2], Castañeda SP[3], Bravo MLJ[1], Espinal CE[1], Gómez MV[4], Moreno MA[1,2]

[1] GENES Ltda., Laboratorio de Genética Forense y Huellas Digitales del DNA. Medellín – Colombia.
[2] Instituto de Biología. Universidad de Antioquia. Medellín – Colombia.
[3] Facultad de Ciencias. Universidad Nacional de Colombia. Medellín – Colombia.
[4] INMUNOGEN Ltda. Cali – Colombia.

The object of this work was to examine a set of 16 Y-STR systems in a population sample from Valle (Colombia) to create a database. In the present study, 150 DNA samples taken from unrelated males were analyzed and PCR amplification of DYS19, DYS385, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS460, DYS461, GATA-A10, GATA-H4 and DYS635 was performed. PCR products were separated in 4% acrylamide-*bis*-acrylamide denaturing gels followed by silver staining. Allele size determination and genotyping were performed according to recommendations of the DNA Commission of the International Society of Forensic Genetic using the allelic ladder manufactured at home. Gene frequencies, gene and haplotype diversity and AMOVA for 16 Y-specific STR loci were calculated using ARLEQUIN version 2000.

One hundred forty six different haplotypes were found, 142 haplotypes of them were found to be unique and the others were shared by two persons. The haplotype diversity was 0.9996. Regarding the minimal haplotype, one hundred twenty four different haplotypes were found (haplotype diversity 0.9970), and one hundred thirty two different haplotypes were found with the GEPY system (haplotype diversity 0.9977). Twenty seven percent of this haplotypes do not match any sample in the Y-STR Haplotype Reference Database which assigned specific region characteristic to these population samples. We compared our data whit a Spain population and another Colombian populations. The AMOVA results show that the percentage of variation is mainly within populations (99.95%) in agreement with previous results in European populations.

By combining the allelic states of the 16 Y-chromosomal STRs we could construct highly informative haplotypes that allowed the discrimination of 94.7% (142 out of 150) of the samples tested. This approach represents a very powerful tool for individual identification and paternity testing in forensic medicine.

contact: genforense@epm.net.co

P-045

Analysis of 16 Y-chromosomal STRs in a Córdoba (Colombia) population sample

Builes JJ[1,2], Castañeda SP[3], Espinal CE[1], Moreno MA[1,2], Gómez JR[4], Bravo MLJ[1]

[1] GENES Ltda., Laboratorio de Genética Forense y Huellas Digitales del DNA. Medellín – Colombia.
[2] Instituto de Biología. Universidad de Antioquia. Medellín – Colombia.
[3] Facultad de Ciencias. Universidad Nacional de Colombia. Medellín – Colombia.
[4] INGEIN Ltda. Medellín – Colombia.

The object of this work was to examine a set of 16 Y-STR loci in a population sample from Córdoba (Colombia) to create a population database. In the present study, 123 DNA samples taken from unrelated males were analyzed and PCR amplification of DYS19, DYS385, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS460, DYS461, GATA-A10, GATA-H4 and DYS635 was performed. PCR products were separated in 4% acrylamide-*bis*-acrylamide denaturing gels followed by silver staining. Allele size determination and genotyping were performed according to recommendations of the DNA Commission of the International Society of Forensic Genetic using the allelic ladder manufactured at home. Gene frequencies, gene and haplotype diversity and AMOVA for 16 Y-specific STR loci were calculated using ARLEQUIN version 2000..
One hundred thirteen different haplotypes were found, 103 haplotypes of them were found to be unique and the others were shared by two men. The haplotype diversity was 0.9987. Regarding the minimal haplotype, one hundred different haplotypes were found (haplotype diversity 0.9896), and one hundred two different haplotypes were found with the GEPY system (haplotype diversity 0.9959). Thirty six percent of this haplotypes do not match any sample in the Y-STR Haplotype Reference Database which assigned specific region characteristic to these population samples. We compared our data whit a Spain population and another Colombian populations. The AMOVA results show that the percentage of variation is mainly within populations (99.95%) in agreement with previous results in European populations.
By combining the allelic states of the 16 Y-chromosomal STRs we could construct highly informative haplotypes that allowed the discrimination of 83.7% (103 out of 123) of the samples tested. This approach represents a very powerful tool for individual identification and paternity testing in forensic medicine.

contact: genforense@epm.net.co

P-046

Analysis of 16 Y-chromosomal STRs in a Cartagena (Colombia) population sample

Builes JJ[1,2], Gómez A[2], Bravo ML[1], Espinal C[1], Aguirre D[1], Montoya A[2], Caraballo L[3], Martínez B[3], Moreno M[1,2]

[1] GENES Ltda., Laboratorio de Genética Forense y Huellas Digitales del DNA. Medellín – Colombia.
[2] Instituto de Biología. Universidad de Antioquia. Medellín – Colombia.
[3]Instituo de Investigaciones Inmunológicas, Universidad de Cartagena. Cartagena-Colombia

Whit this work we stablished a data base of Y-STR, some parameters of forensic importance were calculated. We studied 16 Y-STR (DYS19, DYS385, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS460, DYS461, GATA-A10, GATA-H4 and DYS635) in a population of 173 unrelated males of Cartagena (Colombia). PCR products were separated in 4% acrylamide-*bis*-acrylamide denaturing gels followed by silver staining. Allele size determination and genotyping were performed according to recommendations of the DNA Commission of the International Society of Forensic Genetic using the allelic ladder manufactured at home. Gene frequencies, gene and haplotype diversity and AMOVA for 16 Y-specific STR loci were calculated using ARLEQUIN version 2000.
All men presented different haplotypes. The haplotype diversity was 1.000 +/- 0.0006. Regarding the minimal haplotype, one hundred fifty seven different haplotypes were found (haplotype diversity 0.9974), and one hundred forty different haplotypes were found with the GEPY system (haplotype diversity 0.9952). Forty one percent of this haplotypes do not match any sample in the Y-STR Haplotype Reference Database which assigned specific region characteristic to these population samples. We compared our data whit a Spain population and another Colombian populations. The AMOVA results show that the percentage of variation is mainly within populations (99.95%) in agreement with previous results in European populations.
By combining the allelic states of the 16 Y-chromosomal STRs we could construct highly informative haplotypes that allowed the discrimination of 100% (173 out of 173) of the samples tested. This approach represents a very powerful tool for individual identification and paternity testing in forensic medicine.

contact: genforense@epm.net.co

P-047

Peruvian population study with 16 Y-STR loci

Builes JJ[1,2], Hau J[3], Bravo MLJ[2], Rodríguez J[1,2], Montoya A[2,3], Izarra F[3], Ochoa O[3], Pérez L[3]

[1] GENES Ltda., Laboratorio de Genética Forense y Huellas Digitales del DNA. Medellín – Colombia.
[2] Instituto de Biología. Universidad de Antioquia. Medellín – Colombia.
[3] Laboratorio de Biología Molecular-ADN Forense, Dir. de Criminalística de la Policía Nal. de Perú, Lima, Perú.

The aim of this study was to present the first report of a Peruvian Population Database of 77 samples studied with 16 Y-STR loci including the eight minimal Y-STR haplotype (DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393) and other Y-STR loci (DYS437, DYS438, DYS439, DYS460, DYS461, GATA A10, GATA C4 and GATA H4). PCR products were separated in 4% acrylamide-*bis*-acrylamide denaturing gels followed by silver staining. Allele size determination and genotyping were performed according to recommendations of the DNA Commission of the International Society of Forensic Genetic using the allelic ladder manufactured at home. Gene frequencies, gene and haplotype diversity and AMOVA for 16 Y-specific STR loci were calculated using ARLEQUIN version 2000.

Seventy six different haplotypes were found, seventy five haplotypes of them were found to be unique and only one was detected in two men. The haplotype diversity was 0.9997.

By combining the allelic states of the 16 Y-chromosomal STRs we could construct highly informative haplotypes that allowed the discrimination of 97.4% (75 out of 77) of the samples tested. This approach represents a very powerful tool for individual identification and paternity testing in forensic medicine.

contact: genforense@epm.net.co

P-048

Detection of a 1% to 2% Contributor in a DNA Sample Mixture from Human Milk

Burger, MF, Schumm, JW

The Bode Technology Group, 7364 Steel Mill Rd.; Springfield, VA 22150; USA.

We describe a method to detect very small amounts of DNA in a mixed sample using commercially available multiplexes. We have received a number of breast milk samples from human donors and have been asked by our supplier to determine whether pooled milk samples originate from one donor or from multiple donors.

We determined that it is possible to extract DNA from whole milk samples using the QIAamp® DNA Blood Mini Kit. Total DNA yields from 200 µl of milk were measured using the BodeQuant LCN method described in the accompanying work and ranged from 6.5 ng to 205 ng. The significant observed variability could be due to many causes such as sample age, care in handling by the original donor, or method of shipment to us. The primary cause of variability is likely differences in the numbers of cells shed into the milk by different source individuals. However, in each case, enough DNA was obtained to generate a DNA profile with the Amp*Fl*STR® Identifiler® kit.

We then created volume/volume mixtures of milk samples in ratios of 98:2, 96:4, 92:8, and 88:12 to determine the minimum amount of the minor component that could be detected. Using modified amplification conditions and interpretation guidelines, we can detect the presence of a mixture containing 2% or less of the total DNA content from the minor contributor. Thus, so long as the two donors provide equivalent DNA mass per milliliter of milk the minor component can be scored with as little as one part in 50 contributions.

However, we learned in our initial evaluations that the DNA yield per milliliter of milk varies significantly from sample to sample so that the volume: volume ratio does not always reflect the DNA mass:DNA mass ratio in the sample. In practice, we can generally still detect the minor component of a mixture even when this sample is mixed with 6 other samples and even when the minor component has a lower DNA yield per milliliter of milk.

We will discuss the methods that allow detection of mixtures at these low levels and how these results relate to evaluation of blood mixtures of similar imbalance.

Contact: james.schumm@bodetech.com

P-049

Probability distribution of sibship determination with ABI Identifiler multiplex system using different software

Caenazzo L. [1] , Cerri N.[2], Ponzano E. [1], Sbrignadello S. [1], Benciolini P. [1]Verzeletti A. [2], De Ferrari F. [2], Presciuttini S. [3]

[1]Dept. of Environmental Medicine and Public Health - University of Padua, Italy
[2]Dept. of Forensic Medicine - University of Brescia, Italy
[3] Center of Statistical Genetics - University of Pisa, Italy

Forensic laboratories may be asked to provide genetic evidence that two persons are or are not related, when no other relatives are available for study.
Sibship analysis of the autosome polymorphisms are more complicated since there are no obligatory alleles between siblings that make it possible to exclude a biological relationship with absolute certainty.
In our work forty full-sib pairs were genotyped using the AmpFLSTR Identifiler PCR Amplification kit. All subjects belonged to families that included mother, two or three children, and an alleged father, in which neither the mother nor the alleged father were excluded as biological parents, and no mutational event was observed. In addition, the Y chromosome was investigated to provide further support for the relationship. The probability that each pair was composed of full sibs rather than non-relatives was calculated by standard formulas, and was verified using different published software. The distribution of these probability values was used to ascertain the statistical power of the Identifiler kit to resolve sibship relationships to forensic purposes.

Contact: luciana.caenazzo@unipd.it

P-050

Genetic identification of forensically important Calliphoridae in Portugal

Cainé L[1], Corte Real F[2,3], Lima G[1], Pontes L[1], Abrantes D[1], Pinheiro MF[1,4]

[1]Instituto Nacional de Medicina Legal – Delegação do Porto
[2]Instituto Nacional de Medicina Legal
[3]Faculdade de Medicina – Universidade de Coimbra
[4]Faculdade de Ciências da Saúde – Universidade Fernando Pessoa

Medico-legal Entomology, one area in the broad field of Entomology, is routinely involved in forensic applications. Identifying species is an important first step in the investigation, but morphological identification of immature stages can be difficult and sometimes impossible, due to the similarity between different species, and the possible dead of the insects. The genetic identification provides a rapid and accurate determination of species.
Species from Calliphoridae family are among the first insects to discover and colonize human remains and they give information relating to the estimation of the postmortem interval (PMI). They are attracted to carrion and a large number of eggs are commonly placed in natural body openings and wounds that are exposed. To date many geographical regions were studied, but Portugal presents a total lack of genetic data collected on the main species of forensic interest. The main goal of this study is to improve the genetic data knowledge of cadaveric entomofauna in Portugal. Maggots were collected from different human bodies during autopsy procedures in the National Institute of Legal Medicine. DNA was extracted using two methods: DNeasy® Tissue Kit (Qiagen) or BioRobot® EZ1 workstation (Qiagen). The obtained sequences were used to identify species; they were aligned to the gene sequences entries, using the online BLAST search engine of the National Center for Biotechnology Information (NCBI). The sequences are included in the database of GenBank and the maximum scoring segment pair (MSP) was found. The information content within the nucleotide sequence of the gene enabled the identification of all species used in this study. This study doesn't include all insect species that an investigator might find during autopsy, but it represents their general appearance.

Contact: Biologia@dpinml.mj.pt

P-051

Species identification by Cytochrome b gene: casework samples

Cainé L[1], Lima G[1], Pontes L[1], Abrantes D[1], Pereira MJ[1], Pinheiro MF[1,2]

[1] *Instituto Nacional de Medicina Legal – Delegação do Porto*
[2]*Faculdade de Ciências da Saúde – Universidade Fernando Pessoa*

In routine casework (paternity tests, criminal cases and human remains identification), sometimes is necessary to do the discrimination between human and non-human samples, by identifying the exact specie of the sample. The specie determination can change the overall direction of the investigation. This study presents the determination of the biological origin of unknown casework samples involved in criminal investigations, where the forensic evidence was important to solve the cases. Species identification was carried out by nucleotide sequence analysis of the citochrome b (cyt b) gene, which contains species-specific information. The DNA was extracted using the phenol-chloroform procedure and a fragment of 358 pb was amplified. Sequence determination of PCR products was performed using the PCR primers separately. The electrophoretic separation and detection of the sequencing reaction products were performed using an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). The sequences obtained were used to identify the biological origin of the samples by aligning to the cyt b gene sequences entries using the online BLAST search engine of the National Center for Biotechnology Information (NCBI). The information content within the nucleotide sequence of the cyt b gene enabled the identification of the samples species of the investigated cases.

Contact: Biologia@dpinml.mj.pt

P-052

Haplogroup H in prehistoric osseous remains from the Basque Country as a genetic marker to study the resettlement of Europe

Cardoso S[1] , Amory S[2] , Álvarez M[1], Gómez A[3], Keyser-Tracqui C[2] , Ludes B[2] , Fernández J[3], Martínez de Pancorbo M[1]

[1]*Servicio de Genómica: Banco de ADN, Universidad del País Vasco, Basque Country, Spain*
[2]*Laboratoire d'Anthropologie Moléculaire, Institut de Médecine Légale, Strasbourg Cedex, France*
[3]*Facultad de Geografía e Historia, Universidad del País Vasco, Basque Country, Spain*

Wide studies have been done on how the resettlement of Europe took place after the end of the Last Glacial Maximum (LGM), approximately 15.000 years before the present. The Basque Country is said to have played a major role as a refuge during the LGM and as an expansion focus during the resettlement of the continent (Torroni et al. 1998, 2001; Achilli et al. 2004). These studies have been carried out using mainly mitochondrial DNA data from modern populations. However, these data are influenced by some aspects such as the variation generated along the generations by genetic drift. To overcome these problems it is of great value to use ancient DNA. Working with ancient osseous remains to obtain DNA requires extremely careful manipulation and is not always successful. However, the possibility of analysing ancient mitochondrial DNA it is of great interest for this study, as the variations localized in the control and coding regions would help to understand the movements of the populations along the history.

In this paper we present the preliminary results of our project, based on ancient DNA analysis. The aim of the project is to establish a theory about the importance of haplogroup H and its subhaplogroups in the migratory movements occurred in Europe by comparing data from ancient samples with those from modern populations.

All the samples already analysed belong to the site of Las Yurdinas II, located in the south part of the province of Álava (Basque Country). These samples yielded a radiocarbon date of 4350 +/- 50 years, thus belonging to the Calcolithic period. DNA was extracted from both ulna bones and teeth. Although the region HVI of the mtDNA was successfully sequenced for all the samples, we concluded that the DNA was better preserved in the dental remains. All the samples were classified as belonging to haplogroup H. In order to prevent contamination the samples were processed in specific laboratory for ancient DNA and negative controls for all the steps were included. Moreover, all the persons involved in the processing of the samples were typed. Thus it was possible to discard any false positive result caused by external contamination.

contact: zobcamas@vc.ehu.es

P-053

Analysis of the maternal and paternal lineages of Azores islands population

Carvalho M[1], Brito P[1], Balsa F[1], Antunes H[1], Anjos MJ[1], Andrade L[1], Lopes V[1], Serra A[1], Oliveira C[1], Gamero JJ[2], Romero JL[2], Corte-Real F[3], Vieira DN[3], Vide MC[1]

[1]Forensic Genetic Service. National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal
[2]Faculty of Medicine. University of Cadiz. Fragela s/n, Cádiz 11003. Spain
[3]National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal

The aim of this study was the analysis of the origin of maternal lineage (mithocondrial DNA) and paternal lineage (Y Short Tandem Repeats) of Azores Islands population comparing our data with other populations from Europe and Africa.
The polymorphism of the two hypervariable segments (HVI and HVII) of control region of mtDNA was analyzed in unrelated individuals from Azores Islands, using a amplification method with *primers* refered by Wilson et al.(1995). Sequences have been obtained with ABI PRISM *Big Dye Terminator and dRhodamine Terminator Cycle Sequencing Ready Reaction Kit s, with amplitaq DNA polymerase FS,* and have been detected with ABI 3100 Avant sequencer. We will describe the number of different sequences for HVI and HVII regions in our population data and the polymorphic sites.
The Y-chromosomal haploptype was defined by 17 Y-STRs (DYS19, DYS385, DYS389 I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS460, DYS461, GATA A10, GATA C4 and GATA H4) in a sample of unrelated individuals of Azores islands. The minimal haplotype was carried out according with the *primers* and conditions of the *PowerPlexY PCR Amplification Kit,* de Promega and the other YSTR were amplified with two tetraplexs reactions (GEPY I and GEPY II), using the protocol according to Sanchez-Diz et al.(2003). We will describe the most common haplotype in this population and how many haplotypes will be unique.
The comparison of maternal and paternal lineages from Azores Islands with other lineages from Europe and Africa was performed using the Arlequin software version 2.000.

Contact: geneforense@dcinml.mj.pt

P-054

In-house validation of the PCR amplification kit « Mentype® Argus X-UL »

Castella V, Dimo-Simonin N, Morerod M-L and Mangin P

Laboratoire de Génétique Forensique, Institut Universitaire de Médecine Légale, rue du Bugnon 21, 1005 Lausanne, Switzerland

X chromosome-specific short tandem repeats (STRs) may complement the analysis of conventional genetic markers (autosomal STRs, Y-STRs or mitochondrial DNA), especially when complex relatedness testing cases are analyzed. The Mentype®Argus X-UL PCR amplification kit contains 4 X-STRs : DXS8378, HPRTB, DXS7423 and DXS7132 plus the gender marker Amelogenin as an amplification control. In this study, 50 females and 50 males of Swiss Caucasian origin were analyzed in order to validate the forensic utilization of this kit and to determine some population statistics. Preliminary tests have shown that the diminution of PCR reaction volume from 25.0 to 12.5 □l increased the sensitivity of the kit. With the smaller volume, full profiles were obtained with ≥ 100 pg DNA template. Female/male mixtures produced full profiles from the minor contributor with 10-20-fold excess of the major contributor. Intra and inter-day reproducibility of allele sizing, stutter height and heterozygous balance were comparable to those observed with other amplification kits used by the forensic community. Allelic frequencies and forensic characteristics of individual markers (polymorphism, discrimination power, etc.) are presented in the poster. At the population level, the sample of 50 females enabled to verify that the Hardy-Weinberg equilibrium was respected and that the 4 X-STRs were statistically unlinked. Finally, 4 relatedness testing cases were performed in order to evaluate the efficiency of the Mentype® Argus X-UL kit for solving deficiency cases.

Contact: Vincent.Castella@chuv.ch

P-055

Low diversity in *Cannabis sativa* from Brazil and Paraguay illegal plantations accessed through fluorescent multiplex STRs

[1,2]Castro J, Grattapaglia, D[1,3] and Pereira RW[1]

[1]Graduate Program in Genomic Science and Biotechnology, Catholic University of Brasilia, Brazil; [2]Brazilian Department of Federal Police; [3]Heréditas Tecnologia em Análise de DNA Ltda.

The Cannabis sativa has a long history in the human cultural evolution, starting around 6500 from now, somewhere in the central Asia. Along this relationship the C. sativa have been cultivated as source of fiber, oils, medicines and also as source of a recreational psychoactive. The cannabinoid Δ^9-tetrahydrocannabinol (THC) is the main component responsible for the psychoactive effects. Many countries around the world, faced with the increasing use of C. sativa (marijuana) as a recreational drug, take the decision to turn it illegal, strongly repressing their dealing. But, in some countries the agricultural use of *C. sativa* is allowed. However, the varieties used in this case are THC poor. Methods to differentiate legal from illegal crops have been widely explored, mainly based on THC identification and quantification. The Brazil legal system does not allow any use of *C. sativa* and all levels of our security system repress it. The marijuana that is consumed in Brazil comes mainly from local and from Paraguay plantations. The Brazilian marijuana combat program has the Federal Police as the major action planner. The *C. sativa* banish program effectiveness involves investigation, intelligence actions and effective operations carried out by specialized police task forces aiming the shipping interception and plantations destruction. Any technology that could improve the success rate in the law enforcement is welcome. In this regard, the characterization of polymorphic STRs in C. sativa showed up as a potential toll to establish the geographical origin of marijuana and the identification of marijuana produced clonally, helping in the disruption of criminal network established around the illegal dealing of marijuana. The Brazilian Federal Police and the Graduate Program in Genomic Science and Biotechnology from Catholic University from Brasilia starts a project to develop a multiplex system based on C. sativa STRs characterized by others groups and investigate de genetic diversity in different plantations from the main source areas in Brazil and Paraguay. The fluorescent multiplex standardization starts with 13 STRs. The forward primer to amplify the ANUCS304, the C08-CANN2, the ANUCS201, the H11-CANN1 and the B01-CANN1 STRs loci were labeled with 6-FAM. The forward primer to amplify the ANUCS302, the ANUCS305, the B05-CANN1 and the H06-CANN2 were labeled with 5-HEX. The last four loci, the ANUCS302, the ANUCS202, the H09-CANN2 and the ANUCS301 were labeled with NED. New primers were designed to seven out of the 13 loci listed above. By the end of the optimization we end up with two multiplex set based on primers annealing temperature and with nine microsatellites amplified. Four of them failed to be optimized. These multiplex sets were used to amplify 48 DNA samples from twelve plantations distributed as follow: six plantations from Paraguay, two plantations from Maranhão (Brazil) and four plantations from Pernambuco (Brazil). The PCR products were analyzed in the ABI Prism 377. The sample files were analyzed using Genescan and Genotyper software. The basic diversity indices were computed using Arlequin package. The overall results analysis clearly showed a low diversity in Brazilian and Paraguay plantation. Only the C08-CANN2 and H09-CANN2 had heterozigosity higher than 0.5, respectively 0.8 and 0.56. The ANUCS305 and H06-CANN2 showed only one allele. The fail to optimize all loci may be explained by sequence divergence among our samples and the original sequence from Genbank. All these loci had previously showed to be high polymorphic, mainly in fiber varieties. The low heterozigosity to the majority of the loci we studied shows that more investigation in the classification of new high polymorphic *C. sativa* microsatellites is necessary if we would like to continue testing this tool. contact: rinaldo@pos.ucb.br

P-056

Evaluation of reliability of STR typing for forensic purposes in different types of cancerous tissues

S. Ceccardi[1], M. Alù, F[2]. Lugaresi[1], G. Ferri[1], C. Bini[1], T. Balbi[1], F. Ingravallo[1], S. Pelotti[1]

[1]Department of Medicine and Public Health, Section of Legal Medicine, University of Bologna, Bologna, Italy

[2]Department of Pathological Anatomy and Legal Medicine, Section of Legal Medicine, University of Modena and Reggio Emilia, Modena, Italy

Forensic DNA testing by STR kits validated to have reliable and robust results, might be questionable when cancerous tissues are forcibly used for forensic purposes.

Several studies were performed to elucidate the mechanism underlying gene-environment interaction in carcinogenesis, investigating short tandem repeats. One of the most investigated STR in cancer is the CAG repeats in the androgen receptor gene (AR) used also in forensic application, even if recently Szibor et al. recommended the forensic Community to refrain from its use for the link with disease risks.

In recent studies a number of findings demonstrating DNA instability in tumor DNA also at STRs used in forensic casework, was detected. Partial loss of one allele, complete loss of one allele and microsatellite instability (MSI) were described in esophageal, gastrointestinal, lung, oral, head and squamous cancers and cervical carcinoma.

We analyze 68 sporadic primary tumor samples, including gastrointestinal, urogenital and oral carcinomas, in parallel with near non cancerous tissues for 15 STR loci including in a commercial kit.

To avoid the problem of DNA degradation in paraffin embedded specimens as source of mixture of fragments of diverse length that can lead to misinterpretation of instability; we analyze frozen cancerous tissues compared to frozen normal tissues surgically collected.

The problem of stuttering and complex artefacts in the context of MIN is considered to compare the results and to avoid a false positive diagnosis of MIN. The adopted criteria to classify a sample as MIN positive are those suggested in assessing microsatellite instability (Sobrido M.J. et al. Electrophoresis 2000, 21, 1471-1477).

Besides, the relationship between the pathological stage of cancers and their respective allelic alteration patterns is presented.

Finally, our study may contribute to look for the uniform panel of microsatellites suggested by Sobrido et al. suitable for cancerous tissues analysis.

Contact: susi.pelotti@unibo.it

P-057

Incest by father or by brother? A case report

Cerri N[1], Presciuttini S[2], Notarangelo L[3], Verzeletti A[1], De Ferrari F[1]

[1]Department of Forensic Medicine, University of Brescia, Brescia, Italy

[2]Center of Statistical Genetics, University of Pisa, Pisa, Italy

[3]Department of Pediatric, University of Brescia, Brescia, Italy

Ten years ago a 16 years old girl gave birth to a child who deceased five days later into the hospital. The girl reported to the Prosecutor that she had been raped by a schooolmate.

The molecular analysis to identify cistyc fibrosis mutations in the child, as a screening performed in all new-borns in Italy, allowed to identify the homozigosity status for the mutation N1303K. This mutation is quite rare in Italy (4% of all mutations regarding this disease) so the clinicians suspected that the father could be a member of the girl's family. In fact, the analysis performed on the girl's father, confirmed the presence of the same mutation.

The Prosecutor asked a genetic analysis on the dead child, on the girl and on her father and mother. At the investigation time, only traditional markers such as DQalpha, D1S80, LDLR, GYPA, HBGG, D7S8, GC, TPOX, F13A01, AR, APOB were investigated. Only some years later a genetic profile was obtaind using the commercial kit Profiler Plus (Applera, Foster City, CA, USA).

The DNA for the analysis was obtained from the child's whole blood collected during autopsy and from whole blood from the girl, her father and her mother. DNA was extracted using Phenol/Chloroform method. The amplification of the VNTR was permormed according to the protocols present in Literature and the amplification for the Profiler Plus Kit was performed according to manufacturer's recommendations in a GeneAmp PCR System 2400 (PE).

All markers investigated were consistent with a relationship father/girl except for the APOB and D8S1179 loci.

A research in the Literature regarding the mutation rate at these loci showed no relevant mutation rate, above all for D8S1179. So two alternative hypotesis were considered: a) the girl's father wasn't the child's father; b) the girl's father was the child's father and the two incompatibility were due to new mutations. Using the software for genetyc analysis "Familias", this second hypothesis was excluded because of the inconsistency of the probabilty of two mutations in the two systems considered. Considering another hypothesis, i.e. a a girl's brother as the child's father, the probability index was very strong.

In fact the investigations led to the discovery of a girl's brother who admitted the crime later.

andverz@tin.it

P-058

Frequency data for the STR locus SE33 in a population sample from Brescia (northern Italy)

Cerri N, Verzeletti A, Bandera B, De Ferrari F

Department of Forensic Medicine, University of Brescia, Brescia, Italy

Short tandem repeat (STR) markers are widely used in forensics as well in paternity testing, but before a new locus can be introduced in the current practice a database for the relevant population must be established to evaluate its effectiveness in forensic identification and paternity testing. In Italy there are already data regarding a lot of STR, but few data about SE33. This locus is one of the most informative tetranucleotide short tandem repeat loci used for human identification and paternity testing and due to its extensive polymorphism, the Federal Criminal Police Office of Germany has included SE33 as one of the eight core genetic loci with witch to establish a database.

A total of 90 unrelated individuals from Brescia region were typed. Genomic DNA was extracted using Chelex-100 procedure from whole blood or buccal swabs. PCR was performed in a GeneAmp PCR System 2400 (PE) using the commercial kit AmpFlSTR®SEfiler™ (Applied Biosystems, Foster City, CA, USA) according to manufacturer's recommendations. Typing was performed by capillary electrophoresis (ABI Prism 310 Genetic Analyzer, ABI). Allele scoring for this locus was obtained by comparison to AmpFlSTR®SEfiler™ Allelic Ladder (Applied Biosystems, Foster City, CA, USA) and all alleles were designated according to the recommendations of GEDNAP.

This work provides a picture of allelic and genotypic frequencies for SE33 from Brescia region. As expected the preliminary results in the distribution of allelic and genotypic frequencies in our population sample are close to those found in the caucasian population.

andverz@tin.it

P-059

Population data for 4 X-Chromosomal STR loci in a population sample from Brescia (northern Italy)

Cerri N, Verzeletti A, Gasparini F, Bandera B, De Ferrari F

Department of Forensic Medicine, University of Brescia, Brescia, Italy

Short tandem repeat markers on the X chromosome are the natural counterpart to the well-established Y-chromosome STR loci and they have proven to provide useful tools in paternity cases with female offspring or in forensic identification cases based on the comparison with first or second degree relatives.
But before a new locus can be introduced in the forensic current practice a database for the relevant population must be established to evaluate its effectiveness. Because of the few population data regarding X-chromosme STR loci in Italy, 90 unrelated individuals (50 females and 40 males) from Brescia region were typed for the STR-loci DXS8378, DXS7132, HPRTB, DXS7423.
Genomic DNA was extracted using Chelex-100 procedure from whole blood or buccal swabs. PCR was performed in a GeneAmp PCR System 2400 (PE) using the commercial kit Mentype Argus X-UL (Biotype AG, Dresden, Germany) according to manufacturer's recommendations. Typing was performed by capillary electrophoresis (ABI Prism 310 Genetic Analyzer, ABI). Allele scoring for these loci was obtained by comparison to Mentype Allelic Ladder (Biotype AG, Dresden, Germany).
This work provides a picture of allelic, genotypic and haplotypic frequencies for 4 X Chromosome STR loci from Brescia region. As expected the preliminary results in the frequencies distribution in our population sample are close to those found in the caucasian population.
andverz@tin.it

P-060

Genetic characterization of Y-STR in the Korean populations of the southern region

Byung-Won Chun[1], Sang-Churl Shin[1], Yang-Jung Kim[1], Kyung-Lyong Lee[1], Pil-Won Kang[1], Kwang-Hoon Kim[1], Kyung-Sook Kim[2], Dong-Ho Choi[2], Myun-Soo Han[2]

[1] Department of Forensic Medicine, Southern District Office of NISI, Busan, Republic of Korea; [2] Department of DNA Analysis, National Institute of Scientific Investigation, Seoul, Republic of Korea

Y chromosomal haplotypes of 12 polymorphic loci (DYS391, DYS389l, DYS439, DYS389ll, DYS438, DYS437, DYS19, DYS392, DYS393, DYS390, DYS385 a/b) were analyzed in samples from a total of 762 males in eight Korean sub-populations and 30 Chinese males. 208 Japanese males and 196 randomly sampled Korean males were used to survey the genetic structure among the sub-populations in Korea and the relationship between the northeast Asian populations. The Japanese and the randomly sampled Koreans of these populations were haplotype data. The results showed 589 different types of haplotypes from 762 Koreans with no blood relationship. Of these, 3 haplotypes were found in all 8 groups. They were the haplotype H305: 10-14-12-29-13-14-16-13-13-23-10,19; H311: 10-14-12-29-13-14-16-13-13-23-10,18; and H218: 10-14-12-29-13-14-16-13-13-23-10,17(DYS391-DYS389l-DYS439-DYS389ll-DYS438-DYS437-DYS19-YS392-DYS393-DYS390-DYS385a/b). These three haplotypes also showed the highest frequency, indicating that they are likely to be the genetic type of the common ancestors of the southern Korean population. From the haplotype information of 8 southern Korean populations along with the Chinese and Japanese populations, the Jeonnam population showed the highest number of haplotypes (113/119, 95%), unique haplotypes (108/119, 91%), haplotype diversity (0.9990) and discrimination capacity(0.9495) among the 8 populations. The Geoje population had the lowest number of haplotypes (79/98, 80%), unique haplotypes (67/98, 68%), haplotype diversity (0.9944), and discrimination capacity (0.8061). These results can be explained by the founder effect as shown in the allele frequency distribution analysis. The fact that 509 unique haplotypes were found from 762 southern Koreans suggests that there was a significant influx of outside populations considering that there are only 270 family names in Korea. Within the southern Korean populations, the pairs that had the most shared haplotypes in order were Jeonnam-Andong, Jeonbuk-Geoje, Gyeongnam-Jeonnam & Andong, Gyeongbuk-Gochang and Jeju-Gochang. This shows that there was active interbreeding in the past regardless of the region. The phylogenetic tree analysis using the genetic distance, which is determined by allele frequency, shows that the Honshu-Japanese population had the closest genetic relationship with Jeonbuk, followed in order by Geoje-Gochang-Gyeongnam-Jeonnam-Gyeongbuk-Andong-Jeju populations. The fact that Keoje showed the second closest genetic relationship with the Honshu-Japanese population can be explained by the fact that it had the most shared haplotypes with Jeonbuk. This result genetically supports the historical facts that the Paekje Kingdom, which was based on what is now the Jeolla region, had the most interchange with Japan. The results of this study show that, based on the hypothesis that more than 80% of the Japanese group had migrated to Japan, the Jeolla region, especially Jeonbuk, had the closest relations to the migration of southern Koreans to Japan. The results of this study constitutes the genetic proof that there was a large scale migration to Japan when Korea and Japan was connected during the ice age 10,000~15,000 years ago, and that the Paekje kingdom, which was based in the Jeolla region, was the most influential in the smaller scale migrations since that time. Contact: hmyunsoo@nisi.go.kr

P-061

Short tandem repeat (STR) polymorphisms analysis at 15 loci in Sicilian population: genetic disequilibrium and allelic frequency

I. Ciuna [(1)], Maria Guarnaccia [(2)], E.Ginestra [(1),] Antonella Agodi [(2)], D.Piscitello [(1)], S.Spitaleri [(1)], Giovanni Marcì [(2)], Gianluca Paravizzini [(2)], C.Trapani [(1)], G. S.Travali [(2)], and L. Saravo[(1)]*

[1] *Laboratory of Molecular Biology – Raggruppamento Carabinieri Investigazioni Scientifiche (RaCIS), Messina; Italy.*
[(2)] *Department of Biomedical Science,, University of Catania, Italy*

DNA polymorphic loci are widely utilized for human genome mapping, to perform linkage analysis, paternity testing and forensic investigations. The aim of our work was studying allelic frequencies and distribution within the 15 forensic STR loci in a group of 500 unrelated Sicilian subjects coming from the nine different counties of the island. Afterwards we have evaluated the genetic equilibrium among the most recurrent alleles mapping in the above mentioned loci and have compared our data to those already published by other authors referring to different populations. Results shown in table.

Keywords: DNA STR typing*;* STR-DNA database.
*Corresponding author: rismebiologia@carabinieri.it

P-062

Allele distribution of 6 X-Chromosome STR loci in an Italian Population sample.

Coletti A , Lottanti L , Lancia M , Margiotta G , Carnevali E , Bacci M

Section of Legal Medicine, University of Perugia, Terni, Italy.

Nowadays several research efforts are made to evaluate the allelic frequencies of ChrX STRs: chrX STR loci can be indeed more informative than autosomal loci in such cases as specific paternity deficiency and complex kinship. This is the reason why it needs to increase the population data for ChrX STR allelic frequencies and to create a national or local database to make comparisons with the corresponding population data in a generalized way.

An esaplex PCR was developed to amplify DXS6789, HumARA, DXS7423, DXS6807, DXS101 and DXS8377 in some Italian Samples from Terni. This system represents a protocol for the Chr X analysis with a shorter procedure.
The DNA was extracted from 100 blood samples by using the QIAmp DNA Minikits produced by Quiagen.
The samples were detected using an ABI PRISM 310 genetic analyser (Applied Byosistem), by using the following dye labels: Vic for DXS 6789 and HumARA, Ned for DXS101, Fam for DXS7423 and DXS6807, and Pet for DXS8377, which are the same dye labels used by Kit Identifiler: it means using the same mobility files, matrix files and software parameters.
We performed statistical analyses for all the loci.

contact: baccim@aospterni.it , lancia.massimo@infinito.it or gabriele.margiotta@poste.it

P-063

Tetragametic chimerism in a true hermaphrodite child

Cólica, MV, Rodríguez Cardozo MB, Abovich M, Valente, Ribas N, Di Lonardo AM

Banco Nacional de Datos Genéticos, Unidad Inmunología, Hospital Carlos G. Durand, J. B. Ambrosetti 743 (1405), Buenos Aires, Argentina

Human congenital chimerism is due to the coexistence of two genetically different cell lines either in the whole body or limited to the blood.
In order to prove the generation mechanism of congenital chimerism in a hermaphrodite newborn child, we used DNA polymorphisms of autosomic STRs, chromosome Y and MHC genes in a peripheral blood sample and genital tissues biopsies. All these genetic markers allow us to see the mendelian inheritance of genes.
The results obtained from this patient demonstrated that the most probable cause of congenital chimerism, so called TETRAGAMETIC CHIMERISM, occurred through the fertilization of two ova by two spermatozoa, followed by the fusion of the zygotes and the development of an organism with intermingled cell lines.

* Corresponding author. Tel.: +54 11 4982 1716; fax: +54 11 4982 0625
E-mail address: bndg@infovia.com.ar (A.M. Di Lonardo).

P-064

Ethnic Contributions to the Extant Population of Argentina: as shown by uniparentally inherited genetic markers.

Daniel Corach, Andrea Sala and Miguel Marino

Servicio de Huellas Digitales Genéticas and Cátedra de Genética y Biología Molécular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires
Junín 956 Ciudad Autónoma de Buenos Aires. Argentina.

The population of Argentina is the result of three major ethnic contributions. The original population of South America is of Amerindian ancestry and its arrival from Asia to the New World is accepted to have occurred over 18.000 years ago. A second contribution was provided by the Spanish conquerors that arrived to the now a days territory of Argentina in 1536 and maintained their migration since then. A third contributor was introduced during the seventh century, as a working force, the slaves imported from West Africa. At present, it is not possible to distinguish the presence of African phenotypes in our population; however its genetic contribution can be traced. Finally, during the late XIX and early XX centuries an intense migration wave from Europe and Near East occurred. The history of the admixed Argentina can be traced back to 19 generations and a big deal of admixture might have taken place.
In order to investigate the ethnic contribution to the extant population of Argentina a set of 322 unrelated males inhabiting 10 provinces of Argentina were selected at random from samples of routine forensic casework. Three major geographic regions were considered: Northeastern (Formosa, Chaco, Corrientes and Misiones Provinces, N=102), Center (Buenos Aires, Santa Fe and Entre Rios Provinces, N=120) and South Southwestern (Mendoza, Rio Negro and Chubut Provinces, N=100). DNA was extracted from blood samples. Each sample was analyzed by 15 autosomal STRs included in PowerPlex16 and the uniparentally inhereted genetic markers including: the SNP DYS199, nine Y-STRs (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392 and DYS393); mtDNA D-Loop sequence at HVR I and II; and the ins/del of 9bp at Region V. The use of well defined Amerindian uniparentally inhereted genetic markers could determine the ancestry of the individuals that belong to aboriginal or non-aboriginal patri or matrilineages. Mitochondrial DNA analysis allowed us to detect the presence of the main four Amerindian specific as well as European and African haplogroups. The analysis of Y-chromosome markers allowed to find Amerindian specific polymorphism (such as DYS199 T). The results were analyzed considering the geographical regions from where the samples were obtained in order to assess their similarities. The overall results suggest that 58% of the individuals belong to one of the major Amerindian mtDNA haplogroups (A: 13,44%, B: 35,48%; C: 34,4% and D: 16,66%), 18% showed the DYS199 T variant; 12% belongs to both Amerindian matri and patri lineages and 36% of the total exhibit non-Amerindian lineages. The analysis of these results by geographical areas showed a good correlation with historical and geographical records. The results presented in this work supports previous investigations based on blood groups and autosomal genetic markers analyzed in urban population of different cities of Argentina.

Contact: shdg@ffyb.uba.ar

P-065

Validation of the AmpFlstr® SEfiler™ kit

Cordoba S., Alape J., Camargo M.

Grupo de Genética Forense- Convenio INMLyCF-ICBF. Instituto Nacional de Medicina Legal y Ciencias Forenses. Bogotá – Colombia

The use of new STR markers (SE33) includes at the AmpFlstr® SEfiler™ kit is of grate utility in different cases at the laboratory especially in cases of Paternity and forensic investigations. With this marker is possible to increase the probability due to its high polymorphism.

Recently it has been great improvement in commercial kits that offer large multiplex reactions in a single step, systems whit high discrimination power and reliable and reproducible results.

The AmpFlstr® SEfiler™ kit the recent commercial product of Appiled Biosystems that offers 11 STR from human autosomes chromosomes D2S1338, D3S1358, D8S1179, D16S539, D18S51, D19S433, D21S11, HUMFGA, SE33, HUMTHO1, HUMvWA, and amelogenin.

In this work several aspects were assayed. Differences in extraction methods, differents PCR reactions volume, sensitivity and specificity and application on pathernity cases. The assays were evaluated at the ABI PRISM 3100 genetic analyzer using different qualities controls.

Contact: imlmartha@hotmail.com

P-066

Isolation of DNA using IsoCode Cards

Cordoba S., Prieto A., Camargo M.

Grupo de Genética Forense- Convenio INMLyCF-ICBF. Instituto Nacional de Medicina Legal y Ciencias Forenses. Bogotá - Colombia

The use of new device for DNA isolation (IsoCode Card) for amplification from Blood and saliva, is of grate utility at the laboratory especially in cases of Paternity.

The recent commercial product does not require the use of organic solvents, the procedure is easy and permit a rapid isolation of DNA for use in amplification reactions.

In this work several aspects were assayed: different body fluid samples, different washes and elution volumes, amplification with different commercial kits and others. The assays were evaluated at the ABI PRISM 3100 genetic analyzer using different qualities controls.

Contact: imlmartha@hotmail.com

P-067

The DNA extraction from pulp dentine complex of both with and without carious teeth

Corte-Real A[1], Carvalho M[2], Anjos MJ[2], Andrade L[2], Vide MC[2], Corte-Real F[3]

[1]Faculty of Medicine. University of Coimbra 3000 Coimbra. Portugal [2]Forensic Genetic Service. National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal
[3]National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal

Looking across the various forensic environmental conditions, the teeth constitute a valuable source of DNA and therefore of particular interest for casework analysis.

The main objective of this paper is to show that, despite some adverse forensic condition such as degraded human body remains and exhumed material, the dentine (in pulp dentine complex) keeps, in the majority of cases, its integrity.

In this study we use a sample of thirty human teeth (both with and without carious) after extraction during dental treatment. We analyze fifteen STRs and both high variable regions I and II of mitochondrial DNA.

Each tooth was prepared using a technique that comprises the mechanic removal of the enamel, central pulp and cement. The DNA extraction was carried out with a commercial kit but the protocol was adjusted according to the specificities of the sample. This procedure has allowed us to obtain a genetic profile of mitochondria DNA in all the samples as well as to define a profile of STRs in some of them.

Contact: a_cortereal@yahoo.com

P-068

A single assay for human-specific quantification of less than one picogram DNA and detection of the presence of PCR inhibitors in forensic samples

Costello MT, Schumm, JW

The Bode Technology Group; 7364 Steel Mill Rd.; Springfield, VA 22150; USA.

james.schumm@bodetech.com

We describe the development, validation, and application of a duplex real-time PCR assay for human-specific quantification of DNA samples containing as little as 0.5 pg/µl of DNA. The assay simultaneously detects PCR inhibitors within the sample. It is important to include human-specific quantification of DNA in casework sample analysis to insure successful DNA amplification and profiling. Much recent research has focused on the use of real-time quantitative PCR to achieve this goal. This approach is less labor intensive, less time consuming, more accurate, and lends itself to automation better than previous methods such as slot-blot hybridization (1). Our work builds on that described by Nicklas and Buel (2), Richard et al. (3), and the commercially available Quantifiler™ Kit (Applied Biosystems, Foster City, CA). We have combined the sensitivity and human specificity of *Alu*-based real-time quantification with the presence of an internal positive control allowing detection of PCR inhibitors in the sample. *Alu* sequences are short, repeated elements that are interspersed throughout the primate genome in upwards of 500,000 copies. We selected the Yb8 subfamily of *Alu* genes because of its sequence specificity to higher primates (4). Using this target, we developed primers and a fluorogenic probe for a quantitative real-time PCR assay (5). The assay also contains an internal positive control (IPC) system that is multiplexed with the *Alu* quantification system, consisting of a fixed quantity of non-human DNA template added to each reaction well, and a second set of primers and fluorogenic probe specific for the non-human template. The combination of human DNA quantity data from the *Alu* system and DNA quality data from the IPC system provides the analyst with substantial information to aid in deciding dilution or concentration schemes prior to STR amplification, thereby significantly reducing the number of samples that need to be re-evaluated following initial profiling. Validation work indicates the assay is accurate and precise in the range of 50 ng/µl to 0.5 pg/µl. Thus less than one human genome equivalent can be detected accurately. Species specificity tests indicate the assay is at least 5000 times more specific for higher primate DNA than any other species tested. The IPC system is very sensitive to inhibition observed with addition of hematin, indigo, or humic acid. The assay has been successful with a variety of non-probative sample types.

1. The features of this assay will allow us to apply it very effectively to evaluation of touch evidence samples. With so little sample available in these situations, it is critical to make the right decision to use more or less extracted DNA in the first profiling test.

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6.P-069

Allele distribution at two STR loci (D15S642 and D15S659) in the Croatian population

Crkvenac Gornik K[1], Stingl K[2], Kerhin Brkljacic V[2], Grubic Z[2]

[1]*Department of Genetic and Metabolism, Paediatric Clinic,*
[2]*Tissue Typing Centre, University Hospital Zagreb, Croatia*

Population studies of two STR loci (D15S642 and D15S659) were carried out in a sample of 130 unrelated healthy individuals. After PCR amplification samples were run on 6% polyacrylamide gel in automated sequencer (ALFexpress). Twelve different alleles were identified at D15S642 locus and 11 alleles at D15S659 locus. The most frequent alleles at D15S642 were: allele 2 (16.7%), allele 8 (16.3%) and allele 9 (14.0%), while the most frequent genotype was: 2-2. Among 11 different alleles at D15659 the most frequent were: allele 9 (22.1%), allele 3 (19.1%) and allele 8 (18.4%). Genotype 9-8 showed the highest frequency (9.6%) at D15S659 locus. The observed heterozygosities for these two loci were 0.81818 for D15S642 and 0.83088 for D15S659. PIC was calculated as follows: 0.88 for D15S642 and 0.83 for D15S659. No significant deviations from Hardy-Weinberg equilibrium could be observed for these systems. The results indicate that these two loci are useful genetic markers for paternity testing as well as for prenatal or postnatal diagnosis.

Contact: zgrubic@kbc-zagreb.hr

P-070

Genetic data for the locus SE33 in a South Portuguese population with Powerplex® ES System

Cruz C, Vieira-Silva C, Ribeiro T, Espinheira R

Forensic Genetics Service, National Institute of Legal Medicine, Lisbon

The SE33 (ACTBP2) locus is one of the most informative short tandem repeat systems for human identification.

The aim of this study was to establish the allele frequencies distribution of SE33 locus in a south portuguese population, which can be used for forensic purposes.

Blood samples for paternity testing were obtained in Bloodstain Cards from 328 unrelated individuals, residing in the south of Portugal. DNA was extracted by the Chelex-100 method and the SE33 locus was amplified using the Powerplex® ES System (Promega Corporation, Madison WI, USA) according to manufacturer instructions. The amplified products were separated in an ABI PRISM 3100 Automatic DNA Sequencer. The data were analysed by Genescan® Analysis 3.7 and Genotyper® 3.7 software.

The allele frequencies and forensic parameters of interest were calculated and the Hardy-Weinberg equilibrium was evaluated. A comparison with others populations was performed.

A total of 170 genotypes and 38 alleles were observed. The most common alleles were 16 and 29.2 (73,2%). It was detected an out of ladder allele (39.2).

Heterozygosity and power discrimination values confirm the high degree of polymorphism and discriminating power of this locus.

This study demonstrated that SE33 is a useful locus for forensic identification that should be added to the set of STRs loci routinely studied in order to increase the discrimination potential, namely in complex cases which involve relatives.

contact: genetica@dlinml.mj.pt

P-071

Identification in forensic anthropology and its relation to genetics

Cunha E, Pinheiro J, Soares I, Vieira D N.
Instituto Nacional de Medicina Legal, Delegação de Coimbra.Coimbra, Portugal
cunhae@antrop.uc.pt

The aim of this communication is to call the attention to the fact that DNA can not replace the anthropological analysis. If, in one hand some of the benefits of genetic analysis are their exclusive, on the other, a genetic profile can not provide data on some of the basic parameters of the biological profile, such as age at death and stature. Thus, it is the combination of both anthropological and genetic expertises that can indeed lead to a positive identification. Without a biological profile given by the anthropological expertise, the DNA could be usefulness.

We here present two cases which can be considered antagonic. In the first, identification was confirmed by genetics, while in the second genetics was not conclusive. The former one, concerns a body re-examination in the Pico Island (Azores) by a forensic anthropologist of the National Institute of Forensic Medicine. A complete skeletonized victim of a homicide was recovered from the field after denunciation by one of the murder witnesses. At that time, around one year after the crime had been committed; the victim was autopsied and buried. It was supposed to be a luso-american individual and DNA analysis were done to confirm his identity. Although the genetic profile was accomplished, the prosecutor did not accept it as an unequivocal identity proof, since it could also be compatible with eventual existing brothers. Consequently, further data was required, namely dental charts which were sent to be matched with the victims' one. As this matching was problematic, the victim was exhumed and a second autopsy was then performed. Besides the verification of correspondence between ante and post mortem dental records, a thorough anthropological analysis led to the achievement of a much more reliable characterization of the individual.

In the second case study the body was autopsied by a forensic pathologist and a forensic anthropologist at the Office of Forensic Medicine of Viseu (Gabinete Médico-Legal de Viseu).An almost complete skeleton from an isolated site in the field, missing the bones of extremities, was found superficially buried, 15-20 cm depth by a rural worker. The biological profile, achieved by anthropological and odontological analyses, matched with a missing individual in the area who was disappeared for four years. DNA analyses performed on bone and teeth samples later on, once compared with the one of a relative, confirmed the individual's identity. The victim was suspected to have been murdered by his wife and daughters. However, on the basis of the skeletal remains, it was not possible to establish the cause of death. Since this person was reported as missing, the genetic profile of the victims' relatives was already available at the National Institute of Forensic Medicine for matching leading to an easy confirmation of identity already suspected by the anthropological multidisciplinary expertise.

We argue that it is important for the forensic community and even to the general public to be aware both of the benefits and drawbacks of genetic analysis when leading with non-identified human remains. Genetics is really a fantastic tool in identification. However, it is not the only step. In spite of one of the advantages of genetics is being able to supply a quantitative result, which makes possible to provide the probability that another person shares the same genetic assert, the lack of relatives to compare with, sometimes invalidates its usefulness. In these circumstances the classical anthropological analysis remains as valid as ever.

P-072

LR-calculation of any kinship situation using a graphical interface: generate two or more hypotheses, draw the family trees and assign the DNA-profiles to person symbols

Dajda T, Jung M

bj-diagnostik GmbH, Kerkrader Str. 11, 35394 Giessen, Germany

Based on an idea of Ihm and Hummel (Z. Immun. Forsch. 149, 405-416, 1975) and the kinship-algorithm by C.H. Brenner (Genetics, 145, 535-542, 1997) we developed a graphical interface to allow an intuitively construction of alternative family trees represented by two or more hypotheses. The family tree can be constructed like with a graphics design programme. The LR formulas/results will be generated accordingly to the family tree and hypotheses. Drop person symbols and draw the connection lines between them with a computer mouse. Silent Alleles and mutations can also be treated. A simulation module allows calculations for any kinship scenario (the number of markers and the number of persons can be varied). This module be used to plan a DNA-analysis in a deficiency case (how many markers, which persons should be tested).

Contact: michael.jung@bj-diagnostik.de

P-073

Investigation of single nucleotide polymorphisms associated with ethnicity

Daniel R, Walsh SJ, Piper A

Centre for Forensic Science, University of Technology Sydney, NSW, Australia

Single nucleotide polymorphisms (SNPs) have been widely investigated as markers in human genetic studies ranging from comparative population variation to disease linkage studies. As a result of the low mutation rate of SNPs, they are also considered to be useful markers of biogeographical ancestry.

Recently, in forensic genetics, attention has returned to SNPs, particularly due to their association with ethnicity and physical appearance. Such developments are potentially of great benefit to forensic investigators who are unable to match crime scene samples to database profiles. Unfortunately, physical characteristics are usually polygenic traits that are influenced by a number of genes and, in some cases, by environmental factors. However, many genes contain SNPs that are highly polymorphic in different ethnic groups.

As the first component of an investigation into the utility of SNPs as markers of ethnicity or appearance, we have developed the initial stage of an ethnicity multiplex. From an extensive literature survey, six autosomal SNPs were selected on the basis of strong associations with particular ethnic groups. The SNPs were specifically chosen for their potential to distinguish the major ethnic groups in the Australian population.

The ABI Prism® SNaPshot™ Multiplex kit (Applied Biosystems) based on single base extension of an unlabelled oligonucleotide (extension) primer was utilised for the development of the multiplex. Primer concentration optimisation experiments were conducted prior to genotyping over 200 hundred buccal swab samples collected from participants representing a cross-section of the Sydney community. Allele and genotype frequency data has been used to assess the usefulness of the multiplex as a predictor of ethnicity. Results have been cross-compared to genealogical information, self-declared by the participant over three generations.

The results from this preliminary research are promising in that distinct genotype distributions are evident among the predominant populations under study. Statistical analysis has been applied to empirically evaluate the observed trends.

DNA phenotyping is as yet in its infancy. The development of rapid and robust tests suitable for identification of phenotypes specific to the Australian population will provide a valuable intelligence tool for forensic investigators.

Contact: Runa.Daniel@student.uts.edu.au

P-074

Artificial blood chimerism due to graft-versus-host-disease after liver transplantation

Dauber EM[1], Müller CJ[2], Schöniger-Hekele M.[2], Dorner G[1], Wenda S[1], F.Mühlbacher[3], Mayr WR[1]

[1]*Division of Blood Group Serology, Medical University of Vienna, Austria*
[2]*Division of Gastroenterology and Hepatology, Medical University of Vienna, Austria*
[3]*Division of Surgery, Medical University of Vienna, Austria*

After transplantation of solid organs a small amount of the donor's cells can be detected in the recipient's blood which usually indicates a good prognosis for organ survival in liver transplantation. But in case of graft-versus-host disease (GVHD), however, donor's cells proliferate and produce an immune response against the recipient. In this case a higher degree of chimerism is observed.

Two months after liver transplantation a recipient developed diarrhea and leucopenia, which were interpreted to be side effects of therapy with ganciclovir for cytomegalovirus infection. After cessation of ganciclovir, however, no improvement was observed and a blood sample was sent to our laboratory to exclude graft-versus-host disease as a possible reason for his condition.

Multiplex-STR-typing has been carried out applying the *AmpFlSTR Identifiler PCR Amplification Kit* (Applied Biosystems, Foster City, USA). A blood chimerism with a higher percentage of donor's than recipient's cells was observed. Two buccal swab samples taken inside from each cheek also showed a mixture of the DNA profiles of donor and recipient. Only the eye brows showed the recipient's DNA profile itself. Another blood sample was taken 2 weeks later, three days before the patient deceased. This DNA profile was identical with the donor's profile, which was identified in a sample stored after tissue typing prior to transplantation.

To find out, whether the chimerism could already have been observed in tissue sections of a bone marrow puncture taken on the first onset of clinical symptoms, a singleplex PCR of the ACTBP2 (SE33) locus was carried out: 15% of the nucleated cells derived from the donor. Histopathology had just described hypocellular bone marrow without giving any clues to GVHD. Additionally, material from 21 different biopsies taken during autopsy was investigated. A chimerism was detectable in all samples except the transplanted liver, which only showed the donor's alleles.

In course of progression of clinical symptoms, the recipient increasingly showed the donor's DNA profile and his blood sample was found to be identical with the donor at the zenith of graft-versus-host disease. Just his hairs were found to be free of the donor's DNA genotype and exhibited only his own alleles. Therefore, STR-typing of bone marrow samples should be performed whenever an early stage of graft-versus-host disease is suspected. Hair samples of the recipient and material of the donor, if available, have to be investigated, in order to identify the two cell lines, as the major component does not necessarily represent the recipient's cell line.

contact: eva-maria.dauber@meduniwien.ac.at

P-075

Two apparent mother/child mismatches due to mispriming at the D3S1358 and the SE33 locus

Dauber EM[1], Parson W[2], Glock B[1], Mayr WR[1]

[1]Division of Blood Group Serology, Medical University of Vienna, Austria
[2]Institute of Legal Medicine, Innsbruck Medical University, Austria

We report two cases of apparent mother/child mismatch due to opposite homozygosity. They were observed at the D3S1358 locus after amplification with the *AmpFlSTR IDfiler PCR Amplification Kit* (Applied Biosystems, Foster City, USA) amongst 825 meioses and at the SE33 locus after PCR with the original primers published by Polymeropoulos et al. [1] amongst 1219 meioses.
The D3S1358 results were identical with the *Geneprint Powerplex 16 System* (Promega, Madison, USA). After lowering the annealing temperature in a singleplex PCR at the D3S1358 [2] and the SE33 locus the mendelian inheritance between mother and child was restored in both cases. Therefore a point mutation in the primer binding region had to be supposed.
A PCR with alternative primers lying outside of the primer binding sites of the original oligonucleotides confirmed these results. The alternative amplicons were sequenced and proved a point mutation in the binding site of the original primers. In case of the mother/child mismatch at the SE33 locus the failure of PCR was due a base substitution in the reverse primer region, which was already reported by other authors [3]. A point mutation near the 3' end of the reverse primer was found to be the reason for non-amplification of the D3S1358 allele, which has not been reported so far.
To overcome the problems of isolated parent/child mismatches due to opposite homozygosity a singleplex PCR with lower annealing temperatures can easily be performed to reestablish mendelian inheritance in case of base exchanges in the primer binding site.

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contact: eva-maria.dauber@meduniwien.ac.at

P-076

PCR-based diagnosis of cytomegaloviruses in paraffin-embedded heart tissue

Dettmeyer R, Müller J, Poster S, Madea B

Institute of Forensic Medicine, University of Bonn, Stiftsplatz 12, 53111 Bonn, Germany

Introduction. Immunohistochemical and molecular-pathological techniques have improved the diagnosis, but the incidence of virus-induced lethal myocarditis still remains unclear. Studies of myocarditis in adults demonstrated that numerous cases of acute myocarditis can not be diagnosed, according to the Dallas criteria, by traditional hematoxylin-eosin staining of endomyocardial biopsies. Previously, we reported on detection of enteroviruses (EV) including coxsackieviruses B3 (CVB3), parvovirus B19 (PVB19), adenoviruses (AV) and Epstein-Barr virus (EBV). We analysed cytomegalovirus DNA from paraffin-embedded heart tissue with PCR. Therefore, we established a reliable method to isolate DNA from formadehyde-fixed and paraffin-embedded material.
Materials and methods. Postmortem samples were obtained from 70 cases with suspected sudden infant death syndrome (SIDS). Eight myocardial samples were taken from each heart at standardized locations. Viral DNA was extracted from paraffin-embedded myocardial, liver and spleen samples with the Genial First-DNA-Kit (Genial, Troisdorf, Germany). The prerequisite for virus PCR was the amplification of cyclophilin (cyc). To avoid false-positive results due to contamination, negative controls were performed in all experiments. PCR products were sequenced on a ABI 310 sequencer. Sequence comparison was performed by a BLAST search of NCBI Gen-Bank. PCR products were also analysed on polyacrylamide gels.
Results. Cytomegalovirus-DNA was detected in 2 out of 70 cases of suspected SIDS. In all SIDS cases, the myocardial samples revealed no signs of myocarditis according to the Dallas criteria using conventional histologic stainings.
Discussion. Acute myocarditis can be diagnosed by PCR as a rapid method. Given the fact that in endomyocardial biopsies, the detection of cytomegaloviruses would be regarded as a pathological finding, this can be regarded as the cause of death, especially in SIDS cases presenting immunohistological signs of myocarditis in addition

contact: rdettmey@uni-bonn.de

P-077

Y Chromosome Polymorphisms in Argentine Population

Di Lonardo AM, Santapá O, Valente S, Filippini S
Banco Nacional de Datos Genéticos, Unidad Inmunología, Hospital Carlos G. Durand, J. B. Ambrosetti 743 (1405), Buenos Aires, Argentina

Short tandem repeats (STRs) loci are the most informative PCR based genetic markers available to date for attempting to individualize biological material. The full use of DNA typing technology in forensic science has grown up by the development of National DNA databases. That is the reason why today, many efforts are made to build up Y STRs databases for forensic purposes. Knowledge about mutation rates and mutational process of short tandem repeats (STRs), microsatellite loci used in paternity testing and forensic analysis, is crucial for the correct interpretation of genetic profiles. In our study, we analyzed Y Chromosome Polymorphisms for the loci: DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS439, DYS438, in unrelated Argentine individuals, most of them from Buenos Aires. Statistical interpretation of the results let us create a database of our own population, and we also studied paternity cases to discover genetic inconsistencies in father-son biological relationship testing.

Materials and methods: Blood specimens were collected from 301 unrelated males, most of them from Buenos Aires city, and 63 father-son pairs. DNA was extracted by the salting out method (Miller et. al.). Multiplex PCR amplification of six loci: DYS19, DYS385, DYS389II, DYS390, DYS391 and DYS393 was performed using Y-Plex™ 6 (Reliagene Technologies, Inc.) kit, and the PCR amplification of five loci: DYS389I, DYS389II, DYS392, DYS438 and DYS439 was performed using Y-Plex™ 5 (Reliagene Technologies, Inc) kit, according to the user's manual provided by the manufacturer. The amplified products were detected using an ABI PRISM® 3100 Genetic Analyzer (PE Applied Biosystems).The results were analyzed using GeneScan Analysis v 3.7 software (PE Applied Biosystems) and the alleles were typed using Genotyper v3.7 software (PE Applied Biosystems). The recommendations of the International Society for Forensics Genetics (ISFG) were followed for typing and interpretation.

