Abstracts

Poster presentations

P-1 A Comparison of Three Automated DNA Purification Methods in Forensic Casework

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Manual Chelex®-100 and organic extractions (phenol/chloroform) are used as routine extraction methods at the Swedish National Laboratory of Forensic Science, SKL. The aim of this study was to find an automated DNA purification system to replace the organic method. The following methods were evaluated and compared to each other and to the organic method used routinely BioRobot® EZ1 with EZ1 DNA Investigator Kit and Card (Qiagen GmbH), iPrep™ Purification Instrument with iPrep™ ChargeSwitch® Forensic Kit and Card (Invitrogen), Magnatrix™ 1200 Workstation with the Magnatrix™ gDNA Blood Kit Forensic and Forensic protocol A and experimentally programmed protocol B (Magnetic Biosolutions). Blood on fats, cotton swabs, moist snuff, paper towels and leather, post-mortem blood and tissue were extracted with the different methods. DNA concentration and quality of the electropherograms were examined. Individual comparisons between the three extraction methods showed that iPrep™ and Magnatrix™ 1200 gave significantly lower mean quantities compared to BioRobot® EZ1 and the organic extraction method ($p<0.05$). There was no significant difference between the latter two. BioRobot® EZ1 generated the best results on these types of samples and will be validated for routine analysis at SKL. At this point it will replace the organic extraction for most types of samples.

P-2 Evaluation of the Differex™ System

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Differential extraction is an efficient method to separate sperm cells from epithelial cells. A manual Chelex®-100 based method is used at the Swedish National Laboratory of Forensic Science, SKL. The Differex™ System (Promega) uses a Proteinase K digestion of epithelial cells followed by centrifugation and phase separation. The sperm- and epithelial fractions are further purified with DNA IQ™ System (Promega) or with phenol/cloroform. The Differex™ System in combination with DNA IQ™ System were evaluated and compared to the Chelex®-100 method used routinely. After modifications, the Differex™ System gave comparable results to the Chelex®-100 method. The modifications included additional Protienase K and DTT, longer incubation time and additional steps when removing the solid support from the Digestion Solution. In the Chelex®-100 based method microscopic examination is done on the sperm pellet in a total volume of 50 µl. It was not possible to do a microscopic examination in less than 100 µl using the Differex™ System. Additionally the sperms were in clusters of epithelial cell debris. Microscopic examination is an important part of the differential extraction at SKL. Therefore the Differex™ System will not be implemented at our laboratory.

P-3 A Multiplexed System for Quantification of Human DNA and Human Male DNA and Detection of PCR Inhibitors in Biological Samples

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Forensic analysts routinely encounter samples containing DNA mixtures from male and female contributors. To obtain interpretable STR profiles and select the appropriate STR analysis methodology, it is desirable to determine relative quantities of male and female DNA, and detect PCR inhibitors. We describe a Multiplex Assay for simultaneous quantification of human and human male DNA using the ribonuclease P RNA component H1 (RPPH1) human target and the sex determining region Y (SRY) male-specific target. A synthetic oligonucleotide sequence was co-amplified as an internal PCR control. Standard curves were generated using human male genomic DNA. The SRY and RPPH1 assays demonstrated human specificity with minimal cross-reactivity to DNA from other species. Reproducible DNA concentrations were obtained within a range of 0.023 to 50 ng/µl. The assay was highly sensitive, detecting as little as 25 pg/µl of human male DNA in the presence of a thousand-fold excess of human female DNA. The ability of the assay to predict PCR inhibition was demonstrated by shifted IPC Ct values in the presence of increasing quantities of hematin and humic acid. We also demonstrate the correlation between the Multiplex Assay quantification results and the strength of STR profiles generated using the AmpFlSTR® PCR Amplification Kits.

P-4 Fast PCR amplification of AmpFlSTR Identifiler

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DNA typing serves as a powerful tool in criminal investigations, forensic medicine, paternity testing, and other applications, due to its remarkable specificity and its ability to gather DNA from small amounts of various specimens, including blood stains, sperm, and bone. A recently-established DNA database has contributed significantly to criminal investigations due to its vast data volumes. Increases in the quantity of DNA data stored in the DNA database are closely related to the latest advances in DNA technologies, including automated DNA purification instruments, multiplex PCR, and multi-capillary electrophoresis systems, which have made possible rapid, high-throughput DNA purification and genotyping. Nevertheless, in our view, PCR amplification times need to be reduced even further to achieve greater throughput. In this study, we succeeded in reducing PCR times to 1/3 of those previously achieved to roughly one hour using the AmpFlSTR Identifiler. This reduces the time required for DNA typing to approximately 90 minutes, including the time needed for PCR reaction.

P-5 Development of mRNA tools for body fluid identification

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Current DNA profiling methodology can yield a DNA profile from samples of body fluid that are either too small to see and/or for which existing screening techniques are inadequate. Consequently the emphasis for forensic scientists now is not, from whom did this DNA come from, but from what body fluid or cell type did this DNA profile originate. This may have a significant bearing on the outcome of a forensic case. Body fluid identification at the cellular level is important in determining the likely origin of a DNA profile at a level of sensitivity comparable to the DNA profiling system itself. We have developed methods for the identification of body fluids by measuring the expression of specific genes using reverse transcriptase PCR (RT PCR) and real time PCR analysis. This work includes the co-extraction of mRNA and DNA, design of specific primers and probes, and multiplex PCR as well as sensitivity and forensic application considerations. In this presentation we will describe the results of our work, demonstrating the potential applications to forensic casework and statistical approaches we have taken to data analysis.

P-6 Application of miniSTRs in an Australian Forensic Laboratory

Ms. Alison Sears, Forensic Biology, Division of Analytical Laboratories, Lidcombe, NSW, Australia
Dr. Peter Gunn, FSSB, Forensic Services Group, NSW Police Service, Australia
Professor Claude Roux, Centre for Forensic Science, University of Technology, Sydney, Australia

Identification of deceased individuals is a critical aspect in any coronial and criminal investigation. DNA provides an extra dimension to the identification process, especially for reconciliation of fragmented bodies. Increased prevalence worldwide of natural and terrorism-related disasters has prompted further advancements by scientists involved in human DNA identification. MiniSTRs offer clear advantages in the analysis of compromised samples and compliment existing STR testing systems currently used in Australian forensic laboratories. The modified primers target some routinely analyzed loci, allowing existing DNA databases to be utilized. In addition, non-CODIS miniSTRs can potentially contribute additional information, strengthening the likelihood of individual identification. This is of benefit in kinship reconstruction, especially where victims include multiple members of one family. In this research modified miniSTR primers (based on those developed by Butler et al., NIST) for both CODIS and non-CODIS loci and the recently released AmpFlSTR® miniFiler™ kit (Applied Biosystems) were validated and applied to degraded samples and human skeletal remains of varying ages and histories. MiniSTRs produce reliable results and increase the success rate of DNA testing on degraded samples and skeletal remains. The analytical problems encountered with degraded DNA samples can be reduced through miniSTR technology. This research has demonstrated the potential of miniSTRs to contribute an enhanced DNA analysis component for Disaster Victim Identification (DVI), missing persons as well as criminal and coronial identification cases in Australia.

P-7 Genebench-FX : Microfluidic DNA Separation and Detection for Biochip-Based STR Analysis

Vice-President Product Development, Dr. Eugene Tan, Network Biosystems, USA

Background and Purpose: A major challenge in bringing biochip-based DNA analysis tools to the forensic community has been in developing a robust, easy to operate commercial instrument that offers reliable and reproducible performance. Network Biosystem¹s Genebench-FX system is the first DNA separation and detection instrument designed specifically for forensic identification. Method: Genebench-FX incorporates novel microfluidic, biochip, optical, and instrumentation technologies. Genebench-FX accepts samples of extracted DNA that have been amplified and prepared with commercial STR kits. The system is designed to be operated in a forensic laboratory and has been ruggedized for optional field use. Results: Data presented will include representative results from commercial STR kits, including resolution, sensitivity, and precision. Taken together, these results demonstrate accurate and reproducible STR separations with better than single base pair resolution and will enable efficient integration of sample preparation steps. Conclusion: Genebench-FX meets or exceeds all requirements for analysis of STRs with respect to both technical operation and reproducibility. A first-generation instrument that provides STR or sequence results following conventional DNA extraction and amplification has been developed. Our major goal is to develop a fully integrated instrument that performs DNA extraction from forensic samples, amplification, separation and detection within 45 minutes.

P-8 Internal validation of the AmplFlSTR MiniFiler PCR Kit

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With the aim of generating genetic profiles from aged, compromised, or damaged DNA samples, a validation study of the AmplFlSTR® MiniFiler™ PCR Kit was performed. Using their 5-dye chemistry and mobility modifier technology, Applied Biosystems has developed a miniSTR kit capable of amplifying 8 core STR loci and amelogenin with reduced PCR product sizes relative to current commercial kits. In this study, we present the results of some forensic validation studies including the following aspects : detection limit, evaluation of stutter bands, performance with simulated inhibition, concordance study with SGM Plus data and performance with casework samples.

P-9 PRINCIPLES OF STR MULTIPLEX AMPLIFICATION

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Mr. Christopher A. Cave, Bode Technology, USA
Ms. Heather M. Cunningham, Bode Technology, USA
Ms. Kristen Hancock, Bode Technology, USA

We have studied STR multiplex amplification to understand general principles relating effects of changes in template amount, multiplex primer concentration, amplification cycle number, and amplification volume. The results reveal that, except for stochastic variation with limited template amounts, the same quality, intensity, and accuracy of DNA profiles can be obtained while varying each of these parameters over a broad range. Each parameter can be used to compensate for changes in the others. We will emphasize how this can be used to limit the requirement for sample consumption. Lowering amplification reaction volume increases sensitivity of multiplex amplification reactions. Lowering primer concentrations generates weaker amplification, but, within certain limits, this can be readjusted to standard strength with no loss of quality employing a minimal increase in the amplification cycle number. These principles universally apply across commonly used and commercially available multiplex sets. The basic result of the work demonstrates that many different combinations of primer concentration, amplification cycle number, and amplification volume can provide high quality results. This allows us to provide specific recommendations with use of limited sample material. We will discuss special applications to generate strong profiles with very low copy number templates.

P-10 THE BODECHECKS SOLUTION: A HIGH THROUGHPUT ANALYSIS SOFTWARE COMBINING GENEMAPPER, FSS-I3, LIMS AND ARTIFICIAL INTELLIGENCE.

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Mr. Christopher A. Cave, Bode Technology, USA
Mr. Stephen Stafford, Bode Technology, USA
Mr. David A. Leonard III, Bode Technology, USA
Mr. Robert Griffiths, Bode Technology, USA
Ms. Erin M. Sweeney, Bode Technology, USA
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Mr. Manzar Ahmed, Bode Technology, USA
Mr. Mike Cariola, Bode Technology, USA
Ms. Maureen Loftus, Bode Technology, USA

Computer software systems have provided new tools for forensic data review. Our laboratory evaluates approximately 150,000 samples per year for over 25 clients who provide diverse sample types, and require different extraction, quantification, and STR multiplex systems. This large diverse work load requires efficient analysis and data reporting. By mining the most valuable data and processing strengths of GeneMapper ID and FSS i3 software, combining these data with our internally developed LIMS system information, and adding our own analytical programming (i.e., artificial intelligence), our BodeCHECKS solution accomplishes the following tasks with almost no human effort. •Greater than 99.8% correct allele calls. • Correct allele calls in circumstances that FSS i3 provides uncertainty. •Increased concordance between analyst and software conclusions versus GeneMapper ID or FSS i3. •More refined description of reasons for failing samples. •Unity between analyst and BodeCHECKS rejection code language. •Automated determination of reprocessing pathways for failed samples. • Significantly decreased analysis time. No review of GeneMapper ID electropherograms or FSS i3 "spikograms" is required. We will describe how this is accomplished, show how it increases quality checks in our work, and compare allele determination performance versus GeneMapper ID and FSS i3 software.

P-11 The research of 15 STR loci and ABO gene simultaneous amplification methods by fluorescence labeling

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A simultaneous amplification method of 15 STR loci and ABO gene is developed by using fluorescence labeling technology. The identification information of STRs and category information of ABO bloodgroup type will be obtained simultaneously from a biological evidenceby at one time by this method. The investigation direction will be provided from a biological evidence for suspect screening while STR information is used. The stable results of ABO and STRs are obtained from 35 bloodstains of unrelated individuals, 20 mixture stains of sperm and female vaginal epithelium, 25 cigarette butts from routine criminal cases. The genotyping results of ABO and 15 STR loci fit paternity relationship completely among family tree samples. The method offers a new means for the investigation of criminal cases.

P-12 Novel high-resolution characterisation of ancient DNA reveals C>U-type base modification events as the sole cause of post mortem miscoding lesions

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Dr. Juan J. Sanchez, National Institute of Toxicology and Forensic Science, Tenerife, Spain
Dr. Mark Beaumont, University of Reading, UK
Dr. Ross Barnett, University of Durham, UK
Dr. Jeremy Austin, Adelaide University, Australia
Professor Alan Cooper, Adelaide University, Australia

DNA sequences from forensic samples can only be inferred indirectly following PCR amplification. As these sequences are the primary input into further enquiries, the generation of accurate sequence data is a critical issue and likely to come under increasing scrutiny. Traditional PCR-based methodologies can be inherently unsuited to the job of deriving quantitative conclusions and accurate consensus sequence data from damaged and degraded templates. High levels of non-authentic sequence data can be generated when DNA template numbers are small and/or the target amplification size too large. We therefore present an alternate methodology based on Single Primer Extension (SPEX) amplification that places no pre-defined size constraints on amplification and interacts with only one of the DNA strands at the target locus. SPEX targets a far greater number of highly damaged, up to 60,000 year old, ancient DNA templates than PCR (as small as 35-50 bases in length) and, unlike PCR, is capable of clearly identifying multiple sequences which originated from separate aDNA template molecules. This means that SNP genotyping and sequence inferences, as well as quantitative analyses on aDNA damage, made from SPEX amplification products are likely to be inherently more accurate than those based on traditional PCR amplification.

P-13 Isolating sperm cells from mixed stains by magnetic activated cell sorting

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The most common method for seperating sperm cells from mixed stains is the well established differential lysis. Several methods were described recently to overcome the problem of confined sensitivity and specificity. In our study we tested a new technology of highly specific isolation of sperm cells from a mixture with saliva or blood of a female donor. We used an indirect cell isolation method: First, sperm cells are labelled using a biotin conjugated antibody against an epitope on the surface of the sperm head. In a second step, the labelled cells are captured using paramagnetic particles conjugated to an anti-biotin antibody. Cell separation was performed using MACS MS columns and a MACS separator. MACS columns are packed with an optimized matrix to generate a high gradient magnetic field when placed into the separator, which works as a very strong permanent magnet. The validation of this new technique will be described and first results will be presented.

P-14 Forward to an X chromosomal PCR test system including four STR triples

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The evaluation of 4 pairs of tightly linked chromosome X (ChrX) STRs i.e. DXS10135-DXS8378, DXS7132-DXS10074, HPRTB-DXS10101, and DXS7423-DXS10134 lead to the creation of the Argus X 8 multiplex amplification kit. These 8 STRs are distributed as 4 closely linked pairs over the entire X-chromosome at Xp22, Xq11, Xq26, and Xq28 and for practical reasons they are assigned to 4 linkage groups 1-4. The genetic distance within the STR pairs is assumed to be < 1 cM, whereas the pair to pair space is about 50cM or more. To achieve a further considerable enhancement in discrimination power we suggest to include additional STRs, i. e DXS10148 at Xp22, DXS10079 at Xq11, DXS10103 at Xq26, and DXS10146 or DXS10147 at Xq28. The test system proposed here can be used for haplotyping of 4 ChrX STR clusters which show very low haplotype frequencies at each locus. Hence, when X-chromosomal trekking lineages can contribute to solving of complex kinship cases, STR triple haplotyping may have crucial significance.

P-15 Validation of a 17-locus Y-STR multiplex system

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Internal validation of a commercial 17-locus Y-STR system (AmpFlSTR® Yfiler™, Applied Biosystems) has been performed on the ABI PRISM® 3130 Genetic Analyzer for use in forensic cases. The Yfiler™ kit was validated according to SWGDAM guidelines. Our results show that it is possible to obtain full profiles with as little as 30 pg of male DNA even in the presence of 20,000-fold amounts of female DNA. Reaction volume was optimized for 10 µl. Male-male mixtures yielded full profiles of the minor contributor with 10-fold excess of the major contributor. Stutter values for each locus were determined from data generated for the population study which included Y-STR profiles from 156 caucasian males from the Montreal and Lac St-Jean areas of Québec, Canada. The study recorded 141 different haplotypes of which 131 were unique with a haplotype diversity of 0.9965. A number of non-probative forensic samples from rape kit epithelial fractions and fingernail scrapings were also successfully tested.

P-16 A novel real-time PCR strategy for the quantitation of total human and male DNA

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Heavily multiplexed Short Tandem Repeat (STR) analysis has become the dominant technology in DNA-based human identification. A multiplex assay, Plexor™ HY, has been developed for the simultaneous quantitation of total human DNA and human male DNA. An internal PCR control (IPC) has been included to monitor inhibition in the quantitation process. This assay uses three dyes to detect amplification and a fourth dye to provide a passive reference signal. The autosomal target is a multicopy target on chromosome 17. The Y-chromosomal target is a multicopy target concentrated on the short arm of the Y-chromosome. The IPC target is a synthetic sequence added to all wells. Associated analysis software has been developed to visualize amplification data from multiple instrument platforms, plot standard curves and calculate DNA concentrations of unknowns. A normalization module has been built into the software that allows to compute all necessary dilutions of DNA template samples. Quality control checks are incorporated in to the system as well. In addition to analysis software, automated methods for qPCR set-up, DNA normalization and STR amplification set-up will be described. Data will be presented demonstrating the performance of this assay and the interface of the analysis software.

P-17 Identification of Degraded Skeletal Remains from the Korean War Using a Combination of Improved DNA Typing Methods

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Between 1990 and 1994, the North Korean government unilaterally returned 208 sets of commingled remains claimed to be those of American soldiers considered to be Prisoners of War (POWs), or Missing in Action (MIA) from the Korean War. Due to the highly degraded nature of these samples, the Armed Forces DNA Identification Laboratory (AFDIL) initially utilized mtDNA hypervariable region sequencing. However, a large number of these sequences matched several of the most common HV1/HV2 types in the US "Caucasian" population. This has made both the identification of these remains, and the simple task of determining the number of individuals represented among the 208 boxes, nearly impossible. In recent years, AFDIL has begun to utilize more discriminating DNA typing assays to assist with identification of degraded skeletal remains where standard mtDNA testing has proven insufficient. Our laboratory has begun to apply low copy number (LCN) STR typing, LCN Y-STR typing, mtDNA SNP panels, and an improved DNA extraction protocol to these samples. This poster will present the application of these assays to degraded skeletal remains from the Korean War, and will discuss potential future applications of these methods to increase the efficiency and efficacy of identifying America's fallen military service members.

P-18 Developmental Validation of the AmpFlSTR® SEfiler Plus™ PCR Amplification Kit

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Since its introduction in 2002, the AmpFlSTR® SEfiler™ kit has provided a highly discriminating DNA profiling option to German forensic laboratories by combining the widely used SGM Plus® Kit loci with the SE-33 locus required for the German DNA Database. Whilst proving successful on database samples, laboratories using the SEfiler Kit have reported the need for chemistry better able to handle the ever increasing number of casework samples. New developments in amplification chemistry have resulted in a greater ability to optimize PCR reactions to perform more effectively on compromised samples whilst still providing reliable amplification for database samples. The new AmpFlSTR® SEfiler Plus™ kit contains the same loci and primer sequences as the SEfiler™ kit but uses improved synthesis and purification processes to minimize the presence of dye-labeled artifacts. Other improvements include modified PCR cycling conditions for enhanced sensitivity, a new buffer formulation that improves performance with inhibited samples when compared to the original SEfiler™ kit and a redeveloped allelic ladder with the same improvements in synthesis and purification processes being applied to the amplification primers. Validation studies demonstrating the effectiveness of the multiplex will be presented including sensitivity, mixtures, species specificity, and models of inhibition and casework samples.

P-19 Application of sperm specific antibodies for the separation of sperm from cell mixtures

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This study was designed to test the suitability of monoclonal antibodies (mAb) directed against the testicular isoform of the angiotensin-converting enzyme (tACE), coupled with biomagnetic beads, for the extraction of sperm from mixtures with mucous membrane cells. Nine different mAbs recognizing native and denatured form of tACE were tested for immunohistochemical staining of spermatozoa. Three of these mAbs (1E10, 4E3 and 4E10) showing the best staining were then coupled with magnetic beads, and the efficacy and sensitivity of the biomagnetic separation and extraction of spermatozoa was tested. Although the application was limited to swabs stored in PBS buffer instead of air drying and which contain a sufficient amount of intact sperm with mid-piece and flagellum, we have developed an alternative method for the isolation – or at least enrichment – of sperm from epithelial cell mixtures, which is fast, easy and in contrast to the preferential lysis technique, non-toxic.

P-20 Development of a multiplex system based on Insertion/Deletion polymorphisms distributed throughout the X chromosome for forensic application along with population and case study

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The utilization of markers in X-chromosome bears the potential to efficiently complement the analysis of other genetic markers in forensic science, such as: autosomal, Y-chromosomal or mitochondrial. As the X-chromosomal short tandem repeats (ChrX-STRs) are already a nice option to use in complex kinship testing, this paper suggest a new kind of marker, located in the X-chromosome, to assist forensic investigation - the insertion deletion polymorphism (INDEL). A multiplex polymerase chain reaction (PCR) assay capable of simultaneously amplifying eight X chromosome mini-INDELs markers has been developed to aid human identification testing. This multiplex has been developed to produce PCR products that are reduced in size (named by our group as mini-INDELs) in order to facilitate its use in forensic cases where the samples are not always in a good condition. In addition to that, a Brazilian population study was held in 200 samples from the five geopolitical Brazilian regions. Along with that, kinship case studies were conducted. This new multiplex system of mini-INDELs markers has proven to be useful and its usage on kinship cases as a complement has been approved.

P-21 X-chromossomal mini-INDELs markers – an efficient tool for recovering profile information from low quality forensic samples

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Nowadays, one of the major issues in forensic genetics is the analysis of low quality and/or low quantity biological evidences. Genetic profiles from these samples have their chances to be obtained increased using low copy number methodology, mini-STRs markers and SNPs based systems. Here we describe the use of a multiplex system of eight X-chromosomal mini-INDELs (ranging from 70 to 170 bp) with the purpose of aiding the obtainment of profile information from low quality and/or quantity forensic samples. The mini-INDELs were amplified using forward labeled primers with 6-FAM and VIC dyes and electrophoresed in the ABI 3100. We have amplified high quality samples to serve as a control. After that those DNA samples were diluted in order to have DNA in different concentrations to test the sensibility of the multiplex. We also evaluated their success in amplifying artificially degraded samples. Two combinations of procedures were made, low copy number protocol with the mini-INDELs and whole genome amplification prior to the mini-INDELs amplification. This new multiplex system of X-chromosomal mini-INDELs markers has proven to be successful in the amplification of degraded samples as well as in low quantity DNA samples along with other techniques.

P-22 Single sperm cell isolation by micromanipulation

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M. Rémi Hienne, Strasbourg, France
Mme Sylvie Frackowiack, Marseille, France

This study proposes a new alternative solution in preferential extraction methods or microdissection to isolate and analyse single sperm cells in case of sexual assault : After the transfer of swabs in liquid culture medium, perpetrator's spermatozoas can be physically separated from victim's epithelial cells by using classical techniques of micromanipulation as ICSI (IntraCytoplasmic Spermatozoa Injection), usually applied for In vitro Fertilization. We showed on more than 30 vaginal smears, performed 6 hours after sexual relation for a medical post-coital exploration of infertility, that a single micropipette of 20 ? can very quickly collect several spermatozoas and transfer these gametes in a PCR tube under microscopic control. Moreover, recent methods of low copy number analysis as whole genome amplification (Repli-g ® - Qiagen) allow an efficient DNA amplification from only one cell since a mean of 11,3 +/- 2,4 alleles is obtained for this haploid genotype identification without any amplification of female alleles. This approach seems to be developed not only for vaginal sample but also for others supports as clothes since our results show that this technique of selection can be applied on swabs immediately transferred in liquid medium or on dried swabs after 48 hours.

