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Developments in DNA sequencing methods have advanced rapidly over the past few years, and new technologies that produce large volumes of data at low cost (relative to current platforms) are being broadly applied to various questions in medical genetics, evolutionary biology, molecular anthropology and genomics. These so-called next generation sequencing (NGS) technologies present an entirely new paradigm for DNA sequence data generation and have the potential to completely redefine the acquisition and utility of genetic data in a variety of forensic casework scenarios.

In order to assess the utility of NGS for missing persons applications, we have recently initiated a study of various platforms and target enrichment strategies for sample types regularly encountered in our large-scale identification efforts. Specific laboratory workflows based on target marker enrichment and NGS platform are being considered for different sample types, and the overall effort is being undertaken with a strong emphasis on both raw data and final consensus sequence quality. The current study is first and foremost a general evaluation of these technologies from the standpoint of forensic application, yet the strategy we are pursuing is ultimately intended to facilitate NGS integration into standard casework laboratories. We are, therefore, evaluating NGS workflows and data for the standard nuclear and mitochondrial DNA markers used in forensics, while still allowing for future work that may take greater advantage of the strengths of these technologies. Here, we present an overview of our NGS strategy, along with the preliminary results of these efforts.

O 02 SHORT TANDEM REPEAT SEQUENCING ON THE 454 PLATFORM

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The development of reduced size amplicons for short tandem repeat (STR) markers has increased the success of typing samples with degraded DNA (Butler et al. 2003, Coble and Butler 2005, Grubwieser et al. 2006, Hill et al. 2008). However, when separating these alleles using capillary electrophoresis, the number of loci that can be multiplexed together is limited by the number of available dyes. Those loci with overlapping size ranges must either be labeled with different dyes or amplified in separate multiplexes (Hill et al. 2008). One potential solution to this limitation of standard chemistries and detection platforms is next generation sequencing technology based on clonal amplification followed by emulsion PCR and pyrosequencing (Margulies et al. 2005). With this strategy, many loci with overlapping amplicon size ranges can be multiplexed in one reaction, which allows conservation of valuable sample extract.

In order to investigate the feasibility of next generation sequencing technology for the multiplex detection and sequence production of short tandem repeats, thirteen STR markers (CSF1PO, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D13S317, D16S539, D21S11, D22S1045, TPOX, and vWA) were amplified using an optimized multiplex reaction (Pitterl et al. 2008) with primer sequences designed for reduced size amplicons (Butler et al. 2003, Coble and Butler 2005). Each sample multiplex was barcoded with a different sample-specific multiplex identifier (MID) for subsequent parallel tagged sequencing on the GS Junior System (454 Life Sciences, Branford, CT). Here, we present the results of our preliminary data assessments.

O 03 A NEW ULTRADEEP LT (LOW TEMPLATE) DNA PROFILING APPROACH BASED ON AN EMULSION-BASED CLONAL AMPLIFICATION OF AN STRS MULTIPLEX PCR PRODUCT FOLLOWED BY MASSIVE PYROSEQUENCING

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Due to an insufficient quantity and quality of the template DNA and often to the presence of PCR inhibitory compounds, frequently, evidentiary items collected at the crime scene do not allow to obtain complete or even partial DNA interpretable profiles using standard forensic STRs DNA analyses. This has led to introduce several modifications to the traditional STRs genotyping methodologies known as LT DNA profiling which often allow to recover all the genetic information even from a trace containing DNA at or beneath the stochastic threshold. Nonetheless LT DNA profiling suffers of several disadvantages known as stochastic sampling effects (exaggerated stutter, relevant peak height imbalance, allelic drop-out and allelic drop-in).

In an attempt to overcome these drawbacks and to improve the sample DNA limit of detection of the new STRs multiplexes kits we developed a new approach involving a first step of multiplex PCR amplification by the new generation kits and an emulsion-based clonal amplification of the PCR products followed by a massively parallel pyrosequencing by the FLX-Titanium Genome Sequencer (Roche/454 Life Sciences). This new approach was conducted over several LT DNA samples (both pure DNA and DNA deriving from saliva and blood samples and real traces) at various dilution factors (down to 6 pg) and in comparison with the conventional new generation multiplexes. It revealed capable of genotyping each sample providing in about the 90% of samples full interpretable STRs DNA profiles quantitatively giving, for each sample, the complete STRs alleles population enclosed in a PCR product.

O 04 VALIDATION STUDIES ON THE APPLIED BIOSYSTEMS 3500 GENETIC ANALYZER

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The need to internally validate new technology is an on-going process within the forensic DNA community. Rapid technology developments require confirmation that the new platform or method generates reliable and reproducible results. The Applied Biosystems 3500 genetic analyzer is the newest capillary electrophoresis (CE) instrument that is available to the forensic DNA community. An internal validation was performed using several commercial short tandem repeat (STR) multiplex kits.

Validation experiments to evaluate the performance of this CE instrument consisted of studies/evaluations of precision, sensitivity, genotype concordance, two-person mixtures, and signal normalization. Precision was assessed through multiple injections of the appropriate STR kit allelic ladder, and base pair sizing was evaluated within a single injection (8 capillaries) and across multiple injections. Duplicate injections of amplified DNA template ranging from 1.0 ng down to 0.01 ng were evaluated to establish analytical and stochastic thresholds. Genotype concordance was established through parallel injections on a 3130*x*/ instrument. Two samples, heterozygous at more than 13 loci were evaluated for the mixture study in combination ratios of 1:1, 1:2, 1:3, 1:5, 1:7, 1:9, and 1:10. Lastly, a normalization factor was determined and the application was examined with sample data.

O 05 LASER CAPTURE MICRODISSECTION IN FORENSIC RESEARCH

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Laser capture microdissection (LCM) represents a significant improvement in cell separation methods and offers a valuable tool to generate single-donor cell populations from a mixed trace in order to facilitate DNA typing and identification. Here, an overview will be given of the numerous applications of LCM in forensics. The use of LCM in sexual assault cases is well established for the isolation of spermatozoa from vaginal smears, male cells from male/female mixtures and in DNA-based paternity testing on abortion material of sexual assault victims. In addition, LCM can be very useful to collect single hair follicles, lymphocytes or other cell types mixed with debris or other cells, skin cells from adhesive tape, etcetera.

When working with LCM, special care needs to be taken for staining and DNA extraction protocols. The discriminatory power of different specific and non-specific cell staining protocols as well as their influence on DNA quality will be discussed. Moreover, as the search for desired cells on a slide can be quite time-consuming, image analysis software for automatic detection of target cells will be reviewed. Finally, different DNA extraction protocols to be used on LCM samples will be evaluated, since an important challenge lies in the development of a protocol appropriate for the low amount of cells isolated by LCM.

Despite the technical peculiarities that will be thoroughly discussed, LCM offers a indispensable tool for the investigation of unfavourable forensic mixtures and it is certain that new forensic applications of LCM will be developed in the near future.

O 06 HOT FLAKES IN COLD CASES

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In forensic casework it is sometimes impossible to separate the DNA from an undefined number of different individuals in complex mixed stains. Extracts from such samples obtained, for example by a blanket swabbing method, often contain complex mixtures of ambiguous and/or uninterpretable DNA profiles. Using the method described of comprehensive and targeted selection of single shed cells adhering to tapings of garments or other objects enables the individual components of such mixed stains to be identified. The method has been validated for use in routine casework and has been shown to be rapid, sensitive and reproducible. It can be predicted that many unsolved cases with body tapings which have been archived and not yet examined will benefit from this new or perhaps more appropriate, reanimated technical development. Of particular importance are unsolved serious crimes, the so-called cold cases. The remarkable forensic value of this simple technique is exemplified by some high profile rape- and murder cases – including cold cases – which have already been successfully solved using this approach.

O 07 SINGLE SKIN FLAKES ANALYSIS WITH MITOCHONDRIAL DNA

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As DNA methods improved over the last years typing of skin cells and of single skin flakes becomes more and more important. Using single skin cells or flakes in DNA typing gives a higher success rate in typing individual DNA profiles and thus better possibilities in searching suspects. Besides STR-typing analysis of mitochondrial DNA (mtDNA) can be used for further investigations especially for prior criminal cases ("cold cases") as there are a lot of skin flakes deposited not analysed by now.

In our study we tried to discover the differences and the possible advances of mtDNA typing of single skin cells in crime scenes investigations. Therefore several subjects handled a piece of clothing to leave skin flakes on the surface. Detection and separation of the skin flakes was carried out by binocular microscope. Besides STR typing with a homemade mini-STR PCR (Q11) the skin cells were analysed by sequencing the control region.

Comparing the typing success of STRs and mtDNA sequencing there was a slightly higher typing success for mtDNA typing. Good typing results for STRs led to good typing results for mtDNA. However a minor fraction of single skin flakes leading to poor STR results delivered good mtDNA typing results. Therefore mtDNA typing is a good option to improve the success rate of DNA typing single skin flakes.

Despite the gain of more information at DNA level it is clear that mtDNA has a minor power of individualisation and thus can only be an "add-on" for special cases.

O 08 A FORENSIC DNA PROFILING SYSTEM FOR NORTHERN EUROPEAN BROWN BEAR *(URSUS ARCTOS)*

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The protected brown bears (Ursus arctos) of Northern Europe are often involved in conflicts with humans, livestock depredation as well as subjected to illegal hunting. STR markers are the preferred forensic tools applied in wildlife crime cases and may be used for traceability and as tools for population management. Thus, a validated STR profiling system according to forensic standards is suggested. We have estimated allele frequencies and analysed repeat structure of 13 STR loci (G1D, G10B, Mu05, Mu09, Mu15, Mu26, G1A, G10L, Mu10, Mu23, Mu50, Mu51, Mu59) in 479 individuals of eight Northern European brown bear populations. STR analysis of hair- and faecal-samples (> 5000) collected in the field as well as tissue samples from shot bears (93) were used to genotype the individuals. The success rate for samples collected in the field was approximately 70%. Species specificity testing showed no false positive bear genotypes. These results show that hairs and faecal samples represent an excellent source for bear DNA that may be utilized to sample allele frequency estimates from living populations. For the eight different populations (four from Norway, one from Sweden and one from Finland and two from Northwest Russia) we have determined the observed and expected heterozygosities, departures from Hardy-Weinberg equilibrium, population substructures and probabilities of identity. Our results suggest that samples can be assigned to a particular individual if using a combination of ten or more of the validated markers in this brown bear DNA profiling system.

O 09 WHAT WAS IT? – MICROFLUIDIC SCREENING ASSAY FOR COMMON EUROPEAN ANIMAL FAMILIES

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Enabling a precise control of minute liquid volumes in microstructured channels and reaction chambers, microfluidic reaction platforms offer great potential for biomedical applications. In forensic genetics, enhanced sensitivity by reduced reaction volumes, and the possibility to develop "Lab-on-a-chip" devices with integrated sample analysis and detection, also reducing the risk of contamination, are very intriguing. The project presented wants to transfer the benefits of microfluidic technology to forensic genetic applications in developing a highly sensitive *real-time*-PCR (qPCR) based screening assay for common European animal families on a microfluidic polymer foil disk ("LabDisk") processed on a Rotor-Gene thermocycler. The assay is also interesting for analysis of processed foods, since food chemists often encounter analytical problems similar to forensic samples.

An assay was designed to appropriately meet the needs of forensic genetic analysis and microfluidic demands. It includes a pre-amplification step using primers universally amplifying short fragments of the mitochondrial *12S rRNA* and *cytochrome b* genes also enabling a double-check. This pre-mix is distributed to small reaction chambers where animal families are differentiated by family-specific qPCR using an intercalating dye and melting curve analysis.

The assay allows screening for common European animal families/species (e.g. Bovini, *Sus scrofa*, Caprinae, Cervidae, Equidae, Canidae, Felidae, Mustelidae, Phasianidae, Leporidae, Rodentia) and discrimination from human biological samples. Using an intercalating dye is adequately specific, sensitive, and cost-effective compared to TaqMan probes. The LabDisk approach offers the possibility to pre-load lyophilized assay components before sealing it prospectively enabling fully integrated and automated "ready-to-use" analysis.

O 10 WHERE DOES THIS TIGER COME FROM? — A ROBUST MOLECULAR TECHNIQUE FOR SIMULTANEOUS IDENTIFICATION OF ENDANGERED SPECIES AND SUBSPECIES

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The Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES) controls or bans international trade in endangered animal and plant species, including the tiger. To aid in the investigation of alleged illegal trade in protected species' parts and derivatives, molecular approaches have been developed to aid in their identification. Some countries also require knowledge of the exact subspecies in order to prosecute anyone alleged to be trading in seized products. In this study, we demonstrate the application of a SNaPshot multiplex technique to simultaneously identify tiger species and subspecies; this test is based on identification of SNPs within the tiger mitochondrial genome. The mitochondrial DNA sequence from four of the five extant putative tiger subspecies that currently exist in the wild were obtained and combined with DNA sequence data from 492 tiger and 349 other mammalian species available on GenBank. From the sequence data a total of 11 SNP loci were identified; five were species-specific for tiger and six amplify one of the tiger subspecies, three of which were specific to *P. t. sumatrae* and the other three were specific to *P. t. tigris*. The multiplex assay was able to reliably identify 15 voucher tiger samples. The sensitivity of the test was 15,000 mitochondrial DNA copies (approximately 0.26 pg), indicating that it will work on trace amounts of tissue, bone or hair. This simple and reliable technique can be applied to identify a variety of other species listed in the CITES appendices.

Keywords: CITES, tiger, subspecies, mitochondrial DNA, SNP, SNaPshot

O 11 MIR16 AS A MICRORNA MARKER APPLIED IN SPECIES IDENTIFICATION

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MicroRNAs (miRNAs, 18-25 bases in length) are small, non-coding RNAs that regulate gene expression at the post-transcriptional level. miRNA expression patterns, including presence and relative abundance of particular miRNA species, provide cell-and tissue-specific information that can be used for body fluid identification. Recently, two published studies reported that a number of body fluid-specific miRNAs had been identified. However, they focused on identifying a specific human body fluid and did not consider species specificity. In this study, we selected blood specific miR16 that was reported it can distinguish venous blood from other forensically relevant biological fluids to detect the expression abundance in blood of ten animals (monkey, rabbit, chicken, duck, cattle, sheep, dog, SD rat, mouse and rice field eel) using the optimized extraction kit, high-specificity stem-loop reverse transcription (RT) and high-sensitivity hydrolysis probes (TaqMan) quantitative real-time polymerase chain reaction (qPCR). The results showed that there were different expression of miR16 in ten animals, especially in chicken and duck.

O 12 RAPID DNA ANALYSIS IN THE POLICE BOOKING SUITE: FBI INITATIVE FOR REFERENCE SAMPLE POINT OF COLLECTION ANALYSIS

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Beginning in 2009, the FBI in collaboration with the United States Department of Defense and the Department of Homeland Security incentivized the development of hands free, non-laboratory DNA typing equipment. Prototype machines that perform point-of-collection DNA analysis in less than two hours from buccal swabs are currently under development. The "swab in-DNA profile out" Rapid DNA (R-DNA) devices will produce Combined DNA Index System (CODIS) compatible profiles.

R-DNA technology could allow DNA profiles to be searched from the Booking Suite in a fashion similar to fingerprints. Direct use of DNA technology by non-Laboratory law enforcement personnel will require significant testing and validation. In January 2011, the Scientific Working Group DNA Analysis Methods (SWGDAM) established a Rapid DNA Committee to monitor and address quality assurance and validation issues related to CODIS and the National DNA Database.

The U.S. National Institute of Standards and Technology and the FBI Laboratory are collaborating to test and evaluate the performance of R-DNA platforms. The status of the FBI R-DNA initiative and the NIST-FBI testing plan and strategy for CODIS validation and submission will be presented.

O 13 A SINGLE MULTIPLEX PCR AND SNAPSHOT MINISEQUENCING REACTION OF 42 SNPS TO CLASSIFY ADMIXTURE POPULATIONS INTO MITOCHONDRIAL DNA HAPLOGROUPS

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SNaPshot minisequencing reaction is in increasing use because of its fast detection of many polymorphisms in a single assay. In this work we described a highly sensitive single nucleotide polymorphisms (SNPs) typing method with detection of 42 mitochondrial DNA (mtDNA) SNPs in a single PCR and SNaPshot multiplex reaction in order to allow haplogroup classification in Latin American admixture population. We validated the panel typing 160 Brazilian individuals. DNA was extracted from blood spotted on filter paper using Chelex protocol. Forty SNPs were selected targeting haplogroup-specific mutations in Europeans, Africans and Asians (only precursors of Native Americans haplogroups A2, B2, C1, and D1) and two non-coding SNPs were chosen to increase the power of discrimination between individuals (SNPs positions 16519 and 16362). It was done using a modified version of a previously published multiplex SNaPshot minisequencing reaction established to resolve European haplogroups, adding SNPs targeting Africans (L0, L1, L2, L3, and L*) and Asians (A, B, C, and D) haplogroups based on SNPs described at PhyloTree.org build 2. PCR primers were designed using PerlPrimer software and checked with the Autodimer program. Thirty-three primer-pairs were used to amplify 42 SNPs. Using this panel, we were able to successfully classify 160 individuals into their correct haplogroups. Complete SNP profiles were obtained from 10 pg of total DNA. We conclude that it is possible to build and genotype more than forty mtDNA SNPs in a single multiplex PCR and SNaPshot reaction, with sensitivity and reliability, resolving haplogroup classification in admixture populations.

O 14 DIP-STR MARKERS: A NEW TOOL FOR THE ANALYSIS OF UNBALANCED MIXTURES OF DNA FROM TWO INDIVIDUALS

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In forensic science the genetic characterization of unbalanced mixed stains remains an important area where improvement is imperative. Most cases of aggression, homicide and sexual assaults are likely to produce biological traces with a relatively large amount of the victim's DNA and a small amount of the aggressor's DNA. If this ratio is smaller than 1:10 it is currently not possible to obtain a conventional autosomal DNA profile of the minor contributor, with consequential loss of potentially crucial DNA evidence. To address this recurrent problem, we developed an original method based on a new compound genetic marker formed by a Deletion/Insertion Polymorphism linked to a Short Tandem Repeat polymorphism, that we named DIP-STR.

With this compound locus we can design allele specific primers overlapping the deletion-insertion site that target the minor's DNA under certain genotypes assortments. DIP-STRs are highly heterozygous, widespread in the genome, and they require standard genotyping techniques as for currently used STR markers. This study provides the proof of concept for this new type of genetic marker. Here we describe a preliminary list of DIP-STR loci and their performance at casework resolution of both simulated and real samples.

O 15 FURTHER DEVELOPMENT OF FORENSIC EYE COLOUR PREDICTIVE TESTS

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Two recent studies have analyzed SNP genotypes underlying common European eye colour variation [Mengel-From et al., 2010; Walsh et al., 2010]. Each one arrived at slightly different recommendations for development of a forensic eye colour prediction test aiming to type the most closely associated SNPs. Both identified three SNPs in common in SLC45A2 (MATP), HERC2 and OCA2 genes, and the difference between them in identification of best predictors may partly arise from varying approaches to assigning phenotypes, notably those that are not unequivocally blue or dark brown and therefore occupying an intermediate iris colour continuum that merges into blue and brown iris colour phenotypes.

We have developed two single base extension assays typing 37 SNPs in nine pigmentation-associated genes. These assays were used to test the performance of the forensic eye colour prediction marker sets of these previous studies in 432 phenotyped subjects taken from north and south European populations. We aimed to find if an enhanced SNP combination would lead to improved predictive performance without expanding the SNP assay excessively, in order to maintain the balance between the predictive value of the test and its ability to reliably type challenging DNA in a small, manageable multiplex. To facilitate adoption of SNP based eye colour predictive tests in forensic applications we have also modified an online Bayesian classifier, developed for genetic ancestry analysis, to provide a straightforward system to assign eye colour likelihoods from a SNP profile that includes haplotypes of closely sited SNP loci in HERC2-OCA2.

O 16 ANALYSIS OF VARIATION IN PIGMENTATION GENES FOR FORENSIC PURPOSES

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Prediction of externally visible traits and inference of biogeographical ancestry (BGA) from genetic data may in forensic investigations provide similar types of information, which may allow description of the physical appearance of an unknown perpetrator or a skeletonised body. People of various biogeographical ancestries differ to some degree in phenotypic features and these differences are particularly strong for the pigmentation phenotype. Hence, analysis of variation in pigment related genes may contribute to both prediction of pigmentation characteristics and inference of BGA. Pigmentation was the subject of convergent evolution in Europeans and Asians, meaning that polymorphisms in different genes may have different value as BGA indicators. We examined 31 polymorphisms located within 11 pigment related genes, namely ASIP, HERC2, IRF4, KITLG, MC1R, OCA2, SLC24A4, SLC45A2, TPCN2, TYR and TYRP1 in two population samples from Poland and Japan. The obtained data was used for phenotype-genotype association testing, confirming the significance of a number of polymorphisms in prediction of eye, hair and skin colour. The value of particular polymorphisms as ancestry inference markers was evaluated based on allele frequency differences in both the studied population samples. The role of particular polymorphisms in the prediction of externally visible traits and inference of BGA is discussed.

O 17 GENETIC VARIANTS AND SKIN COLOUR IN DANES

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Introduction and aims
Single nucleotide polymorphisms (SNPs) affect the pigmentation of eyes, skin and hair in humans.
In this study, 34 SNPs in known pigmentation genes were tested for association to quantitative measurements of the skin colour in Danes.

Materials and methods
Objective spectrophotometric reflectance measurements of the upper-inner arm and the buttock were carried out on 188 unrelated Danes. The 188 Danes were typed for 34 SNPs by two multiplex PCRs, two multiplex single base extension reactions and capillary electrophoresis. To further investigate the role of the SNPs, their allelic distribution was compared to those in a population of 36 Somalis.

Results and discussion
Four *MC1R* SNP alleles (R151C, R160W, D294H and 29insA) showed significant associations to light skin pigmentation in individuals that were either homozygous for one allele or had two of these alleles. This confirms the regulatory role of *MC1R* in skin pigmentation.
SNP loci in *SLC45A2*, *SLC24A5* and *TYR* showed large allele frequency differences between Somalis and Danes, which suggested a possible role in skin pigmentation.
SNP loci in *OCA2*, *DCT* and *KIT* did not show significant association to skin pigmentation in Danes nor did they show significant allele frequency differences between Danes and Somalis.
The average buttock pigmentation was significantly lighter than the average upper-inner arm pigmentation. Therefore, the buttock reflectance measurements better resemble the constitutive skin colour compared to upper-inner arm reflectance measurements and will be used in future studies. Males were confirmed to be significantly lighter in buttock skin pigmentation compared to females.

O 18 THE HIRISPLEX SYSTEM: SIMULTANEOUS PREDICTION OF BOTH HAIR AND EYE COLOUR FROM DNA

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The field of predicting phenotypes of externally visible characteristics (EVC's) from DNA genotypes, also known as Forensic DNA Phenotyping (FDP), has started to become established in forensic biology with a promising future. The information yielded is expected to enable the police to further their investigation, termed 'DNA intelligence', if conventional short tandem repeat (STR) profiling fails to provide answers. We previously developed and forensically validated the IrisPlex system for accurate prediction of blue and brown eye colour from DNA, and more recently showed that all major hair colour categories are predictable from DNA markers. Here, we present the new HIrisPlex system capable of simultaneously predicting both hair and eye colour from DNA. It consists of a highly sensitive and reliable 24-SNP multiplex assay as well as an eye and hair colour prediction model. We demonstrate the power of the new HIrisPlex system for accurate simultaneous determination of eye and hair colour, by applying it to DNA samples of individuals for whom we have collected high quality digital eye and hair imagery for colour extraction. As a prerequisite for application of the HIrisPlex system to forensic casework, we also performed the forensic developmental validation assessments recommended by the Scientific Working Group on DNA Analysis Methods (SWGDAM) on the assay's overall proficiency. The use of the new HIrisPlex system in forensic casework is a major step in the next generation of DNA profiling and changes fiction to fact in the term 'eye witness' from DNA.

O 19 MOLECULAR "EYEWITNESS": FORENSIC PREDICTION OF PHENOTYPE AND ANCESTRY

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When a STR DNA profile obtained from crime scene evidence does not match identified suspects or profiles from available databases, further DNA analyses targeted at inferring the possible ancestral origin and phenotypic characteristics (i.e. hair color, skin color and eye color) of the possible perpetrator could yield valuable information. Such a tool would aid in prioritizing suspect processing, corroborating witness testimony, determining the relevance of a piece of evidence to a crime, and ultimately increase the ability to identify individuals related to the crime scene. Single Nucleotide Polymorphisms (SNPs), the most common form of genetic polymorphisms, have alleles associated with specific populations and/or correlated to physical characteristics. We have used Single Base primer Extension (SBE) technology to develop panels which include 100 ancestry and phenotype markers selected from recent literature. Over 250 DNA samples along with corresponding ancestry / phenotype survey information, and spectrophotometric skin color data have been collected from anonymous volunteers of varying ethnicity, gender and age. These DNA samples, and additional samples of known ancestry, have been screened with the SBE panels to assess the predictive value of the candidate SNPs, with the goal of identifying the optimal panel of SNPs to efficiently assess an unknown individual's characteristics. Different statistical approaches are being evaluated for best ancestry and physical trait inference. Preliminary results show good correlation between a small set of SNPs and eye color making it a highly predictable trait, whereas hair and skin pigmentation together with ancestry are more complex characteristics to prognosticate.

O 20 A PANEL OF 46 ANCESTRY-INFORMATIVE INSERTION-DELETION POLYMORPHISMS (AIM-INDELS) IN A SINGLE REACTION

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Ancestry-Informative Markers (AIMs) show high allele frequency divergence between different ancestral or geographically distant populations. These genetic markers are especially useful in inferring the likely ancestral origin of an individual or estimating the apportionment of ancestry components in admixed individuals or populations. The study of AIMs is of great interest in population and forensic genetics, but also in clinical genetics research, particularly to detect and correct for population substructure effects in case-control association studies.

In this work we present a set of 46 ancestry-informative Insertion-Deletion polymorphisms selected to efficiently measure population admixture proportions of four different origins (African, European, East Asian and native American). All markers were analysed in short fragments (<230bp) through a single PCR followed by capillary electrophoresis.

HGDP-CEPH Diversity Panel samples from the four groups, together with Oceanians, were genotyped to evaluate the efficiency of the assay in clustering the populations from different continental origins and to establish reference databases. In addition, other populations from diverse geographic origins (Angola, Portugal, Macau and Brazilian Amazonas) were tested using the HGDP-CEPH samples as training sets. The results revealed that the Indel set is highly efficient in inferring the ancestry of individuals from the studied populations and provides good estimates of ancestry proportions at the population level.

In conclusion, we have optimized the multiplexed genotyping of 46 AIM-Indels in a simple and informative assay, enabling a more straightforward alternative to the widely available SNP typing methods dependent on multi-step protocols and/or implementation of new genotyping technologies.

O 21 BUILDING A COMPACT X-CHROMOSOME ANCESTRY-INFORMATIVE SNP MULTIPLEX

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Recent experiences with assisting major criminal investigations have suggested to us that the inference of the likely ancestry of the parents of a suspect as well as the suspect themselves could provide very useful information from which to redirect investigative leads. This is particularly so when DNA analysis indicates admixed ancestry. Combining uni-parental loci with autosomal ancestry informative markers (AIMs) enables a certain level of inference to be made about parental as well as individual ancestry, but this is very dependent on the differentiation between the markers used and between contributing populations. Adding population divergent X-chromosome markers has the potential to significantly enhance the likelihoods of ancestry component estimates. With the aim of improving the total genetic ancestry information obtained from contact traces we have developed a compact single base extension multiplex of X-chromosome AIM-SNPs. The X-chromosome shows higher levels of Fst compared to autosomes and we were able to collate a candidate list of ~100 highly differentiated SNPs when comparing all five continental population groups. From this pool we selected 16-19 SNPs to construct the multiplex and test the balance of divergence across all population comparisons, overall informativeness and performance with forensic DNA. Throughout SNP selection reference was made to the particularities of X chromosome recombination, so four AIM-SNPs clustered around the centromere have been treated as a single haplotype. The efficacy of the ancestry inferences using this multiplex were then assessed by cross-validation of the CEPH diversity panel and by testing urban admixed African-European donors from the UK.

O 22 NUCLEAR AND MITOCHONDRIAL ANALYSES OF FRENCH NEOLITHIC HUMAN REMAINS

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The Neolithic was a key period in the history of the Europe settlement, and its impact in terms of genetic flow in current populations is still misunderstood and debated among the scientific community. In this context we studied ancient DNA from a 5000 year-old southern French necropolis and succeeded to generate data from nuclear (autosomal and Y-chromosomal) and/or mitochondrial DNA for 29 of the 53 individuals sampled. We highlighted at least 3 kinships among the necropolis and determined maternal and paternal lineages as well as the absence of an allele associated with the emergence of dairy farming. Paternal lineages characterized in this study are the first known Y-data for southern Europe at this period. Furthermore, the Y-haplotypes and their current repartition in European populations confirm for the first time, in a direct way, a greater influence of the Mediterranean than the Central European route in the peopling of southern Europe during the Neolithic transition. It confirms moreover the probable heterogeneity of Neolithic dispersals into Europe. This is of course, only a first indication on the Neolithic lineage dispersal and new data will be needed in the future to have a complete overview on the Neolithic male diffusion through the Mediterranean route.

O 23 WHOLE MITOCHONDRIAL GENOME SEQUENCES FROM PREHISTORIC HUMAN ARCHAEOLOGICAL REMAINS

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New methodologies capable of fully typing mitochondrial genomes from prehistoric human archaeological remains could clearly also be applied to the most challenging forensic samples. The 'time travelling' abilities of ancient DNA research have shown that projecting backwards from present day genetic variation alone can lead to inaccurate reconstructions of past population dynamics (e.g. for bison, bears, humans). By examining DNA from human archaeological remains at depths-of-resolution previously only available for present-day DNA samples, we have therefore begun to test directly - in 'real-time' - long-standing controversies in archaeology. These include the extent and role of genetic continuity vs. discontinuity, migration, and acculturation during major cultural transitions. We have developed novel methodologies to efficiently extract damaged and degraded fragments of ancient DNA from human archaeological remains (5500BC-to-AD1000), to immortalise these extracts as ancient DNA libraries, and to carry out targeted enrichment of mitochondrial genome sequences and selected Y-chromosome SNPs from these libraries. With these advances, we have typed 49 ancient mitochondrial genomes at the highest resolution. These mitochondrial genome sequences have begun to provide detailed insights into Europe's distant past. In particular, they have allowed us to infer the extent of population continuity vs. discontinuity during the spread of Neolithic farming lifestyles and later distinctive Bronze Age cultures in Central and Western Europe. As a last resort for individual identification, these techniques could allow mitochondrial genomes to be fully typed from previously unusable forensic samples, where DNA templates have undergone fragmentation and degradation <50bp, beyond the range of PCR-based protocols.

O 24 MULTIDISCIPLINARY ANALYSIS OF ANCIENT SKELETAL REMAINS AND THE TRANSFER OF EXPERIENCE TO THE FORENSIC CASE WORK

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Historically the first DNA study of an ancient material was performed on the 140 year old museum specimen of quagga skin by sequencing 229 bp part of mitochondrial DNA. The results of the first aDNA analysis of the human origin appeared in 1985 when S. Paabo described his successful attempt to retrieve and analyse nuclear Alu repetitive sequence family DNA from a 2400 years old Egyptian mummy of a child. The invention of PCR boosted aDNA studies, but the majority of studies stick to the sequences of the mitochondrial genome and only a minority focuses on much more difficult studies of nuclear DNA. Last decade improvements in molecular-biological technologies helped to overcome some of the restricting problems that limited the scope of analysis mainly to the mitochondrial DNA that is abundant in the mammalian cells. Especially the transfer of improvements from the field of forensic genetics (inhibitor-free DNA extraction, multiplex PCR, short STR amplicons, qPCR) enabled to extend the testing also on the nuclear DNA, including Y-chromosome STR typing. Currently available techniques for ancient DNA analysis using the "forensic" procedures can be combined with the classical anthropological examination, advanced imaging and CT scanning techniques and isotope analysis to improve the quality of information retrieved from the ancient artefacts. Multidisciplinary approach to the examination of the ancient human remains can thus help to verify the results of DNA analysis and bring more precise interpretation of the findings. "Ancient" methodologies and procedures can be very useful in the forensic case work. The presenting author will also describe, as an example, the complex analysis of 700 years old skeletal remains found in an unusual grave.

O 25 FIRST APPLICATION OF THE INVESTIGATOR DIPplex INSERTION/DELETION TYPING KIT FOR THE KINSHIP ANALYSIS FROM ANCIENT DNA SAMPLES

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Kinship testing in forensic and anthropological fields is commonly based on the analysis of short tandem repeats (STRs). In this study, another type of genetic variations, insertion/deletion polymorphisms (indels) was used through the DIPplex indels typing kit (Qiagen) for such purpose. These polymorphisms have been recently described to show substantial benefits within the analysis of degraded samples. The main objective was here to outline the advantages of indels for the analysis of ancient DNA samples; the second objective was to evaluate the informativeness of this kit for kinship investigation. First tested on modern DNA samples, the assay was then applied on multiple archaeological samples. These samples were selected due to their putative kinship deduced from the analysis of the autosomal STRs, Y chromosomal STRs and mitochondrial haplotypes. Indel typing was successful and highly informative for most of them. Although some limitations have been noticed, these results show that indels are well suited for the analysis of degraded samples and that they might be an interesting strategy additionally to STRs.

O 26 EMPOP QUALITY CONTROL MANAGEMENT FOR THE EVALUATION OF LITERATURE DATA

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Mitochondrial (mt) DNA population studies are prone to error, especially those performed in the earlier years when older versions of sequencing polymerases and buffer systems led to unbalanced and noisy raw lane data. However, problems do also occur with the application of modern chemistry as human error cannot be avoided completely if data are transferred manually without the required scrutiny.

By applying current laboratory protocols and safe data handling processes errors can be significantly reduced to a minimum. If in addition raw lane data are permanently linked to the haplotypes, those data represent "forensic status". The growth of mtDNA databases with forensic haplotypes is relatively slow because the quality control (QC) process is expensive and time consuming. On the other hand, a plethora of population data are available in the literature that have not been generated under such stringent conditions but may represent useful information for investigative frequency searches.

In order to evaluate the quality of such data we developed a software suite that is capable of pinpointing data idiosyncrasies by plausibility and formal checks. Phylogenetic tests play a major role as they are able to uncover errors that cannot be revealed by the inspection of the raw data. When reaching a mature status these programs are also made available through EMPOP for a posteriori mtDNA data QC. The presentation summarizes QC work from the past years, highlights common sources of error and demonstrates software features that can be downloaded from or directly used at the EMPOP website.

O 27 USEFULNESS OF HAPLOGROUP INFORMATION IN FORENSIC GENETICS

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Human mitochondrial DNA (mtDNA) has proven to be a valuable resource in forensics as well as other scientific disciplines. Despite long standing guidelines for generating high quality mtDNA data, errors are still frequently found and mislead interpretation and further investigations. Besides quasi-median network analysis (available via EMPOP) the assignment of haplogroups to individual samples may be helpful to understand the phylogeny and aid the detection of idiosyncrasies in mtDNA data.

The task of assigning haplogroup information to mtDNA sequences is difficult and tedious. A user needs to be cognizant of the up-to-date phylogeny and the comparison of differences to the revised Cambridge Reference Sequence (rCRS) with a haplogroup nomenclature is laborsome and prone to error. A great relief to the scientific community was the development and curation of a standardized phylogenetic tree (Phylotree.org). Soon, software tools for haplogroup assignment became available and thus alleviate the user from the manual look-up of positions.

However, these solutions are not satisfactory for forensic applications as they are either affected by erroneous underlying data or are strongly biased towards the rCRS. We here present a new approach that combines SAM, the string-based search tool in EMPOP, with the estimation of a forensically relevant haplogroup status of the haplotypes. For forensic case work the estimation of the haplogroup status not only represents an additional quality control instrument, it subsequently allows for a more accurate assessment of the strength of evidence through incorporation of geographical information of the haplotype.

O 28 QUALITY EXERCISE IN DISASTER VICTIM IDENTIFICATION

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One of the primary methods for identification of unknown persons not only in mass disasters is the forensic molecular analysis of samples taken from the victims. The quality of these samples depends from the circumstances and can be very low after highly decomposition of the bodies. The first decision in such cases is which samples could be used successfully. An important question is the quality of the results. The analysis can fail, allelic drop out or drop in can be obtained.

Quality exercises are part of the quality control in several parts of the routine case work since many years. For example the German DNA Profiling group (GEDNAP) in forensic stain analysis or several societies of paternity testing established such tests which are part of the accreditation for laboratories too. In mass disaster cases such quality exercises are not established so far. The developments of national and international standards of the DVI process were successfully in the last years. The working group within the German working party of the ISFG decided to establish a quality exercise using typical DVI samples. Therefore a femur was stored under natural conditions in summer for 8 weeks in 2010. The laboratories of the members of the working group took part at this first test and got a bone sample. The methods and kits were not restricted. All laboratories could use their established protocols and available systems. The results of this first quality exercise will be reported.

O 29 KINSHIP ANALYSIS BASED ON SNP DATA FROM MICROARRAY ASSAY

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Pairwise kinship analysis is the fundamental approach in forensic examinations like paternity test and personal identification. The 15 variable STR genotypes are insufficient for the separation of sibship from un-relatedness. In the present study, we evaluated the kinship analysis based on a large amount of SNP data, obtained using the microarray technique. DNA was extracted from peripheral blood or saliva, which was obtained from 16 pairs of various relationships, and was treated according to the manufacturer's instruction. The assay was performed using genome-wide human SNP array 6.0 (Affymetrix), and the data was analyzed using the PLINK software. To infer the pairwise relationship, the index of estimated coefficient of relationship (p), calculated on the basis of identical by descent, was employed. Among the selected SNPs that were more than 0.05 in minor allele frequency and r^2 = 0.5 in linkage disequilibrium, p values (the mean and S.D.) were 0.497 $\pm$ 0.004, 0.505 $\pm$ 0.037, 0.269 $\pm$ 0.002, and 0.016 $\pm$ 0.008 for relationships of parent-offspring, full sibs, second degree relatives and unrelated pairs, respectively. The complete separation from the un-relatedness was obtained in variable r^2 to 0.2 with no significant changes in p values. Moreover, we simulated the minimum number of SNPs to obtain the complete separation. As a result, the SNP numbers of 500, 2,000, and 5,000 were necessary for parent-child, sibship, and second degree, respectively. The SNP assay is beneficial for the analysis of distant relationships like half-siblings.

O 30 A DISTANT RELATIONSHIP RESOLVED WITH GENOME-WIDE SNP ANALYSIS

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Paternity testing is routinely based on the analysis of a limited set of unlinked short tandem repeat (STR) markers. However, the standard loci used in forensic genetics usually fail to resolve the most distant of relationships, particularly those within complex or deficient pedigrees. Theoretical predictions have suggested that distant relationships could be investigated using large panels of linked single nucleotide repeats (SNPs). Here we have used a genome-wide genotyping study based on Affymetrix 6.0 SNP arrays aiming to resolve a second cousin relationship in the absence of any other pedigree members, and for which standard and extended STRs plus SNP panels were insufficiently informative. Genome profiles of the claimed second cousins were compared to profiles obtained from control individuals from the same population and simulated data. Second cousins shared more alleles and their kinship coefficients were much higher than those observed in genomic profiles from unrelated control individuals and simulated data. Results consistently delivered a probability equal to 1 for the claimed second cousin relationship. Important limitations of the high density SNP typing method are discussed — notably when considering multiple relationship models in the absence of a priori data, that would otherwise help suggest the most likely relationship. In this situation near identical likelihoods may be found for each alternative relationship when the claimants are very distantly related across several generations.

O 31 LINKAGE DISEQUILIBRIUM ANALYSIS OF 67 SNP LOCI ON X CHROMOSOME

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Objective
In forensic and medical practice, markers on X chromosome may be more important than autosomal loci in some deficiency cases. When a panel of X-SNPs is used, if linkage disequilibrium (LD) was observed between some loci, frequency of single SNP can not be simly used for calculation of likelihood. The aim of this research is to assess the patterns of LD about 67 SNP loci on X chromosome.

Method
Genomic DNA samples were extracted from blood fluids from 213 unrelated Chinese Han female individuals and 67 X-SNPs were genotyped through multiplex amplification followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). LD was analyzed by SNPAnalyzer 2.0.

Results and discussion
LD were found at two groups and tight LD were observed at six groups. About the loci observed LD, it is proposed to use haplotype frequencies instead of frequencies of single SNP in calculation of likelihood, or to apply them by preventing the linkage groups. From the 67 X-SNP loci, 56 high informative X-SNPs that no deviation from Hardy–Weinberg equilibrium was found and no LD was observed could be chosen. The combination discrimination power of the 56 X-SNPs in female Han population and male Han population were 0.99999999999999999999986 (=1-1.4×10^{-23}) and 0.99999999999999980 (=1-2.0×10^{-17}) respectively. The exclusion probability in duos and in trios were 0.99999974 and 0.999999999987 respectively.

Conclusions
It may be concluded that a panel of 56 X-SNPs loci are valuable in forensic identification.

Keywords: SNP, X chromosome, multiple PCR, MALID-TOF Mass, polymorphism, Linkage disequilibrium

O 32 WHEN THE ALLEGED FATHER IS A CLOSE RELATIVE OF THE REAL FATHER: THE UTILITY OF INSERTION/DELETION POLYMORPHISMS

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The occurrence of Mendelian incompatibilities between true father and child is not an uncommon situation. So, in paternity investigations, when only a few exclusions are observed, there is the possibility that the alleged father is either the real father or a close relative of his.

In this study, we intended to test how a set of autosomal insertion/deletion polymorphisms (indels) can complement the results obtained with a standard Short Tandem Repeat (STR) multiplex analysis in paternity investigations where the alleged father is, in fact, a close relative of the real father.

We analyzed 100 uncle/nephew and grandfather/grandchild unrelated duos. DNA was amplified with AmpFℓSTR Identifiler kit and a set of 38 non-coding bi-allelic indels. For each duo, we determined the number of observed incompatibilities and calculated the Likelihood Ratio (LR) assuming a father/son relationship, for both STRs and indels.

As expected, in a significant proportion of cases, no incompatibilities were found. In these instances, indel's LR contribution was always in favour of the "wrong" hypothesis, strengthening a false paternity. Therefore, in dubious cases, an extended battery of indels can be useful to exclude false fathers but should be taken with care when no incompatibilities are detected.

We estimated that to obtain a satisfactory probability (less than 1 in 500) of finding zero incompatibilities between relatives it is necessary a battery of, approximately, 200 indels, when considering markers with maximum diversity.

O 33 STATISTICAL ASPECTS OF FAMILIAL SEARCHING

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Familial Searching is the process of screening a database for possible relatives of the (usually unknown) donor of a crime stain of which a DNA profile has been obtained, e.g. to identify that donor.

We will present probabilistic models to interpret the results of such a database search, presuming that we have calculated all the appropriate Likelihood Ratios (LR's) between the crime stain's profile and the database profiles.

The first model enables one to calculate posterior probabilities for relatedness with every individual in the database, and to calculate the LR for there being a relative in the database or not.

We will show how these results can be viewed as a generalization of the database controversy in the classical case where one looks for an exact match.

The second model takes the crime stain profile as a starting point and, by viewing the relative's profile as a random variable, can be used to define LR thresholds that enable one to make probabilistic statements about the relative being in a subset of the database, if present in the whole database.

We will show the differences between these models, discuss their (frequentist) interpretation, and how they can be used to arrive at search strategies that control the probability of detection of the relatives.

Finally we present applications of these strategies performed with simulated DNA profiles in the actual Dutch National DNA database and show that the results agree closely with the model predictions.

O 34 A PRACTICAL MODEL TO EXPLAIN RESULTS OF COMPARATIVE DNA TESTING IN COURT

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With ongoing developments in forensic genetics more information is derived from compromised samples, but explaining this information to criminal justice professionals has become increasingly challenging. We developed a practical model to help forensic scientists explain and jurists understand the meaning of reported DNA-based evidence. The model distinguishes four successive steps: (1) analysis; (2) interpretation; (3) DNA-profile comparison and evidential value; (4) consideration of the results in the context of the case. After the alleles are assigned in step 1, the coherence of alleles is established in step 2: how many contributors are represented, can profiles be deduced, are there low-template contributors and is a consensus method based on replicate analyses appropriate? To avoid interpretation bias, step 3 comes only when step 2 is completely finished. The results of comparative DNA testing are classified to one of four categories of scientific evidence: A: exclusion; B: match with statistical interpretation; C: match without statistical interpretation; D: no exclusion or match. Category D is divided into subcategory D1: cannot be excluded; and subcategory D2: inconclusive. Step 4 considers the strength of DNA-based evidence in the context of the case by using sets of two competing hypotheses. Where the previous steps concern source level (which individual does the biological material originate from), step 4 concerns activity level: what activity could have led to deposition of the material? Using this concept reporting officers classify results of comparative DNA testing, including complex low-template mixtures, in a uniform way. Application to casework met with appreciation from jurists.

O 35 THE VALUE OF Y-HAPLOGROUP PREDICTION BASED ON Y-STR HAPLOTYPE DATA: BETWEEN CAUTION AND BENEFIT

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The ongoing discussion on Y-Chromosomal Haplogroup prediction based on Y-STR Haplotype data led to both: caution and promise. We have compared freely available tools and published approaches together with a newly developed *hapred* tool of the YHRD in terms of their robustness and reliability. We examined the scoring function of the different approaches and their corresponding confidence values using different datasets with known Haplogroup information. We will present this survey and focus on the following questions: What impact does the prediction of Haplogroups have for forensic research and casework and for quality control? How do I understand and choose the correct approach for prediction? What parameters do I need to take into account when predicting Haplogroups? What is a feasible Y-STR Haplotype training set?

O 36 COLLABORATIVE EDNAP EXERCISES ON MESSENGER RNA/DNA CO-ANALYSIS FOR BODY FLUID IDENTIFICATION (BLOOD, SALIVA, SEMEN) AND STR PROFILING

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Common enzymatic or immunologic body fluid identification tests lack specificity and are therefore primarily used as presumptive tests. mRNA profiling is an option for the definitive identification of body fluids and is compatible with current DNA analysis methodology. To demonstrate the suitability of mRNA profiling for use in forensic casework, three collaborative exercises on RNA analysis or RNA/DNA co-analysis for body fluid identification and STR profiling were organized within the European DNA Profiling Group (EDNAP).

In previous evaluation studies, promising mRNA markers for the identification of blood, saliva and semen were tested for their specificity and sensitivity. The best candidates were then combined in multiplexes and provided to all participating laboratories. Each study included an evaluation of mock casework samples and body fluid dilution series. Participating laboratories were also asked to include additional samples to further evaluate specificity and performance with forensic casework samples. A majority of the 16-19 participating laboratories were able to confirm the presence of the respective body fluid by mRNA profiling and to obtain autosomal STR profiles of the stain donors. Despite some expected variation in sensitivity between laboratories, the reproducibility and sensitivity of the mRNA profiling method was demonstrated using a range of analysis strategies. The stability of RNA and DNA in dried stains was found to be similar as old and compromised casework samples were successfully analyzed.

The results of these collaborative exercises support the potential use of an mRNA based system for the identification of body fluids along with conventional DNA profiling.

O 37 SPECIFIC MICRO-RNA SIGNATURES FOR THE DETECTION AND DIFFERENTIATION OF BODY-FLUIDS IN FORENSIC STAIN ANALYSIS

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Micro-RNAs (miRNAs) are a class of small non-coding RNA (ncRNA) molecules with a length of 18 to 24 nucleotides which play an essential regulative role for many cellular processes. Evidence suggests that the miRNome is a more precise and meaningful representation of a cells type and condition than the mRNA transcriptome. To identify miRNAs that are suitable for forensic body-fluid identification a global screening by microarray analysis of ~800 miRNAs of forensic samples of blood, saliva, semen, vaginal secretion and menstrual blood was performed and by bioinformatic processing three differentially expressed candidate miRNAs for each body-fluid were selected. These candidates were then extensively evaluated via quantitative PCR. Herein, we present miRNA assays consisting of three differentially expressed miRNAs for the identification of body-fluids and show that miRNA analyses are feasible even in aged samples. We conclude that miRNA extraction from forensic samples is possible and support a "proof of concept" that body-fluid identification by miRNA analysis may become a potent forensic technique.

O 38 IDENTIFICATION OF SKIN IN TOUCH/CONTACT FORENSIC SAMPLES BY MESSENGER RNA PROFILING

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The true nature of touch DNA evidence has remained elusive, generally perceived to be the result of DNA obtained from shed skin cells yet never confirmed with scientific certitude. This is largely due to the perception that it is not possible to ascertain the tissue source of origin of the biological material in touch DNA evidence. Thus far, research has failed to provide crime laboratories with feasible methods to identify the tissue source of origin of touch DNA. The aim of the current work was to identify highly sensitive and specific biomarkers for the identification of skin.

We have previously demonstrated the use of tissue specific messenger RNA (mRNA) profiling assays for body fluid identification. We therefore utilized mRNA profiling to identify potential biomarkers for the identification of skin. From an evaluation of over 100 potential markers, we identified five mRNA markers that demonstrated a high degree of specificity for skin. Using these markers, we have been able to successfully identify skin using as little as 5pg-25pg of input RNA. The presence of skin has been successfully identified in swabs of human skin and in a variety of touch and contact samples. One of the markers is highly sensitive and permits the detection of skin in a majority of known skin containing samples tested. Although further work is needed to produce an assay for routine casework, these initial studies demonstrate that a molecular-based characterization of the biological material recovered from touch and contact samples is possible.

O 39 A MULTIPLEX RNA PROFILING SYSTEM TO DISTINGUISH AMONG BODY FLUIDS AND CONTACT TRACES

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RNA-profiling has emerged as an alternative strategy to examine the biological origin of evidentiary traces. Here we introduce a single multiplex mRNA-based system for discriminating all most relevant forensic body fluids and skin cells. We developed a DNA/RNA co-isolation protocol that results in DNA yields equivalent to our standard extraction procedure which uses silica-based DNA columns. Human-specific DNA quantification results were used to guide RNA input in the cDNA reaction. An endpoint RT-PCR assay was developed that simultaneously amplifies 19 mRNA markers. The multiplex assay targets three housekeeping, three blood, five saliva, two semen, two menstrual secretion, two vaginal mucosa and two skin-specific markers. Full DNA-profiles for blood, semen and saliva were obtained when using >0.1µl body fluid material whereas full RNA-profiles were obtained with >0.05µl. We investigated the specificity of the markers by analyzing 16 different sample sets of which each set consisted of 8 individuals. Both skin markers responded in samplings of hands, feet, back and lips. Three out of five saliva markers showed low expression in these skin samplings. Positive identification (both regarding DNA- and RNA-profiling) was obtained for specimens stored for many years, *e.g.* blood (28 years-old), semen (28 years-old), saliva (6 years-old), skin (10 years-old) and menstrual secretion (4 years-old). We assayed whether RNA-profiling is applicable to existing DNA extracts, allowing examination of the biological origin in old cases. Unfortunately stored DNA extracts did not allow RNA-profiling which is probably due to RNA instability as freshly isolated standard DNA extracts did enable RNA-analysis.

O 40 IMPLEMENTATION OF MESSENGER RNA BODY FLUID TESTING IN FORENSIC CASE WORK

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At ESR we have developed a multiplex PCR system known as CellTyper that utilises messenger RNA (mRNA) and can identify blood, saliva, semen, menstrual blood and vaginal material in individual stains or in mixtures of body fluids. Messenger RNA transcripts specific to each type of body fluid have been identified and a multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) system developed to identify these body fluids along with three housekeeping genes. This multiplex can detect semen and seminal fluid (semen without spermatozoa present). Furthermore, we have targeted the co-isolation of RNA and DNA from the same sample and, using the RT-PCR CellTyper multiplex, we can determine the type of body fluid present while also generating a DNA profile from the same stain. Here we present the results of an extensive validation exercise undertaken prior to casework implementation. This work included a further assessement of sensitivity, variability and specificity as well as more operational requirements such as quality control and quality assurance activities. Aspects of the limitations and reporting of such new technology will be discussed with case work examples.

O 41 BODY FLUID IDENTIFICATION BASED ON TISSUE-SPECIFIC DIFFERENTIAL DNA METHYLATION

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Recent advances in whole genome epigenetic analysis indicate that chromosome pieces called tDMRs (tissue-specific differentially methylated regions) show different DNA methylation profiles according to the type of cell or tissue. Therefore, body fluid-specific differential DNA methylation would be a promising indicator for body fluid identification. Several tDMRs expected to show differential DNA methylation profiles in various body fluids were selected, and DNA methylation profiles for these tDMRs were produced by bisulfite sequencing using pooled DNA from blood, saliva, semen, menstrual blood, and vaginal fluid. The tDMRs with varying degree of methylation according to the type of body fluid were identified and the DNA methylation profiles of four tDMRs for the genes DACT1, DDX4, PRMT2, and USP49 were further analyzed in young and old voluntary male donors because DNA methylation patterns are susceptible to change in response to environmental stimuli and aging. After confirming the stability of the body fluid-specific DNA methylation profile over time, we developed a multiplex PCR system using those tDMRs. This multiplex can successfully identify semen including sperm cells. Because DNA methylation profiling uses the same biological source of DNA for individual identification profiling, the determination of more body fluid-specific tDMRs and the development of convenient tDMR analysis methods will facilitate the broad implementation of body fluid identification in forensic casework.

O 42 EXPLORING THE CAPABILITIES OF MIXTURE INTERPRETATION USING TRUE ALLELE SOFTWARE

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DNA mixtures from sexual assault evidence or high volume crimes such as burglaries can be challenging for the forensic scientist to interpret. The problem is exacerbated when the evidence contains more than two contributors or is highly compromised due to DNA degradation. ISFG guidelines for mixture interpretation developed by Gill *et al.* (2006) have been widely accepted by the global forensic DNA community.

Laboratories have developed "in-house" spreadsheets or have purchased commercial software to rapidly calculate the multiple parameters necessary for mixture interpretation using the Clayton *et al.* (1998) method (*e.g.* peak height ratio, mixture ratio, etc.). Additionally, mixture software can be used to calculate statistics using either Random Man Not Excluded (*e.g.*, combined probability of inclusion, CPI) or Combined Likelihood Ratio (CLR) to evaluate the data.

We have explored the capabilities of the TrueAllele software (Cybergenetics, Pittsburgh, PA, USA) by analyzing an assortment of two-, three-, and four-person mixtures. The software uses quantitative probabilistic genotype modeling of the data to form a joint LR statistic for the weight of the evidence. We examined a series of controlled two-person mixtures with differing contributor ratios and a broad range of allele sharing between the samples to determine the efficacy and reproducibility of the software. For complex mixtures, we examined the gain in information (measured by the log LR) compared to data evaluated with CPI and CLR statistics. We also examined the benefits of analyzing multiple replicate samples of low template DNA mixtures amplified with enhanced techniques (increased cycles and increased polymerase).

O 43 EVALUATION OF GENEMAPPER*ID-X* MIXTURE MODULE

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We have evaluated the mixture module in GeneMapper*ID-X* (Applied Biosystems) to interpret two-person DNA mixtures. This package depends upon terh evaluation of two different parameters – the mixture proportion (Mx) and the heterozygote balance (Hb). We analysed 3,000 heterozygotes in order to determine the characteristics of heterozygote balance versus the average peak height ratio (PHR). This information was used to determine thresholds that were subsequently used to accept or to reject genotype combinations. Apart from the evaluation of likelihood ratios conditioned on suspect's and victim's reference samples, the module can also be used to deconvolve mixtures. This may be important if a suspect or reference sample is not available for analysis. A number of example analyses are provided in order to illustrate the principles. This evaluation forms the basis of a validation exercise to use the GeneMapper*ID-X* mixture module in forensic casework at the Institute of Forensic Medicine in Oslo.

O 44 THE MYTHICAL "EXCLUSION" METHOD FOR MIXTURES – DOES IT MAKE ANY SENSE AT ALL?

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Two different approaches to DNA mixture analysis and computation are commonly called the "exclusion" and "likelihood ratio" methods. The exclusion method is often touted as adequate in principle, and preferable for a variety of practical reasons including ease of use and understanding. But critical consideration shows that none of the claimed advantages of the exclusion method hold much water.

1. Needs **no assumption about number of contributors** is mostly wrong.
2. **Ease of understanding** by judge or jury is an illusion; the method is deceptively easy with emphasis on deception, evading the elusiveness of the concept of "not present" by substituting "not present above 100 RFU" thus losing the intuitive idea that "exclude" means "he didn't do it". The audience who accepts the substitution has been hoodwinked.
3. **Ease of use** is claimed for complicated mixture profiles, but the truth is the opposite. Only the straightforward cases, easy by any method, might permit logical application of the exclusion method.
4. The **exclusion calculation is conservative**, but not for the expected reason. Against the infrequent but important innocent but included suspect, it will usually exaggerate.

The common underlying problem is that the method rests on the impossible premise that we can tell which alleles are "present" and which are not. Without clear and explicit definitions as a foundation, any analytic method can't be more than guesswork. Certainly no one has laid out an explicit and rigorous chain of reasoning from first principles to support the exclusion method. It is at best guesswork.

O 45 LIKELIHOOD RATIO STATISTICS FOR DNA MIXTURES ALLOWING FOR DROP-OUT AND DROP-IN

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Introduction and aims
The likelihood ratio (LR) is the recommended approach for forensic DNA mixture analysis by the DNA commission of the ISFG, as it uses more of the available data than a Random Man Not Excluded (RMNE) approach and parameters for allelic drop-out and drop-in can be incorporated. We have developed and validated a LR method and software for analysis of mixed evidence samples in which drop-out of true contributors' alleles and drop-in of extraneous alleles may have occurred.

Materials and Methods
This method, the Forensic Statistical Tool (FST), employs empirically determined drop-out and drop-in probabilities for single source samples and mixtures of two, three, or four contributors containing 25 pg to 500 pg of template DNA. The LR is computed for pairs of prosecution and defense hypotheses based on sample characteristics specified by the user. Data from up to three amplifications of the evidence sample may be considered simultaneously.

Results and Discussion:
The performance of the program was evaluated with hundreds of profiles generated from a variety of sample types, including blood and buccal samples, purposefully degraded buccal samples, and touched items with one, two, three, and four known contributors. The validation demonstrated that FST assigns an appropriate weight to all types of comparisons. FST is now being used in casework and initial results continue to be consistent with qualitative assessments. Additional work explores the extent to which results vary when different models, especially for number of contributors, are used.

O 46 ESTIMATING DROP-OUT PROBABILITIES IN FORENSIC DNA SAMPLES: A SIMULATION APPROACH TO EVALUATE DIFFERENT MODELS

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Allele drop-out is a well known phenomenon that is primarily caused by the stochastic effects associated with low quantity or low quality DNA samples. Recently, new interpretation models that employ the logistic regression have been utilised in order to estimate the probability of drop-out (Gill et al., FSIG, 2009). The model parameters are estimated using profiles from samples of extracted DNA diluted to low template levels in order to induce drop-out. However, we propose that this approach is over-simplistic, because several sources of variability are not taken into account in this generalised model. For example, in real-life, small crime-stains are analysed where cells are (or were) intact. The integrity of the paired chromosomes of the diploid cell is preserved. In extracted DNA that is diluted to low template levels, we argue that the paired-chromosome integrity is lost. This directly affects the outcome of the logistic model. To date, current experimentation procedures are more akin to haploid cells and thus, different logistic models are needed for haploid and diploid cells. In order to simplify the methodology to evaluate the different features of the statistical models, we propose the use of a simulation model of the entire process associated with the analysis of STR loci as a supplement to the purely experimental approach to support the validation of new methods. We illustrate how the simulation model can be used to evaluate some features of the logistic model of Gill et al, and discuss alternative solutions for modelling the probability of drop-out.

O 47 ESTIMATING Y-STR ALLELE DROP-OUT PROBABILITIES ADJUSTING FOR LOCUS IMBALANCE

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Interpretation of Y-STR results must take the risk of drop-out phenomena into account. We have recently presented a model for the estimation of drop-out probabilities of autosomal STR alleles. Here, we have modeled Y-STR drop-out probabilities using logistic regression adjusting for signal strength, locus, and number of PCR-cycles. The model was developed based on data from 12 different dilutions of DNA from 4 different males. The samples were analyzed in duplicates with the AmpFℓSTR® Yfiler® kit with 28 and 30 PCR cycles. We modeled the signal strength in different ways adjusting for locus imbalance. We used a simulation study to assess the performance of each model. We found that, besides signal strength, both the locus and the number of PCR-cycles had significant effect on the allelic drop-out probability.

O 48 A STATISTICAL MODEL FOR DEGRADED STR SAMPLES AND ADJUSTMENT OF DROP-OUT PROBABILITIES

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DNA samples found at a scene of crime or obtained from the debris of a mass disaster accident are often subject to degradation. When using the STR DNA technology, the DNA profile is observed via a so called electropherogram, where the alleles are identified as signal peaks above a certain level or above a signal to noise threshold. Degradation implies that these peak intensities decrease in strength for longer STR sequences. Consequently, long STR loci may fail to produce peak heights above the limit of detection resulting in allelic or locus drop-outs.

In this talk, we present a method for measuring the degree of degradation of a sample and demonstrate how to incorporate this in estimating the probability of allelic drop-out. This is done by extending an existing method derived for non-degraded samples. The performance of the methodology is evaluated using data from degraded DNA where cases with varying amounts of DNA and levels of degradation are investigated.

O 49 CONSENSUS METHOD FOR LOW TEMPLATE TYPING AFFECTS GENOTYPING RELIABILITY AND DATABASE SEARCH RESULTS

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To analyze DNA samples with very low DNA concentrations, various methods have been developed that sensitize short tandem repeat (STR) typing. Sensitized DNA typing is accompanied by stochastic amplification effects, such as allele drop-outs and drop-ins. To minimize the number of drop-outs and drop-ins in low template (LT) DNA profiles one can try to infer the genotype by a consensus method that uses alleles confirmed in replicate analyses. This study focuses on consensus methods varying for the number of replications and requested level of reproducibility. DNA profiles from mock casework samples were obtained using standard PCR and capillary electrophoresis (CE), increased cycling and enhanced CE injection. Consensus profiles were assembled from two to six replications using four methods: composite (include all alleles), n-1 (include alleles detected in all but one replicate), n/2 (include alleles detected in at least half of the replicates) and 2x (include alleles detected twice). We compared the consensus profiles with the DNA profile of the known donor, studied the stochastic amplification effects and examined the effect of the consensus procedure on DNA database search results. We show that the accuracy of LT DNA typing and the efficiency of database searching improve when the number of replicates is increased and the consensus method is n/2. The most functional number of replicates within this n/2 method is four (although a replicate number of three suffices for samples showing >25% of the alleles in standard STR typing).

O 50 AN AUTOMATED APPROACH FOR GENERATING CONSENSUS PROFILES FROM LOW TEMPLATE STR TYPING RESULTS

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The introduction of new multiplex STR kits such as the PowerPlex ESI/ESX 16/17 (Promega) and AmpFℓSTR NGM (Applied Biosystems) assays, has increased the sensitivity of forensic STR profiling. As a result, more low template forensic DNA samples will become amenable for analysis. However, DNA typing of these samples is often accompanied by stochastic effects such as allele drop-out, heterozygote imbalance and higher stutter levels. A number of procedures have been proposed to increase the confidence of reporting a DNA profile for a low template DNA sample of which replication of the analysis (PCR and capillary electrophoresis) has been adopted by most forensic laboratories. This procedure relies on reporting the alleles observed at least twice in the replicates but is often laborious, especially since the interpretation (determination of the consensus profile) is mostly done manually. Here, we present an automated approach for generating consensus profiles from these replicates, which also includes the allelic balance within an STR result. The method uses data (allele calls and peak height) from a GeneMapper v3.2 output-file to determine the alleles for each STR locus in each profile that fall below and above a certain threshold (currently 50%) of the maximum peak height. STR results from different replicates are then compared to each other and a consensus profile is generated with major and minor alleles, and a separate indication of the alleles that have been observed once. The method was developed in an Access 2007 database and is currently validated with two multiplex STR kits (PowerPlex ESI16 and MiniPlex-9, an in-house developed kit).

Poster Presentations

P 001 NGM SELECT *VS* POWERPLEX ESI 17: RESULTS WITH CHALLENGING DNA SAMPLES

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AmpFℓSTR® NGM SElect™ PCR Amplification Kit (Applied Biosystems) and PowerPlex® ESI 17 System (Promega) enable amplification of 16 autosomic short tandem repeat (STR) *loci*: 10 SGM Plus® kit *loci*, the 5 additional *loci* in the expanded European Standard Set (D10S1248, D22S1045, D2S441, D1S1656 and D12S391) and the highly discriminating SE33 *locus*. The aim of this work is to verify the behaviour of both kits in the presence of challenging DNA samples.

Samples used in this study suffered from afflictions, such as low level DNA, degradation and PCR inhibitors: samples with induced degraded conditions and real crime scene samples, from a wide range of crimes. DNA control samples were also amplified, being the recommended amount of input DNA 1.0ng/µl (NGM SElect) and 0.5ng/µl (PowerPlex ESI 17), although DNA concentrations can differ, depending on the DNA quantification method used. In this study, most of the samples were extracted with PrepFiler Express™ Forensic DNA Extraction Kit (AutoMate Express™ Forensic DNA Extraction System, Applied Biosystems) and quantified with Quantifiler™ Human DNA Quantification Kit (ABI Prism® 7000 Sequence Detection System, Applied Biosystems).

Both analysed kits have been optimized to provide enhanced sensitivity and to generate more consistent results when dealing with challenging DNA samples, compared with other amplification kits. Results obtained (ABI 3130 Genetic Analyzer, GeneMapper ID v3.2, Applied Biosystems) with both kits were very similar, however it is important to refer that PowerPlex ESI 17 has one possible advantage related with the larger volume of DNA input that is allowed in each PCR sample.

P 002 NGM SELECT: AN IMPROVEMENT TO NGM KIT IN DETERMINING GENETIC PROFILES OF LOW LEVEL DNA SAMPLES

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AmpFℓSTR® NGM™ is a 16-*locus* multiplex kit that includes some miniSTRs, claiming better sensitivity and improved robustness than earlier generation kits. NGM SElect™ was released more recently (NGM *loci* plus SE33). With this study we want to determine if the NGM SElect can be an asset compared to the NGM in low level DNA samples and how both kits behave varying the number of PCR cycles.

Kits were tested with DNA total amounts from 1 to 0.0001ng, using 29 and 30 cycles of amplification. Two male samples were used (Control DNA 007, Applied Biosystems, 0.1ng/µl, and a fingernail sample, from a body found in the sea, ~1ng/µl). The recommended amount of DNA for both kits is 1.0ng.

Better results were obtained in the control DNA, being a pure sample; the nail is a degraded material with contaminants derived from the saline environment, despite the use of an extraction kit (PrepFiler Express™ Forensic DNA). For the optimal amount of 1ng we identified the complete DNA profile in both samples. For the amount of 0.1ng, the complete DNA profile of the control sample was identified, but for the nail only 70% of expected alleles were detected, on average. For 0.01ng of DNA, there is a sharp decrease in the percentage of detected alleles with loss of Amelogenin. For quantities of DNA below this value the identification of profiles is negligible or even null. For 29 cycles of amplification, the quality of the profiles obtained is equivalent. With 30 cycles, NGM-SElect profiles are more balanced.

P 003 RAPIDLY MUTATING Y-STRS: EXPANDING THE APPLICATION OF THE Y-CHROMOSOME FOR FORENSIC SCIENCE

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Two of the most conspicuous weaknesses of the current panel of Y-STRs used in forensic biology are their low level of paternal lineage discrimination in populations with low diversity, and their inability to discriminate between males of the same paternal lineage. Both of these limitations can be overcome by adopting Y-STRs with higher average mutation rates than those currently used, effectively promoting greater diversity within each marker, and ensuring that haplotypic links between family members are severed by frequent mutations. The newly proposed set of rapidly-mutating (RM) Y-STRs, composed of 13 markers with mutation rates above $1x10^{-2}$ found by genotyping 2000 father son pairs for 186 Y-STRs, displays higher diversity and greater discrimination capacity than the Yfiler Y-STR set. In a worldwide panel of 604 unrelated males, the RM Y-STRs provided 98.3% discrimination, and diversity of 0.99996 compared to Yfilers' 90.4% discrimination and 0.9994 diversity. The greatest strength of the RM Y-STRs, however, is to discriminate male relatives, with an average rate of 66% between 305 males related by 1—20 generations. This includes 48.7% of fathers and sons, 60% of brothers, and 75% of cousins. This is significantly greater than Yfiler, where only 15% were discriminated across all relative pairs, 7.7% of fathers and sons, 8% of brothers, and 25% of cousins. Thus, the RM Y-STR panel provides significantly greater male relative and lineage differentiation, and is proposed as an alternative or even replacement set of markers, for any cases where non-exclusions are found with the current panels of Y-STRs.

P 004 VALIDATION OF THE HEMOGLOBIN (Hb) HYPSOCHROMIC SHIFT ASSAY FOR DETERMINATION OF THE TIME SINCE DEPOSITION (TSD) OF DRIED BLOODSTAINS

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The ability to determine the time since deposition (TSD) of a dried bloodstain found at a crime scene could prove invaluable to law enforcement investigations, identifying the time frame in which the individual depositing the evidence was present. Although various methods for TSD determinations have been proposed, none have gained widespread use due to poor time resolution and weak age correlation. Previously, we examined the complete UV-visible absorption spectral profile of hemoglobin (Hb) in dried bloodstains of different ages and identified a hypsochromic shift (shift to shorter wavelength) of the Hb Soret band that demonstrated a high correlation with TSD. While this method demonstrated promise for future use with forensic samples, additional validation work was needed.

In the current work, we have further evaluated the effects of temperature and underlying substrate on the ability to obtain reliable TSD measurements using the Hb hypsochromic shift assay. We evaluated bloodstains deposited onto cotton, denim, polyester and paper incubated at various temperatures for up to 3 months. The effects of temperature were consistent with previous studies and no significant effects from underlying substrate were observed. Additionally, we further evaluated the use of a portable spectrophotometer which could be used "on-site" at crime scenes. This would allow not only an estimation of the age of the stain but the rapid positive identification of the presence of blood due to the unique visible spectrum of Hb. The ability to obtain sufficient quantities of DNA and DNA profiles from the original blood extract will also demonstrated.

P 005 THE USE OF EXTRA STR MARKERS IN COMPLEX RELATIONSHIP CASES

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Complex relationship cases can often be unsatisfactorily resolved when testing is performed using just a standard set of commercially available markers, e.g. ESI (Promega) or NGN (ABI). We show here the results that can be obtained when supplementing these tests with other well established markers, as well as a 7-marker in-house set we developed. This 7-marker set comprises 7 highly polymorphic STRs (D9S2157, D12ATA63, D10S1435, D2S1776, D3S4529, D17S974, D1S1627) independent of all existing STR loci commonly used in forensic genetics.

P 006 ASSOCIATION OF GENE POLYMORPHISM OF SEROTONIN-TRANSPORTER-LINKED POLYMORPHIC REGION WITH PARANOID SCHIZOPHRENIA AND ITS FORENSIC SIGNIFICANCE

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Identification of mental illnesses is an important task for forensic scientists; however, no biological marker is recognized so far as an objective indicator of the diseases. Studies have shown that mental illnesses may be caused by genetic factors and may be related to polymorphisms of neurotransmitter genes. In this study, we report an insertion/deletion polymorphism of serotonin-transporter-linked polymorphic region gene in 206 unrelated healthy individuals (102 males and 104 females) and 163 paranoid schizophrenia individuals (81 males and 82 females) in northern China. The results showed that the frequency of allele S was 0.7476, and the power of discrimination, polymorphism information content, and heterozygosity were 0.5422, 0.3062, and 0.3689, respectively, in northern Han Chinese population. The frequency of allele S in paranoid schizophrenic patients was 0.6748, which was significantly different from that of the healthy group ($p=0.0297$). Allele L might be the risk factor (OR=1.288).

Our study indicated that in the patients with hallucinatory, paranoid and impulsive symptoms, the level of serotonin was lower in the cerebrospinal fluid. The allele L exhibited a higher transcription activity. The serotonin transporter with genotype L/L showed higher level of mRNA and protein, and higher level of reuptake serotonin in the synaptic cleft. We therefore hypothesized that the allele L might be associated with paranoid schizophrenia via higher expression of serotonin transporter and transfer efficiency of serotonin, which resulted in the decrease of serotonin in the synaptic cleft. Our data may provide an important objective indicator for identification of mental diseases.

P 007 ASSOCIATION OF GENE POLYMORPHISMS OF TRYPTOPHAN HYDROXYLASE 2 GENE WITH PARANOID SCHIZOPHRENIA AND ITS FORENSIC SIGNIFICANCE

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There has been lack of objective indicators in forensic identification of mental illnesses so far. Studies showed that the mental illness might be caused by genetic factors and be related to gene polymorphisms. In this study, the SNPs within a 905 bp 5' flanking region and a 1104 bp 3' flanking region of tryptophan hydroxylase 2 (*TPH2*) gene were investigated by DNA sequencing in 174 unrelated healthy individuals (93 males and 81 females) and 164 paranoid schizophrenia individuals (87 males and 77 females) in northern China. The results showed that in addition to six reported SNPs, −703G/T, −473T/A, 90A/G, 92999A/G, 93329A/G and 93724C/G, one C/T variant in 92922 site was found in the sequenced region of the *TPH2*. Power of discrimination are both greater than 0.5 in 703G/T and 93329A/G sites, while power of discrimination are all greater than 0.35 in −473T/A, 90A/G and 93724C/G sites in northern Han Chinese population, with a better forensic value. The frequency of allele G in −703G/T site in paranoid schizophrenic patients and healthy group are 0.488 and 0.402. Statistical analysis manifest that there is significant difference between the two groups (p=0.0253). Signifying allele G may be a risk factor in paranoid schizophrenia.

−703 G/T site is located in 5' flanking region of *TPH2*. Whether influences the expression of the *TPH2* or associates with paranoid schizophrenia, further study needs to be performed. The systematic analysis to the related loci may provide important objective indicators for identification of mental disease.

P 008 ASSOCIATION OF GENE POLYMORPHISMS OF 5-HYDROXYTRYPTAMINE RECEPTOR 1A WITH PARANOID SCHIZOPHRENIA AND ITS FORENSIC SIGNIFICANCE

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There is no biological marker for forensic identification of mental illnesses so far. Studies have shown that mental illnesses may be caused by genetic factors and may be related to polymorphisms of neurotransmitter genes. In this study, the SNPs within a 2718 bp region of 5-hydroxytryptamine receptor 1A (*HTR1A*) gene were investigated by DNA sequencing in 182 unrelated healthy individuals (90 males and 92 females) and 161 paranoid schizophrenia individuals (80 males and 81 females) in northern China. The results showed that in addition to the five reported SNPs, rs6295, rs6449693, rs878567, rs6294 and rs34118353, two new SNPs were found in the sequenced region of the *HTR1A*, which were assigned as rs113195492 and rs112846276. The frequency of allele G at rs113195492 and allele C at rs112846276 site were 0.9258 and 0.9863; power of discrimination (DP) was 0.253 and 0.053 in the two sites, respectively. DP values on the rs6295, rs6449693 and rs878567 sites were greater than 0.5 in northern Han Chinese population, suggesting greater forensic implication.

The frequency of allele G at rs113195492 site in paranoid schizophrenic patients was 0.9534, which was not significantly different from that of the healthy group (p=0.1325) when gender was not taken into account. However, we found a significant difference between the frequencies of this allele in the females of the two groups (p=0.0323), suggesting that allele G might be a risk factor in female paranoid schizophrenic.

The newly found rs113195492 is located in 5' flanking region of *HTR1A*. It is not clear whether the G/A variant influences the expression of the *HTR1A* or is associated with paranoid schizophrenia. Further study is needed. Nonetheless, our data may provide an important tool for the biological identification of mental diseases.

P 009 DNA RECOVERY FROM TEETH: A COMPARISON OF TWO DNA EXTRACTION PROTOCOLS

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The high degree of chemical and physical resistance of the dental structure contributes to the DNA preservation, even in extreme forensic conditions. For this reason, teeth are the kind of tissue most frequently used in Human remains DNA typing. However, DNA extraction protocols are more complex in relation to other biological sources and the DNA yield is rather variable.