Results: A total of 301 male unrelated individuals were analyzed for all 10 Y-STR loci and produced 274 haplotypes, of wich 258 haplotypes were unique, 11 were found in two individuals , 3 were found in three individuals, 1 was found in four individuals and the most common haplotype. DYS19 14, DYS389I 13, DYS389II 29, DYS390 24, DYS391 11, DYS392 13, DYS393 13, DYS385 11/14, DYS438 12, DYS439 12, was found in five individuals. The haplotype diversity calculated from the 10 Y-STR loci was 99.92% and the Genetic Identity: 0.0041. Most frequent haplotypes in our population sample have been compared with the Y-STR Haplotype Reference Database (www.yhrd.org, Institute of Legal Medicine, Medical Faculty, Charité, Humboldt University, Berlin-Germany) considering eight loci for the minimal haplotype and ten loci for the extended haplotype. The study of 63 alleged father-child non-exclusion cases with 15 autosomal STRs performed with Amp F/STR® Identifiler™, showed three alleles 14/16/17 at DYS385 locus, in one case. We observed one double mutation displaying two genetic inconsistencies at two different loci: DYS389I and DYS389II, between father (DYS389I: 12, DYS389II: 28) and son (DYS389I:13, DYS389II: 29) with W= 99,9999991 % (15 autosomal STRs). We also found mutational events in two unrelated individuals, three alleles at DYS385 locus: 12/13/14, and a biallelic pattern at DYS19 locus: 15/16.

E-mail: bndg@infovia.com.ar.

P-078

FOUR HIGHLY POLYMORPHIC STR-LOCI AS A "SCREENING TEST" IN PATERNITY CASES

Dorner G, Dauber EM, Wenda S, Glock B, Mayr WR

Division of Blood Group Serology, Medical University of Vienna, Austria

The aim was to design a "screening method" for paternity cases by investigation of 4 loci in a single run. We chose 4 highly polymorphic markers with a high chance of paternity exclusion: SE33 (0.905), D12S391 (0.791), D8S1132 (0.708) and D6S389 (0.845). The expected cumulative CPE (chance of paternity exclusion) for these 4 loci is 99.9%, the calculated probability to find 3 or more exclusions is 81%.

A triplex PCR (SE33, D12S391 and D8S1132), which can be detected simultaneously with a singleplex PCR (D6S389) in the same electrophoresis run, has been established.

74 paternity cases (48 non-exclusions, 26 exclusions), already investigated with conventional markers (red cell antigens, red cell enzymes, protein polymorphisms), 4 VNTR- (D1S80, YNZ, COL2A1, APO-B) and 11 STR- (SE33, TH01, vWA, FGA, D12S391, D8S1132, FES/FPS, F13B, CD4, LPL) loci were included in the study.

All non-fathers were detected in this paternity screening approach with at least 2 exclusions, in 21 out of 26 cases (81%) three or more exclusions were found. A single exclusion at the D6S389 locus, which was probably due to a mutation in the paternal germline, was found in a non-exclusion case.

In 66% of the non-exclusion cases the CPE was between 99% and 99.9%, in 34% the CPE was higher than 99.9%; in 36 of these 47 cases (77%) the probability of paternity was >99.75%, which corresponds to the attribute "paternity practically proven".

contact: gudrun.dorner@meduniwien.ac.at

P-079

A TRIPLEX-PCR FOR SE33, D12S391, D8S1132 AND A SINGLEPLEX-PCR FOR D6S389 IN A SINGLE RUN

Dorner G, Dauber EM, Wenda S, Glock B, Mayr WR

Division of Blood Group Serology, Medical University of Vienna, Austria

A triplex-PCR was developed for the highly polymorphic STR loci SE33, D12S391 and D8S1132. The primers were labelled with different dyes as the amplicons have partially overlapping size ranges. The highly informative STR locus D6S389 had to be amplified separately, as a multiplex-PCR without changing the primer sequences of the other loci was not possible. The D6S389 singleplex-PCR products were labelled with a fourth dye and could therefore be analysed in a single run together with the triplex-PCR products.
In this study and we investigated the triplex-PCR loci and D6S389 in a sample of 342 unrelated Austrian Caucasoid individuals. These data were in concordance with former results obtained after singleplex PCR and native polyacrylamide gel electrophoresis (SE33, D8S1132) or denaturing fragment analysis on an A.L.F. DNA Sequencer (D12S391). Some rare and new SE33 alleles have been detected and sequenced. Population data and statistic parameters were calculated for all loci. No deviation from Hardy-Weinberg equilibrium was observed.

Parameter	SE33	D12S391	D8S1132	D6S389
Observed heterozygosity	0.953	0.898	0.857	0.924
χ^2	86.86	25.91	39.20	48.28
df	78	28	28	36
p	0.234	0.573	0.065	0.078
Polymorphism information content	0.940	0.870	0.840	0.890
Matching probability	0.009	0.026	0.039	0.023
Power of discrimination	0.991	0.974	0.961	0.977
Power of exclusion	0.905	0.791	0.708	0.845
Typical paternity index	10.69	4.89	3.49	6.58

These 4 markers have been used to establish a “screening test” for paternity cases (see presentation of Dorner et al., Four Highly Polymorphic STR-Loci as a “Screening Test” in Paternity Cases)
contact: gudrun.dorner@meduniwien.ac.at

P-080

A new primer set in a SRY gene for sex identification: its implication in forensic applications and prenatal diagnosis

Drobnič K

Forensic Science Centre, Ministry of the Interior, Ljubljana, SLOVENIA

Sex determination can be an important piece of information in various forensic investigations, especially in sexual assault cases, but can be also useful in prenatal diagnosis of foetus with a known family history of genetic disorder affecting only male child. Because of that a gender determination has nowadays become a part of a human identification PCR kits. Although different PCR-based methods are known to identify a sample as originating from a male or a female, the only sex test included in commercially available human identification PCR kits for gender determination is based on the amelogenin sex test described by Sullivan et al., with primers spanning a part of the first intron, which results in two PCR products that vary from each other by 6 bp. The test is quick and effective but new studies showed that is not always reliable. The frequency of a deletion of Y copy of the amelogenin gene occurs between 1.85 % and 0.02 % depending on a population and “*d*eleted-*a*melogenin *m*ales” (designed as *DAMs*) would have been identified as women. In the Slovene national DNA database the number of phenotypic male individuals reached 3713 and one individual showed a sex test failure. The observed frequency of the amelogenin sex failure in our Caucasian sample is thus 0,027 %. Considering the consequences of the result obtained only using an amelogenin marker, we have tried to design a new primer set to facilitate the integration of the SRY sex test into multiplex STR human identification kits. This poster presents strategies and results for solving problems of a sex test reliability. As forensic samples are usually low in quantity and mostly very degraded, we decided to design a primers set which after amplification gave a small amplicon only 96 bp in lengths. Another benefit of the small amplicon is that SRY fragment will not overlap with alleles in multiplex STR kits. At first, different amplification conditions and primers concentrations were tested using DNA isolated from 9947A and 9948 cell-lines. In the end, the amplification resulted in only one band peak for a male sample and no reproducible peaks were observed over minimum threshold in a female sample even with high quantity of female DNA. To evaluate the sensitivity of the primers we tested the minimum required input of male DNA. We obtained peak even with 0.25 ng of template. After optimalization of concentration we tested the amplification under PCR condition of commercially kits AmpSTR SGM Plus (Applied Biosystems) and PowerPlex 16 (Promega) not only using control DNAs from the kit but also DNA isolated from reference samples taken from a man and a woman. We succeeded to coamplify the SRY fragment with STR loci under both condition without any artefact using male DNAs, but it was absent from females. Finally, we tested new primers with a phenotypically normal male, who was genotyped as female, using either the AmpFlSTR® SGM Plus kit or the PowerPlex® 16 kit by lacking the amelogenin Y-specific PCR product. Identical results were obtained by using three different primer sets for amplification of this region of the amelogenin gene. The presence of Y chromosome was determined by using six Y-STR markers. The male genotype of the individual was also confirmed by the amplification of a 96 bp long fragment of the SRY gene. Because it is very important that gender detemination tests give correct prediction in some forensic cases and prenatal diagnosis, we propose that this kind of amplicon from SRY locus is included as an additional safety measure of the sex status, especially in suspicious samples.
Contact: katja.drobnic@mnz.si

P-081

Forensic validation of the X-chromosomal STR-markers GATA165B12, GATA164A09, DXS9908 and DXS7127 in German population

Edelmann J[1], Lessig R[1], Willenberg A[1], Wildgrube R[1], Hering S[2], Szibor R[3]

[1]*Institute of Legal Medicine, University of Leipzig, Germany*
[2]*Institute of Legal Medicine, Technical University of Dresden, Germany*
[3]*Institute of Legal Medicine, Otto-von-Guericke University of Magdeburg, Germany*

The Chromosome X-STRs (ChrX STRs) were recently recognised as useful tools in forensic kinship testing, mainly in solving of complex cases. The highly effective strategy of ChrX microsatellite haplotyping requires the description of numerous STR markers. The aim of this presentation is to add four STRs to the known panel of ChrX markers and to describe their forensic suitability. GATA165B12 and GATA164A09 were characterised recently and population data were published for Korean population samples (Shin SH et al. 2005 and Son JY et al. 2002). DXS9908 and DXS7127 are not forensically evaluated yet to our knowledge.
We report here primer sequences, PCR protocols, allele structures and population data for a German population sample. We investigated for the four STRs up to 766 unrelated individuals and up to 333 meioses. The markers described here revealed a moderate degree of variability (Het = 0.69, 0.67, 0.83, 0.76 and PIC = 0.65, 0.68, 0.72, 0.78, respectively) low mutation rates and no problems in handling when the automated fragment analysis was performed on the ABI PRISM™ 310 Analyzers. Information regarding location on the ChrX are drawn from NCBI and by performing an own recombination study. Performing the exact test for genotype distribution of the STRs we found no significant deviation from Hardy-Weinberg equilibrium. All markers are suitable for forensic purpose.

contact: jeanett.edelmann@medizin.uni-leipzig.de

P-082

Relevant aspects for forensic STR analysis of canine DNA: Repeat based nomenclature, allelic ladders and PCR multiplexes

Eichmann C, Berger B, Parson W

Institute of Legal Medicine, Innsbruck Medical University, Austria

As the dog is deemed to be our closest companion and most popular pet, it can also be considered as the most interesting animal species from a forensic point of view. Canine saliva as well as dog hairs can remain everywhere where contact between dogs and humans have taken place. Canine-specific short tandem repeat (STR) analysis discloses a new approach for investigating dog attacks and other forensically important incidents involving dogs. As forensic identity testing of canine DNA using STRs is becoming commonplace in resolving criminal cases it has become increasingly important to have a set of minimum guidelines, such as common used STR markers and a reliable nomenclature, which enables exchange of data and international collaborations. The majority of canine STR markers described in the literature are not yet characterized with respect to their sequence structure and earlier studies have not been using a uniform repeat-based nomenclature for the STR alleles. Mostly the alleles were reported by the estimated fragment size as determined by electrophoresis of the PCR-products. The lack of a uniform harmonized nomenclature makes the application of these markers difficult. Here we present a nomenclature for a set of forensically useful STR markers that is adopted from the recommendations of the International Society of Forensic Genetics (ISFG) for the nomenclature of human STRs. We describe two newly designed PCR multiplexes sensitive to degraded DNA for 8 polymorphic canine STR markers (FH2087Us, FH2611, PEZ15, FH2054, PEZ2, PEZ6, WILMS-TFs, FH2328l). The sequence structure of selected alleles of these markers was the basis for the implementation of a repeat based nomenclature. Additionally, allelic ladders containing the common alleles for all markers used in both multiplexes are shown, which allow an unequivocal allele designation of unknown samples.

Contact: burkhard.berger@uibk.ac.at

P-083

Molecular analysis of *in vitro* damaged DNA samples

Fattorini P[1], Tomasella F[1], Grignani P[2], Sanchez P[3], Ricci U[4], Carracedo A[3], Previderè C[2]

[1] UCO di Medicina Legale, Dipartimento di Scienze di Medicina Pubblica, Università di Trieste, Italy
[2] Dipartimento di Medicina Legale e Sanità Pubblica, Università di Pavia, Italy
[3] Institute of Legal MedicineUniversity of Santiago de Compostela, Santiago de Compostela, Spain
[4] Azienda Ospedaliera-Universitaria "A. Meyer", U.O. Genetica Medica, Firenze, Italy

A large extraction of DNA was performed from 500 ml of a male donor blood by phenol/chloroform procedure. After spectrophotometric quantification at $O.D._{260}/O.D._{280}$, about 26 micrograms of the sample were aliquoted in 72 different eppendorf tubes. These samples then underwent different treatments with several physical and chemical agents (UV radiation at 254 nm, formic aldeyde, HCl, H_20_2, $FeCl_3$, $CuSO_4$, $FeCl_3$ plus H_20_2, $CuSO_4$ plus H_20_2 and NaOH) for a comparable time (from 1 to 10min). All the treatments were performed in duplicate. After ethanol precipitation, the samples were redissolved in H_2O and analyzed by the following methods: EtBr staining, Alu probing, Real Time PCR, STR typing and SNPs analysis. In addition, to evaluate the degree of chemical damage of the DNA bases, Capillary Electrophoresis (CE) was also performed.
Our data show that most of the above treatments caused a chemical damage of the DNA template. In addition, we observed that PCR fidelity was strongly influenced by the integrity of the template.

(contact: fattorin@univ.trieste.it)

P-084

Analysis of Y chromosome and mtDNA variability in the Madeira Archipelago population

Fernandes AT, Gonçalves R, Rosa A, Brehm A

Laboratório de Genética Humana, Universidade da Madeira

The Atlantic archipelago of Madeira is composed of two islands (Madeira and Porto Santo) with 250.000 inhabitants.These islands were discovered and settled by the Portuguese in the 15th century and played an important role in the complex Atlantic trade network in the following centuries.

The history of local settlements and constrains on the populations mobility especially due to orography is a possible explanation for the differences found between different regions of the Madeira Island. The genetic composition of the Madeira Islands' population was investigated by analyzing Y chromosomal bi-allelic and STR markers in three different regions of the Main Island plus Porto Santo Island. We compared the results with mtDNA data and used the Y chromosome STRs to determine the variability within each haplogroup. A sample of 143 unrelated males divided into four groups (Funchal n=35, West Madeira n=39, North and East Madeira n=46 and Porto Santo n=23) were analyzed.
Significant genetic differences between these regions and the population of Funchal were found. The population of Funchal had a lower gene diversity than expected.

Contact: atgf@uma.pt

P-085

MtDNA analysis of ancient samples from Castellón (Spain): diachronical variation and genetic relationships.

Fernández E.[1,2], Oliver A.[3], Turbón D.[2], Arroyo-Pardo E.[1]

[1)] Depto. Toxicología y Legislación Sanitaria, Facultad de Medicina, Universidad Complutense, Madrid, Spain.
[2)] Unidad de Antropologia. Depto Biología Animal, Facultad de Biología, Universidad de Barcelona, Spain.
[3)] Sección de Arqueología. Museo de Bellas Artes de Castellón, Castellón, Spain.

Thirty seven bone and teeth samples from Calcolithic and Iberian ages from several archaeological sites located in Castellón (Spain) were analyzed for mitochondrial DNA HVRI polymorphism. Despite of the presence of high amounts of PCR inhibitors in the ancient extracts it was possible to recover 150bp fragments in 9 cases. Recovered lineages suggest a close relationship among individuals from the same archaeological site, this suggesting a possible familiar relationship or the presence of an homogenous ethnic group. Moreover, Calcolithic haploypes differed so much from those recovered from Iberian samples. This points out a possible genetic replacement between both periods in the Spanish Levant.

Contact: earroyop@med.ucm.es

P-086

The distribution of Y-chromosomal haplotypes and haplogroups in two population samples from the Romagna region (North Italy): differences between urban (Rimini) and rural area (Valmarecchia)

G. Ferri, S. Ceccardi, F. Lugaresi, F. Ingravallo, C. Bini, A. Cicognani, S. Pelotti

Department of Medicine and Public Health, Section of Legal Medicine, University of Bologna, Bologna, Italy

We have studied the distribution of Y chromosomal haplotypes and haplogroups in two population samples from the Romagna region (North Italy) by analyzing male-specific markers, that reflect past and recent history, like SNPs and STRs. On the basis of previous studies on human Y-chromosomal single-nucleotide polymorphisms (Y-SNPs), the non-recombining part of Y chromosome has been shown useful for the investigation of population movements. These slowly evolving markers permit the detection of differences and similarities among populations without problems due to recurrent mutations in STR-based haplotypes. By contrast, Y-STRs are capable to detect more recent historical events and to resolve population stratification, but they are significantly dependent on an accurate sample collection, especially for neighbouring populations.
Our population samples were collected in the urban area of Rimini, an ancient port in Roman age and in the near rural area of Valmarecchia, that is more isolated and geographically out of ancient trading ways. 100 autochthonous unrelated males from Rimini and 70 from Valmarecchia were selected.
We analyzed 11 Y STRs (DYS391, DYS389I, DYS389II, DYS439, DYS438, DYS437, DYS19, DYS392, DYS393, DYS390 and DYS385) by a commercial kit and 20 binary polymorphisms, grouped in three multiplexes for determining the most frequent haplogroups, by minisequencing analysis. Statistical parameters were calculated using Arlequin 2.0 package.
The aim of this study is to analyse the microgeographic heterogeneity of Y chromosome in a Northern Italian region and to link it to geographical and historical perspectives.

Contact: susi.pelotti@unibo.it

P-087

BPA analysis as a useful tool to reconstruct crime dynamics. Part III

Fratini P[1], Pizzamiglio M[1] , Floris T[1] , Ceneroni G[2] , Talamelli L[1], Sampò G and Garofano L[1]

[1] Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italy

[2] Raggruppamento Carabinieri Investigazioni Scientifiche, Roma, Italy

On 18 June 2001, a 42 year-old man was killed with a single gunshot aimed at his head. The weapon used was a shotgun "Beretta" cal.20 mod.A300. The victim was shot while sitting on a sofa placed in the dining room of his former wife's friend's home.

The lady claimed that her friend, who was a hunter, had come into the room handling his rifle at the end of a long discussion which had taken place beforehand. She also showed her friend's position, added that he just wanted to scare the victim with no intention of shooting him, but that an accidental shot had been fired.

The Prosecutor asked our lab to reconstruct the dynamics of the events, in order to establish what had really happened, especially as regards the exact positions of the shooter and the victim when the shot was fired.

We first examined the gun and all its components in order to exclude mechanical failures or any other fault which could justify the accidental shot. The weapon was in perfect order.

In order to reconstruct the trajectory followed by the bullet and the probable position of the shooter, we examined the report written by the forensic pathologist, analyzed data acquired at the crime scene (i.e. evidence on bullet impact, measurements, etc.), and applied the BPA technique to bloodstains.

In this regard, particularly interesting were the bloodstains which had projected around the victim's head as they allowed to establish the position of the body and both the orientation and height of the victim's head, when it was hit by the bullet. By elaborating the evidence on the bullet impact, it was then possible to trace the second part of the trajectory (from head to wall) and hence estimate the first part (from shotgun barrel to target) on the basis of the conclusions reached by the forensic pathologist.

At the end of our study, by using 3D graphic software (AutoCAD 2000®), we were able to show that at the moment of the shot :

- the shotgun was working perfectly excluding any accidental shot ;
- the victim was sitting on the sofa with his head turned to the right and his legs apart;
- the shooting distance ranged between 2 and 3 meters;
- the position of the shooter was different from the one stated by the lady, indicating the possibility of a voluntary murder. Contact: lugaro@tin.it

P-088

BPA analysis as a useful tool to reconstruct crime dynamics. Part I

Fratini P[1], Pizzamiglio M[1] ,Floris T[1] ,Pierni M[1] and Garofano L[1]

[1] Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italy

This paper concerns a case of two bodies which were found dead in their bedroom, shot several times with a semiautomatic pistol.

It was essential to establish if we were dealing with a double homicide or rather the shooting of the first victim, followed by suicide of the second.

We refer to technical activities we conducted at the crime scene and the analytical approach we adopted, based on DNA as well as on BPA analyses of the bloodstains we recovered, studied and collected during CSI.

Following this method, also supported by ballistic exams, it was possible to establish the exact position of the first victim, as well as that of the shooter and reconstruct the dynamic of the event.

This shows, once more, that to obtain affordable and useful results for investigation we need to look to an integrated analytical approach which uses contributions from all aspects of forensics, especially when DNA and BPA analyses are available.

Contact: lugaro@tin.it

P-089

Adoption of automated DNA processing for high volume DNA casework:A combined approach using magnetic beads and real-time PCR

Frégeau CJ[1], Lett M[1], Elliott J[1], Bowen KL[1], White T[2], Fourney RM[1]

[1]National DNA Data Bank Royal, [2]Evidence Recovery Unit, Canadian Mounted Police, Ottawa, Ontario

In the past five years, the National DNA Data Bank of Canada has successfully processed over 75,000 blood, buccal or hair samples from criminal offenders using a fully integrated and automated approach requiring very little human intervention. As of April 18 2005, the number of offender hits (crime scene to offender) recorded was 3161 and the number of forensic hits (crime scene to crime scene) was 390. In February 2004, an initiative was created between the RCMP Biology Operations and the National DNA Data Bank group to increase the number of profiles contained in the crime scene index of the data bank in order to enhance the number of hits established with serious unsolved crimes. A new DNA extraction process was developed and adapted for our TECAN Genesis 150 Robotic Workstations but optimized to allow processing of break and enter (B&E) samples from non-suspect cases. The magnetic bead extraction technology from Promega (DNA IQ™) was evaluated on a variety of samples from B&E cases (e.g. cigarette butts, chewing gums, swabs from manipulated objects, bloodstains). Our initial work focused on the recommended protocol from the manufacturer based on the company's Lysis Buffer and binding conditions. While blood swabs and trace swabs produced good DNA yields and generated STR profiles, many potential crime scene samples such as chewing gums and cigarette butts failed to produce results. Blood swabs prepared with different types of soil also failed to produce results. Modifications to the lysis step, the DNA binding step onto the magnetic beads, the resin wash and DNA elution step were needed to optimize recovery of DNA using our robotic workstations. Using our modified and automated protocol, as little as 0.003 ng/µl (0.10 ng total) from very compromised samples can be isolated using the Promega DNA IQ™. The DNA yields obtained using the magnetic bead-based approach were equivalent to conventional processes and 3-4 fold higher for samples compromised with soil. Promega DNA IQ™ process produces better quality DNA than organic extraction, and resulted in very balanced peaks across all STR loci tested. To determine the amount of human DNA present in the B&E samples, real-time PCR technology was used. The Quantifiler™ Human Quantification Assay developed by Applied Biosystems 1) quantitates human DNA specifically by using human specific primers for a single copy gene and 2) ascertains the presence of PCR inhibitors in the DNA extract upon failure to amplify an internal PCR control. The amplification assay set up has been incorporated at the end of the extraction routine on our TECAN robotic workstation followed with actual cycling and detection in an ABI PRISM® 7000 instrument. This assay is extremely simple to automate yet is very sensitive detecting reliably down to 0.003 ng/µl of DNA using the Promega K562 standards. The PCR setup of all samples following their quantification is also carried out robotically using the output file from the ABI PRISM® 7000 instrument. This automated protocol combining Promega DNA IQ™ and ABI RT-PCR technology represents a unique way to process B&E samples in a very efficient and cost effective manner. A full batch of 84 samples plus controls (96 in total) can be extracted in approximately two hours 15 min. following lysis overnight, quantitated in approximately two hours as well (30 minutes to set up the reactions on the robotic workstation and 1 hour 46 min for amplification and detection in the ABI PRISM® 7000 instrument) and setup for STR amplification in approximately 1 hour. The TECAN Genesis 150 Robotic Workstations used in our process are equipped with non-disposable Teflon-coated stainless steel tips and a stringent tip washing routine was developed to prevent cross-contamination. A true 2% bleach wash was strategically incorporated within the extraction process as well as after an extraction session using large volumes of system's liquid i.e. distilled water to remove any traces of DNA as well as any traces of residual bleach from the line and tips. The use of the bleach within the extraction does not have any adverse effect on the yield of DNA nor the quality of the STR profiles produced.Our original Sample Tracking and Control System (STaCS™) created for the National DNA Data Bank of Canada was amended to accept B&E type samples. These modifications allowed us to keep the highest standards of quality control while maintaining our capacity 1) to have a tight control over B&E sample traffic and ensure that all samples are processed error-free in the shortest possible time, 2) to batch process B&E samples while maintaining the capability to customize processing conditions for each sample (large scale versus small scale extraction), 3) to re-process B&E samples at any step in the analytical process. This newly developed automated protocol combining Promega DNA IQ™ and ABI RT-PCR technology is currently being evaluated for other more challenging casework investigations (chantal.fregeau@rcmp-grc.gc.ca).

P-090

A novel DNA probe chemistry for HyBeacons®: rapid genetic analysis

French D[1], McDowell DG[1], Thomson JA[1], Brown T[2], Debenham PG[1]

[1]LGC, Queens Road, Teddington, TW11 0LY, UK
[2]School of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ UK

The analysis of single nucleotide polymorphisms (SNPs) and short tandem repeats (STRs) has proven extremely valuable in both healthcare and forensic analyses. However, such analyses have historically been confined to the specialist analytical laboratory both because of the processing and specialist nature of the equipment required as well as the dependence on a skilled analyst for interpretation.

In the field of genetic analysis, homogeneous PCR offers much potential for simplifying the analytical process. However, the majority of such systems are still confined to the specialist laboratory. We describe here a novel homogeneous PCR probe system termed HyBeacons® suitable for rapid genetic analysis.

HyBeacons are synthetic fluorescent oligonucleotides which increase in fluorescence upon hybridisation without the need for quencher moieties, secondary structures, multiple probe interactions or enzymatic degradation. Analytical interpretation is based on the generation of melting peaks post amplification and hence it is the stability of the probe / target interaction which is the basis of the result rather than any increase in fluorescence *per se*.

In SNP analysis, the presence of a base change within the probe binding site can be highly destabilising depending on the nature and position of the mismatch. With careful assay design, melting peaks can be readily produced with delta Tms of 7-11ºC which are easy to interpret. Advantageously the same probe analyses both forms of the sequence and hence both homozygotes and heterozygotes are easily called in a single tube with each sequence effectively acting as a internal positive control for the assay. Data will be presented for a number SNPs commonly typed within the medical profession demonstrating result direct from saliva in as little as 15-30 minutes.

For STR analysis, HyBeacons can be similarly applied since melting temperature is affected by the length of hybridising sequence as well as the presence of any mismatches. Whilst less advanced than the SNP assays, we will again present data indicating the potential for the analysis of STRs direct from saliva.

It is anticipated that HyBeacon based assays, in association with a suitable analytical platform, could be configured for use away from the specialist laboratory in primary healthcare or certain forensic settings, significantly reducing the time to result. In consequence, rapid diagnosis and therapy could be achieved in a healthcare setting, whilst for forensic applications, data could be rapidly obtained to inform and prioritise further investigations. Contact: Jim.Thomson@lgc.co.uk

P-091

Paternity Investigation in Father or motherless cases: how to improve statistical analysis for missing kids DNA databank?

Fridman C, Gattás GJF, Lopez LF, Massad E

Department of Legal Medicine, Bioethics and Occupational Health, Faculty of Medicine-University of São Paulo, Brazil

Paternity investigation of families where the trio mother, child and alleged father is complete is almost well defined nowadays. Different genomic DNA amplification kits are used and at least the genetic markers recommended by the American CODIS are performed. Many times the mother or the alleged father is absent or deceased, and one has to work statistically with other family members or half brothers and sisters, trying to deduct the genetic constitution of the parent. This situation is frequent in DNA databank that is constructed in order to investigate missing kids families. In these cases is necessary to do "reverse paternity determination" where the number of genetic markers used must be calculated in order to get the probability of paternity. Herein, we illustrate this situation describing one fatherless and one motherless cases. In the first one two sisters wished to know if a deceased man was their biological father. The genotype of the unavailable alleged father was reconstructed based on testing his other family that includes a daughter and son, and compared the results with the genotype of the two sisters and their mother. In the second case the paternity investigation was done in a girl case having only the alleged father and a maternal aunt. In both cases the DNA analysis was done using STR loci presented in the AmpFLSTR® Identifiler® PCR Amplification Kit (Applied Biosystems) plus loci HLADQA1, LDLR, GYPA, HBGG, D7S8, GC, F13A01, FESFPS, and D1S80 totalizing 25 markers. We discuss here the importance of the right selection of polymorphic markers in special when we need to deal with similar situations in the DNA databank that was elaborated to identify missing kids in Brazil in a Project that calls "Caminho de Volta". The first seven months of project (106 families) revealed a increased number of families where only one of the parents is present (58% only mother and 16% only fathers) compared to only 13% of families where biological material was collected from both parents.

Contact: cfridman@usp.br

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P-092

mtDNA lineages in two Tunisian Berber communities: comparing diversities between villages and towns

Frigi S[1], Yacoubi B[1], Pereira F[2,3], Pereira L[2], Cherni L[1], Amorim A[2,3], Elgaaied AB[1]

[1]*Laboratory of Molecular Genetic Immunology and Biotechnology, Faculty of Sciences, Tunis, Tunisia*

[2]*IPATIMUP (Instituto de Patologia e Imunologia Molecular da Universidade do Porto), Porto, Portugal*

[3]*Faculdade de Ciências da Universidade do Porto, Porto, Portugal*

Haploid markers are know to be more sensitive to genetic drift, bottlenecks and founder events due to its effective size being ¼ relatively to autosomal. These effects can be dramatic when samplings are carried out in small villages, where inbreeding is very strong, as it has been the case of most studies conducted in North Africa aiming to compare Berber and Arab communities. We can ask, therefore, if this sampling strategy is suitable for the construction of forensic database.

We tried to evaluate the biases introduced by such a sampling strategy by comparing the mtDNA haplotype diversities (HVRI and HVRII) between two north Tunisian Berber communities: 47 individuals from the town of Sejenane (over 41,000 inhabitants); and 33 individuals from the small village of Takrouna (500 inhabitants).

The sampling effort was considerably higher in the small village, where close kinship was more and more difficult to rule out as the sampling proceeded, so that at a certain point for all individuals not yet sampled a male relative had been collected already.

As expected, the diversity was higher in the town sample (haplotype diversity = 0.988 +/- 0.008; mean pairwise differences = 9.521 +/- 4.446) than in the village (haplotype diversity = 0.907 +/- 0.024; mean pairwise differences = 4.625 +/- 2.328). The probability to find a haplotype match was much smaller in the town (1.203%) than in the village (9.280%). And with respect to the haplogroup distribution, the same higher diversity was observed for the town sample (64% Eurasian; 32% Sub-Saharan; and 4% North African), comparatively to the village one (97% Eurasian; 3% Sub-Saharan; and 0% North African).

We assayed also if by pooling small Berber village samples we would get a similar diversity to the town sample. This assay was limited to HVRI diversity because this report will be the first one to describe HVRII diversity in North Africa. When we pooled 47 individuals from the small village of Kesra with 33 from Takrouna we obtained still a lower diversity (haplotype diversity = 0.897 +/- 0.028; mean pairwise differences = 4.909 +/- 2.417) than the town sample (haplotype diversity = 0.979 +/- 0.012; mean pairwise differences = 6.141 +/- 2.973).

These results claim some thought on the sampling strategy to be applied to the construction of forensic databases, not only in Tunisia, but in the rest of North Africa and in other population coverages, where similar sampling strategies are conducted that way.

Contact: fpereira@ipatimup.pt

P-093

The opinion of the Spanish population regarding the procedural situation of the owners of DNA profiles that would justify the inclusion of such profiles in a National Data Base

Gamero JJ[1], Romero JL[1], Peralta JL[1], Carvalho M[2], Vide MC[2], Corte-Real F[3]

[1]Faculty of Medicine. University of Cádiz. Fragela s/n, Cádiz 11003. Spain
[2]Forensic Genetic Service. National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal
[3]National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal

Different questions must to be taken into account with regard to the procedural situation of individuals involved in crime investigation whose DNA profile could be included in a national DNA data base. Among these questions are the following:
1.- Should the DNA profile of an accused individual be included in a national data base only if found guilty in specific lawsuits?
2.- Should the DNA profile of an accused individual be included in a national data base?
3.- Should the DNA profiles of other individuals involved (suspects) or not in a crime or offence be included in a data base?
The intention of this paper is to add complementary information to previously studied aspects of national DNA data bases, from an ethical and social perspective. The point of view or criteria held by Spanish society regarding the procedural situation which an individual must be in to justify the inclusion of their DNA profile in a national data base will be analyzed. Likewise, opinion is also sought concerning the criteria that should be taken into account in future regulations affecting data bases in Spain.

Contact: geneforense@dcinml.mj.pt

P-094

Some social and ethical aspects of analyses and DNA profile databases

Gamero JJ[1], Romero JL[1], Peralta JL[1], Carvalho M[2], Vide MC[2], Corte-Real F[3]

[1]Faculty of Medicine. University of Cádiz. Fragela s/n, Cádiz 11003. Spain
[2]Forensic Genetic Service. National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal
[3]National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal

There is general agreement concerning the fact that research into human genetics can affect the community as a whole, and for this reason it is necessary for society, and not only scientists, to discuss and decide on what they wish to accept and what they wish to reject. It thus seems clear that there is a need in Spain to examine and define the social and individual interests faced. In short, the aim of this study, in accordance with the International Declaration on Human Genetic Data, as well as the plan of action "Science and Society" of the European Commission is to reveal the degree of information and criteria society has with regard to a question that may affect it in specific circumstances.
Indeed, it is of great interest to take into account the opinions of different social groups before adopting legal decisions related with biotechnology given that, in order to reach consensus, information should flow in two directions, Society – Science.
In this paper, the degree of information a representative sample of the Spanish population has with regard to DNA profiles is analyzed, as well as the point of view this population holds concerning the criteria of reliability, quality, precision and security that must be established for the analysis and protection of stored forensic genetic data. Finally, the population's opinion concerning other questions relevant to this subject is also sought.

Contact: geneforense@dcinml.mj.pt

P-095

Haplotype distribution of four new Y-STRs: DYS630, DYS631, DYS634 and DYS635 in a Chinese population

Gao Y[1], He Y[2], Zhang Z[1], Bian S[1]

[1]Department of Forensic Medicine, Medical School of Soochow University, Suzhou, P.R. China
[2]Deparmtent of Anatomy, School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, P.R. China

In this study we analyzed the four new Y-STR loci, DYS630, DYS631, DYS634 and DYS635, investigated haplotype distributions of these Y-STR loci in a Chinese Han population (eastern China), and sequenced alleles of the four loci for clarifying the structure. Extracted DNA was amplified by PCR and the PCR products were analyzed by non-denaturing polyacrylamide gel electrophoresis. Alleles were sequenced on an ABI 3700 using a Dye Terminator Cycle sequencing kit. During the genotyping procedure, no PCR products were found for all the 20 female specimens at the four Y-STR loci which indicated the male specificity of the four Y-STR loci we studied. DYS630, DYS631 and DYS635 were found to be simple repeat systems, while DYS634 was complex repeat systems. Seven alleles at DYS630, four alleles at DYS631, five alleles at DYS634 and seven alleles at DYS635 were observed in our population sample.The gene diversities of DYS630, DYS631, DYS634 and DYS635 were 0.797, 0.418, 0.459 and 0.809, respectively. A total of 50 different haplotypes was observed in 79 males. The haplotype diversity for all the four Y-specific STR loci in Chinese population was calculated to be 98.3% and the stand error (S.E) was calculated to be 0.3%.The results indicate that these four loci are useful Y-linked markers for forensic applications.

(contact: yuzhengao@suda.edu.cn).

P-096

Distribution of Y-chromosomal haplotypes in the Basque Country autochthonous population using a 17-locus multiplex PCR assay

García O[1], Yurrebaso I[1], Uriarte I[1], Pérez JA[1], Peñas R[1], Alonso S[2], de la Rua C[2], Izagirre N[2], Flores C[3], Martín P[4], Albarrán C[4], Alonso A[4]

[1]Area de Laboratorio Ertzaintza, Larrauri Mendotxe 18, E-48950 Erandio, Bizkaia, Spain
[2]Departamento de Genética, Antropología Física y Fisiología Animal, Universidad del País Vasco, Bilbao, Spain
[3]Unidad Investigación, Hospital Univ. "Nuestra Señora de Candelaria", Servicio Canario Salud, Tenerife, Spain
[4]Instituto Nacional Toxicología y Ciencias Forenses, Sección Biología, Luis Cabrera 9, E-28002 Madrid, Spain

Y-STR haplotypes were determined from a sample of 168 unrelated males from the Basque Country autochthonous population (individuals were considered autochthonous if the 8 surnames and birthplace of their grandparents were of Basque origin) using the AmpFlSTR Yfiler PCR Amplification kit (Applied Biosystems) that coamplifies 17 Y-STRs. The panel of markers includes the 9-locus European minimal haplotype (minHT) and the markers DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 (Y GATA C4) and Y GATA H4.
Genomic DNA was extracted by the standard phenol/chloroform extraction procedure. PCR amplification was performed according to the manufacturer's recommendations. Samples were denatured for 5 min at 95ºC and typed on an ABI310 sequencer.
Allele designations were made according to recommendations of the DNA Commission of the International Society for Forensic Genetics.
The number of alleles and haplotypes, the gene diversities, the discrimination capacity and the cumulative haplotype diversity were calculated and compared with results obtained with the minHT-loci only.

contact: gobies01@euskalnet.net

P-097

Basque Country autochthonous population data on D2S1338, D19S433, Penta D, Penta E and SE33 loci

García O[1], Yurrebaso I[1], Uriarte I[1], Pérez JA[1], Peñas R[1], Martín P[2], Albarrán C[2], Alonso A[2]

[1]Area de Laboratorio Ertzaintza, Larrauri Mendotxe 18, E-48950 Erandio, Bizkaia, Spain
[2]Instituto Nacional Toxicología y Ciencias Forenses, Sección Biología, Luis Cabrera 9, E-28002 Madrid, Spain

Before a new marker system can be introduced into forensic casework, a population database for the relevant population must be established for statistical evaluation of the evidence. Therefore, this report presents allele frequency data in a Basque Country autochthonous population sample (n = 204) for 5 STR loci. The loci are: D2S1338, D19S433, Penta D, Penta E and SE33.

Whole blood was obtained from unrelated Basque autochthonous donors. Individuals were considered autochthonous if the 8 surnames and birthplace of their grandparents were of Basque origin. Genomic DNA was extracted by the standard phenol/chloroform extraction procedure.

PCR amplification was performed according to the manufacturer's recommendations using the AmpFlSTR Identifiler PCR Amplification kit (Applied Biosystems) and the PowerPlex 16/ES Monoplex Systems (Penta D, Penta E and SE33) (Promega Corporation). Samples were denatured for 5 min at 95ºC and typed on an ABI310 sequencer.

Allele designations were made according to recommendations of the DNA Commission of the International Society for Forensic Genetics.

Statistical evaluations were performed using the computer program GDA (Genetic Data Analysis) and PowerStats. Analyses included the possible divergence from Hardy-Weinberg expectations and other parameters of forensic importance: observed and expected heterozygosities, mean exclusion chance, polymorphic information content, discrimination power and the possible associations between loci.

contact: gobies01@euskalnet.net

P-098

2004-2005 GEP proficiency testing programs: special emphasis on the interlaboratory analysis of mixed stains.

García-Hirschfeld J[1], Alonso A[1], García O[2], Amorim A[3] and Gómez J[1]

[1]Instituto Nacional de Toxicología y Ciencias Forenses, Departamento de Madrid;
[2]Laboratorio de la Ertzaintza. Sección de Biología, Departamento de Interior, Gobierno Vasco;
[3]Instituto de Patología e Imunología Molecular da Universidade do Porto.

Both the 2004 and the 2005 GEP proficiency testing programs consisted in a simulated paternity case and a simulated forensic criminal case each including 4-5 reference samples (saliva or blood) and 2 forensic samples (mixed stains of semen and saliva or blood and saliva, and clean or contaminated hair shafts).

Due to the widespread use of commercial STR kits, the paternity test is no longer a problem apart from punctual discordances. Nevertheless a theoretical challenge is also included in the paternity test and the results obtained evidenced that rare alleles, mutations and possible silent alleles are treated very differently among participating laboratories.

Moreover the forensic tests have become the more fruitfully of the exercise. The results showed that even in forensic labs, preliminary test are not always performed. Samples management errors, transcription errors and missing a contributor in a mix are also punctually observed.

In the results of the 2004 forensic test (a mixture stain was analyzed consisting of 100 µl saliva from a female and 50 µl of a 1:20 semen dilution subsequently applied to a Whatman® Bloodstain Card) apparently inconsistent results were observed between autosomal STR profiling and mitochondrial DNA sequencing results. Additional validation studies were planned by the GEP Working Group to progress in the interpretation of mtDNA from different mixed stains.

In the present year a new forensic challenge was proposed: an unbalanced mixture stain of saliva and blood (10 µl of saliva and 30µl of blood) from two related contributors (sharing maternal and paternal lineages). Also hair shafts contaminated with blood have been sent to be analyzed.

As a consequence of the high unbalanced presence of the saliva and the low DNA content in this body fluid, no lab detected the minor component in the mixture even when preliminary tests indicated the presence of saliva. This evidence the fact that the detection of a minor contributor in a mixture is still a key outstanding in forensic investigation.

Related to the hair analysis also discussion is going to be generated because of the specific extraction procedures applied at each laboratory and its influence in final mtDNA results.

For the first time in the 2005 exercise all labs were required to send electropherograms and analysis data to better detect the errors source.

Contact: julia.garcia@mju.es

P-099

A comparative study of the sensitivity and specificity of luminol and fluorescein on diluted and aged bloodstains and subsequent STRs typing

Garofano L[1], Brighenti A, Romani F, Mameli A[2], Marino A[1], My D[1], Festuccia N[1], Paolino S[1] , Pizzamiglio M[1]

[1] Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italy
[2] Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Cagliari, Italy

Luminol and fluorescein are very important reagents for diluted and aged bloodstain detection at crime scene. The aim of this study was to carry out a comparative study of the sensitivity and specificity of these two presumptive blood tests using a series of diluted bloodstains (from 1:10 to 1:10000000) on a large variety of substrates, as well as, to evaluate the ability to type STRs on treated samples.

lugaro@tin.it

P-100

"Projeto Caminho de Volta": a Brazilian DNA Program for Missing Kids

Gattás GJF[1], Garcia CF[1], Fridman C[1], Neumann MM[2], Lopez LF[1], Barini AS[1] , Souza APH[1], Boccia TMQR[1], Kohler P[1], Battistella LR[1], Wen CL[3], Massad E[1]

[1]Department of Legal Medicine, Bioethics and Occupational Health, Faculty of Medicine-University of São Paulo
[2] Centro de Pesquisa e Prevenção em Políticas Sociais, CEPESP, São Paulo
[3] Telemedicine, Department of Pathology- Faculty of Medicine - University of São Paulo, Brazil

The Brazilian missing kids´ project called "Projeto Caminho de Volta" that means "coming home" is a program created by our group in collaboration with the public security services of São Paulo State, including molecular biology, genetics, psychology, bioinformatics and telemedicine methodologies. Each year, 8000 kids and teenagers (under 18 years old) disappear from their homes only in São Paulo State. In Brazil the number is about 30.000 per year. The reason for this event is not well addressed in our country and there is no epidemiological data about it, making difficult to establish effective preventive programs. For this reason this system was designed to follow three main goals: 1) to identify, through an epidemiological study, why there is so many cases of missing kids; 2) to help in the identification process of recovered missing kids after years (death or alive) it was developed a DNA data base including biological samples of parents (reference) to be compared to a DNA database of children and teenagers with unknown families (questionable); 3) to give psychological support to missing kids families during the entire process. Since September 13, 2004 this program received 100 families, only in the city of São Paulo, totalizing 48 boys and 52 girls missing, with ages ranging from 2 to 17 years old (average = 11,5 yo). The first analysis showed the mean problems are physical injury against children (30%), domestic violence (20%), alcoholism (20%), drug addiction (9%), and sexual abuse (5%) resulting in running away of the kids (80%) that prefer to be on the street than at home. In fact, about 40% of them are cases of more than one time of disappearance. For the DNA database all the family members are being genotyped using AmpFLSTR® Identifiler® PCR Amplification Kit (Applied Biosystems) that amplify 16 genetic markers (including CODIS loci). The reference database (family members that went to police to notify the disappearance) was mainly represented by only mother DNA (58%) followed by only father (16%) or both (13%); the remaining cases are from other family members like siblings (4,4%), grandmothers (3,5%), aunts (3,5%), and uncles (1,6%). All families data are automatically included in the online project database developed by our group. This program that allows the rapid search and comparison between the genetic and epidemiological information brings a new challenge in missing children identification in Brazil and should provide data to establish future preventive public programs.
Contact: gfgattas@usp.br
Financial Support: Special Human Rights Secretariat - Federal Government/FAPESP.

P-101

Validation of the Mentype® Argus X-UL kit

C. Gehrig and A. Teyssier

Institute of Legal Medicine, Geneva, Switzerland

With the aim of using X-chromosomal polymorphic markers in Swiss crime cases (female DNA on a male background) and particularly in kinship testing, a validation study of the Mentype® Argus X-UL kit was performed. The Argus X-UL kit is a commercial multiplex system which contains Amelogenin for gender determination as well as four uncoupled X-chromosomal STR markers (DXS8378, HPRTB, DXS7423 and DXS7132). In this study, we present the results of some forensic validation studies including the following aspects : detection limit, evaluation of stutter bands, analysis of female/male mixtures, frequency data from a swiss population study, validation of our protocol consisting of blood on FTA cards and amplification in a small PCR reaction volume (10 µl). The use of these markers in a deficiency paternity case will also be shown.

Contact: Christian.Gehrig@hcuge.ch

Christian Gehrig, Institute of Legal Medicine, 9 av. de Champel 1211 Genève 4, Switzerland.
christian.gehrig@hcuge.ch

P-102

Genetic variability at eleven STR loci and mtDNA in NOA populations (Puna and Calchaqui Valleys)

Giménez P[1], Albeza MV[2], Acreche N[2], Castro JA[1], Ramon MM[1], Picornell A[1]
[1]*Laboratori de Genètica, Universitat de les Illes Balears, Spain*
[2]*Facultad de Ciencias Naturales, Universidad Nacional de Salta, Argentina*

Human populations from the Andean region of North Western Argentina (NOA), due to their origin and their historical-demographic peculiarities, constitute an anthropological interesting motive of study. There is little reliable information on the structure of these populations before contact with Europeans in the late 15th century. In addition, the lack of historical data for the post-contact period means that the exact origin and/or the degree of admixture of the inhabitants of this region is also unknown.In this Andean region two zones can be differentiated, from an ecological and human point of view: the Puna and the Calchaqui Valleys. The Puna region in the Province of Salta (NW Argentina) is a typical Andean plateau at high altitude, which is arid or semi-arid. The populations in this region have extreme life conditions: low temperatures, low oxygen pressure and poor soils. In addition, they are distanced from other urban populations by poor and difficult roads. All these factors cause the isolation of these populations. The settlement model in the Puna region, is a dispersed one with a small population density. In this Andean area, San Antonio de los Cobres (altitude 3,880m) is the most populated locality (approx. 3,000 inhabitants). The Calchaqui Valleys are in the East of the Puna, with an altitude between 1,700 and 3,000 m, occupying a band of approximately 200 km of extension in sense north-south (provinces of Salta, Tucumán and Catamarca). In the pre-Hispanic epoch, these valleys were inhabited by the *diaguitas* and these pre-Hispanic societies reached the highest socioeconomic and cultural levels. The population dynamics of this zone is complex, as a consequence of the invasion of the Incas, the European colonization and, finally, the polity of estrangement of the rebels, from half of the XVIth century until the end of the XVIIth, which supposed the disappearance of an important part of the population. The current population (25,000 inhabitants in whole) has a low density and is unequally distributed; the most populated localities are Cafayate (approx. 9,000 inhabitants) and Cachi (6,000). This area has characteristics different from the rest of the Argentine, which are similar to those of the neighboring countries, specially Bolivia and Chile. It is a region of ecological and cultural specific characteristics, combination of Andean and Amazonian, because it was formed with peoples of both high and low lands. Some demographic and only a few genetic data for these populations, mainly based on blood groups and cytogenetic techniques, have been published elsewhere. Also preliminary data on STRs, but no data on mtDNA, have been published to date. The purposes of the present study were: (i) to study the STR variation in Puna and Calchaqui Valleys, (ii) to develop a mtDNA HVRI database from NOA individuals and, (iii) to compare with Europeans and other American populations from the literature.DNA samples from 161 unrelated individuals were analyzed: 106 from different localities of the Puna (Salta) and 55 from Calchaqui Valleys. The STRs studied were: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820 (AmpFℓSTR Profiler Plus, PE Applied Biosystems), HUMF13A1 and D12S391. The mtDNA region subjected to analysis was 15997-16400 (HVRI region). The RFLP motif –7025 *AluI* was also analysed in the samples to determine those that showed haplogroup H. In non-H samples, every control region sequence was assigned to a haplogroup by using the sequence motifs indicated by Richards et al. (Am J Hum Genet 2000, 67: 1251-1276).The eleven STRs studied in the 161 individuals from these populations showed an heterozygosity of 0.762. The number of alleles observed, between 7 and 17 (average 10.2), was high, taking into account that they are small and inbreed populations, probably due to the Amerindian-European admixture in these Andean populations. In mtDNA (HVRI) a total of 34 different haplotypes in 99 individuals were observed, defined by 50 variable positions. The incidence of unique haplotypes (13.1%) was very low. In relation to shared haplotypes, 21 haplotypes were shared by two or more individuals, 17 within the same population, and 4 between both populations. The gene diversity was approximately 0.92, in both populations, and the random match probability was 10%. Amazingly, the haplogroup analysis showed that all the individuals in both NOA populations had Amerindian haplogroups (A, B, C, D). Therefore, there is no indication of European female contributions for these populations. Genetic diversity of the Y-chromosome should be studied in order to estimate the proportion of Amerindian and European genes, and the asymmetrical mating according to sex and ethnic group, in NOA populations. apicornell@uib.es

P-103

Constituting a Y chromosome Short Tandem Repeats loci database in Sicily

E.Ginestra [(1)], I. Ciuna [(1)], Maria Guarnaccia[(2)], Antonella Agodi[(2)], D.Piscitello[(1)], C.Trapani [(1)], Giovanni Marcì[(2)], Gianluca Paravizzini[(2)], C. Romano[(1)], G. S.Travali [(2)] and L . Saravo[(1)]*

[1] Laboratory of Molecular Biology – Raggruppamento Carabinieri Investigazioni Scientifiche (RaCIS), Messina; Italy.
[(2)]Department of Biomedical Science,, University of Catania, Italy
[2] Department of Human Pathology, University of Messina, and
**Laboratory of Molecular Biology*

Many Y chromosome Short Tandem Repeats (STRs) have been studied and characterized over the past years. A few Y-STRs multiplex kits have been placed on the market for forensic and population study purposes. The aim of our work was to analyse a sample of Sicilian male individuals to evaluate the allelic frequency and, thus, the possibility to implement a genetic database. An 11 loci Y-STR Typing kit was used to yield haplotype profiles from male DNA; amplification products were detected on ABI PRISM 310 Genetic Analyzer and examined by Genemapper v 3.2 (Applied Biosystems). The population sample subjected to the screening resulted to be very different with regards to certain loci whereas other loci allelic profile was less variable. Despite a minor discrimination power within the entire population, Y-STRs represent a valid tool to simplify male/female DNA mixture interpretation which is a major challenge when biological traces are found in case of sexual assault. As regards to this, our results indicated that Y-STRs could be used to identify male individuals with a reasonable accuracy.

Keywords: DNA STR typing; Y STR-DNA database, Y aplotype

*Corresponding author: rismebiologia@carabinieri.it

P-104

A SNP-STR LOCUS WITHIN THE HLA CLASS II REGION: SEQUENCE AND POPULATION DATA OF D6S2822

Glock B, Reisacher RBK, Dauber EM, Wenda S, Dorner G, Mayr WR

Division of Blood Group Serology, Medical University of Vienna, Austria

Polymorphic STR markers within the HLA region can be used for a better characterization of HLA haplotypes, determination of disease associations, recombination point mapping or even for forensic testing if no linkage disequilibrium exists.

In this study, population genetics of the tetranucleotide repeat locus D6S2822 (M2_4_25; GATA129G03; GenBank G10435; UniSTS 239167, 464402, 464403; [1], [2]) situated nearby the HLA class II region (6p21.3) were investigated in an Austrian Causasoid population sample of 153 unrelated individuals in order to reveal its specifications.

PCR amplification was performed using the primers described by Matsuzaka et al. [2]. Typing of the amplification products in comparison with a locus-specific allelic ladder containing the most common alleles as well as cycle sequencing applying BigDye chemistry were carried out using denaturing capillary electrophoresis on an ABI Prism 310® Genetic Analyzer.

Sequencing of a total of 34 alleles from the population study and further samples, which were not included into frequency data, revealed 7 different sized alleles ranging from 189 to 213 bp and showing a $(TATC)_{9-14}$ $(CATC)_{1-2}$ repeat pattern. No incomplete repeats were found. Additionally, 17 bp upstream from the repeat region (base 65 of the 5'-flanking region) a A/G SNP, with the minor allele G (23.5%) and the major allele A (76.5%), was observed.

The resulting allele frequencies (n=153) as well as further statistic data are shown below:

Allele designation*	Allele frequency
11	0.026
12	0.261
13	0.510
14	0.183
15	0.017
16	0.003
Rate of heterozygosity:	0.595
Power of exclusion:	0.285
Polymorphism information content:	0.580
Power of discrimination:	0.816
Typical paternity index:	1.230

* the rare allele 10 was only found once in the additional samples and thus not included into frequency data

No deviation from Hardy-Weinberg equilibrium could be detected ($0.4<p<0.5$). Furthermore no mutations were observed in 263 meioses in 71 families.

The tetranucleotide locus D6S2822 showed an interesting sequence structure and an average allele distribution. It might, apart from its applications concerning the HLA system, due to its SNP nearby the repeat region, be a candidate for phylogenetic investigations. Further work was carried out subsequently on the linkage of D6S2822 with other HLA-STR loci, which will be displayed on another poster.

contact: eva-maria.dauber@meduniwien.ac.at

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[1] Cullen M et al. Immunogenetics 2003; 54: 900-910
[2] Matsuzaka Y et al. Tissue Antigens 2000; 56: 492-500

P-105

A multiplex PCR design for simultaneous genotyping of X chromosome short tandem repeat markers

Gomes I[1,2], Carracedo A[2], Amorim A[1,3], Gusmão L[1]

[1]*Institute of Pathology and Molecular Immunology, University of Porto, Portugal*
[2]*Institute of Legal Medicine, University of Santiago of Compostela, Spain*
[3]*Faculty of Sciences, University of Porto, Portugal*

Short tandem repeat markers (STRs) are extensively used as genetic tools in forensic and population studies. Genotyping of STRs located on the autosomes and on the Y chromosome (ChrY) has revealed significant information for application in these fields, whereas X-chromosome STRs have so far played a slender role. However, in the past few years, the human X chromosome has become object of studies that focus on genetic markers suitable for population and forensic analysis. In forensics, X-STRs can complement other STRs located on other chromosomes, especially in complex kinship testing, as in the so-called paternity missing cases, when the probant is female.
In this work, experimental designs were conducted in order to develop a multiplex PCR amplification strategy for X-STR genetic markers. ChrX loci were selected according to the informative power of each locus described in previous population studies. A tetraplex system for the following X chromosome genetic markers, DXS7423, DXS101, DXS8377 and HPRTB (human phosphoribosyl transferase) was optimized in a single PCR reaction. These short tandem repeat markers were typed in 65 individuals (29 female and 36 male samples) from a Galician population (Northern Spain) sample. Locus DXS7423 revealed 5 alleles (alleles 13-17) and DXS101 12 alleles (alleles 17-29). For the DXS8377 marker, 17 alleles were found (alleles 40-57) followed by 12 alleles for the HPRTB locus (alleles 9-16). In this genetic study of the Galician population, allele frequencies were estimated for all loci. We compared our data to those obtained in a German population study (Edelmann et al., 2001; Szibor et al., 2003) and as expected, exposed similar results. The simultaneous typing of ChrX markers for population and forensic studies is a pratical and simple method to obtain large amounts of information as proven in many other studies using autosomal and Y-chromosomal markers.

Contact: igomes@ipatimup.pt

P-106

Y-chromosome lineages from Portugal, Madeira and Azores record elements of Sephardim and Berber ancestry

Gonçalves R, Freitas A, Branco M, Rosa A, Fernandes AT, Brehm A

Human Genetics Laboratory, University of Madeira, Campus of Penteada, 9000-390 Funchal, Portugal

A total of 553 Y-chromosomes were analyzed from mainland Portugal and the North Atlantic Archipelagos of Azores and Madeira, in order to characterize the genetic composition of their male gene pool. A large majority (78-83% of each population) of the male lineages could be classified as belonging to three basic Y chromosomal haplogroups R1b, J, and E3b. While R1b, accounting for more than half of the lineages in any of the Portuguese sub-populations, is a characteristic marker of many different West European populations, haplogroups J and E3b consist of lineages that are typical from the circum-Mediterranean region or even East Africa. Highly diverse haplogroup E3b in Portuguese likely combines sub-clades of distinct origins. The present composition of the Y chromosomes in Portugal in this haplogroup likely reflects a pre-Arab component shared with North African populations or testifies, at least in part to the influence of Sephardic Jews. In contrast to marginally low sub-Saharan African Y chromosome component in Portuguese, such lineages have been detected at moderately high frequency in our previous survey of mtDNA in the same samples indicating to the presence of sex-related gene flow most likely mediated by the Atlantic slave trade.

Contact: ritaAG@uma.pt

P-107

DNA mixtures in forensic casework: report of 32 criminal cases resolved with autosomic STRs

Fabricio González-Andrade [1] • Dora Sánchez. [1] • Miguel Bolea [2] • Begoña Martínez-Jarreta [2]

[1] *Laboratory of Molecular Genetics, Metropolitan Hospital (Ecuador)*
[2] *Department of Legal Medicine, University of Zaragoza (Spain)*

To assess the technical and judicial consequences resulting from the practical application of *DNA testing* in forensic research in the numerous sex crimes in Ecuador. When a sample contains DNA from more than one contributor, the interpretation of its genetic profile becomes complicated. The incidence, complexity, and importance of mixed profiles is increasing due to the sensitivity of polymerase chain reaction (PCR) based typing methods. Mixed DNA samples fromat least two contributors can be originated at the scene of crime, in the course of sample-handling by investigating personnel or others, during forensic examination,or in the laboratory. However, in casework, samples may be degraded, unbalanced, contaminated, etc. thus overriding theoretical approaches from programmed validation assays with laboratory samples. The aim of this work is review our casework results obtained with the mixed genetic STR profiles encountered in our laboratory. Occasionally interpretation guidelines from validation studies are difficult to apply to real forensic casework, especially in the case of mixed samples. Exogenous contamination, an unknown number of contributors or unbalanced proportion of each one in the sample and a varied degree of degradation of the biological materials, contribute to the difficulties in the interpretation of sample profiles. In this paper we have reviewed all the mixed genetic STR profiles encountered in our laboratory and evaluated the problems in the interpretation of the results. From 32 criminal cases with 53 samples typed, 36 showed a mixed profile.

Key words: DNA mixtures • STR • sex offences • Forensic casework • PCR • Justice

Correspondence: fabriciogonzalez@usa.net

P-108

DNA typing in missing persons in Ecuador

Fabricio González-Andrade [1] • Dora Sánchez. [1] • Miguel Bolea [2] • Begoña Martínez-Jarreta [2]

[1] *Laboratory of Molecular Genetics, Metropolitan Hospital (Ecuador)*
[2] *Department of Legal Medicine, University of Zaragoza (Spain)*

Relevant efforts have been continuously made to identify cadavers and human remains after wars, socio-political disturbances, and mass disasters. In many cases, the use of DNA typing techniques offers a definitive answer for identification of victims and thus a direct social benefit is realized. Although DNA analysis is a highly discriminatory method, it is not self-sufficient and could not replace an anthropological evaluation. Amplification and typing of DNA extracted from compact bone of human remains could be useful in establishing the identity of a person, as well as in excluding possible false identifications. Body identification are making by using the results from relatives blood samples and information gathered from family trees, to predict the genotype of the deceased family member, in a paternity style analysis.

There are two types of situations for DNA testing, called close and open studies. Close studies are those in which the remains where a family member has recognized a personal item they believe belonged to the individual the family member claims is missing, or where some form of identification has been found on or near the body, and there is a general agreement on physical characteristics between ante mortem and post mortem data. In other words, when we know personal identity a priori. An open study is when the identities of all the victims is not known a priori. Open cases involve remains where there is a little or no information as to identify individuals. We report 8 cases of missing persons that we resolved by STRs technology.

Key words: DNA typing • STR • Forensic casework • PCR • missing persons • Justice

Correspondence: fabriciogonzalez@usa.net

P-109

Genetic data from Huaoranies Amerindian, the last nomad population from Ecuador, using Power Plex 16 and Power Plex Y

Fabricio González-Andrade [1] • Dora Sánchez. [1] Miguel Bolea [2] • Begoña Martínez-Jarreta [2]

[1] *Laboratory of Molecular Genetics, Metropolitan Hospital (Ecuador)*
[2] *Department of Legal Medicine, University of Zaragoza (Spain)*

Huaoranies, Aucas or Jíbaros are last nomad Amerindians from Ecuador and from Amazonia region. There are only two thousand individuals in small family groups, located Between the Napo river in north, and Curaray river in the south. They speak Hao Tiriro. Linguist studies have been demonstrated that there are not congeners for this language. They are a people in extinction, like all diversity from Amazonia. In a few years, Huaoranies to become extinct by the oil industry. State of Ecuador has recognized different indigenous nationalities, with own identity and language. Ethnicity means cultural practices and moral values to distinguish groups and communities. Individuals from ethnic group see themselves like different to others social and native groups. This concept has two dimensions: cultural and social characteristics (language, religion, faith, location, etc.) and a sense shared of identity and tradition. Indigenous Nationality means a joint of thousand-year old peoples before to Ecuadorian State, that has an historical identity, language and shared culture, that live in a certain territory, among his institutions and traditional forms of social, economical, political organization and practice of his own authority. Amerindians from Ecuador live around all the country.

Adequate evaluation of the DNA forensic evidence need of proper databases on STR polymorphisms distribution. In this paper, we report the allele frequency distribution of STR loci (CSF1PO, TPOX, TH01, F13A01, VWA, D13S317, D16S539, D5S818, D7S820, LPL, HPRTB, F13B), and Y Chromosome STR (DYS 19, DYS 385 a, DYS385 b, DYS, 389 I, DYS 389 II, DYS 390, DYS 391, DYS 392, DYS 393, DYS437, DYS 438, DYS 439) that have proven to be extremely useful for forensic casework, human identification and population genetics in a population sample of Amerindian Huaoranies from Ecuador.