P-23 Autosomal mini-indels markers as a tool to improve analysis on low quality forensic samples

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Methodological improvements allowing the analysis of low quality and/or low quantity biological evidences are the new frontier in forensic genetics. Low copy number procedures try to overcome problems of samples with low quantity. Amplicon lengths reduction from conventional autosomal STR markers along with the development of SNPs based systems are ways to overcome the problems encountered when amplifying degraded samples. Here we present a set of 40 highly heterozygous autosomal short indels amplified in multiplex fashion with amplicon lengths ranging from 70 to 170 bp (named by us as mini-indels) as new tool to improve analysis of low quantity and degraded DNA samples. The mini-indels were amplified using forward labeled primers with the five-dyes technology and electrophoresed in the ABI 3100. The 40 mini-indels were amplified in different multiplex sets and evaluated as their success in amplifying artificially degraded samples and also in samples with low DNA quantity using low copy number protocols or amplified through whole genomic amplification protocols. These mini-indels were successfully amplified in degraded samples as well as in low quantity DNA samples. Low copy number protocols and high quality samples amplified using whole genome amplification protocols were also successfully amplified using mini-indels multiplex systems.

P-24 Large scale analysis of HCM mutations in sudden cardiac death

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Sudden cardiac death (SCD) is a major cause of death worldwide, responsible for half of all heart disease deaths with an incidence ranging from 30-200/100,000 individuals. In addition, an important portion of SCD cases are due to hypertrophic cardiomyopathy and, in some cases, the diagnosis using macro and microscopic findings is difficult. Hypertrophic cardiomyopathy (HCM) is a myocardium primary disease caused by mutations in sarcomeric proteins genes. HCM affect 1:500 adults in general populations and it is the most frequent cause of sudden death in youth and in sportmen. The clinical presentation of the disease is very variable, and the identification of patients at risk for sudden death has low sensitivity and specificity. The prognosis is highly dependent of the specific mutation responsible for the disease, but we have very few data on genotype-phenotype correlations. The establishment of high throughput technologies for SNP typing has allowed the development of mutation analysis screening tools that allow the detection of a large number of mutations related with any disease or character of interest. Specifically, in the case of HCM, more that 600 mutations in 16 genes are already associated with the disease, making the genetic analysis with classical technologies rather difficult and time consuming. Based on this knowledge we have developed a mutation screening tool to quickly analyse the 600 mutations associated with HCM using the Mass Array System of Sequenom. We planed to know the number and spectra of HCM mutations in SCD, by means of a retrospective analysis of HCM mutations in patients that have suffered sudden death or patients with familiar antecedents. More than 1000 HCM affected individuals have been analysed and comparisons of mutation prevalence are been performed between SCD related cases and more benign cases.

P-25 Single cell typing by on-chip Low Volume PCR (LV-PCR)

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In this study commercial PCR chips (AmpliGrid™ AG480, Advalytix) have been used to type single cells in a 1 µL volume consisting of 0.5 µL H2O and 0.5 µL PCR reaction master mix. For this, human male lymphocytes from whole blood were used for cell sorting. 1, 2, 4, 6 and 9 cells were spotted twice onto 44 anchor spots, so that 88 LV-PCR reactions without a sepa-rate DNA extraction could be performed. In consideration of the number of detectable targets the typing success rate increased from 21.7% in single cell amplifications up to 95.7% for typing nine cells. Even with four cells more than two thirds of the targets were detected as well as the first full profiles obtained. These profiles are quite imbalanced but complete compared to a reference profile. With nine cells per LV-PCR a total number of 73 full profiles (84.9%) could be obtained with a consid-erable DNA profile quality. The possibility to detect drop-in alleles in single or sorted cell typing is higher compared to PCR using extracted DNA in comparably low concentrations. Furthermore, the typing results obtained in this study lead to the assumption that a significant proportion of template DNA is lost during the DNA extraction process.

P-26 Mitochondrial DNA typing with the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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The single nucleotide polymorphisms on mitochondrial DNA (mtDNA) were potential markers for analysis of difficult samples in casework and played a role on forensic science. The purpose of our work was to establish a method for analysis of mtDNA based on the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. In order to improve the discrimination power of mtDNA in forensic DNA typing, primer pairs which covered the mtDNA control region were designed. A technique of primer extension was employed for the analysis of the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Our study showed that mtDNA typing with the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry yielded reliable results. The results of our study implied that the analysis of mtDNA was proved to be suitable for forensic application and provided new genetic markers for the forensic purpose.

P-27 SAMPLE COLLECTION USING HYDROPHILIC ADHESIVE TAPE (HAT) METHOD FOR FORENISC DNA ANALYSIS

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We are developing a method that employs a hydrophilic adhesive tape (HAT) for the collection of transferred biological evidence. Certain transferred biological evidence contains human DNA that can be used for forensic DNA analysis. For instance, a suspect's skin cells are potential evidence that can be collected and analyzed at a crime scene. After collection, a DNA profile can be created from the transferred evidence. The DNA profile provides the necessary forensic evidence or "lead" required for the criminal investigation. This type of evidence becomes more useful and important when no other type of evidence (e.g. fingerprints, bloodstains or seminal stains) is collected from the crime scene. In particular, this method would be useful for certain types of crimes that often leave little evidence behind, such as property crimes. The sampling procedure of the HAT collection method was performed by pressing the tape onto a surface followed by lifting it. The pressing-lifting cycle was repeated for each location several times. The tape was then folded over onto itself and stored in a sealed bag at room temperature until needed for DNA extraction. A portion of this tape can be cut off and dissolved directly in an extraction buffer. The resultant solution can be used for DNA quantitation of any nuclear DNA present. Afterwards, an appropriate aliquot can be taken for amplification and subsequent DNA analysis. Since the HAT material itself is dissolved during the DNA extraction process, it minimizes the effect of a physical barrier for DNA isolation. Thus, we expect that the yield of DNA using the HAT method should be higher than those collected using traditional methods and materials. The improved yield of DNA will also increase the success rate in generating DNA profiles. Furthermore, this method produces a dry sample which minimizes the risk of DNA degradation. The application of the HAT method in the collection of potential evidence samples is being studied. This study presently suggests that the application of the HAT method provides a new and potentially useful method for collecting DNA evidence samples for STR analysis.

P-28 Application studies on the direct PCR buffer system (AnyDirectTM) and comparison with other commercial kits.

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There are some commercial direct or semi-direct PCR buffer kits. We had also developed direct PCR buffer system (AnyDirectTM, BioQuest Inc., Korea) which can amplify STR loci without DNA extraction from blood or saliva (manuscript submitted). Here we studied PCR performance from blood, blood (or saliva)-spotted paper and hair roots using three commercial human identification kits and compared with the results of other commercial kits(E-prep?R kit(Prepgene Co., Korea) and Ampdirect?R kit(Shimadzu Biotech, Japan)). Unlikely blood or saliva's case, pre-lysis (with DTT and Proteinase K) was required for hair roots. But even in this case, a simple heat inactivation was sufficient for further PCR reaction without any buffer exchange in AnyDirectTM system. Performance test using STR typing kits showed that the data from AnyDirectTM system were wholly concordant with those from purified DNA and had relatively higher PCR efficiency than other direct kits. In conclusion, AnyDirect PCR buffer system appears to be suitable for fast forensic DNA analysis or criminal DNA databases.

P-29 Nuclease based purification of male DNA from sexual assault cases

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Large amounts of the victim's epithelial cells contaminate the sperm present on swabs taken from rape victims. The standard method for obtaining pure sperm is to digest the epithelial cells with Proteinase K and separate the victim's solubilized DNA from the sperm by pelleting and washing, steps that are labor intensive, difficult to automate, and highly dependent on the technician's skill level. I have found that the alternative approach of destroying the victim's DNA with a nuclease appears to be faster, easier, and better than the standard method. Following ProK digestion of a swab cutting, an aliquot is removed for the victim's fraction and a nuclease is added and then incubated for 1 hour. A nuclease inhibitor and reducing agent are then added to lyse the sperm and release the suspect's DNA into solution. The nuclease degrades more than 99% of the victim's DNA but does not affect the sperm DNA sequestered in the sperm heads. No sperm are lost during the purification, so yields are good. Data will be presented showing that a vaginal swab cutting with 500 sperm yields a profile that is predominantly male. Since only pipetting steps are required, the process can be easily automated.

P-30 Rapid Genomic DNA Extraction (RGDE)

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Background and purpose: Nowadays expanded windows have been opened to the world especially in the area of Biotechnology and Biology science by performance of big projects such as human genome project. Production of great Bio Banks and DNA Banks in national & international levels is one of development pivots, which is very important in medical, agricultural, economical & forensic genetic fields. In this regard, one of the primary and important steps for all is DNA extraction with high quality & quantity in minimum time from cells. By using this method, genomic DNA with high quality & quantity can be acquired in the shortest time which has been presented in the world up to now. Method: In this rapid method using of cell lysis buffer, nuclei lysis buffer (an equal volume), chloroform and absolute ethanol also. Results: Quantity Control DNA extracted to perform by spectrophotometry and showed results that average for DNA yield (µg)= 5.86±2.53 and A260/A280 radio=1.91±0.16 if volume of blood used (µl)=500 (n=20). Quality Control DNA extracted to perform by agarose Gel 0.8% and showed results we have DNA with high quality. Conclusion: In this method genomic DNA can be extracted in the fastest time and preparation of extremely high molecular weight DNA by very simple materials and without any need to special equipment & apparatus.

P-31 A 50 SNP-plex mass spectrometry for human identification

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SNPs are very valuable for relatedness testing and crime case investigations because of their low mutation rates, their abundance in the genome and because SNP loci can be amplified as very short PCR products (<100 bps). We developed a 50 SNP-plex assay for detection on the MALDI-TOF MS platform based on the SNPs in the 52 SNP-plex assay from the SNPforID Consortium. After PCR amplification, the products were purified on Qiagen columns and used as templates in one SBE multiplex reaction. Two different strategies were used to design the SBE multiplex: 1) Small 5'-tags (3-8 nt), which increased the masses of the SBE primers without changing the annealing temperature. 2) Cleavable primers with one RNA nucleotide, that was cleaved by a mixture of RNases in a combined RNase treatment and SBE product purification step following the SBE reaction. The SBE primers were extended with biotin labelled ddNTPs and purified with avidin beads ensuring that only the extended SBE primers were isolated and spotted on the MALDI-TOF anchor target. Detection of the 50 extended primers from was performed in a mass range between 3,000 and 10,000 mass/z. To date, we have typed 200 samples from Somalia.

P-32 A new PCR- Ligase Detection Reaction (LDR) system for forensic SNP analysis

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Forensic DNA analysis is routinely performed using polymorphic short tandem repeat (STR) markers. Nevertheless, for degraded or minute DNA samples, analysis of autosomal single nucleotide polymorphisms (SNPs) in short fragments might be more successful. Furthermore Y-chromosomal SNPs analysis is a more useful tool in forensic investigations. Recently most methods of SNPs typing are based on the principle of minisequencing. Here we developed a new SNPs detection system for limited forensic materials based on the Ligase Detection Reaction (LDR) assay. A set of 8 Y-SNPs have been typed in 232 individuals of two different Chinese populations via multiplexed Ligase Detection Reaction assay through the Applied Biosystem 310 after the multiplexed PCR. Three probes for each Y-SNP were designed including one common fluorescence labeled probe and two allele specific probes different in size. All the amplicons are less than 100 bps for easy detection degraded or minute DNA samples. Even though the number of markers in the current system is limited, it can easily be extended to yield a greater power of discrimination. When fully developed, the PCR-LDR analysis provides a promising system for efficient sensitive SNP analysis of forensic samples in the future.

P-33 Human identification analysis to forensic purposes with two mithocondrial markers in polyacrilamide mini gel

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The classical methods for species identification based on protein analysis are not more applicable to the forensic casework. In the last years the analysis of cytochrome b (cyt b) and other mithocondrial markers 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 this paper we propose a new method for human sample identification based on polyacrilamide mini gel of a duplex PCR product amplified from mitochondrial DNA corresponding to cytochrome b and a new 16S rRNA fragment that is human specific and never used to this purpose so far. Multiplex amplification showed a band of 359 bp for the cyt b and a second band of 157 bp for 16S rRNA fragment for human biological samples. Under the same conditions only cyt b fragment was evidenced for sample of animals different from human whereas 16S rRNA fragment was completely absents This method may be very useful for forensic purposes since 16S ribosomal mtDNA fragment is a small human-specific fragment easily amplifiable even with old and highly degraded specimens.

P-34 Case study of dissolving allele dropout.

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Monoplex PCR amplification represents the earlier mainstream in PCR amplification. The invention of multiplex PCR methods now enables the rapid simultaneous amplification of multiple STR loci, resulting in savings in time, materials, and cost, reducing the risk of contamination and conserving specimens. However, with growing numbers of simultaneously detectable STR loci, the detection of all STR loci has become increasingly difficult. In general, use of extracted DNA from degraded specimen as a template for DNA typing results in less efficient PCR amplification with greater STR fragment sizes. Additionally, using small amounts of extracted DNA for the template poses the risk of allele drop-out. Although the specimen was not degraded and enough amount of extracted DNA was used for PCR reaction, we experienced a case that only one locus can not detect among 16 loci when using AmpFlSTR Identifiler Kit In this study, PCR amplification with a primer mix of the AmpFlSTR Identifiler Kit and QIAGEN Multiplex PCR Kit resulted in the detection of all STR loci.

P-35 Studies on differentially methylated parental allele in imprinted genes

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We have recently developed a detection method of differentially methylated parental allele (DMPA). This allows discriminating either parental allele of SNP(s) in five imprinted genes (H19, SNRPN, MEST, HYMAI and PEG3) so far by using methylation-sensitive restriction enzymes (msRE). To improve its usefulness, a set of seven SNPs considered from allele distributions mostly examined in this study was incorporated in an allele specific minisequencing assay. A multiplex PCR for four loci (without MEST) involving seven SNPs and subsequent minisequencing showed good result of each genotyping not only from native DNA but also from msRE-digested DNA for the DMPA assay. Furthermore, investigation of methylated CpGs around SNP was performed to confirm reliability of the assay. A region including each SNP in bisulfite-treated DNA was amplified, purified, cloned and finally sequenced. Clones from each locus generally showed hypermethylated or hypomethylated CpG status in either parental allele according to expression manner of each gene, however some of clones revealed partly mixed or reverse methylation status. The DMPA assay is useful and reliable method to discriminate parental allele from several imprinted genes, however an ambiguous result might be found using highly sensitive method, especially in PEG3 SNP.

P-36 Validation of DNA typing from Skeletal Remains using the Invitrogen Charge Switch Forensic DNA Purification kit

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The ability to analyze small amounts of DNA from old teeth and bone samples offers the opportunity to identify unknown skeletal remains. However, degradation and contamination of DNA extracted from bone and teeth samples often make that process difficult and so the success of DNA typing mostly depends on selection of appropriate DNA isolation procedure. The Charge Switch technology (Invitrogen) is a very useful method that permits to obtain a rapid and efficient purification of genomic DNA from a large variety of forensic samples, even if in small quantity. The aim of the present study is to validate the application of this technology even to DNA extraction from skeletal remains. In order to develop a reliable method and to verify the reproducibility of results obtained we used different cadavers and from each body we analysed different bones and teeth either using the Charge Switch Forensic DNA Purification kit than the traditional phenol/chloroform procedure. The original Charge Switch protocol was modified in order to maximize the recover of DNA and to remove more efficiently all contaminants in the sample that can interfere with PCR. All samples were then subjected to quantification in Real Time and typing by AmpFlSTR Identifiler kit and AmpFlSTR Y-Filer (Applied Biosystems). DNA profiles from skeletal remains were compared to the DNA profiles of blood samples belonging to living relatives. We observed that DNA profiles obtained with both procedures were clearly readible and comparable between them. Obviously the quality of bone and the conditions under which the remains were exposed had a big influence on the state of DNA. In fact the quality of DNA extracted from teeth was usually higher than that of DNA from bones and the quality of DNA differed between bone types. Our study demonstrates that the Charge Switch technology greatly improves the ability to positively identify skeletal remains by a comparative genetic analysis with presumptive relatives and can be used routinely since it's fast and permits to avoid the use of toxic reagents (phenol/chloroform)obtaining good results.

P-37 Application of less primer method to commercial kits

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Contrast to the conventional PCR product that depends on amount of the template, less primer method has the upper limit. If primer concentration is set at minimum, the locus of higher efficient amplification is reached to the plateau during early PCR cycles and the remaining PCR cycles employ to the production of lower efficient locus. Therefore, PCR product in less primer method is almost constant in every reaction and maintains the reproducibility and good balance among loci. We reported that even PCR products and accurate genotype analysis were obtained by the application of less primer method to Profiler? system (Applied Biosystems, USA) [1]. In this study, commercial kits [PowerPlex? 16 system (Promega, USA) and Identifiler? system (Applied Biosystems, USA)] that are indispensable for a high degree of discrimination are examined. The difference of peak height among loci is due to the ratio of primer concentration in both kits. The primer concentration in Identifiler? system was set at minimum required to the plateau below 6000 RFU without pull-up phenomenon. The peak of D21S11, D3S1358, D13S317, D16S539 and FGA typing were not detected because of lower primer concentration. The ratio of primer concentration is suitable for protocol, but not for less primer method. In the case of PowerPlex? 16 system, an imbalance from locus to locus is described below. Extreme heigh peak with pull-up : Penta E, Penta D, FGA and D8S 1179 typing Lower peak or no detection : TH01, D3S1358 and CSF1PO typing We try to arrange the ratio of primer concentration in PowerPlex? 16 system to improve the balance among loci and add 2 times of Taq Gold polymerase to decrease the extension time. [1] M. Kane, M. Masui, K. Nishi, Progress in Forensic Genetics 11 (2005) 694-696

P-38 STUDY ABOUT THE EFFECT OF HIGH TEMPERATURES ON STRs TYPING

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Environmental factors (UV light, humidity, temperature,etc) speed up DNA degradation. For example, humidity has more of an effect on the quality of the DNA, rather than the quantity, while high temperature shows a significant loss in DNA. The aim of the present study is to evaluate the effect of high temperature on the ability to perform DNA extraction and typing from different biological fluids (blood,saliva,semen) from a male donor. In particular for each biological fluid were prepared 6 slides: around 50 ul of each sample were deposited and smeared onto different slides that were left dried at room temperature for 2 days. 4 slides of each sample were incubated 20’ in a oven at different temperatures 50°C,100°C,150°C and 200°C, an other one was directly exposed to the effect of a flame for few minutes and the last one,without any treatment, was used as reference. All samples were then subjected to DNA extraction by Instant Gene Matrix and Nucleo Spin treatment, quantification in Real Time by Quantifiler™ Human DNA Quantification Kit and typing by AmpFlSTR Identifiler kit (Applied Biosystems) and and Nonaplex II PCR Amplification kit (Biotype). Our data show that it’s possible to type with success biological fluids as blood, saliva,semen exposed to drastic conditions, even if high temperature produces a significant loss in DNA. The chance of obtaining good results from a degraded sample is increased when mini-STRs are analysed because of the small sizes of STRs that are amplified.

P-39 Mini-SGM multiplex in degraded samples

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We have performed a five Mini-SGM multiplex which encompasses TH01, FGA, D18S51, D16S539 and D2S1338, common STR markers in human identity testing. Six cases performed with different biological tissues, such as blood, bones, lung, heart, liver, kidney and teeth were selected to illustrate the usefulness of this technique in forensic casework. DNA was extracted by QIAmp DNA Mini kit or by phenol/chloroform method followed by Microcon purification. Amplifications were performed according to Butler et al (J Forensic Sci., 2003). All cases have been studied previously with routine Powerplex16 and/or Identifiler multiplex systems. The use of routine methodology can sometimes give only a partial genetic profile or no profile at all. However, using the Mini-STR technique, a full profile was obtained for the majority of the degraded samples. But even using this technique, teeth from a much degraded corpse found in Tejo River and heart tissue from a homicide case gave partial profiles because results are still very much sample-dependent. We conclude that the Mini-SGM methodology is more sensible than routine methodology for degraded samples, although a full genetic profile is not obtained in all cases. This Mini-SGM multiplex can be considered a useful tool to complement conventional STR analysis in degraded samples.

P-40 Validation studies of Rapid Stain Identification-Blood (RSID-blood) kit in forensic caseworks

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Various samples are found at crime scenes and some of which may not have human origins. Moreover, human specimens can be mixtures involving blood and other different biological material as saliva, semen, sweat, vaginal secretions. Performing DNA typing without checking real nature and origin of stains can easily lead to mistakes. It highlights the importance of testing before blood to confirm that the samples are indeed originated from humans or are mixtures. The aim of this study is to assess the specificity and sensitivity of Rapid Stain Identification-Blood (RSID-Blood) test that permit the detection of human blood in many mixture specimens using an immunochromatographic assay based on two anti-human Glycophorin-A (GPA) monoclonal antibodies. We tested the kit in forensic caseworks involving aged, degraded stains and potentially bloodstains previously contaminated with reagents of presumptive tests for blood often used in crime scene investigations. One of most common presumptive tests is Luminol that could have deleterious effects on the immunoassay, depending on blood dilution and time of exposure. The genetic analysis of all the confirmed blood stains was subsequently performed using the AmpF1STR Identifiler PCR Amplification Kit (Applied Biosystems).

P-41 DNA typing from handled items

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Background and purpose: To develop in house protocol for DNA analyses of contact traces we conducted a series of experiments using 34-cycle low copy number (LCN) amplification of DNA isolated from touched objects. Method: Seven volunteers held sterile plastic tubes for 10 seconds, 15 min after washing hands. In second experiment, volunteers held each other wrists in order to investigate success of DNA analyses from material transferred by skin contact. The third part of this research examined the effect of the period after the deposition on both quantity and quality of DNA extract. From all experiments, DNA from pooled wet and dry swabs, was isolated by organic extraction and DNA profiles were obtained by LCN amplification, using AmpFlSTR Identifiler kit. Results: From seven volunteers we recovered three full and four partial profiles. One of the partial profiles differed from volunteer's reference profile. In second experiment, mixtures of both persons were obtained from all swabs, and ratio depended both on shedding status, as well as on hand dominance. In the third experiment full DNA profiles were obtained even after 24 hours since deposition for two good shedders. Conclusion: LCN-PCR technology provides a valuable approach in DNA typing of trace amounts of biological material, left on even shortly touched objects.

P-42 Detection limit of the minor component monitoring engraftment after bone marrow transplantation

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Detection limit of the minor component monitoring engraftment after bone marrow transplantation B. M. Dupuy, D. Perchla and M. Stenersen Institute of Forensic Medicine, University of Oslo, Rikshospitalet, 0027 Oslo, Norway Abstract In order to determine the detection limit of the minor component in recipients after bone marrow transplantations, analysis of six different STR polymorphisms (ACTBP2, D11S554, D17S906, D7S1517, PentaE, D12S391) were performed. White blood cells from four voluntaries were isolated and visually counted by microscopy (Bürcher) and diluted (100 000 cells / ml). Mixtures were made (% recipient: 0.6, 1.25, 1.5, 2.5, 5 and 25) prior to DNA extraction by chelex. The STRs were selected based on the following criteria: Both donor and recipient were heterozygous, the 4 alleles were of different sizes (no allele sharing) and avoidance of alleles that could be misinterpreted as stutter bands (n-1, n-2, n+1). The results show that the detection limit is 1% for the minor component when using selected STRs for monitoring chimerisme.