With the aim to assess the efficiency of the DNA IQ System in recovering DNA from teeth, fifty randomly selected samples of our routine case work, were processed following the suggested protocol. Results were compared with that obtained from another group of fifty samples processed with the QIAamp DNA Mini Kit standardized protocol. In both cases an elusion volume of 50 µl was used. Samples were quantified by qPCR and typed with AmpFℓSTR Identifiler (Applied Biosystems), PowerPlex 16HS (Promega), and MiniFiler Kit (Applied Biosystems), when needed.

Results showed a better performance in DNA recovering under the DNA IQ protocol, since in almost 75% of the processed samples, more than 2ng of total DNA were obtained, retrieving a complete DNA profile, whereas, only the 45% of the samples processed following the QIAamp Mini Kit protocol fall into this group.

P 010 SYSTEMATIC CHIMERSIM MONITORING IN DNA FROM BUCCAL SWABS FROM RECIPIENTS AFTER BONE MARROW TRANSPLANTATION: IMPLICATIONS FOR FORENSIC DNA TESTING

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Buccal swabs are the most common way for the collection of forensic DNA reference samples, as this method is straightforward and non-invasive. The reliability of comparative DNA testing using buccal cells as reference is beyond discussion and is based on the fact, that the DNA is the same in every cell of the body. Therefore all exceptions undermining this basic assumption are of vital forensic importance.

It has been shown, that recipients of allogeneic hematopoietic stem cell transplantations contain donor-derived cells in different tissues, e.g. the buccal mucosa, and that a mixed chimerism in the DNA from buccal swabs can result from DNA fingerprinting. However, a systematic documentation of this phenomenon is still lacking.

The chimeric state in a total of 162 buccal swab samples from 77 adult recipients after bone marrow transplantation was quantitatively determined by estimating the chimeric recipient/donor DNA ratios using 16 autosomal STR markers and the mitochondrial control region. Additionally, blood samples and hair roots were analyzed. The median age of the recipients was 50 years, ranging from 18 to 74. From each individual between one and 9 buccal swabs were taken at known time intervals after transplantation, ranging from 17 to 3.361 days (median 394 days). Percental chimerism levels (%Ch) between 0 and 100% were detected with maximal frequencies in the class between 10 and 30%. We discuss the results of the %Ch with respect to age, sex and the post transplantation period.

P 011 IDENTIFICATION OF CARBONIZED CORPSES THROUGH THE ANALYSIS OF STR POLYMORPHISMS OF X CHROMOSOME

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This paper reports the efficiency of the STR polymorphism analysis of the X chromosome in the identification of 13 carbonized corpses in a car accident where the family configuration was not complete, such as lack of the presumed father or mother, as well as the involvement of other relatives. Samples of the corpses and blood relatives were amplified using the polymerase chain reaction (PCR) and a multiplex system that allows co-amplification by PCR of twelve STR markers (12X-STR) linked to chromosome X (DXS7132, DXS7423, DXS7133, GATA172D05, DX7130, DXS6800, GATA31E08, HPRTB, DXS6789, DXS9898, DXS9895, DXS10011). Amplification products were analyzed by an automated sequencer and statistical analysis were performed using 1.7 software Families. The results of this study indicate that multiplex STR-12X is an extremely valuable tool in the analysis of kinship and affiliation of complex criminal cases, being highly polymorphic, the combined power of discrimination of all loci can be safely used for statistical calculation that underlies the conclusion the expert report of cases of complex family relationships.

Keywords: X-STR polymorphism, human identification, carbonized corpses, analysis of forensic cases

P 012 SNP TYPING OF CRIME CASE SAMPLES WITH THE SNP*FOR*ID MULTIPLEX ASSAY

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Introduction and aims
The SNP*for*ID multiplex assay involves amplification of 49 SNPs in one PCR and detection of the SNP alleles by two single base extension (SBE) reactions and capillary electrophoresis. The assay has worked robustly in our laboratory since 2007, where it was validated for paternity and immigration case work according to the ISO17025 standard. It has proved to be a valuable addition to the arsenal of investigations that may be used in cases where the standard investigations of autosomal STRs lead to an ambiguous conclusion.

The aim of this study was to test the assay on typical crime case samples, including mixtures and samples with low amounts of highly degraded DNA.

Materials and methods
Twenty known mixtures, 30 single source samples, and 50 degraded samples were selected from crime cases. The degraded samples were either poorly amplified or not amplified at all by the one of the old STR kits (SGM plus®, MiniFiler™ or Identifiler®).

Results and Discussion
For the study of mixtures, both controlled experiments with samples mixed in known ratios and a blind study were conducted. All mixtures were identified, including samples mixed in 1:40 ratios.

For the study of degraded DNA, the SNP*for*ID assay was optimized by altering the PCR buffer conditions and by increasing the number of SBE cycles. Complete SNP profiles were obtained in 80% of the highly degraded samples and at least 41 SNPs were typed in the last 20%. Furthermore, 30% of the highly degraded samples turned out to be mixtures.

P 013 87 DNA MARKERS FOR A PATERNITY TESTING: ARE THEY SUFFICIENT?

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In a paternity testing a panel of 15 STR is normally sufficient to solve the question. Anyway, in some cases, an increased number of markers are required to confirm existing filiation.

Here we report a judicial paternity testing in which a woman (AD) asserted to be a natural daughter of a deceased man (AF). The typing of alleged father DNA for 15 STRs obtained from paraffin-embedded blocks shows complete compatibility with the legal sons, confirming that profile was genuine, but two exclusions at D2S1338 and vWA loci were found in the comparative analyses with the alleged daughter. Since these results suggested that the true AD father shouldn't be AF, but a close male relative, the AD's mother (M) was included into analysis after exhumation. Alleged daughter, mother and alleged father were also typed for 24 validated STRs, 10 STRs for linkage analysis on 15 and 16 chromosomes, 8 X-STRs and 30 SNPs, for a total of 87 markers. No further exclusions were found.

Paternity index, taking into account mutation rates for D2S1338 and vWA, was 1.45×10^{13} (paternity probability W = 0.99999999999993). The final odds that the true father should be the untyped brother of the alleged father, that refused to provide a sample for DNA profiling, was 1 to 192. To the judge the end of the matter.

P 014 DEVELOPMENT OF A SNP MULTIPLEX ASSAY FOR THE INFERENCE OF BIOGEOGRAPHICAL ANCESTRY AND PIGMENTATION PHENOTYPE

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Intelligence data such as phenotype and ancestry inferences from unknown DNA samples may narrow the collection of potential matches in the absence of adequate reference samples necessary for positive identification. Single Nucleotide Polymorphisms (SNPs) are informative as these have accumulated in global populations, culminating in the classification of SNP motifs characteristic of specific global populations and/ or phenotypes due to geographical and/or cultural isolation. Uni-parentally inherited Y chromosome and mitochondrial DNA (mtDNA) SNPs, as well autosomal SNPs which exhibit strong biogeographical and phenotypic associations in humans have been identified and incorporated in various ancestry and/or phenotype inference assays. These assays usually target only one type of SNP and the predictive power of combining the 3 types of SNPs is yet to be firmly established.

A hierarchical multiplex SNaPshot™ (Applied Biosystems, USA) assay combining 21 mtDNA SNPs, 28 Y-SNPs and 13 autosomal SNPs was developed, where selected SNPs were grouped into 5 multiplexes. The haploid SNPs were combined within multiplexes 1 to 4 where similar ancestry specific SNPs are grouped in the same multiplex. The autosomal SNPs with biogeographical and pigmentation affiliations are grouped separately in Multiplex 5. Volunteer DNA samples collected from population groups which constitute the general Australian population are currently being genotyped to ascertain the predictive power of the assay by comparison to the participants' self-declared ancestry and pigmentation phenotype. The developed assay has the potential to provide valuable intelligence data with possible applications in criminal investigations, disaster victim identification and missing person cases.

P 015 20 SNPs AS SUPPLEMENTARY MARKERS IN KINSHIP TESTING

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Single Nucleotide Polymorphisms (SNPs) are having an increasingly important role in Forensic Genetics. This could become especially important in kinship analysis due to very low SNP mutation rates and the possibility to multiplex a great number of loci to generate a high amount of genetic information. This extra information can make SNPs useful in kinship casework.

The purpose of this study was to evaluate the use of 20 SNPs as additional markers in the resolution of kinship casework where the alleged father was not available for testing and close relatives were used instead. A total of six caseworks which included alleged paternal grandparents, alleged uncles or alleged brothers were studied.

All individuals studied in these cases were typed for 17 autosomal Short Tandem Repeats (STRs) using AmpFℓSTR Identifiler/IdentifilerPlus and Powerplex 16 and 20 SNPs typed using SNaPshot® methodology with two 10-plex, based on SNP*for*ID 52-plex previously shown useful in paternity testing. Likelihood Ratios (LR) were calculated with "Familias" using South Portugal STR and SNP frequency databases.

In all studied cases there was a concordance between results obtained with STRs and SNP analysis. This study confirms that SNP loci analysis, even as few as 20, can be very useful in kinship analysis as a complement to standard methodologies and can be easily implemented in standard Forensic Laboratories.

P 016 COMPLEX CASEWORK USING SINGLE NUCLEOTIDE POLYMORPHISMS

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Forensic geneticists come across casework that is difficult to analyze due to the complexity of family relationships. These cases are among those which can have serious implications for the individuals studied since can bring them criminal consequences. These caseworks can involve maternity by very young adolescents and incest. Routine Short Tandem Repeats (STRs) are not sufficient to give an answer in these cases and therefore more loci are needed to be studied, especially in immigration cases. This work presents two cases studied with routine STRs and Single Nucleotide Polymorphisms (SNPs) as supplementary markers.

In the first case, maternity investigation of a child was requested involving two alleged mothers - a young 29 year old woman, the alleged grandmother, and her absent daughter. The second one was a possible incest case where the maternal grandfather was also the alleged child's father. The individuals of these cases were typed for 17 autosomal STRs and 20 SNPs. STRs were typed with AmpFℓSTR Identifiler/IdentifilerPlus and Powerplex 16. SNPs were typed using SNaPshot® methodology with two 10-plex previously revealed useful in paternity testing, based on SNP*for*ID 52-plex. In the first case, a genetic incompatibility was detected with 17 routine STRs giving a low likelihood ratio value, as also obtained in the second case. In the first casework, the 20 SNP study strongly indicates that the alleged grandmother was not indeed the mother, while in the second casework SNP study reinforced the incest relationship. Therefore, SNPs revealed useful as additional markers in complex kinship testing.

P 017 A PRIMER BINDING SITE MUTATION AT THE D2S1338 LOCUS RESULTING IN A LOSS OF AMPLIFICATION

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An apparent loss of amplification at the D2S1338 locus was disclosed as a by-product of a study on germline mutations leading to an isolated paternal genetic incompatibility.

The allelic loss war detected after multiplex PCR with the AmpFℓSTR Identifiler kit (Life Technologies, Carlsbad, USA) which resulted in apparent false homozygosity at the D2S1338 locus in father (*17, *17) and child (*18, *18) whilst the mother showed a compatible genotype (*18, *18).

As the primer sequences for the AmpFℓSTR kits are not available, a singleplex PCR was carried out with published primers, which gave the same results. Thus, newly designed primers were applied which included the primer binding regions of the published primer pair. As a result, an additional allele appeared in father and child, which restored Mendelian inheritance. These PCR products were sequenced and showed the same C>T transition in both alleles *25 within the binding region of the published forward primer, at the fourth position from its 3' end.

The frequency of this primer binding site mutation (1 event in 2.263 allelic transfers) amounts to 0.44×10^{-3} (95% confidence limits: $0.01–2.46 \times 10^{-3}$). This figure, however, might be an underestimation because such so-called "null alleles" remain undisclosed if the parent/child pair shares the other allele.

P 018 MULTISTEP MUTATIONS AND DOUBLE GENETIC INCONSISTENCIES CAN BE OBSERVED IN RELATIONSHIP TESTING

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In course of a mutation study four multi-step mutations and two double genetic inconsistencies were observed within 50.796 allelic transfers at 23 STR-loci (ACTBP2 (=SE33), CD4, CSF1PO, F13A1, F13B, FES, FGA, vWA, TH01, TPOX, D2S1338, D3S1358, D5S818, D7S820, D8S1132, D8S1179, D12S391, D13S317, D16S539, D17S976, D18S51, D19S433, D21S11) in Caucasoids living in Austria and Switzerland.

Apart from the vast majority of single step mutations two double-step and two four-step mutations occurred, which appear to be very uncommon. Both cases were observed in classical trios at the D18S51 (mother *13,*14; child *8,*14; father *12,*17) or the D2S1338 locus (mother *20,*22; child *20,*21; father *19,*25; in this case sequencing results did not favour the shortest possible two step mutation of the paternal allele *19). Therefore, a four-step mutation had to be assumed in this case, which was encouraged by an unequivocal four-step mutation at the D18S51 locus, where only a nine-step mutation was a possible alternative.

Additionally, two cases with a double genetic inconsistency were observed. The first case was a paternal germline mutation at the vWA locus combined with an allelic dropout in a maternally transmitted SE33 allele. In the other case a combined paternal SE33 and CSF1PO single step mutation contributed to a double germline mutation.

P 019 INSERTION/DELETION POLYMORPHISMS: NEW DIPPLEX KIT FOR FORENSIC IDENTIFICATION AND PARENTAGE TESTING

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Insertion/deletion polymorphisms (INDELs) are diallelic markers derived from a single mutation and have low mutation frequency, which makes them suitable for parentage testing. INDELs combine advantages of both STR and SNP. They have as small amplicon size as SNP (about 100 bp), but could be analyzed by techniques used for routine STR analysis. In our study we genotyped 55 unrelated members of Czech population to make a population study and we also genotyped 11 trios to analyze DIPplex Kit (QIAGEN, Germany) suitability for parentage testing. DIPplex Kit contains 30 diallelic autosomal markers. INDELs in DIPplex Kit were tested with linkage disequilibrium test, which showed that they could be treated as independent markers. We used DNA isolated by QIAmp Blood mini kit (QIAGEN, Germany). DNA was amplified using DIPplex Kit and then analyzed on capillary electrophoresis ABI 310 (Applied Biosystems, USA). Acquired data were analyzed using Genotyper software. All 30 loci fulfill Hardy-Weinberg equilibrium. There are several significant differences between Czech and African populations, but no significant differences within European population. Probability of a random match in Czech population was 1 in 6.8×10^{12}, combined power of discrimination was 99.9999%. Average paternity index was 1.13–1.77 for each locus, combined paternity index was about 21000 for a set of 30 loci. We can conclude that DIPplex kit is useful as an additional panel of markers in paternity cases when a mutation is involved. For application on degraded or inhibited samples further optimization of buffer and primer concentrations is needed.

P 020 SEQUENCING OF MTDNA HV1 AND HV2 REGIONS FROM SAMPLES WITH TRACE AMOUNT OF DNA

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Some degraded biological samples can't be genotyped with routine STR analyzes. At these cases, mitochondrial DNA analyzes can be done to genotype degraded samples. The most diversity between random mtDNA molecules is in the control region of mtDNA. The control region of mtDNA consists of 3 hyper variable regions: HV1 (16024–16383), HV2 (73–354), and HV3 (438–574). In routine, HV1 and HV2 regions are used for forensic analysis. The aim of this work were optimizing and validating the sequencing of mtDNA's HV1 and HV2 regions from samples like hair, nails, earrings, toothbrushes, q-tips, glass edge swabs, gums, razors and cigarette butts. At this work 5 unrelated individual's samples mentioned above were sequenced and other 13 unrelated individual's blood samples were sequenced for the reliability of the procedure. In the first PCR reaction, the target regions were amplified separately with their specific primer pairs, in the second PCR reaction Applied Biosystem BigDye v3.1 Cycle Sequencing Kit was used. The electroforetic separation was carried out on capillary electrophoresis, and results were analyzed with Sequence Scanner (Applied Biosystems) and Sequencher 4.10.1 software's. In this examined Turkish population, most common polymorphic positions were 263, A>G point mutation, and 315.1 C insertion. The HV2 profile "263, A-G 315.1C" observed on 3 individuals and "152, T-C 263, A-G 309.1 C 309.2 C 315.1 C" profile observed on 2 individuals. 2 individual also shared a profile same as rCRS on their HV1 regions. Every analyzed person has unique HV1 + HV2 profiles together.

P 021 NEW MINI STR LOCI D10S1248, D14S1434, D22S1045, D4S2364, D2S441, D1S1677 VALIDATION AND OPTIMIZATION ON BLOOD AND BLOOD SPOTS

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STR loci, which have been used since 1990s, are ideal genetic markers in forensic science. Because DNA analyses with STR markers are sensitive and specific and easy to analyze. STR analyzes give results in short time, allow multiplex PCR, and show high polymorphism. But, it may occur problems with genotyping highly degraded forensic samples and trace amounts of biological samples. To solve these problems National Institute of Standards and Technology (NIST) had developed 26 new miniSTR loci. These loci amplicons are less than 125 bp.

In this study; 6 new mini STR loci (D10S1248, D14S1434, D22S1045, D4S2364, D2S441, D1S1677) were studied, which is based on Coble at all's method (2005) and several modification were made on this PCR method. Primer Dye set should be FAM-TET-HEX rather than FAM-VIC-NED. Primer concentrations were increased to: D1S1677 1.5 µM, D2S441 1.7 µM, D4S2364 1.3 µM, D10S1248 1.6 µM, D14S1434 1.3 µM, D22S1045 1.5 µM. PCR conditions were the same but cycle was raised to 32. Top Taq™ Master Mix Kit(Qıagen) was used for PCR. 6 loci were studied as two triple multiplex sets. PCR amplicons were applied on ABI 310 Genetic analyzer. Compatible with these primer's dye set, DS-34 Matrix Standart and GeneScan™ 350 TAMRA™ Size Standard were used. After the method was optimized with these conditions, validation studies were carried out by working the sensitivity and reproducibility of this procedure and analyzing mixed samples.

P 022 DEVELOPMENT OF AN ANCESTRY INFORMATIVE STR MULTIPLEX

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Genetic ancestry inference using ancestry informative markers (AIMs) can offer considerable additional information to help guide criminal investigations. To date, single nucleotide polymorphisms (SNPs) have been the first choice loci to construct AIM panels for potential use alongside conventional DNA profiling markers. However, typing multiplexed SNPs with SNaPshot™ single base extension chemistry is the only realistic system currently capable of analyzing the small quantities of DNA routinely found in forensic casework. A major shortcoming of SNaPshot is the inability to properly distinguish very imbalanced heterozygote peak patterns from mixed source DNA.

We approached the problem of reliance on SNaPshot by looking at alternatives to SNPs in the battery of AIMs available for forensic applications. Considering short tandem repeats (STRs) as ancestry markers allows use of dye-labelled PCR primers and restores a direct relationship between peak height ratios and input DNA. We examined 783 candidates with global population variability data and eliminated di- and tri-nucleotide repeat STRs, markers lacking proper validation and the least uninformative loci. We aimed to balance the differentiation in allele frequencies shown when comparing the five major population groups of Africa, Europe, East Asia, America and Oceania.

We have built a 12-plex AIM-STR assay with optimized primer combinations, fully characterized repeat unit patterns, sequenced reference ladders and allele frequency databases from the CEPH genome diversity panel. The characteristics and performance of the assay will be outlined with particular reference to classification success measured using the 12-plex alone or in combination with existing SNP-based forensic ancestry panels.

P 023 FLOODS AND MUDSLIDES IN RIO DE JANEIRO AND A PLANE CRASH IN THE BRAZILIAN AMAZON RAINFOREST – A STUDY OF TWO DIFFERENT EXPERIENCES IN DVI

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In DVI there are critical variables that will shape response to incident. In Brazil there were recently two big disasters that had great impact on the society. Floods and mudslides in the mountainous region of the State of Rio de Janeiro caused the biggest natural disaster in Brazil, with 905 deaths and 305 missing. In this event, the victims' identification is still being performed. The other incident was a plane crash in the Brazilian Amazon Rainforest, with 154 deaths. In this case, all victims were identified. The aim of this study is to show the components of the response to each disaster, how specific attributes of the incidents differed, how the victims' identification was performed and the main challenges of each event. In the two disasters, variables such as coordination of the DVI, kind of disaster, different geographic areas, weather conditions, magnitude of the event, number of victims, condition of bodies and remains, degree of fragmentation and decomposition, access and transport to the incident areas, appropriate personnel, integrated professional of forensic sciences and physical resources shaped different responses. In both incidents, fingerprinting, pathology, odontology, forensic anthropology and DNA were used. Concerning DNA testing, this study will show the process of antemortem and reference data collection, sample selection according to each disaster and condition of the bodies, chain of custody, storage and transport of DNA samples, consents, documentation, database, policies on human remains, DNA exams and matching, In the two disasters, DNA testing played a decisive role in the victims' identification.

P 024 FORENSIC PERFORMANCE OF INSERTION-DELETION MARKER SYSTEMS

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The ability to improve amplification and analysis of degraded DNA extracts has been a long standing area of research in forensic genetics. One of the latest approaches is the single multiplex typing of insertion-deletions (InDels), short biallelic length polymorphisms.

InDels share most of the properties of single nucleotide polymorphisms (SNPs) that makes them ideal markers for forensic analysis of degraded DNA. The short amplicon size ranges, high multiplexing capability, and low mutation rate make them an attractive complement to mini-STRs. In addition, as length polymorphism markers, InDels can be analyzed with the same simple end-labeled PCR primer methods as STRs, thus avoiding the multi-step protocols required of SNP typing single base extension assays, as well as providing a more direct relationship between input DNA and peak height ratios.

InDel genotyping should be considered a serious candidate for incorporation into the forensic marker battery. In order to assess the utility of such assays to the forensic community we have conducted a thorough analysis of a set of U.S. population samples. A total number of 68 different InDels [within two kits, a 30-plex 'DIPplex' produced by Qiagen and a 38-plex InDel panel described by Pereira et al., Electrophoresis 30 (2009) 3682-90] were typed for over 600 samples. Allele frequencies were generated for 68 markers on U.S. African American, Caucasian, and Hispanic samples. A thorough assessment of the individual and combined analytical performance of these markers, as well as characterization of artifacts and issues linked to these newly developed assays will be discussed.

P 025 CHARACTERIZATION OF U.S. POPULATION SAMPLES USING A 34-PLEX ANCESTRY INFORMATIVE SNP MULTIPLEX

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The number, scope, and ease of typing of single nucleotide polymorphism (SNP) markers makes them ideal supplements to existing forensic markers sets. SNP typing panels offer additional benefits such as the ability to type degraded DNA, paternity analysis, and the opportunity to infer externally visible traits. SNP also carry the potential to infer the most likely population of origin of an individual using SNPs with highly differentiated allele frequency distributions. One recent example is a 34-plex assay developed by C. Phillips et al. using SNaPshot primer extension reactions[1].

A significant amount of population information has already been generated, published, and uploaded to the SPSmart open access SNP browsers[2]. The original work by Phillips et al. analyzing the CEPH population diversity panel indicated a low error rate of ancestry prediction when confining comparisons to the three major population groups of Europe, Africa and East Asia. However, admixed populations commonly found in the U.S. (e.g. Hispanics and African Americans) represent a significant source of error or at least reduced assignment probabilities when making ancestry predictions. Thorough and wide population surveys in areas where admixture is the predominant pattern, is an important part of the process of assessing this potential source of classification error. In order to contribute to the data already available to end-users, we have generated allele frequencies for a set of U.S. African Americans, Caucasian and Hispanics for 34 ancestry informative SNPs. The data accumulated should contribute to improved characterization of admixed US populations and their analysis through SNP genotyping.

[1] *Phillips, C., Salas, A., Sanchez, J.J., Fondevila, M., Gomez-Tato, A., Alvarez-Dios, J., Calaza, M., Casares de Cal, M., Ballard, M., Lareu, M.V., Carracedo, A. (2007) Inferring ancestral origin using a single multiplex assay of ancestry-informative marker SNPs. FSI: Genetics 1: 273-280.*
[2] *Amigo, J., Salas, A., Phillips, C., and Carracedo, A. (2008) SPSmart: adapting population based SNP genotype databases for fast and comprehensive web access. BMC Bioinformatics 9: 428.*

P 026 SNPs IN MITOCHONDRIAL DNA CODING REGION USED TO DISCRIMINATE COMMON SEQUENCES IN HV1-HV2-HV3 REGION

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Regions HV1, HV2 and HV3 of mitochondrial DNA (mtDNA) are routinely analyzed for forensic and evolutionary purposes due to the high polymorphic rate but sometimes show limited power of discrimination since several polymorphisms are very common in different populations. Genotyping additional SNPs in the coding region of mtDNA has been suggested to increase the power of discrimination between individuals who show common haplotypes in control region. Herein, we intended to evaluate the discrimination power of 26 SNPs from coding region, previously described, in 15 pairs of mother/child who could not be previously individualized and matched by HV1-HV2-HV3 analysis. These 15 pairs were divided into 7 groups of common sequences, of which 2 presented European haplogroup (Hg) H, 2 showed Amerindian Hg B4, 2 Amerindian Hg C1, 2 Amerindian Hg C1b2, 3 African Hg L3e2b, 2 African Hg L3e1 and 2 African Hg L1b. Sequencing was performed using BygDye Terminator v3.1; capillary electrophoresis was performed on ABI3130. The SNPs were determined by comparison of the sequences obtained with reference sequence rCRS. After sequencing we could find 46 SNPs to analyze. Twenty-eight out of 46 SNPs, in different combinations, were able to distinguish all pairs of mother/child except two from Hg L3e2b. The SNP 8047, observed here is not report in MITOMAP. This can be a preliminary result in the direction of having a SNP set that could be used to discriminate common sequences in mitochondrial control region in a highly mixed and heterogeneous population as the Brazilian one.

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P 027 UNUSUAL FGA AND DYS437 OFF-LADDER ALLELIC INHERITANCE FROM FATHER TO CHILD

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We are reporting a case of off-ladder allelic inheritance from the suspected father to questioned child at FGA and DYS437 loci, encountered during routine paternity testing using commercial DNA profiling kits including AmpFℓSTR® Identifiler® kit, PowerPlex® 16 kit, PowerPlex® Y kit and the AmpFℓSTR® Y filer kit. The off-ladder alleles were isolated, cloned and sequenced. The FGA profile of the suspected father was special cleavage peak, which contained 23 and 23.1 allele, the off-ladder allele "23.1" inherited to the son. So, special cleaved profile should be noticed so as to avoid the error of identifying heterozygote as homozygote. The off-ladder profiles in DYS437 locus of the father and son were beyond the category range of DYS437 but before the next locus, and the sequence revealed that no mutation was found in the primer binding region and the repeat motifs displaying Asian specific repeat structure, but a 27 bp insertion was found following the consensus sequence and preceding the repeat region. This is the first report of insertion within the upstream flanking region of DYS437 locus and off-ladder allele inheritance from father to son.

P 028 VALIDATION OF THE NGM SELECT KIT™

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Introduction

The Swiss national database was launched August 2000 based on the 10 SGM Plus loci. With the aim of addressing the needs of the next-generation European STR genotyping systems in Switzerland, we validated the NGM SElect™ kit. This commercial kit contains the SGM Plus loci, therefore compatible with the existing swiss national database, the SE33 locus as well as the 5 new ENFSI/EDNAP loci D10S1248, D22S1045 D2S441, D12S391 and D1S1656.

In this study, we present the results of forensic validation studies including the following aspects: sensitivity, selectivity, performance with simulated inhibition and degradation, stutter and peak height ratio calculations. Many of the validation experiments were also performed in parallel with other STR kits (SGM Plus, ESX 17 and ESI 17) in order to compare their performances regarding diverse forensic samples.

Materials and methods

The amplifications for all the aforementioned kits were performed according to their standard protocols with a reduced PCR volume of 10 µl.

Results and discussion

The NGM SElect, showed comparable performances to the ESX/ESI 17 kits. When compared with the SGM Plus, the NGM SElect showed improved performances, especially in regards to its sensitivity and greater tolerance to high levels of laboratory prepared inhibitors. Making this kit well-suited for challenging casework samples as well as paternity testing.

P 029 HOW USEFUL IS YOUR X IN DISCERNING PEDIGREES?

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It is known that autosomal unlinked markers are unable to distinguish some pedigrees (such as grandparent/grandchild, avuncular and half-siblings). This poses a problem especially in cases of identification in mass disasters, other human remains, or in heritage cases where it is crucial to define kinships.

Theoretically, X-Short Tandem Repeats (X-STRs) analysis should allow distinction of some of those pedigrees and so a practical approach was undertaken in order to quantify the informative power of a set of X-STRs currently available in the forensic community.

Therefore, female/female, female/male and male/male individual pairs, known to be linked by the above mentioned kinships, were tested using Investigator Argus X-12 kit (Quiagen). Likelihood Ratios (LR), between autosomally indistinguishable pedigrees were calculated for 19 duos of each sex combination.

When LRs are assessed considering exactly one pedigree in which incompatibilities are possible (paternal half-sisters or paternal grandmother-granddaughter) satisfactory values are obtained favoring one of the relationships. Apart from these cases, LRs were, in average, low and, in many cases, against the pedigree known to be under analysis.

Nevertheless, the study of X-STRs can be crucial in situations where genetic profiles are the only available information about the individuals. Other types of information (such as age) can help to distinguish between such equally likely possibilities of pedigrees, excluding or weighing differently each possibility.

P 030 RECONSTRUCTION OF PIGMENTATION TRAITS IN A GERMAN POPULATION

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The analysis of autosomal and gonosomal STRs is still the most common method in forensic casework for identification purposes. In most cases the DNA patterns can be compared with those of potential suspects, relatives or from privately used items. However, in some cases no information regarding the investigated individual exists, giving no possibility for identification with the commonly used STR-analysis. In those cases it could be helpful to have information about the phenotype of the unknown individual. Thus, the analysis of externally visible traits represents a valuable tool for forensic case work and historical questions.

In this study 18 SNPs were selected from the literature to determine different haplotypes for the reconstruction of pigmentation traits including eye-, hair-, and skin-color. Therefore DNA samples from 350 German unrelated persons, representing a great variety of phenotypic appearances, were taken. Amplification and detection of selected SNPs was done by using a multiplex PCR assay and the SNaPshot® Kit (Applied Biosystems). Afterwards the individual appearances were compared to the determined genotypes to define specific haplotypes. The statistical evaluation delivered significant SNP-haplotypes for the German population regarding different pigmentation traits.

We will present the results of this study and show first case reports on the application of this method on historical samples.

P 031 ANALYSIS OF TRI-ALLELIC SNPS FOR FORENSIC PURPOSE IN CHINESE HAN POPULATION

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Single nucleotide polymorphisms (SNPs) have been proved to be effective forensic marker for supplementary paternity testing and forensic identification due to their low mutation rate and depending on small amplicon size. However, at least a set of 50–60 binary SNP loci can reach discrimination power of 13 STR loci that were routinely used in forensic practice. Meanwhile, binary SNP loci were ineffective to detect mixed samples, which may result in incorrect genotyping. In this study, we aimed to find out the potential forensic markers, tri-allelic SNP loci in Chinese Han population. Pyrosequencing (PSQ) with pooling samples was used to analyze candidate SNP loci and estimate allele frequencies of them. To confirm the existence of three alleles, individual samples were sequenced with PSQ method. Our results revealed that there were 10 tri-allelic SNP loci resided on 7 autosomal chromosomes in Chinese Han population. These data demonstrated that PSQ was an effective method to detect tri-allelic SNPs and estimate allele frequencies of them.

P 032 DETERMINATION OF SIBLINGS: A SPECIAL CASE REPORT FROM HALLE

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The most common use for sibling tests is to determine the likelihood of individuals having the same parents in common. In situations where one or both parents are unavailable or unwilling to be tested, it is usually not possible to solve such cases with conventional autosomal STR typing alone, because the genetic profiles of siblings can be completely different according the Mendelian law of inheritance.

We present a case of three female children, which were found in a baby hatch of a hospital in Halle during the last three years. The youth welfare office in cooperation with the adoptive parents wanted to know, if the three girls are full siblings, half siblings or unrelated.

Expecting, that autosomal STR typing will be not enough informative, we decided for additionally testing of X-chromosomal STRs (X-STRs), single nucleotide polymorphisms of mitochondrial DNA (mtDNA SNPs) and sequencing of the whole mitochondrial control region.

Genomic DNA was extracted from saliva samples of the three girls according to standard procedures (Qiagen Kit, Valencia, USA). PCR amplifications were performed for 23 autosomal STR loci using PowerPlex16 HS and PowerPlex ESX systems (Promega), 12 X-STR loci using the Investigator Argus X-12 kit (Qiagen) and additionally 15 X-STRs by in-house systems considering X-chromosomal linkage groups. The PCR products were analyzed by capillary electrophoresis using ABI 310® Genetic Analyzer (Applied Biosystems). Furthermore we determined 16 mtDNA SNPs as well as the whole mitochondrial control region sequence structure.

We present the results of all mentioned investigations as well as the results of the calculation of sibling (DNAVIEW – Ch. Brenner).

P 033 AN ITALIAN JEAN JACQUES ROUSSEAU: A COMPLEX KINSHIP CASE

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Since the introduction of haploid markers and binary polymorphisms, forensic DNA typing has no longer been confined to conventional paternity cases. Genetic testing can now effectively support the reconstruction of complex pedigrees, as required for the reunification of immigrant and war-torn families, or the identification of relatives among bodies found in mass disasters.

Here we report the case of four women and a man, all born in an Italian village during and immediately after WWII, that recently contacted our laboratory in order to perform kinship analysis. According to their claim, the propositi were the illegitimate offspring of a country gentleman and a peasant woman, given in adoption immediately after birth. A story that curiously reminded us that of Jean Jacques Rousseau, Theresa Levasseur and their five children.

Alleged siblings were typed with a combination of mtDNA (HV1 and HV2 regions), 21 X-chromosomal STRs including six clusters of linked markers, and a panel of 40 autosomal STRs. The obtained results could not exclude that the tested subjects were full-sibs. They all shared a single mtDNA haplotype, previously unreported in the EMPOP database, and X-chromosomal haplotying also strongly indicated close relationship. Analysis of autosomal STRs with the software Familias showed that full sibship was the most likely hypothesis (LR > 10^8) among more than one thousand alternative pedigrees.

The obtained results and problems connected to kinship analysis in cases where all stated relationship are questioned, and a wide range of different pedigrees could be used as hypotheses in LR calculations are discussed.

P 034 THE POTENTIAL RISK OF FORMALIN FIXED AND PARAFFIN WAX EMBEDDED PROSTATE BIOPSIES FOR HUMAN IDENTIFICATION

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The genetic instability in malignant tumors and the possible DNA degradation in archived histological samples are potencial confounder factors that can interfere in the human identification process using formalin fixed and paraffin wax embedded tissues. Prostate biopsies for tumor diagnostic require a large number of samples that may introduce biological material mix-up, become necessary the forensic investigation in some cases. In the present investigation a total of 49 biopsies from 20 individuals (normal and tumor tissue) were investigated (Identifiler Kit-Applied Biosystems) and the autossomic STR profiles were compared to FTA blood samples. For each amplification, 10ng of DNA hot xylol and phenol/chlorophorm extracted from the biopsies was used. The identification of the entire profile was possible only in 4 cases (20%) and absolute no amplification occurred in other 4 individuals (20%). In 60% of the cases partial profile was obtained and the drop-out occurred mainly at the loci with larger products, probably due to DNA degradation. Considering all the 49 biopsies, it was obtained 13 completed profiles (26%), 18 biopsies with partial profile (37%) and 18 biopsies without DNA amplification. It was not observed genetic instability in the STRs evaluated when compared to the FTA profiles. The DNA degradation due to the size of the prostate biopsies, the laboratory preparation and also the identification process (Identifiler Kit) could be responsible for the partial results observed. The utilization of Minifiler and Yfiler could be better to elucidate suspicions of mix-ups of prostate biopsies.

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P 035 PRENATAL SAMPLES USED AS DNA EVIDENCE IN RAPE CASES

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DNA typing of forensic evidence necessary to prosecute rape cases is routine analysis in criminal forensic casework. Prenatal samples can be used as DNA evidence for such cases by establishing their paternal origin. Herein we report two cases of sexual abuse. The first case regards a thirteen years old girl's rape that resulted in pregnancy. The girl reported the assault after four months and the only available evidence for DNA typing, at that time, was the amniotic fluid. The second case regards a sixteen years old girl's rape by her alleged father. The rape resulted in pregnancy that was terminated three to four weeks after conception. Abortion material was sent to the lab as evidence for DNA analysis. In order to extract DNA from the amniotic fluid sample, the laboratory's protocol for DNA isolation from tissues was slightly modified. In this presentation we report the modified protocol. In addition we present our approach to processing the "abortion specimen" as DNA evidence and the results of DNA analysis.

P 036 USING X-CHROMOSOMAL MARKERS IN RAPE INVESTIGATION

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The X-chromosomal markers are increasingly used in forensic genetics, particularly for relationship testing. Their use has become a valuable tool in complex cases of kinship but rather in criminal caseworks is still quite rate. In this paper the authors present a case of sexual assault in which the use of X chromosome polymorphisms has been crucial.

The victim was a young immigrant woman found dead in her home. The main suspect was her husband. However, the couple lived in a community in which the particular cultural context suggested the involvement of the other males of the husband family.

The suspect lived together with his brother, his father and his uncle.

Generally, a useful tool to solve cases of sexual violence is undoubtedly the use of Y chromosome, but in this case this device could not discriminate between the four males involved.

An additional factor has further complicated the situation: the most important biological evidence (typed with AmpFℓSTR Identifiler and AmpFℓSTR NGM) showed a mixed profile in which was very difficult to discriminate the suspects profiles.

To solve the casework, the authors typed the victim, the suspects and the biological traces with 11 X-STRs in three homemade multiplexes.

The results showed the presence of victim and her husband profiles in the biological trace excluding his brother, father and uncle's profiles.

P 037 IS IT ALWAYS POSSIBLE TO AVOID EXHUMATION IN PARTICULAR DEFECTIVE PATERNITY CASES BY INCREASING AUTOSOMAL STRS NUMBER?

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Genetic markers represent a very important tool in forensic identification caseworks, in family relationships as well as in criminal analyses. The discrimination power of current genetic polymorphisms is so high that the inferential process can be efficiently used even in cases where direct knowledge on the genetic data of one of the terms in comparison is lacking. However in some cases despite the use of Probabilistic Expert Systems (FINEX and Familias) it is not always possible to achieve an acceptable percentage of paternity probability.

Certainly one of these cases is the request to verify the relationship between two siblings of different gender in the absence of data from parents. In these cases it is not possible to use important tools such as polymorphisms of sexual chromosomes, so the only possible approach is to increase the number of autosomal STRs. Therefore the authors decided to investigate 35 pairs of siblings with known relationships from different parts of Italy (13 Perugia; 12 Brescia; 10 Padova), using a high number of autosomal STRs.

The aim of this study is to verify whether, increasing the number of autosomal STRs analyzed, the application of Probabilistic Expert Systems allows to achieve an acceptable value of paternity probability without availability of parents' profiles.

P 038 IDENTICAL BUT NOT THE SAME: THE VALUE OF DNA METHYLATION PROFILING IN FORENSIC DISCRIMINATION WITHIN MONOZYGOTIC TWINS

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Monozygotic twins (MZs) show remarkable resemblance in many aspects including behavior and health, because they theoretically share identical genomic DNA. However, evidence for epigenetic differences within MZs has been accumulated. DNA methylation differences between MZs could partially account for their phenotypic discordance of behavioral traits and diseases. High throughput epigenomic microarray profiling can be a strategy of choice for identification of epigenetic differences in phenotypically different MZs.

In this study, we mapped MZs DNA methylation differences in white blood cells by interrogation of the unmethylated genome on methylation Beadchip. Blood samples were taken from 22 pairs of adult MZs consisting of 13 female and 9 male pairs. Genomic DNA was bisulfite modified by EZ DNA methylation-Gold kit according to the manufacturer's protocols, consequently analyzed with Illumina's Infinium Human Methylation27 Beadchip including more than 27 000 CpG sites distributed on more than 14 000 well annotated genes. The results indicated that MZs exhibited remarkable differences among their genome-wide 5-methylcytosine. Epigenetic distance within MZs was similar to that of unrelated individual pairs. According to a set of selection criteria, 377 CpG sites with significant differences of methylation status within MZs were picked out form the global epigenome, most of which were on the genes interacted with environmental agents. Although DNA methylation shows only partial stability, primary results of this study strongly suggested that the CpG methylation could be a perspective biomarker to distinguish MZs from each other.

Keywords: Monozygotic twins; Individual identification; DNA methylation; Forensic genetics

P 039 PYROSEQUENCING OF A SHORT FRAGMENT OF THE AMELOGENIN GENE FOR GENDER IDENTIFICATION OF HIGHLY-DEGRADED DNA

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Instruction and aims
Gender information is one of the useful information in the forensic casework and archaeological research. For such a purpose we designed and developed an assay to distinguish male and female based on DNA pyrosequencing.

Materials and methods
The PCR products including primers (40bp) and target sequence (5bp) consisting of 3 point mutations and one indel mutation, were sequenced by pyrosequencing. 100 randomly chosen DNA samples of healthy donors of known sex (50 from males, 50 from famales), and a series of validation experiments, such as the sensitivity, accuracy and species specificity, degradation models, bone samples, were performed.

Results and discussion
100 DNA samples got clear typing graphs and correct results. The sensitivity of the technique was 0.5ng template DNA. No specific peak was found in any detected animals or organisms except for monkey. For blood samples that were left outside for 26 weeks and DNA degraded artificially by digesting with DNase I, this method gave more accurate results than the conventional method. Moreover, 4 bone samples analyzed using the method gave clear pyrogragh. This method is easy, quick, cheap and suitable for high-throughput analysis, especially for identifying the gender of highly-degraded DNA samples.

Keywords: Forensic Science; DNA typing; Amelogenin gene; Bone DNA; Degraded DNA; Gender identification; Pyrosequencing

P 040 STUDY ON TYPING OF DNA EXTRACTED FROM DANDRUFF

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Dandruff is composed of epidermal skin cells with different differentiation level. Its diameter is between 0.4–2mm in size. Since some cells in the process of incomplete keratinization may leave which causes the possibility of existing nucleated cells in dandruff and the STR typing of dandruff can be applied into forensic cases as effective complement method. To test the applicability of dandruff in forensic DNA testing, in this study, 10 dandruff and its control samples from adult volunteers were examined. Genomic DNA was extracted using the chelex method and quantified by Quantifiler human DNA Quantification Kit. The DNA content varied between 1.2–7.8ng. 15 STR loci were tested with the Identifiler™ kit. 3–5 STR loci could be successfully detected after storing 10 days and no STR loci could be detected after storing 20 days. 9–11 STR loci were successfully detected for the dandruff stored at 10 days through a series of treatments including purified enrichment with microcon-100 and re-amplification using Minifiler kit which greatly improved the success rate of genotyping for dandruff.

Keywords: forensic genetics; dandruff; STR

P 041 THE STR ANALYSIS OF TWO CONGENITAL CHIMERAS IN PATERNITY CASES

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A congenital chimera is an organism possessing two genetically distinct cell lines either in the whole body or limited to the haemopoietic tissues. Human chimerism is more common than imagine. We described two paternity cases in which two phenotypically normal alleged fathers' blood sample showed abnormal STR profile that more than two alleles hold one locus. To clarify the chimeric form or origin and evaluate its consequence in forensic purpose, We additionally take this two male chimeras' hair follicles, buccal mucosa, semen and urine for test and bring their biological parents involved. The autosomal STR profile from all tissues of both male chimeras revealed the presence of two maternal and paternal alleles at some loci, indicating they were "whole body chimera" who derived from the fertilization of two maternal nuclei and sperms. The first chimera in case 1 was deduced formed from the merging of male and female twins due to existence of a sure paternal X allele at some X-STR loci whereas the second in case 2 might from the merging of two nonidentical male twins due to none of a sure paternal X but double maternal X alleles existing. Y-STR profiles of both male chimeras showed normal and coincided with their fathers. Different intensity of allelic imbalance was also observed in different kinds of tissues. Allele could drop out by significant peak height imbalance (<70%) in some tissue whereas it did not in other tissue. This might lead to a false interpretation in paternity or crime investigation.

P 042 A FORWARD POPULATION IDENTIFICATION TOOL USING Y-SNPs IN FORENSIC CASEWORK AND ANCIENT DNA STUDIES

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Analysis of Y-chromosome single nucleotides polymorphisms (Y-SNPs) is commonly used to reconstruct paternal lineages aiming to find the origin of modern human populations. The application of Y-SNPs in forensic casework has proven to be a valuable method in addition to the established short tandem repeat (STR)-typing. The strength of Y-SNPs lies in their simplicity and population specificity. Because of the single nucleotide difference even highly degraded DNA and minute DNA amounts receives a sufficient Y-SNP profile. Y-chromosome SNPs show a low mutation rate, leading to stable nucleotide sequence differences on population level. Y-SNP analysis is transferable to crime investigation of degraded samples were population background might be a missing lead for individual identification. The aim of this study is to improve the analysis and definition of Y-chromosome haplogroups in forensic and historical samples. 22 Y-SNPs were chosen to define the most common haplogroups in European populations using a hierarchical typing approach. Amplification were performed in multiplex PCRs and analyzed by mini-sequencing using the SNaPshot™ multiplex kit. The result show a sensitive analysis of Y-SNPs giving useful supplementary information even in samples where a full STR-profile was not received. This newly established and highly sensitive assay was then applied on challenging forensic samples from sexual assault cases as well as from ancient skeletal remains, detailed results will be presented.

P 043 GENETIC DISTRIBUTION OF FIVE SNPS IN SULT1A1 GENE IN CHINESE NORTHERN HAN POPULATION

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The purpose of this study is to survey the distribution of five SNPs in sulfotransferase 1A1 (SULT1A1) gene in Chinese northern Han population in order to provide biological data for forensic application.

Blood samples were collected from 202 unrelated individuals. Automated DNA sequencing technique was applied to detect the mutation types and frequencies within the amplified fragment that ranged from the sixth intron to the seventh exon of SULT1A1 gene. Relevant forensic parameters were calculated.

Five SNPs were detected in the amplified fragment (333bp).Frequencies of SNPs were T=0.740/C=0.260 at nucleotide (nt) 557, G=0.740/A=0.260 at nt 578, G=0.740/C=0.260 at nt 600, G=0.752/A=0.248 at nt 638, and G=0.740/A=0.260 at nt 645. Probability of discrimination power (DP) and excluding probability of paternity (PE) of four SNPs (557, 578, 600, 638) were 0.548 and 0.155, and DP and PE of one SNP (645) were 0.383 and 0.131. Three haplotypes out of five SNPs were detected, sequences and frequencies of which were TGGGG, 0.740; CACAA, 0.248; CACGA, 0.012. The DP and PE of haplotypes were 0.566 and 0.103. These data demonstrated a significant difference among this population with populations from other regions of China and African Americans, but no difference was found when compared to Caucasians. Our data suggested that nucleotide 638 in SULT1A1 gene might exhibit geographical and ethnic differences and might be a useful marker in human genetic research. This is the first report on the frequency distribution of T577C, G578A, G600C and G645A, which enriches the database of population genetics.

P 044 A STUDY ON THE SPECIES DIFFERENCE OF HEMOGLOBIN GENE IN FORENSIC APPLICATION

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Introduction

Hemoglobin is one of the most important reagents in the classical methods of species identification and generally found in animals. We observed that some nucleotide sequences in the second exon of hemoglobin beta-globin (HBB) gene exhibited significant difference among many kinds of animals. However, the flanking sequences were conservative. In this study, we expected to establish a simple, economic and reliable method for species identification.

Materials and methods

DNA samples were obtained from fifteen animals blood (including homo sapiens, cattle, sheep, pig, horse, donkey, dog, rabbit, mouse, duck, goose, fowl, coturnix, dove, babbler). The universal primers were designed (5'- GTCTACCCTTGGACCCAG -3'; 5'- TGAAGTTCTCAGGATCCAC -3') and the hypervariable regions in the second exon of HBB gene were amplified. PCR-RFLP technology was used to analyze the PCR products of HBB gene from 15 kinds of animals by two restriction enzymes (AluI,BsaJI). The results were detected by polyacrylamide gel eletrophoresis and silver staining.

Results and discussion

The experimental results showed that all the target sequences from all kinds of animal DNA could be amplified with good efficiency when the annealing temperature was set at 54°C. Six spectra were observed after AluI digestion and five with BsaJI digestion. Only two groups of animals can not be distinguished by these two restriction enzymes (pig and goose, babbler, sheep, and dog). This method was simple and reliable, and proved valuable in forensic applications.

P 045 VALIDATION OF AN AUTOSOMAL SNP-ASSAY FOR THE ANALYSIS OF HIGHLY DEGRADED DNA SAMPLES

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The analysis of single nucleotide polymorphisms (SNPs) provides an effective and useful alternative to current STR-typing methods in forensic trace analysis and historical research. Due to small target regions used for SNP-genotyping it is possible to analyse even highly degraded forensic samples and historical remains exposed to long-time environmental influences. Based on a SNP-panel for human identification reported by Sanchez *et al.* (2006) we selected 50 highly polymorphic autosomal SNPs and separated them into two different PCR reactions to improve sensitivity regarding low DNA concentrations.

The detection was performed by minisequencing using the SNaPshot® multiplex kit. This multiplex-approach needs as little as 0,1ng of genomic DNA. We applied this highly sensitive and informative method on 1) a set of regular and complicated paternity cases, 2) decomposition-altered identification cases and 3) different challenging forensic case samples e.g. single hairs and historical bones and compared the results to commonly used STR-genotyping methods.

In kinship cases this multiplex-PCR has proven to yield optimized combined paternity indices (CPIs) especially for cases involving one- or two-step-mutations, which can be a problem in STR-based paternity analysis. Using this SNP-assay as an additional method we were able to increase CPIs many times over or confirm shaky exclusions. Overall we can show that SNP-genotyping is a valuable supplement to currently established methods in forensic and anthropological case work.

P 046 MUTATIONAL ANALYSIS OF THE MITOCHONDRIAL DNA DETECTED IN SUDDEN DEATH CASES WITH CARDIAC HYPERTROPHY

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A large number of the causes of sudden death, especially in the young, are due to genetic heart disorders, both with structural and arrhythmogenic abnormalities. In this study, comprehensive screening of mitochondrial DNA (mtDNA) was performed in sudden death cases with cardiac hypertrophy (SDCH), in order to evaluate the prevalence of mtDNA mutations in sudden death.

Blood samples were obtained from 20 SDCHs with informed consent of their family members. Mean heart weight was 674 g. A total of 151 preserved samples that presented with no particular diseases were used as controls. DNA was extracted using Quick Gene-800 (FUJIFILM, Tokyo) and stored at 4°C until use. PCR amplification was performed with the mitoSEQr Resequencing System (Applied Biosystems). The resulting sequence data were compared with the reference sequence described by Anderson et al. This study has obtained the examination and recognition of Kitasato University School of Medicine and Hospital Ethics Committee (B01-24).

In the present study, total number of mutations detected in 151 controls was 633. Missense mutations detected only in SDCH were 13 alterations in 8 cases and 3 mutations were new. The m.10506A>G (T13A), m.8435A>G (T24A) and m.14945G>A (A67T) mutations were located in highly conserved domains and resulted in a change of the charge of the altered amino acid. It was considered that these missense mutations in turn affected the ability of the essential components of the oxidative phosphorylation complexes. It may possibly be that these mutations are tended to have increased risk of SDCH compared to the controls.

P 047 ANALYSIS OF 9 MITOCHONDRIAL SNP'S FROM SAMPLES WITH TRACE AMOUNT OF DNA

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Forensic scientists often use nuclear STR loci for identification. But it's not always possible to obtain clear results from nuclear DNA, especially when nuclear DNA is degraded or deficient. In these cases forensic scientists tend to mitochondria's, a cell organelle with its own DNA (mtDNA). The aim of this work is identification of the 9 mitochondrial SNP sites (3010, 5004, 6776, 8592, 10394, 10754, 11864, 15340, 16519) and the polymorphic sites within these SNP's in Turk population, while validating a routine procedure that is optimized to analyze degraded samples. The SNaPshot method is chosen for this work, because of its advantages like: the ease of multiplexing and optimizing, high sensitivity, high reliability, high repeatability and its capability to detect mixed samples and heteroplasmic samples. This work starts with analyzing of blood samples from 30 volunteers to optimize the procedure. After this optimization step, samples taken from 5 volunteers are analyzed with this optimized procedure. These samples contain trace amounts of DNA and consist of hair, nails, earrings, toothbrushes, q-tip, glass edge swabs, gum, razors, cigarette butts and hankies with mucus on it. After the isolation of DNA, the samples which has 1–0,1 ng/µl DNA concentrations give satisfying results and 9 SNP sites can be typed from these samples. The result of 30 volunteers shows that the SNP sites 3010 and 16519 are polymorphic sites among Turks, and the 15340. site is different than the rCRS (revised Cambridge Reference Sequence) at all volunteers.

P 048 EVALUATION OF Y-STR ANALYSES OF SPERM CELL NEGATIVE VAGINAL SAMPLES

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Aim and Introduction

In a large number of sexual assault cases, no sperm cells are visible in the vaginal samples. Failure to detect sperm cells can be due to multiple reasons. In this study, sperm cell negative vaginal samples from 110 sexual assault cases in Denmark were analysed.

Materials and Methods

Sperm cells were detected with Baecchi staining and Sperm Hy-Liter. Samples were non-differentially extracted using Chelex (Bio-Rad) based methods. The amount of extracted Y-DNA was evaluated with the Quantifiler Y Human Male kit (Applied Biosystems (AB)). Y-DNA positive samples were analysed with the AmpFℓSTR®Yfiler (AB). Selected samples were purified and concentrated using the MinElute (Qiagen) procedure.

Results and Discussion

Y-DNA was detected in 49 (45%) cases. Complete or partial Y-STR profiles were found in 32 (29%) cases. Purification and concentration with the MinElute kit (Qiagen) significantly increased the number of cases with complete Y-STR profiles from 3 to 12 ($p < 0.05$). In addition, partial Y-STR profiles with 10 or more Y-STR alleles were obtained in 11 cases (10%). Thus, Y-STR results were obtained in a significant proportion of the cases.

P 049 NEW BIALLELIC POLYMORPHISMS AT THE VWA, D7S820 AND CSF1PO LOCI AS A POTENTIAL SOURCE OF DISCORDANT RESULTS IN STR TYPING

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It is presumed that STR profiles reflect the actual number of tandem repeats at all loci of the tested samples. However, reported genotypes may sometimes contain errors based on the occurrence of SNPs in primer binding sites or insertion/deletion polymorphisms outside the repeat region in PCR products. STR multiplex manufacturers are always seeking conserved regions for primer design. However, in a large sample, there is always the risk of encountering allele dropout or allele shift because of natural polymorphisms. Here, we report several new biallelic polymorphisms that caused discordant results in a concordance study of the Russian population using different primer sets.

Samples from 173 unrelated individuals that had been previously tested with the Identifiler Kit (Applied Biosystems, USA) were retested using an alternative primer set designed by the authors. The results obtained with these two multiplex systems were compared for 13 overlapping STR markers representing the CODIS panel. All discordant samples were sequenced to identify any polymorphisms outside the repeat regions that may have caused allele dropout or allele shift.

Among the 4498 allele calls compared (173 samples x 13 loci x 2 alleles), 29 cases of allele dropout or allele shift occurred in one of the two multiplex systems. In 27 of these cases, the discordances were found at three specific loci: vWA (13 allele calls), D7S820 (7 allele calls) and CSF1PO (7 allele calls). In the case of vWA, a T > C transition was found 7 bp downstream of the last TCTA repeat. This substitution was always linked with allele 14. At the D7S820 locus, allele dropout was caused by two different SNPs. One was an A > C transversion 63 bp upstream of the first TCTA repeat. This polymorphism has been previously described and registered in the dbSNP database under the accession number rs59515969. The second SNP, a previously unidentified G > A polymorphism, was found 11 bp downstream of the D7S820 repeats. In 7 samples, a specific CSF1PO allele was shifted to –2 bp position because of an AG deletion found 152 bp downstream of the last ATAG repeat.

The data obtained were used to modify the alternative primer set to avoid further discordance at the vWA, D7S820 and CSF1PO loci. This information can be used by the developers of other STR multiplex kits in the future for correct primer design.

P 050 MULTI-POINT SNP BASED ANALYSIS OF GENETIC DISTANCES BETWEEN 29 SYNTENIC STR PAIRS IDENTIFIED IN CORE AND SUPPLEMENTARY FORENSIC KITS

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Traditionally, broad-scale family studies have been used to measure the genetic distance between same-chromosome (syntenic) STRs, but these are expensive and time consuming, in many cases uninformative, and lack a reliable means to infer the phase of the haplotypes of paired genotypes. HapMap provides a much more thorough alternative in the form of high density multi-point SNP typing (average inter-SNP distance: ~900bp) analyzing sub-kilobase genetic distances across the whole genome. We have used the most recent HapMap SNP data (release 22) to measure and compare genetic distances, and by inference fine-scale recombination rates, between 29 syntenic STR pairs identified from 39 validated STRs currently available for forensic use. The list of 39 STRs comprises core loci: SE33, Penta D & E, 13 CODIS and 7 non-CODIS European Standard Set STRs, plus those of the recently released Promega CS-7™ and Qiagen Investigator HDplex™. Also included were D9S1120, a marker we developed for forensic use showing less variation than the core loci but unique to chromosome 9, and the novel D6S1043 component STR of SinoFiler™ (Applied Biosystems). The data collated provides reliable estimates of recombination rates between each STR pair, which can then be placed into a standard haplotype frequency calculator for short pedigrees and only requires the addition of allele frequencies. This allows all current STR sets or their combinations to be used in supplemented paternity analyses without the need for further adjustment for physical linkage.

P 051 APPLICATION OF INDELS (INVESTIGATOR DIPPLEX) IN MIXTURE SAMPLES

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The insertion/deletion polymorphisms (indels) have considerable potential in the field of forensic casework. The multiplex amplification of 30 DIPs and Amelogenin (Investigator DIPplex), with the maximal amplicon length restricted to 150 bp, makes them a powerful tool in forensic field, mainly to improve the chances of successful amplification of highly degraded DNA. Hence, in combination with standard markers, these polymorphisms improve discriminatory power and provide a potential supplementation for paternity analyzes. Furthermore, as they do not possess stutter products or microvariants, it could be a good option to solve forensic cases, mainly in the reliable mixture interpretation.

The main objective of this study was to evaluate the performance of the 30 indels included in the Investigator DIPplex Kit (Qiagen) to interpret mixtures linked with forensic cases, essentially involved in the analysis of sexual crime samples. In a preliminary study we used a mixture with 0,5ng/µl of the two controls, DNA XY5 and DNA CCR 9947A.

It was observed that the controls mixture interpretation was straightforward. When the same allele was present in the mixture profile controls the peaks heights were about two times of the correspondent single peak, as expected. However, more experiences must be done using mixture samples, mostly connected with sexual crime cases, related to which the genetic profile of the contributors was known. It will be compared the results from the two mixture type of samples (controls and casework samples) to evaluate the probable interferences during the PCR and amplicon separation, in the last ones.

P 052 INTEREST OF X CHROMOSOME (ARGUS X-12 KIT) IN COMPLEX KINSHIP ANALYSIS

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Paternity trio cases can most easily be solved with autosomal STR markers alone, while test of paternity duos involving a daughter or more complex family relations could gain from X-chromosomal testing. The main feature of X-STR markers, compared to autosomal forensic markers, is that all loci are located on the same chromosome. Thus, linkage and linkage disequilibrium may occur.

The main goal of the present study was to investigate twelve X-STR markers (Argus X-12 kit, Qiagen) based on a North of Portugal population sample. Evaluation of statistical parameters of interest for both forensic and relationship tests was calculated. We analyzed some cases involving the mother her daughter and the mother of the putative father. The amplified products were detected and separated by capillary electrophoresis in an ABI PRISM 3100 Genetic Analyzer (AB). The amplified products were detected and separated in an ABI PRISM 3100: Fragment sizes and genotypes were determined automatically using the GeneMapperID (AB).

All together, the X-chromosome markers included in the Investigator Argus X-12 kit offer the possibility to solve complex kinship cases where autosomal STR markers do not provide the information needed.

Cases were selected from 362 kinship complex ones studied from 2005 to 2010. The use of X-chromosome data offered additional valuable information provided by the autosomal STRs, increasing the LR in all cases. Other statistical parameters also benefit from increased robustness when X-chromosome markers were included in the analysis.

P 053 IMPLEMENTATION OF THE SNP*FOR*ID MULTIPLEX ON THE SEQUENOM® MASSARRAY® ANALYZER 4 SYSTEM

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Introduction and aims
The SNP*for*ID consortium developed a 52plex SNP assay for human identification in 2006. In 2007 the assay was further optimized and validated for forensic genetic testing in our ISO17025 accredited laboratory. The validated assay consisted of one PCR-amplification of 49 SNP loci, two single base extension (SBE) reactions and detection of the SBE products by capillary electrophoresis (CE).

The aim of this study was to implement the SNP*for*ID multiplex on the Sequenom® MassARRAY® analyzer 4 system, a medium-throughput matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) system. The major advantages of this platform are low cost of running, minimal level of maintenance required and dedicated software that allows automated analysis and allele calling.

Materials and methods
Forty-nine SNPs were amplified in a one PCR reaction. The PCR products were purified by the MinElute PCR purification kit. SBE reactions were performed with either the SNaPshot kit (for CE detection) or with the Thermosequenase (for MALDI-TOF-MS detection). DNA/RNA chimeric SBE products were cleaved by RNases prior to ion-exchange purification. Finally, the purified SBE products were dispensed onto the SpectroCHIP array for MALDI-TOF-MS detection. The SNP typing results from the CE- and MALDI-TOF-MS-based platforms were compared.

Results and Discussion
SNP typing results using the MassARRAY® system will be presented and discussed. The following points will be addressed: 1) the sensitivity and reproducibility of the MassARRAY® system, 2) call rates and accuracy, and 3) analysis of DNA mixtures.

P 054 FORENSIC GENETIC CONTRIBUTION TO THE IDENTIFICATION OF INTERNAL CONFLICT VICTIMS IN COLOMBIA

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Due to internal conflict in Colombia, 31 January 2011 reported 35,108 documented cases of forced disappearance which are investigated by the Attorney General's Office (FGN) with the Human Rights Unit and the National Justice and Peace created by Act 975 of 2005, receives scientist and technical support forensic genetics laboratories of the FGN in the process of identifying victims.

Forensic genetics laboratory with their human resource, technology, quality management system and the evolution of one of its strongest lines of work such as supporting the process of identifying human remains on two fronts:

Oriented case: A case analysis of genetic profiles of skeletal remains exhumed as part of an investigative process where the authorities on circumstantial information from various sources are possible identity of the victim and locate possible relatives.

Untargeted Cases: We report a case in which the laboratory of the FGN using the genetic database with profiles of family members reported missing persons were able to locate the possible identity of skeletal remains admitted to the laboratory as NN (possible without identity) and verified this finding with additional genetic testing and ante-mortem data of indices. Additionally present the approach or strategy work in the resolution of cases.

Finally note that the Colombian authorities acknowledged that their laboratories are supportive of the criminal process and the contribution of forensic genetics in the victim identification process, the Colombian laboratories have competent human resources and technology to continue in this process, product state contribution and the continued cooperation of international agencies.

P 055 VISUAL OMP™ SOFTWARE; AN EXCELLENT TOOL FOR EFFECTIVE PROBE/PRIMER DESIGN IN COMPLEX PCR REACTIONS AND ALLELE SPECIFIC ASSAYS

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Visual OMP™ presents a most accurate and comprehensive software tool for the design and simulation of probes/primers, Multiplex PCR, Scorpions, Taqman, Molecular Beacons, Microarrays, Allele Specific and FRET assays, **RNAi**, and new formats. Besides typical primer properties such as primer length, GC content, melting temperature, hetero- and homodimer predictions, hairpin structure and specificity, it also allows the calculation of precise thermodynamic values for the entropy, enthalpy and Gibbs free energy for each primer-template match according to temperature and assay parameters. Here we demonstrate the analysis and simulation of probes with Visual OMP™ for the separation of forensic mixtures by haplotype-specific extraction (HSE). HSE uses the discrimination power of SNPs to separate haplotypes by an allele-specific extension reaction. With Visual OMP™ we could simulate the concentration versus temperature hybridization-profile for 373 probes under match and mismatch conditions. The comparison of the match and mismatch situation for each probe allowed us to predict its specificity and binding properties. Our results show that after the testing of 130 probes in a HSE reaction, 85% of the simulated probes were predicted correctly. Also for the simulation of multiplex PCRs for ABIPRISM®SNaPshot™ assays, the Visual OMP™ software could be applied to calculate primer concentration for a balanced amplification.

P 056 POTENTIAL GENETIC PREDISPOSITIONS FOR SUDDEN INFANT DEATH SYNDROME: A CASE-CONTROL STUDY

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The etiology of the sudden infant death syndrome (SIDS), a major cause of infant mortality, remains rather vague, but is thought to be a multifactorial disorder. In a case-control study, we screened a range of genetic markers from four groups of candidate genes: variants of cardiac ion channel protein genes related to long QT syndrome (LQTS), polymorphisms of genes pertinent to the early development of the autonomic nervous system (ANS), a VNTR of the serotonin transporter gene, and polymorphisms of genes regulating infection and inflammation.

A SNP located in the 3' untranslated region (UTR) of the *SCN5A* gene was found to be significantly associated with SIDS. The 3' UTR of *SCN5A* should therefore be considered a candidate region for LQTS and SIDS-related mutations. An additional missense mutation in *SCN5A*, known to produce the LQT3 phenotype, was found in two cases, underlining a link of SIDS to the sleep related LQTS-type. Further, significant genotype distribution were identified for markers in *EDN1* and *ECE1*, indicating a link between SIDS and ANS embryology. Previously assumed links to congenital central hypoventilation syndrome (CCHS) could, however, not be verified. No associations between SIDS and serotonin transporter gene polymorphisms or infection-related SNPs were found in the German cohort.

This study highlights the importance of genetic studies to define SIDS etiology. The results lend support to the hypothesis that infants dying of SIDS have an underlying genetic predisposition and indicate that genes pertinent to ANS embryology and the LQT3-associated gene *SCN5A* might confer to SIDS risk.

P 057 MUTATIONS ANALYSIS OF 24 AUTOSOMAL STR LOCI IN 11175 DNA-CONFIRMED PATERNITY TESTING CASES

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Introduction and aims
To establish a reliable knowledge on the locus-specific mutation rates and characteristics of 24 autosomal STR loci.

Materials and methods
Fifteen STR loci (D31358, etc) were typed with PowerPlex® 16 system (Promega, USA) for 11175 DNA-confirmed paternity testing cases (8059 trios, 1978 father-child duos and 1138 mother-child duos). Nine more STR loci (D2S1772, etc) were typed with STRtyper®-10F/G system (Condon, China) for 4046 DNA-confirmed paternity testing cases (2115 trios and 1227 father-child duos and 704 mother-child duos).

Results and discussion
A total of 343,959 meiotic transfers were analyzed and 488 mutations were identified. The overall average mutation rate estimate was 1.42×10^{-3} and the locus-specific mutation rate estimates varied between 1.04×10^{-4} and 4.22×10^{-3}. Except for 2 cases with three mutations and 20 cases with two mutations, one mutation was observed in other cases. The maximum mutation step was three and one-step mutation accounted for 98.16%. Significant difference existed among loci in the ratio of repeat gains versus losses. The ratio of paternal versus maternal mutation was 3.66:1. Except for two cases with three mutations, the combined paternity index (CPI) value of 24 STR loci was always >10000 when calculated including the mutation loci.

P 058 NULL ALLELES OF THE X AND Y CHROMOSOMAL AMELOGENIN GENE IN A CHINESE POPULATION

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Introduction and aims
To explore the frequency and cause of null alleles of the X and Y chromosomal amelogenin gene observed in the commercial STR typing kit.

Materials and methods
8087 unrelated Chinese male individuals were typed with PowerPlex® 16 system (Promega, USA). The samples with drop-out amelogenin X-allele *(AMELX)* or Y-allele *(AMELY)* were re-typed with three alternate amelogenin primer sets. The "lost" alleles were separated and directly sequenced. The samples with *AMELY* drop-out were typed with AmpFℓSTR® Yfiler kit (Applied Biosystems, USA).

Results and discussion
Six *AMELX* or *AMELY* null cases were observed with an overall prevalence of 0.074%. Further validation revealed three kinds of different point mutations in the amelogenin priming sites associated with *AMELX* nulls (3 cases, 0.037%), and three kinds of deletions on the Y chromosome encompassing the *AMELY* and other Y-STR loci with 3 *AMELY* nulls (0.037%). These mutations and failure of the amplification of the *AMELX* and *AMELY* alleles have not been reported for the Chinese population. The mutations in the amelogenin gene may result in amplification failure of the *AMELX* or *AMELY* allele, and an additional gender test for unambiguous sex determination may be needed.

P 059 RE-EVALUATION OF THE IDENTICAL-BY-STATE METHOD IN PAIRWISE KINSHIP INFERENCE

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To infer relatedness from short tandem repeat data, the exact method, in which shared allele frequencies are applied to relevant equations, has been conventionally used. An alternative approach is the Identical-by-State (IBS) method that is based on the number of shared alleles between individuals. In the present study, the performance of the IBS method was re-evaluated using simulated data of 10,000 genotype pairs for 15 loci in the ABI Identifiler system. Likelihood ratio (LR) values in allele-sharing of zero, one and two were calculated from joint probabilities to parent-child, full-sibling and non-relative pairs, based on Japanese allele frequencies. The IBS method generally produced lower LR values than the exact method. For instance, 49.6 and 31.8% of simulated full-siblings had the combined index of less than 1,000 in the IBS and the exact method, respectively. However, smaller deviations of distributions of combined indices were evident. To obtain the cut-off value for the combined index that discriminate between full-siblings and non-relatives, the sensitivity and the specificity were examined. The cut-off value in the IBS method was comparable with that in the exact method, indicating that both separative powers were identical. In the IBS method, the LR value depended on the heterozygosity at a locus. Heterozygosities of 15 loci were consistent across various populations, particularly in East Asians, indicating that universal LR values can be established. The convenience of fixed LR values in the IBS method is beneficial in cases like uncertain allele frequencies, and like involvement of rare alleles and variants.

P 060 PRIMARY TEETH AS A DNA REFERENCE SAMPLE IN DISASTER VICTIM IDENTIFICATION (DVI)

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The identification of disaster victims through the use of DNA analysis is an integral part of any DVI response, regardless of the scale and nature of the disaster. DNA analysis is performed to assist in the identification of victims through kinship (familial matching to relatives) or direct (self source sample) matching of DNA profiles. The direct references samples more commonly available are tooth and hair brushes, manual razors or clinical samples, however primary teeth can probably be an alternative reference sample, attending the fact that many communities have the habit of keeping the exfoliated teeth as a memento, and dentin-pulpar complex is well-protected within the pulp cavity.

The aim of this pilot study is to evaluate the possibility of DNA extraction in primary teeth with probative value for identification.

Eighteen anterior primary teeth were collected from 9 people after been stored for 2-18 years. Cryogenic grinding was used to extract DNA from calcified tissues. Total DNA quantification was performed by real time PCR, by using the Human Quantifiler kit. Relevant polymorphisms for genetic identification were analysed with the aid of AmpFℓSTR® Identifiler™ kit. The references samples (buccal swabs) were collected from the same individuals; DNA was extracted using the Chelex100 method and amplified by PCR for the same kit.

In all cases, the results obtained from the swab were in agreement with the results obtained from teeth. The achieved DNA quantity (between 1.33–154 ng/µL) revealed that temporary teeth can be a powerful tool for genetic analysis, especially as reference sample in DVI.

P 061 A 22 MARKER INSERTION DELETION POLYMORPHISM PANEL TO STUDY BIOGEOGRAPHIC ANCESTRY

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Insertion / deletion (indel) polymorphisms have recently received increased interest in the forensic genetics community. This class of markers combines the advantageous genetic properties of single nucleotide polymorphisms (e.g. low mutation rate, genetic stability, short amplicon size) with the technical advantage of short tandem repeat markers (simple detection by fluorescence-labeled PCR and capillary electrophoresis).

For a large number of indel markers significant differences in allele frequencies between the major populations have been reported, making this class of markers suitable for the analysis of biogeographic ancestry.

We present here a multiplex PCR assay designed to establish the biogeographic ancestry of forensic DNA samples based on insertion / deletion polymorphisms. Based on the 16-plex assay presented at the ISFG congress 2009 the panel was increased to 22 short indels with sufficient allele frequency differences between three major population groups (European, African and Asian).

Assay design was keyed towards a multiplex PCR assay suitable for the analysis of forensic samples. All 22 markers are amplified in a single reaction as short fragments with less than 200 basepairs (bp) length, using only two fluorescent labels (FAM and HEX). Sensitivity of the assay is ideal for forensic casework with only 250 pg of DNA needed per 10 µl reaction to obtain full profiles. Increase of reaction volume to 20 µl increases robustness of the assay to larger amounts of template DNA.

Currently, an extensive population genetic study with samples from the major population groups is carried out to assess the performance of the assay for the prediction of biogeographic ancestry. The results will be presented and discussed.

P 062 APPLICATION OF INSERTION/DELETION POLYMORPHISMS IN HUMAN GASTROINTESTINAL TUMOR TISSUES FOR IDENTIFICATION PURPOSE

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Criteria of Likelihood Ratio in traditional personal identification were not suitable to the source identification of body for tumor tissue because of high mutation rate of short tandem repeat. Consequently, it would be extremely necessary to draw a completely new strategy for source identification of body for tumor tissue.

In this study, 30 insertion/deletion polymorphisms (InDel), included in an inhouse 31plex PCR genotyping system, were examined in 69 colorectal cancer and 31 gastric cancer fresh samples and their homologous normal tissue samples. All the samples had been genotyped with Identifiler multiplex system. Consequently, two kinds of InDel mutation type (including pLOH and LOH) and four kinds of STR mutation type (including pLOH, LOH, new allele and additional allele) were found in this group of tumor samples. The frequency of InDel genotypic alteration (ID_{GA}, including LOH) was 0.25%, which was about 1/21 of that of STR genotypic alteration (STR_{GA}, including LOH, new allele and additional allele). At the level of individual, ID_{GA} could be detected in 7.00% of tumor samples, which was about 1/4.57 of that of STR_{GA}. Difference of the frequency or the detectable proportion of the tumor samples between ID_{GA} and STR_{GA} was statistically significant with P values of $2.771x10^{-33}$ and $1.056x10^{-5}$, respectively. Consistency testing revealed that the mutation of the two different genetic markers, InDel and STR, in gastrointestinal tumor was unrelated (P=1.0000). Results of this study suggested that InDel might be more powerful than STR in source identification of body for tumor tissue.

Keywords: Forensic genetics; Short tandem repeat (STR); Insertion/Deletion polymorphism (InDel); mutation; gastrointestinal tumor

P 063 COMPARISON STUDY IN DETERMINATION OF FULL SIBLING WITH IDENTIFILER MULTIPLEX SYSTEM BETWEEN ITO METHOD AND IDENTITY BY STATE SCORING METHOD

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The usual, determination of full sibling can be more difficult than parentage testing in that there are no obligatory alleles between full siblings. According to Mendelian genetics, the probability of autosomal STR allele sharing between full siblings is higher than that between unrelated individuals, which indicates that identity by state scoring system (IBS method) can be utilized in determination of full sibling.

In this study, 2003 pairs of unrelated individuals (UI group) and 280 pairs of full siblings (FS group) were genotyped with Identifiler system. Cumulative full sibling index (CFSI) and the IBS score of Identifiler system (IBSi) between each pair of individuals were calculated with ITO method and direct counting method, respectively. Curve fitting analysis revealed that Log_{10}CFSI was approximately fitted to normal distribution in UI and FS group (R^2=0.9974, 0.9682, respectively). Distribution of IBSi was similar to that of Log_{10}CFSI with R^2=0.9974 in UI and 0.9653 in FS group. Difference of Log_{10}CFSI or IBSi between the two groups was statistically significant (P<0.0001). Consequently, two groups of discrimination functions were developed with Fisher discriminant analysis based on Log_{10}CFSI and IBSi, respectively. Total false conclusion probabilities of the two groups of functions were 3.48% with ITO and 2.98% with IBS method, respectively. High consistency of the two different method was indicated with Kappa index=0.8841 (P<0.0001). Results of this study suggested power of IBS method is similar to that of ITO method, with advantages of convenience in calculation and independence on the allele frequency of STR loci.