Correspondence: fabriciogonzalez@usa.net

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Sub-typing of mtDNA haplogroup H by SnaPshot minisequencing

Grignani P[1], Peloso G[1], Alù M[2], Ricci U[3], Robino C[4], Fattorini P[5], Previderè C[1]

[1]*Dipartimento di Medicina Legale e Sanità Pubblica, Università di Pavia, Italy*
[2]*Dip Integrato Servizi Diagnostici di Lab e di Med Leg, Università di Modena e Reggio Emilia, Italy*
[3]*UO di Genetica Medica, Azienda Ospedaliera-Universitaria "A. Meyer", Firenze, Italy*
[4]*Dipartimento di Anatomia, Farmacologia e Medicina Legale, Università di Torino, Italy*
[5]*UCO di Medicina Legale, Università di Trieste, Italy*

Sequencing analysis of hypervariable regions HVSI/II is the most common approach to mitochondrial DNA (mtDNA) typing in the context of anthropological and medical studies. In the forensic field, mtDNA analysis is particularly important in human identification caseworks where the amount of genomic DNA recovered from samples as skeletal remains and hair shafts is extremely reduced. The presence of multiple copies of mtDNA in any cell can help in collecting a genetic result where typing of conventional STRs fails or gives unreliable results. However, the discrimination power of mtDNA typing is quite low also as a consequence of the maternal inheritance; in fact, about 7% of the Caucasian population shares the same HVSI/II sequence. In order to increase the discrimination of the common sequences for forensic purposes, it could be useful to characterise other mtDNA polymorphisms, such as single nucleotide polymorphisms (SNPs) of the coding region defining the most common European haplogroups. Point mutation detection can be performed by PCR amplification of a fragment containing the polymorphic site and restriction fragment analysis (RFLP) on agarose or polyacrilamide gels. Recently, a SnaPshot minisequencing assay based on ddNTPs single base extension of unlabelled primers immediately adjacent to the polymorphic site was set up; this provided the association of each individual to one of the nine major west European haplogroups. In addition, the SnaPshot method was used to type 7 SNPs allowing sub-typing of haplogroup H, the most common lineage in the European population (about 50%).

In this study we analysed 197 individuals from North-Central Italy (Turin, Pavia, Modena and Florence) by sequencing the hypervariable regions HVI/II. MtDNA haplogroups were then scored by RFLP typing. Haplogroup H was shared by 88 individuals (44,7%), in agreement with the distribution found in other European population samples. The SnaPshot minisequencing multiplex reaction set up by Quintans (FSI, 2004) was then used to sub-characterise the Italian haplogroup H samples. Seven H sub-haplogroups were found with the following frequencies: H*=47%, H1=28%, H2=4.5%, H3=4.5%, H4=3.4%, H5=8%, H6=3.4% and H 7=1.2%. Data on Italian H sub-haplogroups was then compared with the one calculated for the Spanish (Galician) population sample analysed by Quintans and a statistical significant difference (P<.0022) was found in the distribution of the frequencies, probably reflecting a different population history. On the opposite, the 28 (14%) identical rCRS HVSI Italian samples showed the same distribution of H sub-haplogroups, if compared with the Spanish ones. These results confirm the utility of this SnaPshot minisequencing assay to increase the discrimination power of HVSI/II sequencing analysis. In fact, the most frequent Italian mtDNA haplotype (CRS,263G, 315.1C), shared by 8 individuals belonging to the same haplogroup H, was discriminated by the SNPs analysis and subdivided in three H sub-types (H*=3, H1= 4, H4= 1). The SnaPshot approach can be used as a rapid screening method before sequencing, especially if many forensic or reference samples have to be analysed. contact: previde@unipv.it

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Austrian Caucasian population data of 15 STR loci complementing forensic core markers: A highly discriminating set for paternity and kinship analysis

Grubwieser P, Zimmermann B, Niederstätter H, Pavlic M, Steinlechner M, Parson W

Institute of Legal Medicine, Innsbruck Medical University, Austria

We investigated 15 polymorphic STR loci (D1S1656, D7S1517, D8S306, D8S639, D9S304, D10S2325, D11S488, D12S391, D14S608, D16S3253, D17S976, D18S1270, D19S253, D20S161, D21S1437) which are not included in the standard sets of forensic loci (ISSOL, CODIS). The loci were selected according to the complexity of the polymorphic region: Seven of the 15 investigated loci showed a simple repeat structure (D9S304, D10S2325, D14S608, D16S3253, D18S1270, D19S253, D21S1437), three loci (D7S1517, D12S391, D20S161) consisted of compound repeat units and 5 loci (D1S1656, D8S306 , D8S639, D11S488, D17S976) showed a more complex polymorphic region partly including different repeat blocks and incomplete repeat units, which resulted in a relatively high portion of intermediate alleles. A population study on a sample of 270 unrelated persons from Austria was carried out. We did not observe significant deviation from Hardy – Weinberg expectations. The combined PE for the 15 loci was 0.99999998. In combination with the traditional set of STR markers included in commercially available kits (no linkage was observed between these 15 loci and the PowerplexTM 16 System loci) these markers approved as highly discriminating forensic tool, also suitable for the analysis of difficult paternity and kinship constellations.

Contact: petra.grubwieser@uibk.ac.at

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Genetic analysis of autosomal and Y-specific STRs in the Karimojong population from Uganda

Gusmão L[1], Sánchez-Diz P[2], Gomes I[1,2], Alves C[1], Carracedo A[2], Prata MJ[1,3], Amorim A[1,3]

[1] IPATIMUP, Instituto de Patologia e Imunologia da Universidade do Porto, Portugal.
[2]Unidad de Genética Forense, Instituto de Medicina Legal, Univ. de Santiago de Compostela, Spain.
[3]Faculdade de Ciências da Universidade do Porto, Portugal.

The Karimojong are eastern Nilotic pastoral people of northeastern Uganda. They are the largest of a cluster of culturally and historically related peoples, including the Jie, Teso, Dodoth, and Labwor of Uganda and the Turkana of neighbouring Kenya. They speak an Eastern Nilotic language of the Nilo-Saharan language family. Many years ago, a number of groups of people referred to as the Nilotes migrated from near the Nile valley in southern Sudan and Ethiopia toward the south and west. Some of those groups took a south-westerly route, passing through the region that is now Kenya, and they ultimately settled on the high, dry plateau which is the Karamoja of today. The Nilotes are spread in a region that corresponds to the fringe of Bantu migration route, and apparently were not touched by the Bantu influence, keeping a Nilo-Saharan language and maintaining a pastoral lifestyle. However, they still remain almost genetically uncharacterized.

In this work, 90 individuals living in Karamoja region were typed for 17 autosomal STRs (CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPO, VWA, Penta D and Penta E) and 40 males were also typed for 12 Y-STRs (DYS19, DYS385, DYS389I and II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439).

Hardy-Weinberg equilibrium was tested for each autosomal locus and no deviations from equilibrium were observed. The only P value below 0.05 was found for CSF1PO (P=0.01236±0.00017) but, if Bonferroni correction is used, the departure observed at this locus is not significant.

For autosomal STRs, our sample shows a combined matching probability of 1 in 6.5×10^{19} individuals and a combined power of exclusion of 0.999999988. Haplotype diversity for Y-STRs was 0.9859 and 32 different haplotypes were detected out of the 40 samples analysed.

Our sample was compared with available autosomal data from sub-Saharan African samples and significant differences were found with Mozambique in 8 out of 17 loci; Cabinda (Angola) in 5 out of 17 loci; Equatorial Guinea in 4 out of 17 loci; and with Rwanda in one out of 13 loci. Comparisons with the Y-STR data, revealed large genetic distances between the Uganda and Mozambique, Cabinda and Equatorial Guinea (Rst=0.168, Rst=0.195 and Rst=0.183, respectively).

The present work confirms the high genetic heterogeneity between African populations and, therefore, emphasizes the importance of using local forensic databases, for both autosomal and Y-specific STRs.

Contact: lgusmao@ipatimup.pt

P-113

A new legal basis and communication platform for the Swiss DNA database

Haas C[1], Voegeli P[1], Hess M[2], Kratzer A[1], Bär W[1]

[1]Institute of Legal Medicine, Forensic Genetics, University of Zurich, Switzerland
[2]Federal Office of Police, Bern, Switzerland

The Swiss federal DNA profile information system (EDNA) has been launched in July 2000 for a test-period of 4 years. Based on the "EDNA-Verordnung" DNA-profiles of persons who are suspected of having committed a crime according to the Swiss crime catalogue and DNA-profiles of stains from unknown perpetrators were entered into the database. At the end of 2004 the Swiss DNA database contained 53'400 profiles from suspects and 8'554 profiles from stains.

Since 1. January 2005 a new legal basis for the Swiss DNA database is operational (DNA-Profil-Gesetz, DNA-Profil-Verordnung). New criteria for entering a profile into the database were established, no longer based on a crime catalogue. The following DNA-profiles are newly entered into the database: suspects for crimes or delicts, convicted offenders, dead persons, stains, not identified persons, missing persons, relatives of dead or missing persons. Profiles of persons are removed from the database on the basis of the new law.

At the same time the administrative process - from the investigating police unit, to the DNA laboratory, to the database, to the federal police and back to the investigating police unit - has been improved. A new internet-based information system (message handler) was implemented, which allows faster processing and status information.

Contact: haco@irm.unizh.ch

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The AMOVA Analysis of Pakistani Population Y STR Genetic Data

Hadi. S[1] & Goodwin, W. H.[2]

[1,2] Department of Forensic& Investigative Sciences, University of Central Lancashire, Preston, UK

Pakistan is a large country with a diverse population of 140 million consisting of four significant population groups besides many smaller isolated populations. Male population samples were collected from indigenous populations and the extracted DNA was used to amplify Y STRs. The haplotypes were developed for each population and the results of the AMOVA analysis of the data will be discussed in this paper. There were striking results for these populations which even with smaller number of loci when analysed led to historically relevant results. The data shows that the populations are quite diverse as opposed to the popular belief that cousin and other close relative marriages lead to high degree of inbreeding in these populations. The results also show that even with smaller number of loci significant amount of genetic and phylogenetic information can be gained from the data. AMOVA analysis of the population data revealed significant correlations between the population groups and also showed the effects of particular loci on the genetic diversity and ultimately the discrimination power in each population. Results show that Y STRs can be an effective tool to study micro geography. The paper also discusses autosomal & Y STRs in a rare Kalash population. Kalash live in the northwest of Pakistan. The genealogical history of both is quite unknown and subject to mythical reports. The data we present shows for the first time that at least the Kalash population is closely related to other Pakistani populations. The history of Kalash suggested that there would be significant amount of inbreeding and the Y data has shown that in quite a strong way in at least one of the Kalash groups, though similar results could not be detected when autosomal STRs were studied in this population.

contact: shadi@uclan.ac.uk

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DNA and the Innocence Project: Three separate 17 year-old rape cases from Georgia, similar circumstances, different outcomes.

Hampikian, G

Department of Biology, Boise State University, Boise, Idaho, USA

Three men in Georgia appealed to the Innocence Project for help to overturn their convictions. These separate cases had much in common: the convicted men were all African Americans from Georgia, they had each served 17 years, each claimed innocence, and the physical evidence remaining form their trials included smears obtained from the victims which were stored at room temperature.

The author has co-written a book with one of the men, Calvin Johnson (Exit to Freedom, Univ. of Georgia Press, 2003), and was part of the team that investigated the DNA evidence the other two cases: Clarence Harrison and Joseph Lee Brown. Johnson's case was handled by Peter Neufeld and Barry Scheck of the New York Innocence Project, and resulted in the first DNA exoneration in Georgia. However, it took several years to locate the evidence, secure permission to test, and finally obtain a conclusive profile. Finally, DQ alpha testing excluded him at several loci in 1999. The Calvin Johnson exoneration led directly to the Georgia DNA Evidence law, and the formation of the Georgia Innocence Project (GAIP). The new law requires the preservation of potential DNA evidence for 10 years, or until a death sentence is executed.

Joe Brown was the first GAIP case, and the first convicted offender to go to court requesting DNA testing under the new law. After two rounds of STR typing, the partial profile from the smear was consistent with Brown, and his case was dropped by the GAIP (2004). Clarence Harrison, the second GAIP case to request testing from the court, resulted in a complete DNA profile from the 17 year-old slide which exonerated Brown, freeing him within weeks of the test (2004). The District Attorney in the Brown case, with collaboration from the victim who still lives in the area, has pledged to investigate the case and submit the profile to the CODIS database.

contact: greghampikian@boisestate.edu

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Semi-automatic preparation of biological database samples for STR and SNP typing.

Hansen AJ, Simonsen BT, Børsting C, Hallenberg C, Morling N

Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark.

Application of laboratory automation systems (LAS) for preparation of forensic crime-case and database samples is necessary to support the increasing demand for fast, reliable and cheap forensic genetic analysis. The LAS needs to guarantee a high level of security of sample identity and chain of custody. Here, we present a validated (ISO 17025) laboratory semi-automated system for STR and SNP analyses of biological material immobilized on FTA-cards.

The described LAS encompass registration of samples in a LIMS database, export of a sample-file to a puncher, punching of FTA-cards by a BSD600-duet puncher, electronic sample check by means of barcodes, wash of punches by the THEONYX liquid-handler from MWG, PCR-amplification and electrophoresis of amplicons. The system was tested using blood and saliva immobilized on FTA-cards as sources of biological material.

The designed LAS led to:

- Correct STR and SNP typing of individuals with a quality equal to or higher than the profiles produced using chelex-extracted DNA.
- A reduction in the rate of sample-reanalyses by 0.03.
- A reduced risk of mix-up of samples during the laboratory procedure.
- Fewer sample transfers.
- A reduction in the number of PCR-cycles by 4 compared to chelex-extracted DNA from blood.

During the validation process, we did not observe mix-up of samples, loss of FTA-card punches, contamination from extern sources, or cross-contamination between samples.

Contact: Anders.Hansen@forensic.ku.dk

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STR typing of 77-year-old umbilical cord in maternity test

Hara M[1], Kido A[2], Yamamoto Y[1, 3], Takada A[1], Saito K[1]

[1]Department of Forensic Medicine, Saitama Medical School, Saitama, Japan
[2]Department of Legal Medicine, Faculty of Medicine, University of Yamanashi, Yamanashi, Japan
[3]Criminal Investigation Laboratory, Saitama Prefectual Police Headquarters, Saitama, Japan

In Japanese it is customary to preserve umbilical cord, which is presented to parents from maternity clinic, as a sacred materials. The umbilical cord is sometimes available for parentage test and personal identification because it has been preserved for a long time. We performed a maternity test using DNA extracted from an umbilical cord preserved for 77 years. The 15 short tandem repeat (STR) loci included in the AmpFLSTR Identifiler Kit were used for DNA analysis. DNA was extracted from the umbilical cord of the putative mother (already deceased) by ISOHAIR (Nippongene) and from the buccal swab of the child who requested the examination, by the DNA Extractor FM Kit (Wako). Using the AmpFLSTR Identifiler Kit (Applied Biosystems), the 15 STR loci, D8S1178, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA, as well as amelogenin locus were analyzed. For the amplification of the umbilical cord, PCR conditions were modified as followed; denaturation at 94ºC for 1 min, annealing at 59ºC for 5 min and extension at 72ºC for 5 min (40 cycles) using 3% primer. Amplified products were separated by denaturing capillary electrophoresis in the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The results were analyzed by GeneScan Analysis 3.7 software (Applied Biosystems) and Genotyper 3.7 software (Applied Biosystems). The 15 STR loci and the amelogenin locus were determined from the umbilical cord. A contradiction in the mother and child relationship was not observed in the 15 STR loci. This means that the 15 STR loci were correctly typed from the 77-year-old umbilical cord. The maternity probability was 0.999937 and the exclusion probability was 0.999629. Preserved umbilical cord are available for parentage test and personal identification using STR typing.

haramasa@saitama-med.ac.jp

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The Effects of Cleaning Agents on the DNA Analysis of Blood Stains Deposited on Different Substrates

Harris KA, Thacker CR, Ballard D, Syndercombe Court D

Centre for Haematology, ICMS, Barts and The London, Queen Mary's School of Medicine and Dentistry, UK

Potential evidential material at a crime scene is often adulterated. Deliberate attempts to remove biological material (using a variety of cleaning agents) is a problem faced by forensic scientists routinely. The substrates on which the blood is supported can also have an inhibitory role. It has been shown that complete DNA profiles can be obtained from non-visible quantities of blood. Lemire et al (1) reported successful analysis of 100µl of a dried 1:2560 dilution of blood. Sourcing such small quantities of blood at a crime scene is aided by the sensitivity of the Kastle-Meyer presumptive blood test.
Blood samples were obtained from 6 unrelated donors and standardised using white cell count. Blood was applied to a number of different substrates: denim jeans; cotton shirts and carpet. The stains were allowed to dry and were then cleaned with chlorinated bleach, soap or disinfectant until there was no visible trace. Chelex 100 (Sigma) was used to extract DNA from the cleaned areas. PCR was performed at 28 and, if necessary, 34 cycles using the AmpFLSTR® SGM Plus™ PCR Amplification kit (Applied Biosystems). In excess of 250 profiles were examined and characterised using heterozygote imbalance (Hbx), split peak frequency (SPF) and stutter proportion (SP). This information was used to assess the clarity of the electropherograms and the ability to relate evidence and control suspect samples, over a period of 15 days.
It was found that chlorinated bleach had the most pronounced negative effects with respect to the characteristics considered. In particular Hbx increased significantly over the 15 day trial. Such a change has the potential of incorrectly confirming or negating a relationship between evidential and suspect material. The SPF and SP satisfied the allelic designation guidelines set out by Gill et al (2) for STR multiplex systems in successful amplifications. Although dark coloured fabric dyes are anecdotally considered to interfere with DNA amplification, in these tests Khaki fabric (55% cotton, 45% polyester) was found to be the most inhibitive of the materials examined. This may be related to the effect of different chemical agents used in the manufacture of this material.

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(2) Gill P, Sparkes R. Development of guidelines to designate alleles using an STR multiplex system. Forensic Science International (1997); 89: 185-197
Address for Correspondence:Catherine R Thacker
Centre for Haematology- Institute of Cell and Molecular Science
Barts and The London
Queen Mary's School of Medicine and Dentistry
4 Newark Street-London E1 2AT
United Kingdom
E-mail: c.r.thacker@qmul.ac.uk

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An Investigation in to the Genetic Structure of a Barbadian Population

Harris KA, Thacker CR, Ballard D, Harrison C, Musgrave-Brown E, Syndercombe Court D

Centre for Haematology, ICMS, Barts and The London, Queen Mary's School of Medicine and Dentistry, UK

The male-specific inheritance of the Y chromosome and the maternal passage of mitochondrial DNA (MtDNA) allow the genetic features of a population to be investigated. In this study a total of 81 blood stains were characterized using twenty-nine Y-chromosome specific single nucleotide polymorphisms (SNPs) and MtDNA. The ABI PRISM® SNaPshot™ Multiplex System (Applied Biosystems) was used to genotype the Y-SNPs and enabled the paternal characteristics of the population to be assessed. Sequencing of the control region of the mitochondrial DNA loop was carried out using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Haplogroup frequencies and genetic diversity values were calculated and compared to a less well defined UK-resident Afro-Caribbean population. It was found that the Barbados population studied was similar to other populations of African ancestry and it is proposed that further characterization may be possible using population specific SNPs. Historically, Ghana and Nigeria supplied Barbados with black labour during periods of slavery and it may be useful to first target these ancestral populations when attempting to further define the genetic features of Barbados.

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Address for Correspondence:
Catherine R Thacker
Centre for Haematology
Institute of Cell and Molecular Science
Barts and The London
Queen Mary's School of Medicine and Dentistry
4 Newark Street
London E1 2AT
United Kingdom
E-mail: c.r.thacker@qmul.ac.uk

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A Sensitive Issue: Pyrosequencing as a Valuable Forensic SNP Typing Platform

Harrison C[1], Musgrave-Brown E[1], Bender K[2], Carracedo A[3], Morling N[4], Schneider P[2], Syndercombe-Court D[1], The SNP*for*ID Consortium

[1] *Centre for Haematology, ICMS, Barts and The London, Queen Mary's School of Medicine and Dentistry, UK*
[2] *Institute of Legal Medicine, Johannes Gutenberg University Mainz, Germany*
[3] *Institute of Legal Medicine, University of Santiago de Compostela, Spain*
[4] *Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark*

Analysing minute amounts of DNA is a routine challenge in forensics often accompanied by a variety of obstacles resulting in difficulties with analysis. To minimize these events it is important to choose technological platforms with specific criteria in mind. With so many typing technologies available for genotyping single nucleotide polymorphisms (SNPs), the selection of a suitable platform to meet forensic requirements can be difficult; particularly when questioning the sensitivity of an instrument and its ability to optimize detection and the amount of information obtained from forensic samples.

Here we investigate the Pyrosequencing (Biotage) method for genotyping SNPs. The Pyrosequencing method offers intrinsic quantifying capabilities and uniform electropherogram peak heights making it an ideal platform for sensitivity analysis. Using normalised concentrations of DNA and testing five autosomal SNPs, varied amounts of genomic DNA were added to the PCR with the resulting products compared on the two available instrument models: the PSQ™ 96MA and PSQ™ HS 96A systems. In doing so, a detailed comparison of the two models was completed while establishing a lower limit of detection on both instruments to give results supporting the use of Pyrosequencing as a valuable forensic SNP typing platform.

Address for correspondence:
Cheryl Harrison, Department of Haematology, Institute of Cell and Molecular Sciences, Barts and The London, Queen Mary's School of Medicine and Dentistry, 4 Newark Street, London, E1 2AT
Email: c.d.harrison@qmul.ac.uk

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High throughput mitochondrial DNA cloning in forensic and anthropological studies.

Hatsch D[1,2], Amory S[1], Keyser-Tracqui C[1], Hienne R[2], Ludes B[1]

[1]EA 3428, Institut de Médecine Légale, Strasbourg, France
[2]Laboratoire CODGENE, Strasbourg, France

Mitochondrial DNA is widely used in forensic and anthropological investigations. Therefore we developed an in house high throughput mitochondrial DNA cloning method targeting high speed at reduced costs.

A home made T/A cloning vector was obtained after ddTTP tailing of a SalI digested pUC19 vector. Due to this type of tailing, an extremely low background is found. Amplified mtDNA fragments were directly cloned into this vector after gel electrophoresis verification. PCR grade plasmid purification was performed in 96-well blocks according to an adapted alkaline-lysis protocol. The obtained plasmids were further sequenced on both strands and resulting sequencing products were purified through ethanol precipitation.

This method was applied in crime mixture analysis and in heteroplasmy determination in ancient sample from Yakutia graves.

Such a high throughput method is performed in the same time required by commercial kits but with 20 times less costs. Thus it opens possibilities for its routine use in forensic and anthropological laboratories.

Contact: didier@hatsch.net

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Allele frequency data for 12 STR Loci in a population of North Germany

Heide K-G, Krause M

Labor für Abstammungsgenetik, Kiel, Germany

12 Short Tandem Repeat (STR) loci TH01, vWA, D21S11, D18S51, FGA, D8S1179, D3S1358, D7S820, D5S818, D13S317, D16S539, D2S1338 were analysed in a Population geographically located in the north of Germany. We determined a sample of 3300 unrelated persons for paternity cases. Allele frequencies were calculated for all 12 STR loci. No deviation from Hardy-Weinberg and genotype equilibrium was observed.

krause@labor-krause.de

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WHOLE GENOME AMPLIFICATION. A USEFUL TOOL FOR THE INVESTIGATION OF FORENSIC SAMPLES?

Heinrich M[1,2], Brinkmann B[1], Hohoff C[1]

[1]*Institute of Legal Medicine, University of Münster, Münster, Germany*
[2]*present address: Institute of Legal Medicine, University of Freiburg, Freiburg, Germany*

In forensic genetics we are sometimes confronted with the fact that a sample (e.g., a population sample) is running off, although several markers need to be typed. The availability of commercially available whole genome amplification (WGA) kits offer in principle the possibility to amplify the whole DNA in the sample which in turns allows to type as many markers as necessary.

The GenomiPhi kit (GE Healthcare, Freiburg, Germany) amplifies genomic DNA using the bacteriophage Phi29 DNA polymerase. The theoretically exponential amplification of single- or double-stranded linear DNA is performed in an isothermal strand displacement reaction. Due to the proofreading activity of Phi29, the replication of the template DNA should be extremely accurate.

We have investigated whether the amplification of the whole human genome is representative and analysed STR and SNP markers before and after WGA.

Data on our experiences using cell lines, dilution series as well as artificial stains will be presented.

Contact: marielle.heinrich@uni-muenster.de

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A comparison of Y-chromosomal binary polymorphisms in six populations from Germany, the Near and Middle East

Heinrich M[1,2], Nebelsieck H[1], Alkhadam M[1], Brinkmann B[1], Hohoff C[1]

[1]*Institute of Legal Medicine, University of Münster, Münster, Germany*
[2]*present address: Institute of Legal Medicine, University of Freiburg, Freiburg, Germany*

In comparison to short tandem repeats (STRs) single nucleotide polymorphisms (SNPs) are more frequently found sequence variations in the human genome and are thought to offer a lower detection limit due to the possibility of creating very short amplicons. In this study, we have investigated 29 binary polymorphisms on chromosome Y: 26 SNPs (M174, M45, Tat, M2, M170, M217, P25, M201, M304, M38, M207, M123, M35, M128, P31, M216, M119, M173, M96, M122, M75, SRY1532, M168, M9, P2, M33), two short insertions/deletions (INDELs: M17, M175) and the *Alu*-polymorphism YAP.

Except for YAP all markers were analysed in two multiplex reactions, comprising 10 and 18 markers, respectively. The amplification via PCR was followed by a purification step using Exonuclease I and Shrimp Alkaline Phosphatase (SAP). Then, a minisequencing reaction was performed using the SNaPshot kit (ABI, Darmstadt, Germany). After an additional purification with SAP, the diagnostic fragments were analysed using a 3100*Avant* Genetic Analyzer (ABI). The marker YAP was analysed by amplicon sizing using a native 8% PAA gel with subsequent silver staining.

The six population samples are as follows: one sample originated from North-Western Germany (Münster area), three from the Eastern Mediterranean region of Turkey (Turkish and Arabian-speaking *Eti* Turks from Adana, *Gypsies* and Turks from Kahramanmaraş area), one from Syria and one from Afghanistan. We investigated 100-120 as far as we know unrelated males in each population.

The haplogroup determination was performed according to M.A. Jobling and C. Tyler-Smith (2003).

The haplogroup distribution within each population and a population-genetic comparison including Y-STR data in the minimal haplotype format of the six populations will be presented.

Contact: marielle.heinrich@uni-muenster.de

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Pairwise relatedness estimation: accounting for population substructure

Hepler A, Weir B

Department of Statistics, North Carolina State University, Raleigh, North Carolina, USA

The amount of relatedness between two individuals has been widely studied across disciplines. There are several cases in which accurate estimates of this quantity are important in the forensic arena. One common application is in the area of remains identification. In addition, there are several scenarios in which pairwise relatedness estimates may be required in the courtroom. Many estimators of pairwise relatedness have been proposed over the years, however none account for the potential effects of population substructure. This could introduce an additional amount of relatedness between the two individuals under consideration, which should be taken into account when estimating pairwise relatedness. The objective of this research is to develop a new maximum likelihood estimator of pairwise relatedness that accounts for population substructure. We build upon the foundation provided by earlier work in the area. A simulation study compares this new estimator to the previous approach, using simulated populations with and without inbreeding. We also evaluate the new estimator using the CEPH family genotypes available online

contact: abhepler@stat.ncsu.edu

P-126

A cluster of six closely linked STR markers: recombination analysis in a 3.6 Mb region at Xq12 – 13.1

Hering S[1], Augustin C[2], Edelmann J[3], Heidel M[1], Dreßler J[1], Szibor R[4]

[1]Institute of Legal Medicine, Technical University of Dresden; [2]Institute of Legal Medicine, University of Hamburg; [3]Institute of Legal Medicine, University of Leipzig; [4]Institute of Legal Medicine, University of Magdeburg

Forensic use of X-chromosomal markers requires knowledge about their linkage situation. Closely linked markers are inherited as haplotypes. Searching for suitable tetranucleotide tandem repeats, we found a cluster of three unutilized polymorphic markers located in the human X contig NT_011669 (components AL049564 and AL049564) within 280 kb. These markers were evaluated and submitted to the GDB and are now registered as DXS10079, DXS10074 and DXS10075.

To prove the stability of haplotypes within this region of Xq12 we performed a recombination analysis. To obtain more informative constellations, three known STR markers were included: DXS7132, HumARA and DXS981 (STRX1). HumARA, which should not be used in forensic casework, can be included in scientific research. Buccal swabs were collected from 96 males with daughters and grandsons as anonymised samples. Primers were designed according to GenBank information using the Primer3 software. Amplification of the six markers was performed in two sensitive triplex PCR assays. The resulting PCR products were resolved and detected by capillary electrophoresis on the ABI Prism® 310 Genetic Analyzer.

In a German population study (693 males and 328 females) each locus of the three newly established STR markers exhibited 13 (DXS10079) and 14 (DXS10074, DXS10075) alleles by length, respectively. Observed heterozygosity was 0.77 (DXS10079), 0.85 (DXS10074) and 0.67 (DXS10075). Since we cannot obtain any information on recombination in cases of homozygous daughters, we included the three further STRs mentioned above: HumARA is located about 50kb downstream of DXS10079, while DXS7132 and DXS981 are outside the cluster which spans 3.6 Mb. Segregation of haplotypes involving the six STRs mentioned is demonstrated in 96 trios consisting of grandson, mother and grandfather. No recombination event was detected for the cluster at Xq12 investigated here. Hence, it can be concluded that the cluster DXS10079, DXS10074 and DXS10075 segregates into stable haplotypes, providing a powerful tool in kinship testing.

(contact: Sandra.Hering@mailbox.tu-dresden.de)

P-127

Further sequence data of allelic variants at the STR locus ACTBP2 (SE33): detection of a very short off-ladder allele

Hering S[1], Nixdorf R[2], Edelmann J[3], Thiede C[4], Dreßler J[1]1Institute of Legal Medicine, Technical University of Dresden;[2]Saxon State Criminal Investigation Office;[3]Institute of Legal Medicine, University of Leipzig;[4]Department of Internal Medicine, Carl Gustav Carus University Clinic, Technical University of Dresden

SE33 is one of the most powerful STR markers in forensic use. A high number of length and sequence variant alleles have been described, some of which may vary by as little as one bp. The goal of this study is to add the sequence structure of some rare variants to the known data, and examine a very short off-ladder allele which has never been described before.Genetic characterization of more than 15,000 individuals (mainly Caucasians) was carried out using buccal cell swabs or blood. Amplification of SE33 was performed either using the commercially available multiplex PCR kits Nonaplex I and II (Biotype AG, Dresden, Germany) or in a single PCR with the primer pair described by Polymeropoulus et al. 1992. Automated fragment analysis was carried out on the ABI PRISM® 310 or 3100 Genetic Analyzers. The direct Taq-cycle-sequencing method was performed (following standard procedures).The study presents sequence structures of regular alleles ranging from 8 to 38 in comparison with variant alleles. The 120 bp 5'-flanking part and the 20 bp 3'- flanking part of the central polymorphic region defined by Rolf et al. 1997 are included.A very short off-ladder allele was found in a Somalian individual. Amplification with Nonaplex II failed, indicating that there is a variation in the primer binding region. Sequence analysis revealed a deletion of 15 tetranucleotide repeats in the 5' flanking region. A further allele originating from a Portuguese individual with 28 bp deletion in the 5' flanking region resulted in allele length 9. The relatively frequent allele 6.3 was sequenced in four different Caucasians showing an identical repeat structure. We found three classes of X.1 alleles: firstly, alleles ranging from 12.1 to 18.1 resulted from a single A insertion between the AAAG repeats in the central region; secondly, two alleles 15.1* and 18.1* deviated in their structures by a deletion of AAA in the 5' flanking region; and thirdly, by contrast, longer alleles 21.1 and 32.1 resulted from insertion of a single base pair (G or A) in the central repeat region. We found that only half of the variant alleles have insertions or deletions within the central region. Therefore, it is difficult to compare our sequence structures with the existing data. However, although the short X.1 and X.3 alleles are rare, accuracy in SE33 typing analysis is important for distinguishing these from the common alleles.

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(contact: Sandra.Hering@mailbox.tu-dresden.de)

P-128

Allele frequencies for Penta D and Penta E in three populations from Germany and Hungary

C. Hohoff[1], G. Nagy[2], J. Bartsch[1], I. Bajnóczky[2], B. Brinkmann[1]

[1] Institut für Rechtsmedizin, Universitätsklinikum Münster, Germany
[2] Institute of Forensic Medicine, University of Pecs, Hungary

We here present the frequency distributions of the autosomal STR systems Penta D and Penta E in samples from unrelated 188 Germans (Münster area), 115 Hungarian Caucasian and 116 Hungarian Roma (Pecs area).

Genomic DNA was extracted according to standard techniques (e.g., Proteinase K / Chelex-100) and amplified utilizing different amplification approaches (Powerplex16 or a Penta D/E duplex based on the published Promega primer sequences). PCR products were separated by capillary gel electrophoresis on an ABI PRISM 310 Genetic Analyzer and typed by comparison against sequenced allelic ladders.

A variant allele 12.3 was observed in a Hungarian sample and characterized by sequencing after cloning.

Both pentanucleotide STR systems are highly informative markers in the three populations investigated, e.g., the power of discrimination ranges from 0,897 (Penta D, Roma) to 0,977 (Penta E, Germans).

Address for correspondence
Prof. Dr. med. Bernd Brinkmann, Institut für Rechtsmedizin, Universitätsklinikum Münster, Röntgenstrasse 23, D-48149 Münster, Germany, Fax: 00 49 (0) 251 8355158, eMail: remed@uni-muenster.de

P-129

Y-STR analysis of Australian Aborigines

Carsten Hohoff, Ursula Sibbing and Bernd Brinkmann

Institut für Rechtsmedizin, Universitätsklinikum Münster, Germany

We present the frequency distributions of 15 Y-specific STR polymorphisms (DYS19, DYS385, DYS389 I and II, DYS390, DYS391, DYS392, DYS393, YCAII, DXYS156-Y, DYS437, DYS438 and DYS439) and the frequency of the combination of these haplotypes in a population sample of male Aborigines from Australia (Adelaide area).

DNA, that had been extracted from blood of 51 male Australian Aborigines, served as template to amplify the Y-STR loci by means of different multiplex or singleplex approaches. PCR amplicons were analyzed on an ABI PRISM 310 Genetic Analyzer with GenoTyper software (Applied Biosystems) and sequenced allelic ladders.

In the 51 samples, 40 different haplotypes were observed. Of them, 32 haplotypes were unique and the others were shared by 2 or 3 persons.

A YHRD search revealed only 3 matches, most likely due to the fact that until now no entries have been made for the Aborigines population.

Address for correspondence

Prof. Dr. med. Bernd Brinkmann, Institut für Rechtsmedizin, Universitätsklinikum Münster, Röntgenstrasse 23, D-48149 Münster, Germany, Fax: 00 49 (0) 251 8355158, eMail: remed@uni-muenster.de

P-130

Experiences from the *ante mortem* and *post mortem* DNA-analysis in Sweden for the identification of tsunami victims

Gunilla Holmlund, Iréne Lodestad, Helena Nilsson and Bertil Lindblom

The National Board of Forensic Medicine, Department of Forensic Genetics, University Hospital, SE - 581 85 Linköping, Sweden

After the tsunami catastrophe in the Indian Ocean, December 26, 2004 more than 15 000 Swedish citizens were initially reported missing. Planning for DNA-analysis of samples from relatives, *ante mortem* as well as of deceased, *post mortem*, started just two days later.

The collection of reference samples from relatives was started almost immediately and the first samples were received on January the 4th. About 730 samples, of which 113 were from the PKU-bio bank were collected within a few weeks. After an official request by the Swedish police the DNA analysis started on January the 12th. 550 samples from the genetically best references were analysed by mid February. The number of persons missing was by then about 550.

At the beginning of March several laboratories got an initial request, followed by an official at the March 7th to participate in the analysis of *post mortem* samples. We accepted to receive 500 – 600 samples, to be analyzed within 6 months after an initial quality test of 10 samples. Our quality was accepted and on April 5th we got 600 *post mortem* samples to analyse.

Since the work is at best going on we cannot here report results but hope to present an overview of our participation in this work at the meeting.

Address for correspondence: Gunilla Holmlund, The National Board of Forensic Medicine, Department of Forensic Genetics, University Hospital, SE - 581 85 Linköping, Sweden.

E-mail: gunilla.holmlund@rmv.se

P-131

Y-SNP typing with the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Hou YP, Shi MS, Liao LC, Yan J, Zhang J, Wu J, Li YB

Department of Forensic Genetics, Sichuan University (West China University of Medical Sciences), Chengdu, P.R.China

The single nucleotide polymorphisms on Y chromosome (Y-SNP) were potential markers for analysis of mixed biological stains in sexual assault cases and played a role on forensic science. The purpose of our work was to establish a method for analysis of Y-SNP based on the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. To explore the single nucleotide polymorphisms on Y chromosome, a technique of primer extension was employed for the analysis of the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Our study showed that Y-SNP typing with the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry yielded reliable results. The results of our study implied that the analysis of Y-SNP was proved to be suitable for forensic application and provided new genetic markers for the forensic purpose

contact: forensic@mail.sc.cninfo.net

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P-132

Molecular Evidence for the Association of Persian Ethnicities

Massoud Houshmand, Arman Ardalan, Mehdi Shafa Shariatpanahi, Mohammad Hossein Sanati.

National Institute for Genetic Engineering and Biotechnology

Persia is an upland long inhabited by miscellaneous tribes of many languages and cultures. Linguistic remarks point out a close relatedness among majority of the core population, as well as rather farther relations with the marginal aboriginals. However, not only in a medical context accounting for genetic disorders, but also from an evolutionary point of view there has been no adequate genetic data to support this. In the first phase of a bigger project, we sequenced (at least 25 individuals of each ethnic group) the D-loop region of the mitochondrial DNA (mtDNA) from 15 different ethnic groups (Pars [5different area], Kurd, Lor, Bluch, Sistani, Gilani, Mazandrani, Turk, Armani, Jews, Arab, and Turkman). Hypervariable nucleotide sequences were next aligned and compared through a pairwise distance method. The neighbor-joining phylogenetic tree was drawn using MEGA software package for the operational taxonomic units (OTUs) of the average haplotypes in each group, together with some relevant GenBank retrievals.

Contact: massoudh@nrcgeb.ac.ir

P-133

Population genetic analysis in a Libyan population using the Powerplex™ 16 system

Immel U-D[1], Erhuma M[2], Mustafa T[3], Kleiber M and Klintschar M[1]

[1]Department of Legal Medicine, University of Halle, Germany
[2]Institute of Medical Immunology, University of Halle, Germany
[3]University Clinic of Oral and Maxillo-Facial Surgery, University of Magdeburg, Germany

Polymorphic short tandem repeats (STRs) have become the markers of choice for forensic purposes such as paternity testing and personal identification.

In this study we present the results of a survey aimed at investigating the allele and genotype frequency distribution of 15 loci amplified by the GenePrint® PowerPlex™ 16 system (Promega) in Libya. DNA was isolated from blood samples. 103 unrelated individuals were included in the database. Amplification products were analyzed by capillary electrophoresis using the ABI 310® Genetic Analyzer (Applied Biosystems).

Statistical analysis was carried out using various statistical methods (Hardy-Weinberg- Equilibrium, Mean Exclusion Power, Discrimination Power, etc.) to determine allele frequencies and other population parameters of interest.

Address for correspondence:
U.-D. Immel, Institut für Rechtsmedizin, Martin-Luther University Halle-Wittenberg, Franzosenweg 1, 06112 Halle/Saale, Germany, Email: uta.immel@medizin.uni-halle.de

P-134

Y-chromosomal STR haplotypes in an Arab population from Libya

Immel U-D[1], Erhuma M[2], Mustafa T[3], Kleiber M and Klintschar M[1]

[1]Department of Legal Medicine, Martin-Luther Universität Halle-Wittenberg, Halle/Saale, Germany
[2]Institute of Medical Immunology, University of Halle, Germany
[3]University Clinic of Oral and Maxillo-Facial Surgery, University of Magdeburg, Germany

Y-chromosomal microsatellites (STRs) have been established in forensic practice for several years. However, an in-depth evaluation of their population genetic properties requires a large number of haplotypes from different populations. We therefore analysed the Y-chromsome with eight Y-chromosomal STRs (DYS385, DYS19, DYS 389I and II, DYS390, DYS391, DYS392, DYS393) in an Arabic population sample of 64 males from Libya.
DNA was extracted from unrelated male blood samples according to standard Qiagen procedures. Amplifications were performed using fluorescent dye labelled primers according to Elmoznino and Prinz (//ystr.charite.de). The PCR products were analyzed by capillary electrophoresis using the ABI 310® Genetic Analyzer (Applied Biosystems).

The results and the haplotype diversity were compared with data from other Arab populations.

Address for correspondence:
U.-D. Immel, Institut für Rechtsmedizin, Martin-Luther University Halle-Wittenberg, Franzosenweg 1, 06112 Halle/Saale, Germany, Email: uta.immel@medizin.uni-halle.de

P-135

Evaluation of Lewis genotyping by four PCR-based methods

Y. Itoh[1], K. Satoh[1□2], K. Takahashi[1□2], K. Maeda[3], T. Tokura[3] and R. Kobayashi[1,4],

[1]*Department of Forensic Medicine, Juntendo University School of Medicine, Tokyo, Japan*
[2] *Medico-Legal Section, Criminal Investigation Laboratory, Metropolitan Police Department, Tokyo, Japan*
[3]*Atopy Research Center, Juntendo University School of Medicine, Tokyo, Japan*
[4]*Department of microbiology, Tokyo Medical University, Tokyo, Japan*

The antigenic epitope of CA19-9, i.e. sialyl Lewis A antigen, has been used clinically as a tumor marker for pancreatic cancer, colorectal cancer, and certain other malignancies. The synthesis of CA19-9, however, is complex because there are three genes involved; Lewis genes encoding Le transferase (□-1, 4-fucosyltransferase), secretor gene encoding Se transferase (□-1, 2-fucosyltransferase), and the gene encoding sialyltransferase. Through the biosynthetic pathway, Le transferase is thought to be a key enzyme. The activity is genetically controlled by Lewis genotypes. Lewis phenotype Le(a-b+) or Le(a+b-) groups have *Le* allele. The Le(a-b-) group divided into two groups, genuine Le(a-b-) and non-genuine Le(a-b-) by the results of Lewis genotypes. Genuine Le(a-b-) groups have no *Le* allele, while non--genuine Le(a-b-) groups have *Le* allele. *Le* is a functional allele, and *le1* is non-functional allele.

We developed PCR-based methods, confronting two pair primers (PCR-CTPP) and sequence-specific-primers with PCR positive control (PCR-SSPPC) to analyze a SNPs at nucleotide position 59 to reflect Le transferase activity, which were analyzed by ABI PRISM® 3100 genetic analyzer. And we compared 4 kinds of PCR-based methods, sequence-specific-primers (PCR-SSP), restriction fragment-length polymorphism (PCR-RFLP), PCR-CTPP and PCR-SSPPC. We found that all of these methods could be applied to determine Lewis genotyping correctly. The frequencies of *Le* and *le* alleles were 67.2% and 32.8% respectively. Both PCR-CTPP and PCR-SSPPC for Lewis genotyping are simple, reliable and applicable for forensic and clininal investigation.

Address correspondence to:

Dr. Y.Itoh

Department of Forensic Medicine, Juntendo University School of Medicine, Hongo,

Tokyo 113-8421, Japan.

TEL: +81-3-5802-1051

FAX: +81-3-5802-1050

E-MAIL:yitoh@med.Juntendo.ac.jp

P-136

Are tetranucleotide microsatellites implicated in neuropsychiatric diseases?

Jacewicz R[1], Szram S[1], Gałecki P[2], Pokora K[1], Florkowski A[2] and Pepiński W[3]

[1]*Department of Forensic Medicine, Medical University of Lodz*
[2] *Department of Psychiatry and Neurosis Disorders with Crisis Intervention Ward, Medical University of Lodz*
[3]*Department of Forensic Medicine, Medical University of Białystok*

Expecting the significant breakthrough in the diagnosis of complex disorders of neuropsychiatry background, intensive efforts are undertaken to establish genetic markers associated with these disorders. It is known that neurological diseases are correlated with disturbances of the catecholaminergic pathway. The studies within genes involved in the synthesis, neurotransmission and metabolism of dopamine, adrenaline and noradrenaline have not given satisfactory results. Nowadays, great diagnostic expectations are related with sequences of STR type, which are widespread throughout the genome. These microsatellite repetitive sequences do not code proteins, but are supposed to function as regulatory elements in processes of gene transcription and expression. Association of di-, tri- or tetra nucleotide repeats with neurological disorders has been reported earlier in different populations. We have examined association between maniac-depression diseases such as schizophrenia, bipolar and unipolar affective diseases and polymorphism of several tetranucleotide genetic markers from different chromosome positions, including those being candidate in main psychiatric diseases. Results of statistical comparative analysis between neuropsychiatric patients from Poland and their regionally matched healthy subjects are presented

Address for correspondence:
Renata Jacewicz, PhD
Head of Genetic Laboratory
Department of Forensic Medicine
Medical University of Lodz
91-304 Lodz, Sedziowska 18a
Poland
tel. + 48 42 654 45 36
fax + 48 42 654 42 93
e-mail: r.jacewicz@post.pl

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The association of polymorphic TH01 marker with schizophrenia in Poland.

Jacewicz R[1], Szram S[1], Gałecki P[2], Pokora K[1], Berent.J[1], Florkowski A[2] and Pepiński W[3]

[1]Department of Forensic Medicine, Medical University of Lodz
[2] Department of Psychiatry and Neurosis Disorders with Crisis Intervention Word, Medical University of Lodz
[3]Department of Forensic Medicine, Medical University of Białystok

TH01 locus, used for personal identification, is a polymorphic microsatellite region located in the first intron of the tyrosine hydroxylase gene (TH). This gene codes the enzyme limiting synthesis of brain catecholamines. Disturbances in the synthesis and neurotransmission of dopamine and noradrenaline are involved in the pathophysiology of psychiatric diseases such as schizophrenia and affective disorders. The polimorphism in TH01 tetranucleotide sequence correlates with quantitative and qualitative changes in binding by specific protein ZNF191 and may be involved in regulation of TH gene expression. The association between these illnesses and polymorphism of TH01 marker has been reported in a group of neuropsychiatric patients from France, Tunisia, Sweden and the UK (England). Because of scarcity of the investigated samples former reports do not determine unambiguously the case in question. We attempted our own population study to compare distribution of allele frequencies in TH01 locus in a group of neuropsychiatric patients from Poland and their regionally matched healthy subjects. This report presents results of this association study

Address for correspondence:
Renata Jacewicz, PhD
Head of Genetic Laboratory
Department of Forensic Medicine
Medical University of Lodz
91-304 Lodz, Sedziowska 18a
Poland
tel. + 48 42 654 45 36
fax + 48 42 654 42 93
e-mail: r.jacewicz@post.pl

P-138

Evaluation of the genetic affinity between populations based on the comparison of allele distributions in two highly variable DNA regions

Jacewicz R[1], Miścicka- Śliwka D[2]

[1]Department of Forensic Medicine, Medical University of Lodz
[2]Laboratory for Molecular and Forensic Genetics, Medical University in Bydgoszcz

Minisatellite DNA consists of tandem repetitive 9 – 100 base pairs motifs of the length from few hundred to over 20 000 base pairs. These non-coding sequences are the fastest evolving in the genome due to comparatively high frequencies of mutation processes. The investigation of diversity in these hyper variable loci proves to be a valuable source of information ready to be used to characterize different human race and populations, as well as to define their genetic affinity. The aim of this work is to compare the distribution of alleles in the two highly polymorphic mini-satellite DNA regions D7S21 & D12S11obtained from the Polish and other world populations. To achieve this, we used the graphic analysis based on the allele frequencies in the intervals of 100 base pair as well as the statistical analysis. The analysis proved that the distribution of alleles in both the Polish and other Caucasian populations of Europe is similar. Moreover, it revealed significant differences in the structure of distributions when we compared the investigated Polish population, representing Caucasians, with Asian population and Afro-Caribbean population in particular.

Address for correspondence:
Renata Jacewicz, PhD
Head of Genetic Laboratory
Department of Forensic Medicine
Medical University of Lodz
91-304 Lodz, Sedziowska 18a
Poland
tel. + 48 42 654 45 36
fax + 48 42 654 42 93
e-mail: r.jacewicz@post.pl

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Population genetic study of the three minisatellites loci: D7S21, D12S11 and D5S110 in Poland.

Jacewicz R[1], Miścicka- Śliwka D[2]

[1]*Department of Forensic Medicine, Medical University of Lodz*
[2]*Laboratory for Molecular and Forensic Genetics, Medical University in Bydgoszcz*

The VNTR loci: D7S21, D12S11 and D5S110 are the highly polymorphic markers of the human genome. Though they are used in the most difficult cases of kinship analysis, a comprehensive database of these regions has not been set up for the Polish population: such a database is essential for carrying out analysis and performing calculations. The distribution of allele frequency as well as evaluation of the Hardy and Weinberg equilibrium are the subject of this report. The efficiency of forensic evaluation for investigated loci in the population of Poland was compared with similar data for other world populations. The combined values of PD and PE for the three-locus profile in the investigated population were calculated to be at 99.99997% and 99.996% respectively. Our practise indicates that investigated loci are an invaluable help in resolving most difficult forensic cases in kinship analysis, especially when the alleged father or mother are not available, or when there is a risk that the child's father is the defendant's close relative, or when we analyse the relationship between any given people.

Address for correspondence:
Renata Jacewicz, PhD
Head of Genetic Laboratory
Department of Forensic Medicine
Medical University of Lodz
91-304 Lodz, Sedziowska 18a
Poland
tel. + 48 42 654 45 36
fax + 48 42 654 42 93
e-mail: r.jacewicz@post.pl

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Study to compare three commercial Y-STR testing kits

Johns LM, Burton RE, Thomson JA

LGC, Queens Road, Teddington, TW11 0LY

An evaluation study was carried out to test the performance of three commercially available Y-STR DNA profiling kits for their suitability to forensic case work. The three kits assessed were Reliagene's Y-Plex™ 12 kit, Promega's PowerPlex® Y system and Applied Biosystems' AmpFℓSTR® Yfiler™ kit. Four experiments were devised to assess the performance of the three kits. Allelic peak height data was used to measure the reproducibility, sensitivity, male specificity and ability to discriminate male mixtures of the three kits. Samples were processed following the manufacturers recommended protocols. PCR products were run on 3100 electrophoresis platforms and the resultant DNA profiles analysed using GeneScan and Genotyper analysis software packages.

All three kits gave reproducible results with concordant genotypes between replicates and kits. Average peak height data showed the AmpFℓSTR® Yfiler™ kit to be the most reproducible kit during the evaluation study. PowerPlex® Y system was shown to be the most sensitive kit during the evaluation study. All three kits gave full male profiles for all samples processed in the specificity experiment. There was no evidence of female artefacts in the PowerPlex® Y and AmpFℓSTR® Yfiler™ samples however there was evidence of additional female artefacts in all Y-Plex™ 12 samples. The AmpFℓSTR® Yfiler™ kit showed the least degree of variation in peak area ratio's for the expected male mixture ratio's and therefore showed that it was able to discriminate male mixtures better than the PowerPlex® Y and Y-Plex™ 12 kits during this evaluation study.

Contact: Jim.Thomson@lgc.co.uk

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Validation of Quantifiler™ Human Quantification Kit for Forensic Casework

Johns LM, Thakor A, Ioannou P, Kerai J, Thomson JA

LGC, Queens Road, Teddington, Middlesex, TW11 0LY, UK

A study was carried out to test the suitability of Applied Biosystems Quantifiler™ Human Quantification Kit and validate it for forensic casework. The Quantifiler™ assay was performed using an Applied Biosystems 7900HT Real-time PCR system. The validation exercise comprised five parts. (1) Reproducibility (2) Sensitivity (3) Effect of bacterial DNA (4) Effect of reducing reaction volume (5) Back to back comparison with Picogreen® quantification assay. DNA extracts generated using a variety of extraction methods from different forensic sample types were used for the validation exercise. After quantification the DNA extracts were analysed using SGMplus amplification kits. The PCR products were run on 3100 electrophoresis platforms and the resultant DNA profiles analysed using GeneMapperID analysis software.

Quantifiler™ gave reproducible results for samples in the DNA concentration range of 0.1ng/µL - 5 ng/µL. The sensitivity of the assay was demonstrated with DNA concentrations of down to 0.03ng/µL being detected. The presence of increasing ratios of bacterial DNA had no effect on the specificity of the assay. There was no significant difference in calculated DNA concentrations when Quantifiler™ was run at half the recommended reaction volume. The back to back study demonstrated that Quantifiler™ generated SGMplus profiles which were on average of better quality than Picogreen® generated profiles. All extracts for which no SGMplus profile could be obtained had Quantifiler™ DNA concentrations of zero. The number of times a samples requiring a second amplification before an acceptable profile was obtained was three times lower for Quantifiler™ samples compared to Picogreen® samples. The validation exercise demonstrated the suitability of the Quantifiler™ assay for forensic casework.

Contact: Jim.Thomson@lgc.co.uk

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Application of less primer method to multiplex PCR

Kane M1,2, Masui S1,3, Nishi K2

1Forensic Science Laboratory, Shiga Police Headquarters, Japan

2Legal Medicine, Shiga University of Medical Science, Japan

3Legal Medicine, Osaka University, Japan

Multiplex short tandem repeat (STR) analysis have been indispensable for the forensic genotyping because it can use minute amounts of DNA and has a high degree of discrimination. In the case of an imbalance from locus to locus, the manufacturer recommends that reducing the number of PCR cycles and amplification using less templates can improve the balance among loci.

In order to obtain even PCR products as well accurate genotype analysis, reaction conditions including concentrations of primer, amplification cycle number and annealing and extension time were examined. The primer concentration (3 % of commercially available kit, AmFLSTAR Profiler kit, Applied Biosystems) was set at minimum required to the plateau below 8000 relative fluorescent units (RFU) without pull-up phenomenon. Contrast to the conventional PCR product that depends on amount of the template, less primer method has the upper limit. The locus of higher efficient amplification is reached to the plateau during early PCR cycles, the remaining PCR cycles employ to the production of lower efficient locus. Therefore, PCR product in this method is almost constant in every reaction and maintains the reproducibility and good balance among loci. Even if it can not converge on the optimal amounts of PCR products, the sensitivity of this method at 40 PCR cycles has increased more than one of protocol at 28 PCR cycles. When a minute template that has not reached to the plateau is treated, 5% primer is more sensitive than 3% primer. The ordinary primer concentration at 40 cycles results in non#8211; specific PCR because free primer lead to the disordered reaction according to the increase in cycle number. Thus, the cycle number in various kits is limited about 30 cycles. Less primer with higher number of PCR cycles permits the specific amplification.

We think that the annealing and extension time plays a key part because of few opportunities to encounter between template and less primer. In conventional PCR, the excess primer combines with the template immediately. It takes longer to anneal between less primer and the template, likewise, compose of less primer#8212; template and polymerase. The larger yield of low molecular locus is produced at three minutes of the annealing and extension time and five minutes promote dramatically the amount of PCR product of high molecular locus. As molecular weight become higher, the template of locus reduces, especially in degraded sample. Therefore, high molecular locus needs five minutes for both annealing and extension. The accurate genotyping from degraded samples in this method result from the upper limit and the specific amplification with high number of amplification cycle.

Contact: nishi@belle.shiga-med.ac.jp

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DNA analysis as the only solution for identification of remains found in secondary mass graves

Karija Vlahovic M, Furac I, Masic M, Marketin S, Raguz I, Kubat M

DNA Laboratory, Department of Forensic Medicine & Criminology, School of Medicine University of Zagreb

Killed soldiers and civilians, displaced and exiled persons, missing people, destroyed homes and towns; those are consequences of all wars. Unfortunately the same happened in Croatia. More than hundred mass graves were found in Croatia during past ten years after the war ended. For the identification of human remains found in mass graves conventional forensic methods were used, as well as DNA analysis. DNA analysis is the primary tool used for identification of fragmented remains and for the re-association of individual fragments. Here we present results of identification of war victims remains found in the secondary mass grave. 19 civilians were killed and buried in 1991 in Eastern Slavonia. In 1997 the 18 bodies were packed in seven large plastic barrels and transported across the country where they were discovered in a mass grave in the year 2000. After medical experts and antropologist finished the preliminary identification, it was decided to use DNA profile analysis as the final identification method since the body parts were commingled. 37 body parts and 20 blood samples from relatives were analysed at sixteen STR loci using PowerPlex 16 Kit. From 37 analysed samples we managed to obtain full STR profile for 34 samples. DNA profile comparisons enabled us to sort the 34 typed body parts into 17 individuals, as well as identifying the 16 victims for whom reference samples were available.

Contact: mkarija@mef.hr

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Y-chromosome variation in Swedish, Saami and Österbotten male lineages

Karlsson A[1], Götherström A[2], Wallerström T[3], Holmlund G[1]

[1]The National Board of Forensic Medicine, Department of Forensic Genetics, University Hospital, SE-581 85 Linköping, Sweden
[2]Department of Evolutionary Biology, Uppsala University, SE-725 36 Uppsala, Sweden
[3]Institute for Archaeology and Ancient History, University of Lund, SE-223 50 Lund, Sweden

We have analysed 383 unrelated males from Sweden (n=305), Saamiland (n=38) and Österbotten in Finland (n=40). Haplogroups were determined using 16 different Y chromosomal binary markers (M9, Tat, 92R7, M17, M35, M78, M89, M201, M170, M26, M223, SRY10831, M253, M269, YAP and 12f2). The Y-chromosome single nucleotide polymorphisms (Y-SNPs) were typed using Pyrosequencing™ technique. Nine Y-chromosome short tandem repeat loci (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and the separation of DYS385 into DYS385a and DYS385b) were also analysed to get a more detailed view of the variation.

A total of 13 different haplogroups were identified. In Sweden haplogroup I1a* was most frequent (37%), while N3 was the most common haplogroup in both the Saami and the Österbotten population (45% and 68%, respectively).

R_{ST} values were calculated, from haplotype data, in order to analyse the genetic differences between the populations. Using all haplotypes, R_{ST} values revealed that Swedes are more closely related to Saami than to males living in Österbotten. It also showed that Saami lineages are closer to Österbotten than to Swedes.

The Swedish sample consisted of males from seven geographically different regions in Sweden. Västerbotten, a northern Swedish county, was significantly different ($P<0.05$) from the other Swedish regions both comparing haplogroup frequencies and R_{ST} values.

Correspondence: gunilla.holmlund@rmv.se

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STR data for 15 AmpFLSTR Identifiler loci in a Tibetan population (Nepal)

Kido A[1], Dobashi Y[2], Hara M[3], Fujitani N[4], Susukida R[1], Oya M[1]

[1]Department of Legal Medicine, Faculty of Medicine, University of Yamanashi, Yamanashi, Japan
[2]Scientific Crime Detection Laboratory, Yamanashi Prefectual Police Headquarters, Yamanashi, Japan
[3]Department of Forensic Medicine, Saitama Medical School, Saitama, Japan
[4]Department of Biochemistry, Faculty of Science, Okayama University, Okayama, Japan

Allele frequency data for 15 short tandem repeat (STR) loci, D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA, were determined in 122 Tibetan individuals living in Katmandu (the capital of Nepal). DNA was extracted from serum samples**,** which were stored at -20°C for six years, by the QIAamp DNA Mini Kit (Qiagen). PCR amplification of the 15 STR loci was performed using the AmpFLSTR Identifiler Kit (Applied Biosystems) according to the manufacturer's recommended protocol. Amplified products were separated by denaturing capillary electrophoresis in the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The results were analyzed using GeneScan Analysis v3.7 software (Applied Biosystems) and Genotyper v3.7 software (Applied Biosystems). Possible divergence from the Hardy-Weinberg equilibrium was determined using the exact test. Some statistical parameters of forensic interest such as heterozygosity, power of discrimination, mean exclusion chance and polymorphic information content were calculated. Typing of STR loci was impossible in some samples. This tendency was salient in the STR loci with the long fragment. Amelogenin included in the AmpFLSTR Identifiler Kit was detected in all the samples. The agreement with Hardy-Weinberg expectation was confirmed for all studied loci with the exception of FGA. It appears that this departure is caused by the small number of samples. Among the 15 STR loci, FGA showed the highest power of discrimination and the highest mean exclusion chance. The combined power of discrimination and the combined mean exclusion chance for the 15 STR loci were 0.9999999999999999902 and 0.9999988, respectively.

Contact: akido@yamanashi.ac.jp

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Novel Sample Preparation Tool Quickly and Efficiently Prepares Cell Lysates to Facilitate Forensic Genomic Research.

Kirsher S, Dorion R, Chu S

Cartagen Molecular Systems Inc., Seattle, USA.

Plant and insects samples associated with crime scenes are gaining recognition as a source of valuable information related to the overall forensic process. We present data on a novel sample preparation tool for use by the forensic researcher when working with plant and insect samples. The BioMasher sample preparation device was developed by Nippi Inc. (Tokyo, Japan) to prepare bovine brain cell lysates prior to testing for BSE (Bovine Spongiform Encephalitis). We have found that the BioMasher is a versatile tool well suited for preparing PCR ready cell lysates from plant and insect samples. Several plant and animal species were evaluated by PCR using samples prepared with the BioMasher. We compared direct PCR of cell lysates to PCR of DNA isolated using standard genomic DNA extraction methods. We found that the BioMasher consistently provides efficient homogenization of both plant and animal tissue for use in direct PCR analysis or as the front end to commercially available genomic DNA extraction kits

contact: skirsher@cartagen.com

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Old friends revisited: Physical location and linked genes of common forensic STR markers.