P-43 Analysis strategies to establish vWF intron 40 haplotypes

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Sequencing of a 0.65 kb region in the intron 40 of the vWF gene demonstrated a complex variability. Five STRs named Pol K, F, P1, P2-a and P2-b and an indel polymorphisms (I) are present. We established a routine analysing method to puzzle out the Pol K/F/I/P haplotypes which does not require a sequencing procedure. To recognise the combined polymorphisms as haplotypes we performed short and middle range PCRs in combination with Nde I and BsmA I restriction tests. Comparison of the amplicon and restriction fragment length reveals the most likely haplotypes of each person involved a kinship test. Furthermore, a SNP allele specific PCR was employed. Additional information can be achieved by typing Pol P2-a and P2-b. Establishing of intron 40 vWF haplotypes using the methods described here can greatly support the resolution of complex kinship cases. This statement is illustrated by demonstration of a family study.

P-44 Evaluation of DNA extraction and decontamination of skeletal remains

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Background and purpose::DNA from skeletal remains is often degraded due to insults by environmental factors. Moreover, the remains are prone to contamination by exogenous DNA. There are various strategies and methods to remove contamination and extract endogenous DNA from bone samples. In this study, we have used a protocol involving a bleach soak and demineralization by the use of EDTA in high concentration. The purpose was to identify an optimal extraction method, but also to investigate maternal relationship between a set of 70 years old human bone samples and a reference sample. The bones are the disputed remains of a person with close relations to Adolf Hitler. Methods: We have evaluated the use of bleach and EDTA in different concentrations followed by extraction using the Wizard genomic purification kit. All the extracts were further quantified using a real time PCR quantification assay for mtDNA as well as sequenced for the mtDNA d-loop. Results and conclusions: High concentrations of EDTA in pulverised samples yielded most mtDNA copies and soaking whole bones in bleach proved useful to remove possible contamination. Further, there were no sequence difference between the bones and the reference sample.

P-45 Comparative study of D1S80 typing by capillary electrophoresis and sequencing

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The D1S80 locus is very useful for personal identification in Japan. At present, D1S80 typing method of fragment analysis using capillary electrophoresis has been employed however, an allele over the region of the allelic ladder marker is not correctly typed by the method. The allele is estimated to be an allele over 45 similar to that on the previous polyacrylamide gel electrophoresis. This study investigated the repeat structure of 16 bases from an allele over 45 in D1S80 locus. PCR amplification product of the allele over 45 was examined both by direct sequencing and fragment analysis using capillary electrophoresis. We found that it included a noteworthy region of the 16 base repeated sequencing. Based on this region, it could finally be determined as an allele 57 by direct sequencing. However, it was calculated to be an allele 56 using the size marker of capillary electrophoresis. The difference may be attributed to the size marker of capillary electrophoresis. This finding indicates that the direct sequencing method may be useful to determine an allele over 45 in the D1S80 locus.

P-46 Reduce optimisation time and effort: Taguchi experimental design methods

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Development and validation of new methods and technologies can frequently require long periods of time, high costs for reagents and equipment, and complex statistical calculations to determine the optimal system. A common approach to optimisation is the 'one-by-one' method, where each variable is tested at every level of the other variables. An alternative approach to optimisation is to modify the experimental design using a multifactorial approach. The Taguchi design method utilises orthogonal arrays, which distribute the variables in a balanced manner, greatly reducing the number of experiments required. We have applied the Taguchi experimental design method to PCR optimisation, and have been able to reduce the 256 reactions (4 variables, each at 4 levels) required from a one-by-one approach to only 16 with the Taguchi method. Use of level average analysis allows the determination of both the optimal variable levels, and those that are detrimental to reaction success. We have found the Taguchi design method to be a valuable tool for reaction optimisation, which could be applied to many multifactor experiment types.

P-47 SNP TYPING BY USING TAQMAN ASSAYS WITH LIMITED AVAILABILITY OF DNA

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Background and purpose.- Taqman assays are a robust procedure to type SNPs. However, they may be problematic in case of limited supply of DNA because separate DNA aliquots are needed to perform each assay. In order to circumvent this difficulty, we explored the feasibility of reducing the DNA amount and using samples subjected to whole genome amplification (WGA). Methods.- DNA was isolated from the peripheral blood of 5 individuals with a commercial kit (Qiagen). An aliquot was subjected to WGA with the Genomiphi kit (Amersham). The alleles of 10 SNPs distributed through the X-chromosome were determined with Taqman assays (Applied Biosystems). The standard protocol was modified to reduce the reaction volume to 4 microlitres in 96-well plates. Different amounts of DNA were used. Results.- There was a perfect concordance between raw DNA samples and DNA from WGA for the 50 genotypes characterized. On the other hand, assay performance did not change when DNA amount was reduced from 20 to 2 ng. Conclusion.- The reduction of DNA amount down to 2 ng per assay and WGA are useful strategies to type SNPs by Taqman assays when there is a limited availability of DNA.

P-48 Evaluation of the use of a freezer mill to improve DNA retrieval from dried cotton swabs

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Cotton swabs are regularly used within many jurisdictions to collect trace quantities of biological sample from various surfaces for the purposes of generating DNA profiles in criminal investigations. These swabs are often dried after sample collection and prior to DNA extraction. It has previously been shown that significant proportions of the DNA contained by these swabs is not retrieved from them using common DNA extraction methods such as chelex and organic methods. Freezer mills are routinely used to powderise samples such bone and teeth to assist subsequent DNA extraction. This procedure has recently also been shown to be useful in the collection of DNA from glass slides. Here we examine to what extent a pre-extraction preparation of dried cotton swabs using a freezer mill can assist in improving the quantity of DNA retrieval from such samples. Preliminary experiments provided less than satisfactory results.

P-49 OLD CADAVER IDENTIFICATION FROM SEVERELY SPOILED BONES: ANALYTICAL APPROACH TO DEGRADED DNA

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An alleged burial site was identified in the south of Italy thanks to the disclosures of informants. Human skeletonised remains were recovered from a burnt car, interred at a depth of 10 metres. Partially burnt bones were collected from the trunk which was completely submerged with stones, mud and wet soil. Bones were adequately treated to remove contaminants the outer layer of each bone was totally removed. Hence the samples were processed by extraction and amplification protocols adjusted for Low Copy Number DNA (low reaction volume and "low bind" materials). A male STR loci profile was obtained from some of the above reported samples. Reference samples from the relatives of an alleged victim of mafia, mysteriously disappeared several years before, were collected and submitted to the author's lab for kinship testing. All the markers investigated were consistent with the relationship between the alleged victim and his relatives. Confirmation of preliminary informant's statements allowed the prosecutor to start a wide range enquiry on a decade of criminal murders.

P-50 Automatic data processing of reference DNA-profiles from FTA/Non FTA samples

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The new Swedish DNA legislation resulted in a huge increase in reference samples. In 2006 we received approximate 25,000 FTA-cards compared to 5,000 reference samples in 2005. To meet this increase we had to handle these samples in a more automatic way than before. All reference samples are processed as duplicates. The new module in the LIMS system (Forum version 4, Ida Infront AB) combines these duplicate results and form confirmed results if the samples are the same and of enough quality. Of the 25,000 FTA profiles, approximately 22,000 were automatically confirmed by the LIMS system. Some profiles are not automatically confirmed and need to be manually investigated. If discordance occurs between duplicates, they need further consideration. Also if the profiles are of poor quality, have more than two alleles or alleles are unbalanced within a marker. And also if technical problems occur or if the evaluator decides so, samples need to be manually investigated. These samples are directed to a view "Evaluate Results", by certain rules and settings in the LIMS, and are there sorted in different topics. In this view evaluators are able to combine the results to form confirmed results or chose to reanalyze the samples.

P-51 Implementation of a Robotized Real Time PCR Setup for the Use of the AB QuantifilerTM Human DNA Quantification Kit

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To enhance efficiency and quality standards of forensic evidence analysis, a partial automation and LIMS concept was developed at the Landeskriminalamt (Office of Criminal Investigation) Baden-Württemberg, Germany. The automated concept is based on multiple operational steps, hardware components and corresponding applications. DNA quantification results have an important impact on the quality of the STR data. DNA quantification therefore occupies a central role within the analytic process. A STR multiplex amplification with a sub-optimal target DNA concentration range can result in substrate excess inhibition or insufficient signal intensities up to even signal loss. In addition, accurate and sensitive DNA quantification using Real Time quantitative PCR is used as screening method in order to identify samples with sufficient DNA for STR analysis. Therefore, validation of the QuantifilerTM Real Time PCR assay with respect to sensitivity, accuracy and reproducibility is critical. Manual processing for set up of quantification reactions can be time consuming and labor intensive. Automation of Real Time quantitative PCR set up was an important goal of the automation project. Here we show the implementation of a robotized setup for the QuantifilerTM Human DNA Quantification Kit.

P-52 Differential amplification profiles from fresh and archived DNA samples.

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Background and Purpose: The use of mitochondrial DNA in forensic genetics has allowed researchers to answer questions relating to diversity, population scale and movements. Field sampling as well as transportation and storage, can negatively affect the quality of DNA for research. This project aims to investigate the common problem of PCR dropout of the longest mtDNA fragments. Method: Fresh and archived (2002) DNA samples were used in multiplexed PCRs amplifying human HVS1, HVS2 and the intervening region. First stage PCR amplified largest products and shorter products were amplified in the second stage. Amplicons were separated using PAGE and stained with SYBR-GOLD to identify specific amplification profiles. Results: Over all drop-out of larger products was seen for the oldest samples. Fresher stored samples showed predictable amplification patterns for pristine DNA. Less than 25% of older samples yielded products between 506bps-146bps. The dropout rate for the longest products was 1.5 times that of the shortest in older samples. Conclusion: PCR across the HVS1-HVS2 region is significantly hampered in older samples. Follow-up PCRs were victim to the abundance of mtDNA in the cell and the prevalence of smaller fragments in degraded DNA. This study shows that degradation of mtDNA D-loop is progressive, and provides strong indication that the process may be confined to specific regions of the molecule.

P-53 Implementation and validation of robotic extraction of DNA from high yield samples in Forensic Casework

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Keywords: forensic science, robotic DNA extraction, automated DNA purification, silica column
The DNA extraction of forensic evidence samples is a critical step in the analysis process and plays a significant role in the success of downstream applications. The increasing amount of samples treated by the biology department of forensic police laboratory of Lille creates the need for a rapid and automatic extraction method, with a reduced cost and a good yield in order to decrease the time spent to the treatment of casework. To address this issue, we have first compared and estimated the yield of the different commercial kits compatible with an automated system. Then, we validated the selected technique :a silica-based extraction method : Nucleospin®8 - Trace (Macherey Nagel), on our robotic platform: Freedom Evo® 150/8 (TECAN) using blood spots on swabs and checked for cross-contamination. Since june 2006, we are routinely working with this automated procedure which allows us to raise the productivity 2.5 times.

P-54 Development of a novel miniSTR multiplex assay for typing degraded DNA samples

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The ability to type degraded and limited DNA samples has become a necessity with the growing number of submitted evidential skin contact samples and for DNA identification of skeletal remains. Based on the observed locus and allele-dropout for SGM+ and Profiler, we decided to select a number of problematic (D18S51, D21S11 and FGA) STR loci for reduction of the amplicon size and to combine these loci with 4 miniSTR loci (D1S1677, D2S441, D10S1248 and D22S1045) into a novel multiplex together with THO1 and AMEL. Primers in literature were chosen for use of 5-dye technology that allowed us to decrease the length of the amplicons to less than 200 bp for the most frequent observed alleles. The amplification was performed with Qiagen Multiplex PCR kit for 30 cycles and sequenced allelic ladders were developed for the miniSTR loci. A sensitivity study demonstrated that by increasing the number of cycles to 34, it was possible to reproducibly type 30 pg of DNA. The ability to type degraded DNA was demonstrated in an identification case of skeletal remains where no result was obtained for FGA, D18S51 and D21S11 with SGM+. A concordance study of 198 reference samples previously typed with PowerPlex 16 demonstrated no discordance for the common STR loci. Additional validation studies are currently done and the results will be reported. The developed miniSTR assay combined with SGM+ allows the analysis of all ESS-loci and includes also the selected miniSTRs (D2, D10 en D22) that will supplement the current panel of ESS-loci.

P-55 STR, Y-STR and miniSTR markers evaluation for genetic analysis of fingerprints

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When analyzing fingerprints or other LCN evidence, autosomic STR multiplex kits have been widely used. However, in some occasions, great results can be achieved with Y STR, such as those cases perpetrated by men. Additionally, cells from fingerprints develop an apoptotic phenomenon that conduces to fragmentation of DNA in fragments less than 200bp. In these cases miniSTR can be used to recover information of commercial larger-sized PCR products. In the present work we compared the application of autosomic STR, Y STR and miniSTR markers on fingerprints genetic analysis, based on the concept of increased number of PCR cycles as a strategy to accomplish more sensitivity. DNA extracted from 15 fingerprints left on slides was analyzed with AmpFlSTR® IdentifilerTM kit using 34 cycles, AmpFlSTR® YfilerTM kit using 36 cycles and two miniSTR multiplex using 36 cycles. We observed that when miniSTR were used more alleles could be detected, however contamination level was higher. AmpFlSTR® YfilerTM kit revealed high sensitivity to DNA degradation and it seems less robust than AmpFlSTR® IdentifilerTM kit. From all results we concluded that miniSTR are the best choice, though strong guidelines and caution are needed when interpreting miniSTR LCN profiles.

P-56 Genetic analysis of fingerprints – could WGA or nested-PCR be alternatives to the increase of PCR cycles number?

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Increasing number of PCR cycles is the most widely method to perform low copy number (LCN) DNA typing. However, other alternative methods have been described, such as nested-PCR and whole genome amplification (WGA). In the present study we aimed to compare the application of these three strategies on fingerprints genetic analysis. DNA extracted from 15 fingerprints left on slides was analyzed with AmpFlSTR® IdentifilerTM kit using 28 and 34 cycles (normal and LCN condition, respectively) and REPLI-g (WGA) followed by AmpFlSTR® IdentifilerTM using 28 cycles. Nested-PCR was performed with miniSTR primers after amplification with IdentifilerTM kit using 28 cycles. Results were compared for correct alleles, allele dropin, heterozygous peak balance and allelic dropout. WGA provided good results with LCN quantities of human cell line 9947A (Applied Biosystems) however, when analyzing DNA from fingerprints, results were the same as with normal conditions IdentifilerTM using 28 cycles. Nested-PCR gave identical results as IdentifilerTM using 34 cycles, however the drawbacks of this technique makes the increased number of PCR cycles the best way to analyze LCN samples. We concluded that increasing the number of PCR cycles still is the best way to attain the required sensitivity.

P-57 Y-SNPs analysis using two different approaches in a Northern Portugal male population sample

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The non-recombining portion of the human Y chromosome (NRY) has various types of variation, including single nucleotide polymorphism (SNP). In spite of their low discrimination power, an important disadvantage against the use of Y-STRs, they provide a powerful and simple exclusion tool for forensic purposes. A special advantage of SNPs is that it can potentially detect smaller DNA fragments (analysis of degraded DNA). The aim of this work consisted in the analysis of a group of SNP polymorphisms (M2, M9, M35, M89, M45, M170, M172, M173, M207 and P25) in a Northern Portugal male population sample, which allows the determination of the most common European haplogroups, including the Northern Portugal ones. The method used for typing these polymorphisms was the real time PCR with TaqMan probes on the ABI 7000 platform (Applied Biosystems). We had some difficulties in typing some of the markers using this approach. Another problem is that it doesn't permit the use of multiplexes. However, the preliminary results obtained for the defined haplogroups are in accordance with those described in close European populations. To confirm the typing and solve the doubts that emerged from the real time approach, the samples were also typed using SNapShot.

P-58 Implementation of a semi-automated Processing System for DNA Profiling of Forensic Casework Samples

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The concept for a semi-automated processing system was developed at the Landeskriminalamt (Office of Criminal Investigation) Baden-Württemberg, Germany, to handle about 20,000 crime scene samples annually. The complete process comprises the extraction of genomic DNA from human cells by ChargeSwitch® magnetic bead technology, quantification of purified DNA by real-time PCR, amplification of short tandem repeats (STRs) by PCR and PCR fragment length analysis of STRs by capillary electrophoresis. Three liquid handling workstations from Tecan, a real-time PCR device and a 16-channel capillary electrophoresis system, both from Applied Biosystems, are linked with each other by laboratory data network. The server based LIMS enables automated and user-friendly transmission and management of sample and analysis data from the hardware components of the processing workflow. The wide range of different criminal casework samples to be processed includes epithelial cell samples, that often do not contain enough DNA to generate a complete genetic fingerprint. We successfully established a method to exclude these DNA negative samples in an early stage of processing workflow, thus saving time and minimizing costs. Automated barcode sample identification and several measures to avoid risks of cross-contaminations further enhanced efficiency and quality standards of the forensic analysis process.

P-59 The development of visual and chemical methods for predicting the likelihood of obtaining a DNA profile from degraded bone samples

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Many forensic cases involve the identification of skeletal human remains. The relation between the burial environment and the DNA preservation is complicated and predicting whether a bone sample can be successfully analysed is difficult. However, developing an effective system for the sorting of samples based on the likelihood of successful analysis (triage) would be a valuable tool. It could potentially speed up the identification process and also reduce the effort expended on samples that have little prospect of yielding DNA. The morphological and chemical status of the bones has been compared to the amount and quality of DNA that can be recovered. The relationship between three diagenetic parameters gross preservation, histological preservation and nitrogen content has been assessed. Primary results indicate that the best indicator of molecular preservation in forensic samples is gross morphology and histology. Nitrogen content has not proven to be a useful indicator of molecular preservation in samples up to 13 years old.

P-60 Evaluation of a semi-automated, magnetic bead-based DNA-Extraction Method for Genetic Fingerprinting of Forensic Casework Samples

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In order to cope with the demanding workload a concept for a semi-automated processing system was developed at the Landeskriminalamt (Office of Criminal Investigaton) Baden-Württemberg, Germany. As initial step of the analysis process the efficiency of extraction of genomic DNA from human cells is crucial. The applied magnetic bead extraction method is based on ChargeSwitch® Technology from Invitrogen. It was established on a liquid handling workstation Freedom EVO® 150 from Tecan, equipped with an eight-channel liquid handling arm and a barcode sample identification device. A one-for-all protocol was created to handle the whole range of biological sample types, including saliva, blood, semen, and especially the more difficult skin particle samples (epithelial cells). For samples providing only a limited amount of human cell material the method we established exhibits high DNA yield combined with sufficient purity for downward applications. Besides highest sensitivity our application mainly focused on preventing DNA cross-contaminations.

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P-62 Validation of the BioRobot EZ1 system in a routine forensic laboratory environment

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Automation can help forensic investigators to more efficiently process samples and thus enables higher sample throughputs and more rapid data generation. Throughput though is not the only factor why a growing number of forensic laboratories have decided to implement automation. Robotic processing minimizes human interaction with the evidence samples and thus helps to control contamination by the operator. Automation is also an important means to ensure that standard operating procedures are employed. Standardization helps to secure technical quality, reproducibility and allows full compatibility of data generated by different investigators, at different locations or at different times. Internal validation of equipment is an important part of a forensic quality assurance system. Forensic genetics requires analysis of a multitude of sample types, varying greatly in source, DNA quality and quantity, presence of inhibitors, and age. Design of a meaningful and representative but at the same time ressource- and cost-efficient validation plan can be a challenge. The aim of this study is to discuss the rationale for the validation strategy that we chose for the QIAGEN BioRobot EZ1 system and the resulting data.

P-63 Increasing the discrimination power of the mtDNA test through the analysis of a large set of haplogroup H coding region SNPs: forensic applications and validation

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Haplogroup H is by far the most common mitochondrial DNA (mtDNA) lineage in West Eurasian human populations. This haplogroup is poorly defined in the control region, especially for the most common mtDNA fragment analyzed in the forensic routine, namely, the first hypervariable segment (HVS-I). Here we show a minisequencing design that consists of three multiplex reactions that allow interrogating more than 50 SNPs defining different sub-lineages of haplogroup HV, where haplogroup H is nested. The present multiplex has been validated in serial dilutions and typical and problematic forensic samples such as bones and hair shafts. We conclude that the present multiplex reaction is of great utility in forensic casework for two main reasons: (i) it performs well with low and/or degraded DNA samples, and (ii) it allows increasing the discrimination power of samples belonging to the most common haplogroup in Eurasians.

P-64 Visualization and quantification of cell nuclei in telogen hair roots by fluorescence microscopy, as a pre-DNA analysis assessment

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Although nuclear DNA-profiling of human hairs is a well-known technique in forensic investigations, its success rate is quite low. Because the extracted nuDNA from telogen roots is scarce and often degraded, a simple and effective method was developed to estimate the number of cell nuclei. DAPI, a fluorescent, non-destructive DNA-stain, allows visualizing nuclear DNA and does not interfere with subsequent PCR analyses. After staining 3242 telogen roots from 27 volunteers and STR-profiling of a selection of roots, we show that the amount of analysable nuDNA indeed can be predicted. The distribution of the nuclei is sometimes surprising. This screening method allows the DNA laboratory to analyse only the most promising hair roots.

P-65 Forensic validation and implementation of Y-chromosome SNPs for prediction of geographic origin

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Analysis of Y-chromosome haplogroups defined by binary polymorphisms, has became a standard approach for studying the origin of modern human populations and for measuring the variability between them. However, their application in the forensic field, remain under research, principally due to two main features: the lost of forensic validation of the Y-chromosome multiplexes described for SNP typing and the difficulties in understanding the male lineage distribution and their frequencies among the different populations all around the world. In order to solve this problem in the forensic casework of our laboratory we have performed the forensic validation of several multiplexes for SNP typing previously described by our group. We have determined the minor quantity of DNA necessary to get reproducible results, and we have looked for their behaviour in mixed male-female stains. Statistical valuation has also been performed, and the problematic of comparing close populations was also evaluated.

P-66 mtDNA SNPs in a population sample from northwestern Germany

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Here, we describe a rapid and robust assay to simultaneously genotype 22 mitochondrial DNA (mtDNA) coding region SNPs and one non-coding region SNP by minisequencing using the SNaPshot technique. The set of SNPs implemented in this multiplexed reaction allows us to allocate common mitochondrial West Eurasian haplotypes into their corresponding branch in the mtDNA skeleton. Furthermore, it increases the power of discrimination for forensic mtDNA analysis. Haplotype data for a population sample from northwestern Germany will be presented and we will discuss if this approach will lead to a considerable increase in the discrimination power of our current mtDNA analysis that is based on HVI and HVII sequencing in forensic casework (including mass screening).

P-67 Spermatozoa recovered on laundered clothing

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Traces of sperm cells recovered on clothing impose challenges as to the interpretation of such findings in sexual assult cases. The manner of deposition may not be readily determined, clothing may have been washed intentionally, or the findings may not be related to the incidence under investigation. Scarce litterature suggests that spermatozoa can indeed survive machinewash and be useful for DNA typing. In an experimental study, 0.5 mL of thawed semen, from a mixture of five donors, where applied onto each of 30 new cotton briefs. The stains were allowed to dry for 24 hours in open air, before the briefs were split into sets of 10 and laundered in a new AEG® machine on programmes 40°C (80 minutes), 60°C (90 minutes) or 60°C with fabric softener added. In all cases OMO Color detergent was used. DNA was recovered on all 30 items, and sperm cells were visualised under 40 X magnification on 66% of the laundered underwear where of all of the cotton briefs washed on 40°C. Human DNA quantification by Quantifiler (Applied Biosystems) on the ABI 7300 Real-Time PCR System showed that the highets amounts of DNA were recovered when using the 40°C programme.

P-68 Assessment of individual shedder status and implication for secondary DNA transfer

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Low copy number DNA analysis offer the sensitivity to allow for analysis of minute amounts of DNA retrieved from touched objects. Nine persons participated in an experiment desigend to investigate secondary DNA transfer, and where their DNA shedder status were intially unknown. DNA collected from objects by cotton swabs were extracted using the Chelex method, and analysed using the AMPFISTR®Identifiler kit. PCR was performed for 34 cycles on all samples. DNA profiles, partial, full or mixed, were recovered from every item touched (n = 60). We found that a persons shedder status- good, medium or poor- is of consequence in order to deposit enough DNA for a full profile. Furthermore, the vector was not always the one with the most dominant profile on an object (glass beaker or plastic tube), implying that secondary transfer could occur from a better shedder donor through handshaking (up to 30 seconds) or by handling the same object.

