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Oral Presentations

OP001
Introduction and presentation of our projects in genetics of sudden death and post mortem pharmacogenetics

Sajantila A

Laboratory of Forensic Biology, Department of Forensic Medicine, P.O.Box 40, 00014 University of Helsinki, Finland

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OP002
Post mortem pharmacogenetics of opioids

Wong S

Medical College of Wisconsin, USA

OP003
New perpectives in pharmacogenetics

Carracedo A

Forensic Genetics Department, Institute of Legal Medicine, University of Santiago de Compostela

Pharmacogenetics has experienced an important development in the last few years and it's now a reality in clinical practice. The regulatory agencies (EMEA and FDA) advice or request the performance of pharmacogenetic test in at least 8 different products and the number of validated biomarkers for drug response prediction are continuously increasing. However many of these biomarkers are still exploratory and need confirmation. Large scale association studies using SNPs are continuously producing new data and bioinformatic tools (especially PharmGKB) are facilitating the analysis of the information. In this talk the state of the art of clinical pharmacogenetics will be reviewed, the role of regulatory agencies described and lessons that can be extrapolated to the forensic field will be shown.

OP004
Extracting RNA from post-mortem human tissue

Heinrich M, Matt K, Lutz-Bonengel S, Schmidt U

Institute of Legal Medicine Freiburg, Germany

It was shown that the extraction of RNA from post-mortem human tissues can be successfully performed. In several studies it was found that the post-mortem interval does not correlate significantly with the degradation of RNA. Satisfying yields of high quality RNA can be obtained from various tissues after a post-mortem interval of up to 96 h. Thus, it can be stated that gene expression studies could reveal valuable additional information for example to the analysis of the cause of death. Additionally, gene expression data from human tissue would expand the knowledge of physiological pathways and of genes responsible for various diseases. Most of the existing gene expression data were obtained from mouse or rat animal models and a transfer of these data to the human organism is not always possible. Nevertheless, the technique chosen for RNA extraction from various tissues seems to have a strong influence on the retrieval of RNA as well as on its quality, e.g. the nucleotide length of the extracted RNAs. In order to find the most suitable method for RNA extraction of post-mortem material various techniques were tested on six different tissues: brain, muscle, heart, liver, kidney, and spleen. Every technique was tested on tissue of three different individuals with PMI of 15 h, 56 h, and 96 h, respectively. The methods were chosen to span different principles of RNA extraction. Among them were techniques based on phenol/chloroform extraction and binding of RNA to magnetic particles or to a silica membrane in combination with a microcentrifuge protocol and a vacuum protocol. The pros and cons of the different techniques will be discussed. Additionally, the necessity of DNase digestion as well as additional clean-up steps will be addressed. Finally, forensic fields of research based on gene expression studies will be pointed out.

OP005
Mutations in the SCN5A gene: evidence for a novel link between long QT syndrom and sudden death

Kauferstein S, Kiehne N, Bratzke H

Zentrum der Rechtsmedizin, Universität Frankfurt

The long QT-Syndrom (LQTS) is an inherited genetic disorder that can cause sudden death among apparently healthy young individuals due to ventricular arrhythmias. It is a primary cardiac channelopathy with six identified chromosomal loci and seven ion channels implicated so far. Recently, the first non-ion channel protein (ankyrin B) has also been identified. Mutations in these genes produce either a gain or a loss function, resulting in an excess of late inward sodium currents or in reduced outward currents. These changes increase the duration of the action potential which explains the prolonged QT interval, leading to an ventricular arrhythmia. Therefore, cardiac ion channel genes represent viable candidates for the pathogenesis of sudden death. To investigate the impact of changes in these genes on the risk of sudden death, we initiated postmortem genetic analysis. So far, 9 cases of sudden death have been screened, six known mutations in the sodium channel Nav1.5 and the potassium channel KCNH2 have been detected. In a 4-month old infant who experienced sudden infant death syndrom, a novel mutation in the functional pore region of the sodium channel Nav1.5 was identified. A second novel mutation in the sodium channel of an 15 year old boy was also found, which is located in the promoter region of the channel and raises the possibility that altered gene expression may have caused sudden death. These findings suggest a link between LQTS and the cause of death in these cases of sudden death and demonstrate the important role of molecular screening.

OP007
X-Chromosomal Markers: Past, Present, and Future

Szibor R

Institut für Rechtsmedizin, Otto-von-Guericke-Universität Magdeburg, Leipziger Strasse 44, 39120 Magdeburg, Germany

Experience gained in clinical genetics led to the fundamental idea of using X-chromosomal markers in a wide range of forensic applications. All daughters of a male with an inherited X-chromosomal trait, carry the paternal X-chromosome (ChrX) and transfer it to half of the next generation. Moreover, the hemizygote state of the ChrX in males can be used to establish the linkage between several markers and traits. Evidently, knowledge of such simple cognitive contexts is not only valuable to clinical genetics but also to kinship testing. The development of ChrX markers started with the detection of the Xga blood group, followed by typing of SNPs (expressed as RFLPs) using the southern technique, and finally arrived at microsatellite analysis a few years ago. To date more than 30 STRs have been established as forensic markers. Joint typing of very tightly linked STRs yields stable haplotypes, and can be used for establishing the relationship between distant relatives, such as aunt-nice pairs and cousins. For such applications the new ChrX typing kit (ArgusX-8®) which is commercially available now is a powerful tool. This paper is aimed at presenting a brief survey of historical developments and discussing present and future aspects of forensic X-chromosomal testing.

OP008
Kinship testing with x-chromosomal markers: mathematical and statistical issues

Krawczak M

Michael Krawczak, Institut für Medizinische Informatik und Statistik, Christian-Albrechts-Universität Kiel, Germany

Use of X-chromosomal markers for kinship testing is meaningful if the identical-by-descent allele sharing probabilities of at least two individuals involved in the case differ under the different hypotheses made about the composite relationships. In this situation, optimal decision making about one or the other hypothesis should be based upon the likelihood ratio of the genotype data obtained. When more than one X-chromosomal marker is being used, this implies that the patterns of linkage and linkage disequilibrium between the respective loci have to be taken into account. Otherwise, the evidence extracted from the data by means of the likelihood ratio may be misleading. Exact likelihood calculations on complex pedigrees can be performed using available software such as, for example, the „LINKAGE“ programmes widely used in genetic epidemiology. The required genetic maps can be created by combining physical and genetic distance information from public databases. This strategy will be exemplified for a panel of 19 X-chromosomal markers, currently used in forensic practice, which have recently been applied to solve two complex kinship cases in which autosomal DNA information was insufficient.

OP009
Mining the HapMap phase I data release for X-chromosome ancestry informative SNPs

Phillips C, Lareu M, Natera A, Carracedo A

Forensic Genetics Department, Institute of Legal Medicine, University of Santiago de Compostela

We have examined the HapMap project Phase I data release (1), comprising 1.1 million SNPs validated in 60 Africans and Europeans plus 45 Chinese and Japanese, for X-chromosome markers that exhibit large contrasts in allele frequencies between the four populations studied. A substantial number of SNPs were found showing marked allele frequency differences between Africans and non-Africans: giving Fst values, in many cases, between 0.9 and 0.95. In particular the genes: EDA2R; MTMR8; VSIG4; FLJ39827; MSN; ARHGEF9; IL1RAPL2; TRPC5 and AFF2 provided many SNPs with alleles approaching fixation between African and non-African populations. In contrast, SNPs distinguishing Europeans from the other populations and Asians from the others, were uncommon and showed much smaller contrasts in allele frequencies. We genotyped 20 of the most informative X-chromosome SNPs with an independent population sample matching each of the three HapMap continental groups (60 Galician, Mozambican and Taiwanese) to confirm the variability reported by HapMap. The results indicate that these loci would provide an informative marker set for the estimation of ancestry. Typing of the most highly contrasting SNPs would be particularly useful for the analysis of population admixture between African and non-African populations. (1) A haplotype map of the human genome. The International HapMap Consortium. 2005 Nature 437, 1299-1320

OP010
Evaluation of the X-chromosomal Short Tandem Repeat marker DXS10101 for forensic applications

Becker D[1], Rodig H[1], Kloep F[1], Weißbach L[2], Augustin C[3], Edelmann J[4], Hering S[5], Szibor R[6], Götz F[2], Brabetz W[1]

1) Biotype AG, Moritzburger Weg 67, 01109 Dresden, Germany 2) Qualitype AG, Moritzburger Weg 67, 01109 Dresden, Germany 3) Institut für Rechtsmedizin, Universitätsklinikum Hamburg-Eppendorf, Butenfeld 34, 22529 Hamburg, Germany 4) Institut für Rechtsmedizin, Universität Leipzig, Johannisallee 28, 04103 Leipzig, Germany 5) Institut für Rechtsmedizin, Technische Universität Dresden, Fetscherstr. 74, 01307 Dresden, Germany 6) Institut für Rechtsmedizin, Otto-von-Guericke-Universität Magdeburg, Leipzig, Germany

In this study a set of 30 X-chromosomal short tandem repeats (STRs) located within the Xq26 region near the HPRTB locus was evaluated with regard to polymorphism, reliable amplification, and low stutter artefacts using 132 German DNA samples. The highest diversity was found for DXS10101, DXS10102, and DXS10103 (PIC = 0.7174 - 0.8933). DXS10101 as a highly polymorphic marker with reliable amplification and low stutter artefacts was the optimal additional candidate to HPRTB for X chromosomal STR typing in linkage group 3. Therefore, DXS10101 was integrated in a commercial available test system, the Mentype® Argus X-8 PCR amplification kit, which allows the analysis of 2 STR loci per linkage group. A validation of Mentype® Argus X-8 PCR amplification kit was performed with regard to sensitivity and robustness.

OP011
Using the DNA-VIEW kinship program with X-linked markers

Brenner C

DNA-VIEW, also School of Public Health, Forensic Science Group, UC Berkeley

By a „kinship“ problem I mean the problem of trying to decide, using DNA profiles of some of the people, which of several possible pedigrees correctly describes the way a given set of people are related. The DNA-VIEW Symbolic Kinship Program can analyze arbitrary pedigrees such as arise in inheritance, missing body, and paternity disputes. It is most often used with autosomal markers, but it does also have the ability to ccompute the appropriate likelihood ratios for X-chromosomal data. I will show some results, and also discuss limitations and difficulties with the X-linked calculations.

OP012
Identification of a Novel Polymorphism in the X-chromosome Region Homologous to the DYS456 Locus

Hennessy L[1], Chang CW[1], Budowle B[2], Calandro L[1], Mulero J[1]

1) Applied Biosystems, Foster City, CA 2) Federal Bureau of Investigations, Quantico, VA

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, and Y GATA H4. During an extensive multi-population study with Y-STR loci amplified using the AmpFlSTR® Yfiler™ PCR amplification kit, amplification of a 71-bp fragment was observed in 2.32% of the male samples analyzed (N=3141). By direct sequencing of this fragment, it was determined that the primer binding sequences were identical to that of the DYS456 locus. A T to G single nucleotide polymorphism (SNP) enabled amplification of the 71-bp fragment. The SNP is located within an X-Y homologous region at Xq21.31 and was observed with the highest frequency within the African American and Sub-Saharan African populations in our study. Presence of the SNP on the X chromosome did not interfere with the reliability of typing the DYS456 locus and the other Y STR loci typeable using the AmpFlSTR® Yfiler™ PCR amplification kit. Full profiles in a mixture of male: female at 1:4000 were obtained using the current configuration of the AmpFlSTR kit even in the presence of female DNA containing the G variant. In summary, our results demonstrate that the presence of the 71 nt fragment has no impact on the interpretation of a Y-STR haplotype in males. Even when a small mass of male DNA is admixed with a large mass of female DNA containing the G variant, the sample will still yield the correct Y STR haplotype using the current configuration of the AmpFlSTR® Yfiler™ kit. Notably, in the presence of 4000-fold excess female DNA that carries the SNP, 125 pg of DNA were typed correctly at the DYS456 locus, as well as at all other Y STR loci. Lastly, in samples containing male DNA only, this dinucleotide repeat on the X chromosome can be used as an additional marker providing more information for exclusion or for assessing statistical weight of evidence samples.

OP013
Application of novel „mini-amplicon“ multiplexes to high volume casework on degraded skeletal remains

Parsons TJ[1], Huel R[1], Davoren J[1], Katzmarzyk C[1], Pozder A[1], Smajlovic L[1], Coble MD[2, 3], Rizvic A[1]

1) International Commission on Missing Persons 2) National Institute of Standards and Technology 3) Armed Forces DNA Identification Laboratory

The International Commission on Missing Persons (ICMP) performs high volume STR identity testing on skeletal remains exhumed from mass graves from conflicts in the former Yugoslavia during the 1990’s. DNA extraction and STR typing have been successfully performed on over 18,000 samples. Recently, in addition to using standard STR multiplexes (such as PP16), the ICMP has devised, tested, and implemented three novel multiplexes that use reduced length amplicons. These three multiplexes target 13 well characterized STR loci, with one seven- plex, one six-plex, and one five-plex. The target loci overlap at a few loci among the multiplexes to provide confirmatory congruence when combining results. These mini-STR multiplexes have been used now on thousands of bone samples, many of which have also been typed for standard commercial kits. The „minis“ are used for the purposes of increasing allele recovery with difficult samples, as well as providing a quick, robust, and inexpensive means for reassociating skeletal elements from highly commingled mass graves. This talk will present a summary of the performance of these multiplexes on a wide range of bone samples, compare the performance to standard kits, and discuss some important practical considerations and limitations to their use.

OP014
Short amplicon multiplex PCR including the German database systems

Müller K, Klein R, Miltner E, Wiegand P

Institute of Forensic Medicine, University Hospital of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany

Since the beginning of PCR-based DNA analysis of crime scene stains typing of weak stains and degraded material becomes more and more relevant, which necessitate optimised PCR concepts. Nowadays analysing crime scene stains in Germany is focussed on typing of eight database STRs (SE33, D21S11, VWA, TH01, FGA, D3S1358, D8S1179, D18S51) and Amelogenin for which commercial PCR multiplex kits are available. During the last years more often stains with minor amounts of DNA (e.g. epithelial cells) or highly degraded DNA (e.g.

telogen hair roots) have to be investiged. To obtain a higher success rate for such kind of stains, so-called „miniSTR“ concepts have been established which are characterized by reduced amplicon lengths. In the past we started with the developement and validation of a miniSTR multiplex including four STRs (VWA, TH01, FGA, D3S1358) and Amelogenin. In this set of five systems reduced amplicon lengths and different fluorescent dyes were tested. Continuing this concept the STRs SE33, D21S11, D8S1179 and D18S51 were integrated („Q8“). For most of the alleles the amplicon length is <200 bp; only SE33 and D21S11 amplicons both characterized by long and complex repeat stretchtes range between 150 – 300 bp. The forensic validation of the Q8-kit was performed with different sets of stains starting with blood, saliva and sperm up to skin cell mixtures and highly degraded hairs and bones. By comparing the Q8-kit to commercial available kits (MPX2, genfor, Germany; Nonaplex, Biotype, Germany; SGM plus, ABI, USA) a higher success rate was reached using our concept.

OP015
High Efficiency DNA Extraction from Bone by Total Demineralization

Loreille OM[1], Diegoli TM[1], Irwin JA[1], Coble MD[1], Parsons TJ[1, 2]

1) Armed Forces DNA Identification Laboratory, 1413 Research blvd, Rockville MD 20850, USA - 2) Current address: International Commission on Missing Persons, Alipašina 45a, 71000 Sarajevo, Bosnia and Herzegovina

In historical cases, missing persons’ identification, mass disasters, and ancient DNA investigations, bone and teeth samples are often the only, and almost always the best, biological material available for DNA typing. This is because of the physical and chemical barrier that the protein:mineral matrix of bone poses to environmental deterioration and biological attack. DNA is generally best preserved in dense cortical bone, and a recent study indicates that very high quality DNA may be locked away in small, extremely dense crystalline aggregates that are highly resistant to chemical infusion (Salamon et al). Evidence and reason both suggest, then, that the most abundant and best preserved DNA in bone is also the most difficult to access and extract. Most bone extraction protocols utilized in the forensic community involve an incubation period of bone powder in extraction buffer for digestion, followed by the collection of the supernatant, and the disposal of large quantities of undigested bone powder (and unextracted DNA). Alternatively, some bone extraction methods utilize high volume EDTA washes to partly or completely demineralize the bone, resulting in more complete digestion of the bone powder. However, we have demonstrated that DNA is also liberated, and discarded, during the washing steps. We present here an extremely efficient means for recovery of DNA by full demineralization, resulting in full physical digestion of the bone sample. This is performed in a manner that retains and concentrates all the reagent volume, so that released DNA is recovered. Fifteen bone fragments were extracted side-by-side with our new demineralization protocol and the standard extraction protocol in use at AFDIL. A real-time quantification assay based on the amplification of a 143bp mtDNA fragment showed that this new demineralization protocol significantly enhances the quantity of DNA that can be extracted and amplified from degraded skeletal remains. We have used this technique to successfully recover authentic DNA sequences from extremely challenging samples that failed repeatedly using the standard protocol. The better preserved samples were tested for STR analysis and the number of loci characterized almost doubled between our demineralization extract and the standard extract. Reference: Salamon M, T. N., Arensburg B, Weiner S, (2005). „Relatively well preserved DNA is present in the crystal aggregates of fossil bones.“ Proc Natl Acad Sci USA. 102(39): 13783-8.

OP016
Success Rates of DNA Typing from Skeletal Elements

Milos A, Huel R, Katzmarzyk C, Rizvic A, Parsons TJ

International Commission on Missing Persons

The International Commission on Missing Persons (ICMP) performs high volume STR identity testing on skeletal remains exhumed from mass graves from conflicts in the former Yugoslavia during the 1990’s. DNA extraction and STR typing have been successfully performed on over 18,000 samples. This review talk will present the lessons learned from this uniquely large data set regarding the relative DNA preservation in different skeletal elements. We will also examine the effect of different environmental conditions or special circumstances on DNA recovery, and discuss the factors that affect successful typing, and strategies for maximal success.

OP017
Optimization of isolation and amplification of DNA from degraded tissues

Schubbert R, Hell W, Rittler S, Klöpper K

Eurofins Medigenomix

During casework in the past with samples of human and animal origin we found that it was very difficult to judge whether or not the sample was still suited for DNA extraction. Especially when tissue was stored for longer periods of time in humid or warm conditions success rate was low. For optimization of DNA extraction methods and following PCR reactions we set up the following study: To determine degradation of DNA under defined conditions muscle tissue, heart tissue and bones from freshly slaughtered swines was incubated for several weeks at 37°C and 22°C. Samples were incubated 1) at dry conditions, 2) in water and 3) in water with garden mold. At different timepoints between 1 and 12 weeks after start of the experiment DNA was extracted from samples with different commercially available DNA extraction kits. Quality and quantity of extracted DNA was judged by Agarose gel electrophoresis, PCR and RealTime PCR. By PCR and RealTime PCR both, nuclear and mitochondrial genes were amplified. We could show that, even after several weeks of tissue incubation under adversarial conditions our adapted DNA extraction methods resulted in sufficient DNA for PCR and RealTime PCR. With this DNA nuclear and mitochondrial genes up to 1200 bp could be amplified by PCR.

OP018
DNA contamination in bone marrow and tissue adherent to compact bone in tsunami victims

Zehner R

Institute of legal medicine, Kennedyalle 104, D-60596 Frankfurt

As a result of the tsunami in south east Asia in December 2004 a very large number of corpses had to be identified. Many corpses could be addressed to specific persons by morphological markers, but in a connoting number of corpses DNA identification was mandatory. Because of the more laborious preparation of DNA from bone or teeth a DNA extraction from soft tissue may be preferred, so even if there is only a bone sample available it may be appropriate to give an analysis of adherent soft tissue or even bone marrow a try. However, in two identification scenarios of tsunami victims we give examples that only the use of compact bone without remains of soft tissue or bone marrow had given trustworthy results. In these two cases we observed other than the corpses DNA when using bone marrow or pickings of muscle tissue remaining on the surface of the bones. In case 1, only gender discrepancy and the occurrence of a profile indicating more than one individuum indicated that the DNA in the surface-tissue was of other origin. In case 2, typing of bone marrow resulted in a single profile of good quality, but was different to the profile from the bone material (BMT excluded). Until now the origin of the „foreign“ DNA in both cases could not be clarified, different scenarios are subject of discussion. In addition our modified technique of sample collection in the context of published methods is presented.

OP019
The problem of DNA contamination of bone samples for forensic or anthropological research and case analysis

von Wurmb-Schwark N, Heinrich A, Schwark T, Freudenberg M, Gebühr M

Institute of Legal Medicine, University Hospital Schleswig-Holstein

Contamination precautions and quality control are great issues when human bone samples are investigate genetically. This is especially true for historical samples with only minute amounts of usually highly degraded DNA. But also in forensic case analysis, sometimes DNA has to be isolated from bones in equally bad conditions, e.g. from burned victims. In such cases, there are several eventualities to contaminate the sample with foreign DNA, for example caused by the recovery of the bones, by trace investigation on a crime scene, or, of course, during handling in the lab. Here we present a systematic investigation of artificially contaminated bone samples. Historical bone fragments were used as „DNA carriers“ and contaminated with biological traces (saliva stains, touching with bare hands), or pure DNA. The specimens were then treated according to our routine protocol for decontamination and subjected to different PCRs (real time PCR for quantification of human-specific nuclear DNA, Duplex PCR for nuclear and mitochondrial DNA testing, multiplex PCR for STR typing) to check for DNA quality, quantity and origin. We will introduce an experimental design that prevents as many pitfalls as possible when working with challenging bone specimens.

OP020
Isolation of Extended Molecular Haplotypes

Nagy M[1], Entz P[1], Otremba P[1], Murphy N[2], Dapprich J[3]

1) Institute of Legal Medicine, Humboldt-University, Charite, Berlin, Germany, 10115 2) GenoVision, Inc., West Chester, PA, United States, 19382 3) Generation Biotech, Lawrenceville, NJ, United States, 08648

Current typing strategies detect polymorphisms in genomic DNA with sequence specific primers, sequence specific probes, or sequence based typing, however polymorphisms are not assigned to paternal or maternal haplotypes. Haplotype specific extraction (HSE) exploits differences between alleles to physically separate genomic diploid DNA into its haploid components. The separation step is performed utilizing magnetic beads based on specific sequence binding sites. The haplo-separated DNA samples can be directly typed with forensic downstream applications such as SNP-, STR-, or sequence based typing. In this way HSE permits the direct analysis of a haplotype in its individual, separated state without requiring statistical methods for haplotype inference. Consequently, very long sequence regions of separate alleles are available around the extraction binding site for further typing strategies. In the courtroom we are often asked, how sure are you that a specific genotype, or a specific Y haplotype in a mixture, is (or is definitely not) from the suspect and not from another person: One has to resort to often cumbersome statistics to estimate the probability of a given genotype or haplotype in a mixture. This is in contrast to the possibility of simply being able to isolate and determine the true haplotype itself by physical separation from other DNA.

OP021
Investigations on combined single cell, LCN and degraded DNA profiling by redundant on-chip Low Volume PCR (LV-PCR)

Proff C[1], Mann W[2], Rothschild MA[1], Schneider PM[1]

1) Institute of Legal Medicine, University Clinic Cologne, Germany 2) Advalytix AG, Brunnthal, Germany

Commercial multiplex STR typing kits are often used with reduced PCR volumes as a volume reduction of 30-50% normally does not result in a significant loss of typing quality [1, 2]. In this study commercial PCR chips (AmpliGrid™ AG480, Advalytix, Brunnthal) have been used where multiplex PCR can be performed in a 1 µL-PCR volume on a 48 well glass chip in microscopic slide format. Circular hydrophilic wells are separated by hydrophobic regions to prevent cross contamination. Using this technology, it is possible to obtain a full DNA profile in a 1 µl volume consisting of 0.5 µL DNA sample and 0.5 µL PCR reaction master mix. After successful testing of routine casework samples [3], tests on sensitivity, reproducibility, allele balance, allelic dropout and signal intensities, and selected forensic casework samples (mixture, low copy number and degraded DNA) were carried out by dispensing 10 µL of the DNA sample into 20 LV-PCRs of 0.5 µl each. To compare the results from the redundant reactions with those from a standard reaction, 10 µL of the same DNA sample were amplified in a single 25 µL-PCR. The results of the 20 LV-PCRs were combined which, in many cases, led to a much more representative and reliable STR profile of the sample compared to the standard 25 µL-PCR, especially as doubtful allele calls for one DNA sample are often confirmed by one or more of the remaining 19 LV-PCRs. In the present study our primary goal was to check out experimentally how many redundant LV-PCRs are necessary to obtain an optimal DNA profile from various difficult DNA sample types, and in particular from LCN samples. For this objective a large number of different DNA samples were redundantly amplified using commercial STR typing kits by up to 46 LV-PCR for each DNA sample. Serial DNA dilu-tions down to 5-6 pg, single cells obtained by a fluorescence activated cell sorter (FACS) and artificially degraded samples (DNA and single cells) were chosen to obtain sufficient data to evaluate the redundant LV-PCR typing strategy. Taking this data into account, further LCN, mixture and degraded routine casework samples were amplified using the now improved typing strategy. Furthermore, results combining mini-STR typing with redundant LV-PCRs on chip will be presented. Our findings comprising several thousand LV-PCRs demonstrate that combining the results of redundant LV-PCRs into a single DNA profile is a serious option in the investigator's toolbox to get reliable and reproducible results out of difficult DNA samples. [1] Gaines ML, Wojtkiewicz PW, Valentine JA et al. (2002) Reduced volume PCR amplifica-tion reactions using the AmpFlSTR® Profiler Plus™ Kit. J Forensic Sci 47:1224-1237 [2] Leclair B, Sgueglia JB, Wojtowicz PC, et al. (2003) STR DNA typing: increased sensitiv-ity and efficient sample consumption using reduced PCR reaction volumes. J Forensic Sci 48:1001-1013 [3] Proff C, Rothschild MA, Schneider PM (2006) Low volume PCR (LV-PCR) for STR typ-ing of forensic casework samples. In: Amorim A, Corte-Real F, Morling N (Eds.) Progress in Forensic Genetics, Vol. 11. Elsevier, Amsterdam, pp. 645-647

OP022
Genetics of complex traits and its application in forensic science

Carracedo A

Forensic Genetics Department, Institute of Legal Medicine, University of Santiago de Compostela

Physical traits have been a focus of attention amongst forensic geneticists for many years. The use of genetic analysis to predict a phenotype from a biological sample offers clear advantages for forensic investigations over and above identity testing and is creating new expectations in the forensic community, mainly as a consequence of the wealth of detail created from the sequencing and mapping of the human genome. However the majority of physical characteristic traits will be multi-genic and therefore quantitative in expression with a significant environmental component contributing to the variability. For this reason very little progress has been made in the development of tests to date, with only a very few simple genetic traits such as the analysis of red hair color becoming a reality in forensic casework. The situation is nevertheless changing quickly. Complex traits pose special challenges for genetic analysis because of the gene-gene and gene-environment interactions, genetic heterogeneity, low penetrance, and limited statistical power stemming from a genotype comprising multiple loci. We can expect that powerful analytical tools will become widely available in response to this challenge: emerging genome resources and technologies, together with the completion of the first phase of the HapMap project and new approaches such as whole genome admixture mapping are enabling systematic identification of genes underlying complex traits. In this meeting two main presentations in this section, one on hair color identification and another on new genetic analysis approaches will be given, illustrating the new insights that can be expected from the accelerating pace of gene discovery in this field.

OP023
Human Pigmentation - Genotype v. Phenotype

Bilton G

The Forensic Science Service, Trident Court, Solihull Parkway, Birmingham Business Park, Solihull, W. Mids, B37 7YN.

The natural range of hair and skin colour is a continuous spectrum, controlled by multiple genes in a complex fashion. Many of these genes are as yet unknown, but several key pigmentation genes have been characterised, in particular the Melanocortin 1 Receptor Gene (MC1R). Here, the function and known mutations of MC1R and other mammalian pigmentation genes including Tyrp1, dsu, Silver locus, MATP and ASIP will be outlined, and a forensic test based on MC1R SNPs presented. The forensic utility of this and potential future genetic tests will be discussed, in the light of the extensive debate on the ethics of predicting phenotypic traits from crime scene samples.

OP024
In search for markers to predict skin colour: Signatures of positive selection in genes associated with human skin pigmentation

Lao O[1, 2], de Gruijter JM[1, 2], van Duijn K[1, 2], Navarro A[3], Kayser M[1]

1) Department of Forensic Molecular Biology, Erasmus University Medical Centre Rotterdam, The Netherlands 2) Department of Biology, Netherlands Forensic Institute, The Hague, The Netherlands 3) Departament de Ciencies de la vida I de la salut, Universitat Pompeu Fabra, Barcelona, Spain

Phenotypic variation in human skin pigmentation correlates with latitude at the continental level making skin pigmentation one of the most traditionally used markers to classify humans according to groups, despite the biological basis of this grouping is far from being understood. The prediction of skin colour of unknown individuals using biological markers would be highly interesting for forensics (e.g. to trace unknown suspects) but the evolutionary and functional genetics as well as molecular biology of human skin pigmentation first needs to be fully understood. A large number of hypotheses involving genetic adaptation have been proposed to explain phenotypic variation in skin colour, but only limited genetic evidence for positive selection has been presented. To shed light on the evolutionary genetic history of human pigmentation and to find candidate markers for predicting skin colour (e.g. in forensic applications) we inspected 118 genes associated with skin pigmentation in the Perlegen dataset comprising single nucleotide polymorphisms, and analysed 55 genes in detail. We identified five genes involved in the skin pigmentation pathway with statistically significant differences between Europeans, Africans and Asians. In four of these genes we detected patterns of genetic variation compatible with the hypothesis of positive selection in Europeans or Asians but almost never found signals in Africans. A statistically significant correlation of the genotypic variation of six SNPs from these five major pigmentation genes with the phenotypic variation of skin color was revealed in 51 worldwide human populations. To conclude, in humans i) at

least five genes play a major role in skin pigmentation; ii) positive selection has shaped the genetic diversity of at least four of them; iii) light skin is of independent origin in Asians and Europeans, iv) dark skin is of unique origin representing the ancestral state, and v) six carefully ascertained SNPs are informative candidate markers for skin colour prediction at least on the population level.