Klintschar M[1], Immel U-D[1], Kleiber M[1], Wiegand P[2]
[1] *Department of Legal Medicine, University of Halle-Wittenberg, Halle, Germany*
[2] *Department of Legal Medicine, University of Ulm, Ulm, Germany*

Good practice in a forensic DNA laboratory requires knowing the sequence of the alleles, the allelic distribution, the chromosomal location and population genetic data. Nevertheless, as STR markers of forensic interest are also used in medical genetics, for many of the established loci further information, e.g. the exact physical localization and potential gene and disease linkage, is available. One example would be TH01, for which it is known that it is located in intron 1 of the tyrosine hydroxylase gene on the short arm of chromosome 11 (11p15.5), and closely linked to the insuline gene and the Harvey *ras* 1 oncogene. These facts have elicited further genetic studies which found that, either by linkage to one of these genes or by direct influence on the gene regulation, the allele 9.3 seems to be associated to diseases such as hypertension and psychosis. As such phenotypic effects of STRs are highly undesirable in forensic sciences, it appears to be worthwhile to investigate the current extent of information about forensic STR loci in common genetic databases.
To that end, for 16 loci for which only partial information is given in the forensic literature (D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, D19S433, D2S1338, D2S1242, D8S1132, D7S1517, D1S1656, D12S391 and D1S1171) an in silico search in the UniSTS and the EMPOP databases was performed and information concerning the exact physical location and closely linked genes was gathered.
As expected, none of the markers was localized in a coding region. For all markers an exact physical location was found.
Moreover, D3S1358 was found to be located in intron 20 of the leucyl-tRNA synthetase 2 gene and is part of a region on 3p21.3 that is frequently deleted in various tumours. D7S820 is located in intron 15 of the semaphorine 3A gene. D18S51 is located in the B cell lymphoma 2 gene. Also the rarely used loci D1S1656 (Calpain 9 gene) and D7S1517 (hyaluronoglucosaminidase 4 gene) were found to be located in introns, whereas the remaining markers are located outside of genes.
A search in the OMIM database for known linkage between diseases and markers revealed that D8S1179 was linked to Meckel syndrome (type 3) in an Indian family. D18S51 is linked to polyostotic osteolytic dysplasia (McCabe disease). D1S1656 is linked to Kenny-Caffey syndrome type 1 and to hypoparathyroidism-retardation-dysmorphism syndrome in Arabs. D2S1338 is linked to familial pseudohyperkaliemia 2. All these diseases are extremely rare inherited disorders, and linkage does not necessarily allow the conclusion that typing these markers would infer the undesirable diagnosis of an inherited disorder.
Our results allow the reassuring conclusion that up to now for none of these 16 markers a significant influence on the phenotype is known.
Contact: michael.klintschar@medizin.uni-halle.de

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The risk of incorrect typing of D1S80 by unstable minisatellite expansion

R. Kobayashi[1, 3] , N. Iizuka[2□3] and Y. Itoh[3]
[1]*Depatment of Microbiology, Tokyo Medical University, Tokyo, Japan*
[2] *Medico-Legal Section, Criminal Investigation Laboratory, Metropolitan Police Department, Tokyo, Japan*
[3]*Department of Forensic Medicine, Juntendo University School of Medicine, Tokyo, Japan*

The D1S80 locus is very useful for personal identification in Japan. To analyze PCR amplification products at the D1S80 locus, DIG-labeled primer was used for PCR amplifications. After electrophoresis, the PCR products were transferred to a nylon membrane and detected with alkaline phosphatase labeled-anti-DIG antibody (AP-DIG Ab). Numerous extra bands were detected on the membranes, indicating that PCR amplification products at the D1S80 locus contain many extra products which cause the undesirable bands to appear during D1S80 typing. To obtain a correct genotype, it was necessary to perform Southern blotting using an oligonucleotide that includs an internal sequence of the amplification products as a probe.
Introduction: The minisatellite locus D1S80, (location; 1p35-p36), GenBank sequence accession #D28507), is a variable number of tandem repeat (VNTR) locus with a 16 base pair repeat size. With alleles defined by the number of repeat units, the D1S80 locus is highly polymorphic in Japan. However, it is well known extra bands frequently appear during typing. In this paper, we demonstrate that PCR amplification products at the D1S80 locus have numerous extra bands which may cause incorrect genotypes to be obtained and that Southern blotting using an internal sequence as a probe is very helpful to determine D1S80 genotypes.
Materials and Methods: The primer (MCT118F) was labeled with DIG-11-dUTP (Roche, USA) according to the manufacturer's instructions (DIG-MCT118F). The probe (MCT118P: 5'-CTG CGT GTG AAT GAC CCA GGA GCG TAT C-3') was designed and also labeled with DIG-11-dUTP (DIG-MCT118P). PCR amplification was performed as described by Kasai (1990). After electrophoresis of PCR amplification products with DIG-MCT118F and MCT118R using 2% agarose gel, DNA fragments were transferred to a nylon membrane and detected using AP-DIG Ab and NBT/BCIP. The PCR amplification products with unlabeled primers were also transferred to a nylon membrane and hybridized with DIG-MCT118P and detected with AP-DIG Ab and NBT/BCIP.
Results and Discussions: PCR amplification products at the D1S80 locus were analyzed using a DIG-labeled primer. Although only real bands of the products appeared under UV light after ethidium bromide staining, numerous bands were detected when using the DIG-labeled primer. This finding indicates that extra bands are produced under the regular PCR conditions and can be visualized when using the AP-DIG Ab and NBT/BCIP detection. These extra bands may be detected with ethidium bromide staining if additional amplification cycles are performed. This may cause incorrect genotypes to be obtained. However, Southern blotting using an internal sequence as a probe could isolate and detect the real bands. This finding indicates that Southern blotting may be very helpful to determine D1S80 genotypes.
Dr. Y.Itoh Department of Forensic Medicine, Juntendo University School of Medicine, Hongo, Tokyo 113-8421, Japan.Tel: +81-3-5802-1051 Fax: +81-3-3814-9300
yitoh@med.Juntendo.ac.jp

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Mutation typing in Patients with Medium Chain AcylCoA Dehydrogenase Deficiency (MCADD) and PCR based mutation screening in SIDS victims

Krause D[1], Jachau K[1], Mohnike K[2], Nennstiel-Ratzel U[3], Busch U[3], Rosentreter Y[1], Sorychta J[1], Starke I[2], Sander J[4], Vennemann M[5], Bajanowski T[6], Szibor R[1]

[1]*Institut für Rechtsmedizin, Otto-von-Guericke-Universität Magdeburg, Germany*
[2]*Zentrum für Kinderheilkunde, Otto-von-Guericke-Universität Magdeburg, Germany*
[3] *Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit Oberschleißheim, Germany*
[4] *Screening-Labor Hannover, Germany*
[5]*Institut für Rechtsmedizin, Universitätsklinikum, Münster, Germany*
[6]*Institut für Rechtsmedizin, Universitätsklinikum, Essen, Germany*

*In parts of this paper we publish data on behalf of the GeSIDS Group**

We investigated 80 MCADD patients in a German populations and found the following frequencies of mutations: 985A>G (81.9 %); 157C>T (3.1 %), 799G>A (3.1 %), 244-245 ins T (3.1 %), 362C>T (1.3 %) and five rare mutations with frequencies below 0.6%. About 4.4% of the mutations in our patients remained unidentified. After mutation typing procedure we created rapid tests, which are based on the PCR / electrophoresis technology and recognise the four most frequent mutations.(i. e. 985A>G , 157C>T, 799G>A, 244-245 ins T). Using these screening tests we identified one MCADD case under 409 SIDS victims. These investigations indicate that in very few cases MCADD may contribute to SIDS.

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Contact: dieter.krause@medizin.uni-magdeburg.de

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Data analysis of SE33 allele frequencies in the population of province Schleswig-Holstein (North Germany)

Krause M, Heide K-G,

Labor für Abstammungsgenetik, Kiel, Germany

Allele and genotype frequencies for STR SE33 were determined in a sample of 1750 unrelated Germans for paternity cases. We found many “Variants”. No deviation from Hardy-Weinberg equilibrium were observed in the population.

Contact: krause@labor-krause.de

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Laser microdissection and pressure catapulting with PALM® to assist typing of target DNA in dirt samples

Lambie-Anoruo BL[1,2], Prince DV[1], Koukoulas I[1], Howells DW[3], Mitchell RJ[2], van Oorschot RAH[1]

[1]Victoria Police Forensic Services Department, Victoria 3085, Australia
[2]Department of Genetics and Human Variation, La Trobe University, Victoria 3086, Australia
[3]Austin Hospital, Department of Medicine, University of Melbourne, Victoria 3084, Australia

Obtaining a DNA profile from a subset of cells within a mixture of cells where the predominant cells are of a different type and source becomes problematic once the proportion of the target cells becomes very low. This can be difficult even when the total number of minority cells is theoretically sufficient to generate a good DNA profile. Differential extraction methods to separate sperm from epithelial cells are commonly used to assist with mixtures of such cell types (as frequently encountered in sexual assault cases). These methods, however, are inadequate when dealing with mixtures of other cell types, such as saliva and blood, or saliva and shed skin cells. It can also be troublesome to retrieve profiles from small biological samples in debris such as saliva in dirt. Use of the PALM laser microdissection and pressure catapulting process may assist in the retrieval of target DNA and subsequent DNA profiling in these situations.
We tested the capability of PALM to isolate saliva cells (12µl saliva) from mixtures with dirt (8µl of humus rich dirt). DNA was extracted from replicate samples using Chelex and organic extraction methods and compared to DNA retrieved from cells isolated from replicate mixtures using PALM isolation followed by Chelex extraction. Each test was repeated four times on mixtures dried for one day and on mixtures dried for seven days. Extracted DNA was quantified using the Quantifiler™ kit and when found to be positive also amplified and typed with Profiler Plus™ using an ABI PRISM® 3100 Genetic Analyser in conjunction with GeneMapper™ ID.
Results from the one-day-old series of samples demonstrated that typeable DNA from the saliva component of the dirt sample was not retrievable from the samples extracted using either of the standard Chelex or organic extraction methods. In contrast, PALM assisted isolates of 200 saliva derived cells from amongst the dirt of one-day-old samples provided DNA from which the expected full DNA profiles were generated. The DNA extracted from the seven-day-old series of samples, using either the Chelex or the organic method, was also not typeable. The seven-day-old sample examined using PALM revealed that there were fewer whole cells observable and that the retrieval of recognisable cells took significantly longer. The 47 cells (representing a portion of the total available cells) that were isolated from the seven-day-old sample provided a partial profile.
The use of PALM should be considered to identify and isolate target cells from debris that may prevent the generation of DNA profiles using standard DNA extraction methods.

Contact: roland.vanoorschot@police.vic.gov.au

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Allele frequencies of fifteen STR loci in an Italian Population

Lancia M , Coletti A , Margiotta G , Lottanti L , Carnevali E , Bacci M

Section of Legal Medicine, University of Perugia, Terni, Italy.

The study of the STR loci is important for the creation of local human identification databases. In the latest years, European countries have begun to plan studies whose purpose is to create national and/or local databases and to be known with the expression frequency of a great number of these DNA loci. Our research has the aim of creating a local database, according to the recommendations published by the International Society for Forensic Haemogenetics.
The study was carried out on specimens taken from 100 healthy and not related individuals, who were born in Terni and have been living there for at least two generations. The biological material consisted of blood in 96 cases, and of oral swab in the remaining 4. Extraction of the DNA from the blood specimens was carried out by using QIAamp DNA miniKit of the Qiagene company.
The DNA extraction from oral swabs was executed by the Chelex1 method.
The DNA polymorphism analysis was carried out by enzymatic amplification (Polymerase Chain Reaction - PCR).
Allelic frequency determination becomes very important in forensic use when you need to calculate the probability that two DNA specimens derives from the same individual.
In our case, after obtaining the typization of the fifteen STRs studied for the 100 specimens we analysed, we calculated the allelic frequency of every single system. In order to verify if the considered population was in equilibrium with an ideal one having a Gaussian-type distribution, we applied Hardy-Weinberg's law, Pearson's test and p-value calculation.
In our case Terni population turned out to be in equilibrium in all the fifteen systems we studied. The allelic frequencies of the population were compared to the corresponding data of Italian population in a generalized way.

contact: baccim@aospterni.it , lancia.massimo@infinito.it or gabriele.margiotta@poste.it

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DNA recovery from semen swabs with three different extraction methods

Lazzarino F., Laborde L. , Lojo M.M.

Laboratorio de Análisis Comparativo de ADN
Suprema Corte de Justicia, Buenos Aires, Argentina

Efficiently extraction of sperm cells from the solid matrix is an important step in male DNA recovery from cotton swabs. Digestion with proteinase K loosens the attachment of semen to the solid support. Thus, digestion in the presence of the cotton matrix enhance DNA yield. We used simulated samples in order to compare the extraction efficiencies of Qiamp DNA minikit, DNA IQ System and Chelex methods, performing all the extraction steps in the presence of the solid support. Female oral swabs were embedded with serial dilutions of semen samples of known cells density (sperm cells/ ml). The experimental conditions were adjusted in order to use almost the same number of target cells in all the three different protocols assayed. Standard proteinase K digestion without DTT was performed by incubating a 1/4 of the cotton swab. Samples were centrifuged in a spin basket and the following washes, as well as the full extraction procedures were done in the presence of the solid support. DTT was added to both two digestion buffers used in the Qiamp DNA minikit. Str amplicons obtained by Profiler Plus PCR amplification were run on an ABIprism 310 Sequence Analyzer and the recovery of peak intensities were compared. No significant differences were observed in the extraction efficiency between Quiagen and DNA IQ systems, whereas the detection limit (number of target cells) were higher in the extraction performed with Chelex. A male DNA profile could be still recovered by Chelex extraction from the swabs previously extracted with both Qiamp and DNA IQ methods, suggesting that both treatments were not able to fully release the sperm out of the fiber.

mercedeslojo@hotmail.com

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Selection of Y-STR loci and development of a PCR multiplex reaction for use in South Africa.

Leat N, McCabe M, Kleyn E, Cloete K, Benjeddou M, Davison S

Biotechnology Department, University of the Western Cape, Cape Town, South Africa

The objective of the present study was to examine the properties of a set of single-copy Y-STR loci to assess their suitability for forensic case work in three South African populations. Three criteria were used to select markers for assessment. Firstly, the single-copy markers of the minimal haplotype were selected based on their established use in forensic studies. Secondly, eight markers were selected on the basis of high gene diversity values reported for several population studies, and thirdly 19 markers were chosen from a survey of Y-chromosome sequence with selections made primarily on the basis of the number of repeated elements present. Samples were typed from English-speaking Caucasians, Xhosa individuals and Asian Indians. Gene diversity values, the number of alleles identified and the average stutter was determined for each locus. The data has been used to select a subset of highly polymorphic Y-STR loci. A PCR multiplex reaction is currently being refined and to facilitate the analysis of the selected loci in forensic studies.

Contact: nleat@uwc.ac.za

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Haplotypes and mutations of 17 Y-STR loci from Korean father-son pairs

Hwan Young Lee[1], Han Young Lee[2], Ukhee Chung[1], Myung-Jin Park[1], Ji-Eun Yoo[1], Kyoung-Jin Shin[1,3], Sang-Ho Cho[1,3], Woo-Ick Yang[1]

[1]*Department of Forensic Medicine, College of Medicine, Yonsei University, Seoul, Korea*
[2]*Department of Forensic Medicine, National Institute of Scientific Investigation, Seoul, Korea*
[3]*Human Identification Research Institute, Yonsei University, Seoul, Korea*

We have investigated 17 Y-STR loci-DYS19, DYS385a/b, DYS389-I, DYS389-II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439, DYS437, DYS448, DYS456, DYS458, DYS635 (Y GATA C4), Y GATA H4-in 365 father-son pairs (6,205 meioses) of 355 families. Of 338 different haplotypes obtained from 355 fathers, 326 haplotypes were observed once, 10 haplotypes two times and the other two haplotypes were observed 4 and 5 times, respectively. The overall haplotype diversity was 0.9996. In 365 father-son pairs, a total of 21 mutations were observed at 12 Y-STR loci (DYS19, DYS385a/b, DYS389-I, DYS389-II, DYS390, DYS393, DYS439, DYS437, DYS456, DYS458, DYS635 (Y GATA C4), Y GATA H4). Sequence analysis for mutant alleles demonstrated 21 single step mutations: 8 gains and 13 losses. However, there was no significant surplus of gains or losses. The locus-specific mutation rate estimates were between 0.0 and 8.2 X 10^{-3} and the average mutation rate estimates were 3.4 X 10^{-3} (95% C.I. 2.1~5.2 X 10^{-3}) across all 17 Y-STR loci. Mutation rates differed strongly between loci depending on the molecular structure of the respective STR locus, and the locus-specific mutation rate estimates also showed differences between populations. However, in contrast to the case of autosomal STRs, no noteworthy correlation was observed between mutation rate and the father's age at child's birth.

Contact: hylee192@yumc.yonsei.ac.kr

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Retrieval of DNA and genetic profiles from swaps taken inside cars.

Lenz C, Flodgaard LR, Eriksen B, Morling N.

Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark.

In a survey of crime case samples collected from the interior of cars in the period 2003-2004 the success rate of retrieving a genetic profile from cotton swaps was estimated. A total of 241 samples were analysed and DNA profiling was performed using the AmpFlSTR®-SGM-plus™ kit with 28 cycles PCR. Only 23 % of the samples showed a DNA concentration >0.02 ng/µl as quantified with a slot blot method. STR profiles were retrieved for all but three of these samples (overall success rate 22 %). The samples were collected by five different police units and the success rate for the units varied from 14 % to 34 %. This indicated that the sampling technique played a major role for the success rate.
In a controlled experiment, we tested if the amount of water and the storage conditions of the swaps influenced the retrieval of DNA. One policeman made swaps from steering wheels and spokes in 14 different cars. All swaps were taken from delimited areas. A total of 56 samples were collected from the cars. In 89 % of the samples, a DNA concentration > 0.04 ng/µl was retrieved (range 0.01 – 5.6 ng/µl) using quantification with the Quantifiler™ kit. DNA analysis was performed on 25 samples and a DNA profile was obtained for all of them. No significant difference regarding the amount of DNA retrieved from the swaps was seen when 1 versus 4 drops of water were used for the swaps. Also, no significant difference was seen when swaps were air dried versus frozen. The high success rate for the samples from the controlled experiment compared to the crime case samples could be contributed only to the sampling technique where the swaps were taken by thoroughly wiping a delimited area.

Contact: Camilla.Lenz@forensic.ku.dk

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Tsunami 2004 – experiences, challenges and strategies

Lessig R, Thiele K, Edelmann J

Institute of Legal Medicine of the University of Leipzig, Germany

The Tsunami after the sea quake in Southeast Asia on the 26th of December 2004 represents the largest disaster in the modern World. More than 280,000 people in the countries around the Indian Ocean have been reported missing. Especially Thailand and Sri Lanka as major tourist centres demand a large number of victims from different European countries. Twenty international Disaster Victim Identification (DVI) teams were present in Thailand to help identifying the recovered bodies. Such a number of different teams and the circumstances in the area of Khao Lak were a great challenge for the organisation. It was necessary to adapt the different teams to a common strategy of investigations. The established international centre established the guidelines for the forensic-medical, forensic-odontological and forensic genetic investigations. The fast decomposition of the bodies was a great challenge. The collection of the post mortem data was done by forensic specialists. The guidelines for the DNA analysis request a collection of different samples from every investigated body – two healthy teeth, rib, bone or similar tissues – for examination. The biggest problem seems to be the expected rapid degradation of the DNA. So the suggested strategy in such cases should be to test samples very soon to assess the suitability for genetic typing. After knowing the possibilities the second step would be the decision about the best markers to be used. So a small number of samples were investigated in our laboratory. A high level of degradation of the DNA was observed and special procedures of extraction were necessary to get a result.
So an important conclusion for further work in this field is an agreement on international standards and also the training of specialists who are able to coordinate the analysis. The Tsunami shows also that the DNA analysis can be very helpful in such mass disaster case work as a part of the forensic fields.

contact: Ruediger.Lessig@medizin.uni-leipzig.de

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HVI and HVII Sequence Polymorphisms of the Human mtDNA in the North of Portugal: Population Data and Maternal Lineages

Lima G[1], Pontes ML[1], Abrantes D[1], Cainé L[1], Pereira MJ[1], Matos P[1], Pinheiro MF[1,2]

[1] *Instituto Nacional de Medicina Legal – Delegação do Porto*
[2] *Faculdade de Ciências da Saúde – Universidade Fernando Pessoa*

The analysis of mitochondrial DNA (mtDNA) control region is of great importance in forensic casework. The aim of this study was to create a population database for the HVI and HVII regions of the mtDNA in the population of North Portugal and to analyse these two segments in maternal relatives from this population. For the population study, the HVI and HVII segments were analysed in unrelated and healthy individuals, chosen randomly, from the North of Portugal. For the maternal relatives analysis, those two regions were studied in a set of families (mother/child, grandmother/grandchild or sibling pairs) from that region of Portugal. The DNA was extracted from peripheral blood and oral swab samples, using different methods (phenol-chloroform, Chelex and Chelex + phenol-chloroform). The HVI and HVII segments were amplified by PCR using specific primers. These two segments were direct sequenced on both strands using the universal primers M13 and two different sequencing kits (dRhodamine and BigDye v1.1, Applied Biosystems). The HVI and HVII sequences were studied between positions 16033bp – 16391bp and 57bp – 408bp, respectively. Nucleotide substitutions (transversions and transitions) and insertions / deletions were found using Anderson's reference sequence. Length and position heteroplasmy were observed. The genetic structure of the population was analysed by calculating the number of different haplotypes, nucleotide diversity, genetic diversity and mean number of pairwise differences. The match probability and discrimination power values were calculated. The classification into haplogroups was also made.

Contact: Biologia@dpinml.mj.pt

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Polymorphisms Analysis of Mitochondrial DNA in Coding Area

LIU YC[1]□HAO JP[2]□TANG H[1]□YAN JW[1]□WANG J[1]□REN JC[1]

[1]*Forensic Medical Examination Center of Beijing Public Security Bureau*
[2]*school of Forensic Medicine,Shanxi Medical University*

Mitochondrial DNA (mtDNA) sequencing has allowed investigators to derive genetic informations from forensic samples where nuclear-based analyses have failed, for example degraded samples, old bone fragments or hair shafts without roots. Currently mtDNA for forensic testing consists primarily of portions of the control region, most often targeting the hypervariable regions one and two (HV1/HV2) ,but poor discrimination power remains a problem. The only solution would appear to be to find more polymorphic sites within mtDNA. The suggestion has been made that besides the mtDNA control region, the polymorphisms within mtDNA coding area should be used for forensic biologists in order to greatly increase the discrimination power of mtDNA .

In this study, we have sequenced the mtDNA coding area nt8162-8483 and nt13070-13299 of 100 unrelated healthy Han Chinese individuals. We have presents the single nucleotide polymorphisms (SNP) sites and 9-bp length-polymorphism of the mtDNA intergenic COII/tRNA^{Lys} region, which may be of crucial importance to forensic testing. The lengths of the amplicons were 322bp and 230bp respectively. There were 24 mitochondrial haplotypes defined by 21 variable positions in both regions. The gene diversity was estimated at 75.11%, and the probability of two randomly selected individuals having identical mtDNA types was 25.64%.

Conclusions

The polymorphic sites within mtDNA coding area can be useful in combination with mtDNA control region in order to increase the discrimination power.

Contact: yachengliu@163.com

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Study of microvariation of allelic frequency distribution of 17 STR's in each of the Azores islands population

Lopes V[1], Carvalho M[1], Andrade L[1], Anjos MJ[1], Serra A[1], Balsa F[1], Brito P[1], Oliveira C[1], Batista L[1], Gamero JJ[2], Corte-Real F[3], Vieira DN[3], Vide MC[1]

[1]*Forensic Genetic Service. National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal*
[2]*Departament of Legal Medicine, Faculty of Medicine, University of Cádiz, Spain*
[3]*National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal*

We performed a study of the allelic frequency distribution of 17 STR's along each of the islands to look out for statistical differences among islands. From the global population we selected only those individuals whose both parents were born in the same island.

DNA was extracted by Chelex from air-dried blood stains of healthy and unrelated individuals from Azores archipelago and amplified with two commercial *multiplex kits*: AmpF*l*STR® Identifiler™ (Applied Biosystems) and PowerPlex® 16 System (Promega). The detection was carried out on ABI Prism™ 310 Genetic Analyzer with internal standards (LIZ-500 and I.L.S. 600 respectively) and allelic *ladders* supplied with each *kit*.

The allele frequency distribution of the seventeen STR's present in the *multiplex* systems in each of the islands is in equilibrium of Hardy-Weinberg.

The microvariation study of the allelic frequency distribution among the islands was performed with software Arlequin 2.000 to obtain the genetic distances between the islands and the correspondent P values by the sum of squared size difference (RST) method. The phylogenetic tree derives from Phylip 3.5c software using the Neighbor-joining method.

There were no significant differences among the islands with the exception of Flores (in the most occidental group) with some P values not reaching 0.05.

Contact: geneforense@dcinml.mj.pt

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Y-STR polymorphisms from Basque-speaking region of Cinco Villas (Navarra) in the context of the Pyrenean genetic landscape.

López-Parra AM[1], Tavares L[2], Gusmão L[2], Mesa MS[4], Prata MJ[2], Amorim A[2,3], Arroyo-Pardo E[1]

[1] *Depto. Toxicología y Legislación Sanitaria, Facultad de Medicina, Universidad Complutense, Madrid, Spain.*
[2] *Instituto de Patología e Imunologia Molecular da Universidade do Porto (IPATIMUP) Portugal.*
[3] *Faculdade de Ciências, Universidade do Porto, Portugal.*
[4] *Depto Zoología y Antropología Física, Facultad de Biología, Universidad Complutense, Madrid, Spain.*

The Iberian Peninsula presents a complex geographical landscape with mountain ranges as in Northern Spain. That enhances the genetic isolation of small populations and consequently significant differentiation. We have studied a set of 42 samples from the Basque-speaking region of Cinco Villas (Navarra), located at the western part of the Pyrenees, for a total of 15 Y-STRs (Minimum Haplotype plus DYS460, DYS461, DYS437, DYS438, DYS439, GATA H4, GATA C4). Thirty five different haplotypes were detected (haplotype diversity: 0.9919± 0.0069) and only seven were found in two individuals. Data from this population were compared with those from other Pyrenean and Iberian populations. Statistical analyses revealed that Cinco Villas region clusters with other samples of the Basque Country and also with other non Basque-speaking population from Pyrenees. This fact suggests a common genetic background throughout the whole Pyrenean mountain range or an important gene flow between these mountain populations, irrespectively of the language presently spoken.

Contact: earroyop@med.ucm.es

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Microgeographic mitochondrial DNA patterns in the South Iberia

López-Soto M[1], Salas A[2], Sanz P[1], Carracedo A[2]

[1] *National Institute of Toxicology and Forensic Sciences, Depart. of Seville, Ministry of Justice, Spain*
[2] *Unidad de Genética, Facultad de Medicina, Universidad de Santiago de Compostela, Galicia, Spain*

Along history, Andalusia (South of the Iberian Peninsula) has been a territory occupied by many civilizations coming from Europe and North Africa. Here we aim to identify its mitochondrial composition by analyzing the two hypervariable regions (HVS-I and HVS-II) and selected coding region SNPs of the mitochondrial DNA (mtDNA). A total of 419 individuals from 28 villages (belonging to different provinces and with more than 200 years of history) have been sampled. This sampling has been design in order to uniformly cover the geographic area of South Iberia. Historical record indicates that these villages have experience little recent migration. Preliminary results revealed that 94% of the haplotypes belong to typical European haplogroups, 2.1% are Sub-Saharan lineages, and only 1.6% North African. AMOVA analysis indicates that the main percent (97.6%) of the variability in these populations is found between individuals, 2.2% between villages of the same province, and 0.25% between provinces. In addition, haplotype diversity is high (0.99) in Andalusia in comparison with other Iberian and European populations. The results point to a lack of significant demographic impact (at least in the maternal mtDNA side) of North Africa despite the close geographic proximity and eight centuries or Arabian colonization.

Contact: manuel.lopez@mju.es

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Multiplex STR and mitochondrial DNA testing for paraffin embedded specimen of healthy and malignant tissue: Interpretation issues

Lu C[1,2], Budimlija ZM[1], Popiolek DA[3], Illei P[3],West BA[3], Prinz M[1]

1 Office of Chief Medical Examiner, Department of Forensic Biology, New York, NY

2 John Jay College of Criminal Justice, New York, NY

3 New York University School of Medicine, Department of Pathology, New York, NY

DNA-based short tandem repeat (STR) typing is a powerful tool for the confirmation of suspected sample mix-ups or the presence of contamination in histology material (1). Histology specimens also have potential as reference samples in body identification efforts. But microsatellite analysis of tumor DNA has shown substantial allelic instability (2,3) which might impair correct sample associations. The aim of this study was to compare STR typing results for healthy and malignant tissues to mtDNA data for the same sample sets, evaluate the results and formulate interpretation rules, for example, for the determination of Loss of Heterozygosity (LOH). Ten different types of carcinoma were represented in the anonymous study material (endometrial adenocarcinoma – types I and II, granulosa cell tumor, adenosarcoma, malignant mixed Mullerian tumor, adenocarcinomas of prostate, lung, colon and cecum and cutaneous melanomas). Healthy and carcinogenic tissues were collected from each individual and embedded in paraffin. All slides were set up in a double blind manner where the researchers did not know which tissue was healthy or cancerous. After standard organic extraction, samples were typed using the PowerPlex®16 multiplex STR system (Promega Corp., Madison, WI) and the Linear Array Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit (Roche Applied Science, Indianapolis, IN). Many of the tested samples yielded partial profiles that showed characteristics of degraded DNA. Tissue fixation and embedding have been shown to negatively affect DNA quality. DNA degradation results in reduced peak intensity for high molecular weight alleles and increased stochastic effects causing heterozygous peak imbalance and allelic drop out. Several samples did display additional STR alleles and LOH. The mtDNA assay is less affected by DNA degradation but more prone to detect DNA contamination. Another critical issue for mtDNA testing that must be addressed during interpretation is heteroplasmy (4). In order to distinguish LOH from degradation based allelic drop out, the interpretation guidelines need to incorporate signal intensity and molecular weight of affected alleles. For RFU values below 300, LOH cannot be determined for loci > 350bp. Alleles smaller than 350bp should still display full types and a heterozygote balance ≥ 0.7. For mtDNA testing on clinical specimen mutation and heteroplasmy issues will be difficult to establish unless the histology samples can be processed under ultra clean conditions. Contact: PRINZ@ocme.nyc.gov

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P-164

Disparity between self-identified ethnicity and mtDNA ancestral lineages: a case study in Kenyan populations

Luiselli D[1], Boattini A[1], Flamigni ME[1], Castrì L[1], Pettener D[1]

[1]Department of Biology, Unit of Anthropology, University of Bologna, Bologna, Italy

Genetic studies of African populations can be frequently biased by different sampling criteria or erroneous assessment of ethnicity. In this study we analyze mtDNA variation of Turkana, Samburu and Rendille populations, three pastoralist nomadic ethnic groups of Kenya, with the aim of proving that genetic data should always be associated to individual biodemographic information. The simple use of self-identified ethnicity is often misleading. The social structure of African Pastoralists is patrilineal and requires a detailed reconstruction of the real marital migration patterns. In fact, in exogamous marriages, the brides loses her ethnicity and acquires the groom's one, creating a disparity between the real ancestral maternal lineage and the declared one.

The data were collected in the Loyangalani village, district of Marsabit, and in Morijo village. Buccal swab samples were obtained from 107 individuals, and the geographic and ethnic origin of each subject as well as of his four grandparents was carefully ascertained by oral interviews. All mtDNAs were subjected to sequencing of the control-region hypervariable segment I (HVS-I), and surveyed for 13 RFLPs polymorphic markers in the coding region.

Biodemographic results show consistent admixture between the three ethnic groups, with different patrilineal and matrilineal migration patterns. As concerns maternal lineages, admixture between Turkana, Samburu and Rendille are 27%, 9% and 42% respectively. The genetic data are analyzed both taking into account the self-identified ethnicity and the genealogic reconstruction of real ancestral lineages up to the third generation. In the former case AMOVA (Fst=0,036; p=0.029, 1000 iterations) shows absence of genetic homogeneity among the three groups, while in the latter (Fst=-0,016; p=0,819, 1000 iterations) homogeneity is high.

The striking difference in results using the two clustering criteria suggests caution in the analysis and interpretation of mitochondrial genetic data of African populations. When samples are collected without detailed biodemographic information, the use of self-identified ethnicity can lead to cultural groupings that are largely independent from their genetic origin**.**

Contact: donata.luiselli@unibo.it

P-165

Enzyrim: a new additive to increase the DNA yield from different materials such as teeth, blood or saliva

Mályusz V[1], Schwark T[1], Simeoni E[1], Ritz-Timme S[2], von Wurmb-Schwark N[1]

[1]*Institute of Legal Medicine, University of Kiel, Kiel, Germany*
[2]*Institute of Legal Medicine, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany*

Enzyrim is an enzyme mixture normally used for bone maceration. It is cheap, easy to handle, non-toxic and disposal is simple. When extracting DNA from Enzyrim treated teeth we discovered that the amount of extracted DNA was unexpectedly high.
We then systematically investigated different biological materials using three extraction kits, the Invisorb Forensic kit, the SPS Spin Swab kit (both Invitek, Germany) and the NucleoSpin Blood Quick pure kit (Macherey Nagel, Germany).
DNA was extracted from buccal swabs, dried blood spots on filter paper, whole blood and toothpowder. All DNA extractions were performed according to the manufacturer´s recommendations as well as after addition of Enzyrim to the lysis step of each kit.
DNA quality and quantity was tested on ethidium bromide stained agarose gels. Absolute quantification was done using real time PCR. The DNA samples were also employed to genetic fingerprinting using the Powerplex ES and the AmpF*l*STRIdentifiler kit.
The application of Enzyrim greatly improves the DNA yield from forensically important materials and does not hamper DNA amplification. Thus Enzyrim apparently is a very useful additive for the optimisation of DNA extraction in the forensic routine.

Contact: vm@rechtsmedizin.uni-kiel.de

P-166

Amplification of very small amounts of DNA in sub-µl volumes in routine: A new platform for on-chip PCR

Wolfgang Mann, Ulrike Schön, Tanja Schmitt, Thomas Zacher, Kerstin Hagen Mann

ALOPEX GmbH, Fritz-Hornschuch-Str. 7, D-95326 Kulmbach, Germany

Amplification of small amounts of nucleic acids is a challenge for a number of questions in genetics and forensics. In terms of commercially available kits for typing DNA sensitivity of those products is given in amounts of pg / sample to be analysed with or without allelic drop outs (ADOs). A technical reason for generation ADOs is simply the fact that a sequence might be missing because of the nature of a DNA dilution series. Small numbers of molecules cannot be distributed homogenously into seperated reaction vessels. Approaches like the digital PCR try to circumvent this obstacle by analysing the total volume that was amplified and try to detect any reaction with at least one starting molecule in a very large number of amplification reactions.
We have adressed the challenge by introducing a new amplification platform (AmpliGrid) that is suitable for amplifying small amounts of nucleic acids on a glass chip. The advantage by using this new platform is the optical inspection of the biological sample via microscope (since it is a glass substrate) immediately before starting the amplification in a one µl reaction. It is no problem to determine e.g. the number of starting cells each reflecting one single genome equivalent. Now the challenge to amplify one, two or three copies of a single sequence is no longer dependent on dilution series as described above. Furthermore, the frequency of ADOs is proportional to amplification parameters and no longer the analysis of technical ADOs based on the fact that there was not any starting target copy of the sequence to be analysed.
Hundreds of single cell PCRs have been carried out and a systematic study on the ADO phaenomenon based on commercially availabe multiplex typing kits will be presented.

Contact: wmann@alopexgmbh.de

P-167

Detection of microchimerism using short tandem repeats in patients submitted to blood transfusion

Mardini AC[1], Schumacher S[1], Albarus MH[1], Rodenbusch R[1], Giugliani R[1,2], Matte U[1], Saraiva-Pereira ML[1,3].

[1]*Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil*
[2]*Genetics Department, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*
[3]*Biochemistry Department, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*

DNA analysis is a common method to diagnose several genetic and infectious diseases. Identification of microsatellite (short tandem repeats – STR) marker sets is normally used in many laboratories for human identification, helping in solving paternity as well as forensic cases. All of these studies use polymerase chain reaction (PCR) to amplify DNA extracted from peripherally drawn blood. As PCR is highly sensitive procedure, capable of amplifying even 1 molecule of DNA, sources of contamination have to be eliminated. However, transfusion might be a source of DNA contamination in ill patients since there is a period that donor cells are present in the patient system. In order to prevent this contamination, several procedures are performed to eliminate white cells from blood, such as irradiation. As our laboratory is a reference center for both diagnosis of genetic disorders and DNA paternity tests, we decide to determine whether STR from different sources can be detected in blood samples from patients that underwent blood transfusion. Samples analyzed were from two different sources. Ten anonymous blood samples were mixed and generated five blood mixtures, each of the in five different dilutions. Besides being tested as a mixture, all these samples were also tested for each marker before being mixed. We also evaluated 20 transfused patients. In this group, patient cells were typed before and up to 7 days after transfusion. In addition, donor cells were also typed prior transfusion. Polymorphic markers tested were D3S1358, D16S539, TH01, TPOX, CSF1PO, and D7S820. DNA isolation was performed from 300µl of each sample using the Wizard® Genomic DNA Purification Kit (Promega), according to manufacture instructions. Regions of interest were amplified by multiplex-PCR using fluorescent primers, using the Applied Biosystems CofilerSTR™ kit. Amplification products were analyzed in ABI Prim® 3100 Genetic Analyzer, and GeneScan® and Genotyper® software. In these samples analyzed in the conditions described above, no microchimerism was identified. We concluded the microchimerism from blood transfusion is unlikely to have major effects on the genotype results of common polymorphisms, even when blood sample is taken within a day after transfusion. contact: mlpereira@hcpa.ufrgs.br

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Evaluation of allelic alterations in STR in different kind of tumors and formalyn fixed tissues- possible pitfalls in forensic casework.

Margiotta G , Coletti A , Lancia M , Lottanti L , Carnevali E , Bacci M

Section of Legal Medicine, University of Perugia, Terni, Italy.

Nowadays, the use of formalyn fixed tissue for forensic identification is frequently requested. This is why forensic genetics laboratories must often study normal or tumour tissue specimens that are usually archived with this method.
The somatic instability of tumour tissue on STR (short tandem repeats) loci and the DNA damages caused by formaldeide are well described. These conditions can cause an incorrect allelic determination that makes a forensic identification fail.
In order to evaluate the real incidence of the genetic alterations caused by somatic instability of tumour tissue, and the incidence of the DNA damages caused by formalyn, we studied 50 specimens of patients who have been operated for neoplasia.
For each patient, we studied a specimen of fresh tumour tissue and a specimen of formalyn-fixed tumour tissue, and the results of these analyses were compared to a specimen of fresh normal tissue and to a specimen of formalyn-fixed normal tissue of the same patient.

contact: baccim@aospterni.it , lancia.massimo@infinito.it or gabriele.margiotta@poste.it

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On-Line Autosomal and Y-STRs Genetic Marker Reference Data Base of Argentina.

Miguel Marino, Andrea Sala and Daniel Corach

Servicio de Huellas Digitales Genéticas and Cátedra de Genética y Biología Molecular. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. Junín 956 Ciudad Autónoma de Buenos Aires. Argentina. shdg@ffyb.uba.ar

Autosomal and Y chromosome-specific short tandem repeats (STRs) became the genetic markers of choice for individual identification. In addition, these markers also became powerful tools to assist molecular anthropologists. The availability of internet on-line reference databases may contribute either with forensic scientists or molecular anthropologists to obtain genetic information that may be continuously updated. At the Servicio de Huellas Digitales Genéticas (Genetic Fingerprinting Service, University of Buenos Aires) we constructed an interactive reference database that includes a set of fifteen autosomal STRs (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX and FGA) as well as a set of Y-STRs (DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393 and DYS385a/b). The complete set of data corresponding to 2003 includes 2710 samples typed with autosomal markers and 239 samples typed with the minimal haplotype (nonaplex) Y-STRs. The search can be done by choosing all the country or by choosing a particular province. In the database are included 10 provinces: Buenos Aires, Santa Fe, Rio Negro, Chubut, Mendoza, Misiones, Corrientes, Formosa, Chaco and Salta. To evaluate allele or haplotype frequencies in a given province the cursor selects the province from the map of Argentina, the genetic marker is selected by clicking on the ideogram of a metaphase graph in which the markers are located. During July the previous year information is being updated. The frequencies can be determined for a particular year or as combined information. In addition since it is a modular program the number and type of markers can be increased or included. It also includes mutation frequency of the markers described. This contribution offers a rapid tool for assessing genetic information on-line in order to improve data access.

Contact: shdg@ffyb.uba.ar

P-170

Population data at fifteen autosomal and twelve Y-chromosome short tandem repeat loci in the representative sample of multinational Bosnia and Herzegovina residents

Marjanovic D[1], Bakal N[1], Pojskic N[1],Drobnic K[2], Primorac D[3],Bajrovic K[1], Hadziselmovic R[1]

[1]*Institute for Genetic Engineering and Biotechnology, Kemalbegova 10, 71 000 Sarajevo, B&H*

[2]*Forensic Laboratory and Research Center, Ministry of the Interior, Stefanova 2, 1501 Ljubljana, Slovenia*

[3]*Laboratory for Clinical and Forensic Genetics, University Hospital Split, Spinciceva 1, 21 000 Split, Croatia*

In DNA analysis of forensic biological evidence, we have used 15 STR loci (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, FGA) included in the *PowerPlex 16® System*, as well as twelve Y-chromosomal short tandem repeats loci (DYS19, DYS385a, DYS385b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439) incorporated in the *PowerPlex® Y System*, both manufactured by Promega Corp., Madison, WI. Success of this process depends on various factors, but one of the most important is existence of reference database that will create representative picture about molecular-genetic diversity of local population. Therefore, we have tested unrelated healthy individuals born in the Bosnia and Herzegovina, from three main ethnical groups. For the autosomal STR analysis we choose 100 male and female individuals (Bosniacs - 44%, Serbs - 31%, Croats - 17% and others 8%), but for the Y-STR analysis 100 males, voluntary donors, have been tested. Buccal swabs and blood samples (blood spots) have been used as the DNA source. Qiagen Dnaeasy™ Tissue Kit was used for DNA extraction. Amplification was carried out as described previously. The total volume of each reaction was 10µl. The PCR amplifications have been carried out in PE Gene Amp PCR System Thermal Cycler (ABI, Foster City, CA) according to the manufacturer's recommendations. Electrophoresis of the amplification products was preformed on an ABI PRISM 377 genetic analyzer (ABI, Foster City, CA), using 5% bis-acrilamide gel (Long Ranger® Single® Packs). Raw data have been compiled and analyzed using the accessory software: ABI PRISM® Data Collection Software and Gene Scan®. Numerical allele designations of the profiles were obtained by processing with Powertyper16 and PowertyperY Macro. Deviation from Hardy-Weinberg equilibrium, observed and expected heterozygosity, power of discrimination and power of exclusion were calculated for autosomal STR loci, as well as exact test of population differentiation. Also**,** **w**e have compared B&H data with data obtained from geographically closer (neighboring) European populations. In comparison of B&H and southern Croatian data no significant difference ($P<0.05$) is found at any individual locus. The same statistical parameters are obtained in comparison with pooled Slovenian data. Significant differences ($P<0.05$) are found at FGA locus in comparative analysis of B&H and pooled Austrian data. In addition, 81 different Y-STR haplotypes: (from total number of 100 obtained) were detected: 69 of them were unique, 7 appeared twice, 4 appeared three and only 1 five times. Allele frequency distribution, the most frequent haplotypes, observed haplotype diversity as well as major allele frequency and gene diversity for the PowerPlex® Y loci are calculated. Joint result of this study are going to be used as guidelines in additional investigation of genetic relationship between recent B&H and neighboring human populations, originated in our previous studies on Y chromosome bi-allelic markers. Contact: damir.marjanovic@ingeb.ba

P-171

Application of Mini-STR Loci to severely degraded casework samples

Martín P [1], Albarrán C [1], García P [1], García O [2], Alonso A [1]

[1] *Instituto Nacional de Toxicología y Ciencias Forenses. Servicio de Biología. Luis Cabrera, 9. 28002 Madrid. Spain*

[2] *Area de laboratorio Ertzaintza. Larrauri Mendotxe 18, E-48950 Erandio, Bizkaia, Spain*

Two PCR-multiplexes of mini-STR loci (Big Mini multiplex: TH01, FGA, CSF1PO, D21S11, TPOX and D7S820 and Miniplex 5: Penta D, Penta E and D2S1338) (Butler et al. J. Forensic Sci(2003) 48(5) 1054-1064) have been used to get a nuclear DNA profile from different severely degraded casework biological specimens that generated negative PCR results or partial profiles when commercial STR kits (Identifiler and PowerPlex 16) were employed. These biological specimens included:

- Bone and soft tissue fragments fixed and long-term storage (3 years) in formalin.
- Exhumed remains (teeth and compact bone) from a formalin-embalmed cadaver
- Formalin fixed and paraffin embedded biopsies
- Old bone remains from mass graves of the Spanish Civil war (1936-1939)

In all cases, mini-STR technology allowed to retrieve additional genetic information with very high efficiency especially for those STR loci with allele sizes less than 150 bp. However, due to the high degradation degree, an extremely peak imbalance was observed between the smaller (60-100 bp) and bigger-sized (120-170 bp) STR markers or even between the smaller and the bigger-sized alleles of the same STR in heterozygote samples. Therefore, in some cases singleplex-PCR amplifications were carried out using different amounts of DNA template and different PCR cycles (28-32 cycles) to improve the quality of STR profiles. On the other hand, different artefactual peaks were observed that were removed by filtration of PCR reactions with Centricon centrifugal devices.

In conclusion, our data indicate that the mini-STR technology is an effective strategy to improve DNA profiling from severely degraded casework human DNA samples that are refractory to amplification of DNA fragments bigger than 200-300 bp. At the present time, we are undertaken a similar study with the new Mini-STR loci recently described by Coble et al. (J Forensic Sci. 2005. 50:43-53)

p.martin@mju.es

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Reference Database of Hypervariable STR Loci in Entre Ríos Province of Argentina

Martínez GG [1 2], Schaller LC [1], Vázquez LE [1], Bolea M [2] and Martínez Jarreta B [2]

1 Servicio de Genética Forense, Superior Tribunal de Justicia, Provincia de Entre Ríos, Argentina.
2 Laboratorio de Genética Forense e Identificación Humana, Universidad de Zaragoza, España.

Allele frequencies of twelve Short Tandem Repeats (STR) loci, CSF1PO, TPOX, TH01, F13A01, FESFPS, vWA, D16S539, D7S820, D13S317, D5S818, F13B and LPL, were determined over six mayor regionally groups in Argentinean province of Entre Ríos. No deviation was observed in the total population analyzed and so in subpopulation for all loci. There was also no evidence of correlation of alleles between loci. The combined matching probability and the combined mean of exclusion chance in Entre Ríos population was 2,44 x 10^{-13} and 0,99993 respectively. Frequencies, statistical parameters and a filogenetics inference based on distance matrix for all populations group are provided. We analyzed allele frequencies distribution by Pairwise Fst Genetic Distance to construct a tree based on Neighbor-Joining method, and obtained one that is well coincident with their geographical distribution. This study demonstrates that these loci are a useful and convenient tool for forensic identification and parentage testing in this Argentinean province.

Contact: ggmartinez@arnet.com.ar

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LMD as a forensic tool in a sexual assault casework: LCN DNA typing to identify the responsible

D. Di Martino [(1)], G. Giuffrè [(2)], N. Staiti[(1)], A. Simone[(2)], G. Sippelli[(2)], G. Tuccari[(2)], and L . Saravo[(1)]*

[1] Laboratory of Molecular Biology – Raggruppamento Carabinieri Investigazioni Scientifiche (RaCIS), 98128 Messina – ITALY.
[2] Department of Human Pathology, University of Messina.

We have previously studied the sensitivity of laser microdissection (LMD) techniques and have tested our capability to yield a complete genotype from 30 aploid sperm cells. Partial but significant genotype information have been obtained also from 5-10 aploid sperm cells. This experimental procedure has important applications both in pathological and in forensic fields. A growing number of sexual assault caseworks occur in the South of Italy, often on behalf of teenagers, and it is more and more important to determine the responsible's profile, which is usually difficult as biological residuals are mixed and DNA is present in low copy number.
LMD can offer us the chance to distinguish sperm cells of assaulter's origin from diploid epithelial/ lymphoid cells of victim's vaginal origin even though both these biological residuals are present on a solid substrate, like car carpets, sofas, skirts or underwear.
A real casework of a teenager sexually assaulted in a car in a small town in Sicily has induced us to evaluate the possibility to transfer the biological residuals present on a cut off from the car seat to a laser microdissection prepared microscope slide. Biological traces have been former analyzed with Crimescope CS-16 in order to evaluate the kind of traces we were to process; to avoid the loss of the forensic traces, we have reproduced the same conditions in several experimental procedures: we have settled several samples distinct for amount of biological mixed traces, age of the traces and their exposure to different atmospheric agents. DNA typing was performed both with Identifiler STR loci kit and with another forensic kit based on shorter STR amplicons.
Laser microdissection techniques, coupled to high sensitivity DNA typing methods as short STRs,
allows forensic operators to isolate the different cell residuals whenever in front of mixed traces.

Keywords: DNA STR typing; Forensic casework; Mentype®, LMD, LCN.
*Corresponding author: rismebiologia@carabinieri.it

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Genetic Population Data from Araraquara region (SP State, Brazil) using PowerPlex® 16 Systems Kit

Martins JA, Paneto GG, Pereira GA, Alvarenga VLS, Cicarelli RMB

Laboratório de Investigação de Paternidade, FCF-UNESP, Araraquara, São Paulo, Brazil

Introduction: Because of the fast evolution in molecular biology techniques and in statistical calculations studies, DNA analysis is today the most sensible and specific method for human identification being extremely used in solving most of different forensic cases and paternity tests. As the genetic population data of polymorphic markers are still less known in Brazilian population, the aim of this work was to study allele frequency distributions for the 15 STR loci using PowerPlex®16 Systems (Promega) in a population of Araraquara region (SP, Brazil) and to report some statistical parameters of forensic and paternity interest. **Methods:** Blood samples were obtained from 55 unrelated individuals living in Araraquara region. DNA was extracted using GenomicPrep™ Blood DNA Isolation kit (Amersham). The amplification was performed using PowerPlex® 16 Systems kit (Promega) in a PTC-100 PCR Systems (MJ Research), following the manufacturer's recommendations. The amplified products were run on denaturing 6% polyacrylamide gel in an ABI PRISM® 377 DNA Sequencer (Applied Biosystems) and analysed with the GeneScan ver.2.1 analysis software (Applied Biosystems). The frequency of each allele for each locus tested was calculated using the number of observed genotypes in the sample by POWERSTATS ver. 12 (Promega) software. The exact test for the Hardy-Weinberg equilibrium was carried out using GENEPOP ver. 3.4 software and the forensic and paternity parameters (Power of Discrimination, Power of Exclusion, Matching Probability, Polymorphism Information Content, Typical Paternity Index, Observed heterozygosity, Expected heterozygosity) were performed using POWERSTATS ver. 12 and GENEPOP ver. 3.4 softwares. **Results and Discussion:** After all calculations it was observed that no deviations from Hardy-Weinberg equilibrium were detected for all markers in this study. Moreover, all loci were highly polymorphic and loci as PENTA E (90,5%), TH01 (89,0%), D18S51 (87,2%) and FGA (86,8%) had the highest observed heterozygosities, and the locus TPOX (64%) showed the lowest observed heterozygosity. TPOX also presented lower discrimination power (85,0%) while D18S51 (96,0%), PENTA E (95,92%) and FGA (94,3%) were greater discrimination power systems. The combined power exclusion was 0.99999987, ranging from 0.34 (TPOX) to 0,81 (PENTA E). **Conclusion:** Results indicated that the 15 loci studied would be useful as genetic markers for forensic identification and paternity testing in Araraquara region

SP, Brazil – contact: cicarell@fcfar.unesp.br

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Distribution of four specific STRs Y-chromosome in Iranian ethnic population

Marvi M[1,2] , MirzazadehNafe R[1,2] ,Moshiri F[2] , Bayat B[2] , Mesbah A[2] , Sanati MH[2] , Mirzajani F[2]

1) Islamic Azad University of Tehran, Science & Research campus
2) National Institute for Genetic Engineering & Biotechnology

The Y chromosome is one of the smallest human chromosomes , with an estimated average size about 60 million base pairs (Mb).STRs are short tandem repeated arrays with 2-6 bp in length on the Y chromosome and transmitted from father to son's .

The polymorphism of human Y-specific STRs has an important role in genetic mapping, evolutionary biology, forensic analysis and is very sensitive for genetic drift.

Human genome diversity project in Iran (HGDPI) aims to collect biological samples from different ethnic groups of Iran in order to build up a representative database of human genetic diversity in Iranian population.

In this report we analyzed a set of four Y-STR markers contain: one trinucleotide (DYS 392) and three tetranucleotide (DYS 393, DYS 389I, DYS 389 II) markers in 129 samples from three ethnic groups to determine the allelic frequencies of these markers.

Contact: nice_roze5408@yahoo.com

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Molecular Genetic Diversity in North India: Forensic and Paternity implications.

Mastana SS[1], Papiha SS[2] , Sachdeva MP[3] , Singh PP[4], Singh M[4]

[1]Department of Human Sciences, Human Genetics Lab, Loughborough University, Loughborough, UK.
[2]Department of Human Genetics, University of Newcastle upon Tyne, Newcastle upon Tyne, UK.
[3]Department of Anthropology, Delhi University, Delhi, India;
[4]Department of Human Biology, Punjabi University Patiala Punjab, India;

The primary objectives of this investigation were to assess level and extent of genetic variation at Minisatellite (VNTRs) Microsatellite (STRs), and ALU Insertion loci in five endogamous caste populations of the Punjab state, North India, and to establish a database of allele frequencies which is suitable for population genetic and forensic investigations As north Indians form a large proportion of migrant population in the UK and Europe, a secondary objective of this study was to assess if there is any subpopulation heterogeneity which could affect the forensic calculations. Blood samples (600) were collected at random from the Jat Sikh, Brahmin, Khatri, Lobana and Scheduled Caste populations. Using standard molecular genetic techniques, we analysed MS1, MS31, YNH24, MS43a VNTRs and HUMTHO1, F13A, F13B, FES, LPL, VWA31 and CSF1PO STRs. Alu insertion polymorphisms studied included, ACE, TPA, PV92, D1, APO and FXIIIB. General pattern of genetic variation at these highly polymorphic loci is compatible with many European and Indian populations, though some loci have low level of polymorphisms in some populations. Overall efficiency of these loci for forensic and paternity work in Punjabi populations is at par with many Caucasian populations. Average value of PE is more than 0.999 and cumulative PM was extremely low with some variation for different castes. Brahmins, contrary to expectations show higher level of variation at a number of loci. Overall comparisons provided interesting results suggesting caution should be exercised in usage of pooled or general Indian population databases for forensic and paternity investigations.

Address for Correspondence
Dr Sarabjit Mastana
Human Genetics Lab.,
Department of Human Sciences
Loughborough University
Loughborough LE11 3TU.
UK
Tel: 01509223041
Fax: 01509223941
e-mail: S.S.Mastana@lboro.ac.uk

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ALU Insertion polymorphism variation in India: Genetic Variation and Forensic applications.

Mastana SS[1] and Papiha SS[2],

[1]Department of Human Sciences, Human Genetics Lab, Loughborough University, Loughborough, UK.
[2]Institute of Human Genetics, University of Newcastle upon Tyne, Newcastle upon Tyne, UK.

Alu polymorphisms provide a useful tool to population and forensic geneticists for understanding the population dynamics and its usage in forensics. We report here a study of Alu insertion loci variation from 20 main endogamous caste and tribal populations representing North, Western, Central and East India. In addition two main populations from Sri Lanka were also analysed. Overall spectrum of variation in these populations is interesting at different geographical and cultural levels. High level of insertion frequencies was observed in some highly inbred groups. Average levels of heterozygosities were found to be relatively high in these populations (range 41% to 49.8%). The genetic diversity coefficient G_{ST} among this group of populations was observed to be high (0.049). We also compiled published and unpublished data on other Indian populations to assess the level and extent of genetic diversity at various ethno linguistic, geographic and climatic levels. Overall phylogenetic trees and principal components analysis (PCA) computed from Alu frequencies provide support for socio-cultural and geographical assignment of these populations in Indian population structure. Results are discussed with reference to population origins, forensic applications and human evolution in India using multivariate analyses.

Address for correspondence:
Dr Sarabjit Mastana
Human Genetics Lab.,
Department of Human Sciences
Loughborough University,
Loughborough LE11 3TU. UK
Tel: 44-1509-223041
Fax: 44-1509-223941
E-Mail: S.S.Mastana@LBORO.AC.UK

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Dynamics of microsatellite genetic variation in the India: Forensic implications and applications.

Mastana SS [1] , Sun G [2], Papiha SS [3], Chakraborty R[2],Deka R[2].

1) Dept Human Sciences, Human Genetics Lab., Loughborough University, Loughborough, England;
2) Department of Environmental Health, University of Cincinnati, Cincinnati, OH 45267. USA;
3) Department of Human Genetics, University of Newcastle on Tyne. England.

We have analysed genetic variation at 13 STR loci (CODIS core loci) in a sample of 16 ethnically and geographically diverse endogamous caste and tribal populations of the India. A wide spectrum of allelic distribution at different loci was observed in different geographical and ethnic populations. Overall populations within geographical regions showed greater degree of similarity. Statistically significant differences were observed in a large number of inter-population comparisons. FGA, D18S51 and D21S11 loci were the most polymorphic in a majority of populations. FGA locus had the highest average heterozygosity (86%) and the lowest was observed for TPOX (69%). Average heterozygosity for all loci was 0.79. Coefficient of genetic diversity showed a narrow range for different loci (0.007 to 0.026) with an average of 1.4%, which indicates that these populations are at an early stage of micro-differentiation. Phylogenetic trees and principal component analysis computed from microsatellite allele frequencies provide support for socio-cultural and geographical assignment of these populations. Lowest match probability and highest exclusion probability was observed for the FGA locus in majority of the populations. Combined match probability was low (1.55E-15 to 7.47E-16), and combined exclusion probability was > 99.999%. There was no evidence of association of alleles between loci studied, so these loci seem to comprise a suitable group of markers for population genetic purposes and for paternity and forensic testing.

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Address for Correspondence
Dr Sarabjit Mastana
Human Genetics Lab.,
Department of Human Sciences
Loughborough University
Loughborough LE11 3TU.
UK
Tel: 01509223041
Fax: 01509223941
e-mail: S.S.Mastana@lboro.ac.uk

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Introduction of DNAase in forensic analyses

Melean G, Ricci U, Genuardi M

Azienda Ospedaliera-Universitaria "A.Meyer", U.O. Genetica Medica, Florence, Italy

Laboratory DNA contamination (plastic, tube etc.) is a frequent problem in forensic genetics, especially when extra PCR cycles are used to obtain acceptable amplified products from low copy number DNA samples. Many authors have suggested that the use of DNAase may reduce the possibility of DNA contamination, but this approach is normally limited for anthropological DNA analysis. Here, we have evaluated the use of the DNAase in combination with a commercially available kit for forensic genetics study.

Initially, we introduced 1 ng genomic DNA in the manual amplification mix without primers in order to simulate DNA contamination. The contaminated mix was incubated with DNAase at different temperatures to obtain the optimal action time of the enzyme. Different times and temperatures of DNAase inactivation were also tested to obtain the most efficient inactivation conditions. The primers were added only after DNAase inactivation to avoid their degradation. The PCR reaction was performed without adding any DNA, following the indication of the authors, using 40 cycles of amplification. PCR products were analysed by electrophoresis in a vertical polyacrylamide gel in an automated DNA sequencer (LICOR IR2 4200 DNA sequencer). Inactivation times were considered effective when no amplification was observed. Inactivation times and temperatures were considered effective when the primer's band was evident in the polyacrilamide gel.

Using optimal values obtained in the first experiment, we performed the amplification of low copy number DNA samples, quantified by Real-Time PCR, recovered from gloves and objects touched by known donors. The same experiments were applied using the commercial kit AmpF*l*STR® Profiler Plus™ (Applied Biosystems), with and without DNAase, using 34 cycles of amplification. The use of DNAase in these conditions didn't modify the efficiency of the PCR.

We argue that the use of DNAase may be useful to reduce the possibility of laboratory DNA contamination when low copy number DNA samples are amplified. In these situations, introduction of the DNAase may be possible considering that the optimal conditions, found in the present work, are easily applicable in standard PCR protocols.

contact: u.ricci@meyer.it

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Flemish population genetic analysis using 15 STRs of the Identifiler® kit

Mertens G, Mommers N, Cardoen E, De Bruyn I, Jehaes E, Rand S, Van Brussel K, Jacobs W

Forensic DNA Laboratory, Antwerp University Hospital, B-2650 Edegem, Belgium

Allelic frequencies for the short tandem repeat systems CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, vWA, FGA, TH01, TPOX, D2S1338 and D19S433 were determined in a Flemish population sample of 231 individuals, using the Identifiler kit (Applied Biosystems). No deviations from Hardy-Weinberg equilibrium were observed. Combined, the 15 loci yield a Matching Probability of 1 in 111×10^{12} and a Power of Exclusion of 99.999995 %.

1. Introduction

The aim of this study was to establish a database of the Flemish, i.e. the Dutch speaking population of the northern half of Belgium. We therefore applied the AmpFlSTR Identifiler PCR Amplification (Applied Biosystems) kit, that co-amplifies the 13 Combined DNA Index System (CODIS) STR loci (CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, vWA, FGA, TH01, TPOX) and in addition the two tetrameric markers D2S1338 and D19S433, as well as the amelogenin locus for gender identification. Here we present the allelic frequencies and parameters of forensic efficiency in a sample of 231 unrelated Flemish individuals.

2. Material and Methods

Buccal swabs were collected from 231 unrelated Flemish individuals, representing the mother (115 females) or alleged father (116 males) from paternity cases. DNA was extracted using the Qiamp DNA kit (Qiagen). PCR amplification and subsequent capillary electrophoresis were performed according to the manufacturer's manual, on the PE 9700 thermal cycler (Applied Biosystems) and the ABI 3100 Genetic Analyzer (Applied Biosystems) respectively. Alleles were named according to the recommendations of the DNA Commission of the International Society for Forensic Genetics [1]. Allelic frequencies were estimated by direct gene counting. Conformity of the observed genotype frequencies with Hardy-Weinberg expectations (HWE) was examined by the exact test from Guo and Thompson [2] using the Arlequin software [3]. The parameters relevant for forensic casework (matching probability, power of exclusion, mean paternity index and polymorphism information content) were determined using the Powerstat worksheet (Promega).

3. Results and Discussion

Allelic frequencies in the Flemish population sample typed for the 15 Identifiler STRs are given in Table 1; results of testing for HWE and the statistical parameters of forensic interest are shown in Table 2. Regarding the test results for HWE, a p value > 0.05 was obtained for all STRs except one. For D7S820 the exact test yielded a p value of 0.015. To judge whether to reject the null hypothesis (population equilibrium) based on the magnitude of the smallest of multiple p values, it is necessary to apply the Bonferroni [4] correction to the chosen significance threshold, which is typically 0.05. Considering the Bonferroni procedure and the fact that 15 tests for HWE were simultaneously performed on the same population sample, the significance threshold is adjusted from $\alpha = 0.05$ to $\alpha = 0.05 / 15 = 0.0033$ which is clearly below the p value of 0.015 that was observed for D7S820. Hence this single p value gives no reason to reject the null hypothesis.

Combined, the 15 STRs result in a Matching Probability of 1 in 111×10^{12} and a Power of Exclusion of 99.999995 %, which should be effective in the resolution of most forensic and paternity cases.

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Polymorphisms of 4 Y-chromosome STRs in three ethnic groups of Iran

MirzazadehNafe R[1,2],Marvi M [1,2],Bayat B[2],Moshiri F[2], Mesbah SA[2],Sheydaie M[1], Sanati MH[2] , Mirzajani F[2]

1-Science &Research Campus, Islamic Azad university of Tehran
2- National Institute for Genetic Engineering & Biotechnology

Y-chromosomal microsatellites or short tandem repeats (STRs) are of increasing interest in paternity testing, forensic casework, anthropological and evolutionary studies. This study reports Y-chromosome STR allele frequencies data in three Iranian ethnic groups. Four Y-chromosome STRs (DYS19, DYS388, DYS390and DYS391) have been analyzed in 129 males from these ethnic groups in three provinces (Azerbaijan,Fars and Kurdistan)of Iran.

Contact: r_nafe@yahoo.com

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Amelogenin Y negative males: multiple origins

Mitchell RJ[1], Kreskas M[1,2], Baxter E[1,2], Buffalino L[1,2], van Oorschot RAH[2]

[1]Department of Genetics and Human Variation, La Trobe University, Victoria 3086, Australia
[2]Victoria Police Forensic Services Department, Victoria 3085, Australia

Many forensic laboratories routinely test for gender of a biological sample by typing for an Amelogenin sequence that is incorporated in many of the DNA profiling kits. Amelogenin is a locus found on both X and Y chromosomes but differs in size and sequence which allows the alleles to be distinguished. Occasionally a sample known to be from a normal male is scored as a female ie X allele only, i.e. Amelogenin Y negative. It is of interest to know if this phenomenon results from primer mismatch(es) or deletions and if the mechanism is the same for all such samples.

We tested five Amelogenin Y negative male samples with a series of DNA markers on Yp to determine the approximate size of the deletion(s) as well as with STRs to determine the Y haplotypes associated with these samples to evaluate their level of relatedness.

We show that there are at least two different deletions that cause the phenomenon. A deletion of 304 to 731Kbp was identified in two samples and a deletion of 712 to 1001Kbp was identified in the other three samples. Each sample had a different Y 11-locus haplotype. Whereas the haplotypes of the samples with the smaller deletion were closely related/similar to each other, one of the haplotypes of the samples with the larger deletion was very distant from the other two. These data suggest that Amelogenin Y negative males have arisen through multiple, independent evolutionary events.