P-69 Sieving DNA samples with mesh size H: simultaneous typing of the mtSNPs A2706G and C7028T using allele specific PCR followed by dissociation curve analysis

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Some 40-50% of the West Eurasian mitochondrial (mt) lineages belong to haplogroup (hg) H. Thus, a reliable, low-cost pre-screening tool for high throughput differentiation between hgH and non-hgH samples allows a fast exclusion of a major portion of specimens in high volume investigations such as mass screenings. We developed a homogeneous duplex PCR assay (ARMS) specifically amplifying either the derived 2706G allele (hgH samples) or the ancestral 7028C allele (non-hgH samples) in the presence of the dsDNA binding fluorescent dye SYBR Green I. Allele calling was based on the determination of the product-characteristic Tm values by on-line recording of the decrease in fluorescence-signal during a post-PCR temperature ramp from 60°C to 90°C (dissociation curve analysis, DCA). ARMS-DCA worked over a broad range of initial DNA concentrations, was successfully applied to the typing of DNA extracted from a more than 200 years old tooth, and enabled a single lab-technician to analyse more than 2.000 samples within two regular working days without using robotic equipment. Furthermore, by performing the amplification in a real-time PCR machine instead of a standard thermal cycler, the ARMS-DCA assay also allowed for absolute DNA quantification on the basis of CT values and calibration curves for both amplicons.

P-70 Improved resolution of the Y-chromosomal haplogroup R1b by the recently introduced Y-SNPs U106, U152 and U198 within a geographically well defined sample from Tyrol (Austria)

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Haplogroup R1b is the most common Y-chromosomal lineage in Europe showing a complex spatial distribution with a frequency peak in the western parts of the continent and a continuous decline towards east and south-east. Although several sub-groups within R1b have been defined by the YCC2003 Tree, they were only observed in very small percentages of R1b-lineages. Recently, a set of three previously unknown phylogenetically informative binary markers (U106, U152 and U198) that define new sub-groups of hg R1b was published. In order to analyse the frequency of these new haplogroups in a geographically well defined sample, Y-SNP haplotyping was performed in 135 individuals from Tyrol (Austria). The derived states of U152 (defining R1b3h), U106 (R1b3i) and U198 (R1b3i1) were found within the Austrian R1b population sub-sample (n=42) in 9 (21.4%), 25 (59.5%), and 1 (2.4%) individuals, respectively. Only 19% of the hg R1b Y chromosomes remained unresolved. The novel R1b sub-haplogroups were more abundant in the Austrian R1b-sample compared to an R1b-sample of European-Americans. The new SNPs proved to be promising tools for distinguishing R1b lineages in the evolutionary as well as in the forensic context.

P-71 Application of BioRobot M48 to forensic DNA extraction

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The development of a nucleic acid extraction method based on magnetic separation has opened up possibilities of full automation of DNA extraction. BioRobot M 48 is a robotic station applicable to automated DNA extraction in forensics. However, each new method should be thoroughly validated before application to routine casework. Our aim was to compare the effectiveness of the currently utilized organic Microcon 100 based extraction procedure and magnetic extraction with BioRobot M48. The DNA concentration of DNA extracts obtained from different kinds of typical forensic material was evaluated followed by amplification with the SGM Plus kit and capillary electrophoresis using ABI 3100 A. A performed validation confirmed that in the case of regular traces, results obtained with both manual and automated methods were equally robust. DNA concentrations obtained for corresponding samples were significantly lower in the case of magnetic DNA extraction, but this did not affect the ultimate result. However, our experiments showed that in the case of heavily degraded samples and bone material better results are obtained with the standard organic method. We can conclude that BioRobot M48 is a very effective instrument for DNA extraction from most specimens and can be successfully applied in forensic laboratories.

P-72 Using the new Phadebas® Forensic paper to find crimescene saliva stains suitable for DNA analysis

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The Phadebas® Forensic paper is a new, commercially available product that detects saliva stains by reacting with amylase. When the paper is pressed against a positive saliva stain a blue spot occurs. To test the sensitivity of the paper, a set of dilution series (1:1, 1:5, 1:50, 1:100, 1:200, 1:500) was prepared on cotton fabric. Blue spots could be seen for dilutions of 1:100 when incubated at room temperature, and 1:200 in 37°C. However, incubation at room temperature provided a better reproducibility between runs compared to 37°C. The Phadebas® Forensic paper was compared to four different fluorescent lightsources, Quaser 2000/30, Crimescope® CS-16, Polilight® and Labino® UV Spotlight, with respect to the ability of finding known saliva stains (1:1, 1:5, 1:25, 1:100) on different materials. The materials tested were cotton fabric (T-shirt), denim, suede, leather, painted wood and untreated wood. On denim, no stains could be seen with the lightsources, but with the Phadebas® Forencic paper stains were visible for both pure saliva and a 1:5 dilution. DNA analysis (AmpFlSTR® SGM Plus™) was performed on both the detected stains on the different materials and on the corresponding spots on the Phadebas® Forensic paper.

P-73 The mtDNA sequencing analysis without DNA purification from blood, saliva and hair roots

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In general, the purified DNA template is required for an amplification of hypervariable region in routine mtDNA sequencing. We have directly amplified ~ 1 kb mtDNA template using direct PCR buffer system (BioQuest Inc., Korea) from blood, saliva and hair roots and followed by sequence analysis with BigDye?R terminator cycle sequencing kit. The resulting HV1 and HV2 sequence data showed fairly good resolution without particular noise peaks and whole sequence concordance with data from routine method. No purification step except sample dilution is required for blood or saliva. In case of hair roots, pre lysis with DTT and proteinase K is necessary, but needs no further purification step. Our new method is so fast and easy-to-use that this direct PCR buffer named AnyDirectTM appears to be applicable for fast forensic mtDNA analyses.

P-74 A fast and efficient DNA extraction method using pulverizing device

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A fast and efficient DNA extraction method using pulverizing device Kwang Man Woo and Seung Hwan Lee As only the limited amount of DNA typing material can be obtained from many crime scenes, more efficient DNA extraction method can be very useful for forensic scientist. Here we report an alternative method which can be applied to tiny amount of sample and dramatically reduce lysis time. Using pulverizing device, Precelly? 24 (Bertin Technologies, France), DNA extraction protocols were established for single hair, tiny blood stain, saliva spot on cigarette butt and semen stain. Its performances were checked using commercial STR typing kits. Couples of minutes were sufficient for lysis and subsequently followed by further DNA purification step. The resulting STR data were all concordant with those from routine DNA extraction method. We could get successful data from even a single plucked(or sometimes shed) hair which is hard to be typed successfully in many cases. In conclusion, it appears to be useful for many forensic cases which need high sensitivity and rapid analysis.

P-75 Development of a new multiplex STR system with high discriminating power in Korean population.

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We have developed a highly discriminating fluorescent dye labeled STR system, which includes 12 autosomal STR loci(D5S818, D13S317, D19S253, D3S2406, D2S1371, D8S1477, D12S391, D20S470, D6S1043, D9S925, D7S821, D4S2368). The combined Probability of Identity(PI) value was calculated as 3.8x10-16 in Korean population. Especially D3S2406 was extremely polymorphic and had a complex type of repeat unit. As result, this multiplex system appears to be a supplement tool in forensic science with other commercial kits.

P-76 Optimization of DNA-extraction and -typing procedures for contact stains

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If samples of secretions such as blood, saliva, or sperm are available, generally the common short-tandem repeat system as a means of DNA-typing is utilized to identify a potential crime suspect. However, the analysis of so-called contact stains (shedded epithelial cells) left on the surfaces such as a hand, etc. continues to prove difficult with the above mentioned method. Generally, fingerprints (contact stains) are regarded as unsuitable due to the low amount of DNA that can be extracted from such stains,. In addition, the DNA sample found in fingerprints is often treated with different reagents such as Ninhydrin for visualization or is contaminated with soot powder. The aim of this study was to develop an improved procedure for obtaining a complete DNA profile from a suboptimal sample amount (contact stains).The experimental procedure included the isolation of DNA from epithelial cells collected off of stearing wheels and shift sticks of cars, as well as surfaces of mobile phones, letters, and other objects. Three different collection methods for retrieving the stain material and three different DNA-isolation techniques were compared. The extracted DNA was quantified by Real-Time PCR using SYBR Green on a Light Cycler 2.0 with amelogenin primers. The DNA-typing was performed by using mainly STR-systems with reduced amplicon length. Best extraction results were obtained using Qiagen Mini Kit in combination with QiaShredder columns. DNA-profiles consisting of 8 STRs could be obtained from approximately 90% of all samples.

P-77 Validation of Biomek® NX Laboratory Automation Workstation in the Preparation of Forensic Casework Samples for Human DNA Quantitation

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Background and purpose: Human DNA quantitation of evidentiary DNA samples helps to estimate the concentration of human DNA in the sample, which is essential for the subsequent step of PCR-based DNA typing. In this study, we tested a newly launched automation workstation-Biomek® NX equipped with an automated tube barcode reader in preparing quantitation reactions for the QuantifilerTM Human DNA Quantification System for forensic casework samples on 96-well plate with the aim at improving efficiency, saving manpower, keeping track of the samples and minimizing the risk of human error during the liquid transfer process. Methods: The areas on pipetting accuracy, delivery accuracy, cross contamination of samples and reproducibility of quantitated DNA results on the robotic system were assessed. Results and Conclusion: Our findings revealed that i) the pipetting accuracy and reproducibility studies of the robotic system gave satisfactory results, characterizing by low %CV values ii) no cross contamination of samples was detected in a series of tests and iii) the robotic system succeeded in verifying the identity of samples before delivering them to the designated positions on the 96-well plate. Further development on using the Biomek® NX in automating other process of forensic DNA profiling will also be discussed.

P-78 STR and Y-STR genotyping assays for 25-year-old semen stains

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Introduction The DNA extracted from old samples is often decomposed and fragmented, making it difficult to use in short tandem repeat (STR) and Y-chromosome-specific STR (Y-STR) genotyping assays. Here we investigated STR and Y-STR genotyping assays for old semen stains using commercial STR assay kits. Method We used 20 samples (gauze with semen stains) that had been stored for 25 years at room temperature. DNA was extracted from semen stains on 0.5 × 0.5-cm pieces of gauze using a QIAmp DNA mini kit (containing 1.0 M DTT). For the STR genotyping assay, PCR reactions were performed using an AmpF1STR Identifiler kit and a PowerPlex 16 kit. The Y-STR genotyping assay was performed using an AmpF1STR Yfiler kit and a PowerPlex Y System kit. Amplified DNA was separated by capillary electrophoresis using an ABI Prism 310 Genetic Analyzer. The separated PCR products were identified using GeneScan and Genotyper software. Results and Discussion The STR genotyping detected 11–15 of 15 loci using the AmpF1STR Identifiler kit and 9–12 of 16 loci using the PowerPlex 16 kit. Y-STR genotyping detected 15–16 of 16 loci using the AmpF1STR Yfiler kit, and 11 of 11 loci using the PowerPlex Y System kit. The longest PCR products detected by each kit were 320–360 bp, suggesting that the DNA fragment sizes from old semen stains are smaller than 360 bp.

P-79 A novel method for efficient analysis of STR loci from a single sperm captured by laser microdissection

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Introduction DNA analysis of trace samples is important for individual identification in forensic medicine. In particular, identification analysis from mixed or trace samples is critical. In this study, short tandem repeat (STR) analysis of single sperms captured by laser microdissection was performed with the reduced-primer method using an AmpFlSTR Identifiler kit. Materials and Methods Semen samples were collected from three volunteers, washed in TE buffer, and mounted on glass slides. Smeared sperm samples were visualized by hematoxylin and eosin staining, and a single sperm was captured by the laser microdissection method. Fifteen STR loci and the amelogenin locus were analyzed using the AmpFlSTR Identifiler kit. We used primers at the original concentration and at 3% dilution. PCR was carried out, and the amplified products were detected with an ABI Prism 310 Genetic Analyzer. Fragment sizes were estimated using GeneScan software, and the alleles were typed using Genotyper. Results and Discussion Using the original primer method, the STR genotyping assay was impossible in the 15 loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA). However, using less primer (3% dilution), the 15 STR loci and the amelogenin locus were perfectly determined in all of the preserved single sperms. The reduced-primer method is thus more efficient for analyzing STR loci from a single sperm captured by laser microdissection.

P-80 Automated DNA extraction from large volumes

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Automation of DNA extraction with DNA-IQ™ is a very efficient process when dealing with small extraction volumes but requires special adaptations when trace amounts of DNA must be recovered from large substrates requiring large volumes of extraction buffer (e.g. clothing). We have compared several approaches for concentrating DNA after the initial treatment in a large volume. These include : 1) elution of intact cells from the substrates followed by centrifugation, 2) treatment of substrates with hypotonic buffer to induce cell lysis but recovery of intact nuclei and 3) complete lysis with DNA extraction buffer (containing SDS or Sarkosyl, Proteinase K, DNA-IQ™ lysis buffer) followed by batch DNA recovery through incubation with DNA-binding magnetic beads. Tests were performed with blood, semen, saliva and manipulated or worn objects on cotton, polyester and nylon substrates. The best results were obtained with lysis of cells directly on the substrate followed by batch DNA recovery with magnetic beads. The process was optimized for incubation time, temperature, detergent and Proteinase K concentrations. Non-probative forensic samples were tested and the procedure integrated into the robotic scripts.

P-81 Comparison of STR loci profiles obtained from extracted FTA discs using different Taq DNA polymerases

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Five commercially available Taq DNA polymerases together with their respective buffers were compared as to their ability to generate profiles of commonly used short tandem repeat loci. Discs were cut from FTA paper containing dried human venous blood and treated to extract the genomic DNA. The discs were used in two fluorescent multiplex PCRs containing the same number of units of Taq DNA polymerase to amplify twenty STR loci and the resultant alleles were visualized after electrophoresis on a capillary sequencer. Significant differences were observed upon comparing the profiles: one polymerase failed to amplify larger alleles and presence of additional peaks varied according to the enzyme used. The use of hot-start polymerases did not affect the quality of the resultant profiles in this comparison.

P-82 Quadruplex real-time PCR for forensic DNA quantitation

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Forensic DNA quantitation is an important initial step preceding PCR amplification of the STR loci even though information concerning the quality of the DNA is not revealed. A quadruplex real-time PCR (qPCR) assay was developed to quantify four DNA targets: 1) the human RB1 gene in nuclear DNA 2) the DAZ gene present on the human Y chromosome 3) the ATPase8 gene present in human mitochondrial DNA 4) an artificial internal positive control to reveal possible PCR inhibition. Primers labelled with four different fluorophores are used together with a single quencher using the antiprimer quenching-based qPCR method in one reaction, in which the resultant amplicons are less than 127 bp in size. Sensitivity was shown to be less than ten copies for all four targets in the absence of amplification inhibition. The amplification remained sensitive in the presence of an excess of non-human DNA.

P-83 Simultaneous detection of ABO and secretor-nonsecretor blood groups from forensic biological samples by fragment analysis

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ABO and secretor-nonsecretor blood groups can be very useful information concerning forensic samples such as body fluid spots, however, it is occasionally difficult to decide ABO grouping by serological methods because of the small quantity or non-secretor typing. The purpose of this study is to establish the simultaneous detection of ABO and secretor-nonsecretor blood groupings using DNA materials by means of fragment analysis. Simultaneous detection of ABO and secretor-nonsecretor blood groupings from biological samples was successful. For the determination of A, B and O alleles, we examined three nucleotide positions at 261, 796 and 803 of transferase cDNA. For the determination of Se, se1, se2 and se5 alleles, we examined three nucleotide positions at 428 and 385 of transferase cDNA and the fusion gene. We correctly typed six ABO genotypes, AA, AO, BB, BO, AB and OO, and five secretor-nonsecretor genotypes, SeSe, Sese2, Sese5, se2se2 and se2se5. Genotyping was performed using fragment analysis of multiplex PCR products. The results corresponded to the serological determination. We were not able to find se1 non-functional alleles among our Japanese samples. Our method is an effective tool for ABO and secretor-nonsecretor blood groupings from forensic biological samples.

P-84 The determination of Lewis and secretor-nonsecretor functional alleles for clinical application of urinary CA19-9

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The synthesis of CA19-9 is complex. Three genes namely, Lewis (Le) genes encoding Le transferase, secretor-nonsecretor (Se) gene encoding Se transferase, and the gene encoding alpha 2,3-sialyltransferase involved. In the biosynthetic pathway, Le transferase is thought to be a key enzyme. The activity is genetically controlled by Le genotypes. The effect of the Le and Se gene dosages on the urinary levels of CA19-9 and DU-PAN-2 was further examined. As a result, the urinary CA19-9 level can be an effective diagnostic tool in bladder cancer patients with both Le and Se functional alleles. For clinical application of urinary CA19-9, determination of Le and Se genotypes is necessary. The purpose of this study is to establish simultaneous genotyping of Le and Se using PCR-based methods. Genotyping was performed using fragment analysis of multiplex PCR products. Simultaneous genotyping of Le and Se was successful. The frequency of the patients with both Le and Se functional allele are about 76% in Japanese. Le is a functional allele, and le is non-functional allele. Se is a functional allele, and se1, se2 and se5 are non-functional alleles described together as se for the data processing.

P-85 Proteinase K challenged by a Novel Protease

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Proteinase K is used in forensic DNA extraction methods for cell lysis and degradation of proteins. Here we investigate if a novel protease, hereafter called "Protease X", can improve or speed up this treatment. Proteinase K has endolytic activity and an optimum at 56°C. Protease X has both endolytic and exolytic activity with an optimum at 37°C and was provided by Johan Sjödahl and Johan Roeraade at the Royal Institute of Technology in Stockholm, Sweden. Samples were prepared from human tissue and processed at two occasions. The proteases were tested individually and as a mixture, in 37°C or 56°C and for different incubation times. Samples were purified either by BioRobot® EZ1 or by phenol-chloroform followed by Centricon®-100 filtration. DNA recovery was significantly higher from samples treated with Proteinase K at 56°C than Protease X or the mixture incubated at 37°C. The majority of samples generated complete DNA profiles. At 56°C, the mixture gave significantly higher DNA recovery than Protease X, but not compared to Proteinase K. We conclude that there is no need to exchange Proteinase K in our methods, but for short incubation times at 56°C the mixture seems to be more efficient compared to Proteinase K alone.

P-86 Low Copy Number (LCN) DNA analysis at SKL, Sweden

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During the last three years, LCN DNA analysis has frequently been used in casework at SKL. Successful items include bomb remains, threatening letters, the inner side of envelopes and electronics (wires and skimmers). In our laboratory LCN DNA analysis means using 30-34 cycles instead of 28 cycles as in standard DNA analysis. There are two ways for the laboratory to handle samples containing minute amounts of DNA. First choice: Samples collected and analysed in a specially designed area from items mostly without visible stains. Material can contain DNA just from having been touched. Second choice: Samples collected and analysed as a standard analysis (28 cycles) but showing results too weak to interpret. The number of cycles for the existing extract is then increased. Thus protective clothing and awareness of contamination is always important. In 2006 we received 235 cases. One was a serious robbery case, the largest police investigation in Sweden that year. 67 LCN DNA analyses were performed. One DNA profile of importance matching a reference sample was obtained from a swabbing of electric wires in a bomb replica. We estimate a continuous increase in years to come and further development of the analyses.

P-87 A mitochondrial DNA SNP multiplex assigning Caucasians into 32 haplo- and subhaplogroups

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Mitochondrial DNA (mtDNA) is maternally inherited without recombination events, and has a high copy number, which makes mtDNA analysis feasible, even when genomic DNA is sparse or degraded. Here, we present a selection of 34 previously described coding region mitochondrial SNPs for haplogroup assignment of Caucasians. Samples are ascribed into the 9 major Caucasian haplogroups (H, I, J, K, T, U, V, W, X), and 23 of the most frequent Caucasian subhaplogroups. PCR and single base extension (SBE) primers were designed to function in one multiplex PCR and SBE reaction, respectively. The optimised assay is being validated in 209 unrelated Danish individuals, who were previously sequenced for the mitochondrial HV1 and HV2 regions. We believe that a fully functional assay can be used as a screening tool in forensic investigations assisting well-established methods such as genomic STR analysis and mtDNA sequencing.

P-88 Population data for 8 X-Chromosome STR loci in a population sample from Northern Italy and from the Sardinia island

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Short tandem repeat markers on the X chromosome are the natural counterpart to the well-established Y-chromosome STR loci and they have proven to provide useful tools in paternity cases with female offspring or in forensic identification cases based on the comparison with first or second-degree relatives. But before a new locus can be introduced in the forensic current practice a database for the relevant population must be established to evaluate its effectiveness. Because of the few population data regarding X-chromosome STR loci in Italy, 150 unrelated individuals (60 males and 90 females) from Northern Italy and from the Sardinia island were typed for the STR-loci DXS8378, DXS7132, HPRTB, DXS7423, DXS10134, DXS10074, DXS10101, DXS10135. Genomic DNA was extracted using Chelex-100 procedure from whole blood or buccal swabs. PCR was performed in a GeneAmp PCR System 9700 (AB) using the commercial kit Mentype Argus X-8 (Biotype AG, Dresden, Germany) according to manufacturer's recommendations. The amplification products were loaded on the Abi Prism 310 Genetic Analyser and analysed by GeneMapperID V3.2. This work provides a picture of allelic, genotypic and haplotypic frequencies for 8 X Chromosome STR loci in a popultation sample from Northern Italy and from the Sardinia island. As expected the preliminary results in the frequency's distribution in our population sample are close to those found in the Caucasian population.

P-89 Population data for MiniNC01 in a population sample from North-eastern Italy and their use in neoplastic tissues fixed in formalin and embedded in paraffine

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A number of studies have demostrated that successful analyisis of degraded DNA specimes from mass disaster or forensic evidence improves with smaller sized PCR products (MiniSTRs). Because of the few population data regarding MiniNC01 loci in Italy, 100 unrelated individuals from North-eastern Italy were typed for the three miniSTR-loci D10S1248, D14S1434 and D22S1045. Genomic DNA was extracted using the Chelex-100 procedure from whole blood or buccal swabs. PCR was performed in a GeneAmp PCR System 2400 (PE) using the protocol suggested by Coble (www.cstl.nist.gov./biotech/strbase/miniSTR). The amplification products were loaded on the Abi Prism 310 Genetic Analyser and analysed by GeneMapperID V3.2. This work provides a picture of allelic frequencies for three mini-STRs loci in a population sample from North-eastern Italy. As expected the preliminary results in the frequency's distribution in our population sample are close to those found in the Caucasian population. To verify the value of these markers in neoplastic tissues, they were tested on different tumoral specimes fixed in formalin and embedded in paraffin (gastric, colorectal and breast cancer and mesothelioma) that sometimes are used to forensic purposes. For each case the genetic profile obtained fron the neoplastic specimen was compared to that obtained from normal tissue.

P-90 Analysis of 16 Y-STRs in a sample of Colombian male

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We studied 16 Y-STR (DYS19, DYS385, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS460, DYS461, GATA-A10, GATA-H4 and DYS635) in a sample of 133 unrelated Colombian males selected of cases of paternity from the Laboratorio de Genética y Biología Molecular - Bogotá. PCR products were separated in 4% acrylamide-bis-acrylamide denaturing gels followed by silver staining. Allele size determination and genotyping were performed according to recommendations of the DNA Commission of the ISFG with allelic ladder manufactured at home. Gene frequencies, gene and haplotype diversity were calculated using ARLEQUIN version 3.1. In general a good allelic representation in all the systems was observed, between three and seven alleles, with the exception of the DYS385 with 35 allelic classes. DYS385 displayed the greater genic diversity (0,9012), whereas the system that displayed the smaller (0.4570) was the DYS393. The rest of markers presented a genic diversity between 0,4923 and 0,7114, thus demonstrating its great utility by the high degree of genetic information. One hundred thirty one different haplotypes were found, only one was detected in three men. The haplotype diversity and haplotypic discrimination was 0.9997 and 0.9849, respectively. This approach represents a very powerful tool for individual identification and paternity testing.