Keywords: Forensic Genetics; Short tandem repeat (STR); Full sibling; discriminant analysis; ITO method; IBS method

P 064 EYE COLOUR AND SNPs IN DANES

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Introduction and aims
Single nucleotide polymorphisms (SNPs) affect the pigmentation of the eyes, skin and hair in humans. In this study, 4 SNPs hypothesized to be important for the eye colour were evaluated in a Danish population sample.

Material and methods
A total of 187 unrelated Danes were typed for 4 SNPs located in the genes *HERC2* (rs12913832), *OCA2* (rs1800407), *SLC45A2* (rs16891982) and *IRF4* (rs12203592). Photos of the participants' eyes were taken. The eye colour was categorized as (1) either dark or light and (2) brown, blue or intermediate (neither blue nor brown). The weight of the evidence for predicting eye colour was estimated using the likelihood ratio (LR).

Results and discussion
It was shown, that the highest LR = 38 for dark versus light eye colour was found when the genotypes rs12913832 AG and rs1800407 GG were considered together. Furthermore, it was shown that the rs12913832 homozygote genotype GG alone gave the highest LR = 30 for blue versus brown eye colour. The rs12913832 genotypes AG and AA were exclusively found in Danes with intermediate or brown eyes colours.

In conclusion, rs12913832 was found to be the major determinant for eye colour in Danes, whereas rs1800407 GG darkened the eye colour in individuals with the rs12913832 AG genotype. rs16891982 and rs12203592 had minor effect on the eye colour in Danes.

The results will be compared with the eye colour prediction made from the 6 SNPs of the IrisPlex (rs12913832, rs1800407, rs16891982, rs12203592, rs12896399 and rs1393350).

P 065 MELANOCORTIN 1-RECEPTOR GENE POLYMORPHISMS ANALYSIS REVEALS ASSOCIATION WITH FRECKLES AND HUMAN PIGMENTATION IN A BRAZILIAN ADMIXED POPULATION SAMPLE

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Among the known genes influencing eye, skin and hair pigmentation variation, the melanocortin 1-receptor (MC1R) gene is the best characterized so far. It encodes a transmembrane protein in melanocytes, responsible for regulating the production of melanin, determining the ratio of eumelanin (brown/black color) and pheomelanin (yellow/red). This study aims to analyze known SNPs of the MC1R gene in order to evaluate their influence on features like freckles and variation of eyes, skin and hair pigmentation on an admixed population. We analyzed 29 known SNPs from the MC1R coding-region in 284 individuals from the São Paulo State, Brazil. The MC1R coding-region (951bp) was amplified in a single PCR fragment, which was sequenced on a ABI PRISM-310 genetic analyzer, employing the same primers used for amplification. Many associations were found on allelic and genotypic levels. Eleven of the 29 SNPs evaluated were associated with pigmentation characteristics. Alleles of four of them were associated with dark skin and two others had significantly high frequencies in fair skin. Blue eyes had association with one allele, while three others were associated with green eye color and two others with dark brown eyes. Darker hair color was associated with seven different alleles, whereas red hair was associated with other four. Finally, the presence of freckles was significantly related to three alleles. This study shows significant associations between SNPs and eye, hair and skin pigmentation. The results presented here confirm that this gene plays a relevant role in the pigmentation variation in the admixed Brazilian population.

P 066 TOWARDS MORE ACCURATE DNA BASED PREDICTION OF INTERMEDIATE EYE COLORS

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Prediction of externally visible traits from genetic data may speed up criminal investigations by narrowing down the number of potential suspects or leading an investigation concerning identification of human remains in a particular direction. Pigmentation is one of the most variable human phenotypic features, showing continuous distribution of eye, hair and skin colour from dark to light. The genes and polymorphisms which have been associated with pigmentation enable accurate prediction of extreme eye colours, but are insufficient to predict intermediate categories. We assumed that implementation of nonlinear effects between known predictors in prediction models may improve the prediction accuracy of these intermediate eye colour states. We genotyped selected polymorphisms located in 11 pigmentation genes in two population samples from Poland and Japan. Using various mathematical models including the Bayesian approach, multinomial logistic regression and neural networks, we were able to show that iris colour can be predicted with high accuracy at the level of light and dark without prior knowledge concerning biogeographical ancestry. The study revealed strong epistatic effects of a redundant and synergistic character with *HERC2*, a major eye colour gene, as a component of all interaction models. It was found that introduction of the detected gene-gene interactions into the prediction models may slightly improve the accuracy of green eye colour prediction.

P 067 ANALYSIS OF CANDIDATE POLYMORPHISMS FOR ANDROGENIC ALOPECIA – PREDICTION POSSIBILITY OF MALE BALDNESS

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Male baldness affects a significant portion of the human male population and shows a heritability of over 80%. Numerous studies performed on male baldness including recent GWAS analysis have indicated that the locus on chromosome X containing two genes *AR* and *EDA2R* is associated with the most popular form of male baldness known as androgenetic alopecia. Two independent GWA studies detected additional susceptibility locus for male-pattern baldness on chromosome 20p11. Some studies have also linked vitamin D pathway genes with alopecia. Since baldness is an externally visible trait, its prediction can be considered to be useful in forensic sciences, especially in suspect-less cases and cases concerning identification of human remains. Therefore, we decided to further investigate this issue and launched a population association study examining polymorphisms within the candidate genes and regions on a group of males. Using the SNaPshot protocol, so far seventeen candidate SNPs located on the chromosome X, the chromosome 20p11 and in the *VDR* gene were analysed. Multivariate logistic regression confirmed the significant association of rs6625163 and rs12558842 within the AR/EDA2R locus with alopecia indicating that both SNPs, although in LD, may have an independent effect on this trait. Additionally, our analysis indicated that two SNPs in *VDR* (rs2228570, rs731236) are significantly associated with androgenetic alopecia. Using MDR analysis supported by logistic regression we were able to detect some interactions between the analysed loci.

P 068 PIGMENT ASSOCIATED SNP MARKERS IN A NEW ZEALAND FORENSIC CONTEXT

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Forensic DNA techniques and eye witness accounts have long been integral tools that aid criminal investigators in identifying a person of interest. The forensic applications for DNA found at a crime scene may now include the inference of externally visible characteristics (EVCs) of the donor, particularly in instances where a DNA database match or an eye witness account is unavailable. Loci associated with particular variations of a trait can be identified using specific DNA markers. Likelihood probabilities can be calculated, indicating the phenotype for a particular trait using a multiplex of SNP markers. Potential characteristics could include pigmentation, ancestry, facial characteristics, height and even chronological age. Knowledge of the likelihood of a donor's appearance through a collection of these characteristics may prove to be vital clues to their identity, helping to exonerate inappropriate suspects and improve investigative efficiency.

A SNP multiplex reaction designed to reveal pigmentation haplotypes of an individual's probable hair and iris pigmentation could prove to be a new and valuable tool to add to the spectrum of forensic DNA analysis in New Zealand. To investigate this new form of DNA analysis, we have assessed the association of known pigment markers in a New Zealand population. Using this data, we are assembling these associated markers within robust multiplexes to analyze pigmentation haplotypes of a New Zealand population and access the accuracy of marker revelations and pigmentation probabilities.

P 069 SINGLE MULTIPLEX SYSTEM OF TWELVE SNPs: VALIDATION AND IMPLEMENTATION FOR ASSOCIATION OF SNPs WITH HUMAN EYE AND HAIR COLOR

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Predictions of human traits from stains with genetic methods have nowdays gained tremendous interest in criminal investigations. Single nucleotide polymorphisms (SNPs) within the *HERC2, OCA2, MC1R, SLC24A5, SLC45A2* and *TYR* genes have been strongly associated with the variation of pigmentation traits in human populations. Among them, we have selected twelve SNPs which have high prediction value for determination of eye and hair color in different European populations. The study encompassed 105 unrelated male and female Slovenes, who signed a written consent for the use of their DNA in scientific research. Single multiplex assay works optimally between 1.0 ng and 4.0 ng of template DNA, allelic drop outs were observed only at 62 pg of DNA. This assay was done in duplicates for all samples and was till now oriented on 4.0 ng of template DNA, with heterozygote (average peak height: 500–3000 RFU) and homozygous (average peak height: 1000–5500 RFU) allele calls determined for each locus. The allele frequencies for all SNPs were in a comparable range, as provided by HapMap for the Caucasian population. When sequencing the short DNA region surrounding the selected SNPs for some of the samples, we managed to confirm the results which had been obtained with twelve new SNPs assay. Sensitive, specific and reliable multiplex genotyping assay of twelve SNPs offers promising association, especially with hair and eye colors of examined individuals.

P 070 EVALUATION OF THE IRISPLEX ASSAY IN ADMIXED SAMPLES

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The development of assays predicting externally visible characteristics (EVCs) is an important investigative tool and a growing field in forensic genetics. The IrisPlex assay uses six single nucleotide polymorphisms to predict blue and brown eye colour in humans with over 90% precision. However, the accuracy of this system has not been tested in samples with known genetic admixture.

We tested the IrisPlex assay in 24 samples with known and varying levels of Asian-European genetic admixture. Self-declared eye colour information was obtained and no further visual assessment was performed.

The overall accuracy rate for eye colour assignment was 78.57%, however only 14 samples achieved classification above the 0.7 probability threshold employed. The correct eye colour was predicted in 100% of both blue and brown eye colour samples although none of the green eye colour samples were accurately predicted. When the probability threshold is removed, the accuracy decreases to 66.7% for all 24 samples, with only 76.9% and 85.7% of brown and blue phenotypes predicted correctly. There was also a trend for decreasing accuracy with increasing number of generations since admixture, with 1:1 admixed individuals predicted correctly in 71.4% of cases, and 1:7 and 1:15 individuals in 66.7% of cases.

These results suggest that the prediction of EVCs in admixed individuals, although possible, may be more challenging than originally envisaged. However larger sample sizes and different genetic backgrounds need to be studied to obtain a better understanding of EVCs' prediction accuracy in admixed individuals.

P 071 EVALUATION OF THE IRISPLEX EYE COLOR PREDICTION TOOL IN A GERMAN POPULATION SAMPLE

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Predicting human visible characters by means of genotyping informative molecular markers opens up new perspectives in the forensic case work. Besides hair color, eye color is a complex phenotypic character of interest which is determined by multiple genes e. g. *OCA2, HERC2, TYR, SLC24A4*. Recently, Liu et al. 2009 and Walsh et al. 2010 developed and evaluated a multiplex SNaPshot genotyping assay, named IrisPlex, consisting of six predictive SNP markers. The statistical prediction model provided in the publications is based on the results of 3804 Dutch Europeans and was verified with the typing of 2364 additional Dutch samples, in which the IrisPlex assay revealed over 90% accuracy for the prediction of blue and brown eye color. In our study we present one of the first German population study consisting of about 100 individuals aiming to evaluate the handling of the method as well as the plausibility of the prediction results. Since eye color prediction will most probably applied as an intelligence tool to trace unknown persons as well as criminal offenders or victims of a crime, accident or mass disaster, we also show the applicability of the method to real casework. We used the IrisPlex to determine the eye color of a skeleton, for which no phenotyping information is available and the typing results of autosomal markers did not match with any samples in the reference database for missing persons. Because of the sensitivity of the IrisPlex and its robust design, profiles could be generated from picograms of DNA and highly degraded DNA.

P 072 HAIR COLOUR PREDICTION IN NORTH AND SOUTH EUROPEANS

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DNA profiling is a key tool in the arsenal of forensic investigators, however current methods of DNA analysis require the identification of a suspect for comparison purposes so that potential offenders must either be known or be identified by a DNA-database search. In cases with unidentified suspects, the evidence DNA profile only provides limited information. Here the prediction of visible physical traits e.g. pigmentation, body height and facial features of the source of a crime scene sample could provide probative information.

The project presented here aims to design a genetic analysis system which may be able to derive information about the hair colour of an individual. A panel of 34 SNPs for which association to hair pigmentation has been published in the recent literature was selected to develop multiplex assays based on SNaPshot technology. A first genotyping study has been performed on European population groups (i.e. 129 Spanish, 104 Germans, and 21 Austrians). Phenotyping was accomplished by matching donor's hair to a graded colour category system of 30 reference shades grouped into five categories: fair blond, blond, dark blond, brown, and brown-black.

The predictive approach developed uses a naïve Bayesian classifier to provide hair colour assignment probabilities for the SNP profiles of the key SNPs. Results show that estimated success ratios are best for blond and black haired individuals. Nevertheless several steps remain before sufficient predictive accuracy will be established and DNA-based hair colour predictions can be made to help guide a criminal investigation.

P 073 PREDICTION OF IRIS, HAIR AND SKIN COLOR IN THE HUNGARIAN POPULATION

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Introduction and aims
Several previous studies identified genetic variants in several genes associated with human iris, hair and skin color and quantitative measures were taken for the categorization of the pigmentation. The aim of this study is the generation of a tool based on genetic information for prediction of the color of the eyes, hair and skin in the absence of the person.

Materials and methods
The iris, hair and skin color were photographed and quantitatively measured in high resolution with the Pigment v1.0 software that was made for this project, it processes the pictures resulting in median HSV values and SD and tells a color based on the more than 50000 pixels. 21 SNP loci (rs4778138, rs12203592, rs12896399, rs12913832, rs1800407, rs16950987, rs2240204, rs4778241, rs7183877, rs7495174, rs8039195, rs16891982, rs1667394, rs1393350, rs258322, rs12931267, rs1805008, rs1805007, rs1545397, rs2031526 and rs1426654) were tested on more, than 200 Hungarians between the age of 18–45 and 100 Hungarian Romanies with TaqMan probes on ABI7500. 100 Malaysian Indians and 100 Mongolians were tested only for fewer SNPs (e.g. rs1681982, rs1545397, rs2031526 and rs1426654). The prediction modeling was performed as in Liu et al. (2009).

Results and discussion
In comparison to the NCBI database, most of the European allele frequencies did not differ from the Hungarians using G-tests (p=0.01), but were different from the other populations. The autosomal SNP tool was tested on the DNA of persons with known phenotype and it proved to be useful in forensic genetics.

P 074 MRNA EXPRESSION AND DNA METHYLATION BIOMARKERS FOR ESTIMATING CHRONOLOGICAL AGE FROM BLOOD

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Establishing the age of unknown person may provide important leads in police investigations, disaster victim identification, identity fraud cases, etc. Current methods mostly rely on odontological or skeleton analysis. The establishment of molecular methods for forensic age estimation using samples that possess no morphological information, such as bloodstains, would be extremely valuable as this type of samples is commonly found at crime scenes. Previously, age-dependent accumulation of mtDNA deletions and telomere length measurement were investigated for DNA-based age estimation. However, dedicated forensic studies showed that both of these molecular approaches suffer from low sensitivity and/or poor reliability and accuracy. Recently, we proposed a new DNA-based method for forensic age estimation from blood stains that is based on the quantification of T-cell specific DNA rearrangements (sjTREC). Although the accuracy of this new method allows the reliable prediction of age categories spanning 20 years from blood-derived DNA, the standard error of the individual-based age estimation (about ±9 years) is too high for accurate point age estimation. In search for additional biomarkers for molecular age estimation we performed genome-wide profiling of gene expression along with genome-wide profiling of DNA methylation on a set of whole blood samples obtained from various individuals of different age. Using statistical analysis we identified candidate mRNA and CpG methylation markers that are highly informative for chronological age. Preliminary results indicate that the newly identified biomarkers have a potential to predict a person's age from blood with a higher accuracy than any other method proposed so far.

P 075 A FORENSIC CASE: HAIRS AS A MATERIAL EVIDENCE

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Is well known the usefulness of genetic polymorphisms in forensic studies in the identification of material evidence, when the victim has been killed or disappeared. It has been considered that biological trace evidence as blood, hair, semen, saliva, etc., derived from any criminal act is susceptible of a forensic genetic analysis. Forensic genetic analysis of hairy filaments as evidence, has taken great importance due to the information that they provide in critical cases when the proper collection and treatment conditions has been well set.
During the year 2007, the criminalístic group of forensic genetics at the INML y CF (Bogotá-Colombia) solved around twenty cases where hairy filaments were the only material evidence to probe the cases.

Case Report
Homicide of a 51 year's old men who died by sharp weapon's wound which generates a mechanical obstruction of the respiratory system. Hairy filaments were collected on the neck's victim, in the victim's room and the hall entrance and over a sisal found at the scene. They were classified as human hairs. The hairs were analyzed to extract DNA using organic extraction. The DNA was quantitated using the Quantifiler human kit of Applied Biosystems. Amplification of STR's genetic markers using the Identifiler® kit of Applied Biosystems was performed. Analysis using the ABI 3130 was done and genetic profiles for the 15 STR's were obtained from the four different scene evidence collected.
They were compared with a reference sample from the suspect and the victim. It was found that the on of the hair found in the victim's room matched the genetic profile of the suspect. The match probability was $1.8x10^{12}$. The hair on the neck's victim matched the victim's profile and the two other hairs (the one found on the hall entrance and the one found over the sisal correspond to an unknown male and female individuals.
The success of the hair extraction depends on the evidence collection and the amount of cells recovered from the bulb's hair and the type of extraction used. DNA. To obtain clean genetic profiles that can be compared with reference evidence is not always easy. In this case in particular, the results obtained helped to solve the investigation.

P 076 A *DE NOVO* MUTATION IN CAVEOLIN-3 GENE MAY CONFER GENETIC SUSCEPTIBILITY TO LONG QT SYNDROME

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Long-QT syndrome (LQTS) is a potentially lethal, inheritable arrhyithmia syndrome affecting about 1 in 3000 persons. The pathogenetic basis for LQTS has focused on ion channels but recently 2 LQTS-susceptibility genes encoding for two non-ion channel proteins have been discovered (ankyrin B and caveolin-3). Mutations in both genes result in secondarily ion channels disruption as consequence of altered localization or function. In this study we investigate whether a *de novo* CAV3 mutation, found in 1 out of 50 unrelated Italian patients with LTQS, negative to mutational analyses of other LQTS-associated genes, may be implicated in this syndrome. It is a missense mutation (V82I), localized to the intra-membrane domain of caveolin-3, in a highly conserved position across species. The human wild-type CAV3 and the V82I-CAV3 mutant were cloned and transfected in BHK cell line. The expression and the distribution of V82I-CAV3 and WT-CAV3 proteins are quite similar, however cells expressing the mutant CAV3 are significantly more susceptible to the death under stressing conditions. Preliminary data indicate that the mutant V82I-CAV3 affects the sodium-calcium exchanger (NCX1): transfecting V82I-CAV3 in BHK cell line stable expressing NCX1, the activity of the exchanger resulted compromised. These results could be clinically relevant considering that NCX1 localized to caveolae, due to its association with caveolin 3, and that abnormal distribution or activity of NCX1 can lead to an arrhythmic response.

These data suggest that the V82I mutation found in CAV3 can be potentially involved in congenital Long-QT syndrome, affecting survival signaling pathways and/or the NCX1 functionality.

P 077 A QUANTITATIVE SURVEY OF EXISTING AND NEW DNA EXTRACTION TECHNIQUES FROM ANCIENT AND DEGRADED TEETH AND BONES

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DNA extraction is a critical step for the efficient recovery of highly-degraded and damaged DNA from subfossil/archaeological remains and for forensic DNA profiling of missing person remains. Recent ancient DNA and forensics research has shown that simple modifications to extraction techniques can dramatically improve DNA typing success. These studies also highlight the complexities of method comparisons and improvement, and the need for further work and cross-disciplinary collaboration to test results. We conducted a broad, quantitative survey of six existing organic- and silica-based DNA extraction methods and two new silica-based protocols on animal and human remains. Nuclear and mtDNA recovery was quantified using real-time PCR. Age and preservation quality of the material varied drastically from forty to four thousand years old and from environments as diverse as the Alaskan permafrost to soil-derived samples from temperate climates. We developed two novel techniques (built around silica and chaotropic salts) that produce optimal recovery of both mitochondrial and nuclear DNA. Additionally, these techniques remove PCR inhibitors and are essentially free of contaminating human DNA. One, non-kit-based, method can be rapidly scaled from 10mg to several grams of starting material. Importantly, in addition to mitochondrial DNA, both new methods recovered the less abundant nuclear DNA, which is important for forensic genotyping applications. As sequencing costs are decreasing and whole-genomes are becoming accessible, obtaining the maximum amount of clean, genomic DNA from samples becomes the critical laboratory step.

P 078 OPTIMIZATION OF DRIED STAIN CO-EXTRACTION METHODS FOR EFFICIENT RECOVERY OF HIGH QUALITY DNA AND RNA FOR FORENSIC ANALYSIS

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Forensic analysis of biological material recovered from crime scene stains can aid in the determination of the body fluid or tissue from which it originated (mRNA profiling) as well as identifying the donor of the biological material (DNA - STR analysis). Many of these stains will involve mixtures of different body fluids. As a result of stain heterogeneity, separate sub-sampling for DNA and RNA analysis may result in a misleading estimate of the ratio of the body fluids present and even fail to identify one of the body fluid constituents. Therefore, a requirement for the successful implementation of mRNA profiling methods is the ability to co-extract RNA and DNA from the same biological stain.

While methodologies exist for the co-isolation of DNA and RNA from biological material, few studies include a thorough evaluation of these methods for use with forensic samples. Frequently, modifications to standard methods need to be implemented in order to accommodate the challenging nature of forensic biological samples. We therefore evaluated several co-extraction methods for ease of use and ability to recover DNA and RNA of sufficient quantity and quality for forensic analysis. From the five methods evaluated, two methods were selected for subsequent optimization (an in-house manual organic co-extraction and the Qiagen AllPrep DNA/RNA Mini kit). Modification of the incubation temperature and time and the inclusion of additional purification steps were found to improve the efficacy of nucleic acid recovery. Details of the modified methods will be presented.

P 079 GETTING READY FOR VOLUME CRIME DNA ANALYSIS

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Many countries are implementing systematic sampling for burglaries or car theft and other minor crimes also called volume crimes, resulting in a major jump in the number of samples to analyse. The throughput of the Laboratoire de Police Scientifique de Lyon will have to increase from 35.000 casework samples/year to about 70.000 samples/year within the next 2 years. To deal with such major throughput increase we looked at ways of optimising the sample workflow and chemistry without changing equipment and staff needed for the actual throughput. The current casework workflow uses 2 Hamilton STARlet workstations for DNA extraction, Quantification, STR setup and Normalisation already integrated with the LIMS for complete traceability.

To optimise workflow, traceability and user hands on time, we will show in details the optimisation of the Lysis step, the introduction of 2D Barcoded tubes processed directly in racks of 24 with minimal user hands on time and reduced risks of contamination.

To improve yield and success rate, the ABI PrepFiler™ DNA extraction protocol has been implemented and optimised on the Hamilton STARlet workstation. Preliminary results on controlled samples show a 2 to 3 fold increase in DNA yield compared with the Silica membrane kit actually in use. An additional study with mock contact samples results in a 2 fold increase of the success rate compared to results obtained with routine samples.

All these changes will allow a more efficient use of resources and reduced hands on time for operator to cope with the future throughput increase without compromising sample integrity and traceability.

P 080 RNA CAN DO BETTER – AN IMPROVED STRATEGY FOR RNA-BASED CHARACTERIZATION OF DIFFERENT BODY FLUIDS AND SKIN

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RNA-based identification has proven to work for cellular material from various sources of bodyfluids. This study addressed to the questions whether simultaneous coextraction of DNA and RNA yields similar amounts of DNA compared to established DNA extraction methods, whether RNA tests have an at least comparable sensitivity as conventional characterization tests, whether also skin epithelial cells can be characterized by specific transcripts and whether all used markers show specificity or cross reactivity.

Markers for blood, saliva, sperm, seminal fluid and menstrual blood were adopted from Haas et. al 2009, markers for vaginal secretion from Fleming, Harbison 2010. Markers for skin epithelial cell identification (CDSN, CST6, DSC1) were self-designed.

Extraction was performed with an optimised protocol of AllPrep RNA/DNA Mini. DNA yield was compared to that from QIAamp® DNA Mini extraction. Marker sensitivity and specifity were tested on RNA extracts, in comparison to conventional tests, using singleplex RT-PCR. At the end, three multiplexes were established, a triplex for blood and saliva, a tetraplex for sperm, seminal fluid, menstrual blood and vaginal secretion and a triplex for skin epithelial cells.

Results showed that modification of the coextraction protocol by overnight lysis at 56°C yielded an at least comparable DNA amount to that obtained from QIAamp® DNA Mini. RNA markers were as sensitive as conventional characterization methods and no evidence of cross-reactivity was observed except for the skin markers which showed a positive result for vaginal secretion and CDSN also for menstrual blood and saliva.

P 081 APPLICATION OF FORENSIC DNA FINGERPRINTING METHODS FOR GENETIC DISCOVERY OF MEDIEVAL HUMAN SKELETAL REMAINS

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In 2001/02 the municipal Archaeology of Hall accomplished by order of the Institute of Archaeologies (University of Innsbruck) successful excavations of an early Middle Age cemetery (dated between the 6th and 12th centuries CE) in Volders (Tyrol), which was a major settlement area in the Inn Valley since pre-historic times. On the basis of radiocarbon dating and grave situations, the majority of burials are assumed to range from the late 6th to the early 7th century. For this region and time period only scarce historical information is available. This cemetery represents one of the largest ancient series of human remains in Tyrol and the Alpine region in general. Of scientific interest is the burial situation in a tight area and the orientation of the skeletons with a prevalent restriction to East-West and only few North-South directions. There is a heterogeneous pattern of sparse grave goods and dress elements which indicate origin from different social structures and the religious denomination of the buried individuals. Because of their excellent preservation state the historical remains lend themselves perfectly to the application of biomolecular methods to gain increased insight for a better interpretation of the findings. We present first results regarding kinship analysis by autosomal Short Tandem Repeats and haplotypic markers as well as ancestry informative SNP typing.

P 082 IS THERE A CORRELATION BETWEEN METHADONE NEED AND CYP2D6 GENOTYPE?

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Introduction and aims
CYP2D6 belongs to the family of human cytochrome P450 enzymes, which play a major role in drug metabolism. These enzymes play also a critical role for the metabolism of methadone, which is used as a potent substitution drug of former heroin addicts. Because methadone shows strong variability for its pharmacokinetic a dose optimization has to be found for each patient to prevent both intoxication and withdrawal. In most cases the administered dose of methadone depends on the subjective need and well-being of the person. In this study we tried to find out whether there exists a correlation between the methadone need and the gene dose for CYP2D6.

Materials and methods
We have established an assay for typing five of the most relevant polymorphisms of CYP2D6 by SNP analysis. Deletion or amplification of the CYP2D6 gene was analyzed by long distance PCR. Copy number variation (CNV) was determined by real time PCR. From German methadone maintenance (MMT) program 105 people were analyzed. Validation was done with 404 samples of a clinical study. Enzyme kinetics was analyzed for same people of the MMT program.

Results and discussion
For both collectives the calculated genotype frequency distributions were in good agreement with published data. By Real time PCR analysis up to six gene copies were found. Because of parallel consumption of other drugs, from Enzyme kinetics no correlation can be shown for methadone need and CYP2D6 genotype. Therefore Methadone demand cannot be estimated by genotyping only.

P 083 DNA TYPING FOR THE IDENTIFICATION OF 8 VICTIMS OF SPANISH CIVIL WAR REPRISALS IN THE CANARY ISLANDS: THE CASE OF "LOS TRECE DE FUENCALIENTE" MASS GRAVES (FUENCALIENTE, LA PALMA)

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During the Spanish Civil War (1936–1939), Canary Islands suffered the highest levels repression by the insurgent side, although there were no battles in the islands. More than 50 people were killed in La Palma Island between July 1936 and June 1937. The "Association for the Recovery of Historical Memory in La Palma", made up of relatives of people who went missing during the Civil War, located "The Fuencaliente mass graves" in 2004. The excavation process recovered eight victims skeletal remains. The aim of this work was the genetic identification of the reprisal victims.

In general, obtaining nuclear DNA profiles from old skeletal samples is assumed to be difficult, due to the age and conservation conditions of the remains. For these reasons, we firstly try to analyze the mithocondrial DNA (mtDNA). Although the mtDNA control region sequence was obtained for the eight skeletal remains, the limited number of suspected maternal relatives donors complicated the identification based on mtDNA.

Taking into account the problems in stablishing the identity using mtDNA, a few years later we decided to analyze the nuclear DNA using the new forensic PrepFiler BTA DNA Automatic extraction methodology. This extraction method, in combination with the new AmpFℓSTR NGM PCR Amplification Kit (Applied Biosystems), allowed us to obtain the nuclear DNA full profile of eight skeletal bodies.

In conclusion, the use of protocols designed for old DNA samples, such as PrepFiler BTA Extraction Kit and NGM PCR Amplification Kit was crucial for obtaining full profiles that allowed high significance results.

P 084 THE CONTRIBUTION OF DNA ANALYSIS TO THE DISASTER VICTIM IDENTIFICATION (DVI) RESPONSE TO THE 2009 BUSHFIRES IN VICTORIA, AUSTRALIA

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On Saturday 7th February 2009 firestorms with temperatures in excess of 1500°C devastated 78 townships in Victoria, Australia. Human victim identification was supported by a number of scientific disciplines at the Victorian Institute of Forensic Medicine (VIFM), including DNA profiling. This abstract describes the contribution of DNA analysis to the DVI response and comments on the discriminatory power of ProfilerPlusTM.

Ten victims died in hospital, and in an effort to identify the remaining 163 missing persons VIFM's DNA laboratory received 182 post-mortem (PM) samples and 236 ante-mortem (AM) reference samples. Due to the severity of the heat of the fires it was not possible to recover remains from all victims. DNA extraction and quantification were performed using Qiagen's QIAcube, and Applied Biosystems' Quantifiler™, respectively. PCR and DNA profiling were performed using Applied Biosystems' ProfilerPlus™ and 3100 Genetic Analysers, respectively.

Matching of PM and AM DNA profiles assisted in the identification of 67 deceased. The majority (82%) of these identifications were kinship matches with relatives of missing persons, with only 18% of identifications achieved by direct comparison to DNA from Guthrie cards. ProfilerPlus™ (with nine loci) resulted in a number of matches between unrelated individuals, highlighting the importance of a minimum of 12 loci for DVIs relying heavily on kinship matching. However, the locations affected by the bushfires were spread over geographically independent areas and could therefore be treated as individual mini-disasters. The availability of such circumstantial evidence overcame the limitation of ProfilerPlusTM in terms of the number of loci available.

P 085 MOLECULAR INVESTIGATION OF CARDIAC ARRYTHMIA AND SUDDEN DEATH USING NEXT GENERATION SEQUENCING

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Aim: to use next generation sequencing (NGS) in order to screen for genetic mutations causing cardiac arrhythmia and sudden unexpected death (SUD).

NGS refers to new DNA sequencing technologies that can rapidly sequence DNA on the gigabase scale. An alternative approach involves resequencing of the protein coding regions (the exome). The exome comprise ~1% of the human genome (approximately 30 megabases). It has been estimated to constitute about 85% of the disease-causing mutations and sequencing of the exome is an efficient strategy in the search for candidate genes for different disorders.

In collaboration with the Forensic medicine department and the Cardiology clinic, both at Uppsala university hospital, several families suffering from SUD and showing an elevated ST pattern have been identified. None of these patients had any previous history of cardiac disorder. These patients have been analyzed using Whole exome sequencing and the SoliD-platform (Applied Biosystems). The huge amount of generated data is analyzed using bioinformatics and appropriate filtering against public SNP databases and HapMap genomes.

This project will enable us to identify genetic factors contributing to disease. It will also allow identification of mutations among asymptomatic relatives that can be prophylacticly treated, thereby preventing sudden death.

In forensic medicine, a genetic test would mean increased legal security by eliminating an unnatural cause of death and it will be an important tool for the forensic department when performing molecular autopsies. Another essential aspect is the help that unequivocal information about the cause of death would offer to grieving family members.

P 086 TWO CASES OF SEX CHROMOSOME ABNORMALITIES FROM DNA LABORATORY CASEWORK: XX MALE SYNDROME AND XXY KLINEFELTER IN MURDER AND RAPE CRIMINALS

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Study of gender markers is a part of routine forensic genetic examination of crime scene and reference samples. Amelogenin gene markers are included in majority of commercial forensic STR kits. Sex chromosomes abnormalities in forensic casework samples are revealed rarely, mainly there are male samples with no amelogenin Y or X amplification products. We have met about 10 such cases in 11-year practice.

Two special cases were discovered over the past six months. One was a severe murder case where man killed his mother-in-law and his son. During DNA study we found out the murderer was not the biological father of the boy. He showed heterozygous state in seven out of eight X-chromosome loci, no amelogenin Y allele, only X one was present. Only two loci of Y chromosome (DYS456 and DYS393) were amplified by 16 loci Y-filer kit. The man had male phenotype, he even had served in the army with no abnormalities detected. He was stated to be ill with schizophrenia several years ago.

Another case was a homosexually pedophilic rape. Sperm stains without spermatozoa were found on victim's and offender's clothes. DNA analysis revealed X-chromosome peak to be twice higher than the Y-chromosome one in saliva and sperm reference samples. Additional study of X- and Y-chromosome STRs showed heterozygous state in seven out of eight X-chromosome loci and amplification of full 16 Y-chromosome profile.

Autosomal and Y-chromosome DNA analyses were performed with Applied Biosystems kits, X-chromosome DNA analysis – with Argus X-8 Biotype kit.

P 087 THE TRANSFER OF SKIN EPITHELIAL CELLS VERSUS VAGINAL MUCOUS MEMBRANE CELLS BY DIRECT CONTACT

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Female cellular material may be detected on swabs from the surface of the defendant's penis in rape cases including vaginal penetration. The defendant may display alternative explanations to the findings, i.e. that he touched the offender's skin with his hands prior to manual transfer of the cellular material to his penis. It is not possible to decipher if the female DNA profile detected originates from skin or mucous epithelial cells.

Based on case work experience we hypothesized that vaginal mucous epithelial cells will be transferred to a greater extent by direct contact than skin epithelial cells.

To test the hypothesis, 11 male-female pairs were tested as follows: Each male participant swabed his penil surface on 3 specified anatomical locations in two different situations: 1. Following vaginal intercourse with his female partner. 2. Following manually touching of his penis succeeded by his touching of his female partner's skin. Reference profiles were produced from self harvested oral swabs.

DNA was extracted by Chelex 5% followed by Quantifiler® Duo™ quantification and AmpFℓSTR® NGM™ kit DNA profiling. The results of the penil swab profiling were compared to the reference profiles of each couple.

The quantification results indicate that swabbing of the penil surface following intercourse produce higher DNA concentrations than after manual touching. Moreover, our results also give indications as to the best penil anatomical region for the sampling of female epithelial cells. The DNA profiling results show a preponderance of female profiles over male profiles following intercourse compared to manual touching.

P 088 VALIDATION OF THE POWERPLEX® ESX17 AND ESI17 KITS FOR STR TYPING OF TELOGEN HAIR ROOTS IN FORENSIC CASEWORK

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Telogen hair roots are usually characterized by only a small amount of nuclear DNA which is frequently also degraded. STR analysis of telogen hair roots is therefore often complicated. However, significant improvement has been achieved in the past. Due to the requirements to analyze the new 16 STR markers of the European Standard Set of loci (ESS) and SE33 in forensic casework, we investigated the new PowerPlex® ESX17 and ESI17 kits from Promega for their application to telogen hair roots in comparison to the genRES® MPX SP-4 kit from Serac and the AmpFℓSTR SEfiler Plus™ kit from Applied Biosystems. Apart from the advantage to be able to analyze 17 loci in one multiplex PCR to implement the new European Standard Set of loci into the routine investigation of telogen hair root in forensic casework, the PowerPlex® ESX17 and ESI17 kits from Promega yielded reproducible results and showed the tendency to be even more sensitive than the genRES® MPX SP-4 kit from Serac and the AmpFℓSTR SEfiler Plus™ kit from Applied Biosystems. Considering the possible amount of detectable allels, allelic drop outs seemed no to appear increasingly in PCR systems with amplicon sizes more than 200 base pair.

P 089 CYTOCHROME P450 GENETICS SUPPORTING RISK FACTORS FOR MOLECULAR AUTOPSY

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Medicolegal investigations assist in determining the cause and manner of death. Autopsy is a primary tool of the investigation for assessing cause and manner of death. Some autopsies, however, do not resolve the cause of death unambiguously. In these cases, there is no evident pathology to determine the cause of death and toxicology is negative or difficult to interpret. This is particularly so in cases of sudden unexplained death and some drug-related deaths. The genetic constitution of victims at specific genes may clarify some of these unexplained deaths or at least suggest susceptibility to triggering affects. We report on the progress of a major collaborative project to develop a baseline that defines the predictive value of genetic variation at selected known drug metabolizing enzyme genes. The data comprise complete sequencing on an Genome Analyzer IIx (Illumina, Inc.) next generation sequencer in unrelated individuals from four population groups (African Americans, Caucasians, southwestern Hispanics, and Finns) at selected genes. The Cytochrome P450 (CYP450) genes that encode the enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5, metabolize about 90 percent of drugs, and are the current targets. Polymorphisms, found in the genes encoding CYP450 enzymes, can result in four categories of metabolizers (in increasing order of activity): poor (PM), intermediate (IM), extensive (EM), or ultra-rapid metabolizers (UM). The underlying genetic variation provides insight into evolutionarily conserved regions that pose higher risk. The baseline predictive data are assessed on autopsy case samples with complete toxicology screens.

P 090 A SNP MULTIPLEX FOR THE SIMULTANEOUS PREDICTION OF BIOGEOGRAPHIC ANCESTRY AND PIGMENTATION TYPE

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DNA analysis of ancestry informative markers (AIMs) and physical trait markers from biological stains can help to provide investigative leads in cases without suspects. To enhance the resolution and informative value of two previously developed single nucleotide polymorphism (SNP) multiplexes, the 34-plex and *Eurasiaplex* assays (Phillips et al., 2007, Phillips et al., manuscript in preparation) differentiating European with Near and Middle East populations, we have selected an additional set of 23 AIM-SNPs. The selected markers offer discriminatory potential mainly among Europeans and Asians, and we also supplemented this set with a panel of 10 recently published pigmentation markers informative for eye, hair and skin colour (Walsh et al., 2011, Branicki et al., 2011, Sturm 2009). Preliminary results using a range of population reference samples indicate this multiplex can readily distinguish the major continental groups of Europe, Africa, and East Asia. Using the "Snipper" web portal (http://mathgene.usc.es/snipper/), ancestry predictive likelihoods in the range of 10^3 were achieved when samples from Turkey and Germany were compared, using East Asian, West African, Central European, and Turkish individuals as refererence populations. In a small pilot study using voluntary donors, the *IrisPlex* SNP markers (Walsh et al., 2011) built into our multiplex were successfully used to make predictions about likely eye colour. We intend to increase the number of test samples per population, as well as the range of populations by recruiting more sample donors with reliable information about eye, hair and skin colour, as well as ancestry.

P 091 MOLECULAR AUTOPSY IN TRAUMATIC DEATH

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Introduction
The heart of the forensic pathology is provided by the classical morphology, which can be enriched by a range of additional analyses, including molecular techniques, that recently had a growing interest in the determination of the causes and circumstances of death, with the new chapter of "molecular autopsy". Molecular biology is currently limited to sudden cardiac death, and only at specialized centers.

Materials and methods
A 16 years old boy died for hemorrhagic shock in a distractive trauma of shoulder reported the day before, during a volleyball match.

Results and discussion
The histopathological data showed a large hematoma in the left axillary region, a lung collapse with massive hemothorax in pleural cavity and the laceration of the left axillary artery. A marked vascular fragility, with vascular formations that have been ripped at the slightest mechanical drive, required a verification of diseases characterized by vascular fragility. Genetic screening revealed the presence of heterozygous mutation c.1474G> A in exon 21 of COL3A1 gene, diagnostics for Ehlers-Danlos syndrome. The possibility that the boy had been beaten, as supposed by the investigators, was excluded.

This case report highlights the extreme utility of molecular biology in forensic investigations and the need to establish clear guidelines for post-mortem molecular study with uniform protocols for sampling and for the preservation of biological material collected during the autopsies. Due to the inheritance of many diseases, must be taken into account the possible implications of a missed diagnosis that could delineate professional responsibility for the sector itself.

P 092 RAPID ANALISYS FOR CONFIRMATION OF AMELOGENIN NEGATIVE MALES CHARACTERIZED BY A YP11.2 DELETION

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In humans, the amelogenin gene is present on both the X and the Y chromosomes. However, there are size differences in this gene between these chromosomes, which have been utilised for sexing in forensic casework and prenatal diagnosis. The assay typically generates a 106 bp long fragment from the X chromosome and a 112 bp long fragment from the Y chromosome.

However, several studies have shown that the amelogenin gender test may not always be concordant with true male gender in forensic casework or in prenatal diagnosis.

Deletions of *AMELY* can result in no amplification product and these null *AMELY* alleles can occur in different percentage in different population groups.

The literature data support that the null allele is the result of a larger deletion on the short arm of the Y chromosome.

Considering the consequences of the result obtained using only the amelogenin marker, and potential interpretation difficulties in the few cases where gender misinterpretation may be problematic, in this paper we propose a method for the identification of samples with deleted *AMEL* based on a small polyacrylamide-gel electrophoresis of a duplex PCR product of a novel marker residing in the *SRY* gene, which results in a 197 bp long PCR product in combination with primers for *AMEL*.

This method can be applied, as an additional assay, in case of doubt regarding the presence of deleted AME in the DNA profile.

P 093 MITOCHONDRIAL DNA ANALYSIS OF FORMALIN FIXED PARAFFIN EMBEDDED TISSUE SAMPLES: EFFECT OF FORMOL IN DNA STABILITY AND ITS IMPLICATIONS FOR GENETIC STUDIES

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Formalin Fixed Paraffin Embedded Tissue (FFPET) samples represent one of the most important sources of biological material in Molecular Epidemiology and Forensic Genetics. Notwithstanding that the use of FFPET samples is widely held, several studies have demonstrated that formaldehyde (H2CO), the principal component of formol, causes alterations in nuclear DNA. However, its effects over mitochondrial DNA (mtDNA) still remain unexplored. Thus, we aimed at determining the presence of alterations in mtDNA caused by the process of fixation with formol. Samples were collected from 14 autopsies for a total of 105:66 FFPET samples, 10 blood samples from heart cavity and 29 from fresh tissue. Segment HVSIa within the displacement loop (Dloop) and a segment of the coding region of the mtDNA were amplified and sequenced. Changes were not observed in the coding region. However, analysis of HVSIa revealed the existence of numerous differences between FFPET samples and their corresponding reference sequences from blood and/or fresh tissue. The majority of these alterations were point heteroplasmies by transition, although point heteroplasmies by transversion and base transition were also observed. Similarly, length heteroplasmies were observed in FFPET samples not present in their reference samples. These variations might be a direct consequence of the fixation process with formol. These results point to readdress the utility of FFPET samples in studies of the Dloop of the mtDNA and urge to act with caution in the resolution of practical cases in Forensic Genetics.

P 094 NUCLEIC ACID OPTIMIZER FOR FAST RECOVERY OF DNA FROM FORENSIC SAMPLES

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Introduction
Investigating crime scene requires devices that can recover the entire sample collected. Copan developed flocked forensic collection devices (FFCDs) to collect and preserve minimal sample present at crime scene. For recovering all DNA from the FFCDs Copan developed the Nucleic Acid Optimizer (NAO), consisting of a semi-permeable basket used with the FFCDs to efficiently release all sample. The objective was to validate the performance of the NAO for direct and fast recovering all nucleic acid directly from the collection device.

Materials and Methods
The Nucleic Acid Optimizer (NAO) is a semi-permeable basket designed to be used with 1.5 and 2 ml microtubes. NAO has been tested for retaining 500 µl of liquid (water, saline, CyMol, Ethanol, Methanol, buffers) after storage at 22°C and 37°C followed by vortexing at 3000 rpm/1min. Fluid release was tested by centrifuging at 10000 xg/1min. with/without FFCDs. Nucleic acid extraction platforms interference and recovering was tested using FFCDs inoculated with DNA. FFCDs were broken into a NAO and fluid holding, releasing and nucleic acid extraction and amplification and quantization were tested by real time PCR validating all specifications. FFDCs tested with traditional NA extraction were used as comparison.

Results and Conclusions
No liquids were released by the NAO after 22°C and 37°C storage and vortexing. The entire sample was released after high speed centrifugation. Double the amount of DNA was recovered from the FFCDs tested with the NAOs compared to NA extraction. The Copan SNAOs are performing as per designed specifications, reducing time/manipulations while recovering small amount of nucleic acid.

P 095 DEVELOPMENT OF AN 8 MINISTR AND AMELOGENIN FLUORESCENT-MULTIPLEX SYSTEM

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In this study, we had established a fluorescent multiplex system of 8 non-CODIS MiniSTR and Amelogenin for forensic purpose. Eight highly polymorphic non-CODIS MiniSTR, D20S1082, D6S474, D12ATA63, D9S1122, D2S1776, D1S1627, D3S4529 and D2S441 were selected, and a fluorescent multiplex system including these loci and Amelogenin was developed through redesigning primers, labeling fluorescence dye, and optimizing experiment conditions. 204 randomly selected individuals of Guangzhou Han population, members of 30 families and 30 degraded samples were genotyped using this system. Tested by the new multiplex system, all the loci can generate stable and full profiles, with amplicons less than 200bp in size, which resulted in an increased overall typing success rate for degraded DNA samples. And its cumulative power of discrimination and probability of exclusion were 0.99999993 and 0.992287 respectively in Guangzhou Han population. Conclusively, the established fluorescent multiplex system was robust, sensitive and stable, with high value in forensic application, and it can serve as a new approach for the analysis of degraded DNA as well as an effective supplementary of commercial kits.

P 096 ESTABLISHMENT OF MTDNA SNPS MULTIPLE SYSTEM AND ITS APPLICATION IN FORENSIC MEDICINE

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Objective
To develop the method typing SNPs of mitochondrial DNA with fluorescence labeling and apply to the forensic science practice.

Methods
SNP markers 709, 1719, 15607, 13928, 7028, 12438, 10398, 16519, 4833, 5250, 4529, 4580, 477, 7202, 11719, 4793, 11914, 12633, 12858, 10873 and 13263 were selected and developed a multiplex mtSNPs typing system based on multiplex PCR amplification, single base extension and capillary electrophoresis technology. A total 236 samples from Guangdong area were typed with the multiplex mtSNPs system and all the allele frequencies were determined.

Results
All of the mtDNA SNPs are polymorphism except for 477, 12438. The genetic diversity (GD) of mtDNA SNPs ranged from 0.0085~0.4980, and the haplotype diversity(HD) was estimated to be 0.9705. 90 haplogroup were found. The 19-plex mitochondrial DNA SNPs minisequencing reaction was developed with high sensitivity, simple operation, high accuracy.

Conclusion
The 19-plex mitochondrial DNA SNPs has a favorable prospective in the forensic science.

P 097 MUTATION RATES OF 15 X-CHROMOSOMAL SHORT TANDEM REPEAT MARKERS: FINAL RESULTS OF A LARGE-SCALE STUDY

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X-chromosomal short tandem repeat (X-STR) markers have recently been recognized as useful tools to supplement traditional kinship testing in the forensic setting. Development of assays allowing the multiplex detection and analysis of various combinations of X-STRs has spawned numerous publications reporting the standardization of repeat structure and distribution of allele frequencies in a number of populations across the globe. However, far fewer studies have been published regarding the mutation rates of the commonly used markers, and data that have been published are typically based upon limited population groups and only a subset of the markers used by the community. In order to enhance the practical application and interpretation of X-STR markers, the rate of mutation should be determined through examination of a substantial number of meioses, and the dependence of mutation rates upon the origin, length, and structure of the allele should be investigated. Therefore, more studies using relevant populations are necessary before the full potential of these markers can be realized. At the Armed Forces DNA Identification Laboratory, two multiplexes consisting of a total of 15 markers (DXS6789, DXS9902, DXS7132, DXS7130, DXS6795, DXS10147, DXS8378, DXS7423, HPRTB, DXS101, DXS7424, GATA31E08, GATA172D05, GATA165B12, and DXS6803) have been characterized and allele frequencies determined for several different populations. A preliminary study examining a subset of 6960 meioses in established family trio samples from several different population groups revealed an overall mutation rate for X-STR markers of 7.18×10^{-4}. Here, the authors present the results of the final large-scale mutation rate study.

P 098 CHARACTERIZATION OF THE QIAGEN® INVESTIGATOR ARGUS X-12 KIT IN U.S. POPULATIONS

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Though a large number of multiplex assays using X-chromosomal short tandem repeat (X-STR) markers have been described in the literature, there is currently only one commercial kit available, the Qiagen® Investigator Argus X-12 kit, which simultaneously detects 12 X-STR markers plus Amelogenin. Several recent publications have described its performance on high quality samples and presented allele and haplotype frequency data from populations in Germany [1], Morocco [2], China [3], and Hungary [4]. Further study of these 12 markers is required before the potential of the kit can be fully realized for use in the forensic setting. To this end, 975 samples from the four major U.S. population groups (African Americans, Asians, Caucasians, and Hispanics) were typed using this commercial kit. Resultant profiles were examined for kit performance measures such as stutter and peak balance. Alleles that were off-ladder, unpublished, or null were noted and many were sequenced to confirm repeat structure and number. Additionally, a concordance study between the X-12 kit and the four overlapping markers also present in a published mini X-STR assay [5] was completed. Nearly all of the samples typed were concordant, with only 1 sample (0.1%) exhibiting discordance due to a null allele. The forensic utility of markers present in both the X-12 kit and the mini-X assays (23 markers total) in these U.S. populations was also compared, demonstrating that the highest heterozygosity values corresponded to those X-12 kit markers composed of the most complex repeat structures.

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P 099 THE CHANGE IN HUMAN DNA CONTENT OVER TIME IN THE ARTEFACTS OF THE BLOWFLY *LUCILIA CUPRINA* (MEIGEN) (DIPTERA: CALLIPHORIDAE)

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Adult blowflies can act as vectors of human DNA by depositing faecal or regurgitation spots (termed artefacts) after feeding on meals of human biological fluid. Consequently, there is the potential for fly artefacts to contaminate genuine DNA evidence if crime scene personnel inadvertently collect them while swabbing for trace DNA. Alternatively, artefacts may be deliberately targeted as a source of DNA, particularly in circumstances where a body that has not shed blood has been moved, or where an offender has cleaned up the scene.

In situations where artefacts have been targeted, consideration must be given to how long the human DNA contained within remains stable. Given the backlog of cases in many forensic laboratories, it is important to know the maximum time period fly artefacts can remain untested before the human DNA becomes unviable. This is particularly crucial if artefacts are the only potential source of DNA available.

This study investigated whether the human DNA content in fly artefacts deposited by the blowfly, *Lucilia cuprina*, changed over time.

DNA from blood- and semen-based artefacts was extracted after periods of 3, 10, 30 and 60 days and the human component was quantified. Fly artefacts derived from blood demonstrated no change in the amount of human DNA over two months. However, the human DNA extracted from semen-based fly artefacts significantly increased ($p < 0.001$) over this period. Randomly selected samples from each period were genotyped. The DNA profiles from all samples tested corresponded to the profile of the biological donor.

These findings will assist forensic biologists seeking to obtain DNA profiles from fly artefacts to more effectively process their samples.

P 100 C667T MUTATION IN MTHFR: POPULATION DATA IN FVG (NORTH-EAST ITALY)

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The enzyme 5,10-Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetra-hydrofolate, the predominant circulatory form of folate and carbon donor allowing the re-methylation of homocysteine to methionine. The gene coding for MTHFR maps on 1p36.3 and it is composed by eleven exons. A point mutation (C>T) on site 667 of the exon 4 causes the substitution of Alanine by Valine leading to a reduced enzyme activity and increased thermolability. This mutation is recognized to be associated to several diseases. Since the frequency of the mutated allele *C667T* differs among populations, we created a database on a healthy control population (n=143) living in our area (North-East Italy). A simple and inexpensive method based on the *HinfI* RFLP of the 198 bp long PCR products was employed. The results (wild allele=0.577; mutated allele=0.423) are discussed.

P 101 ANALYSIS OF SNP INVOLVED IN CENTRAL NERVOUS SYSTEM IN COMPLETED SUICIDE VICTIMS

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Suicide represents a significant category of preventable death involving forensic practice.

Reliable tools for identifying those at risk for suicide, being clinical or laboratory tests are still lacking. Evidence suggests that genes play an important role in predisposition to suicide behavior. Thus, based on case reports, twin and family studies estimates of heritability for suicide range from 20% to 50%. Hence, identifying the relevant mutations or polymorphism is quite important for developing a preventive strategy.

A set of nine SNPs located in genes involved in serotonergic, dopaminergic and noradrenergic systems were selected to develop a reliable test based on multiplex PCR and minisequencig by using SNaPshot kit, useful for forensic samples.

About 60 complete suicide individuals aged from 20 to 95 years old, classified according to the method of suicide, were sampled. The control subjects were recruited from anonymous DNA samples, previously selected for absence of psychiatric disorders and substance addiction (nicotine, alcohol, cannabis).

Deviations from Hardy-Weinberg expectations were tested by the use of Arlequin v3.1. Preliminary results based on logistic regression model show significant values with higher Odds Ratio for risk alleles for rate-limiting enzymes. Given the complexity of suicide behaviors, a systematic effort for analyzing and interpreting the obtained data should be constantly performed.

P 102 GENETIC VARIANTS RELATED TO NICOTINE DEPENDENCE

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Smoking behavior is a worldwide epidemic that leads to severe health troubles for related illnesses, with high social and individual costs together with reported comorbidities with principal psychiatric disorders, raising important medico-legal issues.

Estimates of heritability for nicotine addiction range from 50% to 70% and recent genetic association studies as well as large scale GWA approaches supported the evidence of candidate genes behind tobacco consumption and different smoking phenotypes, highlighting a robust role of variants in nicotinic receptor subunits.

We have selected 10 single nucleotide polymorphisms (SNPs) located in eight different genes, which were reported as showing a strong association with high dependence risk. Sequence information for PCR primers including allelic variants, counting positions, heterozygosities and site functions were obtained from the NCBI dbSNP database, preferring those SNPs with minimum allele frequencies of 10%.

An European population sample of 454 volunteers was analyzed by minisequencing assay through SNaPshot kit (AB). The Fagerström test for nicotine dependence (FTND) was used to asses nicotine dependence. Cases were chosen according as current and former nicotine dependent (FTND ≥ 4 and Smoking Quantity, SQ ≥ 15), while controls were smoking-exposed but non-dependent and never smoker individuals (FTND = 0 and SQ ≤10 and FTND = 0 and SQ = 0, respectively).

The method of the *multivariate* analysis was based in a logistic regression by using the R project (v2.12.2). Preliminary results shows that the SNPs rs2023239, rs16969968 and rs1051730, located in the genes CNRA1, CHRNA5 and CHRNA3, respectively, could be associated to risk of developing nicotine dependence. Factors as age, sex, and exposition to smoke were also found as possible factors of risk of nicotine addiction.

P 103 EVALUATION OF THE RELATIONSHIP BETWEEN MITOCHONDRIAL HAPLOGROUP AND DEVELOPMENT OF HEART FAILURE IN BRAZILIAN SAMPLE

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Recently, some studies have been suggested an association between mitochondrial haplogroups (mtDNA Hg) and pathogenesis of cardiovascular diseases, showing an influence of ethnic origin with the development and prognosis of patients with heart failure (HF). Herein, we intended to evaluate the existence of a possible association of specific mtDNA Hg in 188 patients with HF, compared with 188 healthy individuals. Sequencing of regions HV1, HV2 and HV3 was performed using BygDye Terminator v3.1 and capillary electrophoresis was performed on ABI3130. Polymorphisms were determined comparing the sequences obtained with the Cambridge Reference Sequence (rCRS). In patients group, 92 individuals showed sequences of African Hg (49%) (L0 5%; L1 25%; L2 27%; L3 43%), 54 showed Ameridian Hg (29%) (A 39%; B 24%; C 24%; D 13%) and 42 individuals showed European Hg (22%) (H 55%; U 12%; K 10%; J 7%; T 7%; HV 5%; R 2%; W 2%). For control group, 68 individuals presented Ameridian Hg (36%) (A 31%; B 32%; C 24%; D 13%), 67 individuals showed African Hg (36%) (L0 11%; L1 25%; L2 22%; L3 40%; L4 2%) and 53 individuals showed European Hg (28%) (H 41%; HV 9%; T 9%; U 9%; I 8%; J 8%; K 6%; R 4%; X 4%; W 2%). Although African Hg was more prevalent among patients, we found no statistically significant difference between groups (P=0.10). The sample size is being increased in order to confirm these preliminary results.

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P 104 PROFILING OF DNA TRACES ON FIRED HANDGUN AMMUNITION

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Cartridge casings recovered from a crime scene are common objects of forensic investigations. However, the success rate of using DNA traces on fired ammunition for human identification is variable and little is known about the key parameters involved. A series of collaborative studies was started aiming to improve current strategies for the forensic routine. The handgun used for these experiments was the semiautomatic pistol ZCZ M57, which is associated with a number of crimes in Vienna. Initially, we assessed the impact of bullet firing on traces (blood/saliva) that were applied to ammunition (Tokarev 7,62 x 25 mm) either laterally, at the case mouth or at the bottom. DNA was harvested from fired cases using the double swab technique and the DNA yield was determined with the Quantifiler Duo kit. STR profiles were obtained with the PowerPlex S5 (PPS5) kit. Shooting caused no apparent loss of DNA quantity or quality. Transfer of DNA was observed with traces applied to the bottom (but not with laterally- or case mouth-labeled ammo) and occurred between labeled and successively fired clean ammo, involving the firing pin of the gun, but not the barrel. In attempt to simulate casework, random individuals handled clean shotshells for 60 seconds resp. 5 seconds and as long as needed to load and fire the weapon. STR typing was performed with the kits PPS5 and PPESX17. DNA profiling success seemed to primarily reflect the "shedder" type and the duration (intensity) of contact, but again, bullet firing had no gross impact.

P 105 WHERE SEXUAL ASSAILANTS LEAVE THEIR DNA PROFILES: HIGHLIGHTING THE IMPORTANCE OF TESTING Y-CHROMOSOMAL MARKERS

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Investigations in sexual assault cases are often confined to body fluids, hairs and fingernails taken from the body of the victim. However, sexual assault covers also cases of unwanted sexual contact that stops short of rape or attempted rape. This includes sexual touching and leaves contact stains at the clothes or the skin of the female victim. Investigators often expect insufficient information from these stains mainly because of the obligate mixture character and the low DNA quantity. In our study we show that by using Y-STR analysis in combination with autosomal analysis and Y-SNP analysis 70% of the cases with contact stains give an informative DNA profile of a male contributor. Y-STR profiles whether complete or partial are indicative and allow for exclusion or the direct comparison with a suspect. To identify serial offenders attacking females in a certain neighborhood, in a certain period of time and mostly with only short interaction (no rape) we propose to establish a database collecting Y-STR profiles from traces to identify recurrent patterns and to support pertinent police actions. We see the justification of such a database in the fact that sexual offenders will likely commit future attacks and thus pose a significant risk. To assess the weight of the Y chromosome evidence such a database could include an estimate of the frequency of the haplotype in the reference population as well as biogeographical information encrypted in the haplogroup.

P 106 GENETIC MARKERS FOR BODY FLUID AND TISSUE IDENTIFICATION IN FORENSICS

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Several studies have demonstrated mRNA profiling as a promising tool for the identification of biological sources in forensic case work. Recently, analysis of DNA methylation patterns for tissue detection has also been tested. Both are highly, sensitive cell-specific methods representing alternatives to conventional assays for tissue and body fluid identification. Additionally, they have forensic technical advantages permitting the use of assays already implemented in routine work and the detection of several body fluid markers in multiplex reactions.

The current work aimed at the study of mRNA markers for forensically relevant body fluids and tissues. mRNA analysis was conducted for the following tissue-specific genes selected from previous studies: for skin epidermis LOR, KRT9 and CDSN; for blood HBB and SPTB; for saliva STATH and HTN3; for semen PRM1 and PRM2; for vaginal secretions HBD-1 and MUC4 and for menstrual blood MMP-7 and MMP-11. The method used was a multiplex RT endpoint PCR followed by CE detection. The mRNA markers were successfully amplified in multiplex reaction revealing sensitivity and specificity to the biological material studied. Furthermore, comparison of mRNA-based tissue identification with detection methods based on DNA methylation patterns will be carried out.

Additionally, successful and efficient identification of 23 year old blood stains using the mRNA blood specific marker HBB was demonstrated. HBB revealed to be highly stable representing a useful marker for mRNA-based identification of old blood stains. Using RNA/DNA coextraction, material from the same stain sample could also be used for successful genetic typing of STR markers in forensics.

P 107 PRACTICAL VALUE OF THE MARKER MUC4 FOR THE IDENTIFICATION OF VAGINAL SECRETION IN PENILE SWABS

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The mRNA profiling method combined with a reverse transcription endpoint PCR method has proved a suitable technique for the identification of vaginal secretion. The reliability of the most common vaginal secretion mRNA-marker MUC4 to detect vaginal secretion in vaginal swabs has been confirmed. Because the cross-reactivity of MUC4 with some other samples has been reported, its specificity should be taken into consideration. The correct identification of biological source of samples from sexual assault cases is of vital importance. Having been aware of that issue, we evaluated the practical value of specificity of MUC4 for the identification of vaginal secretion by testing five types of penile swabs taken after different sexual intercourses. Buccal swabs from tonsils of men and women and penile swabs were used as a negative control, while markers statherin and histatin 3, specific for saliva, served as positive markers. All experiments were repeated five times. The paper demonstrates that the correct identification of biological source of samples as vaginal secretion is possible under two conditions. Firstly, markers specific for saliva must be tested coincidently with marker MUC4. Secondly, the cut-off value of MUC4 marker must be defined in a way that negative results below that value allow the conclusion that the sample was indeed not vaginal secretion. Other options to avoid wrong identification will be also discussed.

P 108 THE PERSISTENCE OF SPERMATOZOA: A RETROSPECTIVE OVERVIEW FROM 2010

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The literature on sexual assault cases describing the persistence and detection of spermatozoa in combination with acid phosphatase (AP) test results on samples from different female anatomical locations is limited. Samples from the victim's genitals were collected for the detection of semen in 181 of the 371 cases of alleged rape examined by our laboratory in 2010. All the swabs from vulva, vagina and cervix, in addition to samples from the female lingerie, were tested with AP and underwent Christmas tree staining followed by microscopy to confirm the presence of spermatozoa. In 67 cases (37%) spermatozoa from a presumed perpetrator were detected in one or more samples, including 9 cases in which spermatozoa were found only in the female garment. For a selection of samples differential extraction and quantification (Quantifiler® Duo) were performed prior to the DNA analysis (AmpFℓSTR® SGM Plus®) in order to determine the DNA profiles. The average time between the alleged assault and the medical examination was 12.4 hours, with some variation between the three different sampling areas, respectively 11.1 h (vulva), 13.7 h (vagina) and 14.4 h (cervix). 74 samples (46%) with microscopically verified spermatozoa displayed a negative AP reaction, and the results indicate that vulva samples more frequently produce a negative reaction despite verified semen compared to samples from vagina and cervix.

P 109 EXTRACTION OF NUCLEAR DNA OF RELIABLE FORENSIC EVIDENTIARY VALUE FROM HUMAN TEETH

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Identification of unknown bodies, especially in a mass disaster event, remains an ongoing challenge for societies worldwide. When conventional identification methods fail DNA may provide the only link to identity. Teeth, rather than bone, are becoming increasingly relied upon as a source of nuclear DNA for identification of human remains, however the results from DNA extraction from these tissues are extremely variable. Aims: To develop appropriate protocols for tooth selection and handling and DNA extraction from teeth of different stages of decomposition. Methods: Molar teeth of varying post mortem age were examined both for DNA content and histologically utilising nuclear staining techniques. Variable decontamination and sampling techniques as well as DNA extraction techniques were trialled. Results: Teeth of shorter post mortem age displayed a different pattern of DNA location than those subjected to a longer post mortem interval. Fresher teeth yielded higher quantities of DNA when not treated with traditional decontamination protocols and when not subjected to decalcification processes whereas the reverse was true for teeth of a longer post mortem interval. Teeth affected by dental disease yielded reduced DNA from dentine but not from cementum. This research demonstrates that the peri mortem health status and post mortem state of the tooth has an effect on the DNA location within the tooth and its relationship with the tooth mineral dictating that different protocols for handling and sampling are required to obtain useful genetic information. It also shows that careful selection of tooth and tooth tissue to sample is important.

P 110 CONCORDANCE TESTING COMPARING STR MULTIPLEX KITS WITH A STANDARD DATA SET

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Concordance evaluations are important to detect allelic dropout or "null alleles" present in a data set. These studies are performed because there are a variety of commercial STR multiplex kits with different configurations of STR markers available to the forensic community. The electrophoretic mobility of the markers can vary between kits because the primer sequences were designed to amplify different polymerase chain reaction (PCR) product sizes. When multiple primer sets are used, there is concern that allele dropout may occur due to primer binding site mutations that affect one set of primers but not another. These null alleles become evident only when data sets are compared. Null alleles are a concern because this could result in a false-negative or incorrect exclusion of two samples that come from a common source (only if different PCR primers are used).

Multiple concordance studies have been performed at NIST with a standard sample set (~1450 in-house U.S. population samples) using various STR multiplex kits from Applied Biosystems, Promega and Qiagen, including many of the new generation European kits. Various discordant results have been identified using concordance software developed at NIST, confirmed by DNA sequencing and reported to the forensic community on the null allele web page of STRBase.

A summary of the results, including discordance and sequencing results, will be shown in order to help assess the benefits of performing concordance testing using a standard data set with STR multiplex kits that have different primer sequences for the same markers.

P 111 TWINS: ANALYSIS OF X CHROMOSOME STR MARKERS

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Twin studies have been a valuable source of information about the genetic basis of complex traits. To maximize the potential of twin studies, large, worldwide registers of data on twins and their relatives have been established. X chromosome STR (X-STR) assays are helpful in complex kinship testing cases where autosomal and Y chromosome analyses convey little information. X-STR analysis has the power of exclusion/inclusion needed to resolve an alleged mother-son/father-daughter parentage and sibship. The purpose of this study was to apply autosomal STRs and X-STRs to confirm paternity and sibship on 272 sets of female twins and three sets of triplets from the Region of Murcia in Spain. It also seeked to announce the creation of a twin population database from the Region of Murcia. Zygosity was confirmed through the use of autosomal STRs with I-DNA1 multiplex system followed by capillary electrophoresis separation on ABI PRISM® 3130 Genetic Analyzer. From the 272 sets of twins, 143 pairs were monozygotic and 129 were dizygotic. One set of triplets was determined as monozygotic. The remaining triplets were determined as one pair of monozygotic and a dizygotic twin. Sibship and shared paternity of dizygous twins was determined through X-STR amplification with the GHEP-ISFG X-Decaplex followed by capillary electrophoresis separation. Statistical analysis included allele frequencies, polymorphism information content (PIC), heterozygocity (HET), power of discrimination (PD), and Hardy-Weinberg equilibrium.

P 112 ANALYSIS OF VENTRICULAR MYOSIN LIGHT CHAIN GENES IN CARDIOMYOPATHY

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Introduction
Cardiomyopathy is defined as a heart disorder with cardiac dysfunction and is known to cause the sudden death. Hypertrophic cardiomyopathy (HCM) and Dilated cardiomyopathy (DCM) are general disease in cardiomyopathy. Since molecular genetic studies have identified several genes encoding sarcomeric proteins, genetic heterogeneity was considered as the principal cause of disease. In this study, ventricular myosin regulatory light chain gene (MYL2) and ventricular myosin essential light chain gene (MYL3) were analysed in order to elucide the cause of cardiac sudden death caused by cardiomyopathy.

Materials and methods
DNA was extracted by QuickGene-800 (FUJIFILM) from blood specimens obtained from the cases of 15 HCM, 18 DCM and 3 ARVC (Arrhythmogenic right ventricular cardiomyopathy) with informed consent of their family members. The 200 cases that presented with no particular disease were used as control. Sequence analysis was essentially the same as those described previously (Legal med.2010;12:280-283). This study has been reviewed and approved by the Hospital Ethics Committee of Kitasato University School of Medicine (B01-24).

Results and discussions
In this study, missense mutation including, SNPs, one-base deletion and one-base insertion were detected. In a HCM case, Ala57Gly missense mutation was identified in MYL3. This mutation was located in the EF-hand domain, where the alignment of amino acid across the species and isoform are highly conserved. It was suggested that the cardiac hypertrophy by decreasing force generation was a primary mechanism for the pathogenesis of HCM associated with Ala57Gly mutation.

It is necessary to analyze the other sarcomeric genes and clarify the relationship with etiology.

P 113 MOLECULAR GENETIC INVESTIGATIONS OF SKELETON FINDS BURIED IN THE GROUND

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In the year 2003, several largely complete skeletons were found on various neighbouring spots during construction works in the vicinity of a Chemnitz detention centre, which had been used for decades. The Authority of the Inquiry did not assign systematic excavation, and so the skeleton material, which was sent in for investigation from the various finding places, also contained skeleton remains of several individuals per finding place. Police investigation, the burial place and some minor clothing remains resulted in the assumption that the graves had been laid out in the first years after World War II. In addition, it was suspected that one of the buried bodies might have been a male person who had been missing at that time.

After appropriate mechanical preparation of selected bone materials the DNA-isolation followed, and subsequently quantification with the Quantifier™ Human DNA Quantification kit of the firm Applied Biosystems. The amplification of DNA was carried out with commercially available autosomal and gonosomal amplification kits of various firms. DNA fragment separation was done with ABI Prism™ 3130 Genetic Analyzer of the firm Applied Biosystems.

Compared to the pre-investigation, much better and forensically usable results of the analysis were achieved by modified methodology, as will be presented in detail.

P 114 A STUDY OF DIFFERENTIAL DNA METHYLATION PROFILE BETWEEN TWO NEW BORN MONOZYGOTIC TWINS

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Instruction and aims
The human identification technology currently can identify persons all over the world except monozygotic (MZ) twins. Several studies have identified epigenetic differences either at selected genes of MZ twins or in the overall epigenome. In this study we mapped DNA methylation profile of a pair of new born MZ twins, hoping to discover some different regions for forensic analysis.

Materials and methods
Genome DNA was extracted from cord blood of a pair of confirmed MZ twins. DNA methylation profile was obtained by using methylated DNA immunoprecipitation sequencing (MeDIP-seq).

Results and discussion
According to the MeDIP-seq results, 3.6Gb data containing 73 million reads was obtained for each sample. By mapping onto the human genome reference, each sample got 48 million and 50 million unambiguous reads respectively. The reads cover 33.25% and 38.76% of the genome and appeared in every gene region, and the maximum number of reads was in repeat elements. Among the repeat elements, the SINE/Alu sequence was the most widely distribution area. Peak information showed that 257 thousand and 197 thousand peaks were obtained for each sample, covered 6.53% and 5.29% in the genome. These peaks had a widely distribution among gene regions but had a maximum number in the internal introns. Totally, we found 2253 different DNA methylation regions through whole genome, 643 located in the gene regions and 1610 in the internal gene regions. From all of the regions, we finally selected 113 regions as markers for following forensic study.

Keywords: Monozygotic twins; DNA methylation; Forensic genetics; Epigenetics; Methylated DNA immunoprecipitation sequencing; High-throughput sequencing

P 115 VALIDATION OF A NOVEL DNA CAPTURE METHOD (C-POD) IN COMPARISON TO STANDARD BUCCAL CELL COLLECTIONS

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Introduction
C-POD is a first generation DNA sample collection device that stabilizes and preserves DNA prior to laboratory testing. It simplifies and automates the process of collecting samples. The device claims key features such as a tamper-evident closure and embedded radio frequency identification (RFID) read/write datachip capturing and tracking forensic information automatically thus maintaining absolute chain-of-custody. Study objectives: Patients' evaluation of C-POD when collecting buccal swabs; Laboratory evaluation of C-POD when processing samples for analysis.

Materials/Methods
Users collected equal numbers of samples using standard swab methods and C-POD. Patients and Laboratory staff completed a questionnaire evaluating various aspects of C-POD i.e.: Ease of use (human factor evaluation); Integration into lab flow and sample management/automation; DNA quality and quantity; Stability of collected sample stored in the C-POD; Value analysis with respect to RFID as a data management tool. Buccal cells were collected with the FLOQSwabs (Copan Italia) and placed inside C-POD. Nucleic acid was extracted using the Genomic DNA Isolation Kit (Norgen Biotek). DNA quality and quantity was assessed with the Nanodrop spectrophotometer and by real time PCR using the Factor V Leiden DNA Assay Kit (Roche Molecular).

Results/Conclusions
All users found it easy to collect samples with the flocked swabs and their placement inside C-POD. The device with sample easily integrates with laboratory automation and was quantified favourably when compared to DNA isolated using standard methods. The study presently suggests that forensic applications of C-POD provides a new secure, tamper-evident method for collecting DNA samples while maintaining continuity of evidence.

P 116 MITOCHONDRIAL HETEROPLASMY IN DIFFERENT HUMAN TISSUES

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With improved methods, the occurrence of mitochondrial heteroplasmy seems to increase. In the past, tissue specificity of point heteroplasmy has been discussed.

In the present study, different tissues of 100 individuals were collected and analysed. The aim of this systematic study was to determine the frequency of heteroplasmy and to investigate tissue differences.

In a first step, the whole control region of five tissues (blood, buccal cells, liver, skeletal muscle and brain) of the mitochondrial DNA has been sequenced. If point heteroplasmy appeared, another five tissues (heart muscle, bone marrow, bone, lung and hairs) were analysed. Additionally, nuclear markers were typed to exclude sample mix-up and contamination.

In summary, tissue specific differences in frequency of point heteroplasmy were found. Furthermore, different heteroplasmic positions were observed to be more common and tissue-dependent.

P 117 CHARACTERIZATION AND DEVELOPMENT OF A TOOL TO DETECT A SPECIFIC BIOMOLECULES OF VAGINAL FLUID

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In rape and sexual assault cases, we want to find a biological link between the victim and the perpetrator. For that, indicative tests are used. Today, there is test for blood, saliva and semen detection. In the context of sexual assault, the semen tests are the most investigated. But, when semen is not found, how to show the event of the vaginal intercourse? One option would be the vaginal fluid detection. The problem is that there is no means to detect their specific presence.

The aim of this research will be to find a technique to detect vaginal fluid. The approach is different of that used usually with semen tests, because with this tool, we would detect vaginal fluid on objects used for sexual assault, or on the suspect's body directly.

The experimental procedure consisted to detect a vaginal fluid specific biopolymer with immunodetection analysis. This experiment was made by Dot-Blot, Western-blot and 2D-Electrophoresis.

The impact of this research is essential for the forensic sciences and for the justice, because it could allow evaluating the nature of the sexual activity.

P 118 NOVEL AND RARE Y-CHROMOSOME SHORT TANDEM REPEATS IN AUSTRALIAN ABORIGINES

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Y-chromosome short tandem repeats (Y-STRs) have become a vital tool in forensic casework. Forensic Science South Australia (FSSA) has utilised the Applied Biosystems AmpFℓSTR® Yfiler™ PCR Amplification kit to obtain male DNA profiles from relevant case work samples since 2007. FSSA have identified novel and rare variant Y-STR alleles which are specific or present at a higher frequency in Australian Aboriginal males residing in South Australia. Many of the observed variant alleles fall outside the range of the AmpFℓSTR® Yfiler™ allelic ladder. This prevents accurate typing of these alleles and impedes comparison between profiles where similar or identical variants exist.

In this study, a total of 43 off-ladder variant alleles at DYS456, DYS458, DYS385, DYS635 , DYS392 and GATA H4 were characterised. Through sequencing, the 19 variants at DYS 456 were determined to contain 11 repeat units and the 11 variants at DYS635 contained 17, 29 and 30 repeat units. The 29 and 30 repeat units identified at DYS635 have not previously been reported and contained a rare $(TGTA)_3$ block in the repeat motif. No sequence variations were observed in the repeat motif flanking regions. The remaining 13 variants at DYS458, DYS385, DYS392 and GATA H4 are currently being sequenced. FSSA intends to use the information gained in this study to develop an in-house allelic ladder to accommodate these rare and novel alleles.