Whilst Amelogenin Y null males are rare, routine screening of forensic samples of unknown gender for the presence of the Y chromosome, using other methods, should be considered.

Contact: roland.vanoorschot@police.vic.gov.au

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Validation of five X-chromosomal STR DXS6800, DXS6807, DXS6798, DXS8377 and DXS7423 in an Antioquian population sample

Moreno MA[1,2], Builes JJ[1,2], Jaramillo P[2], Espinal C[1], Aguirre D[1], Bravo MLJ[1]

[1]Genes Ltda. Medellín-Colombia.

[2]Instituto de Biología, Universidad de Antioquia. Medellín–Colombia.

The X linked short tandem repeats (STR) markers have proven to be very useful tools for paternity testing when the disputed child is female.

The aim of this study was to describe the polymorphism of five X-chromosomal STR loci (DXS6800, DXS6807, DXS6798, DXS8377 and DXS7423) in an Antioquian (Colombian) population sample, and evaluate their efficiency in forensic practice and paternity testing.

PCR products were separated in 4% acrylamide-*bis*-acrylamide denaturing gels followed by silver staining. Allele size determination and genotyping were performed according to recommendations of the DNA Commission of the International Society of Forensic Genetic using the allelic ladder manufactured at home and based on DNA controls including K562 (Promega Corporation) and 1331-1, 1331-2, CEPH family members. Gene and haplotype frequencies were calculated using ARLEQUIN version 2000.

Population genetic data were obtained by analyzing 300 unrelated males from Antioquian (Colombian) population. The comparisons of the allele frequencies distributions for Antioquia population are similar to Europe populations. The forensic efficiency values demonstrate that especially DXS8377 and DXS6798 are highly informative markers for kinship analysis and deficiency cases

contact: genforense@epm.net.co

P-184

Human DNA bank in Sao Miguel Island (Azores): a resource for genetic diversity studies

Mota-Vieira L[1,3], Pacheco PR[1,3], Almeida ML[2], Cabral R[1,3], Carvalho J[2], Branco CC[1,3], de Fez L[1,3], Peixoto BR[1,3], Araujo AL[2], Mendonça P[2].

[1] Molecular Genetics and Pathology Unit, Hospital of Divino Espirito Santo, São Miguel, Azores, Portugal
[2] Hematology Department, Hospital of Divino Espirito Santo, São Miguel, Azores, Portugal
[3]Instituto Gulbenkian de Ciência, Oeiras, Portugal.

The peopling of São Miguel Island in the 15th century was made by Portuguese and settlers of foreign origin, (Flemish, Jews, Moorish prisoners and black slaves), generating an admixture signature. Thus to unravel São Miguel's population genetic background and to characterize its population's polymorphisms, we decided to establish a human DNA bank.

Here, we describe the construction of the DNA bank, and analyse the information of 1000 samples obtained from healthy blood donors. The bank follows the international ethical guidelines, which include Informed Consent, confidentiality, anonymity of personal data, and abandonment in case of expressed will. DNA was isolated from blood samples, coded and immediately stored in a locked refrigerator. The identifiable DNA bank has self-reported data concerning sex, age, birth, current place of living, and parental birthplaces. The samples are representative of all the island's municipalities ($r=0.995$, $p<0.01$). The majority (87%) of the participants are male, with mean age of 36.3 y (18-64y). Birthplace analysis reveals that 902 (90%) have both parents born in São Miguel. Moreover, 477 (54%) have their parents born in the same locality, confirming high rate of consanguinity in rural area.

To date, this DNA bank was used to assess the Y-chromosome phylogeny and diversity in Azorean population (Pacheco et al, *Ann Hum Genet* **69**:145-156, 2005). Now, we are analysing autosomal STRs for the better understanding of the gene pool and genetic structure of the archipelago's population

contact: lmotavieira@hdes.pt
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Haplotype studies of germline mutations in short tandem repeats using flanking markers

Mueller M[1], Klintschar M[2], Hohoff C[1], Brinkmann B[1]

[1]*Institute of Legal Medicine, University of Muenster, Muenster, Germany*

[2]*Institute of Legal Medicine, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany*

In our routine case work for parentage testing we have observed more than 150 cases in which a *de novo* mutation had occurred. The paternity was highly validated with a paternity value W of greater than 99.99% including the mutation. Germline mutations occur in short tandem repeats as deletion or expansion of repeat units. The following example from STR system VWA shows the difficulty to assess the mutation's origin: child: 14/17; mother 14/18; father 14/16. According to this, the child's allele 17 could be due a maternal expansion or a paternal deletion. With routinely used STRs it was not possible to determine whether the mutation occurred in maternal or paternal germline.
We here report our results from 37 families with mutations at one of five different loci (i.e., D8S1179, D18S51, D21S11, ACTBP2 and VWA) to specify the origin of the observed mutation.
We chose four to six polymorphic flanking markers in each case and typed these markers by, e.g., amplicon sizing on a ABI PRISM 310 Genetic Analyzer.
The results of our study will be presented and the consequences for the analysis of STR mutations will be discussed.

Contact: marielle.heinrich@uni-muenster.de

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Characterization of a novel stutter product in the Y-STR marker DYS392 and a rare polymorphic variant in the DYS456 homolog identified using the AmpF□STR® Yfiler™ PCR Amplification Kit

Mulero J, Chang C, Calandro L, and Hennessy L

Applied Biosystems, Foster City, CA 94404

Y-chromosome short tandem repeat (STR) markers yield a high degree of confidence that only the male contributor is being analyzed in male-female mixtures. The AmpFℓSTR® Yfiler™ PCR amplification kit is a commercial multiplex system designed for the simultaneous amplification of 17 Y-STR markers (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 (formerly known as Y GATA C4) and Y GATA H4).

A by-product of the amplification of the trinucleotide repeat locus DYS392 is the formation of N-3 and N+3 stutter products. Sequence analysis of the novel N+3 stutter band demonstrates that its sequence is one TAT repeat longer than that of the corresponding main allele. Both N-3 and N+3 stutter percentages increased as (1) the main allele repeat number increased, as (2) the magnesium concentration was increased in the reaction or if (3) the initial amount of DNA template was decreased. Since both stutter products behave in a similar and reproducible fashion, we propose that the same rules that apply to the interpretation of N-3 stutter products could be applied to N+3 stutters.

During an extensive population study conducted using the AmpFℓSTR® Yfiler™ PCR amplification kit, we identified a 71-bp FAM™-labeled fragment in 2.1% of the samples analyzed. We determined that the DYS456 primers amplified the fragment. Direct sequencing of these fragments indicated a T to G single nucleotide polymorphism (SNP) in the primer binding site of the affected individuals. The SNP is located within a X-Y homologous region on chromosome Xq21.31 and was observed with the highest frequency within the African American population (7.7%). This fragment is outside the Y STR allele size range and does not interfere with allele calls. In addition, we demonstrate that the AmpFℓSTR® Yfiler™ kit is capable of yielding full profiles of the minor male contributor (male: female=1:4000) even in the presence of female DNA containing the variant G.

Contact: Julio.J.Mulero@appliedbiosystems.com

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Use of Fluorescence In Situ Hybridisation and Laser Capture Microdissection to isolate male non-sperm cells in cases of sexual assault

Murray C, McAlister C, Elliott K

The Forensic Science Service, Trident Court, Birmingham Business Park, Birmingham, B37 7YN, UK.

In cases of rape where vaginal swabs taken from the victim test positive for the presence of semen, but no sperm can be found (i.e. the semen is azoospermic), other cells originating from the perpetrator may still be present. These could include cells from the urethral tract, white blood cells and epithelial cells from the penis. However, lysis of the cell harvest is likely to yield only the victim's profile due to the large number of vaginal cells present on the swab. Analysis of Y chromosome markers could be carried out, however a profile with which to search the National DNA Database may be preferable in some cases.

Here we describe a method to identify male (non-sperm) cells using Fluorescence In Situ Hybridisation (FISH) and subsequently isolate them using laser capture microdissection. Cell harvests from post-coital vaginal swabs were fixed onto glass microscope slides and fluorescently labelled probes were hybridised to the X and Y chromosomes. The slides were searched and any cells containing both X and Y signals were collected using laser capture microdissection. DNA was extracted from the collected cells in a direct lysis procedure and then amplified using Low Copy Number conditions.

In this study, fifteen samples have been tested, where time since intercourse (TSI) ranged from 1 hour to 24 hours. Positive results (at least 75% of the male profile) were obtained from 10 of these samples, with 16 hours being the highest TSI to give a result. The remaining 5 samples had too few male cells present to produce a profile.

Contact: Caroline.Murray@fss.pnn.police.uk

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Mixture interpretation using SWaP SNPs and non-biallelic SNPs

Musgrave-Brown E[1], Anwar N[2], Elliott K[2], Phillips C[3], Syndercombe Court D[1], Carracedo A[3], Morling N[4], Schneider P[5] and McKeown B[2]

[1]*Centre for Haematology, ICMS, Barts and The London, Queen Mary's School of Medicine and Dentistry, UK*
[2]*Orchid BioSciences Europe Ltd, Abingdon, UK*
[3] *Institute of Legal Medicine, University of Santiago de Compostela, Spain*
[2] *Institute of Legal Medicine, Johannes Gutenberg University Mainz, Germany*
[4] *Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark*

Improved analysis of degraded samples, increased throughput, and a wider choice of typing platforms are some of the significant advantages offered by single nucleotide polymorphism genotyping over current short tandem repeat (STR)-based systems. However, DNA mixtures present a considerable problem to SNP analysis as there is currently no generally accepted method that allows recognition of the presence of a mixed profile or identification of the individual contributors.
We describe a multiplex approach to solving this problem that is based upon the use of two rare subsets of SNPs: SWaP™ SNPs and non-biallelic SNPs. The SWaP SNP technique relies upon the use of modified PCR primer tails to generate 'mirror' copies of the SNP under analysis so that the resulting amplicon contains a real SNP that is flanked by two copies. The mirror copies are generated in known ratios, so that comparison of peak height or peak area ratios following SNaPshot enables an estimate of the relative contributions of each allele to the mixture to be made.
An assay comprising eight such SWaP SNPs combined with three non-biallelic SNPs is described and its value for forensic mixture analysis is discussed.

Address for correspondance: Department of Haematology, Institute of Cell and Molecular Sciences, Barts and The London, Queen Mary's School of Medicine and Dentistry, 4 Newark Street, London, E1 2AT
email: e.musgrave-brown@qmul.ac.uk

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Introducing a highly polymorphic STR at the D12S391 locus valuable for use in forensic application

Massoumeh Nadji, Zahra Lashgary, Hadi Namazi, Massoud Houshmand.

Legal Medicine Organization

Different ethnic groups live in Iran, among which Farsis, Kurds, Lors, Balooches, Bakhtiaris, Azari Turks, Taleshes, Turkamans, Qashqais and Arabs may be pointed out. Smaller ethnic groups also live in Iran. In this study we have investigate allel frequency distribution for D12S391 locus of 354 Persian different ethnic groups. PCR and ALFexpress DNA sequencer was used as methods in this study. Allele's number was found from 2-15 in our study for D12S391 locus in total 708 chromosomes. The results were compared by means of statistic to evaluate confirmation to Hardy-Weinberg predictions. Statistical investigation showed that this polymorphic STR with its simple structure can be used as valuable STR locus for forensic purposes in Iranian Population.

Contact: h_namazi@yahoo.com

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Analysis of the HVI, HVII and HVIII regions of mtDNA in 400 unrelated Japanese

Nagai A, Nakamura I, Bunai Y

Department of Legal Medicine, Graduate School of Medicine, Gifu University, Gifu, Japan

Sequence polymorphism of the hypervariable regions HVI, HVII and HVIII of mitochondrial DNA (mtDNA) was analyzed in a sample of 400 unrelated Japanese individuals living in Gifu Prefecture (central region of Japan) by PCR amplification and direct sequencing.

A total of 306 different haplotypes resulting from 199 polymorphic positions was found in our Japanese population sample. The most common haplotype (16129A, 16223T, 16362C, 73G, 152C, 263G, 309.1C, 315.1C, 489C) was shared by 10 individuals. The genetic diversity and the genetic identity were calculated to be 0.9975 and 0.0050, respectively. The length heteroplasmy in the homopolymeric C-stretch regions located at nucleotide positions 16184-16193 in HVI, at positions 303-315 in HVII and at positions 568-573 in HVIII was observed in 26.1%, 8.6% and 4.1% of individuals, respectively.

Contact: anagai@cc.gifu-u.ac.jp

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Interpreting DNA evidence isolated from a self made firearm in a homicidal case

Nagy G[1], Angyal M[2], Czömpöly T[4], Nyárády Z[3], Bajnóczky I[2]

[1] *Institute of Forensic Medicine, University of Pécs, Hungary*
[2] *Forensic Examiner Unit, Baranya County Police Department, Pécs, Hungary*
[3] *Department of Oral and Maxillofacial Surgery, University of Pécs, Hungary*
[4] *Institute of Immunology and Biotechnology, University of Pécs, Hungary*

A woman was brutally murdered. She was shot in head but no bullet was found in the head. Later the Baranya County Police Department arrested a man and charged to murder the woman. A self made firearm was found and secured for further investigation. The firearm works with blank cartridge which lunches a hollow steel spike out of the firearm's tube. After the shoot the steel spike was jerk back to the tube by a spring device. The weapon used to slaughtering pigs. After the murder at least six pig were slaughtered with the weapon.
From the cavity of the hollow steel spike biological material were secured from different depth. From the samples genomic DNA was extracted according to standard techniques (Chelex-100 method) and amplified utilizing different amplification approaches (AmpFlSTR SGM Plus produced by Applied Biosystem). PCR products were separated by capillary gel electrophoresis on an ABI PRISM 310 Genetic Analyzer and typed by comparison against standard allelic ladders.
With the firearm ballistic tests were also made by the Forensic Examiner Unit of Baranya County Police Department which proves that the firearm could be the perpetrator's weapon. From the biological samples the victim's full DNA profile was detected.
The suspect was sentence to prison for 25 yeas based on the DNA and ballistic evidences.

Address for correspondence
Prof. Dr. med. István Bajnóczky, Institut of Forensic Medicine, Medical Faculty, University of Pecs, Hungary, Szigeti u. 12, H-7624 Pécs, Hungary, Fax: 00 36 72 536242, eMail: gergely.nagy@aok.pte.hu

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Allele frequencies for 15 STR loci in two populations from Hungary

Nagy G[1], Nagy Zs[1], Nyárády Z[2], Bajnóczky I[2]

[1] *Institute of Forensic Medicine, University of Pécs, Hungary*
[2] *Department of Oral and Maxillofacial Surgery, University of Pécs, Hungary*

To enlarge our understanding of genetic variation in Hungarian population, a population genetic study was carried out on unrelated 115 Hungarian Caucasian and 116 Hungarian Roma (South-West Hungary area). We here present the frequency distributions of 15 highly polymorphic autosomal STR systems (CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S539, D16S539, D18S51, D19S433, D21S11, FGA, THO1, TPOX, VWA) from the two population.
Genomic DNA was extracted according to standard techniques (Chelex-100 method) and amplified utilizing different amplification approaches (Powerplex16 produced by Promega Corp. and AmpFlSTR SGM Plus produced by Applied Biosystem). PCR products were separated by capillary gel electrophoresis on an ABI PRISM 310 Genetic Analyzer and typed by comparison against standard allelic ladders.
The overall pattern of allél frequencies was similar to many Caucasian and Indian (compare to Roma) populations and heterozygosity varied from 57% (TPOX, Caucasian) to 92% (FGA, Caucasian). For all fifteen, no deviations from the Hardy-Weinberg equilibrium hypothesis were detected. The mean exclusion probability ranged from 25% (TPOX, Caucasian) to 83% (FGA, Caucasian). All tetranucleotide STR systems are highly informative markers in the three populations investigated, e.g., the power of discrimination ranges from 0,744 (TPOX, Caucasian) to 0,969 (D18S51, Caucasian).
The results suggest the usefulness of these loci for anthropogenetic, paternity and forensic investigations in Hungarian populations.

Address for correspondence
Prof. Dr. med. István Bajnóczky, Institut of Forensic Medicine, Medical Faculty, University of Pecs, Hungary, Szigeti u. 12, H-7624 Pécs, Hungary, Fax: 00 36 72 536242, eMail: gergely.nagy@aok.pte.hu

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Y chromosome haplotypes in Roma and Caucasian populations from Hungary

Nagy G[1], Nagy Zs[1], Nyárády Z[2], Bajnóczky I[2]

[1] *Institute of Forensic Medicine, University of Pécs, Hungary*
[2] *Department of Oral and Maxillofacial Surgery, University of Pécs, Hungary*

To enlarge our understanding of genetic variation in Hungarian population, a population genetic study was carried out on unrelated 50 Hungarian Caucasian and 50 Hungarian Roma male (South-West Hungary area). Eleven Y chromosome STR polymorphisms (DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439) were analyzed in the samples.
Genomic DNA was extracted according to standard techniques (Chelex-100 method) and amplified utilizing different amplification approaches (PowerplexY produced by Promega Corp). PCR products were separated by capillary gel electrophoresis on an ABI PRISM 310 Genetic Analyzer and typed by comparison against standard allelic ladders.
A general STR allelic frequency pattern in the Roma and Caucasian population from Hungary corresponds to other European populations. Thirty six haplotypes were observed in single copy. Twenty one Hungarian haplotype were not previously observed in the Y STR Haplotype reference Database among the set of European populations.

Address for correspondence
Prof. Dr. med. István Bajnóczky, Institut of Forensic Medicine, Medical Faculty, University of Pecs, Hungary, Szigeti u. 12, H-7624 Pécs, Hungary, Fax: 00 36 72 536242, eMail: gergely.nagy@aok.pte.hu

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Characterization of mtDNA SNP typing using quantitative real-time PCR for forensic purposes with special emphasis on heteroplasmy detection and mixture ratio assessment

Niederstätter H[1], Coble MD[2,3], Parsons TJ[2], Parson W[1]

[1] *Institute of Legal Medicine, University of Innsbruck, Müllerstrasse 44, 6020 Innsbruck*
[2] *Armed Forces DNA Identification Laboratory, Armed Forces Institute of Pathology, Rockville, MD, USA*
[3] *Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD, USA*

The analysis of mitochondrial DNA (mtDNA) has proven to be highly useful in forensic casework, especially when samples comprising degraded DNA are analyzed which are not amenable to the typing of the highly informative nuclear short tandem repeat markers (STRs). However, unlike autosomal STRs, mtDNA testing does not provide definitive identification of individuals because all members of a matriline are expected to match each other due to maternal inheritance of the mitochondrial genome and the lack of recombination. Therefore, the principal limitation associated with forensic mtDNA typing is the low power of discrimination that is obtained when common mitochondrial types are present. Current mtDNA testing typically targets the variable base positions in the non-coding control region of the mitochondrial genome by sequencing both strands, and most laboratories restrict their investigations to one or two hypervariable regions (HV1 and HV2), comprising approximately 600 base pairs (~3.6 % of the entire mitochondrial genome). In the last couple of years it has become increasingly recognized that assays targeting single nucleotide polymorphisms (SNPs) are well suited for efforts to gain additional information in mtDNA testing. We investigated the forensic applicability of real-time detection PCR using TaqMan probes targeted to the highly discriminatory mitochondrial control region SNP 16519 T/C for several reasons: 1) it's large linear dynamic range in terms of target-molecule input number allows – along with the short amplicons that are obtained - the analysis of a broad spectrum of samples differing in DNA quantity and quality with a single protocol, 2) the homogeneous format of the assay avoids potential cross-contamination of samples with PCR products and makes it easy to automatize, 3) the quantitative information that can be obtained aids the formulation of objectively-based criteria for distinguishing between authentic signal and contamination and 4) the multicolor capability of real-time PCR instruments enables the simultaneous interrogation of both base-states of the SNP under investigation. The last point is particularly important because of the potential of mtDNA to manifest heteroplasmic mixtures in continuously varying proportions. The results of a study on 135 paternity trios with known control region sequences showed that 16519 can be reliably typed with the TaqMan approach without a need to run samples in replicates. For both alleles the linear dynamic range was at least 5 orders of magnitude with a lower end sensitivity of approximately 10 double stranded target molecules. The apparent single-cycle PCR efficiencies during the exponential phase of the amplification were close to 100% for complex genomic DNA as well as for non-linearized plasmids used as templates. Defined mixtures of plasmids containing 16519T and C could be detected and quantitated reliably down to the 5% level for either variant with a lower end sensitivity of a total of 100 - 200 double stranded target molecules. Finally, the estimated mixture ratios for three heteroplasmic and two homoplasmic paternity DNA samples were consistent with the results obtained by typing approx. 300 bacterial colonies/sample containing PCR amplified mitochondrial control regions.
Contact: harald.niederstaetter@uibk.ac.at

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Comparison of six DNA quantification methods

Nielsen K[1], Mogensen HS[1], Eriksen B[1], Hedman J[2], Parson W[3], Morling N[1]

[1]*Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark*

[2]*Swedish National Laboratory of Forensic Science, Linköping, Sweden*

[3]*Institute of Legal Medicine, Medical University of Innsbruck, Austria*

Six commercial preparations of human genomic DNA were quantified using six quantification methods, including UV spectrometry, SYBR-green dye staining, slotblot hybridization with the probe D17Z1, and three TaqMan real time PCR assays: Quantifiler™ Human DNA Quantification kit, Quantifiler™ Y DNA Quantification kit, and RB1 rt-PCR. In general, all methods measured higher DNA concentrations than expected based on the information by the suppliers of the human DNA preparations. The Quantifiler™ Human DNA Quantification kit gave the highest measures of the DNA concentrations of five of the six human DNA preparations compared to the other five quantification methods. With the Quantifiler™ Human DNA Quantification kit, the ratio of measured DNA/expected DNA ranged from 1.9 to 2.8. When the Quantifiler human DNA standard was replaced by a different commercial human DNA preparation (G147A, Promega) to generate the DNA standard curve in the Quantifiler™ Human DNA Quantification kit, the DNA quantification results of the human DNA preparations were comparable to the results of other DNA quantification methods. The ratio of measured DNA/expected DNA ranged from 1.1 to 1.6. Human DNA preparations were quantified by the Swedish National Laboratory of Forensic Science in Linköping, using the Quantifiler™ Human DNA Quantification kit. The results confirmed the Quantifiler™ results obtained in Copenhagen. Samples of the same human DNA preparations were quantified by the Institute of Legal Medicine in Innsbruck, using RB1 rt-PCR, and the ratio of measured DNA/expected DNA ranged from 1.1 and 1.5. The quantification results using RB1 rt-PCR were comparable to those obtained with the other quantification methods.

The results indicate a calibration problem with the Quantifiler™ human DNA standard for its use with the Quantifiler™ Human DNA Quantification kit. The possible reasons of the problem are discussed, and a solution is suggested. The results emphasise the need for standard reference DNA material and standard methods for DNA quantification.

Contact: helle.smidt@forensic.ku.dk

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Effect of soil environment on detectability of SGM profiles in selected tissue samples

Niemcunowicz-Janica A [1], Pepinski W [1], Janica JR [2], Skawronska M [1], Janica J [1], Koc-Zorawska E [1], Soltyszewski I [3]

[1] *Department of Forensic Medicine, Medical University of Bialystok, Poland*

[2] *Department of Radiology, Medical University of Bialystok, Poland*

[3] *Central Forensic Laboratory of Police, Warsaw, Poland*

Processes of autolysis and decomposition have always been a concern to forensic specialists. In cases of decomposed bodies, estimation of time of death – very crucial for evidential reasons – is often impossible due to effect of various environmental conditions. The authors attempted to assess capability to type AmpFlSTR SGM Plus (Applied Biosystems) loci in tissue material stored in sand, garden soil and peat in view of estimation of time of death. Tissue material was collected during autopsies of five persons aged 20-30 years with time of death determined within the limit of 14 hours. Heart muscle, liver and lung specimens of dimensions 2x2x2cms were placed in 40ml containers filled with sand, garden soil or peat and stored at 21°C. DNA was extracted by organic method from tissue samples collected in 7-day intervals. Recovered DNA was quantitiated fluorometrically and by hybridization with human DNA-specific probe (QuantiBlot) with chemiluminescent detection. DNA quality was assessed by 2% ethidium bromide agarose gel electrophoresis. 2-10ng target DNA was amplified according to the manufacturer's instruction. ABI 310 and reference sequenced ladders were used following the manufacturer's instructions. As a threshold value a signal of ≥150 was assumed. Storage of liver specimens in garden soil for more than 14 days resulted in allelic drop-out and after 21 days no profiles were typeable. Heart muscle specimens were typeable in all SGM systems after 35-day storage in sand, while allelic drop-out and subsequent lack of profiles were noted after 14 and 35 days, respectively. Lung specimens stored in garden soil exhibited allelic drop-out and subsequent lack of profiles after 7 and 21 days, respectively. All SGM loci were typeable in the latter material stored in sand up to day 35 with gradual decline of longer amplicons (D2S1338, D16S539 and D18S51).

Contact: pepinski@amb.edu.pl

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Effect of water environment on detectability of SGM profiles in selected tissue samples

Niemcunowicz-Janica A [1], Pepinski W [1], Janica JR [2], Skawronska M [1], Janica J [1], Koc-Zorawska E [1], Soltyszewski I [3]

[1] *Department of Forensic Medicine, Medical University of Bialystok, Poland*

[2] *Department of Radiology, Medical University of Bialystok, Poland*

[3] *Central Forensic Laboratory of Police, Warsaw, Poland*

Processes of autolysis and decomposition have always been a concern to forensic specialists. In cases of putrefied bodies, estimation of time of death – very crucial for evidential reasons – is often impossible due to effect of various environmental conditions. The authors attempted to assess capability to type AmpFlSTR SGM Plus (Applied Biosystems) loci in tissue material stored in water environment in view of estimation of time of death. Tissue material was collected during autopsies of five persons aged 20-30 years with time of death determined within the limit of 14 hours. Heart muscle, liver and lung specimens of dimensions 2x2x2cms were placed in 40ml containers filled with pond water and sea water (0.8% salt) and stored at 21°C. DNA was extracted by organic method from tissue samples collected in 7-day intervals. Recovered DNA was quantitiated fluorometrically and by hybridization with human DNA-specific probe (QuantiBlot) with chemiluminescent detection. DNA quality was assessed by 2% ethidium bromide agarose gel electrophoresis. 2-10ng target DNA was amplified according to the manufacturer's instruction. ABI 310 and reference sequenced ladders were used following the manufacturer's instructions. As a threshold value a signal of ≥150 was assumed. Liver specimens were typeable in all SGM loci within 100 days of storage in pond water with gradual allelic drop-out at D18S51 in sea water. Heart muscle specimens stored in pond water exhibited allelic drop-out at THO1, FGA and D18S51, while all loci were typeable in sea water stored samples. For lung specimens allelic drop-out was noted throughout the profile. The authors conclude that the course of complex postmortem processes is variable. Dynamics of tissue and internal organs decomposition in an intact corpse is different than that in tissue specimens placed in a water environment which delays decomposition processes promoted by bodily and microbial enzymes.

Contact: pepinski@amb.edu.pl

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DNA quantity variation in shed hairs, plucked hairs and contact traces

Nilsson M, Andréasson H, Allen M

Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Sweden

Different categories of forensic evidence materials are known to vary considerably in their DNA content, affecting the probability of a successful identification analysis. In this study, the mitochondrial and nuclear DNA content were quantified in 46 head hairs, 61 body hairs and 76 contact trace samples using a previously reported real-time PCR method. In this TaqMan assay, two specific probes, labelled with different dyes, enables quantification of the nuclear Retinoblastoma 1 gene and the mitochondrial tRNA Lys gene.

DNA quantification in the roots and distal sections of plucked head hairs revealed large variations in DNA content between the root and the shaft. Furthermore, large intra- and inter-individual variations were found among hairs. The overall difference in mean mtDNA content in the first cm between shed and plucked hairs was 74-fold. In the first cm of shed hairs no nDNA copies were detected whereas plucked hairs contained an average of 25,800 nDNA copies. Furthermore, variation in mtDNA content was evaluated at different lengths of the hairs. The decrease in average mtDNA content per cm was 2-fold between the first cm and the following 1-4 cm part in shed hairs. In contrast, an 80-fold decrease in the average mtDNA content per cm was observed between the first cm and the following 1-4 cm of plucked hairs. This large difference is only seen for the root part as the first cm of shed hairs contain approximately equal average mtDNA amounts per cm as the second part (1-4 cm) of plucked head hairs (45,700 and 41,700 mtDNA copies per cm, respectively). Thus, most of the DNA content difference between shed and plucked hairs is observed in the first cm and is likely to reflect the different growth phases of hair. In other types of plucked body hairs evaluated, beard contained on average 2-fold and 6-fold more nDNA compared to eyebrows and arm hair respectively.

In addition, DNA content was estimated in epithelial cells collected from fingerprints and accessories. The quantification of epithelial cells on various items or from fingerprints also displayed large variations, with some material categories containing large amounts of nuclear DNA while no detectable nuclear DNA and only limited amounts of mitochondrial DNA were seen in others. Fingerprints visualised by black powder contained slightly more mtDNA and nDNA compared to magnetic powder treated prints. The quantification results illustrate that some prints contain up to 700 nDNA copies and the majority of the prints contain sufficient DNA amounts for an mtDNA analysis. The quantification analysis of DNA collected from accessories showed that earrings contained most DNA, 144,400 nDNA copies on average while rings, bracelets, necklaces and charms contained the lowest amounts of DNA with 80-300 nDNA copies on average. Samples collected from glasses and watches contained on average 4,200 and 1,800 nDNA copies respectively. In addition to the large variations seen between different sample categories the DNA content were shown to vary largely between samples taken on the same category.

In conclusion, the use of real-time DNA quantification in this study has revealed several important insights regarding DNA content in various forensic materials. Information regarding inter- and intra- individual variation, variation in content within plucked and shed hairs at different lengths, the average DNA content in different types of hairs as well as in other materials is highly relevant for forensic applications.

Contact: martina.nilsson@genpat.uu.se

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Sensitive forensic DNA analysis using the Pyrosequencing technology

Nilsson M, Styrman H, Andréasson H, Divne A-M, Allen M

Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Sweden

DNA from casework samples are in some cases degraded and not in sufficient amounts for a routine STR analysis. Analysis of mtDNA or reduced size PCR fragments of nuclear targets is often necessary for a successful analysis of these samples. We have developed several sensitive, rapid, flexible and easy-to-use mtDNA, SNP and STR typing systems based on the Pyrosequencing technology. This is a non-electrophoretic, single-tube sequencing-by-synthesis method in which a cascade of enzymatic reactions yields detectable light. If a nuclear DNA analysis is permitted on degraded or limited samples, STR analysis is preferred due to the large number of alleles at each locus. As a complement to the routinely used STR assays, a pyrosequencing-based analysis of short PCR fragments covering only a few bases outside the actual repeat unit, have been developed. Ten widely used autosomal STR loci with short repeat units with short maximum allele lengths have been analysed using pyrosequencing. Since no size separation based on overlapping fluorescence spectra is necessary using this method, all amplicons have been kept short, between 66-175 bp. Discrimination of different alleles is possible by the use of a termination-recognition base (TRB) and a sequence-directed dispensation order. The TRB represents the first occurring downstream base in the template that is not part of the repeat unit. For heterozygous genotypes, the signal is reduced by half when the shortest allele terminates while no signal reduction is observed for homozygous genotypes. Pyrosequencing was found suitable for analysis of short simple repeat markers and a few markers could be interpreted using DNA input concentrations of 25 pg.For analysis of mixtures, eight commonly used Y-STR markers have been analysed in PCR fragments between 72 bp and 233 bp. The Y-STR markers were easily interpretable and all markers could be analysed using 100 pg of input DNA, while half of the markers could be analysed at 25 pg input DNA. When the Y-STR analysis fails in degraded or limited samples, SNP analysis is likely to be more successful as the amplicons can be designed very short. A system for analysis of 17 SNP markers on the Y-chromosome has been developed. The PCR products are between 50 and 96 base pairs in size and the most informative markers have been optimised to allow analysis in triplex PCR and pyrosequencing reactions. The Y-SNP analysis could be performed on 10 pg of input DNA for some markers. For severely degraded DNA samples an mtDNA analysis system has been developed. Two PCR fragments covering the HVI and HVII regions in the D-loop are analysed rapidly in eight pyrosequencing reactions. Furthermore, 17 fragments in the coding region of the mitochondrial genome can be used for additional discrimination. Each fragment covers multiple polymorphic SNPs with an average read length of 74 nucleotides in the pyrosequencing reactions. In order to save valuable material multiplex PCR and pyrosequencing reactions are under development. Although it will be possible to analyse STR markers in duplex pyrosequencing reaction by further developments, the multiplex capability is the major limitation of pyrosequencing. Consequently, the pyrosequencing method is more suited for analysis of a limited set of markers in challenging samples allowing short amplicons on degraded DNA samples rather than analysis of a large set of markers. Since the actual sequence is determined rather than the repeat length in this assay there is a possibility to achieve additional information such as the nature of a mutational event. Furthermore, a rapid compilation of population databases and evaluation of novel less complex STR markers can be performed. Pyrosequencing is a robust and flexible system that can handle SNP analysis, STR analysis or sequencing of short stretches of DNA with two hours post-PCR handling.

Contact: martina.nilsson@genpat.uu.se

P-200

Projeto Paternidade Social

[1] NOGUEIRA, G. C.; [1] MONTEIRO, E. H. G; [1] SILVA, L.S ; [1].NASCIMENTO, D.S.; [1] TOMMASI, B. O;

[1] Instituto Tommasi;

The main objective of the present work is the familiar reorganization of children and adolescents of low income. In each year, about 2,5 million of births are registered in Brazil. Of these, an average of 750 thousand is made without identification of the father. As consequence, these children do not have officially father declared, what frequently represents a serious emotional, social and economic problem. This work counts with a multidiscipline team formed by biochemists, biologists, biomedicine doctors, judges, lawyers, promoters and psychologists aiming to help 132 families in 12 months. In this research, paternity investigation are done analyzing 16 markers of type STR and for the psychological assistance are being used focal psycotherapy and the clinical methods of Piaget. Until now 110 cases for DNA had been carried through, and the molecular examinations had proven paternity with 99,999 % of probability in 82 cases (74,55%) and had excluded paternity in 27 cases (25,45%).

Tommasi Institute and Canadá Embassy support this work

[1]gabriela@tommasi.com.br

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Non-human mtDNA helps to exculpate a suspect in a homicide case

Nussbaumer C, Korschineck I

Ingenetix Ltd., Vienna, Austria

In January 2005 the dead body of a young female was found close to a highway in the South of Austria. Since the corpse had been set on fire after the woman was killed, the victim could not be identified so far. Part of the wool pullover the woman was wearing was left and showed a few short animal hairs. The investigations led the police to a young man who was already on remand due to a property offense.
Morphological comparison of the hairs found on the pullover of the victim were suspected to derive either from an animal out of the group of minks and martens (*Mustelidae*) or from a dog (*Canidae*). Since light microscopy did not show any differences, the court gave the order to analyze the DNA of the few animal hairs collected from the victim's pullover and to compare these hairs to the hairs found in the car the suspect had been driving and the material from the dog which belongs to the owner of the car. The question was firstly which species the hairs came from and secondly if they derive from the same individual.

DNA from the hairs was purified using QiaAmp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol except for minor changes. The gene of cytochrome b was amplified by PCR, the products were purified and amplified by cycle sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA). Sequencing was done on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Sequence data were analyzed using Sequencing Analysis Software and SeqScape Software (Applied Biosystems).
The results revealed that all of the hairs which could be analyzed derived from the species *Canis familiaris* (dog). Only one single hair, found in the trunk of the car, came from *Felis catus* (cat). Since only very few of the hairs showed hair roots, STR analysis for identification of the individual could not be applied. Thus, the second question had to be answered by further analysis of the mtDNA. Although the hypervariable region (HV) in the non-coding control region of the mtDNA in dogs is not as variable as in human it is used to compare individuals within this species. A 350 bp product within the region HV1 was amplified from all samples. All the hairs from the pullover of the victim showed the same sequence. The hairs from the car showed two different mtDNA haplotypes. These sequences differed (at least 2 mutations) from the sequence of the hairs found on the victim's pullover. The sequence of the dog of the owner of the car matched the sequence of one of the two haplotypes found in the car. The other haplotype derived from an unknown dog.

Thus, we were able to show, that the hair from the victim's pullover doesn't belong to the dog associated with the suspect. This evidence amongst other investigations of the police helped to exculpate the young man. Neither the identity of the victim nor the identity of the murderer could be resolved up to the present.
Contact: christa.nussbaumer@ingenetix.com

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Simultaneous detection of DNA length and sequence variations by liquid chromatography electrospray ionization time-of-flight mass spectrometry

Oberacher H[1], Niederstätter H[1], Casetta B[2], Parson W[1]

[1]*Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria*
[2]*Applied Biosystems, Monza, Italy*

The combination of ion-pair reversed phase high-performance liquid chromatography and electrospray ionization quadrupole time-of-flight mass spectrometry is presented as an efficient method for the fast and accurate detection of sequence variations. The chromatographic separation enables the highly efficient purification of nucleic acids prior to their mass spectrometric analysis. Therefore, sample preparation is limited to PCR only. As separations are performed at elevated temperatures (65-80°C), liquid chromatography represents an elegant way for denaturing double-stranded nucleic acids into the corresponding single strands. The denaturation is advantageous because of the division in half of the masses of the detected species and the possibility to identify base substitutions by measuring mass differences ranging in size between 9.01 and 40.02 amu, which would have been missed by measuring the nearly composition independent molecular masses of double-stranded nucleic acids. Furthermore, as the allelic state derived from one single strand is confirmed by the result obtained from the complementary single strand, the reliability of the mass spectrometric genotyping assay is increased. Taking advantage of the high mass spectrometric performance of the time-of-flight mass analyzer all kind of single base exchanges were detectable in PCR amplicons with lengths up to approximately 250 base pairs. Consequently, the described hyphenated technique represents one of the most powerful mass spectrometric genotyping assays available today.
The mass spectrometric genotyping assay was applied to the characterization of two simultaneously amplified PCR products covering the homopolymeric stretches of cytosines within the hypervariable regions 1 and 2 of the non-coding mitochondrial control region. Based on the high performance of the assay length and sequence variants were simultaneously identified. Additionally, relative quantification of the individual allele frequencies was obtainable by measuring and comparing individual peak intensities. Mass spectrometric results were checked by sequencing.

Contact: herbert.oberacher@uibk.ac.at

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Population Study of Four X Chromosomal STR Loci in the UK Population

Oguzturun C, Thacker CR, Ballard D, Syndercombe Court D

Centre for Haematology, ICMS, Barts and The London, Queen Mary's School of Medicine and Dentistry, UK

X-chromosome STR typing can complement existing DNA profiling being carried out by laboratories and can also offer very useful information in cases of complex kinship analysis. In this study a database relevant to the UK population was compiled. The four X-chromosome short tandem repeats DX8378, DX7132, DX7423 and HPRTB were used to generate allele frequencies in a population sample of 600 unrelated males and females from the UK population. The population was composed of three subsets of data: 200 individuals who described themselves as Irish Caucasian (originating from and resident in Southern Ireland); 200 individuals who described themselves as British Caucasian (originating from and resident in mainland Britain) and 200 individuals who described themselves as South Asian (originating mainly from the countries of Bangladesh and Pakistan, resident in mainland Britain). Amplification was performed using the Mentype® Argus X-UL PCR amplification kit (Biotype AG) which enabled the four X chromosome markers and the sex marker Amelogenin to be amplified simultaneously. Slight modifications were made to the amplification conditions recommended by the manufacturer. Products were detected using ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). Allele frequencies were calculated and the three population subsets compared. The Mentype® Argus X-UL PCR amplification kit was found to be a robust system and a useful addition to autosomal markers routinely used in forensic and paternity testing applications.

Address for Correspondence:
Catherine R Thacker
Centre for Haematology
Institute of Cell and Molecular Science
Barts and The London
Queen Mary's School of Medicine and Dentistry
4 Newark Street
London E1 2AT
United Kingdom
E-mail: c.r.thacker@qmul.ac.uk

P-204

Preliminary studies of individual genetic identification of domestic dogs (*Canis familiaris*)

Oliveira AC[1], Balsa F[1], Brito P[1], Lopes V[1], Serra A[1], Carvalho M[1], Anjos MJ[1], Andrade L[1], Corte-Real F[2], Vide MC[1]

[1]Forensic Genetic Service. National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal
[2]National Institute of Legal Medicine. Largo da Sé Nova, 3000 Coimbra. Portugal

Human traces are not the only source of biological samples that can be collected from a crime scene. Biological traces from cats, dogs and other domestic animals can be found related to suspects or victims being themselves the protagonists of the forensic case.

According to Portuguese Legislation (D.L. 313/2003 of 17 of December art 6th) is obligator the individual identification of dogs dangerous or potentially dangerous.

It seems important to follow this subject with the implementation of genetic identification.

So, this work has the aim to study STR's of DNA for individual genetic identification of dogs.

DNA was extracted by Chelex™100 (Walsh, et al) from saliva of different dogs races. The DNA was amplified 10 STR *loci* with StockMarks® Kit - Canine Genotyping - PEZ 1, FHC 2054, FHC 2010, PEZ 5, PEZ 20, PEZ 12, PEZ 3, PEZ 6, PEZ 8 and FHC 2079.

The detection was carried out on ABI Prism™ 310 Genetic Analyser with internal standard (Rox 350), the DNA sample control and an allelic cocktail.

Contact: geneforense@dcinml.mj.pt

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Outcome in acute lymphobastic leukaemia: influence of thiopurine methyltransferase genetic polymorphism

Oliveira E[1,2]; Alves S[3]; Quental S[1], Ferreira F[4]; Norton L[5]; Costa V[5]; Amorim A[1,2]; Prata MJ[1,2];
[1]*IPATIMUP, Porto, Portugal*
[2]*Faculdade de Ciências da Universidade do Porto, Porto, Portugal*
[3]*Unidade de Enzimologia, Instituto de Genética Médica Jacinto Magalhães, Porto, Portugal*
[4]*Serviço de Hematologia Clínica, Hospital Geral S. João, Porto, Portugal*
[5]*Serviço de Pediatria, Instituto Português de Oncologia, Porto, Portugal*

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer accounting for 25-30% of all childhood malignancies. Presently, due to the introduction of multiagent chemotherapy, the cure rate nearly reaches 80%. However, a wide inter-patient variability in tolerability to drug regimens does exist and these differences highly influence the success of ALL therapy. One current treatment strategy for improving cure rate is to modify the intensity of drug dosages, but usually the genetic host characteristics are not used for assigning ALL patients to risks groups or for individually tailoring the therapy.

One of the best known functional polymorphisms in genes involved in ALL drugs action or metabolism is that in thiopurine methyltransferase (TPMT), for which the impact of TPMT genotypes in 6-mercaptopurine (6MP) toxicity is well documented.

In order to assess the influence of this polymorphism in ALL outcome in patient from North Portugal, we have used PCR/HCSGE based methods to characterise molecularly a sample of 110 children with ALL who were diagnosed and followed-up in Hospital de São João or IPO, both from Porto. Four distinct alleles associated with TPMT deficiency, TPMT*3A, *3C, *2 and *8, were found in heterozygous individuals representing 11.8% of the sample.

Several parameters related with ALL outcome were compared in subsamples of children homozygous or heterozygous for TPMT. In the heterozygous group, 6MP dosages were lower comparatively to the other group, and higher the number of whole interruptions during treatment or interruptions due to haematological toxicity as well as the number of relapses or deaths.

These findings qualitatively replicate previous reports relating TPMT with a poor outcome in ALL, although probably due to the low sample size of our study, differences between the two groups do not reach statistical significance.

Since TPMT represents a determinant of 6MP response and ALL outcome, this study reinforce the relevance of introduce the prospective analysis of TPMT prior to any ALL treatment, in order to individually optimise 6MP therapy and avoid adverse reactions to this drug.

Contact: mquental@ipatimup.pt

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Power of Exclusion of 18 autosomic STR loci in a Brazilian Center-West region population sample

Oliveira SF[1], Trindade-Filho A[2], Mendes CRBO[2], Paula KAA[2], Maia FAS[2], Pak HI[2], Dalton GC[2]

[1]*Departamento de Genética e Morfologia, Universidade de Brasília, Distrito Federal, Brazil*
[2] *Forensic DNA Research Institute, Policia Civil do Distrito Federal, Distrito Federal, Brazil.*

The purpose of this work was analyze the Power of Exclusion (PE) in paternity investigation cases from Distrito Federal of Brazil (Center-West region of Brazil) using a local population sample in comparison with Promega Corporation published databank. We used 300 cases where the alleged father was excluded and a databank of 917 individuals from Distrito Federal, both analyzed for eighteen STRs loci (D16S539, D7S820, D13S317, D5S818, CSF1PO, TPOX, TH01, vWA, Penta-E, D18S51, D21S11, D3S1358, FGA, D8S1179, F13A01, FESFPS, F13B e LPL). The expected and observed values in the paternity exclusion cases were compared for each locus and then for each multiplex system employed (PowerPlex 1.1, PowerPlex 2.1 and FFFL). The number of times that each locus was used in the exclusion paternity cases implicated in the number of times it participated in the exclusion (observed value) which was compared with the expected value, according to its Power of Exclusion using both databanks: the population sample from and the one by Promega Corporation. The values of χ^2 show a P higher than 0.05 for each locus, except in TPOX and D16S539 ($0.05 > P > 0.01$). For both loci in both databanks, the observed number of exclusions was considerably higher than the expected ones. Complementarily, the combined Power of Exclusion obtained for each multiplex system for each databank was compared and results showed no significant difference.

Contact: silviene@unb.br

P-207

Characterization of a novel variable number of tandem repeats (VNTR) polymorphism in *CIAS1* gene

Omi T[1,2], Kumada M[1,2], Okuda H[1,2], Gotoh T[1,2], Kamesaki T[1,2], Kajii E[1,2], Sakamoto A[1] and Iwamoto S[1,2]

1. Division of Human Genetics, Center for Community Medicine, Tochigi, Jichi Medical School, Japan
2. Division of Legal Medicine, Center for Community Medicine, Tochigi, Jichi Medical School, Japan

Cold-induced autoinflammatory syndrome 1 (MIN 606416; *CIAS1)* gene encodes cryopyrin/NALP3 (NACHT-LRR-PYD-containing protein-3)/PYPAF1 (PYRIN-containing Apaf1-like protein) protein, predominantly expressed in peripheral blood leukocytes. The function of these proteins is to regulate apoptosis or inflammation through the activation of NF-□B and caspase. Recent genetic analyses showed an association between inflammation and oxidative stress-related genes in the development of hypertension. We performed the single-candidate-gene approach study of *CIAS1* for essential hypertension and identified a novel VNTR polymorphism in this gene. The VNTR polymorphism was consisted of a 42-bp repeat core sequences in the *CIAS1* intron 4 (*CIAS1* 42bp–VNTR). Four novel alleles containing 12, 9, 7, and 6 repeats were detected with frequencies of 0.577, 0.008, 0.248, and 0.167 from the 507 unrelated Japanese individuals, respectively. Case-control study showed that the frequency of 12-12 genotype was significantly higher in 1087 patients with hypertension compared with 1033 control subjects (P=0.007; Odds ratio=1.24). Association study between the VNTR genotype and blood pressure revealed that the systolic blood pressure level of 12-12 subjects was significantly higher in the random population (n=285, men, P=0.009). The real time PCR analysis showed that among healthy young adults, 12-12 subjects expressed CIAS1 mRNA in peripheral leukocytes significantly more abundantly than X-X (X: 9, 6, and 7) subjects (P<.05). Reporter gene assay of the *CIAS1* 42bp-VNTR in HL60 stimulated by lipopolysaccharides showed that the intronic sequence involving 12 repeat increased the expression of luciferase compared with 9, 7, and 6 repeats. These results suggest that the *CIAS1* 42bp-VNTR modifies the expression level of the *CIAS1* transcript. Thus, we propose that *CIAS1* is a new candidate for the hypertension-susceptibility locus.

Contact: t-omi@jichi.ac.jp

P-208

Y-chromosome genetic structure in a sub-Apennine population of the Marches (central Italy): analysis by SNP and STR polymorphisms.

Onofri V, Alessandrini F, Buscemi L, Pesaresi M, Turchi C, Tagliabracci A

Institute of Legal Medicine, Università Politecnica delle Marche, Ancona, Italy

The male-specific region of the Y chromosome (MSY) region spans many repetitive (STR) and sequencing (SNP) Y-markers, which represent a precious tool for both human evolutionary studies and forensic identification purposes.
This study was carried out on subjects living in Fabriano and Urbino, two small towns in the upland area of the Marches, speaking different dialects and submitted to a limited genetic flow. The origins of these two micro-populations might be the long-term divergence from a common group of settlers in the Apennine mountains of the Marches region, or separate evolution from other geographical areas of the Italian peninsula.
The aim of this work was to compare the genetic structure of Y-chromosome in the Apennine populations with other Italian populations in order to obtain information on the settlement of the Marches.
81 healthy male donors, unrelated and with different surnames, were selected from two hinterland areas of the Marches region, 44 from Fabriano and 37 from Urbino. DNA was extracted from whole peripheral blood using phenol-chloroform protocol. 37 Y-SNPs were analyzed using primer extension reaction. Two multiplexes - arbitrarily named MY1 and MY2 - were developed to explore the basal branches of the tree encompassing all the major clades A-R: MY1 for markers M35, M89, M172, M170, M9, M173, M45; and MY2 for markers M52, M216, M174, M181, M201, M91, M96, M214. To obtain a more discriminative haplogroup assignment, a large number of the shallowest SNPs for the most frequent haplogroups in Italy were selected: R1, E3b, J2 and I. Four distinct multiplex PCRs were set up, capable of typing the more superficial branches typical of these haplogroups, named MY-E3b (M78, M107, M224, M165, M148, M81), MY-J2 (M158, M68, M47, M102, M137, M67), MY-R1 (M17, M269, M18, P25, SRY10831.2) and MY-I (M72, M223, M26, M21, M161).
All samples were genotyped for Y chromosome microsatellites using AmpFlSTR® Yfiler™ (AB) that allow the co-amplification of the core set of European Minimal Haplotype, and other eight loci (DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 and Y GATA H4).
Pairwise differences among haplogroups, gene frequency and haplotype diversity were estimated.
contact: a.tagliabracci@univpm.it

P-209

A comparative study of STRs and SNPs typing efficiency in highly degraded forensic samples

Onori N, Onofri V, Alessandrini F, Buscemi L, Pesaresi M, Turchi C, Tagliabracci A

Institute of Legal Medicine, Università Politecnica delle Marche, Ancona, Italy

DNA recovered at a crime scene often results as damaged; this represents enormous difficulty for the correct typing because of fragmentation or the lack of DNA region of interest. It is well known that DNA is subject to enzymatic and chemical post-mortem damage: DNasi I randomly cuts the histon-free double strand DNA and hydrolysis, oxidation, alkylation and cross-linking phenomena produce alterations at single nucleotides, hindering DNA polymerase activity or introducing mismatches.
The STR markers commonly used in forensic casework give only partial positive results with degraded samples, yielding a discrimination power not sufficient for a certain identification. Forensic geneticists have recently focused their attention on SNPs, which can be analysed in short amplicons, thus allowing typing of degraded DNA.
No experimental studies exist that have monitored STRs and SNPs genotyping efficiency during the DNA degradation process. For this reason, a set of biological samples was prepared in order to simulate the effects of natural DNA degradation on: dry and wet bloodstains, 20g of male muscle tissue stored in the open air, buried, immersed in river and sea water. The DNA was extracted by the phenol-chloroform method every month for nine months and electrophoresed on agarose gel in order to evaluate the fragmentation degree. Human DNA quantification was accomplished by Quantiblot (AB). All DNA samples were submitted to amplification of nuclear microsatellites, using AmpFlSTR Identifiler PCR Amplification kit (AB), and Y chromosome biallelic polymorphisms, employing a set of multiplex PCRs developed in our laboratory.
As expected, microsatellite analysis showed a progressive allelic and locus drop-out for the higher molecular weight STR loci in all DNA samples. SNPs amplification reactions gave results depending on the different sample type and storage conditions, and a nucleotide alteration was also observed for the locus M269.
The results suggest that further studies are necessary to establish the biochemical alterations which can affect SNP allele typing, the frequency of their occurrence and the consequent implications in forensic science.
contact: a.tagliabracci@univpm.it

P-210

The Y-chromosome in the Azores Islands: phylogeny and diversity

Pacheco PR[1,2], Branco CC[1,2], Cabral R[1,2], de Fez L[1,2], Araújo AL[3], Peixoto BR[1,2], Mendonça P[3], Mota-Vieira L[1,2]

[1] *Molecular Genetics and Pathology Unit, Hospital of Divino Espirito Santo, São Miguel, Azores, Portugal*
[2] *Instituto Gulbenkian de Ciência, Oeiras, Portugal*
[3] *Hematology Department, Hospital of Divino Espirito Santo, São Miguel, Azores, Portugal*

The Azores, a Portuguese archipelago located in the North Atlantic Ocean, had no native population when the Portuguese first arrived in the 15th century. The islands were populated mainly by the Portuguese, but Jews, Moorish prisoners, African slaves, Flemish, French and Spaniards also contributed to the initial settlement.
To understand the paternal origins and diversity of the extant Azorean population, we typed genomic DNA samples from 172 individuals using a combination of 10 Y-biallelic markers (YAP, SRY-1532, SRY-2627, 92R7, M9, sY81, Tat, SRY-8299, 12f2 and LLY22g) and the following Y-chromosomal STR systems: DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS385 a/b.
We identified nine different haplogroups, most of which are frequent in Europe. Haplogroup J* is the second most frequent in the Azores (13.4%), but it is modestly represented in mainland Portugal (6.8%). The other non-European haplogroups, N3 and E3a, which are prevalent in Asia and sub-Saharan Africa, respectively, have been found in the Azores (0.6% and 1.2%, respectively) but not in mainland Portugal. A Y-chromosomal haplotype was constructed for each individual using the seven loci. In total, 118 different haplotypes were observed in the 172 sample set (68.6% discriminatory capacity). Haplotype diversity value was high (0.9994), due to high variability of the Y-STRs. Moreover it is important to notice the great genetic diversity observed within the Azorean group. Microsatellite data indicate that the mean gene diversity (D) value for all the loci analysed in our sample set is 0.590, (values range from 0.4592 for DYS393 to 0.8212 for DYS385).
Taken together, our analysis suggests that the current paternal pool of the Azorean population is, to a great extent (59,3%), of Portuguese descent with significant contributions from people with other genetic backgrounds

contact: paularpacheco@hdes.pt

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P-211

Identification of the Finnish Tsunami Victims

Palo JU, Hedman M, Sajantila A

Department of Forensic Medicine, University of Helsinki, Helsinki, Finland

The Asian Tsunami on December 26th 2004, has been estimated to have caused the death of more than 173 000 people in eleven countries around the Indian Ocean. Among the casualties were 178 Finnish tourists, belonging to 75 extended families. As numerous complete families were affected, the age distribution of the missing persons is clearly bimodal with peaks around ten and forty years. The identification process of the Finnish victims, led by the Disaster Victim Identification (DVI) team of the National Bureau of Investigation, started on Dec 28th. Large amount of diverse AM data has been collected by the local section of the team, including DNA reference samples from surviving relatives and personal items. Four months after the catastrophe, 117 of the victims have been identified and repatriated solely with the aid of AM dental records and fingerprints. However, majority of the missing children lack conclusive dental AM data due to intact teeth. This has led to a bias in the age distribution of the identified victims – 75% of the persons missing after four months of the disaster were under 18 years of age. For these, the identification can be achieved only through DNA-based methods. As direct DNA profiles are largely lacking, reference samples from the relatives were required. Graphical pedigrees constructed at the early phase of the identification process have helped in planning effective sampling. The closest relatives for most of the missing children were among the victims, therefore PM femur bone samples have been taken in the autopsies performed for all repatriated bodies, immediately followed by DNA extraction and profiling for sixteen STR loci. The careful sample handling and phenol-chloroform-based DNA extraction has ensured a very high success rate in recovering the DNA profiles. The data handling, current state of the identification as well as the relative importance of different types of data for the identification will be discussed.

Contact: jukka.palo@helsinki.fi

P-212

Improved Y-STR analysis of degraded DNA using reduced size STR amplicons

Myung Jin Park[1], Ji-Eun Yoo[1], Ukhee Chung[1], Hwan Young Lee[1], Chang-Lyuk Yoon[2,3], Kyoung-Jin Shin[1,3]

[1]*Department of Forensic Medicine, College of Medicine, Yonsei University, Seoul, Korea*
[2]*Department of Oral Medicine and Forensic Odontology, College of Dentistry, Chosun University, Kwangju, Korea*
[3] *Human Identification Research Institute, Yonsei University, Seoul, Korea*

To increase PCR sensitivity in amplification of degraded DNA, we have developed two new multiplex PCR sets for 17 Y-STR loci (DYS19, DYS385a/b, DYS389-I, DYS389-II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, GATA C4 and GATA H4) by reducing the sizes of some amplicons in a commercial Y-STR kit, AmpFlSTR® Yfiler™ (Applied Biosystems). Among 17 Y-STR loci, DYS385a/b, DYS390, DYS391, DYS392, DYS438, DYS439, DYS448 and GATA C4 have been redesigned to produce reduced size amplicons by moving primers as close as possible to the STR repeat region. DYS393, DYS456, DYS458 and GATA H4 have small PCR product sizes (99~165 bp) in AmpFlSTR® Yfiler™, and newly designed PCR primers for those STRs in the new multiplex also generated amplicons of similar sizes. In addition, we redesigned the amplification primers for DYS19, DYS389-I, DYS389-II, and DYS437 which have relatively large PCR products (150~286 bp) in AmpFlSTR® Yfiler™, but they could not be made to generate amplicons of smaller sizes than those of AmpFlSTR® Yfiler™. We performed concordence study for 100 unrelated Korean samples, and the genotypes obtained from two new multiplex were the same as the genotypes obtained from AmpFlSTR® Yfiler™ kit. To assess the effectiveness of new multiplex, we have tested these primer sets with enzymatically degraded DNA and compared the amplifications to AmpFlSTR® Yfiler™. We also conducted sensitivity test in blood genomic DNA, and this showed that all Y-STR loci in two new multiplex were reliable and sensitive at template concentrations as low as 31 pg/10 µl. Moreover, comparison studies in 30 samples of 50-year old skeletal remains demonstrated that the multiplex were capable of producing more complete profiles in comparison with AmpFlSTR® Yfiler™. Overall, our data verified that two new multiplex can provide fully concordance results to commercial STR kits and can produce improved signal from degraded DNA than AmpFlSTR® Yfiler™.

Contact: hylee192@yumc.yonsei.ac.kr

P-213

Organizing The Argentinian Combined DNA Index System (CODIS)

Penacino GA

Sociedad Latinoamericana de Genetica Forense (Latin American Society for Forensic Genetics): www.slagf.org Unidad de Analisis de ADN (DNA Analysis Unit) - www.adn.ac - COFyBCF - Rocamora 4045, Buenos Aires, ARGENTINA - Phone/fax (5411) 4862-1142 - E-mail presidente@slagf.org

As proposed our Latin American Society for Forensic Genetics in June 17th, 2003, last year the Argentinian Minister of Justice, Security and Human Rights signed the Resolution # 415/2004 (see the full text in spanish in *www.slagf.org*), that create the DNA Fingerprint Registry, similar to American CODIS.
The Registry blends computer and DNA technologies into an effective tool for fighting violent crime. The system uses two indexes to generate investigative leads in crimes where biological evidence is recovered from the crime scene. The Convicted Offender index contains DNA profiles of individuals convicted of felony sex offenses (and other violent crimes). The Forensic index contains DNA profiles developed from crime scene evidence. The Registry utilizes computer software to automatically search these indexes for matching DNA profiles.
Like the American CODIS, this is a system of pointers; the database only contains information necessary for making matches. Profiles stored contain a specimen identifier, the sponsoring laboratory's identifier, the initials (or name) of DNA personnel associated with the analysis, and the actual DNA characteristics. Matches made among profiles in the Forensic Index can link crime scenes together; possibly identifying serial offenders. Based on a match, police can coordinate separate investigations, and share leads developed independently. Matches made between the Forensic and Convicted Offender indexes ultimately provide investigators with the identity of the suspect(s).
Following are the major enhancements planned for next years:

- There are differences among province laws, so the first step is to make compatible them.
- Optimize software performance for Local, Province and National Indexes.
- Select the labs with high-quality standards. Quality controls in Latin America are conducted by the Latin American Society for Forensic Genetics and the Spanish and Portuguese Speaking Group of the ISFG.
- Training courses to DNA analysts from participating laboratories.
- Begin operation of the National DNA Index System.
- Proliferate the installed base to include all crime laboratories performing DNA analysis.

Contact: gpenacino@wwiecorp.com

P-214

Development of a bidirectional exchange between the Sapphire LIMS and analytical softwares to drastically increase the throughput of a forensic laboratory

Pene L, Barsacq S, Gleizes A, Paléologue A
Laboratoire Police Scientifique de Lyon

During the recent years, in France, different laws extended the number of offences categories that are concerned by the DNA national database. This extension of the DNA database has led to an increase of the number of caseworks and database samples that the french laboratories have to analyse. In order to ensure the analysis of this increasing samples number, we received, in 2004, a grant, dedicated to buy new analytical devices and a Laboratory Integrated Management System (LIMS). The LIMS, implemented in the laboratory, is a Labvantage product called Sapphire, distributed in France by SpectraLIMS. Based on the practical experience of a first home-made LIMS and the current use of robotic platforms since four years, we have decided to design a global project that both automate the processing of analytical data and samples. We would like to reach a medium throughput: 15000 database samples and 21000 casework samples analysed per year. In order to achieve this goal, we are trying to automate all the repetitive tasks for the laboratory members where the error risk is high. Moreover, we are trying to use all the flexibility and subtlety wich offer the use of a LIMS that exchanges data with analytical softwares. We are going to illustrate our philosophy with examples along the description of the database samples analytical pathway. In our analytical pathway, all the reference samples must be on FTA paper to be analysed. Two punches of one FTA card are distributed in two wells of two different FTA plates, thanks to a puncher. The output file of the puncher is imported in the LIMS which triggers the creation of an "FTA plate". When the object "FTA plate" is created in the LIMS, different analysis are associated with this object. One analysis defines one step which undergoes the "FTA plate". All the steps are automatised on pre-PCR and post-PCR platforms, thanks to input-files, called work-lists. These work-lists define the treatment of each well of the plates. In order to check the quality of robotic platform work, out-put files are created at the end of robotic method. These files are attached in the LIMS to the object "FTA plate". In order to analyse the FTA amplicons, a second plate object is created in the LIMS : the "Electrophoresis Capillary plate" ("EC plate"). The analyst has the choice to merge different complete "FTA plates" in one "384 EC plate" or to pick-up few samples of "FTA plates"to dispense in one "96 EC plate". This last option allows to re-migrate, on the genetic analyser, only the samples wich fail during the first analysis. This possibility is very convenient to optimize the use of multicapillary genetic analyser. Following the electrophoresis, a GeneMapper project is generated with sample files grouped by "FTA plate" origin or "EC plate" origin. The GeneMapper software affects numerous quality factors for each locus genotyped. The importation of alleles in the LIMS is conditioned by the quality factors associated with each locus. Moreover, the LIMS affects a label for each global genotype. All this importation algorithm allows the analyst to only review, in a final table, the genotype loci that are not automatically labelled by the GeneMapper analysis method.