OP025
Evaluation of a novel SNP genotyping system and a 49-plex forensic marker panel

Phillips C[1], Ballard D[2], Fondevila M[1], Harrison C[2], Musgrave-Brown E[2], Proff C[3], Ramos E[1], Sobrino B[1], Fang R[4], Furtado MR[4], Syndercombe Court D[2], Carracedo A[1], Schneider PM[3], SNPforID Consortium[5]

1) Forensic Genetics Department, Genomic Medicine Group, University of Santiago de Compostela, Galicia, Spain 2) Department of Haematology, Queen Mary School of Medicine & Dentistry, London, UK 3) Institute of Legal Medicine, University of Cologne, Germany 4) Applied Markets Group, Applied Biosystems, Foster City, US 5) www.snpforid.org

Using a 52-SNP marker set developed for forensic analysis (1), a 49-plex assay has been developed based upon a modification of SNPlex™ chemistry (Applied Biosystems, Foster City, USA) using oligo ligation of pre-amplified DNA with dye-labeled, mobility modified detection probes. This allows detection of genotypes using automated capillary electrophoresis analyzers calibrated for standard dye sets and gives highly predictable electrophoresis of the allelic products generated from the assay. The loci chosen comprised the 48 most informative autosomal SNPs from the SNPforID core discrimination set plus a gender marker developed independently by Applied Biosystems. The SNPs are evenly distributed across all 22 autosomes, exhibit balanced polymorphisms in three major population groups (2) and have been previously shown to be effective markers for forensic analysis. We tested the sensitivity and reproducibility of this novel SNP genotyping system in three laboratories using both 3130 and 3730xl Genetic Analyzers. The performance of this system in forensic analysis was assessed by comparing results obtained from standard STR typing systems and with SNaPshot™ primer extension based assays (Applied Biosystems) developed previously by SNPforID (1). Genotyping concordance was checked by comparison to alternative SNP typing chemistries and detection platforms. (1) Sanchez JJ, Phillips C, Børsting C, et al. (2006) A multiplex assay with 52 single nucleotide polymorphisms for human identification. Electrophoresis 27(9):1713–1724 (2) http://bioinformatics.cesga.es/snpforid/search.php

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OP026
Forensic validation of the SNPforID 52-plex SNP assay

Musgrave-Brown E[1], Ballard D[1], Balogh K[2], Bender K[2], Berger B[3], Bogus M[2], Børsting C[4], Ramos-Luis E[5], Fondevila M[5], Harrison C[1], Oguzturun C[1], Parson W[3], Proff C[6], Sanchez J[4], Sánchez-Diz P[5], Thacker C[1], Carracedo A[5], Morling N[4], Syndercombe Court D[1], Schneider PM[6]

1) Centre for Haematology, Institute of Cell & Molecular Science, Queen Mary, University of London 2) Institute of Legal Medicine, Universitsy of Mainz 3) Institute of Legal Medicine, Innsbruck Medical University, Austria 4) Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark 5) Institute of Legal Medicine, University of Santiago de Compostela, Spain 6) Institute of Legal Medicine, University of Cologne, Germany

The advantages of single nucleotide polymorphism (SNP) typing in forensic genetics are well known and include a wider choice of high-throughput typing platforms, lower mutation rates, and improved analysis of degraded samples. However, if SNPs are to become a realistic supplement to current short tandem repeat (STR) typing methods, they must be shown to successfully analyse the challenging samples commonly encountered in casework situations. The European SNPforID consortium, supported by the EU GROWTH programme, has developed a multiplex of 52 SNPs for forensic analysis[1], with the amplification of all 52 loci in a single reaction followed by two single base extension reactions which are detected with capillary electrophoresis. In order to validate this assay, a variety of extracts were chosen to represent the main problems of low copy number, degradation, and mixtures commonly seen in forensic casework. A total of 40 extracts were used in the study, each of which was sent to two of the five participating laboratories for typing in duplicate. Laboratories were instructed to carry out their analyses as if they were dealing with normal casework samples. Results were reported back to the coordinating laboratory and compared with those obtained from traditional STR typing of the same extracts using Powerplex® 16 (Promega). These results indicate that, although the ability to successfully type low copy number extracts is reduced, the 52-plex SNP assay performed better than STR typing on degraded samples, and also on samples that were both degraded and of limited quantity, suggesting that SNP analysis can provide advantages over STR analysis in forensically relevant circumstances. Results from the analysis of mixed extracts originating from more than one person will also be discussed. [1] Sanchez et al. (2006) A multiplex assay with 52 single nucleotide polymorphisms for human identification Electrophoresis 27: 1713-1724

OP027
Parallel analysis of 52 forensic SNPs using a new biochip technology

Heim S, Rupprecht M, Jung M, Brabetz W

Biotype AG, Moritzburger Weg 67, 01109 Dresden, Germany

The development of a DNA-chip for human identification using single nucleotide polymorphisms (SNP) meets the growing demands for genotyping systems that are independent of the amplification of short tandem repeats (STR) and variable number of tandem repeats (VNTR). The advantages of SNPs over tandem repeats are justified by their lower mutation rate and their biallelic nature. The most important benefit is that SNP loci can be amplified out of partially degraded DNA. For these reasons SNP genotyping systems are a very useful tool especially in the analysis of crime scene samples with very little amounts of even highly degraded DNA. Based on the publication of Sanchez et al. (1) and the recommendations of the SNPforID Consortium (www.snpforid.org) we developed a DNA-microarray for genotyping 52 biallelic SNPs. Our SNP-Chip enables the user to genotype 52 SNPs simultaneously. The experimental procedure starts with a multiplex amplification reaction of 52 SNP loci. The chip is spotted with a set of specific oligonucleotides which become elongated by one labelled ddNTP according to the corresponding SNP allele in the template. Due to the covalent bonding of the labelled terminators to the 3' end of the immobilised extension oligonucleotides, we are able to apply stringent washing conditions and therefore minimize background signals. In our presentation we will show genotyping data of standard DNA samples (K562, NA9947A, NA9948 and NA3657) and compare them with data obtained with the ABI Prism SNaPshot multiplex kit. (1) Juan J. Sanchez, Chris Phillips, Claus Børsting, Kinga Balogh, Magdalena Bogus, Manuel Fondevila, Cheryl D. Harrison, Esther Musgrave-Brown, Antonio Salas, Denise Syndercombe-Court, Peter M. Schneider, Angel Carracedo, Niels Morling. A multiplex assay with 52 single nucleotide polymorphisms for human identification. Electrophoresis. 2006 27:1713-24.

OP028
The development of a Minisequencing-SNP-Assay for forensic casework

Senge T, Junge A, Madea B

Institute for legal medicine, University of Bonn, Stiftsplatz 12, 53111 Bonn

The present work shows the development of a Minisequencing-assay which contains five SNPs, named TSC0171847 (Chr.1), TSC0582423 (Chr.2), TSC0741184 (Chr.3), TSC0126548 (Chr.4) and TSC0191459 (Chr.6), which were selected from the database of the SNP-consortium, a foundation organized for the purpose of providing public genomic data, which had discovered and characterized nearly 1.8 million SNPs and published the corresponding sequences. The main criteria for choosing the SNPs named above were a balanced allele distribution of at least 40% Allele 1 to 60% Allele 2 and a location on different chromosomes to warrant an independent distribution. As a first step singleplex-assays for each SNP were developed based on the SNaPshot-method from Applied Biosystems. In all cases the singleplex-reactions showed the correct alleles as determined by comparing with sequenced samples of at least five unrelated persons. The next step was the gradual development of multiplex-reactions: First the singleplex-PCR-reactions were combined and used as template for a multiplex Minisequencing-reaction. All alleles of the examined samples could be typed correct despite a higher background as in the singleplex-reactions. The final step was a multiplex of the 5 PCR-reactions followed by a multiplex of the 5 Minisequencing-reactions. All alleles of the examined samples could be typed correct, too and the background was as low as in the singleplex-reactions. But additional peaks were observed which could disturbe the correct typing in some cases and the integration of other SNPs to these Minisequencing-assay. Further tests showed that the PCR-primers of at least 2 SNPs can form dimeric structures and cause these additional peaks. Redesigning one primer of one of these SNPs solve the problem and results in a multiplex reaction with distinguished SNP-Allele-Peaks and no disturbing additional peaks. In further studies this developed Minisequencing-assay will be passed a thorough validation process to show its applicability for forensic casework and will be compared with 5´Nuclease-assays which are also developed for typing the same SNPs to get knowledge of the effectiveness and robustness of the two methods. In the end this 5 SNP-Minisequencing-assay will be expanded to a 10 SNP-Minisequencing-assay.

OP029
Amelogenin gender determination via Pyrosequencing of 48 bp PCR products

Tschentscher F, Frey U, Bajanowski T

Institut für Rechtsmedizin und Institut für Pharmakogenetik Universitaetsklinikum Essen, Germany

The amelogenin sex test has become indispensable in forensic casework. Implemented in almost all commercially available PCR kits, there are numerous advantages, especially because of the usually short amplicon length of around 100 base pairs. However, in respect to highly degraded DNA even 100 bp might be a stretch too long for sufficient analysis. Therefore, we developed a PCR test based on the published sequence of the amelogenin-loci on chromosomes X and Y. The only prerequisite of the search for suitable regions was a maximal length of about 50 bp, including primers. We identified a region of 48 bp, that includes a central 3 bp deletion on the X-chromosome. Typing of the respective alleles was performed by Pyrosequencing. Pyrosequencing is a relatively new, and rapidly evolving DNA sequencing method based on a chemiluminescent enzymatic reaction. Initially mainly used for very fast sequence determinations of short DNA fragments, several applications are at hand today. In an initial blind study we reliably typed 100 randomly chosen DNA-samples of known healthy donors. We then tried to resolve the gender of seven bone samples, which were difficult to type with regular forensic PCR kits. The sexes of all but one of the samples were successfully resolved. Further experiments will show if the test is suitable for routinely diagnostics of regular or even very problematic samples like ancient remains.

OP030
mtDNA haplogroup structure in West Eurasia as revealed by complete sequence data

Kivisild T

Estonain Biocentre, Tartu

Human mtDNA variation in Eurasia derives from a small subset of African mtDNA lineages nested in haplogroup L3. Virtually all of the mitochondrial variants in Europe trace their origin back to a single founder haplotype in haplogroup N within the last 40-60 thousand years. Three major haplogroups, JT, U, and HV characterize most Europeans and there is little difference in the frequency of those broad haplogroups between the populations inhabiting East, West, South or Northern Europe. With the help of accumulating complete mtDNA genome sequence data it is possible now to differentiate within the basic haplogroups in Europe sub-clades that have more restricted geographic spread and can therefore bear information about peopling of different geographic zones of the continent. More than a third of the mtDNA population structure in Europe is fragmented into a number of subclades of haplogroup (hg) H. While there is considerable recent progress in studying complete mitochondrial genome variation in Europe, in particular hg H, little data of comparable resolution is so far available for regions like the Caucasus and the Near and Middle East – for areas where hg H has likely emerged. We have analyzed 545 samples of hg H from these regions at high resolution, including 15 novel complete mtDNA sequences. Significant differences between the distribution of hg H subclades in Europe and in the Near East and South Caucasus imply limited recent maternal gene flow between these regions.

OP031
Translating DNA data tables into quasi-median networks for parsimony analysis and error detection

Bandelt H-J[1], Brandstätter A[2], Dür A[3]

1) Institute Dept. of Mathematics, University of Hamburg, Bundesstraße. 55, 20146 Hamburg, Germany 2) Institute of Legal Medicine, Innsnbruck Medical University, Muellerstr. 44, A-6020 Innsbruck 3) Institut for Mathematics, University of Innsbruck, Technikerstr. 44, A-6020 Innsbruck

Every DNA data table can be turned into a quasi-median network that faithfully represents the data. For (weighted) condensed data tables the associated network harbors all most parsimonious reconstructions for any tree that connects the sampled haplotypes. Structural features of this network can be computed directly from the data table. The translation of a table into a network enhances the understanding of the properties of the data in regard to homoplasy and potential artifacts. The total number of nodes of such a network measures the complexity of the data. In particular, networks that display the results of filter analyses by which hotspot mutations are removed help to detect data idiosyncrasies and thus pinpoint sequencing problems. Examples drawn from human mtDNA illustrate these points.

OP032
EMPOP - a forensic mtDNA database

Parson W

Institut of Legal Medicine, Innsbruck Medical University, Muellerstr. 44, A-6020 Innsbruck

Mitochondrial DNA (mtDNA) has become a wide-spread genetic marker. Its mode of inheritance (maternal transmission and lack of recombination) makes it suitable for population genetic and anthropological studies to reconstruct human history. The molecule has been extensively investigated in the medical genetics field to determine its role in genetic disease. The forensic field takes advantage of the high copy number per cell that makes it the most sensitive method for human identification available to date. All these disciplines require collections of mtDNA data – usually mtDNA databases – that help interpretation of the results in the light of the established mtDNA variation. In the forensic setting the database builds the basis for frequency estimations of mtDNA sequences that became relevant in a case. The establishment of mtDNA databases sounds trivial, however, it has been shown in the past that this undertaking is prone to error for several reasons, particularly human error. We have established a concept for mtDNA data generation, analysis, transfer and quality control that meets forensic standards. The data are stored and made publicly available on the internet in the form of the EDNAP mtDNA Population Database, short EMPOP. The talk presents the concept and software features of EMPOP.

OP033
Development and Expansion of High Quality Control Region Databases to Improve Forensic mtDNA Evidence Interpretation

Coble MD[1], Irwin JA[1], Just RS[1], Saunier JL[1], Diegoli TM[1], Strouss KM[1], Sturk KA[1], Parsons TJ[1, 2]

1) Armed Forces DNA Identification Laboratory (AFDIL), 1413 Research Blvd., Rockville, MD 20850, USA 2) International Commission on Missing Persons, Alipasina 45 A, 71000, Sarajevo, Bosnia

Phylogenetic analyses of published mtDNA databases have been utilized to identify sequencing „phantom mutations“ and other artifactual errors that have plagued forensic databases. After a series of high profile discussions in the scientific literature debating the seriousness of these errors, culminating in a court case (US v. Ida Chase) that challenged the admissibility of the US SWGDAM mtDNA database, we have sought to rectify the current situation by developing high-quality mtDNA control region sequences for the forensic community. At the Armed Forces DNA Identification Laboratory (AFDIL) we have developed a high-throughput automated system that utilizes robotic instrumentation for all laboratory steps from pre-extraction through sequence detection, and a rigorous 5-step, multi-laboratory data review process with entirely electronic data transfer. Since 2004 we have generated over 5000 control region sequences from both US populations and underrepresented global populations (such as several from Central Asia). The strength of this project is based on our collaborations with international colleagues, who provide the laboratory samples for sequencing and co-author any resulting publications. Furthermore, we have developed a strong relationship with the EDNAP Mitochondrial Population Database (EMPOP) team to provide these data to the forensic community. In this presentation, we will present our current progress on an effort being funded by the US National Institute of Justice to generate over 3500 control region databases per year from US populations. In addition to the high quality data generated from this effort, one principle aim of this effort will be to provide a framework within which to examine the substructure and heterogeneity of various regional or named population groups (e.g. „Hispanics“) within the United States and component source populations.

OP034
Control Region Sequence Variations in the Hungarian population: Analysis of population samples from Hungary and from Transylvania (Romania)

Egyed B[1], Irwin JA[2], Brandstätter A[3], Szamosi G[1], Pádár Z[1], Saunier JL[2], Parsons TJ[2], Parson W[3]

1) Department of Haemogenetics, Institute for Forensic Sciences, POB 314/4, 1903 Budapest, Hungary 2) Armed Forces DNA Identification Laboratory, 1413 Research Blvd., Rockville, MD 20850 USA 3) Institute of Legal Medicine, University of Innsbruck, Müllerstrasse 44, 6020 Innsbruck, Austria

The main aim of this study is to present mtDNA databases of the Hungarian population, an European population that has not been previously studied using mtDNA analysis. In order to assess the mitochondrial DNA polymorphisms of the Hungarian population in the Carpathian basin and to facilitate forensic mtDNA testing control region sequence databases have been generated from two population sets from Hungary and from two population sets from a Hungarian speaking region of Transylvania (Romania). 211 individuals were sampled from

the mixed (heterogeneous) population of the Hungarian capital Budapest, 208 individuals were sampled from a Roma („gypsy“) population in Baranya county (Hungary), 178 individuals were sampled from the Szekler population living in Csíkszereda (Miercurea Ciuc, Romania) and 183 individuals were sampled from the Csango population living in Gyimesfelsőlok (Lunca de Sus, Romania). Entire control region sequencing was performed by an absolutely automated process of laboratory manipulations (including robotics for each step of amplification and sequencing) and data export which did not involve any manual transcription. The entire control region approach involved a single amplification step reducing the potential for „phantom recombination“ to near zero. Generating sequence data highly redundant analysis was performed because our aim was to minimize any potential errors in the sequence evaluation. The random match potential and pairwise comparisons within and between the datasets and with that of neighbouring countries like Austria and other European databases is reported. This study highlight the importance of considering population structure when generating reference databases for forensic testing. Comparisons between our population samples indicate the need for heightened caution when sampling, and using mtDNA databases of, small endogamous populations.

OP035
Mitochondrial DNA composition of Slavonic populations of Europe revealed by complete genome sequencing

Grzybowski T[1], Malyarchuk BA[2], Derenko MV[2], Wozniak M[1]

1) Nicolaus Copernicus University, Collegium Medicum, Forensic Medicine Institute, Bydgoszcz, Poland 2) Institute of Biological Problems of the North, Genetics Laboratory, Magadan, Russia

As a result of the previous studies on mitochondrial DNA diversity in Slavonic populations of Europe (Poles, Russians, Ukrainians, Slovenians, Bosnians) it was found that both haplogroup frequency patterns and haplotype composition in Slavs were similar to those characteristic of other Europeans. In this study, DNA sequences of entire mitochondrial genomes have been obtained from a sample of 21 individuals (Poles, Russians, Ukrainians and Polish Roma), representing selected mitochondrial subhaplogroups. The analysis of a maximum resolution allowed us to describe the specific mtDNA components which seem unique for Central and Eastern European populations, being found predominantly in Slavs. The most important of them, mitochondrial subhaplogroup U4a2, which has been dated here with coding region molecular clock, is probably of a central-eastern European origin. Based on the U4a2 coalescence time to the most recent common ancestor (TMRCA, 7 117 ± 1 677 years) one may suggest that expansion of this subcluster can be explained by a dispersal of the Corded Ware (Battle Axes) culture in Eastern and Central Europe. Sequencing of selected mtDNAs bearing control-region mutation T16311C has shown that these genomes represent paraphyletic group HV3*, observed so far with highest frequencies in some populations of northwestern part of Russia. Within HV3*, we are able to distinguish a novel monophyletic subcluster HV3a, defined here by a coding region transition G8994A. The coalescence time estimate for HV3a is 8 581 ± 3 854 years. Previous phylogenetic analyses of a haplogroup K in Poles and Polish Roma exhibited the presence of haplotypes with control region motifs 16224-16234-16311-114, 16223-16224-16234-16311 and 16223-16224-16234-16311-114. Complete genome sequencing performed in this study has revealed that these mtDNAs belong to Ashkenazi-specific cluster K1a1b1a. This finding is a first direct proof of the presence of Ashkenazi-specific mtDNA lineages in non-Jewish European populations.

OP036
mtDNA haplogroup determination as an additional tool for authenticating ancient East Asian mtDNA

Lee HY[1], Yoo JE[1], Park MJ[1], Chung U[1], Kim CY[1, 2], Shin KJ[1, 2]

1) Department of Forensic Medicine, Yonsei University College of Medicine, Seoul, Korea 2) Human Identification Research Center, Yonsei University, Seoul, Korea

DNA was extracted from the skeletal remains of 35 museum samples excavated from 11 local burial sites scattered throughout southern Korea. The meticulously designed PCR was performed on two independent extracts from the ancient Korean DNA using 8 small PCR fragments (133 - 177 bp). mtDNA control region sequences (HV1, HV2 and HV3) were successfully determined for 12 samples, and none of them shared the control region polymorphisms with those from the persons involved in the analyses. Among these, two were from the Paleolithic Age; three were from the Neolithic Age; two were from the Bronze Age; one was from Baecje Dynasty (BC 18 - AD 660); and four were from Goryeo Dynasty (AD 936 - 1392). Since mitochondrial DNA control region sequences were obtained from 8 overlapping PCR fragments, the screening for cross-contamination or sample mix-up was also required for assessing authenticity. Therefore, each of 12 mtDNA was assigned to the appropriate East Asian mtDNA haplogroups or subhaplogroups according to the haplogroup-specific mutation motif present in the control region sequence. Depending on the results, diagnostic coding region SNPs for the corresponding haplogroups were confirmed using monoplex or multiplex SNaPshot reactions. The successful

East Asian mtDNA haplogroup determination for each ancient Korean mtDNA and the absence of abrupt polymorphisms substantiated that there are no mosaic structure or other errors in our mtDNA sequences. These procedures suggest an additional strategy that would be effective and successful in assessing ancient East Asian mtDNA authenticity besides the 9 criteria suggested by Cooper and Poinar.

OP037
Base-numbering challenges of insertion/deletion variation in the human mitochondrial DNA control region and relevance to database searches

Melton T[1], Nelson K[2], Sturk KA[3], Irwin JA[3]

1) Mitotyping Technologies, LLC, State College, PA, USA 2) Department of Biology, The Pennsylvania State University, University Park, PA, USA 3) Armed Forces DNA Identification Laboratory, Rockville, MD, USA

Besides large numbers of simple nucleotide polymorphisms, the human mtDNA control region contains rich length variation. In casework done between February 1999 and June 2006, Mitotyping Technologies observed 42 different insertion/deletion variants, not including those commonly observed in homopolymeric C-stretches such as 16193.1, 309.1, 309.2, and 315.1. Variants fell into several categories: 1) single nucleotide insertions or deletions [e.g. 44.1C, 247D, 291.1A, 16263.1A, 16298D], 2) deletions of a group of nucleotides [e.g. 105-110D, 335-337D, 523-524D], and 3) rearrangements of nucleotides within a short stretch [e.g. variation in regions 55-73, 308-320, 16182-16193]. Many variants are rare, having been observed a single time, however, some are readily recognizable by most practitioners, such as the triple deletion of 249, 290, and 291 or the 523/524 deletion pair. Current mtDNA databases include AFDIL's Casework and Family Reference Databases, the FBI's SWGDAM database, and EMPOP. The search mode of each employs a list of a sample's rCRS numbered polymorphic sites to locate matching samples in the database. However, because length variation may be subject to alternative rCRS numbering schemes depending on sequence context (such as heteroplasmy) as well as a laboratory's previous experience with length variation, this flawed approach may prevent location of matching sequences. Unless a laboratory can conceive of and search all possible schemes, an underestimate of the database frequency may result. AFDIL has identified at least nine cases where ambiguity prevents straightforward database searches due to possibility of alternative numbering. Example: The sample has an apparent loss of 16194A and 16195T, but also extreme length heteroplasmy due to the 16189 transition. The rCRS is on the top line, two alternative calling schemes of the sample are below.

rCRS:	`TCAAAACCCCCTCCCC-ATGCT`
Lab #1 calling scheme:	`TCAACCCCCCCCCCCCNddGCT`
Lab #2 calling scheme:	`TCAACCCCCCCCCCCC-CCGCT`

Base numbering per rCRS: [16178/79/80/81/82/83/84/85/86/87/88/89/90/91/92/93/93.1/94/95/96/97/98] where (-) is no base, N is unknown, and d is deletion. While rules have been suggested for consistent rCRS numbering of mtDNA length variants, these rules occasionally abrogate a common-sense approach based on understanding of mtDNA biological mutation mechanisms. The incorporation to all database search engines of a feature that would permit searches of un-numbered nucleotide strings containing complex length variants would constitute a superior system.

OP038
Studies of mtDNA heteroplasmy in single cells

Lutz-Bonengel S[1], Parson W[2], Sänger T[1], Heinrich M[1], Müller H[3], Mann W[3], Ellwart JW[4], Bonengel B[5], Schmidt U

1) Institute of Legal Medicine, University hospital of Freiburg, Freiburg, Germany 2) Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria 3) Advalytix AG, Eugen-Sänger-Ring 4, 85649 Brunnthal, Germany 4) GSF- National Research Center for Environment and Health, Institute of Molecular Immunology, Marchioninistrasse. 25, 81377 München, Germany 5) HLS Offenburg (BTG), Zähringerstrasse 41, 77652 Offenburg, Germany

The reliable analysis of highly degraded or low copy number nuclear DNA samples is still a challenge in forensic sciences. As genomic DNA typing is often unsatisfactory in such cases, analysis of mitochondrial genomes ensured a higher probability for successful PCR amplification due to the high copy number per cell. An additional enhancement of PCR sensitivity can be attained by reduction of the reaction volume. Low volume (LV) amplification of mtDNA can be performed on the surface of chemically structured chips, i.e. modified microscopic glass slides. In combination with fluorescence activated cell sorting (FACS), it is possible to deposit sorted single cells on the glass slide and analyze them individually by PCR based typing. In addition, microscopic control of the sample prior to amplification is possible. We have tested the application of several methods, which can be useful for a chip based analysis of single cells: amplification of mtDNA with a fluorescence-labelled primer pair, amplification of mtDNA with subsequent minisequencing, amplification of mtDNA with subsequent cycle

sequencing and multiplex STR analysis of genomic DNA. Combining these methods, we studied mitochondrial heteroplasmy on the level of single cells.

OP039
Forensic Application of the Affymetrix Human Mitochondrial Resequencing Array

Vallone PM[1], Jakupciak JP[1], Coble MD[1, 2]

1) National Institute of Standards and Technology, Biochemical Science Division, 100 Bureau Drive, Gaithersburg, Maryland 20899-8311 2) Armed Forces DNA Identification Laboratory, 1413 Research Boulevard, Building 101, Rockville, Maryland 20850(2)

In the field of forensic DNA testing, coding region polymorphisms in the mitochondrial genome can be useful for resolving individuals who have the identical HV1 and HV2 control region sequence. Sequencing regions of the mitochondrial genome is performed when insufficient genomic DNA is present for traditional autosomal short tandem repeat (STR) testing. Various methods and strategies have been established to interrogate coding region polymorphisms. These range from SNP assays probing sites most likely to differentiate individuals based on their HVI/HVII sequence to the use of mass spectrometry to pyrosequencing. Here we evaluate the potential of the Affymetrix GeneChip Mitochondrial Resequencing Array (ver 2.0) for forensic applications. The GeneChip Mitochondrial Resequencing Array is a means to perform full genome sequencing on an array-based platform. The amount of DNA needed for the resequencing array is much greater than that required for autosomal DNA typing (1 ng versus 10-30 ng). Because of this relatively high sample requirement the array may have limitations for running a limited quantity of casework sample. However the platform should have utility in running family reference samples for the elucidation of SNPs that will help resolve individuals. These array-determined polymorphisms found in reference sample can then be probed in the limited casework sample. A set of 10 U.S. Caucasian samples found to contain the same control region sequence by traditional fluorescent sequencing were run on the GeneChip platform. All 10 samples were fully resolved after comparing coding region sequence data from the GeneChip. The reproducibility of GeneChip experiments was evaluated by running samples in triplicate for two of the samples. A sensitivity study was also conducted in which 10 to 0.3 ng of template DNA (nuclear) was amplified for the array experiments. Two challenging samples were also examined to test the array’s ability to successfully call a relatively large number of sequence differences compared to the revised Cambridge Reference Sequence. All results were compared to traditional dideoxy fluorescent full genome sequencing experiments. A typical array experiment resulted in approximately 95% sequence coverage (the remainder being Ns or no calls). Comparisons between the GeneChip and traditional sequencing indicated the array platform had difficulty calling insertions and deletions as well as some closely spaced polymorphisms.

OP040
Forensic mtDNA Mixture Fractionation by Denaturing High-Performance Liquid Chromatography

Danielson PB[1], Kristinsson R[1], Melton T[2]

1) University of Denver, Department of Biological Sciences, Denver, Colorado 80210 USA 2) Mitotyping Technologies, LLC, State College, Pennsylvania 16801 USA

Mitochondrial DNA (mtDNA) sequencing can provide crucial information to forensic investigators when the quantity and quality of DNA would otherwise be limiting. The difficulty of analyzing mtDNA mixtures, however, has been a significant obstacle to its broader use in forensics. Unlike short tandem repeats, mtDNA amplicons are identical or nearly identical in length and thus must be characterized by direct nucleotide sequencing. The presence of a natural (heteroplasmic) or situational (multi-contributor) DNA mixture complicates the interpretation of the sequencing electropherograms. In these cases, DNA sequencing data are usually characterized by multiple mixed base positions or regions of sequence that are out of register (and thus unreadable) following subtle length polymorphisms. In both cases, it becomes difficult and very often impossible to accurately determine the complete haplotype of an individual contributor. Use of a Denaturing High-Performance Liquid Chromatography (DHPLC) system (WAVE® System, Transgenomic Inc.) in combination with linkage phase analysis is a promising approach for rapid, low-cost fractionation and analysis of DNA mixtures without secondary amplification or excessive sample manipulation. Using the same standard operating procedures that have already been validated for use in forensic laboratories, this approach enables sequence-specific fractionation of natural or situational DNA mixtures in advance of direct DNA sequencing. The sensitivity, reproducibility and accuracy of the approach have been assessed using a population of over 1200 pair-wise mixtures of 88 distinct mitotypes. Based on a strong correlation between changes in DNA contributor ratios vs. changes in electrophoretic peak height ratios, ≥20% enrichment by DHPLC of either contributor to a mixture makes it possible to accurately determine the linkage phase and thus the correct mitotypes of the individual contributors to a mixture. Finally, the capability of DHPLC to simultaneously quantify and purify DNA makes it possible streamline the processing of all mtDNA samples. This

improves efficiency by eliminating the need for separate yield gels or PCR clean up steps. Thus, DHPLC aids criminal investigations by making it possible to obtain conclusive mitochondrial DNA results from mixtures that would not otherwise be amenable to analysis by direct sequencing.

OP041
21 years of Y-chromosomal DNA studies

Tyler-Smith C

The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK

The first Y-chromosomal DNA polymorphisms were described in 1985, 21 years ago, so this anniversary provides an opportunity to review our current understanding of Y-chromosomal DNA variation and pose questions about future directions for the field. Y-STRs have been extensively used in forensic and evolutionary genetic analyses. The adoption of a standard set of markers and nomenclature, the availability of commercial typing kits and the establishment of publicly-accessible databases led to the rapid development of this area. Now that we have a near-complete set of Y-STRs and information about their variation in different populations, current issues concern the extent to which we should expand the standard Y-STR sets for different purposes. Y-SNPs have been equally, or more, important in evolutionary studies, but have only contributed in minor ways to forensic work. Nevertheless, they can potentially provide information from degraded samples and insights into male geographic origins. Sequence information from many sources is identifying vast numbers of candidate Y-SNPs, and these should enrich the Y-chromosomal phylogeny by resolving multifurcations and increasing resolution. However, current coordination of the Y-SNP phylogeny and nomenclature is inadequate and needs to be improved, and standardisation of marker usage is desirable. A combination of Y-STR and Y-SNP data is often useful and a database that combines both types of marker would be very valuable. Publicly-available data on Y-chromosomal variation is accumulating steadily from small-scale studies by many individual labs, and to some extent from larger international collaborations such as the HapMap project and analysis of the HGDP-CEPH panel. In addition, interest in genealogy has led to the generation of large datasets, although their sample ascertainment and inaccessibility limit their usefulness. The largest current study of Y-chromosomal variation is the Genographic Project (2005-2010), which will type both self-selected paying participants with Y-SNPs and 100,000 males from indigenous populations around the world with a combination of Y-STRs and Y-SNPs. After 21 years, the field has reached maturity, and we look forward to the next 21 years.