P 119 IDENTIFICATION AND SECONDARY STRUCTURE ANALYSIS OF A REGION AFFECTING ELECTROPHORETIC MOBILITY OF THE STR LOCUS SE33

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SE33 is one of the most informative markers in forensic use due to its high power of discrimination. During the course of developing the AmpFℓSTR® NGM SElect™ PCR Amplification Kit several SE33 primer designs were screened with one primer pair yielding a high frequency of discordant alleles when compared to the AmpFℓSTR® SEfiler Plus™ PCR Amplification Kit. This discordance was mostly specific to samples of African descent with an estimated frequency of 5.1% and was a result of a mobility shift of approximately +0.84 nt. The sequence analysis of the affected alleles revealed that the only difference from the wild type sequence was a SNP outside of the SE33 repeat but within the amplicon of this particular set of experimental primers. In total, we identified three different SNPs all within 9 nt of each other, each of which could cause the mobility shift individually. Further characterization of this region via site directed mutagenesis and thermostability measurements strongly suggests that this polymorphic region contains a secondary structure that, when disrupted due to the presence of a variant SNP, results in a mobility shift relative to the wild type sequence. To overcome this problem, the SE33 primers used in the final configuration of the NGM SElect™ Kit avoided the amplification of this polymorphic region yielding in turn results highly concordant with the SEfiler Plus™ Kit.

P 120 ANALYSIS OF THE SARCOMERE PROTEIN GENE MUTATION ON CARDIOMYOPATHY – MUTATIONS IN THE TROPONIN COMPLEX GENES

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Introduction

Developments in the molecular genetic studies of cardiomyopathy (CM) have led to discovery of a large number of mutations in the genes encoding the sarcomeric proteins. In this study, comprehensive screening of three genes encoded troponin complex (TNNT2, TNNC1 and TNNI3) were performed in 36 consented autopsy cases diagnosed as CM, in order to evaluate the prevalence of gene mutations in sudden death caused by CM.

Materials and methods

Blood samples were obtained from 15 cases of hypertrophic cardiomyopathy (HCM), 18 cases of dilated cardiomyopathy (DCM) and 3 cases of arrhythmogenic right ventricular cardiomyopathy (ARVC), with informed consent of their families members. The 200 cases that presented with no particular diseases were used as controls. DNA was extracted using Quick Gene-800 (FUJIFILM) and stored at 4°C until use. Sequence analysis was carried out as described murakami et al. (Legal med. 2010; 12:280-283). This study has been reviewed and approved by the Hospital Ethics Committee of Kitasato University School of Medicine (B01-24).

Results and discussions

Three missense mutations, two silent mutations, twenty single nucleotide polymophisms (SNPs) and one-base delition were identified. The missense mutation Pro16Thr in TNNI3 detected in DCM was located on N-terminal region. This region contains serine residues, which are phosphorylated by PKA. Alterations of cardiac troponin I phosphorylation levels are suggested to be important in the development of CM, further study will be needed to determine the functional role of the phosphorylation of this mutation.

P 121 DEVELOPMENT OF A LONG QT-SYNDROM MUTATION DETECTION METHOD

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Sudden cardiac death (SCD) especially in younger adults with no previous symptoms is a challenging problem in forensic diagnostics, occurring with an incidence of about 80,000 per year in Germany. Long QT-Syndrome (LQTS) and other cardiac disorders associated with abnormality of cardiac rhythm triggered by mutations of cardiac ion channels have to consider if no cause of death is detectable during autopsy. A molecular genetic screening can help to ensure.

Meanwhile twelve genes are known in which more than 700 different mutations are associated with LQTS. The three major LQTS-susceptibility genes are KCNQ1, KCNH2 and SCN5A. They are responsible for 75% of all congenital LQTS cases.

We developed a rapid, sensitive and reasonable method for the simultaneously screening of the most common LQTS mutations, focused on these three genes.

Using the SNaPshot® minisequencing primer extension assay a total of 91 Single-Nucleotide Polymorphisms (SNPs) were examined in six multiplex assays. The data suggest that this technique is applicable, solid, flexible and a good alternative to complete sequencing.

P 122 DNA DEGRADATION ANALYSIS IN POST-MORTEM SOFT MUSCLE TISSUES IN RELATION TO ACCUMULATED DEGREE-DAYS (ADD)

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After the death of an organism DNA starts to degrade and as the organism's cell structure breaks down, nucleases are released and directly cause DNA degradation. Subsequent colonisation and action of insects and microorganisms also contributes to the DNA degradation. As the post-mortem interval (PMI) increases DNA continues to degrade until no high molecular weight DNA (HMW-DNA) remains.

In order to assess DNA degradation in the model organisms chosen (pig and rabbit), two nuclear genes, Connexin 43 and RAG-1, were aligned to identify conserved regions. Primers were designed to amplify 70 bp, 194 bp, 305 bp and 384 bp amplicons. The primers were also designed to amplify human DNA, which allowed the use of commercially purchased DNA standards to be used as controls. Following DNA extraction PCR analysis was performed using the four primers sets in a multiplex (4-plex): the PCR was optimised so that it worked over a wide range of template amounts (0.1 ng to 75.83 ng). The multiplex PCR was found to work efficiently in triplicate samples of all three species down to 0.3 ng of DNA template.

This multiplex has been used to assess whether DNA degradation can be predicted by accumulated degree-days (ADD), which provides a measure of both time and temperature.

Full 4-plex profiles were generated until day 7 (ADD 111.93) from whole carcasses and body fragments and up to day 11 (ADD 172.48) from insect-activity-free muscle samples.

Future work will include; development of real-time PCR quantification assays, DNA Fragment analysis and DNA preservation.

P 123 POSTMORTEM MOLECULAR ANALYSIS TO SIDS VICTIMS

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The sporadic occurrence of SIDS appeared not to be attributable to heritable factors. To date, dominant genetic disorders via *de novo* mutation at meiosis can be involved in various diseases. In the present report, our recent molecular analyses to SIDS victims are introduced. Congenital central hypoventilation syndrome (CCHS) is characterized by apnea during sleep with onset in the neonatal period. The responsible gene has been identified as *PHOX2B*, and the majority carried the polyalanine repetitive expansion on exon 3. In contrast to the presence of the expansions in all clinically diagnosed CCHS patients, no abnormalities of the gene sequence were evident in 67 SIDS cases, indicating that CCHS was unlikely to be associated with SIDS. Long QT syndrome is occasionally fatal through syncope with critical arrhythmia, so called torsades de pointes. Several responsible genes, including ion channel genes such as *KCNQ1, KCNH2* and *SCN5A*, have been identified. A total of six non-synonymous substitutions were detected from direct sequencing of these genes in specimens from the victims. A genome-wide association study revealed an SNP in *NOS1AP* as a determinant of the QT duration on the ECG. The T allele in a recessive manner might be associated with the occurrence of SIDS. We therefore considered that SIDS might be partly explainable by fatal arrhythmia. Postmortem molecular analysis is not omnipotent in disease diagnosis, but this approach is effective for searching for a number of disorders in which mainly physiological dysfunction is exhibited.

P 124 MUTATIONAL ANALYSIS OF STR-FGA LOCUS

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Introduction

The microsatellite FGA-FIBRA locus, a tetranucleotidic STR, of complex structure. Because the high polimorphism it represents one of the more usefull loci in forensic investigation. It's mapped on 4q28 region, in the third intron of the Human alfa fibrinogen gene (Acceso code in *Genbank* M64982).

Materials and methods

DNA extraction was made from blood samples on FTA® cards. The amplification was performed using routine identification kits: Identifiler®, Powerplex 16®, SEfiler® or Minifiler®, and the amplicons was detected on ABI3130XL and ABI3100, analysers. The mutational events was selected like mendelian incompatibilities, found during 30 months. All of the other analized markers were compatibles with expected segregation pattern. The paternity probability was higher than 99,999%.

Results and discussion

In 19860 cases submitted for paternity or maternity DNA análisis, were detected 60 mutational events in the 70%, they come from the father. 10% of cases were assigned to mathernal origin. In 12 cases (20%) was not possible to establish the origin. In general, 6 alelles are involved on 97% of mutational events. All of them were complete gains or losses. The difference between gain or loss was no significant (27 vs 25 events). However, the gain increase if the allele size increase too, this is observed only until the 25 allele. On the other hand, since 26 allele, the loss is three times more frequent than gains. The mutation rate by locus and allele was calculated. The sequence analysis showed that all mutations involved the variable zone (CTTT)n of the STR.

P 125 FORENSIC QUANTITATION OF DNA – COMPARISON OF DIFFERENT TECHNIQUES

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In forensic genetics quantitation of DNA plays an important role in adjusting DNA contents for successful downstream-analysis (e.g. STR-profiling). Three different methods are frequently used to quantitate DNA: Fluorometry, UV/Vis spectroscopy and quantitative PCR (qPCR). The aim of this study was to determine and compare general features of these methods in particular regarding sensitivity. Also, the reliability of measurements in the presence of degraded DNA or of interfering substances that are often found in forensic casework samples was evaluated.

We measured solutions of dilute DNA (100 ng/µl – 10 pg/µl), DNA that was artificially degraded by UV-light and DNA solutions containing different interfering substances (proteins, dyes and PCR-inhibitors).

Fluorometry and qPCR showed the highest sensitivity. Especially high amounts of protein (BSA) and Indigo had a great effect on DNA quantitation using UV/Vis spectroscopy.

Interestingly, in contrast to fluorometry and qPCR, the extent of DNA degradation did not show any impact on UV/Vis-spectroscopic measurements.

All methods tested showed different features as to their benefits and drawbacks and thus, the decision on the best quantitation technique is mainly dependent on the type of sample investigated.

P 126 THE INFLUENCE OF FORMALIN FIXATION ON THE DETECTION AND QUANTIFICATION OF TOTAL AND SPECIFIC DELETED HUMAN MITOCHONDRIAL DNA

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The investigation of formalin fixed and paraffin embedded tissue is well used in forensic case work. These paraffin blocks are regularly stored for a long time, providing a detailed archived tissue bank for scientific research.

Several reports show that it is difficult to recover high-quality DNA from formalin fixed and paraffin embedded tissue and that there can also be an inhibiting influence of this chemical on the amplification of target molecules in the PCR.

Mitochondrial DNA (mtDNA) analysis is another tool in forensic case work. Sequencing of the D-loop region is often still possible when autosomal DNA is too severely degraded. The common 4977 bp deletion of mtDNA might be used as a marker molecule for premortum stress and as a result of this as an indicator for the cause of death (e.g. hypoxia, hypothermia).

This study aims to investigate whether it is possible to obtain reliable results regarding quantification of mitochondrial DNA out of formalin-treated tissue samples.

For this purpose post mortem tissue samples (cerebral cortex and cerebellum) of six different individuals were sampled (n=136 samples) and exposed to formalin for different durations (30 min – 3 months). Absolute quantification of nuclear, total mitochondrial and 4977 bp deleted mtDNA was done using self-made real time PCR assays.

The first results show that the content of mtDNA as well as the amount of deleted mtDNA decreases proportionally to the formalin incubation time. This could lead to problems and wrong results in scientific research.

P 127 MOLECULAR CHARACTERISATION OF A MUMMIFIED BODY FOUND IN GLACIER

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A mummified male body was found emerging from the melting ice of the *Scersen Glacier* (Retiche Alps, elevation m 3,050). Near the body well preserved mountain equipment with topographic maps written in German dated back to the 1920's, a compass, a lantern, a pocket watch, some climbing items were found; the skeleton was still wearing rotting hobnail boots and wool gloves with an embroidered cross reminding the Swiss national one. Inside an internal pocket of the clothes, some newspaper cuttings written in German dated 1928 were discovered. The body was carefully dug out the glacier and taken to the hospital for the autopsy which revealed internal organs such as heart, liver, kidney, lung still well recognizable. Sampling of those tissues was performed for the histological and DNA analysis together with nail clippings and sampling of a section of the right femur. After extraction from different tissues, DNA was quantified by Real-Time PCR using the *Quantifiler Human DNA Quantification* kit and amplified using the *Amp FℓSTR MiniFiler kit*. A male complete genetic profile for the autosomal STR markers was obtained from the analysis of most of the tissues. In order to provide the most complete genetic survey of the mummified body useful for a possible identification, the Y-chromosome haplotype and haplogroup were determined using the Amp FℓSTR Yfiler PCR amplification kit and a Y-SNP multiplex PCR, respectively. Finally, the mtDNA HVR I region was sequenced.

P 128 GHEP-ISFG PROFICIENCY TEST 2011: PAPER CHALLENGE ON EVALUATION OF MITOCHONDRIAL DNA RESULTS

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Nowadays, the analysis of mitochondrial DNA (mtDNA) for forensic purposes is widely extended among forensic laboratories (labs) in the world. A lot of information is available about the different analysis strategies and protocols that forensic labs use, but little is documented how they evaluate the results. In order to obtain a realistic picture on the communication of mtDNA evidence at court, the GHEP-ISFG has proposed a paper challenge in the 2011 edition of its proficiency test regarding to this topic.

GHEP labs were asked to evaluate a common simulated forensic case (homicide in Barcelona) in which the mtDNA haplotype (16024-576) of a hair shaft found in the hand of the victim matched the suspect's haplotype (victim's haplotype was also known and different from the suspect's one). The different databases that labs used and the different types of searches in those databases will be discussed in this presentation.

In addition, labs were asked to calculate the likelihood ratio (LR) under the following two hypotheses: Hp (the hair shaft comes from the suspect or from a maternal relative of the suspect) and Hd (the hair shaft comes from a random man of the European population not related to the suspect). The presentation discusses how the labs calculated the LR and evaluated the evidence.

P 129 IDENTIFICATION OF BODY FLUIDS USING MRNA MARKER PROFILING

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Numerous studies have been published describing co-extraction of RNA and DNA and the subsequent use of mRNA markers for body fluid identification in the forensic context. Markers have been described for the identification of blood, menstrual blood, saliva, semen, and cervico-vaginal fluid (CVF). The majority of the published assays use only one or two markers for identification of each body fluid. However, several recent publications have shown expression of some markers in non-target body fluids. Therefore, in some instances, body fluid identification using only one or two markers could lead to false positive results.

For this work, the protein and mRNA expression literature and databases were reviewed to identify additional candidate markers for each of the body fluids. A large number of samples (>200) were then analyzed to determine the suitability of the new markers and further characterize the specificity of published markers. The majority of the markers are not absolutely specific to the target body fluid. To minimize false positives, at least five markers per body fluid were used to design four multiplex assays (semen, blood, menstrual blood (MB), and SaCVF (saliva and CVF). Based on the expression of the markers in known samples, every marker has been given a numerical value related to its target body fluid expression and a minimum threshold (sum of individual markers) has been determined for each body fluid. This scoring method minimizes the chances that a body fluid is incorrectly identified.

P 130 INVESTIGATOR QUANTIPLEX KIT – FOR RELIABLE QUANTIFICATION OF HUMAN DNA IN FORENSIC SAMPLES

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Commonly short tandem repeat (STR) analysis is performed for human identification, although recently alternative approaches like the analysis of deletions and insertions (DIPs) have been become commercially available. However, these multiplex assays used for human identification are complex systems that require a defined range of template input. Accuracy of quantification, even of low concentrated samples and an assessment of the presence of PCR inhibitors are key requirements to ensure successful genotyping on the first try. Quantitative real-time PCR has become the standard method for DNA quantification in forensic samples. However, there is a need for advanced solutions further streamlining the forensic workflow by increasing the accuracy of the quantification results, especially for low concentrated samples, and reducing the time for analysis by faster procedures. Here we present a novel human DNA quantification assay – the Investigator Quantiplex Kit – which provides fast and accurate quantification of human DNA in forensic database and casework samples. The assay provides sensitivity down to 0.3 pg/µl, with highly accurate quantification in linear range of standard curve down to 4.9 pg/µl. Detection of inhibitors is ensured by a balanced internal amplification control. The Investigator Quantiplex makes use of PCR fast-cycling technology allowing fast time to result .Using the Rotor-Gene Q system, quantification can be performed in 48 minutes. To further streamline the workflow and to minimize time consuming and error-prone manual steps, it is possible to combine Investigator Quantiplex with the QIAgility instrument, a benchtop automation platform for routine procedures in the forensic PCR laboratory.

P 131 MOLECULAR STRUCTURE AND GENEALOGICAL CHARACTERIZATION OF THE DYS458.2 ALLELIC VARIANTS FOUNDED IN TURKEY POPULATION SAMPLES

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We found a 38 intermediate alleles of DYS458 locus in a 249 Turkish population samples. To determine molecular structure and genealogical characterization of human Y-chromosome DYS458.2 Short Tandem Repeat (STR) alleles, we have examined these allelic variants by sequence analyses. All of these variant alleles show an incomplete repeat caused by a AA insertion or GA deletion in front of the third repeat form from the last. When individuals who have these alleles were tested with M267 SNP markers, it is determined that all of DYS458.2 variants were on the J1* cluster.

We have also observed that all of the 38 samples have been shared the same alleles at some YSTR loci when we have examined 17 YSTRs loci in our population data. These alleles are DYS392*11, DYS393*12, DYS438*10 and DYS458.2 variants. Arlequin software was used to measure the degree of association between alleles at DYS392, DYS438, DYS393 and DYS458 in the form of standardized linkage disequilibrium (D') values. When all loci were analyzed in combination, using the D' values, complete dependency was noted between all of the DYS458.2 allelic variants and DYS393*12 (D'=1.00), DYS392*11 (D'=1.00) and DYS438*10 (D'=1.00).

It is reported that the shared molecular structure and the inclusion in the same Y genealogy branch were considered as supportive to common origin for the .2 allelic variants. Our data is supporting this findings. Furthermore, complete dependency was detected noteworthy between DYS458.2 variants and three loci; DYS392*11, DYS393*12 and DYS438*10 respectively, related with the same origin. However, further studies must be done with more additional samples.

P 132 FORENSIC FLOCKED COLLECTION DEVICES TO MAXIMIZE CRIME SCENE SAMPLE PROCURAMENT

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Introduction
Cotton, polyester or rayon swabs are used for sample collection from crime scenes. Samples collection devices that are preventing or neutralizing microbial contamination while preserving nucleic acids (NA) integrity are essential. Copan developed a line of forensic flocked collection devices, 4N6 FLOQSwabs™ (FFCDs) that are facilitating crime scene sample procurement. The aim is to compare Copan FFCDs to traditional swabs for collecting and preserving NA for forensic investigation.

Materials and Methods
For this study samples collected with regular, flat, round/rims, nails FFCDs, rayon (RS) and polyester swabs (PS) were tested for NA collection/release, microbial contamination, extraction platforms compatibility, and DNA quantity and quality for profiling. Dried saliva spots were used for NA collection and release; bacteria/fungi/viruses neutralization after one week at 22°C and 37°C. NA were extracted with silica columns and magnetic beads, amplified with Quantifiler Human DNA Quantification and sequenced with Identifiler Plus PCR Amplification kits (Applied Biosystems).

Results and conclusions
The Copan FFCDs when compared to saliva recovered 90% DNA versus 15% and 10% by, RS and PS respectively. FFCDs recovered 100% DNA after 5 days at 220C and 370C in presence of mixed microbes compared to 10% and 10% by RS, and PS respectively. No amplification or DNA profile differences were found by all extraction methods. Copan FFCDs are increasing/preserving DNA collection 9 times more than the traditional swabs without the need of drying the swabs or using drying devices during transport. FFCDs can be the proper tools for crime scene samples collection and preservation.

P 133 COMPARISON OF MIRNA EXTRACTION SYSTEM USING TWO RNA ISOLATION KITS FROM FORMALIN-FIXED TISSUES

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MicroRNAs (miRNAs) are a novel class of short stranded RNA that controls the post-transcriptional regulation of gene expression. Recently, miRNA has been reported to regulate aging in cells. Doleshal et al. reported that miRNA could be extracted from formalin-fixed paraffin-embedded tissue. These reports suggest that miRNA could be a useful method for estimation of aging from formalin-fixed tissues. The analysis of quality and quantity of miRNA from this kind of sample can be an important step. In this study, we compared the manipulation of two commercially available RNA isolation kit for formalin-fixed tissues.

Five tissues (brain, heart, lung, kidney, liver) excised from an ICR mouse (1 year) were used. Tissues were soaked in 10% formalin and incubated at 4°C for 7 days after resection. MiRNA isolation was performed with RecoverAll Total Nucleic Acid Isolation Kit and RNeasy FFPE Kit according to the manufactures' instructions. The quality and quantity of miRNA measured using the NanoDrop ND-1000 and the Agilent 2100 Bioanalyzer.

miRNAs were successfully extracted from all 10 samples. The highest value of miRNA yield was obtained from liver (328.78 ng/µl) with RNeasy Kit. The lowest value was obtained from brain (2.30 ng/µl) with RNeasy kit. The OD_{260}/OD_{280} ratios of all samples ranged from 2.04 to 2.09 for RecoverAll kit, from 1.94 to 2.04 for RNeasy kit. This study indicates that no significant differences between two kits ($p > 0.01$, t-test). In general, our results suggest that the kits are useful for miRNAs extraction from formalin- fixed tissues.

P 134 AN INVESTIGATION INTO THE PROTECTIVE CAPABILITIES OF NUCLEOSOMES ON FORENSIC STRS

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Partial DNA profiles are often obtained from degraded forensic samples due to allelic and locus dropout, particularly at the high molecular weight loci. It is hypothesized that nucleosomes could offer protection to the bound DNA by limiting access to enzymes. Choosing and incorporating STRs that are protected into a "high nucleosome binding" multiplex could mean that a better DNA profile could be obtained from degraded samples.

58 STRs were evaluated for their nucleosome-forming potential (NFP) using two computer programs – NXSensor and nuScore. Ten mini-STR primer sets with varying NFPs were designed and optimised. They were used to amplify degraded saliva samples and simulated casework samples to determine if there was any correlation between NFP and DNA survivability, indicated by DNA concentration.

The effect of nucleosome protection was not observed for both sample types ($r^2 = -0.166$, $p = 0.647$). High-NFP loci did not perform better than low- or medium-NFP loci. It was possible that nucleosome protection might only work in living cells and not in forensic stains, as histones could be removed during necrosis by lysosomal proteases, and subsequently DNA would be degraded by endo- and exonucleases. Other processes such as chromatin remodelling and DNA methylation could shift nucleosomes away from the predicted sites, thereby changing NFPs. It was concluded that nucleosome protection did not exist for degraded saliva samples, given that NFP accurately represented the probability of finding a nucleosome.

P 135 POST-MORTEM GENE EXPRESSION I: EXPRESSION OF THREE HYPOXIA-RELATED GENES IS INFLUENCED BY THE CAUSE OF DEATH

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Gene expression studies have the potential of complementing current conventional autopsy techniques as a molecular tool for the determination of the cause of death. This is the first study following MIQE guidelines that comprises an analysis of specific changes of gene expression patterns according to the cause of death. A total of 30 autopsy cases were grouped following similar causes of death: Asphyxia (n=10, comprising deaths due to mechanical airway obstruction or external chest compression), Cardiac death (n=8, comprises cases with macroscopically visible signs of myocardial infarction or ischaemia) and Controls (n=12, comprising cases of traumatic events leading to immediate death). Total RNA was extracted from brain (frontal lobe), cardiac muscle (left ventricle, working myocard) and skeletal muscle (M. iliopsoas) and reverse transcribed. Three genes that are known to be activated in cases of hypoxia were analysed by reverse transcription quantitative PCR (RT-qPCR): hypoxia induced factor 1α (HIF 1α), vascular endothelial growth factor (VEGF) and Glucose transporter 1 (GLUT1). Quantitative data were normalised against a set of four previously validated endogenous control genes. Results comprise statistically significant increases of expression for these genes in different tissues according to the cause of death.

The focus will be on the outcome of the study, highlighting the biologically meaningful results after correct data normalisation. This presentation is closely related to our second presentation: "Post-mortem gene expression II: Normalisation against single "housekeeping" gene results in erroneous data interpretation".

P 136 POST-MORTEM GENE EXPRESSION II: NORMALISATION AGAINST SINGLE "HOUSEKEEPING" GENE RESULTS IN ERRONEOUS DATA INTERPRETATION

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Quantitative results for three hypoxia related genes were obtained from 29 autopsy cases grouped according to their cause of death (asphyxia, cardiac death and controls). The relative quantities were normalised against a commonly used "housekeeping" gene, GAPDH. Furthermore, the same results were normalised against a set of four validated reference genes using qBase Plus software. Results show that after normalising against GAPDH only, a downregulation of hypoxia induced genes was observed, while after normalisation against the validated set of four stably expressed genes an upregulation was noticed. To explain this discrepancy, GAPDH was further investigated and quantitative data for this gene were also normalised against the set of four reference genes, resulting in a clear co-regulation of GAPDH. Thus, this gene cannot be regarded a suitable normaliser. Due to the strong increase in GAPDH expression in the causes of death studied here, a downregulation of the genes of interest was mocked. This clearly shows that single, non-validated "housekeeping" genes are unsuitable for normalisation of gene expression data and can lead to misinterpretation of gene expression levels.

This presentation focuses on the necessity of correct data normalisation. Furthermore, it highlights the risk of wrong data interpretation due to simplified normalisation against a single, non-validated "housekeeping" gene. This presentation is closely related to our presentation "Post-mortem gene expression I: Cause of death induces changes of expression in three hypoxia related genes".

P 137 mRNA BASED SKIN IDENTIFICATION

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The identification of the cellular origin of biological traces is highly relevant in forensic case work. mRNA-based multiplexes are now being developed for simultaneous identification of various forensically relevant body fluids. However, no mRNA markers for skin identification have yet been included, and there also is no widely used presumptive test for the positive identification of skin cells. In search for suitable mRNA markers for forensic skin identification we investigated 11 skin candidate genes as well as 5 candidate reference genes in different forensically relevant tissues, including skin material. Three mRNA markers, *CDSN, LOR* and *KRT9*, analysed together with the most suitable reference gene *ACTB*, showed strong over-expression in skin samples compared to samples from forensic body fluids. Quantitative PCR analysis of full, half and quarter thumbprint skin samples indicated high sensitivity of the applied assays with successful detection of minute amounts of sample. Parallel forensic STR genotyping provided poor quality DNA profiles from the partial thumbprint skin samples indicating that our skin-specific mRNA assays are more sensitive than commercial forensic STR profiling. Thumbprints stored for 6.5 months showed no significant decrease in mRNA signal detection, implying a reasonable time-wise stability of the three skin-specific mRNAs as well as the reference *ACTB* mRNA. Our study provides several suitable mRNA markers together with a suitable reference gene for successful identification of minute amounts of skin material, and contributes to the further completion of suitable multiplex mRNA assays for the reliable identification of forensically relevant tissues.

P 138 DISTRIBUTION OF STR LOCI (CSF1PO, TPOX AND TH01) USEFUL FOR DNA TYPING IN TWO SUBPOPULATIONS OF PAKISTAN

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The distribution of three STR loci CSF1PO, TPOX and TH01 was investigated and analyzed in two major subpopulations of Karachi, the largest city of Pakistan. These subpopulations are *Muhajir* and *Punjabi*. Both the subpopulations were defined through a pre- set criteria based on two variables i.e. paternal ethnicity and mother tongue. Allele frequencies were calculated through the gene count method. *Pearson's chi square test, likelihood ratio test* and *exact test* were used to check Hardy Weinberg expectations of genotypic data. Non random association of the loci was also tested by calculating the variance of the number of heterozygous loci. The loci are independent of each other in the subpopulations under study. Forensic efficiency of the markers was determined by estimating the parameters defined in the literature. Results showed that these markers have a high level of heterozygosity, power of discrimination and polymorphism information content in both the subpopulations. Independent sample t-test reveals a significant ($p<0.05$) difference in the distribution of TPOX alleles across the two subpopulations. Phylogenetic efficiency of the three STRs was also evaluated through the allele frequency data. For this purpose the data of the present study was added to the frequency database of sixty two populations/subpopulations of the world. These populations/subpopulations have different racial and linguistic affiliations. Clustering of both the subpopulations in the phylogenetic trees is worth noticing. Results exhibited that the three STRs are efficient in differentiating the racial and linguistic boundaries between the populations.

Keywords: Pakistani subpopulations, CSF1PO, TPOX, TH01 distributio

P 139 ALLELE FREQUENCIES OF ALL CODIS AND FOUR NON-CODIS STR LOCI IN AN IMMIGRANT BRAZILIAN POPULATION LIVING IN LISBON

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Introduction: As well as it occurs all over Europe, in Portugal, and particularly in Lisbon, immigrant populations are increasing. According to official data, from Portuguese Foreign Affair Services, specifically concerning to Brazilian immigrants, between the 1999 and 2005, this immigrant group increased from 11000 up to 16000 individuals with fixed residence in Lisbon.
To calculate some parameters of biological kinship relations, allele frequencies on the population of origin are determinant.
We intend to verify if the observed allele frequency distribution in the studied group of Brazilian immigrants show significant differences from the Lisbon population were they are actually fixed.

Material and Methods: DNA from blood samples of 178 unrelated immigrant Brazilian individuals, living in Lisbon, involved in routine paternity testing were extracted using the Chelex® method. The co-amplification of the *loci* D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, VWA, D8S1179, TPOX, FGA, D2S1338 and D19S433 was performed using available commercial kits. The amplified products were detected and separated by capillary electrophoresis. The results were analyzed and allele designations were automatically determined with the GeneMapper® software.

Results: The allele frequencies will be presented in adequate tables.

Discussion and Conclusions: Allele frequencies of seventeen STRs were determined in the studied immigrant Brazilian individuals, living in Lisbon.
If the observed allele frequencies distribution in the studied Brazilian immigrant population shows significant differences in comparison to Lisbon population, it will be necessary to considerate it, in future biological kinship parameters calculation.

P 140 MITOCHONDRIAL CONTROL REGION DATA OF 3 ETHNIC GROUPS FROM ANGOLA

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Angola population has origin from Bantu people expanding to the south along the Atlantic coast beginning region between Nigeria and Cameroom and other Bantus that came from the great lakes region. Linguistic ethnic groups Bakongo, Kimbundo and Ovimbundo represent ¾ of the Angolan population and the remaining are represented by other Bantu groups. The polymorphic nature of mtDNA hypervariable control region is important to clarify the history and demographic past of a population since they can reflect phylogenetic relationship between populations.

The aims of this work were to obtain the mtDNA variability, classify the haplotypes into haplogroups, and infer the phylogenetic relationship of the Bakongo, Kimbundo and Ovimbundo ethnic groups from Angola.

Blood samples were obtained from 150 individuals, 50 of each ethnic group from Angola. PCR amplification was performed with primers L15971 / H017 / L16450 / H599 and cycle sequencing was performed using the BigDye® Terminator v.3.1. Obtained haplotypes with 3130 ABI were compared with the Cambridge Reference Sequence and typed following the nomenclature recommendations of the IUPAC. Haplogroups were determined on the mtDNAmanager. Statistical analysis of the control region and genetic affinities among the populations were computed using Arlequin v.3.0 software.

Preliminary results showed great variability with high frequency of unique haplotypes and significant values of nucleotide and sequence diversity parameters. All mtDNA sequences were included into specific African mtDNA haplogroup.

P 141 IDENTIFICATION OF AZORES ISLANDS HAPLOGROUPS BY MITOCHONDRIAL DNA ANALYSIS

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Mitochondrial DNA (mtDNA) haplotypes analysis found an important role in forensic genetics, especially when nuclear DNA analysis does not give a conclusive response; also mtDNA haplogroups assignment became noteworthy to clarify the history and demographic past of a population. Due to higher overall mutation rate, control region is comparatively enriched in sequence variation, and therefore its analysis is important to establish haplotypes and haplogroups. However, it is pertinent to corroborate predicted haplogroups based on control region sequences using informative SNPs from the coding region. Traditional mtDNA sequencing and Single Nucleotide Polymorphisms (SNP) analysis techniques were combined to establish the mtDNA variability of the Azorean population, and classify the haplotypes into haplogroups.

Blood samples were obtained from 50, healthy and unrelated, individuals from Azores. DNA was extracted using Chelex 100 protocol. PCR amplification for mtDNA HV1 and HV2 regions was made according to Wilson et al (1995) and segments sequenced using BigDye Terminator v1.1 Cycle Sequence Kit (AB). Analysis was done with ABI DNA Sequencing Analysis V5.2 and SeqScape v2.5. Sixteen mtSNPs were amplified in two multiplex reaction using primers proposed by Bradstatter et al (2003) and amplification protocol published by Parson et al (2008), followed by a mini-sequencing reaction with SNaPshot. Analysis was done using GeneMapper ID v3.2.

In the study 48 haplotypes were identified. All the mtDNA haplotypes were included into specific haplogroups: 43 haplotypes belonging to macrohaplogroup R; 3 haplotypes belonging to macrohaplogroup N; 2 haplotypes belonging to macrohaplogroup M.

P 142 GENETIC DATA FOR THE LOCUS SE33 IN A SOUTHERN ITALY POPULATION WITH AmpFℓSTR NGM SElect™ PCR AMPLIFICATION KIT

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A genetic population study for SE33 locus has performed from a sample of 100 individuals coming from South of Italy (Calabria) since at least 3 generations, using the next generation multiplex AmpFℓSTR NGM SElect™ PCR Amplification Kit by Applied Biosystems.

Allele frequencies and statistical parameters of forensic interest (Power of Discrimination, Power of Exclusion Matching Probability, etc.) were calculated using PowerStats v1.2 software.

Results demonstrate the usefulness of SE33 for forensic identification, which should be added to the set of STRs loci routinely studied in caseworks and in complex paternity cases.

P 143 GENETIC CHARACTERIZATION OF SIX SNP IN CATALONIA POPULATION

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It will only be a matter of time before physicians can screen patients for susceptibility to a disease by analyzing their DNA for specific SNP profiles.

The study of SNPs associated with dopamine and serotonin systems has a strong interest because of their roles in controlling behaviour and mental status.

According the increasing understanding of the genetic bases of human behaviour, the possible role of SNPs in developmental psychopathologies could be important in forensic studies. Furthermore, the growing interest of forensic researchers in autosomal SNPs is due to the potential advantages in human identity and paternity testing, because of the low rate of mutation and the possibility of analyzing highly degraded samples.

The purpose of this study is to report allele frequency data of a Catalonian population sample (n=408) from Spain for six SNP, related to a neurotransmitter pathway genes (ANKK1, DRD2B, HTR1B, TPH1, ADRA2A and OPRM1).

No significant deviations from Hardy-Weinberg expectation were found for all SNPs. From a forensic point of view the heterozygosity value, power of discrimination and the a priori chance exclusion value were calculated.

P 144 INFERRING ETHNICITY FROM THE X-CHROMOSOME *ALU* INSERTIONS: DATA FROM WESTERN MEDITERRANEAN HUMAN GROUPS

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Ethnicity plays an important role in forensic investigation and can be inferred by several genetic markers. The analysis of human *Alu*-insertion polymorphisms has been used to address several questions about human origins and demography. The homoplasy-free characteristics and the identical-by-descent nature of *Alu*-insertion polymorphisms, make them the unique and best servers for this purpose, compared with other type of genetic polymorphism, such as VNTR, RFLP or SNP. In the present work, we have analyzed the genetic profiles of 645 individuals from 11 Western Mediterranean populations of South Europe and North Africa, using 9 human-specific polymorphic X-chromosome *Alu*-insertions. All populations showed remarkably high gene diversity values. When compared to other populations, Western Mediterranean groups form a visibly differentiated cluster. The genetic relationships agree with the geographical and ethnic pattern of differentiation between these populations, with some peculiar features. Moroccan Berbers and Sahrawi showed the lowest genetic distance (0.002749); meanwhile, Ibiza, in spite of its geographical location, lies far from the other Spanish samples. Some markers are specially discriminatory for some geographical-ethnical affiliations. In this way, Yd3JX437 has a remarkably low frequency in the Arabic population (0.038), compared with the rest of the studied populations and Ya5DP62 presents the highest frequency in the Italian population (0.916) and the lowest in a sub-Saharan Africa population (0.634). Our results demonstrate that these X chromosome *Alu* elements comprise a reliable set of genetic markers that have a good potential for population discrimination and for inferring ethnic background in forensic investigations.

P 145 MtDNA LINEAGES D1g AND D1h: NEW INSIGHTS INTO THE PIONEER PEOPLING OF SOUTH AMERICA'S SOUTHERN CONE

Bodner, M.[1], Perego, U.A.[2,3], Salas, A.[4], Röck, A.W.[1], Zimmermann, B.[1], Fendt, L.[1], Huber, G.[1], Gómez-Carballa, A.[4], Woodward, S.R.[3], Bandelt, H.-J.[5], Achilli, A.[6], Torroni, A.[2], Parson, W.[1]

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It is now widely agreed that the Native American founders originated from a Beringian source population ~15–18 thousand years ago and rapidly populated all of the New World on a main coastal route. Details about the migration into the Americas and the routes pursued on the continent still remain unresolved, despite numerous genetic, archaeological and linguistic investigations.

To examine the pioneering peopling phase of the South American continent, we screened literature and mtDNA databases and identified two specific mitochondrial clades of the pan-American founder lineage D1, here named D1g and D1h. They both show overall rare occurrences, but local high frequencies, essentially restricted to populations from the Southern Cone of South America (Chile and Argentina). According to highest forensic quality standards, we identified, selected and then completely sequenced 43 D1g and D1h mtDNA genomes.

Phylogeographic analyses revealed extensive variation within each of the two lineages and distinct dispersal patterns, thus suggesting an ancient separation, at the Andean barrier, of population groups which reached South America, and a very early arrival of Paleo-Indians in the Southern Cone.

This study confirms that major sampling and sequencing efforts are mandatory for uncovering all of the most basal variation in the Native American mtDNA haplogroups by targeting, if possible, both the general mixed population of national states and autochthonous Native American groups, especially in South America.

This work received support from the Austrian Science Fund FWF, project L397.

P 146 MITOCHONDRIAL DIVERSITY IN KICHWA AND MESTIZO POPULATIONS FROM ECUADOR

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Mitochondrial DNA (mtDNA) analysis is a very useful tool to infer the history of human migrations and for forensics studies. Because of its ethnic and genetic diversity, the study of South American human groups has awakened a growing interest in the field. Here we report the mitochondrial sequencing study of the two main ethnic groups of Ecuador: Amerindian Kichwas and Mestizos. A total of 103 samples were typed for the control region of the mtDNA. DNA was extracted from blood stains on FTA® cards using Chelex method. The control region was amplified and sequenced by capillary electrophoresis on an ABI Prism 310 Genetic Analyzer. Sequences were aligned and compared to the rCRS using the software SeqScape. Genetic parameters were calculated with ARLEQUIN software. A total of 43 different haplotypes were identified, with 34 being unique and 5 shared between groups. Sequence diversity was 0.9793 and 0.7901 in Mestizos and Kichwas respectively, being higher in Mestizos as a consequence of a major gene flow. Results also showed that the population sample was entirely composed by Native American haplogroups, with a distribution as follows: 46.77% D, 40.32% A, 9.68% C and 3.23% B for Kichwa; and 36.59% B, 26.83% A, 24.39% D and 12.20% C for Mestizo population. These data showed the absence of maternal admixture with Caucasian or Africans in these groups and, together with the results of previous studies on autosomal and Y-chromosomal STRs, confirmed the genetic asymmetry in Mestizos, with a Native American female substrate and European paternal lineages.

P 147 POPULATION DATA FOR 38 AUTOSOMAL INSERTION/DELETION (INDELS) AND 50 SNPS POLYMORPHISMS IN ARGENTINEAN POPULATION

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Forensic laboratories must add as much genetic information as possible in order to solve the broadened variety of post-mortem paternity and kinship cases now routinely presented. Often, use of STR autosomal markers alone is not enough to reach a high discrimination power, especially when dealing with degraded post-mortem material or complex family pedigrees. In order to expand the set of genetic markers used to solve challenging cases, and since reference databases are required in forensic application, allele frequencies and forensic parameters for 38 InDel and 50 SNP binary markers were calculated from a sample of thirty unrelated individuals from the two most populous cities of Argentina: Córdoba and Buenos Aires. DNA was extracted with a modified salting out procedure and amplification products were detected on an ABI 3130 Genetic Analyzer (Applied Biosystems). No significant deviations from Hardy–Weinberg expectations were found. In the analyzed sample, no pairwise linkage disequilibrium between InDels and SNPs was found neither between the 88 binary markers and 17 STR markers from commercial kits. Comparisons were also made with previously studied populations. The present database will be useful for forensic and paternity purposes for the above mentioned cities of Argentina. Moreover, these additional markers can help forensic laboratories to solve parentage testing as well as to improve the analysis of degraded DNA samples.

P 148 FORENSIC APPLICATION OF 15 AUTOSOMAL SHORT TANDEM REPEATS ANALYSIS IN LITHUANIAN POPULATION

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Introduction
The research data presents the first comprehensive autosomal STR analysis in the Lithuanian population for the purpose to compile an autosomal STR DNA database of natives from Lithuania, evaluate autosomal STR diversity, infer the genetic relation between Lithuanians and neighbouring populations and introduce population reference data for forensic and population genetics issues.

Materials and methods
Autosomal STR data were collected from the blood samples of 300 unrelated individuals distributed throughout the country. The amplification of 15 autosomal STRs was performed in one multiplex PCR by the use of AmpFℓSTR® Identifiler® PCR Amplification Kit for human identity and parentage testing. Statistical analyses were carried out to determine the basic parameters of population genetics and forensic efficiency for 15 autosomal STRs.

Results and discussion
The results indicated that autosomal STR profiles enable to achieve high-resolution essential for forensic DNA casework particularly human identification analysis, though AMOVA for autosomal STRs between Lithuanians and neighbouring populations suggests an influence of gene admixture that correlates with the geography and history of the Lithuanian population.

P 149 PATAGONIAN NATIVE AMERICAN GROUPS: GENETIC CHARACTERIZATION

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Aiming to investigate the genetic features of two potentially related Patagonian aboriginal groups: Mapuche (N=75) and Tehuelche (N=28), 17 Y-STRs as well as one Y-SNP (M3-Q1a3a) and the entire mitochondrial Control region (16024-540) were analyzed.

The results obtained demonstrate that 100% of the individuals analyzed belonged to one of the Native-American specific mitochondrial haplogroup (hg). HgA2 was absent in Tehuelche group meanwhile hgD1 was the most frequent (53%). In Mapuche the four Native American hgs were observed: hgA2 (8%), hgB2 (36%), hgC1 (27%) and hgD1 (29%). More than 80% of the individuals belonged to Q1a3a haplogroup. The minimal haplotype corresponding to the most frequent haplotype in Mapuche was observed in one sample out of 89804 haplotypes included within YHRD (real. 35). The most frequent minimal Y haplotype observed in Tehuelche was not detected in Mapuche individuals. The mitochondrial nucleotide diversity observed was 0.0124 and 0.011, and the Y-chromosome haplotype average gene diversity was 0.57 and 0.593, in Mapuche and Tehuelche, respectively Mitochondrial and Y-chromosome genetic distances between both groups were non significant, according to the history of both ethnicities.

Related aboriginal groups from Chile previously investigated by means of mtDNA sequencing, such us Pehuenche, Mapuche and Yaghan were compared with our data. Significant differences were observed between the Argentinean groups respect to Yaghan, and between Mapuche fron Argentina respects to Mapuche from Chile.

This work provides additional genetic information of two out of the three southern Argentinean aboriginal ethnicities.

P 150 INFERRING ADMIXTURE PROPORTIONS AND RECENT ADMIXTURE EVENTS

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The identification of admixture proportions for forensic DNA samples has recently had some success in criminal investigations (Phillips *et al.* (2009), PLoS ONE 4: 8). If researchers were able to differentiate first generation admixture events, this might shed new light on the identity of samples. We describe a new method, based on maximum likelihood estimation, for simultaneously inferring admixture proportions in a SNP-genotyped individual, and the genetic divergence of their unobserved parents with respect to the general population, based on allele frequencies from reference groups. This is achieved by examining the reduction in homozygosity that one expects to see after first generation admixture.

We use simulated individuals, under various parental admixture scenarios, to validate the method. The simulations showed that, with 80,000 independent SNPs, most parental admixtures could be accurately identified at the intercontinental level of divergence (Fst>0.1), although large effects such as pure first generation admixture can be predicted for more closely related populations (Fst>0.02). These results take into account the sampling errors in the reference allele frequencies.

P 151 HAPLOTYPE FREQUENCY DATA OF THE CHROMOSOME X CENTROMERE REGION

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A lot of closely linked markers are known on the human X-chromosome (ChrX). In the case of genetic linkage or linkage disequilibrium it is necessary to use haplotype frequencies which have to be estimated directly from the appropriate male population.

The centromere region of the human ChrX is known as an area with particularly low crossing-over-rates. Because tightly linked and non-recombining STRs are required for kinship testing, we validated a cluster of six STRs in the region between 56 and 64 Mb distanced from the Xp telomere at Xp11.21-Xq11.1 for forensic use, and described a multiplex PCR setup for simultaneous amplification of the six markers DXS10161, DXS10159, DXS10162, DXS10163, DXS10164 and DXS10165. Typing of male DNA enables direct recognition of the ChrX marker haplotypes. X-chromosomal variability and significant differences in haplotype-frequencies between several populations are expected. We present data of population samples from different regions (Estonia, Latvia, Lithuania, Vladivostok, Japan and Rwanda) as a supplement to previously published data for Germany, Ethiopia, Egypt and Somalia [FSI: Genetic Supplement Series 2 (2009) 398-399].

P 152 GENETIC ANALYSIS FOR 17 Y-CHROMOSOMAL STR LOCI IN A POPULATION FROM EASTERN LIBYA (BENGHAZI REGION)

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The seventeen Y-STR loci included in the AmpFℓSTR® Yfiler™ PCR Amplification kit (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b, DYS438, DYS439, DYS437, DYS448, DYS458, DYS456, DYS635, and Y-GATA-H4) were used to type a sample population of 238 males from eastern Libya (Benghazi region). Of 238 observed haplotypes, 214 were unique (90%) and 24 (10%) were found more than once. The 17 loci gave a discriminating power of 0.999. DYS458 showed the highest diversity as a single-locus marker (0.73). Allelic frequencies and gene diversities for each Y-STR locus were determined. The high haplotype diversity and discrimination capacity (0.90) demonstrate the utility of these loci for human identification in forensic applications. Comparative analysis with Y-STR datasets of relevant populations and submission of the haplotypes to the Y-STR Haplotype Reference Database (YHRD) was undertaken.

Keywords: Forensics; Y-STR (short tandem repeats); Libya; Benghazi.

P 153 ALLELE FREQUENCIES OF NC02 MULTIPLEX STR LOCI (D1S1677, D2S441, D4S2364) IN TURKEY

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For all countries it is necessary to know statistical analysis of their population data. For this aim, we choose to study with mini STR loci because those loci have features like have a small place (amplicons less than 125 bp), don't be affected the refraction on DNA.

In this study, three new mini STR loci, (D1S1677, D2S441, and D4S2364) were analyzed in a sample of 200 unrelated healthy Turkish individuals. First of all, DNA from blood spots was extracted by QIAamp DNA Mini Kit (Qiagen). Secondly, quantition of ds DNA was made by using Qubit® ssDNA Assay Kit, for use with the Qubit® 2.0 Fluorometer. After that, 1 ng target DNA was amplified using TopTag Master Mix Kit (Qiagen) and multiplex PCR system was performed for these loci. Then, DNA Typing was done using an ABI Prism 310 Genetic Analyzer. Allele frequencies and forensic parameters were calculated with the Powerstats Version 1.2 (Promega Corp.). The frequency distributions in the three STR loci showed no deviations from Hardy–Weinberg equilibrium expectations. Regarding analysis of data, expected heterozygosity (He), observed heterozygosity (Ho) and population differentiation tests were carried out with the Matching probability (MP), power of discrimination (PD), polymorphism information content (PIC), probability of exclusion (PE) and typical paternity index (TPI).

P 154 HIERARCHICAL ANALYSIS OF 15 Y-CHROMOSOME SNPs AND DEMOGRAPHIC HISTORY OF AFRO-DERIVED ISOLATED COMMUNITIES IN ALAGOAS, BRAZIL

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The slave trade across the Atlantic brought an estimated four million Africans to Brazil, thus contributing to the significant miscegenation of Brazil's population. Presently, despite this population's elevated heterogeneity, small isolated groups can still be found, as is the case of the *Quilombos*, who are the product of the resistant movement against slavery. In order to improve knowledge about the genetic composition and the origin of paternal lineages in 9 Afro-derived communities of Alagoas, Brazil, 15 Y-SNP markers were analyzed by applying the SNaPshot™ method. Nine haplogroups were identified in a total of 209 individuals. The haplogroup R1b1b2*-M269 was observed in all populations at a frequency of 5.26-79.17%, and F*(xK)-M213 was found at a rate of 4.17% and 36.84%, suggesting an origin of European male ancestry in these individuals. E1b1a1*-M2, of Sub-Saharan African descent, was found in 7 populations, which presented a frequency above Alagoas' admixed population, varying from 13.3% to 90.0%. However, the Amerindian haplogroup Q1a3a*-M3 was observed in only 2 chromosomes. Sub-clades of Haplogroup E, of African descent but frequents among the Portuguese males, including E1b1b1b1*-M81, E1b1b1a1*-M78 and E1b1b1c*-M123 were observed. AMOVA test revealed heterogeneity among Alagoas' *Quilombola* populations (FST=0.23964, P=0,00000±0,00000), with a genetic intrapopulation variation of 23.96%. The study revealed no significant genetic distances between populations from Alagoas, Rio de Janeiro, Portugal and four Afro-derived communities of Alagoas. However, other five communities presented distinct genetic constituents. These results highlight the hypothesis that the aforementioned communities were originally places of refuge for slaves.

P 155 POPULATION GENETIC DATA FOR 15 AUTOSOMAL STR DNA MARKERS IN FOUR VENEZUELAN STATES (ARAGUA, CARABOBO, ZULIA AND TACHIRA)

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Fifteen autosomal DNA markers (D3S1358, TH01, D21S11, D18S51, PENTA E, D5S818, D13S317, D7S820, D16S539, CSF1P0, PENTA D, VWA, D8S1179, TPOX and FGA) were genotyped in a total of 1290 unrelated individuals living in Aragua (232 individuals), Carabobo (321), Táchira (431) and Zulia (306) States of Venezuela. STR amplification was carried out using GenePrint Fluorescent Systems (Promega Corp.) and the analysis of amplicons was performed on an ABI Prism™ 3130 Genetic Analyzer (Applied Biosystems). Statistical evaluations were performed using GENEPOP version 4.0 and PowerStats softwares. Minimum frequencies were calculated according to Budowle et al (1996). All markers obey Hardy-Weinberg law in the States of Aragua, Tachira, and Zulia ($p>0.05$). However, CSF1P0 marker deviates from Hardy-Weinberg expectations in Carabobo State ($p=0.029$), although equilibrium could be considered after sequential Bonferroni correction (Rice, 1989). When each pair of populations is compared at the genic and genotypic level, there are no differences among Aragua, Carabobo and Zulia, but all these populations are different to Tachira population. Allele, Minimum, and null allele frequencies were estimated, as well as the main forensic parameters. These are the first population studies concerning these four States, and they will contribute to the conformation of a Venezuelan national Data base.

P 156 AUTOSOMAL SNP VARIATION IN CHILE: ANALYSIS OF THE SNP*for*ID 52-PLEX MARKERS IN NORTH, MID-LATITUDE AND SOUTH CHILEAN POPULATIONS

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Single nucleotide polymorphisms (SNPs) provide an important tool for the analysis of highly degraded DNA due to their short-amplicon size. In addition they offer a low mutation rate and relatively straightforward multiplex development. Since the establishment of the SNP*for*ID 52-plex marker set, several populations around the world have been studied. Nevertheless, for Chilean population there was no available data on autosomal SNPs genetic diversity. In the present study, we have characterized a representative sample for the Chilean population for 52 autosomal SNPs from samples in north, mid-latitude and south Chile, therefore including the full longitudinal axis of the populous part of the country. Allele frequency estimation, Hardy-Weinberg equilibrium, linkage disequilibrium and statistical forensic parameters were analyzed. Genetic characterization of these 52 SNPs in the Chilean population has provided additional South American population data for the SNP*for*ID frequency browser (http://spsmart.cesga.es/snpforid.php) and helped establish an ID-SNP reference database in Chile to contribute to the implementation of these markers in forensic laboratories.

P 157 MUTATIONS ANALYSIS AT FORENSIC STRs USED IN PATERNITY TESTING IN MOROCCO

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Paternity tests are carried out by the analysis of hypervariable short tandem repeat DNA loci. These microsatellites sequences mutate at a higher rate than that of bulk DNA. The occurrence of germline mutations at STRs loci poses problems in interpretation of resulting genetic profiles.

We recently analyzed 445 confirmed paternity cases in the laboratory of Genetics of Royal Gendarmerie at microsatellites loci included in AmpFℓSTR® SGM plus and AmpFℓSTR® Identifiler™ kits (Applied Biosystems). Four cases of allelic loss at the vWA and the D18S51 loci were described after multiplex PCR with the AmpFℓSTR® Identifiler™ kit. In this case the failure of PCR resulted in genetic inconsistencies due to opposite homozygosity. After multiplex PCR with Powerplex® 16 Kit, additional alleles were observed and Mendelian inheritance was restored. We discovered 16 mutations for the loci D21S11, D13S317, D18S51, D8S1179, vWA, FGA, D7S820, D5S818, THO1 and D16S539. The highest mutation rate was observed at D21S11 loci with 4 mutations at different alleles. The event was always single repeat related. Mutation events in the male germ line were eight to ten times more frequent than in the female germ line. The mutation event is very crucial for forensic DNA testing and accumulation of STRs mutations data is extremely important for genetic profile interpretation.

Keywords: STRs, Mutations, null allele, Paternity testing, SGM, AmpFℓSTR® Identifiler™ kit, Powerplex® 16 Kit, Morocco.

P 158 MICRO-GEOGRAPHICAL ANALYSIS OF Y CHROMOSOME LINEAGES IN SOUTH IBERIA

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Analysis of the Berber and Arab influence into Iberia has received little attention in the literature. Due to the its mode of inheritance, analysis of variation at the Y-chromosome represents a convenient way to assess the possible genetic contribution of North African populations to the present-day South Iberian genetic pool and reconstructs other demographic events that could have influenced the Iberian southern region.

We have sampled males from the eight different provinces from Andalusia (South of Spain): Almería, Cádiz, Córdoba, Granada, Huelva, Jaén, Málaga and Seville. A total of 151 samples were genotyped for 27 Y-SNPs and 17 Y-STRs in order to assess the male genetic composition and the level of substructure of male lineages in this area. Y-SNPs were genotyped using a set of 27 SNPs as implemented in multiplexes previously reported in the literature. The *AmpFℓSTR® Y-filer™* kit (AB) was used for genotyping the 17 Y-STRs according to the manufacturer's instructions.

Haplogroup and haplotype frequencies were estimated. Analysis of Molecular Variance (AMOVA) and genetic distances at both Y-SNP and Y-STR levels were calculated on two different geographical scales: on a micro-geographical scale considering the different regions sampled, and on a broader scale that considers Andalusia as a whole in an European and North African context.

We here show preliminary results, including the frequency patterns of main European haplogroups in South Iberia. Our results will contribute both elucidating the genetic composition of South of Spain and reconstructing the complex demographic history of the Mediterranean Basin.

P 159 THE EUROPEAN DNA DATA EXCHANGE PROJECT

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It is now more than ten years since STR multiplex systems were introduced into EU forensic DNA laboratories. Since then, national DNA databases have been set up in many countries and have grown considerably in size. The Prüm Treaty [1] enables participating countries to compare unidentified DNA profile data with other databases in order to facilitate cross-border data exchange. Historically, there were only seven STR loci common to the available STR multiplex kits [2]. Gill et al. (2006) suggested five loci which could increase the standard set of loci from seven to twelve and these were adopted by an EU Council recommendation in 2009 as the new European set of standard loci (ESS). The five additional loci were selected based on previous studies indicating their polymorphic nature and the ability to make them into 'mini-STRs' [3,4].

The European Network of Forensic Science Institutes (ENFSI) DNA working group has been coordinating an exercise to facilitate the transition to the new ESS loci. A collaborative project was organised in order to assess the new multiplex kits available. This exercise has now been completed, and we present data for 26 European populations. We have prepared allele frequency databases from 26 EU populations; concordance studies have been carried out to determine if genotyping results varied between the different kits; and population genetics studies have been conducted to determine if there is disequilibrium within different populations, and to determine the level of sub-structuring by analysis of Fst.

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P 160 THE DISCRIMINATORY POWER OF THE HYPERVARIABLE REGIONS HV1, HV2 AND HV3 OF MITOCHONDRIAL DNA IN THE BRAZILIAN POPULATION

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Analysis of mitochondrial DNA (mtDNA) has been widely applied in the field of human identification, and features such as large number of mtDNA molecules per cell, the exclusively maternal inheritance, lack of recombination and high mutation rate found in the control region, ensure the high level of polymorphism, mainly in hypervariable regions HV1, HV2 and HV3. However, despite highly polymorphic, one of the limitations of using these regions is that many polymorphisms are highly common, resulting in the presence of sequences shared by more than one individual, or maternal lineages, in different populations. The aim of this study was to evaluate the discrimination power of the analysis of hypervariable regions HV1 (16024–16365), HV2 (73–340) and HV3 (438–574) of mtDNA in a Brazilian sample containing 290 unrelated individuals. Sequencing was performed using BygDye Terminator v3.1 and capillary electrophoresis was performed on ABI3130. All samples were validated by EMPOP and were classified into 15 haplogroups. Eighty out of 290 individuals presented European haplotypes, 108 showed Amerindian haplotypes and 102 individuals presented African haplotypes. Sixty-nine individuals (23.8%) could not be discriminated by the analysis of hypervariable regions HV1, HV2 and HV3 of mtDNA, and they were distributed in 25 different groups of common sequences. The most common sequence belonged to European haplogroup R0 with 10 individuals showing the same sequence. Trying to increase the discrimination power of common haplotypes and, therefore, individuals, it has been done analysis of SNPs in the coding region. Supported: FAPESP, HC-LIM40.

P 161 ABSTRACT WITHDRAWN

P 162 THE POTENTIAL FORENSIC UTILITY OF TWO SNPS IN PREDICTING BIOGEOGRAPHICAL ANCESTRY

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DNA-based prediction of an individual biogeographical ancestry would provide some investigative lead value in specific forensic cases. Recent studies have shown that single nucleotide polymorphisms (SNPs) in pigmentation-related genes such as SLC24A5 and OCA2 can be considered as ancestry informative markers (AIMs). These markers, usually expected with large differences in phenotype and allele frequency, need to be assessed and validate on the basis of comprehensive distribution data in specific populations. In the present work we typed these two SNPs (rs1800407 located in exon 12 of the OCA2 gene and rs16891982 in the SLC45A2 gene) in 413 individuals including 210 Hans and 203 Uygurs, who are closely related for geographic range in China. Our study generates new allelic data of Chinese population and reveals the significant different distribution of pigmentation-associated SNPs between Hans and Uygurs. It has been shown that the Han has higher frequency of homozygote than the Uygurs within two SNPs, especially heterozygous genotypes were only observed in the latter group. Furthermore, our research confirms the earlier results and supports SNP rs1800407 and rs16891982 can potentially be selected for AIMs.

P 163 MITOCHONDRIAL DNA POLYMORPHISM AND MATRILINEAL GENETIC COMPOSITION OF CHAOSHAN POPULATION IN CHINA

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Chaoshanese, a Chinese population residing in Chaoshan region in southern China, is an admixture population whose gene pool is derived from the Central China Han and southern aboriginal natives, as determined by our previous autosomal short tandem repeat (STR) study. To determine the matrilineal genetic composition of this population, we investigated the mitochondrial DNA (mtDNA) of various Chinese populations and analyzed the genetic relationship between Chaoshanese and other Chinese populations from the perspective of maternal inheritance. mtDNA polymorphisms in the hypervariable segment regions (HVS-I and HVS-II) and the COII/tRNA^{Lys} intergenic region were typed in 201 Chaoshan individuals. The mtDNA HVS-I sequence and haplogroup frequency data of other Chinese populations were collected and used for population comparison. Population relationships were examined by principal component, multidimensional scaling and median-joining network analyses. In addition, admixture analysis was performed to estimate relative contribution of northern Hans and southern natives to the Chaoshan population. Our results showed that the Chaoshanese, along with other southern Hans, is well separated from the northern Hans and occupies an intermediate position between northern Hans and southern natives. In matrilineal gene pool of the Chaoshan population, genetic composition of southern natives and northern Hans accounts for about 50%, respectively. Chaoshanese is a typical southern Han population. The matrilineal genetic composition of Chaoshan population consists of both northern Han and southern native origin.

P 164 PERUVIAN GENETIC STRUCTURE AND THEIR IMPACT IN THE IDENTIFICATION OF ANDEAN MISSING PERSONS: A PERSPECTIVE FROM AYACUCHO

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In the process of identifying around 15000 missing persons by the armed violence in Peru (1980 to 2000), the major problem is the high probability of random matches in especial of Andean Ayacucho population which represents the largest number of missing persons. To that end, in a first analysis, we analyzed the relationships and genetic structure in 880 individuals from 20 regions of Peru (including Ayacucho) with IDENTIFILER Kit and in a second analysis; we studied 203 individuals of Ayacucho using the ARGUS-X and ESSplex SE Kit with the aim to confirm the intra-population structure found in the first analysis. In the first analysis using the delta-mu parameter, clearly shows three genetic groups at the national level (North, Central and South) with a low variability among groups but significative with AMOVA (0.47%** ($P<0.01$)). In the tree, Ayacucho population is located in the south group and interestingly showed a significative FIS (0.10** ($P<0.01$)). The second analysis confirm this FIS but lowest (FIS=0.06) and this FIS could be explained by the evidence of a recent bottleneck found under the IAM, SMM and TPM models ($P<0.01$). On the other hand, the X-STR show low probability of random match than in Autosomic STR which may be related to content of admixture among native and foreign population (30% foreign content in Peruvian population). The results in this study are consistent with the demographic history (processes of migration, immigration, inbreeding), which contribute to the increase of IBDs and may be related to the random match DNA identification.

P 165 ANALYSIS OF X-CHROMOSOME STR MARKERS IN AMERICAN POPULATIONS OF THE BASQUE DIASPORA

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The pressures behind human migration are numerous; within those the most salient push factors behind the Basque emigration included: Spanish colonization of the Americas, demand for clerics, military personnel and tradesmen in the colonies, economic opportunities in the New World and wars of the last centuries. The Basque Diaspora is an interesting human population because its founders spring from a population known to be genetically unique. X chromosome genotyping is a genetic tool that has recently gained relevance because it can complement autosomal and Y chromosome STR results in complex cases in paternity and forensic testing. The aim of this study was to perform X chromosome STR analysis on four populations of the Basque Diaspora in Argentina and the United States of America. Its scope was to characterize a well-known migrant population through the use of a recently developed in-house X chromosome STR kit (GHEP-ISFG X-Decaplex). Samples included 55 autochthonous Basques and 56 mixed donors collected in Buenos Aires, Argentina, and 94 autochthonous Basques and 95 mixed donors collected in Chino, California; Boise, Idaho; and Reno, Nevada. All samples were collected via mouthwash, extracted using Gentra Puregene DNA Isolation Kit for mouthwash samples, quantified using Quant-iT™ Pico Green® dsDNA and amplified with the GHEP-ISFG X-Decaplex followed by fragment separation on ABI PRISM® 3130 Genetic Analyzer. Statistical analysis included allele frequencies and population pairwise genetic distances, Hardy-Weinberg equilibrium (females), linkage disequilibrium (males), power of discrimination and power of exclusion.

P 166 CHROMOSOME X MARKERS DXS6795, DXS9907 AND GATA144D04: REPEAT STRUCTURE AND ALLELE DISTRIBUTION IN A GERMAN POPULATION STUDY

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X chromosomal markers have been shown to be quite useful in forensic genetics, particularly in solving complex kinship cases. Some of these published markers are poorly described with regard to their molecular structure and the available population data sets are sparse. This is the case for 3 STRs in particular, located on the short arm in the region Xp22.11 to Xp11.23.

These loci were typed from buccal swab samples from about 500 unrelated German individuals (161 male, 362 female). Additionally, DNA from 90 males with one ore more daughters and their 135 grandsons were typed to check for mutability of these loci.

German population and sequencing data for these three loci as well as statistical parameters of forensic interest are presented.

DNA typing patterns of cell lines were used as intra- and inter-laboratory standards to calibrate allelic ladders. We found 8 different alleles in DXS9907 and GATA144D04, whereas DXS6795 exhibited only 6 alleles. Allelic distribution patterns are identical for males and females. There is no evidence for deviation from the Hardy-Weinberg equilibrium in female samples. Furthermore, no mutations were detected within 90 paternal and 135 maternal meioses. The three microsatellites are moderately variable with PIC values of 0.61, 0.56 and 0.70.

In our study, we could confirm the repeat structure and allelic distribution of DXS6795 reported for U.S. Caucasians. Comparison of our allele frequencies with those published in Asian population studies failed due to the missing allele nomenclature and cell line DNA typing.

P 167 MITOCHONDRIAL DNA POPULATION FREQUENCY OF HV1 AND HV2 FROM A SAMPLE OF INDIVIDUALS FROM THE CITY OF SÃO PAULO. DNA EXTRACTION FROM BLOOD, HAIRS AND PUBIC HAIRS

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This study analyzed the most common haplotypes and the frequency of heteroplasmy of mtDNA extracted from head and pubic hair samples compared to the mtDNA extracted from blood from the same individuals. Samples of blood, puckled hair and pubic hair were obtained from 200 non-related individuals submitted for autopsy in the *Coroner Service* of São Paulo City (Serviço de Verificação de Óbitos). DNA from blood was obtained by saline extraction. We selected head and pubic hair samples of 25 males to perform the analysis of mtDNA heteroplasmy. Each three hair shafts were grouped and divided into 0.5 cm sections: with the bulbs, and the other with 2 cm, the proximal portion, without the bulbs. The DNA extraction of hair samples was performed using QIAamp DNA Micro Kit (QIAGEN). The extracted DNA was quantified by spectrophotometry. We performed PCR amplification of HV1 and HV2 and analysis on 1% agarose. The amplified products were purified and sequenced using Big Dye™ Terminator and ABI 3130 (Applied Biosystems). To insure that hair samples were from the same individual, the AmpFℓSTR Identifiler Kit® (Applied Biosystems) was used. Analysis of HV1 and HV2 of mtDNA haplotypes are shown and the heteroplasmy is discussed. One hundred ninety six different haplotypes were detected, three persons had the same haplotype and two persons each had the same other three haplotypes. The lineage values associated with African, European and American Native ancestries for São Paulo sample were 35%, 32.5%, and 29.5%, respectively. Support: LIM HC-FMUSP, Capes, FAPESP 2008/06369-8.

P 168 GENETIC STRUCTURE OF STR VARIANT AND NULL ALLELES DETECTED IN KOREAN LIVING IN JEJU ISLAND

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Jeju island is the largest island in Korea and is apart from the main peninsula. For individual identification of buried old bones, familial relationships has been screened for 668 Juju island habitants using AmpFℓSTR® Yfiler® PCR Amplification Kit and an AmpFℓSTR® Identifiler PCR Amplification Kit. Y-STR tests showed 23 samples with unusual allele patterns at 5 Y-STR loci (1 sample: two alleles at DYS389II, 3 samples: null alleles at DYS392, 10 samples: null alleles at DYS448, 2 samples: off-ladder alleles at DYS438 and 7 samples: off-ladder alleles at DYS458). In autosomal STR tests, 31 samples showed off-ladder alleles at the D21S11 locus. In order to understand the genetic structure of these variant alleles, we sequenced all these unusual alleles using direct PCR for the PCR product or cloning for the bi-allelic loci. Some of results are as follows, cause of null allele in DYS392 was a G→A mutation at 19 bp downstream of the repeat region, and cause of null allele in DYS448 was deletion from G66018 to sY1206 of the AZFc region. Furthermore three types of off ladder alleles at the D21S11, 30.3, 33.1 and 34.1, were sequenced. These results were compared with previously reported. This study would be useful for a better understanding of the Korean genetic structure, and of practical value for identification by adding up the discrimination power.

P 169 GENETIC CHARACTERIZATION OF MALE LINEAGES OF PATHANS FROM PAKISTAN

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To better characterize and understand the male lineages of Pakistani populations, 22 Y-STRs and 12 Y-SNPs were analyzed in 270 unrelated Pathans from North West Frontier Province and Federally Administered Tribal Areas of Pakistan. Twenty two Y-STRs (DYS19, DYS385, DYS388, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS446, DYS447, DYS448, DYS449, DYS456, DYS458, DYS464, DYS635, and GATA H4.1) were analyzed using AmpFℓSTR® Yfiler™ PCR amplification kit and an in-house multiplex PCR system. Twelve Y-SNPs (M40, M89, M201, M69, M304, M9, M20, M45, M242, M207, M173 and M17) were typed using two newly developed multiplex PCR systems followed by SBE (single base extension) reactions for assignment of the Y-chromosomal haplogroups E, F, G, H, J, K, L, P, Q, R, R1 and R1a1a. In haplotype analysis for the 22 Y-STRs, 230 different haplotypes were observed with overall haplotype diversity of 0.9968; 211 haplotypes were unique and the most frequent haplotype occurred 4.4% of the samples. With the developed multiplex SBE reactions, more than six different Y-chromosomal haplogroups were defined within our samples, and the R1a1a haplogroup of South Asian origin was most frequently observed.

P 170 GENETIC ANALYSIS OF 16 X-STRS LOCI IN XINJIANG UIGHUR AND NORTHERN HAN POPULATION FROM CHINA

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In this study, a total of 1250 samples obtained from 941 Northern Hans Chinese (549 females and 392 males) and 309 Xingjiang Uighur (231 females and 78 males) were successfully analyzed using a homemade new multiplex polymerase chain reaction (PCR) system which can analyze simultaneously 16 X-STR markers including DXS10011, DXS101, GATA165B12, DXS6795, DXS6800, DXS6801, DXS6803, DXS6808, DXS7132, DXS7133, DXS7423, DXS7424, DXS8377, DXS8378, DXS9898 and HPRTB. The allele frequencies and statistical analysis were performed using Powerstats and Arlequin software. A total of 105 alleles for all the loci were observed by this multiplex PCR system. Polymorphism information content was 0.3864-0.9004, and power of discrimination in females was 0.6317-0.9841. Hardy-Weinberg equilibrium tests demonstrated no significant deviation from expected values (P>0.05) for all of the 16 X-STR loci in Xinjiang Uighur and Northern Han population from China. There were no statistically significant differences between Xinjiang Uighur and Northern Han populations in allele distribution of the 16 X-STR loci, in line with the results of AMOVA. The results show the 16 X-STR loci in the multiplex systems may provide high polymorphism information for kinship testing.

Keywords: X-STR; Multiplex PCR;. Population; Northern Han; Xinjiang; Uighur

P 171 POPULATION GENETIC INVESTIGATION OF FOUR X-STR LOCI IN HAN POPULATION OF NORTHERN CHINA

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Introduction and aims
Due to the unique inheritance mode, X-STRs have attracted much more attention of forensic scientists. The purpose of this project is to find more available X-STRs for forensic application.

Materials and Methods
Four X-STR loci were selected form NCBI GENE database, namely GATA151A05, GATA2B05, DXS7129, HUMUT1595, which located in the linkage group II. DNA was multiplex amplified for these four X-STR loci. Population genetic investigations were performed on 214 healthy unrelated individuals of Han population in Northern China.

Results and discussion
Besides GATA2B05 locus, No deviation from Hardy-Weinberg equilibrium was observed in other three loci in the female samples (P>0.05). Allele frequencies between female and male samples were no differentiation in the 3 X-STR loci. Each kind of alleles of 3 X-STR loci is sequenced and forensic genetic parameters were calculated base on the population genetic data of pooled frequencies. Linkage disequilibrium was tested, separately, in both female and male samples and association was not detected between alleles in any of the pairs of the 3 X-STR loci. The heterozygote observed (Ho) and polymorphism information component (PIC) of DXS7129, HUMUT1595, GATA151A05 locus were respectively 0.29358, 0.60550, 0.54128 and 0.36858, 0.62258, 0.58478. The power of discrimination for females (PDfemale) and the power of discrimination for males (PDmale) were respectively 0.593145, 0.761857, 0.731926 and 0.385692, 0.602436, 0.573134. The mean exclusion chance (MEC) for ChrX markers in trios involving daughters and MEC for ChrX markers in father/daughter dous were respectively 0.356211, 0.522350, 0.487274 and 0.226958, 0.380390, 0.346576.

P 172 GENETIC DIVERSITY OF 17 Y-CHROMOSOMAL SHORT TANDEM REPEAT LOCI IN NORTHEAST GERMANY

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Y-chromosomal profiling is widely recognized as an invaluable and powerful tool to complement the information provided by autosomes, particularly in forensic cases with mixtures of high level female DNA and low level male DNA, as well as application in genealogical and kinship testing of paternal lineages even into the past. Population genetic investigations from inhabitants of Mecklenburg-Vorpommern in the northeast of Germany have been characterized in a small resident sample, so far [1]. The aim of this study was to evaluate particular regional variations of Y-chromosomal STR loci and increase population data of the Y-Chromosome Haplotype Reference Database [2]. Genetic diversity for the 17 short tandem repeat loci included in the AmpFℓSTR® Y-filer™ PCR amplification kit (Applied Biosystems, Foster City, USA) DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 and GATAH4 was obtained from a population sample composed of 531 unrelated males resident in Mecklenburg-Vorpommern. A total of 531 haplotypes were identified, and among these, 480 were unique, 21 where found two times and three haplotypes were observed three times. Allele frequencies were estimated and the overall haplotype diversity was 0.9993. We report some non-standard characteristics, including the infrequent microvariant alleles 16.2, 17.2 and 19.2. Furthermore 257 father-son pairs, previously confirmed by autosomal STR analysis with paternity probability >99.9%, were genotyped twice, and among these 4369 allele transfers 16 one-step mutation events were discovered, giving an average mutation rate of $3.6x10^{-3}$ per locus per generation, similar to results of other working groups obtained for Y-STRs [3-6].

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P 173 ABSTRACT WITHDRAWN

P 174 PRELIMINARY RESULTS OF MITOCONDRIAL DNA SEQUENCE VARIATION IN JUJUY POPULATION (ARGENTINA)

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Introduction and aims: Jujuy is a province of Argentina, placed in the extreme northwest of the country, close to the borders with Chile, Bolivia and the Catamarca province of Argentina. Different Studies on South American population have proposed that actual population are composed by Native American maternal lineages and European paternal lineages with a different proportions among regions. The principal aim of this work is to study the mitochondrial genetic variability of the Jujuy population and also the different contributions of European and American-Native settlers.

Materials and methods: DNA extraction was carried out from peripheral blood using a standard Phenol-chloroform method. Polymorphisms of the mitochondrial DNA were determined by restriction fragment length polymorphism (RFLPs) in samples from autochthonous individuals from Jujuy (Argentina) (n=278). Haplogroups assignments of H, U, Pre-V, V, K, X, Pre-HV and HV were analyzed by primers and RFLPs described in Turchi et al. (2008). Haplogroups assignments of A and B, were confirmed by primers and restriction fragment length polymorphism (RFLPs) described in Stone and Stoneking (1993), Wrischnik et al. (1987), respectively. Finally, haplogroup assignments of A and B were done according to Parr et al. (1996).

Results and discussion: Results showed that the population sample was mainly composed by Native American haplogroups. Genetic contribution of European origin is very low. Haplogroups distribution in the province of Jujuy was related to other neighboring Native American populations. These preliminary data could be helpful for understanding the present distribution of the mitochondrial DNA haplogroups in this region.