Contact: laurent.pene@laposte.net

P-215

Polymorphism of four X-chromosomal STRs in a religious minority of Old Believers residing in northeastern Poland

Pepinski W [1], Niemcunowicz-Janica A [1], Skawronska M [1], Janica JR [2], Koc-Zorawska E [1], Janica J [1], Soltyszewski I [3]

[1] *Department of Forensic Medicine, Medical University of Bialystok, Poland*

[2] *Department of Radiology, Medical University of Bialystok, Poland*

[3] *Central Forensic Laboratory of Police, Warsaw, Poland*

Old Believers are a fraction of the Russian Orthodox Church who came into existence as a result of schism introduced in 1653-1666 by Patriarch Nikon in opposition to the Russian Church Reform, adopting the liturgy and practices of the Greek Church. The Old Believers who resisted the Reform were condemned and declared dissidents, in 1667 they were separated from the Russian Orthodox Church and severely persecuted under the tsars sought shelter in the most remote corners of Russian Siberia as well as abroad, including USA, Lithuania and Poland. In the 19th century they moved to Suwalki Region (NE Poland), where they founded several villages and have struggled to maintain their religious identity and traditional ways of life, such as farming and continuing to speak Russian. In an effort to preserve their culture, some parents pressured their children to marry young. Many communities lived in almost complete isolation for centuries. Allele frequencies for four X-chromosomal STR (DX8378, DX7132, HPRTB and DX7423) were determined in a population sample of 140 unrelated males and 70 females by multiplex PCR and subsequent automated fluorescent detection (ABI 310) using a commercially available multiplex PCR kit Mentype Argus X-UL (Biotype, Germany). For each locus, allele frequencies were calculated separately for males and females. Comparison of allele frequencies between males and females was performed by the exact test of a RxC contingency table analysis. Possible divergence from Hardy-Weinberg equilibrium (HWE) was tested using the exact test based on 3200 shuffling experiments using the GDA software v1.2. The following statistical parameters were calculated: observed and expected heterozygosity (Ho, He), polymorphism information content (PIC), mean exclusion chance (MEC), expected probability of exclusion (PE) and discrimination power in males (DP_M) and in females (DP_F). The genotype distributions among the females conformed with Hardy-Weinberg equilibrium except for DX7423 (P=0.0013). A pairwise comparison using the exact test disequilibrium analysis yielded no indication of allelic dependence ($0.2438<P<0.6981$). No significant differences were observed between allele distributions in males and females ($0.4230<P<0.9940$), therefore the two groups were pooled into single frequency distributions for respective loci. Kinship tests revealed a typical X-linked inheritance with no mutation. For the quadruplex evaluated, the combined MEC is 0.9919, and the combined DP values are 0.9954 and 0.9999 (for males and females, respectively). A pairwise testing for heterogeneity using the RxC contingency table exact tests for population differentiation according to Carmody revealed significant differences between the group of Old Believers and the autochthonous Polish population at HPRTB and DX7132 (P=0.0010 and P=0.0110, respectively).

Contact: pepinski@amb.edu.pl

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Y-chromosome variation in northeastern Poland

Pepinski W [1], Niemcunowicz-Janica A [1], Skawronska M [1], Janica JR [2], Koc-Zorawska E [1], Janica J [1], Soltyszewski I [3]

[1] *Department of Forensic Medicine, Medical University of Bialystok, Poland*

[2] *Department of Radiology, Medical University of Bialystok, Poland*

[3] *Central Forensic Laboratory of Police, Warsaw, Poland*

Ethnically, Poland has a largely homogeneous population, its percentage of national or ethnic minorities being one of the lowest in Europe, officially estimated at between 3-4% of the inhabitants, which is equivalent to about 1.5 million people. Podlasie in northeastern part of Poland is a frontier region where the influences of various countries and cultures have been clashing for centuries. The region differs from the others due to its scanty population (1.2 million) and ethnical and cultural diversification. It is estimated, that northeastern corner of Poland is inhabited by 200,000-300,000 Belorussians, 20,000-30,000 Lithuanians and also 2,500 Polish Tatars and 600 Old Believers. Lithuanians compose one of the most emancipated, best organized and least assimilated minority communities in the country. Tatars who presently live in Poland are Sunni Muslims. Old Believers are a fraction of the Russian Orthodox Church who came into existence as a result of schism introduced in 1653-1666 by Patriarch Nikon in opposition to the Russian Church Reform. DNA was extracted using the Chelex 100 and proteinase K protocol. The quantity of recovered DNA was determined spectrophotometrically. DNA was amplified in PCR System 9700 (Applera) using commercial kits: PowerPlex Y System (Promega) or genRES DYSplex-1 and genRES DYSplex-2 (Serac). The SWGDAM recommended Y-STR minimal haplotype was considered. Electrophoresis and typing were performed in the ABI 310 Genetic Analyzer (Applera). Reference ladders included in the kits were used for genotype classification. The nomenclature according to the Y-STR Haplotype Reference Database (http://www.yhrd.org) was used. Allele frequencies for each locus were calculated by simple gene counting method. Gene or haplotype diversity/discrimination (GD) and discrimination capacity (DC) values were calculated. AMOVA was performed using the Monte-Carlo test included in the Arlequin software ver. 2.000. The combined values of GD were 0.9836, 0.9750, 0.9815, 0.9638 and 0.9938 for Poles, Belorussians, Lithuanians, Polish Tatars and Old Believers, respectively with corresponding values of DC=0.84, 0.86, 0.82, 0.81 and 0.79 respectively. The pairwise population comparisons between autochthonous Poles and the studied minorities revealed statistically significant differences (P=0.0180, 0.0360, 0.0090 and 0.0000, respectively) and relatively small values of interpopulation variation (R_{ST}=0.0064, 0.0162, 0.0127 and 0.0311, respectively) indicating a certain degree of genetic differentiation. The resulting data are consistent with the idea of a genetic proximity of Belorussians and Lithuanians to the Polish population due to the common Slavic origin and historical-political contacts. xxx and support the concept of a Polish admixture in Y-chromosomal lineages of Polish Tatars. Old Believers appeared to be more distant from autochthonous Poles which may reflect their different history, religious affiliation and long-established principles of living. We suggest that the differences in some haplotype frequencies should be taken into consideration in certain trace-donor match analyses within the population of northeastern Poland.

Contact: pepinski@amb.edu.pl

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Sampling efficiency for Amerindian female lineages

Pereira L[1], Goios A[1,2], Amorim A[1,2]

[1]IPATIMUP (Instituto de Patologia e Imunologia Molecular da Universidade do Porto), Porto, Portugal; [2]Faculdade de Ciências da Universidade do Porto, Porto, Portugal

lpereira@ipatimup.pt

Characterisation of mtDNA lineages in South American urban populations has revealed that as much as one third of its gene pool is of Amerindian ancestry, showing the introgression of native American females in the highly mixed (with Eurasian and sub-Saharan) new cosmopolitan communities. By opposition, only a small fraction of the male pool of urban South American populations is of Amerindian ancestry (almost 98% is of Eurasian background). The old native communities were drastically reduced in its effective size, and the remaining few are small and scattered. We could then hypothesize the following scenarios: the native female lineages picked up for the constitution of the new cosmopolitan mixed populations were from communities around the location of the new town and probably still diverse; while the native female lineages observed in present small native communities went through severe demographic forces, such us bottlenecks, genetic drift and founding events. Being these scenarios true, a sampling effort would reveal much more diversity when surveying cosmopolitan populations than small tribes, leading to a reconstruction of the Amerindian mtDNA phylogeny much more promising in the first case than in the second. We compiled published data for South and Central Amerindians and compared their diversity with the cosmopolitan pool of Amerindian ancestry, in order to check if the above described scenarios are discerned in the data: - for native populations: 120 Cayapa from Equator [1]; 129 Yanomami from Venezuela and Brazil [2]; 39 Mapuche from Argentina [3]; 34 Mapuche, 24 Pehuenche and 15 Yaghan from Chile [4]; 46 Ngobe from Panama [5]; 27 Huetar from Costa Rica [6]; 44 Emberá and 31 Wounan from Panama [7]; 22 Arequipa, 61 Tayacaja and 22 San Martin de Pangoa from Peru [8];- for cosmopolitan populations: 44 from Chile and 20 from Colombia [9]; and 82 from Brazil [10]. The Amerindian haplogroup (hap) distribution in the pooled tribal samples was equivalent to the one in the pooled cosmopolitan samples, but the diversity was higher in towns. Haps A and B where the main contributors for the higher diversities observed in towns, while for haps C and D, diversities were almost equal in tribes and towns. This trend of higher hap A and B diversities in towns (as well as the equivalent diversity for haps C and D) was also observed when randomly resampling 4 sub-groups (the same size of towns) inside the pooled tribal sample, showing that this effect is not due to a bias resulting from disproportionate sample sizes. Network analyses showed that in the pooled tribes, haps B and C present a star-like phylogeny, while A and D show several equally frequent haplotypes, being one-step departed in A but many steps in D (leading to a high mean pairwise difference). This testifies the diverse effects acting upon different haps. In pooled towns, networks show a much diverse phylogeny for all haps, as resulting from the picking up of divergent lineages. In conclusion, a considerable amount of information for the native Amerindian lineages can be inferred by studying the descendents of the newly constituted populations after the arriving of Eurasians. This is true for the mtDNA, but, unfortunately, cannot be applied to the Y-chromosome, for which many lineages are lost forever.

[1] Rickards et al. (1999) [2] Merriwether et al. (2000) [3] Ginther et al. (1993) [4] Moraga et al. (2000) [5] Kolman et al. (1995) [6] Santos et al. (1994) [7] Kolman et al. (1997) [8] Fuselli et al. (2003) [9] Horai et al. (1993) [10] Alves-Silva et al. (2000)

P-218

The Islamization of Iberian Peninsula: a demographic shift or a cultural change? Search for an answer using extant and ancient DNA from Mértola (Southeast Portugal)

Pereira L[1], Morales AC[2], Goios A[1,3], Duarte R[1], Rodrigues C[2], Endicott P[4], Alonso A[5], Martín P[5], Torres C[2], Amorim A[1,3]

[1]IPATIMUP (Instituto de Patologia e Imunologia Molecular da Universidade do Porto), Porto, Portugal; [2]Campo Arqueológico de Mértola, Portugal; [3]Faculdade de Ciências da Universidade do Porto, Porto, Portugal; [4]Henry Wellcome Ancient Biomolecules Centre, Department of Zoology, University of Oxford, UK; [5]Instituto Nacional de Toxicologia y Ciencias Forenses, Servicio de Biologia, Madrid, Spain

A classical view of the Iberian Peninsula history used to correlate the Islamization of the Iberian Peninsula with significant demographic migrations from North Africa, but, more recently, acculturation phenomena were advanced as being more significant in places like Mértola, an important roman and medieval fluvial port in southeastern Portugal. The Islamization phenomenon has been intensively studied there since the 1980's. Archaeological excavations lead to the discovery of the "Rossio do Carmo", a funerary area outside the city walls, where Islamic burials overlap Paleo-Christian ones. A pacific conversion of the inhabitants of Mértola after the Islamic conquest may explain this continuity of use of the early medieval place of burial, better than a massive introgression of North African people. In order to evaluate which of the scenarios fits better the available data, we followed two lines of research on both: (a) from bones recovered from this necropolis; and (b) in the extant population of Mértola.With respect to the ancient mtDNA, three Paleo-Christian individuals (right second metartarsal; 3 upper second premolars and 1 lower second premolar) and one Islamic (right first metartasal and deciduous lower left canine and first molar) were analysed. These samples span a time range from 5^{th}-13^{th} centuries A.D. The amplification of mtDNA, in all the appropriate conditions for the study of ancient DNA, resulted unsuccessful for fragments longer than 123 bp (quantities of DNA templates were minimal), what pointed to degraded sequences, and the presence of multiple sequences in independent PCRs (some of the results could be real, but it wasn't possible to infer which sequences were endogenous). This last fact could be due to 3 plausible causes: post-excavation contamination (excluded by absence of matching between spurious sequences and the ones obtained from the survey of the archaeological team); damaged DNA, causing jumps between templates and generating novel sequences (not cleared up by cloning); and contamination during the burial period, presumably by percolation. This last explanation looks the most probable cause for no reliable DNA sequences being achieved from the Paleo-Christian and Islamic cemeteries of Mértola. So, we were left with the results from the extant Mértola district population. We sampled 43 individuals from the town and from three small villages (Alcaria Ruiva, ancient pre-Islamic foundation; Alcaria dos Javazes, Islamic foundation; and Santana de Cambas, Christian post-Islamic foundation). MtDNA survey revealed that its feminine genetic composition is significantly different from North and Central Portugal, and even from the South of the country. North African lineages are more frequent in Mértola (11.6%) than in any other region of the Iberian Peninsula (the highest is 5% in North Portugal), and the sub-Saharan ones are scarcer than in Southern Portugal (7% comparatively to 11%), while Near/Middle Eastern lineages are much more common (37.2% relatively to 10.9% in Portugal). We are now enlarging the sample in order to obtain a sufficient number of Y-chromosome lineages. Thus, the female lineages from the extant population of Mértola bear a higher proportion of typical components of the Southern and Eastern Mediterranean when compared to other regions of Portugal. Unfortunately, we cannot safely conclude on the time scale for the arrival of these typical southern Mediterranean lineages in the Mértola gene pool. lpereira@ipatimup.pt

P-219

Seventeen Y-chromosome specific short tandem repeat haplotypes study in Brazilian populations

Pereira RW[1], Hirschfeld GC[2], Wang AY[2] and Grattapaglia, D[1,3]

[1]Graduate Program in Genomic Science and Biotechnology, Catholic University of Brasilia,
[2]Applied Biosystems, Brasil,
[3]Heréditas Tecnologia em Análise de DNA Ltda.

The Y-chromosome haplotypes based on high polymorphic STRs are broadly used in forensic laboratories, mainly applied in case of rapes where they drop the number of profile contributors just to male/males without differential DNA extraction necessity. The ultimate commercial set of Y-STRs available to forensic community allows the amplification of 17 loci in a multiplex fashion. This set amplifies loci from the European minimal haplotype, loci from the SWGDAM-recommended Y-STR panel and six additional highly polymorphic loci. The AmpF□STR® Yfiler™ kit has showed in populations studied so far a power of individual discrimination greater than 95%. Here we show the haplotype diversity and some population genetics parameters found with the AmpF□STR® Yfiler™ kit in 274 males from the five geopolitical Brazilian regions. The 274 samples were distributed in 77 samples from the North, 49 samples from the Central west, 49 samples from the Northeast, 36 samples from the Southeast and 63 samples from the South. All males were resident in such regions when they submitted themselves to paternity investigation. The DNA samples were amplified and the PCR products analyzed in the ABI Prism 3100 according to the manufacturer's protocol. The ABI Prism 3100 sample files were analyzed using the Genescan and Genotyper softwares and once the table with individual STRs genotypes was generated, it was used to create Arlequin package input data file. The Arlequin package was used to automatically investigate haplotype diversity, haplotypes uniqueness and the molecular variation partition among regional groups and within them. The global sample analysis showed 258 unique haplotypes out of the 274 Brazilian Y chromosomes sampled (94.42 % of individual discrimination power). The number of unique haplotypes / total chromosomes for each geopolitical region was 74/77 to North, 48/49 to Central west, 46/49 Northeast, 34/36 Southeast and 62/63 to the South chromosomes. The haplotype diversity in the global sample was 99.95 % (S.D +/- 0.0004). All geopolitical regions samples showed haplotype diversity greater than 99 %. The analysis of molecular variance showed that 99.72 % of the molecular variation was due variation within each geopolitical region group and that 0.28 % was due variation between them. The results found in this work showed that the AmpF□STR® Yfiler™ kit has a high power of individual discrimination and that there is no Y-chromosome structure in Brazilian population, allowing the use of a unique database of Brazilians chromosomes. As described by others we also found some loci with more than one allele. The multi-allelic pattern occurred frequently at the DYS385 loci, twice at the DYS389II and DYS439 and once at the DYS437. One sample showed two alleles at the DYS389II, DYS439 and DYS437. As pointed out by others authors this multi-allelic pattern frequency must be better understood, as they might be taken as sample mixture. Regarding the three loci double allele pattern, at least two independent authors described it in one sample from Spain and other from Bahia, Brasil. The three loci are located in the AZFa segment and its duplication, followed by STRs mutation must the culprit for the double allele pattern. As forensic community broadly uses these three STRs, the understanding of duplication uniqueness or recurrence is important to realize the real consequence of multi-allelic pattern in forensic casework. contact: rinaldo@pos.ucb.br

P-220

Microsatellite polymorphisms in two Taiwanese aboriginal groups

A.M. Pérez-Miranda [1, 3], M. A. Alfonso-Sánchez [2], R. J. Herrera [1]

1 Molecular Biology and Human Diversity Laboratory, Department of Biological Sciences, Florida International University, Miami, FL 33199, USA

2 Departamento de Genética y Antropología Física, Universidad del País Vasco, Apartado 644, 48080 Bilbao, Spain

3 Anglia DNA Bioservices Limited, Norwich Research Park, Colney Lane NR4 7UH, Norwich, UK

In the attempt to reconstruct the prehistory of Pacific and Indian Ocean populations, Taiwan's aborigines appear to be of particular interest. Linguistic and archeological evidence indicates that the dispersal of Austronesian speakers throughout the islands of Oceania and Southeast Asia may have originated from Taiwan about 5,000 years ago. In the island of Taiwan, formerly known as Formosa, nine indigenous groups have coexisted (Tsou, Bunun, Paiwan, Rukai, Atayal, Saisiat, Ami, Puyuma and Yami), which are highly homogeneous within each tribe, but diversified among the different tribes probably due to long-term isolation. The aim of the present study was the genetic characterization of two of these tribal groups (Ami and Atayal) based on the short tandem repeats (STRs) sanctioned by CODIS (D3S1358, TH01, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, CSF1PO, vWA, D8S1179, TPOX, and FGA). The sample included 108 unrelated healthy individuals: 40 Ami and 68 Atayal. Significant departures from genetic equilibrium were detected at the D8S1179 and TH01 loci in the Ami, which persisted even after applying Bonferroni-type corrections. Gene diversity (GD) values ranged from 0.5377 (TPOX) to 0.8674 (FGA) in Atayal, whereas in the Ami subpopulation GD oscillated between 0.6409 (TPOX) and 0.8764 (D21S11). A notable heterozygosity was observed in both tribal groups, although it was slightly higher in the Ami group (average: 0.7867) than in Atayal (0.7036). The information provided by the STR loci was analyzed using distance-based methods (Neighbor-Joining trees and multidimensional scaling), to assess the genetic relationships of these Taiwanese groups with others Asian populations.
Contact: ana@angliadna.co.uk

P-221

Automation of post-mortem or non-standard reference samples genotyping using FTA

Pesquier B, Taillé A, Garcin G, Frackowiak S, Coiffait P-E

Laboratoire de Police Scientifique de Marseille – Section Biologie – 13245 Marseilles Cedex 04 – France

In forensic biology, reference samples take a growing place in the analysis pool. Today, in France, the vast majority of these are saliva samples on FTA® paper, which can be easily automated*. However, some of them, like post-mortem samples or buccal swabs and brushes, are not standardised.
In this poster, we describe our work toward automation of the analysis of such non-standard reference samples, using FTA® cards (*Indicating FTA Microcard, Whatman*).
The first step consists of manual spotting of the samples on the FTA® card. For autopsic blood, 50µL are directly applied. Other post-mortem tissues are strongly rubbed on the paper, and swabs or brushes are moistened before application. All the samples are allowed to dry at least overnight.
The second step consists of the genetic analysis of the samples, according to the high-throughput process we developed for standardised reference samples.
The genotypes of the spotted samples are compared to those obtained from the same original samples typed after non-organic extraction – each sample is tested 6 times, to estimate the reproducibility of the results.
Then, the impact on the genotypes' quality of the amplification of 1, 2 or 3 FTA® punches in the same well is assessed.
Our results show that
1) the quality of the profile from a FTA-spotted sample is positively correlated to the quantity of DNA in the reference extract of the corresponding sample.
2) None of the samples lead systematically to acceptable results. At best, the optimal quality of profile is obtained for 4 of the 6 successive tests.
3) In a few cases, the addition of more than one punch in the same reaction can ameliorate the profiles. However, the improvement does not seem to be correlated to the quantity of DNA in the original samples (maybe due to polymerase inhibition by excess of paper in the PCR mix).
In conclusion, the use of FTA cards for non-standard reference samples can be a convenient alternative for DNA typing as it offers all the advantages of the subsequent automatised treatment. Yet, in some cases, multiple analysis may be necessary to obtain a reliable profile (≥ 5 loci meeting the validation criteria), because of the lack of reproducibility of the FTA technology (as already noted for saliva).
* Delpech and coll, INTERPOL Meeting, Lyon, october 2004 "*Use of an automated process for DNA typing of buccal samples to supply the french national database*"
Contact: Benjamin.PESQUIER@interieur.gouv.fr, Sylvie.FRACKOWIAK@interieur.gouv.fr

P-222

MALDI-TOF MS analysis of Y-SNPs in ancient samples

Petkovski E[1,2], Keyser-Tracqui C[1], Hienne R[2], Ludes B[1]

[1]*EA 3428, Institut de Médecine Légale, Strasbourg, France*
[2]*Laboratoire CODGENE, Strasbourg, France*

Studying ancient central Asian, Siberian and South American populations with classical markers (nuclear microsatellites and mitochondrial DNA sequence polymorphisms) allowed us to investigate parental relationships among individuals from burial sites revealing funeral practices. Ancient DNA can also provide information on the origins and the history of population from the past. Focussing on biallelic markers which have a lower mutation rate than repeat polymorphism it is possible to address events corresponding to longer periods of time. Working on ancient DNA samples from Mongolia, Siberia, Yakutia and South-America we concentrated on three Y chromosomal SNPs (TAT, M242 and RPS4Y) known to have specific allelic distributions in these populations or to be informative regarding the peopling of America (M242 and RPS4Y).
The TAT-C allele is observed at very high frequencies in Yakuts (Pakendorf et al., 2002). The M242 derived allele occurs in all indigenous American Y chromosomes that do not carry the RPS4Y mutation (Seielstad et al., 2003) and also at a non-negligible frequency in central Asian, Indian and Siberian populations. The M242 mutation is widely distributed in Eurasian populations and it arose after the M45 and M74 mutations but before M3 which is before the first migration into the Americas. The $RPS4Y_{711}$ mutation is restricted to eastern Asia and America (Bergen et al., 1999) raising a Native American founder lineage outside M45 characterized by differentiated STR alleles (Lell et al., 2002).
Facing ancient samples where DNA is strongly degraded and scarce requires the use of technologies which can provide information from only short fragments of intact template. We developed a primer extension and MALDI-TOF MS based triplexed reaction for the investigation of these polymorphisms. This sensitive method, based on an intrinsic property of the oligonucleotides not requiring any product labelling, allows taking particular questions in hand as it can be adapted to the sample and its informativity.

contact: elizabet.petkovski@ulp.u-strasbg.fr
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P-223

Forensic DNA typing of human nails at various stages of decomposition

Piccinini A[1], Cucurachi N[2], Betti F[1], Capra M[1], Lorenzoni R[1]

[1]*Istituto di Medicina legale. Università degli Studi di Milano*
[2]*Dipartimento di Anatomia umana, farmacologia e Scienze medico-forensi. Università degli Studi di Parma*

Forensic scientists often face the problem of extracting and typing human DNA from highly degraded materials such muscle and bones from decomposed bodies.
Bone samples are particularly difficult and time consuming to be analysed and other body tissues suffer from rapid deterioration.
Nails are a well-known source of DNA and their composition makes them less predisposed to decomposition compared to other soft tissues.
The aim of this study was to evaluate the usefulness of DNA extracted from aged human nails in forensic cases.
We analysed human nails taken either from exhumed and partially skeletonised bodies or from nail clippings stored at room temperature for more than 10 years.
DNA was extracted with phenol-chlorophorm and typed with STRs using commercial kits.
The adopted DNA extraction procedures yielded enough DNA for reliable PCR results even when no results were obtained either from soft and bone tissues.
This study thus confirms the usefulness of nails as a good source of DNA even in cases when PCR failed to amplify DNA extracted from bones

contact andrea.piccinini@unimi.it

P-224

Y-STR typing in the identification of genetic profile of the semen

Pinheiro MF[1,2], Pereira MJ[1], Cainé L[1], Lima G[1], Pontes L[1], Abrantes D[1]

[1] *Instituto Nacional de Medicina Legal – Delegação do Porto*
[2]*Faculdade de Ciências da Saúde – Universidade Fernando Pessoa*

The Genetics and Biology Forensic Laboratory of the National Institute of Legal Medicine (Oporto Delegation) has been asked to solve criminal cases, among other analysis, being the majority of them sexual female assaults. For a variety of reasons, some victims of sexual aggressions provide vaginal samples more than 24-36 h after the incident. In these situations, the ability to obtain an autosomal STR profile of the semen donor reduces as the post-coital interval is extended. Therefore, we have used Y-STR *loci* to obtain a genetic male haplotype even when the autosomal STRs failled. DNA was extracted from samples collected in sexual female cases using the organic phenol-chloroform-isoamylalcohol method. The *loci* were co-amplified using the PowerPlex® 16 System (Promega) or the AmpFℓSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems) for the autosomal STR systems and, for the Y-STR systems, the PowerPlex® Y System (Promega) and, in some situations, the AmpFℓSTR® Yfiler™ (Applied Biosystems). The amplified products were detected and separated by capillary electrophoresis on an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). Fragment sizes were determined automatically using the Genescan® Anlysis Software v 3.7 and allele designations using Genetyper® Software v. 3.7 (Applied Biosystems) typed by comparison with an allelic ladder. In this study we demonstrate, through some examples, that Y-STR systems provide a complete male haplotype despite the cytological absence of spermatozoa, the negative acid phosphatase reaction and without the male autosomal profile. These evidences show the efficacy and high sensivity of Y-STRs for discerning the genetic profile of the male donor in admixtures of body fluids, mainly when the female component was present in vast excess.

Contact: Biologia@dpinml.mj.pt

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BPA analysis as a useful tool to reconstruct crime dynamics. Part II

Pizzamiglio M[1], Fratini P[1], Floris T[1], Cappiello P[1], Matassa A[1], Festuccia N[1] and Garofano L[1]

[1] Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italy

This paper concerns a case of a gruesome double murder committed by two minors, a girl and her boyfriend, who killed a 40 year old woman and her son, who was just 12. The victims were the mother and the young brother of the girl and the murder was committed in their house, as the victims came back from the gym.
We refer to technical activities we conducted at the crime scene and the analytical approach we adopted, based on DNA as well as on BPA analyses of the bloodstains we recovered, studied and collected during CSI.
Following this integrated analytical approach, also supported by fingerprint and footprint exams, it was possible to understand the role of the two young killers and thus reconstruct the dynamic of the event.

lugaro@tin.it

P-226

The use of mini STRs on degraded DNA samples

Pizzamiglio M[1], Marino A[1], Coli A[1], Floris T and Garofano L[1].

[1] *Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italy*

Forensic laboratories, much more frequent than in the recent past, have to face degraded evidence, which usually contains small amounts of DNA (LCN DNA). With these exhibits, even relying on the most efficient extraction system, or amplifying with increased number of PCR cycles, the STRs profiles are often incomplete or exposed to stochastic effects.
In this paper we refer to the use of a mini pentaplex (FGA, D21S11, CSF1PO, D7S820 and TH01) and a mini quadruplex (Penta D, D2S1338, Amelogenin and D18S51) used to analyse casework samples which gave negative or very partial results, when analysed by the kits commercially available.
The results we obtained, still preliminary, really encouraged us to continue these studies because evidence, negative to the quantification procedures or exhibiting DNA concentration of 50 picograms or less, gave reliable data.

lugaro@tin.it

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STRs typing of DNA extracted from cigarette butts soaked in flammable liquids for several weeks

Pizzamiglio M[1], Marino A[1], Maugeri G[1] and Garofano L[1]

[1] *Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italy*

This paper refers to a case of arson, in which we analyzed three cigarette butts, apparently smoked, collected from a crime scene when they were still soaked in a petroleum blend used to ignite the fire. DNA extraction was carried out using QIAamp 96 DNA Swab BioRobot kit procedure. The amount of human DNA recovered was then quantified by slot-blot hybridization with the chemiluminescent signals recorded by GeneGnome CCD imaging system, whose values ranged from approximately 0.01 to 0.1 µg/µl. Two complete male profiles based on 17 STRs were obtained from two out of three cigarette butts, while a mixed profile compatible to the previous two individuals was obtained from the third butt. Chemical analyses suggested that the cigarette butts had been left soaking in a mixture of diesel and kerosene oils for at least 45 days before they were collected and sent to the lab.

Due to these situations, we decided to carry out two experimental trials in order to establish the possibility of extracting and successfully typing DNA from cigarette butts, smoked by the same individual, under the same conditions as we faced with the evidence described above. The trials were carried out as follows:

1) The first set of three cigarettes were left soaking in three common flammable liquids (alcohol, gasoline and diesel oil) for 12, 24 and 72 hours ;

2) The second set of three cigarettes were soaked in the same liquids, but for a longer periods time of 1, 2 and 3 months.

A total of 54 cigarette butts underwent STRs analyses. The results of the two trials were as follows: all samples were successfully typed, showing the great possibility of DNA analysis, even when exhibits are recovered from very critical situations and DNA is present in very low quantities.

lugaro@tin.it

P-228

The importance of a well defined analytical strategy to solve complex murder cases

Pizzamiglio M[1], Marino A[1], Maugeri G[1], Stabile M[1] and Garofano L[1]

[1] Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italy

Forensic techniques are becoming more and more powerful and affordable. This allows labs to utilise precise strategies, permitting multiple analytical approaches on the same evidence, thus obtaining precious information to solve criminal cases.

This paper refers to a murder in which we received a plastic bottle and a latex glove. These items were collected near a stolen car used in the commission of the murder, and then burnt in order to destroy evidence linked to the murder.

Regarding the bottle, we collected samples of saliva from the neck of the bottle. The glove underwent three different analyses, which were:

- sampling and genetic analyses of sweat traces taken from the internal surface of the glove, corresponding to the lower palm area ;
- development of palm-prints from the internal surface of the glove, corresponding to the upper palm area ;
- collection of gun shot residues (GSR) from the edge of the glove.

Two fully genetic profiles were obtained from the biological traces collected from the glove. The analyses of the glove was instrumental in permitting the identification of the shooter who had played an important role in the murder.

lugaro@tin.it

P-229

Robotic DNA extraction system as a new way to process sweat traces rapidly and efficiently

Pizzamiglio M[1], Marino A[1], My D[1] Bellino C[1] and Garofano L[1]

[1] *Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italy*

A modified DNA IQ™ System (Promega Corporation, USA) was used on a variety of exhibits collected at different crime scenes, potentially interested by sweat traces and analysed by means of a fully automated extraction (Multiprobe II plus EX by ABD, USA).

As is well known, sweat usually soaks large portions of fabrics and clothes. Variable preliminary treatment steps are needed to isolate the few cells eventually still present in each item.

The goal of our application consisted in setting up a dedicated robotic extraction in order to manage large volumes of lysis buffer, to facilitate processing of large portions of evidence.

We started with a melted volume of 10-15 ml, with a recovery of purified DNA between 500 and 2000 picograms, which allowed us to obtain full STR profiles, saving time, improving the rate of success on LCN samples and reducing the risk of possible contamination.

lugaro@tin.it

P-230

Multiplexing autosomal and Y-STRs loci as a powerful tool for solving old a new criminal cases

Pizzamiglio M[1], Marino A[1], Stabile M[1] and Garofano L[1]

[1] *Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italy*

In this paper we refer to a partial DNA match, from evidence linked to two different robberies occurring almost three years apart, in two different and distant towns in Northern Italy.

According to these results, we decided to reanalyse the evidence still available, with both Identifiler™ by ABD and Powerplex 16™ by Promega, in order to verify a possible parentage. We also submitted our evidence to the new Yfiler Kit by ABD. The results we obtained were the following:

- 12 STRs loci out of 17, identical in both sets of evidence ;
- 5 STRs loci exhibiting one allele in common ;
- 16 Y-STRs loci identical in both evidence sets, showing the same haplotype.

This supported the hypothesis that parentage was more than likely and gave strong support to the investigation, allowing police to identify and arrest the guilty parties at a later date.

Once more, it is to be stressed that when we are faced with criminal investigations it is strongly recommended to examine a large number of both autosomal STRs as well as Y-STRs. This is the only way for forensic scientists to obtain the most complete genetic information possible and be able to assist in difficult and complex investigations, especially when only degraded or very small samples are available.

lugaro@tin.it

P-231

The use of Y STRs in rape cases associated to kinship relation

Pizzamiglio M[1], Marino A[1], Tempesta P[1] and Garofano L[1].

[1] *Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italy*

The aim of this study is to show the usefulness obtained by combining autosomal and Y STRs in casework, especially when faced with rapes in which we commonly have to analyse male/female mixed material. In particular, we refer to two rape cases in which, starting from the partial match coming from the autosomal STRs, applied on the exhibits, we decided to run the Y STRs. This allowed us to provide additional information based on parentage hypothesis that proved to be essential for investigation and the subsequent identification of the culprits.

lugaro@tin.it

P-232

DNA typing from a persimmon helps solve a murder case

Pizzamiglio M[1], Marino A[1], Tullio V[1], Denari D[1] and Garofano L[1].

[1] *Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italy*

As is well known, saliva is nowadays considered good evidence from which to obtain full DNA profiles. In this case we report about a murder in a small village near Venice in which, among other exhibits, a persimmon was collected from the garden outside the crime scene. The fruit had been bitten into by someone linked to the murder, just before he entered the victim's house.

Due to the consistency of the persimmon, and the DNA degradation caused by bacteria and fungi easily proliferated in the sugar content of the fruit, we decided to sample the small amount of saliva left by the suspect, with three dacron swabs, gently rubbed on the fruit, as soon as the evidence came into our lab. We then proceeded with DNA extraction, quantification, amplification and typing by multiplex STRs analyses, using the commercially available kits. A complete STR profile was obtained from two out of three of the swabs used. This profile was then compared with several individuals and allowed us to identify the person who bit into the fruit. This person was then interrogated, and he not only admitted his guilt but also gave to the police new leads which allowed them to catch the rest of the gang.

lugaro@tin.it

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AmpFℓSTR® Y-filer™: a new tool for rapid Y-STR forensic haplotyping

Pontes ML[1], Abrantes D[1], Lima G[1], Cainé L[1], Pereira MJ[1], Matos P[1], Pinheiro MF[1,2]

[1]Instituto Nacional de Medicina Legal – Delegação do Porto
[2]Faculdade de Ciências da Saúde – Universidade Fernando Pessoa

In addition to the standard panel of autosomal *loci* used in forensic genetics, Y-STR haplotyping gives the ability to sensitive typing of male-specific DNA especially in sexual assault cases or other situations where mixtures of male and female cells are present. Within the last years a number of Y-STR multiplex assays have been developed, most of them involving six or fewer *loci*, with some exceptions. Recently a new commercial kit has been available, the AmpFℓSTR® Y-filer™ PCR amplification kit (Applied Biosystems) that permits the simultaneous amplification of 17 Y-STR *loci*, including all the markers in the actually used European "extended haplotype" (DYS19, DYS189I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385I/II, DYS438 and DYS439) and also DYS437, DYS448, DYS456, DYS458, DYS635 and Y GATA H4. The DNA was extracted from healthy unrelated males from peripheral blood and oral swabs samples, using different methods (Chelex and phenol-chloroform). The amplified products were detected on the ABI PRISM® 3100 genetic analyser (Applied Biosystems). Fragment sizes were determined automatically using the Genescan® Anlysis Software v 3.7 and allele designations using Genetyper® Software v. 3.7 (Applied Biosystems). We present population data using this new kit, in order to apply it in our routine work.

Contact: Biologia@dpinml.mj.pt

P-234

Beware; gloves and equipment used during the examination of exhibits are potential vectors for transfer of DNA-containing material

Poy A, van Oorschot RAH

Victoria Police Forensic Services Department, Victoria 3085, Australia

Gloves are worn during the examination of exhibits in forensic biology laboratories. They are worn to protect the wearer from harmful agents and to protect the exhibit from contamination by DNA-containing material derived from the surface of the hand of the examiner. Changing of gloves between handling of different exhibits is common practice. Gloved hands however are capable of picking-up DNA-containing material from exhibits being examined and transferring this to other areas of the exhibit and/or tools being utilised whilst examining. If these tools are not adequately cleaned after examination of a particular exhibit they may become a potential vector for future pick-up and transfer of DNA derived from an exhibit examined earlier to a subsequently examined exhibit. Items that come into direct contact with exhibits such as scissor blades and forcep tongues are routinely cleaned between exhibit examinations, however, their handles, containers, tissue boxes, pipettes, examination lamps etc, that are touched by gloved hands during examination, may not.
Swabbings or tape-lifts of numerous objects that may be used or touched during the examination of an exhibit revealed that only a few provided sufficient DNA from which profiles could be generated. None of those in the highest risk category (i.e. those that could pose a direct transfer risk) contained sufficient DNA from which a full DNA profile could be generated. We found that gloves can pick-up DNA-containing material while examining exhibits and when touching other surfaces known to be contaminated with DNA-containing material. We highlight a situation where a swabbing of the top of a flexible examination lamp attached to an examination bench revealed a strong Profiler Plus DNA profile from a single individual that matched the profile from samples taken from a jacket that was examined on the bench associated with the lamp three months prior to the swabbing of the lamp.
It is recommended that examiners of exhibits from which samples may be taken for DNA analysis regularly change their gloves whilst examining exhibits, avoid contact with areas of the exhibit that are likely to be sampled for DNA analysis and to regularly clean tools and objects that they may come into contact with while examining exhibits. This is especially so for cases involving trace quantities of DNA.

Contact: roland.vanoorschot@police.vic.gov.au

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High-Resolution analysis of Y-SNPs in three populations from São Tomé and Príncipe

Prata MJ[1,2], Tavares L[1,2], Trovoada MJ[1,3], Gusmão L[1], Beleza S[1], Alves C[1], Amorim A[1,2]

[1]*Instituto de Patologia e Imunologia Molecular da Universidade do Porto*

[2]*Faculdade de Ciências da Universidade do Porto*

[3]*Departamento de Antropologia da Universidade de Coimbra*

São Tomé e Príncipe is a small archipelago situated in the Gulf of Guinea whose population history traces back to the end of the XV century when Portuguese navigators discovered the previously uninhabited islands. The first settlers were a few Europeans, mainly from Portugal, but the major contribution to the peopling of São Tomé e Príncipe was from African slaves brought, originally, from the mainland Western coast.

A very informative tool for recovering the origin and history of human populations is the analysis of patterns of genetic variation in contemporaneous populations. In previous studies we have used mtDNA and Y-STRs to characterise Angolares, Forros and Tongas, three population groups from São Tomé e Príncipe.

In order to obtain a better understanding of the demographic history of the archipelago, here we have analysed 20 Y-chromosome biallelic polymorphisms (YAP, SRY8299, 92R7, SRY1532, SRY2627, Tat, sY81, M9, LLy22g, 12f2, M109, M112, M150, M168, M213, M170, M201, M81 M78 and M269) in samples from Angolares (N=56), Forros (N=39) and Tongas (N=44).

As expected, most male lineages belonged to sub-Saharan haplogroups. However, in the whole sample from the archipelago the component of European origin reached approximately 20% in the male pool of SNP-defined lineages, contrasting with the virtual absence previously reported for the mtDNA female counterpart. The lowest percentage of putative male European ancestry was registered among the Angolares, reflecting the long history of relative isolation of the group. Globally, the Y-SNP data now obtained, afforded a significant contribution to improve the knowledge about the admixture process between Europeans and Africans that took place in São Tomé e Príncipe.

Contact: ltavares@ipatimup.pt

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Y-chromosome haplotypes and male isonymy: genetic and genealogical study in a small town of Tuscany (Buti, Italy)

Silvano Presciuttini[1], Chiara Toni[2], Donato Epiro[1], Isabella Spinetti[2], Fabio Marroni[1], Anna Rocchi[2], Ranieri Domenici[2]

[1]*Center of Statistical Genetics, University of Pisa, Italy*

[2]*Unit of Legal Medicine, School of Medicine, University of Pisa, Italy*

Introduction. In societies that use patrilineal family denominations, surnames and Y-chromosome haplotypes (YCH) follow a common pattern of inheritance. Therefore, isonymous individuals are expected to carry the same YCH, and observed inconsistencies can be ascribed to gene mutations, illegitimacies, surname polyphyletic origin, or transcription errors in the civic records. To investigate these issues in a well-defined population, we selected the village of Buti, near Pisa. This small town (about 5,000 inhabitants) maintains lively historical traditions; oral anecdotes report high level of reproductive isolation from the neighboring area. The mayor and the municipality council encouraged participation in the research.

Material and methods. A consent form has been approved by the IRB of the University of Pisa. Volunteers sign it when they donate blood or saliva for the present project at a local health facility. The entire civic records of the municipality have been acquired. In addition, the parochial archives of the main church of the village are almost intact, and were consulted for tracing specific surname genealogies back to the sixteenth century. YCHs of 12 STR markers were determined by the Y-system Promega ® commercial kit.

Results. To date, 69 males participated in the study; the sampling campaign is still open. The vast majority of the volunteers declared that all four grandparents were born in the village. Here, we report on the subjects whose surname occurred more than once in the sample, and who were unable to specify the degree of relationship with their isonymous fellow citizens. This subsample included 36 subjects distributed among 13 different surnames. Seven surnames (20 subjects) did not show variation of YCH among isonymous individuals, whereas 6 surnames (16 subjects) showed one or more locus difference, as follows:

- surname Blue included 5 subjects; two of whom carried an allele of DYS391 differing of one repeat unit from the allele of the other three, whereas another subject carried an allele of DYS392 differing of one unit from the other four; their genealogy has been reconstructed back to a founder individual (the most recent common ancestor, MRCA) who moved into the village at the end of the 16th century, the total number of meioses separating the MRCA from these five present-day descendants was 37;
- surname Pink included 3 subjects, two of whom were identical but a single step difference at locus DYS390, the other totaling 14 and 15 step differences with the first two at seven loci;
- surnames White, Brown and Yellow included two subjects each, and showed a single step difference for the loci DYS390, DYS391, and DYS385, respectively;
- surname Green included two subjects, differing for a total of 11 mutational steps in nine loci. Interestingly, one of these haplotypes was identical to a haplotype observed in Pink.

Conclusions. Among 13 different surnames including at least a pair of isonymous individuals (36 total subjects), we were able to identify six single-step mutations (two in DYS391, the others in DYS385, DYS390, DYS392 and DYS393) and two cases of historical illegitimacies. Our approach shall allow us to estimate mutation rates at STR loci with high accuracy.

Contact: sprex@biomed.unipi.it

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An unusual case of disputed paternity: predicting the effect of typing multiple siblings

Silvano Presciuttini[1], Chiara Toni[2], Isabella Spinetti[2], Anna Rocchi[2], Ranieri Domenici[2]

[1]*Center of Statistical Genetics, University of Pisa, Italy*
[2]*Unit of Legal Medicine, School of Medicine, University of Pisa, Italy*

The following case of disputed paternity has come at our attention recently. The claimant pretended to be the natural daughter of a man long deceased, survived by six legitimate children. The judiciary question verged on the probabilities of paternity exclusion (in case of false parenthood) or attribution (in case of true parenthood), provided that all the six defendants contributed their DNA for genotyping.
In case of false parenthood, incompatibility can occur only when four different alleles are present among the legitimate siblings and none of these is present in claimant's genotype. We calculated the probability of this occurrence both by an analytical method and by computer simulation. We assumed that 20 STRs were available for the analysis. The two methods produced overlapping results; in about 78% of the cases there was incompatibility in at lest one locus, in 41% there was incompatibility in at least two loci, and only in 14% of the cases there was incompatibility in at least three loci.
In case of true parenthood, we estimated the probability distribution of the paternity index (PI) by computer simulation. We obtained a probability of paternity higher than 0.999 in 99.7% of cases.
In conclusion, while in the hypothesis of false kinship only in a small percentage of cases is it possible to obtain at least three incompatibilities, in the opposite case of true fatherhood is it almost certain to reach a value for the probability of paternity > 99,9%.

Contact: sprex@biomed.unipi.it

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Experiments on the DNA contamination risk via dactyloscopy brushes

Proff C, Schmitt C, Schneider PM, Rothschild MA

Institute of Legal Medicine, University Clinic Cologne, Germany

One of the crucial tasks of a crime scene investigation is the search for latent fingerprints. In addition to using chemicals, lasers, alternate light sources, and other physical means, one of the most common techniques for the detection and development of latent fingerprints is the use of carbon black powder.
As the analysis of small amounts of DNA has been improved especially in the last years using "low copy number" typing strategies, the police more frequently requests DNA testing of latent fingerprints that are not analyzable for dactyloscopy because of smearing or incompleteness. Often enough this is the last possibility to obtain crucial information leading to a suspect by database search or to match evidence to a suspect.
In contrast to other contact stains taken directly from the evidence, latent fingerprints have normally been treated with powder using dactyloscopy brushes made from glass fibers or bird feathers. According to SOCO´s from the Cologne Police Department these brushes are typically used for several weeks up to months on numerous different crime scenes. This led us to the assumption that DNA from powder-treated fingerprints may be contaminated by DNA from other crime scenes or other evidence from the same crime scene. The area for visualization of fingerprints is selected arbitrarily which means even huge surfaces (e.g. doors) are treated with powder, and even when no fingerprint was found, human cells (e.g. skin debris, saliva) may adhere to the brush.
In a first study 14 used fingerprint brushes were obtained from police investigators and subjected to DNA analysis using standard multiplex STR typing kits. We found human DNA traces on 12 of these, partially with high amounts of DNA. Typing results using standard STR multiplex analysis mostly showed DNA mixtures from two or more persons. In one case, however, a full DNA profile from a single person was detected with high signal intensities suggesting that it may have arisen from a blood or saliva stain.
However, these results are not suitable to demonstrate the transfer of DNA adhering to the brush to a fresh fingerprint. To address the question whether such a secondary transfer from dactyloscopy brushes to other surfaces and fingerprints can be observed, experiments with artificially contaminated and used fingerprint brushes were carried out.

Address for correspondence:
Dr. Carsten Proff, Institute of Legal Medicine, Melatenguertel 60-62, 50823 Cologne, Germany;
phone +49 221 47886623, fax +49 221 4783496, e-mail: carsten.proff@uk-koeln.de

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Work in progress – Applied Biosystems GeneMapperID®

Proff C

Institute of Legal Medicine, University Clinic Cologne, Germany

The GeneMapperID® software solution for forensic identity testing is finding its way into more and more laboratories worldwide. Replacing the time consuming GeneScan® and/or Genotyper® software one expected to purchase a modern easy-to-use software that performs high quality genotyping and provides a wide range of options in the field of forensic DNA testing.
After giving a short overview on the software options, our experiences after working with the GeneMapperID® for more than one year are going to be presented. These experiences comprise the advances in comparison with formerly used GeneScan® and/or Genotyper®, the software handling, observed software bugs and improvement suggestions.
Besides this, a critical appraisal on Applied Biosystems' policy concerning the ongoing restrictions through so-called 'integrated solutions' is made. The user is forced to spend a lot of time to adopt previously used protocols, matrices and run methods on their machines for use with the newer software versions and technical solutions, such as new Matrix Standards, Data collection or GeneMapperID® versions 3.1 and 3.2. In some cases, "integration" appears to offer predefined solutions which complicate or even prevent user-defined control of the sample processing. As many users are confronted with these developments a critical discussion is necessary.

Address for correspondence:
Dr. Carsten Proff, Institute of Legal Medicine, Melatenguertel 60-62, 50823 Cologne, Germany;
phone +49 221 47886623, fax +49 221 4783496, e-mail: carsten.proff@uk-koeln.de

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Monte Carlo Bayesian Identification Using STR Profiles

Promish DI

The method described here bears out the premise that a complete CODIS 13 STR profile contains all the information needed to establish the probability of identity of two individuals having the same profile. In order to do so, the method combines three concepts: the concept of the culprit as a member of a group; the concept of the suspect as a one-person group; and the concept of groups, other than the suspect, which are distinguishable only by their homozygosity. This presentation shows how to perform a Bayesian analysis of an STR profile with respect to the suspect group and a random sample of non-suspect groups. It demonstrates the robustness of the method with respect to a varied selection of real profiles and with respect to evidence other than the profiles.
The Monte Carlo Bayesian (MCB) method described here has several features worth noting.
(a) The method is case-specific. Both evaluation of and adjustment for substructure are automatic, and they are unique to the STR profile at issue.
(b) The method accommodates variation in prior probabilities according to the investigator's judgment regarding non-profile data.
(c) The method produces probabilities not likelihood ratios.
(d) The method does not rely on reference group allele frequency data. The investigator can use the method when she/he lacks either knowledge of, or immediate access to, suitable frequency data.
In addition to the features mentioned above, the case analysis results shown in this presentation lead, through a series of subordinate conclusions, to one major one.
Here are the subordinate conclusions.
(a) The results of a Monte Carlo Bayesian (MCB) analysis appear to be consistent with what an experienced investigator might infer by inspection.
(b) Because MCB relies only on within-locus allele differences, it can analyse encrypted (real) profiles. It thus has potential for use where personal privacy is of concern.
(c) Consequently, MCB can also analyse synthetic profiles, such as the extreme case of an individual who is homozygous at all of the CODIS 13 loci. The results of such a case lead to the following major conclusion.
It seems safe to say that any "cold hit", regardless of substructure, is at least a very good investigative lead; and that a profile match coupled with a "minimum probable cause" prior amounts to an investigative, if not a juridical, certainty.

Because the initial work on this subject tacitly assumed that crime scenes yield complete CODIS 13 profiles, this presentation will explore the sensitivity of MCB to reduction of the profile, particularly by elimination of the more informative loci.

Contact: DonaldPromish@cs.com

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Efficiency comparison of seven different *Taq* polymerases used in hemogenetics.

Purzycka JK [1], Olewiecki I [1], Soltyszewski I [1], Pepinski W [2], Janica J [2]

[1] *Central Forensic Laboratory of Police, Warsaw, Poland*[1]
[2] *Department of Forensic Medicine, Medical University of Bialystok, Poland*

Currently, STR typing is the most efficient and the fastest way for identification of biological material samples from humans. Automated analysis of dye-marked PCR products processed during capillary electrophoresis provides with accurate and precise results. However, efficacy of DNA analysis largely depends on DNA degradation which is related with sample aging and storage conditions. Objective of the study was to compare the enzymatic efficiency of polymerases used for analysis of degraded low concentration DNA templates. Nine different polymerase brands have been used: AmpliTaq Gold DNA Polymerase (Applera), OptiTaq DNA (EURx), Taq DNA Polymerase (EURx), Perpetual Taq DNA Polymerase (EURx), DNA Polymerase (Biotools), DNA Polymerase - Gel form (Biotools), Platinum Taq DNA Polymerase (Invitrogen), Taq DNA Polymerase, Recombinant (Invitrogen), JumpStart Taq DNA Polymerase (Sigma). DNA was extracted by organic method from dried blood samples collected from 30 non-related individuals in 1955 at the Department of Forensic Medicine, Medical University of Bialystok. The samples were stored in paper envelopes at room temperature and constant humidity. Recovered DNA was quantitiated fluorometrically using PicoGreen dsDNA Quantitation Reagent (Molecular Probes) and Fluoroscan Ascent FL (Labsystems). DNA quality was assessed by 2% ethidium bromide agarose gel electrophoresis. Different amounts of DNA from 0.25 to 1.25 ng were used as a PCR template. AmpFlSTR SGM Plus kit and ABI 3100 Sequencer (Applera) were used to obtain genetic profiles. Significant differences in polymerase efficiency in relation to DNA template degradation were observed.

Contact: pepinski@amb.edu.pl

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Cytochrome b. An alternative to cytochrome oxidase as a species-specific marker in Forensics

Ramos de Pablo R[1,2], Saloña M[2], Sarasola E[1,2], Sergio Cardoso S[1,2] and Martínez de Pancorbo M[1,2]

[1]*Servicio de Genómica: Banco de ADN. Universidad del País Vasco. Vitoria-Gasteiz. Spain*
[2]*Dpto. de Zoología y Biología Celular Animal. Universidad del País Vasco. Leioa (Bizkaia). Spain*

Several DNA sequences such as those belonging to the mitochondrial genome (e.g. cytochrome b) comprise species-specific information which has shown to be a quick and reliable choice in forensic as well as in phylogenetic investigations (Parson et al. Int J Legal Med. 2000; 114: 23-28). Methodologies based on these type of sequences, allow the identification of carrion flies specimens which is a fundamental first task in forensic entomological studies. Unfortunately, species are frequently rather difficult to establish, since (a) adults may have not been collected during a forensic prospect and (b) eggs and larvae are fairly hard to distinguish morphologically at specific level until they have not been developed to adults.

The purpose of this work has been to develop a proper methodology based on mitochondrial cytochrome b gene to allow the identification of entomological species pertaining to forensic casework.

To achieve this goal, seventeen third-instar larvae previously identified as *Steribia nigriceps* by morphological means, were utilized. DNA was extracted as follows: cell lysis was performed using proteinase K and SDS. Next, DNA was purified using the phenol/chloroform method. A 358 bp fragment of the cytochrome b was amplified via PCR under adjusted conditions after Parson et al. (2000). All the samples were sequenced in an automatic ABI Prism 310 DNA sequencer. PCR products were separated by electrophoresis in agarose gels stained with ethidium bromide and visualised under UV-illumination.

A consensus sequence of 305 bp was obtained from the aforementioned procedure. Then, this sequence was submitted to a BLAST search at NCBI (www.ncbi.nlm.nih.gov/BLAST). This process permitted to locate the nearest species, and so work on phylogenetic studies. It is important to highlight that there is a sequence of 86 bp which is equal in all the studied specimens genome. This unique characteristic could be utilized as a species-specific marker for this species, as it only possesses dissimilarities at the interspecific level. These results indicate that the characterization of insects via the mitochondrial cytochrome b gene is fairly trustworthy.

Contact: vitoriajulio2004@yahoo.es

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Common Y-chromosomal STR database for three closely related European populations

Rębała K[1], Mikulich AI[2], Tsybovsky IS[3], Siváková D[4], Szczerkowska Z[1]

[1]*Department of Forensic Medicine, Medical University, Gdansk, Poland*
[2]*Institute for the Study of Arts, Ethnography and Folklore, National Academy of Sciences, Minsk, Belarus*
[3]*Institute of Problems of Criminology, Criminalistics and Forensic Expertise, Minsk, Belarus*
[4]*Department of Anthropology, Comenius University, Bratislava, Slovakia*

A unique inheritance pattern and specificity to males has made the human Y-chromosomal short tandem repeat (Y-STR) markers an excellent tool in male kinship analysis, genealogical studies and discrimination of male DNA in male/female stain mixtures. However, extensive analysis of Y-STR polymorphism throughout Europe has shown significant differences in allele and haplotype distribution even between closely related human populations. Therefore, establishment of a common Y-STR haplotype frequency database for different European populations appeared to be impossible. Since homogeneity of paternal lineages determined by analysis of minimal haplotypes has been shown between 6 regional populations in Poland, the aim of this study was to compare usefulness of 18 Y-chromosomal microsatellites in forensic practice in the Polish population and two other closely related Slavic populations of Belarus and Slovakia, and to check for the possibility of the creation of a common Y-STR haplotype frequency database for forensic purposes.Y-chromosomal microsatellites were genotyped in 568 randomly selected, unrelated males: 196 Belarusians, 208 Poles, and 164 Slovaks, by means of a multiplex (octadecaplex) PCR reaction and capillary electrophoresis using an ABI Prism 310 Genetic Analyzer. The loci analysed included DYS19, DYS388, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS426, DYS437, DYS438, DYS439, DYS460, GATA H4.1, DYS385 a/b, and YCAII a/b. Allele designation was based on comparison with the constructed allelic ladder. In order to check for a marker's potential for resolution of similar haplotypes, contribution of each system to the discrimination capacity was calculated. Analysis of molecular variance (AMOVA) was performed using Arlequin 2.000 software. Markers responsible for the differences between populations were identified using a chi-square test for homogeneity and locus-by-locus AMOVA. The overall haplotype diversity value ranged from 0.9982 in the Polish population to 0.9992 among Belarusians and Slovaks, while discrimination capacity was 93.4%, 92.3%, and 94.5% for Belarusians, Poles and Slovaks, respectively. The most polymorphic system was DYS389 in the Belarusian population, whereas among Poles and Slovaks, the highest gene diversity was found in DYS385. The most valuable marker in discrimination of similar haplotypes was DYS389 while DYS426 and DYS438 did not affect the discrimination power of the multiplex in all three populations. AMOVA revealed significant differences between the populations and excluded possibility of one common Y-STR database. Analysis of haplotypes defined only by markers showing homogeneity within the three populations (DYS388, DYS389I, DYS389II-I, DYS393, DYS426, DYS460, YCAII a/b) showed that the whole genetic variation was attributable to the variation within populations and enabled establishment of a common database with haplotype diversity equal to 0.9632. For databases combined for pairs of populations, the number of available loci increased (up to 13 loci in case of a Belarusian-Slovak database) and the power of discrimination was higher. The studied Y-STR loci define very informative haplotypes for population-genetic and forensic investigations. A constantly growing number of Y-chromosomal microsatellites available for research enable selection of markers for haplotype databases common for closely related populations, so that discrimination power of such haplotypes may reach a level acceptable in forensic casework.

Prof. Zofia Szczerkowska, PhD Department of Forensic Medicine, Medical University of Gdansk
ul. Marii Skłodowskiej-Curie 3A, 80-210 Gdańsk, Poland e-mail: szczerko@amg.gda.pl

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SampleCheck, an information management system for quality assurance of DNA-profile analysis in parentage testing

Reitz P, Jung M

bj-diagnostik GmbH, Kerkrader Str. 11, 35394 Giessen, Germany

To increase the validity of laboratory results SampleCheck performs plausibility checks on DNA profiles for every batch processed. This platform independent programme interacts with a database storing DNA profiles and further information. The main function of SampleCheck is tp perform plausibility checks on DNA profiles of a batch to detect pipetting errors, contaminations with other DNA and if samples during collection or pipetting may have been interchanged. Checks can be performed batchwise and/or against the whole database. Samples of each family in a batch can also be checked for exclusions and exchanges. Every sample is monitored for correct gender by comparing expected and measured gender. At the same time an alignment of the measured profiles and the profiles of coworkers and positive controls takes place. Additionally a single sample or the samples of a whole batch can be checked for identical profiles and profiles sharing a common allele on each marker like in father-child and mother-child relations. Allele frequency tables for diferent populations, mutation rates, coworkers and positive control profiles can be stored. SampleCheck enables the user to import this information from different file formats to the database. LR-values can be calculated using three different methods.

Contact: michael.jung@bj-diagnostik.de

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Quantification of human DNA by Real Time PCR in forensic casework

Ricci U[1], Marchi C[2], Previderè C[3], Fattorini P[4]

Azienda Ospedaliera-Universitaria "A.Meyer", Florence, Italy
[1]U.O. Genetica Medica
[2]Laboratorio di Analisi Chimico Cliniche e Microbiologia[2]
[3]Dipartimento di Medicina Legale e Sanità Pubblica, Università di Pavia, Italy
[4]Istituto di Medicina Legale di Trieste, Italy

The estimation of the amount of human DNA is a recommended procedure in forensic casework. We extensively employed a Real-time quantitative PCR system together with a commercially available kit (Quantifiler™ Human DNA Quantification Kit, Applied Biosystems), for the quantification of human DNA in a large variety of samples. Preliminarly, fifty DNA extracts from blood, thirty from bone marrow, ten from fresh bone, twenty from saliva and various swabs from gloves used by a known donor, were dosed with this kit and compared with the spectrophotometric determination at 260 nm. The method was then used to quantify forensic DNA extracts from blood stains, sperm, vaginal swabs, hairs, old bones and teeth and DNA recovered from touched objects. In many cases duplicated analyses were performed to evaluate the reliability of the results. Generally, a low deviation standard for the same sample was observed. However, when the inhibitors in a sample were in high concentration and/or DNA degradation was present (like in post-mortem matrices), we observed variable results of quantification in the same extract. In forensic samples from touched objects or containing low copy number DNA, PCR amplifications with commercial and home-made kits using dosed template DNA with Real-time, were performed. In these samples it is very important to know the DNA concentration to state how many replicate analyses are required for statistically reliable results. Moreover, DNA extracts from vaginal swabs containing sperm and vaginal cells were also dosed with a Y-specific human DNA system (Quantifiler™ Y - Human DNA Quantification Kit, Applied Biosystems), to evaluate the proportion of the male component in these samples.
PCR amplification reactions were prepared from these samples, using AmpF*l*STR® Profiler Plus™ (Applied Biosystems) and a Y-specific amplification kit (PowerPlex® Y System, Promega). Here, we discuss the correlation between DNA quantification and PCR amplification in different forensic samples normally recovered in forensic casework.

contact: u.ricci@meyer.it

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Analysis of twelve X-chromosomal short tandem repeats in the Northwest Italian population by means of two multiplex PCRs

Robino C, Giolitti A, Gino S, Torre C

Department of Anatomy, Pharmacology and Legal Medicine, University of Turin, Turin, Italy

The analysis of short tandem repeats (STR) located on the X chromosome can effectively complement autosomal STR data in selected cases of kinship testing: deficiency paternity cases; paternity cases involving close blood relatives as alternative alleged fathers; maternity testing of male children. The use of X-STRs in forensic practice requires a precise knowledge of the population genetics properties of these markers: degree of polymorphism and allelic distribution in different population samples, testing of Hardy-Weinberg equilibrium, possible evidence of linkage disequilibrium. At present, population data on X-STRs available in forensic literature are however rather limited.
In this study, twelve of the most commonly used X-STRs were analysed in a sample of 140 unrelated Italians (70 females and 70 males) residing in Piedmont (Northwest Italy) by means of two multiplex PCRs. Multiplex PCR "I" included markers DXS6789, HumARA, GATA172D05, DXS101, DXS8378, and DXS8377; PCR "II", loci DXS7132, DXS6800, DXS6803, DXS7424, HPRTB, and DXS10011. Typing was done on ABI PRISM 310 Genetic Analyzer in comparison to sequenced allelic ladders and control DNA (K562 cell line).
Allelic frequencies of the twelve X-STR loci in the Northwest Italian population were determined by direct gene counting: a duplication at the locus DXS6789 was observed in a male individual. All loci were found to be in Hardy-Weinberg equilibrium. No significant differences in the allelic frequencies of female and male samples were observed by exact test. Parameters of forensic interest - heterozygosity, polymorphism information content (PIC), power of discrimination in females (PD^F) and males (PD^M)- were calculated. Heterozigosity of X-STR loci ranged between 0.942 (DXS10011) and 0.686 (DXS8378); PIC between 0.935 (DXS10011) and 0.605 (DXS8378). Locus DXS10011 showed the highest values of PD^F (0.992) and PD^M (0.934), while DXS8378 the lowest: PD^F (0.832) and PD^M (0.646). Inter-marker linkange disequilibrium was analysed in the male sample: significant linkage disequilibrium ($p < 0.01$) was observed for the closely linked loci HumARA and DXS7132.

Contact: carlo.robino@unito.it

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Gene frequencies of six miniSTR in Tuscany (Italy)

Anna Rocchi[1], Isabella Spinetti[1], Chiara Toni[1], Silvano Presciuttini[2], Ranieri Domenici[1]

[1]*Unit of Legal Medicine, School of Medicine, University of Pisa, Italy*
[2]*Center of Statistical Genetics, University of Pisa, Italy*

In a recent work [1], six STR loci with amplification products smaller than 150 bp have been characterized using a protocol involving two triplexes. These loci, called "miniSTR", represent promising tools for recovering genetic information from degraded DNA samples for which the currently used loci generate partial profiles.

Here, we report preliminary population data from a sample of unrelated subjects born in Tuscany. The following table shows the number of alleles detected and the expected heterozygosity.