P-91 A DYS438 null allele observed in two generations of a large family

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Background and Purpose: Typing of Y-chromosomal STR loci using the AmpFISTR Yfiler kit showed a DNA profile lacking the DYS438 allele in an Austrian Caucasoid brother pair. An investigation was performed in order to reveal the inheritance and the underlying cause of this finding. Method: Buccal swabs were collected from males of two generations and different branches of the family. Subsequent typing of Y-chromosomal STRs was performed using the AmpFISTR Yfiler kit and an ABI prism 310 Genetic Analyser (Applied Biosystems) as well as the Powerplex Y System (Promega). Subsequently, new DYS438 primers were designed and the PCR products were sequenced. Results: Two generations of males from different branches of this family did not show a DYS438 allele in their Yfiler profile. Using the Powerplex Y system an allele with dramatically reduced peak height was amplified. Sequencing of the DYS438 locus exhibited no deletions or duplications but an unknown transition upstream of the repetitive region. Conclusion: Two generations of males in an Austrian family carry a DYS438 allele, which is not detectable with AmpFISTR Yfiler kit and hardly detectable with the Powerplex Y System. Such null alleles have to be taken into consideration, especially when interpreting mixed samples.

P-92 Population data of 8 X-STRs in South Italy (Calabria) using

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X-STRs have been proven to be useful in case of deficiency paternity testing and in effective mother-son kinship and father-daughter testing. Male individuals inherit their one X-Chr from their mother, while female individuals receive one X from the mother and the other one from the father. So, female individuals fathered by the same man share their paternal Chromosome X. Hence in case of deficiency paternity in which the mother is available for typing, the possible X alleles of the putative father can be determined and the paternal profile can be reconstructed. In the present study we investigated the distribution of 8 X-STRs loci DXS8378, HPRTB, DXS7423, DXS7132, DXS10134, DXS10074, DXS10101, DXS10135 in an Italian population sample, using the Mentype® Argus X-8 PCR Amplification Kit (Biotype). Samples for the study were obtained form 200 unrelated healthy individuals belonging to Calabria (South Italy)population since at least 3 generations. All samples were quantified by the Quantifiler™ Human DNA Quantification Kit using a 7300 Real Time System and then amplified according to the Mentype® Argus X-8 PCR Amplification Kit (Biotype) protocol using GeneAmp PCR Systems 9600,9700,2400,2720 thermal cyclers (Applied Biosystems). Female and Male positive controls and negative controls were used during all amplification steps. Amplified products were analyzed by capillary electrophoresis using an ABI PRISM 3130 Genetic Analyzers employing GeneMapper 3.2 software(Applied Biosystems).

P-93 Polymorphism of eight X-chromosomal STRs in a Japanese population

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X-chromosomal STRs (X-STRs) have been recognized as important tools in forensic analysis, particularly in complex cases of kinship testing. In this report, we investigated 8 X-STRs in 258 unrelated Japanese (144 males and 114 females) and built a X-SNPs Japanese database for identification. X-STRs multiplex amplification was performed by the MentypeR Argus X-8 PCR Amplification Kit (Biotype AG) which contains eight X-STRs, that is DXS7132, DXS7423, DXS8378, DXS10074, DXS10101, DXS10134, DXS10135 and HPRTB, as well as Amelogenin for sex determination. Electrophoresis and allele typing were carried on ABI PRISM 310 Genetic Analyzer and GeneMapper ID ver.3.7 software (Applied Biosystems). Allele frequencies and polymorphism information content (PIC) were calculated from the combined data of males and females, whereas power of discrimination (PD) was figured out for each data. The DXS10135 locus proved to be highly polymorphic (PIC:0.945), DXS7423 showed the lowest value (0.453). The value of PD for male indicated the value ranging from 0.500(DXS7423) to 0.905(DXS10135), while PD for female ranged from 0.527(DXS8378) to 0.986(DXS10135). Furthermore, no significant deviation from Hardy-Weinberg Equilibrium was detected. We concluded that these eight X-STR markers offer high effectiveness for forensic application.

P-94 Analysis of the Polymorphism of STR Loci of Human Genome in Indigenous Population of Armenia

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Even among representatives of one nationality reliable distinctions were testified by comparative analysis of the genetic variability of Short Tandem Repeats (STR) loci of unrelated individuals. In this respect, the use of data from foreign population in the examinations by national DNA laboratory is a compulsory step. To reveal character of distribution of the allele frequencies in the Armenian population an analysis of polymorphism of the STR loci of human genome was conducted for the first time in Armenia. The Polymerase Chain Reaction method was used to analyze the polymorphism of STR loci - CSF1PO, TPOX, THO1, F13A01, FESFPS, vWA, D16S539, D7S820, D13S317 (Promega Corporation, USA) and their combinations in indigenous Armenian population. The Republican Scientific-Practical Center of Forensic Medicine conducted this research using biological samples of living persons and cadavers, during 2000-2007years. The results revealed that tested loci are highly polymorphous for the Armenian population. The character of distributions of the allele frequencies of tested loci and their distinctions from similar data on Caucasian–Americans, African-Americans, and Hispanic-Americans were revealed. Application of revealed values of allele frequencies of tested STR loci in probability calculations increases the reliability of examinations on identification of a person and of consanguinity testing.

P-95 Population data of eight X-chromosomal STR markers in Ewe individuals from Ghana

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The investigation of the X-linked DNA markers are well established in the forensic routine case work. The study of X-chromosomal STRs is particularly useful in case of paternity testing. Population genetic data from Africa are not widely available. That's why we studied a population from Ghana. The eight X-chromosomal STRs DXS8378, HPRTB, DXS7423 and DXS7132, DXS10134, DXS10074, DXS10101 and DXS10135 were analyzed in 183 Ewe individuals (108 females and 75 males) from the region of Sogakofe (Ghana). About 13% of the Ghana population are Ewe. The samples were typed using the Mentype® Argus X-8 multiplex kit (Biotype, Germany) following manufactures instructions. Electrophoresis was performed on the ABI Prism 310 Genetic Analyzer (Applera, USA). Allele frequencies and statistical parameter as well as comparison with known data from Germans and with data from an Amharic population (Ethiopia) are presented.

P-96 Genetic analysis of the skeletal remains attributed to Francesco Petrarca

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We report the mitochondrial DNA (mtDNA) analysis of the supposed remains of Francesco Petrarca exhumed in November, 2003 from the S. Maria Assunta church, in Arquà Padua, where he died in 1374. The optimal preservation of the remains permitted the retrieval of sufficient mtDNA for genetic analysis. DNA was extracted from a rib and a tooth and mtDNA sequences were determined in multiple clones using the strictest criteria currently available for validation of ancient DNA sequences. MtDNA sequences from the tooth and rib were not identical, suggesting they belonged to different individuals consistent with morphological investigations Historical records indicated that the remains were violated in 1630, possibly by thefts. These results confirm once again the importance of using both molecular and morphological approaches in investigating historical remains.

P-97 mtDNA diversity in Sudan (East Africa)

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East African populations present the highest genetic diversity levels, namely for mitochondrial DNA (mtDNA). It is known that the vast majority of mtDNA haplotypes in a population are unique, rendering that mtDNA databases must have a considerable size in order to be informative. Here we report the mtDNA diversity (for hypervariable regions I and II) in 103 individuals from Sudan, an Eastern African country so far poorly characterised for mtDNA diversity. As expected, levels of diversity were high, attaining a value of 0.989±0.004 for haplotype diversity in HVI, comparable with values in neighbouring populations: 0.993±0.005 in Egypt (n=68), 0.977±0.008 in Nubia (n=80), and 0.993±0.001 in Ethiopia (n=270). The random haplotype match probability in HVI was of 2.04% in Sudan, 2.16% in Egypt, 3.54% in Nubia and 1.06% in Ethiopia. The haplogroup distribution in Sudan was: 23.3% of Eurasian ancestry, 4.9% of the East African M1 lineage, and 71.8% of sub-Saharan affiliation. In the sub-Saharan pool, a proportion of 40.5% is represented by the haplogroup L3, the ancestor of the worldwide mtDNA diversity outside Africa. Given the recent interest in the alternative routes for Out-of-Africa migration(s) (Levant versus Southern), these L3 Sudan haplotypes will contribute information to shed light on this issue.

P-98 Genetic Profile in Annobon Population (Equatorial Guinea)

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The aim of this study is to characterise genetically the Annobon Island population to compare it with other ethnic groups from Equatorial Guinea. Annabon Island is volcanic, with an area of 17 km2 and 5008 inhabitants (according to the 2001 census). Haplotype frequencies for sixteen Y chromosomal STR loci, included in the Y-FilerTM kit, were determined in 31 unrelated healthy males. 23 different haplotypes of Y chromosome were identified and 17 were unique. Haplotype diversity was 0.9785. Allele frequencies for fifteen STR loci, included in the AmpFlSTR? IdentifilerTM kit, were analysed in 69 unrelated healthy individuals. For all polymorphisms, the observed genotype frequencies are in good agreement with the expected distribution under Hardy-Weimberg law. From a forensic point of view the heterozygosity value, power of discrimination and the a priori chance exclusion value were calculated to be >0.9999, >0.9999 and 0.9999995 respectively. The population data obtained for these loci were compared with the Fang and Bubi population from Equatorial Guinea, previously published by our group.

P-99 Haplotypes of mtDNA Control Region in Yao Ethnic from China

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Sequence polymorphism of control region of mitochondrial DNA was analyzed in a sample of 105 unrelated Yao ethnic individuals living in Guangxi province of China by PCR amplification and direct sequencing. A total of 84 haplotypes resulting from 117polymorphic positions was found. The genetic diversity and discrimination power were 0.9973 and0.9842, respectively.
Keywords: Mitochondrial DNA, haplotype, control region, Yao ethnic of China

P-100 Genetic relationship and ancestry inference on five population groups of South Africa with Y-STRs

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The genetic ancestry of the metropolitan population of Cape Town is highly diverse, being represented by many local native groups and descendants from European, Indian, SE Asian, Indian and Middle East immigrants. The admixture among these generated a local Creole group locally called "coloured". In this work, we present the genetic relationship and inference of paternal ancestry among 5 of these ethnicities: coloured, Indian, Caucasian English and Afrikaans, and the native Xhosa. We genotyped a total of 504 individuals at the 9 loci of the Minimal Haplotype haplotype (DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393). A total of 382 different haplotypes were detected, the highest haplotype diversity was found in the "coloured group" (HD = 0.69) whereas the lowest was present in the Xhosa group (HD = 0.55). The coloured group showed its higher proportion of shared Y-haplotypes with the Caucasian group (16%) and the lowest with the Xhosa (0.9 %). A Factorial Component Analysis shows a similar result where the position of the coloured individuals overlaps or is closer to the European individuals. These results indicate the predominant European paternal ancestry of the local Creole community of Cape Town.

P-101 Multiplex PCR of three dinucleotide markers in the HLA Class I Region:

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Three dinucleotide marker systems located in the HLA Class I Region (D6S2792 and D6S273 near the HLA-B and HLA-C locus, D6S2972 near the HLA-A locus) were investigated in a population sample of 290 healthy, unrelated Caucasoid individuals. The primers for the multiplex-PCR were chosen according to Gourraud et al. 2004. The forward (D6S2792, D6S2972) or reverse primers (D6S273) were labelled with a fluorescent dye (6-FAM, VIC, NED) to allow detection by capillary electrophoresis on an ABI Prism 3130 Genetic Analyser. The alleles were assigned by comparison with sequenced reference samples, which contained the most common alleles, using the Genemapper software (Applied Biosystems). At the D6S2792 locus 14 different (AC)n alleles (size range: 97-123 bp, observed heterozygosity 0.86), at the D6S273 locus 8 (GT)n alleles (size range: 128-142 bp, observed heterozygosity 0.75),and at the D6S2972 locus 13 different (AC)n alleles (size range: 120-152 bp, observed heterozygosity 0.82) have been found. More data on population statistics and statistic parameters will be presented. Due to the high degree of polymorphism these loci can be used as additional markers for genetic identity applications. A possible linkage disequilibrium between some alleles, however, has to be considered. Gourraud PA et al. 2004, Tissue Antigens 64: 543-555

P-102 Haplogroup H sublineages with mitochondrial SNPs

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From previous studies sequencing HVI and HVII control regions, the most common European lineage (haplogroup H) represents more than 50% of South Portuguese population samples, which are poorly resolved for forensic casework. Mitochondrial DNA has some informative polymorphisms at coding-region positions, which complement sequencing of hipervariable regions. To detect the frequency of sublineages in 36 haplogroup H samples typed by sequencing methods, we have studied seven mitochondrial DNA coding region SNPs – 3010, 3915, 3992, 4336, 4769, 4793 e 6776, by minisequencing reaction using SNaPshot methodology (Quintáns et al, Forensic Sci. Int., 2004). Even though all samples were not subtyped with these seven mtDNA SNPs, several sublineages of haplogroup H were obtained. In South Portuguese population, the most frequent sublineages were H1(3010A), H2(4769A) and H3(6776C). These results are in agreement with other Portuguese and Spanish population studies. H7(4793G) haplogroup was also detected in our study, although it is rare in some Spanish populations. As more than 50% of our samples belong to haplogroup H, the SNaPshot methodology provides a simple, rapid and informative method to detect sublineages of haplogroup H.

P-103 Study of MtDNA Sequences with Length Heteroplasmy in the Population of Alagoas, Brazil

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Sequencing analysis of the two hypervariable mtDNA regions is used mainly for forensic and anthropological applications. HV1 and HV2 have two citocine segments (np16184-16193 and np303-315, respectively), which in accordance with CRS possessed a timine at np16189 an np310. In our population data, 22% (37/167) of mtDNA have a transition T-C at np16189 which was easily identified because of the dramatic decrease in sequence quality beyond the heteroplasmic region. The discrimination of length heteroplasmy in HV2 is significantly more difficult since heteroplasmic ratios vary considerably between individuals. However, 11% (19/167) of the mtDNA sequences were considered to be heteroplasmic for HV2. The 44 mtDNA sequences with length heteroplasmy were resequencied with internal primers of Imaizumi et al. (2002) and allocated to specific mtDNA haplogroups using HV1/2 motifs. The classification into haplogroups showed that 16 mtDNA sequences belong to the Native-american haplogroup B and three, two and one sequences to the haplogroups C, A and D, respectively. The African haplogroups are represented by six sequences belonged to the haplogroup L1C, five to L2a and seven to L3. Additionally, three and one sequences belong to the European haplogroups H and U, respectively. The weighty network displayed little reticulation.

P-104 Polymorphism of Five STR Markers Linked of X-Chromosome in the Population of Alagoas, Brazil

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In forensic science, X-chromosomal short tandem repeats (ChrX STRs) bear the potential to efficiently complement the analysis of other genetic markers, especially in complex cases of kinship testing and in paternity testing, when the disputed child is a girl. One of the advantages of ChrX typing is that in men, it reveals the haplotype directly. The allele frequencies for five ChrX STRs (DXS10079, DXS10074, DXS10075, DXS7424 and DXS101) were determined in a population of 90 unrelated males and 74 females living in the state of Alagoas, Brazil, by multiplex PCR and subsequent automated fluorescent detection (ABI 310). For each locus, allele frequencies were calculated using the Arlequin software ver. 3.11. The allele frequencies of the five X-STR ranged from 0.004 to 0.328 and the most frequent alleles were 20, 18, 17, 15 and 24 for the markers DXS10079, DXS10074, DXS10075, DXS7424 and DXS101, respectively. None haplotype was found more than once in our data set. The power of discrimination ranged from 0.782 to 0.888 (male) and from 0.921 to 0.977 (female). The combined mean exclusion chance in trios involving daughters was 0.9998 and the polymorphism information content ranged from 0.751 to 0.878. Significant deviations from the HWE were not established.

P-105 Population study of three miniSTR loci in Veneto (Italy)

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New mini-short tandem repeat (mini-STR) loci that produce small amplicon size (less than 125 bp) have been developed and characterized to improve the forensic analysis of highly degraded DNA specimens. Three of these new markers (D10S1248, D2S441 and D22S1045) have recently been recommended by the European DNA community as new core loci for forensic testing [1,2]. This study investigated allele frequencies and forensic parameters of these three mini-STRs in a sample of 198 unrelated subjects born in Veneto, Italy, using a protocol involving one triplex. The loci showed no significant deviations from Hardy-Weinberg equilibrium, the observed heterozigosity was > 0.7, the combined power of discrimination and the combined power of exclusion were of 0.9990 and 0.8490 respectively. [1] Gill, P., Fereday, L., Morling, N., Schneider, P.M. (2006) The evolution of DNA databases-recommendations for new European loci. Forensic Sci. Int. 156:242-244. [2] Gill, P., Fereday, L., Morling, N., Schneider, P.M. (2006) "Letter to the Editor: New multiplexes for Europe – Amendments and clarification of strategic development." Forensic Sci. Int .163:155-157.

P-106 Paternity investigation analysis in artificial mother-deficient cases using STR loci of Cofiler/Profiler Plus™ and Identifiler™ Kit in the Brazilian population

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This study addresses the issues of analysis of only the father and the child, in the absence of the mother in paternity investigations, considering the increase of these types of cases that are solicited in laboratories in Brazil. Two hundred and fifty-nine paternity investigations composed of mother, child and the alleged father were analysed, as well as the same 259 cases, in which the results of the mother were removed, and only the results of the alleged father and child were analyzed, using the AmpFlSTR Cofiler™/Profiler plus™ Kit and Identifiler™Kit. Paternity index (PI) was calculated using population data on STR allele frequencies for the Brazilian population. Using the AmpFlSTR Cofiler™/ Profiler Plus™ kit, the total 139 cases analyzed were as follow: a) for the 85 non-exclusion cases the average PI was 37,260,561.78, b) in the case of artificial mother-deficient cases, the average PI was 124,730.30, c) in 54 cases exclusions in 12 to 4 loci were found and d) when artificial mother-deficient cases were simulated, exclusions were found in 11 to 2 loci. Of the total 120 cases analyzed using the Identifiler™ Kit: a) in the 85 non-exclusion cases, the average PI was 14,314,531,006.54, b) in the case of artificial mother-deficient cases, the average PI was 24,350,873.41, c) in 35 cases, exclusions in 13 to 6 loci were found and d) when artificial mother-deficient cases were simulated, exclusions in 10 to 3 loci were found.

P-107 Population and mutation analysis of 7 Y-STR loci from São Paulo city (Brazil). The forensic implications of double alleles.

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The haplotypes of seven Y-chromosome STR loci (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393) were determined in a sample of 634 healthy Brazilian males (190 adult individuals and 222 pairs of fathers and respective sons), all 412 adults (whose data were used for the estimation of haplotype and individual gene frequencies) were unrelated and all 222 investigated father-son pairs included in the study had their biological relationship confirmed using the typing results of autosomal STRs (LR>10,000). A total of 264 different 7-loci haplotypes were identified, 210 of which were unique. The most frequent haplotype (14/13/29/24/11/13/13) was detected in 31 instances, occurring with a frequency of 7.52%. In the 222 father/son pairs and among 1.554 allele transfers, six mutations were observed, taking place with an average overall rate of 3.86 x 10-3 per locus. In our sample of father/son pairs a haplotype with double peaks at the DYS389I locus and another with double peaks at DYS389I, DYS389II, and DYS439 were detected in both fathers and sons. (LIM-HC-FMUSP-Brazil)

P-108 AMOVA for large population samples

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Since the Y-Chromosome Haplotype Reference Database (YHRD) [1] is continuously growing (current release 21 consists of 51,253 haplotypes in 477 populations), there is a need for improved population analysis methods to compute larger population samples (>> 1000 individuals) as well as larger sets of populations (> 20 populations). In this poster we announce a new implemented program for Analysis of Molecular Variance (AMOVA) [2,3] of Y-chromosomal STR (short tandem repeat) haplotypes, which is up to 16 times faster than other software and not limited to small sets of populations. To reach such a performance gain, we had to exhaustively optimize the brut-force algorithm regarding the following points: (i) Use advanced vector instructions of modern CPUs through compiler intrinsics [4] or the assembler programming language [5], (ii) avoid redundant calculation of haplotype distances, (iii) avoid redundant calculation of the total variance, (iv) use pre-calculated integer squares and square roots, (v) randomization without copying haplotypes. For evaluation of this program, we analyzed a dataset of 38,812 haplotypes (9 STR loci) within 291 populations and 21 population groups [1]. We made this program online available enabling the user to quickly perform AMOVA calculations using spreadsheet files together with population samples from the YHRD.
References: [1] S. Willuweit, L. Roewer (2007) Y chromosome haplotype reference database (YHRD): Update FSI: Genetics in press. [2] Excoffier L, Smouse PE, Quattro JM. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131(2):479-91. [3] Roewer L, Kayser M, Dieltjes P, Nagy M, Bakker E, Krawczak M, de Knijff P (1997) Analysis of molecular variance (AMOVA) of Y-chromosome-specific microsatellites in two closely related human populations. Hum Mol Genet. 5(7):1029-33. [4] GNU GCC 4.1 Manual (http://gcc.gnu.org/onlinedocs/gcc-4.1.2/gcc/X86-Built_002din-Functions.html) [5] Bartlett J (2004) Programming from the Ground Up. Bartlett Publishing Group ISBN 0975283847-

P-109 Genetic characterisation of 6 miniSTR loci in an Italian population sample

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In the forensic practise, it is well known that DNA recovered from particular forensic specimens may sometimes show incomplete results in terms of partial genetic profiles with loss of the larger-sized STR loci, PCR inhibition due to co-extracted contaminants or with amplification artefacts as allelic imbalance and allele drop-out. These results are generally referred to the DNA degradation and chemical modification of the genetic material occurring in the forensic sample exposed to the environmental conditions. In order to recover the genetic information from these degraded forensic samples, the most widely used approach is the reduction of the size of the PCR products, setting up multiplex PCR reactions with primers selected very close to the STR polymorphic regions. These reduced STRs, defined miniSTRs, have been developed either redesigning CODIS STR loci primer sets or selecting new STR candidates from public genome databases. We selected six new miniSTR loci (namely D1S1677, D2S441, D4S2364, D10S1248, D14S1434 and D22S1045) to analyse 100 unrelated northern-western Italian individuals and 20 family trios with paternity confirmed with autosomic STRs. No statistically significant differences were seen in the allelic distributions. The forensic usefulness of the selected miniSTRs was verified by analysing different bone samples from skeletal remains

P-110 Autosomal Variation in Subsaharan African Populations: Report of New Alleles

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With the aim to contribute to a better knowledge of the sub-Saharan populations, a general survey of the genetic polymorphism of autosomal STRs was carried out. The study encompasses a total of 18 STRs (15 belonging to the AmpFLSTR Identifiler Kit TM) and three additional ones (HUM F13B, CD4 and LPL). Up to 6 population groups were analysed: Bamileke, Fon, Tutsi, Chaga, Sidama and Antandroy (from Cameroon, Benin, Rwanda, Tanzania, Etiopía and Madagascar, respectively). After the screening of 13,744 alleles, six new variants, not previously described in the literature, have been found. After DNA-re-extraction and subsequent typing of these variants, the results were always concordant, with no exception. Alleles were characterized according to their size/ electrophoretic mobility using the ABI PRISM R 3100 Genetic Analyzer, and tentatively named as CSF1PO* 13.1, D3S1358* 16.1, D2S1338*19.2, D2S1338*20.2, D2S1338*21.2 and VWA*19.2. Population connotations and genetic significance are also discussed.

P-111 Y chromosome J2 subtyping in an Italian sample: population and forensic implications.

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Background and purpose: In the recent years the Y chromosome genealogy has been refined by a number of newly discovered SNPs. The non-random distribution of the Y chromosome lineages world-wide makes fundamental the dissection and characterisation of haplogroups associated with specific geographic areas. In Southern Europe the haplogroup J2, as defined by the M172 marker, can reach frequencies up to 35%, making the dissection of such lineage critical for population studies. Here we present a study on J2 chromosomes from the Italian peninsula. Populations and forensic implications are discussed. Method: A total of 900 individuals were previously genotyped for a number of SNPs, including M172 (Capelli et al, in press, Onofri et al, Ferri et al). More than 200 of these have been now genotyped for 7 SNPs within the J2 lineage using a multiplex SNaPshot approach. Results: The following lineages were tested and identified in the Italian sample screened for J2 sublineages: J2a1a, J2a1b*(xJ2a1b1), J2a1b1, J2b*, J2*(xJ2a1a,J2a1b*,J2a1c,J2a1d,J2a1e,J2b*). Conclusion: The different distribution of the various lineages in different geographic areas probably reflects different historical demographic events and points to differential Y chromosome haplotype distribution, with implication for forensic application of this genetic marker.