P 175 EVALUATION OF POWERPLEX ESI 17® AMPLIFICATION KIT IN A ADMIXED HISPANO-AMERINDIAN POPULATION SAMPLE OF VALPARAÍSO, CHILE

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The PowerPlex ESI 17® (PP17) amplification Kit was designed for European population (ENFSI and EDNAP recommendations). Routinely, DNA testing in our population was performed using the AmpFℓSTR® Identifiler® PCR Amplification kit (ID), that includes the 13 CODIS *loci* (FBI recommendations). Our population is an admixture between Hispanic and Amerindians. To evaluate the usefulness of the *loci* included in PP17 we analyzed 150 unrelated individuals' samples obtained with previous informed consent, who attend to paternity testing. The sample size was calculated according to Chakraborty (1992) to obtain frequencies higher than 0.1 (α=0.95). The DNA extraction was automated performed and the STR *loci* was PCR amplified using PP17 and ID amplification kits. Allelic, genotypic frequencies, Power of exclusion (PE) and Match Probability (MP) were calculated using PowerStat v1.2 software (Promega). The SE33 *loci* MP was the lowest (2.20×10^{-2}) and the PE (0.788) was the third highest. The other 5 new *loci* (D10S1248, D22S1045, D2S441, D12S391 and D1S1653) have similar informativeness than CODIS *loci*. The combined MP and PE for PP17 were 3.71×10^{-19} and 0.9999998 respectively, while for ID the parameters were 5.93×10^{-17} and 0,9999993. This study showed a high percentage of heterozigocity in PP17® *loci* in the population studied, despite being designed for European population. In conclusion, the use of the PP17 system has better informativeness than ID. The combined use of these two systems could resolve complex DNA testing, where ID alone is not sufficient to get a strong conclusion.

P 176 ALLELE FREQUENCIES OF 15 STRS IN A REPRESENTATIVE SAMPLE OF ENTRE RÍOS PROVINCE OF ARGENTINA POPULATION

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Allele frequencies for 15 short tandem repeat (STR) loci present in AmpFℓSTR® Identifiler® PCR Amplification Kit (Applied Biosystems) were obtained from a sample of 839 unrelated individuals undergoing paternity testing. This sample includes individuals from all regions in Entre Rios province of Argentina. The most polymorphic loci were FGA, D18S51 and D2S1338. All the analyzed loci meet Hardy-Weinberg equilibrium after Bonferroni correction. Total non-discrimination probability and combined power of exclusion for the 15 tested STR loci were 1.91×10^{-18} and 0.9999995, respectively. Comparative analysis between populations from different Argentinean provinces populations is presented.

P 177 GENETIC DATA 10 X-STR IN THE DEPARTMENT OF BOLIVAR, COLOMBIA CARIBBEAN POPULATION

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X-chromosome markers have been used in human identification and paternity testing since the seventies, among the best known is the red cell antigen "Xg [a]", encoded by the gene locus Xp22.32, and some enzymes erythrocytes, the best known is the G6PD. Molecular markers linked to chromosome X and used in forensic genetics are the STR, have been identified over 200 loci, among trinucleotide and tetranucleotide. However, only in the past years the forensic community has assessed the informational power of the X-STR for human identification and paternity investigations, encouraging their study in different populations around the world.

We present the results of a population study in a sample of the Department of Bolivar, Colombia, with 10 X-STR: DXS6809, DXS7423, GATA172D05, DXS6789, DXS9902, DXS7132, GATA31E08, DXS7133, DXS9898 and DXS8378 contained in GHEP Decaplex. 279 chromosomes were analyzed from 101 men and 89 women, biologically unrelated, allele frequencies were reported for the 10 markers studied. None of the loci studied showed deviation from Hardy-Weinberg. There was no linkage disequilibrium between the loci studied. The 10 X-STR showed a cumulative discrimination for men and women of 99.9999% and 99.9999999%, respectively, and a cumulative power of exclusion greater than 99.9% in father/mother/daughter trios and in father/daughter duos. These findings validate the GHEP Decaplex to be used in cases of identification and establishment of biological relationships in the Bolivar Department, creating a new database for the region and Colombia.

P 178 Y CHROMOSOME HAPLOTYPES AND SURNAMES IN THE SPANISH POPULATION

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In most societies, surnames are passed down from fathers to children, just like the Y chromosome. So, theoretically, men sharing the same surnames would also be expected to share similar Y chromosomes. Previous investigations have challenged such relationship but data has been collected so far only from the British Isles. In order to provide additional insights into the correlation between surnames and Y chromosomes, we focused our attention on Spain and investigated Y-chromosome SNP/STR variation by analysing a total of 1,766 DNA samples from unrelated male volunteers belonging to 37 surnames and 355 controls. Our results suggested that the degree of co-ancestry within surnames was highly dependent on surname frequency. However, geographic distance between samples' place of origin influenced significantly this correlation. Within surname variation, number of descent clusters and TMRCAs correlated well with surname frequency, though exceptions were found. Studies evaluating the link between Y chromosome and surnames could be potentially helpful in forensic investigations as Y-chromosome profiles could be used to predict surname in forensic caseworks.

P 179 INSERTION/DELETION POLYMORPHISMS AT THE X-STR DXS10146 AND DXS10147 FLANKING REGIONS

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Some short tandem repeat (STR) loci have insertion/deletion (INDEL) polymorphisms in their flanking regions. We analysed INDEL polymorphisms at the X chlomosomal STR DXS10146 and DXS10147 loci in a Japanese population. DNA was obtained from 146 unrelated Japanese males. All samples were amplified by singleplex PCR, followed by sequencing. For the DXS10146 locus, we observed a 17-bp INDEL polymorphism located 35 bp downstream of the core repeat unit, as described by Sim et al (2010). Allele frequency for the long type allele containing the 17-bp INDEL element was 0.932 and that for the short type allele, in which the INDEL element was deleted, was 0.068. All the short type alleles had six repeat number polymorphisms at the first repeat structure $(TTCC)_n$ in the core repeat unit. On the other hand, none of the long type alleles had six repeat number polymorphisms at $(TTCC)_n$. For the DXS10147 locus, a 3-bp INDEL polymorphism was observed 86 bp downstream of the repeat structure $(AAAC)_n$, as described by Edelmann (2008). The long type allele frequency was 0.822 and the short type allele frequency was 0.178. The deletion of the 3-bp INDEL element was observed only at $(AAAC)_6$. The frequencies of the long and short type alleles at $(AAAC)_6$ were 0.395 and 0.605, respectively. Thus, analysing INDEL polymorphisms together with STR polymorphisms for both loci (DXS10146 and DXS10147) may be useful in forensic genetic investigations.

P 180 DISTRIBUTION OF H19HP HAPLOTYPES IN ASIAN POPULATIONS

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Introduction
We previously reported a H19HP haplotype system composed of 23 SNPs upstream of the H19 imprinted gene, which is maternally expressed. The parental origin of this polymorphism is detectable by locating all SNPs in differentially methylated region, and twenty haplotypes showing high diversity have been observed from three ethnic groups; Africans, Germans and Japanese. In this study, we examined the H19HP haplotypes in several Asian populations.

Materials and methods
Genomic DNA samples were obtained from Japanese subjects residing in four areas, Turkish volunteers residing in Germany, as well as residents in China, Thailand and Myanmar. A 1.2 kb region of DNA was amplified and directly sequenced. In some samples, the methylated paternal sequence was selectively determined from *Hpa*II-digested DNA.

Results and discussion
Five haplotypes, 2, 6, 10, 15 and 17, were found in all Asian populations as major components. In some haplotypes, there was a tendency for there to be either increasing or decreasing frequencies from west to east Asian populations. Group-specific sequences were detected from several Thai and Myanmar samples. Interestingly, haplotype 19 was observed only in Japanese samples with significant frequencies of 8.0% on average. This system might therefore be useful as a forensic tool, not only for human identification purposes, including discrimination of the parental origin, but also for determining ethnicity.

P 181 EFFECT OF LINKAGE BETWEEN VWA AND D12S391 IN KINSHIP ANALYSIS

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The expanded European Standard Set of loci includes D12S391, which is found on the short arm of chromosome 12 and is only 6.3 megabases (Mb) from the established vWA locus that is widely used in Europe and the U.S. Ideally for use in forensic analyses, genetic markers on the same chromosome should be more than 50 Mb in physical distance in order to ensure full recombination and thus independent inheritance. Recent studies have shown no significant linkage disequilibrium between vWA and D12S391 in U.S. and worldwide populations [O'Connor et al., 2011; Budowle et al., 2010; Phillips et al., 2011], although genetic linkage has been found (estimated recombination fraction of 0.108 using 28 meioses) [Budowle et al., 2010]. We evaluated genetic linkage in six multi-generation families (109 meioses) using the program LINKAGE. The recombination fraction was estimated at 0.089 (95% CI 0.044-0.158). The effect of linkage is an increased tendency for alleles at physically close loci to be transmitted together during meiosis. For linked loci, the use of haplotype frequencies and the recombination fraction has been recommended in likelihood calculations for kinship testing [Krawczak, 2007]. Moreover, ignoring linkage in likelihood calculations may lead to incorrect conclusions of kinship in some instances [Nothnagel, et al. 2010]. Using pedigree simulations, we evaluated the impact of including or ignoring linkage between vWA and D12S391 on likelihood ratio values. We will demonstrate the case-specific impact on likelihood ratio values and provide error rates when the "incorrect" model is used.

P 182 A PHYLOGENETIC STUDY OF THE MONGOLIANS AND BURYATS

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Introduction and aims
The population history of Central Asia offers several issues on which population genetics can shed light. The present study discusses population history, the results of Y chromosomal SNPs and haplotypes in the non-recombining region of the Y chromosome in samples from two Central Asian population groups: the Mongolians and the Buryats.

For approaching this issue Y-STR and Y-SNP loci of 124 Mongolian and 87 Buryat males from the Outer Mongolia were tested and compared to other Eurasian populations.

Materials and methods
Genomic DNA samples were amplified with the PowerPlex Y kit and analyzed by capillary electrophoresis. Testing of Y-SNP loci were performed with TaqMan assays on ABI7500. Haplotype and haplogroup diversity values were calculated. Genetic distances to Eurasian populations were calculated based on haplogroup frequencies with AMOVA implemented in Arlequin2.0. Based on the distances, a multidimensional scaling (MDS) plot was constructed with ViSta 7.9.2.4 software. Networks were constructed using the Network 4.5.1.0 program.

Results and discussion
The haplotype and haplogroup diversity values were the followings: Mongolian: 0.98503 and 0.63729; Buryat: 0.87427 and 0.68235.

Based on genetic distances of Eurasian populations including Mongolians and Buryats an MDS plot was constructed where Mongolian speaking ethnic groups and Central Asian populations clustered together. For STR variation within haplogroups networks were constructed.

P 183 POLYMORPHIC STUDY ON NINE SNPS LOCATED ON SEVEN SUSCEPTIBLE PARKINSON DISEASE GENES BY MULTIPLEXED PCR-RFLP ANALYSIS*

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Parkinson's disease (PD) is one of the most common neurodegenerative disorders. More than hundreds of SNPs have been found on the 16 susceptible PD genes and part of the SNPs have been reported to be associated with human PD. At the same time, increasing results from population studies indicated that the genetic information of aforementioned loci not only exhibited their significances in human genetics but also possessed a potentiality applied in forensic medicine. To investigate potential genetic risk factors of PD and obtain genetic parameters, nine SNPs (rs56092260, rs1801582, rs35802484, rs33949390, rs34778348, rs76718524, rs11107, rs12564040, rs4538475) located on seven susceptible PD genes were multiplex- or singleplex-amplified in four independent PCR systems and subsequent genotyped by RFLPs with either endonuclease *Hae* III or *Pst* I digestion in 215 PD patients and 212 matched controls in northern Han Chinese population. The results showed that multiplexed PCR-RFLP assays, high throughput in nature, are able to accurately identify all nine SNPs. The genotypic distribution of rs34778348 site is significantly different between case and control groups; however ones of the remained loci are not associated with PD. Heterogeneity were not observed in rs76718524 and rs12564040 sites and was less in rs35802484 site. The combined power of discrimination showed the values of 0.7723 and 0.7841 and the combined power of exclusion are the value of 0.3945 and 0.4054 in control and case groups, respectively. The nine SNPs investigated in this study are useful tools for the research of genetic diseases and forensic medicine.

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P 184 STR DATA FOR AMPFℓSTR® IDENTIFILER FROM FORENSIC AND ANTHROPOLOGICAL SAMPLES OF THE POPULATION OF MAR DEL PLATA, BUENOS AIRES PROVINCE (ARGENTINA)

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Allele frequencies for the 15 short tandem repeats (STRs) loci included in the AmpFℓSTR® Identifiler kit (Applied Biosystems, Foster City, USA) were estimated in a sample of 160 unrelated individuals inhabiting in the city of Mar del Plata (Southeast of the Province of Buenos Aires, Argentina). Of the sample, 80 were from voluntary donors and 80 from forensic cases. All voluntary donors were adequately informed about the aims of the study, and gave consent to perform this research. Between the two groups were not significant differences ($P > 0.05$) for the population parameters analyzed, and can be considered as belonging to the same sample. Population showed to be in Hardy-Weinberg equilibrium, using the Bonferroni correction for the number of loci analyzed, except for vWA locus. D18S51 was the most informative system in the population of Mar del Plata, with a power of discrimination (PD) of 0.969, and the least discriminating locus was TPOX with PD = 0820. Also, there were no significant differences ($P > 0.05$) between our population data and those previously obtained by other authors in the province of Buenos Aires, Argentina. The allelic frequency data obtained are potentially useful for identity testing purposes and for anthropological researchs.

Keywords: Autosomal STR; Forensic and anthropological data; Mar del Plata.

P 185 GENETIC POLYMORPHISM OF THE NEW POWERPLEX® ESI 17 SYSTEM IN A TIBETAN POPULATION FROM DHARAMSALA (INDIA)

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Allele frequencies for the 17 autosomal-STR loci included in the PowerPlex® ESI 17 kit (D22S1045, D2S1338, D19S433, D3S1358, Amelogenin, D2S441, D10S1248, D1S1656, D18S51, D16S539, D12S391, D21S11, vWA, TH01, SE33, FGA and D8S1179) in a sample of 80 randomly selected unrelated individuals from a Tibetan ethnic group living in Dharamsala in the state of Himachal Pradesh (Northern India) were investigated. Due to the relative novelty of the ESI 17 system including the 5 new ENFSI recommended loci (D2S441, D10S1248, D22S1045, D1S1656, D12S391) together with the highly polymorphic SE33 locus, no information regarding these loci allele frequencies are available for Tibetan population. The aim of the present study was to determine the genetic polymorphism of forensic validated markers and to increase the ethnical population database. Genetic parameters of forensic interest were calculated. Our data are useful for forensic applications and population genetics studies.

P 186 GENETIC CHARACTERIZATION OF SOMALI AND IRAQI POPULATIONS USING A SET OF 33 X CHROMOSOME INDELS

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Insertion/deletion polymorphisms or Indels are, as the name suggests, length polymorphisms created by insertions or deletions of one or more nucleotides in the genome. The genotyping of Indels has gained increased importance in population studies and human identification over the last few years and so has the study of the X chromosome markers.

In the present work, a total of 245 samples were collected from unrelated individuals from Somalia (148 males) and Iraq (58 males and 39 females). Samples were analyzed for 33 X-chromosome Indel markers. The aim was to characterize their diversity pattern, contributing at the same time to a better understanding of the genetic landscape of the Horn of Africa and the Middle East.

Diversity measures for the Somali and Iraqi populations were similar and significant genetic distances were obtained between the groups (F_{ST}=0.0627). No departure from Hardy-Weinberg equilibrium was detected, using the genotypic data of 39 Iraqi women. Linkage disequilibrium analysis revealed significant association between pairs of markers in Somalis and Iraqis: MID3719–MID2089 and MID357–MID356 were detected in both populations, whereas MID3690–MID2089 was detected only in Somalis. The power of discrimination in males and females and the exclusion power in father/daughter duos and in father/mother/daughter trios presented high overall values. Therefore, the analyses of these statistical parameters for forensic evaluation suggested that the 33plex system has a potential for application in human identification and kinship testing in the Somali and Iraqi populations and most likely in other populations as well.

P 187 STUDY OF 25 X CHROMOSOME SINGLE NUCLEOTIDE POLYMORPHISMS IN AFRICAN AND ASIAN POPULATIONS

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There is a growing interest in the study of X chromosome markers in population and forensic genetics. Most of the studies focus on Short Tandem Repeats (STRs) and more information is needed concerning Single Nucleotide Polymorphisms (SNPs).

In the present work, male samples from Africa (Angola: n=46; Mozambique: n=42) and Asia (Taiwan n=21; China: n=43; Bangladesh: n=58) were analysed for 25 SNPs located on the X chromosome to evaluate the diversity pattern of the populations from the two continents. Data were also compared with previous results from Somalia and populations from the Mediterranean area to assess their genetic affinities.

All the markers were polymorphic in the studied populations. Minimal allele frequencies ranged from 4.8% (at X036 marker) to 50%. Gene diversities were similar in all populations, and no single haplotype was shared among individuals within or between populations. Pairwise linkage disequilibrium analysis revealed significant association between X029–X047, X036–X056, X036–X134 and X029–X165 in the Taiwanese population, even after correction for multiple analyses (p=0.000167).

The results showed clear genetic differences between the Sub-Saharan populations and those from North Africa and the Mediterranean area. The differentiation between East and South Africa was also recognizable, with Angola and Mozambique being statistically different from Somalia but not from each other. Likewise, the Asian samples formed a separate cluster with Bangladesh exhibiting a statistically significant difference from China and Taiwan.

P 188 EXPLORING 1000 GENOMES DATA FOR FORENSIC PURPOSES USING THE *ENGINES* SNP BROWSER

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The 1000 Genomes project aims to build a broad-scale catalogue of human SNP variation showing minor alleles frequencies at 1% or above. The project's Pilot 1 data comprises low coverage sequencing (2–6x) of 270 whole genomes from the four original HapMap population samples, now expanded to 629 genomes from 12 populations in the 1000 Genomes Interim Phase I release of late 2010. In contrast, Pilot 2 data comprises high coverage sequencing (>60x) of two trios, across multiple platforms and centres. While a browser exists for the six Pilot 2 genomes, none has so far been developed for Pilot 1, preventing users from exploring genome-wide SNP variation across the widest range of populations. We have developed an open-access browser termed *ENGINES* (ENtire Genomes INterface for Exploring SNPs) based on the Interim Phase I data and permitting users to find established and new SNP sites in defined chromosome segments or genes, or by submitting a list of rs-numbers. *ENGINES* provides a simple interface to browse the positions, allele frequencies and other genome details of a list of SNPs. Genotype data can then be directly downloaded for inclusion in STRUCTURE or Arlequin. Some potential forensic applications are demonstrated, including: exploration of forensic SNPs where HapMap data was previously unavailable (e.g. SNPforID or Kiddlab loci), exploring low frequency SNPs in genes of forensic interest (e.g. MC1R) and finding previously uncharted SNPs that might interfere with primer binding sites of established forensic loci.

ENGINES is available at: http://spsmart.cesga.es/engines.php

P 189 X-CHROMOSOMAL HAPLOTYPE FREQUENCIES OF FOUR LINKAGE GROUPS IN A NORTH AFRICAN POPULATION

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X-STR markers have a series of special characteristics that justify its increasing interest in forensic practice, population genetics and anthropology. Compared with autosomes, the X-chromosome has lower recombination rate, lower mutation rate and a smaller effective population size, resulting in a faster genetic drift. In consequence, both linkage disequilibrium (LD) and population structure in the X chromosome, are expected to be stronger than those in autosomes. The X-STRs are particularly useful in paternity testing and kinship analysis, specially in deficiency cases, such as grandmother-granddaughter, aunt-niece and cousins. Owing to the complexity of kinship investigation, a new approach of substituting single STRs by stable haplotypes of closely linked loci, has been recently suggested. The new investigator Argus X-12 kit, allows simultaneous amplification of 12 X-chromosomal STR loci, clustered into 4 linkage groups with 3 closely linked markers per group. Here we present haplotype frequency data of a Moroccan sample, typed according to the manufacturer's instructions. Amongst the 97 males analyzed, the 4 X-STR trios of linkage group 1–4 revealed 88, 64, 72 and 76 haplotypes, respectively. Most of them (79%) were only observed once, and the other haplotypes were shared by two to five men. The *discrimination capacity* and the *match probability* ranged from 90.72% (lg 1) to 65.98% (lg 2) and between 0.21% (lg 1) and 1.03% (lg 2), respectively. The present work demonstrates that haplotypes for the four X-STR trios are highly discriminating and, therefore, provide a powerful tool for solving complex kinship cases in Moroccan population.

P 190 MOLECULAR CHARACTERIZATION AND STATISTICAL ANALYSIS OF SHORT TANDEM REPEATS (STR) USED FOR FORENSIC APPLICATIONS IN BRAZILIAN POPULATIONS: BAHIA, ESPIRITO SANTO AND MINAS GERAIS

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Short tandem repeat (STR) loci are the most informative forensic DNA markers .We developed and used two multiplexes systems with 18 markers (D2S1338, D3S1358, D3S2387, D3S2406, D5S818, D5S2503, D7S820, D9S938, D10S1237, D12S391, D13S317, D16S539, D16S753, D21S1437, D22S534, D22S689, SE33, TH01) that include common, a subset of commercial markers, and non-common markers, new markers or markers little used, analyzed their forensic informativeness and characterized the molecular nature of this markers. Data from 11127 individuals from Three Brazilians states (Bahia, Espirito Santo and Minas Gerais) were chosen for characterization of the multiplexes systems. Two alleles of the most common alleles were sequenced, and forensic metrics such as power of exclusion (PE), random matching probability (RMP), polymorphic information content, allele frequencies and mutation rate) and population estimations (Fst, Hardy-Weinberg equilibrium (HWE) and heterozigosity) was calculated for all 18 markers. We determined that for most of the STR the repeat pattern is are perfect. The combined PE was 0,999967and average RMP was 0,061. The population estimation demonstrated that these populations didn't show genetic differentiation when these markers are used. We didn't observed deviation for the HWE and all populations showed similar values for heterozigosity. These sets of markers are a suitable tool for forensic analysis

P 191 ITALIAN POPULATION DATA FOR THE NEW ENFSI/EDNAP LOCI D1S1656, D2S441, D10S1248, D12S391, D22S1045. THE GeFI COLLABORATIVE EXERCISE AND CONCORDANCE STUDY

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Five new ENFSI/EDNAP loci, D1S1656, D2S441, D10S1248, D12S391, and D22S1045 were investigated in Italy in a collaborative exercise organized by the ISFG Italian Working Group GeFI. Fifteen laboratories participated to the project, each one contributing at least 50 DNA samples from unrelated individuals. Two blind blood stains were provided by the Organizing Committee as a proficiency test. Each laboratory was also requested to type its samples in duplicate, freely choosing a combination of at least two of the following kits: *PowerPlex ESX, PowerPlex ESI, AmpFℓSTR NGM* and *Investigator ESSplex*. The electropherograms obtained by the labs were collected by the Organizing Committee together with a questionnaire aimed at investigating the concordance of the various kits in allele detection. A total of 752 samples were finally collected in the excercise database. Statistical analyses were performed using Arlequin 3.11. Among 75 HWE tests (5 loci x 15 labs), three showed P values <0.05 (consistent with expectation). Number of alleles and expected heterozygosities ranged 10 to 18 and 0.70 to 0.90, respectively. None of the 105 pairwise tests of population differentiation was significant at the nominal 0.05 level. Analysis of Wright F statistics showed a high degree of genetic homogeneity of all these five markers across Italy.

P 192 ANALYSIS OF Y-CHROMOSOME SNPs IN MALES FROM PUEBLO LLANO (VENEZUELA)

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While several regions in Europe and North America have been analyzed in detail for Y polymorphisms, South American populations remain poorly characterized at the genetic level.

In this work, Y-chromosome haplogroups defined by binary polymorphisms were analyzed in 76 unrelated males from a specific region of Latin America. These individuals were sampled in Pueblo Llano, a region of the Venezuelan Andes that remained isolated until the 60s, representing an endogamic population in which different genetic diseases has been described so far. Within this population, people have been described to have physical appearance that goes from white-almost-European to dark-Native forms, while African influence has not been detected yet.

SNP genotyping was carried out using a set of 27 SNPs grouped into different multiplexes previously published in the literature, in order to determine the most frequent haplogroups, by means of only 1 or 2 multiplexes. We only need to type 10 SNPs at best, and at worst no more than 16 SNPs to define the haplogroup. This way, to define the final haplogroup, we can avoid genotyping the unnecessary SNPs and therefore we save cost and effort. The selected method for allele discrimination was a single base extension using the SNaPshot multiplex kit (Applied Biosystems, Foster city, USA).

In this work, we show preliminary results for the frequency distribution of main haplogroups in a population sample from Venezuela. Our results will contribute both elucidating the genetic composition of Venezuela and reconstructing the demographic history of South American populations.

P 193 DEMOGRAPHIC INFERENCES FROM HIGH-THROUGHPUT MTSNP GENOTYPING IN POPULATIONS FROM THE LAKE CHAD BASIN

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Lake Chad Basin is located in Northern Central Africa; it covers almost 8% of the continent and spreads over several countries. Previous studies based almost exclusively on the analysis of control region mitochondrial DNA (mtDNA) data have defined the region as a bidirectional corridor connecting West and East Africa, as well as a meeting point for populations coming from North Africa through the Saharan desert. Here we carry out an analysis at deep phylogenetic resolution considering 230 mitochondrial SNPs (mtSNPs) selected from the coding region and using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry technology. A total of 542 samples belonging to 12 different ethnic populations were genotyped.

Several diversity indices were calculated both individually for HVS-I and mtSNPs, and both data sets in combination. Moreover, several Bayes-based estimates were obtained concerning migration rate, effective population size and growth rates. High heterogeneity for these estimates was observed in the Chad Basin mirroring the different and individual demographic histories of these ethnic groups. Differences in the patterns of variability were also observed between nomadic and sedentary populations. The present study indicates that analysis of mtSNPs at high resolution could be a fast and extensive approach for screening variation in population studies where other techniques such as entire genome sequencing remain unfeasible.

P 194 ADMIXTURE IN PRESENT-DAY URBAN VENEZUELANS AS INFERRED FROM MTDNA VARIATION

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Analysis of mitochondrial DNA (mtDNA) variation has demonstrated to be a useful tool for the reconstruction of past demographic events and for unraveling admixture patterns that have shaped different human populations. Here we aimed to analyze admixture patterns of modern urban Venezuelans as inferred from mtDNA variation. Two different populations were sampled: (i) the cosmopolitan city of Caracas (n = 131), and (ii) Pueblo Llano (Estado Mérida; n = 219), a population for which episodes of isolation and consanguinity were documented. All the samples were analyzed for the control region and selectively genotyped for coding region mtDNA Single Nucleotide Polymorphisms (mtSNPs). The Native American component of admixed Venezuelans accounted for 80% of all mtDNAs. African lineages represented about 10% of the Venezuelan lineages which are mainly of west-central sub-Saharan African origin. The European component made-up ~10% of the urban Venezuelan mtDNAs; some lineages can be clearly allocated to particular European source populations; this is the case of e.g. the U6b1 mtDNAs, that most likely came from the Canary Islands, one of the main Spanish contributor to urban Venezuelans according to the documentation. A total of 17 entire mtDNA genomes were sequenced and five new branches were revealed; some of them seem to be autochthonous when inspecting these profiles in a large survey of American mtDNAs. Coalescence ages could be obtained for some of them, ranging from 2.6 ky (95% C.I: 0.1–5.2) to 3.9 ky (95% C.I: 0–7.8). Mitochondrial DNA variation in Pueblo Llano shows clear evidences of severe genetic drift and/or consanguinity, reveled by control region variation and entire genome sequences, explaining the high prevalence of certain Mendelian and complex multi-factorial diseases in this region.

P 195 EXPLORING MTDNA PATTERNS OF RECENT ADMIXTURE ARGENTINA

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Despite being one of the largest countries of South America, Argentina is yet poorly characterized from the genetic point of view. The present Argentinean population is the result of a complex amalgamation of people coming from different continents that superposed or replaced the Native American component of the country in Colonial times. Contemporary migrations involved masses of people coming from different European countries (e.g. Spanish, Italian, French, German, Polish or Jewish people) and, to a lower extent, sub-Saharans arriving as a consequence of the African slave trade. The main goals of present study were (i) improving the genetic characterization of different regions of the country by genotyping both control regions (HVS-I and HVS-II) and two sets of mitochondrial diagnostic SNPs, and (ii) exploring the existence of population stratification in the country, with special focus on urban populations.

Our results suggest that the distribution of Native American haplogroups was substantially different in the main Argentinean regions, North, Central and South, especially when looking at urban populations. The results also agree well with the census and documentation signaling Spain and Italy as the main European contributors to the country. The impact of the Trans-Atlantic slave trade in the mtDNA pool of Argentina was much lower than in other American regions. Forensic implications of the present study will be also discussed.

P 196 PATTERNS OF MTDNA VARIATION IN BOLIVIA

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Study of the variation patterns of the mitochondrial DNA (mtDNA) molecule has been very useful to unravel the history of Native and urban/rural admixed American populations. We have sampled several regions in Bolivia, representing the largest study carry out to date in this region. We focused specifically in metropolitan areas, including the departments of Pando, Beni (including the locality of San Ignacio de Moxos), Santa Cruz, Cochabamba and La Paz (including the Yungas and Copacabana regions), and Chuquisaca. We will show preliminary results concerning patterns of variation at the entire control region segment and comparison with a large American dataset. As inferred from the data, the great majority of the Bolivian mtDNAs belong to the main Native American haplogroups, with little presence of sub-Saharan and/or European lineages. The data obtained would be useful for those interested in population genetics, but also forensic geneticist given the lack of data available from this country. Moreover, patterns of variation in Bolivia differ between different departments, a fact that should be considered when evaluating the weight of the mtDNA evidence in forensic casework.

P 197 ANALYSIS OF Y CHROMOSOME LINEAGES IN A BOLIVIAN POPULATION

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Up to this date, SNPs have been the ideal markers for genetic characterization in order to study parental or maternal lineages, many of which had been analyzed in South American populations. Thus, Bolivian population is poorly characterized in these terms and currently there is very few data concerning to this kind of polymorphisms.

In this work, we have performed an analysis of Y chromosome biallelic polymorphisms in order to define haplogroups, in a representative sample of 220 individuals from 5 different departments along Bolivia, specifically: Pando, Santa Cruz, La Paz, Cochabamba and Beni.

Genotyping of the samples were carried out amplifying a set of 26 SNPs grouped in 4 previously published multiplexes, which define the most frequent haplogroups in this population.

Haplogroup frequencies were calculated for all the departments. Genetic distances between all the regions as well as between the whole sample and other South American populations were estimated by means of *Fst*. The existence of genetic structure in this sample representative of Bolivian population was also assessed.

The results obtained here will contribute to the knowledge of the most important demographic processes of male lineages that happened on this region, which is of essential interest not only for the understanding of the genetic history of Bolivians, but also from the forensic point of view.

P 198 ABSTRACT WITHDRAWN

P 199 Y-STR DIVERSITY IN THE SWEDISH POPULATION AND ITS IMPLICATION ON FORENSIC CASEWORK

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17 Y-chromosome STR loci included in the AmpFℓSTR® Y-Filer kit were typed for 302 males originating from seven geographically different regions in Sweden. In the complete dataset, 287 different haplotypes were identified of which 274 were unique. The most common haplotype was found 3 times corresponding to a population frequency of 1.0%. The haplotype diversity was computed to be 0.9996 in the total dataset, and was comparable among the different regions. The overall F_{ST} value among the different regions was computed to be 0.0016, and the highest pairwise F_{ST} value was observed between the regions Västerbotten and Uppsala (F_{ST}=0.003, P<0.001). The diversity increased substantially and the interregional differences decreased using Y-Filer compared with the earlier used Y-STRs included in the PowerPlex® Y System. The implications of these findings on forensic casework will be discussed.

P 200 ANCESTRY PROPORTIONS IN URBAN POPULATIONS OF ARGENTINA

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Ancestry-Informative Markers (AIMs) have demonstrated to be a valuable tool to assess ancestry proportions in admixed populations, which is relevant in the statistical interpretation of forensic caseworks and in association studies. In this work we aimed to evaluate the African, European and Native American ancestral contribution to different Argentinean populations. A set of 46 ancestry-informative insertion-deletion polymorphisms (AIM-Indels) was used to type 279 unrelated individuals from urban populations of six provinces located in distant geographical regions of Argentina. Allele frequencies were estimated. No departures from Hardy-Weinberg equilibrium were detected. AMOVA results showed a low, but significant, interpopulation variation (*Fst*=0.0106; *P*<0.00000). When estimating the African, European and Native American admixture proportions, using the software STRUCTURE, the African contribution presented the lowest values, with no significant differences among the studied regions (between 2–3%). As expected, population from Buenos Aires appears to have a higher European ancestry contribution (0.868) when compared to populations from other provinces (between 0.580 and 0.811). In contrast, the Native American component is better represented in urban samples from the northern and southern regions of the country, reaching almost 40% in both Tucuman and Santa Cruz. These differences are also revealed by the *Fst* analysis results, where most of the significant genetic distances are between Buenos Aires and the other populations. The results are in agreement with previous reports based on other type of genetic markers (e.g. the uniparental) and supported by the demographic history of Argentina.

P 201 FORENSIC EVALUATION OF THE INVESTIGATOR DIPPLEX TYPING SYSTEM

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Although the short tandem repeats (STRs) have become markers of choice in human identification, recently other types of genetic variations such as Deletion/Insertion Polymorphisms (known as DIPs or INDEL) have been considered for their possible application in forensic field.

However, to date studies evaluating the potential use of INDEL markers in forensic practice are scarce. We employed a newly developed commercial kit the Investigator DIPplex kit to genotype a sample of 200 unrelated healthy individuals living in North-East Italy. The Investigator DIPplex kit allows the amplification of 30 biallelic DIP loci (distributed over 19 autosomes) and amelogenin in a single PCR reaction.

Allele frequencies and other statistical parameters of forensic interest such as discrimination power (DP) and random match probabilities (RMPs) for each locus and profile were calculated. Given the results obtained in this preliminary study, the panel of 30 biallelic DIPs seems to be very efficient and it could represent a useful new tool for paternity analysis.

P 202 MITOCHONDRIAL CONTROL REGION SEQUENCES OF THE CZECH REPUBLIC POPULATION AND A COMPARISON TO OTHER POPULATIONS

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The correct use of mitochondrial DNA (mtDNA) testing in the forensic context requires appropriate population databases to determine the relative rarity of the haplotype of the tested sample. The aim of this study was to evaluate the results of full HVRI and HVRII mtDNA sequences of 230 unrelated individuals and to compare the data to the previously published Czech population data obtained using PCR-RFLP. The Genetic diversity (GD) and Random match probability (RMP) of the Czech mtDNA population data were also compared to the other European populations. The results indicate that the full control region sequencing can bring more precise population information that is useful for the comparison with other data sets and also for the forensic identification purposes.

P 203 POPULATION DATA FOR 8 Y-CHROMOSOMAL STRS (NOT INCLUDED IN Y-FILER™ KIT) IN A POPULATION SAMPLE OF CZECH REPUBLIC

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The newly designed Y-chromosome miniSTR pentaplexes I and II include 8 "non-core" Y-STR loci DYS388, DYS426, DYS444, DYS446, DYS447, DYS449, DYS459, DYS481 plus additional 2 Y-STR loci DYS392 and DYS438 that overlap with the of Y-filer™ kit. The amplicon sizes were designed as "miniSTRs" so the pentaplexes can be also used for degraded and ancient DNA typing. The Y-pentaplexes I and II were used to obtain the allele frequencies and gene diversities for the population sample of more than 140 unrelated individuals from the Czech Republic. The data show that the additional Y-STR loci (on top of Y-filer) are extremely useful not only in the complex genealogical studies but also as a research tool for the Y-chromosome and surname correlation studies.

P 204 ALLELE FREQUENCIES OF THE NEW EUROPEAN STANDARD SET (ESS) LOCI IN THE POPULATION OF CZECH REPUBLIC

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Allele frequencies of 5 STRs loci (D10S1248, D2S441, D1S1656, D12S391 and D22S1045) included in the new European Standard Set (ESS) were calculated for the population of Czech Republic. The samples were collected on the flocked swabs (COPAN Innovation, Italy), extracted using Quick gDNA MicroPrep (Zymo Research, USA), quantified using the qRT-PCR assay with 4N6 Quant ALU (Amplicon, Czech republic), amplified using AmpFℓSTR NGM kit and analyzed on ABI PRISM 310. Allelic frequencies of the new ESS loci obtained for the tested population sample were compared to the previously published population data and no significant differences were found.

P 205 AN IN-DEPTH POPULATION GENETIC ANALYSIS OF FORENSIC SHORT TANDEM REPEAT LOCI IN INDONESIA

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Estimating the strength of forensic DNA evidence requires an understanding of the population genetic features of all major populations within the relevant region. Limited information is available regarding population sub-structure in areas neighbouring Australia, including Indonesia, Papua New Guinea and the Pacific Islands. These are remote island communities expected to display pronounced sub-population effects. In particular, Indonesia is an archipelago with more than 17,000 islands encompassing a number of distinct ethnic and linguistic groups.

STR data from this study will assist in forming genetically appropriate sub-population groupings for the purpose of constructing defensible forensic STR databases which display minimal sub-structure within populations. Ultimately, these sub-population databases can be used to assist practitioners with forensic casework match probability assessments.

Samples from 1500 unrelated Indonesian individuals, representing 31 sub-populations and spanning 22 distinct geographical regions, have been analysed using Identifiler (Applied Biosystems). Preliminary results from pair-wise population F_{ST} comparisons suggest that fine genetic structure may be masked by simplistic east/west or north/south divides. A number of loci have also been identified as being significantly out of Hardy-Weinberg Equilibrium ($p < 0.01$) in a number of sub-populations.

Additional analysis of ancestry informative Y-, mitochondrial- and autosomal single nucleotide polymorphisms (SNPs) will assist in identifying the biological ancestry of the current sample set and provide guidance in selecting the most informative marker combinations for the identification of population sub-structure within this region.

This project will investigate the population genetic features of Indonesia and provide useful STR genotype data to forensic practitioners and research institutions.

P 206 POPULATION GENETIC DATA FOR F13A01, FES/FPS, F13B AND LPL IN THE SOUTH PORTUGUESE POPULATION

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DNA parentage testing are currently performed using several highly polymorphic short tandem repeats. In our routine casework, we applied two validated STRs kits, in order to have results in the 13 codis loci plus D2S1338, D19S433, PENTA E, PENTA D and Amelogenin.

According to ISFG, all bioestatistical calculations shall be based on a likelihood ratio requiring the evaluation of two relevant mutually exclusive hypothesis. This principle remains the same regardless of the complexity of the paternity cases. In theses cases, in order to obtain conclusive likelihood ratios, it is often necessary to increment the number of STRs. For this reason, we introduced in our laboratory GenePrint® FFFL Multiplex kit, which can provide results in F13A1, FES/FPS, F13B and LPL. It is our aim, study allele frequencies in these loci for further use in biological kinship relations.

Samples in this study were collected from 150 unrelated healthy individuals collected from South Portugal population. PCR amplifications were performed using the FFFL multiplex kit and amplified products were analysed, using an ABI 3130*xl*. Allele designations were made according ISFG recommendations. Statistical evaluations were performed using Arlequin 3.5.1.2.

Statistical evaluations, Hardy-Weinberg equilibrium, allele frequencies and forensic parameters such as power of discrimination, and a priori change of exclusion, for each loci were estimated.

In conclusion, a south Portuguese population database, for these four STRs has been established. In the near future it is also important to estimate mutation rates for these loci.

P 207 FAMILY SELECTION STUDY AMONG DNA SAMPLES COLLECTED FROM AN AMERINDIAN ETHNIC GROUP (WAYUU) IN NORTHERN COLOMBIA

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Introduction
Among the cell bank of RIKEN BioResource Center, there is the Sonoda-Tajima Cell Collection, in which blood samples were collected from various ethnic minority groups around the world, especially in South America. The center immortalized about 500 samples with the Epstein-Barr Virus and established B lymphoblastoid cell lines (B-LCLs). All of them were anonymized. However, it is important to investigate the kinships among those samples to study on more accurate genetic relationship among those groups. In this study, we selected kinships among the maximum number of samples in an ethnic group in the collection using autosomal and Y-STRs, and investigate how the kinships influence population genetic analysis.

Materials and methods
DNA from 98 immortalized B-LCLs originated from the Wayuu in Colombia were analyzed for 21 autosomal STRs and 17 Y-STRs using three multiplex kits. Parentage and sibling relationships were selected using Geno-Proof and Familias software. Population genetic analyses were performed using Arlequin and MEGA software.

Results and discussion
Totally 17 kinships including 4 mother-child, 6 one-parent-two-child, 3 sibling relationships, and 4 kinds of complicated kinships were selected. All the paternal linage were confirmed by the Y-STR haplotypes. When compared between the 98-sample population and the 78-sample population in which the parentage relationships were omitted, no significant difference was observed at allele frequency distributions. Topology in a NJ-tree based on Fst-distance with the other 20 ethnic groups was also no difference. It seemed that about one-fifth of parentage relationship in such population size didn't influence population genetic analysis.

P 208 GENETIC POLYMORPHISMS OF EIGHT X-CHROMOSOMAL STR LOCI OF MENTYPE ARGUS X-8 KIT IN KOREANS

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We have examined 45 Korean haplotype transfers in 41 families at eight X-linked STRs (DXS10135 and DXS8378 in linkage group 1, DXS7132 and DXS10074 in linkage group 2, HPRTB and DXS10101 in linkage group 3, DXS10134 and DXS7423 in linkage group 4) of Mentype Argus X-8 kit. The allele frequencies and haplotype frequencies within the four linkage groups were determined for males and females, respectively. Deviation from Hardy-Weinberg expectation could not be detected ($p < 0.05$). No recombination was observed within each 4 linkage group, however, six cases of mutation were founded in DXS10135, DXS10101, and DXS10134. Details of X-STR haplotype study in Koreans would be useful in kinship testing and forensic applications.

P 209 STUDY OF TWO X-LINKED MICROSATELLITE BLOCKS: ALLELIC FREQUENCIES IN MIXED AND ISOLATION POPULATION GROUPS

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Introduction and aims
X-chromosome microsatellites have emerged in recent years as a useful tool in kinship analysis and forensic cases. When cases include people from isolated populations, it is important to know if allelic frequencies differ from the general population databases. Hence, the purpose of this study was to compare allelic frequencies of 6 X-linked microsatellites grouped into two haplotypic blocks (DXS10079, DXS10074, DXS10075, DXS6801, DXS6809 and DXS6789) in two different areas of Cantabria, corresponding to well mixed and isolated populations, respectively.

Material and Methods
We studied female subjects living in the coastal area (mixed population, n=142) or in the Pas valley (n=30, isolated population). The latter is a mountainous area with limited communications which used to difficult the interaction with people from other areas. DNA was isolated, quantified and amplified by PCR using fluorochrome-labelled primers. The size of amplified fragments was determined in an ABI 310 DNA sequencer. Allelic frequency distributions were tested by a Monte Carlo extension of Fisher's exact test using SPSS software.

Results and Discussion
There were significant between-population differences in the allelic frequency distributions of loci DXS10079 (p=0.002), DXS10074 (p=0.030) and DXS6809 (p= 0.025). One allele located in the DXS10079 locus was unique in the Pas valley population.

Although given the small sample size these results must be considered as preliminary, they suggest that care should be taken to interpret X-linked markers in cases involving individuals for relatively isolated populations.

P 210 VERIFICATION OF ALLELES BY USING PEAK HEIGHT THRESHOLDS AND QUALITY CONTROL OF STR PROFILING KITS

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In the autumn of 2010 SKL performed in-house validation of PowerPlex ESX 16 System (Promega). As the validation showed that very low amounts of DNA (< 10 pg) may provide correct allele callings (peaks above 50 rfu), we investigated the linear range, i.e., the interval of DNA amounts where a profile is well balanced and does not contain drop-outs and/or drop-ins. The linear range as indicated by our results is approximately from 0.5 ng (manufacturer's recommendation) to 2.0 ng of DNA. Profiles generated by less than 0.5 ng contained intra locus imbalances and/or drop-outs. Above 2.0 ng "bleed through" occurs due to overload of template-DNA.

A way to verify the correctness of a profile, without knowing anything about the condition of the template-DNA, is to use peak height thresholds adjusted to each marker and batch of kits used. SKL performs a quality control and adjust thresholds for each batch of kits. Three main tests are performed; detection limit, inhibitor tolerance and signal repeatability. The detection limit is examined to identify at which concentration intra locus imbalances and drop-outs start to increase. The ability to overcome inhibition is checked by analysing varying amounts of blood extracted with Chelex. Finally a set of replicates of control DNA is amplified (0.5 ng template-DNA) to calculate the mean peak height and standard deviation at each locus. Generally, the peak height thresholds vary between 200 and 250 rfu for heterozygote peaks. To verify allelic peaks below the set peak height thresholds, SKL uses consensus analysis.

P 211 EFFICIENT DNA EXTRACTION FROM HAIR SHAFTS

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Hairs are common biological samples in crime scene investigation. However, most of hairs are fragments without root. As the majority of DNA is located in the root, hairs are usually problematic samples in forensic analysis. For these reasons, hair DNA typing is directed to mitochondrial DNA (mtDNA), which is present in high copy numbers in each cell, instead of nuclear DNA profiles.

There are some protocols for DNA isolation from hair shafts as phenol/chloroform, proteinase K, silica based methods, chelex, alkaline digestion. Some of these methods can involve a number of steps, each of which add time to the procedure and increase the risk of contamination. Other of these methods reduces the processing time, but leads to the DNA degradation.

In our laboratory, we have used the PrepFiler BTA extraction method for routinely processing difficult samples as old bones or cigarette butts, obtaining in all cases clean DNA. The use of automatic extraction methods has been progressively introduced in forensic laboratories. For these reasons we decided to apply DNA extraction from hair shafts using the PrepFiler Express BTA extraction method in combination with AutoMate Express equipment.

In order to determine the efficiency of the method, DNA extractions were quantified using a real-time PCR approach, and mtDNA fragments of different length were amplified to determine DNA degradation. Moreover, we have processed several types of hairs with different characteristics (gender, antiquity, hair dyeing) and from diverse ethical groups.

P 212 NEXT GENERATION STR KITS APPLIED TO POST-MORTEM DNA EXTRACTS

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The development of enhanced PCR buffers has led to the release of new STR kits that allegedly provide increased levels of both robustness and sensitivity. The performance of these next generation STR kits has previously been tested on common forensic samples. Although these tests are informative they fail to reproduce the complexity of extracts obtained from skeletal remains that often contain only minute amounts of degraded DNA mixed with bacterial DNA and PCR inhibitors.

The ICMP is involved in the identification of persons missing as a result of armed conflict and victims of mass disasters. To date more than 34,700 STR profiles have been obtained from skeletal remains in the process of the identification of the victims of the Western Balkans conflicts. Therefore the next generation kits appeared as a potential source of improvement for the typing of challenging post-mortem samples.

Three next generation STR kits: PowerPlex® 16 HS System, PowerPlex® ESX 17 System and AmpFℓSTR® Identifiler® Plus were tested on regular and problematic post-mortem samples. Performance was assessed based on the balance between loci, the occurrence of amplification artifacts, the sensitivity against low-template extracts and the performances versus inhibitors.

Our results show that the new generation kits under assessment tend to produce STR profiles with increased imbalance between short and large molecular weight markers. Furthermore, the amplification of bacterial DNA appears to be more problematic when compared to the "classic" versions of these kits.

P 213 *PHADEBAS® PRESS TEST* AND THE PRESENCE OF AMYLASES IN BODY FLUIDS NATURALLY DEPOSITED ON TEXTILE

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In forensic DNA casework saliva stains with epithelial cells can be very useful even presenting the key evidence. Tests for amylase activity, like *Phadebas® Press test*, help locate stains and indicate presence of saliva. Sensitivity is high, with positive amylase tests obtained prior to detectable levels of DNA and saliva diluted to 1:100 readily generate a positive reaction with *Phadebas® Press test* for presence of amylase. The salivary amylase activity varies on individual basis over time as well as it does between individuals. In addition some individuals secrete high levels of amylases [1,2]. However, amylases are present in other body fluids as well, generally too much lower levels than saliva. Due to sensitivity of amylase tests there is a potential interference by other fluids when using them to verify the presence of saliva. Other studies also demonstrate that e.g. faeces can give positive reactions.

For underwear the presence of several different body fluids might have natural causes, including vaginal secretions, (menstrual) blood, urine, faeces, as well as semen and saliva. Here we present the use of *Phadebas® Press test* on underwear with naturally deposited body fluids and single source body fluid mock samples including one individual with higher levels of amylase activity. Our results and implications are discussed.

[1] J. Hedman, E. Dalin, B. Rasmusson, R. Ansell (2011). Forensic Science International; Genetics, 5, 194–198.
[2] J. Hedman, K. Gustavsson, R. Ansell (2008). Forensic Science International; Genetics Supplement Series, 1(1), 430–432.

P 214 THE EFFECT OF INCREASED CYCLE NUMBERS FOR QUANTIFILER® DUO DNA QUANTIFICATION KIT ON THE DETECTION OF SMALL AMOUNTS OF MALE DNA IN MIXTURES AND THE USE IN ROUTINE CASE WORK

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In this study the effect of increased cycle number conditions for Quantifiler® Duo DNA Quantification Kit (AB) on the detection of small amounts of male DNA in male/female mixtures was tested. First male/female mixtures with different sample ratios were prepared from standard control DNA's. Each mixture was quantified 10 times with Quantifiler® Duo DNA Quantification Kit using both 40 and 45 cycles and the results were compared. Afterwards from our routine case work samples more then 400 mixtures of different male/female ratio were quantified using the 45 cycle conditions. Depending on the mixture ratio and the over all DNA amount, the samples were grouped and typed with 17 autosomale and/or 16 Y-chromosomale STRs. Each profile was confirmed by a second multiplex PCR. The quality of the profiles refers to allelic and locus drop outs were estimated. The reliability of the quantification results refer to the detection of minimal amounts of male DNA was compared with the results of Y-chromosmale STR analysis.

P 215 PRELIMINARY VALIDATION OF PREPFILER EXPRESS™ EXTRACTION KIT IN AUTOMATE EXPRESS DNA EXTRACTION SYSTEM

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Introduction and aims
A variety of challenging biological samples, including blood stains, saliva, semen, hair, bones, finger nails, among others, are often a part of our casework investigation.
In this study, semen, blood samples and saliva swabs were extracted by several methods in order to optimize and validate the Prepfiler Express™ Extraction kit and the AutoMate Express DNA Extraction System.
Results obtained with the robot (using silica-coated magnetic beads) were compared with methods based on a chelating resin, silica membranes and paramagnetic resin.

Materials and methods
A set of 30 samples (10 blood stain, 10 semen stain and 10 bucal swabs) were extracted with QIAamp®DNA Investigator (Qiagen), Chelex®100 method (Walsh *et al.*, 1991) and Prepfiler Express™ Extraction kit (Applied Biosystems). The samples were quantified with Human Quantifiler™ kit (Applied Biosystems), according to manufacturer's instructions using an ABI Prism® 7000 (Applied Biosystems). The DNA extracts were amplified with the AmpFℓSTR® Identifiler® Direct PCR Amplification kit and the amplified products were analyzed on a 3500 Genetic Analyzer using GeneMapper® ID-X 1.2 Software as described in the User Guides (Applied Biosystems).

Results and discussion
Comparing the several extraction methods, the AutoMate™ Express DNA Extraction System allows to reduce time consuming, improve the quality of extracted DNA and to increase efficiency.
In a preliminary study we were able to conclude that the robot enables to provide a quantity of amplifiable pure DNA to obtain a complete genetic DNA profiles in samples such as blood stains, semen samples and bucal swabs.

P 216 VALIDATION OF AmpFℓSTR NGM SElect™ PCR Amplification Kit ON FORENSIC SAMPLES

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The AmpFℓSTR NGM SElect™ is a next generation kit developed by Applied Biosystems that contains the same 16 loci as the NGM Kit, plus the highly discriminating SE33. This permits exchange of data with several central European countries using SE33 routinely.

We performed a validation study of the NGM SElect Kit with evaluation of critical parameters as species specificity, sensitivity, degradation/inhibition study, performance on a wide variety of forensic samples, mixture sample analyses.

Our study confirmed the inclusion of mini STR loci in the multiplex, together with a robust PCR chemistry allows better analysis of degraded/inhibited forensic samples.

P 217 VALIDATION OF BTA™ LYSIS BUFFER FOR DNA EXTRACTION FROM CHALLENGED FORENSIC SAMPLES

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Isolation of DNA from forensic samples is strongly conditioned by many factors such as wide variety of samples, degradation by environmental exposure, presence of PCR inhibitors or limited quantity of starting material.

BTA™ lysis buffer, is a new formulation reagent. By Applied Biosystems that is designed for challenged forensic sample types such as Bone, Teeth, and Adhesive-containing substrates since it destroys more efficiently complex matrices.

BTA performance in the extraction of genomic DNA has been evaluated from real casework powdered bones, teethes, chewing gum, cigarettes, tape adhesive lifts.

DNA extracted from these samples was in sufficient quantities for downstream applications; the method was successfully able to remove inhibitors producing highly purified DNA; IPC values of Quantifiler® Human DNA Quantification Kit were consistent and within the normal range.

The procedure also provided conclusive, reliable profiles using the AmpFℓSTR® NGM® PCR Amplification kit even when working with difficult samples.

P 218 COMPARISON OF Identifiler®, Identifiler Plus® AND Minifiler® PERFOMANCE IN A FIRST PATERNITY TESTING STUDY FROM OLD SKELETAL REMAINS IN THE FORENSIC AND LEGAL MEDICINE AREA OF THE GOVERNMENT OF ANDORRA (PYRENEES COUNTRY)

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The problem that forensic scientists most often face when working with DNA extracted from exhumation and skeletal remains is either DNA degradation or DNA contamination. Various methods have been used to improve the identification of skeletal remains by DNA technology. Most of these systems include short tandem repeat (STR) analysis.

We describe here our work on the identification of individuals involved in a paternity testing in which all parents, mother and two alleged fathers, had died between 10 and 58 years ago.

Trace amounts of highly degraded human DNA were successfully extracted from each bone. Despite the presence of DNA inhibitors in the amplification, microsatellite alleles could be reproducibly amplified from the femur. DNA was extracted from bone which was turned into fine powder and proteolytic digested followed by phenol/chloroform purification and Centricon®-100 filtration.

Our experience indicated that the AmpFℓSTR® MiniFiler™ used as a complement to the AmpFℓSTR® Identifiler Plus™ Kit is extremely sensitive multiplex STR amplification systems. They have been successfully used to obtain multilocus STR profiles from bone samples with minimal amounts of human DNA.

We report genotyping results obtained from each kit and biomathematical results of paternity testing case.

P 219 IMPROVING THE SNPforID 34-SNPPLEX AND OPTIMIZATION FOR POP7™

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The 34-SNPplex designed by the SNPforID consortium for inferring biogeographical ancestry [1] was designed for POP4™ and POP6™ electrophoresis polymers. When this polymer is used on a 3130xl Genetic Analyzer, closely spaced peaks within the first 30–40 bp cannot be resolved due to the higher viscosity of the polymer leading to peak blobs on the electropherogram. We have adjusted the 34-SNPplex assay for use with POP7™ and optimized its performance.

Buccal swab DNA-extracts were amplified using an enhanced version of the 34-plex PCR assay with increased final extension time (60 min) and Taq and magnesium concentration (3 units and 8 mM respectively) to increase signal/noise ratios compared to the original assay. After spin-column purification, PCR amplicons served as template for single-base extension using a 16 and 18-plex SNaPshot™ multiplex assays with increased annealing time (15 sec) and adjusted primer concentrations to decrease peak height differences between SNPs. Reaction products were cleaned up using SAP and fragments separated on a 3130xl Genetic Analyzer. Results were interpreted with GeneMapper v3.2.1.

Separating and redesigning the 34-plex into two individual multiplex SNaPshot™ assays allowed the use of POP7™ for separation of the fragments and resolved the single SNP blob at the beginning of the electropherogram. Validation of the assay included reproducibility, sensitivity (complete DNA-profiles up to 0.5 ng) and specificity studies (no cross-species interaction). A small population study (n=68), including 50 Belgian samples, further validated the assay for future forensic use.

[1] C. Phillips, A. Salas, J. J. Sanchez, M. Fondevila, A. Gomez-Tato, et al., Inferring ancestral origin using a single multiplex assay of ancestry-informative marker SNPs, Forensic Sci Int Genet 3-4 (2007) 273-80.

P 220 ANALYSIS OF COMPLEX LOW TEMPLATE MIXTURES USING CONSENSUS METHODS AND POOL-PROFILES

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Forensic casework samples regularly contain cell material of multiple contributors. Analysis of mixed DNA profiles is especially complicated when the LT mixture is unbalanced. For these samples, methods that sensitize DNA typing meet with serious constrains as increased cycling or higher injection settings will result in over-amplified DNA profiles. To assist profile interpretation notwithstanding LT effects like allele drop-out and allele drop-in, one can try to infer the genotype(s) by a consensus method. An alternative approach may be to generate a pool-profile by injecting a blend of independently amplified PCR products on a capillary electrophoresis instrument. In this study, we compared the performance of the consensus method n/2 and pool-profiles using three or four NGM amplifications of a large set of complex LT mixtures (various ratios, up to four contributors of (un)related individuals, some with sporadic contamination). The n/2 consensus-profiles and pool-profiles showed similar percentages of detected alleles. Both approaches rarely included drop-in alleles when using pristine DNA samples. The pool-profiles allowed more methods to estimate the number of contributors or the mixture proportion than the consensus approach as pool-profiles contain peak height information. A striking observation was that the consensus and pool-profiles could contain different alleles from the LT component(s). Consensus profiles include alleles that are reproducibly amplified; pool-profiles contain alleles that have sufficient peak height for detection in a blend of independent amplifications. Therefore, both strategies (or a combination there-of) may be useful in the analysis of complex LT mixtures.

P 221 RECOVERY OF DNA PROFILES FROM SALIVA AND TOUCH EVIDENCES AFTER POSTAL BOMB EXPLOSION

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In the last ten years the world has had deal with an increasing terrorist crime both in national contexts (recently in Italy in Greek, Swiss and Chile embassies in Rome and in an Italian Army barrack in Livorno) and in military missions abroad (Iraq, Afghanistan). In national environments the most diffused improvised explosive devices were postal bombs often inflicting severe injuries and even the death on people.

Based on the experience gained in several forensic investigations concerning terrorist attacks, we evaluated the possibility to recover after the explosion biological traces useful to identify the postal bomb manufacturer's. We tested saliva and touch evidences, usually recoverable from a postal package, deposed on different parts of the postal bombs, both inside and outside the device, charged with deflagrating (black powder) or detonating explosives (TNT, RDX) in different quantities. After the explosions postal bomb fragments were collected and analysed by STRs multiplex PCR to discover DNA profiles useful for identification purposes. For every kind of explosives the percentage of correctly assigned genotypes was relevant and increasing depending on the location of the stain with respect to the position of the explosive. Full STRs profiles derived in the 75% of cases from traces located on the circuit of the device (especially on the tapes) and, almost always, from traces on the external envelope (stamps) suggesting the relevance of a accurate recovery of the postal bomb debris at the crime scene.

P 222 PRESUMPTIVE IDENTIFICATION AND STR DNA TYPING ANALYSES OF BLOOD EVIDENCES IN LOW TEMPLATE DNA CONDITIONS

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Bloodstains are the most widespread kind of biological evidences at the crime scene and one of the most used reagents for the presumptive identification of blood for forensic purposes is tetramethyl-benzidine. In our laboratory we have introduced and validated the tetramethylbenzidine-based Combur[3] Test® E, a colorimetric catalytic test based upon the detection of the peroxidase-like activity of the haemoglobin, due to its high sensitivity, easiness of use and capability to maintain the complete structural and morphological integrity of the bloodstain. Particularly we investigated the possible positive interferences of the test on the subsequent short tandem repeats (STRs) DNA typing analyses, especially in Low Template DNA (LT DNA) blood traces when some of the test reagents may soak the stain and their interference may be much more relevant than in non-LT DNA conditions.

Several samples of bloodstains stored in different environmental conditions since 1975 were diluted down to 10^{-7} and spotted on several surfaces, then tested by Combur[3] Test® E and STRs genotyped by new generation PCR multiplexes.

Contrary to previous results obtained by other authors on a similar test and unlike other presumptive and immunochromatographic confirmative tests, we have demonstrated that the Combur[3] Test® E did not significantly affect the STRs DNA typing analyses even in LT DNA situations where the blood material is poor and no confirmative tests were applicable, thus providing reliable information on both the donor's genetic profile and the presumptive nature of the bloodstain.

P 223 DNA TYPING OF LATENT BLOODSTAINS TREATED BY A NEW LUMINOL TEST FORMULATION BASED ON TITANIUM DIOXIDE NANOPOWDER CHEMILUMINESCENCE ENHANCER

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Luminol assay is undoubtedly the most common technique that is used by forensic scientists to investigate on latent blood traces. In the recent years several formulations, using either patented luminol molecule modifications or luminol blood-dependent chemiluminescence enhancers, have been proposed to quantitatively improve intensity and endurance of the emission. We recently developed, patented and validated for the specific forensic use a new luminol formulation method involving three steps: a preliminary application of a titanium dioxide (TiO_2) nanopowder water suspension over a suspected area followed by its drying and, finally, by the spraying of a luminol-based formulation containing carbamide peroxide, as oxidant agent. This method provides a strong increase in the intensity, sensitivity and duration of luminol chemiluminescence. In this work we evaluated the possible positive interference of this method with the new generation STRs DNA typing analyses by preparing several blood dilutions, spraying them with TiO_2 suspension and, after 30 minutes, with the luminol preparation and, in parallel, with TiO_2 alone or with luminol alone. Quantification data and DNA typing electropherograms demonstrated the lack of damage of this new luminol treatment on the recoverable genetic material and suggested its compatibility with the DNA typing analyses. Thus we endorse the operational forensic use of this new luminol method for its relevant chemical performances in the detection of minutes amounts of blood without provoking alterations in quality and quantity of the genetic material or affecting the subsequent STRs DNA typing procedures.

P 224 DNA TYPING STRATEGY TO OVERCOME POST-MORTEM BONE MACERATION

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Chemical maceration is an useful procedure to remove soft tissues and to bleach bones facilitating the forensic anthropologist examination. However, prolonged exposure to chemicals often compromises DNA recovery and its amplification via PCR. Our purpose was to test the new generation multiplexes to type nuclear DNA and contemporarily to verify the possibility of mt-DNA sequencing. We used a femur belonging to human skeletal remains that had been buried many years in a moist soil and, after exhumation, had been immersed in a hydrogen peroxide 30% solution for 12 hours.

A total amount of a hundred grams of bone powder was submitted to organic extraction, Qiagen manual and automatic extraction, Promega Maxwell 16 extraction. After many attempts, we obtained a partial profile from the 8 smallest loci of nuclear DNA by using 1 g of bone powder decalcified in EDTA, lysed by Maxwell 16 Biorobot extraction, concentrated with the Vivacon 500 device (30 kDa MWCO) and finally amplified with the NGM multiplex kit (AB); no other kits and extraction strategies were able to provide any acceptable results. Otherwise, mt-DNA sequencing was easily performed as described by Eichmann and Parson (2008).

Finally we suggest not to use chemicals to macerate human bones for the purpose of identity or at least to save a fragment of femur from the treatment and we indicate the analytical strategy, described in the lines above, as the best method to obtain a nuclear-DNA profile.

P 225 COMPARISON OF FOUR DNA EXTRACTION METHODS FOR FORENSIC APPLICATION

Bogas, V.[1], Balsa, F.[1,2], Carvalho, M.[1,2], Anjos, M.J.[1,2], Pinheiro, M.F.[2,3,4,5], Corte-Real, F.[2,6,7]

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Introduction and aims: Challenging biological samples found in crime scenes are often brought to our lab. Several factors, such as degradation and the presence of inhibitors, can difficult the analysis of these samples. Chelating resine, silica membranes, silica-coated magnetic beads and paramagnetic resine are DNA extraction techniques widely used in our lab.
Our aim was to find out the DNA extraction method more suitable to overcome problems raised by these samples and to enable the identification of its genetic profile.

Material and methods: Blood was collected from three non related donors and blood stains were made in fabrics such as denim, cotton and lycra, previously washed and decontaminated. Blood stains were dried during 3 days at room temperature before being placed in different degradation places, like indoors and buried in different types of soil (sand, marsh and clay). Small pieces of each stain were collected after 1, 3 and 7 days.
Positive controls (blood stains of each individual) were made in all type of fabrics.
DNA extraction was performed using Chelex 100 method, QIAamp®DNA Investigator kit, DNA IQ™System kit and with AutoMate *Express*™ Forensic DNA Extraction System. DNA was quantified with an ABI Prism®7000 using *Quantifiler™ Human DNA Quantification kit*. Samples were amplified with *AmpFℓSTR®IdentiFiler™*, *AmpFℓSTR®MiniFiler™* and *PowerPlex*®ESi17 System kits and analyzed in ABI Prism®3130 and ABI Prism®3500 Analyzers.

Results and discussion: The AutoMate *Express*™ Forensic DNA Extraction System was the best extraction method, which enabled the recovery of more quantity of high molecular weight DNA, probably by efficiently removing the PCR inhibitors, such as the humic acids.

P 226 VALIDATION OF THE AMPFℓSTR® IDENTIFILER® DIRECT PCR AMPLIFICATION KIT IN A LABORATORY ACCREDITATED ACCORDING TO THE ISO17025 STANDARD

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Introduction and aims
At the of Section of Forensic Genetics, Copenhagen, buccal samples deposited on FTA® cards are used as sample materials in paternity case work and for crime DNA database samples. In the standard protocol, the FTA® cards are punched, washed and dried prior to PCR amplification. The AmpFℓSTR® Identifiler® Direct PCR Amplification kit (Identifiler® Direct) was developed to amplify the 16 loci directly without washing and drying of the FTA® card punches. Here, we describe the validation of the Identifiler® Direct kit for routine case work in a ISO17025 accredited laboratory.

Materials and methods
A total of 249 FTA® card samples were investigated with the Identifiler® Direct kit.PCR amplification was performed according to the manufacturer's protocol with modifications in the reaction volume (10–25µ, the number of PCR-cycles (25–27) and the number of FTA-card punches (1–2).

Results and discussion
Full concordance was observed between the results from the Identifiler® Direct kit and the washed punches typed with the Identifiler® kit. The success rate with one 1.2mm punch in a 15 µl PCR reaction volume and 25 PCR-cycles was 93.3%. After retyping the failed samples, the combined success rate was 98.7%. Overall, the quality of the results was reduced when the number of punches was increased or the PCR reaction volume was decreased. The Identifiler® Direct master mix may be used in combination with other primer sets for direct amplification of other loci, e.g. the 49 SNPs using the SNP*for*ID multiplex assay.

P 227 AMPLIFICATION OF NON-FTA SAMPLES WITH AMPFℓSTR® IDENTIFILER® DIRECT PCR AMPLIFICATION KIT

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Introduction and Aims
The AmpFℓSTR® Identifiler® Direct PCR Amplification Kit is a short tandem repeat (STR) multiplex assay, optimized to amplify 16 loci directly from biological material (blood or buccal cell samples) stored in FTA paper, without resorting to DNA extraction. However, preliminary experiences have shown the versatility and robustness of this amplification kit, when applied to other biological sample supports. The aim of this research work is to analyze the results of this kit when applied to non FTA paper or other support for biological samples such as a buccal swab.

Materials and Methods
To carry out this study, non-FTA blood samples (Whatman® blood stain cards) and its respective buccal swabs (Whatman® Sterile Omni Swabs) were amplified, for AmpFℓSTR® Identifiler® Direct PCR Amplification (Applied Biosystem), in the direct form. The chosen samples had been previously extracted with Chelex® 100 and amplified for AmpFℓSTR® Identifiler® Plus PCR Amplification Kit, in order to validate the results. The results were compared with samples amplified directly from FTA paper.

Results and Discussion
After analyzing the results, it is possible to verify that full profiles were obtained with a high success rate in both non-FTA samples (blood stains and buccal swabs). The amplification quality is very similar when using FTA paper.

P 228 NEW ALTERNATIVE FOR HUMAN IDENTIFICATION. REPRODUCIBILITY KIT INVESTIGATOR IDPLEX – QIAGEN®: STUDY INTERLABORATORY LATIN AMERICA

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Currently there are several types of genetic markers for human identification and establishment of biological relationships, among them are commonly known as STRs. From 2010 QIAGEN® made available to eight kits of different combinations of STRs, including the Investigator IDplex Kit. This kit allows amplification in one PCR reaction markers D2S1338, D19S433, the 13 CODIS loci and amelogenin.
The aim of this study was to evaluate the reproducibility of IDplex Investigator Kit-QIAGEN® among Latin America laboratories.
In the framework of the III INTERNATIONAL THEORETICAL-PRACTICE COURSE IN POPULATIONS GENETIC AND BIOLOGICALS FILIATIONS in Medellín-Colombia, invited participants to evaluate the reproducibility of the kit provided to each the necessary materials for the study. The results reported by participating were tabulated for the study of concordance and reproducibility. One result is concordance when at least three laboratories reported results and at least 70% of them agree on the value.
Results and comments were received on the agreed date of 12 of the 22 laboratories registered, a participant submits comments only. There was a 100% concordance for the 16 markers by 10 laboratories, one laboratory showed concordance for 14 markers (87.5%) included in the kit.
Some laboratories reported greater sensitivity IDplex Investigator Kit-QIAGEN® compared with other existing containing similar markers, also highlight the easy adaptability to existing conditions in laboratories, without involving major changes to its implementation.
This work showed the reproducibility of the kit Investigator IDplex-QIAGEN® and provides an alternative for human identification with DNA from different sources.

P 229 MODIFICATION OF THE PROTOCOL "SALTING-OUT" TO ISOLATE DNA FROM URINE SAMPLES

Builes, J.J.[1], Manrique, A.[1], Ortiz, I.[2], Martínez, L.M.[2], Agudelo, C.A.[2], Vásquez, D.A.[2], Toro, A.E.[2], Barguil, I.[2], Cuesta, D.[2], Aguirre, D.[1], Puerto, Y.[1], Bravo, M.L.[1]

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DNA isolation from samples of different tissues is required for identification and analysis in complex cases or crimes scenes when we have not access to classical samples. We have made a modification in the classic protocol for isolate DNA "salting-out" from Miller (1988) with the aim of isolate DNA present in urine fluid from healthy unrelated male samples of students of Universidad Pontificia Bolivariana in Medellín Colombia.

The DNA quantification was made with a spectrophotometer and the DNA concentration was between 10 to 40 ng/per µl. For analyze the quality of the DNA we have made a PCR with 5 STRs markers; D16S539, D7S820, D13S317, D5S818 and Penta D, PCR products were separated by electrophoresis in denaturing polyacrylamide gels silver stained and allele designation was performed with positive controls like and allelic ladder constructed in-house. All the markers were successful typed in a rate of 65%, and the rate was increasing when decreasing the number of markers. For this reason we propose use mini STRs for typing this kind of samples due they can allow a more accurate typing.

P 230 SE33 VARIANT ALLELES: SEQUENCES AND IMPLICATIONS

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Among the 23 short tandem repeat (STR) loci commonly used in commercial STR kits, SE33 is by far the most polymorphic locus possessing 58 alleles and 343 genotypes in 1443 U.S. population samples [1]. A review of the SE33 literature has found more than 175 alleles when internal sequence rearrangements are included [2]. The high degree of variation with SE33 can potentially impact PCR primers and amplicon mobility. Sample sets have been tested with PowerPlex ESX 17 and ESI 17 as well as NGM SElect to explore any concordance issues between kits possessing primers in different positions. A G≠A mutation 68 bp downstream of the repeat region has been detected in several samples that can cause a mobility shift in PowerPlex ESI 17 relative to PowerPlex ESX 17 and NGM SElect SE33 alleles. The observed frequencies and potential implications of flanking region differences will be discussed.

[1] C.R. Hill, et al., Concordance and population studies along with stutter and peak height ratio analysis for the PowerPlex® ESX 17 and ESI 17 Systems, Forensic Sci. Int. Genet. (2011). DOI:10.1016/j.fsigen.2010.03.014.
[2] J.M. Butler, Appendix 1 in Advanced Topics in Forensic DNA Typing: Methodology (2011). Elsevier Academic Press: San Diego.

P 231 ALTERNATIVE POLYMERASES FOR LOW TEMPLATE DNA USING DIRECT PCR

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AmpliTaq Gold is a standard polymerase which is used in many of Applied Biosystem's forensic amplification kits, for example SGMPlus and Identifiler. Studies have shown that AmpliTaq Gold is not necessarily the best polymerase to be used for samples with inhibitors, though no studies have been conducted for low template DNA or Direct PCR. This study was aimed at looking into the efficiency of seven PCR polymerases and buffers for low template DNA using Direct PCR and the suitability of these polymerases for multiplexing. 1 ng total DNA was used with two sets of primers for polymerase optimisation. A total of 0.1 ng and 0.05 ng of DNA was used in the amplification reaction to represent low template DNA and amplified with the polymerases and their corresponding buffers. The results were then compared with AmpliTaq Gold DNA polymerase. In the second part of the experiment, the diluted DNA samples were placed on glass microscope slides and dried overnight. A sterile swab was used to retrieve the DNA deposited on the glass, and the swab was subjected to Direct PCR using two primer sets with the seven different polymerases. Finally a set of swabs were prepared for Direct PCR using SGMPlus by substituting AmpliTaq Gold with the best performing polymerases and their buffers. Initial results indicate that alternative polymerases are more efficient at amplification low template DNA than AmpliTaq Gold. Further experimentation is needed to definitively test these findings.

P 232 TWO 16S rRNA MITOCHONDRIAL MARKERS FOR SPECIES IDENTIFICATION IN FORENSIC SCIENCE

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In the recent years, with the development of molecular biology technology, new methods for human identification to forensic purposes, based on genetic differences among the animal species have been proposed. The analysis of cytochrome b (cyt b) showed a good feasibility to species determination and individual human identification.

The use of cytochrome b is well-known for species detection, even if sequence analysis and comparison in BLAST made this analysis troublesome in case of degraded samples from which it is difficult obtained a good sequence.

In this paper we propose a method for the identification of human samples based on the amplification of a duplex PCR corresponding to two 16S rRNA mitochondrial fragments, an universal fragment of 236bp and a human-specific fragment of 157bp. A rapid analysis of the PCR products can be performed in a mini poliacrylamide gel.

The primers for both 16S rRNA fragments were designed by us. Since these two 16S rRNA fragments are small, they amplify easily even in the presence of old and highly degraded specimens in a single round PCR; for these reasons, this method could result very useful for forensic purposes in case of human species identification.

P 233 ROBUSTNESS AND INTEGRATION OF A LYSIS BUFFER FOR DNA EXTRACTION FROM CALCIFIED TISSUES AND OTHER COMPLEX SAMPLE TYPES

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Previously, we described a novel lysis reagent (B&T Lysis Reagent) that significantly improved the yield and purity of DNA extracted from complex sample types involving challenging substrates such as calcified tissue, adhesive, paper and gum matrices. Further work on this reagent has culminated in the development of a lysis buffer that is optimized and validated for high quality DNA isolation for Bone, Tooth and Adhesives ("BTA"). The BTA lysis buffer is compatible with existing PrepFiler® extraction chemistry and processing workflows.

Comparative chemistry studies were conducted with BTA lysis buffer extraction of a set of standardized mock forensic samples consisting of bone, tooth, chewed gum, E-cell-laden adhesive tape, envelope flaps and cigarette butts. Endpoints for performance evaluation included: DNA yield and STR profile quality as determined by Intra-Color Balance (ICB) ratios and percentage of alleles recovered. Overall, samples extracted with BTA lysis buffer returned results that were either comparable or superior to other methods tested, which included Phenol/Chloroform/Isoamyl Alcohol (PCI) and comparable commercial extraction kits.

The BTA extraction process yielded several advancements in addition to the performance criteria described. First, relative to PCI, it significantly reduces the likelihood of error and difficulty involved in DNA extraction, as it involves substantially less sample handling and tube transfers. Second, in comparison to comparable commercial kits, the protocol is more streamlined and universal over this diverse set of sample types, enabling sample batching and automation integration. And finally, with a two hour lysis incubation time, even calcified tissue samples can be robustly processed from extraction to genotype profile generation within a single day.

P 234 DNA EXTRACTION USING THE QIASYMPHONY: EVALUATION OF PCR INHIBITOR REMOVAL

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Increased forensic applications and demands are causing many laboratories to consider high throughput technologies such as robotic platforms for DNA extractions. However, in a forensic laboratory the routine application of an automated DNA extraction and purification robot has to fulfill several conditions, like efficiency of DNA recovery, high quality DNA suitable for profiling, no cross-contamination, fast and reproducible. Ideally the throughput technology should perform as well as, if not better than the manual method it is replacing.