OP042
Future prospects of the Y chromosome Haplotype Reference Database (YHRD)

Roewer L[1], Willuweit S[1], de Knijff P[2]

1) Dept. Forensic Genetics, Institute of Legal Medicine Berlin, Charite - University Medicine Berlin, Germany 2) The Forensic Laboratory for DNA research, MGC Dept. of Human Genetics, Leiden University Medical Center, The Netherlands

The Y chromosome is a multi-purpose tool in forensic genetics. Because it records male history in different time-depths it allows a thorough description of the genetic structure of contemporary human populations and a prediction of the geographical or ethnic ancestry of male lineages. Currently, the YHRD collects rapidly evolving Y-STR haplotypes with high polymorphism information content and thus a broad applicability in forensic casework. Possibilities to calculate minHt frequencies on basis of metapopulations as well as to map haplotype variety will be reported. Despite a remarkable forensic interest and feasible technology deep-rooting binary polymorphisms are not yet added to the database due to inherent problems of quality assurance. Since YHRD is not a repository of published data but relies on the submission of original data generated by quality assessed laboratories, an expansion of the haplotypes collected within YHRD by haplogroup information should maintain this custom of provable quality by blind sample testing and self-control by standard reference samples. To approach a Y-STR/Y-SNP reference database we propose the following strategy on occasion of the V. Forensic Y-User Workshop in Innsbruck 2006: (i) establishment of a decentralised YHRD reference panel of Y-STR/Y-SNP typed DNA from all major haplogroups and world populations; original DNA of high quality might be amplified by WGA if necessary and tested on suitability afterwards, it should be stored locally and distributed on request (ii) judgement on the level of tree resolution, technology and a minimal number of binary polymorphisms to identify the major haplogroups of the world (see e.g. Brion et al. 2005 using a minimal number of 29 SNPs chosen from about 250 in a single reaction to define 31 haplogroups) (iii) appointment of a panel of scientists with expertise on the expected haplogroup variety in different parts of the world (iv) implementation of a Hg search tool in the YHRD (v) a plea to the Forensic Y-User group to add binary markers to their haplotyped and already submitted chromosomes after an initial quality check

OP043
Towards a Y-Haplogroup Database

De Knijff P

Department of Human Genetics, Leiden University Medical Center, 2300 RC Leiden, The Netherlands

Y-SNP information, enabling the attribution of males to a specific Y-haplogroup, has been published from at least 40.000, but perhaps even as much as 60.000 globally dispersed males. Yet, it is very time consuming to get access to these data in an efficient manner. Ideally, one would like to have a central and flexible database with Y-STR and Y-SNP information in the same format as the current Y-STR-only YHRD (http://www.yhrd.org/index.html). Until such exists, it would already be a big improvement to be able to use most of the already published Y-haplogroup information instantaneously. In order to facilitate this, we have collated Y-SNP information into a single database which will be made available to all interested parties. This database contains Y-SNP and Y-Haplogroup information from 30.000 globally dispersed males from which genetic data and geographical coordinates could be extracted from publications. In addition, we have also genotyped all males DNA samples from the CEPH-HGDP panel for Y-STRs and Y-SNPs in order to provide a reference frame work of genotypes from DNA samples used by many other laboratories. We will discuss the power and pitfalls of such an article based Y-SNP database in the context of the new YHRD plans.

OP044
Y-chromosome diversity in Sweden - A long-time perspective

Karlsson AO[1], Wallerström T[2], Götherström A[3], Holmlund G[1]

1) The National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden 2) Department of Archaeology, University of Tromsø, Norway 3) Department of Evolutionary Biology, Uppsala University, Sweden

Sixteen Y-chromosomal binary markers and nine Y-chromosome short tandem repeats were analyzed in a total of 383 unrelated males from seven different Swedish regions, one Finnish region and a Swedish Saami population, in order to address questions about the origin and genetic structure of the present day population in Sweden. Haplogroup I1a(*) was found to be the most common haplogroup in Sweden and accounted, together with haplogroups R1b3, R1a1 and N3, for over 80% of the male lineages. Within Sweden, a minor stratification was found in which the northern region Vasterbotten differed significantly (P<0.05) from the other Swedish regions. A flow of N3 chromosomes into Vasterbotten mainly from Saami and Finnish populations could be one explanation for this stratification. However, the demographic history of Vasterbotten involving a significant male absence during the 17th Century may also have had a large impact. Immigration of young men from elsewhere to Varmland at the same time, can be responsible for a similar deviation with I1a(*) haplotypes. Y chromosomes within haplogroup R1b3 were found to have the highest STR variation among all haplogroups and could thus be considered to be one of the earliest major male lineages present in Sweden. Regional haplotype variation, within R1b3, also showed a difference between two regions in the south of Sweden. This can also be traced from historical time and is visible in archaeological material. Overall this Y chromosome study provides interesting information about the genetic patterns and demographic events in the Swedish population. European Journal of Human Genetics advance online publication, 24 May 2006; doi:10.1038/sj.ejhg.5201651.

OP045
Does after-war migration explain Polish Y chromosome homogeneity?

Woźniak M, Grzybowski T, Starzyński J

Nicolaus Copernicus University, Collegium Medicum, Forensic Medicine Institute, Bydgoszcz, Poland

As shown in a study by Kayser et al. (Hum Genet 2005: 117, pp. 428 – 443), Polish population seems to be very homogenous as far as Y chromosome polymorphism is regarded. The hypothesis that explains this phenomenon is based on the assumption that massive migrations that took place in Poland after World War II could evoke such an effect. Thus, knowledge of the pre-war frequencies of Y chromosome haplotypes in different parts of the country would be a useful tool in testing such a hypothesis. We have collected 228 DNA samples, together with family history data, from males living in the rural area of Podhale and Beskid Sądecki near southern Polish border. Based on donor's family history we were able to extract an „ancestral" subpopulation of 109 males whose male ancestors lived in the area before both World Wars. We have analyzed 11 Y-STR loci: DYS19, DYS385, DYS389I&II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439 in all the collected samples. AMOVA analysis showed that there is no significant difference between contemporary population of the region under investigation and the population of males who lived there ca. 100 years ago as represented by their descendants. Additional comparisons of our contemporary and „ancestral" population samples with other Polish

and Central European populations showed that the population of Podhale and Beskid Sądecki is very closely related to other Polish and Slavonic populations. The abovementioned observations suggest that Polish population could have been highly homogenous even before World War II and call for a more detailed study of the phenomenon of Polish Y chromosome lineages homogeneity.

OP046
Y-chromosomal DNA variation in the Kuwaiti Bedouin Tribes of the Persian Gulf

Mohammad T[1], Xue Y[2], Evison M[3], Tyler-Smith C[2]

1) University of Sheffield, Division of Genomic Medicine, The Medical School, University of Sheffield, Sheffield, S10 2RX, United Kingdom 2) The Wellcome Trust Sanger Institute, Cambridge, U.K. 3) Department of Anthropology, University of Toronto, Canada

The study of the genetic origins and history of Bedouin populations is important both for questions relating to early Arab ancestry and for understanding the settlement of the Arabian Peninsula and Persian Gulf. There is historical agreement that Arab and Bedouin tribal populations share a common paternal ancestor of either Adnan or Qahtan (Joktan). To investigate the origin of paternal lineages of Bedouin populations, 153 Y chromosomes representing six tribes (three Adnani, three Qahtani (Joktani)) were analyzed hierarchically with a set of 97 Single-Nucleotide Polymorphic (SNP) markers and a 17 Y-STR multiplex. 83% of the Y chromosomes belong to Haplogroup J and the rest are distributed among R, G and E. Our Y-STR results show that diversities are generally low within the six Bedouin tribes, possibly reflecting genetic drift. Based on both the Y-chromosomal haplogroups and results of Y-STR analysis, we observed clustering of the Bedouin populations with other Arabs, but statistically-significant genetic differentiation between several of the six populations.

OP047
Genetic characteristics of 22 Y-STRs in Koreans: Intermediate, null, and duplicated alleles and deletions in the DYS385 flanking region

Park MJ[1], Lee HY[1], Chung U[1], Kim NY[1], Yang WI[1, 2], Cho SH[1, 2], Shin KJ[1, 2]

1) Department of Forensic Medicine, Yonsei University College of Medicine, Seoul, Korea 2) Human Identification Research Center, Yonsei University, Seoul, Korea

Twenty two Y-STRs were analyzed in 708 unrelated Korean males using 3 multiplex PCR systems: Multiplex I contains DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393; Multiplex II, DYS385a/b, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, and GATAH4; Multiplex III, DYS385a/b, DYS388, DYS446, DYS447, DYS449, and DYS464. A total of 693 different haplotypes were observed with overall haplotype diversity of 0.9999, and among these, 680 haplotypes were unique. The atypical alleles present at 22 Y-STRs were scrutinized and characterized by sequencing analysis. Microvariant alleles were observed at DYS449, DYS464, and DYS458 in 16, 6, and 2 samples, respectively. Null allele at DYS448 was found in six times, and it was consistent with the result of AmpFISTR® Yfiler™ kit. Duplicated alleles were occurred once at DYS19, DYS390, and DYS447, respectively. An unusual long allele 33 at DYS449 was also observed once. For DYS385a/b, 4 or 8 bp deletions was discovered at the 2 different sites in the flanking region of 8 samples, and the separate analysis of DYS385a and DYS385b allele showed that the deletions are not linked at a specific locus. In the case of the flanking region deletions, the DYS385a/b primer sets of other previous reports as well as those of Powerplex® Y and the AmpFISTR® Yfiler™ systems encompass these deletion regions and accordingly mask the real repeat units based on the ISFG recommendations for allele nomenclature. This scrutinizingly established Y-STR database will be a useful reference for the Y-STR genotyping and its interpretation in the forensic investigation of Koreans.

OP048
Y-haplotype screening to find „kinship“ cold hits in offender database

Brenner C

DNA-VIEW, also School of Public Health, Forensic Science Group, UC Berkeley

If a man commits a crime but he is not in the offender database he may have a relative who is. A „kinship“ search in the database, using the usual autosomal STR markers, would typically produce a number of leads. To the extent that family crime patterns follow male lineage, follow-up Y-haplotyping will reject all the false leads nearly all the time.

OP049
50 novel simple Y-chromosomal microsatellites: global genetic diversity and mutation rates

Kayser M[1], Vermeulen M[1, 2], de Knijff P[3], Tyler-Smith C[4]

1) Department of Forensic Molecular Biology, Erasmus University Medical Centre Rotterdam, The Netherlands 2) Department of Biology, Netherlands Forensic Institute, The Hague, The Netherlands 3) Forensic Laboratory for DNA Research, Leiden University Medical Center, The Netherlands 4) The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK

Although, the set of 9-16 Y-STRs usually applied in forensics provides high haplotype diversity, there are cases with identical haplotypes and the question of identity-by-state (unrelated Y-chromosomes) versus identity-by-descent (related Y-chromosomes) needs to be answered. This can be achieved by typing additional Y-STRs that are able to increase the haplotype resolution provided by the previously studied loci. Also, the frequency of identical haplotypes given the usual 9-16 Y-STRs is known to be increased in certain populations (e.g. Fins, Pakistani) and can be expected for others that also underwent a male-mediated bottleneck in their recent history. Recently, we identified 166 previously unknown Y-STR markers but molecular characterization was not studied in detail. As first attempt to provide molecular characterization of previously identified new Y-STRs we ascertained 50 single-copy simple loci consisting of only one stretch of repetitive sequence. Simple Y-STRs carry the advantage over complex loci that allele designation via fragment length analysis is unequivocal which is important for forensic but also evolutionary studies. This presentation will provide preliminary data and analyses on global and regional genetic diversity using a worldwide panel of DNA sample as well as mutation rates using deep-rooting pedigrees.

OP050
High degree of Y-chromosomal divergence within Finland – forensic aspects

Palo JU[1], Hedman M[1], Ulmanen I[2], Lukka M[2], Sistonen P[3], Sajantila A[1]

1) Department of Forensic Medicine, P.O.BOX 40, FIN-00014, University of Helsinki, Finland 2) National Public Health Institute, Mannerheimintie 166, FIN-00300 Helsinki, Finland 3) Finnish Red Cross Blood Transfusion Service, Kivihaantie 7, FIN-00310 Helsinki, Finland

Among the Finns, the levels of autosomal STR and mtDNA variation are relatively high, and evenly distributed throughout the country. In contrast, the Y-STR variation is markedly lower than observed in most other European populations, showing notable geographical substructure within Finland. The pattern is most plausibly explained by drift, affecting especially the eastern and north-eastern parts of the country. Consequently, there are striking interregional differences in haplotype frequencies – the coastal, inner and south-eastern parts of Finland segregate into a clearly delineated subpopulations, with FST values comparable to the highest divergences among European populations (Western Europeans vs. Slavic). The low Y-STR diversity reduces the discriminative power of Y-chromosomal markers among Finns, and, furthermore, the geographical substructure complicates the assessment of profile probabilities. Here, we discuss he observed Y-STR diversity pattern and how it should be taken into account in forensic casework conducted in Finland.

OP051
Argentinean Patagonian Mapuche Ancestry Analysed by Surnames and Genetic Markers

Corach D[1], Sala A[1], Marino M[1], Bravi CM[2], Bobillo MC[1]

1) Servicio de Huellas Digitales Genéticas and Cátedra de Genética y Biología Molecular, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina 2) Multidisciplinary Institute of Cell Biology (IMBICE), 1900 La Plata, Argentina

A reduced number of Amerindian ethnic groups still remains in the nowadays territory of Argentina. The Mapuche population that inhabits many Central and Southern Argentinean provinces derived at least partially from the Chilean Araucanians. Its origin might be the result of an admixture processes between populations that inhabited both sides of the Andes. The culture developed by the Mapuche included a language restricted to its spoken expression. Its linguistic affinities are unclear and it was developed prior to the Spanish arrival to Southern South America, occurred in early XVI century. Lineages adopted linguistic elements to identify their family members (e.g. cura -stone- define members of a certain family group, calfu cura -blue stone- was a member of the Cura family). Although, in Argentina, the Mapuche descendant are considered to be almost eliminated by different wars, populations limited in size still persist and many of their members use their ancestral names included in the surnames. At present the family names are inherited patermally according to the Argentinean laws, when the offspring is recognized by its father, but if it is not the case, the offspring receives their mother´s surname. From the forensic point of view the feasibility to identify ancestral lineages becomes relevant since aboriginal

organizations claimed for their ancestral territories to the governments. In order to investigate the correlation between cultural (surnames) and genetic markers suitable for ancestry tracing, a set of volunteer male unrelated donors (N=139) from the Patagonian provinces of Rio Negro (N=79) and Chubut (N=60) were considered. Within each geographical region two subsets were selected based in their surnames. Individuals with indigenous surnames from Rio Negro (N=37) and from Chubut (N= 31) and those with European surnames from Rio Negro (N=42) and Chubut (N= 29) were investigated. Surnames, the Native American Y-specific haplogroup (hg) M3-Q3, determined by typing locus DYS199 and mtDNA hg A, B, C and D, determined by Control Region sequencing, were employed as independent ancestry tracing parameters. In addition, 15 autosomal STRs (13 CODIS core plus Penta D and E) and the nine Y-STRs included in the YHRD minimal haplotype, were used to determine the Fst values and genetic distances for each ancestry evaluation approach. In both provinces a 97% of the individuals with Mapuche surname also depicted Amerindian matri- and/or patrilineage. Meanwhile, in Rio Negro and Chubut only 18% and 17%, respectively, of the sample denoted neither aboriginal surname nor Amerindian genetic ancestry markers. The most prevalent West Eurasian mtDNA haplogroup were H (6.5%), U5 (4.3%) and K (2.8%), followed by T (1.4%), V (0.7%), X (0.7%) and M1 (0.7%). Sub-saharan African matrilineages were detected in two individuals (1.4%). Our results confirmed the close correlation between surnames with Mapuche lingüistic elements and the genetic ancestry markers. However, it must be emphasized that this correlation is not Mapuche-specific, but Amerindian specific, at present efforts are being made by our group, to identify ethnic specific informative genetic ancestry markers. Concerning the forensic relevance it should be proposed the combined use of both, cultural (surnames) and genetic ancestry markers when an ancestry diagnosis is required.

OP052
Population origins in South Siberia: mitochondrial and Y chromosome analysis of Bronze and Iron age human specimens

Keyser C[1], Amory S[1, 2], Bouakaze C[1], Crubézy E[2], Ludes B[1, 2]

1) Laboratoire d'Anthropologie Moléculaire, Institut de Médecine Légale, 11 rue Humann, 67085 Strasbourg Cedex, France 2) Anthropobiologie, Université Paul Sabatier, CNRS, UMR 8555, 39 allées Jules Guesde, 31000 Toulouse, France

Southern Siberia has long been seen as the area of intense admixture of West and East Eurasian groups. According to paleoanthropological data, the Europeoid traits predominated in the steppes of the Tuva, Altai and Khakassia regions at least since the Bronze Age. Subsequent migrations from Central and Eastern Asia led to the formation of anthropological traits seen in the contemporary populations of South Siberia. Mitochondrial DNA studies, made on present-day populations of South Siberia, revealed an east-to-west cline in the frequencies of West Eurasian-specific mtDNA haplotypes. Similarly Y chromosome data revealed contrasting patterns of Y-chromosome variation from Balkai and Altai-Sayan regions. Nevertheless, the substantial assimilation underwent by the Siberian populations since the arrival of Russians in the 16th century could have obscured their ancestral gene pool. For this reason, information provided by the analysis of ancient specimens appears to be a good way to validate ethnogeneis hypotheses inferred from anthropological or modern molecular data. In the present work, 28 ancient specimens from the Krasnoyarsk area (Lower Yenisei) were typed for uniparental DNA markers : mitochondrial DNA (HVI region) and the non-recombining portion of the Y chromosome (SNP and STR). Among these specimens, 13 were dated from the Bronze age, whereas the 15 other ones were from the Iron age. The results obtained are consistent with paleoanthropological view about the presence of Europoid-specific traits in the populations of the Southern siberian region since the Bronze age and the presence of Mongoloid component becoming prevalent in modern times. Indeed, the mitochondrial gene pool of the Krasnoyarsk ancient specimens was characterized by a combination of European-specific and Asian-specific mtDNA haplogroups, with a proportion of European-specific haplogroup close to 85% for the Bronze age specimens. Concerning the male lineages, they were largely represented by the R1a1 haplogroup, which is common in Central and Eastern Europe as well as in some populations of Western and Central Asia. All these results are discussed in the light of molecular data obtained from modern populations.

OP054
Haplotype discrimination capacity of 35 Y-chromosomal Short Tandem Repeat loci

Rodig H[1], Roewer L[4], Gross A[1], Richter T[2], De Knijff P[5], Kayser M[3], Brabetz W[1]

1) Biotype AG, Moritzburger Weg 67, 01109 Dresden, Germany 2) Qualitype AG, Moritzburger Weg 67, 01109 Dresden, Germany 3) Department of Forensic Molecular Biology, Erasmus University Medical Centre Rotterdam, PO Box 1738, 3000 DR Rotterdam, The Netherlands 4) Institute of Legal Medicine, Charité - Universitary Medicine, Hannoversche Str. 6, 10115 Berlin, Germany 5) The Forensic Laboratory for DNA research, MGC Dept. of Human Genetics, Leiden University Medical Center, The Netherlands

The discrimination capacity of the 9 European minimal haplotype (MH) loci, 2 loci recommended by the SWGDAM, and further 24 single-copy loci was analyzed in this study. For this evaluation 279 DNA samples from Germany, 72 samples from The Netherlands, and 40 samples from Turkey were used. Highest gene diversities were shown for the well-known Y-chromosomal short tandem repeat loci DYS385, DYS389-II, DYS390 and for the new loci DYS449, DYS481, DYS570, DYS447, DYS576 (D = 0.7518-0.8746). According to our findings these 5 new Y-STR loci in addition to the European MH loci individualized all male haplotypes in each population group (h = 1.000000). With regard to the pooled DNAs we were able to differentiate 387 of 391 samples (h = 0.999974). For the individualization of the pooled DNA samples, the supplementary analysis of DYS446 or DYS505 and DYS406S1 or DYS522 will be recommended.

OP055
Impact of Additional Y-STR Loci on Resolving Common Haplotypes and Closely Related Individuals

Decker AE, Vallone PM, Kline MC, Butler JM

National Institute of Standards and Technology, Biochemical Science Division, Gaithersburg, MD 20899-8311 USA

Commercial Y-STR kits such as Promega’s PowerPlex Y and Applied Biosystems’ Yfiler permit laboratories to go beyond the original nine minimal haplotype loci (MHL) and to discover the advantage of additional Y-STR loci in resolving common haplotypes. In an effort to discover the impact of Y-STR markers beyond the 17 loci now available in commercial kit form, we have been investigating new loci on a common set of samples representative of the major U.S. population groups. A set of 27 Y-STR loci across 656 U.S. Caucasian, African American, and Hispanic samples was described in Butler et al. (2006) Forensic Sci. Int. 156:250-260. Since that publication an additional 41 loci have been examined on a subset of our U.S. population samples. The approach for selecting and evaluating these loci will be described including their ability to resolve samples with common types that cannot be resolved with commercial Y-STR kit loci. Efforts in constructing additional multiplex PCR reactions with the new loci will be detailed. The additional loci examined in our studies could potentially assist in increasing the power of discrimination between closely related male lineages—and with enough loci may even help resolve males separated by one or a few generations from one another due to mutation. In almost 300 father:son sample pairs, we observed 14 differences between father and son with the 17 Y-STR loci in the Yfiler kit. Five mutations resulted in the loss of a repeat in the son and 9 loci gained a repeat. All samples resulted in single repeat mutations except one sample, which was a two repeat loss at Y-GATA-H4. Also, one sample pair was found to have two mutations (DYS635 and DYS458). Within our 656 U.S. population samples, a total of 26 samples (~4 %) were found to have the most common type and to be unresolved with the nine MHL. Adding the two SWGDAM loci of DYS438 and DYS439 to the MHL breaks these samples into three groups. The 12 PowerPlex Y loci further resolve the samples into seven groups, whereas the 17 Yfiler loci separate all but three of the samples from one another. A screen of these unresolved samples with 20 additional Y-STR loci found that either one of the two of the loci DYS522 or DYS576 (when combined with the 17 Yfiler loci) were capable of resolving all 26 samples with the most common type.

OP056
Casework Approach and Casework Experience using the Yfiler™ kit for analysis of YSTRs

Gross AM

Minnesota Bureau of Criminal Apprehension, Forensic Science Laboratory, 1430 Maryland Ave East, St. Paul, MN 55106, USA

Y-STRs have been used in forensic casework at the Bureau of Criminal Apprehension Forensic Science Laboratory (MN BCA) since 2003. At that time two separate amplifications were required to type the Scientific Working Group on DNA Analysis Methods (SWGDAM) recommended loci (DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS438 and DYS439) [Forensic Scence Communication, July 2004] . The Yfiler™ kit co-amplifies these loci as well as DYS437, DYS448, DYS456, DYS458, DYS635 and Y GATA C4. The Yfiler™ kit was validated following the internal validations outlined in the SWGDAM revised validation guidelines [Forensic Science Communication, July 2004]. This validation is essential in establishing the limitations of the technology as well as to assist in the development of interpretation guidelines. Our studies demonstrate the Yfiler™ kit is extremely sensitive, does not exhibit any cross-reactivity with female DNA and successfully types male DNA in the presence of overwhelming amounts of female DNA. This presentation will briefly describe some of the validation studies which determined our casework approach. In addition, data from some of the 30+ cases that have been analyzed using this technology will be presented.

OP057
The forensic evaluation of the Y-STRs DYS448, DYS452 and DYS459

Howe A, Edelmann J, Thiele K, Lessig R

Intitute of Legal Medicine, University of Leipzig, Germany

The Y-chromosomal Short-Tandem-Repeats DYS448 (hexanucleotid repeat), DYS452 (pentanucleotid repeat) and the multicopy STR DYS459 (tetranucleotid repeat) were characterized in unrelated male samples from Germany (West Saxony), Estonia, Latvia, Lithuania and Vladivostok. Sequence date was obtained to designate a repeat number nomenclature. The discrimination capacity of these three markers by their own is similar to markers like DYS391, DYS392, DYS393 or DYS19. In combination with the commonly used „minimal or extended haplotype“ these markers can increase the discrimination capacity. Additionally, a combination with the multicopy marker DYS464 is really significant as well. However, there is the problem that information about the infertility may be discovered as a side effect, because 7 % of all infertility cases are caused by a deletion of the AZFc (Azzospermiefactor c), were DYS464 and probably the DYS459 are located.

OP058
Evolutionary-Genetic Architecture of Normal Variation in Human Pigmentation

McEvoy B[1], Beleza S[2], Bradley DG[1], Shriver M[3]

1) Smurfit Institute of Genetics, Trinity College Dublin 2, Ireland 2) IPATIMUP, Porto, Portugal 3) Dept. of Anthropology, The Pennsylvania State University, PA, USA

Skin pigmentation varies substantially across human populations in a manner largely coincident with ultraviolet radiation intensity. Natural selection in response to sunlight is likely to be an important force in explaining the evolution of global pigmentation diversity. Sexual selection and additional environmental pressures may have also played a role and signatures of these selective events may be a useful source in identifying genes that explain this variability. We have analyzed SNP data from the International HapMap project in 77 pigmentation candidate genes for such signatures. Based on these results and other similar work, we provide a tentative three-population model (West Africa, East Asia and North Europe) of the evolution of human pigmentation. These results suggest a complex evolutionary history with selection acting on many different gene targets at various times and places in the human past. These selection-nominated genes are candidates for follow-up functional studies, including linkage and association analyses, to confirm and determine their role in explaining normal variation in human pigmentation.

Poster Presentations

PP001
Correlation of metabolite ratios of amitryptiline in hair samples of 23 children and genetic polymorphism of CYP2D6 and CYP2C19

Schmid D, Thieme D, Rolf B

Institut für Rechtsmedizin München

We investigated a case of illegal administration of amitriptyline to a group of children by a female childminder. The woman was working as day mother. By the child welfare office, she was allowed to take care of 4 children in her apartment. To increase her income, she took up more then 10 children at the same time over a period of several month. The children were sedated by amitriptyline and had to sleep in a cellar room. After the suicide of the woman, the illegal administration was uncovered and the hair of the children was investigated for amitryp-tiline to prove the intoxication during the past month. Significant variations of hair concentra-tion ratios were observed. Each of the ratios nortriptyline/amitryptiline, 10-hydroxynor¬tryp¬tiline/nortryptiline, 10-hydroxyamitryptiline/amitrypti¬line and E10- hydroxynortryptiline/Z10- hydroxynortryptiline varied by a factor of 5-10 between individuals. The variations were sta-ble and reproducible over the time period covered by the hair samples. The metabolism of amitryptiline is known to be influenced by polymorphisms of the enzymes involved. CYP2C19 is mainly responsible for the demethylation of amitryptiline, while hy-droxylation is supposed to be predominantly controlled by CYP2D6. Attempts to associate metabolite ratios in hair with polymorphism of the CYP2C19 and CYP2D6 genes revealed that the amount of nortryptiline in hair (relative to amitryptiline) is highly correlated with the CYP2C19 genotype and the conformation of hydroxyl metabolites (i.e. E10/Z10-hydroxy isomers) is governd by CYP2C19 rather than CYP2D6. Furthermore there is no obvious correlation between CYP2D6 phenotype and hydroxylation of amitrip-tyline.

PP002
Genetics of Sudden Cardiac death: Analysis of HCM mutations by Sequenom MassArray genotyping system

Brion M[1], Allegue C[1], Monserrat L[2], Hermida M[2], Castro-Beiras A[2], Saukko P[3], Carracedo A[1]

1) Grupo de Medicina Xenomica, CEGEN-IML, University of Santiago de Compostela, Spain 2) Juan Canalejo Hospital, A Coruña, Spain. Red RECAVA 3) Department of Forensic Medicine, University of Turku, Turku, Finland

Perhaps the most important unsolved challenge in the practice of forensic pathology is the failure to determine a cause of death, particularly in a previously healthy young person who has died suddenly and unexpectedly. Sudden cardiac death (SCD) is a major cause of death worldwide, responsible for half of all heart disease deaths with an incidence ranging from 30-200/100,000 individuals. In addition an important portion of SCD cases are due to HCM and, in some cases, the diagnosis using macro and microscopic findings is difficult. Negative CSD and HCM autopsies can benefit from the so-called „molecular autopsy" by the analysis of genes involved in the condition (including HCM, WPW and ion-channel disorders, among others). There is an increasing understanding of the clinical genetics of sudden cardiac death. Hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular dysplasia (ARVD), long QT síndrome (LQTS) and other abnormalities of rhythms such as Wolff-Parkinson-White (WPW) syndrome all have an association with genetic disease. The analysis of genes implicated in these syndromes with simple tests after the autopsy, could decrease the percentage of negative autopsies and provide essential information to relatives, helping in the prevention of further cases of CSD. HCM affects 1:500 adults in general populations and it is the most frequent cause of sudden death in the young and in sportsmen, even though it may cause sudden death at any age. Sudden death is the mode of presentation for more than 50% of patients with HCM. Patients at risk of sudden death are those with a family history of sudden death and those with a history of syncope or presyncope. Molecular basis at the DNA level, of the genes involved in some causes of CSD is increasingly being discerned and at least 10 genes for HCM and at least other 14 genes for other genetic causes of CSD are characterised. DNA technologies for analyzing single nucleotide polymorphisms and mutations have experienced an impressive recent development. The flexibility and variability of genotyping chemistries and platforms improves the choice of approach, so making the analysis of mutation, on a large scale, easier and cheaper. Based on this knowledge we planed to know the number and spectra of HCM mutations in SCD, by means of a retrospective analysis of HCM mutations in patients that have suffered sudden death and show signs of HCM after the autopsy. More than 400 described mutations will be detected with an efficient mutation detection system based on semi-automated MALDI-TOF mass spectrometry using Sequenom.

PP003
Endogenous controls in gene expression studies of post-mortem human tissues

Matt K, Schmidt U, Lutz-Bonengel S, Heinrich M

Institute of Legal Medicine Freiburg, Germany

It was shown that the extraction of RNA from post-mortem human tissues can be successfully performed. In several studies it was found that the post-mortem interval does not correlate with the degradation of RNA. Thus, it can be stated that gene expression studies could reveal valuable additional information for example to the analysis of the cause of death. Additionally, gene expression data from human tissue would expand the knowledge of physiological pathways and of genes responsible for various diseases. Most of the existing gene expression data were obtained from mouse or rat animal models and a transfer of these data to the human organism is not always possible. Recently, several studies were published on the suitability of various endogenous controls for their use in gene expression studies. It was found that the expression of some of the well established endogenous controls is not invariant but may differ among tissues. Our study included several endogenous controls (GAPDH, β-Actin, 18sRNA and others). It was checked whether the real-time PCR results vary among tissues and among post-mortem intervals. For this study, various human tissues from individuals with post-mortem intervals between 15 h and 96 h were used. The suitability of the different endogenous controls will be discussed with respect to their stability over various post-mortem intervals and their variance in expression among tissues.

PP004
Application of X-chromosomal short tandem repeats in a case of sexual assault

Turrina S, Atzei R, Filippini G, Zaglia E, De Leo D

Department of Medicine and Public Health, Division of Legal Medicine, Forensic Genetic Laboratory, University of Verona, Italy

In sexual assault involving youthful girls which yields pregnancy, after the abortion DNA examinations can be performed from the aborted foetal material to provide evidence of paternity of the suspected perpetrator. This study presents a case of rape against a 13-year-old nomad girl. The suspected perpetrators were an adult nomad man and his son, both unrelated with the girl. Immediately after the assualt, the victim was admitted in a local hospital, but any kind of procedure was performed to obtain an objective confirmation of sexual contacts (vaginal, anal and oral swabs) and to prevent pregnancy. As established by the Jugde, 10 weeks later the girl was admitted to the same hospital to interrupt her pregnancy and, in order to identify the father of the product of conception, the authors were charged to determine DNA-short tandem repeat (STR) profile from products of abortion or directly from foetus. The genetic analysis, performing for 15 autosomal STRs (AmpF1STR Identifiler PCR Amplification Kit, Applied Biosystems), demontrated that the adult man was the biological father of foetus (probability of paternity of 99.99999%). Nevertheless, probability of paternity of the second young man was 99,9998%. In fact, the young man shared with the foetus 13 STRs of the 15 STRs analized. So, to solve the problem reaching stronger evidences about the paternity, because the foetus was female, we carried out another genetic analysis evaluating 12 X-chromosomal STRs. The findings clearly revealed that the biological father of foetus and then the perpetrator of sexual assault was the adult man. This case shows that X-STR screening results can be very useful in the detection and objective confirmation of patenity in cases of sexual assaults when the perpetrators are relatives and the foetus is female. Moreover, this type of evidence can corroborate child testimony and spare the victim from further trauma caused by prolonged forensic investigations and court proceedings.