P-112 NRY Haplogroups Distribution in Sub-Saharan Africa and Bantu Expansion

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PhD. María Regueiro
Professor Jose Luis B. Caeiro
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The general consensus among the scientific community is that the Bantu expansion, starting from Central West Africa, entailed several waves along two major routes: a southwest trajectory, and a more complex arc comprised of an easterly transversal component towards Central Africa and a later progression through a southeastern corridor. This scenario is supported by results from genetic markers and archeological and linguistic evidence. We have investigated the male component of this demic expansion, by analyzing informative biallelic markers (SNPs: Single nucleotide polymorphisms) on the nonrecombining region of the Y chromosome (NRY). The 434 samples analysed were obtained in seven sub-Saharan African populations from Benin, Cameroon, Rwanda, Kenya and Tanzania. The overwhelming presence of M2 haplotypes in the Central West African populations analyzed in this study reinforces the role of this area as the core of the Bantu expansion. On the other hand, the clinal distribution towards the East of typical Bantu haplotypes, supports a mass dispersal of Bantu people replacing previous inhabitants, which corresponds with the eastern transversal expansion component above mentioned.

P-113 Population data for six X-Chromosome STR loci in a Rio de Janeiro (Brazil) sample: Usefulness in forensic casework

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This study presents data for the X-chromosome STR loci DXS7133, DXS7424, DXS8378, DXS6807, DXS7423 and DXS8377. In order to establish a database, unrelated individuals (males and females) from Rio de Janeiro were typed for the above loci. No significant differences were observed between allele frequencies in male and female samples (non-differentiation exact P values = 0.156). Hardy-Weinberg equilibrium was tested in the female sample and no significant deviations were found. All six markers have shown to be highly polymorphic in our sample, with gene diversities varying between 0.6797, for DXS7133, and 0.9260 for DXS8377. Pairwise linkage disequilibrium analysis did not allow discharging a possible association between DXS7133 and DXS7424 alleles in Rio de Janeiro population. Parameters of forensic interest, like PDM, PDF. Hetobs, Hetexp, were calculated for each X-STR. The high discrimination power estimated in both male and females, as well as mean exclusion chance in father/daughter duos and in father/mother/daughter trios, demonstrates the usefulness of these six markers in forensic investigation. Keywords: X-chromosome, STR, database, Rio de Janeiro, Brazil *Corresponding author. Rua São Francisco Xavier, 524, 20550-013, Rio de Janeiro, Brasil. Tel.: 55 21 25877902, Fax: 55 21 25877662 E-mail address: elizeufc@hotmail.com (Elizeu F de Carvalho)

P-114 Analysis of 8 Y-Chromosome STR Loci in Population from Alicante (Spain)

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ANALYSIS OF 8 Y-CHROMOSOME STR LOCI IN POPULATION FROM ALICANTE (SPAIN) Introduction:The allelic frequencies and the haplotype distribution were studied in 8 Y-Chromosome STR loci: DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, and DYS 385. Data was obtained from 104 unrelated autochthonous subjects from the province of Alicante, located in the Southeast of Spain. Method: Buccal swabs samples were obtained from 100 autochthonous volunteers. DNA was isolated by the DNA IQTM System (Promega) and approximately 1 ng of genomic DNA was amplified. Genetic typing was performed using ABI 3100 Genetic Analyzer (Applied Biosystems). Allele and haplotype frequencies were estimated by gene counting. Results: No new allele were identified in the analysed loci. The most frequent allele found were the following: DYS19 (14, freq. 0.712), DYS389I (13, 0.567), DYS389II (29, 0.423), DYS390 (24, 0.510) DYS391 (11, 0.490), DYS392 (13, 0.538), DYS393 (13, 0.692), DYS385 (11,14, 0.337). A total of 84 haplotypes were observed, among which 71 were unique Conclusion: The obtained data will contribute to further progress in the studies on chromosome Y in Spain. Comparison with other Spanish populations showed some differences which were analysed.

P-115 The distribution of Y-Chromosomal STRs in Dominican Population

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We have studied the distribution of Y chromosomal STRs and established a database with in population samples from Dominican Republic by analyzing male-specific markers. The population samples were collected in by Laboratorio Clinico Lic. Patria Rivas and this population are truly representative of the entire country. We analyzed 12 STRs loci included in PowerPlex Y (Promega Corporation).

P-116 The distribution of allele frequencies of 15 STRs in Dominican Population

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The most effective tools for individual and population genetic characterization that we'll use today is STRs. This work presents the results of a population study of 15 STRs loci included in PowerPlex 16 (Promega Corporation) that has the aim of creating a local database. Buccal swabs, or blood were obtained about two thousands unrelated individuals, who were born in Dominican Republic and lived there for at least two generations.

P-117 Genetic Analisys of Libyan Population Using Y STR Markers and mtDNA Control Region

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GENETIC ANALISYS OF LIBYAN POPULATION USING Y STR MARKERS AND mtDNA CONTROL REGION Abdulwahab A. Al-Deib1, Turán P. Urmenyi1, Edson Rondinelli1,2, Rosane Silva1, Rodrigo Moura-Neto3,4 1Instituto de Biofísica Carlos Chagas F., UFRJ, RJ, 2Faculdade de Medicina, UFRJ, 3Departamento de Genética, IB, UFRJ, 4Instituto de Pesquisa e Perícia em Genética Forense, PCERJ, Rio de Janeiro, Brazil Background and purpose: Libya is a North Africa country, with Arab culture since the seventh century, nowadays there are a mixture of populations, including the Native Berbers and Africans. In order to generate a database on Libyans, we undertook a preliminary genetic background study on actually Libyan population. Method: We have surveyed a preliminary genetic analysis on Libyan population, using Y STR markers and the control region of mtDNA. Using the AmpFlSTR Y Filer 17 STR systems from Applied Biosystems, we analyzed 26 unrelated males, and we have also sequenced the mtDNA control region (HVI and HVII) from 49 unrelated persons. Results and Conclusion: The Y STR profiles have shown four unique haplotypes for Libya (15%), and duplication (15/16) at DYS19 locus, that was confirmed by the presence at father and son DNA. We have compared our results to YHRD worldwide database, using the minimal haplotype (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393). The majority of haplotypes came from Europe (61%), and there are unique profiles from Africa and Asia. The control mtDNA region analysis had shown a pattern compatible with North Africa and Middle-East profiles. All 49 mtDNA sequences were found homology from Morocco to Syria. We are now performing a more extensive analysis with both lineage markers, and comparing to other reference populations to infer amalgamation and genetic distance. Supported by CNPq (Brazilian Council for Scientific and Technological Development) FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro).

P-118 Allele distribution of three X-chromosome STR loci in an Antioquian population sample

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The X linked short tandem repeats (STR) markers have proven to be very useful tools for paternity testing when the disputed child is female. The aim of this study was to describe the polymorphism of three X-chromosomal STR loci (DXS6797, DXS6800 and HPRTB) in an Antioquian (Colombian) population sample. PCR products were separated in 4% acrylamide-bis-acrylamide denaturing gels followed by silver staining. Allele size determination and genotyping were performed according to recommendations of the DNA Commission of the International Society of Forensic Genetic using the allelic ladder manufactured at home and based on DNA controls including K562 and 9947A (Promega). Gene frequencies were calculated using ARLEQUIN version 3.11. Population genetic data were obtained by analyzing 127 - 400 unrelated males and 135 unrelated females from Antioquian (Colombian) population. Distribution of the allele frequencies of these systems for Antioquia population is similar to the European populations.

P-119 Diversity of 17-locus Y-STR haplotypes in Upper (Southern) Egyptians

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The analysis of multiple Y-chromosomal STR loci has proven to be highly informative in forensic situations where male-specific profiles are needed and also provides information on geographical or ethnic affiliation. Population databases utilizing these markers have grown rapidly over the past few years, but are still lacking in Middle Eastern countries including Egypt. To address this, 17 Y-STR loci included in the AmpFlSTR® Y filer PCR Amplification kit (contains Extended European haplotype loci plus the highly polymorphic markers DYS437, DYS448, DYS456, DYS458, DYS635 and Y-GATA-H4) were analysed in a sample of 208 males from south (Upper) Egypt. A total of 197 haplotypes were identified among which 194 were unique (97%) and three were found twice each. The 17 loci gave a discriminating power of 0.9998. DYS458 showed highest diversity as a single locus marker (h=0.869) along with a high frequency of microvariants and novel alleles (22% of the sample). Other loci in the set revealed duplicate and null alleles which are being further characterized. Comparative analysis with Y-STR datasets of relevant populations and the submission of haplotypes to the Y Haplotype Reference Database will be undertaken.

P-120 Regional Patterns of Genetic Admixture in South America

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The colonization histories of Argentina, Brazil and Colombia (South America) have had main contributions from Amerindian, European and African populations. Despite its similar beginning, this peopling process developed in distinct ways. Several studies using the CODIS STRs have already taken place, as these are consolidated in forensic practice and provide a rich data source for population studies. Aiming to compare these populations' constitution at the present time, admixture and genetic distance analysis for five geographic regions in Brazil, five in Argentina and four in Colombia have been performed. Frequency data of the 13 CODIS STRs was compiled from already typed 43397 Brazilian individuals, 2248 Argentineans and 4150 Colombians. The European contribution predominates on these three countries and mainly in Brazil, but not in the North Colombian Pacific Coast and Northwest Argentina. The African presence in Brazil and Colombia was significant, meanwhile the lowest Amerindian contribution was observed in Brazil, reflecting the heterogeneous assimilation of these parental groups. The distribution pattern of Amerindian contribution in these countries points out that, in the regions of major economic development by colonization time, the Amerindian contribution has been smaller than elsewhere, agreeing with historical and demographic data.

P-121 Genetic characteristics of 22 Y-STRs in Koreans: Atypical alleles, deletions in the DYS385 flanking region, and null alleles associated with AZFc microdeletions

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To construct a Korean Y-chromosomal STR database for 22 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), 708 DNA samples from unrelated Korean males were analyzed using three multiplex PCR systems. During analysis, atypical alleles were observed and characterized by sequence analysis. At DYS385, one or two repeat unit discrepancies were observed in eight samples according to the primer binding sites. Sequence analysis revealed deletion mutations at two sites in the upstream of the DYS385 core repeat units. At DYS448, a null allele was observed in six individuals that resulted from the deletion of the entire DYS448 locus. The deletion pattern was further characterized by arrangement analysis of STSs aligning along the AZFc region. Results showed that the deletion patterns of DYS464 as well as DYS448 are involved in AZFc rearrangement. In haplotype analysis for the 22 Y-STRs, a total of 693 different haplotypes were observed with overall haplotype diversity of 0.9999, and of these, 680 haplotypes were unique. The combined haplotypes of DYS447, DYS458, DYS635, GATA H4.1, and SWGDAM Y-STR loci was comparable to haplotypes of 17 loci in the AmpFlSTR® Yfiler™ kit.

P-122 Analysis of mtDNA HVIII length heteroplasmy

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Length heteroplasmy has been observed in homopolymeric tract of cytosines (nt 568-573) of human mitochondrial HVIII region. We analyzed the HVIII length heteroplasmy in a Japanese population using size-based PCR product separation by electrophoresis. DNA was extracted from peripheral blood samples of 437 unrelated Japanese individuals living in Gifu Prefecture (central region of Japan). All samples were amplified using primer pair F182/R619 (Bini et al. 2003) and directly sequenced for the HVIII region. Samples that showed length heteroplasmy were amplified a second time using the same primer pair except that R619 was fluorescently labelled with Texas Red or 6-FAM. The fluorescently labelled PCR products were then digested with Hae III restriction endonuclease and separated by electrophoresis on a SQ5500-S DNA Sequencer (Hitachi) or an ABI 310 Genetic Analyzer (Applied Biosystemes). In this study, samples from 19 of 437 individuals (4.3%) showed HVIII length heteroplasmy. Every heteroplasmic sample was composed predominantly of the 9, 10 or 11 cytosines in the homopolymeric tract. In order to identify each length variant within the heteroplasmic sample, we carried out cloning analysis.

P-123 Analysis of 16 Y-chromosomal STRs in an African descent sample population of Chocó (Colombia)

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Whit this work we stablished a data base of Y-STR and some parameters of forensic importance. We studied 16 Y-STR (DYS19, DYS385, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS460, DYS461, GATA-A10, GATA-H4 and DYS635) in a population of 298 unrelated males African descent of Chocó (Colombia). PCR products were separated in 4% acrylamide-bis-acrylamide denaturing gels followed by silver staining. Allele size determination and genotyping were performed according to recommendations of the DNA Commission of the International Society of Forensic Genetic using the allelic ladder manufactured at home. Gene frequencies, gene and haplotype diversity were calculated using ARLEQUIN version 3.11. A total of 257 haplotypes were identified using the present set of Y-STR markers, of which 224 were represented only once in the database. 26 hapotypes were presents two times, six haplotypes were presents in three individuals and one haplotype in four men. The haplotype diversity was 0.9987 +/- 0.0004. By combining the allelic states of the 16 Y-chromosomal STRs we could construct highly informative haplotypes that allowed the discrimination of 86.2% of the samples tested. This approach represents a very powerful tool for individual identification and paternity testing in forensic genetic.

P-124 Results of Y-SNP Typing in three different populations

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The Y-STRs are well established in forensic routine case work since several years. The usefulness of these markers is described in many papers and widely accepted. As additional possible polymorphisms the Y-SNPs are in focus of the forensic community. More than 200 SNPs are compiled in the phylogenetic tree and reported by the Y-Chromosome Consortium. The potential of these markers is the detection of additional information about the haplogroups which may reveal information about the geographic origin of the paternal lineage. In our study samples of 400 unrelated males were typed in three populations collected in Leipzig (Germany), Wladiwostok (Russia), and Wroclaw (Poland). The samples have been typed previously with the minimal and extended core set of Y-STRs. A set of Y-SNPs (29 markers) published by Brion et al. (Electrophoresis 2005, 26, 4411–4420) was used. The genotyping was performed with the single base extension reaction (SNaPshotTM, Applied Biosystems) and detection by capillary electrophoresis using an ABI310 or ABI3100 genetic analyzer. For all samples, haplotypes could be assigned, and their frequencies followed the expected geographic distribution. Our data confirm that using the 29 Y-SNPs it's possible to detect the major haplogroups. These may help to get additional information in the context with the Y-STR typing results.

P-125 Minisatellite variant repeat analysis shows Asian population specificity in Thai, Chinese and Japanese

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Background and purpose: Minisatellite MS32 (D1S8) shows vast diversity by digital minisatellite variant repeat analysis using PCR (MVR-PCR). Virtually all of the alleles in the populations surveyed were found to be different. However, distinct alleles exhibit significant similarities in repeat organization. Method: We mapped MS32 alleles in Thai and Han Chinese and the structural similarities of these and other Asian—as well as Caucasian and African—populations, were investigated by heuristic dot-matrix algorithms. Results: Slightly more than half of the mapped Thai alleles could be grouped of which 35% were predominantly related to only Thai alleles and 54% to both Thai and other Asian alleles. Only 6% were shared by Caucasian alleles and no Thai alleles possessed similar motifs to African alleles. The results obtained from Chinese alleles demonstrate a close resemblance to Thais, while Japanese alleles display a strong tendency (72%) of intrapopulation similarity in comparison to other Asian alleles. Conclusion: Some MS32 alleles in Thai and Chinese populations also reflect ethnicity in their repeat arrays. As opposed to mitochondrial DNA and Y-specific STR which are vulnerable to sexual limitations, MS32 is indiscriminate in its ability to determine ethnicity and individual identification, making it an invaluable tool.

P-126 Analysis of mtDNA control region using mitoSEQr™ resequencing system and its forensic application

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The analysis of mitochondrial DNA (mtDNA) has become a powerful tool for forensic investigations. The presence of thousands of copies per cell make it relatively convenient for the genetic analysis of highly degraded material where DNA typing with nuclear markers would not be successful, such as bones, teeth, hair shafts, etc. The mitoSEQr™ resequencing systems, mitoALL™ and mitoCR™ (Applied Biosystems), are designed to identify sequence variations in the control region or the entire human mitochondrial genome quickly and easily. In the present study, we analysed mtDNA control region using mitoALL™ resequencing system and its forensic application was also examined. Blood samples were obtained from 100 unrelated healthy Japanese individuals living in Kanagawa. DNA was extracted using Quick Gene-800 (FUJIFILM, Japan). PCR amplification was performed with mitoALL™ primer sets (RSA001145133, RSA001250252, RSA001250250, RSA001145161, RSA001250241) according to the user's manual. The sequencing analysis was done using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with M13 forward and reverse primer. The PCR products were electrophoresed by ABI 310 Genetic Analyzer. The polymorphic profiles in the present study were essentially the same as those obtained from Japanese former studies. From the present study, the mitoSEQr™ resequencing system was applicable to routine casework.

P-127 Analysis of 8 STR of X-chomosome in two Italian regions (Umbria and Sardinia)

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Eight X-chromosome STRs were investigated in two groups of individuals from two Italian regions, Umbria and Sardinia. These two regions have had a very different history. Umbria, being in the centre of Italy and not having defensive natural barriers, in the course of the centuries it has been object of many invasions and appropriations by foreign people, on the contrary, Sardinia, being an island in the centre of the Mediterranean sea, it has endured a few number of foreign invasions. The ChrX markers were amplified in a pentaplex (DXS6789, DXS7423, DXS6807, DXS101, DXS8377) and a triplex (DXS10074, DXS10075, DXS10079) that represents an haplotype. The DNA was extracted from 200 unrelated blood samples using the QIAmp DNA Minikit (Qiagen™). Amplifications products were detected on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems), using the same dye labels, run conditions, standard (GeneScan 500 Liz) and matrix file of AmpFlSTRIdentifiler. Statistical analyses for all the loci and intergroup comparison between the two regional groups of individuals were performed.

P-128 Study on polymorphism at DCP1 locus in three populations in China

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An Alu sequence's insertion/deletion (I/D) located at intron 16 of dipeptidyl carboxypeptidase 1 (DCP1) gene, which involves in the regulation of blood pressure in renin-angiotensin system, exhibits higher polymorphism. However, population data in China has been rarely reported. In present study, the I/D polymorphic information at the DCP1 locus is investigated in three Chinese nationalities. In addition, the most parameters in forensic application are calculated. Genomic DNAs from 101 unrelated individuals from Han, 98 from Uygur and 112 from Tibetan populations in China were amplified. PCR products were separated and typed by polyacrylamide gel electrophoresis and silver staining techniques. The results of genotype frequency and other polymorphic information in forensic application are shown in Table 1. The genotype distribution is consistent with Hardy-Weinberg equilibrium. Table 1. The insertion/deletion polymorphism at the DCP1 locus in three Chinese populations. population Genotype Allele DP EP II ID DD I D Han 0.428 0.253 0.319 0.5550.4450.6510.186 Uygur0.280 0.460 0.260 0.5100.490 0.6420.187 Tibetan0.294 0.329 0.377 0.4590.5410.6630.187 The Alu I/D system at DCP1 locus demonstrates higher polymorphism in the investigated three populations in China and may be applied in forensic and human genetics fields.

P-129 A Study on Short Tandem Repear ACTBP2 (SE33) in an Sicilian Population Sample

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Human ß-actin pseudogene is currently investigated for its applications in real casework and population genetics. In this study a total of 250 unrelated members of the sicilian population were analysed. Forensically relevant parameters were calculated. The distributions of the genotypes were in good agreement with Hardy-Weinberg equilibrium. The power of discrimination (Pd) of this single locus was 0.99691 with an observed heterozygosity of 0.93081, making the SE33 a good alternative marker for individual identification and paternity testing. Results confirmed ACTPB2 potential to support genetics analysis in closed population and in forensic casework involving relatives. Reliability of capillary electrophoresis separation and accuracy in microvariant allelic designation are also discussed.

P-130 Diversity and geographic heterogeneity of Y-chromosomal STR haplotypes in Belarus

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Nine loci defining so-called minimal haplotypes and nine additional Y-chromosomal short tandem repeats (Y-STRs) were analysed in the Belarusian population samples from Brest, Homiel', Hrodna, Mahilioú and Viciebsk. The haplotype diversity for the minimal Y-STR set ranged from 0.9891 in Viciebsk to 0.9977 in Homiel', indicating presence of identical haplotypes among unrelated males, which could be resolved with the additional nine Y-STRs in all but one population. Among 196 unrelated males, 183 different haplotypes were identified and 173 of them were observed only once. The highest gene diversities were found in two-locus systems DYS385 and YCAII (H = 0.83 and 0.72, respectively). The 18-locus haplotype diversity was 0.9992. DYS385, DYS389 and GATA H4.1 were the most valuable markers in discrimination of similar haplotypes, whereas DYS388, DYS391, DYS426 and DYS438 did not have any impact on the haplotype discrimination power. Analysis of molecular variance (AMOVA) revealed regional stratification within Belarus. Comparison of the results with data for Belarusians residing in northeastern Poland excludes the use of the Y-STR database for Polish Belarusians as representative for the Belarusian population as a whole in forensic practice.

P-131 Molecular characterisation and population genetics of the DYS458 .2 allelic variant.

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Background and purpose We recently found a number of intermediate DYS458 alleles, indicated as .2. This allelic variant is distributed in several populations, but currently no information is available regarding the molecular structure and the genealogical correlation of chromosomes with this variant. The molecular characterisation of such allele, its worldwide distribution and the correlated evolutionary history are the subject of the present paper. Method: More than 100 chromosomes bearing the DYS458 .2 allele were screened for both Y-STRs and SNPs. Literature data provided additional 30 chromosomes. Different alleles associated with the .2 variant were sequenced for both strands. Haplogroup identification was based on SNPs analysis performed by minisequencing. Network microsatellite analysis was conducted using the Network 4.2.0.1 software (www.fluxus-engineering.com). Results: All the screened alleles bearing the .2 variant were characterised by the common repeat sequence structure [GAAA] nAA [GAAA]2. All chromosomes defined by this uncommon allele were derived at the M267 marker and genealogically part of the J1 haplogroup. Conclusion: Molecular and genealogical data are suggestive of a single origin for the .2 variant. Phylogeographic analysis points to either a Middle East or East African origin, but additional data is necessary to clarify this point. Our results suggest that the .2 variants is a stable polymorphism and that it could be used for population studies.

P-132 Y-SNP and Y-STR analysis in a Japanese population

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[Introduction] We investigated haplotype frequency and regional differences among Japanese, with the 14 loci or11 loci of Y-STR and 4 Y-SNPs as biallelic polymorphisms. [Materials and Methods] DNA samples from four areas (33 people living in Gunma prefecture, 25 people in Fukui prefecture, 28 people in Kagoshima prefecture, and 121 people in Tokushima prefecture) were used for this study. On Y-SNP, we detected three lineages (O (M122, P31), D (M174), and C (M216)) which are common types among Japanese. Cycleave PCR Core Kit (Takara Bio Inc.) was used for detecting these lineages. On Y-STR of 3 areas (Gunma, Fukui and Kagoshima), we detected 14 loci which consist of PowerPlex(R) Y (Promega Co.) and YCAII a/b. Data in our previous paper was used on Y- STR data for Tokushima. [Results and Discussion] 186 haplotypes were detected from Y-STR data of 207 Japanese men who don't have any blood relationship with each other. HD value was estimated at 0.9981. HD value was calculated by adding YCAII a/b to minimal haplotype. Y-SNP data showed that the D rate (42%) was the highest in Gunma. But in other areas the O rate was higher than the D rate.