Marker	N. alleles	Heterozygosity
D10S1248	8	0.794
D14S1434	5	0.777
D22S1045	5	0.785
D1S1677	7	0.841
D2S441	7	0.834
D4S2364	5	0.751

1. Michael D. Coble, John M. Butler. Characterization of New MiniSTR Loci to Aid Analysis of Degraded DNA. J Forensic Sci. 50 (1):43-53 (2005)

Contact: sprex@biomed.unipi.it

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Allele frequency of 12 Y-STR loci in the Brazilian population from South Brazil

Rodenbusch R[1], Mardini AC[1], Estivalet AAF[1], Gastaldo AZ[1], Schumacher S[1], Albarus MH[1], Giugliani R[1,2], Saraiva-Pereira ML[1,3].

[1]*Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil*
[2]*Genetics Department, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*
[3]*Biochemistry Department, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*

Genetic individuality is based on genome variability among individuals. Analysis of short tandem repeats (STR) polymorphic markers is commonly used in paternity testing as well as forensic cases. Although autosomal STRs are commonly used in these cases, STRs in Y-chromosome can provide useful information in paternity investigation cases where alleged father cannot be tested and in other investigation cases where paternal lineage identification can be assessed. The aim of this study was to determine, in the regional population, allele frequency of 12 *loci* (DYS19, DYS385a, DYS385b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, and DYS439) included in PowerPlex Y kit (Promega) and mutation rate in each loci. Population tested was composed by 162 pairs father/son that paternity was previously tested and confirmed using autosomal STRs, with a minimal probability of 99,99%. DNA isolation was performed from 300µl of each sample using the Wizard® Genomic DNA Purification Kit (Promega), according to manufacture instructions. Regions of interest were amplified by multiplex-PCR using fluorescent primers, using PowerPlex Y kit (Promega). Amplification products were analyzed in an ABI Prim® 3100 Genetic Analyzer, and GeneScan® and Genotyper® software. In this sample population, we have found 151 distinct haplotypes and mutation was reported in the following *loci*: DYS19, DYS390, DYS439, and DSY437. These results allowed the establishment of allele frequencies for the above Y-STRs in our population. These frequencies can be now used in our lab, a reference center for paternity cases, mainly in paternity cases where the alleged father cannot be tested. We were also able to determine the limited mutation rate in our population using this set of markers. Finally, this study allowed the establishment that these STRs show a high discriminating rate in our population, being valuable to be employed in either cases of paternity testing as well as forensic genetic.

contact: mlpereira@hcpa.ufrgs.br

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Evaluation of 12 single-copy and 2 multi-copy Y-chromosomal STR loci in five German populations

Rodig H[1], Grum M[1], Grimmecke H-D[1], Roewer L[2]

[1]*Biotype AG, Moritzburger Weg 67, 01109 Dresden, Germany*
[2]*Institute of Legal Medicine, Charité - Universitary Medicine, Hannoversche Str. 6, 10115 Berlin, Germany*

The Minimal Haplotype (MH) loci, definitely DYS19, DYS385, DYS389-I, DSY389-II, DYS390, DYS391, DYS392, and DYS393 were evaluated by the International Forensic Y-User Group as a standard for Y-chromosomal STR-typing. In the meantime the complete sequence of the Y chromosome was screened for microsatellites and further highly discriminating Y-STR loci were identified [1, 2].
We have developed two multiplex applications for Y-STR typing. One multiplex which is also commercial available is suitable for the analysis of the MH loci, named Mentype® Argus Y-MH PCR Amplification Kit.
A second multiplex was designed for DYS446, DYS447, DYS448, DYS449, DYS463, and DYS464 typing. These Y-chromosomal STRs were evaluated with regard to gene diversity (D) in 5 German population groups. The resulting data showed DYS464 (D = 0.7733 - 0.9446), DYS449 (D = 0.7844 – 0.8511), and DYS385 (D = 0.7511 – 0.8433) on the top of the newly established ranking list. The need for further loci was shown in one forensic case where the two haplotypes of the piece of evidence and the suspect person could only be discriminated by DYS446.

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Correspondence to

Dr. rer. nat. Heike Rodig, Biotype AG, Moritzburger Weg 67, 01109 Dresden, Germany
Tel.: +49-351-8838400, Fax: +49-351-8838403
E-Mail: h.rodig@biotype.de

P-250

The male genetic history of the Sorbs – a Slavic island population in Germany

Roewer L[1], Willuweit S[1], Rodig H[2], Groß A[2], Weidlich S[2], Kayser M[3], Nagy M[1]

[1]Institute of Legal Medicine, Charité-University Medicine Berlin, Hannoversche Str. 6, 10115 Berlin, Germany
[2]Biotype AG, Moritzburger Weg 67, 01109 Dresden, Germany
[3]Department of Forensic Molecular Biology, Erasmus University Medical Centre Rotterdam
PO Box 1738, NL-3000 DR Rotterdam, The Netherlands

The about 67.000 Sorbs living in the provinces of Upper and Lower Lusatia represent the only autochthonous Slavic-speaking population in Germany. Linguistically the Sorbs belong to the Western Slavs and are closely related to the Poles and Czechs. Whereas the ancient Sorbs living in Poland underwent complete assimilation, they managed to exist as a bilingual minority in Eastern Germany until today.
The Y-chromosome haplotype analysis in a sample of 30 unrelated Sorbian males reveals a significant distance to the German neighbouring populations and a close vicinity to the Polish population samples. Nevertheless, the most frequent Sorbian Y-chromosomal minimal haplotype 17,13,**31**,25,10,11,13,10-14 typed in roughly 14 % of the analysed 30 Sorbs is relatively rare in Poland (Fig.1, Fig.2).
A closer look reveals a one-step deviation of this Sorbian modal haplotype from that of the Poles (17,13,**30**,25,10,11,13,10-14; highest frequencies in Wroclaw – 7.4%, Krakow – 6.5 %, Warsaw 5.41 %, Gdansk 4.2 %).
The working hypothesis is to assume that the isolation of the early Sorbian (*Surbi*) families on the Eastern Franconian territory (colonization of Lusatia by the Franks starts in 1104) and later in Germany and its separation from Poland and the Bohemian/Czech territories led to the observed differentiation. Only half of the Sorbian population survived the Thirty-year-war 1618-48. This bottleneck may further explain the current reduced haplotype diversity.
The in-depth analysis of the Sorbian modal haplotype and its neighbours by up to 39 Y-STR markers reveals a recent split of the Sorbian-Polish lineages. This fits well with the hypothesis of a separation of the Lusatian Sorbs from the Western Slavic Poles within recent historical time. Contact: lutz.roewer@charite.de

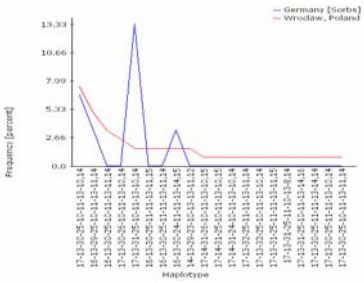

Fig. 1. 20 most frequent haplotypes in Sorbs and Poles (Wroclaw)
Fig.2 Distribution of the Sorbian modal haplotype among YHRD population samples

P-251

A novel approach for genotyping of LCN-DNA recovered from highly degraded samples

C. Romano, E. Di Luise, D. Di Martino, I. Ciuna and L .Saravo*

[1] *Laboratory of Molecular Biology – Raggruppamento Carabinieri Investigazioni Scientifiche (RaCIS), 98128 Messina – ITALY.*

Nowadays Short Tandem Repeats Polymorphisms are the most utilized genetic markers in forensic biology. They have become the markers of choice for human identification protocols applied in case of mass fatality disaster. Unfortunately in the latter event STR-based methods easily result in amplification failures due to the extremely low DNA quality together with the presence of contaminants.

A new kind of STRs profiling system is based on the amplification of shorter fragments compared to the conventional STR multiplexes; because of their length, these "Mini" STR amplicons could be obtained even from extremely degraded DNA and/or from very few copies of template DNA. Mini STR kits for Human Identification have been already validated and placed on the market; moreover they are related to those loci which are commonly detected by traditional STRs kits, allowing comparison between data yielded with both methods. For all the above mentioned reasons, "Mini" STR represents more suitable and robust markers system which could be applied even in case of spoiled completely burnt and anyway degraded DNA samples.

In this paper we report an homicide casework occurred in the South of Italy: a young guy was killed in a farmhouse and then burnt. Nothing inside could be collected apart a burnt stub which was found totally submerged in mud and wet ash. It was the only sample we could process in short time in order to perform a comparison with the likely family of origin and then with the burnt organic residuals. Hence a comparative genotype analysis between STRs and "Mini" STRs was carried out in order to verify reliability, efficiency and discrimination power of the latter ones.

Our results show that "Mini" STRs permit a reliable determination of 9 human allelic loci whereas traditional STRs typing protocols offer only a partial resulting genotyping profile heavily reducing the chance of identification.

In our experience "Mini" STRs turned out to be more robust and sensitive markers than the traditional ones. Our next aim will be extending this method to several kinds of degraded samples in order to confirm the above mentioned observations.

Keywords: DNA STR typing; Forensic casework; Mentype®.

*Corresponding author: rismebiologia@carabinieri.it

P-252

Validation of the AmpFℓSTR® Yfiler™ kit

Romero R.E., Lizarazo R

Grupo de Genética Forense. Instituto Nacional de Medicina Legal y Ciencias Forenses. Bogotá - Colombia

The use of Y chromosome STR markers is of grate utility in forensic cases, especially in sexual assault investigations. Recently it has been great improvement in commercial kits that offer large multiplex reactions in a single step, systems whit high discrimination power and reliable and reproducible results.

The AmpFℓSTR® Yfiler™ kit is the most recent commercial product of Appiled Biosystems that offers 17 STR from human Y chromosome DYS19, DYS385a, DYS385b, DYS389 I, DYS389 II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS448, DYS456, DYS458, DYS635, Y GATA H4, DYS438 and DYS439.

In this work several aspects were assayed. Differences in equipment used for PCR amplification, sensitivity and specificity and application on forensic cases with low concentrations of human genomic DNA.

Contact: rosa.romero@javeriana.edu.co

P-253

The genetics of pre-Roman Iberian Peninsula: a mtDNA study of ancient Iberians

Sampietro M.L[1], Caramelli D[2], Lao O[1], Calafell F[1], Comas D[1]., Lari M[2]., Agustí B[3], Bertranpetit J[1], Lalueza_Fox, C[1*]

[1] *Unitat de Biologia Evolutiva, Dept. Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, Barcelona,Spain*
[2] *Dipartimento di Biologia Animale e Genetica, Laboratori di Antropologia, Università di Florence, Italy*
[3] *Museu d'Arqueologia de Catalunya, Girona*
[*] *Present address: Unitat d'Antropologia, Dept. Biologia Animal, Universitat de Barcelona, Barcelona*

The Iberians developed a surprisingly sophisticated culture in the Mediterranean coast of the Iberian Peninsula from the 6th century BC to their conquest by the Romans in the 2nd century BC. They spoke and wrote a non-Indo-European language that still cannot be understood; their origins and relationships with other non-Indo-European peoples, like the Etruscans, are unclear, since their funerary practice was based on the cremation of the bodies, and therefore, anthropology has been unable to approach the study of this people. We have retrieved mitochondrial DNA (mtDNA) from a few of the scarce skeletal remains preserved, some of them belonging to ritualistically executed individuals. The most stringent authentication criteria proposed on ancient DNA, such as independent replication, aminoacid analysis, quantitation of template molecules, multiple extractions and cloning of PCR products, have been followed to obtain reliable sequences of the mtDNA hypervariable region 1 (HVR1) as well as some haplogroup diagnostic SNPs. The phylogeographic analyses show that the haplogroup composition of the ancient Iberians was very similar to that found in modern Iberian Peninsula populations, suggesting a long-term genetic continuity since pre-Roman times. Nonetheless, there is lesser genetic diversity in Iberians than among modern populations, a fact that could reflect the small population size at the origin of the population sampled and the heterogenic tribal structure of the Iberian society. Moreover, the Iberians were not specially close to the Etruscans, which points to a considerable genetic heterogeneity in Pre-Roman Western Europe.

contact: lourdes.sampietro@upf.edu

P-254

The Human Genome Diversity Project of Iran

Mohammad Hossein Sanati, M. Houshmand, M. M. Banooi, F. Mirzajani, F. Mahjoubi.

National Institute for Genetic Engineering and Biotechnology-Tehran, Iran

Iran is situated on the route to Central Asia and Turkey as well as western countries. Different ethnic groups live in Iran, among which Farsis, Kurds, Lors, Balooches, Bakhtiaris, Azari Turks, Taleshes, Turkamans, Qashqais and Arabs may be pointed out. Smaller ethnic groups also live in Iran. Turkamans, who live in Turkaman Sahara and north of Khorasan, are different from other Iranian ethnic groups in appearance, language, and culture. Qashqais, who are of Turkish origin, live in the central part of Iran. Arab clans mostly live in Khuzistan and are scattered along the coastlines of Persian Gulf.

Some groups of colored people, who are the descendants of slave trade with Zanzibar, are scattered in the southern provinces of Iran. The existing minority in the south of Iran also descends from Indian traders of past times.

Samples (1981 Individuals) from individuals within each of these populations were collected (1336 males and 612 males) and the DNA content was analyzed to produce data on the frequency of occurrence within the population of an agreed set of alleles or other genetic markers. In order to establish a resource that would be available for many years and that would allow future scientists to study any polymorphism, and in order to provide a back-up source of original sequence DNA, all blood samples were used to develop cell lines.

Contact: massoudh@nrcgeb.ac.ir

P-255

Peopling, demographic history and genetic structure of the Azores Islands: Integrating data from mtDNA and Y-Chromosome

Santos C[1,2], Montiel R[3], Bettencourt S[3], Prata MJ[4], Abade A[2], Aluja MP[1], Lima M[3]

[1]Unit of Anthropology, Department BABVE, Autonomous University of Barcelona, Spain
[2]Department of Anthropology, University of Coimbra, Portugal
[3]Center of Research in Natural Resources (CIRN), University of the Azores, Portugal
[4]Institute of Pathology and Molecular Immunology of the University of Porto (IPATIMUP), Portugal

Nine islands, clustered in 3 geographical groups, Eastern, Central and Western, form the Archipelago of the Azores. The archipelago has an area of 2 344 Km^2 and a total population of 237 315 inhabitants, distributed in a very asymmetric way among the three groups of islands (134 885 inhabitants in the Eastern, 98 101 in the Central, and only 4 329 inhabitants in the Western group). The Islands were discovered uninhabited by Portuguese navigators in the early 15th century. Starting from the islands of the Eastern group, the process of settlement was initiated by 1439 and lasted for over a century. Main imports came from Mainland Portugal and Madeira Island; immigrants from other European regions (Flanders, Spain, Italy, France, England, Germany, and Scotland) made up part of the first groups of settlers but, with exception of the Flemish, their overall contribution is thought to be less than that of the Portuguese groups. An African influence, derived mainly from the contribution of Sub-Saharan and Moorish slaves, is also reported. Furthermore, there is evidence that Sephardic Jews, expelled from the Iberian Peninsula, also contributed to the peopling of this Archipelago. Historical documents, however, are not sufficient to provide accurate information concerning the demographics of settlement, and do not report on how these distinct contributions were distributed among the 3 groups of islands. Genetic characterization of the Azorean population should allow the reconstruction of a more comprehensive picture of these processes. We have conducted studies to assess the variability of mtDNA and Y-chromosome markers and found that for both genetic systems the Azores Islands, as a whole, fit well into the pattern of variation described for other Western European populations. Phylogeographic analysis of mitochondrial DNA (mtDNA) showed a major contribution from Mainland Portugal as well as evidences of influxes from Northern Europeans, Africans, and Jewish groups. Characterization of Y-chromosome (NRY) markers has shown a main component of European chromosomes and also the presence of North African chromosomes in frequencies similar to those described for mainland Portugal. On the other hand, both mtDNA and Y-chromosome analysis have shown differential demographic histories for the three groups of islands forming the archipelago, especially on what concerns the Western group. This group showed a very atypical distribution of mtDNA haplogroups attributable to genetic drift but not to a differential female settlement history. However, an assessment of the NRY variability, and its comparison with mtDNA variability, evidenced a differential composition of males during the settlement of the three groups of islands, contrary to what has been previously deduced for the female settlers using mtDNA data.

Contact: mcbettencourt@notes.uac.pt

P-256

Subtyping of D7S820 alleles in African-American population using two SNPs: rs7786079 and a new one described in this work

Sarasola E[1], González-Fernández MC[1], Ferández del Pozo V[1], Cardoso S[1], Builes JJ[2], Moreno MA[2], Bravo MLJ[2], Martínez de Pancorbo M[1]

[1]Servicio de Genómica: Banco de ADN, Universidad del País Vasco, Vitoria-Gasteiz. Spain
[2]GENES Ltda, Laboratorio de Genetica Forense y Huellas Digitales del DNA, Medellin, Colombia.

The locus D7S820 is located 65 bp downstream the SNP rs7786079. The design of new primers with the aim to obtain minialleles of this locus showed a new SNP in African individuals. This new SNP is located 115 bp upstream the D7S820. This work is based on the study of these two SNPs, one of them described for the first time. The aim of this study is the differentiation between D7S820 alleles carrying the same number of repetitions. The SNP rs7786079, with A and C alleles, has been studied using allele-specific polymerase chain reaction. The second one, not yet included in the databases (NCBI SNP database or www.ensebml.org) presents C and T alleles and was studied with a simple PCR followed by digestion with Sml I restriction enzyme.

Sixty four African-American samples and 34 Antioquian (Colombia) samples were analyzed. The heterozygosity of rs7786079 was 0.198 in African-American sample, whereas the Antioquia sample was A homozygous. The other SNP showed a heterozygosity of 0.167 in African-American sample and 0.019 in Antioquian individuals. These results indicate that the SNPs here studied are not discriminative of D7S820 alleles in Antioquian population. This is coincident with rs7786079 informativeness in Caucasian populations.

The disequilibrium test in African American population sample has shown that these SNP loci are closely linked, being this linkage A-C and C-T. This data is indicative that only one or both SNPs can be used to perform allelic discrimination between D7S820 alleles with the same number of repetition units.

In addition, these SNP loci do not show close linkage with D7S820 alleles, probably due to the higher mutation rate of the microsatellite loci. Taking this into account, it is possible to discriminate between D7S820 alleles combined with these SNPs. The Power of Discrimination values observed range from 0.252 to 0.315 for different alleles.

In conclusion, the genetic identification based on D7S820 locus in African American population could be improved combining the analysis of this microsatellite locus with the SNPs close to it.

contact: zobsadie@vc.ehu.es

P-257

Fetal sex determination from maternal plasma by nested PCR of the Amelogenin gene.

Sarasola E[1], Martínez de Pancorbo M[1], Martín-Vargas L[2], Melchor JC[3], Rodríguez-Alarcón J[2]

[1]*Serviciode Genómica: Banco de ADN. Universidad del País Vasco. Vitoria-Gasteiz. Spain*
[2]*Dpto. de Pediatría, Hospital de Cruces, Barakaldo. Bizkaia. Spain*
[3]Dpto. de Obstetricia y Ginecología, Hospital de Cruces, Barakaldo. Bizkaia. Spain

Fetal sex determination from circulating DNA in maternal plasma has been reported to be a reliable way to avoid invasive prenatal tests, such as amniocentesis or chorionic villus sampling, in pregnancies with high risk of X-linked disorders. This plasma circulating DNA is highly degraded, which makes more difficult its detection. Highly degraded DNA is common in forensic casework. The experience accumulated in manipulating forensic samples has allowed us to develop the methodology described here. The study was carried out in 22 samples from both male/female pregancies, fact which was unknown by the investigator while developing the tests. Cell-free fetal DNA was extracted from maternal plasma using phenol-cholorophorm method and the amelogenin gene was amplified by PCR. This amplification results in one fragment of 106 bp when only the X chromosome copy of the gene is present, or two fragments of 106 and 112 bp when both copies from X and Y chromosome are present. This 6 bp difference is the base of the next step, which consists in the reamplification of the previous PCR products with a primer whose 3'-end only anneals to that different 6 bp. From the 22 samples analyzed, 10 where correctly sexed as male carrying pregnancies and 11 as female carrying pregnancies. Only one female pregnancy resulted in nested PCR positive amplification. The method described here has detected one false positive, but no false negative so it could be considered a reliable approach to fetal sex determination.

contact: zobsadie@vc.ehu.es

P-258

Ancient DNA Analysis from medieval and Etruscan bones

L . Saravo*, S.Spitaleri , N. Staiti, E. Di Luise, C. Trapani and C. Romano

[1] *Laboratory of Molecular Biology – Raggruppamento Carabinieri Investigazioni Scientifiche (RaCIS), 98128 Messina – ITALY.*

DNA extraction and genotyping from ancient bones represent a great challenge for scientists investigating both on archaeological and population genetics topics. The possibility to obtain an amplifiable nuclear DNA from such items is still fully debated. Here we report our experience on some medieval and Etruscan bones
found during the excavation of different burial sites in Tuscany. Bones were extremely spoiled in outer layer but well conserved as regards to the inner tissue. Nevertheless we had to dramatically improve extraction efficiency by means of an experimental procedure. Firstly we had to purify DNA from environmental pollutants, as humic acids, and obviously from exogenous DNA. Secondly particular extraction adjustments were performed to eliminate shorter DNA fragments in order to avoid the annealing mismatch during the amplification phase. PCR was carried out by using both a 15 loci traditional STRs system and another forensic kit based on shorter STR amplicons (i.e. "Mini" STRs). Result showed that "Mini" STRs are less affected by DNA quality and/or number of copies than the traditional 15 loci STRs kit. In fact, in our experience the latter multiplex system resulted in an amplification failure whereas "Mini" STRs still provided a significant outcome. Despite the genotype profile is incomplete, it can furnish extremely important information and a promising starting point for anthropological and forensic investigation.

Keywords: DNA STR typing; Forensic casework; Mentype, LCN..

Corresponding author: rismebiologia@carabinieri.it

P-259

Forensic ABO blood grouping by 4 SNPs analyses using ABI PRISM® 3100 genetic analyzer

K. Satoh[1,2] and Y. Itoh[1]

[1] *Department of Forensic Medicine, Juntendo University School of Medicine, Tokyo, Japan*

[2] *Medico-Legal Section, Criminal Investigation Laboratory, Metropolitan Police Department, Tokyo, Japan*

Serologic ABO blood grouping is well established. Grouping from forensic samples such as bloodstains is usually limited to the serologic detection of ABH antigen. However, these are still some problems with establishing the grouping. Since Yamamoto et al reported the molecular genetic basis of the three major alleles (A, B and O) at the blood group ABO gene locus, 114 alleles based on the nucleotide sequencing have been identified and described in the blood group antigen gene mutation database. We now face the problem of whether to examine all of these alleles as part of the forensic ABO blood grouping strategies.

Most of the published ABO genotyping strategies are restricted to the detection of several known ABO alleles. However, it is exceedingly important to classify four phenotypes such as A, B, O and AB to establish forensic ABO grouping by the analysis of the nucleotide sequencing. We previously proposed the analysis of four SNPs at nucleotide positions 261, 796, 802 and 803 to reflect serologic specificity.

This paper reports effective PCR-based methods, such as sequence specific PCR with positive control (SSPPC) and confronting two pair primers (CTPP) for ABO groupings using the fragment analysis by ABI PRISM® 3100 genetic analyzer.

Our data showed that 105 of 114 alleles in the database corresponded to the three major alleles by assaying the 4 nucleotide positions 261, 796, 802, and 803. The remaining 9 alleles, the two Aw08 and O03 (initially called O^2), and the seven O08 (initially called O^3), O14 (initially called O301), O15 (initially called O302), O19 (initially called R102), O20 (initially called R103), O39 and O40 are difficult to determine whether each is involved in a A or O allele. These O alleles differ from the common O allele by the absence of one nucleotide deletion of a G at position 261. Although these frequencies are extremely low in Japan, more detailed investigation accompanied by serological prevalence data will be necessary. The common O alleles share a deletion of 261G. This deletion induces a frameshift and creates a premature stop codon. The O allele corresponds to a silent allele of the ABO gene. To determine the silent allele without deletion is difficult only by analysis of the nucleotide sequence. Accordingly, both serologic and PCR-based testing should be applied to classify the four phenotypes, such as A, B, AB and O, in the practice of forensic ABO blood grouping. In addition, both PCR-SSPPC and PCR-CTPP methods using fragment analysis by ABI PRISM® 3100 genetic analyzer are an effective method, because these methods include a PCR control to examine whether the target DNA obtained from the forensic specimen can be amplified or not.

Address correspondence to:
Dr. Y.Itoh
Department of Forensic Medicine, Juntendo University School of Medicine, Hongo,
Tokyo 113-8421, Japan. TEL: +81-3-5802-1051 FAX: +81-3-5802-1050
E-MAIL: yitoh@med.Juntendo.ac.jp

P-260

PI (paternity index) vs. Residual PI in real cases. Inferences about Exclusion Power and Real Exclusion Rates over 11 STR polymorphic systems in Entre Ríos population of Argentina

Schaller LC [1], Martínez GG [1,2], Vázquez LE [1], Bolea M [2] and Martínez Jarreta B [2]

[1] *Servicio de Genética Forense, Superior Tribunal de Justicia, Provincia de Entre Ríos, Argentina.*

[2] *Laboratorio de Genética Forense e Identificación Humana, Universidad de Zaragoza, España.*

Eleven polymorphic systems were analyzed in 107 trios compound by alleged father, mother and son. IP, Residual IP (RIP), and the respective distribution descriptive parameters were obtained. In those cases where the exclusion of paternity was determined, the exclusion percentage was evaluated for each system and it was compared with the calculated Exclusion Power. Only three IP values were observed to be inside of the curve of RIP values, although this doesn't happen for more than 99.5 % of the cases. We conclude, like other authors over other populations, that in this population more polymorphic systems must be analyzed when IP values smaller than 1000 were observed. Total Exclusion Power for this polymorphic systems was 0,99973, and D13S317 was the system with highest exclusion power (0,6183), however the highest real exclusion rate in this population was observed in F13A01 system (0,7000), calculated over the number of F13A01 exclusions above the total paternity exclusions observed.

Contact: ggmartinez@arnet.com.ar

P-261

Multiplex typing of 5 Y-chromosomal SNPs

Schell D, Klein R, Miltner E, Wiegand P

Dept. of Legal Medicine, University Hospital of Ulm, Prittwitzstraße 6, 89073 Ulm

Within the last years PCR based SNP (single nucleotide polymorphism) typing has become more and more important in forensic DNA analysis. Many different methods have been established for SNP detection and especially minisequencing has often been used. In the year 2000 a new kit (SNaPshot Kit, Applied Biosystems, Darmstadt, Germany) was introduced which is based on the principle of minisequencing and especially designed for SNP detection using capillary electrophoresis. For our study we have selected 5 Y-chromosomal SNPs (M9, M17, M45, M170, M173) based on the degree of polymorphism. PCR primers were designed with the aim to get amplicon lengths < 200 bp. The Y-SNPs were optimized in singleplex reactions and then combined to multiplex approaches by sequential optimization. Finally, we were able to analyse these 5 Y-chromosomal SNPs in one PCR/SNaPshot reaction. To validate this method for forensic stain analysis we investigated bones, bloodstains, cigarette butts and epithelial cells. Additionally, we typed 50 unrelated Caucasian individuals to obtain our regional haplotype frequencies.

Contact: peter.wiegand@medizin.uni-ulm.de

P-262

CYP2D6 polymorphism and methadone metabolism –a pharmacogenetic study

Schmid D, Anslinger K, Rolf B

Institute of Legal Medicine, Ludwigs-Maximilians-Universtiy of Munich, Munich Germany

CYP2D6 is a highly polymorphic enzyme of the cytochrome P450 family involved in the metabolism of many drugs. Due to the polymorphism of this enzyme, pharmacokinetically distinct phenotypes can be distinguished that show different enzymatic activity of the CYP2D6. For many alleles of the CYP2D6 gene, the resulting phenotype can be predicted.

Methadone is often used for substitution therapy of heroin dependence. The dose of methadone is highly variable between patients. This great interindividual variability is probably based on a pharmacokinetic difference in the phase I metabolism. It is known that the first step of methadone degradation is catalyzed by several cytochrome P450 enzymes, among others CYP2D6.

To determine the effect of CYP2D6 on the metabolism of methadone, we analysed the most common CYP2D6 polymorphism in a sample of 96 heroine abusers that were undergoing a substitution therapy with methadone.

In this sample, poor metaboliser (PM), intermediate metobolizer (IM), extensive metabolizer (EM) and ultra rapid metabolizer (UM) were observed. The phenotypes are compared to the methadone dose and the methadone concentration in the blood of the patients.

Contact: Burkhard.Rolf@med.uni-muenchen.de

P-263

Absolute DNA quantification of forensic casework samples

Schulz I, Schneider PM, Rothschild MA
Institute of Legal Medicine, University of Cologne, Germany

In forensic casework, identification systems deal with routine DNA typing of minute amounts of DNA, characteristically found in small blood stains, saliva stains, and other old or degraded biological material. Fragment size analysis using multiplex short tandem repeat (STR) systems yields excellent results from small quantities of DNA with high discrimination power. However, validation studies on the STR systems have shown that the amount of template DNA must be controlled to ensure optimal amplification and subsequent identification of the PCR product. The STR systems have an optimum DNA range of 0.5 to 2.0 ng. Thus, it is essential to determine the concentration of extracted human DNA prior to amplification. In addition to recently introduced real-time PCR methods, routine quantification of forensic samples is still predominantly done either by subjective assessment, by time- and labor-intensive slot blot procedure, or by sample-consuming and often unreliable UV-spectroscopy.
In this study, two DNA quantification methods were validated and compared in their quantification efficiency using the ABI PRISM® 7000 Sequence Detection System (SDS, Applied Biosystems). In addition to the Quantifiler™ Human DNA Quantification Kit (AB), a "home made" Telomerase Assay was designed based on sequence information provided by Applied Biosystems, and having a 98 bp target fragment. The assays are based on fluorescence resonance energy transfer and the 5'-3' exonuclease activity of Taq DNA polymerase, respectively. Both assays detect a non-translated region of the human telomerase reverse transcriptase gene (hTERT). In contrast to the home made assay, the minor groove binder (MGB) probe of the Quantifiler™ Human DNA Quantification Kit has a length of 62 bp, and is thus 36 bp shorter than Telomerase Assay probe which is directly reflected in sensitivity differences. After establishing the within- and between-run precision, the range, the sensitivity limit and human specificity for both assays, a panel of forensic casework samples (n = 1000) was quantified, compared and investigated to assess the potential correlation with the subsequent typing results.
Contact: iris.schulz@uk-koeln.de

P-264

A novel method to quantify deleted mitochondrial DNA in a real time PCR

Schwark T, Fisch-Kohl C, von Wurmb-Schwark N

Institute of Legal Medicine, University of Kiel, Kiel, Germany

The quantification of deleted 4977 bp mitochondrial DNA (dmtDNA) may be of interest for the forensic as well as clinical pathologist. However, the determination of dmtDNA in two separate PCR reactions may lead to imprecise if not false results due to pipetting inaccuracies, deviant PCR conditions, etc. A conventional duplex PCR with subsequent fragment analysis yields only relative quantities of dmtDNA based on the analysis of PCR end products. To eliminate these factors, we established a duplex real time PCR using FAM- and VIC-labeled MGB probes. The PCR was carried out on an ABI Prism 7000 Sequence Detection System using standard chemistries. Amplicon sizes were 123 bp for deleted and 113 for total mitochondrial DNA. Serial dilutions showed a detection limit of 10 copies for both fragments.
In our opinion, the presented duplex PCR is an efficient means to reliably and easily quantify the mitochondrial 4977 bp deletion without the limitations of (conventional) singleplex PCRs.

Contact: NvonWurmb@rechtsmedizin.uni-kiel.de

P-265

The development of three SNP-assays for forensic casework

Senge T , Junge A , Madea B
Institute of Legal Medicine, University of Bonn, Germany

Several groups of forensic researchers work on the development of SNPs as a new marker generation for DNA classification.
The present work introduces three novel validated SNP-assays and describes their applicability in forensic casework. For this study three unlinked and non-coding SNPs named TSC0582423 (Chromosome 2), TSC0171847 (Chromosome 1) and TSC0741184 (Chromosome 3) with a balanced allele distribution were selected from the database of the SNP-consortium. SNP-detection was based on the 5'Nuclease-system from Applied Biosystems. Before the use of this SNP-detection-system in the laboratory routine, validation studies must be performed including determination of SNP-genotypes by sequencing, sensitivity studies, population studies and analysis of artificial stains. For each SNP DNA of eight unrelated persons was sequenced by Taq Cycle Sequencing to determine their SNP-alleles. These sequenced samples with known genotypes served as standard probes to establish the 5´Nuclease-assays and were used as positive controls in all analyses. For each SNP the genotype of each person determined by sequencing was identical with that determined by the 5´Nuclease-assays. Sensitivity studies were carried out with template DNA-amounts ranging from 5ng to 50pg. The test results show that an amount of at least 250pg genomic DNA could be reproducibly typed. The population studies, which were done with a group of 40 unrelated persons, show an equated allelic distribution of 58% T-Allele to 42 % C-Allele for TSC0582423, 40% T-Allele to 60% C-Allele for TSC0171847 and 59% T-Allele to 41% C-Allele for TSC0741184. The allele frequencies were comparable with those published by the SNP-consortium. The artificial stains consisted of two cigarette butts, two chewed-on chewing gums, a bottle neck abrasion as well as a scalp abrasion taken from under a fingernail. DNA extracted from the stain-material gave the expected genotypes as determined by the analysis of the corresponding saliva samples. In order to type stains with a low DNA content, an upstream multiplex PCR was developed. For each SNP a singleplex PCR with the 5´Nuclease-primers was established to get the optimal conditions for the multiplex PCR. Since only short PCR products -of about 100bp- are detected by SNP-typing using the 5´Nuclease-method, this method could be needful to detect degraded DNA. To assess the correctness of this hypothesis different approaches will be applied in the future: e.g. DNAse I-digestion and environmental studies. Also a further aspect of this work will be the comparison of the 5´Nuclease-system with the so-called Minisequencing, another method for SNP-detection, to get knowledge of the effectiveness and robustness of the two methods.
Contact: b.madea@uni-bonn.de

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Y-STR loci multiplex amplification and haplotype analysis in a Chinese Han population

Shi MS, Li YB, Wu J, Hou YP

Department of Forensic Genetics, Sichuan University (West China University of Medical Sciences), Chengdu, P.R.China

We have developed a multiplex PCR assay dealing with simultaneously amplifying 9 STR loci on Y chromosome to aid human identity testing. These Y- STR markers included DYS434, Y-GATA-A10, DYS438, DYS439, DYS531, DYS557, DYS448, DYS456 and DYS444. Efforts were made to design three sets of the tailed primers to improve the efficiency of the multiplex PCR as well as close packing of PCR product size ranges in order to keep all alleles less than 300 bp through careful examination of known allele ranges. A total of 101 different haplotypes was found among 120 unrelated males in the Chinese Han population by using the Y-STR-9-plex system, 91 of them being unique. Gene diversity ranged from 0.4394 at DYS434 to 0.7975 at DYS557. The haplotype diversity value calculated from all nine loci combined was 0.9968. The minimum amount of input DNA that could be used to obtain a full 9 Y-STR profile was 1 ng. For the male/male mixtures, the minor component in the mixture could be identified to a ratio of 1:9. In male/female DNA mixtures, the Y-STR-9-plex proved to be highly specific for the Y chromosome in that no significant female DNA products were observed up to 300 ng of female DNA. Our results revealed that the Y-STR-9-plex system was useful for forensic analysis and paternity tests in the Chinese Han population

contact: rechtsme@wcums.edu.cn
forensic@mail.sc.cninfo.net

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Characterisation of Y Chromosome SNPs Duplications

Silva MR[1] , Serra S[1] , Ribeiro T[1] , Geada H[2]
[1]*Forensic Genetics, Lisbon Delegation, National Institute of Legal Medicine*
[2]*Faculty of Medicine, University of Lisbon*
(hgeada@dlinml.mj.pt)

Single nucleotide polymorphisms (SNPs) have become widely used for population genetics. Multiple analysis of SNP markers can be important in future for human identity testing, specially in parentage testing and forensic casework due to low mutation rates of SNPs and analysis of 40-50 bps from heavily degraded DNA. Automation of SNP technology is also key demand. The lack of recombination along most of the Y chromosome makes it a useful tool in male population studies and in special forensic situations, e.g., in cases of mixtures of DNA and unavailable alleged father. Furthermore, Y-SNP markers offer additional information to that obtained by STR typing. SNP typing method was based on multiplex PCR and minisequencing reaction with SNaPshot™ Multiplex Kit followed by capillary electrophoresis on an AB Prism 3100 Genetic Analyser. In a total of 102 unrelated South Portuguese males typed, 28 were from Faro District (Algarve) and 19 from Beja District. 20 Y-SNPs have been studied in three multiplex (MP) reactions – MP1/M22, P25, 92R7, SRY1532, M173, M70, Tat, M213, M9; MP2/M170, M62, M172, M26, M201; MP3/M34, M81, M78, M35, M96, M123. The most frequent haplogoup was R1b* and the haplogoup diversity was 0.6720 and 0.8304, respectively, in Faro and Beja populations. E3b1 haplogoup was only encountered in these Portuguese population samples.
In Forensic Genetics, it is important that SNP markers have only one polymorphic site for results interpretation in forensic casework. However, two of the most widely used Y-SNPs for population studies and for forensic purposes present two signals – P25 and 92R7. These duplicate segments, called Paralogous Sequence Variations (PSVs), occur in the same multiplex reaction (MP1), which at a first glance could interfere in the results interpretation. P25 polymorphism is considered to be a C-A transversion, while 92R7 is a G-A transition. These two SNP markers are implicated in the definition of the P, Q or R haplogrups in the phylogenetic tree of the Y-chromosome. Almost all samples studied presented duplication in one of these SNP markers. Two situations have been well characterised – 92R7GA, M173A, P25C for the ancient state in these SNP markers and 92R7A, M173C, P25CA for defining haplogroup R1b*(0.4901 in the Portuguese population). In very few samples characterising haplogroups R1* or R1a no duplication was detected in the two Y-SNP loci (92R7A and P25C). The polymorphism of the two Y-SNPs will be considered as C – CA for P25 and GA – A for 92R7. No P25A or 92R7G has been detected in the Portuguese population. However, in a latter African population study a 92R7G was detected, defining the ancient state as 92R7G detected in association with M173A and P25C. Another duplication was detected in the Y-SNP M78 when studying MP3. M78 polymorphism was defined as a C-T transition. While in the ancient state (M78C) the duplicated peak was in the M96 peak area, but when defining the E3b1 haplogroup (M78T), a double T peak occurred. Singleplex defined the M78 polymorphism as CC – TT. These different types of polymorphims should be capped in mind when performing SNP typing, as by any chance, a different genetic mechanism can occur, which enable a different number of peaks in a SNP electropherogramme. Rather than an obstacle, these problems should be a challenge when using this useful new methodology for SNP typing.
Contact: ARebelo@inml.mj.pt

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Results of the 2005 Paternity Testing Workshop of the English Speaking Working Group

Simonsen BT, Hallenberg C, Morling N

Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark

Since 1991, The English Speaking Working Group (ESWG) of the International Society of Forensic Genetics (ISFG) has, once a year, offered an exercise involving genetic analysis in a paternity case. In 2005, the laboratories were invited to calculate a paper challenge in addition to performing paternity testing of a mother, two children and an alleged father. Blood samples were sent to 67 laboratories together with information about the paternity case. Also, a questionnaire concerning the techniques and routines in the laboratories were distributed.
Here, we present the results of the 2005 Paternity Testing Workshop. The evaluation includes concordance/discordance in typing results, collation of the systems used by the laboratories as well as methods used for DNA-typing. Furthermore, we present a comparison of the requirements given by the laboratories to issue a report with an excluded man and with a non-excluded man, respectively. As laboratories used different systems for typing as well as different frequency-databases in calculations, comparison of calculated PI-values in the performed paternity test was not possible. Therefore, the paper challenge constituted a valuable tool to compare calculations and to compare how laboratories deal with genetic inconsistencies, silent alleles, rare alleles as well as Y-chromosomal haplotypes. Laboratories were encouraged to treat results in the paper challenge as they would do in a paternity case as well as in an immigration case. The results of the paper challenge are presented and discussed.
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Contact: Bo.Simonsen@forensic.ku.dk

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Validation of multiplex STR systems for the investigation of familial relationships in immigration cases

Sippel H, Hedman M, Sajantila A

Department of Forensic Medicine, University of Helsinki, Finland

The purpose of this study was to evaluate the efficiency of three multiplex STR PCR Kits: SGM Plus and Profiler from Applied Biosystems and Penta BEC Multiplex from Promega in family reunion testing. The SGM Plus Kit is composed of the sex marker Amelogenin and 10 STR systems: D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01 and FGA. The second Kit, designed as Profiler combines Amelogenin and 9 STR loci: D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820. Finally, the third kit Penta BEC Multiplex combines three STR loci, Penta B, Penta E and Penta C, and provides additional power to resolve difficult cases.

Practically any first-degree biological family relationship can be established with the same technique as that for paternity investigation. Conventional paternity testing usually assumes that the mother is the true biological mother. However, in family reunion testing the aim is to investigate whether the family is a true biological family, so there are several potential constellations, including the maternity and sibship, to test.

The paternity, maternity, sibling and avuncular indices and the likelihood ratios were calculated using the DNA-view immigration program (Brenner C, Berkeley, USA), and this calculation was based on a database constructed from the respective ethnic group. For those situations where a specific population was not available a Finnish database was used. For correcting for population substructure, an inbreeding coefficient $\Theta = 0.01$ was used.

On the basis of this study the use of 18 STR loci is recommended for difficult familial relationship cases. Often cases with mutations, single-parent paternity cases and sibling testing without parents cannot be sufficiently resolved with SGM Plus and Profiler systems alone.

Helmuth.Sippel@helsinki.fi

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Molecular variation and genetic structure of variable drug response in a worldwide population sample

Sistonen J[1], Fuselli S[1], Barbujani G[2], Sajantila A[1]

[1]*Department of Forensic Medicine, University of Helsinki, Helsinki, Finland*

[2]*Department of Biology, University of Ferrara, Ferrara, Italy*

Pharmacogenetics is the study of genetically determined variation in drug response. The polymorphic Cytochrome P450 2D6 (debrisoquine 4-hydroxylase) is one of the best characterized genetic factors underlying abnormal responses to drugs. It belongs to the group of drug-metabolizing enzymes and it is responsible for about 25% of the metabolism of commonly used drugs belonging to classes like antidepressants, neuroleptics, beta-blockers and antiarrhytmics. *CYP2D6* is highly polymorphic and the phenotypic consequences are considerable: the enzyme activity ranges from complete deficiency, possibly giving rise to profound toxicity of medication, to ultrarapid metabolism, which can lead to therapeutic failure with recommended drug dosages. SNPs and sequence polymorphisms of the *CYP2D6* gene have been studied earlier in several populations but no systematic analysis of SNPs at the intercontinental level has been attempted to date. Here, we present the first population-genetic study of the *CYP2D6* data collected in 1060 individuals from a worldwide sample of 52 populations (HGDP-CEPH panel). In the whole set of individuals, we genotyped 12 SNPs and two major rearrangements of the gene using a combination of long PCR and multiplex single base extension reaction (SNaPshot). The data was analysed to describe the geographic distribution of the gene diversity and to identify a possible spatial structure. Analyses of genetic distances and spatial autocorrelation suggest the absence of major genetic barriers and the existence of a clinal pattern. The observed partition of molecular variance shows that the percentage of global genetic variation due to intercontinental differences agrees with the value commonly obtained for neutral markers. This variation turned out to be sufficient to identify by means of a Bayesian analysis a stable population structure. Considering the relevance of *CYP2D6* variation in drug metabolism and the worldwide distribution of alleles coding for enzymes with abnormal metabolic activity our results have applications in clinical as well as in forensic medicine.

Contact: johanna.sistonen@helsinki.fi

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Mitochondrial DNA variability in populations from East Timor (Timor Leste)

Souto L[1], Rocha A M[2], Pires A[2], Ferreira E[2], Kayser M[3], Amorim A[4,5], Côrte-Real F[6], Vieira D N[6]

[1]Centro de Biologia Celular, Departamento de Biologia, Universidade de Aveiro, Aveiro, Portugal
[2]Departamento de Biologia, Universidade de Aveiro, Aveiro, Portugal
[3]Department of Forensic Molecular Biology, Erasmus University Medical Centre Rotterdam, The Netherlands
[4]IPATIMUP, Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal
[5]Faculdade de Ciências, Universidade do Porto, Portugal
[6] Delegação de Coimbra, Instituto Nacional de Medicina Legal, Coimbra, Portugal and Faculdade de Medicina da Universidade de Coimbra, Portugal.

East Timor (Timor Leste) represents a region with high linguistic diversity where two major language groups: Austronesian and non-Austronesian (or Papuan) languages are found. The purpose of our study is to reveal whether the linguistic diversity is reflected in the genetic diversity and whether genetic data can shed light into the human population history of East Timor. In this study we continue the genetic characterization of human populations from East Timor, as previously started for autosomal STRs and Y STRs, with a preliminary report on mitochondrial DNA (mtDNA) diversity. A sample of 110 individuals collected from all the districts of Timor and representing 12 linguistic groups was studied. Analyses of the mtDNA sequence from the hypervariable region 1 (HVS1) and the presence of the 9-bp deletion (intergenic regions COII-tRNA lys) were performed.
These genetic data allowed us to detect the presence of the P and Q haplogroups, typical of non-Austronesian (Papuan) speaking populations from New Guinea, along with a significant frequency of haplogroup B (namely B4a and B4b) generally associated with the expansion of Austronesian-speaking groups from East Asia along with several M sub-branches previously reported to the Southeast Asian region. Thus, our preliminary data show a correlation between linguistic and genetic diversity in East Timor and further more detailed analyses will reveal insights into the population history of the region. In addition our genetic data (mtDNA, STRs) may serve as prerequisite for forensic genetic applications in East Timor.

Contact: lsouto@bio.ua.pt

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Y-chromosome haplotypes in East Timor: evidences of population differentiation

Souto L[1], Gusmão L[2], Ferreira E[3], Pires A[3], Rocha A M[3], Amorim A[2,4], Côrte-Real F[5],Vieira D N[5]

[1]Centro de Biologia Celular, Departamento de Biologia, Universidade de Aveiro, Aveiro, Portugal
[2]IPATIMUP, Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal
[3]Departamento de Biologia, Universidade de Aveiro, Aveiro, Portugal
[4]Faculdade de Ciências, Universidade do Porto, Portugal
[5] Delegação de Coimbra, Instituto Nacional de Medicina Legal, Coimbra, Portugal and Faculdade de Medicina da Universidade de Coimbra, Portugal.

The Y-chromosome markers, due to their peculiar characteristics are considered useful tools in detecting populations' differentiation.
The population of East Timor is known to gather a high number of ethnolinguistic groups. From linguistics and classical anthropological studies, the different groups in east Timor are generally assigned to Austronesian or Papuan ancestry.
We analysed a total of 346 males from East Timor for 12 Y-chromosome specific STRs (DYS19, DYS389I and II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS437, DYS438 and DYS439). According to anthropological and linguistics information, samples were classified in major linguistic subgroups: **Timoric** (Timorese-Austronesian), which includes Group A ("Fabronic", N=92) and Group A1 ("Ramelaic", N= 130), and a third group including languages that are related to a **Trans-New Guinea phylum (**Papuan) and supposed to represent aboriginal (pre-austronesian) signature (Group B, N= 124).
The highest haplotype diversity value was found in Group A, "Fabronic" (0.9931), while the "Papuan" group B shows the lowest value (0.9882).
Comparison of the 3 samples suggests that the "Ramelaic" group differentiates from the "Fabronic" and "Papuan" ones (Rst values of 0.02710, P= 0.00366 and Rst of 0.05839, P= 0.0000).
These genetic results, while putting in evidence some accordance with the proposed linguistics classification, and strengthening a high homogeneity of "Papuan" speaking populations, they also suggest that the Papuan/Austronesian linguistics criteria is not sufficient to differentiate East Timor populations and deeper analyses at ethnolinguistic groups and geographic districts should be considered.
Despite the uniqueness of the vast majority of the haplotypes found in our east Timor sample, in an extensive search in the international Y Chromosome Haplotype Reference Database (YHRD – www.yhrd.org) most of the "matches" were produced with South East Asia and Oceania samples, although "matches" with some European haplotypes were also observed.

Contacto: lsouto@bio.ua.pt

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Genotyping of human DNA recovered from mosquitoes found on a crime scene

S.Spitaleri, C. Romano, E. Ginestra and L.Saravo*

[1] *Laboratory of Molecular Biology – Raggruppamento Carabinieri Investigazioni Scientifiche (RaCIS), 98128 Messina – ITALY.*

Many mosquitoes species are man parasitical biters commonly found in the south of Italy by crime scene investigators. Some authors already demonstrated the possibility of amplifying human DNA from blood-meals of Diptera species using various methods for epidemiological issues. Here we decribe a casework occurred in Sicily: a person was killed in a room where no traces were found apart from a fresh mosquito blood-meal stain which could be probably referred to who had been sleeping in that place during the previous nites. Hence an optimized DNA extraction was carefully carried out in order to yield deoxyribonucleic acid uncontaminated by insect-specific molecules. PCR amplification and STRs profiling at 15 human genetic loci was then performed on the extracted DNA, using AmpFLSTR Identifiler kit by Applied Biosystems. Additionally a DNA sample extracted from a mosquito of the same genus was processed, with equal conditions, to assess eventual unspecific detection. Results showed that it is possible to successfully amplify and obtain a complete genetic profile even if DNA is recovered from small and biologically-contamined traces. The applied analitical strategy represented a powerful tool for the investigations and allowed to address the profile to the major suspect of the murder.

Keywords: DNA STR typing; Forensic casework; Identifiler, Mosquitos.

*Corresponding author: rismebiologia@carabinieri.it

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Single Human Telogen Hair Analysis: Multiplex Amplification of 8 STR loci

Spitzer E, Borck C, Grosse R

Institute of Medical Molecular Diagnostics GmbH, Schoenstrasse 90, 13086 Berlin, Germany

Hairs in the telogen phase represent the majority of trace hairs collected in connection with criminal cases. However, until now nuclear DNA profiling has not yet become routine in forensic casework because of PCR problems related to low amount and high degradation of DNA isolated from telogen hairs. There are no published procedures for efficient genotyping of single hair shafts.

Here we describe a protocol for simultaneously amplifying 8 STRs in 3 triplex amplification reactions. DNA has been purified from single hairs by phenol –chloroform extraction yielding a total of 15µl of DNA.

The primers were designed to produce amplicons of 50-150 bp. SE 33 has been excluded from triplexes due to its fragment size of 200 bp and more.

The three triplexes designated as MPX1, MPX2 and MPX3 consisted of the STRs TPOX/THO1/D18, FGA/vWA/D3, and Amelo/D8/D21 respectively. Fragment analysis has been performed by means of the 310 Genetic analyzer.

We show a number of examples to demonstrate usefulness and efficacy of the multiplex approach for single hair DNA analysis. Also, we give examples showing the necessity to perform STR typing of short DNA fragments in a special clean area apart from laboratory.

Finally, we show that our approach is suitable for other stain analysis, particularly if degraded DNA of low amounts is to be expected.

Contact: spitzer@immd.de

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Molecular analysis of genomic low copy number DNA extracted from laser-microdissected cells

N. Staiti [(1)], G. Giuffrè [(2)], D. Di Martino [(1)], A. Simone[(2)], G. Sippelli[(2)], G. Tuccari[(2)], and L . Saravo[(1)]*

[1] *Laboratory of Molecular Biology – Raggruppamento Carabinieri Investigazioni Scientifiche (RaCIS), 98128 Messina – ITALY.*

[2] *Department of Human Pathology, University of Messina.*

Tissue microdissection techniques, allowing a correlation between the topologic organization of the cells and the molecular analysis of nucleic acids, offer the chance to characterize the several kinds of cells present both in pathologic and in forensic samples cutting small tissue fragments as well as single cells by an ultraviolet laser beam under direct microscopic visualization. The low number of target cells among a wide spectrum of cell types in heterogeneous tissue samples may complicate the analysis of biological residuals found on crime scenes, while the possibility to perform a genomic analysis of low copy number DNA from few cells harvested by laser microdissection represents a valid aid in order to solve forensic problems.

In the present report we evaluate the sensitivity of the above described method in order to perform genomic analysis from low copy number DNA. Laser-microdissection was performed using a Leica AS LMD system (Leica Microsystems, Germany) on fresh smears of aploid (spermatozoa) and diploid (lymphocytes) cells, on sections of routinely formalin-fixed and paraffin-embedded tissues, on cryostatic sections obtained at surgery and post-fixed with cold methanol. The samples were stained with specific procedures (Haematoxilin-Eosin, Giemsa, Papanicolau, Picroindigocarmine-Nuclear fast red) and an increasing number of cells (from 1 to 100) was harvested from them in different PCR tubes. DNA extraction was performed by Chelex™ 100 (Biorad), QIAmp DNA Micro Kit (Qiagen) and DNA IQ™ System (Promega Corp.). DNA extracted from each sample was amplified to identify a specific genetic profile by the most common microsatellite loci of pathologic and forensic interest. PCR products were separated by capillary electrophoresis with 3100 AB Prism Genetic Analyzer, and analyzed by Genemapper Software v 3.2 (Applera Europe B.V.).

A complete genotypic profile was obtained down to 10 aploid and 5 diploid cells as to pathological and forensic genetic markers; different results - concerning the integrity of the extracted DNA - were achieved according to the kind of histological stainings performed.

Keywords: DNA STR typing; Forensic casework; Mentype®, LMD, LCN.

*Corresponding author: rismebiologia@carabinieri.it

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AZF deletions of the Y chromosome and failed amplification of commonly used Y-STRs

Stein B[1], Willuweit S[1], Nagy M[1], Vogt PH[2], Roewer L[1]

[1]Institute of Legal Medicine, Charité – University Medicine Berlin, Hannoversche Str. 6, 10115 Berlin, Germany

[2]Section of Molecular Genetics and Infertility, Department of Gynecological Endocrinology and Reproductive Medicine, University of Heidelberg, Im Neuenheimer Feld 328, D-69120 Heidelberg

Deletions of the Y chromosomal azoospermia factor called *AZFa*, *AZFb* and *AZFc* have shown to be associated with impaired spermatogenesis. *AZFc* deletions are the commonest of the deletions found in infertile men, with a frequency estimated to be 1 in 4000. *AZFa* deletions are particularly rare (< 1 : 100 000), whereas AZFb deletions are intermediate in frequency. Sequence analysis of the complete Yq11 region reveals a 1.5 Mb overlap of the *AZFb* and *AZFc* regions (*AZFb+c*). Several widely used as well as new Y-STRs can be affected by the AZF deletions. For example, the multicopy Y-STR DYS464 lies within the ampliconic r1-r4 repeats of the *AZFc* region. The locus DYS389 resides in the *AZFa* region, and DYS385 and DYS392 in *AZFb.*

In a Y chromosomal "minimal" haplotyping study of 48 German males deleted for *AZFc*, *AZFb* and *AZFb+c* we found several incomplete haplotypes lacking PCR products at the loci DYS392 and DYS385. Two *AZFc* diagnosed male DNAs failed to amplify at the locus DYS392. This points to a wrong AZF classification, an *AZFb+c* deletion is more probable for this Y chromosome. One *AZFb* male lacks only the loci DYS385. Two *AZFb* males lack both DYS392 and DYS385. All three *AZFb+c* males show PCR failure at the loci DYS392 and DYS385. The remaining 41 *AZFc* diagnosed males show complete minimal haplotypes.

Recently, King et al. report deletions of DYS464 in three *AZFc* males (f = 3/3255) and 9 out of 19 Y-STR amplification failures for an *AZFa* deleted chromosome(f = 1/5374).

The core set marker set of the Y-STR haplotype reference database (YHRD) currently includes the markers DYS19, DYS389I+II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS438 and DYS439. All of these STRs are well established in forensic and genealogical routine and are included in most commercial kits. Of these STRs the markers DYS389, DYS392, DYS385, DYS438, DYS439 could fail to be amplified due to AZF deletions, thus generating incomplete haplotypes. King et al. have recommended to skip DYS464 testing for genealogical and forensic routine because of the relative high frequency of the *AZFc* deletion and its association with infertility. Probably because of its rarity, incomplete haplotypes pointing to the *AZFa* and *AZFb* deletions have not yet been reported to the YHRD currently comprising about 28.650 minimal and 6.281 extended haplotypes.

A consequence of inadvertent diagnosis of male infertility through genealogical or forensic Y-STR haplotyping should be information and possibly warning of customers by companies and institutions using Y-STR markers for identity testing.

Contact: lutz.roewer@charite.de

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TSUNAMI-Disaster: DNA typing of Sri Lanka victim samples and related AM matching procedures

Steinlechner M, Parson W, Rabl W, Grubwieser P, Scheithauer R

Institute of Legal Medicine, Innsbruck Medical University, Austria

More than 30.000 individuals of varied racial and ethnic background lost their lives when Sri Lanka was affected by the South Asian tsunami. DVI-Teams from many countries were involved in examining victims, taking DNA-samples from victims for identification procedures. The principal role of our laboratory in this process was to provide barcoded sampling kits, to perform DNA typing of human remains and ante mortem (AM)/kinship samples and to accomplish DNA matching services to support DNA-based identifications.
Until now (22.04.2005) more than 400 samples related to 101 victims were sent to our laboratory. The barcoded sampling kits allowed for the collection of 2 swabs (from intact inner surfaces like muscle, urinary bladder etc.), 1 4 cm piece of long bone and 1-2 teeth, which were all shipped on ice.
A high throughput DNA extraction and STR analysis procedure for the swabs were developed in order to provide STR profiles within 24 hours. For the skeletal remains and teeth a highly sensitive DNA extraction procedure was set up in order to maximise DNA recovery. Both processes included electronic accessioning to maintain the numbering system of the DVI team in Sri Lanka and the data exchange via Interpol and PLASS Data Software.
AM DNA profiles related to missing persons from Sri Lanka and Austria were generated in our laboratory, AM DNA profiles related to missing persons from other countries were transmitted via Interpol or directly sent to our laboratory for matching purposes. DNA matching procedures were facilitated by using the Mass Disaster Matches option of Charles Brenners DNAVIEW software.
In all, the established DNA typing procedures proved to be highly efficient. The swab typing procedure produced successful results in 47% of the completed cases (n=81). For the remaining cases skeletal remains had to be typed via the sensitive DNA extraction procedure and produced useful results in all investigated samples so far, obtaining 34% full profiles (AmpFLSTR® Identifiler® PCR Amplification Kit) and 66% partial profiles (>=7 STR loci). In nine cases the DNA investigations were stopped due to successful dental identifications. The analyses of the remaining eleven cases are currently underway.
Until now, the DNA matching procedures enabled 22 DNA-based identifications of victims from eight countries.

Contact: martin.steinlechner@uibk.ac.at

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Norwegian population data for 2 autosomal STR loci; D12S392 and D17S906

Stenersen M, Perchla D, Dupuy BM

Institute of Forensic Medicine, University of Oslo, Norway

Autosomal STR polymorphisms at 2 loci (D12S392 and D17S906) are presented. Samples from current paternity analysis (n=713) were analysed. The observed heterozygosities were 0.891 (D12S392) and 0.934 (D17S906). No significant deviation from Hardy-Weinberg equilibrium was observed. 19 different alleles were observed at D12S392 whereas a total of 56 different alleles were observed at D17S906. The repeat numbers ranged from 15 to 27 at D12S392. For D17S906 the allele sizes ranged from 331 to 437 bases. Sequence variation at both loci will be presented as well as frequency databases and other relevant forensic genetic parameters.

Corresponding author: Margurethe Stenersen
Mailing address: Rettsmedisinsk Institutt
Rikshospitalet
N-0027 Oslo
Norway
Telephone number: 00 47 23 07 13 17
Fax number: 00 47 23 07 13 18
E-mail: margurethe.stenersen@labmed.uio.no

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Norwegian population data for 15 autosomal STR loci: PowerPlex 16

Stenersen M, Perchla D, Dupuy BM

Institute of Forensic Medicine, University of Oslo, Norway

Autosomal STR polymorphisms at 15 loci (D3S1358, THO1, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta E, vWA, D8S1179, TPOX and FGA) are presented. Samples from current paternity analysis (n=1380) were analysed. The observed heterozygosities ranged from 0,610 (TPOX) to 0,896 (Penta E). One significant deviation from Hardy-Weinberg equilibrium was observed at Penta D. The number of observed alleles ranged from 7 (THO1 and TPOX) to 19 (D21S11 and FGA). The shortest tandem repeat observed was 2.2 at Penta D and the largest 44.2 at FGA. The power of discrimination and exclusion ranged from 0.787 (TPOX) to 0.967 (FGA) and from 0.303 (TPOX) to 0.788 (Penta E) respectively. Frequency databases and other relevant forensic genetic parameters will be presented in detail.

Corresponding author: Margurethe Stenersen
Mailing address: Rettsmedisinsk Institutt
Rikshospitalet
N-0027 Oslo
Norway
Telephone number: 00 47 23 07 13 17
Fax number: 00 47 23 07 13 18
E-mail: margurethe.stenersen@labmed.uio.no

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A Comparison of various methods used in extraction of DNA in Sexual assault cases

Student B [2], Fox S [1]

[1]LGC, Queens Rd, Teddington, TW11 0LY, UK
[2] King's College London, London WC2R 2LS, UK

For the past two decades the method of choice for the enrichment of sperm cells from sexual assault cases has been that documented by Gill *et al* (1) in 1985, the preferential extraction. Following a sexual assault DNA evidence is usually comprised of vaginal swabs or semen stains. The problem with these samples is that the spermatozoa are mixed in with vaginal epithelial cells from the victim. Separating spermatozoa from epithelial cells results in more simple interpretation and increased success of individualisation. The traditional method is relatively inefficient and often does not produce complete separations especially when there is low numbers of spermatozoa which is often the case in sexual assault samples. This study has considered different techniques of separating the spermatozoa from epithelial cells. The first one is the Differex™ system by Promega which essentially uses the same chemistry as the traditional method but employs a more sophisticated method of separation based on a combination of phase separation and differential centrifugation. Further consideration has been given to filtration methods based on the differences in size and shape of the two cell types.