PP005
Analysis of 10 X-STRs in three African populations

Gomes I[1, 2], Alves C[1], Maxzud K[3], Pereira R[1], Prata MJ[1, 4], Sánchez-Diz P[2], Carracedo A[2], Amorim A[1, 4], Gusmão L[1]

1) IPATIMUP, Institute of Pathology and Molecular Immunology of the University of Porto, Porto, Portugal 2) Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain 3) CEPROCOR Unit - Agencia Córdoba Ciencia. Complejo Hospitalario Santa María de Punilla, Córdoba, Argentina 4) Faculty of Sciences of the University of Porto, Porto, Portugal

Although genetic markers on the X chromosome are infrequently used, they have revealed their usefulness in particular cases of parentage investigations. For example, in the absence of the alleged father, these markers allow for higher likelihood ratios in paternal half-sister investigations, even when other members of the family are not available. Nowadays, there are many STR databases for autosomal and Y chromosomes. However, for X chromosome STRs there are still few populations sampled and, for forensic purposes, there is no data on African

populations. In this work we have analysed 10 X-STRs in a decaplex PCR reaction (DXS8378, DXS9898, DXS8377, HPRTB, GATA172D05, DXS7423, DXS6809, DXS7132, DXS101 and DXS6789) in unrelated individuals belonging to three African population groups: Angola (N=74), Mozambique (N=112) and Uganda (N=51). They all present a high genetic diversity for all the markers studied, in the same order of magnitude as the previously observed for European and Asian populations. No significant associations between alelles of any pair of loci were found in these three population groups. Population differentiation tests revealed no significant differences between Angola and Uganda, but both groups present significant values in relation to Mozambique. Allele frequencies together with parameters of forensic interest for each X-STR were estimated for each population group and will be reported. The overall mean exclusion chances (MECs) for the 10-plex in trios are quite high, since the probability of finding the same profile is over 1 in 4.2 million (Angola: 1 in 4 233 907.117; Uganda: 1 in 4 272 661.995), being the highest in Mozambique (1 in 5 301 841.332); in duos these values drop down considerably to around 1 in 60 thousand. Concerning the overall power of discrimination (PD), this decaplex can discriminate 1 in nearly 41 million Ugandan men and 1 in around 30 million Angolan and Mozambican men. When calculated on the female samples, these PD values shoot to an order of magnitude of over 13 digits in all population groups, being higher in the Mozambican sample.

PP006
Development and forensic validation of a new multiplex PCR assay with 12 X-chromosomal Short Tandem Repeats

Turrina S, Atzei R, Filippini G, De Leo D

Department of Medicine and Public Health, Division of Legal Medicine, Forensic Genetic Laboratory, University of Verona, Italy

One multiplex system for the coamplification of twelve X-chromosomol short tandem repeats (STRs) DXS7132, DXS8378, DXS6809, DXS7133, DXS6789, DXS7424, GATA172D05, HPRTB, DXS7423, GATA31E08, DXS101, DXS6807 and amelogenina were analysed in a sample of 200 unrelated healthy individuals (100 males and 100 females) living in North Italy, in 40 family trios with female children and in 10 father/daughter duos (previously confirmed by autosomal STR analysis). To avoid overlap between allele ranges, loci were labelled with 6-FAM, VIC, NED and PET days. The multiplex amplification conditions were optimized on the Gene Amp PCR system 9700 thermal cycler. The amplification products were separated on ABI PRISM 3100 Avant Genetic Analyzer in comparison to sequenced allelic ladders and control DNA (K562, 9948 and 9947A cell lines). The chi-square test for genotype distribution of the X-chromosomal STRs showed no significant deviation from the Hardy-Weinberg equilibrium. Allele frequencies between female and male samples were not significantly different in all examined markers. Heterozygosity, polymorphism information content (PIC), power of discrimination in females (PDf) and males (PDm), mean exclusion chance in trios involving daughters (MECI) and father/daughter duos lacking maternal genotype information (MECII) were determined. The applicability and usefulness of our multiplex approaches in paternity deficiency cases is demonstrated by a combined power of discrimination (PDc) for both females and males with PDc > 0.999999.

PP007
Validation of the Mentype® Argus X-8 kit

Gehrig C, Teyssier A

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-8 kit was performed. The Argus X-8 kit is a commercial multiplex system which contains Amelogenin for gender determination as well as eight X-chromosomal STR markers (DXS8378, HPRTB, DXS7423, DXS7132, DXS10134, DXS10074, DXS10101 and DXS10135). At any one time, two markers belong to the four coupling groups of the X-chromosome. Thus two markers of each group have to be handled as haplotype for genotyping. 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, 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.

PP008
DXS10079, DXS10074 and DXS10075: new alleles and SNP occurrence

Hering S[1], Rodig H[2], Augustin C[3], Edelmann J[4], Heidel M[1], Dreßler J[1], Szibor R[5]

1) Institute of Legal Medicine, Technical University of Dresden 2) Biotype AG, Dresden 3) Institute of Legal Medicine, University of Hamburg 4) Institute of Legal Medicine, University of Leipzig 5) Institute of Legal Medicine, University of Magdeburg

The recently introduced X-chromosomal STR markers DXS10079, DXS10074 and DXS10075 form closely linked haplotypes useful for pedigree analysis. Here, we examined SNP occurrence in their flanking regions. We sequenced a variety of samples from Germans, Asians and Africans with respect to different STR alleles. For all three marker systems SNPs were detected in the repeat flanking regions. Mainly we found differences between Africans and the other ethnic groups. The new SNP information will be useful for avoiding allele dropouts.

PP009
Forensic validation of three closely linked STR markers located within the Xq28 region

Edelmann J[1], Lessig R[1], Hering S[2], Augustin C[3], Rodig H[4], Szibor R[5]

1) Institute of Legal Medicine, University of Leipzig, Germany 2) Institute of Legal Medicine, Technical University Dresden, Germany 3) Institute of Legal Medicine, University Hospital Hamburg-Eppendorf, Germany 4) Biotype AG, Dresden, Germany 5) Institute of Legal Medicine, Otto-von-Guericke University-Magdeburg, Germany

Searching for further suitable and closely linked STRs on the X-chromosome we evaluated three new polymorphic markers within the human X contig NT_011726, clone AL034384 in the region Xq28. The markers were characterised and evaluated for their forensic efficiency in a population sample of more than 300 unrelated German individuals and registered in the GDB as DXS10134, DXS10146 and DXS10147. We report here primer sequences, PCR protocols, allele structures as well as allele and haplotype frequencies. Performing the exact test for genotype distribution of the STRs we found no significant deviation from Hardy-Weinberg equilibrium. The stability of haplotypes was tested in 90 three-generation families. Hence, the Xq28 STR cluster reported here is suitable for forensic purpose and can contribute solving complex kinship cases. One of the markers, DXS10134, was integrated in the commercial available test kit Mentype Argus X-8, for typing of linkage group 4, in addition to DXS7423.

PP010
X-chromosomal polymorphism data for the ethnic minority of Polish Tatars and the religious minority of Old Believers residing in northeastern Poland

Pepinski W[1], Skawronska M[1], Koc-Zorawska E[1], Niemcunowicz-Janica A[1], Janica J[1], Soltyszewski I[2]

1) Department of Forensic Medicine, Medical University of Bialystok, Poland 2) Department of Criminalistics and Forensic Medicine, University of Warmia and Mazury, Olsztyn, Poland 3) Central Forensic Laboratory of Police, Warsaw, Poland

Ancestors of contemporary Polish Tatars arrived to Poland in the 14th century from the Golden Horde and Khanates of Kazan, Crimea, Volga and Astrakhan. Tatars who presently live in Poland are Sunni Muslims. They number about 2,500 and all speak Polish. Islamic centre of Polish Tatars is Bialystok in the region of Podlasie (NE Poland) where an intensive settlement of Tatars in late 17th and early 18th century took place. Despite several centuries of cultural and religious assimilation and separation from Tatar-Turkic roots Polish Tatars living in separate, closed communities, preserved their ethnic and religious identity. 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. The Old Believers were severely persecuted under the tsars and sought shelter in the most remote corners of Russian Siberia as well as abroad, including Poland. Many communities lived in almost complete isolation for centuries. Presently, not many more than 600 Old-Believers inhabit several villages in Suwalki Region in northeastern Poland, where they have struggled to maintain their religious identity and traditional ways of life. Buccal swabs were collected from 210 unrelated volunteers (140 males and 70 females) belonging to each minority. Mentype Argus X-UL kit (Biotype AG, Germany) was used to co-amplify X-STR loci: DXS8378, DXS7132, HPRTB and DXS7423. Electrophoresis and typing were performed in the ABI 310 Genetic Analyzer. For each locus, allele frequencies were calculated separately for males and females (140 chromosomes, respectively). The genotype distributions among females conformed with HWE for all analyzed loci except for DXS7423 and HPRTB (P=0.0001 and 0.0440, respectively) in Old Believers. No significant differences were observed between allele distributions in males and females. No mutation was detected at any of the four loci, based on 54 paternal and 48 maternal transfers. For the quadruplex evaluated, the combined values of DPM are 0.9945 and 0.9957, the combined values of DPF are 0.9998 and 0.9999 and the combined values of MEC are 0.9808 and 0.9919 (Polish Tatars and Old Believers, respectively). Since a pairwise

comparison using the exact test disequilibrium analysis yielded no indication of allelic dependence. A pairwise testing for heterogeneity using the RxC contingency table exact tests (Chi2 and G-statistics) for population differentiation revealed statistical differences at HPRTB and DXS7132 for the both minorities in relation to the autochthonous Poles (0.0000<P<0.0110). Prominent differences in allelic frequency distributions were also revealed at HPRTB between the Old Believers and Polish Tatars (P=0.0000). Scarcely represented in Polish population alleles 8 at DXS8378 and 17 at HPRTB (0.28%, respectively) were absent in the both minorities datasets which, on the other hand, exclusively exhibited HPRTB allele 10 (0.4% and 3.2%, respectively).

PP011
Hungarian population data of eight X-linked markers in four linkage groups

Zalán A, Völgyi A, Pamjav H

Institute of Forensic Medicine, Budapest, Hungary

The X chromosomal STR markers DXS10135 and DXS8378 in linkage group 1, DXS7132 and DXS10074 in linkage group 2, HPRTB and DXS10101 in linkage group 3, and DXS10134 and DXS7423 in linkage group 4 were studied in the Hungarian population. After genotyping unrelated men (219) and women (165), forensic efficiency parameters were calculated. Between the linkage groups deviation from the Hardy–Weinberg equilibrium could not be detected. Different population data were compared with G-tests showing significant difefrences as expected. The loci were tested in deficiency and normal paternity cases.

PP012
As to the problems of molecular-genetic research of stored bone samples exemplified by a skeleton find

Thiele K[1], Uerlings H, Lessig R, Edelmann J, Placke R, Bindl R, Amaro Suarez FF[3], Braun S[1], Bruchhaus H

1)Institute of Legal Medicine, University of Leipzig, Germany 2)Institute of Human Genetics and Anthropology, Friedrich-Schiller-University Jena, Germany 3)Institute of Legal Medicine, Havana, Cuba

In July 1999, mushroom seekers discovered a human skull in a woodland near Johanngeorgenstadt. Research of the criminal investigation department led to a male person who had been missing since April 1999. Using existing stomatological comparable results, the skull could definitely be assigned to this missing man. In March 2001, numerous remains of human skeletons were found in a woodland near Steinbach. Osteological investigations of femur determined on a male individual sized 174 cm of over 40 years of age. Amidst the skeleton remains a lower jaw was found which could be identified by stomatological comparative research. It came from this male person, who had been reported missing in April 1999. In the beginning, molecular-genetic investigations to identify the discovered skeleton remains of the postcranium were not arranged. When in early 2006 the widow strove for the funeral of the skeleton remains, the responsible public prosecutors ordered the molecular-genetic identification of the postcranium. For this, bone samples of tibia, pelvis, femur, skull, and teeth were cut up, the DNA was isolated by the First DNA kit of GEN-IAL Ltd. and later quantified (Quantifiler® Human DNA Quantification Kit of Applied Biosystems Company). Various multiplex kits were used for PCR and STR fragment analysis. The poster demonstrates the results.

PP013
A study of 12 autosomal STRs in a population sample of Cuba

Amaro Suarez FF[1], Lessig R[2], Thiele K[2]

1)Institute of Legal Medicine, Havana, Cuba 2)Institute of Legal Medicine, University of Leipzig, Germany

The Cuban population originates from successive migrations of people from Spain, Africa and the Caribbean Islands, and, in lower quantities, also from China. These basic groups lived under conditions which did not suffer from isolation. They were frequently a mixture of Whites and Blacks. These conditions were not necessarily the same in all the regions, and for this reason it is necessary to obtain the allele frequencies in a population sample which is more representative of the country. This work presents the results obtained from a genetic population study of 12 systems (which included the GenePrint Kits for silver staining of Promega Corporation) in a sample of the Cuban population from various parts of the country who attended the Institute of Legal Medicine for paternity tests. We selected mothers and fathers who did not have any filial relationship. We studied the three principal groups of the Cuban population, classified as Whites, Blacks and mulattos. The number of persons who were included in each system varied between 821 as the highest (D13S317 in the STR III multiplex systems) to 102 as

the lowest (F13A01 in FFv multiplex systems in Blacks). The results that we obtained in this study have convinced us that this sample of the Cuban population is very similar to other population reports of Promega and one sample of the Cuban population reported by Leonard and coworkers , insofar as the more frequent alleles are the same, but the changes of the order of alleles with a higher frequency and the absolute value of frequency are slightly different. When we compare our results of allelic frequency with the Caucasian and Hispanic groups of Promega, we can observe that this sample of the Cuban population is more similar to the Caucasian group than to the Hispanic group, because the Hispanic group have been influenced by Latin American peoples whose ancestors were native Indians (such as Mexicans, Peruvians, Bolivians etc.). When we compare the results with the sample reported by Leonard and coworkers, we can observe differences insofar as the more frequent allelic frequency changes in some markers, but in general the more frequent alleles are the same, except for TPOX in Blacks, LPL in Whites, F13A01 in mulattos and Blacks, and TH01 in mulattos. We came to the conclusion that this study is more representative of the Cuban population because it includes persons of different regions of Cuba, and because in this study the sample is higher and included three markers (STR III Systems) more than Leonard and coworkers.

PP014
A single approach to the recovery of DNA and FDR (firearm discharge residue)

Fairley M

Strathclyde Police Forensic Support Department, Glasgow G2 4JS, Scotland, United Kingdom

The DNA Section of the Strathclyde Police Forensic Support Department in Glasgow extracts DNA from cellular material recovered from garments and other evidence items using a „Mini-Tape method. This method can be used to simultaneously recover both DNA and FDR (firearm discharge residue) particles. This presentation will focus mainly on the DNA application. The Mini-Tapes“ are prepared in the laboratory and are cheap and easy to use. Each batch of tapes is quality control checked for DNA contamination prior to use. The method uses a small square of tape to recover and concentrate „invisible“ cellular material such as skin cells, buccal cells and vaginal cells. This makes the recovery fast and effective. Examples will be given of the use of Mini-Tapes in respect of Murder, Rape, Robbery, Drugs, Firearms, Terrorism and other types of crime. This is a low tech method that can be taken up very quickly by a laboratory, preparation and extraction of the tapes is described.

PP015
Apparent exclusion of maternity at the RHCE locus potentially including RHD

Klintschar M[1], Dauber EM[2], Glock B[2], Mayr D[2], Mayr WR[2]

1) Institute of Legal Medicine, Martin Luther University Halle-Wittenberg, Halle, Germany 2) Division of Blood Group Serology, Medical University of Vienna, Vienna, Austria

RHCE phenotyping of a Caucasoid mother/child pair revealed an isolated genetic mismatch: the mother was typed as R1R1 (CCDee), whereas her offspring was typed as R2R2 (ccDEE). All other red cell antigens tested (ABO, RHD, MNS) and 18 short tandem repeat (STR) polymorphisms (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA, SE33, D12S391 and D8S1132) were inherited in the Mendelian way. Therefore a deletion, a silent gene or an amorph gene at the RHCE locus, potentially including RHD, (involving D-- or --- haplotypes) was taken into consideration. Furthermore, uniparental isodisomy 1 (UPD1) could not be ruled out. Testing the mother's RBC with Anti-C and Anti-e and the child's RBC with Anti-c and Anti-E reagents revealed weaker reactions, while the reactions with Anti-D reagents were of equal strength in mother and child compared with homozygous controls. Consequently, single dose of RHCE was to be supposed. A PCR-RFLP and long-range PCR revealed apparent RHD homozygosity, because the genetic pattern typical of normal RHD heterozygous individuals could not be detected. But the results could not rule out RHD hemizygosity. Additional chromosome 1 markers were tested: FY and PGM1 were inconclusive because mother and child were homozygous, whereas they were heterozygous at the D1S80 locus. Therefore, UPD1 could be excluded as the reason for the mother/child mismatch. Additionally, 15 polymorphic dinucleotide STR marker systems all over chromosome 1 (D1S468, D1S2890, D1S2635, D1S2836), but most of them close to the RHD and RHCE locus were investigated (D1S507, D1S2697, D1S2644, D1S199, D1S2864, D1S234, D1S2236, D1S1466, D1S233, D1S255, D1S2892), the latter encompassing an interval from 11 Mb telomeric to 14.5 Mb centromeric of RHCE/RHD. D1S234 and D1S233 exhibited heterozygous results flanking the RHD and RHCE genes at a distance of 0.5 cM telomeric and 5.7 cM centromeric, respectively. Consequently, an alteration in between these two markers was suggested. Further analysis including markers close to the RHCE and RHD genes and real time PCR to test for RHD heterozygosity will be carried out in future to define the underlying genetic structure of the observed phenomenon.

PP016
Definition of HLA haplotypes by STR polymorphisms

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

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

In a previous study (S. Wenda et al., International Congress Series 1288, 804-806, 2006), the alleles of 284 HLA haplotypes at the loci HLA-A, -B, -C, -DR, D6S2931 (C1_4_4), D6S2939 (C2_4_4) and D6S2906 (C3_3_6) could be defined. The analysis of the linkage disequilibria between the alleles of these 7 loci showed only one Caucasoid superhaplotype: HLA-A*01, B*08, Cw*07, DRB1*03, D6S2931*10, D6S2939*9, D6S2906*12. The analysis of the HLA alleles and the STR alleles on the 284 haplotypes showed that 7 out of 8 haplotypes with HLA-A*01,B*08,Cw*07,DRB1*03 also carried D6S2931*10, D6S2939*9, D6S2906*12; out of the 16 D6S2931*10, D6S2939*9, D6S2906*12 haplotypes, only 7 showed HLA-A*01,B*08,Cw*07,DRB1*03. Analogous results have been obtained by analyzing the second Caucasoid superhaplotype, HLA-A*03,B*07,Cw*7,DRB1*02, which shows some linkage disequilibrium with D6S2931*19, D6S2939*10, D6S2906*9. In this case, 9 out of 16 haplotypes with HLA-A*03,B*07,Cw*7,DRB1*02 also carried D6S2931*19, D6S2939*10, D6S2906*9; out of the 17 D6S2931*19, D6S2939*10, D6S2906*9 haplotypes, only 9 showed HLA-A*03,B*07,Cw*7,DRB1*02. In spite of the linkage disequilibria observed between STR alleles and HLA alleles, it is not possible to define HLA genotypes by using only the 3 above mentioned STR polymorphisms. For this reason, it is not probable that the typing for a limited number of STR polymorphisms, which are localized within the HLA complex, gives a clear-cut indication for the presence of HLAA, -B, -C and -DR alleles.

PP017
Investigation of four X-chromosomal STR markers in Amharic individuals from Ethiopia

Thiele K[1], Reißig D[2], Assegedech B[2], Yared W[2], Worku N[2], Edelmann J[1], Lessig R[1]

1) Institute of Legal Medicine, University of Leipzig, Germany 2) Department of Anatomy, University of Gondar, Ethiopia

X-linked DNA markers are a powerful tool in forensic case work, especially in forensic kinship testing and deficiency cases. They have been found to be highly informative and significant different in several ethnic populations. We investigated a sample of 350 unrelated Amharic individuals, which are the main population in Gondar, Ethiopia. The tests were performed for the four X-chromosomal STR loci DXS8378, HPRTB, DXS7423 and DXS7132 by using the Mentype Argus X-UL multiplex kit (Biotype, Germany) following manufactures instructions. Electrophoresis was performed on the ABI Prism 310 Genetic Analyzer (Applera, USA). Allele frequencies and statistical parameter as well as comparison with known data from Germans for the above-mentioned STRs are presented.

PP018
Population data of X-STR in Tuscany

Riccardi LN[1], Ricci U[1], Peloso G[2], Grignani P[2], Genuardi M[1]

1) Azienda Ospedaliero-Universitaria „A.Meyer“ U.O. Genetica Medica 2) Dipartimento di Medicina Legale e Sanità Pubblica, Università di Pavia, Italy

Due to their informativeness X-STRs are useful to solve selected forensic caseworks; in particular, deficiency paternity cases can be better solved using these STRs. Here, we describe population data obtained from the analysis of 5 X-STR (DXS6789, DXS6795, DXS7423, DXS8377, DXS9898) of 100 Tuscan unrelated subjects, 50 males and 50 females, to calculate allele frequencies. Data compared by Fischer test were similar in males and females and no deviations from HWE were found in females. Statistical parameters were calculated according to Desmarais et al. [1]. These data were compared with a previously analysed population sample from Pavia (Northwest Italy) [2] to observe allele frequency distribution. [1] Desmarais D. et al. J Forensic Sci 43 (1998) 1046-1049. [2] Peloso G. et al. International Congress Series 1261 (2004) 260-262.

PP019
Discrimination between human and non-human remains by species-specific DNA pyrosequencing

Karlsson AO, Holmlund G

The National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Artillerigatan 12, SE - 581 33 Linköping, Sweden

In forensic investigations it can be a problem to deduce whether a forensic sample (e. g. skeletal remain or internal organ) is of human origin or not. A number of commercial kits with human specificity are today available, but a negative result from such a kit can either mean that the sample is of non-human origin or that the analysis failed due to PCR inhibition or poor DNA template quality. To overcome this, we present a new assay for identification of human remains with the advantage of obtaining a DNA sequence for further identification of the species using a nucleotide database (GenBank, EMBL etc). The method is based on PCR amplification of two short (~100 bp) mtDNA fragments of the ribosomal genes 12S rRNA and 16S rRNA using universal primers specific for mammals. The nucleotide sequence is determined by means of the pyrosequencing technique. For validation, a number of higher mammals common in the Swedish fauna (for example human, moose, roe deer, fallow deer, red fox, dog, cow, horse among others) were tested for primer specificity and also to obtain reference sequences from species not presented in the nucleotide databases. Furthermore, a blind test of samples from different species was also performed for assay verification.

PP020
Victim Of Medical Malpractice: DNA Typing as Tool For Linking Biological Specimen and Patient

Varljen T[1, 2], Djuric M[3], Stanojevic A[1], Stojkovic O[1, 2], Savic S[1]

1) Institute of Forensic Medicine, School of Medicine, University of Belgrade; 2) International Commission on Missing Persons; 3) Security Information Agency – Institute of Security, Belgrade

An earlier study (Gephardt and Zarbo, 1996) showed that nearly 3% of the microscopic slides in surgical pathology contain extraneous or foreign tissue. This fact, along with the possibility of a pathology specimen mix-up, may create a serious problem for the pathologist. Forensic DNA typing has been suggested as an ideal system for rapid identification of clinical specimens in such cases. Here we report a STR typing based resolution of a incorrect diagnosis which followed from a clinical sample mix-up. Male patient, aged 43, has been hospitalized due to cough, high temperature, general weakness, numbs in left arm, and pains in left hemithorax. After initial bronchoscopy, and secondary histopathologic analysis of bioptic samples of bronchial tissue, he has been diagnosed with invasive planocellular carcinoma of bronchus, grade II. Upon these results, patient has undergone left pulmonectomy in another hospital. However, unexpected pathohistological findings of sarcoidosis, without any evidence of carcinoma in more than 30 samples from removed lung tissue, clearly excluded the necessity for pulmonectomy. These discrepant findings could be explained either as a rare case of complete carcinoma removal on biopsy, or as a human error, introduced during the tissue processing. In order to discern between these two possibilities, both preoperative and postoperative samples, along with reference blood samples from the patient, were submitted to the Institute of Forensic Medicine. Pathohistologic analysis confirmed both the preoperative diagnosis of bronchial planocellular carcinoma and the postoperative diagnosis of sarcoidosis. Also, no microscopic evidence of contamination could be seen on any of the glass slides. STR typing was performed on all samples, and a complete match was observed between the reference sample and postoperative samples. Moreover, in DNA analyses on preoperative samples, a mismatching DNA profile was obtained, supporting the scenario of specimen mix-up.

PP021
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

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 roots. Here we describe a protocol for simultaneously amplifying of 8 STRs in 3 triplex amplification reactions. DNA has been purified from single hairs by combining a modified digestion procedure with the BioRobot automat (Qiagen). For digestion and concentration Micro kit/DNA Tissue

kit from Qiagen and Microcon 30 have been used correspondingly. The primers were designed to produce amplicons of 50-150 bp. SE33 has been excluded from triplexes due to its fragment size of 200 bp and more. The three triplexes designated MPX1, MPX2 and MPX3 consisted of the STRs TPOX/THO1/D18S51, FGA/VWA/D3S1358 and Amelogenin/D8S1179/D21S11 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 multiple approach for single hair DNA analysis. Also, we give examples, showing the necessity to perform STR typing of short DNA fragments in special clean area apart from routine laboratory and discuss problems connected with this case of low copy number (LCN) PCR amplification. Finally, we show that our approach is suitable for other stain analysis, particularly if degraded DNA of low amounts is to be expected.

PP022
Quantitation of latent DNA traces and correlation with DNA typing: preliminary results of a large casework study

Reuss E, Zehner R, Kauferstein S, Bratzke H

Zentrum der Rechtsmedizin, Universität Frankfurt am Main, Germany

Over the past years more and more swabs containing unknown traces of biological material are submitted for forensic DNA analysis. To handle this increasing amount of samples it became necessary to establish reliable screening methods for promising DNA typing results. Moreover for the police it becomes essential to select which samples are worth being collected at the site of crime in order to achieve most beneficial DNA evidence. To analyse and optimise collection of DNA evidence, we are performing a field study in collaboration with the Frankfurt police departement since the beginning of this year. Aim of this study is to find out which sample type (slight contact, handling of an item, body fluids etc.) leads to most significant typing results. The field study comprises burglary and theft. Most of the swabs were taken from handled items such as tools or exhibit contact stains. As a screening method we applied DNA quantitation using the TaqMan technology and the ABI human DNA Quantifiler kit. Meanwhile the study comprises 1700 swabs for which sample type, DNA amount and typing results were correlated. Statistical analysis of these results will be presented and discussed.

PP023
Quantitative Determination of human tumor load in murine lungs with Real-time PCR using amelogenin primers

Stübke K[1], Augustin C[1], Püschel K[1], Schumacher U[2], Thies A[2]

1) Institute of Legal Medicine 2) Institute of Anatomy II: Experimental Morphology both: University Hospital Hamburg-Eppendorf, Germany

Malignant melanoma is a very aggressive tumor as malignant cells very early separate from the primary tumor, degrade the extracellular matrix and invade blood and/or lymphatic vessels. This is followed by adhesion to the endothelial cells and extravasation to form clinically detectable distant metastases. To develop new rational strategies for melanoma treatment the mechanisms underlying metastatic spread have to be understood. Human melanoma cell/ scid mouse xenograft models have been developed to analyze metastatic behavior. At the moment the number of lung metastases is assessed microscopically in H&E stained serial lung sections. The aim of the present study was to establish a method to replace the time-consuming and laborious cell-counting method by a DNA-quantitation approach. Therefore, a Real-time-PCR method using SYBR Green technology was developed. After the DNA extraction the metastatic load in the lung tissue sections was investigated by quantitative PCR using forensically established primers specific for the XY-chromosomal amelogenin locus and for the Y-chromosomal DYS391 locus. A close correlation between the metastatic rate counted in H&E sections and the DNA amount measured by real-time PCR could be shown. This correlation was proved in 67 sections of murine lungs with a tumor cell load ranging from 1 to about 6000 cells. The Real-time PCR assay developed here shows high sensitivity for single melanoma cells and additionally a highly discriminative power for the assessment of the specific tumor load. This method presents a tool for future quantitative assessment of lung metastases in animal models aiming at therapeutic strategies.

PP024
Who is my grandfather? - An illicit love affair in Nazi Germany

Milde-Kellers A[1], Schuchardt S[2], Krawczak M[3], Boomgaarden-Brandes K[1], Simeoni E[4], Kaatsch HJ[4], Mühlbauer B[1]

1) Institut für Pharmakologie, Labor für forensische Analytik, St. Jürgen Str. 1, 28177 Bremen 2) Institut für Pathologie, Campus Kiel, Niemannsweg 11, 24105 Kiel 3) Institut für Medizinische Informatik und Statistik, Campus Kiel, Brunswiker Str. 10, 24105 Kiel 4) Institut für Rechtsmedizin, Campus Kiel, Arnold-Heller-Str. 12, 24105 Kiel

Objective: We were recently commissioned to affiliate paternity in a complex case of kinship analysis 63 years after an illicit love affair had taken place in Nazi Germany. Setting: E.H. was married to a Wehrmacht soldier who was serving on the Eastern front. The couple had two sons. While her husband was doing his service E.H. had a love affair with a Polish slave worker. She became pregnant while her husband was on leave. Both of the men had to be considered for parentage. After the affair was made public E.H. was imprisoned at the concentration camp Ravensbrück in early 1943 and presumably the Polish slave worker was sentenced to death. In September 1943 E.H.`s daughter was born, handed over and fostered by the maternal grandparents. E.H. died in Ravensbrück in 1944 at the age of 29. E.H.`s husband survived the World War II, remarried and became father of an additional son. E.H.`s daughter was brought up in his family and in 1965 she became mother of a son. E.H.`s daughter and grandson requested our Institute to resolve their kinship. Material/Method: No biological material was available from E.H., her husband and the Polish slave worker. For DNA analysis blood samples were provided by one of E.H.`s son, E.H.`s daughter, the daughter`s husband and their son, the second wife and this wife`s son. This deficiency case could finally be solved by means of DNA typing using a total of 16 STR loci and formal pedigree analysis. Two competing hyotheses were setup and calculated with references to a database comprised of individuals in the North German population using the computer program LINKEAGE. Results: Based on our results the following statement can be made: the Polish slave worker is 19 times more likely to be the father of E.H.`s daughter than her husband.

PP025
Single-cell-picking directly from material evidence with the help of the „Lumar.V12“ stereomicroscope from Zeiss and the aura optik work platform

Verhoff MA, Brück S, Pipper V, Evers H, Weiler G, Heidorn F

Department of Legal Medicine, University of Gießen, Germany

When preparing DNA from material evidence with little cellular material or with mixed samples from more than one individual, the isolation of single cells, for example epithelial cells, can be of advantage. So far in forensic DNA analysis, cells for lysis and the preparation of DNA could only be obtained with the aid of swabs taken from surfaces or destruction of material evidence. Smears had to made first to obtain single cells. A direct isolation of single cells from the surface of material evidence was restricted by the optical resolution of stereomicroscopes. With the aid of the high-resolution stereomicroscope „Lumar.V12“ from Zeiss and a work platform from „aura optik“ (Jena) it was possible to detect and isolate single epithelial cells on various surfaces (glass, plastic, metal and paper) and to afterwards transfer them to a reaction tube for DNA analysis.