P-133 Contribution to the understanding of the population history of East-Timor (Timor-Leste)

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In East-Timor, the numerous languages are usually assigned to an Austronesian or non-Austronesian (Papuan) family. However, a detailed analysis of the relationship between genes and languages in East Timor is lacking so far. In addition, searching for population substructure is important when considering forensic applications. Following the previous studies with autosomal, mitochondrial and Y-STRs, Y-SNP haplogroup distribution and comparisons of both STRs and SNPs are presented here providing a deeper characterization of the human gene pool of East-Timor. Pairwise Fst distances based on haplotype and haplogroup distributions shows that the general population from East-Timor is significantly differentiated from all the considered neighbouring populations, but the lowest differentiation is obtained from Papuan samples (besides samples from the same geographical provenience as Nusa Tengaras). In East-Timor we detected a total of 10 haplogroups, with M38, K-M9 and RPSY-4 representing more then 2/3. The European R1bP25 is also present (3%). Besides a clear predominance of Papuan assigned haplogroups, the presence of Austronesian Y markers was also evident. When ethnolinguistic groups are considered, not only is it possible to detect several non-differentiation values with reference samples from Pacific islands but AMOVA analyses reveals that geography is better explaining Y-chromosome genetic diversity than linguistics.

P-134 Exploring mitochondrial DNA variation in the Italian Peninsula

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The genetic structure of Italy appears to be mainly shaped by pre-Roman historical events. The studies carried out so far show a major North-South cline, possibly the result of two distinct main demic processes: the first colonisation of the area during the Palaeolithic period and the subsequent Neolithic expansion from the Middle East. However, the demographic contribution of these events is still a matter of debate. We here report mitochondrial DNA (mtDNA) data from nine population groups covering the main Italian regions: Central Liguria (N = 50), East Friuli (N = 51), South Latium (N = 48), Central Marche (N = 53), West Calabria (N = 50), Central Campania (N = 50), South Apulia (N = 53), and two populations from Sicily (East and West Sicily, N = 40 each). Haplogroup frequency spectra indicates clear differences at a regional level for instance, haplogroup J makes-up ~20% in South Apulia (Southeast Italy) but only account for ~2.5% and ~7.5% of the mtDNAs in West and East Sicily (Southwest Italy), respectively. Haplotype sharing among populations is low, which is consistent with limited gene flow along the Italian Peninsula and supports the existence of substantial levels of population stratification. An evaluation of the forensic implications in the observed population stratification will be discussed.

P-135 Polymorphism of four X-chromosomal STR loci in Belarusians and Slovaks

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We have investigated four X-chromosomal STR loci (a tetranucleotide repeat marker HPRTB and trinucleotide repeat systems DXS101, DXS7423 and DXS8377) in population samples of unrelated Belarusian and Slovak males (N = 180 and 116, respectively). Markers were amplified in a multiplex PCR reaction with primers labelled with fluorescent dyes 5-FAM and 5-JOE (dye set F). The separation and detection of PCR products were performed by capillary electrophoresis on the a 3130 Genetic Analyzer (Applied Biosystems), using positive controls (K562 and NA9947A) and the GeneScan-500 ROX internal lane standard. Allele designation was based on comparison with the constructed allelic ladder. We performed statistical analysis estimating power of discrimination for males (PDmale). Our data was compared with European populations from Poland and Germany. The obtained data are useful in forensic practice and they contribute to creation of national X-STR databases in Belarus and Slovakia.

P-136 Sequence variation at 3 X chromosomal short tandem repeats in Caucasian and African populations

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Sequence variation for the X chromosome short tandem repeats (X-STRs) DXS9898, DXS6789 and GATA172D05 was studied in two major population groups, namely Caucasians (Northern Portugal) and Africans (Uganda, Angola and Mozambique). Allele selection in both groups was based on previous population genetic studies. DXS6789 revealed two different subtype sequence polymorphisms: for shorter alleles, less than 17 repeats, results showed a simple composition with the following structure: (TATG)n-(TATC)m. For longer alleles, a constant TATC insertion was observed at the beginning of the variable repeat unit. Other sequence polymorphisms were also encountered at DXS6789 in both groups: alleles identical in size revealed structural variations regarding the TATG/TATC proportion. Africans showed a higher intra allelic variation at this locus than the Caucasian population group. In spite of the hidden sequence variation observed at DXS6789, no exclusive structure type was found for any of the two major groups. For DXS9898 and GATA172D05, no sequence variation was found in or among Africans and Caucasians. The increasing use of X-STRs in population and forensic genetics enhances the importance of analysing repeat motifs and sequence structures of these genetic markers, in order to establish a consistent nomenclature.

P-137 Allele frequencies of five miniSTR loci (D1S1677, D2S441, D4S2364, D10S1248 and D22S1045) in Han population from China

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MiniSTR assays, which is to reduce the size of the PCR products by moving primers as close as possible to the repeat region, produce a higher success rate for genotyping with degraded DNA samples. In the present study, the genetic polymorphisms for D1S1677, D2S441, D4S2364, D10S1248 and D22S1045 five miniSTR loci were investigated with blood samples of 115 unrelated healthy Chinese Han individuals. Each locus was successfully genotyped with the length of the amplicon less than 118bp. 7, 8, 5, 8, 8 alleles and 12, 21, 10, 15, 18 genotypes were detected and no deviation from Hardy-Weinberg equilibrium was observed in the five loci. The cumulative power of exclusion and power of discrimination for the five miniSTR loci were respectively 0.9459 and 0.999953. Nine highly degraded DNA samples were successfully genotyped by miniSTR assays, and the success rate was up to 84%, which was significantly higher than the results from the conventional commercial STR kit, the success rate of which was only 3%. In conclusion, the five miniSTR loci have good polymorphism and have a special value in forensic individual identification and paternity test for highly degraded samples.

P-138 A simulation-based approach to evaluate population stratification in Argentina

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The analysis of STR markers typically used in forensic genetics has shown that substantial level of population stratification exists across different Argentinean populations nevertheless, most of the differences between any pair of urban populations reside in a few markers. In order to determine to what extent there is a real impact in forensic casework, we performed a comparison of combined likelihood ratios (CLRs) of 1,906 genetic profiles from eight different populations from Argentina. CLRs were computed using 15 and 5 Short Tandem Repeats (STRs) profiles and eight different (reference) population frequency databases. A random sampling of individuals in each population was performed in order to generate a mean value and standard deviations for the CLRs of every profile. One-way-ANOVA and a post hoc test (Dunnet) were applied to asses the significance of the differences between the mean CLRs values. ANOVA P-values lower than 0.05 were obtained at different proportions depending on the populations considered. Thus, differences were most notable when comparing CLRs from urban populations with those from Native populations, and appeared to become more important when comparing 5-STRs CLRs. Results are consistent with previous findings on population stratification and support the use of local reference databases in forensic casework.

P-139 Similarities and differencies between three Slavic populations on the basis of allelic frequencies for 15 STR loci

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Allelic frequencies of 15 short tandem repeat (STR) markers (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, VWA, TPOX, D18S51, D5S818 and FGA) were analyzed using AmpFlSTR Identifiler PCR Amplification Kit in Belarusian (N=176) and Slovak (N=164) individuals. No deviation from Hardy-Weinberg equilibrium was found for all loci. These results were compared with data available for northern Poland and Belarusian minority residing in northeastern Poland. Statistically significant differences were observed between Belarusian and Slovak populations and other compared populations. The values of heterozygosity, polymorphic information content (PIC), power of discrimination (PD), power of exclusion (PE), paternity index (PI) and matching probability (pM) were calculated. The combined power of discrimination and cumulative power of exclusion for the 15 studied loci in both populations were greater than 0.9999999999999999 and greater than 0.99999, respectively. The results demonstrated the forensic usefulness of the analyzed loci.

P-140 Results of GeFI's collaborative exercise on three miniSTR (Italy)

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Twelve laboratories of Central and Northern Italy participated in the forensic validation of the so-called "NC-01 triplex", which consists of the three miniSTR D10S1248, D14S1434, D22S1045. The method was published in the Journal of Forensic Science (50:43, 2005) the allelic ladder was kindly supplied by M. Coble. Two blind stains were typed as a quality control by each lab. More than 1,000 subjects from seven Italian regions were typed. Hardy-Weinberg equilibrium was tested for each locus and each sample using the "exact" test implemented in the software ARLEQUIN. A total of 36 tests were performed, and none was significant of deviation after applying the Bonferroni correction. The allele frequencies were generally consistent with those published for other populations.

P-141 Microgeographic variation of Y-chromosome haplotypes in Italy

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Within an Italian collaborative exercise on the extended haplotype of the Y-chromosome, 1,288 subjects were typed by the AmpFlSTR YFiler Amplification Kit (AB Applied Biosystems) and other 526 were typed by the PowerPlex Y® System (Promega). The sampling scheme included either a "regional" or a "local" recruitment, the first referring to individuals born in the region of the participating lab, the second referring to individuals coming from small villages. Total sample sizes were N = 954 and 860, respectively. A significant decrease of haplotype diversity was found in the local samples. The results may be of interest in forensic applications of the Y-chromosome.

P-142 Genetic Analysys of Libyan Population on Using Y STR Markers and mtDNA Control Region

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Background and purpose: Libya is a North African country with Arab culture since the seventh century nowadays there are a mixture of populations, including the Native Berbers and Africans. In order to generate a database on Libyans, we undertook a preliminary genetic background study on the present Libyan population. Method: We have surveyed a preliminary genetic analysis on Libyan population, using Y STR markers and the control region of mtDNA. Using the AmpFlSTR Y Filer 17 STR systems from Applied Biosystems, we analyzed 26 unrelated males, and we have also sequenced the mtDNA control region (HVI and HVII) from 49 unrelated persons. Results and Conclusion: The Y STR profiles have shown four unique haplotypes for Libya (15%), and duplication (15/16) at DYS19 locus, that was confirmed by its presence in father and son DNA. We have compared our results with YHRD worldwide database, using the minimal haplotype (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393). The majority of haplotypes came from Europe (61%), and there are unique profiles from Africa and Asia. The control mtDNA region analysis has shown a pattern compatible with North Africa and Middle-East profiles. All 49 mtDNA sequences were found homologous with those from Morocco to/and Syria. We are now performing a more extensive analysis with both lineage markers, and comparing to other reference populations to infer amalgamation and genetic distance. Supported by CNPq (Brazilian Council for Scientific and Technological Development) and FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro)

P-143 Genetic characterization of 52 autosomal SNPs in the Portuguese population

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Routine forensic casework constantly challenges genetics labs to implement the use of new genetic markers in order to answer a range of demands from police investigations and the courts. In this context, there has been great interest in Single Nucleotide Polymorphisms (SNPs), especially applied to the analysis of degraded DNA, and therefore SNP sets with equivalent discrimination power to existing STR multiplexes have been developed for identification purposes. Nevertheless, for the Portuguese population there were no available data on autosomal SNPs genetic diversity and population structure. In this study, 52 autosomal SNPs were characterized in a Portuguese population sample using the SNPforID project multiplex assay. Allele frequency estimation, Hardy-Weinberg equilibrium and linkage disequilibrium analyses were performed. From these data the population comparisons made between the three main study regions and statistical parameters of forensic efficiency are presented. Genetic characterization of these 52 SNPs in the Portuguese population constitutes a contribution to the SNPforID population frequency browser (freely available online at http://bioinformatics.cesga.es/snpforid/) and the first step in the implementation of SNPs assays in Portuguese forensic genetics laboratories, establishing a population database for further reliable statistical analyses.

P-144 Allele frequencies of six miniSTR loci in Northern Germany

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MiniSTR have demonstrated to be a powerful tool in forensic casework analysis. The reduction in fragment size leads to an increase in sensitivity that allows for positive typing results even in cases where highly degraded DNA or stains with Low Copy Number DNA are used for DNA-analysis. For almost all common database STRs new primer sets leading to much shorter fragments have been published recently. Additionally, new miniSTR loci have been suggested for forensic purposes. In the present study the six miniSTR-loci D10S1248, D14S1434, D22S1045, D1S1677, D2S441 and D4S2364, originally published by Coble and Butler (Int J For Sci 2005) were typed in a population sample of 150 unrelated adults from Northern Germany. Allele frequencies were determined and HET, PD, PIC and MEC values were calculated. Forensic validation Furthermore critical stains containing only low amounts of DNA (fingerprints, swabs from tools and car wheels etc.) or degraded DNA were typed with the six miniSTRs to test their robustness and sensitivity.

P-145 Genetic characterization of 52 autosomal SNPs in two sub-Saharan African populations

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Population data regarding autosomal Single Nucleotide Polymorphisms (SNPs) variation on African populations are very limited. At the present time only three African populations (Somalia, Mozambique and Nigeria) are genetically characterized and included in the SNPforID browser, a tool for population frequency searches of the 52 SNPs included in the SNPforID forensic identification multiplex. In this work, the aim was to provide enhanced sampling and a more representative coverage of the African continent. We studied two sub-Saharan African populations (Uganda and Angola) using the SNPforID 52-plex. Allele frequencies, Hardy-Weinberg equilibrium and linkage disequilibrium analyses are reported and discussed for both populations. Genetic distances between populations and parameters of forensic interest are also presented.

P-146 Analysis of 11 SNPs of Human mtDNA in Individuals from the North of Portugal: a preliminary study

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Human mitochondrial DNA (mtDNA) has specific features that make its analysis very useful in forensic casework and human population studies. However, the principal limitation associated with mtDNA typing is the low power of discrimination that is obtained when common mitochondrial types are present in two unrelated samples. The analysis of the hypervariable segments I and II represents only a small portion of the genetic information contained in the mtDNA molecule. The direct sequencing is a time-consuming and labour-intensive method. In this sense, the sequencing of the entire mitochondrial genome is not a practical method. The typing of single nucleotide polymorphisms (SNPs) located throughout the mitochondrial genome can increase the informative and discrimination powers of the human mtDNA analysis. Also, it can provide important information on the mitochondrial haplogroup affiliation. A set of 11 mtDNA SNPs proposed by Vallone et al. (2004) was chosen for starting the study of mitochondrial SNPs in our laboratory. The analysis was done using a multiplex PCR followed by minisequencing.

P-147 Y miniSTR: new aplication for compromised samples

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STRs are the most used genetic markers for evidence typing. However, there are some cases in which samples are so badly degraded that it is impossible to get good results with the use of commercial kits. One possible approach is the application of miniSTRs. There are already some papers recognizing the efficiency of this technique in autosomic markers. Based on the same principles, miniSTRs from the Y chromosome are appearing that can play an important role in the forensic field in the future. Asamura et al. published a work describing the conception and optimization of two quadruplexes for Y-chromosome that result in amplicons with less than 150bp. From their primers, and with little changes occurring, we have recreated two "new" quadruplexes and optimized them to be used in our laboratory conditions. Additionally we have collected some population data. Since these markers are not incorporated in any of the commercial kits, one big advantage arises that is the fact that a wealth of information is obtained even when high quality samples are used. We believe that this new methodology is starting to earn its place and that it will soon be recognized as an essential tool for Forensic Genetics.

P-148 Study of 16 X-STRs in a prostate cancer population sample

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Prostate cancer, like numerous other cancers is a result of genetic alterations that accumulate during disease progression. Study of short tandem repeats (STRs) have already demostrated that this type of polimorphism could provide a mean to rapidly scan genomes at know or unknown predisposing loci for some diseases. Scan genomic DNA from patients with prostate cancer in comparison with normal controls for X-STRs. In this study, samples of unrelated males with prostate cancer and apparently healthy and unrelated males were analysed with Argus X-8 (Biotype®) and with a new X Decaplex used in a collaborative study of GEP-ISFG. Samples were run in ABI PRISM 310 or in ABI PRISM 3100 Genetic Analyser. For allelic determination, we used a commercial allelic ladder (Argus X-8) or a home made allelic ladder (X-Decaplex). Statistical analysis was performed using SPSS for a significant level of 95%. Xu et al, have already reported a correlation between DXS1113 and prostate cancer. Studying more X-STRs is a possibility to scan chromosome X to find some predisposition loci for this disease.

P-149 The Study of Single Nucleotide Polymorphisms (SNPs) in the Arabic Populations – a Tool for the Analysis of Degraded DNA

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Biological materials that are recovered from crime scenes have often been exposed to sub-optimal environmental conditions such as high levels of UV light, high temperatures, high humidity and the action of microorganisms. These environmental conditions can lead to high levels of DNA degradation, which in turn can lead to incomplete DNA profiles. Single nucleotide polymorphisms (SNPs) are the most common genetic polymorphism in the human genome. They can provide a valuable tool for human evolution studies, forensic identification and clinical testing. During profiling of heavily degraded forensic samples, the small amplicon required for SNP analysis has an advantage over the analysis of larger STR loci which are routinely used in forensic casework. The aim of the project is the identification and characterisation of informative SNPs within an Arabic population. For initial screening, to obtain a large amount of data of the SNP frequencies in an Arabic population, 10 unrelated individuals from Kuwait and United Arab Emirates (UAE) were typed using the Affymetrix® 250K chip. One hundred polymorphic SNPs were identified from autosomal chromosomes and accurate allele frequencies were estimated using the SNaPshot® technique (Applied Biosystems). Previously published polymorphic SNPs are also being examined.

P-150 The Mediterranean basin: A population genetic study based on 25 X-chromosome SNPs

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The X-chromosome has valuable characteristics for population genetic studies. In order to investigate the genetics of the human Mediterranean populations further, we developed a 25 X-chromosome SNP-plex typing system. The system was based on PCR multiplex amplification and subsequent multiplex Single Base Extension with the SNaPshot reaction, capillary electrophoresis and multicolor fluorescence detection. We investigated 11 Mediterranean populations with the 25 X-chromosome SNPs. A high overall homogeneity was found among the Mediterranean populations except for Moroccans, who differed genetically from the rest of the populations in the Mediterranean area. This result supports the hypothesis of a low incidence of the South-North genetic interchange at the western shores of the Mediterranean basin. A low genetic distance was found between populations in the Middle East and the western part of the Mediterranean area most likely reflecting the strong effect of the Neolithic wave. A certain level of background linkage disequilibrium among the 25 SNPs on the X-chromosome in Ibiza and Cosenza was observed, possibly as a consequence of their demographic history.

P-151 Microgeographic genetic variation of Y chromosomes in a population sample of Ravenna's area in the Emilia-Romagna region (North of Italy)

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The analysis of the genetic structure of regions with a complex demographic history shed light on the various topographic, linguistic and historical influences which form the present genetic landscape of Europe. In the Emilia-Romagna region (North of Italy) Ravenna is a geographical area with a historical complex background: it was an important seaport on the Mediterranean sea, the capital of the Ostrogothic kingdom of Italy and the seat of the Byzantine governor of Italy. The purpose of this study was to investigate the microgeographic variation of Y chromosome haplotypes of the area of Ravenna by analyzing seventeen Y-chromosome short tandem repeats (Y-STRs) in 122 unrelated males. 100% of all haplotypes were different. A comparison with neighbouring Italian as well as with European and Levante root populations was done by AMOVA and visualized by a phylogenetic tree. The two main haplogroups found in this area were R1b and E3b1. The results of the present study add to the data for the forensic databases and can be useful also for anthropological studies.

P-152 Mitochondrial HVI and HVII Polymorphisms and Heteroplasmies Inheritance in Brazilian Pairs of Mother/Child

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The mitochondrial DNA has been used in forensic identification areas due to its maternal inheritance, high mutation rate, and the high number of copies. Even the highly polymorphic regions, HVI/HVII, sometimes are of low discriminate value due to the presence of common population polymorphisms. Our aim is evaluate the inheritance of these polymorphisms and heteroplasmies in HVI/HVII using known pairs mother/child analyzed in blind-test, and characterize the Brazilian population. DNA sequencing was performed in 98 pairs to HVI, with match of 71.4%, and in 88 pairs to HVII with 45.5% of match. For the joint analysis (HVI+HVII=88 pairs) the match was of 86.4%, with 82 haplotypes: 76(92.7%) unique and 6 seen in 2 individuals. Mothers and respective children showed length heteroplasmies in ~28.6% to HVI with 16 different types of sequences and ~62.8% to HVII with 5 types of sequences only HVII showed point heteroplasmies. HVI showed discriminate power much higher than HVII the joint analysis was able to match only 5 pairs more than HVI (all belong to the group of 18 pairs that were not matched not even in HVI or HVII). In a real situation of identification the choice of HVI to initial screening become obvious. Support:FAPESP,LIM40-HC.

P-153 The analysis of UAE Populations using the AmpFlSTR® Y-Filer™

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The analysis of Y chromosome polymorphisms has become commonplace in forensic genetics, both for the identification of male component in sexual assault samples and also in paternity cases. The AmpFlSTR® Y-Filer™ kit was developed to allow the amplification of 17 STR loci on the Y chromosome including the core set defined as the European Minimal Haplotype and the loci recommended by the Scientific Working Group on DNA Analysis Methods (SWGDAM). The AmpFlSTR® Y-Filer™ kit was used to create a Y STR database for the three major populations (UAE Arab, Indian and Pakistani) of the United Arab Emirates to help in forensic casework. Blood was collected from 414 unrelated male individuals and DNA was extracted using phenol chloroform. All samples were then amplified and analysed using the ABI Prism® 310. Genotyping was done against an allelic ladder supplied with kit. Allelic and haplotype frequencies were calculated from the three populations: haplotype diversities ranged from 0.9981 in the UAE Arabic population to 0.9996 in the UAE Indian population. Several new alleles not present in the allelic ladder were observed. Null alleles in the non Arabic samples were detected at four loci.

P-154 Population Data for Y-Chromosome Haplotypes defined by 17 STRs (AmpFlSTR YFiler) in Central Portugal

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Background and purpose: Y-Chromosome Short Tandem Repeats (Y-STR) are very useful in forensic practice, with application on kinship investigation, reconstruction of male lineage and male-female mixture analysis. The purpose of this work was to create a population database for the Y-STRs. Methods: AmpFlSTR YFiler PCR Amplification kit (Applied Biosystems) uses the 5-dye chemistry for the amplification of 17 Y-STR including the European Minimal Haplotype loci (DYS19, DYS389I, DYS389II, DYS390, DYS456, DYS391, DYS392, DYS393 and DYS385 a/b) and 7 additional loci (DYS458, DYS439, DYS635, GATA H4.1, DYS437, DYS438 and DYS448). In this study 100 samples from Central Portugal were analysed. DNA was extracted using Chelex 100 protocol. The electrophoresis was carried out on ABI PRISM™ 310 Genetic Analyser using GeneScan Analysis. The nomenclature is according to the ISFG recommendations for Y-STR. Results: A total of 99 different haplotypes were found, with only two individuals sharing the same haplotype. Gene frequencies and gene diversity was calculated. The overall haplotype diversity (HD) was 0.9899. DYS458 non-consensus alleles were present in 4 samples and a null allele was found on locus DYS456. Conclusions: Y-STR polymorphisms from YFiler in Central Portugal population provides a powerful discrimination tool for routine forensic applications.

P-155 Mitochondrial DNA (mtDNA) Sequence Analysis of Native Bolivians Population

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Background and Purpose: Andean region can reveal some History of human colonization of New World. MtDNA is remarkable useful for population genetics by its own characteristics: high copy number, maternal inheritance, high mutation rate and absence of recombination. Haplotypes of a Bolivian population has been assessed analysing the polymorphisms in mtDNA HV1 and HV2 regions of 111 individuals from La Paz, in order to study genetic interpopulations variation. Method: Blood samples were obtained for healthy and unrelated individuals. DNA was extracted using Chelex®100 method. HV1 and HV2 were amplified using primers L15997/H16401 and primers L408/H048, respectively. Sequencing chemistry: ABI Prism® dRhodamine Terminator Cycle Sequencing Ready Reaction Kit. Sequences were analysed in a ABI PRISM® 3130 – Genetic Analyser with ABI DNA Sequencing Analysis Software v.5.2 and SeqScape® Software v.2.5. Results: 99 haplotypes were identified with 125 polymorphic nucleotidic positions, 93 haplotypes were unique. Nucleotide and sequence diversity were estimated in 0,016068±0,008186 and 0,9988, respectively. Haplogroup distribution was: 47,75% B, 14,41% C, 6,31% A, 4,5% D, and 27,03% undetermined or compound haplogroups. Rate of heteroplasmy was 37,83% in HV1 and 53,15% in HV2., Conclusions: Native Bolivians exhibited haplotypes from four haplogroups observed in Native Americans. Genetic variability was high, suggesting isolation after an early colonization of the population.