The goal of this study was to compare the quantity and quality of DNA extracted using the QIAsymphony robot with that of casework-validated manual extraction procedure QIAamp DNA mini kit, currently in use in our laboratory.

We found that the yield of manually prepared DNA was still 1.5 to 3.5 times higher than that from the automated procedure, however QIAsymphony is 6 to 20 times more efficient at removing PCR inhibitors than the manual protocol and showed no cases of DNA cross-contamination among samples of a series.

In conclusion, the comparison of manual QIAamp DNA mini kit and automated QIAsymphony DNA extractions confirmed a known advantage for automated methods based on silica-coated magnetic beads, which is the efficiency in eliminating PCR inhibitors from extracts. Such feature may compensate for the lower DNA yield observed when the procedure is automated. Less hands-on time and the fact that a greater number of samples can be processed at once make the QIAsymphony an alternative to QIAamp extractions.

P 235 HOW TO MIMIC REALISTIC CASE WORK SAMPLES FOR SMALL SCALE IN-HOUSE VALIDATION – A SERVING SUGGESTION

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Validation of in-house and commercial protocols for DNA-extraction and PCR analysis has become a central issue in improving and amending laboratory techniques in forensic DNA laboratories, especially those focussing on accreditation requirements. In order to compare a manual (GenIal First-DNA-Kit) with a semi-automated (QIAGEN M48) DNA-extraction protocol, we established a procedure that mimics realistic casework samples suitable for comparative study. The primary objective was to establish a means by which one could acquire a number of samples sufficient for several extractions while ensuring equal amounts of target DNA, inhibitors and other compounds with the potential to affect DNA extraction and yield quality.

Suitable amounts of source material (swabs, cloth, etc.) were incubated in a TE/SDS buffer in a 1,5 ml reaction tube. Subsequently the samples were vortexed, punctured with a glowing hot needle and placed on a new tube. Suspended source material was collected by sharp centrifugation and re-suspended before being applied to new swabs. Air-dried swabs underwent DNA-extraction according to the manufacturers guidelines, yielding equal volumes of purified DNA. PCR analysis was carried out with AmpFℓSTR SGM+ followed by a pre-analytic slab gel and capillary electrophoresis (ABI PRISM 310).

Comparing the band intensity on the slab gels, peak height in CE-data and completeness of expected STR profiles, manual DNA extraction both with and without subsequent purification clearly provided the highest quality results. Semi-automated DNA extraction employing QIAGEN M48 however appeared to better remove inhibitory compounds, but was coupled with reduced signal intensity in post-PCR analysis.

P 236 INVESTIGATION OF PATERNITY LOSS: IS STATISTICAL EVALUATION RELIABLE? A CASE REPORT

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Some years ago, the expert witness of a laboratory was called by the court to conduct an analysis to verify the paternity of a woman whose mother was alive, while the father was deceased. However, the father had three siblings (two females and one male) that were available for genetic analysis, allowing the reconstruction of the deceased father's genetic profile.

The comparison between the obtained results performed by Identifiler (ABI), the woman and her mother's genetic profiles, revealed two genetic incompatibilities, and also among the genetic profiles of the three siblings one incongruence for a marker was evidenced. The data were processed with the computer program "Familias" to evaluate whether these discrepancies were due to a paternity exclusion rather than a double allelic mutation.

The evaluation suggested a paternity exclusion, also supported by the study of four Xmarkers on the using ArgusX (Mentype). Per request of the mother, a second expert witness of laboratory was called by the court to carry out the exhumation of the father for a direct comparison of the genetic profiles among, mother, daughter and father's samples.

The expert of the second laboratory concluded for an exclusion of the paternity on the basis of incompatibilities regarding 8 polymorphisms. This result confirmed what previous analysis conducted by laboratory a had evidenced, thus highlighting the value of the first analysis even without the necessity of exhumation.

Starting from this experience the authors discussed, in general terms, the reliability of statistical evaluation in deficiency paternity testing.

P 237 A NEW METHOD FOR ABO BLOOD TYPING USING CONVENIENT DNA CHIP TECHNOLOGY

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Although DNA profiling using short tandem repeat (STR) and single nucleotide polymorphisms (SNPs) is currently most commonly performed in forensic analysis, ABO blood typing is still performed as one of the crucial tests for rapid individual identification of the subject in crime investigations. As replacements for conventional analytical methods (hemagglutination test, heat-elution test, hemadsorption test, etc.), various new ABO genotyping methods using ABO glycosyltransferase genes have been reported in recent years, including PCR-SSCP, SNaPshot®, and TaqMan® Probe Real Time PCR. However, because the new methods require special equipment and expensive reagents, it is difficult to perform these methods in a normal environment. Therefore, in this study, we evaluated a simple ABO genotyping method based on convenient DNA chip technology that does not require specific equipment or reagents. We selected three nucleotide positions in the cDNA of ABO glycosyltransferase genes, 261 and 297 in exon 6 and 796 in exon 7, and visualized the SNPs of each position by the staining method using avidin–biotin complex and alkaline phosphatase–BCIP/NBT. We confirmed that the ABO genotypes of all experimental subjects were consistent with their ABO phenotypes that we had previously detected by the hemagglutination test. This new method for ABO blood typing using convenient DNA chip technology may become an effective method for the individual identification of unidentified cadavers.

P 238 TEST OF THE RAPID PCR METHOD USING AmpFℓSTR IDENTIFILER KIT

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Current forensic DNA typing is conducted in approximately 8∼9 h. Whole steps included DNA extraction step, Quantification, PCR amplification step, electrophoresis process through capillary separation with fluorescence detection, data analysis and DNA profile interpretation. Among them, we have tested rapid PCR method of AmpFℓSTR Identifiler PCR to reduce running time. We have altered several PCR conditions of ordinary AmpFℓSTR Identifiler PCR method and used 9947A control DNA to cut the time for the PCR reaction. In the results of this study, the critical step of the PCR reaction was the annealing step and also we reduced PCR running times by 1/3 to 2/3 (approximately 60–90 minutes) with complete concordance of STR allele calls using standard reference material 9947A.

Keywords: DNA typing, Identifiler, Rapid PCR

P 239 LONG TERM ROOM TEMPERATURE HUMAN CORPSE SOFT TISSUE PRESERVATION: AN EFFICIENT STRATEGY FOR LONG-TERM TISSUE SAMPLE STORAGE

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Preservation of biological samples is a key aspect when any biomedical analytical procedure needs to be performed. The aim of this work was to develop a simple and reliable strategy for human soft tissue storage at room temperature that ensures extraction of DNA quality suitable for PCR-based DNA typing.

Fragments of human psoas muscle were exposed to three different environmental conditions for diverse time periods at room temperature: a-preserving medium, b-without any additional substance and c-soil. Time intervals for environmental exposure ranged from 1 to 365 days. After Real Time PCR quantitation (*Plexor, Promega*), a set of forensic autosomal and Y chromosome STRs were typed. mtDNA and Y haplogrouping was also performed on samples exposed to condition a- and b-.

DNA recovery from samples in contact with preserving medium were similar along 365 days of storage: 397.9 ± 131.5 ng/mg and 200.7 ± 73.29 ng/mg of tissue for autosomal and Y-chromosome DNA, respectively, compared with the samples stored without any substance: 81.6 ± 123.6 ng/mg and 41.1 ± 68.9 ng/mg of tissue for autosomal and Y-chromosome DNA, respectively. No DNA was recovered from samples exposed to soil after 2 days. Y-SNPs and mtDNA SNPs haplogrouping was successful in all the tested samples. As qualified by the expert software *GeneMapperID-X*, typing of autosomal and Y-STR showed an excellent electropherogram quality in all samples submitted to preservation medium compared with samples without any substance.

The procedure proposed is a cost efficient alternative for storage of human remains sampled in challenging environmental areas such as mass disaster locations, mass graves and exhumations.

P 240 EFFECTS OF PRESUMPTIVE TEST BUFFER ON DNA EXTRACTION

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Presumptive tests for detecting blood, saliva or seminal fluid represent an advisable step in forensic samples analysis, helping to make decisions concerning DNA extraction strategies.

Despite the protocol suggested by some comercial diagnostic kits that state the compativility of buffer solutions for samples treatment and further DNA extraction and amplification, these buffers might seruosly impair the following analytical steps.

Aiming to evaluated the potential buffer effect on DNA extraction a set of complex samples were artificially prepared, including three contributors (two blood and one seminal fluid donors). Ability to efficiently extract DNA using the presumptive test buffer supplemented with DTT (0.1mM) versus TEC-SDS (Tris/HCl 10mM pH=7.6, EDTA 1mM pH=8.0, NaCl 100mM – SDS 2%) supplement with DTT (0.1 mM). Both extractions were carried out using semi-automated DNA extractor Maxwell 16 (Promega, USA).

Although DNA quantitation using real time PCR (Plexor, Promega) showed a DNA concentration range between 0.54 and 3.5 ng in all samples, using both buffers, different results were obtained.

Autosomal and Y-chromosomal STRs amplifications detected only two genetic profiles from the sample extracted with the presumptive buffer-DTT, whereas TEC-SDS-DTT allowed detecting the three components of the mixture.

Our results emphasize the need of using a separate sample fraction for presumptive tests. The observation is particularly relevant for samples containing seminal fluid.

P 241 TOOTH PORTION PROFILE IN CRIMINOLOGY

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Up to date the mineralized tissue is the only option, in many extreme forensic conditions, to achieve a genetic profile.
In several samples, tooth type and portion were studied in order to optimize the methodology, to reduce time and costs. Results from a wisdom tooth (natural degraded sample) and topographic drawings of Gaytmenn and Sweet's study from 2003, are presented.
DNA from the root tooth (apical and remaining portions) was extracted by using the ArchivePure DNA Tissue kit (5Prime). Total DNA quantification was performed by real time PCR, by using the Human Quantifiler kit (Applied Biosystems). Depending on the DNA quantification value, the most relevant polymorphisms for genetic identification, autosomic STRs, were amplified with AmpFℓSTR® Identifiler™ and AmpFℓSTR® Minifiler™ kits (Applied Biosystems).

The two root tooth portions presented different results (in quantity and quality), with respect to DNA quantification and genetic profile. The apical portion allowed an autossomic profile while in the remaining portion a mitochondrial profile.
Our results can be explained based on the fact that apical canicular obliteration through the formation of terciary dentin can preserve some of the pulpar contents in the topographic region; on the other hand cementogenesis in the apical portion can occur by invagination into the canalicula and canaliculi in a rapid and disorganized fashion enabling the trapping of cementocites in its lacunae. Furthermore we must add that it is the apical portion that through its cellular content potentially presents us with the best results in genetic analysis.

Keywords: genetic ID, root tooth portion

P 242 INDELS IN Y CHROMOSOME HAPLOGROUP DEFINITION

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The characterization of Y chromosome haplogroups is currently done by genotyping SNPs and a few InDels. However, InDels are increasingly gaining importance, so the aim of this work was to create an InDel PCR multiplex allowing a fast, simple and straightforward characterization of the main Y-haplogroups. For this, we have selected the InDels already accepted by the Y Chromosome Consortium, listed in Karafet *et al.* (Genome Research, 2008, 18:830). However, due to their position in the Y chromosome phylogenetic tree, they only allow classifying chromosomes from 6 of the 20 main Y haplogroups. Thus, we have extended the search to already described and validated InDels in dbSNP (http://www.ncbi.nlm.nih.gov/snp/) and MGS (http://research.marshfieldclinic.org/genetics/home/index.asp). All 154 InDels retrieved from that search were subjected to multiple screenings and just 10 were found to be new, potentially polymorphic and Y specific. Their typing in samples for 13 distinct haplogroups confirmed only 2 polymorphisms (named M2 and M14). M2 is polymorphic in R haplogroup but it also shows a reversion within the R1b1b2 sub-haplogroup. Therefore, it is not recommended for the characterization and distinction between R and the other haplogroups. M14 shows variation in R and Q and so it can be used to identify samples belonging to the paragroup P, which was not possible using the InDels in Karafet *et al.* (2008). The detailed analysis of all the available information allowed us to conclude that the creation of the aimed multiplex will only be possible with the detection and phylogenetic characterization of new InDels.

P 243 CONCORDANCE TESTING WITH THE NEW STR KITS AND THE INFLUENCE ON THE SWISS NATIONAL DNA DATABASE

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The European Network of Forensic Science Institutes and the European DNA Profiling Group recommended the extension of the European Standard Set by 5 additional loci regarding international DNA data exchange based on the treaty of Prüm. This recommendation launched the development of new STR Kits by the key market players Promega and Life Technologies (LT). In the last 10 years the Swiss forensic DNA labs have routinely used the same Kits (AmpFℓSTR® SEfiler Plus™ and the AmpFℓSTR® SGMPlus™). Despite not being part of the EU nor the treaty of Prüm Switzerland has decided to implement the new STR Kits (including SE33). Several international laboratories reported difficulties with the concordance of the new STR Kits. We decided to conduct an in-house population study with roughly 1600 samples analyzed with AmpFℓSTR® SEfiler Plus™ and AmpFℓSTR® NGM SElect™ (LT) and PowerPlex® ESX 17 and PowerPlex® ESI 17 (Promega). On a low percentage level (so far 3 deviations in 550 samples), the results of the analyzed samples show that differences can occur using the different Kits. It is important to note that the extracted DNA from reference samples in Switzerland must be destroyed by law within 3 months, for this reason retyping of reference samples in case of a discordance is not possible. As a feasible consequence the Swiss Forensic DNA community is currently discussing the determination of one common single Kit for reference samples in the Swiss National DNA Database.

P 244 FORENSIC ANALYSIS OF MAGNETIC STRIPE SKIMMER DEVICES

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The number of cases with a magnetic stripe skimmer device, or skimmer, has increased since 2009 at the Swedish National Laboratory of Forensic Science (SKL). The primary function of a skimmer is to capture and store the magnetic stripe card track data. Additionally to this, the corresponding PIN must be retrieved to complete the information. Sweden is facing three major skimmer targets where magnetic stripe cards are found: pay-at-the-pump solutions, point-of-sale terminals (POS terminals) and automated teller machines (ATMs). Several skimmers with highly sophisticated miniaturised technology have been observed in our cases. A consequence with such tiny devices is the difficulties in handling, finding and securing DNA and fingerprints in combination with a technical analysis. Hence a successful multidisciplinary collaboration has been developed between the three disciplines; fingerprints, DNA and IT forensics and the initial investigation is performed with staff from the different disciplines. Different parts of the skimmers are analysed for DNA and for fingerprints. These parts are chosen in consultation with the personal at the IT department knowing how they are constructed. During the years 2007-2010 there were 36 cases that included skimmer devices. For DNA analysis 211 samples were collected and they were analysed with the LT DNA technique, normally 31 cycles (AmpFℓSTR® SGM Plus®). In 23 of the cases at least one sample gave a result with a useful DNA profile, which give a 69% success rate. This is a high success rate compared to other groups of items examined with LT DNA analysis.

P 245 SUCCESS RATE OF LCN/LT DNA ANALYSES

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Since 2004 low copy number (LCN) DNA analysis has been used in casework at the Swedish National Laboratory of Forensic Science (SKL). LCN or LT (low template) analysis is from our perspective defined as "interpretation of samples where the results are below the thresholds that are in use for conventional interpretation" and will include consensus analyses with 28 or 30–33 cycles. Documentation of results obtained has been compiled according to four different criteria: "A useful DNA profile important for the case", "A useful DNA profiles but not important for the case", "Too complex" and "Too weak". Along with the results, a description of the item and sample collection is enclosed. The items are grouped into nine different characters such as "Tools", "Electronics" and "Letters and envelopes". From 2007 to 2010 results were compiled in 419 cases. The overall success rate for these cases was 37%. A successful case is defined as having at least one sample with "A useful DNA profile important for the case". In these documented cases a total of 2033 samples were collected and analysed. A success rate value can also be received for samples according to the groups of characteristics. For example 498 samples were grouped into "Weapons and knives" and gave a success rate of 15,2%, which correspond to the most successful group. The success rate data generates information that can be of helpful use as guidance to select and recover trace DNA in pending cases.

P 246 DEVELOPMENTAL VALIDATION OF A FULLY AUTOMATED GENOTYPING ASSAY CAPABLE OF DETECTING LENGTH AND SEQUENCE VARIATION IN THE CODIS STR LOCI

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Single nucleotide polymorphisms (SNPs) have been observed in STR loci, but are not detected with conventional electrophoretic analyses. We have validated a high-throughput assay providing base composition analysis of the thirteen CODIS STR loci plus the amelogenin locus. Alleles are amplified with an 8-well PCR panel and the PCR products are directly analyzed on an automated electrospray ionization – mass spectrometry (ESI-MS) platform. The resulting mass determinations are converted to base compositions specifying the number of each of the nucleotides in the PCR amplicons, and STR profiles are derived from the base compositions. Notably, the accuracy of the mass measurements supports detection of SNP variants of STR alleles. Correct profiles were obtained with input levels up to 50ng DNA per well. Sensitivity on a per well basis was typical of PCR. No signals were observed with high levels of microbial DNA or nonhuman vertebrate DNA, and non-human DNA did not interfere with the analysis of human DNA. Results for over fifty samples were compared to results obtained with the Identifiler™ kit (Applied Biosystems). The accuracy and reproducibility of the assay were typical of other PCR/ESI-MS assays that we have developed. Sample panels were analyzed to characterize allele frequencies, the inheritance of SNP alleles, the occurrence of germ line mutations in trio sample sets, and the complex profiles derived from DNA mixtures. Results indicate that the assay is generally concordant with current methods, but the detection of SNPs provides additional diversity within many of the CODIS loci, thus increasing the discriminatory power of the assay.

P 247 DEVELOPMENT OF A GENOTYPING ASSAY FOR THE UK, EUROPEAN, AND CODIS CORE STR LOCI IDENTIFYING LENGTH AND SEQUENCE VARIATION IN THE TARGET LOCI

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We have developed a high throughput assay covering the twenty one markers that span the UK, European, and CODIS core STR loci. In this assay, sample DNA is amplified with an 8-well PCR panel, and the resulting products are analyzed on a fully automated electrospray ionization – mass spectrometry (ESI-MS) platform. The accuracy of the mass measurements made with ESI-MS is high enough to assign a base composition to the PCR products, specifying the number of their constituent dA, dG, dC, and dT residues. The base compositions in turn define the STR profile. Single nucleotide polymorphisms (SNPs) have been observed in many of the STR loci, and the accuracy of the mass measurements supports the identification of SNPs with this assay. Parameters for the evaluation of the assay include species specificity, sensitivity, reproducibility, accuracy, differential amplification effects in the multiplexed reactions, concordance, and allelic and interlocus balance. Microbial DNA and nonhuman vertebrate DNA were used to evaluate the specificity of the assay. The assay accurately typed samples across a wide range of DNA input levels and showed typical sensitivity on a per well basis. The profiles of a panel of human DNA samples were determined and the nominal alleles (ignoring the SNP determinations), allelic balance, and interlocus balance were compared to results obtained with the NGM SElect™ kit (Applied Biosystems). Preliminary evaluations indicate that assay results are generally concordant with existing assays while providing additional information by virtue of the detection of SNP variants of STR alleles.

P 248 THE FREQUENCY AND DNA YIELD OF NUCLEI ASSOCIATED WITH TELOGEN ROOTS

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Telogen hairs are commonly recovered as trace evidence, however contain very little nuclear DNA. In recent studies, it has been revealed that telogen roots may be shed with epithelial material adhering to the root, which may contain cells that can be used as a source of nuclear DNA. By screening telogen roots using a histological stain, it can be confirmed whether nuclei are present. Using a protocol for staining telogen roots, the frequency of roots with nuclei was determined and a correlation of DNA yield to nuclei number established, which can be linked to STR profiling outcome. In this study, 999 hairs from 143 volunteers were stained with haematoxylin and nuclei were counted and categorised according to nuclei number. DNA was extracted using Chargeswitch® and quantified using quantitative PCR. STR profiling was performed using AmpFℓSTR ProfilerPlus™. Sixty five percent of the roots yielded no nuclei and 22% contained one to ten nuclei. Six percent of all hairs screened contained more than 100 nuclei. Forty percent of roots had visible epithelial material, however this was not correlated to nuclei number. For STR profiling at least 20 nuclei were required to obtain an informative partial profile and 70 or more for a full profile. This research demonstrates the importance of utilising a simple staining protocol to confirm the presence of cells within epithelial material. By using this protocol telogen hairs may be able to be utilised as a source of nuclear DNA, thus increasing the usefulness of telogen hairs in casework.

P 249 A COMPARISON OF METHODS FOR STR PROFILING OF LOW TEMPLATE AMOUNT DNA

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The amplification of low template amount (LTA) DNA is usually performed using 34 amplification cycles (LCN profiling). While this method has been widely researched and scrutinized, it is problematic as PCR artefacts and allelic imbalance can complicate interpretation. Previous studies have explored other methods including reduced volume reactions and alterations to capillary electrophoresis conditions, but this research has been limited. Using AmpFℓSTR ProfilerPlus™, four strategies were compared – a standard PCR method, post-PCR Qiagen MinElute™ cleanup, LCN profiling and a reduced volume reaction. DNA was extracted from nuclei recovered from epithelial material associated with telogen roots, and concentration was determined using quantitative PCR. All four PCR methods were performed on each sample. Overall profiling success for each method was determined by comparing allele dropout, peak heights, heterozygote balance and the presence of artefacts. Compared to the standard method, LCN profiling generally increased peak height but not number of alleles, and artefacts and heterozygote imbalance were exacerbated. The MinElute™ method reduced artefacts, however in some cases DNA loss was substantial resulting in lost peaks. The greatest number of allele peaks was seen using the reduced volume reaction and compared to the LCN method, peak imbalance was improved. This study demonstrates that the use of the 34-cycle LCN method may not be optimal for maximising profiling success on LTA samples and a reduced reaction volume is more efficient. Furthermore, despite the development of advanced statistical methods for interpretation, this study demonstrates the importance of PCR optimisation.

P 250 COMPETITION FOR DNA BINDING SITES USING THE PROMEGA DNA IQ™ PARAMAGNETIC BEADS

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The adoption of automated DNA IQ™-based protocols in May 2004 to process casework samples enhanced our capacity to produce high quality DNA profiles in a shorter turn-around time compared to the manual organic extraction. Unexpectedly, some hemochromogen-positive samples submitted for DNA analysis failed to produce DNA due to some chemical components present on the Hemastix® reagent strip that were competing for sites on the beads. This situation prompted us to examine the competition that could exist between DNA and other components often present in casework specimen lysates (e.g. heme, denim dyes) for the binding sites on the DNA IQ™ magnetic beads. It was determined that heme from heavily blooded samples interfered slightly with DNA binding and that an increase in the concentration of Proteinase K did not enhance DNA recovery. Results also reveal that dye/chemicals from black denim lysates interact directly with the DNA IQ™ beads and mask DNA binding sites. The competition from the black denim chemicals appear to be different from that previously reported for the Hemastix® reagents. This study strongly suggests that reducing the size of the portion from the specimen submitted to DNA IQ™ extraction will optimize DNA capture. In addition, it was found that a simple two or three-fold dilution of the sample lysate following the overnight lysis prior to DNA extraction overcame the reduction noted in DNA yield and preserved portions of the lysates for subsequent analysis when required. This represents an option to counteract competition and optimize DNA IQ™ extraction.

P 251 VALIDATION OF THE FLUORESCENCE-BASED SPERM HY-LITER™ KIT AS A MEANS TO STANDARDIZE SPERMATOZOA IDENTIFICATION IN SEXUAL ASSAULT CASES

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In an attempt to enhance searching and standardize the identification of spermatozoa in sexual assault exhibits, the Sperm Hy-Liter kit from Independent Forensics was evaluated and validated using a large number of sexual assault type specimens and the RCMP 6 mm circle slides. The practicality and sensitivity of the fluorescence-based protocol was compared to our current phase contrast microscopy protocol and examined in the context of an improved workflow for processing sexual assault cases. Experiments performed indicate that the Sperm Hy-Liter™ assay is 1) highly specific and valid (no interference from semen obtained from various animals and absence of positive fluorescence signals in all control slides prepared from swabs with only blood, yeast, urine, vaginal epithelial cells or fecal material), 2) sensitive and reliable (spermatozoa detected in vaginal swabs soaked in 1:1000 and 1:10,000 semen dilutions; spermatozoa detected more effectively in real casework samples containing few spermatozoa compared to phase contrast microscopy), 3) fast (1 min versus average of 10 or more min using phase contrast microscopy for <10 spermatozoa), 4) robust (spermatozoa detected from 24-year old suspected semen stains, no interference from spermicides, lubricants, fluorescent and non-fluorescent condoms and anti-fungal creams) and 5) simple to use for the detection of spermatozoa in a variety of sexual assault samples. The optimized Sperm Hy-Liter™ kit combined with the use of RCMP 6 mm circle slides should expedite and standardize the search for spermatozoa in specimens containing a limited number of spermatozoa.

P 252 AUTOMATED SCORING OF SPERM HY-LITER™-STAINED SPERMATOZOA BY THE METASYSTEMS METAFER IMAGE ANALYSIS SOFTWARE SYSTEM IN SEXUAL ASSAULT SPECIMENS

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The MetaSystems Metafer image analysis software system was purchased three years ago in the hope of developing a routine approach in the RCMP Forensic Laboratories to automate the scoring of human spermatozoa in sexual assault exhibits. This would enhance case throughput, increase assay sensitivity and standardize the search for spermatozoa. The development of appropriate classifiers was challenging but essential to teach the software system to specifically recognize human spermatozoa fluorescently stained using the Sperm Hy-Liter™ kit (Independent Forensics). Optimized classifiers were tested/validated using a diverse sample of slides prepared from mock sexual assault samples containing a limited or a large number of spermatozoa (fecal swabs, vaginal swabs, all mixed with different semen dilutions in addition to urine, blood and yeasts for a subset of those swabs). The performance of Metafer was recorded with respect to false positive counts, false negative counts and time required for the detection of spermatozoa in each sample. Automated spermatozoa counts were further compared to manual spermatozoa scoring in addition to comparing the time spent executing the identification. An excellent concordance was noted between automated and manual counts. The results of this study indicate that automated scoring of fluorescently-stained spermatozoa in mock sexual assault exhibits can be carried out reliably and reproducibly using well-developed classifiers for the MetaSystems Metafer image analysis software system. The automated scoring of spermatozoa combining Sperm Hy-Liter™/MetaSystems Metafer will be tested on a large number of sexual assault cases as part of a pilot project within an operational setting.

P 253 RELIABLE NUCLEAR AND MITOCHONDRIAL DNA QUANTIFICATION FOR LOW COPY NUMBER AND DEGRADED FORENSIC SAMPLES

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DNA quantification is a prerequisite for both low copy number (LCN) forensic analysis and ancient DNA studies. Moreover, if nuclear quantification is focused on the amelogenin locus, it also allows for sex determination. Some of the problems of these techniques are allelic drop-out phenomenon in amelogenin locus and mitochondrial DNA (mtDNA) quantification biases, due to the occurrence of mutations affecting the annealing of primers and/or probes.

The method presented here combines two multiplex TaqMan real-time PCR for nuclear and mtDNA quantification in degraded or limited samples. Nuclear DNA detection is based in the independent amplification of X and Y chromosome specific fragments in the amelogenin locus, and a internal PCR control to recognize inhibition problems. The small length of the fragments (71 bp) favors the quantification of severely degraded DNA, whereas the use of two distinct primer sets for X and Y chromosome amplification is directed to reduce allelic drop-out in LCN analysis.

MtDNA quantification is based on the amplification of three PCR fragments located in the mtDNA 16S region. Two of them are amplified with conservative primers and probes, allowing a world-wide application for this technique. Moreover, their length difference (95 and 314 bp respectively), provides information about the DNA degradation level. As these conserved fragments could be amplified in other primate species (e.g. *Pan troglodytes*), a specific human mtDNA fragment (167 bp) was also designed to recognize non-specific amplification.

P 254 STATISTICAL EVALUATION OF PRE-LABORATORY AND LABORATORY FACTORS THAT INFLUENCE DNA RECOVERY FROM ARCHAEOLOGICAL MATERIAL

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We evaluated the influence of different factors over the efficiency of DNA amplification and PCR inhibition from archaeological samples. We studied the effect of sample age and location of the archaeological site as well as some macroscopic features, like colour and fragmentation. We also measured the efficiency of four different extraction protocols.

We analyzed 89 samples from different locations and ages (8700-1300 B.P.), obtaining 116 DNA extracts. Four different extraction methods were tested: a silica-based protocol, a modified phenol/chloroform protocol and two modifications of the protocol designed by Yang et al. (1998). Two short overlapping fragments from mtDNA HVRI were amplified and sequenced in these extracts. Amplification and sequencing efficiencies were evaluated considering all the variables mentioned by a bivariate statistical analysis using SPSS 15.0 software.

Among all the variables studied, neither age nor macroscopic preservation had a direct effect on the recovery of endogenous DNA, while sample origin showed to significantly influence extraction and amplification success.

However, the extraction method employed seemed to be a determining factor, influencing both inhibition and efficiency. An overall analysis of the results identified the silica-based protocol as the most efficient method for extracting DNA from archaeological material.

P 255 DNA TYPING FROM SKELETAL REMAINS: EVALUATION OF TWO DNA EXTRACTION METHODS

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This study aims to evaluate two different methods of DNA extraction in the yield and the possibility of amplification by PCR of DNA extracted from skeletal remains (teeth and bones). Seven (7) cases, including 11 bones and teeth samples which the post-mortem periods ranged approximately from 16 to 52 years are described. We evaluated yield and quality of DNA samples obtained with the use of two different methods, the standard organic and modified ethanol precipitation based protocol, by using real time PCR. The performance of these methods to successfully extract DNA from bones and teeth was evaluated by Nuclear DNA typing using AmpFℓSTR® Minifiler™ kit (Applied Biosystems) and mitochondrial (mtDNA) mitotyping.

DNA quantification results showed that the modified ethanol precipitation based protocol extracted on average 0.022 to 0.92 ng of DNA of bone, compared with only 0.0025–0.382 ng by the organic method extracted. Although, the amounts of samples are limited, using the modified ethanol precipitation based protocol followed by DNAIQ™ purification system, we have been able to amplify efficiently not only mtDNA but also several nuclear STRs from bone DNA (DNA was successfully extracted in 60% of cases analyzed). Indeed, mtDNA sequencing could be carried out in four (4) cases among the seven (7) cases analyzed and was able to produce DNA fragments up to 340pb in size. Complete STRs typing was possible in 3 cases and partial STR in the two others cases but not confirmed. The procedure based on modified ethanol precipitation based protocol followed by DNAIQ™ purification system with substantial protocol modifications, yields pure DNA that can be used for further analysis such as STR typing and mitochondrial DNA sequencing from skeletal remains.

Keywords: Skeletal remains, Bone, DNA extraction, Real Time PCR, DNA typing, STRs, Mitochondrial DNA.

P 256 A NEW ALGORITHM FOR MTDNA SEQUENCE CLUSTERING

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MtDNA sequence assignment into haplogroups is a common procedure in forensic and phylogenetic analyses. The first step in haplogroup assignment is usually tedious and subjective especially for those with little knowledge of the common variation in mtDNA, because it requires going through a phylogenetic tree in search for polymorphisms. Several online databases now provide haplogroup prediction by inserting a list of polymorphic sites, but they have some limitations, either because they focus only on the control region or because they need an exact match to provide a prediction. The use of the control region for major haplogroup definition also has its limitations due to ambiguous alignments and homoplasy.

We present an alternative method for preliminary sequence clustering that allows a major haplogroup assignment by grouping the query sequence with sequences already assigned into haplogroups. In order to avoid ambiguous sites, the method relies on protein coding region polymorphisms. The program here presented extracts and concatenates gene sequences, and applies a new algorithm for estimating genetic distances between the sequences. The algorithm is based on converting sequences into vectors before subtracting them, which allows for a shorter run time.

This allows for a first assignment of the sequences into major haplogroups, which can then be further subtyped into sub-haplogroups by a detailed analysis of additional polymorphisms.

P 257 HUMAN Y-STR PROFILING USING FULLY-AUTOMATED ELECTROSPRAY IONIZATION TIME OF FLIGHT MASS SPECTROMETRY

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Polymorphic Y-chromosomal markers are useful for studying the male-specific complement of human DNA. In forensic analyses, Y-chromosomal short tandem repeats (Y-STRs) are typed to produce a haplotype profile that is shared among male members of a lineage and can be useful in parentage and relationship testing of male family members. Y-STRs can also be valuable in cases involving a male suspect's DNA with an excess of female DNA, such as rape cases. An automated Y-STR profiling assay has been developed for the Ibis Biosciences PLEX-ID™ mass spectrometry platform that reveals sequence polymorphisms within Y-STR alleles and requires only DNA template is added to a pre-fabricated plate prior to thermocycling. The assay analyzes sixteen loci: DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, and Y-GATA-H4. Samples from Caucasian, African American and Hispanic population groups were analyzed via PCR followed by direct ESI-MS. Correct allele assignments were confirmed for a subset of samples by comparison to truth data produced using standard Y-STR typing techniques. Allelic variants differing by sequence polymorphisms were revealed, expanding the allele base for several loci. Preliminary validation has shown sensitivity to approximately 125-250 pg of DNA per reaction. The assay has been characterized for sensitivity, reproducibility, species specificity, and specificity to male DNA in the presence of a 100- to 1000-fold excess of female DNA. The ESI-MS platform is capable of running Y-STR, autosomal STR, mitochondrial DNA, and SNP analyses on a single instrument within the same automated run.

P 258 HUMAN AUTOSOMAL SNP PROFILING USING FULLY-AUTOMATED ELECTROSPRAY IONIZATION TIME OF FLIGHT MASS SPECTROMETRY

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Single nucleotide polymorphisms (SNPs) represent a simple yet powerful tool for individual identification. Efforts by Pakstis and Kidd, *et. al.* to produce an ideal panel of genetically unlinked binary SNPs with high heterozygosity, low population bias, and uniform distribution over global populations have resulted in a 40-SNP panel analyzed across 40 global populations and a more-recent highly-unlinked 45-SNP panel analyzed across 44 global populations (Pakstis, *et. al.* 2007, Pakstis *et. al.* 2010). A fully-automated PCR / Electrospray ionization mass spectrometry (ESI-MS) assay that genotypes the first 40-marker SNP panel has been developed for the Ibis PLEX-ID™ platform and developmentally validated. A 64-SNP assay that incorporates the union of the two SNP panels has been developed for the Ibis PLEX-ID™ and is undergoing validation. Concordance with standard TaqMan assays for a panel of samples has been demonstrated for all loci. The assay has been characterized for sensitivity, reproducibility, species specificity, and the ability to detect when genotyping results indicate a pure sample or a mixture / contaminated sample. Validation studies suggest sensitivity close to125 pg DNA per reaction. A convenient software interface has been developed for visual review of automated data analyses. The Ibis PLEX-ID™ ESI-MS platform is capable of running Y-STR, autosomal STR, mitochondrial DNA, and SNP analyses on a single instrument within the same automated run.

Pakstis, *et. al.* (2007) Hum. Genet. 121: 305-317.
Pakstis, *et. al.* (2010) Hum. Genet. 127: 315-324.

P 259 HUMAN STR GENOTYPING OF DNA EXTRACTED FROM THE STOMACH CONTENTS OF ROOF RATS

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Introduction and aims
In this study, human STR genotyping of DNA extracted from the stomach contents of roof rats was performed. In this case, an 81-year-old female was found dead with one side of her face skeletonized. Roof rats were also found dead in her urine bottle near the corpse. It seemed likely that the rats had been feeding off this poor woman.

Methods
Preparation of samples; undigested food (23g) from the rat's stomach was separated into three samples; sample 1 (greasy feed, yellow in color), sample 2 (lean feed, brown in color), sample 3 (texture of hashed meat, white in color). Sample 4 was a blood sample from the corpse. DNA was extracted from the samples and amplified using a commercial kit (ISOGEN). STR genotyping of the extracted DNA was performed using the AmpFℓSTR Identifiler PCR Amplification kit (Applid Biosystems). PCR amplified products were separated in capillary electrophoresis ABI Prism 310 Genetic Analyzer and analyzed with GeneScan software v3.7 and GeneMapperID v3.7 (ABI). Total human DNA was determined by real time PCR analysis in samples 1, 2 and 3. Histopathological analysis of the samples from the rat stomachs was performed with H&E and sudan IV staining.

Results and discussion
Human endothelial cells, hair roots, and adipocytes in three samples were identified by histopathological analyses. STR genotypes of 15 loci in sample 1 were matched with sample 4 (the corpse); however, there was no match for samples 2 or 3. Thus, the roof rats had been feeding from the human face. In this case, it was possible to detect human DNA in the stomach contents of the rats using human STR genotyping.

P 260 A COMPARATIVE STUDY ON THE TECHNIQUES OF SNPS ANALYSIS FOR DEGRADED DNA SAMPLES

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Introduction
The analysis of degraded DNA samples is crucial for forensic genetics. Studies have described MiniSTR analysis. This technique accounts for the DNA fragmentation induced by degradation and is capable of efficiently analyzing degraded samples by obtaining shorter polymerase chain reaction (PCR) products. On the other hand, sex chromosomal polymorphisms are useful for identifying the relatedness of individuals in anomalous cases such as deficiency cases. In present study, we focus on sex chromosomal SNPs analysis from degraded DNA samples and estimate the two techniques (TaqMan assays and single base extension reactions) by obtaining shorter PCR products.

Materials and methods
We extracted DNA using SDS-proteinase K treatment followed by phenol/chloroform extraction from male various bone materials, and performed analysis with the AmpFℓSTR Yfiler kit. Based on the results, we had defined as degraded DNA samples more than twenty samples of which allele typing was unsuccessful in seven or more of 16 loci. Then, sex chromosomal SNPs typing for these degraded samples was performed using TaqMan assays and multiple single base extension reactions (SNaPshot assays). To make the techniques more useful for analyzing degraded DNA samples, we newly designed primers to render amplicons of 180 bp or shorter.

Results and discussion
Results of tests on degraded DNA samples have confirmed the usefulness of both the techniques in such samples, although present study is now in progress. We will make a more detail report, including a comparative result between the two techniques, after conducting further investigations.

P 261 OPERATION EARTHQUAKE: THE FORENSIC DNA RESPONSE TO THE CHRISTCHURCH EARTHQUAKE

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At 12.51pm on February 22nd 2011, the City of Christchurch, in New Zealand's South Island was struck by an earthquake measuring 6.3 on the Richter scale. This was the second major earthquake to hit Christchurch, New Zealand's second most populous city, in a short time. The previous earthquake of magnitude 7.1, on September 4th 2010, caused damage to buildings and infrastructure but no direct deaths were recorded. Both quakes were the result of the movement of a previously un-identified fault running beneath the city and surrounding areas. The February 22nd event, a shallow quake centred 10 kilometers south east of the city centre, close to the small town of Lyttelton, caused widespread damage and multiple fatalities. Following international best practise, Disaster Victim Identification (DVI) teams were set up in which forensic biologists worked closely with other specialists in a temporary mortuary, including pathologists and forensic dentists under the direction of the Chief Coroner.

This presentation will provide an overview of the damage caused by the earthquake and its impact on the identification process. Sample selection and collection processes, the logistics of transporting samples to the laboratory for analysis and DNA success rate data for various sample types will be presented. One half of the deceased were international visitors, mainly students, and some of the challenges of such an international operation will be highlighted for future consideration.

P 262 DEVELOPMENT OF AN ELECTROCHEMICAL SENSOR FOR THE DETECTION OF BODY FLUIDS IN FORENSICALLY IMPORTANT SAMPLES

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Discovery of body fluids such as blood, saliva and semen at a forensic scene require identification and analysis. Conventional methods for the identification of these body fluids can be labour-intensive and use technologically diverse techniques. This can prove a problem due to the low volume typically encountered in forensic samples. Therefore, there is a place within forensic science investigation for a selective and sensitive sensor able to identify body fluids. An approach to nucleic acid sensing based on an electrochemical sensor previously developed within our group, coupled with a reverse transcription PCR based multiplex developed by ESR was used to design a system to perform body fluid identification of forensic samples. This sensor technology uses the conducting polymer polypyrrole as both sensing element and transducer of sensing events – namely the hybridization of target oligonucleotide to probe oligonucleotide. Detection is performed using electrical impedance spectroscopy (EIS). Initial research focused towards creating a stable, reproducible sensor surface for sample investigation. This presentation will discuss the effect of electropolymerisation conditions (solvent and electrolyte used, electropolymerisation method) and post-growth treatment (cycling and EIS experiments) on sensor applicability and stability. Subsequent DNA studies will also be presented.

P 263 APPLYING A CUSTOMISED DNA POLYMERASE BLEND IN FORENSIC DNA PROFILING

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Crime scene stains often contain extraneous compounds that may interfere with PCR-based DNA analysis, resulting in partial or negative/blank DNA profiles. Extensive DNA purification may remove PCR inhibitors, but involve a risk of DNA loss and introduction of contaminations. Customising the chemical content of the PCR reaction is a strategy that may increase PCR inhibitor tolerance without manipulating the sample. Previously we have shown that crime scene stain analysis can be significantly improved by replacing the commonly used DNA polymerase Ampli*Taq* Gold with either individual alternative DNA polymerases or a blend of such enzymes [1,2].

Here we present the validation of AmpFℓSTR SGM Plus with a modified PCR chemistry for routine casework, applying a 1:1 blend of the DNA polymerases Ex*Taq* Hot Start and PicoMaxx High Fidelity. Allele callings are identical to standard analysis, and stutters sizes and balance values are indistinguishable. The modified chemistry provides increased resistance to PCR inhibitors, resulting in an elevated number of detected alleles for crime scene stains of both blood and secretion/saliva. Additionally, the detection limit is improved.

[1] Hedman, J., Nordgaard, A., Rasmusson, B., Ansell, R. and Rådström, P. (2009) Improved forensic DNA analysis through the use of alternative DNA polymerases and statistical modeling of DNA profiles. *Biotechniques*, 47, 951-958.
[2] Hedman, J., Nordgaard, A., Dufva, C., Rasmusson, B., Ansell, R. and Rådström, P. (2010) Synergy between DNA polymerases increases polymerase chain reaction inhibitor tolerance in forensic DNA analysis. *Anal Biochem*, 405, 192-200.

P 264 EVALUATION OF FOUR NEW FORENSIC DNA PROFILING KITS ON COMPLEX CRIME SCENE STAINS

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In 2009–2010, several forensic DNA profiling kits accustomed for Europe and the Prüm Treaty were commercially released. The manufacturers have made efforts to increase the PCR inhibitor tolerance compared to older kits, as shown by their increased resistance to known inhibitors such as humic acid and hematin. However, the inhibitor content in true crime scene stains is more complex. Inhibitors may be unknown or not well characterised and various troublesome compounds may be mixed.

Here we evaluate four new 15 STR-marker profiling kits on 29 inhibited crime scene stains from routine casework with DNA concentrations ranging from 0.026 to 0.11 ng/µL. For AmpFℓSTR SGM Plus, used as reference, analysis of 7 samples generated negative/blank DNA profiles, whereas 22 samples produced partial profiles. The four investigated kits were AmpFℓSTR NGM (Applied Biosystems), PowerPlex ESI 16, PowerPlex ESX 16 (Promega) and Investigator ESSplex (Qiagen). All four new kits produced DNA profiles of significantly improved quality compared to AmpFℓSTR SGM Plus. No profiles came out negative/blank. However, the kits were affected by the complex samples and often failed to produce complete profiles. Matrices such as cigarette butts and moist snuff seemed especially troublesome. The new kits have raised the bar for PCR inhibitor tolerance, but the problem still needs to be considered.

P 265 EXPANDING THE CAPABILTIES OF DIRECT AMPLIFICATION FOR PROCESSING DATABASING SAMPLES

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Laboratories worldwide are developing or expanding forensic DNA databases and are looking for simple, robust, high throughput workflows to efficiently process their single source samples. Direct amplification from blood or buccal cells samples deposited onto FTA® cards is routinely being done using the AmpFℓSTR® Identifiler® Direct PCR Amplification Kit. To further expand the direct amplification workflow to samples deposited on non-FTA® substrates, we have developed a lysis buffer to simplify the upstream sample preparation protocols. The lysis buffer eliminates any incubation or heating steps and generates STR profiles of higher quality as compared to other on-market lysis protocols for the direct amplification workflow. Addition of 1.2 mm punches to the lysis buffer from buccal samples collected on the Bode DNA Collector™ or a sampling of cellular material collected from swabs added to the lysis buffer yielded results comparable to profiles obtained with samples on FTA® cards. Results from external testing of the lysis buffer using the Identifiler® Direct kit showed a >90% first pass success rate using a peak amplitude threshold of 50 rfu. Quality STR profiles were obtained with the average intralocus balance at >65% and the average intracolor balance at >40%. Comparative studies were performed to demonstrate that the new lysis chemistry and workflow generated higher first pass success rates and better data quality over other current on-market lysis protocols for the direct amplification workflow. Additional experiments demonstrate an improvement in profile quality using the lysis buffer for direct amplification in conjunction with the AmpFℓSTR® NGM™ and NGM SElect™ PCR Amplification kits.

P 266 ON THE PRACTICE OF SINGLE CELL DNA TYPING APPLIED FOR FORENSIC BIOLOGICAL EVIDENCE

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Practices of DNA typing of single cells with reference to forensic biological evidence may have peculiarities.

As an example, laser micro dissected (LMD) single cell preparations from the mixed traces on a cigarette butt were analysed for contributor's DNA typing. However, no PCR products were obtained from a few individual cells.

Such a result was in controversy with what we had with macro preparations where no technical difficulties occurred and the only problem was an irresolvable mixed profile.

Eventually, when a series of 16 preparations was tested, we managed to amplify individual DNA in five cells, but still had no amplification product in the rest 11 cells.

Certainly, not all the cells individually retrieved from the sample are suitable for the analysis, e.g. due to degradation. For example, a check with ethidium bromide staining indicated that only a small proportion of the buccal cells deposited on the butt retained genetic material.

It can explain the observation that one can get a result from macro preparation (where only a proportion of the whole bulk of analyzed cells is good enough for typing), but when going for single cell preparations a bottleneck occurs, thus reducing chances for successful analysis.

It is not an issue how many single cells need to be isolated to obtain a result. But such a need must be taken into account as a prerequisite for a useful single cell analyses in forensics.

More comments would be made on single cell DNA profile recovery dependency on the cell type and sample condition.

P 267 STR ANALYSIS USING TWO METHODS OF DNA EXTRACTION FROM BONES EXPOSED TO BRAZILIAN TROPICAL CLIMATE

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Brazil is the 5th most populous country in the world and has the 6th highest rate of homicides (25 per 100 000 inhabitants). In this context, many cases of post mortem human bones that have been exposed to adverse environmental conditions and contaminants are the only materials available for analysis. The humid tropical climate with high rainfall and temperature has a direct influence on the bone material that has been exposed to those conditions, resulting in cell loss and degradation of DNA. The aim of this study was to extract DNA using a commercial kit and the organic extraction; both methods to evaluate their success in amplifying 15 STR markers from those human skeletal remains submitted to taphonomic tropical conditions. Fragments of compact bone from the femoral diaphysis of 20 skeletonized corpses, found in the period 1998 to 2007 in the micro-region of Ribeirão Preto, São Paulo, Brazil, were used. These fragments were ground and pulverized in a blender (Waring Product, CT, USA). The DNA was extracted from 200 mg of powder bone using: 1) QIAamp DNA Mini Kit (QIAGEN); 2) phenol chloroform and alcohol precipitation, quantified with the Duo DNA Quantifiler kit (Applied Biosystems) and amplified by PowerPlex® 16 HS System (Promega). The DNA could be quantified in 70% of the samples, using commercial kit extraction. Partial profiles were obtained using the STR Powerplex16 in samples extracted with the two methods. Our results show the feasibility to obtain short DNA amplicons and the need of the mini STR analysis in these samples.

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P 268 DEVELOPMENT OF FORENSIC-QUALITY MTDNA DATA USING NEXT-GENERATION SEQUENCING

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We have recently argued for the creation of entire mitochondrial genome (mtGenome) databases appropriate for forensic use, given new assays and technologies which will facilitate the generation of mtDNA coding region data from even the most difficult forensic specimens (Irwin et al. 2011). However, the development of complete mtGenome profiles by Sanger sequencing is laborious and costly, requiring thoughtful strategy design, automated laboratory processing, fully electronic data handling and extensive data review to avoid errors. Next-generation sequencing (NGS) promises rapid, cost-effective data production when samples are sequenced in parallel, and has potential utility for the development of forensic reference profiles. But NGS presents substantial challenges to consensus sequence determination at the level of surety required in forensics, given the massive amount of data generated by NGS platforms, sequencing errors in general and technology-specific drawbacks (e.g. 454 homopolymer-length errors), and considerations related to heteroplasmy detection and reporting.

As a first step toward developing NGS data handling strategies for forensic mtDNA applications, we sequenced reference samples enriched for the mtGenome on two NGS platforms (Illumina and 454) and compared these data to Sanger-generated control profiles. Our results indicated that region-specific alignment parameters and a method for distinguishing sequencing errors from true heteroplasmy could be incorporated in the secondary processing of NGS data to improve the development of consensus sequences appropriate for use in forensics.

P 269 AN ALLELIC LADDER FOR AN OPTIMISED SNAPSHOT ANALYSIS

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The analysis of SNPs via SNaPshot technique (Applied Biosystems) is established in many forensic laboratories. The high sensitivity and the cost effective application are important aspects of this technique. Nevertheless the interpretation of the sequencing results is up to some experts, because there exist no references to distinguish possibly false alleles from the correct ones.

Here we present an allelic ladder based upon reference alleles for the SNaPshot technique. Therefore we cloned 84 alleles that belong to 42 mtDNA SNPs that can be amplified in one multiplex PCR and one subsequent SNaPshot reaction. This ladder will help to discriminate between correct and false alleles, due to the fact that the SNaPshot technique is known to produce artefacts, dye blobs or pull ups. We recommend the use of allelic ladders/reference alleles for SNP analysis to maintain high quality analysis standards.

P 270 DNA TYPING OF SINGLE SPERM CELLS POST WHOLE GENOME AMPLIFICATION

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DNA typing of mixed stains in forensic casework encounters difficulties or even lacks sufficient validity in particular if the suspect's traits are underrepresented. This is particularly true for rape investigations when the time since intercourse exceeds several days and, hence, the number of sperm cells to be analyzed from vaginal swabs is limited.

To circumvent the drawback of sample contamination we developed a procedure to unambiguously identify haplo-identical cells isolated from mixed cell suspensions using low-volume on-chip DNA typing at the single cell level. Thereby, a whole genome amplification (WGA) step is applied to single cells isolated by means of laser microdissection and pressure catapulting. Aliquots of these WGA products are then used for multiple analyses of the respective single cell.

For prove of principle we microdissected sperm cells from cytospin preparations and forwarded them either directly to DNA typing or to WGA followed by DNA typing using PowerPlex 16 and PowerPlex 17 ESX and ESI, respectively. We will present the details of our work comparing the PCR efficiencies of single sperm cells analyzed by direct DNA typing to pre-amplified single sperm cells followed by multiple DNA typing.

P 271 EVALUATION OF DNA EXTRACTION EFFICIENCY FROM BONE MATERIAL USING THE PREPFILER™ FORENSIC DNA EXTRACTION KIT

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In the genetic identification of human remains, the standard material for analysis – due to its durability – is skeleton fragments. The degree of DNA degradation and also the presence of PCR inhibitors in DNA samples obtained in the process of extraction have a decisive influence on later results of amplification of polymorphic systems. A key element of genetic analyses is thus application of an appropriate method of extraction guaranteeing maximum efficiency of the process and lack of inhibitors in the obtained DNA samples. The aim of the presented research was to compare the efficiency of DNA extraction from bone material by the organic method and with the help of the PrepFiler™ DNA Extraction Kit. 30 skeleton fragments were collected for analysis: 10 femurs, 10 teeth and 10 petrous portions of temporal bones, from bodies found after an elapse of from several to several dozen years from death in various environmental conditions. Bone fragments were extracted by the organic method and with the PrepFiler Kit. The quantity of human DNA in the extracted samples was analysed using the Quantifiler™ Human DNA Quantification Kit. Samples were also amplified with the NGM kit. The following were assessed: the amount of total isolated DNA, the presence of PCR inhibitors and results of STR amplification.

The efficiency of extraction of bone fragments with the PrepFiler was lower than comparable extraction by the organic method. The presence of PCR inhibitors was not ascertained in samples extracted with the help of the PrepFiler.

P 272 COMPARISON STUDY OF FOUR DIFFERENT 16-LOCUS "EXPANDED ESS" STR KITS

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New 16-locus multiplex STR kits incorporating the five additional loci in the expanded European Standard Set (ESS) are now available from three manufacturers. These kits offer a number of advantages including an increased number of loci and improved performance over previously available kits. An assessment of four of the kits by means of comparison to each other and to the Applied Biosystems AmpFℓSTR® SGM Plus® kit was carried out. The four kits assessed were the Applied Biosystems AmpFℓSTR® NGM™, Promega PowerPlex® ESI16 and ESX16 and the Qiagen Investigator™ ESSPlex kits. All the kits showed an increase in sensitivity and in ability to overcome inhibition as compared to SGM Plus® and with no increase in stutter proportions or heterozygote imbalance. The next generation kits showed very similar performance to each other and the differences seen are discussed. The implications of the different kit configurations are also considered.

P 273 THE EFFECTS OF DIFFERENT ADHESIVE TAPES AND OF HEMASTIX TEST STRIPS ON DNA RECOVERY USING MAGNETIC SILICA BEADS

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Adhesive tape is used in a number of forensic applications, including immobilising fragments of biological material and retrieval of cellular material from exhibits. A large range of adhesive tapes are available and used within the forensic community and we have assessed the effects of two types commonly used within our laboratories on DNA processing methods. Single-sided 3M Scotch™ tape and double-sided 3M tape are used to retrieve cellular material from exhibits for subsequent DNA analysis. The adhesive from the single-sided Scotch™ tape was seen to interact with the silica magnetic beads during the DNA extraction process (Qiagen Investigator chemistry on the QIAsymphony SP instrument) and to cause a reduction in the quality of the results obtained while the double-sided tape showed little effect. This effect is most marked with low levels of cellular material and is evident from even relatively small amounts of tape. Optimisation of the lysis procedure was carried out to reduce the adhesive effect but it was not possible to entirely overcome it.

In addition, we have also investigated the previously reported effects of reagents from the Hemastix® test strips (Bayer), commonly used for presumptive identification of blood at crime scenes. We demonstrate that these too can reduce DNA recovery using the Qiagen Investigator bead chemistry and offer recommendations for their use to prevent this.

P 274 ABSTRACT WITHDRAWN

P 275 IMPROVED PERFORMANCE FOR FORENSIC CASEWORK: EXTRACTION AND ISOLATION UPDATES FOR THE MAXWELL® 16 INSTRUMENT

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The DNA IQ™ System is an established approach to the recovery of DNA from casework samples. The recovery of DNA from casework samples depends upon the efficiency of two separate processes. Extraction efficiency refers to the recovery of samples that are removed from solid supports, such as swabs, or fabrics. Isolation efficiency refers to the purification chemistry.

We have recently improved the performance of the DNA IQ™ System on the Maxwell® 16 instrument. We accomplished this enhancement through independent improvements in extraction and isolation chemistries. First, we designed a new LEV plunger of a proprietary material in order to increase the post-extraction isolation efficiency of the DNA IQ™ System chemistry, as performed on the Maxwell® 16 instrument. This design change significantly reduced variability and improved isolation efficiency. We can demonstrate the effect of our LEV plunger redesign upon isolation efficiency using liquid samples. Second, we have recently improved upon the extraction chemistry that precedes the isolation process through the optimization of the extraction buffer.

These changes resulted in increased DNA yield across a variety of samples, which are demonstrated through comparisons to organic extractions.

P 276 CASE REPORT: POST-MORTEM PATERNITY TEST PERFORMED WITH A BABY TOOTH AND NEONATAL HAIR FROM ALLEGED FATHER

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In a post-mortem paternity case where only the mother of the alleged father was available, the alleged father DNA profile was obtained from a Baby tooth and a lock of neonatal hair. DNA was extracted with QIAamp DNA Mini Kit (QIAGEN) and typed with IdentiFiler, YFiler and MiniFiler Kits (Applied Biosystems). Results did not exclude the male profile recovered from the tooth as belonging to the real father ($PI=7.8 \times 10^6$).

The identity of the recovered genotype was tested by using the alleged grandmother DNA profile ($MI=3.1 \times 10^4$). From the lock of hair typed with the MiniFiler STR panel it was recovered a mixed DNA profile, compatible with the alleged grandmother genotype plus that corresponding to the tooth sample.

P 277 APPLICATION OF NEXT GENERATION SEQUENCING TECHNOLOGIES TO THE IDENTIFICATION OF HIGHLY DEGRADED UNKNOWN SOLDIERS' REMAINS

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In 1951 the US Department of Defense started repatriating deceased soldiers in the midst of active combat operations for the first time in military history. Today, the U.S. Government is still committed to recovering, identifying and returning to their families, the remains of all military service members. Included among these efforts are over 860 unidentified American soldiers from the Korean War buried in the National Memorial Cemetery of the Pacific in Hawaii. Since 1999, several caskets have been disinterred in hopes of using mtDNA to establish identification. Unfortunately, the Korean War era mortuary procedures that were performed on these remains involved aggressive embalming.

Despite improved DNA extraction protocols and aggressive techniques to amplify mtDNA, we have been unsuccessful in obtaining authentic DNA with our standard amplification protocols that target amplicons as small as 124 bp. Preliminary data based on modified methods suggest that the surviving human mtDNA fragments are approximately 70 bp or less in length. As a result, they present a challenge for conventional PCR amplification and Sanger sequencing methods.

Thanks to high quality publications describing the recovery of authentic nucleic acids from very old remains via next generation sequencing technologies (Neanderthals, etc.), we are confident that NGS coupled with efficient enrichment for human mtDNA and restriction enzyme treatment to reduce contaminating DNA will permit the recovery of reliable, authentic mtDNA data that can be used for identification.

Here we present our promising preliminary results to obtain authentic mtDNA sequences from these Korean War remains using NGS technologies.

P 278 NEW RAPID ABO GENOTYPING USING DIRECT EXTRACTION KIT

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Introduction
The ABO blood group is one of the most important system in DNA profiling. Since Yamamoto et al. elucidated the molecular genetic basis of ABO blood system, many genotyping techniques have been reported. In this study, rapid and high specificity ABO genotyping by using TaqMan Sample-to-SNP Kit followed by fast real time PCR was developed and allele frequencies in Japanese population were also examined.

Materials and Methods
Genomic DNA was extracted from blood, bloodstain, muscle, buccal swab by TaqMan Sample-to-SNP Kit. Primers and probes were designed to detect 6 SNPs (nucleotide positions 261, 297, 467, 703, 829 and 1061) of exon 6 and 7 in ABO genes. 2x TaqMan GTXpress Master Mix was used for fast PCR and the final volume of the reaction mix was 6.0 µl. Thermal cycling was undertaken in a Step One Real-time PCR (Applied Biosystems) using the following conditions: Initial denaturation at 95°C for 20 sec followed by 40 cycles of 95°C for 3 sec, 60°C for 20 sec.

Result and Discussion
ABO genotyping developed in this study demonstrated clearly in scoring 6 SNPs to identify 9 alleles, A101, A102, A104, A201, A204, B101, O01, O02, and O06. TaqMan Sample-to-SNP Kit could extract only by adding the reagent directly and incubating the sample lysate for 3 min. Moreover, this method needs only 40 min to analyze ABO genotype in single plate. Therefore, this study indicated that our new ABO genotyping method would be applicable in forensic caseworks.

P 279 RECOVERY OF DNA IN PARAFFIN EMBEDDED TUMOR SAMPLES FOR ANALYSIS OF PEDIATRIC SARCOMAS

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Extraction of DNA from old paraffin embedded tissue sections is a main problem to obtain analyzable DNA. The objective of this work was to recover DNA from paraffin embedded tumour samples. We analyzed 30 tumours pieces included in paraffin from 20 to 25 years old to research the existence of deletion/duplication in INI1/SMARCB1 gene. This is a retrospective study to improve clinical control in patients. Pediatric sarcomas are a heterogeneous group of tumours with monomorphic cytologic features that hampers the accurate diagnosis to apply specific tumour-depend therapies. Some of these neoplasias have copy number alterations of the sequence in INI1 gene, a tumour suppressor gene located in the long arm of chromosome 22 (22q11.2). These molecular alterations are associated with loss gene expression, aggressive tumour behaviour and lack of clinical response.

The tumours pieces included in paraffin and 10% hystoplast, were obtained by curettage. After that, DNA extraction was performed with TEC/SDS pK and posterior treatment with organic solvents. Concentration and purification was made using microcon-Y-100 (Amicon). We obtained analyzable DNA in all tumours to apply MLPA technique (Multiplex Ligation Probe Amplification). The products were analyzed by capillary electrophoresis (ABI3130) and then were processed using GeneMarker Software vs1.6.

The obtained results allowed to characterize tumours depending on the existence or not of variation in copy gene number of INI1/SMARCB.

The molecular characterization of tumours in retrospective studies is a useful complementary tool to make accurate tumour diagnosis and apply the better therapeutic option for current patients.

P 280 PRELIMINARY STUDY OF STR TYPING FROM MIXED BLOODSTAINS BY SUPPRESSION SUBTRACTIVE HYBRIDIZATION PCR TECHNIQUE

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The exact profiling of each component in mixed samples depends not only on the differences of genotypes, but also on the quantity and proportion of the components. In this study, we investigated the possibility of STR genotyping of trivial amount of DNA component by selectively inhibiting the predominant amplification of major DNA component in the mixed bloodstain.

Two blood samples with different STR genotypes were mixed in proportions of 1:3, 1:5, 1:7, 1:9, 1:19, 1:29, 1:39 to form mixed samples. The higher genomic DNA composition was suppressed and substracted by suppression subtractive hybridization PCR (SSH-PCR) technique. The experimental procedure was as follows. The drivers were made from the PCR products of mixed samples which were tagged with different adaptors; the PCR product of high composition was used as the tester. Subsequently, the subtractive hybridization was done twice to allow binding between the drivers and the tester. Finally, suppression PCR was done using the residual genomic DNA as template, the product of which was detected using polyacrylamide gel electrophoresis.

All samples were accurately genotyped by STR-PCR or SSH-PCR when the ratio of mixed bloodstain was 1:9 or less, while only higher genomic DNA composition could be identified by routine STR-PCR when the ratio was more than or equal to 1:9. However, low genomic DNA composition was successfully detected through SSH-PCR. The SSH-PCR technique may provide a new tool for analyzing the low copy composition of DNA in mixed samples.

P 281 THE APPLICATION OF THE DNA REPAIR ENZYMES IN THE DNA POLYMORPHISM ANALYSIS OF THE FORMALIN-FIXED AND PARAFFIN-EMBEDDED TISSUE

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Formalin-fixed and paraffin-embedded tissue (FFPET) can be kept for a long time for medical research. Because of its damaged genomic DNA, FFPET is not suitable for the traditional PCR or STR analysis. In this study, we used DNA repair enzymes to treat the damaged DNA in the FFPET in order to improve the quality of DNA templates for future analysis.

FFPET samples that were fixed with 10% neutral formalin solution for 7 days were used. DNA was extracted with phenol/chloroform method, and was treated with Taq DNA polymerase, T4 DNA ligase, and the combination of Taq DNA polymerase and T4 DNA ligase, respectively. Both the treated group and the non-treated group were amplified with the CSF1PO primers, and PCR products were genotyped by the polyacrylamide gel electrophoresis (PAGE) (T=6%, C=5%).

PAGE showed that the samples treated with either Taq DNA polymerase or the combination of Taq DNA polymerase and T4 DNA ligase produced stronger bands compared with those untreated ones, and the result of combination treatment was superior. There were no significant differences between the treated and non-treated groups when T4 DNA ligase was employed. These data suggested that the DNA repair enzymes could be used to process the damaged DNA from the FFPET samples, and different repair enzyme demonstrated varied repair capacity.

P 282 POST MORTEM SAMPLES ON FTA-CARDS

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Analysis of DNA is one of the primary methods for identification in mass fatality incidents. It is also a common method to identify a missing person found, and whose identity is not clear. The most common type of sample for identification arriving the department is bone material, preferably femur. Bone material is a reliable source of DNA with a good yield of high molecular DNA. However, it is tedious and expensive to extract the DNA and it is not always necessary to analyze bone. Therefore, it is desirable to facilitate the process. Each year, we analyze a large amount of buccal swabs on FTA-cards in kinship cases with a fast and comparatively cheap method. One earlier report describes analysis of Post-Mortem (PM) samples on FTA-cards, with a success rate of 47% for an Identifiler profile. The aim for this study has been to find out in which cases FTA-cards are suitable as sample type for PM-samples, and where the limits are for its suitability.

Samples from identification cases from the Department of Forensic Medicine were analyzed. For each case, we received bone, muscle, one buccal swab (if available), and swabs from two internal organs. Information on the location where the human remains were found, weather conditions and estimated time for death accompanied the samples. Preliminary results show that FTA-cards are suitable for human remains found in fire accidents, but less suitable on human remains found days or weeks after death in homes with room temperature.

P 283 INTERNAL VALIDATION OF TECAN ROBOTS (Freedom EVO® 150 and 75) FOR PCR- AND CAPILLARY ELECTROPHORESIS-SETUP

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We validated and established an automated method for PCR- and capillary electrophoresis-setup (CE-setup), using the Tecan Freedom EVO® 150 and 75 automated liquid handling workstations powered by Freedom EVOware®. The PCR-setup-script of the Freedom EVO® 150 was based on the HID EVOlution™ System. However, substantial changes in the HID-script were required before putting the system on stream in the routine. The CE-Setup of the Freedom EVO® 75 was based on a script made by application specialists of Tecan. For internal validation of the two robots we investigated repeatability, reproducibility and occurrence of contaminations. The analysis of the reproducibility-data demonstrated that electropherograms of samples pipetted by the robots had a lower mean sizestandard-peakheight, than the samples pipetted by hand, whereas the mean sample-peakheight didn't differ between the two methods. This was mainly due to precision-differences between the robots and the hand-pipettes. The investigation of the occurrence of contamination showed, that with the PCR-setup-robot no sample got contaminated. The CE-setup-robot on the contrary revealed carryovers of samples from one well to the subsequent well. After intense examination we discovered that the tip-travel-height was set too low, therefore the tip touched the rim of the 96 well plate, which led to the carryovers. The contact between rim and tip couldn't be seen by naked eye, but after increasing the tip-travel-height no further contamination was observed. Our validation demonstrates that robots, even when setup by application specialists, need to undergo a proper internal validation, before being put on stream in the routine.

P 284 EXPERIENCES AND SURPRISES WITH POWERPLEX ESI AND AMPFℓSTR NGM SELECT IN ROUTINE CASEWORK

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The introduction of five "new markers" (D1S1656, D2S441, D10S1248, D12S391, and D22S1045) in forensic DNA analysis called for the development of new Multiplex PCR kits. The aim was to combine the widespread SGM+ marker combination with the new markers to be investigated in a single kit. In addition, SE33 was also to be included in the new kits. We present some practical experiences that have been gained since the use of PowerPlex ESI and AmpFℓSTR NGM SElect at our laboratory.

Separation on the ABI 310: Separation on the ABI 310 under standard conditions (POP-4, 36 cm length to detector) can lead to problems (inadequate resolution of 1 bp differences in the longer fragments). The use of POP-6 has a serious disadvantage in that there is a drastic increase in run times (approx. 30 minutes with POP-4 and approx. 50 minutes with POP-6). The use of capillaries with a length to detector of 40 cm instead of 36 cm permits the required POP-4 separation with a minimal change in run time (33 minutes).

Artifacts in ESI: During amplification with PowerPlex ESI there were repeated reports of reproducible artifacts that occurred neither in PowerPlex ESX nor in AmpFℓSTR NGM SElect. These artifacts chiefly appear with samples where microbial DNA is to be expected. They particularly affect the red channel, but artifacts have already been reported in green and yellow as well. These artifacts can erroneously suggest genuine alleles and are often difficult to recognize.

NGM SElect: If too much DNA is used, very distinct split peaks appear. They can largely be eliminated by prolonging the final extension time in the PCR to 60 minutes. The reduction in PCR volume from 25 µL to 12.5 µL surprisingly resulted in a reduced sensitivity to humic acid.

P 285 SPOTTING THE HAYSTACKS WITH THE RIGHT NEEDLES

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Introduction
PCR is sometimes referred to as the method that turns the proverbial needle in a haystack into a needlestack. Its specificity and sensitivity paved the way for the revolution witnessed in forensic individualization of biological material. STR markers are the "stars amongst the needles" and their multiplexed typing represents the gold standard in human identification, but the analysis of other genetic markers, such as mitochondrial or Y-chromosomal DNA, fills vital niches. The speed and simplicity of these analyses enable the typing of large sample numbers e.g. in population studies or DNA mass screenings. However, testing the entire sample set becomes highly uneconomical when the investigation aims only at a particular part of the sample. Under such framework conditions affordable and reliable pre-screening assays for the high throughput exclusion of e.g. innocents in a DNA dragnet or samples not attributable to the mitochondrial or Y-chromosomal haplogroup under study are desirable to avoid unnecessary STR genotyping or sequencing analyses.

Materials and methods
Multiplexed fluorescent allele-specific PCR and subsequent mtDNA SNP allele calling based on melting curve analysis as well as non-allele-specific mtDNA amplification and allele scoring by means of high resolution melting curve analysis were used for the homogeneous pre-screening of a large population sample from Tyrol (Austria).

Results and discussion
Both SNP-typing approaches fulfilled the requirements for a low-cost, reliable and high throughput pre-screening technique for the dissection of a large sample set. Results for both strategies are presented and relevant parameters characterizing the methods are discussed.

P 286 EFFECTS OF THE MOST COMMON METHODS FOR THE ENHANCEMENT OF LATENT FINGERPRINT ON DNA EXTRACTION FROM FORENSIC SAMPLES

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The aim of the research was to understand if the use of chemicals compounds used to enhance latent fingerprints, might interfere with the extraction and amplification of DNA from biological samples on crime scenes.

Only three methods were used: powders (black and white ones, used on non porous surfaces, and here applied on glass), cyanoacrylate (used on non porous surfaces, and here applied on plastic, silver, plastic-coated paper, panty hose and glass too) and DFO (only used on porous surfaces and here applied on white, colored and recycled papers).

The biological samples put on surfaces included blood, saliva and fingerprints applied to all substrates by pressing for 5 s. Finds were analyzed not only upon 24 hours, but also after 7, 30 and 60 days. "Untreated" samples have been used as control. DNA coming from each model was quantified by using three different kinds of techniques: the first is a qualitative one (amplification of the beta-globin gene) and the other two (Real Time PCR and Nano Drop) are quantitative. The obtained results from each sample are very similar for blood and saliva: independently from the presence of chemical compounds or trace age DNA extracted was useful for typing complete genetic profile with STR markers. Only for latent fingerprints, were noticed important differences between spectrophotometer analysis and the techniques based on PCR. To resolve these ambiguities, STR amplification and mtDNA sequencing were performed. Only mtDNA sequencing could be classified as a good technique to extract DNA from this kind of fingerprints.

P 287 HUMAN SEX DETERMINATION BY AMELOGENIN PADLOCK PROBES

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In this study, we utilized a technique called ligation-mediated rolling circle amplification (L-RCA) that combined padlock probe ligation and rolling circle amplification techniques to determine human sex. Amelogenin X and Y specific padlock probes were designed based on polymorphisms and the 6-bp indel of the amelogenin X and Y alleles. Lengths of DNA target for padlock probe detection were 40 and 33 bases of amelogenin X and Y alleles, respectively. Analysis of female DNA samples produced only L-RCA product from the AmelX padlock probe, whereas 2 different types of products were amplified from the male DNA samples, each generated by AmelX and AmelY padlock probes. Utilization of padlock probes *via* L-RCA technique provided an alternative tool for human sex determination in highly degraded DNA analysis.

P 288 DNA TYPING FROM FLUORESCENT-DUSTED LATENT FINGERPRINTS

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DNA profiles were successfully typed from four latent fingerprints deposited on a glass plate, glossy magazine paper, and a plastic surface that were dusted with red, green, and yellow fluorescent powders. No or partial DNA profile was generated from one latent fingerprint samples. In addition, two DNA extraction methods, i.e. QIAamp® DNA Mini Kit and Chelex® 100 extraction were compared. Experiments demonstrated that the absorbance measurements by spectrophotometery were interfered by colors of the fluorescent powder extracts, although these did not affect DNA profiling and detection of the PCR-amplified products by the genetic analyzer instrument. Results demonstrated that QIAamp® DNA Mini Kit was more suitable than Chelex® 100 extraction method for recovery of DNA from fluorescent powder dusted fingerprints. The quality of partial DNA profiles obtained from fluorescent powder dusted fingerprints was improved through the application of low copy number (LCN) typing, in which the number of PCR cycles was increased from 28 to 34. The type of surfaces, on which latent fingerprints were deposited, had a subtle effect on the profile quality obtained.

P 289 SIMPLE DETERMINATION OF EAST ASIAN Y CHROMOSOMAL HAPLOGROUPS USING MULTIPLEX ALLELE-SPECIFIC PCR ASSAY

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A multiplex allele-specific PCR system was developed for simple and reliable scoring of single nucleotide polymorphisms (SNPs) on Y chromosome in large number of samples. A set of 16 Y-chromosomal SNPs was selected to hierarchically designate haplogroups D, C, K, N, O, O1a, O2, O2a, O2b, O2b1, O3, O3a3, O3a3b, O3a3c, O3a3c1, and O3a4 for the identification of Y-haplogroups frequent in East Asians. The multiplex allele-specific PCR assay was optimized for simultaneous detection of 16 SNPs followed by fragment analysis on an automatic DNA sequencer like general forensic STR typing method. The primer set was consisted of combinations of two allele-specific primers and a fluorescently labeled common primer for each SNP marker, and designed to produce amplicons of less than 160 bp. A total of 300 Korean males were tested by this assay and the results were compared with those from single base extension (SBE) reaction in our previous study. In addition, this assay was validated using serially diluted DNA, artificially degraded DNA and DNA samples from old skeletal remains. The results and implications of the newly developed multiplex allele-specific PCR assay will be presented.

P 290 REVEALING THE VARIABILITY OF STR PEAK HEIGHTS FOR FTA DATABASE SAMPLES

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The main objective of this study is to rank the factors which could affect the peak heights of genetic profiles obtained by analysing database samples collected on FTA cards. The factors could be: (i) biological matrices (blood or buccal cells), (ii) individual sampled, (iii) area of FTA card punched, (iv) position of a genetic marker in the PCR multiplex kit.

Twenty-two subjects were split in two groups. Eleven buccal and eleven blood samples were collected using FTA cards. Ten punches of each FTA card were analysed: five for the PowerPlex ESI 16 kit and five for the PowerPlex ESX 16 kit. Electrophoresis of all samples was performed on the same genetic analyzer. We studied the peak heights for all loci (except AMEL) and all conditions. The two PCR kits used have the advantage to contain the same genetic markers but not in the same order.

We compared for each subject, each genetic marker, peak heights variances between kits for each biological matrice. For buccal cells samples (resp. blood samples), 19% (resp. 8%) of intra-subject variance comparisons showed significant differences. Separated normed principal component analyses on genetic markers showed no structure for punches and, more global variability for buccal cells than blood samples whatever the kit is. Grouping the kits per biological matrice revealed a size effect on peak heights on the first axes, some specific loci: D12S391 and D16S539 for buccal cells and, D1S1656, D16S539, D19S433, D18S51 and D22S1045 for blood on the second axes.

P 291 COMBINING DNA EVIDENCE FOR GREATER MATCH INFORMATION

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Most fields of scientific enquiry routinely combine data from multiple experiments. These experiments can be repetitions drawn from one item, or involve different items entirely. The motivation is to elicit maximal information from an experimental design. The statistical mechanism is the joint likelihood function.

A likelihood function mathematically quantifies how well alternative hypotheses explain a fixed data result. A joint likelihood function assesses these hypotheses on multiple data items simultaneously. Typically, the data are drawn from independent experiments. Therefore the joint likelihood simply multiplies together the likelihoods from separate experiments, jointly conditioned on a particular explanatory hypothesis.