PP026
Effects of reduced reaction volumes on DNA amplification

Schmidt U, Heinrich M, Lutz-Bonengel S

Institute of Legal Medicine, University Hospital of Freiburg, Freiburg, Germany

As has been previously stated, reduction of the amplification volume is a possible way to enhance sensitivity and efficiency of the PCR reaction. Multiplex DNA amplification in 1-µL volumes on chemically structured chips yields strongly increased signal intensities at capillary electrophoresis compared to conventional in-tube reaction. In this study, several experiments were performed to observe the effects of reduced reaction volumes on the DNA amplification. Reaction volumes of 25, 20, 15, 10, 7, 5, 4, 3, 2, and 1 µL were tested using identical amounts of starting template. Multiplex STR amplification as well as real time PCR analysis was carried out. Results indicated higher yields of PCR product per µL in reduced reaction volumes, but signal intensities showed greater variation compared to the regular 25-µL amplification. Real time PCR analysis resulted in lower Ct values in reduced reaction volumes suggesting better amplification efficiency. Implications of these observations for forensic DNA analysis will be discussed.

PP027
Evaluation of fourteen Short Tandem Repeat marker for forensic applications

Becker D[1], Götz F[2], Bender K[3], Edelmann J[4], Henke L[5], Hering S[6], Hohoff C[7], Klintschar M[8], Much M[9], Rolf B[10], Szibor R[11], Weirich V[12], Brabetz W[1], Jung M[1]

1) Biotype AG, D-01109 Dresden 2) Qualitype AG, D-01109 Dresden 3) Inst. Legal Med., Uni. Mainz, D-55131 Mainz 4) Inst. Legal Med., Uni. Leipzig, D-04103 Leipzig 5) Inst. for blood group serology, D-50672 Köln 6) Inst. Legal Med., Uni. Dresden, D-01307 Dresden 7) Inst. Legal Med., Uni. Münster, D-48149 Münster 8) Inst. Legal Med., Uni. Halle, D-06112 Halle 9) Inst. for blood group serology and genetics, D-22081 Hamburg 10) Inst. Legal Med., Uni. München, D-80337 München 11) Inst. Legal Med., Uni. Magdeburg, D-39120 Magdeburg 12) Inst. Legal Med., Uni. Rostock, D-18055 Rostock

In this study 14 short tandem repeats (STRs) were evaluated for forensic applications as well as for paternity testing. The STR systems comprise of 8 common STR loci (D3S1744, D12S391, D2S1360, D7S1517, D8S1132, D5S2500, D18S51, D10S2325) and 6 recently new described loci (D4S2366, D6S474, D19S246, D20S480, D21S226, D22S689) [1] mostly including non CODIS loci. The markers were chosen with regard to their high discrimination power especially for related individuals and low stutter artefacts during PCR amplification. DNA samples from trios (consisting of parents and one child) from different regions in Germany were included in this study. New alleles, allele frequencies and primer site mutations were analyzed and population statistics were calculated. PD values for the different STR loci ranged from 0.76 for D21S226 to 0.97 for D12S391. In a total of 488 meioses 16 isolated exclusions were observed at 8 different loci and were confirmed by sequencing. Thereby, 12 of these mutations were proven to be caused by repeat gain or loss. In the other cases genotyping discrepancies were due to primer binding site mutations. The combination of the 14 STR loci is a suitable tool for difficult forensic applications and kinship analyses and recommended in combination with commercially available kits. [1] D. Becker, D. Vogelsang, W. Brabetz, Population data on the seven short tandem repeat loci D4S2366, D6S474, D14S608, D19S246, D20S480, D21S226 and D22S689 in a German population. Int. J. Legal. Med. (2006), in press

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PP028
Evaluation of slab gel versus capillary electrophoresis and POP4 versus POP6, for mitochondrial DNA sequencing

Desmyter S, Hoste B

NICC-Brussels

For more than five years our lab has gained experience in mitochondrial DNA sequencing for forensic purposes. Recently the method was upgraded in different ways. A major change was the switch from slab gel to capillary electrophoresis. In the scope of this modification the type of polymer also had to be considered. POP6 is recommended by the manufacturer as a ‘sequencing specific’ polymer, but in our lab POP4 was already in use for forensic STR-analysis of nuclear DNA. So it was interesting for us to compare sequencing results from ABI377-LR5% not only with those from ABI3100-POP6 but also with those from ABI3100-POP4. The method was validated for DNA-extracts from buccal scrapes and from liquid blood samples. A selection was made from samples that had been analysed before with the former method. Samples with specific sequencing problems in the C-stretch of HV1 and HV2 were included. For each sample the complete control region was amplified, and this amplicon was used as template for the sequencing of HV1 and HV2 in four different reactions. The purified sequence reactions were split up at the final stage and loaded on ABI377-LR5%, ABI3100-POP4 and ABI3100-POP6. Sequencing results from ABI377 and ABI3100 were analysed with Sequence Navigator v1.0.1 and SeqScape v2.5, respectively. The validation program treated different criteria like repeatability, reproducibility, detection limit and mixed base detection. The sequencing results were evaluated in terms of correct profile and length. The latter is split up in a double-stranded and a single-stranded sequencing part. The upgraded method was considered to be suitable for mitochondrial DNA sequencing of HV1 and HV2, with all three electrophoresis types tested. Nevertheless, the sequencing protocol had to be extended with additional primers to obtain a full double-stranded profile of samples with specific sequencing problems in the C-stretch of HV1 and HV2. The major differences between the electrophoresis types tested were observed in the detection limit and the automatic mixed base detection. But also primer to sequencing start-distance and the number of mixed bases in the single stranded lecture zone are method dependent. The work reveals that besides the evident advantages of capillary to slab gel electrophoresis its detection limit is better. Considering both POP6 and POP4, a better analysis of diluted samples was observed with the ‘sequencing specific’ polymer POP6. This advantage is not crucial in the polymer choice for the analysis of reference samples, which contain sufficient amounts of DNA, but can be very important for the analysis of samples with lesser amount of DNA (hair or bone samples). The far more accurate automatic detection of mixed bases for capillary electrophoresis compared to slab gel electrophoresis is dependent on the resequencing program used. The expected mixed base positions that we were not able to identify using Sequence Navigator after slab gel electrophoresis were clearly identifiable by visual control of the corresponding electropherograms. In this study POP4 and POP6 were evaluated to be both suitable for mitochondrial DNA sequencing of reference samples, with a preference for the latter.

PP029
May CNBR1 and COMT gene patterns allow to predict individual sensitivity to cannabinoids ?

Castella V[1], Augsburger M[2], Favrat B[3], Mangin P[1, 2,3], Giroud C[2]

1) Laboratoire de génétique forensique 2) Laboratoire de toxicologie et chimie forensiques 3) Unité de médecine du trafic institut universitaire de médecine légale, rue du Bugnon 21, 1005 Lausanne, Switzerland

During a study designed to evaluate the effects of oral administration of cannabinoids on the ability to drive, 2 out of 8 volunteers over-reacted to medium doses of D9-Tetrahydrocannabinol (THC) by developing transient psychotic symptoms. Their blood levels of psychoactive substance were similar to those observed in the other 6 volunteers during the experiment and they had no psychiatric history. Genetic tests were therefore undertaken to check whether particular gene patterns at the cannabinoid receptor type 1 (CNBR1) and/or at the Catechol-O-Methyltransferase (COMT) may explain such individual difference in cannabinoids sensitivity. These two genes are thought to play roles in drug reward, drug dependence and are associated with several mental disorders. A 3-SNP haplotype located in CNBR1 intron 2 and a point mutation located in COMT codon 158 were investigated with TaqMan and RFLP technologies, respectively. Genetic polymorphism was detected at these sites, but no apparent association was found between genotypes and THC sensitivity. Work remains to be done in order to identify relevant genes and to understand the genetic (and environmental) mechanisms underlying individual cannabinoids response.

PP030
Population data of 10 X-STRs in a Spanish population sample

Aler M[1], Sánchez-Diz P[2], Gomes I[2,3], Gisbert M[1], Carracedo A[2], Amorim A[3, 4], Gusmao L[3]

1) Laboratorio de Genética Forense U.D. Medicina Legal, Universidad de Valencia E.G., Valencia,Spain 2) Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain 3) IPATIMUP, Institute of Pathology and Molecular Immunology of the University of Porto, Porto, Portugal 4) Faculty of Sciences of the University of Porto, Porto, Portugal

The use of X-chromosomal STRs in forensic science powerfully complements the analysis of other genetic markers. In particular, they are very helpful for solving deficiency cases of parentage investigations and cases involving blood relatives. In spite of this, available population data from these markers are scarce compared with those on autosomes and Y-chromosome. In this work we presented population genetic data of 10 X-STRs (DXS8378, DXS9898, DXS8377, HPRTB, GATA172D05, DXS7423, DXS6809, DXS7132, DXS101 and DXS6789) obtained from a sample of 145 unrelated female individuals belonging to Valencia (Spain), a region located in the east of the Iberian Peninsula. All the markers studied present high genetic diversities, similar to those previously reported in other European population samples. No significant associations between alleles of any pair of loci were found in this population. No deviations from Hardy-Weinberg equilibrium were observed with the exception of the DXS101 locus. Allele frequencies and parameters of forensic interest for each X-STR were calculated and will be reported. Population comparisons (exact test of population differentiation; pairwise genetic distances) were carried out and no significant differences were observed between our data and those previously published for other Spanish regions.

PP031
Three X-Chromosome STR Loci DXS7133, DXS7424 and DXS 8378 Frequency Data from a Brazilian Population.

Tavares CC[1], Gusmão L[2], Silva DA[3, 4], Carvalho EF[3]

1) Departamento de Biologia Celular e Genética, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brasil 2) IPATIMUP, Instituto de Patologia e Imunologia da Universidade do Porto, Porto, Portugal 3) Laboratório de Diagnósticos por DNA, Departamento de Ensino de Ciências e Biologia, Instituto de Biologia, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brasil 4) Departamento de Patologia e Laboratórios, Faculdade de Ciências M

X-chromosome STRs markers have been used in supplementation to autosomal STRs markers in forensic analysis to stablish kinship, specially in cases of deficiency of paternity when females are under investigation. Men transmit to his daughters one X-chromosome they received from their mothers. Paternal grandmother and uncles are considered in kinship determination as well as sisters and half-sisters who share the X-chromosome haplotypes with females under investigation. X-STRs tend to present a mean exclusion chance higher than that of autosomal STRs. This work presents X-STR allele frequencies using a PCR approach with a triplex including DXS7133, DXS7424 and DXS8378. Genomic DNA was extracted from blood samples of unrelated Brazilian individuals (male and female). It was confirmed that DXS7424 is a polymorphic marker which is one of the most

informative studied till now. This marker presents a linkage disequilibrium compared to DXS7133. These two markers are in close association in the Brazilian population. Hence, alleles from both markers ought to be seen as haplotypes segregating together, and a frequency for the pair should be considered in such special situation. Although the variation of alleles showed to be distinct in males and females concerning the three markers, the prevalent alleles are the same in both sexes.

PP032
Forensic use of canine mtDNA – how many differences between individuals of one breed could be found?

Poetsch M, Bockholdt B

Institute of Forensic Medicine, University of Greifswald

Domestic dogs are often involved in forensic case work, for example as attacker, as cause for a car accident or as a link between a suspect and the scene of the crime. Mostly, only dog hairs could be retrieved resulting in the need for an mtDNA analysis. A variety of canine mtDNA sequences of the D-loop have been published, but only few data exists on frequent German dog breeds like German shepherd, Labrador, or Golden Retriever. Here, we present sequencing data of nt15470 to nt16109 of the canine mtDNA control region from 19 female and 17 male individuals, belonging to these three breeds, in comparison to published data and own results from other dog breeds (21 individuals). These preliminary data revealed 33 polymorphisms in comparison to the first complete mtDNA dog sequence published by Kim et al. (1998; GeneBank accession number: NC_002008) and 23 different haplotypes.

PP033
Genetic Analysis of Autosomic STR-PCR Polymorphisms of the mestizo population from Nicaragua (Central America)

Sánchez D[1], González-Andrade F[1], Martínez-Jarreta B[2], Gascón S[2]

1) GENética, Laboratorios de ADN, Hospital Metropolitano, Quito (Ecuador) 2) Legal Medicine Department, University of Zaragoza

Allele frequency data for the 15 STR systems, Amelogenine and 12 STRs from chromosome Y were determined in a population sample of healthy Mestizo individuals from Nicaragua. All loci met Hardy-Weinberg expectations and the high discrimination power of the combined system showed the forensic efficiency of these genetic markers. Population Sample: whole blood was obtained in EDTA vacutainers tubes by venipuncture from healthy unrelated mestizo born and living in Managua (Nicaragua). DNA extraction and Quatinfication: DNA was extracted using Wizard Genomic DNA Purification Kit System© (Promega Corporation, Madison WI, USA), and the quantity was estimated by UV absorbance (Gene Quant Calculator, Pharmacia, Uppsala, Sweden). PCR: amplification was performed in a Perkin Elmer Thermal Cycler, model 9600 following the manufacturer's recommendations. Typing: by ABI Prism 310. Fragment size and allele designation of different loci was determinated by comparation with allellic ladders provided by Promega with the kit PowerPlex 16. The recommendations of the DNA Comission of the International Society of Forensic Genetics for analysis of STRs systems were followed. Analyses of data: evaluation of Hardy-Weinberg expectations and determination of statistical parameters of forensic interest were carried out by using the computer programme HWE-analysis version 3.3, as previously described.

PP034
Genetic analysis of different soft tissues from decomposed bodies

Schwark T, Stein K, Mályusz V, Simeoni E, von Wurmb-Schwark N

Deptartment of Legal Medicine, University Hospital Schleswig-Holstein

The identification of putrefied bodies is a common task in forensic routine work. The deceased are usually identified by dental records, fingerprinting, or – in cases were no such data are available – DNA analysis. However, with progressive putrefaction, DNA integrity is rapidly decreasing. DNA fingerprinting may then be greatly impaired, if not impossible. The aim of our study was to establish an efficient procedure to successfully extract and amplify DNA from soft tissues of bodies in different stages of putrefaction. Soft tissues – unlike teeth or bones – usually allow the application of fast and easy to use extraction protocols. Approximately 500 mg of aorta, kidney, liver and skeletal muscle were taken during forensic autopsies of 16 different putrefied bodies. DNA was extracted using two different commercially available DNA extraction kits, and DNA quality and quantity was controlled by agarose gel electrophoresis and real time PCR, respectively. Presence of mitochondrial DNA was tested using a highly sensitive duplex PCR. STR analysis was done using the AmpFlSTR Identifiler kit. Additionally, mtDNA sequencing was performed. STR typing of all 16 bodies was possible after DNA extraction from at least two different tissues – preferably kidney and aorta – with an extraction kit based on the nucleobond method. The results in detail will be presented.

PP035
Genetic structure of the Rimini area (North of the Italian Peninsula) in the context of the historical background

Bini C, Luiselli D, Ceccardi S, Lugaresi F, Lombardini L, Falconi M, Pelotti S

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

Variation in the sequence of mtDNA and Y chromosome analysis are used to reveal the maternal history and the paternal legacy of human populations and to clarify by phylogenetic analysis the gene flow during prehistory. In Europeans there has been evidence for population homogeneity, however different frequencies of mtDNA types were shown at local level. Besides mtDNA for its maternal inheritance and the high evolutionary rate is useful for forensic studies that however require high quality mtDNA database. In the present study mitochondrial DNA was analyzed for the control region in an Italian population sample from Rimini, an ancient port in the North of Italy during Roman age, famous for amber exchange in the Mediterranean basin. In 268 B.C. at the mouth of the Ariminus river, in an area that had previously been inhabited by the Etruscans, the Umbrians, the Greeks and the Gauls, the Romans founded the colony of Ariminum. Rimini was a road junction with connections to central (Via Flaminia) and northern Italy (Via Emilia and Via Popilia) and it also opened up trade by sea and river. This population sample was previously studied for Y chromosome polymorphism by STRs and SNPs analysis showing 92 different haplotypes and 10 haplogroups also founding a rare cluster β of E3b1 haplogroup in two unrelated individuals. Samples from 98 unrelated individuals from the urban area of Rimini with known ancestry were analyzed for HVI and HVII sequences following the guidelines for mitochondrial DNA typing of the International Society for Forensic Genetics and performing phylogenetic analyses that reconstructs ancestral sequences. The aims of this study were: to identify the mitochondrial composition of the Rimini population, to compare its maternal and paternal lineages and finally to investigate the influence of the particular historical background on the genetic structure of this area.

PP036
Haploid markers in Finnish forensic casework

Hedman M, Palo JU, Sajantila A

Laboratory of Forensic Biology, Department of Forensic Medicine, P.O.Box 40, 00014 University of Helsinki, Finland

Forensic DNA investigations within Finland are centralized in three laboratories – Crime Laboratory (National Bureau of Investigation), Paternity Laboratory (National Public Health Institute) and Laboratory of Forensic Biology (University of Helsinki, Department of Forensic Medicine). Y-chromosome markers are in use or under validation in all of the three laboratories, mtDNA HVS-analysis is in use only in the Laboratory of Forensic Biology. X-chromosomal markers are not yet in use in any of the laboratories. In Laboratory of Forensic Biology mtDNA-analysis has been used in forensic casework since 1998 and Y-chromosomal markers since 2000. Y-chromosomal markers have limited value in Finnish forensic casework compared to other European countries due to extreme drift, which have reduced variation within Finland, especially in Eastern part of the country. A found Y-

chromosomal haplotype in a sample reveals a male contributor, but identification based on solely on Y-chromosomal information, especially in case of common haplotypes, is doubtful. However, Y-chromosome markers have been in use for genealogy studies to reveal several centuries ago lived common forefathers. MtDNA HVS-analysis has not shown such a drastic reduction of variation within Finland compared to Y-chromosome. MtDNA sequencing has been used with high success to identify remains of WW II soldiers. In these identification cases it has been possible to trace a living relative (even very distant) of the deceased solder, who has been sharing the same mtDNA lineage and identification could have been completed. In refugee family reunion cases autosomal STR markers are sometimes insufficient, especially in cases with only one parent or when siblings only are tested. Y-chromosomal and mtDNA-analysis have added a valuable new information about common maternal or paternal lineages of two subjects.

PP037
Heterogeneous mt-DNA haplogroup distribution in immigration countries - call for local database feasibilities for forensic purposes: the EMPOP efforts

Sala A, Marino M[1], Brandstätter A[2], Bobillo MC[1], Parson W[2], Corach D[1]

1) Servicio de Huellas Digitales Genéticas, Cátedra de Genética and Biología Molecular, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina 2) Institute of Legal Medicine, Innsbruck Medical University, Austria.

The American continent is a good example of immigration territory. It received three major ethnic contributions. The first immigrants came from Asia, determining the peopling of the continent; this process took place between 18-20 Ky ago. The immigrants widespread from Alaska to Tierra del Fuego along 8-10 ky ago. During this period peculiar traits were developed and some of them fixed in the immigrant populations. The second major immigrant wave come from Europe and was produced only 0.5ky ago and its effects were dramatic on the aboriginal Asia-rooted „natives“. Although, a massive reduction of the aboriginal population was produced a big deal of admixture took place. Accordingly, an important genetic contribution provided by the aboriginal remained. The third component was brought as working force from Africa during XVII through XIX centuries. Directional mating determined that aboriginal female genetic contribution widespread and admixed with European male immigrants. Accordingly, the resulting extant populations are far from being genetically homogeneous, although some countries like Argentina and Uruguay claim to be of almost „pure“ European ancestry. By typing Amerindian-specific Y chromosome haplogroups it became apparent that about 17% of the population have aboriginal patrilineage ancestry. In contrast, when analyzing mtDNA D-Loop sequences it was demonstrated that over 60% of the randomly sampled individuals depicted Amerindian-specific haplogroups (hgs; A, B, C and D). Meanwhile, less than 40% denoted non-Amerindian mtDNA hgs, within this subset, the most frequent were H (12,9 %), U (6.3%), T (2.8%) and only 1.4% exhibited hg L of possible African ancestry. The overall heterogeneous hg-distribution represents a serious challenge for estimating mtDNA haplotype frequencies required for forensic purposes. The pretended use of foreign mtDNA references database may lead to serious frequency estimation bias and determine erroneous conclusions. The continuous efforts made by the EDNAP Mitochondrial Population Database (EMPOP) to improve and optimize the quality of the accepted sequences might warrant a reliable tool for frequency estimation and haplogroup determination. Due to the restriction imposed by the heterogeneity of the haplogroup frequency distribution and hence the overall haplotype diversity in the different world regions, the opportunity to create local reference databases hosted in a worldwide major reference facility as EMPOP, will strongly contribute with the forensic scientist all over the world.

PP038
Identification of biological samples in the case of potential contamination of a cytological slide preparation

Junge A, Dettmeyer R, Madea B

Institute of Legal Medicine der Rheinischen Friedrich-Wilhelms-Universität, University Bonn, Stiftsplatz 12, 53111 Bonn, Germany

Several reports have been published on the identification of mixed up histological slides. In the case of potential contamination of a cytological slide preparation DNA analysis with STR (short tandem repeat) systems may also be helpful for correct identification and assignment of the material to a patient. On behalf of a pathological institute we carried out STR analysis on a cytological slide preparation of a healthy female person and on pleura liquid of a female tumour patient who was obviously laid up with an ovarian carcinoma. Microscopic examination of the slide preparation revealed that a discreet section of the preparation was obviously contaminated with tumour cells. The remaining area contained only epithelium cells of the portio cervicis and endocervix of the healthy woman. Two samples were prepared from the cytological slide preparation, one from the section containing the mixture of tumour and healthy cells and the other from the remaining area containing only healthy cells. DNA extraction was performed with the All-tissue DNA kit (Genial) for the pleura liquid and with Chelex100 for the slide material.

Amplification was carried out with the MPX2-kit (SERAC) according to the manufactures instructions. Analysis of the PCR products was done on the capillary electrophoresis CE310 using the GeneScan and Genotyper software. Analysis of the electropherograms showed reproducible results for all samples. The section of the healthy cells and the tumour cells of the pleura liquid showed DNA profiles which came from two different female persons. The DNA profile of the cell mixture could be assigned both to the tumour and healthy patient. On account of the application of STR analysis the potential contamination of a cytological slide preparation was clearly verified and the assumption of the pathologist, that a little drop of the pleura liquid caused the contamination during the staining process of the slide, was confirmed.

PP039
Length heteroplasmy in HVII homopolymeric region of mtDNA leads to ‘different’ haplotypes in different tissues of the same body

Borovko SR

State Medical Forensic Service of the Republic of Belarus, Minsk, Belarus

We faced an unexpected problem when working with a murder case. The dead woman’s body identification with her sister’s blood sample after HVI and HVII regions of mitochondrial DNA sequencing leads to questionable result. At the same time nuclear DNA results seemed to prove the relationship. Nuclear DNA analyses were performed with AmpFlSTR Identifiler Kit, mitochondrial DNA – with BigDye Terminator v.3.1 Kit from Applied Biosystems on ABI Prizm 3100 Genetic Analyzer. Woman M killed woman B by hitting her on the head by an axe after drinking alcohol together in woman’s B house in the village P. The dead body and the axe were found on crime scene probably two months after the accident, the body was in a stage of decomposition and could not be identified without DNA analysis. Muscle and bone fragments as well as blood samples from alleged victim’s sister were delivered to the laboratory. Blood which was found on the axe matched body according to nuclear DNA typing, but identification issue was still unresolved after comparison of HVI and HVII sequences of mtDNA that was isolated from victim’s muscle and sister’s blood sample. Only one HVII 309.2C insertion observed in reference blood sample and not found in muscle differed the body from the sister. Law enforcement authorities were asked to find other relatives of woman B. Blood sample of the victim’s daughter was delivered for investigation. An additional study of nuclear and mitochondrial DNA isolated from muscle, bone, daughter’s blood sample was performed. It revealed HVI 16224C; 16311C, HVII 73G; 146C; 263G; 309.1C; 309.2C; 315.1C in the sister’s, daughter’s blood and victim’s bone and again, in difference, HVII 309.1C in muscle. Identifiler 15 loci profiling proved maternity, LR=357142. After careful examination of both strands of HVII region of mitochondrial DNA isolated from the sister’s, daughter’s blood and victim’s bone we came to the conclusion that there were signs of length heteroplasmy in position 309.2C (309.2C>309.1C) mostly noticeable in the bone and sister’s sequences and not so clear in the daughter’s sequence. The HVII sequence of mtDNA isolated from the victim’s muscle looked like ‘normal’ 309.1C without significant signs of expected 309.2C. So mitochondria with the different in length mtDNA haplotypes: HVII 309.1C; 309.2C and 309.1C were inherited by the victim, her sister from their mother; victim in her turn ‘gave’ such heteroplasmic cocktail to her daughter. But the victim herself, in muscle tissue, had 309.1C mtDNA haplotype. Thus it can be concluded that relationship and maternity was finally proven, we have got our own illustration of different mtDNA types in different tissues of the same individual.

PP040
Looking for the identity of missing-in-action soldiers during the first world war along the italian front. A case report

Piccinini A[1], Lovisolo A[1], Coco S[1], Cattaneo C[2], Galassi A[3], Barbazza R[4], Parson W[5]

1) Forensic Genetics Lab., Institute of Legal Medicine, University of Milan, Italy 2) Forensic Anthropology and Odontology Lab., Institute of Legal Medicine, University of Milan, Italy 3) UOS Necroscopic Medicine and Forensic Anatomical Pathology ULSS 6 Vicenza, Italy 4) UOA Anatomical and Histological Pathology Service, ULSS 2 Feltre, Italy 5) Institute of Legal Medicine, Innsbruck Medical University, Austria

We here report the results of the genetic identification of remains supposed to be those of an Italian famous soldier, a hero of World War One who was killed in battle along the Italian front. Offspring of both maternal and paternal lineage were available for testing so that Y-STRs and MtDNA were analysed. No match was found either after MtDNA nor Y-STRs analysis. This is the first effort of identification of the remains of soldiers who perished during the First World War under the aegis of a multidisciplinary project aimed at the retrieval of the historical and cultural heritage linked to World War One, and to the systematic anthropological and genetic study of the remains of soldiers and ultimately their identification; this last steps involving Italian and Austrian laboratories.

PP041
Mentype® NonaplexQS Stain: One-Tube Mini Multiplex PCR for the Amplification of Amelogenin and 8 STR markers of the German DNA Database (DAD)

Forbriger R, Kalusniak J, Brabetz W

Biotype AG, Moritzburger Weg 67, D-01109 Dresden, Germany

Multiplex polymerase chain reaction (PCR) based DNA typing of polymorphic short tandem repeats (STRs) is the current standard in human identification. The analysis of forensic stains is often hampered by PCR inhibitors and the occurrence of low quantities of highly degraded DNA. Thus, the amplicon size should be kept as small as possible to optimize PCR performance and gain of complete DNA profiles. In addition, a high degree of multiplexing is desirable to save sample material for further investigations. Using capillary electrophoresis, the capability to set up mini STR multiplex PCRs is limited by the allelic range of the loci and the number of fluorescent dyes which can be analyzed in parallel. To overcome this drawback we have developed a new STR typing strategy which allows after multiplex amplification the differential analysis of loci with overlapping allelic ranges and the same fluorescent dye. For this purpose, the labelled primers of two overlapping mini multiplexes were tailed at their 5'-ends with sequences encoding recognition sites for two different restriction endonucleases, respectively, and combined into one large multiplex PCR. After amplification, the reaction was split into two separate tubes and subjected to differential restriction digests to cleave off the labels from STR loci with overlapping allelic ranges and the same colour prior analysis. The approach was used to design the multiplex PCR Mentype® Argus-SQS for simultaneous amplification of an InDel for gender determination in the amelogenin locus and the 8 polymorphic STRs D3S1358, TH01, SE33 (ACTBP2), vWA, FGA, D18S51, D8S1179, D21S11 with reduced amplicon sizes. The sensitive dyes 6-FAM and HEX were applied in the test system and it was validated for forensic stain analysis with sequencing automates from Applied Biosystems.

PP042
Mitochondrial DNA control region analysis and cancer genotyping.

Bini C[1], Ceccardi S[1], Lugaresi F[1], Raspanti ME[1], Micheletti S[1], Ferri G[2], Alù M[2], Luiselli D[3], Pelotti S[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
3) Department of Biology, University of Bologna, Bologna, Italy;

Mitochondrial DNA (mtDNA) is being widely analyzed in order to investigate its potential role as an active marker of tumorigenesis in various types of cancer. Mitochondrial DNA instability in different types of human cancers was observed, in particular in the hypervariable regions of mt D-loop. Since the D-loop regulates the transcription and replication of mtDNA, alterations in this region might modify the rate of mtDNA replication. In cancerous tissues genetic alterations, including loss of heterozygosity (LOH) and microsatellite instability (MSI), have been reported also for short tandem repeats used in forensic analysis. Recently a critical reassessment of the role of mtDNA in tumorigenesis was presented as systematic errors in mtDNA sequence are often found in the anthropological, forensic and clinical investigations. The medical field seems to be most strongly affected by missequencing and misdocumentation, for instance, the majority of mutations, found to be unstable in tumours in some studies, are instead common polymorphisms in human populations. To provide further data especially for forensic applications, we directed our study towards the analysis of the mitochondrial DNA control region in a panel of 50 gastrointestinal sporadic cancers with their corresponding normal tissues, already analyzed for 15 STRs used for forensic identification and of these showing MSI and LOH. A phylogenetic approach was performed to assess the accuracy of mtDNA data. The correlation between mtDNA instability and sex, tumor staging, STRs instability was evaluated. As observed in previous clinical genetics studies, we found the majority of the alterations in the poly-C tract of HVII control region. Since different amounts of heteroplasmic length variants cannot be used alone to support an interpretation of exclusion, the expert must take into account this fact when the reference sample is a cancerous tissue which may also show STRs alterations.

PP043
Mitochondrial DNA control region polymorphism in the population of Alagoas, northeastern of Brazil

Barbosa ABG[1], Da Silva LAF[2], Azevedo DA[1], Balbino V[3], Mauricio-da-Silva L[3]

1/3) Department of Genetics, Federal University of Pernambuco,BR 2) Forensic DNA Laboratory, Federal University of Alagoas,BR

The analysis of human mitochondrial DNA (mtDNA) sequences has become a useful tool in forensic genetics due to its special features like haploid maternal inheritance, lack of recombination and high copy number per cell. The objective of this work was to determine the polymorphism of hypervariable regions 1 and 2 of the human mtDNA in the population of Alagoas, to form a referencial forensic database for the northeastern region of Brazil. The sequences of the two hypervariable segments of the mitochondrial DNA (mtDNA) control region were generated for 167 unrelated individuals from this population. DNA sequences of the PCR amplicons were determined from both forward and reverse sequence data using the primers described by Imaizumi et al. (2002). The mtDNAs were classified into the haplogroups based on the HV1/2 motifs of haplogroup-specific sequences previously described to detect erroneous haplotypes by phylogenetic methods. Length heteroplasmy in the c-stretch HV1/HV2 regions was observed in 22% (37/167) and 11% (19/167) of the samples, respectively. Of the total of 123 individuals, 110 different haplotypes were found as determined by 128 variable positions. A total of 13 haplotypes (11%) occurred in at least two individuals. The most frequent haplotype in this study, defined by 16111, 16223, 16290, 16319, 16362, 73, 146, 153, 235, 263, 309.1C, 315.1C (haplogroup A), could be found in 5 individuals (4%) followed by 263, 315.1C (haplogroup H) found in 3 individuals (2%). One haplotype was shared by 2 individuals in seven different occurrences (frequency of 1.6%) belonging to haplogroups C, L1c, L3e1, H and U. The genetic diversity was estimated to be 0.997 and the probability of two random individuals showing identical mitochondrial DNA (mtDNA) haplotypes was 0.011. Based on the results of mtDNA profiles, 45% of mtDNA sequences could be classified as African, 27% as Native American and 25% as European haplogroups. Approximately 3% of the haplotypes could not have been classified in haplogroups. All sequences showed high quality values and phantom mutations were not detected. In conclusion, these results suggest that sequence polymorphism of the mtDNA control region can be highly informative for forensic cases in the population of Alagoas.