Reference**:** Gill, P. Jeffreys, A.J. and Werret, D. J. (1985) Forensic Application of DNA 'fingerprints'. *Nature* **318**, 577-9

Contact: Jim.Thomson@lgc.co.uk

P-281

Coding region mtDNA analysis for increased forensic discrimination using Pyrosequencing technology

Styrman H, Andréasson H, Nilsson M, Allen M

Department of Genetics and Pathology, Rudbeck laboratory, Uppsala University, Uppsala, Sweden

Analysis of mitochondrial DNA (mtDNA) is very useful for samples where a nuclear DNA analysis fails due to degradation or insufficient DNA amounts. However a drawback of mtDNA analysis is its limited discrimination power. The D-loop sequencing performed in routine analysis today might therefore result in different individuals showing identical HVI and II sequences. In order to resolve identical mtDNA types from different individuals, additional discrimination might be achieved by analysis of coding region variation as a complement to the sequencing of the HVI and II regions. We have previously developed a Pyrosequencing-based system for both mtDNA control region and coding region analysis. Pyrosequencing is a fast, non-electrophoretic DNA sequencing technique that uses PCR products as templates and is based on a four enzyme reaction to monitor DNA synthesis. In this study, the coding region assay has been further expanded to cover additional informative positions for increased discrimination. The entire coding region analysis comprises 17 pyrosequencing reactions on 15 PCR fragments. Coding region analysis was performed on all 15 fragments in 135 samples, with an average read length of 83 nucleotides. A total of 52 SNPs with frequencies ranging from 1% to 47% were identified. Of these, 18 variants were found in a single individual, illustrating the potential to detect highly informative SNPs. The revised Cambridge reference sequence (rCRS) has the most common HVI/HVII mtDNA sequence and belongs to haplogroup H. As the rCRS HVI/HVII sequence is very common in Caucasians, this sequence is often observed in cases with unresolved sequences samples from different individuals. In a group of 60 samples with identical sequences to rCRS or with a single difference in the D-loop, only 12 samples could not be resolved by at least two differences using pyrosequencing analysis of these coding region fragments. Thus, the use of this pyrosequencing mtDNA coding region analysis system has the potential to increase the discriminatory power of mtDNA analysis. Coding region analysis proved very useful in a case involving the bishop election in Sweden in 1952. Prior to the election, approximately 500 anonymous letters were sent to the 190 voting priests. The letters contained propaganda for one of the five candidates, Dick Helander and slander about the other candidates. Soon after Helander was assigned as bishop by the Swedish government, the police initiated an investigation regarding the letters. Helander became a prime suspect and was convicted on a chain of circumstantial evidence. DNA from the sealing of a total of four anonymous letters were analysed by sequencing of HVI and HVII. The results were compared to DNA from four official letters written by Dick Helander, resulting in a single C/T difference at position 16239. Due to this inconclusive result, further analysis of coding region variation was performed. All 15 PCR fragments were amplified and three additional variable positions (3010, 7028 and 16519) were identified by pyrosequencing analysis. In conclusion, the use of this mtDNA coding region analysis system has demonstrated the potential to reduce the number of unresolved individuals with similar or identical HVI/HVII sequences by 80%. Furthermore, this pyrosequencing-based system was shown to be informative in resolving samples with a single difference in the HVI/HVII sequences in an old forensic investigation. The assay is very easy to use, rapid and highly flexible, facilitating analysis of different target combinations. Analysis of the mitochondrial coding region by pyrosequencing can provide a useful tool in cases where different individuals share identical HVI/II sequences or when only a single difference is detected between samples.

contact: hanna.styrman@genpat.uu.se

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STR sequence variants revealed by Pyrosequencing technology

Styrman H, Divne A-M, Allen M

Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden

Pyrosequencing has proven to be useful to evaluate allelic variants of STR repeats. Pyrosequencing is a fast real time non-electrophoretic sequencing-by-synthesis method, based on a cascade of four enzymatic reactions, which enables nucleotide incorporation and release of pyrophosphate (PPi), to yield detectable light. The produced light is proportional to the number of incorporated nucleotides and shown as peaks in a pyrogram. As the actual sequence is determined rather than the fragment length in pyrosequencing variant alleles resulting in the same fragment length can be revealed in addition to length variation.

In a population study of 10 autosomal markers (CSF1PO, THO1, TPOX, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539 and Penta E) several allelic variants were detected. At the THO1 locus the incomplete allele 9.3 was detected in 61 genotypes of which 19 were homozygous. Furthermore, additional variation was found at loci D13S317, D7S820, D5S818 and D8S1179. At locus D13S317, we found alleles carrying a T/A SNP in the last repeat, resulting in the repeat unit AATC. The possibility to detect this variant allele increases the resolution. For instance, four different combinations of alleles (12/13, 11AATC/13, 11AATC/12AATC and 12/12AATC) were observed that all generate the same fragment length. At the DYS720 locus a C/T SNP in the first sequenced upstream flanking nucleotide (***C/T***GAACTAAC[GATA]n) was observed in 9/114 genotypes (8%). The SNP was found in the flanking region of allele 8 (8 out of 9 samples) and allele 9 (1 of 9 samples). At the D5S818 locus a four base pair deletion, involving a CTCT motif next to the ATCT repeat was observed in 45/114 genotypes. Finally, at the D8S1179 locus a variant allele, due to a G/C SNP, resulted in the repeat structure [TCTA][TCTG][TGTA][TCTA]$_{11}$ in four genotypes.

Furthermore, in a population study of eight Y chromosome markers (DYS19, DYS389 I-II, DYS390, DYS391, DYS392, DYS393 and DYS438) variants were detected at four loci. At the DYS391 locus a G/A SNP was observed in one out of 70 individuals. One nucleotide upstream the repeat, TCTA was seen resulting in TCTG. At the DYS390 locus a G/A SNP was observed in one individual, upstreams of the first repeat unit. At the DYS393 locus a A/C SNP was observed in the first repeat unit, converting the repeat from AGAT to CGAT in 10/70 individuals. Finally, at the DYS389 II locus an A/G SNP resulted in (TCTG)$_6$ instead of (TCTG)$_{4-5}$ in one genotype.

All detected autosomal and Y-chromosome variants were confirmed by Sanger sequencing. In conclusion, pyrosequencing is a useful tool for rapid compilation of population data with higher resolution for some markers. As allelic variants were easily detected in most cases, pyrosequencing is a suitable method for sequence evaluation of known or novel markers of less complex nature.

contact: hanna.styrman@genpat.uu.se

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Length Heteroplasmy in the HVI Control Region

Sucena A[1] , Ribeiro T[1] , Geada H[2]

[1]*Forensic Genetics, Lisbon Delegation, National Institute of Legal Medicine*
[2]*Faculty of Medicine, University of Lisbon (hgeada @dlinml.mj.pt)*

MtDNA is generally accepted as a tool for forensic identity testing and evolutionary studies. It is used to analyse biological samples where the quality and/or quantity of nuclear DNA content is low. The evaluation of results obtained by mtDNA typing is continuously subjected to scientific scrutiny and some genetic issues have to be considered when establishing an effective methodology. In order to properly interpret results, one needs to appreciate mtDNA's features such as nomenclature, heteroplasmy, recombination and haploid maternal inheritance. Forensic community has adopted a common language to describe the variation observed in human populations naming mtDNA sequences by referring to a standard sequence, CRS. In a forensic case, the weight of evidence is primarily based on the number of times a profile is observed in a reference data set. The relevance of these databases should be considered for forensic applications.

HVI and HVII mtADN regions have two citosine segments (np16184-16193 and np303-315, respectively), which in accordance with CRS possessed a timine at np16189 and np310. In our population data, 15% of mtADN sequences have a transition T - C at np16189. In 20% of these sequences occurs a C – T transition at np16186 (2 samples) and at np16187 (1 sample). In five samples besides a 16189T, there were also a 16188T (1sample), a 16193T (2samples) and a 16192T (2samples). Sequence variability was referred by the number and position of Cs and Ts (CxTx).

The variability in np16184-16193 HVI region presents two different types: sequence variability detected in eight samples representing six distinct timine positions, not interfering with results and length variability with length heteroplasmy with no timine position in this region. The different types of length heteroplasmy were identified as Lh1I (A3C10), Lh2I (A3C11), Lh3I (A3C12), Lh4I (A3C13), Lh5I (A2C11), Lh6I (A2C12) and Lh7I (A2C13), considering also adjacent np16182 and 16183. Twenty-seven samples in our studied have been detected with length heteroplasmy.

For forensic purposes, the HVI homopolimeric regions should be well defined with a correct classification of the homopolimeric track defined by As and Cs encountered in the electropherogrammes and the various population types involved identified by the three nuclear adjacent positions to the homopolimeric track.

Contact: ARebelo@inml.mj.pt

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Genetic studies of seventeen X –STR in the Japanese population

Tamura A, Iwata M, Takase I, Fukunishi S, Takagi T, Tsuboi K, Miyazaki T, Nishio H, Suzuki K

Department of Legal Medicine, Osaka Medical College, Japan

Introduction Short tandem repeat (STR) loci are useful for personal identification and paternity testing because the number of repeats within STRs tends to be highly variable and these STR polymorphisms can be rapidly analyzed using PCR. Autosomal STR makers are widely applied to personal identification and paternity testing. However, in some kinship or deficiency paternity cases typing of sex chromosomal STRs may be more informative than autosomal STRs. Typing of X chromosomal STRs (X-STRs) could be of great usefulness to the paternity where the child is female because fathers transmit their X-STRs to all their daughters. We studied 17 X-STR (DXS101, DXS6789, DXS6800, DXS6803, DXS6807, DXS7132, DXS7133, DXS7423, DXS7424, DXS8377, DXS8378, DXS9895, DXS9898, DXS10011, HUMARA, HPRTB, GATA172D05) polymorphism in 99 unrelated Japanese individuals (55 males, 44 females) and mutation rate in 22 true biological trio families.

Materials and Methods Genomic DNAs were recovered from buffy coat by proteinase K digestion followed by sodium iodide extraction. The 17 X-STRs were separately amplified using each primer set as previously reported.

Results and discussion Power of discrimination (PD) of the 17 X-STRs ranged from 0.331 to 0.933 (male) and from 0.447 to 0.997 (female). Allele frequencies and number of alleles were 0.007-0.853 and 4-35, respectively. High PD values were observed at DXS101 (0.822 male, 0.933 female), DXS6789 (0.799 male, 0.956 female), DXS8377 (0.888 male, 0.981 female), DXS9895 (0.748 male, 0.902 female), DXS10011 (0.930 male, 0.997 female), HUMARA (0.881 male, 0.975 female), and GATA172D05 (0.715 male, 0.912 female). DXS10011 showed the highest PD value among them.

We have detected two *de novo* alleles at the DXS10011 locus in two paternity trio cases (probabilities: >0.999999). No alleles of the mothers were found to be shared with the daughter in one case and with the son in the other case. The mutation events seem to have occurred during oogenesis, probably by replication slippage. Further studies are needed to evaluate the mutation rate at the locus.

contact: leg003@art.osaka-med.ac.jp

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Mutation analysis in fatal pulmonary thromboembolism - Postmortem validation study and beyond

Tang, Y.[1], Kim, Y.[1], Jeudy, S.[1], Roman, K.[2], Sansone, M.[1], Shaler, R.[1]

[1]Department of Forensic Biology, [2]Department of Forensic Pathology, Office of Chief Medical Examiner, New York, NY

Sudden fatal pulmonary thromboembolism (PE) is a common finding in forensic pathology practice, usually presented as a complication of deep venous thrombosis (DVT). The current view of the aetiologies of DVT is multifactorial, where inherited genetic predisposition interplays with acquired risk factors, such as surgery, pregnancy, inactivity of any cause, malignancy, obesity. There are several genetic risk factors involved in the predisposition of individuals to develop DVT. The most common mutation in the Caucasian population, but less in other ethnic groups, is the Factor V Leiden (G1691A) mutation. Heterozygotes for the Factor V Leiden (G1691A) mutation have an approximately 4-fold to 7- or 8-fold increased risk for DVT as compared to individuals without the mutation. Homozygotes for the Factor V Leiden (G1691A) mutation have an approximately 80-fold increased risk for DVT as compared to individuals without the mutation. The second most common mutation is the G20210A mutation in the prothrombin (Factor II) gene. Heterozygotes for the prothrombin G20210A mutation have an estimated 2 to 4-fold increased risk for DVT as compared to individuals without the mutation. In addition, individuals carrying both the Factor V Leiden and the prothrombin G20210A mutations have a 20 fold more likely chance of having DVT than individuals without either mutation. Another risk factor for DVT is associated with the homozygous state of a nucleotide variant (C677T) in the methylenetetrahydrofolate reductase (MTHFR) gene.

Molecular testing for these common mutations is one of the most frequently ordered laboratory tests in a clinical setting, but is not routinely done by forensic pathologists. Molecular testing for fatal PE can confirm autopsy results, provide insights regarding disease effects and provide knowledge for genetic counseling of family members. Clinical diagnostic testing methodologies for the common mutations associated with DVT are well established but there are limited data about the reliability of these procedures for various post mortem samples. The aim of this study was to establish and validate a genetic test for DVT in a forensic setting.

To investigate the genetic risk factors in the fatal PE cases, we focussed the validation work on the three common mutations described above. The in-house molecular testing methodology is based on the polymerase chain reaction (PCR) and automated DNA sequencing technologies. The method was validated for postmortem tissue samples, such as heart, spleen, and liver. Tissues are stored in *RNAlater*® solution for up to two years. The method was also validated for blood specimens collected in tubes with or without anticoagulant. Blood was spotted on staincards, dried and stored at room temperature for up to two years. All samples were extracted using a magnetic bead capture and release chemistry. We obtained results for all tested specimens, including those displaying varying extents of decomposition.

One of the first cases, submitted less than a month after the DVT mutation assay had been approved for routine casework, involved a 63 years old Caucasian woman who was presented as a sudden death and PE at autopsy. She was diagnosed as a carrier for the homozygous MTHFR C677T mutation. Given the potential benefits of the test results to the surviving family members, pursuing molecular testing to investigate the genetic cause of fatal PE should be a common practice in forensic pathology.

Contact: PRINZ@ocme.nyc.gov

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The Effect of Whole Genome Amplification on Samples Originating From More Than One Donor

Thacker CR[1], Balogh K[2], Børsting C[3], Ramos E[4],Sanchez-Diz P[4], Carracedo A[4], Morling N[3], Schneider P[2], Syndercombe Court D[1] SNP*for*ID Consortium

[1]Centre for Haematology, ICMS, Barts and The London, Queen Mary's School of Medicine and Dentistry, UK

[2]Institute of Legal Medicine, University of Mainz, Germany

[3]Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark

[4]Institute of Legal Medicine, University of Santiago de Compostela, Galicia, Spain

Limited starting material is a common problem in forensic science. Samples are often compromised in terms of quality or quantity (sometimes both) and the possibility of contribution by more than one donor is a necessary and frequent consideration. Whole genome amplification (WGA) offers the opportunity to create a 'stock' of starting substrate on which to perform subsequent testing and provides an interesting avenue of investigation for the forensic scientist. Its potential to deal with mixed samples is of particular interest and the research presented here looks at the ability of WGA (using the GenomiPhi™ DNA Amplification Kit, Amersham Biosciences) to cope with samples originating from more than one individual.

Blood samples were taken from four individuals (A, B, C and D) and DNA extracted using the QIAamp® DNA Mini Kit (Qiagen). The extracts were quantified (in duplicate) using the Quantifiler™ Human DNA Quantification Kit (Applied Biosystems) on the ABI PRISM® 7700. Following quantification, the extracts were normalised and extract A was mixed with B whilst extract C was mixed with D. In each case the samples were combined in the ratios 1:1, 1:3, 1:7 and 1:15. The mixture proportions were verified by performing routine amplifications using both the AmpFLSTR® SGM Plus® PCR Amplification Kit (Applied Biosystems) and the PowerPlex® 16 System (Promega). Peak areas were used to calculate observed ratios. WGA was performed by adding the minimum concentration of starting material recommended by the manufacturer (1ng/ µL). The reaction was also performed by adding DNA at concentrations known to exceed this minimum value. The remainder of the protocol was performed according to manufacturer's guidelines.

Relative proportions were found to be maintained in the 1:1 and 1:3 ratios following WGA; the observed peak ratios were found to match the expected peak ratios regardless of the starting concentration of DNA. With samples mixed in the ratio of 1:7 and 1:15, and when the concentration of starting material was at the lower limit, too few minor component peaks were found to allow for statistical analysis. With an initial template exceeding 1ng/ µL there was an increase in problems associated with profile interpretation but the results obtained indicated that mixture proportions could be quantifiably maintained. To check the reproducibility of these findings, initial mixture preparations were shipped to collaborating laboratories for WGA. The results of these extra replicates are presented and the findings discussed.

Address for Correspondence:
Catherine R Thacker, Centre for Haematology, Institute of Cell and Molecular Science, Barts and The London
Queen Mary's School of Medicine and Dentistry, 4 Newark Street, London E1 2AT, UK
E-mail: c.r.thacker@qmul.ac.uk

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An Investigation into Methods to Produce Artificially Degraded DNA

Thacker CR, Oguzturun C, Ball KM, Syndercombe Court D
Centre for Haematology, ICMS, Barts and The London, Queen Mary's School of Medicine and Dentistry, UK
c.r.thacker@qmul.ac.uk

DNA samples recovered from a crime scene are often subjected to detrimental environmental conditions before they can be collected for analysis. Environmental sources of degradation, which can include heat, light and bacterial decomposition, are by their very nature random in the effect they have on the DNA deposited at the scene. These effects further test the scientist's ability to produce an evidentially valuable profile from a sample already compromised in terms of quantity. The facility to produce a 'stock' of degraded DNA on which to optimize existing protocols would go some way to help in the preparation of standard practices to follow when faced with an environmentally degraded sample. The knowledge gained from the preparation of such stocks has the potential to benefit those asked to give their Expert opinion in a court of law. Experience gained on the behaviour of DNA stored in a variety of hostile conditions (albeit in controlled environments) could help with the interpretation of results produced from degraded samples and may also be useful if asked to consider storage conditions of that sample prior to generating the resultant profile. Blood samples were collected from volunteers and blood stains on cotton cloth squares prepared. The prepared stains proved difficult to process in terms of laboratory space required for drying and maintaining sterility whilst monitoring degradation over an extended time frame. An alternative sampling source was found and subsequent experiments were performed using blood stained Salivettes® (Sarstedt). Cigarette ends and chewing gum were also collected for examination. Control samples (Day 0) were taken from each 'exhibit' and the DNA extracted. Throughout the course of the work a number of different extraction techniques were investigated: Chelex® 100 (Sigma); Charge Switch™ (Invitrogen); Invisorb® Forensic Kit I (Invitek) and Qiagen. DNA profiles were generated using the AmpFLSTR® SGM Plus® PCR Amplification Kit (Applied Biosystems) run under standard conditions. A previous study used sonication and DNAse I treatment to artificially degrade DNA (1). Our aim was to mimic as closely as possible environmental conditions and as a consequence UV light, humidity and temperature were investigated as degradation agents. In the case of chewing gum, prepared samples left outside in direct sunlight were also analysed. Sections were taken from the samples at timed intervals throughout the period of degradation. The DNA was extracted and amplified. The resultant electropherogram was analysed and, if necessary, amplification was repeated with slight modifications to improve the quality of the profile. UV light caused a clear 'drop-out' of heavier alleles. This increased as exposure to UV light increased. Cigarette analysis yielded inconsistent results but partial profiles were produced that could assist in excluding a suspect. Chewing gum was an excellent material for obtaining profiles. Full profiles were obtained even after exposure to 30 hours of sunlight. Humidity degradation experiments seemed to produce the most controlled method of degradation. The performance of different extraction techniques varied according to the extent of degradation.

(1) Bender K et al. Preparation of degraded human DNA under controlled conditions. Forensic Science International 139 (2004) 135-140

Catherine R Thacker, Centre for Haematology, Institute of Cell and Molecular Science, Barts and The London Queen Mary's School of Medicine and Dentistry, 4 Newark Street, London E1 2AT, UK

P-288

Population genetics of Y-chromosomal STRs in Amharic males from Ethiopia

Thiele K[1], Reißig D[2], Assegedech B[2], Yared W[2], Edelmann J[1], Lessig R[1]

[1]*Institute of Legal Medicine, University of Leipzig, Germany*
[2]*Department of Anatomy, University of Gondar, Ethiopia*

The Y-STRs are well established in the forensic routine case work. The investigation of Y-STRs in different populations is very important to get informations about the distribution of the haplotypes especially in relatively closed populations worldwide. So the main population in Gondar (Ethiopia) are the Amharics.

Samples from 173 unrelated males of this population were analysed. The samples were typed using the Y-PowerPlex-Kit (Promega) containing the markers of the so called "minimal haplotpye" and additional the STRs DYS437, DYS438 and DYS439. For allele typing a denaturing PAG and the ABI PRISM™ 377 DNA Sequencer were used.

The allele and haplotpye frequency data, the exclusion power of the STRs according to Nei and the haplotype diversity index to Takayama were calculated.

The most frequent haplotypes are obtained with a frequency of 0.0231. The haplotype diversity was estimated with 0.99 and the power of discrimination with 0.99. The allele diversity of the analysed markers differs between 0.068 and 0.747.

contact: yp2@gmx.de

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Usefulness of X-chromosome markers in resolving relationships among females, with reference to a deficiency case involving presumed half sisters

Chiara Toni[1], Silvano Presciuttini[2], Isabella Spinetti[1], Anna Rocchi[1], Ranieri Domenici[1]

[1]*Unit of Legal Medicine, School of Medicine, University of Pisa, Italy*

[2]*Center of Statistical Genetics, University of Pisa, Italy*

The use of X-chromosome (Chr-X) markers in forensic practice has played a minor role so far, probably because of its peculiar transmission rules, which reduce their potential use in forensic analyses to cases involving females only. However, the probability of excluding a false father in standard trios is higher for Chr-X markers than for autosomal loci with comparable values of polymorphic information content, and there are special circumstances in which they may resolve cases with deficiencies more efficiently than conventional loci. Therefore, Chr-X genotyping can efficiently complement the analysis of other genetic markers, and may resolve cases that otherwise would remain inconclusive. We were interested in the probability that two women with deceased parents were half sisters rather than unrelated. We first typed 16 autosomal markers commonly used in forensic practice, and obtained a cumulative likelihood ratio (LR) of 701.3, in favour of the hypothesis that they were half sisters, corresponding to a P value of 99.86% (assuming equal priors). As we usually present more compelling evidence in court cases, we typed the four unlinked Chr-X markers DXS101, HPRTB, STRX1, and DXS8377. Formulas needed for calculating likelihood ratios were obtained by Bayesian analysis (see Table below).

We compared the power of discriminating relationships between Chr-X and autosomal markers of equivalent informativeness in relation to the case at hand. All possible genotype configurations of any two individuals were listed for each marker, and for each of these configurations the LR that they were half sisters rather than non-relatives was obtained, using both the autosomal and the Chr-X formulas. LRs were converted into probabilities as usual [P = LR/(LR+1)], and the mean value of these probabilities was computed separately for the autosomal and the Chr-X cases. The ratio of the two mean values was chosen as a measure of the relative power of discrimination. The following ratios Chr-X/Autosomal were obtained: HPRTB, 1.19; STRX-1, 1.26; DXS8377, 1.37;DXS101, 1.33. In fact, the LR computed using these four markers in the casework was 495.8, not much lower than that obtained with 16 autosomal markers (701.3). The final (combined) probability value was 99.9997%, thus providing sufficient proof.

	Autosomal markers	Chr-X markers
Genotype configuration	LR	LR
AA,AA	$\frac{1}{2} + 1/2p_A$	$1/p_A$
AA,AB	$\frac{1}{2} + 1/4p_A$	$1/2p_A$
AA,BB	$\frac{1}{2}$	0
AB,AB	$\frac{1}{2} + 1/8p_A + 1/8p_B$	$1/4p_A + 1/4p_B$
AA,BC	$\frac{1}{2}$	0
AB,AC	$\frac{1}{2} + 1/8p_A$	$1/4p_A$
AB,CD	$\frac{1}{2}$	0

Table 1. Formulas used for calculating the likelihood ratios (LR) that two females are half-sisters rather than unrelated

Contact: sprex@biomed.unipi.it

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Variability in the detection of mixed profiles in four commercial autosomic STR multiplexes.

Torres Y, Sanz P

Instituto de Toxicología y Ciencias Forenses. Sevilla. Spain.

In some samples of forensic casework autosomic STR allelic mixtures can be not detected depending of the commercial kit used. Since it consumes time and money, the reference samples can be typed with one multiplex, but each evidence must be analyzed by duplicate with two different multiplexes, resulting in the confirmation of the results upon some markers.

The variability in the detection of mixed profiles with four multiplexes of a same manufacturer in the same DNA extracts of casework evidences were analyzed. DNA extracts of 55 evidences in forensic cases previously typed and reported as allelic mixtures were amplified by AmpF*l*STR® Profiler Plus™, Cofiler™, Identifiler™ and SGMPlus™ (Applied Biosystems), electrophoresed in an ABIPrims™ 310 and analysed with Genotyper® software v2.5.2 and GeneScan Analysis software 3.1. Re-injections of 15-20 second were made in the same tube when it was considered necessary. Only alleles over 100 r.f.u. were considered.

SGMPlus™ has been the multiplex that has identified allelic mixtures in a greater number of casework samples. With Identifiler™ we detected a lack of detection of 21% in the same DNA extracts. The presence/absence of some markers seems to be most decisive in the mixtures detection that the number of markers included in each commercial kit. The greater percentages of three or more allele determinations in the 55 samples tested were found in D8S1179 and VWA. CSF1PO, D2S1338, D7S820, TPOX and D13S317 showed the lower capacity of detection of mixed profiles.

contact: yolanda.torres@mju.es

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The inclusion of profiles of evidence of sexual aggressions in DNA databases: The viewpoint of a forensic genetics laboratory

Torres Y[1], Gamero JJ[2] , Sanz P[1], Romero JL[2]

[1]*Institute of Toxicology and Forensic Sciences. Sevilla. Spain*

[2]*Faculty of Medicine. University of Cádiz. Fragela s/n, Cádiz 11003. Spain.*

In Spain, although there exist norms that will enable the elaboration of future regulations regarding DNA profile databases, no text has yet appeared that could give those institutions directly involved in identification processes using DNA profiles some indication of the imminent coming into force of such regulations

We consider that it is necessary to examine and define confronting social and individual interests in order to obtain a legislative answer that would bring about the regulation of said databases, before an agreement on the final text is reached. In this sense, any future law that may come into force should be drawn up taking the following criteria into account: the way society conceives and values the subject, specialist advice that is obtained on the subject (based on prevailing scientific knowledge regarding forensic genetics), and finally the law itself

In this paper the possibilities, advantages and inconveniences of the inclusion in DNA profile databases of the results of the casework of sexual aggression obtained in our laboratory are analysed. The aim would be to provide objective data that may serve to aid the drawing up of future database regulations in Spain.

Keywords: National DNA database; DNA profile; sexual aggression; Forensic genetics; Ethics.
yolanda.torres@mju.es

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Genetic variability of 17 Y chromosome STRs in two Native American populations from Argentina

[a]Toscanini U, [b]Gusmao L, [a]Berardi G, [bc]Amorim A, [d]Carracedo A, [d]Salas A, [a]Raimondi E

[a]*PRICAI-FUNDACIÓN FAVALORO, Buenos Aires, Argentina*
[b]IPATIMUP, Instituto de Patología e Imunología Molecular da Universidade do Porto, Portugal
[c]*Faculdade de Ciências, Universidade do Porto, Portugal*
[d]*Instituto de Medicina Legal, Facultad de Medicina, Universidad de Santiago de Compostela, Spain*

Seventeen Y-STRs (DYS19, DYS389I, DYS389II, DYS389I, DYS390, DYS391, DYS392, DYS393, DYS385, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, GATA H4) were analyzed in two Native American populations, namely Tobas (N = 47) and Collas (N = 28), settled in the north and northwest regions of Argentina respectively. Standards diversity indices and haplotype frequencies were estimated. Genetic distance between both population was estimated by mean of Fst (Rst) test. Statistical tests were performed using Arlequin software Ver 2.000. Thirty three and fifteen different complete haplotypes were observed for the Tobas and Collas respectively. Haplotype diversity was 0.9769 +/- 0.01 for Tobas, and 0.9497 +/- 0.02 for Collas. These values are lower than those observed in other populations. A new variant, present in thirteen haplotypes was identified at DYS385 loci in Tobas. Two alleles were found in two samples from Toba population and in one sample from Collas at DYS448. No shared haplotypes were found between the two populations. A significant Fst value of 0.1466 was obtained at the pairwise comparison between the two populations ($P = 0.00$ +/- 0.0).

Contact: utoscanini@ffavaloro.org

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Forensic considerations on STR databases in Argentina

[a]Toscanini U, [a]Berardi G, [bc]Amorim A, [d]Carracedo A, [d]Salas A, [b]Gusmao L, [a]Raimondi E

[a]PRICAI-FUNDACIÓN FAVALORO, Buenos Aires, Argentina; [b]IPATIMUP, Instituto de Patología e Imunología Molecular da Universidade do Porto, Portugal; [c]*Faculdade de Ciências, Universidade do Porto, Portugal;* [d]*Instituto de Medicina Legal, Facultad de Medicina, Universidad de Santiago de Compostela, Galicia, Spain*

A genetic comparison study was conducted between populations from different regions of Argentina in order to determine if a pooled population STR database could be used for general forensic purposes. Samples were from urban populations of six geographically distant provinces of Argentina, namely, Tucumán (N = 51), San Luis (N = 42), La Pampa (N = 147), Buenos Aires (N = 879), Neuquén (N = 355) and Santa Cruz (N=82), and two Native American populations from the North and northwest region of the country, namely, Tobas (N = 129) and Collas (N = 43). A total of fifteen autosomal markers (D3S1358, TH01, D21S11, D18S51, PENTA E, D5S818, D13S317, D7S820, D16S539, CSF1PO, PENTA D, vWA, D8S1179, TPOX, and FGA) were analyzed. Exact tests P-values did not show deviation from Hardy-Weinberg equilibrium for both, urban and Native American populations ($0.005 < P < 0.987$, SD < 0.016). Regarding population differentiation, low Fst values were observed for the population pairwise comparisons; however, only significant differences were found when comparing Buenos Aires with Neuquén, and Santa Cruz (P = values between 0.000 and 0.024). Concerning Native American populations, Fst P-values were statistically significant when comparing Toba and Collas with every urban populations ($P = 0.000 \pm 0.000$). Furthermore, the two Native American populations themselves appeared to be significantly different ($P = 0.000 \pm 0.000$). Single locus comparisons showed some significant differences when comparing Neuquén and Buenos Aires, namely at D5S818, FGA and Penta D ($0.000 < P < 0.002$). However, no significant differences were found between the four remaining urban samples. When comparing urban populations with Amerindians and European populations, significant P-values were observed at 12 to 15 locus comparisons ($0.000 < P < 0.0498$). The four non differentiated urban populations studied were pooled in a single population database (N = 322). Exact test for Hardy-Weinberg equilibrium, frequencies estimates and forensic parameters were computed for the pooled sample as well as for Buenos Aires and Neuquén. P-values showed no deviation for Hardy-Weinberg equilibrium in the global sample ($0.057 < P < 0.991$). The combined matching probability and *a priori* chance of exclusion were 2.0×10^{-18} and 0.9999995, respectively. These results suggest that it would be possible to use a combined database for Tucumán, San Luis, La Pampa and Santa Cruz, provided that no significant differences were found between any of these populations. Caution should be taken concerning small isolates where Native American component could be much more relevant. In addition, it is remarkable that when comparing Argentina urban population with two Iberian samples (a major population source of the country European stock), some significant differences were found. Therefore, an Iberian database, might not adequately represent the Argentinean genetic makeup, although the real impact in forensic casework would require further investigation. utoscanini@ffavaloro.org

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Chromosome Y Haplotypes Database in a Venezuelan Population

Tovar F[1], Chiurillo MA[2], Lander N[1], and Ramírez JL[1]

[1].*Centro de Biotecnología, Fundación Instituto de Estudios Avanzados - MCT, Caracas; Venezuela.*
[2]*Decanato de Medicina, Universidad Centro Occidental Lisandro Alvarado, Barquisimeto; Venezuela.*

The non-recombining portion of Y chromosome is a source of polymorphic regions for the analysis of male DNA. The PowerPlex® Y System consisting of 12 Y-STR markers (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439 and DYS437) is a useful tool for database creation. These databases allow us to determine and correlate different allelic distributions, and the reconstruction of phylogenetic relationships among human populations. In this work, a chromosome Y database corresponding to 100 individuals living in Caracas city-Venezuela is presented. This is the first step leading to the implementation of this important forensic tool in our country. As in other studies, our results indicate that the 12 Y-STR makers here studied are useful markers for forensic and paternity testing.

contact: jramirez@reacciun.ve

P-295

Sperm DNA extraction from mixed stains using the Differex™ System

Tsukada K, Asamura H, Ota M, Kobayashi K, Fukushima H

Department of Legal Medicine, Shinshu University School of Medicine,0'
Asahi 3-1-1, Matsumoto, Nagano 390-8621, Japan

DNA typing is a very important and powerful tool in criminal investigations, especially those revolving around sexual crimes. Although nearly all specimens from crime scenes are of blood or blood stains, in cases of rape many of the specimens are of mixed stains, such as sperm with oral cells or sperm with vaginal cells. In recent years, with the development of multiplex Y-STR PCR kits, it has become possible to type many sperm loci within a short time. However, with autosomal DNA typing of sperm from mixed stains, it is necessary to separate out the sperm DNA from the mixed stain via a two-step method (two-step differential extraction procedure). However, this two-step method requires a long time, at least 1 to 2 days.

Recently, a new kit named the Differex™ System was newly supplied by Promega Co. (Madison, WI, USA). The Differex™ System uses a combination of phase separation and differential centrifugation for the separation of sperm and epithelial DNA. By use of this system the time required for sperm DNA extraction from mixed stains is greatly shortened (to approximately 2 hours) as compared with another methods.

In this study, mixed stains were created on pieces of cotton by mixing female epithelial cells with sperm of various concentrations; we compared the extraction efficiency of the Differex™ System with that of the two-step method. The sperm DNA extracted from the mixed stains was amplified using the AmpF*l*STR Profiler PCR Amplification Kit (AppliedBiosystems, Foster City, CA, USA). Electrophoresis was performed using an ABI 310 Genetic Analyzer, and alleles were determined with GenoTyper 3.7 software.

(contact: tuk-lab@mx1.avis.ne.jp)

P-296

Evaluation of an Autosomal SNP 12-plex Assay

Vallone PM, Decker AE, Coble MC, Butler JM

National Institute of Standards and Technology, Gaithersburg, Maryland, USA

SNPs have potential to play a useful role in human identification testing. Small PCR amplicon sizes associated with SNP typing technologies make SNPs attractive for typing degraded DNA or other low copy number situations. SNP markers can be useful in combination with STRs for resolving complex paternity issues (e.g. incest), identifying victims of mass disasters where insufficient family references are available and possibly inferring population of origin. Important considerations for SNP markers are the larger number required to equal the discriminatory power compared to traditional STRs, their inability to resolve complex mixtures, issues related to databasing new loci, and the availability of a standard analysis platform. However, in appropriate situations SNPs can be useful as a supplementary tool complementary to STR markers.

Various SNP typing platforms exist, but at this time there is not a universally accepted platform for SNPs and human identity testing. Currently we are typing SNPs with multiplex allele specific primer extension (ASPE) reactions. The assay is comprised of an initial step of PCR followed by primer extension and subsequent fragment separation and detection by capillary electrophoresis. ASPE multiplex panels can routinely type 6-12 SNPs in a single tube and have reported to go as high as 35 SNP markers. We have recently developed a 12-plex SNP assay that has been used to type over 600 U.S. population samples. The 12 markers are a subset of 70 bi-allelic SNP markers that were previously typed in our laboratory [1]. The amplicons range between 62-110 base pairs. The 12-plex assay has been used to successfully type DNA from shed human hairs. Samples typed by commercial and novel multiplex STR panels allow for a direct comparison of SNP and STR markers.

Practical and inherent characteristics of SNP markers will prevent them from replacing traditional STR typing methods. However, SNP markers can provide valuable complementary roles in human identity testing. Small autosomal panels of SNPs for typing challenging DNA samples is an example of where SNPs can benefit the forensic community. (contact: petev@nist.gov).

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P-297

Haplotypes analysis of the PowerPlex® Y System in northeast population from Italy

Turrina S, Atzei R, De Leo D

Department of Medicine and Public Health, Institute of Legal Medicine, University of Verona, Verona, Italy

Y-chromosome analysis is a useful tool in evolutionary study, paternity testing and personal identification.

Every year an increased number of Y markers are being reported in literature, nevertheless to evaluate their efficiency in forensic science it is necessary to investigate a large number of different populations.

Recently, a new multiplex set of 12 Y-STRs loci (PowerPelx® Y System, Promega) that includes the 9 Y-chromosome loci of the European minimal haplotype (DYS19, DYS385 a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393) plus two loci recommended by SWGDAM (DYS438 and DYS439) and DYS437 locus, was commercially released.

In the present study we evaluated allele frequencies and others statistical parameters of the PowerPlex® Y System in a population sample of 155 unrelated autochthonous healthy males from northeast Italy. In the totally of the observed haplotypes, there were 143 different haplotypes and among these, 134 were unique, while 9 haplotypes were observed more than one times. The haplotype diversity (HD) of 12 Y-STR multiplex was 0.9987.

Contact: deleodom@tin.it

P-298

Evaluation of an automated system for amylase detection in forensic samples

Da Vela G[a], Bertino MG[a], Ricci U[b]

Azienda Ospedaliera-Universitaria "A.Meyer", Florence, Italy
[a] Laboratorio di Analisi Chimico Cliniche e Microbiologia
[b] U.O. Genetica Medica

The amylase enzyme is present in human saliva and its detection in forensic samples is a very important step for the identification of the origin of a biological sample. The methods used in the forensic context employ chromatic reactions with visualisation of a colour or with spectrophotometric detection at an established wavelength. Here, we used an automated system normally employed in the clinical chemistry laboratory of to measure the activity of amylase in forensic samples. This method is perfectly integrated with DNA typing. Samples with a known concentration of amylase were tested with a manual system (BNP-Amylase test, Sclavo Diagnostics). Visual detection and spectrophotometric detection at 405 nm were performed, in comparison with the automated system. A calibration curve for sensitivity study using a commercial preparation of amylase was also performed to verify the linearity range. The automated method was employed for various samples containing human saliva (cigarette butts, chewing gum, stamps, etc). We used this detection system also on biological samples containing human saliva contaminated with different materials commonly recovered in forensic casework (ground, plaster, lipstick, glue).

The sensitivity of the system is superior to the other systems and offers an objective evaluation of the amylase in forensic samples.

contact: u.ricci@meyer.it

P-299

South Portugal population Genetic analysis with 17 loci STRs

C. Vieira - Silva, C. Cruz, T. Ribeiro, and R. Espinheira

National Institute of Legal Medicine, Forensic Genetics, Lisbon Portugal

STRs are the standard genetic markers mainly used in forensic cases. In routine casework it is important to establish a population genetic database for further reliable statistical analyses.

AmpF1STR®Identifiler™ (Applied Biosystems) and Geneprint Powerplex 16® (Promega Corporation, Madison WI, USA) are multiplex kits wich co-amplifie 17 STR - loci including de segment of X-Y homologus gene Amelogenina routinely used in our laboratory. 13 core short tandem repeat loci standardized under the combined DNA Index System (Codis): CSF1PO, D3S1358, D5S818, D13S317, D16S539, D18S51, D21S11, vWA, FGA, TH01, TPOX, two additional tetranuceotide loci - D2S1338 and D19S433 – and two additional pentanucleotides – Penta E and Penta D.

The purpose of this study is to determine the allele distribution data of the 17 STR loci in 2445 caucasian unrelated individuals from the south of Portugal, 176 unrelated individuals from Cabo Verde and 102 unrelated individuals from Angola and compare it with the values of the all the population resident in the same area.

Allele frequencies for each locus, observed heterozigoty, expected heterozygoty, power of exclusion, power of discrimination and p values of chi square test for departures from Hardy-Weinberg expectations were calculated.

contact: genetica@dlinml.mj.pt

P-300

Evaluation of the 4-year test-period of the Swiss DNA database

Voegeli P, Haas C, Kratzer A, Bär W

Institute of Legal Medicine, Forensic Genetics, University of Zurich, Switzerland

The Swiss federal DNA profile information system (EDNA) is operational since July 2000, using the CODIS Software provided by the FBI. The database holds DNA-profiles of suspects, single stains and also mixed stains (presumably consisting of not more than 2 persons). The genetic criteria for entering profiles into the database are the 10 SGMplus loci for suspects, at least 6 SGMplus loci for single stains and at least 8 loci for mixtures. At the end of the 4-year test-period (31 december 2004) the database contained 61'954 DNA profiles, 53'400 profiles from suspects (89% male, 11% female) and 8'554 profiles from stains (90.7% single profiles, 9.3% mixtures). Thereof 548 profiles are from foreign countries. Stains which are assigned to a suspect are removed from the database day-to-day.

During the test-period the database provided excellent results. 6'825 stains could be assigned to a suspect (offender hits), about 2'000 crime-sites could be connected (forensic hits) and 35 criminal monocygotic twin-pairs were identified (offender duplicates). 44% of the single stains that were entered into the database resulted in an offender hit, allowing the identification of the unknown perpetrator. About 50% of the mixtures revealed one offender hit and another 15% were solved with two persons.

The 6'825 offender hits can be subdivided into the following crime categories: the major group with 85.2% was burglary/theft/wilful destruction, followed by homicide/bodily harm with 4.2%, robbery with 4%, sexual offenses with 2.4% and 4.2% other delicts.

Voluntarily the DNA profiles of laboratory staff and involved police members could be entered into a separate index, in order to detect contaminations. Thus, 36 stains could be identified as contaminations. A special search mode (allowing 2 errors) helps finding incorrect profiles. 33 additional hits were discovered using this search mode.

haco@irm.unizh.ch

P-301

The extent of substructure in the indigenous Australian population and its impact on DNA evidence interpretation.

Walsh SJ[1], Mitchell RJ[2], Curran JM[3], Buckleton JS[4]

[1] *Centre for Forensic Science, University of Technology, Sydney, Australia*
[2] *LaTrobe University, Melbourne, Australia*
[3] *University of Waikato, Hamilton, New Zealand*
[4] *ESR, Auckland, New Zealand.*

Indigenous Australians have a unique evolutionary history resulting in a complex system of inter- and intra-tribal relationships. Although European colonisation has disrupted to a varying extent these and other features of Aboriginal life, forensic DNA evidence has recently been called into question with respect to the impact of this evolutionary past on issues associated with population genetics and the estimation of DNA match statistics. The extent of substructure within the indigenous Australian sub-population raises two main questions relevant the interpretation process; 1) what is the appropriate value of the co-ancestry coefficient, theta or Fst?, and 2) what is the effect of sub-population substructure on the performance of the sub-population model? This paper describes research that focuses on these issues. Research examining classical markers as well as DNA SNPs has shown evidence of considerable heterogeneity within the indigenous Australian population. The question is, to what extent do autosomal microsatellites used in contemporary forensic testing show such structuring effects? Autosomal microsatellite diversity within the indigenous Australian population has been examined through the analysis of genotype data from a large number of geographically distinct tribal groups and urban centres. Genotypes included highly polymorphic loci from the Profiler Plus™ and Identifiler™ PCR systems. Autosomal STR Fst values have been estimated from these data, and were found to be considerably lower than some values from previous research that has focussed more often on SNPs or blood group and protein loci. The performance of the sub-population model was also investigated by simulation under circumstances where the assumption of equilibrium in the sub-population is violated. The results imply that departures from equilibria at the sub-population level do very little to alter the inherent conservativeness of the model.

Simon.Walsh@uts.edu.au

P-302

Analysis of single nucleotide polymorphisms and its application to a disputed paternity case

Wang X, Ito S, Sawaguchi A, Sawaguchi T

Department of Legal Medicine, Tokyo Women's Medical University, School of Medicine, Japan

There has been recent progress in the areas of research and applied development in the genetic analysis of the single nucleotide polymorphisms (SNPs) employing fluorescent dye labeling technology. SNPs are places along the chromosomes where the genetic code tends to vary from one person to another by just a single base. They are estimated to occur about once every 1000 bases along the 3-billion-base human genome. SNPs are an increasingly important tool for genetic and biological research. SNPs analysis is becoming increasingly important studies of drug resistance, evolution, and molecular epidemiology in mycobacterium tuberculosis, human immunodeficiency virus, and other organisms. Although current genomic databases contain information on several million SNPs and are growing at a very fast rate, the true value of a SNP in this context is a function of the quality of the annotations that characterize it. The most common application of SNPs is in association studies that look for a statistically significant association between SNP alleles and phenotypes, in order to find pinpoint candidate causative genes. For this reason, large databases of well-annotated SNPs have been developed, and are growing at an ever-increasing rate. Data derived from analysis of SNPs are being applied in many diverse fields, from medical studies of disease mechanisms and individual drug response, to population genetics for tracking migration and mixing of ancestral groups and also in forensic science for the identification of human remains and identification of individuals from bodily samples.

In this study, we investigated distribution of allele frequencies for 16 SNPs loci (G63767, G63754, G65359, G63748, G65275, G65270, G65266, ss4019224, ss4947490, ss4974676, ss4974689, ss4974729, ss4974915, ss5013903, ss6658727) in 120 unrelated healthy Japanese individuals using multiplexed single nucleotide primer extension by ABI PRISM SnaPshot Multiplex Kit and ABI PRISM 310 Genetic Analyzer (Applied Biosystems). A total of 32 alleles and 48 genotypes were observed in these samples for 16 SNPs loci. The combined power of discrimination and the combined power of exclusion for SNPs in 16 loci were 0.9954 and 0.92653, respectively.

We applied these databases to analyze a case of special paternity testing. In this case, the putative father and the child's mother were decease. The DNA of the deceased putative father was only extracted from a formalin-fixed liver tissue. In this inspection result of 16 SNPs, the paternal rights affirmative probability to the deceased putative father's child was 0.9912, and the possibility of existing related to the deceased putative father and child was not denied in genetics. The result demonstrated that analysis of 16 SNPs are an extremely effective method for the diagnosis of paternity with formalin-fixed liver tissue in paternity testing of a deceased parent. This study also indicated that if DNA fragment length is longer than 100bp, that could be enough to using for analysis of SNPs from the liver tissue, even if the liver had been fixed for a long time with formalin solution.

Contact: xiuling@research.twmu.ac.jp

P-303

Analysis of Mitochondrial DNA Polymorphisms based on Denaturing High-Performance Liquid Chromatography

Wang XD, Liao LC, Li YB, Wu J, Hou YP

Department of Forensic Genetics, Sichuan University (West China University of Medical Sciences), Chengdu, P.R.China

The purpose of this study is to establish a novel method for the detection of polymorphism of mitochondrial DNA (mtDNA) based on denaturing high-performance liquid chromatography (DHPLC) and to explore the new mitochondrial DNA polymorphism in coding region in order to improve the discrimination power of mtDNA in forensic DNA typing. We explored the polymorphism of the sequence in the coding region, which covered 1435bp. A total of seven pairs of primers for PCR were designed to analyze the region of mtDNA, so that it was nominated as seven loci. To explore the polymorphism of the region of mtDNA, a technique of sample pool was employed for the analysis of DHPLC. All of seven loci were analyzed by DHPLC in a Chinese population sample. Our study revealed that there were 53 haplotypes at seven loci in the coding region with covering 1435bp and the haplotype diversity was 0.8775 in our Chinese population sample. Among these, four loci with higher diversity were proved to be suitable for forensic application and provided new genetic markers for the forensic mtDNA typing

contact: rechtsme@wcums.edu.cn
forensic@mail.sc.cninfo.net

P-304

Linkage disequilibria between 6 STR loci situated in the HLA region on Chromosome 6

Wenda S, Dauber EM, Dorner G, Reisacher RBK, Glock B, Mayr WR

Division of Blood Group Serology, Medical University Vienna, Austria

Six STR polymorphisms coded for by the HLA region of chromosome 6p21.3 have been investigated: three tetranucleotide repeat loci D6S389, D6S1051 (GenBank G08553) and D6S2822 (M2_4_25) situated in the HLA Class II region (D6S2822 located between HLA-DQ and DP; D6S1051 4 cM centromeric of HLA-DP and D6S389 1.3 cM centromeric of D6S1051), as well as two tetranucleotide repeat loci, C1_4_4 (D6S2931) and C2_4_4 (D6S2939), and one trinucleotide repeat locus C3_3_6 (D6S2906) located in the HLA class I region (C3_3_6 0.4 cM and C2_4_4 1 cM centromeric of HLA-A, respectively; C1_4_4 0.2 cM centromeric of HLA-B). 284 haploytpes from 71 Austrian Caucasoid families could be defined. The analysis of the linkage disequilibrum between alleles of the 3 STR loci located in the HLA class I region (C1_4_4, C2_4_4, C3_3_6) showed several significant values (the p values have been corrected by multiplying them with the number of comparisons made). No linkage disequilibria could be found between alleles of the 3 STR loci next to the HLA class II region (D6S389, D6S1051, D6S2822) and between alleles of these 3 loci and of the loci situated in the HLA class I region.

C1_4_4	C2_4_4	C3_3_6	χ^2	Significance Corrected p-value
	*20	*17	34.88	p<0.001
	*9	*12	27.36	p<0.001
*10	*9		153.13	p<0.001
*12	*11		83.29	p<0.001
*16	*16		25.74	p<0.001
*7	*16		24.86	p<0.001
*8	*10		23.24	p<0.001
*19	*10		20.85	p<0.001
*10	*10		19.27	p<0.01
*18	*17		18.69	p<0.01
*7	*17		14.29	p<0.05
*10		*12	31.34	p<0.001
*19		*9	15.05	p<0.05

Within the HLA class I STRs, only one haplotype with a significant three-locus-disequilibrium could be observed: C1_4_4*10, C2_4_4*9, C3_3_6*12. These 3 alleles are situated on the common Caucasoid superhaplotype HLA-A1,B8,Cw7,DR3. The absence of a high degree of linkage disequilibrium between the alleles of the HLA STRs is probably due to the fact that the STR loci, in contrast to the phenotypically expressed HLA alleles, are not subjected to selective forces. The lack of significant disequilibria between the HLA class II STRs is also caused by the higher physical distance between the loci.

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P-305

Validation and Evaluation of the ABI 3100 Genetic Analyser for Use With STR Analysis of CJ Buccal Swabs - Systematic Differences Between the AB13100 and ABI377

Warren T, Johnston I, Johns LM, Bardill SC

LGC, The Heath, Runcorn, WA7 4QX, UK

For many years ABI377 DNA sequencers have been used in the production of short tandem repeat (STR) profile data for forensic human identification (HID) applications. These instruments provided a 36-96 lane, slab gel, based electrophoresis system. However accurate, using this system resulted in a relatively high level of re-work due to the inconsistent nature of the polyacrylamide gels used. The operation of the ABI377 was also labour intensive and hazardous due to the requirements for gel production. The development of capillary electrophoresis genetic analysers, e.g. ABI3100, suggested potential improvements in sensitivity, reliability, and flexibility. In this study a comparison has been made between the ABI 377 DNA sequencer and the ABI 3100 genetic analyser when performing STR profiling of forensic Buccal swabs. The criteria used to evaluate/validate the ABI 3100 are outlined and the assessments made are described.

Contact: Jim.Thomson@lgc.co.uk

P-306

Variability of mitochondrial DNA mutagenesis in human blood

von Wurmb-Schwark N[1], Jelkmann I[1], Bruhn HD[2], Oehmichen M[1]

[1]*Institute of Legal Medicine, University of Kiel, Kiel, Germany*
[2]*Clinic for Internal Medicine, University of Kiel, Kiel, Germany*

The 4977 bp deletion of mitochondrial DNA (mtDNA) is known to accumulate with age in post mitotic tissues. Meanwhile, this mutation can also be detected in tissues with a fast turnover like blood or skin. From a forensic point of view, it is interesting to elucidate the possible correlation between age and the amount of mutated mtDNA (dmtDNA) in blood to estimate the age of an individual.
We investigated mtDNA mutagenesis in blood from 10 persons (22-60 years) over a time period of 6 month. During that time, we monitored exogenous factors that might influence the integrity of mtDNA, like smoking habits, alcohol consumption, and medicine intake. A blood sample was drawn from each proband every other week, and the following criteria were investigated: 1. The amount of total mtDNA/cell was measured using a real time PCR; 2. the occurrence and relative quantification of deleted mtDNA was carried out in a Duplex-PCR with subsequent fragment detection in an ABIPrism310; 3. a blood cell count was done.
Real time PCR results showed values between 1003 and 3275 mtDNA copies/cell (average 2127) with a very strong variation within one individual from time point to time point. The occurrence of the dmtDNA also varied considerably, showing ratios from 0 – 0,6621 (dmtDNA/undeleted mtDNA) in the same individual on different days.
Consequently, the quantity of dmtDNA in blood is not a suitable measure to determine the age of an individual for forensic purposes, since mitochondrial mutagenesis seems to be influenced by too many exogenous factors.

NvonWurmb@rechtsmedizin.uni-kiel.de

P-307

Fast and simple DNA extraction from saliva or sperm cells obtained from the skin or isolated from swabs

von Wurmb-Schwark N, Mályusz V, Fremdt H, Koch C, Schwark T, Oehmichen M, Simeoni E

[1]*Institute of Legal Medicine, University of Kiel, Kiel, Germany*

The forensic scientist often has to cope with problematic samples from the crime scene due to their size and thus the amount of extractable DNA. The retrieval of DNA from swabs taken from the surface of the skin, for example in cases of strangulation, can be especially difficult.

We systematically investigated swabs taken from the skin (to obtain a genetic profile from the victim and also from a possible offender) and from sperm containing swabs using two extraction kits: the Invisorb Forensic and the Spin Swab kit (both Invitek, Germany). DNA quality and quantity was tested on ethidium bromide containing agarose gels and a highly sensitive Duplex-PCR which amplifies fragments of mitochondrial and nuclear DNA. Absolute quantification was done using real time PCR. Samples which were positive in the Duplex-PCR were also employed to genetic fingerprinting using the Powerplex ES and the AmpF*l*STRIdentifiler™ kit. Additionally a 433 bp fragment of the mitochondrial HVI region was generated and sequenced.

Our study shows that the easy-to-use Spin Swab kit is very suitable for DNA isolation from swabs taken from the skin and surprisingly also from sperm cells. We will present modifications which greatly improve the isolation of DNA and additionally simplify the whole process of trace handling and DNA extraction.

NvonWurmb@rechtsmedizin.uni-kiel.de

P-308

Newly designed multiplex amplification and genotyping system at four pentanucleotide repeat STR loci useful for degraded mixed DNA specimens

Yamamoto T, Uchihi R, Ando Y, Suzuki M, Yoshimoto T, Katsumata Y

Department of Legal Medicine and Bioethics, Graduate School of Medicine, Nagoya University, Nagoya, Japan

Short tandem repeat (STR) markers with tetranucleotide repeat are globally utilized for personal identification and kinship analysis in forensic field, and give us very useful information in almost practical cases. In case of mixed samples, however, the 'stutter peaks' sometimes make it difficult to interpret whether they are exact 'stutter peaks' or minor peaks originated from the another individual, because of more than about 10 % of stutter percentage for tetranucleotide STRs which is the ratio of the stutter peak height to a main peak height. In general, it is said that the stutter percentages become lower with the more numbers of repeat units. In the present study, we selected four pentanucleotide STR loci (Penta E, Penta D, Penta B and D10S2325) to construct a multiplex PCR and genotyping system with multicoloured fluorescently labelled primer sets newly designed for degraded DNA samples, of which the amplicon sizes are smaller than about 200 bp. Sequence analysis was performed for all alleles at these all STR loci observed in a Japanese population, and also the allelic ladder markers with these alleles inserted into the plasmids in a commercially available kit were constructed for semi-automated genotyping by making a template of Genotyper 2.5 software. Using the present multiplex system, 300 unrelated Japanese (Nagoya city) were genotyped with written informed consent, and calculated the allele frequencies at each locus. Three tests for Hardy-Weiberg equilibrium (HWE) were performed, and the allele distributions at those four loci did not deviated from HWE. The heterozygosities and the power of discriminations (PD) at all the four loci were more than 0.80 and 0.93, respectively. The combined PD and MEC (mean exclusion chance) were 0.9999962 and 0.9900966, respectively. The stutter percentages at all alleles for these four loci, where stutter peak heights were more than 50 RFU, were calculated, and it was found that the stutter percentages were almost directly proportional to the numbers of repeats at each locus, and that almost all the stutter percentages were distributed within the regression lines from ± 3 times values of SD (standard deviation) of at each allele for each locus. Accordingly, this could be a statistical standard to decide whether a one-repeat small peak from a main peak is the stutter peak or the peak originated from mixed individual sample. It was suggested that this system is one of the useful multiplex typing system, especially for mixed DNA specimens.

yamachan@med.nagoya-u.ac.jp

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STR loci analysis of buccal cavity cells captured by laser microdissection

Yamamoto Y[1, 2], Hara M[2], Kido A[3], Takada A[2], Saito K[2]

[1]Criminal Investigation Laboratory, Saitama Prefectual Police Headquarters, Saitama, Japan
[2]Department of Forensic Medicine, Saitama Medical School, Saitama, Japan
[3]Department of Legal Medicine, Faculty of Medicine, University of Yamanashi, Yamanashi, Japan

In the present study, we have attempted to analyze short tandem repeat (STR) loci for buccal cavity cells isolated from saliva. The buccal cavity cells were captured from smear of saliva stained by Harris Hematoxylin and Eosin by a laser microdissection method using PALM Microlaser Systems (P.A.L.M). DNA was extracted from the buccal cavity cells using DNA Extraction FM Kit (Wako). After the whole genome amplification of DNA extracted from the buccal cavity cells carried out by the improved PEP PCR method, the 15 STR loci, D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1339, D19S433, vWA, TPOX, D18S51, D5S818 and FGA, as well as amelogenin locus were amplified using AmpFLSTR Identifiler Kit (Applied Biosystems). Amplified products were separated by denaturing capillary electrophoresis in the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The results were analyzed using GeneScan Analysis v3.7 software (Applied Biosystems) and Genotyper v3.7 software (Applied Biosystems). The 15 STR loci and the amelogenin locus were determined from 20 buccal cavity cells, the 13 STR loci and the amelogenin locus from 10 buccal cavity cells, the nine STR loci and the amelogenin locus from five buccal cavity cells, and the five STR loci and the amelogenin locus from two buccal cavity cells. This method is feasible for the STR loci and the amelogenin locus analysis of a few buccal cavity cells. In further studies, we should investigate the STR loci analysis of single cell from mixed seminal/vaginal secretion stains and tissue slice specimens by technical improvement.

Contact: CW4Y-YMMT@j.asahi-net.or.jp

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ANALYSIS OF SIX TETRANUCLEOTIDE POLYMORPHISMS OF THE X-CHROMOSOME IN DIFFERENT SPANISH REGIONS

Zarrabeitia MT[1], Alonso A,[1], Martín J[2], Gonzalez-Gay MA[3], Martin-Escudero JC[4], Martinez de Pancorbo M[5], Martín P[6], Ruiz-Cabello F[7] and Riancho JA[8]

1 Unidad de Medicina Legal. Universidad de Cantabria. Santander. 2 Instituto "Lopez Neyra", CSIC. Granada. 3 Complejo Hospitalario Xeral-Calde. Lugo. 4 Hospital del Río Ortega. Universidad de Valladolid. 5 Facultad de Farmacia. Universidad del Pais Vasco. Vitoria. 6 Instituto Nacional de Toxicología. Sevilla. 7 Hospital Virgen de las Nieves. Universidad de Granada. 8 Departamento de Medicina. Universidad de Cantabria.

X-linked markers are particularly useful in paternity deficiency cases, as all daughters of a father inherit the same X chromosome. We studied 6 X-linked microsatellites in a large group of Spanish individuals (n=614) from five different regions located in northern, central and southern Spain. All the markers had tetranucleotide repetition units (DXS9895, DXS9898, DXS7130, DXS7131, GATA172D05, and DXS6789). They were amplified in two triplex PCR. There were no significant sex- or region-related differences in allelic frequencies, suggesting that general national databases can be adequate as a reference in X-linked markers. Fst coefficients varied between 0.002 (DXS6789, GATA172D05) and 0.066 (DXS7130).
The forensic efficiency parameters are shown in the table.

	DXS7130	DXS7132	DXS6789	DXS9895	GATA172D05	DXS9898
PIC	0.716	0.739	0.816	0.727	0.790	0.753
PD female	0.894	0.903	0.904	0.894	0.934	0.911
PD male	0.733	0.758	0.752	0.748	0.805	0.772
PE trio	0.699	0.719	0.718	0.705	0.777	0.735

PIC: polymorphism information content
PD: power of discrimination
PE: power of exclusion

The 6-locus combined analysis in 316 males revealed 300 different haplotypes, 283 of which were found only once. There was no evidence for statistically significant linkage disequilibrium among the loci studied.
Therefore these markers are quite polymorphic and useful for forensic purposes
contact: zarrabet@unican.es
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Estimating the postmortem interval by determining the age of fly pupae: Are there any molecular tools?

Zehner R, Mösch S, Amendt J

Institute of Legal Medicine,
J W Goethe-University Frankfurt, Germany

Forensic entomology, the use of insects in medicolegal investigations mainly focus on the estimation of the postmortem interval (PMI) by calculating the age of necrophagous specimens. Usually the age of an insect developing on a corpse is determined e.g. by the measurement of the length of the larvae and using species-specific growth rates in consideration of the temperature conditions at the scene of crime. The estimated age represents the minimal PMI because the necrophagous insects do not oviposit before death. While staging the age of the larvae is possible at a quite detailed scale, the age of the pupae is not to specify without rearing up to the adult stage, which is time-consuming or might be difficult.

However, the pupal stage represents about 50% of the immature development time and the pupal age may serve as an important tool in entomological PMI estimation if no larvae are present..

Our approach was to study gene expression patterns of transcripts, which are differently expressed during pupal development and estimate its usefulness in estimating the pupal age compared to morphological characters of the pupae after removal of the puparium (outer shell).

Total RNA was extracted from single pupae of different ages, reversely transcribed and subjected to differential display PCR. PCR products were separated by PAGE and stained with silver. The banding profiles from pupae of different ages were compared and evaluated with regard to their use as age marker.

Using ddRT-PCR as a single tool, estimating the pupal age is not more accurate than using morphological characters alone. The significance of molecular techniques for entomological PMI estimation is discussed.

Contact: zehner@em.uni-frankfurt.de

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STR typing with High Performance Liquid Chromatography

Zhu QF, Li YB, Liao LC, Wu J, Hou YP

Department of Forensic Genetics, Sichuan University (West China University of Medical Sciences), Chengdu, P.R.China

The purpose of our work was to study the STR typing method with high performance liquid chromatography and to comprehend the rules of STR typing with HPLC. Firstly, all alleles of the STR marker at D10S2325 locus were sequenced and mixed to construct an allele ladder. Secondly, the HPLC conditions to separate each allele at D10S2325 locus were optimized. Thirdly, STR typing of D10S2325 was carried out by comparing the retention time of the allele ladder on HPLC with that of a sample. Our method was validated by typing same samples with the polyacrylamide gel electrophoresis. Lastly, the sensitivity of this method and the ability to analyze mixed samples were tested. The results showed that the method of STR typing with HPLC established by us was successful. The results of our study implied that it was important to consider both the conditions of chromatography and the variation of retention time caused by the sample concentration when STR typing was carried out with high performance liquid chromatography

contact: rechtsme@wcums.edu.cn
forensic@mail.sc.cninfo.net

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Haplotype distribution of the mitochondrial control region in the native Canary Islands population.

Zurita A[1], Hernandez A[1], Sanchez J[2], Cuellas JA[1]

[1] *National Institute of Toxicology and Forensic Sciences, Canary Islands, Spain*
[2] *Department of Forensic Genetics, University of Copenhagen, Denmark*

Canary Islands constitute a crossroads territory, with a considerable flow and settlement of different human groups, mainly from Europe, North Africa and South America. This introduces a complex forensic scenario that makes often difficult the interpretation of population genetic data and statistical models applied in forensic genetics casework. To understand the genetic composition and origen of the present-day Canary Islands population, we studied the mitochondrial control region of a total of 210 voluntary native islanders, i.e. individuals who were born in the archipelgo with at least two known Canarian ancestor generations, in order to lessen the contribution of the last fifty years immigration.

Both HV1 and HV2 regions were sequenced by standard procedures and haplotype frequency distribution was determined. From the data obtained some useful forensic statistical parameters were inferred, like nucleotide diversity, gene diversity or mean number of pairwise differences. Furthermore, the sequences were compared with other population groups, like Europeans, North and Sub-Saharians, and aborigens (from ancient samples), and phylogenetic relatioships were established. Data were compared with those obtained with chromosome Y SNPs.

Contact: ahernandez@canariastelecom.com