In forensic DNA science, human data interpretation is usually performed on data derived from only a single item. This practice is a consequence of thresholding quantitative peak height data into all-or-none qualitative allele possibilities, in order to simplify human review. Combining profiles after interpretation for "consensus" has little statistical foundation.

Quantitative computer interpretation, however, does not share these artificial limitations. It is therefore natural to mathematically preserve identification information by inferring a genotype using a joint likelihood function, examining all the independent data simultaneously.

This talk describes the joint interpretation of DNA evidence. We show how likelihood functions can be used to rigorously explain DNA evidence, and how joint likelihood functions can combine evidence. We present data that shows how the number of assumed contributors affects the inferred result, and why appropriately constructed likelihood ratios cannot overstate the inferred DNA match information. We illustrate these concepts on representative DNA mixture cases and experiments.

P 292 PHYSICAL AND STOCHASTIC VARIABILITY OF INSTRUMENTAL SENSITIVITY IN QUANTITATIVE GENETIC TYPING FOR FORENSIC PURPOSES

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In forensic genetics the interpretation of mixtures and low template DNA relies on qualitative and quantitative analysis of electropherograms.

The discrimination between PCR artifacts and real alleles is mainly based on the measurements of the height of the peaks (Relative Fluorescence Units) and strongly dependent on instrumental sensitivity.

The aim of this study was to verify the existence of variability in the instrumental sensitivity as a function of time, temperature and other undetermined factors.

A positive control, amplified using AmpFℓSTR® Identifiler® PCR Amplification Kit, was analyzed and detected by capillary electrophoresis (CE) on ABI PRISM® 310 Genetic Analyzer, once a week, within a period of eighteen weeks and under the same conditions.

We quantified the relative fluorescence units (RFU) of the peaks of the sample as well as of the Internal Size Standard.

The mean of peaks heights calculated for each analysis showed non linear values ranged from 345 RFU to 2700 RFU, suggesting the presence of high variability in the sensitivity of the instrument.

These results were probably due to the capillary wearing state conservation and other factors that need to be further investigated.

Since little differences in the quantification of the fluorescence signal could have a deep impact on results interpretation, these data suggest the need to use a quantitative positive control as an intra laboratory indicator of instrument sensitivity.

P 293 COMPARISON AND OPTIMIZATION OF SPERM VS. EPITHELIAL CELLS DNA RECOVERY USING LASER CAPTURE MICRODISSECTION TECHNOLOGY AND IMMUNOFLUORESCENT STAINING SYSTEM

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Introduction and aims
Laser Capture Microdissection (LCM) is an accurate and robust technology used for separation of pure cell populations from a heterogeneous mixture via direct visualization and collection of elements. It offers an additional approach to process sexual assault samples with low sperm count and it is especially valuable in cases for which the only remaining evidence is a microscope slide. In this study, the capability of the Zeiss P.A.L.M.® LCM to be used as a separation method for sperm and epithelial cells, combined with Immunofluorescent staining kit SPERM HY-LITER™ was assessed.

Materials and Methods
The technique was evaluated in regards to its sensitivity, specificity, reproducibility and ability to function for mixed samples with low spermatozoa content. The specified immunofluorescent dyes were tested on single source and mixture cell titrations.

Results and discussion
The applied LCM technology effectively separated and recovered sperm and epithelial cells with an average recovery rate of 99.50% and 89.90% for single source(s) and 147% and 104.60 % for mixed sperm and epithelial cell samples, respectively. The median percent yields for slides stained with SPERM HY-LITER™ ranged from 90% to 123%. Separation of sperm and epithelial cells using LCM is a robust method to generate readily interpretable single source DNA profiles from mixed samples. The immunofluorescent staining method is reliable, but time consuming and not suitable for high through-put. A flow chart for incorporating these techniques into routine forensic casework was developed.

P 294 COMPARISON OF TWO WHOLE GENOME AMPLIFICATION METHODS FOR MINUTE DNA

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Objective
To apply multiple displacement amplification (MDA) and modified improved primer extension preamplification (mIPEP) for whole genome amplification (WGA), and compare their effects on forensic DNA analysis.

Methods
REPLI-g kit was for MDA. MIPEP was developed on the basis of improved primer extension preamplification by utilizing Expand High Fidelity^{PLUS} Enzyme Blend which is a kind of enzyme of higher fidelity and increasing the final concentration of random primers. DNA samples of varying amount were for WGA based on MDA and mIPEP. WGA products yield was evaluated by real-time quantitative PCR and STR genotyping performance was determined with AmpFℓSTR® Identifiler® kit.

Results
The DNA quantity was increased about 103–106 folds by MDA and 160–1220 folds by mIPEP. The least genome DNA amount was 1ng for MDA and 0.025ng for mIPEP to obtain accurate genotypes of all loci. MDA products and mIPEP products of 0.01ng-0.1ng DNA exhibited more loci observed than original DNA that was not for WGA, but allele imbalance and allele drop-out was frequently observed.

Conclusion
The yield of MDA is higher than mIPEP. The sensitivity of mIPEP is higher than MDA. MIPEP may be better for forensic trace DNA analysis, but should be carefully utilized.

P 295 SEQUENCES OF MICROVARIANT/"OFF-LADDER" STR ALLELES

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Introduction and aims
Commercial STR muliplex kits and capillary electrophoresis platforms are widely used for human identification. These methods are highly reliable and offer a fast and fairly economic solution for DNA profiling large amount of samples. However, simple analysis of the lengths of the PCR amplicons cannot reveal any information about sequence variations within the amplicons and, thus, important information may be overlooked. We have recently developed a sequencing method and an algorithm specially designed to process data reads of repetitive sequences from high-throughput Roche Genome Sequencers. The aim of this study was to investigate the sequences of microvariant/"off-ladder" alleles.

Material and methods
DNA was extracted and PCR-amplified. The amplicons were tagged, converted into libraries, pooled and sequenced on Roche Genome Sequencers (FLX or GS Junior). Using a new, specific algorithm, the data was sorted according to (1) the tags that identified the individuals and (2) the selected STR target sequences. For each sample, the sequences were aligned to create consensus sequences that were compared to reference sequences.

Results and discussion
The results showed high degree of sequence diversity between the micro-variants and the reference sequence for several of the investigated STR loci. Variations were also seen between the micro-variants within the same STR locus. The variations included base substitutions, insertion/deletions and differences in repeat structures.

P 296 DNA COLLECTION FROM NEWBORN SKELETAL REMAINS USING PHENOL: CHLOROFORM: ISOAMYL ALCOHOL

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The application of organic extraction techniques for samples with small amount of bone and in poor condition has been conclusive in resolving cases with a high degree of complexity, taking into account the time of burial and bone loss.

These are two cases forensic results: one of exclusion and other not exclusion where long and flat bones used to implement the protocol cutting, cleaning and spraying, application of a lysis buffer overnight, followed by organic extraction, real-time quantification and amplification of different systems being the Power Plex 16 HS most efficient for handling this type of samples and other systems with fewer obtaining genetic markers.

P 297 A COMPARISON OF AMPFℓSTR® IDENTIFILER KIT VERSUS AMPFℓSTR® IDENTIFILER PLUS KIT IN CHALLENGING BONE SAMPLES BY USING NORMAL AND INCREASED PCR CYCLE NUMBER

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With the aim to increase the chance of obtaining DNA profiles from challenging forensic samples, several strategies are tested. One of the most powerful tools used in forensic DNA typing are commercial amplification kits. Enhanced buffers are provided with these kits allowing amplification of highly degraded or inhibited samples. For samples with low DNA concentration, sensitivity can be increased by raising the PCR cycle number.

Fifteen bone samples between 35 and 40 years postmortem were tested with Identifiler using 28 and 34 PCR cycles and Identifiler Plus with 29 and 32 PCR cycles in order to compare both amplification kits.

A high percentage of samples showed higher number of successfully amplified loci with Identifiler Plus than with Identifiler. This effect is more evident when increased PCR cycle number was used.

Also, there were samples that exhibited identical number of successfully amplified loci by using both kits. These samples showed highly degraded DNA characteristics and the successfully amplified loci were not the same for each amplification kit.

For a group of samples that displayed flat profiles in normal PCR cycles for both kits, increased PCR cycles allowed a profile improvement that was higher using Identifiler with 34 PCR cycles than using Identifiler Plus with 32 cycles. Characteristics of highly degraded DNA or low DNA concentration were found in these samples.

On the basis of our results, Identifiler Plus offers a more increasing chance of DNA typing than Identifiler does, based on an enhanced buffer which mainly allows overcoming PCR inhibition.

P 298 INVESTIGATOR ESSPLEX PLUS KIT – FAST, SENSITIVE AND ROBUST AMPLIFICATION OF THE EUROPEAN STANDARD SET LOCI

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Forensic DNA laboratories are challenged by the requirement to provide results on the identity of genetic evidence within a very short time. Thus, in addition to crucial quality parameters like sensitivity and robustness, speed becomes an increasingly important feature of STR PCR assays. We have developed a next generation Investigator ESSplex kit that combines all critical features necessary for fast and reliable analysis of demanding forensic samples.

Based on our fast-cycling PCR technology, we have introduced a novel reaction mix that allows completing a standard 30 cycle amplification in as little as 90 minutes. Using this protocol, well balanced full profiles can reliably be obtained with 100pg of DNA template. Even a single genomic DNA copy gives rise to peak heights well detectable applying commonly used analysis thresholds. The assay is very robust towards potential PCR inhibitors. It can tolerate concentrations up to 200ng/µl humic acid, or up to 1000µM hematin without showing allelic drop outs at any of the 16 amplified loci. The Investigator ESSplex Plus kit furthermore provides a clean baseline. No dye artefacts are present anymore that may interfere with the analysis of low copy number samples. The combination of all features mentioned above helps to reduce the number of samples that have to undergo re-analysis, which further contributes to more streamlined and efficient laboratory workflows.

P 299 VISUALIZATION OF LATENT BIOLOGICAL TRACES VIA 5-METHYLTHIONINHYDRIN (5-MTN) STAINING FOR FORENSIC DNA TYPING

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Advance in forensic DNA analysis has made it possible to assign very small DNA amounts to a specific person and to analyze even biological traces invisible to the naked eye such as skin abrasions. As latent traces by nature include the risk of being overlooked, often large areas are sampled. Unfortunately, this approach very often generates mixed DNA profiles, which are associated with several disadvantages. Therefore investigators try to detect latent biological traces e.g. by the use of tuneable light sources.

Within this study a dyeing technique for the visualization, and hence controlled analysis, of latent biological traces is introduced. By a series of experiments the performance limits and a possible impact on subsequent STR-analysis were examined. On staged and authentic casework exhibits the efficiency of the screening aid was again tested and the usability of the new procedure demonstrated.

P 300 ANALYSIS OF SKELETAL REMAINS SEVERELY COMPROMISED BY THE USE OF THE LATEST GENERATION KITS

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In recent years, European societies (ENFSI - EDNAP) have strongly encouraged the development of new amplification kits that allow to obtain DNA profiles even on difficult samples (degraded samples, presence of inhibitory substances, LCN, mixtures, etc.).

To enable the amplification of difficult biological samples the new loci were designed to achieve greater resistance to inhibitors and most robust and uniform amplification.

With the aim of evaluating the performance of latest generation kits in real forensic caseworks the authors retested some bone samples previously analyzed in our laboratory over the past five years with the old commercial kits.

The authors compared the performance of AmpFℓSTR® NGM™ PCR Amplification kit (Applied Biosystems) with PowerPlex ESX 17 systems (Promega).

The twelve analysed samples came from eight exhumed corpses and four bodies found outdoor in the advanced stage of putrefaction. For the genetic investigation the authors used a piece of bone taken from the femur.

In conclusion, the authors can claim that the latest generation kits have proved decisive in all cases, including those where the previous use of traditional kits did not produce reliable and uniquely interpretable results.

P 301 A PROTOCOL FOR DIRECT AND RAPID MULTIPLEX PCR AMPLIFICATION ON FORENSICALLY RELEVANT SAMPLES

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Forensic DNA typing involves a multi-step workflow taking around 10-12 hours. For several scenarios it may be valuable to obtain an interpretable DNA profile, suited to search a DNA database, within a few hours. We reduced the total DNA profiling time to 2–3 hours by a direct and rapid PCR approach that uses the following elements: 1) the inhibitor-tolerant, highly processive Phusion" Flash DNA polymerase; 2) a modified, not-adenylated allelic ladder; 3) the quick PIKO" thermal cycler system; 4) profile interpretation guidelines with increased allele calling threshold, modified stutter ratios and marked low-level artefact peaks and 5) regulation of sample input by mini-tapes that lift a limited amount of cell material from swabs or fabrics. The procedure is specifically effective for single source samples like saliva, blood or semen stains and hair roots. Success rates, defined as a complete DNA profile, depend on stain type and surface. Due to the use of tape lifting as the sampling technique, the swab or fabric remains dry and intact and can be analyzed at a later stage using regular procedures. The protocol effectively instructs researchers unfamiliar with the procedure. Direct and rapid PCR was found to be compatible with various STR kits. We have incorporated direct and rapid PCR in a "DNA-6hours" service that can assist police investigations. This procedure rapidly derives DNA information from trace evidence, searches the STR profile against a DNA database and gives a brief report to police or prosecution.

P 302 DNA FROM FINGERPRINTS; EFFECT OF COMMON FINGERPRINT DETECTION TECHNIQUES ON SUBSEQUENT STR PROFILING

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DNA and fingerprints are the two main types of forensic evidence considered to be able to individualize traces of persons. We examined the effects of seven fingerprint detection techniques on the DNA in fingerprints. Fingerprints were placed on plastic or paper, cut in half and divided in treated and untreated categories. The full DNA isolate was used in a single PCR, which was achieved by ethanol-precipitation of the isolate. Treatment of fingerprints on plastic with cyanoacrylate, vacuum metal deposition or a combination of these two techniques did not affect STR-profiling, while application of basic yellow or safranin (both after cyanoacrylate-fuming) did reduce DNA analysis for which the washing steps seem responsible. Treatment of fingerprints on paper with physical developer was destructive for DNA analysis; the use of ninhydrin or DFO (1,8-diazafluoren-9-one) did not decrease STR-typing but these treatments regularly introduced DNA contamination. We observed a large variation in the DNA content between fingerprints which can have several reasons: the number of (DNA-containing) cells that are shed in a fingerprint can vary greatly or saliva contamination from face-touching can add DNA to fingerprints. An additional complexity is the uncertainty whether the DNA belongs to the donor of the fingerprint since DNA from a second donor can be left either on the surface or on the hand due to interpersonal contact. Therefore, although DNA analysis after the application of certain fingerprint detection methods is possible, we advise to interpret the results of the DNA investigation of the treated fingerprints with caution.

P 303 EXTENDED PCR CONDITIONS TO REDUCE DROP-OUT FREQUENCIES IN LOW TEMPLATE STR TYPING INCLUDING UNEQUAL MIXTURES

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The most common approaches to sensitize DNA profiling involve increasing the amplification level by a higher cycle number or enlarging the amount of PCR products analyzed during capillary electrophoresis. These methods have limitations when unequal mixtures are genotyped, since the major component will be over-amplified or over-loaded. To increase PCR efficiency and improve detection of the minor component in mixtures, we extended the primer annealing time several folds. When the AmpFℓSTR® Identifiler® amplification parameters are changed to an annealing time of 20 minutes during all 28 cycles, the drop-out frequency is reduced for both pristine DNA and single or multiple donor mock case work samples. In addition, increased peak heights and slightly more drop-ins are observed while the heterozygous peak balance remains similar as with the conventional Identifiler protocol. By this extended protocol, full DNA profiles were obtained from only twelve sperm heads (which corresponds to 36pg of DNA) that were collected by laser micro dissection. Notwithstanding the improved detection, allele drop-outs do persist, albeit in lower frequencies. Thus a LT interpretation strategy such as deducing consensus profiles from multiple independent amplifications is appropriate. The use of extended PCR conditions represents a general approach to improve detection of unequal mixtures as shown using four commercially available kits (AmpFℓSTR® Identifiler, - SEfiler Plus, - NGM and - Yfiler). The extended PCR protocol seems to amplify more of the molecules in LT samples during PCR, which results in a lower drop-out frequency.

P 304 MORPHOLOGICAL AND DNA ANALYSIS IN HUMAN SKELETAL REMAINS UNDER HARSH ENVIRONMENTAL CONDITIONS IN BRAZIL

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Investigations of genetic kinship and human identification through DNA analysis of human skeletal remains have been required for several types of cases. A molecular study of this kind of sample is a challenge because of the small amount of cells available. In Brazil, there is a dominating humid tropical climate which can exert a direct influence on the microscopic morphology of bone and consequently on the DNA. The objective of this study was to analyse the microstructure of femoral compact bone tissue samples that have been under harsh environmental conditions in Brazilian territory, correlating it with the amount of human DNA extracted. We used fragments of compact bone of the femoral diaphysis of 20 skeletonized corpses found in the period 1998 to 2009 in Ribeirao Preto, São Paulo, Brazil .The samples were fixed in formalin and immersed in decalcifying solution, inclusion in paraffin blocks and the slices obtained were stained with H & E. From the most cellular area, were selected 10 consecutive fields by a pathologist and the software Image Tool (UTSCH-USA) and Image J (NHI-USA) were used for cellular analysis. Quantification was performed with the Quantifiler Duo DNA kit (Applied Biosystems). The average cellularity by area was 0,000041. Were observed osteocytes in all cases ranging from 1 to 40 (mean 6,45). The DNA was extracted in samples in which more than three nuclei were found. These results indicate preservation of osteocytes in bone material exposed to tropical environmental conditions, indicating the feasibility of obtaining DNA for genetic studies.

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P 305 AUTOMATED EXTRACTION OF DNA FROM CLOTHING

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Introduction and aims
Presence of PCR inhibitors in extracted DNA infer with the subsequent quantification and Short Tandem Repeat (STR) reactions used in forensic genetic DNA typing. We have compared three automated DNA extraction methods based on magnetic beads with a manual method.

Material and methods
Two stains from 120 clothing items from crime cases were manually isolated. Each stain was divided in four equal sized parts and placed in sample tubes. Parts from the two different stains from the same item were combined in the same sample tube resulting in four sample tubes per item. DNA was extracted using a manual Chelex based extraction method, a QIAsymphony SP using a modified lysis protocol, an AutoMate Express or QIAsymphony SP using the recommended lysis protocol. Extracts were quantified using the Quantifiler® Human DNA Quantification Kit. Extracts showing indications of inhibition were diluted four-fold and re-quantified. Extracts with DNA concentrations higher than 20 pg/µL were set up for STR analysis using the AmpFℓSTR® SEfiler Plus™ PCR Amplification Kit.

Results and discussion
Samples extracted using the manual Chelex method showed the highest degree of inhibition. Of 120 samples, 84 (70%) were inhibited. Following dilution of inhibited samples, 61 (51%) of Chelex extracted samples were inhibited. Samples extracted using the three automated methods showed less inhibition (0.0%, 3.3% and 0.0%, respectively). Reportable STR profiles were obtained from 23%, 60%, 70% and 40% of the samples without dilution and in 55%, 60%, 75% and 40% following dilution indicating significant differences in extraction yield and performance.

P 306 AUTOMATED ADDITION OF CHELEX SOLUTION TO TUBES CONTAINING TRACE ITEMS

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Introduction and aims
Extraction of DNA from trace items for forensic genetic DNA typing using a manual Chelex based extraction protocol requires addition of Chelex solution to sample tubes containing trace items. Automation of addition of Chelex solution may be hampered by high viscosity of the solution and fast sedimentation rate of the Chelex beads. Here, we present a simple method that can be used on an Eppendorf epMotion liquid handler resolving these issues.

Material and methods
All trace items were isolated manually and those suitable for DNA extraction were placed in individually labeled sample tubes in Eppendorf Thermorack TMX sample racks. A 20% Chelex solution pH>10.6 was manually prepared in advance and aliquoted into 50 mL Falcon tubes. The Chelex solution was added to the samples using an Eppendorf epMotion 5070 automated liquid handler.

Results and discussion
Two scripts were developed enabling processing of up to either 24 or 48 samples per run. Implementation of the automated addition of the Chelex solution enabled a more flexible and easily scalable sample processing procedure. Furthermore, the automated procedure removed many repetitive pipetting steps associated with muscular ailments to the technical staff. Aliquoting the Chelex solution resulted in low risk of contamination of the Chelex. Any remaining Chelex was discarded after each run. Furthermore, automated addition of Chelex reduced pipetting variations between individual members of staff ensuring standardized and reproducible addition of Chelex to the samples.

P 307 RE-EXTRACTION OF INHIBITED DNA EXTRACTS – A METHOD TO OVERCOME INHIBITION IN OLD CRIME CASE EXTRACTS

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Introduction and aims
Some DNA extraction methods are not capable of removing PCR inhibitors. More recent extraction methods based on magnetic beads are optimized to minimize co-extraction of PCR inhibitors. We have tested and compared three different automated extraction instruments for their ability to remove PCR inhibitors in inhibited DNA extracts.

Material and methods
DNA extracts previously extracted with a manual Chelex based method (Bio-Rad) and purified with Amicon Ultra 4 100k (Millipore) that were too inhibited to be successfully quantified were re-extracted using three automated magnetic bead based methods, the AutoMate Express™ (Applied Biosystems - AB), the QIAsymphony® SP and the BioRobot® EZ1 (Qiagen). Following re-extraction, samples were quantified using the Quantifiler® Human DNA Quantification Kit (AB).

Results and discussion
Out of 22 samples, re-extracted with the Automate Express, 20 samples (91%) showed no sign of inhibition. Nine of the 22 samples (41%) contained more than 200 pg DNA per 10 µL eluate, which is considered sufficient to generate reportable DNA profiles. For samples re-extracted with the BioRobot® EZ1, 22 of 22 samples (100%) showed no sign of inhibition. Four of the 22 samples (18%) contained more than 200 pg DNA per 10 µL. Out of 120 samples re-extracted with the QIAsymphony SP, 115 (96%) showed no sign of inhibition. A total of 55 samples (46%) contained more than 200 pg DNA per 10 µL. In conclusion, we found that automated re-extraction of inhibited extracts can remove the PCR inhibitors. However, the methods showed significant differences in terms of amount of DNA recovered.

P 308 REPEATED EXTRACTION OF DNA FROM THE SAME FTA CARD

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Introduction and aims
Extraction of DNA using a magnetic bead based technique on automated DNA extraction instruments provides a fast, reliable and reproducible method for DNA extraction from various matrices. However, the yield of extracted DNA from FTA-cards is typically low. Here, we present a method that increases the yield from the nanogram range to the microgram range.

Material and methods
Buccal and blood samples were collected and stored on FTA-cards. Approximately 1 cm^2 of the card containing biological material was manually isolated with a scalpel and placed in a sample tube. Following initial extraction, the FTA-card pieces were left in the sample tubes and either immediately subjected to a second round of sample pre-treatment and extraction or stored for one year at 4°C. Samples were subjected to up to six rounds of extractions. Samples were quantified in triplicate and typed using the AmpFℓSTR® Identifiler® PCR Amplification Kit.

Results and discussion
Repeated extractions of FTA-card pieces indicated that the bound DNA was not eluted in the first extraction. For buccal samples, the amounts of eluted DNA in the subsequent rounds were 63%, 29%, 21%, 15% and 13%, respectively. For blood samples, the numbers were 131%, 115%, 80%, 86% and 76%, respectively. Combining the extracted DNA resulted in an increase in yield of 242% and 589% for buccal and blood samples, respectively. The results also demonstrated that FTA-card pieces may be archived for a prolonged period and used for another round of extraction if the initial extraction procedure fails.

P 309 PURIFICATION AND CONCENTRATION OF PCR PRODUCTS LEADS TO INCREASED SIGNAL INTENSITIES WITH FEWER ALLELIC DROP-OUTS AND ARTIFACTS

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Introduction and aims
Capillary electrophoresis of amplified DNA isolated from trace evidence samples occasionally results in inadequate STR profiles due to artifacts caused by e.g. primers and dNTPs. Removal of artifacts by purification and concentration of the PCR products may increase the sensitivity and the quality of the DNA profiles without re-amplification of the sample. We have validated and implemented an automated method to purify PCR products.

Material and methods
DNA extracted from various biological samples and DNA from a known control was amplified with the AmpFℓSTR® SEfiler Plus™ PCR Amplification Kit. The known, amplified control was diluted 27 times before electrophoresis. All samples were analyzed on ABI3130xl. Amplificates with allelic peaks below 50 RFU were purified with the MinElute PCR Purification Kit on a QIAcube automated spin-column instrument. Following purification, the samples were re-analyzed by capillary electrophoresis.

Results and discussion
Post-PCR purification and 2-fold concentration of amplified DNA from 34 trace evidence samples resulted in an increase in the median number of allelic peaks from 12 to 25. The allelic heights were increased from an average of 68 (injection time 30 s) to 127 RFU (injection time 10 s) when a diluted, known control was purified and concentrated. The number of artifacts was reduced from 22 to 1. In conclusion, purification and concentration of amplificates yielded higher allelic peaks, fewer drop-outs and a reduced number of artifacts. Purification does not require the use of additional sample material and the method can be automated enabling reproducible and standardized processing.

P 310 EVALUATION OF HIGH-THROUGHPUT EXTRACTION METHODS FOR LOW QUANTITY SAMPLES

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Forensic laboratories, including the Armed Forces DNA Identification Laboratory (AFDIL), are continuously interested in ways to improve productivity and efficiency. AFDIL processes thousands of reference, case and population samples every year as part of large-scale missing persons identification efforts. Automated processing of a variety of sample types, including low quantity forensic specimens, offers increased throughput, lower cost and a reduction in human error. To this end, AFDIL has acquired a Hamilton STARlet Liquid Handling Workstation with 8 independent channels and a 96-probe head. This customized instrument will be utilized to automate pre-amplification processes such as sample preparation, extraction and amplification.

For new mitochondrial DNA databasing efforts at AFDIL, blood serum specimens will be used to generate sequence data. Serum is a challenging sample type for DNA-based applications because it contains primarily proteins, lipids and other substances while retaining few cellular components. As is often the case with the extraction of low quality forensic samples, it is difficult to isolate trace amounts of genomic DNA in the presence of inhibitors. With the need to develop a high-throughput process, the chosen extraction protocol must be both robust and automatable for the target sample set. To determine which method was best suited to meet these requirements, serum and other forensically-relevant samples were used to evaluate several silica-based extraction chemistries. Based on results from quantitative and standard polymerase chain reactions, the optimal extraction method was selected, automated, and then applied to the serum samples used for databasing projects at AFDIL.

P 311 NON INVASIVE PRENATAL DIAGNOSIS: A NEW TOOL IN FORENSIC GENETICS

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Introduction and aim
Genetic prenatal diagnosis can have forensic applications, in particular in cases of rape resulting in pregnancy. Currently the option for prenatal genetic analyses in these cases are limited to the invasive approaches on amniotic fluid or chorionic villi. A convenient non invasive prenatal diagnostic approach has long been sought. We report preliminary results of a non invasive prenatal paternity test on circulating fetal cells of peripheral blood of pregnant women.

Materials and methods
Following approval by the Research Ethics Board and consent for the enrollement of the couple, pregnant blood samples were taken in the first trimester. Nucleated red blood cells of embryonic origin were enriched by FACS (fluorescent activated cell sorting, BDFACSAria III) with the following combinations of monoclonal antibodies: anti CD45, anti CD71 and anti-fetal hemoglobin (HbF).
After delivery a sample of the new born was taken as control.
DNA was extracted from blood of the couple, nucleated red blood cells of fetal origin, and of new born with Prepfiler kit (Applied Biosystems, AB) and genetic profiles were obtained after DNA amplification with NGM Kit (AB). Amplified fragments were analyzed on ABI Prism 310 Genetic Analyzer (AB).

Results and discussion
Comparison of the genetic profiles showed that all amplified fetal alleles matched the alleles of their putative fathers. We carried out a non invasive prenatal paternity testing on DNA of rare fetal cells circulating in pregnant blood. This is the first report on nucleated fetal red blood cell analysis for forensic purpose.

P 312 RECOVERY OF HUMAN DNA PROFILES FROM POACHED DEER REMAINS: A FEASIBILITY STUDY

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Poaching is a worldwide crime that can be difficult to investigate due to the nature of the evidence. Previous studies have focused on the identification of endangered species in cases of poaching. Difficulties arise if the poached animal is not endangered. In the UK deer have hunting seasons whereby they can legally be hunted. Therefore, identification of deer alone has little probative value as samples could have originated from legal hunting activities in season. After a deer is hunted it is usual to remove the innards, head and lower limbs. The limbs are removed through manual force and represent a potential source of human 'touch DNA'.

We investigate the potential to recover and profile human autosomal DNA from poached deer remains. Samples from the legs of ten culled deer were obtained (40 in total) using minitapes. DNA from samples was extracted, quantified and amplified to determine if it would be possible to recover human STR profiles. Low quantification data led to the use of an extended PCR cycling protocol of 34 cycles. Samples from five deer gave match probabilities ranging from 6.37×10^{-3} to 9.53×10^{-11}.

This study demonstrates the recovery of human touch DNA from poached animal remains. There is the potential for this test to be used in relation to other species of poached remains or other types of wildlife crimes. This is the first time that human STR profiling has been successfully applied to touch DNA in regards to wildlife crime.

P 313 FAST PCR AMPLIFICATION OF AMPFℓSTR YFILER

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DNA typing is used to identify victims following calamities such as earthquakes and tsunamis, as well as in criminal investigations, forensic medicine, and paternity testing. Due to its high power of discrimination, DNA typing represents a powerful tool, allowing detection of DNA type from small amounts of various specimens, including blood stains, semen, and bone. Particularly in cases of accidental death or calamity, identifying DNA type rapidly is essential. At previous conferences (ISFG2007 and ISFG2009), we reported on fast PCR cycling for AmpFℓSTR Identifiler by three methods: The first is the QIAGEN method using QIAGEN Fast Cycling PCR Master Mix and GeneAmp PCR System 9700. The second is the 9800 method, which involves amplification according to the manufacturer's instructions for the AmpFℓSTR Identifiler kit using 9800 Fast Thermal Cycler. The third method is T-Go-Fast for the Idf method using AmpliTaq Gold Fast PCR Master Mix, UP(x2).

For the present study, we reduced PCR running times by half (to approximately 110 min.) using AmpFℓSTR Yfiler — that is, even when not using AmpFℓSTR Identifiler.

Materials and Methods
We performed PCR amplification with a primer set of AmpFℓSTR Yfiler kit, AmpliTaq Gold Fast PCR Master Mix, UP(x2), and GeneAmp PCR System 9700.

Results and Discussion
We reduced PCR running times by half (to approximately 110 min.). No unbalanced peak heights were observed between the loci with the new method, called the T-Go-Fast for Yfiler method. Reducing PCR amplification time is essential and highly useful for DNA typing.

P 314 INFLUENCE OF PRESUMPTIVE REAGENTS ON DNA TYPING

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Various specimens are found at crime scenes, including blood, semen, and saliva. However, even specimens left at crime scenes that appear to be blood (colored red, for example) may not be blood. Conversely, body fluids may be present even in cases when they appear to be absent. In such cases, for presumptive tests performed before DNA typing, it is critical to find any body fluids that may be present and to infer the conditions under which a crime may have been committed. Performing DNA typing for all specimens without presumptive testing entails unnecessary time and cost. To the best of our knowledge, no previous reports have addressed the use of leucomalachite green (LMG), a presumptive reagent for blood in DNA typing, although reports have discussed the use of luminol (LM) with DNA extraction and typing.

In this study, we performed DNA extraction from blood stains treated with LM or LMG, successfully DNA-typing not just LM-treated blood stains, but LMG-treated blood stains.

Materials and Methods
We prepared DNA extracted from blood stains by placing diluted blood on cotton cloth and allowing it to stand to air-dry. We then performed DNA typing using AmpFlSTR Identifiler.

Results and Discussion
We achieved successful DNA typing using DNA extracted from LM- and LMG-treated blood stains. Due to the ease in assessing positive reactions, LMG is a highly useful and convenient presumptive reagent not just in the forensic laboratory, but at actual crime scenes. We believe LMG represents a new and powerful presumptive tool for DNA typing.

P 315 DIRECT PCR AMPLIFICATION OF STR LOCI: PROTOCOLS AND PERFORMANCE

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The option to perform robust STR typing of a single-source reference sample while bypassing extraction and quantitation saves time and money. Several commercial direct PCR kits and enzyme systems have been specifically developed for this purpose (e.g. Identifiler Direct and PowerPlex 18D). These new direct PCR typing kits contain PCR master mix components not typically found in traditional STR kits. The direct PCR master mixes are optimized to overcome PCR inhibitors commonly found in blood such as heme, immunoglobin G, and lactoferrin.

A cohort of 50 blood samples was spotted on FTA and 903 collection cards. An aliquot of each blood sample was also extracted and purified with a standard forensic protocol (Qiagen EZ1 Advanced platform) for non-direct genotyping and STR performance comparisons. Successful STR amplifications (full profile) were obtained from 1.2 mm punches of blood adhered onto FTA and 903 paper substrates without prior extraction. A series of pilot experiments involving the transfer of freshly collected buccal cells onto the paper substrates was also performed. STR profile characteristics such as N-4 stutter products, heterozygote peak height ratios, and genotype concordance (with non-direct PCR methods) were determined for each direct PCR system.

P 316 COMPARISON OF EXTRACTION METHODS FOR SPERMATOZOA RECOVERED USING LASER MICRODISSECTION

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Many factors could impact the retrieval of DNA from cells collected using laser microdissection (LMD), including; cell type, age and condition, staining method. When these are constant it is the DNA extraction method that could impact on the quantity and quality of DNA retrieved. From a forensic perspective, when dealing with small numbers of cells, it is imperative that the extraction method employed is efficient.

Comparisons of profiling success of different DNA extraction methods reported in the literature are difficult to make as details are limited relating to: cell numbers, extract volume, amplification template volume, amplification volume, profiling kit, number of amplification cycles, allele peak height cut offs.

As DNA IQ is the DNA extraction method routinely used at our laboratory three modified versions of DNA IQ were tested along with a one-tube method developed by ESR for use on spermatozoa collected using LMD. These were compared on serially diluted semen and known numbers of stained spermatozoa collected using LMD.

Each of the extraction methods provided full profiles from the serial dilutions (neat, 1:10, 1:100, 1:1000, 1:5000). However whilst the one-tube method provided full profiles from the 50, 100 and 150 spermatozoa samples the other methods provided mainly partial profiles. The one-tube method was also the best performer after determining the theoretical peak height if 100% of the DNA product were utilised in the amplification.

It is of value to develop and implement DNA extraction methods that have been optimised specifically for low numbers of stained cells collected using LMD.

P 317 LASER MICRODISSECTION: CHECKING THAT THE DISSECTED CELLS ARE RECOVERED FOR DNA EXTRACTION

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From a forensic application perspective it is essential that users of laser microdissection (LMD) equipment can be confident that the selected cells have indeed been captured, therefore facilitating the acquisition of a DNA profile from the target cells and ensuring that dissected cells do not contaminate subsequent cell collections.

We conducted three sets of tests: 1) Compared the number of dissected cells to that observed in the collection cap; 2) Analysed and compared the DNA profiles generated by sampling from multiple slides, containing cells from different individuals, into multiple caps; 3) Analysis of profiles taken from potential contamination risk surfaces of the LMD equipment and work area.

Results of our initial cell recovery and cross contamination studies revealed that not all cells were being recovered and that some could end up in unintended tubes. Repeat analyses of cell recovery after moving the equipment to a new dedicated work area with less airflow revealed a 100% recovery of both epithelial cells and the smaller sperm cells. It is recommended that LMD users check their recovery rate to ensure that their environment and procedures are optimal for forensic applications. No DNA profiles were found from high contamination risk surfaces, however, DNA profiles were obtained from some other uncleaned surfaces. To reduce the risk of contamination it is recommended that only one collection tube is placed in the tube rack per collection session and that the LMD equipment is cleaned before and after use.

P 318 GENOMIC DNA EXTRACTION PROTOCOLS FOR BONE SAMPLES: THE COMPARISON OF QIAGEN AND ZYMO RESEARCH SPIN COLUMNS

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DNA analysis of bone samples is considered as very difficult especially due to the numerous factors influencing the success or failure of testing. Composition of soil, age of the specimen, soil humidity, presence of microorganisms, pH and temperature, as well as the treatment after exhumation influence the success rate of the DNA extraction from bone samples and therefore the need of multidisciplinary approach to solve problems connected with DNA analysis of ancient material is understandable. The aim of this study was to develop an extraction protocol for bone samples based on ZR Genomic DNA Tissue MicroPrep kit (Zymo Research) and compare it to the already published (Davoren and Vanek 2007 and Vanek 2009) standard Qiagen based extraction protocol. The newly developed extraction protocol is faster, requires lower starting amount of bone powder, and provides similar or higher yields of DNA if compared to the standard protocol.

P 319 EVALUATING THE EFFICIENCY OF DNA EXTRACTION FROM DIFFERENT SUBSTRATES: A COMPARISON OF CHELEX AND AUTOMATED DNA IQ EXTRACTION METHODS

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The efficiency of extracting DNA directly from substrates varies according to a range of factors including the type of substrate and the extraction technique. Experiments were performed to determine how both automated DNA IQ and manual Chelex extraction (two routinely used DNA extraction methodologies) from a range of blood volumes (0.1–30 µL), on plastic and cotton, differed in efficiency, and to evaluate the threshold effect of DNA IQ. The efficiency of extracting DNA from plastic appeared to be lower than from cotton for both methods, but was only statistically significant (p=0.03) for Chelex extractions. Pairwise comparisons of blood volumes extracted using DNA IQ showed statistically significant (p=0.03) differences. Comparisons of Chelex extractions of different blood volumes also showed significant differences for cotton, but not for plastic. DNA IQ extractions were less variable than those using Chelex. The threshold effect, a major concern when using DNA IQ for experiments where total DNA quantities must be obtained, was only demonstrated at blood volumes above 15 µL. This preliminary research highlights discrepancies between extraction methods and that extraction from different substrates and amounts of biological substance can produce varied results. It demonstrates that laboratories should be aware of the limitations of their analysis techniques, as knowledge of extraction efficiencies may assist in optimisation of methodologies and procedures. Extraction efficiency analysis will also allow for more accurate assessment of the influence of used methodologies in studies relating to determination of DNA transfer rates, and, should these transfer studies be put into practice, in casework.

P 320 THE PINPOINT DNA ISOLATION SYSTEM AS A NOVEL DNA SAMPLING METHOD IN FORENSIC BIOLOGY

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The Pinpoint DNA Isolation System (Zymo Research) uses a dissolved polymer compound to remove and extract DNA from slide mounted pathology specimens. This polymer is applied to a non-porous substrate on which biological material is deposited, allowed to dry, and the polymer, containing cells and DNA, is peeled off. The polymer dissolves into solution during extraction, theoretically releasing more DNA into the extract than standard cotton swabs; a notion supported by preliminary data. Comparison of the Pinpoint extraction method and DNA IQ showed that extracting with DNA IQ gave a tenfold increase in the quantity of DNA. DNA was extracted using DNA IQ for all further experiments. We performed preliminary experiments to test its effectiveness in comparison to wet/dry swabbing methodology for forensic samples, including 4 replicates of 10 µL of saliva on glass slides and a pitted, non-porous surface. Results from the glass substrates showed that the initial application method of spreading with pipette tips generated significantly (p=0.03) less DNA than the swabbing method. The mode of polymer application is being investigated further with the aim of improving DNA collection. Sampling from a pitted surface with Pinpoint exhibited significantly (p=0.004) less variability than swabbing, but the mean quantity of DNA obtained from both collection methods was comparable. The Pinpoint system, combined with an optimised application method, may be another effective way to sample DNA in forensic casework. It has the potential to collect higher quantities of DNA than traditional methods which may be especially advantageous in casework involving trace DNA.

P 321 SYNOVIAL FLUID AS AN ALTERNATIVE SOURCE IN FORENSIC DNA

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Introduction
In decomposed human body, bloods or other biological tissues have been recognized as demanding sources for forensic DNA identification whether the yield of success is confined according to an influence of degradation process affected to cellular structure as well as nucleic acid. Synovial fluid is considered in its potentiality of forensic DNA recovery comparing to decomposed blood or other human biological tissues.

Materials and methods
In this study, synovial fluid and blood or other tissue samples are collected from 12 decomposed bodies performed medico-legal autopsy. Two different extraction methods, QIAamp micro kit and DNAIQ extraction kit, are particularly chosen depend upon sample's volume collected. Quantification, amplification and electrophoresis are followed through manufacturer's guide.

Results and discussion
Decomposed tissues or bloods have presented negative or partial DNA while 5 out of 12 of synovial fluid samples could demonstrate full recovered DNA profiles. However, degraded synovial samples that were quantified with a concentration below 0.1 ng/μL should be considered in theirs accomplishment. It's the point to be further discuss that additional pre-treated synovial sample with alkaline hydrolysis is allowed to increase an opportunity to recovered DNA. This is the first study of synovial sample which is an alternative source for forensic DNA especially in decomposed body.

P 322 NEW METHODS FOR THE STUDY OF POLYMORPHISMS IN MITOCHONDRIAL DNA CODING REGION BASED ON DENATURING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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The purpose of this study is to establish a novel method for the detection of mitochondrial DNA (mtDNA) polymorphism based on denaturing high-performance liquid chromatography (DHPLC) and to explore the new mitochondrial DNA polymorphisms in coding region in order to improve the discrimination power of mtDNA in forensic DNA typing. MtDNA coding region loci were amplified by the polymerase chain reaction (PCR). Using a technique of DNA pools and performing the analysis of pairwise combining samples, the DHPLC methods were evaluated and optimized. The methods established were employed to investigate the polymorphisms of mtDNA. Finally, two methods that based on the most homologous and retention times of cartridge were established respectively. Using the two methods, we explored the polymorphism in the coding region sequence with covering 1435bp which was nominated as seven loci. The number of alleles observed at the seven loci ranged from 4 to 13, yielding 53 haplotypes in 120 unrelated individuals. The values of allele diversity for each locus ranged from 0.0782 to 0.5701. The haplotype diversity using all these loci was 0.8775. Among these, four loci with higher diversity were proved to be suitable for forensic application and provided new genetic markers for the forensic mtDNA typing. The new methods that we established for the investigation of polymorphisms in coding region loci were the foundation for exploring polymorphism in entire mitochondrial genome and were expected to be important methods for forensic mtDNA identification.

P 323 IN-HOUSE VALIDATION OF NGM WITH EMPHASIS ON DETERMINING THE STOCHASTIC THRESHOLD, -1 AND +1 REPEAT STUTTER RATIOS AND LOW-TEMPLATE DNA METHODOLOGY

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The AmpFℓSTR® NGM™ kit shows increased sensitivity compared to previous AmpFℓSTR® kits, which we found largely due to the addition of a 29th PCR cycle. During in-house validation, we evaluated whether the increased sensitivity requires elevation of the stochastic threshold below which single peaks may represent one allele of a heterozygous pair with the sister allele dropping out due to low-template amplification effects. The stochastic threshold was set when 99% of over 500 of such alleles (from degraded or low-template DNA-samples) were below it. For comparison, we assayed the peak heights of true homozygous alleles in the same sample set. Next, we empirically determined the locus-specific stutter ratios using about 2000 Dutch reference samples and compared these with the ones provided by Applied Biosystems. In order to comprise 99% of the –1 repeat stutters, thirteen stutter ratios could be lowered with 0.46 to 1.79% and two had to be elevated with 0.04 and 0.06%. Lowering the –1 stutter ratios is especially interesting to assist minor contributor allele detection in mixtures with (a) low-template DNA-component(s). At all loci, +1 repeat stutters were visible for the higher DNA inputs and for lower inputs at the tri-nucleotide repeat locus D22S1045 as well. The overall +1 stutter ratio was set to 2.5% and for D22S1045 to 7.27%. For samples with <20 pg DNA-input 29+5 PCR cycles could be used as low-template method, but capillary electrophoresis at 9 kV for 10 seconds was the optimal strategy to sensitize genotyping of most low-template DNA-samples.

P 324 SINGLE-TUBE PCR AMPLIFICATION OF 21 AUTOSOMAL STR LOCI

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In more recent versions of commercially available human identification kits there has been a tendency to increase the number of autosomal STR loci that are amplified in a single multiplex PCR; in one example 17 loci together with the amelogenin locus are amplified simultaneously. This increase in the number of markers from 13, to then 15, and now 17 has been welcome, especially in parentage testing where certain cases led to paternity indices of insufficient magnitude. These situations often comprise motherless cases (duos) together with the presence of paternal mutations, and the analysis of more loci is usually required to enable a safer conclusion. To confront these scenarios we have been using and now report a single-tube multiplex PCR in which 21 STR loci together with amelogenin are amplified, using four different fluorophores. The reaction is designed to work with dried blood present in ordinary filter paper discs without any prior genomic DNA extraction. Nearly all the alleles of all the loci have been cloned and are amplified to make allelic ladders which are electrophoresed alongside the sample reactions. We have also used this amplification for forensic casework.

P 325 USE OF MATRIX STANDARDS FOR NEW FLUOROPHORES IN CAPILLARY SEQUENCERS

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Most laboratories presently carrying out forensic identification and parentage testing use capillary DNA sequencers to separate and visualize the alleles that are produced by multiplex PCR amplification, whether they be of STR, SNP or DIP loci. Because at least four, but usually five, different fluorophores are used simultaneously, each instrument must undergo its own spectral calibration in which a matrix is created and used to correct the overlapping of fluorescence emission spectra of the mixture of dyes. For this users must acquire matrix standards in addition to the multiplex PCR kits and it is not clear what these standards consist of. In our efforts to develop new multiplex STR and DIP amplification kits using different fluorophores from those commonly used, we were obliged to produce new matrix standards to calibrate our multicapillary sequencers, and we describe how this was done.

P 326 USE OF UNIVERSAL REPORTER PRIMERS IN MULTIPLEX PCR OF AUTOSOMAL LOCI

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At present forensic identification and parentage testing is invariably carried out using multiplex PCR amplification kits in which many loci, whether they be STRs, SNPs or DIPs are analysed simultaneously using primers labeled with at least three, but usually four, different fluorophores. Should existing loci need to be substituted by newer, for instance, more polymorphic loci, new fluorescent primers need to be synthesized; indeed, the dye labels of several loci may need to be switched to allow a re-accommodation of the allele sizes in the single multiplex, and this can be costly. We sought to facilitate these substitutions and reduce costs by using universal reporter primers which were described some years ago for use in the multiplex amplification of SNP loci. In this case we use only four universal primers each labeled with a different fluorophore and employ these in a test multiplex PCR amplification of 12 DIP loci. The advantages and disadvantages of this method are discussed.

P 327 COMPARISON OF TWO STR MULTIPLEXES FOR THE ANALYSIS OF CHIMERISM AFTER HEMATOPOIETIC STEM-CELL TRANSPLANTATION

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Introduction and aims
Short-tandem repeat (STR) genotyping is widely used for forensic purposes and it is also used to determine the proportions of donor and recipient cells after hematopoietic stem-cell transplantation. New STR multiplexes have recently been developed to analyze the complete CODIS plus D19S433 and D2S1338, such as

AmpFℓSTR® Identifiler® Plus PCR Amplification Kit (Applied Biosystems) and Investigator™ IDplex® (Qiagen). The objective of this study was to compare the sensitivity of both kits in the analysis of chimerism.

Materials and methods
200 blood samples from patients who received a hematopoietic stem-cell transplantation were amplified with the Identifiler® Plus kit and analyzed with an 310 ABI Genetic Analyzer. Fifteen of those samples that showed evidence of chimerism were also analyzed by using the Investigator™ IDplex® to compare the sensitivity of both kits.

Results and discussion
The Investigator™ IDplex® allowed quimerism detection only in 10 (67%) of the 15 cases with chimerism detected by using the Identifiler® Plus kit. This difference between the two kits appeared to be due to some allelic losses in the longest STR alleles with the Investigator™ IDplex® . Nevertheless, the average number of loci showing three or four alleles was 8.6±3.4 using Identifiler® Plus, and 7.7±3.5 with the Investigator™ IDplex®, when only the individuals with chimerism were included.

In conclusion, although this study should be considered as preliminary due to the relatively small sample size, it suggests that the sensitivity to detect donor/recipient chimerism is higher when the Identifiler® Plus kit is used.

P 328 UTI PREVENTING DNA DEGRADATION OF STORING URINARY SAMPLES FOR GENOTYPING

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In forensic practices, individual identification of urinary samples is necessary when sample switching or handling are suspected. DNA degradation with time elapsing and the low yield of extracted urinary DNA prevent its application. Storage of urine prior to analysis is increasingly advocated yet no best practice has emerged. To improve the genotyping based on storing urinary samples, we employed UTI (Urinary Trypsin Inhibitor) to prevent urinary DNA degradation. Urinary samples from 10 (5 females and 5 males) healthy volunteers from China were stored at –80°C with different concentrations (0–0.8 μg/mL) of UTI. Urinary DNA was extracted and quantified by Quantifiler Human DNA Quantification Kit. Genomic DNA was genotyped with Identifiler Kit at days of 1,3,7,9 and 30 after storage. 30 alleles were all detected in

all urinary samples in 1 day with or without UTI. Loci losses were observed at 3 days and no loci were detected at 9 days of female urinary samples preserved without UTI while all loci were detected when preserved with UTI during 9 days. No loci were detected at 7 days of male urinary samples preserved without UTI while mean 18 alleles were detected at 7 days when preserved with UTI. There were no significant differences among the average detection rate of STR loci in female urinary samples preserved with UTI at different concentrations. Detection rate of urinary samples preserved with UTI increased significantly, which result in prolonging the storage periods of urinary samples for personal identification.

Keywords: forensic genetics; Urine; UTI; DNA genotyping; individual identification

P 329 ESTIMATING STUTTER RATES FOR Y-STR ALLELES

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Interpretation of Y-STR results must take stutter phenomena into account. We have investigated 360 reference samples in duplicates with the AmpFℓSTR® Yfiler® kit. We estimated the stutter rates using weighted linear regression with intercept to allow for stutter rates varying with the peak height. The inverse peak heights were used as weights to incorporate the fact that the variance grows with the signal strength. We estimated the stutter rate at the allelic level. The stutter rates seemed to increase with increasing numbers of Y-STR repeats.

A poor fit for stutter rate of allele 23 of DYS635 was observed. Further analysis demonstrated two groups of DYS365-23 alleles with different stutter rates. Samples from each group were Y-STR typed and analyzed again, and the result confirmed the existence of two stutter groups of DYS365-23 alleles. The nucleotide sequences of the DYS365-23 alleles are being investigated, and the results will be presented at the conference.

P 330 TOWARDS AN OPEN-SOURCE FRAMEWORK TO INTERPRET COMPLEX DNA EVIDENCE

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Web-based collaborative projects have become essential to knowledge construction and dissemination in all scientific communities. For example, STRBase (http://www.str-base.org/) was originally developed in order to facilitate the compilation of frequency STR databases from across Europe. STRBase has now become the preferred platform to accept allelic frequency data from current initiatives to validate new generation multiplexes.

It is proposed to complement STRBase with an extension of the Forensim software (http://forensim.r-forge.r-project.org), in order to act as an 'open-source' platform for programs dedicated to the statistical interpretation of DNA samples. As an open source software, Forensim is inherently transparent and collaborative. This allows equal and rapid access to the general forensic community to tools that enable complex mixture analysis. Furthermore, open-source software can provide community based educational support.

Working within the guidelines originally outlined by the ISFG DNA commission on mixture interpretation, we demonstrate how an open-source platform can be used as an exploratory tool that assists with decisions about optimizing the biochemistry, as well as the statistical analysis itself. Forensim (already) offers interpretation tools for low template DNA samples that are based on the calculation of likelihood ratios by incorporating probabilities of drop-out and drop-in. Because the model is open-source, it will facilitate continuous improvement by the forensic community. To quantify improvements, it will be necessary to introduce tests of robustness. The two most important parameters to consider in this context are the levels of false positive and false negative error rates. Simulation methods (Tippett plots) are described that enable these comparisons to proceed.

P 331 AN EVALUATION OF POTENTIAL LINKAGE DISEQUILIBRIUM BETWEEN THE STRS VWA AND D12S391 WITH IMPLICATIONS IN CRIMINAL CASEWORK

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A brief evaluation was carried out to determine the effect of linkage between STRs D12S391 and vWA that are co-located on the same arm of chromosome 12. It has been suggested that linkage disequilibrium could result in over-estimations of strength-of-evidence calculations. In this paper we use computer simulation to generate a small population with haplotypes that are in significant disequilibrium. It is demonstrated that there is rapid decay of disequilibrium at the predicted recombination rate of c=0.117. If linkage disequilibrium is observed in a population generally the causal effect is unknown. We confirm that genetic drift is the most important driver that increases the level of disequilibrium that is observed in populations. The theta correction (θ = 0.02) can be applied in order to ensure that overestimates of strength-of-evidence are minimized.

P 332 FORENSIM: AN OPEN-SOURCE INITIATIVE FOR THE STATISTICAL EVALUATION OF FORENSIC DNA SAMPLES

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Forensim is a software package that provides a comprehensive set of tools for the statistical interpretation of forensic DNA samples. It implements standard statistical methods for the interpretation of DNA evidence, such as likelihood ratios and the random man not excluded probability, along with more original methods dealing with problems such as mixture deconvolution and the estimation of the probability of allelic drop-out. Furthermore, the package offers a collection of simulation tools that allow the generation of realistic datasets that can assist forensic scientists with the design of experiments. *Forensim* uses the R statistical programming language and is open-source. It is freely available for all platforms from: http://forensim.r-forge.r-project.org. The manuals and tutorials can also be found on the website.

P 333 ANALYSIS OF PATERNITY TESTING BY SIMULATING FAMILIES FROM STR RESULTS OF FULL SIBLINGS WITH AMPFℓSTR IDENTIFILER™ KIT

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The aim of this study was to evaluate the validation of paternity exclusions and the evidential power of relative chance of paternity (RCP) in paternity investigations when alleged fathers are biological brothers. The analysis was based on the genotyping results using AmpFℓSTR Identifiler™ kit. Results of STR genotyping using AmpFℓSTR Identifiler™ for 115 pairs of full siblings of Chinese were analyzed according to general international standard. Simulated couples were created by generating unrelated individuals (putative wives) for each sibling. 10 putative children for each couple were constructed whose STR genotypes were derived from 'father' and 'mother' using a random number table, which resulted in 2300 simulated 'father-child-mother' trio families. The genotypes of the 'Mother-Child' /or 'Child' were compared with those of the 'Uncle' (the brothers of putative fathers) at 15 STR loci.

'M-C-Uncle' trio investigations showed that 0, 1 and 2 STR mismatch(es) occurred in 10, 68, and 220 putative families, respectively (298 in total). 'C-Uncle' diad investigations showed that 0, 1 and 2 STR mismatch(es) occurred in 78, 295, and 510 putative families, respectively (883 in total). It is worth noting that the values of RCP were greater than 99.99% in all 0 STR mismatch comparisons from trio investigations, while from 'C-Uncle' pairs, 44 showed RCP values greater than 99.99%, 11 greater than 99.99% to 99.975%, and 23 less than 99.975%. Since non-monozygotic siblings have higher genetic similarities compared to unrelated individuals, caution should be used when employing AmpFℓSTR Identifiler™ kit to validate paternity in such cases.

P 334 "BLAME THE BROTHER"-ASSESSMENT OF FORENSIC DNA EVIDENCE WHEN ALTERNATIVE EXPLANATIONS HAVE DIFFERENT LIKELIHOODS

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In a crime case where a suspect is assumed to be the donor of a recovered stain, forensic DNA evidence presented in terms of a likelihood ratio is a clear course as long as the set of alternative donors contains no close relative of the suspect, since the latter has a higher likelihood than has an individual unrelated to the suspect. The state-of-art today at several laboratories is to report the likelihood ratio but with a reservation stating its lack of validity if the stain originates from a close relative. Buckleton et al [1] derived a so-called extended likelihood ratio for reporting DNA evidence values when a full sibling is present in the set of potential alternative donors. This approach requires consideration of prior probabilities for each of the alternative donors to be the source of the stain and may therefore be problematic to apply in practice. Here we present an alternative way of using prior probabilities in the extended likelihood ratio when the latter is reported on an ordinal scale of conclusions. Our example show that for a 12 STR-marker profile using the extended likelihood ratio approach would not imply a change in the level reported compared to the ordinary likelihood ratio approach, unless the close relative has a very high prior probability of being the donor compared to an unrelated individual.

[1] Buckleton JS, Triggs CM, Champod C., Science & Justice 46: 69-78

P 335 ESTIMATING COANCESTRY FROM GENOTYPES USING A LINEAR REGRESSION METHOD

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The estimation of the coancestry coefficient associated with a pair of individuals, given their genetic types, has been a matter of great theoretical and practical importance, namely in forensic kinship tests.

Generally considered as a condensed measure of relatedness, it is quite often assumed that there is a loss of information when this measure is taken in consideration instead of Jacquard's identity-by-descent partitions. Nevertheless, considering non-inbred individuals, it can be proved that, excluding the pedigrees parent-child and full-siblings, both measures are equivalent for pedigrees that relate two individuals by exactly one path.

In this work, the coancestry coefficient between two (not necessarily non-inbred) individuals is straightforward inferred from their genetic types through the probability of finding two identical alleles each one randomly chosen from each individual, or, alternatively, through the expected homozygosity of their virtual offspring. The presented method is rooted in the algebraic expression for the probability of finding an individual homozygous in the population.

It is shown that the coancestry coefficient of two individuals related by a given kinship can be mathematically modeled by a linear function depending on (a) the expected homozygosity of their virtual offspring, and (b) the average homozygosity in the population; the slope and the y-intercept of the straight line carrying information about the gene diversity of each database. Therefore, the theoretical (and unobservable) measure can be obtained through an estimation of the observable measure.

P 336 QUESTIONABILITY OF RESULTS IN DEFICIENCY CASES

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Judging siblinghood without involving parents the strength of evidence has to be treated with reservation due to random distribution of hereditary traits. Genetic exclusion of siblinghood between two persons, without parental findings, is basically not possible, both the positive and the negative proof of descent can only be provided by statistics. Our aim is to evaluate the range of Likelihood ratios (LR) in favour of non-sibship based on unrelated men and in favour of siblinghood based on full-sibs. Further, we are interested in the influence of the number of analysed STR-systems on the LR-value. Therefore we did the analysis for 1225 unrelated couples and 61 pairs of full-sibs and tested following hypotheses: full-sibs:half-sibs:unrelated. In 14 unrelated pairs, the LR-value is in favour of full-sibship, 2 of them come within the verbal predicate "sibship very likely". 93 couples show an LR-value in favour of half-sibship (2 of them as "half-sibs highly likely"). The average LR-values (18 STR-systems) in favour of unrelated persons are $39x10^5$ (unrelated:full-sibs) and 209 (unrelated:half-sibs). The number of false positives increases with reduction of STR-systems whereas the average LR-value in favour of non-relation decrease. Among the 61 sibpairs the average LR-value (15 STR-systems) in favour of full-sibship is 84x109 (full-sibs:unrelated) and 3x105 (full-sibs:half-sibs). The LR-value is against full-sibship in 2 pairs (full-sibs:unrelated) and 10 pairs (full-sibs:half-sibs), respectively. These results show the possibility of false conclusions and that under certain conditions the analysis of autosomal STR-systems is not sufficient for a doubtless statement.

P 337 MIXSEP: AN R-PACKAGE FOR STR DNA MIXTURE SEPARATION

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This poster presents an implementation of a mixture separating algorithm based on a statistical model for STR peak intensities. The implementation is freely available in the open source software R. A graphical user interface (GUI) eases the data importing, specification of known profiles and analysis of the STR data. Furthermore, data can be acquired directly from a database using the build-in connection feature.

The statistical model formulated for the peak intensities satises properties of the data generating process, and assumes that the contribution to shared alleles is additive in terms of the total amount of DNA. The model and algorithm for separating DNA mixtures was validated and gave similar results as those of three experienced forensic geneticists. The advantages of the model based approach are consistency in case reports within a laboratory, increased speed and objective measures for comparing different proposed combinations of DNA profiles. Furthermore, the statistical model can be used to compute expected peak intensities, which plotted together with the observed peak intensities provide a powerful tool for communicating the conclusions of an analysis.

P 338 BONAPARTE: APPLICATION OF NEW SOFTWARE FOR MISSING PERSONS PROGRAM

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In collaboration with SNN at Radboud University Nijmegen, the Netherlands, the Netherlands Forensic Institute (NFI) has developed new software for pedigree matching which can handle autosomal, Y chromosomal and mitochondrial DNA profiles. Initially this software, called Bonaparte, has been developed for DNA DVI. Bonaparte has been successfully applied in a real DVI case: the Afriqiyah Airways crash in Tripoli, Libya on 12 May 2010 in which 103 persons perished. The software performed excellently in terms of computational performance, stability and user-friendliness. This showed that Bonaparte is a reliable and time-saving tool which significantly simplifies and enhances a large-scale victim identification process.

Bonaparte will now also be applied in NFIs missing person program. For this, the software is connected to the NFI's missing persons database (CODIS). Since Bonaparte uses XML as import format, data from any source can be imported. In the new configuration, CODIS data is automatically imported in Bonaparte. Then the software performs automatically a set of direct searches, as well as searches against both partial and full family trees. For all cases, exact likelihood ratios are computed. Finally, match reports can be generated on demand by Bonaparte's customized reporting modules. In this way, an advanced search strategy combined with a modern, efficient work flow is realized in NFI's missing person program.

P 339 CONTAMINATION MONITORING IN THE FORENSIC DNA LABORATORY AND A SIMPLE GRAPHICAL MODEL FOR UNBIASED EPG CLASSIFICATION

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In this work we present a procedure for contamination monitoring in a trace search and recovery area and a graphical classification model. The recent launch of more sensitive and robust amplification kits increases the possibility to detect minute amounts of trace DNA. As a consequence this enhances our need to establish elimination databases and demands for an increased awareness on how to avoid contamination. DNA contaminating the evidence somewhere along the forensic process has the potential to destroy the evidence or totally confuse and mislead the crime investigation. In the forensic laboratory specific areas are designated for different parts of the process: trace search and recovery, pre-PCR, post-PCR etc. Work procedures and cleaning routines are adapted to minimise the risk of contamination. Monitoring presence of DNA in the laboratory environment, on specific surfaces or instruments of interest, is one way to assess these risks and will in addition increase our knowledge on how to improve cleaning routines and behaviour in the lab. A monitoring process needs to some extent be standardised in order to become unbiased and independent on an individual level, regarding both where and how samples are taken and how the results are classified. The graphical model constitutes a linear transformation of a three-dimensional "credit system" based on alleles, markers and peak heights, into a two dimensional classification. The standardisation allows results to be compared over time, and if applied to other work-areas comparison between different parts of the process will be possible.

P 340 LATIN-AMERICAN SOCIETY OF FORENSIC GENETICS (SLAGF) RESULTS OF THE INTERLABORATORY QUALITY CONTROL EXERCISE 2010-2011

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Here are presented the preliminary results of the Interlaboratory Quality Control exercise 2010-2011 of the Latin-American Society of Forensic Genetics (SLAGF). At that time the exercise was composed of a practical part to determine the genetic profiles of 3 blood samples on FTA card, and a theoretical part. The theoretical exercise consisted in two paternity cases for calculations, including an obligatory case with alleged father, mother and child; the other one was a paternity case with a dead alleged father with 3 children and their mother, and an extramarital child with the biological mother. All samples and the CD report formats were sent to 85 participant laboratories on January 14, 2010. The deadline for submission of results was April 15, 2010. To date, 56 reports have been submitted by 14 different countries in Latin America and one from Spain, distributed as follows: 13 from Colombia, 10 from Brazil, 7 from México, 6 from Argentina, 4 from Peru, 3 from Venezuela, Costa Rica and Ecuador, 2 from Bolivia and 1 from Nicaragua, El Salvador, Spain, Uruguay and Dominican Republic. The results will be presented in the poster. With this Interlaboratory Quality Control exercise 2010-2011 of SLAGF, we are going to strengthen the collaboration between all participating countries in Latin America helping to promote scientific exchange, improving the development and knowledge in the Forensic Genetics Science field.

P 341 COLOMBIAN RESULTS OF THE INTERLABORATORY QUALITY CONTROL EXERCISE 2009-2010

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Colombian Reference National Laboratory, **GENES LTDA**, have organized and coordinated for the past two years (2009 and 2010) the Quality Control Exercise for laboratories undertaking paternity, maternity and forensic tests with DNA markers.

There have been some similarities in the two controls: A practical exercise including 3 blood samples on FTA cards, and theoretical exercises including optional and obligatory cases. For the theoretical exercises, the participating laboratories should calculate the partial and final BRI (Biological Relationship Index). Twenty-two laboratories have participated in 2009, increasing the number to 27 in 2010. From the 1909 genetic profiles reported in 2009, forty-nine markers were under consensus and distributed in autosomal, Y and X chromosomes STR. In 2010, 52 markers were under consensus on similar distribution the past year, two thousand thirty-two genetics profiles were reported; only two laboratories analyze mitochondrial DNA. The rate of reporting error was 2.9% in 2009 while in 2010, 4.7% error was reported. In 2009 all the 22 laboratories reported results for the obligatory exercise: 14 the optional # 1 and 16 the optional # 2. In 2010 from the 27 participating laboratories, 25 reported results for the obligatory exercise and 12 the optional exercise.

The Proficiency Test conducted through the Colombian National Reference Laboratory has become a useful tool for quality assurance of all Colombian laboratories and some of Latin America that do DNA testing to establish biological relationships and an excellent opportunity for ongoing training of experts from the region.

P 342 AN INVESTIGATION OF THE PRESENCE OF DNA ON UNUSED LABORATORY GLOVES

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The presence of background DNA in forensic laboratories can be a significant source of contamination and poses a risk to criminal investigations. Our Environmental Monitoring program revealed potential contamination risks which resulted in amendments to procedures.

A laboratory glove investigation was undertaken to determine a) the presence of DNA on unused gloves in open boxes in examination areas, b) the presence of DNA on unused gloves from closed boxes (3 boxes/3 brands. Gloves were from the top, middle and bottom) and c) the origin of reddish-brown and black stains on unused gloves.

Results

a) 3 of 6 gloves from open boxes produced partial DNA profiles (8, 2 and 14 alleles respectively).
b) Of the 56 gloves from closed boxes, one brand revealed the presence of DNA on 4 gloves (12, 4, 2 and 20 alleles respectively).
c) Hemastix, Hematrace and Outchterlony testing indicated that the stains were not of human or animal origin. Further testing of the reddish-brown stains indicated the presence of iron oxide (possibly rust). The black stain tested for grease was negative.

These findings resulted in

a) Open boxes of gloves being covered in examination areas.
b) The brand of gloves on which DNA was present was removed from use.
c) Laboratory staff were advised to discard any stained gloves from boxes.
d) Investigation of alternate gloves appropriate for use in a DNA laboratory.

This study indicates the importance of regular monitoring of laboratory consumables to ensure their appropriateness for use in a forensic DNA laboratory.

P 343 STATISTICAL METHODS FOR ROUTINE QA ANALYSIS OF FORENSIC MTDNA TYPES

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The observation that polymorphisms in mitochondrial DNA (mtDNA) often appear in patterns forms the basis for using these patterns in double-checking a mitotype for accuracy. The comparison of sample types to patterns has been useful identifying missing or erroneously-reported polymorphisms. It is clear from this work that **"It is time to provide the mtDNA research community with analysis tools that efficiently check their sequences for potential problems, such as sequencing errors or unusual variations."** [Pereira, L, Samuels, D C. Response to Yao et al. Am J Hum Genet. December 11, 2009]

The use of phylogenetically-derived patterns for quality analysis has required expertise, software, and reference data that is inaccessible to the everyday practitioner and also has imposed rigid coverage requirements. The work presented here describes the development and use of routine tools for statistical analysis of mitotypes and individual polymorphisms.

We present a series of analyses in which forensic examiners can compare an mtDNA sequence to the discovered patterns in a body of existing data. The impact of differing clustering methods and reference datasets and coverage ranges will be discussed. The techniques will be presented using examples of historical-suspect polymorphisms and mitotypes. These tools can highlight novel polymorphisms, individual polymorphisms that may be suspect, potentially-missing polymorphisms, and HV1/HV2 exchanges, allowing users to efficiently double-check any suspected data. The freely-available Mitotyper™ web software will be demonstrated in some examples. The methods presented can be used by everyday or occasional practitioners without locking them into a particular comparison set or coverage range.

P 344 A FORENSIC AND PHYLOGEOGRAPHIC VIEW ON A GHANAIAN POPULATION AND ITS APPLICATION AS ETALON DATASET FOR QUASI-MEDIAN NETWORK CONSTRUCTION OF WEST AFRICAN SAMPLES

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Archaeological recordings evidence noncontinuous human presence in the area of modern Ghana dating back approximately 150,000 years before present. Today's Ghanaian population is mainly descendent from settlers that came from the North as early as in the 13th century. The aim of the study was to establish a high quality Ghanaian mitochondrial (mt) DNA dataset for forensic database purposes and for the application as etalon dataset for quasi-median network construction of a comprehensive West African sample. We further evaluated the relation of the Ghanaian population to those of surrounding regions.

We sequenced full mt control regions of 193 Ghanaian individuals according to high forensic standards. As a further quality measure, we identified closely maternally related individuals due to preceding kinship testing and excluded two samples from our data. The remaining dataset comprising 191 sequences was applied as etalon for quasi-median network analysis. Resulting networks were inspected for positions introducing complexity while lacking sufficient phylogenetic information. For phylogeographic considerations, the Ghanaian haplotypes were compared to those of 18 neighbouring populations resulting in a total number of 6,198 HVS1 haplotypes.

Based on the Ghanaian sequences we defined a filter for quasi-median networks specific to the West African phylogeny. This filter proved to be robust when displaying starlike networks of our etalon incorporating 99 West Africa sequences. Population genetic comparisons revealed extensive genetic admixture between the Ghanaian lineages and those from adjacent populations diminishing with geographical distance. Finally, all sequences were incorporated into the EMPOP database enriching the severely underrepresented African mtDNA pool.

P 345 GENETIC KINSHIP ANALYSIS: A CONCORDANCE STUDY BETWEEN CALCULATIONS MADE WITH SOFTWARE FAMILIAS AND ALGEBRAIC FORMULAS OF THE AMERICAN ASSOCIATION OF BLOOD BANKS

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Statistical analysis of genetic kinship performed manually can be very complex and error prone. The use of software tools can solve this problem. However, studies of concordance between the results of calculations made with software and algebraic formulas already established, based on Mendelian inheritance and probability of transmission, are needed to verify the possible existence of errors in the software itself or in its improper use. Recently it was proposed by O'Connor et al. (2010) the use of family data reference called "Candidate Family Reference Data (CRFD) (www.strbase.nist.gov/strbase/kinship.htm) for software validation. To validate the use of software Familias 1.97, a concordance study was done between the likelihood ratio (LR) calculations made with Familias (LR_F) and with the algebraic formulas published by the American Association of Blood Banks (LR_{AABB}). We analyzed the following cases of family relationships using 21 microsatellites CRFD genotypes: paternity; motherless; and kinship (Mother/Child/Uncle, Mother/Child/Grandmother, Mother/Child/Alleged Father Wife/Alleged Father Children, and Mother/Child/Alleged Father Children). To investigate the concordance of results LR_{AABB} value was subtracted from the LR_F value for each of the 21 markers analyzed in all cases. The results were considered concordant when $LR_F - LR_{AABB}=0$. In the present study there was complete agreement for each marker analyzed in all cases. Cases with mutation or null allele were not considered. The results confirm the efficiency and reliability of software Familias for LR calculation in cases of genetic relatedness.

P 346 RESULTS OF THE GEDNAP PROFICIENCY TESTS 2008–2010

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The GEDNAP (German DNA Profiling) Proficiency Testing (PT) program is the largest in Europe with more than 200 participants. It offers certification of stain characterization, DNA typing (autosomal STR, Y-STRs, X-STRs), mtDNA sequencing and biostatistical calculations.

This paper will focus on the recent developments (e.g., implementation of the 5 new European loci) and on typical errors. One of the major aims of the GEDNAP PT program is to understand the cause for an error (e.g., a transcriptional error, a drop out-related error, a contamination/drop-in, false typing of variant alleles) and to prevent this error in the future, thus to improve the overall quality of forensic DNA analyses.

P 347 QUALITY CONTROL OF DNA FROM FORMALIN FIXED AND PARAFFIN WAX EMBEDDED PROSTATE BIOPSY FOR FORENSIC ANALYSIS

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The harmonization and sensitivity tests are necessary when the material is reduced and previously manipulated, as formalin fixed and paraffin wax embedded biopsies. The harmonization of the thermal cyclers and the sensitivity were here determined using prostate biopsy from normal tissue of three individuals using Applied Biosystems kits: Identifiler, Minifiler and Yfiler. The amplification of 10ng of DNA from two biopsies was performed in the Perkin Elmer 2400 (Perkin Elmer), GeneAmp Thermal Cycler 9700 (Applied Biosystems) and Mastercycler Eppendorf (Eppendorf). Three biopsies were amplified, in duplicate, using 100, 10, 1, 0,5, 0,25 and 0,125ng of DNA. The efficiency of the thermal cyclers was DNA sample and Kit dependent, although the best performance was achieved with the Applied's equipment. The minimal quantity of DNA to get results was 10ng (10 times higher than suggested by the manual), although even this concentration was not guarantee of the entire success of the amplification. When 10ng of DNA and the Minifiler are used, all samples were totally amplified what confirms the best performance of this kit. The Identifiler failed to amplify the larger alleles even with 100ng of DNA. As not expected, the Yfiler results were not satisfactory for all biopsies, even with DNA up to 10ng. Considering we use prostate biopsy, the Y-STR information was not useful for all samples mainly due to DNA conditions. These results suggested the importance to previously know the laboratory and sample variables before performing forensic analysis in samples like prostate biopsies.

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P 348 THE NEW STANDARD REFERENCE MATERIAL® 2391C: PCR BASED DNA PROFILING STANDARD

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Standard Reference Material 2391c (SRM 2391c) is the fourth generation material for PCR based DNA profiling. The first generation material SRM 2391 was released in 1995, and since then the subsequent generations have all had minor modifications in the type and number of loci certified but always used the same genomic DNA samples. SRM 2391c has been produced with an entirely new set of genomic DNA samples and has two dry storage matrices including 903 paper, which is part of all the previous versions, and FTA paper.

SRM 2391c consists of six components: three are single source genomic DNA samples that are labeled A, B, and C, with the fourth genomic sample (component D) as a mixture of components A and C (1:3 ratio). Component E consists of two 6 mm punches of 903 paper that have been spotted with approximately 75,000 cells / spot. Component F consists of two 6 mm punches of FTA paper that have been spotted with approximately 75,000 cells / spot of a different cell line.

The six components representing 5 different DNA samples plus the mixture component have been analyzed using 22 commercially available STR typing kits, obtained from three different vendors, as well as the 26plex STR multiplex developed at NIST. In total there is data for 51 autosomal STRs and 17 Y-STRs included in the certificate of analysis.

P 349 IMPORT AND DIRECT REDUCTION OF ANALYSIS DATA IN FORENSIC CASEWORK – SUGGESTIONS FOR HIGH QUALITY DATA

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The reduction and tabulation of analysis data in forensic casework is time consuming and has a lot of sources for possible errors. To eliminate known sources of errors and to reduce the direct labour we established an MS-Access database relying on visual basic programming.

The software supports the laboratory workflow of the Institute in Münster in a way to provide a consistent and useful user-interface. It presents the common features of a LIMS (**L**aboratory **I**nformation **M**anagement **S**ystem). Besides these features, the software manages a direct data import from the capillary electrophoresis sequencer (3130 Avant Genetic Analyzer) into the MS-Access database. The data is reduced immediately for the output in a survey. The criteria for reduction are variable and may depend on expert opinion. A check for contamination against the DNA-characteristics of the laboratory personel and/or the DNA-characteristics stored in the database will help to find false DNA-profiles.

The **G**erman **DNA P**rofiling (GEDNAP) interlaboratory testing of 2010 has once again shown the problems of transmission errors. Those can be avoided by using software that imports and reduces analysis data directly. Furthermore contamination has become a hot topic in forensic genetics. This may be discovered by using software that is able to check DNA characteristics of selected samples against a subset of samples or against the whole database.