PP044
Mitochondrial DNA sequence variation in Spanish Pyrenean populations

López-Parra AM[1], Fernández E[1], Baeza C[1], Arroyo-Pardo E[1], Tirado M[1], Gusmão L[2], Gamba C[1,3], Mesa MS[3]

1) Laboratorio de Genética Forense y Genética de Poblaciones. Dpto. Toxicología y Legislación Sanitaria. Fac. Medicina. Complutense University of Madrid, Spain. 2) IPATIMUP, Instituto de Patologia e Imunologia da Universidade do Porto, R. Dr. Roberto Frias s/n, 4200 Porto, Portugal. 3) Unidad Docente de Antropología. Departamento de Zoología y Antropología Física. Facultad de Biología, Complutense University of Madrid, Spain.

The Pyrenees are a mountain range which spans 430 km from the Cantabric see to the Mediterranean shore. Its widest section (160 km) lies in the central part and contains also the highest mountains, most over 3000 meters. Because of this orography, the Pyrenees could have been responsible for the isolation of the populations located in their valleys. However, the Pyrenees are one of the more important ways of entrance of other European populations to Iberia. The main objective of this work was to study the genetic variability of some populations from the Pyrenean valleys. Polymorphisms of the mitochondrial DNA Hypervariable Region I were determined by direct sequencing, in autochthonous populations from East Pyrenees -Cerdanya (n=26) and Alt Urgell (n=23)- Central Pyrenees –Aran Valley (n=18)-, and West Pyrenees -Jacetania (n=8)-. We detected 54 different HVI haplotypes, 6 in Jacetania, 13 in Aran Valley, 15 in Alt Urgell and 20 in Cerdanya. Only CRS (Cambridge Reference Sequence) haplotype was present in all populations. This was by far the most frequent haplotype in all populations except for Jacetania. The most frequent haplogroup in the four populations studied was H/HV/pre-HV/U/R, with frequencies ranging from 50 % (in Cerdanya and Aran Valley) to 75% (Jacetania). Haplogroup V/pre-V was also present in all populations, being 69% in Cerdanya and 17.39% in Alt Urgell. Other European haplogroups were also present in these populations: K (Jacetania, Cerdanya and Alt Urgell); J (Cerdanya, Alt Urgell and Aran Valley), I (Cerdanya, Alt Urgell), T (Cerdanya, Aran Valley), U5 (Alt Urgell and Aran Valley) and X (Cerdanya). By comparing haplogroup structure in these populations to the one from other Iberian populations, we observed a high degree of similarity between Aran Valley and the Basque Country, especially in haplogroups H/HV/pre-HV/U/R, V/pre-V, J, and U5a. In conclusion, results from mitochondrial DNA may well support some degree of differentiation between the Pyrenees and the rest of Iberia, and a certain degree of differentiation between West Pyrenees and East-Central Pyrenees.

PP045
Mitochondrial DNA variation in northern French population

Odabachian MR, Stevanovitch A, Brahiti H, Bouzaid E, Haro D, Giacometti C, Coiffait PE

Laboratoire de Police Scientifique de Marseille - Institut National de Police Scientifique

In a rape case, investigators decided to proceed to a massive DNA typing of male individuals. Previously to an accurate nuclear typing for comparison with the questioned samples, a first screening based on mitochondrial DNA (mtDNA) was realised, to detect possible genetic links between questioned and comparison samples. Among the 277 individuals originated from France typed in this case, 201 were born in Picardie region, 40 in Nord/Pas de Calais region and 36 from other French areas. We propose in this study to report the mitochondrial DNA variation of these French populations. Both the sequencing of hypervariable segments I and II (HVS-I and HVS-II) and the analysis of mitochondrial SNPs were used to distribute different haplotypes in mitochondrial haplogroups. Haplogroup H and macro-haplogroup U were the most frequent in each population. We noticed the presence of some non-European haplogroups (as M1 and U6 haplogroups) which could be due to recent migrations from Africa. These populations represent a good example of current French population (data have been added to EMPOP database). Statistical and phylogenetical analysis were used to compare these three French populations to other Europeans populations (including previously published French, Italian, German, Austrian, Spanish and Scandinavian populations).

PP046
Multiplex amplification of mitochondrial DNA for species identification in forensic science

Fang YQ, Yun LB, Li YB, Wu J, Yan J, Zhang J, Hou YP

Institute of Forensic Medicine, Sichuan University (WCUMS),Chengdu 610041, China

Mitochondrial DNA (mtDNA) allows for the unambiguous species identification for its high copy number in cells. Employing the sequences of appropriate genes in mtDNA to develop reliable system, we have established a method combining in a single-round polymerase chain reaction (PCR) amplification of both cytochrome b (cyt b) and Nicotinamide Adenine Diuncleotide Hydrogen dehydrogenase subunit 6 gene (ND6) to differentiate human origin from animals. Using two sets of primers, one was universal for cyt b gene fragment, another was human-specific for ND6 gene. Following the amplification step, amplicons were analyzed by horizontal non-denaturing polyacrylamide gel electrophoresis (PAGE) with discontinuous buffer system and visualized by silver staining. The presence of only one band of 358 base pair (bp) showed that the sample was not human, while the presence of two bands, 358 bp and 181 bp, indicated a human origin. To validate it in forensic application, several tests were adopted. Sensitivity of the system established was 0.25 pg mtDNA samples. Bloodstains stored in 4°C, room temperature and 37°C for more than 120 days got correct results. Samples stored in room and dry condition for more than 90 days and in high-humidity for more than 50 days also got desirable results, but those stored in high-humidity for 90 days could not be identified. Various carriers of bloodstain, such as soil, cloth, wall, etc. were detected no impact on the system. Then the system was employed to discriminate the species of bloodstains stored in room for 7 to 9 years, and bone for nearly 10 years. Both got satisfactory results.

PP047
Multiplexed SNP Detection System for Mitochondrial DNA: A Tool for Genotyping of Aged Forensic Type Samples

Fang R[1], Bzroska P[2], Ma P[2], Furtado MR[1]

1) Applied Markets and 2) Molecular and Cell Biology Division, Applied Biosystems, Foster City CA 94404

Single nucleotide polymorphisms (SNPs) are the most prevalent form of genetic variations in the human genome. Because of their low mutation rate, SNPs have the potential to be used as genetic markers in human identity applications such as paternity testing, genealogy studies, and human population genetics. In addition, the ability to be genotyped using very short DNA fragments, SNP genotyping may become a very important tool to analyze highly degraded and aged samples commonly seen in the forensic field. For the analysis of aged sample (e.g. bones) or samples that contain a small amount of biological material (e.g. hair), SNP markers on mitochondrial DNA are preferred over SNP markers on nuclear DNA because of multiple copies of mitochondrial DNA in a cell. The major goal of present study was to develop a sensitive, easy to use, rapid, and robust SNP detection method for forensic applications. We have optimized a detection system for identification of mutations and SNPs. The method involves a multiplexed PCR amplification of as many as 48 regions in the mitochondrial genome, followed by detection of mutations or SNPs in these amplicons using an oligonucleotide ligation assay (OLA). Optimization of the multiplex PCR was achieved using primer titration and minimizing primer-dimer formation. The ligated products were hybridized to coded sequences with mobility modifiers and detected by capillary electrophoresis.

Direct detection by CE following ligation is also possible. To define the accuracy of the system we also sequenced total mitochondrial genomes from 22 individuals using M13 tailed primers for PCR amplification designed to cover the entire genome. A SNP detection multiplex assay comprised of 11 SNPs outside the hypervariable region was developed for mitochondrial SNP detection. Performance of the assay was evaluated by comparison with sequence approach. Complete concordance for more than 200 allele calls made with the PCR/OLA method and sequencing method was observed. The method was further evaluated for forensic application by using forensic type samples, such as vaginal swab, saliva, hair, urine, and blood stains. The results indicate that the SNP system is accurate, sensitive, fast (< 7 hour time for analysis), easy to perform, medium through-put, and robust.

PP048
Species Identification through DNA „Barcodes“

Ferri G, Alù M, Sola N, Corradini B, Beduschi G

Department of Diagnostic and Laboratory Service and Legal Medicine, Section of Legal Medicine, University of Modena and Reggio Emilia, Italy

Classical methods for species identification of casework stains are mainly based on immunological procedures that have limited applications for old and degraded specimens. Between molecular methods, the mitochondrial cytochrome b gene sequence emerged, allowing the reliable identification of a species by comparative sequence analysis. The sequence information and the genetic differences between organisms were used to establishing phylogenetic links and to identify the biological origin of casework sample through an enhanced information content. Also genomic approaches to taxon diagnosis exploit diversity among DNA sequences to identify organisms, and in a real sense, these sequences can be viewed as genetic „barcode“ that are enclosed in every cell. Therefore „barcoding“ is a standardized approach for characterizing species using short DNA sequences as a diagnostic „biomarker“ for organisms (www.barcodinglife.org). The cytochrome c oxidase subunit 1 (COI) is emerging as the standard barcode region for higher animals because it appears to posses a greater range of phylogenetic signal than any other mitochondrial gene. Similar technique are also used routinely in forensic investigation by cytochrome b and 12S rRNA gene analysis as above, so the aim of this study was to evaluate the potential of „barcode“ gene as COI and 16S rRNA gene as a forensic tool, not only in casework but also in food products, poaching, illegal trade of endangered species, fish processing and in further evaluation, for the identification of forensically important insects in death investigation. Seven fragments ranging from 157 bp to 541 bp were selected from COI and 16S rRNA genes by different re-designed set of primers suitable for forensic purposes. These regions are defined as having high levels of diversity flanked by conservative regions. Some primers have been made degenerate for expected most common mutations because mismatches at the 3' end are critical for successful amplification of different species. Biological specimens were obtained from 50 different vertebrate animals from five principal different classes by home-made sampling. Extracted DNA was subject to PCR and direct cycle sequencing. The study confirmed the high degree of versatility of the applied set of primers. All but one amplicons do not shown evident differences in PCR efficiency depending on the species studied, confirming the high conservation of the selected annealing regions. To verify the informativeness of the variable regions and the degree of resolution for species identification, the sequences of fragments of different species were submitted to a BLASTn sequence similarity search in the NCBI database. Inter and intraspecific sequence comparison using phylogenetic analysis for COI gene were obtained by submission of the sequences in FASTA format to the BOLD Identification System (IDS) (www.barcodinglife.org/views/idrequest.php) that returns a species level identification of unknown sample by neighbour-joining tree of genetic distance based on Kimura's two-parameter. The present results show that an identification system based on COI and 16S rRNA genes will be highly effective and robust not only in forensic analysis of higher animals, but also in wildlife enforcement, in feedstuff control and with further development, can provide a mean to estimate the post mortem interval.

PP049
Use of mini-STR typing systems to identify victims of apartheid era violence

Leat N, Kleyn E, Benjeddou M, Davison S

University of the Western Cape, South Africa

A missing person's task team has been established in South Africa. The team aims to trace individuals who were murdered during the apartheid era. Anthropologists working for the team have exhumed the remains of fifteen individuals in three cases dating to the mid 1980s. The cases involve four activists whose bodies were dismembered with explosives, a single activist killed in an ambush, and ten youths drugged and burned in a minibus. Skeletal remains from the exhumations have been analyzed using mini-STR typing systems. Data will be presented showing the efficacy of mini-STR typing systems for the analysis of the relevant skeletal elements.

PP050
Opposite homozygosity in the STR system FGA due to mutations in the primer binding region

Müller M, Schürenkamp M, Brinkmann B, Hohoff C

Institut für Rechtsmedizin, Universitätsklinikum Münster, Röntgenstr. 23, 48149 Münster

The STR system FGA (= FIBRA) is located at chromosome 4 (4q31.3) in the 3rd intron of human alpha fibrinogen gene and is one of the most informative markers used in forensic genetics. In our routine casework of paternity testing covering more than 10 000 meiotic transfers at FGA by multiplex PCR, two cases with opposite homozygosity were observed. The first case was a deficiency case in which only the samples of mother and child were available for testing. Analysis using the Profiler kit (Applied Biosystems) led to exclusion in a single STR system, FGA. In the mother the allele 25 was detected while in the child the allele 21 was found. After performing a singleplex PCR using a different pair of FGA primers we found an additional allele 24 in both samples. We thus hypothesised that a mutation in the primer binding site of FGA had led to a 'silence allele' 24. Saliva samples from the putative father, mother and child were analysed in the second case using the Identifiler kit (Applied Biosystems) and allele 22 in the putative father and allele 25 for the samples of mother and child were detected. This was also an apparent exclusion of the putative father. Using another multiplex kit (MPX-2, Serac) the following phenotypes were observed: 22/25 (putative father), 25 (mother), 25 (child). We hypothesised again a mutation in the primer binding site of FGA which led to a 'silence allele' 25 in this case. Thus, the child must carry iso-allele 25 with the variant inherited from the father showing the predicted primer binding site mutation. The apparently homozygous samples were identified as truly heterozygous by reanalysing these samples in a singleplex PCR using the PowerPlex16 primers (Promega) that generate an amplicon which is longer than the amplicon from the Profiler/Identifiler kits. Sequencing verified the assumed mutation in both cases as a T to G transversion at a distance of 25 base pairs 3' of the first base following the forward primer (Promega). This must be a critical position (e.g., the ultimate base) in the binding site of the Applied Biosystems forward primer. With a lower annealing temperature for the multiplex kits it was also possible to overcome the problem. To test if additional silent FGA alleles were present in our samples we have examined 100 additional samples from cases with at least one homozygous finding, but no other case showing this mutation was found.

PP051
Eva: A new Bioinformatic system for analysing and storing mtDNA for the identification of missing persons.

Caetano LHT[1], Ferreira da Silva LA[2], De Almeida ES[1]

1) Federal University of Alagoas – Institute of Computation 2) Federal University of Alagoas – Institute of Biological and Health Sciences

The mtDNA analysis has proven to be a powerful tool in the identification of human remains and widely used to characterize forensic biological specimens, particularly when there is insufficient nuclear DNA in samples for typing. Forensic scientists analyse the HVI and HVII mtDNA genetic variations to help resolve identity in missing persons and criminal cases. Population databases are being generated and used to estimate and determine the rarity of mtDNA profiles obtained in forensic cases. There is a need for specific softwares for managing the massive amount of sequence data that are generated. The present work presents a Bioinformatic system that contributes to the process of automated analysis, transcription, storage and comparison of mtDNA profiles for human identification. The development of the proposed tool is based on the patterns of literature, taking in account the consistency of aligning mtDNA sequences, which generates the haplotype and annotates its polymorphisms by the appropriate nomenclature, and also facilitates the inspection and validation of the polymorphisms due to the errors that can be present on the raw DNA sequence. The analysis of polymorphisms is a tool to help prevent errors that may be generated during the entire process of manipulation, extraction, amplification, sequencing and aligning mtDNA sequences. Hence this enables us to create, store and estimate the frequency of mtDNA profiles, in forensic and population databases, with efficiency and high quality. The Bioinformatic system proposed is a web-based application that contributes to integrate, standardize and enable the communication between the national forensic DNA laboratories, thus allowing them to share mtDNA profiles to be compared among the states of Brazil, through a mutual database. The system has been modelled to manage and compare the stored mtDNA profiles of missing persons with their relatives and can be used to solve mass disasters.

PP052
Automating the mtDNA analysis with the MitoTyper software

Orekhov V, Markov A, Ivanov P

Russian Center of Forensic Medical Expertise, 12 Polikarpov st., Moscow, Russia

Sequence variation analysis in human mtDNA is an important and powerful tool for forensic applications. The recent progress in sequencing techniques allows to perform mtDNA sequencing on a routine basis with high throughput. However, alignment, comparative analysis of obtained sequences and documentation of the revealed haplotypes into database still make up a time consuming process, which remains a bottleneck of the whole study. Currently available software for multiple sequence alignment (like ABI SeqScape Software) substantially optimizes this stage, but still some manual steps are required before the haplotype is recorded in a database. The researcher can easily make a mistake while manipulating the data. Because of this, a lot of errors occur in published population and forensic databases. In the worst case scenario such errors can affect the results of the case study. Here we present the newly developed MitoTyper software that automates the comparative analysis of the mtDNA sequences and minimizes the risk of human errors. MitoTyper has user friendly interface designed for non-expert users. The software performs the following steps of the analysis: - viewing and editing sequencing electroforegramms in ABI format, - aligning the sequences with electrophoregramms along the reference sequence, - marking mismatches for further confirmation, - making database entry, - performing database search for revealed haplotype with wide range of options. The alignment performed with Needlman-Wunsch algorithm modified for better performance with mtDNA sequences. A centralized haplotype database is shared by multiple user sessions. MitoTyper was validated by reanalysis of a set of old sequences. The performance of MitoTyper software will be shown.

PP053
Rapid and Sensitive Mitochondrial Haplogroup Determination using Pyrosequencing

Patel E, Harrison C, Musgrave-Brown E, Ballard D, Syndercombe Court D

Department of Haematology, Institute of Cell and Molecular Sciences, Queen Mary's School of Medicine and Dentistry, London, UK

Mitochondrial DNA (mtDNA) analysis is a useful tool in forensic science. It is particularly valuable when analysis of genomic DNA is a challenge (e.g. shed hair or highly degraded material), establishing a relationship between distantly related individuals or determining information regarding ancestry. Sequencing all or of part of the control region has been the traditional method used for mtDNA analysis but this can be time consuming and labour intensive, especially when deciphering haplogroup information or when working with poor quality samples. Recent studies looking at mtDNA SNPs that differentiate haplogroups demonstrate how detailed sample information may be more easily obtained[1]. In this study, mtDNA SNP analysis using Pyrosequencing technology was evaluated for its ability to be used for screening samples in comparison to traditional methods. Pyrosequencing is an efficient and highly sensitive technology that meets the requirements for rapid and reliable genotyping of samples. It is a sequencing based method that uses real-time bioluminetric detection of DNA synthesis. By analysing a selection of eight SNPs, chosen to differentiate between the major haplogroup types commonly encountered in the laboratory, unknown samples could rapidly and accurately be assigned to haplogroups. When combined with the sequencing results, the addition of the 8 SNPs increased discrimination and made haplogroup assignment possible for some samples with ambiguous control region sequences. When the technology was tested for its reproducibility and accuracy in genotyping poor quality samples, the results showed that the method was sensitive and reliable. It was concluded that as a screening method, SNP analysis using the pyrosequencing technology was well suited for forensic mtDNA casework, particularly compared to traditional methods used in the laboratory. 1. Quintans, B., et al., Typing of mitochondrial DNA coding region SNPs of forensic and anthropological interest using SNaPshot minisequencing. Forensic Sci Int, 2004. 140(2-3): p. 251-7.

PP054
Considerations for a Text String Based Search Engine for Forensic Mitochondrial Control Region Databases

Irwin JA[1], Sturk KA[1], Saunier JL[1], Coble MD[1], Parsons TJ[1, 2]

1) Armed Forces DNA Identification Laboratory (AFDIL), 1413 Research Blvd., Rockville, MD 20850, USA 2) Current address: International Commission on Missing Persons, Alipašina 45a, 71000 Sarajevo, Bosnia and Herzegovina

The utility of mitochondrial DNA testing is dependent on a search of the questioned haplotype against specific reference databases, in order to estimate the rarity of the haplotype in question. For all forensic mtDNA reference databases available, the search methodology is the same – the questioned haplotype is translated into a list of differences from the rCRS, and then searched against a database of haplotypes stored in the same format. In most cases, generally those for which no length variation is observed, these searches are rather straightforward. However, when novel length variation is observed, the subjective placement of insertions and/or deletions (indels) relative to the rCRS can quickly complicate searches, particularly when the nomenclature for identical haplotypes differs between the questioned profile and the reference database. At best, these nomenclature differences will be reflected in hypervariable C-stretch regions that are generally ignored in evidence interpretation. At worst, these differences will underestimate the frequency of particular haplotypes. While recommendations have been provided to standardize the interpretation of length variants [1,2] these recommendations, unfortunately, cannot encompass all of the unique situations encountered. There are simply too many possible variants, and in some cases, multiple interpretations are equivalent with respect to the suggested hierarchy of recommendations. Furthermore, there are particular situations for which the evolutionary history of a length variant haplotype provides additional information upon which interpretation can be based. In some cases, this information conflicts with standardized recommendations, but provides relevant and sensible data to guide interpretation. Given the complications of length variant interpretation and nomenclature, the Armed Forces DNA Identification Laboratory is investigating the potential of a modified mtDNA search engine that is based solely on text strings. The principle of searching text strings against text strings would obviate the need to position or interpret indels relative to the rCRS. Moreover, this type of search paradigm may provide additional flexibility to incorporate phylogenetic data, site-specific mutation rates, and other biologically relevant information that would refine the interpretation of mitochondrial DNA evidence. We will report on the development and further considerations of a string comparison-based mtDNA search and reporting tool. [1] Wilson MR, Allard MW, Monson K, Miller KW, Budowle B (2002) Recommendations for consistent treatment of length variants in the human mitochondrial DNA control region. Forensic Sci Int 129: 35-42 [2] Wilson MR, Allard MW, Monson K, Miller KW, Budowle B (2002) Further Discussion of the consistent treatment of length variants in the human mitochondrial DNA control region. For Sci Comm 4(4).

PP055
Optimization of genetic analysis of DNA from teeth for forensic research

Mályusz V, Karwacik U, Heinrich A, Schwark T, von Wurmb-Schwark N

Institute of Legal Medicine, Christian-Albrechts-University of Kiel, Kiel, Germany

When human remains are found that are in advanced stages of decomposition, e.g. skeletonized or burned, the forensic expert may try to establish a potential identification by using dental records or skeletal features. If these methods do not lead to a positive identification, DNA typing from bones or teeth would be the method of choice. Furthermore, identifications based on genetical analyses provide a more objective probability of identity than those performed only by the comparison of dental records. In this study we compared different standard DNA extraction methods concerning DNA yield, expenditure of time, use of hazardous chemicals etc. to improve DNA extraction from teeth. For all pretests powdered teeth provided by multiple donors were pooled and then divided into aliquots of 0.5 g each. DNA was extracted either applying a commercially available kit (Invisorb Forensic kit, Invitek) or an organic extraction method using phenol-chloroform-isoamyl alcohol according to a standard protocol. Additionally, both methods were modified by applying Enzyrim® or Proteinase K to the lysis step. The modified extraction methods were repeated several times on different days and also used for the isolation of DNA from single teeth. DNA quality and quantity was tested on ethidium bromide stained agarose gels. Absolute quantification was done using real time PCR. To test the amplifyability of the extracted DNA, all samples were first subjected to a highly sensitive Duplex-PCR which amplifies fragments specific for mitochondrial and nuclear DNA. DNA samples from single teeth were also employed to genetic fingerprinting using the Powerplex ES and the AmpFlSTR Identifiler kit. In comparison with DNA extraction using phenol-chloroform, the Invisorb Forensic kit led to a significantly higher DNA yield. Application of Enzyrim® or Proteinase K to the lysis step resulted in an even higher DNA yield (that exceeded that mentioned by the manufacturer). Our study shows that the easy-to-use Invisorb Forensic kit (with or without modifications) is an excellent tool for DNA isolation from teeth. Furthermore it is less time consuming than organic DNA extraction and avoids the use of hazardous chemicals.

PP056
PCR and SBE multiplexes for determination of SNPs associated with hair and eye colours in humans

Mengel-Jørgensen J, Wachter A, Børsting C, Sanchez J, Morling N

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

Differences in hair, eye and skin colour are mainly genetically determined and are results of the amount, type and cellular packaging of melanin. In the mouse, more than 100 genes affect the regulation of melanin synthesis and many of these genes have homologues in humans. The best-described gene encodes the melanocortin 1 receptor (MC1R). MC1R is a key regulator of the tanning response to UV-light. Activation of MC1R in humans induces synthesis of the brown/black form of melanin and several mutations in MC1R have been associated with hair colour and skin type. Two other genes involved in the regulation of pigmentation are the OCA2 gene (oculocutaneous albinism type II gene or P gene) and the MATP gene (OCA4). It has been shown that mutations in OCA2 and MATP are associated with oculocutaneous albinism and hypopigmentation of the eyes. However, some mutations in these genes are also associated with normal variations in pigmentation. Here, we present a multiplex PCR and a multiplex single base extension (SBE) reaction for typing of 16 mutations in MC1R and two mutations in MATP and OCA2, respectively. All the amplification products in the PCR multiplex are shorter than 200 bp to allow amplification of degraded DNA samples. Genotypes in 392 Danes were compared to the hair- and eye colours of each individual. Furthermore, the frequencies of the mutations in additional populations were investigated. The DNA from individuals with two mutations in MC1R were amplified by allele specific PCR and the haplotypes were determined.

PP057
Current populations living in the area of the Franco-Cantabrian Refuge: regions HVI and HVII of the mitochondrial DNA

Cardoso S[1], Odriozola A[1], Calvo N[1], Alfonso-Sánchez MA[2], Peña JA[2], Pérez-Miranda AM[2], García-Obregón S[2], Zarrabeitia MT[3], Riancho JA[4], Herrera R[5], de Pancorbo MM[1]

1) Genómica: Banco de ADN. Facultad de Farmacia. Universidad del País Vasco, UPV/EHU. Vitoria-Gasteiz (Spain) 2) Dpto. de Genética y Antropología Física. Universidad del País Vasco. Leioa (Spain) 3) Unidad de Medicina Legal, Universidad de Cantabria. Santander (Spain) 4) Dpto. de Medicina, Universidad de Cantabria. Santander (Spain) 5) Molecular Biology and Human Diversity Laboratory, Department of Biological Sciences. Florida International University, Miami (USA)

Due to the orographic characteristics of the Cantabrian Coast many human populations have evolved in an isolated manner. In this study a total of 165 unrelated individuals belonging to three populations located in the area known as Franco-Cantabrian refuge were analysed: 55 individuals autochthonous from the Basque Country, a historically isolated human group; 61 individuals autochthonous from Valle del Pas, in Cantabria, another isolated community; and 49 individuals autochthonous from the north of Navarra. The majority of the mtDNAs in the Basque population fall into six European-specific haplogroups (H, J, K, U, V and X). The highest frequency is found for haplogroup H (0.509) as described for most of the European populations. The population from Valle del Pas shows one of the highest frequencies of haplogroup V found in Europe (0.213), except for the Skolt Saami. It coincides, but with a greater value, with the peak described by Torroni et al. (Am. J. Hum. Genet. 2001) in the Basque Country. Intrapopulational diversity of haplogroup V in this population is similar to that found in Basques (0.497 vs. 0.486) and higher to the diversity showed by the Skolt Saami (0.074). Thus, its possible to state that this frequency is not due to a recent founder event. On the other hand, the population from Valle del Pas shows one of the lowest frequencies of haplogroup H (0.262) found in Europe. The haplogroups showing the higher frequencies in the population from north of Navarra are four: H, U5a, J and T2 (0.286, 0.163, 0.163 and 0.111, respectively). Haplogroup U5a is also found in Basques and haplogroup T2 in Valle del Pas; however, in both cases these haplogroups show very lower frequencies suggesting that haplogroups U and T have specifically evolved to U5a and T2, respectively, in the population from Navarra. These results induce to think that, even being isolated populations, their maternal lineages are all descendant from the Franco-Cantabrian refuge, with a different evolution for each of them. Thus, these populations could provide additional insights about the postglacial human colonization of Europe.

PP058
Dual use of a mtDNA amplification: quantitation and sequence analysis

Hoppe K, Brinkmann B, Hohoff C

Institut für Rechtsmedizin, Universitätsklinikum Münster, Germany

While for nuclear DNA commercial quantitation kits are already available, there are only few reports on quantitation assays for mitochondrial DNA (mtDNA). We have developed a new method to quantify mtDNA in minimal stains (i.e., minute amounts of DNA and/or highly degraded DNA) by real-time PCR: a part of the HV1 region (np 16262 - 16439) is amplified in the presence of an internal positive control (Applied Biosystems) and detected by means of a Black Hole Quencher (BHQ) double-dye probe (Eurogentec). Absolute quantification is carried out using plasmid DNA containing the cloned mtDNA target region on an ABI PRISM 7000 Sequence Detection System. Afterwards the real-time PCR products are purified using the Qiaquick PCR Purification kit (Qiagen) and then sequenced on both strands with the Big Dye Terminator kit v.1.1 using an ABI PRISM 310 Genetic Analyzer and POP-4 polymer (Applied Biosystems). We discuss here the results and compare the determined sequences after real-time PCR (40 cycles) with the conventional direct sequencing of PCR products (30 cycles).

PP059
The development of five 5´Nuclease-SNP-Assays for forensic casework

Senge T, Junge A, Madea B

Institute for legal medicine, University of Bonn, Stiftsplatz 12, 53111 Bonn

This study should show the applicability of 5´Nuclease-assays as one method of typing DNA extracted from forensic materials by SNPs: 5 SNPs, TSC0171847 (Chr.1), TSC0582423 (Chr.2), TSC0741184 (Chr.3), TSC0126548 (Chr.4) and TSC0191459 (Chr.6) were selected from the database of the SNP-consortium, a foundation organized for the purpose of providing public genomic data, which had discovered and characterized nearly 1.8 million SNPs and published the corresponding sequences. The main criteria for choosing the SNPs named above were a balanced allele distribution of at least 40% Allele 1 to 60% Allele 2 and a location on different chromosomes to warrant an independent distribution. Assays were developed based on the 5´Nuclease-system from Applied Biosystems and passed a thorough validation. The validation studies include the comparison of sequenced samples with the assays, sensitivity- and reproducibility studies and population studies. These studies confirm the postulated balanced allele distribution and show that an amount of at least 250 pg genomic DNA is sufficient to type DNA by SNPs. To find out if these assays are applicable for forensic purposes two approaches with artificial stain-material were performed: The first approach showed that typing of DNA extracted from fresh stain-material was possible in all cases. The other approach gave the result that typing of DNA extracted from old stain-material which were aged under natural conditions was possible for a longer period of time in relation to a commercial available STR-typing-Kit which is routinely used for typing stain-material in our laboratory. Further studies should confirm the consequential hypothesis that maybe SNPs (5´Nuclease-assays) are the better chosen marker when working with old stains containing degraded DNA-material.

PP060
Forensic Mitochondrial DNA Analysis: Casework Experience in Rio de Janeiro, Brazil

Silva DA[1, 2], Silva MD[1], Costa GA[1], Almeida FM Junior[1], Carvalho EF[1]

1) Laboratório de Diagnósticos por DNA, Departamento de Ensino de Ciências e Biologia, Instituto de Biologia, Universidade do Estado do Rio de Janeiro, Brasil 2) Departamento de Patologia e Laboratórios, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Brasil

Mithocondrial DNA (mtDNA) analysis is an important and growing area of forensic testing. This methodology may successfully being used in cases with limited biological evidence, such as hairs and degraded skeletal remains. DNA Diagnostic Laboratory, State University of Rio de Janeiro, Brazil, is a public laboratory that works on forensic analysis since 1996 and began mtDNA forensic casework testing in 2001, as such; it has over five years of mtDNA casework experience. DNA Diagnostic Laboratory was the first Brazilian public laboratory to start a Program on the basis of mtDNA analysis to identify cadavers and human remains which could not be identified by the use of traditional forensic approaches. The main objective of this work is present the statistics were kept for five years on all aspects of mtDNA forensic cases, including types of cases, types of samples, levels of samples success and failure, rates of failures to exclude, and match statistics using a mtDNA sequence database. Low sample failure rate was observed, the data collected from mtDNA haplotypes developed in casework showed extremely high diversity of haplotypes consistent with other formally developed databases. MtDNA forensic

analysis was succefully applied to many different types of samples overall even to degraded DNA, contaminated DNA and minimal samples. Support: Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (Faperj). Programa DNA – Universidade do Estado do Rio de Janeiro e Tribunal de Justiça, Brasil.