P 350 RESULTS OF THE 2011 RELATIONSHIP TESTING WORKSHOP OF THE ENGLISH SPEAKING WORKING GROUP

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The English Speaking Working Group of the International Society for Forensic Genetics offers an annual exercise involving genetic analysis in a relationship case with the objective to compare the results obtained in the participating laboratories.

In 2011, blood samples from a man and two children were distributed to 62 laboratories. Assuming that the man is the biological father of both children, the laboratories were asked to investigate if the children were half or full siblings. As in previous years, the workshop included a questionnaire and a paper challenge. The paper challenge included typing results of a mother, a child and the biological full brother of the alleged father. Database information about the allele distribution in the tested systems was provided, and the laboratories were asked to submit results of the biostatistical calculations and the formulas used.

The presentation of the 2011 Relationship Testing Workshop will include concordance/discordance in typing results, collation of systems and kits used by the laboratories and an evaluation of methods and strategies applied for dna-typing. Furthermore, the results of the biostatistical calculations of the paper challenge will be presented and discussed.

P 351 CAPILLARY ELECTROPHORESIS ANALYSIS OF DNA PRIMARY STRUCTURE: TOWARD A QUALITY CONTROL TEST FOR THE RELIABILITY OF THE STR-TYPING FROM FORENSIC SPECIMENS

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Although PCR technology has been greatly improved in the last two decades, PCR fidelity remains a serious concern in damaged samples. Several studies showed that DNA decay accumulates inexorably in post-mortem specimens thus producing an alteration of its primary structure. Capillary electrophoresis (CE) has been shown to be a powerful tool to study DNA damage, identifying both canonical and un-canonical DNA bases.

In the present study, DNA samples were extracted from different aged specimens and other forensic samples. In addition, known extent of apurynic-apirimidinic (A-P) lesions were introduced *in vitro* on a trial DNA sample. The integrity of the DNA primary structure of all these samples was investigated using CE and molecular assays such as agarose gel electrophoresis, UV spectrophotometry and qPCR. PCR amplification of autosomal STR and mini-STR markers was carried out on the quantified DNA samples. Unreliable DNA profiles and PCR artefacts such as allelic drop-out and drop-in were seen in DNA samples with the most altered primary structure.

The data of the CE analysis together with the molecular tests routinely applied in forensic DNA investigations, can be evaluated for their ability to bring to trustful decisions on the reliability of STR profiles obtained from forensic and artificially degraded DNA samples. The genetic typing obtained from an ancient or severely degraded sample should be supported by a critical evaluation of its DNA primary structure as it is unlikely that a reliable result can be obtained from a genetic template lacking a certain degree of integrity.

P 352 A METHOD FOR SIMULTANEOUS DETECTION OF PSA AND SEMEN

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Textile stains are essential for crime scene investigators to identify potential suspects by fibre analysis or genetic profiling. For the crime investigation it is also crucial to determine the body fluid type (especially semen) as well as the genetic profile. Normally two tests must be done to show the presence of semen. One possibility is the indirect proof of semen by PSA. However, only microscopic verification of semen is evidentiary. Standard procedures describe the examination with separate textile samples for every test. This is problematic as most stain material is very limited and must be used economically to facilitate reproduction of the results.

Here we describe a method that requires minimal amount of stain material for simultaneous detection of PSA and semen. The applicability of this method for the analysis of DNA will be discussed.

P 353 ORAL SWAB, AS SIMPLE AS THAT? A CASE REPORT

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Introduction and aims
Oral swabs are very cheap, easy to perform, require no special training and provide an excellent source of DNA. However, it's important to keep in mind possible sources of contamination, fraudulent too. We report a case of voluntary contamination of oral swabs to alter a paternity testing.

Materials and methods
An alleged father came to our attention for a paternity testing disposed by the Court. Two oral swabs were collected, in two different areas of the oral cavity. The results for a 15 loci profile revealed a mixed profile regarding two individuals and only the analysis of a small fragment of one of the two swabs allowed to highlight a single profile.

Results and discussion
The results, including indirect confirmations, were indicative of the fact that the alleged father tried to alter the oral cavity through scattering some biological material of another person. Even if the procedure of the oral swab is very simple, it should always be kept in mind that it can be easily fraudulently altered. This is often not considered: sometimes the involved parties run the swab by themselves, even in an isolated place. Therefore: carefully evaluate the oral cavity before collecting the swab, collect directly the sample, perform at least two oral swabs in two different portions of the oral cavity.

P 354 FALSE HOMOZYGOSITY AT D12S391 LOCUS: A CASE REPORT

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Introduction and aims
STRs analysis is currently used in paternity testing. We report a case of an apparent paternal inconsistency due to low discrimination power of the heterozygosis status at D12S391 locus.

Materials and methods
DNA extraction: chelex 100. PCR: AmpFℓSTR Identifiler and AmpFℓSTR NGM (Applied Biosystems) kits, according to manufacturer's recommendations. Typing and analysis: ABI 310 and GeneMapper ID V3.2. At D12S391 locus an apparent inconsistency was found (alleged father 20–20; mother 17–19.3; child 19.3–19.3). In subsequent analysis, the child was 20–20 in some runs and 19.3–19.3 in others.

Results and discussion
AmpFℓSTR NGM kit was validated for new instruments (like ABI-3130). Using the kit with ABI-310 resulted in different genotyping in different runs for the child sample. Technical support from Applied Biosystems attributed the problem to low discrimination power for old instruments such as ABI-310, when the instrument set up, environmental and temperature condition are not perfect. Only modifications of GeneMapperID parameters allowed the discovery of the heterozygosis status in the child (19.3–20). This case underlines the necessity to always carefully evaluate the electropherograms, to pay attention to different runs and to keep in touch with the manufacturer's technical support when some alerts occur, above all using kit (such as NGM) not validated for old instruments (such as ABI-310) that are however still used in a lot of labs. Applying a very strict and regular maintenance schedule, together with fresh reagents could help too in order to avoid the case of modifying the analysis method.

P 355 THE FUTURE OF FORENSIC SCIENCE STANDARDS

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In 2010 the Australia New Zealand Policing Advisory Agency National Institute of Forensic Science, with funding from the Australian Government, established a project to set up a sustainable mechanism for the long-term development and maintenance of Standards, across a broad science and technology base that is relevant to general law enforcement, as well as the forensic science community.

The project includes four core general forensic Standards covering the collection, analysis, interpretation and reporting of forensic evidence and discipline specific Standards.

One specific Australian Standard already at late stage development covers the analysis of ignitable liquid residues and the second specific Standard identified covers the manufacture of products used to collect biological material for forensic analysis. This is a significant issue as contamination events in the manufacturing process have resulted in non-case related DNA profiles obtained during case work analysis hampering investigations. This has occurred both in Australia and internationally.

The core Standards continue to be developed as Australian Standards however the contamination minimisation Standard is being progressed through ISO. The development of the Standards and potential impact in Australia and internationally will be discussed.

P 356 MITOCHONDRIAL DNA CONTROL REGION VARIATION FROM SAMPLES OF THE MOROCCAN POPULATION

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To guarantee high quality mitochondrial DNA reference population data to facilitate forensic mtDNA testing in Morocco, control region sequences were generated using a comprehensive laboratory processing and data review system from 509 individuals. This large dataset, comprised of random samples of various Moroccan population groups (Arabs speaking, Berbers speaking, Sahrawi's speaking and Moroccan mixed), exhibited a low random match probability (0.52%) and a mean of pairwise comparisons of 13.24. Among all individuals, 372 different haplotypes were observed, of which 315 were unique. The most common haplotype (16519C 263G 309.1C 315.1C 573.1C; Haplogroup R0) was shared by 32 individuals. The mtDNA pools for the studied populations show different compositions of West Eurasian haplogroups (55,64%) and African haplogroups (44,35%). The data presented here are available for forensic mtDNA comparison.

P 357 DIRECT PCR BY THE AMPFℓSTR NGM™ KIT FOR DATABASE PURPOSE

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To fully maximize the utility of DNA databases for tracing the origins of criminal activity, the streamlining of database sample processing must also be accompanied by high performance chemistries. Blood and buccal samples are often used for convicted-felon DNA profile databases, as these samples are easy to obtain. The AmpFℓSTR NGM™ Kit amplifies the 5 new markers approved by the European Union Council for the expansion of the European Standard Set (D10S1248, D22S1045, D2S441, D1S1656 & D12S391) together with the previous well established loci (D2S1338, D3S1358, D8S1179, D16S539, D18S51, D19S433, D21S11, FGA, TH01, vWA, Amelogenin).

Because of NGM™ Kit enhanced performance on degraded and inhibited samples and tolerance to PCR inhibitors we decided to test the kit ability to amplify directly blood and buccal samples deposited on special collecting cards.

Saliva/blood samples from donors were collected using the IsoCode sample collection device. A 1.2 mm disc punch from the card was added to the tube together with PCR reagents and go straight to amplification.

The ability to amplify stored DNA directly would allow the lab to get results more efficiently by eliminating the DNA purification step, minimizing the possibility of contamination reducing costs and time accelerating testing by as much as 30% so that lab faster would send consistent results to national database .

P 358 AN INTEGRATED SYSTEM FOR PRE-PCR PUNCHING AND LIQUID HANDLING

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Governmental agencies everywhere in the world are ramping up their efforts for building reference databases. With the increased throughput, the projects are suffering from the fact that there is no instrument available which is able to automate the complete pre-PCR workflow. This leads to extra costs due to complex integration of different instruments and/or further labour time and cost. In addition, it leads to plate logistics between separate instruments and reduces traceability and process stability. A further issue is the limited sample recognition capability of current automated punchers or the biased choice of punch position for the manual ones, influencing the quality of PCR results.

To overcome these problems, we have developed a system with integrated punching and liquid handling. The core part of the system is a module for card transport and a module for punching sampling cards both integrated in the well established Hamilton Microlab STARlet workstation. This allows for seamless integration of the punching in the pipetting workflow for pre-PCR preparation. In addition, we included a camera with up to date image recognition software to evaluate sampled indicating cards. This enables us to detect saliva sample spots and calculate the best position for punching within the sample spot which can potentially improve the success rate of the reaction. Positive recognition of the punched discs in the wells of the target plate improves traceability of the samples.

The integration of punching and pipetting into one system leads to a significant improvement of the workflow, decreased overall costs and improves process reliability.

P 359 BGBX: X-CHROMOSOME BRAZILIAN GENETIC DATABASE

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Polymorphic Short Tandem Repeats (STRs) are the markers most commonly used in human identification classified as autosomal, Y and X-chromosome STRs. The latter are of recent use and very efficiently complement autosomal analysis in complex cases of biological relationships (when the alleged father is not available, in paternity cases involving close blood relatives, etc.). The authors developed the X-Chromosome Brazilian Genetic Database (BGBX) with the aim of compiling all published Brazilian data and provides another available tool for the Brazilian Scientific Police and DNA Laboratories. The database system was implemented using the PHP manager with MySQL, both free, and used the JavaScript Framework. BGBX consists of different sections: Home – X-STRs in Brazil – Ideogram of the X-chromosome – Data of the X-STRs, containing data published on X-STRs markers for the Brazilian population such as: allelic and haplotype frequencies, the statistical parameters of forensic interest and paternity tests, data markers in linkage disequilibrium, and other information (physical and genetic location of the X-STRs, sequence composition, primers described, etc.). There is also a section for researchers to submit their data since these are published in scientific journals. These data will be checked and assessed by the coordinator of the project before to be accessible. The database will be publicly available in next May (www.bgbx.com.br). This initiative will facilitate the data search and access, disseminating the researches with X-STRs in Brazil, increasing our scientific recognition by the international community, as soon as it will be translated into English.

Support: FAPESP, CAPES, PADC/FCF-UNESP.

P 360 ESTABLISHMENT OF ITALIAN NATIONAL DNA DATABASE AND THE CENTRAL LABORATORY: SOME ASPECTS

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On 27 May 2005 in Prüm was signed a convention between the Kingdom of Belgium, the Federal Republic of Germany, the Kingdom of Spain, the French Republic, the Grand Duchy of Luxembourg, the Kingdom of the Netherlands and the Republic of Austria on the stepping up of cross-border cooperation, particularly in combating terrorism, cross-border crime and illegal migration (hereinafter the Prüm Treaty) improving the exchange of information and data resulting by DNA, dactyloscopic and vehicle data to regulate forms of closer cooperation between law enforcement and judicial authorities.

On 23 June 2008 the Council of the European Union adopted the Prüm Treaty under the Treaty on European Union with the Decisions 2008/615/JHA and 2008/616/JHA.

On 14 July 2009 the Italian parliament has passed the act n.85/2009 published on the Official Journal (G.U.) n.160 Supp.Ord.n.108/L G.U. General series with the title: "Adhesion of the Italian Republic to the Prüm Treaty. Establishment of national Dna database (NDNADB) and the central laboratory for the NDNADB" in order to facilitate the identification the perpetrators of crimes (chapter 2).

The purpose of this paper is to illustrate some aspects described on the chapters of the law as the organization, the rules, the types of activities of NDNADB and the central laboratory, the categories of the person for inclusion in the DNA database, the methodology of analysis of evidence and reference sample, etc.

P 361 Y-SNP ANALYSIS IN AN ANGOLA POPULATION

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Introduction and Aims
From the study of Y chromosome, it can be evaluated the paternal lineages of an individual or a population. In this study, the polymorphisms of Y chromosome analyzed are biallelic markers of the Y chromosome – SNPs (Single Nucleotide Polymorphisms) – characterized as a single mutation event. The analysis of these polymorphisms allows the assignment of lineages to different groups, defining the haplogroups that characterize a population. This information can reveal the origin of a population, essential to population genetics and of significant importance to forensic genetics. The major advantage is that these markers can be studied in very short amplification products (50bp or less), being very useful in the analysis of highly degraded DNA samples. The aim of this research work is to characterize an Angola population, defining the haplogroups present in this population, for subsequent application to forensic genetics.

Materials and Methods
In this study, were used samples from unrelated individuals of an Angola population. The samples were extracted by the Chelex®100 method, amplified for the Multiplex E system (P2/M154/M293/M81/M85/M78/M35/M96/V6/M191/M33/M123/M2) and followed by the Snapshot minisequencing method. The detection was performed on the ABI PRISM® 310 Genetic Analyzer.

Results and Discussion
After analyzing the results we observed that the samples fit mostly in haplogroup E, which is characteristic of African populations. The most frequently sub-haplogroup observed in this population was E1b1a * (xE1b1a4, 7). It was still necessary to resort to other systems to characterize samples that did not fall into haplogroup E.

P 362 ANALYSIS OF CHROMOSOME Y SUGGEST SEVERAL DATABASES FOR COLOMBIAN POPULATION

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The Y-linked markers have traditionally been used for population studies and forensic genetics, among these markers for this use are the SNP and STR. For the latter, the STR, it has a large amount of information from populations around the world. For this reason they are very useful to compare populations with each other and thus to define the most appropriate data bases for each population of interest.

In this study we report the results of comparing the information the haplotypes of 12 Y-STRs (DYS19, DYS389i, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, GATAH4 and DYS635) in 1762 Colombian men. R_{ST} values were found statistically significant and no-significant for distances between 15 populations studied (Antioquia, Bolívar, Chocó, Córdoba, Valle, Santander, Cauca, Nariño, Magdalena, Cesar, Sucre, Guajira, Atlántico, La Po-Emberá Chamí, Guambiano Cauca), suggesting that caution should be taken when using the database of Y-chromosome haplotypes to perform any inference.

These results are consistent with historical and genetic data existing about Colombia, which has a population with a high degree of mixing caused by the colonization process occurred over 500 years ago.

P 363 GENETIC STRUCTURE OF MOROCCAN POPULATION USING 15 STRS OF NGM KIT

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Polymerase chain reaction (PCR) amplification using the AmpFℓSTR® NGM™ Kit ("NGM", an acronym for "Next Generation Multiplex") was performed in random sample of 200 unrelated individuals from three Moroccan population groups (Arab Speaking, Berber Speaking, and Sahrawi) Allele frequency and other forensically relevant statistics data were generated for the NGM™ multiplex kit includes the original 10 STR loci from the AmpFℓSTR SGM Plus® kit (D3S1358, vWA, D16S539, D2S1338, D8S1179, D19S433, TH01, FGA, D21S11, D18S51) together with five additional STRs (D10S1248, D22S1045, D2S441, D1S1656 and D12S391) and the Amelogenin sex-determination locus. Population study was conducted to evaluate usefulness of the loci (especially the five new microsatellite systems) in forensic genetic identification examinations. All Fifteen autosomal STR loci were found to be in Hardy-Weinberg equilibrium. Discrimination power was particularly high in case of D1S1656 and D12S391 STR loci.

We present the results of our developmental validation studies of critical performance parameters such as sensitivity, specificity, performance with inhibited PCRs and degraded DNAs, mixture sample analyses.

Keywords: Allele frequency, Degraded DNA, Morocco, NGM, STRs.

P 364 NEW VALIDATED ANALYTICAL PROCESS FOR CONVICTED OFFENDER SAMPLES SUBMITTED TO THE CANADIAN NATIONAL DNA DATA BANK

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In preparation for potential changes in Canadian legislation, the DNA analytical processes currently used for convicted offender (CO) samples collected on FTA® cards for the National DNA Data Bank (NDDB) have been modified to ensure optimal efficiencies with the implementation of new equipment and STR kits. Amplification conditions were optimized to perform direct amplification of single source blood, buccal and extracted hair roots directly from FTA® paper without the need for sample purification prior to amplification. Validation was performed using AmpFℓSTR® Identifiler® Direct (IDD) and PowerPlex® 16 HS (PP16HS) on a wide collection of blood, buccal and extracted hair roots collected from volunteer donors. In addition, 55 CO blood samples were selected to assess the robustness of the new analytical process (i.e. 0.53 mm FTA disks, not washed and amplified using IDD and PP16HS in 10 µL PCR volume). These represented CO samples that required rework when originally processed using the current NDDB protocols i.e. 1.5 mm FTA disks, washed three times and amplified using AmpFℓSTR® Profiler Plus® and COfiler® in 15 µL PCR volume. Another group of 36 CO samples was used to verify the stability of DNA on FTA paper (10 yr old samples). Conditions were also optimized for the separation and detection of amplicons on the high-throughput AB 3730 DNA Analyzer (48 capillaries). Studies such as cross-contamination, precision, reproducibility, robustness, sample stability and concordance were carried out. The new analytical process produced reliable and high quality profiles from all CO samples and volunteer samples which support its implementation.

P 365 EVALUATION OF COPAN NUCLEICARD™ FOR THE STORAGE OF BIOLOGICAL SAMPLES

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Introduction

The Copan NUCLEICard™ device (Copan Flock Technologies, Italy), a chemically treated paper, allows the room temperature storage of body fluids, preserves DNA integrity and eliminates bacterial proliferation. The objective of this study was to evaluate the NUCLEICard™ for DNA storage and preservation from biological samples for forensic analysis.

Materials and Methods

For this evaluation, saliva, buccal samples collected with forensic FLOQSwabs™ and blood were deposited on both NUCLEICard™ and Whatman FTA® card. After room temperature drying, 1.2mm discs were punched and tested with Quantifiler® Human DNA Quantification kit and for STR profiling: directly with Identifiler® Direct PCR Amplification Kit and after purification washes, with Identifiler® Plus PCR Amplification kit (Applied Biosystems).

Results and conclusions

When averaging replicate results, obtained from 10 different testing times, similar DNA concentrations (ng/ul) were obtained from samples stored in NUCLEICard™ and Whatman FTA® card; buccal swab (**1.65 vs. 2.00**), saliva (**3.95 vs. 2.60**) and blood (**2.95 vs. 3.10**). 100% of the profiles were successful with NUCLEICard™ and FTA®, with no significant differences in the quality parameters evaluated (intracolor balance and PHR). The Copan NUCLEICard™ can be a good device for storing saliva and blood samples for DNA profiling. Buccal FLOQSwabs™ allows non invasive sample collection and NUCLEICard™ device enables room temperature storage of nucleic acids.

P 366 COMPLETE AUTOMATED DNA PROCESS TO FACILITATE DNA DATABASE COLLECTION

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To address the requirement of DNA database workflow, approximately 200 000 FTA cards per year in duplicate, a complete automated procedure is necessary. Analysis by direct PCR on FTA punches is possible, however, since the quantity of extracted DNA is highly variable from one sample to another, genotyping by PCR with common commercial kits lead to a very high rate of invalidated genetic profiles (20 to 25%). In order to be in the best amplification conditions, it is important to isolate good quality DNA in a normalized concentration. To meet this requirement in one step, we used calibrated magnetic particles that are suitable with a fast and completely automated procedure, from DNA extraction to injection plate preparation. Extraction and quantitative normalization of DNA were performed from 257 FTA cards in duplicate using the Smart DNAdem Kit for Profiling (ADEMTECH). Automated extraction and PCR plates preparation were performed twice on three different Hamilton STAR robots. Extracted DNAs were amplified using Identifiler Plus PCR kit (Applied Biosystems). PCR products were injected on 3500xL genetic analyzer and genetic profiles were analyzed with GeneMapper ID-X v1.2 (Applied Biosystems). The success rate of this procedure is 96.5% in first analysis (average PHR of 85.9%). Furthermore, GeneMapper software validates in a reliable way a large number of genetic profiles without human intervention (80.7%). This automated process is a real alternative to the direct PCR on FTA punches in order to obtain, in first analysis, the best success rate of good quality genetic profiles.

P 367 STUDIES ON STR ALLELIC DISTRIBUTION AND MICROVARIANT ALLELE FREQUENCIES IN KOREAN DNA DATABASE SAMPLES

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According to DNA database law which has been enforced in 2010, Supreme Prosecution Service (Rep. of Korea) gets and manages DNA profiles from convicted offenders to compare with unsolved crime scene DNA cases. In databasing process using Identifiler kit, we analyzed allelic distribution of 15 Short Tandem Repeat (STR) markers and microvariant allelic frequencies from 16,828 individual samples.

Saliva spots of 16,828 people who were applicable to the DNA database were taken through the FTA cards. Extraction and quantification step of genomic DNA were processed using PrepFiler™ (Applied Biosystems) kit and 7500 Real-Time PCR System (Applied Biosystems).

Sample was normalized with automated robotic system and then amplified with AmpFℓSTR® Identifiler® Kit (Applied Biosystems). Genotypes of samples were analyzed ABI 3730 DNA Analyzer (Applied Biosystems) and GeneMapper ID v3.2 software (Applied Biosystems).

The Genotype and allelic frequency of each 15 STR loci was determined as usual. No statistically significant deviation from Hardy-Weinberg Equilibrium (HWE) was observed in Fisher's exact test using GDA v1.1 (Genetic Data Analysis v1.1) software with over 100,000 shuffling steps.

Trisomic or microvariant allelles were observed at 12 loci (CSF1PO, D13S371, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D7S820, D8S1179, FGA, TPOX). Except D21S11 (0.7%) and D7S820 (0.6%), the microvariant allele frequencies of other 8 loci were under 0.0005.

P 368 HAPLOTYPE FREQUENCIES AND MUTATION RATES FOR 17 Y-STRS IN A SAMPLE FROM MENDOZA PROVINCE (ARGENTINA)

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A sample of 566 unrelated males from Mendoza province was typed for 12 Y-STRs loci, 237 of these males were typed for 17 Y-STRs.

When analyzing only the minimal haplotype of 9 Y-STRs we found a total of 398 different haplotypes, of which 317 were unique, and the Haplotype Diversity was 0,9931.

If we analyzed the 12 Y-STRs, which make up the extended haplotype, we observed that 87% of the total of 470 different haplotypes are uniques, and the Haplotype Diversity was of 0,9971.

In the 237 males typed with 17 Y-STRs included in the AmpFℓSTR YFiler Amplification Kit (AB Applied Biosystems), we found 226 different haplotypes of which 96% are unique and the most frequent haplotype appears only 3 times in the study population.

On the other hand, in a total of 173 father/son pairs, mutations were observed in DYS389I/II, DYS385a/b, DYS439 and DYS458 markers. In addition, there were duplications on DYS19, DYS437 and DYS439 markers. However, the mutation rates are similar to those already described.

The comparison between our data and previous one (data) from Mendoza, other Argentinean provinces, and different world populations, reflects a similar haplotype distribution to that depicted by European populations. The most frequent haplotype is the modal for the European haplogroup R1b, this supports its European origin.

P 369 GENETIC DATA OF 17 AUTOSOMAL STRS IN MENDOZA POPULATION (ARGENTINA)

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In the present study the allele frequencies, together with some parameters of forensic interest, were estimated from a sample set of 1933 unrelated individuals from Mendoza province. We analyzed the 17 autosomal STRs included in the Powerplex-16 System (Promega Corp.) and AmpFℓSTR Identifiler Amplification Kit (AB Applied Biosystems) (TPOX, D2S1338, D3S1358, FGA, D5S818, CSF1PO, D7S820, D8S1179, TH01, VWA, D13S317, Penta E, D16S539, D18S51, D19S433, D21S11 and Penta D). No deviations from Hardy-Weinberg equilibrium were observed using the Bonferroni correction for the number of loci analyzed.

The most informative loci in our data set was the Penta E, with discrimination power larger than 0,98 and typical paternity index of 5,2059.

If we compare these results with previously published data, we observed a great similarity with the population of other Argentinean provinces and Caucasian populations in the world.

On the other hand, we estimated the mutation rate for these markers by analyzing a total of 596 trios mother / son / father. We found 14 mutations in different markers, most of them were observed in D8S1179 and FGA markers.

It is known that the creation of local reference databases it is extremely important for solving forensic caseworks and paternity testing, so this represents an important contribution to the local forensic genetics.

P 370 FAMILIAL SEARCHING – A REVIEW OF METHOD REFINEMENTS AND CASEWORK APPLICATIONS

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Searching of large national DNA databases to identify possible close familial relatives of unknown crime profile donors, often termed familial searching, has become increasingly common since its first use in 2003. LGC Forensics developed, validated and implemented a familial searching tool for interrogation of the UK national DNA database (NDNAD) in 2007 to identify possible siblings or parent/child relatives of the donor. This incorporated, for the first time, prioritisation of partial matches by likelihood ratio to rank the candidate relatives.

In casework applications, access to the NDNAD for searching is strictly controlled by the Database custodian, and the search itself is closely integrated into the investigative process with close liaison between the laboratory and the investigating officers. Filtering of possible relatives using demographic information (provided to the investigating officers by the NDNAD), coupled with Y-STR or mtDNA screening of potential relative's samples provides a framework for the effective application of this searching in serious crime investigations.

Here we review the development and use of familial searching by LGC Forensics and its application in a number of key cases.

P 371 STRUCTURAL POLYMORPHISM AT THE X CHROMOSOMAL SHORT TANDEM REPEAT LOCI DXS10134, DXS10135, DXS10146 AND DXS10148

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Sequence analysis of the X chromosomal short tandem repeat loci DXS10134, DXS10135, DXS10146 and DXS10148 was performed in 140 unrelated Japanese males. For the DXS10134 locus, 12 differently sized alleles were identified. Two types of sequence structures were observed in the repeat regions. The structural polymorphism for the proportion of repeat number of two repeat blocks, $(GAAA)_m$ and $(GAAA)_n$, in the sequence type was found only in one allele. For the DXS10135 locus, 16 differently sized alleles were identified. Three types of sequence structures were observed in the repeat regions. The structural polymorphism for different sequence types in an allele that was identical in size was found in eight differently sized alleles. For the DXS10146 locus, 14 differently sized alleles were identified. Two types of sequence structures were observed in the repeat regions. The structural polymorphism for the proportion of repeat number of two repeat blocks, $(TTCC)_m$ and $(CTTT)_n$, in the sequence type was found in two differently sized alleles. For the DXS10148 locus, 16 differently sized alleles were identified. Three types of sequence structures were observed in the repeat regions. The structural polymorphism for different sequence types in an allele that was identical in size was found in seven differently sized alleles. Discrepancy between allele designation based on size and repeat number was observed in two samples each of DXS10146 and DXS10148. We conclude that sequencing is effective in identifying the alleles of DXS10134, DXS10135, DXS10146 and DXS10148 where sequence polymorphism is observed.

P 372 DISCREPANCIES BETWEEN FORENSIC DNA DATABASES

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Evaluation of findings from forensic DNA analysis is made by the use of a reference database of DNA profiles compiled from a sample of individuals assumed to represent a specific population of interest. In several countries there are also databases of DNA profiles compiled from real casework. Such databases can consist of profiles obtained either from crime scenes for which no information about the donor is available, or from individuals suspected or convicted for a crime. The purpose with the latter is primarily to search them for hits against a new recovered profile from a crime scene for purposes of intelligence and investigative work. However, these databases are continuously growing (in comparison with a more steady-state reference database) and one might ask whether they would constitute a more accurate mirror of the population of potentials donors of a crime stain than does the reference database. Here we present a comprehensive statistical comparison between a number of reference databases used in Sweden and two databases of DNA profiles from casework. Our results show no substantial differences with respect to various measurements of overall discrepancies but reveal significant differences for individual alleles at several markers.

P 373 DATABASE SAMPLE SIZE EFFECT ON ALLELE MINIMAL FREQUENCY ESTIMATION: COMPARATION BETWEEN DATABASES ANALYSIS WITH 4652 AND 560 INDIVIDUALS RESPECTIVELY ON TWENTY MICROSATELLITES COLOMBIAN POPULATION DATA

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Introduction and aims
Two Colombian (Antioquia's region) population databases with 560 and 4652 individuals respectively were compared to test for the effects of database size on allele minimal frequency estimation and rare alleles frequency.

Material and methods
Blood Samples from 560 and 4652 non related Individuals from two mestizo population were analyzed using 16 STRs markers (AmpFℓSTR® Identifiler kit, Applied Biosystems). Samples were run on genetic analyser 3130 (Applied Biosystems), data collection and allele calling were performed using Data Collection v. 3.0 and Genemapper v.3.2. Population statistics for each marker was done using PoweStat and allele minimal frequencies were calculated using both Chakraborty's (Chakraborty, 1992) and Weir's method (Weir, 1992).

Results and discussion
When database size was increased, the total number of aleles increased in a 25%, going from 187 (n=560) up to 251 alleles (n=4652). A marked effect was also detected for de number of alleles with frequencies below the minimal frequency m.f, while for the smaller database xxx alleles have frequency < m.f, for the bigger database it happened for only one allele. These results indicates that Antioquia's mestizo population is well represented in the latter database.

Chakraborty R (1992) Sample size requirements for addressing the population genetic issues of forensic use of DNA typing. Hum Biology 64:141–159.

Weir BS (1992) Independence of VNTR alleles defined by fixed bins. Genetics 130 : 873–887.

P 374 INVESTIGATIVE DNA DATABASES THAT PRESERVE IDENTIFICATION INFORMATION

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A DNA database can link crime scenes to suspects, providing investigative leads. These DNA associations can solve cold cases, track terrorists, and stop criminals before they inflict further harm. However, current government databases do not fully preserve DNA identification information, and cannot maximize public safety.

DNA data is summarized in a genotype. The genotype can be stored on a database, and compared with other genotypes to form a likelihood ratio (LR) match statistic. Data uncertainty, present in most evidence, translates into genotype probability.

Highly informative interpretation uses all the quantitative DNA data, placing higher probability on more likely genotype values. Most evidence, though, is interpreted by qualitative human review, which diffuses probability across infeasible solutions. Since the LR is proportional to the true genotype probability, weaker interpretation methods lead to weaker (or nonexistent) DNA matches.

The weakest DNA interpretation method is RMNE, which thresholds quantitative data into all-or-none qualitative "allele" events. The current DNA databases (including CODIS) use an RMNE allele representation that discards considerable genotype information, losing sensitivity and specificity.

The "probabilistic genotype" representation is part of the new ANSI/NIST-ITL data exchange standard. Unlike allele lists, this database representation can preserve all DNA identification information, and be quantified dynamically into LR match statistics. Every interpretation method has a corresponding genotype probability representation.

ISFG's 2006 mixture guidelines recommend the more informative LR over RMNE. Unfortunately, current databases transform hard won LR genotypes into less informative RMNE alleles. This talk shows how genotype probability can preserve identification information for DNA investigation.

P 375 APPLICATION OF DIRECT PCR TO CRIMINAL DNA DATABASE IN KOREA

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Since Korea DNA database was launched in 2010, we have concentrated on establishment of automated DNA database profiling system which analyzes STR loci with high throughput and cost effective. Direct PCR is the technology that makes it possible without DNA extraction and quantification. Therefore, direct PCR has the advantage of reducing time, labor and the risk of cross contamination. For implementation of direct PCR into DNA database, we performed the optimization studies with buccal FTA® indicating card (Whatman). It was conducted by simply replacing AmpFℓSTR Identifiler® PCR Reaction Mix (Applied Biosystems) with commercial direct PCR buffer, AnyDirect F PCR Mix™ (BioQuest Inc.). The 15 Identifiler STR profiles were obtained from 3,076 individuals and followed by comparison of the results with those of corresponding purified DNAs. The direct PCR method showed not only whole concordance with purified DNA but also excellent results in the point of PCR success rate and multiplex PCR quality. Intra/inter loci peak height ratio showed even more balanced shape in direct PCR. In conclusion, our established direct PCR system can be very useful tool for constructing time and cost saving DNA database system.

P 376 MITOCHONDRIAL DNA-CONTROL REGION SEQUENCE VARIATION IN THE NE PORTUGUESE JEWISH COMMUNITY

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The cultural phenomenon of Crypto-Judaism, defined as the secret adherence to Judaism while publicly professing another faith, arose in Portugal in the beginning of the 16th century after the Decree of Expulsion and the establishment of the Inquisition. Surprisingly, the scientific community acknowledged the persistence of Crypto-Judaic communities at the beginning of the 20th century in central and north-eastern regions of the country (e.g. Bragança).

In the present work we aimed at characterizing the maternal lineages (mitochondrial DNA – mtDNA) of these communities.

We have sampled 56 unrelated individuals from 5 different communities of the Bragança district, namely: Argoselo, Bragança, Carção, Mogadouro and Vilarinho dos Galegos. A 3348bp mtDNA fragment was amplified and sequenced in two overlapping fragments using mitochondrial-specific primers in order to obtain the entire control region (from 16024bp to 576bp, according to the rCRS). Haplogroup classification was performed according to current nomenclature.

High frequencies were found for haplogroups H (35.7%), HV0 (14,3%), T2 (12,5%), U5 (10,7%) and N1 (7,1%) which, when compared to respective frequencies in the Portuguese host population (H – 41,1%, HV0 – 5,4%, T2 – 5,2%, U5 – 7,2%, N1 – 0,6%), indicate some degree of admixture with Western Europe populations along with a remarkable signature of a Near East ancestry.

In accordance with previous results on the male lineages these data confirm that the Crypto-Judaic communities from Bragança were able to maintain not only their cultural identity but also some ancestral genetic identity, showing a significant population substructure with forensic relevance.

P 377 X-CHROMOSOME IN ITALY: A DATABASE OF 29 STRS

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In order to establish a database of X-chromosome markers typed by different forensic laboratories in Italy, we collected published and unpublished data from 16 contributing groups participating in the GeFI (Italian Forensic Genetists). The total number of typed markers was 29, scattered along the X-chromosome physical map in several clusters; the number of typed markers per lab was 4 to 12. The total number of typed subjects was 1120 males and 781 females, coming from 12 regions of North, Center and South Italy. The most used marker was DXS7423 (1020 males, 781 females); the mean number of subjects typed per marker was 356 for males and 223 for females.

P 378 POPULATION GENETIC EVALUATION OF 12 X-CHROMOSOMAL SHORT TANDEM REPEATS OF INVESTIGATOR ARGUS X-12 KIT IN NORTH-EAST ITALY

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The aim of this work is to extend the evaluation of suitable X-Short Tandem Repeat (STR) in complex kinship cases when the autosomal STR do not lead to provide informative results.

For this purpose we evaluated genetic population data of 12 X-Chromosomal STRs (DXS10103, DXS8378, DXS7132, DXS10134, DXS10074, DXS10101, DXS10135, DXS7423, DXS10146, DXS10079, HPRTB, DXS10148) using Investigator Argus X-12 PCR Amplification kit. A total of 207 unrelated healthy individuals (89 females and 118 males) living in North-East Italy were analyzed.

Allele frequencies of the 12 markers and parameters of forensic interest for each X-STR were calculated. Some new microvariant alleles in the loci DXS10148, DXS10101, DXS10079 have been detected.

The Polymorphism Information Content (PIC) of the 12 X-STRs ranged from 0.642 (DXS8378) to 0.942 (DXS10135).

P 379 CONCORDANCE STUDY AND ALLELE FREQUENCIES FOR 5 NEW EUROPEAN STANDARD SET (ESS) LOCI IN THE NORTH-EAST ITALIAN POPULATION

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Recently new commercial kits included five new additional autosomal short tandem repeats (D1S1656, D2S441, D10S1248, D12S391 and D22S1045) were developed to meet the European Network of Forensic Science Institutes (ENFSI) and European DNA Profiling (EDNAP) Group recommendations.

The aim of this work was to performe a concordance study and population genetic data of these five new ESS loci using four different kits: AmpFℓSTR® NGM™ PCR Amplification Kit, PowerPlex® ESX and PowerPlex ESI Systems and Investigator ESSplex SE kit®. Buccal swab sample were collected from 266 unrelated healthy individuals from North-East Italy and allele frequencies were calculated and compared to previously published population data. Forensic parameters were calculated using the software package PowerStats. No significant differences were found in comparison with Italian and European population data.

Full concordance between the typing results for the four kits was observed in 99,925% (2658 out of 2660).

P 380 DOING MORE WITH LESS: IMPLEMENTING DIRECT AMPLIFICATION WITH THE POWERPLEX® 18D SYSTEM

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"No good deed goes unpunished ..." goes the saying about being noticed for effective performance. The state and national DNA databasing program demonstrates increasing value to law enforcement and has subsequently seen rapid growth in workload. Development of single-amplification STR kits, multicapillary electrophoresis and automation has provided great efficiency gains over the past decade. Still, many database laboratories seek further process improvements to keep pace with the dramatic growth in submissions.

Currently, sample processing and thermal cycling are two of the longest steps in the STR analysis process for databasing laboratories. The PowerPlex® 18D System has been developed to significantly reduce the STR analysis process with direct amplification and rapid PCR technology. Additionally, simplifying or removing sample preparation also reduces manipulations and saves extraction reagents. Performance and workflow impact of PowerPlex® 18D will be shared for processing of common sample types including GE/Whatman FTA® cards, Omni Swabs and Bode DNA Collectors™.

P 381 CAN MICROBES ON SKIN HELP LINKING PERSONS AND CRIMES?

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Linking persons to crimes throughout DNA analysis is well-established approach for more than 25 years. While enormous numbers of cases all over the world have been solved based on DNA, there is still need for additional tools for improvement in physical evidence choice and collection. Unfortunately not all samples collected from crime scenes, are suitable for linking persons to crimes due to the quantity and quality of the human DNA collected. Recent studies have shown personality of bacterial community on human body surface that may open new perspectives for forensics.

The aim of the study was evaluation of variability of bacterial communities on skin of palm and fingers between and within individuals as well as transfer of bacterial DNA during contact to the object and persistence of community parameters during storage. Four volunteers were recruited and samples collected during five days and afterwards once per week during 1 month in the morning and in the afternoon by swabbing of palm and fingers after holding sterile object for 1 min with persons dominant hand. The samples were also collected from the handled objects, except the first object, which surface was quartered and sampled by quarter zone with one-week interval after storing the object at room temperature. DNA was extracted and metagenomic analysis of bacterial community using 16S rRNA gene hypervariable regions was performed on Roche/454 platform. Our preliminary results are promising however foresee need for more elaborative studies to be able to implement this approach into the routine practice.

P 382 IDENTIFICATION OF AN ANIMAL CONTRIBUTOR WITHIN MIXTURE TRACES BY INTERPRETATION OF THE MITOCHONDRIAL DNA SEQUENCE COMPONENTS: CASEWORK REPORTS

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Species identification of non-human traces is well established in the forensic community and supplements the commonly used human specific DNA analyses. The routinely used Sanger sequencing analysis of the cytochrome b gene proved to be one of the most suitable methods to infer the species of an unknown sample over the last years as it is robust and in a lot of cases successful due to the high copy number of mitochondrial (mt) DNA per cell.

However, as a consequence of potentially high human background in forensic evidentiary samples the analysis of the cytochrome b gene may occasionally result in a DNA mixture. So far, mtDNA mixtures were either interpreted as inconclusive or the mixture components were separated using laboratory technologies such as cloning that are time consuming and cost-intensive.

We here present two case work reports where we were able to resolve mtDNA components in forensic mixture samples by phylogenetic interpretation of the components. The haplotypes of a minor (in both cases human) and a major contributor (once pigeon and once cat) were interpreted based on differences in allele peak height at several mixed positions within the cytochrome b gene. Species identification was then performed using a BLAST (Basic Local Alignment Search Tool) search and the results were confirmed by cloning and sequencing of the control regions using species specific primers.

P 383 A MULTIPLEX PCR APPROACH FOR IDENTIFICATION OF HUMAN BODY FLUIDS BY THEIR MICROBIAL "SIGNATURE"

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The discrimination of body fluids in forensic examinations is crucial for the comprehension of the events happened on the crime scene. Conventional methods rely on the detection of antigens or enzymatic activity, with limits on the level of sensitivity and specificity, especially on old forensic samples. Methods based on RNA analysis are not easily applicable to aged samples due to the rapid degradation of the template. For these reasons a new approach based on the identification of prokaryotic genomes was developed. Specific bacterial communities are typical of different human non-sterile body fluids: the molecular characterization of the microbial signature can effectively lead to univocal identification of these fluids.

Bacterial DNA was extracted from 10 vaginal, 5 oral and 2 faecal swabs. In addition 2 forensic vaginal swabs were processed. We set up a multiplex real time PCR using an oligonucleotides mixture annealing on genomes sequences specific for a selected group of bacteria. The samples were also processed in forensic STR profiling.

Vaginal samples showed a strong specific signal for bacteria of the female genital tract (Ct 18–27). Oral samples showed only signal for bacteria present in saliva (Ct 25–33); in faecal samples the main signal was from *Enterococcaceae*. Vaginal forensic samples showed results comparable to the fresh ones; moreover the DNA extracted was efficiently used in STR tests.

In conclusion, an easy and robust technical approach for the discrimination of human body fluids was developed. The stability of DNA allows investigators to apply this method also to aged forensic samples.

P 384 IDENTIFICATION OF PINUS SYLVESTRIS BY MULTIPLEX STR ANALYSIS IN CRIME SCENE INVESTIGATION

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Genetic analysis tools were validated for human identifications and relations study. Sometimes identification of other species is necessary in crime scene investigation. For some animals, commercial kits for individual identification are available.

In our case on the edge of the forest complex, in the south of Poland a man's body was discovered. In the trunk of the suspect's car a fragment of pine branches was revealed. The second part of the branch was located within the components of the chassis, and the third one within the area of the car engine. Traces of a car running through the broken young pine trees were also revealed at the crime scene which extracts were secured as reference material. It was founded that the fragments of the pine branches found during an inspection of the suspect's car come from the damaged trees from the crime scene. In order to verify the hypothesis of accusations genetic study were performed.

Multiplex system was establish for genotyping *Pinus silvestris* STR: SPAG7.14, SPAC12.5, PtTx2146, PtTx3116, PtTx3032 and PtTx4001.

Population study was performed in 200 individuals for origin of Poland.

Allele frequencies were calculated for each STR locus. HI and PIC was established. Hardy-Weinberg equilibrium was determined. No correlation between and across loci. PD and MEC was calculated for single loci and for multiplex.

High values of heterozigosity, polimorphic information contens, power of discrimination and mean exclusion chance demonstrate that establish multiplex system is valuable tools for individual identifications and relationship study in *Pinus sylvestris* for forensic casework.

P 385 FORENSIC IDENTIFICATION OF 15 MAMMALIAN SPECIES BASED ON PCR AMPLICONS OF MITOHONDRIAL CYTOCHROME B GENE

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Species identification of biological samples is one of the most important aspects in forensic science not only in routine casework analysis with human origin, but also in particular cases that include non-human remains. Conventional methods for investigation of biological samples that are based on immunological and morphological tests discriminate are less and less used for discrimination between different species, because of the limited stability and the commercial support of antibodies against various species. In our study, we have used new approach based on nucleotide sequence diversities among species in particular regions on mitochondrial cytochrome *b* gene. The high copy number of mtDNA compared to nuclear DNA makes this locus ideal for the analysis of forensic samples. Analysis of DNA samples from 15 mammals (samples from domestic animals were obtained from veterinarians and samples of wild animals from a biological institute) based on different sizes of PCR amplicons on agarose electrophoresis. The species-specific primers were designed so that they will only react with the species for which they were designed (except dog and wolf). Primers specific for domestic animals were taken from S.S. Tobe, but for wild animals that live specifically in our area we created alone. The differences in the sizes of the polymerase chain reaction ranging from about 90 to 340 bp permitted us to identify all species, even the most similar roe and red deer. We will discuss also about species identification using multiplex PCR in combination with capillary electrophoresis.

P 386 DEVELOPING EQUINE MTDNA PROFILING FOR FORENSIC APPLICATION

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Horse mtDNA profiling can be useful in forensic and archaeological work investigating degraded samples. In this study we search for the most discriminatory sections within the hypervariable horse mtDNA control region. Among a random sample of 39 horses, we identified 32 different sequences in a stretch of 921 nucleotides. The sequences were assigned to the published mtDNA types A-G, and to a newly labelled minor type H. The random match probability within the analysed samples is 3.61%, and the average pairwise sequence difference is 15 nucleotides. In a "sliding window" analysis of 200-nucleotide sections of the mtDNA control region, we find that the known repetitive central motif divides the mtDNA control region into a highly diverse segment and a markedly less discriminatory segment. We apply these high-quality sequences to scan for and rectify mistakes in published equid mtDNA.

P 387 MITOCHONDRIAL GENES ALLOW DISCRIMINATION BETWEEN THREE CYNEGETIC SPECIES FOR FORENSIC PURPOSES

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Efficient tools for consistent species identification are essential as it can provide information of the implication of animals in forensic-related events. The development of better assays for precise species determination are always in demand. This is particularly due to different legal situations in which animals can be implicated, such as accidents where biological traces are required by law to reconstruct the crime scene. Other related cases include quality control in the food industry and wildlife poaching. DNA analytical techniques based on selected mitochondrial genes, such as Cytochrome b (CYTB), are powerful alternative tools for specie identification (Pfeiffer et al. 2004; BMC Genetics 5:30). The objective of this study was to evaluate the effectiveness of the mitochondrial CYTB, cytochrome c oxidase subunit I (COX1) and 16S rRNA genes for discriminating between three important cynegetic species. Samples included deer (*Cervus elaphus*), roe buck (*Capreolus capreolus*) and wild boar (*Sus scrofa*). Five miligrams of muscle were used to extract DNA followed by PCR amplification with in-house designed primers and separation via capillary electrophoresis on the ABI PRISM® 3130 Genetic Analyzer. The sequences were edited with Chromas Pro 1.34 and aligned with Clustal X v2.0. The alignment of the consensus sequences of CYTB, COX1 and 16S rRNA genes revealed interspecific differences between the three game species under study. The sequence variations encountered across the three genes allow the proper discrimination of each species.

P 388 PCR-BASED DETECTION OF THE WHITE-TAILED EAGLE

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The white-tailed eagle (*Haliaeetus albicilla*) is listed in Annex I of the European Birds Directive (Directive 2009/147/EC on the conservation of wild birds) and in the Austrian Red List as "critically endangered". This large apex predator is not only threatened by the bioaccumulation of environmental pollutants, but also by targeted poisoning and poaching. In connection with a forensic case of illegal gamebird hunting, we developed a novel PCR assay for the sensitive and specific detection of *H. albicilla*-DNA traces in mixtures. The assay amplifies a fragment of the popular phylogenetic marker gene cytochrome b. Primers were designed to bind sites with relatively high variability between homologous sequences from *H. albicilla* and other related local birds of prey. The assay was tested *in vitro* and did not cross-react with DNA extracts from humans and the following raptors: common buzzard *(B. buteo*), northern goshawk (*A. gentilis*), red kite (*M. milvus*) and black kite (*M. migrans*). Cytochrome b is encoded in the mitochondrial DNA, which exists in high copy numbers in eukaryotic cells. Therefore, the assay provides enough sensitivity to work with DNA extracts of low quantity and quality. Its applicability for the analysis of poor quality samples was demonstrated with DNA extracts from small molted feathers collected in Austrian wetlands in close proximity to eagle nests. Amplicons of the expected size were generated, purified and sequenced. Sequence data were subjected to BLAST analysis and affiliated with cytochrome b from *H. albicilla*.

P 389 APPLICATION OF DIRECT PCR IN A FORENSIC CASE OF TOXIC PLANT POISONING

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Intoxications with yew (*Taxus spp.*) pose a challenge to forensic toxicology, because a variety of *Taxus* ingredients have been associated with its toxic effects. To provide preliminary evidence in cases where plant material is available, we introduce a novel direct PCR assay for the detection of DNA traces from *Taxus spp.*. This assay has been successfully applied to a forensic case of suicidal poisoning via ingestion of *Taxus* leaves. PCR primers were designed to target a sequence located in the internal transcribed spacer 1 (ITS1) of nuclear ribosomal DNA, which is relatively well-conserved among species of the genus *Taxus* and can therefore be exploited to discriminate between *Taxus* and other related conifers. Because ITS1 exists as a multicopy sequence within the plant genome, the assay provides enough sensitivity to work with trace amounts that are below the DNA content of a single cell. Specificity of the assay was tested with DNA extracts from *Taxaceae* and selected representatives from other related plant families (*Cephalotaxaceae*, *Cupressaceae* and *Pinaceae*). When combined with the commercial Phire® Plant Direct PCR kit (Finnzymes), the primers allowed application of a 2-step cycling protocol (without the annealing step), and because direct PCR requires only little sample pre-treatment, results from PCR could be obtained within 1.5 hours after analysis had begun. Direct PCR was performed with diluted gastric content from the forensic case. Amplification products of the expected size were purified and sequenced. Sequence data were subjected to BLAST analysis and affiliated with ITS1 from *Taxus baccata*.

P 390 THE POSTMORTEM BEHAVIOR OF RAT MICRORNA (MIRNA) AS DETERMINED BY THE COMPREHENSIVE MICROARRAY ANALYSIS

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Introduction and aims
Non-coding and small single-stranded RNA molecules, microRNAs (miRNAs), have been found to regulate a variety of physiological functions and to be stable at postmortem. We focused on the behavior of miRNAs at postmortem as a biomarker to quantify the physical stress. In this preliminary study on rat, we determined a number of miRNAs that exist after death using comprehensive microarray analysis of rat miRNAs.

Materials and methods
Fifteen 10 weeks-old Sprague-Dawley rats were randomly divided into five groups. Heart tissue specimens (n=3) were taken from just after death (0h), 12, 24, 36 and 48 h after euthanasia with anesthetic drug. Total RNA was isolated using a mirVana™ miRNA Isolation Kit (Ambion, Foster City, USA). Complementary RNA were amplified and labeled with Cy3 fluorescent dye using miRNA Complete Labeling and Hyb Kit (Agilent Technologies, Santa Clara, USA). The labeled samples were hybridized with microarray with 350 rat miRNA probes. The hybridized images were detected and analyzed by a DNA MicroArray Scanner (Agilent).

Results and discussion
The total 15 samples were divided into early (0–24 h) and late (36–48 h) stage groups by hierarchical clustering and principal component analyses. Using the *k*-Means method analysis, 237 miRNAs were under the threshold level and 105 were expressed until 48 h after death. In addition, eight miRNAs showed the unstable level with postmortem. Further investigations using Real-Time PCR analysis and animal experiments are required.

P 391 MULTILOCUS VNTR ANALYSIS AS A MICROBIAL FORENSIC TOOL FOR SUBTYPING CHINESE ESCHERICHIA COLI O157:H7 STRAINS

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In microbial forensics arena, the large number of microbes been used as potential bioterrorist agents in a criminal attack, especially many foodborne pathogens such as *Escherichia coli* O157:H7 caused serious zoonotic disease involved. Further development of high resolution genotyping assays is needed to rapidly and accurately analyze microbiological evidence from a crime for attribution purposes. Some recent approaches support multilocus variable number tandem repeat analysis (MLVA) is a PCR-based subtyping method to discriminate amongst different strains of a bacterium. In our study, MLVA combined with automated capillary electrophoresis was used to analyze genetic relationships and potential population structure within 31 *E. coli* O157:H7 isolates from humans and animals in China and standard strains. Alleles of each multilocus variable number tandem repeat (VNTR) were validated by sequencing. MLVA resolved 29 distinct genotypes respectively, and were able to largely separate genotypes from humans and all kinds of animals among zoonotic strains. Microvariation events occurred in two VNTR loci. In the light of the advantages of highly discrimination, MLVA can be regarded as powerful tools for detailed tracking of *E. coli* O157:H7 and could also prove useful in forensic investigations.

P 392 FORENSIC ENTOMOLOGY: NUCLEAR AND MITOCHONDRIAL MARKERS FOR DIPTERA AND COLEOPTERA'S IDENTIFICATION

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The presence of entomological evidences can be of great importance to solve some forensic investigations. Hence, insect species identification found in corpses is extremely important. In this direction becomes necessary to create a database containing molecular information about the insect diversity in different geographic regions. Implementing such molecular database requires the establishment of used molecular markers suitability to discriminate different species. COI (cytochrome c oxidase subunit I) is the most used *locus* to insects identification, but CytB (cytochrome b) have already been used with this purpose. Due to mitochondrial markers' limitations, some studies were performed using nuclear markers, e.g. ITS2 (internal transcribed spacer 2). In this study it was used two mitochondrial markers (CytB and COI) and one nuclear marker (ITS2), on which it was intended to infer about its suitability for Diptera and Coleoptera identification.

Insect specimens were collected from decomposing vertebrates. CytB, COI and ITS2 fragments were amplified by PCR methodology, and sequenced. Sequences were matched in the Basic Local Alignment Search Tool (BLAST). Nucleotide sequence divergence and phylogenetic analyses were performed.

Insect specimens were properly identified through online tool BLAST, using COI and ITS2. However, CytB demonstrated some limitations leading to some incorrect identifications, possibly due to the lack of information from this molecular marker in the database.

Obtained results demonstrate that combining nuclear and mitochondrial markers constitute a valuable and efficient complement for insect species identification, being more effective than an identification using a single marker.

P 393 FORENSIC WILDLIFE: MOLECULAR IDENTIFICATION OF BLOWFLY SPECIES (DIPTERA: CALLIPHORIDAE) IN PORTUGAL

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Forensic entomology applies knowledge about insects and other arthropods to legal issues. Calliphoridae adults are one of the earliest sarcosaphagous to infest a corpse. Traditionally identification has been carried out based on morphological characters and requires specialized taxonomic knowledge. Interspecific differences in morphology are frequently subtle and sometimes even in adult specimens identification is very difficult or even impossible. However, molecular characterization provides a rapid, precise and reliably method which can be performed at all developmental stages.

Blowflies of the family Calliphoridae (*Calliphora vicina, C. vomitoria, Lucilia caesar, L. illustris* and *L. sericata*) were collected from Serra da Estrela, Portugal, using carrion-baited traps, and identified by morphological characterization. DNA was extracted using EZNA® Insect DNA Isolation kit. Two molecular markers were amplified and sequenced, one mitochondrial and one nuclear marker, cytochrome c oxidase subunit I (COI) and internal nuclear spacer 2 (ITS2), respectively. Sequences were analyzed using BioEdit® software, and compared with ClustalX to find interspecific differences. All samples were matched by Basic Local Alignment Search Tool (BLAST).

Using COI, all species were correctly identified in the BLAST searches except for *L. caesar* and *L. illustris*, both species difficult to identify using morphological characteristics. When comparing the sequences of both species it was obvious how very similar they were, not being distinguishable using the analyzed COI sequences. However, this problem was overcome using ITS2 that was suitable to correctly identify these species. Our results show how helpful it was the study of an additional nuclear gene to provide extra information.

P 394 DISCRIMINATION OF 'FIBER-TYPE' AND 'DRUG-TYPE' CANNABIS SATIVA L. BY FLUORESCENT DUPLEX PCR

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A fluorescent duplex-PCR test was developed based on polymorphisms of the THCA synthase gene in order to discriminate the fiber- and drug-type *Cannabis sativa* L. and to indicate the presence of *Cannabis* trace in suspected materials by the numbers and sizes of PCR-amplified products. DNA analysis of drug-type *Cannabis* resulted in two different PCR-amplified DNA fragments of 94 and 158 bp, whereas only the 94-bp PCR product was amplified from the fiber-type DNA. DNA test results of another 6 *Cannabis sativa* L. collected from the field agreed with chemotype determined by GC-MS. However, it was noted that the only intermediate drug-type tested gave a drug-type results for DNA testing. Specificity of the duplex PCR was shown by testing with DNA from species that may be related to *Cannabis* abuse, i.e. common hop (*Humulus lupulus* L.), 2 narcotic plants (*Papaver somniferum* and *Mitragyna speciosa*), and human. Sensitivity of detection was as low as 10 pg of genomic DNA.

P 395 ADULTERY IS EASIER IN THE CITY – EVEN IF YOU ARE A GREAT TIT (*PARUS MAJOR*)

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Short tandem repeat (STR) analysis has become a widespread method not only in forensic science, but also in zoology. Paternity analysis has fundamentally changed our view on mating strategies in birds: it revealed that many songbirds – which were traditionally assumed to be monogamous – engage in extra-pair copulations producing clutches with more than one father. Nevertheless, the reasons for unfaithfulness – mostly initiated by the female – are still not fully elucidated.

One possibility is that females evaluate the "quality" of their partner by the amount of food that he provides during egg-laying. Males in poor habitats would then be at higher risk of being cheated, because they find less food to "satisfy" their female mates. To test this hypothesis, we investigated great tits, a widely distributed song bird species, from three different habitats: an urbanized city quarter ("poor" habitat; 146 samples), an isolated park within the city ("intermediate" habitat; 704 samples) and a forest at the town border ("good" habitat; 336 samples) by means of STR analysis with 9–11 STRs highly variable in *Parus major*. The percentage of offspring being the result of an infidelity was highest in the urbanized city quarter (41%) and lowest in the forest (24%). The proportion of unfaithful females was also higher in the city (65%) than in the two other habitats (forest: 55%; park: 58%). These results are in agreement with the hypothesis that females have a greater tendency to cheat if their social partners are not "convincing" enough before the kids are born.

P 396 A SNaPshot® ASSAY FOR THE IDENTIFICATION OF FORENSICALLY IMPORTANT BLOWFLIES

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Introduction and Aims
A dead body is an attractive habitat for many insect species but it is members of the Blowfly family (Calliphoridae) that are usually the first to arrive, using the body as an oviposition site. The stage of larvae found on a body can be a useful indicator of time since death but in order for species specific life cycle data to be applied, accurate species identification is critical. Damaged, unviable or immature specimens can be difficult to identify morphologically and recent work has focussed on genetic identification. The aim of this study is to assess the Cytochrome Oxidase I (COI) gene within the mitochondrial genome as a suitable marker for the Calliphoridae and develop a single assay for species identification.

Materials and Methods
DNA was extracted from wild-caught specimens and COI amplified and sequenced in house. Alignments allowed informative SNPs to be identified and a SNaPshot® assay designed.

Results and Discussion
A 6-plex SNaPshot® assay revealed a unique haplotype for seven different blowfly species. Alignment of COI sequences revealed sufficient variation for species identification with low levels of heteroplasmy and intraspecies variation. However identifying conserved regions across species for SNaPshot® primer design can be problematic. The lack of genomic data for this group of insects needs to be addressed and the analysis of additional genes would enhance a single assay approach to species identification.

P 397 ASSIGNING CONFIDENCE TO SEQUENCE COMPRISONS FOR SPECIES IDENTIFICATION: A DETAILED COMPARISON OF THE CYTOCHROME B AND CYTOCHROME OXIDASE SUBUNIT I MITOCHONDRIAL GENES

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Species identification is a highly used tool in forensic science; particularly in the investigation of wildlife crime. The two most commonly used genetic loci in species identification are the cytochrome oxidase I gene (COI) and the cytochrome *b* gene (cyt*b*), and identification is generally carried out through the use of DNA sequencing. However, there is currently no standard method to quantify sequence comparisons for presentation in reports and to courts in legal proceedings. There have been no detailed studies of the expected levels of inter- and intraspecific variation.

For the first time this study provides a detailed comparison of the effectiveness of these two loci. Interspecific and intraspecific variation is assessed and statistical confidence is applied to sequence comparisons. Comparison of 217 different mammalian species reveals that cyt*b* more accurately reconstructs their phylogeny and known relationships, and gives better resolution when separating species based on sequence data.

Intraspecific variation was assessed using three model species and showed variation ranging from 0–1.16% (Kimura 2-parameter p-distance (K2P) x100%), indicating that some level of variation should be expected. Interspecific variation was greater in cyt*b* than in COI (28.79±1.01 and 24.54±0.75 K2P x 100 respectively). Using a K2P (x100) threshold of 1.5–2.5, cyt*b* gives a better resolution for separating species with a lower false positive rate and higher positive predictive value than those of COI. This study allows, for the first time, application of statistical confidence to sequences comparisons for species identification.

P 398 A RAPID SINGLE STEP ASSAY FOR IDENTIFYING ENDANGERED SPECIES IN TRADITIONAL EAST ASIAN MEDICINE

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Despite renewed public interest, education, research and legislation, Traditional East Asian Medication (TEAM) continues to incorporate animal parts from endangered species and can be obtained openly in many countries. Products sold as having medicinal properties include plasters, pills, ointments, tonic wine and others. Due to the low population levels of some endangered species other more common species may be substituted or very small amounts may be used. This results in low levels of DNA which can be difficult to identify. Most species identification tests rely on sequence comparison with a known reference sample. Most TEAM contains mixtures of several different species making this technique unsuitable.

We describe a novel single step assay to rapidly and simultaneously identify rhino, tiger, bear, leopard, pangolin, musk deer and several non-endangered mammals often substituted in TEAM. The test targets the mitochondrial genome to amplify species-specific fragments that can be separated easily using a genetic analyzer. Each fragment is of a different size so that none can be confused. The specificity of each primer pair allows for species identification to be made even if a mixture of several species is present. Further, if more than one of the target species is present then all the species will be amplified and identified simultaneously.

The test is sensitive to very low levels of DNA equating to several hundred mitochondrial copies (a fraction of single cell). The test has been validated using voucher specimens and TEAM seized by customs and now offered to the wider scientific community.

P 399 MOLECULAR ANALYSIS OF BOTANICAL EVIDENCE BY DNA THERMAL DISSOCIATION TEMPERATURE

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This work was based upon earlier studies (Ward *et al*. 2005, 2009), which exploited indels located in the mitochondrial genome to discriminate between different varieties of grass. The *nad7* and *nad5* indels were amplified from the DNA of nine different varieties of amenity grass species typical of lawn and recreational use. Discrimination was achieved by differences in T_m values as shown by dissociation curve and supported by gel electrophoresis. Experiments were also implemented to determine the applicability of a genetic test using samples that were exposed to different environmental conditions. These were designed to include simulated conditions experienced by real forensic samples and consisted of grass leaves, stored dry inside paper envelopes at room temperature, desiccated grass leaves contaminated with fungal growth, and also stains made from grass leaves on cotton cloth to simulate grass marks that might be found on clothing. These stains were stored in a variety of conditions including, contact with soil, water logged, dry and exposed to sunlight and also stored dry in the dark and at 4° centigrade for comparison. All samples were extracted using a Qiagen DNeasy Plant kit and amplified using primer sequences reported by Ward *et al* using Agilent Brilliant III Green qPCR amplification reagent. All samples except the soil and the fungus contaminated samples gave amplification products that could be distinguished by means of both gel electrophoresis and by T_m determined by a dissociation curve.

P 400 POPULATION ASSIGNMENT IN SEVEN PORTUGUESE DOG BREEDS AND IBERIAN WOLVES

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Seven Portuguese dog breeds (n = 344) and Iberian wolves (n = 44) were analysed using a previously characterized set of 9 autosomal short tandem repeat loci. The total dataset was used to estimate genetic diversity, parameters of forensic interest and genetic distances among and within populations. Furthermore, clustering analyses were performed to investigate the genetic similarity of individuals belonging to the same morphological population. The levels of genetic divergence varied among dog breeds and may reflect historical differences in the gene flow between them. Departures from Hardy-Weinberg equilibrium were not consistent over loci, but instead occurred within different markers in different populations and were most likely due to limited sample size and non-random mating. The results showed that dogs and wolves could be discriminated on the basis of 9 autosomal STR genotypes. Clear clustering of dogs into groups according to their breeds of origin using our genotype dataset was not possible. Dog breeds showed relatively heterogeneous genetic constitutions indicating that they consist of populations derived from several genetically different ancestral populations and their crossbreeds rather than from a single origin. Also, inbreeding practices appear to have not yet been established. Thus, individual dogs could not be readily assigned to their breeds of origin on the basis of these genotypes alone. Nevertheless, genetic discrimination between wolves and dogs was possible with a high probability (>98%) of correct assignment of each individual to its population of origin.

P 401 INDIVIDUAL IDENTIFICATION OF FOX (*VULPES VULPES*) IN FORENSIC WILDLIFE INVESTIGATIONS

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As in human forensics, wildlife investigations previously deemed impossible have come within reach due to new or improved DNA-techniques. In a recent case the relatedness of several foxes (*Vulpes vulpes*) disrupting an island ecosystem was questioned, as the manner of their relatedness may indicate the intentional release of one or more individuals. To this extent a fox-genotyping technique was pursued.

Multiple microsatellite markers (STRs) designed for individual identification of dogs (*Canis lupus familiaris*) were tested on foxes to determine which markers could be amplified reliably and scored unambiguously. For this subset of markers the possibility of multiplex amplification was tested, as were the variability and allele range of these markers.

Approximately two third of the tested STRs were amplified and scored readily in foxes, of which some were combined in multiplex reactions. Differences in marker variability and marker range between dogs and foxes will be presented, demonstrating which markers met our criteria for suitable individual identification markers.

After genotyping the case samples with this subset of markers a conclusion was formulated expressing the likelihood of a familial relationship of all individual foxes. The obtained genotypes are stored to compare with biological material found if a suspect of an intentional release were to be found.

P 402 AUSTRALIAN MARSUPIAL SPECIES IDENTIFICATION

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Wildlife crime, the illegal trade in animals and animal products, is a growing concern and valued at up to US$20 billion globally per year. Australia is often targeted for its unique fauna, proximity to South East Asia and porous borders. Marsupials of the order Diprotodontia (including koala, wombats, possums, kangaroos) are sometimes targeted for their skin, meat and for the pet trade. However, species identification for forensic purposes must be underpinned by robust phylogenetic information. To date, a Diprotodont phylogeny containing a large number of taxa generated from nuclear and mitochondrial data has not been constructed. Here the mitochondrial (COI and ND2) and nuclear markers (APOB, IRBP and GAPD) are combined to create a more robust phylogeny to underpin a species identification method for the marsupial order Diprotodontia. Mitochondrial markers were combined with nuclear markers to amplify 27 genera of Diprotodontia. Data was analysed using parsimony and likelihood methods. These combined data sets resolved two suborders: Vombatiformes and Phalangeriformes. Phalangeriformes was subsequently split into two clades. The first clade contained the Macropodiformes and Burramyidae. The second clade contained Petauridae, grouping with Phalangeroidea. Phylogenies to date have generally resolved either three suborders grouping all possums in one suborder, or two suborders dividing the possums into two clades, with one clade grouping Burramyidae and Phalangeroidea with Macropodiformes and the other containing the remaining possums. Of the markers tested, ND2 provided the greatest level of diagnostic accuracy and could be used as a forensic species identification tool for Diprotodonts, with appropriate validation.

P 403 A LEGAL GAP IN SPANISH LAW AND UNSOLVED ETHICAL QUESTION IN PATERNITY TESTING DIAGNOSTIC

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Article number 18 of the current law of the Spanish Constitution (since 1978) defends the right to privacy of all citizens. Based on this statement, the new Code of Ethics of the Spanish Medical Association includes several articles on studies about genetic information and their acceptance by all the individuals involved. So there is a serious problem in paternity testing cases in the investigation of the alleged father-child relationship without the consent of the biological mother to gain access to her genetic information.

The problem of lack of permission from a parent, in the laboratory that investigates these cases, is greater when the child is a minor or mentally incapacitated or psychologically incapable, because Spanish law currently in force (LEY 41/2002, de 14 de noviembre, básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica.) requires informed consent of legal representatives. This law doesn't typify what happens when one progenitor consent (putative father) and the other one (mother) doesn't agree.

At the moment there are no Spanish judicial records on this issue. Depending on the spirit of the law is logical to understand that the team of forensic genetics laboratory could be sued for performing a biological paternity test without mother's permission, and a lawyer may win the case. The aim of this study is to provide legal solutions to avoid potential legal problems.

P 404 FORENSIC GENETICS LABORATORY OF THE UNIVERSITY OF BARCELONA: EVOLUTION OF PATERNITY TESTING CASES IN 35 YEARS OF HISTORY

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Biological paternity research in Catalonia (North-east Spain) began in 1976, when Drs. Castillo, Gelabert and Huguet studied the first case to the Department of Legal and Forensic Medicine, University of Barcelona in collaboration with the Haemostasis and Hemotherapy service and Blood Bank of Clinic Hospital of Barcelona. With the knowledge that each human is genetically unique as result of genetic polymorphism, blood group antigen typing and other immunological techniques including the human histocompatibility leukocyte antigen (HLA) typing became widely used for paternity testing and forensic science application until the end of the 1970s. But the biological tests were not accepted by the courts in Spain until 1981 with the reform of the civil code. With the support of Dr. Carracedo, University of Santiago, in 1982 Drs. Huguet and Gené, who are still active, set up the Haemogenetic Forensic Laboratory in the University of Barcelona and this area of Legal Medicine Unit was consolidated. It was the first Forensic Genetics Laboratory in Catalonia. The aim of this study is a retrospective analysis paternity testing cases that have been done along the last 35 years.

We report the social and legal profile evolution of the analysed cases grouped in different periods of time. The results about paternity and non-paternity claims were studied, included exclusion and non-exclusion rates. Biomathematical values of performance were reported for each methodology stage (number of exclusions, averages values of probability of paternity, population index, etc.).

P 405 COLD CASES – NEW TECHNOLOGY FOR DNA ANALYSIS ALLOW THE REOPENING AND SOLUTION OF OLD UNSOLVED CRIMES.

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In recent years considerable progress in the field of forensic science (latent prints, ballistics and biology) have occurred, causing the reopening investigation of old unsolved murders, called "Cold Cases", especially when its findings can still be analyzed and / or re-analyzed taken into account new technologies and new scientific methodologies.

To this purpose in July 2009, the Anti-Crime Central Directorate of the Italian National Police has established a working group known as UDI (Unsolved Crimes Unit), which collects scientific and technical expertise (Forensic Science Police Service) and investigators (Operations Central Service) to assess which unresolved cases could be selected in relation to new investigative hypotheses and in the presence of not analyzed samples.

During these two years about 30 cases were selected (which occurred between 1985-2005).

In particular, the automation of DNA extraction processes, combined with the next generation PCR kits, led in eight cases to the identification of one or more genetic profiles which were not highlighted before and were resulted to be different from the victim's profile.

These genetic profiles are considered of great interest from the investigation point of view, allowing the identification of the murderers in three different cases.

These results therefore indicate a real possibility to identify the perpetrator of a crime, even after several years, in accordance with the sense of justice due to the family of the victim and the community.

P 406 ETHNIC ORIGIN IN FORENSIC CASEWORK AND PATERNITY TESTING

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Historically, Native American communities have been subdued by conquerors and displaced by migratory waves, mostly coming from Europe. Argentina has always been claimed to be the most European country of Latin America, nevertheless genetic studies proved that the extant population has still an important Native American genetic component.

The broad spectrum of criminal cases has been closely related to those individuals that have been socially marginalized. Hence, the aim of the present work was to establish whether there is correlation between social stigmatization and ethnic origin. For this purpose, forensic casework and routine paternity sample sets were analyzed for Native American-specific Y chromosome and mitochondrial (mt) haplogroups (hg). Over 600 unrelated male and female samples from routine casework, including victims and offenders, were screened for Y and mt-hgs by multiplex Real Time PCR and High Resolution Melting analysis. The analyzed Y-hgs included Q1a3a, R1b1b2 and I, and mt-hg C and sub-hgs A2, B2 and D1.

Native American-specific mt-hg proportions were not significantly different between both groups (Paternity= 71.5%, Forensic=78.5%). However, Y-hg analysis did show a significant difference in Native American hg Q1a3a proportion between forensic and paternity cases (Paternity=8.2%, Forensic=20.5%; p=0.003). Interestingly, when considering both maternal and paternal Native American origins together, the difference between both groups was significantly increased (Paternity=7.4%, Forensic=20.7%; p=0.002).

The present results add new insights in the consequences of the former conquest and the later immigration in Argentina, reflecting the impact of social stigmatization and marginalization of native populations.

P 407 PCR AMPLIFICATION STRATEGIES INFLUENCE THE DETECTION OF MITOCHONDRIAL DNA HETEROPLASMY IN HUMAN HAIR SHAFTS

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Hairs of an individual are known to differ in their mitochondrial DNA (mtDNA) sequence, mostly affecting ratio levels of mixed nucleotides at individual positions (point heteroplasmy). However, also complete base exchanges between hair shafts have been observed that lead to a full difference in the sequences. We here systematically investigated the effects of PCR amplification strategy, mtDNA quantity and hair colouration on the detection of mtDNA point heteroplasmy.

We examined 249 different fragments (2 cm) of 20 plucked telogen hairs from ten female individuals. The mtDNA control region was amplified using four amplification strategies comprising 16 primer pairs that yielded PCR amplicons with sizes between 259 bp to 1,475 bp.

As expected, quantification gave highest mtDNA concentrations in the proximal hair segments (excluding the root) with a continuous decrease towards the hair tips. The reduction in quantity was greater in coloured hair compared to untreated hair, maybe caused by repeated hair dyeing. As a result, permanently coloured hair fragments yielded only weak amplification success rates and bad sequence quality, especially with larger amplicon sizes. Interestingly, this resulted in a higher number of observed point heteroplasmatic situations following established interpretation rules. When analysed with smaller amplicon sizes, some of the point heteroplasmies could not be confirmed indicating their artefactual nature. This study clearly demonstrates that both mtDNA amount and amplicon size influence sequence quality, which is the basis for point heteroplasmy interpretation. In order to avoid erroneous interpretation it is of great importance to adjust PCR amplicon length to quantification results.

P 408 NON MEDICAL APPLICATIONS OF NON INVASIVE PRENATAL DIAGNOSIS: ETHICAL ISSUES

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Introduction and aim
Non invasive prenatal diagnosis (NIPD) is becoming increasingly important and is reaching consensus in the scientific research community on its application as a practical diagnostic tool.
We discuss the opportunities and ethics of non invasive prenatal testing for non medical purposes, particularly in forensic genetics.

Discussion
NIPD provides a source of information about the genetic make-up of the fetus, avoiding the small but significant risk of pregnancy loss related to invasive testing such as amniocentesis or chorionic villi sampling.
A number of ethical issues arise from non clinical applications of NIPD, such as sex determination and paternity testing in earlier gestational age and subsequent off spring selection.
On all instances, clinical and non clinical, NIPD is characterized by several ethically favourable features: safety, earlier detection and easy sampling.
All these positive features increase the opportunity of non invasive prenatal testing for non medical reasons.

Conclusion
A number of elements qualify NIPD as a good practice even if prenatal diagnosis tout cour is a topic of ethical judgements. The non clinical use of NIPD will benefit from an informed and open debate involving both pregnants and physicians.

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ABBOTT SATELLITE SYMPOSIUM

Wednesday, August 31st 2011
Lunch Symposium 12:00–14:00
University of Vienna, Room name: Audimax

TALK 1 **Forensic DNA Analysis with the PLEX-ID**
Dr. Steven A. Hofstadler & Dr. Tom Hall, Ibis BioSciences R&D, Carlsbad, United States

TALK 2 **PLEX-ID Case Study:**
Paternity testing on PLEX-ID using mtDNA, STRs, SNPs
Prof. Dr. Lili, Institute of Forensic Sciences, Ministry of Justice, Shanghai, China

TALK 3 **From "Which LIMS Should I Choose?" to "Go-Live":**
Insight into Successfully Navigate these Challenges
Dr. Robin Gall, STARLIMS, Hollywood, United States

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