PP061
Haplotyping assists in the study of germline mutations at short tandem repeat loci

Hohoff C, Müller M, Sibbing U, Brinkmann B

Institut für Rechtsmedizin, Universitätsklinikum Münster, Röntgenstr. 23, 48149 Münster, Germany

In the course of routine parentage testing, that covers approximately 15 000 allelic transmission, we have observed 22 cases with a Non-Mendelian transmission at D3S1358. These transfers were regarded as de novo mutations after additional STR markers had been analyzed and the mutational event had been included in the biostatistical evaluation still leading to a paternity probability value of W > 99.99%. In some cases it could not be decided whether the mutation had occurred in the male or female germ line. Therefore, we have selected on the basis of various parameters (e.g., distance to D3S1358, repeat type and heterozygosity value) six flanking microsatellite markers, i.e., D3S2407, D3S2304, D3S3582, D3S688, D3S1767 and D3S2420, and have established specific typing procedures using fluorescence-labelled primers. The resulting genotypes were used for haplotype construction. These haplotypes helped in assigning the mutational event to the male or female germ line and in turn allowed to categorize the mutations with respect to various parameters (e.g., gain/loss, age of parent with the mutation). The results of our study will be presented and the consequences for the analysis of STR mutations will be discussed.

PP062
The problem of deficiency paternity investigations with related putative fathers: Final clarification of a paternity case using three different methods

von Wurmb-Schwark N, Mályusz V, Schwark T, Simeoni E

Institute of Legal Medicine, University Hospital Schleswig-Holstein

Recent investigations have shown that paternity analysis without investigation of the mother - so called deficiency cases - can be very challenging to elucidate. We present the case of a 56 year old man, who was looking for his biological father. His mother named one putative father but refused to participate in any genetic analysis. Thus, only a deficiency investigation was possible. DNA extracted from buccal swabs was subjected to a multiplex PCR amplifying 15 STRs simultaneously (AmplSTR Identifiler kit, Applied Biosystems). Only two exclusions were detected between „child“ and prospective father; the paternity probability (W) without these non-matching STRs was 99.857757 %. Additional analysis of four RFLP single locus probes presented four more inclusions and led to a combined W of 99.994361 %, sufficient for the verbal predicate „fatherhood practically proven“. Nevertheless, there were two excluding STRs, raising doubts about this conclusion. It could be assumed that child and prospective father belonged at least to the same family. This assumption was supported by analysis of ten Y-specific STRs, since both individuals shared the same Y-haplotype. However, further investigations resulted in three more exclusions (one STR, two single locus probes). After extensive inquiries, a brother was found, who indeed „had known“ the child`s mother. He could definitely be included as being the biological father, after investigation of 16 STRs, six single locus probes and ten Y-STRs. This case shows how complicated paternity analysis can be, especially without inclusion of the mother. Even by completely following the German guidelines, the brother of the biological father could have been wrongly included.

PP063
Application of an Optimized SNP Detection System for Human Identification: Comparison with STR Profiling Methods

Fang R, Hyland F, Furtado MR, SNPforID Consortium

Applied Markets, Applied Biosystems, Foster City CA 94404 and the SNPforID consortium (www.snpforid.org)

The past two decades have seen enormous growth in the use of DNA profiling methods in crime scene investigations as well as paternity testing. Currently, multiplexed analysis of short tandem repeats (STRs) provides the best solution with high power of discrimination and rapid analysis speed. However, single nucleotide polymorphism (SNP) profiling is also very powerful for human ID testing purposes especially in instances where DNA is highly degraded, as with disaster victim identification (DVI), and in typing mitochondrial DNA. SNPs have also been used for paternity testing, genealogical studies and more recently in population based stratification of patient groups in clinical trials. We have optimized a detection system for identification of known mutations and SNPs. The method involves a multiplexed PCR amplification of ~ 48 regions in the human genome, followed by detection of mutations or SNPs in these amplicons using an oligonucleotide ligation assay (OLA). The ligated products were hybridized to coded sequences with mobility modifiers and detected by capillary electrophoresis. Direct detection by CE following ligation is also possible. To test the accuracy of the method and to compare relative utility of SNP analysis to STR analysis for human identification and paternity testing, we genotyped a panel of 41 individuals from 3 different CEPH families spanning three generations. The SNPs used were based on a study by the SNPforID consortium, who selected a set of highly discriminative SNPs suitable for forensic analysis based on criteria described in Sanchez et al., (Electrophoresis 2006,27:1713). We also designed a gender specific deletion marker. The test samples were genotyped using 49 SNPs with the SNP detection system, and also with IdentifilerÔ. To determine accuracy, the SNPs were also genotyped using the TaqManâ allelic determination method to yield 100% concordance. The results indicated that the overall informativeness is comparable, with about 45 SNPs being equivalent to Identifiler. The SNP panel was more informative for identity but less informative for paternity exclusion. In this data set, the SNP panel exhibited greater number of genotypic differences (mean ~19) compared to Identifiler (mean ~9) between closely related individuals. The SNP panel also exhibited a greater number of genotype differences (mean ~ 28) compared to Identifiler (mean ~ 14) between any two unrelated individuals. This trend was consistent in both full sibs and unrelated samples. And, while Identifiler has a higher probability of paternity exclusion, (about 10-7 for Identifiler and 10–5 for the SNPs when the mother's genotype is known), the SNP panel was better able to distinguish between close relatives for paternity identification. The results indicate that carefully selected SNPs can be as useful as STRs in human ID testing and related applications. The development of highly multiplexed SNP detection systems enabling lower cost, higher automation and higher throughput will result in increased use of SNP profiling in a number of applications. The barrier to quick adoption is the existing national STR databases that have been in use for a decade, and are not likely to be changed in the near future.

PP064
Population data of 12 Y-STR in Tuscany

Carboni I, Ricci U, Genuardi M

Azienda Ospedaliero-Universitaria „A.Meyer" U.O. Genetica Medica Florence Italy

Y-chromosome markers are used for specific applications because of their male-specificity. Y-STR plays an important role in forensic science, especially in sexual assault cases, deficiency paternity cases involving male offspring and genealogical and population studies. Here we report on the results of 100 unrelated males in Tuscany for 12 short tandem repeats loci of the Y chromosome. We also compared 10 father-son pairs Y haplotypes, for which real paternal relationship had been previously confirmed by autosomal analysis. Allele frequencies and gene diversity were estimated at each locus.

PP065
Population Structure in the Kuwaiti Bedouin Tribes of the Persian Gulf based on Autosomal DNA Polymorphisms

Mohammad T[1], Xue Y[2], Evison M[3], Tyler-Smith C[2]

1) University of Sheffield, Division of Genomic Medicine, The Medical School, University of Sheffield, Sheffield, S10 2RX, United Kingdom 2) The Wellcome Trust Sanger Institute, Cambridge, U.K. 3) Department of Anthropology, University of Toronto, Canada

Population genetic substructure is of anthropological interest but can be a major source of confusion in forensic and medical genetic studies. Although the markers used in forensic work were chosen to be variable in all populations, they may still exhibit geographic structuring. Here, 153 Bedouin individuals from Kuwait were genotyped with 15 forensic autosomal markers and their population substructure investigated. The Bedouin individuals originated from six different populations belonging to two main lineages: Adnan or Qahtan (Joktan). We analysed population substructure by clustering individuals into subgroups using the programme STRUCTURE (Pritchard et al., 2000a). When we compared our data with other populations ('Caucasians', 'Hispanics' and African Americans) typed with the same 15 autosomal markers, the Bedouin formed a distinct cluster. When the six Bedouin populations were analysed alone, only subtle traces of substructure were found and there was no clear division according to Adnan or Qahtan (Joktan) lineages. Thus, for forensic purposes, the Bedouin appear to form a single population, distinct from the other geographically-distant populations examined.

PP066
Slovakian Romani, Jats of Haryana and Jat Sikhs Y-STR population data in comparison with other Romani haplotypes and preliminary Y-SNP study for determining Romani haplogroups

Volgyi A[1], Nagy M[2], Henke L[3], Henke J[3], Chatthopadhyay PK[4], Zalán A[1], Pádár Z[5], Füredi S[5], Pamjav H[1]

1) Institute of Forensic Medicine, Budapest, Hungary 2) J. Selye University, Komárno, Slovakia 3) Institut für Blutgruppenforschnung, Köln, Germany 4) Amity Institute of Advanced Forensic Science Research & Training; E-25, Defence Colony, New Delhi, India 5) Institute of Forensic Sciences, Ministry of Interior

Haplotype frequencies for eleven Y-STR markers (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS437, DYS438 and DYS439) in a Romani population (n=63) from Slovakia, Jats of Haryana (n=84) and Jat Sikhs (n=80) from India were determined. The Slovakian Romani, the Haryana and Sikh populations were endogamous based on their unique haplotype ratio and haplotype diversity values, although the Sikh population appeared to be more diverse. AMOVA revealed nonsignificant differences between the Romanies and significant differences with non-Romani populations. The Macedonian Romani population differed from all Romani populations examined. Frequent Y-STR haplotypes observed in Romani populations were sporadic in northwest Indian populations. A mixed Hungarian population was used as reference for statistical analysis. 13 out of 316 populations worldwide were found to share the six most frequent Y-STR haplotypes of the Slovakian Romanies when the screening conditions were narrowed based on the population size to be over 40 males, the occurrence of the haplotypes was more than one and the sum frequencies of the most frequent haplotypes was at least 0.02. The most common Slovakian Romani haplotypes were also observed in other Romani groups. In cases of searching with the most frequent haplotypes of two Indian (Malbar and Malaysian Indian) populations under the same conditions matches could be detected in all Romani populations except for the Macedonian Romanies. The search with the Jat Sikhs and Jats of Haryana most frequent haplotypes resulted no matches in Romani populations. M52 and M82 SNP loci specific for haplogroup H were tested for Hungarian and Slovakian Romani populations.

PP067
Testing algorithms to predict binary states on the human Y chromosome from STR data

Tofanelli S, Taglioli L, Bertoncini S, Caciagli L, Paoli G

Dipartimento di Biologia, Università di Pisa, Pisa, Italia

STR and binary markers covariate on the human Y chromosome. Theoretically, this makes possible the assignments of binary states defining „haplogroups“ only by analysing the combination of STR alleles, or „haplotypes“. Descriptive and inferential methods could be applied once suitable background information is available. We manually-constructed a geographically unbiased database of 3,672 reference Y chromosomes with West Eurasian origin where both, binary and STR data, were contemporary available. One super-haplogroup [F(xK)], 4 haplogroups (I, L, N, Q), 14 sub-haplogroups (E3a, E3b1a, E3b1b, E3b3, G1, G2, I1a, I1b, I1c, J1, J2, R1a, R1b, R2) and 8 among the most widely used set of STR loci (DYS19, DYS389I, DYS389II, DYS390,

DYS391, DYS392, DYS393, DYS388), encompassing barely the 99% of West Eurasian variability, were considered. We designed a Bayesian method implemented in an Excel-based program, WYZARD, to which our database provided the background information to calculate the final posterior distribution. Estimators based on exact matching, Bayesian (Rannala & Mountain, 1997; Badouin & Lebrun, 2001), frequency (Athey, 2004; Paetkau et al., 1995), IAM- (Cavalli-Sforza & Edwards, 1967) and SMM-based (Goldstein et al., 1995) distance algorithms were also computed. The effectiveness of the diverse approaches was validated comparing probability outputs for 193 different haplotypes with Balkan ancestry (Bosch et al 2006) against the true haplogroup. Independently of the method used, haplogroup predictions based on Y-STR data retain 5-15% of incorrect assignments. However, the introduction of standardized set of STR loci in routine hierarchical protocols would shortcut the diagnosis of binary mutations with costs 40-80% lower than standing-alone approaches. This research was supported by a EC INTERREGIIIa grant to G.P.

PP068
The role of Y in identification of war victims in Croatia

Furac I, Masic M, Karija Vlahovic M, Marketin S, Raguz I, Kubat M

DNA Laboratory, Department of Forensic Medicine and Criminology, School of Medicine University of Zagreb, Salata 11, 10000 Zagreb, Croatia

During and after the war in Croatia many war victims' remains have been successfully identified by conventional methods. However, a number of human remains could not be identified by conventional means because either premortal records were missing or remains were badly decomposed. In these cases, DNA analysis has played a significant role especially when reassociation of body parts was necessary or to exclude possible false identification when presumptive identity of the victim was not correct. As the time goes by, DNA analysis is taking over the most important role in the identification process because bodies exhumed 12-15 years after the death could hardly be identified by any other method. In our lab, we usually extract DNA from teeth or bones and type nuclear STR markers using different multiplex kits. But, in some identification cases, even when genomic DNA was successfully amplified, the additional information was still needed for final conclusion. Then we performed Y chromosome or mtDNA analysis. Since the majority of war victims were males, YSTRs analysis was quick and extremely useful method especially when only male relatives were available for testing. For ID cases we use commercial Y-STR multiplex kits. We got started with Y-Plex 5 and Y-Plex 6 (ReliaGene Technologies) followed by DYSplex-1 and DYSplex-2 (Serac) since those were the first multiplexes on the market. Now we use PowerPlex Y (Promega) and/or YFiler (Applied Biosystems). Here we will present several identification cases where YSTRs typing results were very helpful in establishing the identity of the human remains.

PP069
Typing of Y-chromosomal binary polymorphisms in a Finnish population using a chemically structured chip

Braun T, Lutz-Bonengel S, Sänger T, Saukko P, Heinrich M

Institute of Legal Medicine Freiburg, Germany

The analysis of Y-chromosomal polymorphisms has already become a routine technique in most laboratories involved in forensic identity testing and kinship analysis. Although short tandem repeat (STR) loci are the most commonly used markers on the human Y chromosome, an increasing interest in single nucleotide polymorphisms (SNPs) is evident in the field today. The aim of our study was to combine the efficiency of a multiplex approach for SNP-typing with the high sensitivity of a low-volume amplification on a chemically structured chip. For this study, PCR was performed in 1 µL- approaches on-chip followed by a purification using Exo-Sap IT (GE Healthcare, Freiburg, Germany) and minisequencing with the SNaPshot system (Applied Biosystems, Darmstadt, Germany), both performed on-chip. After treatment with SAP (Roche, Mannheim, Germany), the samples were analysed on a 3100 Avant Genetic Analyzer (Applied Biosystems). The distribution of Y-chromosomal haplogroups in a Finnish population will be analysed. The special situation and the particular pitfalls of SNP-typing in a low-volume approach will be discussed.

PP070
Y chromosome diversity in ancient and modern Yakut populations

Amory S[1, 2], Keyser C[1], Bouakaze C[1], Crubézy E[2], Ludes B[1]

1) Laboratory of Molecular Anthropology, Institute of Legal Medicine of Strasbourg, Strasbourg, France 2) Laboratory of Anthropobiology, University Paul Sabatier, CNRS, UMR 8555, Toulouse, France

Yakutia is the largest autonomous Republic of the Russian Federation. This 3 millions km² territory is localized in the north east of Siberia, between Mongolia in the South and the Artic Ocean northward. This vast region is peopled by different ethnic groups from various origins. Among this mosaic of peoples, the Yakuts contrast strikingly from their neighboring groups. Indeed, the Yakuts are semi-nomadic cattle and horse breeders and speak a language composed by Turkic and Mongolic words whereas the surrounding populations are mostly hunters-gatherers and reindeer herders belonging to the Tungustic language family. The very cold and dry climate of this region combined with specific taphonomic conditions (presence of permafrost) and inhumation practices generally induce a good preservation of the bodies and also of the DNA integrity. These characteristics make Yakutia an amazing field of investigation for anthropologists and ancient DNA scientists. An interesting feature of the modern Yakut population is a very low Y-chromosome diversity (Pakendorf et al, 2002). This reduced genetic diversity could be in relation either with a bottleneck or a founder effect and an influence of the Russian colonization during the 17th century has also been suspected but not clearly established. Therefore, the possibility to analyze samples dated from different periods of the pre-colonization era can bring interesting elements to understand this low variability. In this prospect we have studied 40 ancient male subjects from Central Yakutia dated from the 3rd century BC to the 19th century AD and compared the results with 97 modern individuals from the same regions. Sixteen Y-chromosome STRs have been studied using the AmpflSTR® YFiler™ kit. Complete and reliable profiles were obtained for more than half of the ancient specimens. Haplotype diversity among the two sample sets has been evaluated and the data have shown a reduced variability in both groups. Two haplotypes, belonging to the TAT C haplogroup (haplogroup assignation has been made by SNaPshot™ minisequencing of 11 informative SNPs), have been retrieved with very high frequencies in ancient and modern Yakuts indicating a transmission through time of these paternal lineages. Interestingly, all the individuals excavated from the richest graves belonged to one of these two haplotypes. During the Middle Age large clans founded by rich individuals and their male relatives as well as their respective families dispersed through extended territories (Gogolev, 2000). This patrilineal mode of dispersion could have been responsible of the preferential expansion of paternal lineages belonging to rich individuals. Therefore, the comparison between archaeological and genetic data has leaded us to conclude that this reduced variability could have been the consequence of an early founder effect rather than a later influence of the Russian colonization.

PP071
Y Chromosome Polymorphisms in Argentine population

Di Lonardo A M[1], Santapa´ OA[2], Valente SF[2], Filippini SE[2]

1) Banco Nacional de Datos Genéticos, Unidad Inmunología 2) Hospital Carlos G. Durand

Abstract 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, DYS458, DYS635, Y GATA H4, DYS437, DYS456 and DYS448. Multiplex PCR amplification of 16 loci was performed using AmpFℓSTR® Yfiler™ kit (Applied Biosystems) in unrelated Argentine individuals, most of them from Buenos Aires. Statistical interpretation of the results let us create a database of our own population.

PP072
Y Chromosome Variation in a Sample of Kurdish Population

Useli A[1], Castrì L[1], Fabbri C[1], Ferri G[3], Bini C[2], Pettener D[1], Luiselli D[1]

1)Dipartimento di Biologia Evoluzionistica Sperimentale, Lab. Antropologia Molecolare, Università di Bologna, Italy
2)Dipartimento di Igiene e Sanità Pubblica, Sez. di Medicina Legale, Lab. di Genetica Forense, Università di Bologna, Italy
3)Dipartimento di Scienze Morfologiche e Forensi, Università di Modena, Italy

Kurdistan is a wide mountainous region of Western Asia where about 30 millions of Kurds live and represent the principal population. During the millennia, different populations established their rule in this region, in consequence of its geographic position and natural resources. The latest subdivision of this region has occurred in 1923 (Losanna Treaty) when it was annexed to Turkey, Iraq, Iran and Syria. Kurdish population suffered ethnic persecutions and cultural repression by the different central rules that determined Kurdish communities dispersion within and outside Kurdistan. The Kurdish diaspora has involved about 1,5 millions of refugees and migrants that moved towards ex-U.R.S.S. territory, Canada, U.S.A., Australia and Western European countries. Nevertheless this population, speaking an Indo-European language and with still unclear origins, has maintained a marked ethnic, cultural and linguistic identity. Analysis of Y chromosome variability represents an useful tool to infer relationship among populations and their demographic history. In this study, 11 multiallelic markers (STRs) and 34 biallelic markers were typed by different methods (RFLPs, SNaPshot etc.) in a Kurdish population sample composed by 45 individuals, actually living in Italy, but coming mainly from Southern Kurdistan (Iraq); for comparison we also analysed a sample of Persian population (n= 19) from South-Western Iran. Aims of this study were to test if the strong Kurdish ethnic feature corresponds to an homogeneous pool of male chromosomes and to analyze the phylogenetic relationships with populations of the same geographic area. The Y chromosomes typed were assigned to 15 haplogroups spread in Western Asia; haplogroups J and R were particularly represented. Comparison to other Kurdish samples, coming from different geographic areas, has revealed a genetic differentiation probably due to limited genetic flow among tribes. Moreover, the Kurds share the main Y chromosome lineages with the other Middle Eastern populations; these results suggest their participation to the most important demographic events of Middle East and, together with historical data, an ancient settlement of Kurdish population in this region.

PP073
Y chromosome variation in the Oromo and Amhara of Central Ethiopia

Luiselli D[1], Useli A[1], Fabbri C[1], Castrì L[1], Pelotti S[2], Ceccardi S[2], De Stefano GF[3], Pettener D[1]

1) Dipartimento di Biologia Evoluzionistica Sperimentale, Lab. Antropologia Molecolare, Università di Bologna, Italy 2) Dipartimento di Igiene e Sanità Pubblica, Sez. di Medicina Legale, Lab. di Genetica Forense, Università di Bologna, Italy 3) Dipartimento di Biologia, Università di Roma „Tor Vergata“, Italy

Previous studies has indicated Ethiopia as an interesting region situated at the gateway of the „out of Africa“ genetic route. The closeness to the Arabian peninsula made easier gene flows with non-African people, so the composition of the Ethiopian population is the result of a complex and extensive intermixing. Two of the main groups inhabiting the region are the Amhara, descended from Arabian conquerors, and the Oromo, the most important group among the Cushitic people. The basic ancestry of the Amhara is Semitic, like their language, although they intermarried and assimilated some of the Cushitic groups who preceded them in this area. A set of 25 biallelic polymorphisms and 11 STR loci were genotyped in 83 males from the two populations (46 Amhara and 37 Oromo). The aim of the study was to highlight the complexity and substructure of the EthiopianY-chromosome, analyzing two kinds of genetic markers (SNPs and STRs) with different mutation rates. Preliminary analysis of biallelic markers has provided in both populations the following main results: the presence of several Y-chromosomes belonging to the African-specific haplogroup A has confirmed these populations shared a common past with the Khoisan population; the high frequency of E3b subclades suggests the male Ethiopian population is a branch of the East African population; the presence of J*-12f2 is a possible marker of the Neolithic expansion. No significant differences in the haplogroup distribution have been highlighted between Amhara and Oromo (p=0,271). Nevertheless, the presence of E-M34 and K2- M70 chromosomes in the Amhara group underlines the close genetic affinity with the Middle Eastern populations, according to the Arabian origin and suggesting a much larger Eurasian genetic component of the Amhara. STR variance, averaged over eleven loci on binary haplotype background, will be carried out to assess the relative level of diversity and phylogenetic substructure in Central Ethiopia, Eastern Africa and Middle East.

PP074
Effect of soil environment on detectability of PowerPlex Y (Promega) 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) Department of Criminalistics and Forensic Medicine, University of Warmia and Mazury, Olsztyn, Poland

Processes of autolysis and decomposition have always been a concern to forensic specialists. In cases of decomposed bodies Y chromosomal STR markers may be useful in identification of a male relative. The authors attempted to assess capability to type PowerPlex Y (Promega) 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 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 Powerplex Y 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 PowerPlex Y loci were typeable in the latter material stored in sand up to day 35 with gradual decline of longer amplicons (DYS19, DYS385, DYS389II and DYS392).

PP075
Y-Chromosomal STR haplotypes in two mediterranean populations from the iberian peninsula and Morocco

Gaibar M, Santiago C, Gómez-Gallego F, Bandrés F, Esteban E, Moral P, Luna F, Harich N, Fernández-Santander A

Unidad de Biomedicina, Facultad de Ciencias de la Salud, Universidad Europea de Madrid, Madrid, Spain

The non-recombination of human Y-chromosome is transmitted unchanged from father to son except by the accumulation of mutations. Markers on this region will be inherited within a haploid state which makes them a powerful tool to trace easily paternal lineage and to use in human population evolutionary studies. The 17 Y-chromosomal short tandem repeats (STRs) included in the AmpFlSTR® YfilerTM PCR Amplification Kit (Applied Biosystems) (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385I, DYS385II, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 and GATA H4) were typed in two western Mediterranean populations, an Andalusian from Alpujarra (South Spain) and a Berber community from Khenifra (Central Morocco). Typing was achieved using an ABI Prism automatic sequencer. Allele and haplotype diversities were estimated in both populations. The detection of the most common haplotypes in these groups may be useful in forensic special cases and also supplies new data to consider for the study of the genetic history of populations settled on both shores of the western Mediterranean. This work has been supported by the Universidad Europea de Madrid (project OTRI 2006/UEM17).

PP076
Effect of water environment on detectability of Powerplex Y 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) Department of Criminalistics and Forensic Medicine, University of Warmia and Mazury, Olsztyn, Poland

Processes of autolysis and decomposition have always been a concern to forensic specialists. In cases of decomposed bodies Y chromosomal STR markers may be useful in identification of a male relative. The authors attempted to assess capability to type Powerplex Y (Promega) 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 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 Powerplex Y loci within 100 days of storage in pond water with gradual decline at DYS392 in sea water. Heart muscle specimens stored in pond water exhibited allelic loss at DYS19, DYS385, DYS389II and DYS392, while all loci were typeable in sea water stored samples. For lung specimens allelic loss 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.

PP077
Y-SNPs and Y-STRs pitfalls in alleged father-child pairs

Serra S, Vieira-Silva C, Ribeiro T, Geada H

National Institute of Legal Medicine, Lisbon Delegation and Faculty of Medicine, University of Lisbon

Single nucleotide polymorphims (SNPs) have became widely useful in population genetics as they are the most frequent sequence variation encountered in DNA. In the near future, analysis of SNP markers can be important in human identity testing, especially in parentage testing and forensic casework due to SNP low mutation rates and the possibility of analysing short amplicons in severely degraded samples. After performing Y-SNPs in an African origin population from Cabo Verde Islands, the second most important population in our routine casework, a study has been performed in 52 alleged father-child pairs. For characterization of Y-chromosome haplogroups, twenty Y-SNPs - M22, P25, 92R7, SRY1532, M173, M70, Tat, M213, M9, M170, M62, M172, M26, M201, M34, M81, M78, M35, M96 and M123, have been studied in three multiplex reactions, followed by single base extension reactions with the SNaPshot Multiplex Kit. The 12f2 insertion/deletion was detected with PhastGel. Capillary electrophoresis detection of single base extension products was carried on an ABI Prism 3100 Genetic Analyser (Brion et al, Int J Leg Med, 2004). The most frequent haplogroups in children are R1b*(0,3269) and E*(0,3462), including E3a. The clade BCD was detected with a 0,0096 frequency. A special sample – no several Y-SNPs(0.0192), was also detected in this study with no haplogroup assigned. Concerning the 52 alleged father-child study performed with PowerPlex16 and Identifiler, in ten(83%) of twelve exclusion cases, we have detected different Y-SNP haplogroups between the alleged father and the child and identical haplogoups in two exclusion cases(17%). In 40 non-exclusion cases with PI >1.000.000, all haplogroups between the alleged father and the child were identical except for a special case where we have detected a de novo mutation – deletion of AZFb and AZFc regions of Y-chromosome in the child. These deletions were detected when performing Y-SNPs as P25, M70 and M9 were absent in the first multiplex. In multiplex 2, M26 was also absent, where in multiplex 3 no Y-SNPs have been detected ((M96, M35, M78, M81, M123, M34). In this case, Y-STRs have also been studied to try to detect any anomaly in Y-STRs. The study performed with PowerPlexY revealed no DYS385 and DYS392, while that with Y-Filer revealed no DYS385, DYS392 and DYS448. We have typed a set of 20 Y-SNPs and 15 Y-specific microsatellites – ten single nucleotide polymorphisms and three Y-STRs failed to amplify. All Y-STRs and Y-SNPs which have not amplified are located in the AZFb region, except P25 and DYS448 which are located in the AZFc region. These two regions are near by and involve around 7Mb zone in the Y-chromosome. Y-chromosome microsatellites within the AZF regions are well established and are typed by all companies carrying out commercial Y-chromosome tests. Deletions in the AZF regions are associated with male infertility, and as they are not that rare, can rise ethical questions in Forensic Genetics. Although some ethical problems can be raised with these methodologies, it is crucial to face these new challenges with a renewed attitude.

PP078
Y-STR typing in a genealogical study along the Camino de Santiago

Rolf B

Institut für Rechtsmedizin, Frauenlobstr. 7a, D-80337 München, Germany

As a highly valuable indicator of the origin of a man's ancestral paternal lineage, Y-STRs were used in a genealogical project to reveal a potential relationship between an Italian and a Spanish family sharing the same surname. Historical records exposed a pilgrimage of male members of the Italian family to Santiago de Compostela in medieval times after having survived an epidemic pestilence. Nowadays, the same family surname is still found in Spanish individuals living close by the Road to Santiago (Camino de Santiago). Therefore, it was assumed that the Spanish individuals with the same name might be offspring of the pilgrims. Due to the lack of further historical supportive sources the hypothesis of an identical ancestry was inferred by Y-STR analyses of several Spanish and Italian family members.

PP079
SNP-pentaplex as a tool for screening analyses of degradated DNA samples

Babol-Pokora K, Prosniak A, Berent J

Department of Forensic Medicine, Medical University of Lodz, Poland

SNPs serve as excellent biological markers as they occur frequently throughout the genome to the number of over 2 milion, which is why they are known to be the most powerful genetic tools for paternity testing and personal identification in forensic genetics. Moreover SNP genotyping is the best method for analysing evidences from crime scenes, for it doesn’t require large amplicon size, thus increases the possibility of amplification of degradated DNA samples. Although single SNP is not polymorphic enough we can obtain sufficient results by examination of several SNPs. Moreover the general world’s tendency in implementing new markers is to increase the chance of amplification highly degraded DNA using even less polymorphic markers like SNPs, rather than to increase the discriminating power of the current techniques.This paper shows the results of Central Poland population studies with using SNP-pentaplex, containing 5 biallelic loci (rs2294067, rs2282160, rs2070764, rs2277216, rs2101039)and the example of forensic case, impossible to solve by means of standard methods using STR markers, because of highly degradated DNA samples, which was however effectively analysed with using SNP-pentaplex kit. DNA fragments were amplified in one multiplex PCR reaction, which contained 5 primer pairs and SNPs were identified by minisequencing method. The combined PD of the SNP-pentaplex used was 0,990663. The genotype obtained from degradated evidential sample analyse was identical with the profile of the suspect. The genotype frequency was 0,0175. Our research with using SNP-pentaplex revealed the possible presence of suspect’s DNA at a crime scene. Although the discrimination power of 5 SNP is not enough to obtain sufficient evidential value, it seems to be a proper screening method for forensic applications.

PP080
Characteristics of Y-Haplotypes in Kazakhstan

Rothämel T, Turmanov N, Tröger HD

Institute of Legal Medicine, Medical School of Hannover, Carl-Neuberg-Strasse 1, D-30625, Hannover, Germany

Kazakhstan stretches over a vast expanse of central Asia. Though it is the ninth largest country in the world, it is sparsely populated with about 6 persons per sq km. Traditionally, Kazakhs track back their tribal roots for at least 7 generations. Most of them belong to one of three juz: Higher, Middle and Junior juz. Stemming from pastoral nomads, immigrated more than two millennia ago, the population has been shaped by patriarchal structure with patrilocal settlement. We determined the Y-haplotypes of Kazakh males from 4 provinces of Kazakhstan, spanning members of all three juz, to investigate the haplotype distribution between the regions and the juz. Samples and methods Province region capital Zhambyl southern Kazakhstan Taraz: N = 184 Qyzylorda southern Kazakhstan Qyzylorda: N = 100 Shymkent southern Kazakhstan Shymkent: N = 95 Qaraghandy central Kazakhstan Karaganda: N = 95 Among these: Higher juz: N = 234 Middle juz: N = 131 Junior juz: N = 76 Others: N = 33 Lower Saxony, Germany: N = 212 Regional distribution of juz: Taraz (99 %Higher), Qyzylorda (27% Middle, 61% Junior), Shymkent (55% Higher, 28% Middle, 15% Junior), Karaganda (82% Middle, 8% Junior). All samples were typed with Applied Biosystems‘ AmpFlSTR® Yfiler™, with the fragments of 16 Y-chromosomal STRs being separated by capillary electrophoresis (310 Genetic Analyzer). Genetic distances were calculated with ARLEQUIN (V.2; by Stefan Schneider, David Roessli, Laurent Excoffier) Results Most frequent haplotypes: DYS19, 385ab, 389I, 389II, 390, 391, 392, 393, 437, 438, 439, 448, 456, 458, 635, Y GATA H4 Taraz: 23,4% 16 12,13 13 29 25 10 11 13 14 10 10 22 15 17 21 11 Qyzylorda: 14,0% 16 12,12 14 31 25 10 11 13 14 10 11 20 15 17 23 10 Shymkent: 6,3% 16 12,13 13 29 25 10 11 13 14 10 10 22 15 17 21 11 Karaganda: 21,1% 13 13,17 14 29 23 10 12 13 16 10 13 22 16 15 20 11 Lower Saxony: „almost individual“ Diversity index (h; Tajiama 1989) and discrimination capacity (HD, Kayser et al. 1997) in %: Taraz h = 94,23 HD = 61,41 Qyzylorda h = 98,00 HD = 79,00 Shymkent h = 99,15 HD = 78,95 Karaganda h = 94,36 HD = 55,79 Higher juz h = 95,21 HD = 58,92 Middle juz h = 96,93 HD = 60,45 Junior juz h = 96,62 HD = 73,49 Lower Saxony h = 99,99 HD = 99,53 Genetic distances (AMOVA): Qyzylorda Shymkent Karaganda Lower Saxony Taraz 0,098 0,032 0,200 0,146 Qyzylorda 0,085 0,212 0,087 Shymkent 0,181 0,119 Karaganda 0,188 Middle juz Junior juz Higher juz 0,116 0,113 Middle juz 0,159 Discussion The genetic distances between the different tribes within Kazakhstan resemble those distances, found between Kazakhstan and Lower Saxony. There are little genetic distances in case of similar region and juz (Taraz/Shymkent) and large distances with increasing tribal and regional difference (Qyzylorda/Karaganda). There are unique regions with low diversity and characteristic most frequent haplotypes (>=20%; Taraz/Karaganda).

PP081
Y-STR typing of an Albanian population sample using a 17-loci multiplex PCR system

Robino C[1], Inturri S[1], Varacalli S[1], Gino S[1], Di Gaetano C[2], Crobu F[2], Piazza A[2], Grignani P[3], Previderè C[3], Ricci U[4], Torre C[1]

1) Department of Anatomy, Pharmacology and Legal Medicine, University of Turin, Turin, Italy 2) Department of Genetics, Biology and Biochemistry, University of Turin, Turin, Italy 3) Department of Legal Medicine and Public Health, University of Pavia, Pavia, Italy 4) Genetics and Molecular Medicine Unit, University of Florence, A. Meyer Hospital, Florence, Italy

Over the last decade, the analysis of Y-chromosomal STRs has emerged as a powerful tool for paternity testing and forensic casework. More recently, single multiplex PCR assays including new Y-STRs, which complement those comprised in the Y Haplotype Reference Database (YHRD) minimal haplotype (minHt) and the Scientific Working Group on DNA Analysis Methods (SWGDAM) recommended haplotype, have been validated for forensic casework and made commercially available. However, due to the peculiar pattern of inheritance of the Y chromosome, a precise knowledge of the distribution of these extended haplotypes in human populations is required in order to obtain reliable frequency estimates for quantitative assessment of observed matches in kinship analysis and forensic studies. Following a large flow of immigrants from neighbouring Albania during the 1990's, both paternity and criminal cases involving Albanian individuals have lately became a common occurrence in Italian forensic laboratories. Because of past political isolation, population genetics data from this area of Europe have been historically scarce. As a consequence, there is an almost complete lack of information as to the distribution of DNA polymorphisms of forensic interest in Albanians. Bearing this in mind, a population sample of 108 unrelated first generation male Albanian immigrants residing in Italy was typed using the AmpFlSTR Yfiler PCR amplification kit (Applied Biosystems). This multiplex PCR system includes, apart from the minHt loci, the markers DYS456, DYS458, DYS439, DYS635, Y GATA H4, DYS437, DYS438 and DYS448. A total of 95 different 17-loci Y-STR haplotypes were observed in the Albanian population sample and 86 of them were unique. The commonest haplotype was shared by five individuals. A haplotype diversity of 0.996 and a discrimination capacity of 0.880 were calculated. Among the additional markers, gene diversity ranged between 0.743 (DYS458) and 0.549 (DYS439). Significant differences in allelic distribution and gene diversity were observed within single supplementary Y-STR markers when comparing groups of individuals belonging to distinct Y-SNP haplogroups.

PP082
Cluster aggregation of Y chromosome haplotypes in a random sample of 189 Polish citizens studied with AmpFlSTR Yfiler Amplification Kit

Opolska-Bogusz B, Kaczmarczyk G, Piniewska D, Sanak M

Department of Forensic Medicine, Jagiellonian University Medical College, Grzegorzecka 16, 31-531 Krakow, Poland

Human STR loci of the Y chromosome (Y-STR) are frequently co-amplified and haplotyped to test paternal inheritance and to confirm identification in forensic cases. By use of Y-STR a history of a population can be also traced and ethnic diversity studied. The aim of this study was to haplotype 17 Y-STR in a sample of unrelated males of Polish nationality and to look for aggregation of the most common haplotypes into clusters. The place of birth of tested individuals was analyzed for its spatial distribution in relation to the most prevalent haplotype. Y-STR loci: DYS456, DYS389I, DYS389II, DYS390, DYS458, DYS19, DYS385I/II , Y GATA H4, DYS437, DYS438, DYS448, DYS393, DYS391, DYS439, DYS392 and DYS635 were haplotyped in 189 unrelated males (age: 26±1.41 years) using AmpF1STR Yfiler PCR Amplification Kit (Applied Biosystems) and 377 DNA Sequencer. Participants of the study were subjects for paternity testing in the years 2004-2006. Genetic analysis was performed on the DNA samples blinded to the technician. Statistical calculations included gene diversity indices (Arlequin v. 2.0), allelic frequencies at tested loci, cluster analysis of haplotypes (Matlab v. 7) and spatial distribution comparison. Tested sample had 183 different haplotypes with overall haplotype diversity 0.995. Average mismatch between the haplotypes was 8.96 repeats. Cluster analysis based on „cityblock" distance algorithm arranged the genotypes into 30 groups. The most prevalent haplotype group contained 56.6% of individuals and was characterized by alleles (modal:range): DYS456 16:15-18, DYS389I 13:12-15, DYS390 25:24-26, DYS389II 30:29-32, DYS458 16:15-17, DYS19 16:15-17, DYS385I 11:10-12, DYS385II 14:13-15, DYS393 13:13-14, DYS391 10:8-11, DYS439 10:8-12, DYS635 23:23-25, DYS392 10-13, Y-GATA H 12:11-13, DYS437 14:14-14, DYS448 20:19-21. This most prevalent haplotype was reported in the PowerPlex Y Haplotype Database (Promega) with the frequency 0.31% in Caucasians. Out of 6 haplotypes shared in the sample by two different individuals 3 identical pairs of haplotypes belonged to this most prevalent haplotype group. The second most common haplotype group consisted of 13.2% individuals. Twenty out of 30 haplotypes were different enough not to belong to any haplotype group. Spatial distribution of the place of birth for males whose haplotypes consisted the most prevalent haplotype group did not differ from birth locations of the others. However, a limitation of this study is that our sample enrolled mostly inhabitants of the Southern-Eastern Poland. Imperfect repeat alleles were noted in DYS437 (13.1), DYS385I/II (14.2; 17.2) and were present as a single copy alleles. No duplications in loci other than DYS385 were observed. Multiplex Y-STR typing proved useful method for forensic

studies in the Polish population. Relatively high frequency of haplotypes within the common haplotype group, however, requires a cautious reasoning. The most common haplotypes were shared by 3.17% unrelated individuals, and composed 5.6% of the most prevalent haplotype group.

PP083
Y-STR typing of large families using a 17-loci multiplex PCR assay

Caenazzo L[1], Ponzano E[1], Rampazzo A[2], Verzeletti A[3], Cerri N[3]

1) Department of Enviromental Medicine and Public Health – University of Padua –Italy 2) Department of Biology – University of Padua –Italy 3) Department of Forensic –Medicine University of Brescia – Italy

Because of their unique transmission properties and male specificity, analysis of Y chromosome haplotypes have become an important tool in forensic investigation. Y-chromosomal STR haplotypes were determined from male individuals belonging to large Italian families using the AmpFlSTR Yfiler PCR amplification kit (Applied Biosystems) that coamplifies 17 Y-STRs. The aim of the present study is to typing the male haplotype in families with a lot of members to highlight possible mutation transmission in the male lineage.

PP084
Diversity and Mutation Analysis of Minimal Haplotype Y-Microsatellites Loci Plus DYS447, DYS458 and DYS464 in Alagoas, Northeastern Brazil

De Azevedo DA[1], Da Silva LAF[2], Barbosa ABG[1], Mauricio-da-Silva L[1]

1) Department of Genetics, Federal University of Pernambuco,BR 2) Forensic DNA Laboratory, Federal University of Alagoas,BR

The State of Alagoas is located in Northeast Brazil and the population of this region can be characterized by intense admixture of three ethnical groups, Caucasian, African and Native Amerindians. In this work, we studied the nine microsatellites loci of the minimal haplotype (DYS19, DYS385, DYS389I and II, DYS390, DYS391, DYS392 and DYS393) plus DYS447, DYS458 and DYS464. Blood samples from 255 unrelated males and his sons were provided from the paternity caseworks of the whole State of Alagoas, with personal consent. DNA was extracted through Chelex method. The amplifications were performed in four PCR reactions: 1) DYS19, DYS389I and II; 2) DYS392, DYS393; 3) DYS385, DYS390, DYS391; 3) DYS447, DYS458, DYS464. Each PCR reaction used 20 ng of genomic DNA, in a total reaction volume of 25 µl, using published primers. PCR cycling protocol was: 95° C, 2min; 28 cycles: 94° C, 1 min.; 56° C, 2 min.; 72° C, 30 min. Amplified DNA was analyzed by denaturing 6% polyacrylamide gel silver stained. Home made allelic ladders were used for allele designation. For the minimal haplotype, loci allele calibration was made by using the 9948 male DNA control (Promega Corporation). For DYS447, DYS458 and DYS464 allele designation were confirmed by sequencing at least two alleles of each locus. Analysis of data was carried out using Arlequin ver. 2000. Gene diversities (GD) and haplotype diversities (HD) were estimated according to Nei. A total of 230 haplotypes were observed, where the three most frequent ones occurred three times, 19 occurred two times and 207 were represented only once. In the same sample for minimal haplotype, the most frequent haplotype was observed 16 times. The markers DYS464 and DYS385 were the most polymorphic ones, with a GD respectively of 0.9398 ± 0.0080 and 0.8930 ± 0.0147. The most polymorphic single copy was DYS458 with a GD of 0.7756 ± 0.0129. The HD increased considerably when the DYS447, DYS458 and DYS464 loci was added to the minimal haplotype loci. Eight mutations were observed among 3,825 allele transmitions. All mutations were confirmed by reanalysis and, for DYS390 and DYS458, by DNA sequence analysis, and they were found to have occurred inside the repetitive sequence structure. Only one step mutation was observed and find in only one locus for the same father/son pair. The overall mutation rate estimate, across the 15 loci, was $2.09 \times 10\text{-}3$ (95% confidence interval (CI) $9.03 \times 10\text{-}4$ to $4.11 \times 10\text{-}3$). The data show that the haplotype composed by this markers set is highly informative and discriminative for male lineages in Alagoas, with potential for application in forensic casework. This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq

PP085
Forensic Y-STR DNA Analysis: Casework Experience in Rio de Janeiro, Brazil

Silva DA[1, 2], Neves-Manta FS[1], Carvalho EF[1]

1) Laboratório de Diagnósticos por DNA, Departamento de Ensino de Ciências e Biologia, Instituto de Biologia, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brasil 2) Departamento de Patologia e Laboratórios, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Brasil

Y-chromosome short tandem repeats (Y-STR's) are useful forensic DNA markers in investigation of sexual assault cases when a mixture of male and female DNA is present in a sample when DNA of the male contribuitor is present only in very small amount compared to the DNA of female victim. This work presents the methods and the frequency statistics were kept for seven years on sexual assault cases, in the DNA Diagnostics Laboratory, State University of Rio de Janeiro, Brazil. The statistics included types of cases, types of samples, levels of samples success and failure, rates of failures to exclude, and match statistics using a Y-STR database. The most repercussion cases are presented to ilustrate the usefulness of Y chomosome specific human DNA markers. On the basis of Y-STR analysis, low sample failure rate was observed, the data collected from Y-STR haplotypes developed in casework showed extremely high diversity. Y-STR forensic analysis was succefully applied to different types of samples even to contaminated and minimal samples. Support: Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (Faperj). Programa DNA – Universidade do Estado do Rio de Janeiro e Tribunal de Justiça, Brasil.

PP086
Exclusion criteria for Y chromosome datasets

Myres NM, Ritchie KH, Angerhofer NR, Ekins JE, Hughes RE, Nelson AA, Perego UA, Woodward SR

Sorenson Molecular Genealogy Foundation

Y-chromosome haplotype datasets are typically compiled from males who report themselves as paternally unrelated. However, self reported relatedness can be unreliable due to various religious, cultural, and/or historical factors that can limit a subject's knowledge concerning his true genealogy. Consequently, datasets may contain related males and/or exclude unrelated males resulting in statistically skewed samples for population genetics studies and forensic databases. Herein, we use documented genealogical records correlated with 37 Y-STR haplotypes from 1,200 males of European descent to assess the degree of allelic identity among individuals of various levels of paternal relatedness. We additionally present criteria by which haplotypes can be included or excluded with confidence from a Y-chromosome dataset.

PP087
Genetic Attributes of the YHRD Minimal Haplotype in 10 Provinces of Argentina

Marino M, Sala A, Corach D

Servicio de Huellas Digitales Genéticas and Cátedra de Genética y Biología Molecular, Faculty of Pharmacy and Biochemistry, University of buenos Aires, Argentina.

In the last few years, the Y-STRs became a powerful complement for the DNA-based forensic identification procedures. Due to the inherent attributes of the Y chromosome: lack of recombination ability and, hence, patrilineage inheritance, the Y-STRs allows to identify a male lineage but not individuals. The source of variation is provided by mutation and its rate is similar to that exhibited by the autosomal STRs. This may provide a sensitive tool for addressing recent historical events. Nowadays population of Argentina is the result of the genetic contribution of diverse ethnic groups, mainly aboriginal and European. The beginning of the admixture process can be precisely dated in 1536, when the first Spanish conquerors settled in the territory that is, at present, occupied by Argentina. In less than 20 generations a remarkable change occurred in the demography of the region. Since these markers has been used routinely in forensic casework, since 1996 in our lab, the quantity of available haplotypes allowed us to analyze their genetic attributes in a countrywide extent. Nine Y-STRs, representing the minimal YHRD haplotype, were investigated in 959 unrelated males from 10 provinces of Argentina (Buenos Aires, Santa Fé, Mendoza, Río Negro, Chubut, Corrientes, Chaco, Formosa, Misiones and Salta). Additionally, three different Amerindian tribes were included as out groups. Members of the Guaraní tribe (N=68), from the Misiones province, and Tobas (N=32) and Pilagas (N=45) tribes, from the Formosa province, were selected. Haplotype frequency distribution was determined either for Argentina as a whole or for each population. This parameter was also determined for the Amerindian populations in order to establish if geographical differences were detectable. 609 different haplotypes, out of 959 individuals, representing 63.5% were detected. Two of them denoted relatively high frequency (~3%). The overall Argentinean population

exhibited a considerable Gene Diversity (GD= 0.996), similar to the European populations. However, when the GD values of the populations were compared, the Northern and the aboriginal groups denoted lower GD values. By AMOVA it was determined the extent of population stratification. No significant differences were detected among the populations. But, when the Amerindian populations are also compared, the differences became significant. Genetic distance determined by Rst, showed three clusters, one of them, included Southern region (Chubut and Río Negro provincies) together with Santa Fe province (Center), the second one included Central and Northern region provinces (except Santa Fe) and the last one included the aboriginal groups, with no significant differences among them. The results presented herein showed the impact of the European male genetic contribution on the native aboriginal populations. It has been shown, by Y-SNP typing, that only about 17% of the male lineages are of Amerindian ancestry. Accordingly, it becomes apparent that a more informative platform might be required for forensic purposes as that offered by the combined use of Y-STRs and Y-SNPs, as may be provided by the YHRD in a near future.

PP088
Multiplex miniSTR System of Chromosome Y for Forensic Science

Yun LB, Li YB, Wu J, Yan J, Zhang J, Hou YP

Institute of Forensic Medicine, Sichuan University (WCUMS),Chengdu 610041, China

Analysis of degraded DNA samples is a great challenge for forensic genetics. It is expected that reduced-size STR amplicons, so-called „miniSTR“ assays can recover information from degraded DNA samples. The purpose of this study is to establish a multiplex system with three miniSTR loci on chromosome Y and to provide a new technology for testing degraded DNA samples. We redesigned the primers of three loci DYS385a/b, DYS389I/II and DYS392 to reduce the size of amplicons for developing miniSTR assay on chromosome Y, that was miniY-STR. The allelic ladder for eachminiY-STR locus was prepared by simplex PCR with redesigned primers. A multiplex PCR was carried out using the primers of three loci. The PCR products were detected by ABI PRISM 310 Genetic Analyzer. A series of validation experiments were performed for the miniY-STR multiplex system according to the recommendation of the Scientific Work Group DNA Analysis Methods (SWGDAM). A miniY-STR multiplex system with three miniY-STR loci, DYS385a/b, DYS389I/II and DYS392, was constructed. The results showed that this system had excellent male and species specificity. The results of DNA typing with the miniY-STR multiplex system were in accordance with that of PowerPlex® Y kits. The results of the sensitivity studies showed that DNA template in a range from 0.25ng to 30ng could be analyzed by the miniY-STR multiplex system. For the male/male mixtures test, the minor component in the mixtures could be identified up to a ratio of 1:9. The miniY-STR multiplex system can obtain complete DNA information from some of degraded DNA samples, which can not be gotten by the commercially available kit.

PP089
Fine mapping of a polymorphic 3Mb deletion at Yp11.2 of Chromosome Y in East Asian (Malaysia and Singapore) populations confirms common ancestral origins

Yong RYY[1], Gan LSH[1], Chang YM[2], Yap EPH[1]

1) Defence Medical & Environmental Research Institute, DSO National Laboratories, 27 Medical Drive #12-00, Singapore 117510 2) Forensic DNA Laboratory, Department of Chemistry, Petaling Jaya, Malaysia

Y-STR haplotyping studies revealed the phenomenon of Amelogenin Y null allele in both Malaysia and Singapore populations. The frequency of Amelogenin Y null allele is 1.7% and 0.55% in Singapore Indian and Malay, and 3.2% and 0.6% for Malaysian Indian and Malay respectively. This null allele was not detected in 210 Singapore Chinese, nor 331 Malaysian Chinese. Y-STR data confirmed all Amelogenin Y null samples were of independent individuals. Majority of them are of haplogroup J2e1, while haplogroups F* and D* were also observed, suggesting common founders, but at least 3 independent deletion events. Low resolution STS mapping confirmed a large deletion of about 3 to 3.8 Mb size located at the Yp11.2 region, eliminating at least three transcripts in this region, including Amelogenin Y (AMELY), Transducin-beta-like 1 Y-linked (TBL1Y) and Protein Kinase Y-linked (PRKY). This deletion amounts to about 12% of the euchromatic Y chromosome. High resolution mapping suggested the deletion mechanism likely to be non-allelic homologous recombination involving the TSPY (Testis-specific Protein Y) minor and major arrays. The 5’ breakpoint has been resolved to within 400bp to ~ 4kb per resolution. Samples of similar haplogroup shared similar 5’ breakpoints, further supporting common ancestry origin for each deletion. Further work is in progress in attempting to resolve breakpoints to nucleotide level, and the extent of TSPY repeat variation in local populations. This work has confirmed the necessity to include other sexing marker besides Amelogenin XY for gender determination in our regional populations. This work also provided evidence to support common ancestral origin of this large scale deletion, but also supported at least 3 independent deletion events. The normal phenotype of these individuals suggested the redundancy of the 3 protein homologues in chromosome X. This deletion event could act as an example of structural variation of

human chromosome Y. Understanding it to sequence level helps to add information to present knowledge / hypothesis of genomic DNA rearrangements due to higher order genomic architecture.

PP090
Application of low copy number Y-STR typing to degraded skeletal remains

Irwin JA[1], Loreille OM[1], Los CW[1], Leney M[2], Barritt SM[1], Haliniewksi DE[1], Coble MD[1], Parsons TJ[1,3]

1) Armed Forces DNA Identification Laboratory (AFDIL), 1413 Research Blvd., Rockville, MD 20850, USA 2) Joint POW/MIA Accounting Command- Central Identification Laboratory (JPAC-CIL), 310 Worchester Ave. Bldg 45, Hickam AFB, HI 96853-5530, USA 3) International Commission on Missing Persons, Alipasina 45 A, 71000, Sarajevo, Bosnia

One of the primary missions of the Armed Forces DNA Identification Laboratory (AFDIL, Rockville, MD, USA) is to assist in the identification of degraded skeletal remains believed to be associated with missing U.S. military personnel, by employing DNA technologies. Due to the highly degraded nature of these samples, AFDIL typically sequences the mtDNA hypervariable (HV) regions. However, on occasion, no maternal references with which to compare mtDNA sequence can be obtained. In these unique cases, alternative DNA typing methods must be employed to assist the identification efforts. The application of low copy number (LCN) STR typing to forensic casework samples has been previously described, and LCN protocols for autosomal loci are now in regular use in some forensic laboratories (1). Using this previous research as a guide, we have successfully developed and implemented a low copy number (LCN) DNA approach for Y-STRs to assist in the typing of degraded skeletal remains encountered during routine casework. By increasing both the concentration of Taq polymerase and the number of amplification cycles recommended for the AmpFlSTR® Yfiler™ PCR Amplification Kit (2), we have obtained Y-STR profiles from degraded skeletal remains submitted to our laboratory. The LCN Y-STR typing protocol has been applied to two interesting cases recently processed at AFDIL. In the first case, AFDIL obtained a Y-STR profile from skeletal remains linked to the Vietnam War, and compared the results to the Y haplotype from the son of the missing individual. In the second case, the LCN Y-STR protocol was used to assist with sex determination of a set of skeletal remains which were previously believed, through anthropological analysis, to be female. Each of these cases demonstrates that the utility of low copy number Y-STR typing in the identification of degraded skeletal remains. In the future, AFDIL will likely utilize LCN Y-STRs as part of a series, or panel, of DNA typing methods which may consist of mtDNA HV sequencing, LCN STRs (i.e. PowerPlex16™), and/or multiplex mtDNA SNPs. The employment of these methods will likely provide the required discrimination to exclude degraded skeletal remains from, or confidently associate them with, available reference specimens in order to identify the missing. 1. Gill, P. Application of Low Copy Number DNA Profiling. Croatian Medical Journal 2001;42(3):229-232. 2. Mulero JJ, Chang CW, Calandro LM, Green RL, et al. Development and Validation of the AmpFlSTR® Yfiler™ PCR Amplification Kit: A Male Specific, Single Amplification 17 Y-STR Multiplex System. J Forensic Sci. 2006;51(1):64-75.

PP091
Mutation events at 12 Y-STR loci among German father-son pairs

Schulz I[1], Proff C[2], Schneider PM[2], Rothschild MA[2]

1) Institute of Legal Medicine Munich 2) Institute of Legal Medicine Cologne

Knowledge about reliable mutation rates of Y-chromosomal microsatellites are indispensable tools for the correct interpretation of genetic profiles in paternity testing and forensic casework. In the present study we analyzed 270 German father-son pairs at 12 Y-chromosomal STRs using one singleplex polyermase chain reaction for YCAII and the commercial PowerPlex® Y system (Promega, Mannheim). Nine single step mutations were observed at DYS391, DYS439, DYS19, DYS385, DYS390, DYS389II and YCAII. Furthermore, two duplications at DYS437 and DYS389I and a „null allele" at DYS389II were found. In total, 263 haplotypes were identified in 270 pedigrees.

PP092
Northern and eastern Africa: a Y-chromosome-based phylogeographic analysis

Cruciani F[1], La Fratta R[1], Trombetta B[1], Santolamazza P[1], Sellitto D[2], Beraud Colomb E[3], Dugoujon JM[4], Scozzari R[1]

1) Università „La Sapienza", Dipartimento di Genetica e Biologia Molecolare, Rome, Italy 2) CNR, Istituto di Biologia e Patologia Molecolari, Rome, Italy 3) Hopital de Sainte-Marguerite, Marseille, France 4) Centre d'Anthropologie, CNRS, Toulouse, France

The screening of 27 new and 74 previously described Y chromosome single nucleotide polymorphisms in 893 male subjects from 23 populations in northern and eastern Africa resulted in the detection of 40 different binary haplogroups. The analysis of molecular variance revealed a high and significant degree of Y-haplogroup interpopulation diversity ($\Phi st = 0.23$, $P<0.0001$). Upon grouping of the populations according to a geographic criterion, we obtained a $\Phi ct = 0.12$, ($P<0.0001$) and a $\Phi sc = 0.16$ ($P<0.0001$), indicating a high level of heterogeneity both among and within groups. The northeastern group of populations showed the highest degree of internal variation, a finding that could be only partially explained by genetic drift alone. The majority of the populations analyzed speak languages belonging to 4 different branches of the afroasiatic linguistic family (berber, semitic, cushitic and omotic). When afroasiatic speaking populations were grouped following their linguistic affiliation, we observed a low and not significant level of apportionment of the variance between groups, suggesting that the afroasiatic linguistic branches spread independently with respect to genes. Haplogroup E-DYS271, a haplogroup commonly found in sub-Saharan Africa, was found in most populations from northern Africa at frequencies around 5%. However, the analysis of 11-microsatellite-based network showed a very different haplotype distribution among the two regions, a finding consistent with the occurrence of relatively old trans-Saharan human movements. Finally, the overall phylogeographic profile of E-M78 chromosomes revealed geographic partitions of sub-haplogroups that may indicate source and direction of human migrations between eastern and northern Africa.

PP093
Y-Chromosome-Surname Coinheritance in Ireland

McEvoy B, Bradley DG

Trinity College, Dublin 2, Ireland

The Y-chromosome has proved a useful means of exploring the origin and history of paternally inherited Irish surnames. It is clear from these studies that men of the same surname are substantially more likely to share a Y-chromosome than the random population, a consequence of the foundation of most Irish surnames by a single or limited number of males ca. 1000 years ago. The shared paternal ancestry of surnames may also be useful in a forensic context by allowing some degree of surname prediction or exlusion from male DNA samples. However, such a potential application is complicated by both multiple Y-chromosomes within a single surname and the sharing of Y-chromosomes between surnames. An important consideration in the latter case may be pre-exisiting patrilineal kinship within early medieval (ca. 500AD) tribal groupings as many surnames may have emerged from each. This possibility is already known to substantially affect haplotype sharing in the Northwest region of Ireland. We have examined early medieval patrilineal population structure and surname inter-relationship using 17 STR Y-chromosome haplotypes in 250 men from southwest Ireland. We are currently investigating haplotype sharing in 1400 men encompasing multiple samples from 70 Irish surnames to assess the prospects for Y-chromosome/surname co-inheritance as a forensic tool.

PP094
Y-STR haplotypes in Amerindian cromosomes from Mexican populations: genetic evidence to the dual origin of the huichol tribe

Rangel-Villalobos H[1], Sandoval-Ramírez L[4], González-Martín A[2], Muñoz-Valle JF[3], Figuera LE[4], Ibarra B[4], Nuño-Arana I[1], Páez-Riberos LA[1]

1) Laboratorio de Genética Molecular, Centro Universitario de la Ciénega, Universidad de Guadalajara (CUCiénega-UdeG), Ocotlán, Jalisco, México. 2) Departamento de Zoología y Antropología Física, Universidad Complutense de Madrid (UCM), Madrid, España. 3) Laboratorio de Inmunología, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Jalisco, México. 4) División de Genética, Centro de Investigación Biomédica de Occidente (CIBO-IMSS), Guadalajara, Jalisco, México.

We studied six Y-linked short tandem repeats (Y-STRs) to describe the internal diversity of the Amerindian haplogroup Q-M3 in 129 males from eight Mexican populations. The low gene diversity in the Huichol tribe demonstrated the effects of genetic drift, attributable to geographic isolation and founder effect. The presence of two principal paternal lineages supported historical and anthropometric records, which indicate that Huichols were formed by the fusion of two ancestral Mexican tribes. Moreover, genetic distances and close relationships of haplotypes between Huichols and Tarahumaras were in agreement with their linguistic affiliation. The high genetic diversity of the Purépechas and wide distribution of haplotypes along the constructed network-joining tree suggest that the present genetic composition was influenced by Purépecha dominance in western Mesoamerica. The Y-haplotypes shared between populations suggest that, among the Amerindian tribes studied herein, the paternal genetic pool of Nahuas could have contributed more importantly to the European-admixed population, the Mexican-Mestizos.

PP095
Inferring the population of origin of DNA evidence within the UK by allele-specific hybridization of Y-SNPs and Y-STR profiling

Wetton JH, Tsang KW, Khan H

The Forensic Science Service Ltd, R&D, Trident Court 2960, Solihull Parkway, Birmingham Business Park, Solihull B37 7YN, UK

Marked differences in Y-SNP allele frequencies between continental populations can be used to predict the biogeographic origin of a man's ancestral paternal lineage. Using 627 samples collected from individuals within the UK with pale-skinned Caucasian, dark-skinned Caucasian, African/Caribbean, South Asian, East Asian or Middle Eastern appearance we demonstrate that an individual's Y-SNP haplogroup is also strongly correlated with their physical appearance. Furthermore, experimental evaluation of the Marligen SignetTM Y-SNP kit in conjunction with the Luminex 100 detection instrument indicates that reliable and reproducible haplogrouping results can be obtained from 1 ng or more of target template derived from a variety of forensic evidence types including, blood, saliva and post-coital vaginal swabs. The test proved highly male-specific with reliable results being generated in the presence of a 1000-fold excess of female DNA, and no anomalous results were observed during degradation studies despite a gradual loss of typable loci. Hence, Y-SNP haplogrouping has considerable potential forensic utility in predicting likely ethnic appearance. Y-STR haplotypes were also found to cluster within haplogroups and offer an alternative route to ethnic prediction with the additional advantages of higher discrimination power between individuals, easier detection of mixtures and greater suitability for small batch sizes. Furthermore, haplotype sub-clusters revealed biogeographic sub-structuring beyond the resolution of the available Y-SNPs.

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Organiser
Institute of Legal Medicine, Innsbruck Medical University
Director Prof. Dr. Richard Scheithauer
Muellerstrasse 44
6020 Innsbruck, Austria
Phone +43 512 9003 70600
Fax +43 512 9003 73600
Email gmi@i-med.ac.at
Web www.gerichtsmedizin.at