P-156 Cabo Verde population study with 17 STR loci

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In Lisbon Metropolitan region, one of the most representative immigrant communities comes from the Cabo Verde Archipelago and is also the second most studied population in our Department. We have studied 295 unrelated individuals and 88 paternity investigation cases from an African Cabo Verde population. PCR amplification was performed using AmpF1STRâIdentifilerÔ and Geneprint Powerplex16â multiplex kits and PCR products analyzed by capillary electrophoresis. Allele and minimum frequencies were determined for each locus and forensic statistic parameters were estimated. Significant differences in several STR loci were encountered when comparing this population with a South Portuguese one. Concerning TH01 and PentaE, alleles 7 and 8 are, respectively, the most frequent ones, while TPOX allele 6 and PentaD alleles 2.2 and 3.2 have significant higher frequencies in Cabo Verde population. Several rare alleles were also detected - in D18S51 alleles 10.2, 13.2 and 15.2, in D3S317 alleles 9 and 10, in D21S11 alleles 21 and 34.1 and in D16S539 allele 7. Exclusion and non-exclusion paternity investigation cases, including a heterozygotic twin case, have also been studied and mutation rates estimated when applied. A 17 autossomic STR loci database for the most frequent African population in our laboratory was established for forensic casework.

P-157 Haplotype data for 12 Y-chromosome STR loci from Costa Rica

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Haplotype data were obtained from a sample of 100 unrelated male individuals from Costa Rica, for 12 Y-chromosome STR loci (DYS19, DYS385, DYS389 I, DYS389 II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439). A total of 86 different haplotypes were identified, of which 76 were unique and the most frequent haplotype was found in 4 individuals. High haplotype diversity was found (99.6%), being DYS385, representing two loci, the most diverse marker, and DYS393 presented the lowest gene diversity value. Genetic distances were calculated between our and previously published haplotype data for the same set of Y-STRs. The lowest distances were obtained when comparison was performed with Rio de Janeiro (Brazil). Although higher, low Rst values were also found in the comparison with Iberian origin samples, from Portugal and Spain, as well as with Cartagena (Colombia). Samples from African origin revealed highly significant differences with Costa Rica.

P-158 STR data for the 15 AmpFlSTR® IdentifilerTM loci in the Brazilian population of São Paulo State

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The settlement of Brazil began in São Paulo State, in 1532, with the arrival of the portuguese. The ethnical variety, which characterizes this population, has an historical context related with the arrival of african groups and subsequent migratory waves of spanish, italian, german and japanese. Allele frequencies and statistical parameters of forensic interest for the 15 STRs loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818 and FGA) from the AmpFlSTR® IdentifilerTM PCR Amplification Kit, were estimated in 294 healthy unrelated individuals of the brazilian population of São Paulo State. DNA from bloodstain samples was extracted with the Chelex® 100 method, amplification was performed in accordance with the manufacturer´s instructions, samples were genotyped using an ABI Prism® 310 Analyser and the GeneScan® Analysis 3.1 software. With the exception of the CSF1PO system (p=0.03667), no deviations from the Hardy-Weinberg equilibrium were found. The combined probability of exclusion (PE) and the combined power of discrimination (PD) for the 15 studied loci were 0,99999945 and above 0,999999999999999, respectively. A phylogenetic tree was elaborated using population data from european, african, asian, south-american and central america populations. The results are in agreement with other population studies and historical data.

P-159 Mitochondrial DNA genetic relationships at the ancient Neolithic site of Tell Halula

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Mitochondrial DNA genetic relationships at the ancient Neolithic site of Tell Halula Fernández E., Ortiz J.E.1, Torres T. 1, Pérez-Pérez A. 2, Gamba C., Tirado M., Baeza C., López-Parra A.M., Turbón D.2, Anfruns J.3, Molist M. 3, Arroyo-Pardo E. Dpto. Toxicología y Legislación Sanitaria. Facultad de Medicina. Universidad Complutense de Madrid. 28040-Madrid, Spain. 1 Dpto. Ingeniería Geológica. Escuela de Ingenieros de Minas. Universidad Politécnica de Madrid. Spain. 2 Unidad de Antropología. Dpto. Biología Animal. Facultad de Biología. Universidad de Barcelona. Spain. 3 Dpto. Prehistoria. Facultad de Letras. Universidad Autónoma de Barcelona. Spain. Background and purpose A Tell –meaning "hill"- is an archaeological site resulting from accumulation of material deposited by human occupation over a long time. The aim of the study was to establish familiar relationship among individuals buried at different archaeological phases and houses from the Neolithic site of Tell Halula (Syria) by using ancient DNA techniques. Method Fifty six samples belonging to 50 different individuals from Tell Halula (8800 BP) were studied. A fragment of 300 bp of mitochondrial DNA HVRI was amplified in the whole sample. Results were authenticated through 1) Independent replication from the same or different extracts, 2) Aspartic acid racemization, 3) Real Time PCR quantification and, 4) Bacterial cloning of some amplification products. Results It was possible to recover 23 authenticated mitochondrial DNA sequences (47%) in the analyzed sample. Conclusion The obtained mitochondrial DNA results suggest possible relationships not only among individuals buried into different archaeological phases of the same house but also through different houses from different archaeological phases. This suggests an homogeneous population structure of this Neolithic community. The amount of recovered sequences indicates the extent of preservation of critical samples in a dry and hot soil.

P-160 Polymorphisms of the D-loop mitochondrial DNA in Rimini and Valmarecchia areas in the North of Italy.

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The sequence polymorphisms of the hypervariable segments I and II (HVI and HVII) of the human mitochondrial DNA (mtDNA) in two population samples from Romagna region in the North of Italy, the urban area of Rimini and the near area of Valmarecchia, were analyzed. The aim of this study was to create a population database for mtDNA HVI and HVII regions of these two areas. A total of 83 and 80 samples of unrelated individuals from Rimini and Valmarecchia respectively were collected. A phylogenetic analysis was performed following EMPOP program. The principal European haplogroups were found, being the haplogroup H the most frequent. No point heteroplasmy was observed in any of individuals tested, but the majority of samples showed length heteroplasmy in HVII region. For forensic purpose the observed data may be included in the general Italian database.

P-161 Genetic structure of the population of Beni Department (North Bolivia).

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Genetic structure of the population of Beni Department (North Bolivia). López-Parra A.M., Tirado M., Baeza C., Bert F.1., Corella A.1, Pérez-Pérez A.1, Gamba C., Fernández E., Arroyo-Pardo E. Dpto. Toxicología y Legislación Sanitaria. Facultad de Medicina. Universidad Complutense de Madrid. 28040-Madrid, Spain. 1 Unidad de Antropologia. Depto Biología Animal, Facultad de Biología, Universidad de Barcelona, Spain. Background and purpose Variability of Y-STRs is still scarce for the populations of some regions of the world. In this work, we analyze a sample of Y-chromosomes of 87 Bolivians of Beni department (Northern Bolivia). Results allow to determine the genetical structure of the Amerindian populations of the Amazonian basin. Method Samples were typed with AmpFlSTR® Yfiler® PCR Amplification Kit (Applied Biosystems). A database of Y-STRs frequencies (minimum haplotype except DYS385) was constructed with a total of 12 South American samples plus our population. Rst were calculated using Arlequin ver 3.01 and distance tree was drawn using Phyllip package ver. 3.65. Geographical coordinates of each population and absolute frequencies of Y-STR haplotypes were used to estimate groups of relative low invariance through software SAMOVA ver 1.0. Multidimensional Scaling (MDS) over frequency data were carried out through SPSS ver 13.0. Results and conclusion Y-STR structure of the database reveals, first, the degree of differentiation of our sample and, second, the close relatedness of most of the populations considered. Differences can be due in part to the low level of admixture with European Y-chromosomes. Results also allow to determine the usefulness of South American databases in forensic casework.

P-162 Y-STRs and forensic parameters in African populations.

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Y-STRs and forensic parameters in African populations. Baeza C., López Parra A.M., Fernández E., Gamba C., Tirado M., Mesa M.S.(1), Arroyo-Pardo E. Dpto. Toxicología y Legislación Sanitaria. Facultad de Medicina. Universidad Complutense de Madrid. 28040-Madrid, Spain. (1) Depto Zoología y Antropología Física, Facultad de Biología, Universidad Complutense, Madrid, Spain. Background and purpose Short Tandem Repeat polymorphisms have been widely studied in the world, since specific databases are required to produce correct estimates of forensic statistical parameters. In this regard, Y-chromosome STRs have been studied mainly in Europe but are relatively scarce for sub-Saharan populations, despite these populations are represented in most of the Western countries. The aim of this work is to detect groups of populations with the lowest genetic variability within the African context. This allows to establish the relative homogeneity of Y-STRs databases for forensic casework and eventually to provide a wider insight into the African genetic history. Method A database of Y-STRs (minimum haplotype except DYS385) frequencies was constructed with a total of 23 African populations. A matrix of Rst was calculated using Arlequin ver 3.01. Groups of highest variance were separated using program Barriers ver.2.2. Geographical coordinates for each population and Y-STR haplotypes were used to calculate clusters of populations with the lowest variability through software SAMOVA ver 1.0. Results and conclusion Results reveal the presence of groups with relatively low genetic variability within the African continent. Forensic parameters within each of these groups can be reliably calculated for any of their members.

P-163 Analysis of the Genetic Substructure in Argentina using fifteen microsatellite loci

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It has been shown by Y-SNPs and mtDNA-hg that the population of Argentina is genetically heterogeneous, with relevant Native American and European contributions to its extant population. In order to test if genetic substructure can be detected by autosomal markers, a set of 15 STRs (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX y FGA) were analyzed in 5,282 individuals inhabiting 10 provinces: Buenos Aires, Santa Fe, Mendoza, Río Negro, Chubut, Misiones, Formosa, Chaco, Corrientes and Salta. Allele frequencies, Hardy-Weinberg equilibrium and genetic distance, determined by Fst, were establish between the sampled populations. The results obtained indicate that the genetic distance are non significant between most of the sampled populations, being Salta the only one that significantly differs respect to most of the compared provinces (Fst: 0.00484-0.01601, p: 0.0498-0.0000). In order to broaden the comparison, previously published information of five additional provinces (N=1,994), were included in the analysis. The extended results showed that genetic distance are non significant between these fifteen provinces, with the exception of Salta sample. These results might suggest that genetic sub-structure can't be detected by the autosomal STRs in the 15 populations considered. Accordingly, a database of the whole country is needed, for the correct interpretation of the result in paternity testing and forensic casework.

P-164 Population genetics and DNA preservation in ancient human remains from Eastern Spain.

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Population genetics and DNA preservation in ancient human remains from Eastern Spain. Gamba C., Fernández E., Tirado M., Baeza C., López-Parra A.M., Oliver A.1, Arroyo-Pardo E. Dpto. Toxicología y Legislación Sanitaria. Facultad de Medicina. Universidad Complutense de Madrid. 28040-Madrid, Spain. 1) Sección de Arqueología. Museo de Bellas Artes de Castellón, Castellón, Spain. Background and purpose: Eastern Spain has undergone genetic flow from immigrant and surrounding populations at least since Neolithic times. Present work evaluates this hypothesis in ancient samples from Castellón and Valencia provinces, dated back in Roman, Iberian, Calcolithic and Bronze ages. Preservation of these critic samples has taken place in a dry carbonated soil and alkaline pH (˜ 8.0). This study aims to establish the genetic relationship between the different settlers and also to determine the conditions of DNA preservation. Method: We studied two overlapping sequences (16126-16251 and 16256-16369) from mitochondrial HVR-I in 36 bone and teeth samples from 16 archaeological sites of Spanish Levant. Consistence of the results was established by repeated replication of amplifications. Results: Approximately, 50% of the samples yielded reproducible results. The obtained overall sequences belonged to haplotypes whose frequencies are quite homogeneous within the different populations and periods. Haplogroup V, an alleged marker of Paleolithic newcomers in Europe, has been found in an unusual elevated frequency (1 Calcolithic and 2 Iberian samples). Conclusion: Results suggest a possible genetic continuity between the Calcolithic and Iberian inhabitants. The conditions of preservation allowed a significant recovery of ancient sequences, probably due to the lack of humidity.

P-165 Discriminating European and South Asian Individuals Using SNPs and Pyrosequencing Technology

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The population specific SNP set developed by the SNPforID consortium comprised of 34 SNPs enabling accurate discrimination between three major population groups: White European, African and East Asian. If a South Asian population (from the Indian subcontinent) was also analysed, the assignment error rate increased due to a difficulty with separating this population from the European samples. It was thus necessary to find alternative SNPs to distinguish this population. An assay has been designed using Pyrosequencing technology for eight candidate SNPs selected from the literature. Using this small SNP panel, a set of samples has been typed from both European and South Asian populations. The frequency data obtained was submitted to the web portal (http://mathgene.usc.es/snipper/) allowing a discrimination algorithm to be produced. A number of samples were then run through this algorithm to assess the success rate of population identification.

P-166 Development of a system pentaplex for the study of the polymorphism of chromosome X in loci DXS7133, DXS7424, DXS981, DXS8378 and HPRTB in samples of the population of the Bahia, Brazil.

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In forensic genetics, the polymorphism of chromosome X constitutes important tool to the personal identification, mainly, in complex situations of paternity, including cases where if it verifies the absence of the paternal ancestor. In the state of the Bahia (Brazil), the number of these cases is raised by not existing normative that it regulates of civil register of the biological father. The system pentaplex was developed for loci DXS7133, DXS7424, DXS981, DXS8378 and HPRTB, with amplicons of until 330bp. Data were obtained from a sample of 100 unrelated Brazilian individuals using the system pentaplex developed by PCR. The polymorphism detections occur in 3100Avant Genetic Analyze. The allele's assignment was made comparing the genotypes gotten with those known for cellular ancestries. The system revealed efficient for typing of samples with 0,1ng 10ng of DNA. The distribution of the alleles and genotypes frequencies and too population statistical parameters of study are presented. Keywords: chromosome X, forensic genetics, Bahia-Brazil.

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P-167 Population substructure by social stratification

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Brazil is known for its high genetic admixture rates. The Federal District population has been recently formed (1960) by migrants from all other Brazilian regions. Thus, it may be considered a representative sample of the country's genetic composition. Commonly, the individual social origin is not considered during sampling in Brazil. Our aim was to evaluate whether the social origin of a sample influences its genetic composition. The proportion of each parental population (Amerindians, Africans and Europeans) contribution was estimated, using STRs data, for a public forensic laboratory sample and compared to the estimates obtained by a private laboratory (compiled from the literature). Results showed a huge difference between the two samples concerning parental contributions. In the public laboratory sample, European and African contributions were equivalent, around 40%. In contrast, the private laboratory sample showed higher European contribution (71%) and a much lower African one (18%). The Amerindian contribution proportion, observed in our sample was almost twice as much as the one estimated by the private laboratory. While lower social classes make use of public services, higher classes often go to private laboratories. Therefore, our results showed that the parental contribution of Brazilian lower classes is different from the higher ones.

P-169 Forensic Genotypes and Genetic Landscapes of Extant Argentinean Population

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Thomas F. Wienker, Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Germany

Forensic studies provide copious genetic information. Specific sets of neutral markers, independent autosomal markers and Y-haplotypes, are genotyped, conditioned to the characteristics of each investigation. Forensic markers are neutral, in consequence, forensic STRs constitute an appropriated data set to explore genetic composition of extant populations. Argentina covers extensive and differentiated geophysical regions. Along the Argentinean territory, diverse patterns of population admixture are expected. Indigenous and predominantly European groups mixed in different proportions in the distinct geographical areas. Spatial genetic heterogeneity of extant Argentinean population is assessed based on forensic genotypes. Population genetics is combined with geodesic analysis to determine spatial patterns of genetic heterogeneity, the genetic landscapes. A landscape is an area that is spatially heterogeneous in at least one factor of interest. Geostatistic analysis and population genetics can be adequately integrated within the open source GRASS geographic information system (GIS) in combination with the R environment. A genetic geographical Information system (GenGIS) set the framework to model and analyze genetic landscapes. Clines, gradients of genetic heterogeneity, are detected by quantifying landscape slope. Transects allow a precise profile of the detected gradient. GenGIS assessment of genetic landscapes provides an insight into the spatial composition of extant, highly admixed Argentinean population.

P-170 Genetic diversity of 4 X-STRs in a Romanian population sample

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The X-chromosomal STRs DXS8378, DXS7132, HPRTB and DXS7423, located in four different X-chromosomal linkage groups, were studied in a Romanian population sample comprising 256 unrelated individuals, 116 males and 140 females. The study was performed using the commercially available kit Mentype Argus X-UL (Biotype AG, Germany) and an ABI 3100 Avant Genetic Analyzer. All the 4 X-STRs revealed high genetic diversities, similar to those reported in other European population samples. Population comparisons (exact test of population differentiation, pairwise genetic distances) were carried out and low genetic distances were found between our sample and those from other European regions. The parameters of forensic efficiency, deviations from HW equilibrium for the female genotypes and mutations on 65 proven parent–child pairs were further evaluated. Our study confirms that the 4 X-STR markers are highly informative and therefore useful for forensic applications and anthropological research.

P-171 The contribution of the HVIII region to mitochondrial polymorphism in Bahia (Brazil) population.

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In the mitocondrial genome the biggest diversity between people is in the control region, particularly, in the HVI and HVII. Region HVIII has been pointed as an alternative with respect to distinction between sequences when the analyses of regions HVI and HVII are coincident. The HVRIII has presented little polymorphic in the populations of Malaysia, Germany and Japan. Considering that the population of the Bahia(Brazil) show high indices of polymorphism for regions HVI and HVII in relation to other populations, the study for HVIII one becomes necessary to know the forensic utility of this region in the population of the Bahia (Brazil). The HVIII mtDNA sequences from 150 individuals had been compared with Cambridge Reference Sequence (CRS) and the registered differences as recommended by the ISFG. The analysis of these sequences disclosed low polymorphism of comparative region HVIII to regions HVI and HVII. Of the eight identical samples for HVI and HVII, only one was distinct in HVIII due to insertion of 1C in position 571 (571.1C). The differences were predominantly transitions.
Keywords: mtDNA, HVIII, forensic genetics, Bahia-Brazil.

P-172 Y-chromosome lineages in São Tomé e Príncipe and Cabo Verde islands: different input of European influence

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The Y-chromosome haplogroup composition of the population of São Tomé e Príncipe and Cabo Verde Archipelagos was profiled by using 32 biallelic markers, and compared with populations from Europe, Africa and the Middle East. According to the traditional view, these archipelagos colonized by the Portuguese in the 15th century were settled mainly by West African slaves, with the addition of a minor fraction of male colonizers from Europe. Although the major proportion of the founding population of São Tomé e Príncipe cluster in haplogroup E3a (84.2%), very common among sub-Saharans, this lineage was observed at a frequency of only 15.9% in Cabo Verde. Haplogroups I, J and R1, characterized of populations of Europe and the Middle East account for more than half of the paternal lineages of Cabo Verdeans (53.5%). These West Eurasian haplogroups are found at a frequency of only 12.5% on the population of São Tomé e Príncipe. Our findings suggest that despite the sub-Saharan genetic background of these archipelagos, a relevant contribution of European paternal lineages is present in nowadays populations indicating that gene flow from multiple sources have been important in the formation of the diversity of the islanders, nevertheless with a different degree of admixture.

P-173 Development of a system pentaplex for the study of the polymorphism of chromosome X in loci DXS7133, DXS7424, DXS981, DXS8378 and HPRTB in samples of the population of the Bahia, Brazil.

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In forensic genetics, the polymorphism of chromosome X constitutes important tool to the personal identification, mainly, in complex situations of paternity, including cases where if it verifies the absence of the paternal ancestor. In the state of the Bahia (Brazil), the number of these cases is raised by not existing normative that it regulates of civil register of the biological father. The system pentaplex was developed for loci DXS7133, DXS7424, DXS981, DXS8378 and HPRTB, with amplicons of until 330bp. Data were obtained from a sample of 100 unrelated Brazilian individuals using the system pentaplex developed by PCR. The polymorphism detections occur in 3100Avant Genetic Analyze. The allele's assignment was made comparing the genotypes gotten with those known for cellular ancestries. The system revealed efficient for typing of samples with 0,1ng 10ng of DNA. The distribution of the alleles and genotypes frequencies and too population statistical parameters of study are presented. Keywords: chromosome X, forensic genetics, Bahia-Brazil.

P-174 Y-chromosome haplotype mismatch in different haplogroups: coincidence or evidence of SNP mutation?

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The analysis of 240 individuals from the populations of the Madeira and Azores archipelagos for both Y-chromosome STRs and SNPs allows the determination of a correlation between the haplotype and haplogroup for each individual. The variability within each haplogroup were analysed and coalescence age were calculated. These results allow detecting some founder effects in both populations. It was found that some individuals share the same haplotype but belong to different Y-chromosome haplogroup suggesting that SNP mutations may occur frequently. We propose using SNP and STR information together that provides a more reliable characterization of an individual in terms of Y-chromosome origin, minimizing the risk of misassigment due to mutation.

P-175 Population Genetic Data of 10 X-STRs in East-Timor (Timor-Leste)

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In order to understand the genetic variation of a population it is important to analyse different classes of markers. The present study provides population genetics data from a multiplex system of 10 X-Chromosome STRs applied to the population of East-Timor. A collection of 46 unrelated males from Timor-Leste were subjected to common procedures of DNA extraction and PCR amplification of the X-STRs: HPRTB, GATA17D05, DXS8378, DXS7423, DXS7132, DXS6809, DXS6789, DXS101, DXS8377, DXS9898, followed by typing in ABI 310 DNA sequencer. Arlequin software ver.3.0 was used for several population genetics tasks such as: allele frequencies calculations and gene diversities. Genetic parameters useful in forensic science calculated for this sample show high overall values of Mean Exclusion Chances and Power of Discrimination. The most polymorphic marker was DXS8377 and the least one was HPRTB. The present data, although preliminary, may contribute both to forensic applications, namely in kinship analyses in Timor-Leste (a country with a tragic history) and to an integrated uderstanding of its population history.

P-176 Population genetic analysis in african-american population from Venezuela

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In this study we present the present the frequency distributions of the autosomal STR systems of 15 loci (CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPO, VWA, Penta D and Penta E) amplified by the GenePrint® PowerPlexTM 16 system (Promega) in an african american population from Barlovento, in the north caribbean coast of Venezuela. The study was carried out on blood simple taken on FTA Gene Card (Whatman) from 100 not related individuals, who were born in barlovento area and have been living there for at least two Generations. Amplification products were analyzed by capillary electrophoresis using the ABI 310® Genetic Analyzer (Applied Biosystems). Statistical analysis calculated using Arlequin 2.0 package to determine allele frequencies and other population parameters of interest . The allelic frequencies of the population were compared to the corresponding data of Venezuelan population and other African American population.

P-177 Y Chromosome SNP analysis in a Portuguese population sample, using a multiplex PCR and minisequencing strategy

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PhD. L. Gusmão, IPATIMUP Institute of Pathology and Molecular Immunology of University of Porto, Portugal
PhD. F. Corte-Real, National Institute of Legal Medicine. Largo da Sé Nova, Portugal

The study of single nucleotide polymorphisms located on the Y chromosome specific region can be helpful in forensics, since Y haplogroups show regional specificity, providing useful information about geographic origin of an individual or evidence under investigation. A multiplex with nine Y-SNPs (92R7, M70, M22, Tat, P25, SRY10831, M173, M213 and M9) was used to characterize a population sample from Central Portugal, in order to investigate the frequency distribution of the male lineages and to compare the observed results with those obtained to other Portuguese regions. The genotyping strategy was according to the described by Brion et al. [IJLM 119 (2005) 10-15]. This multiplex was used to type 46 individuals from a population sample from Central Portugal and a typical Western European haplogroup composition was found. The majority of samples (almost 70%) were assigned inside haplogroup R. As for other Iberian populations, the most frequent haplogroup was R1b-P25 (52.2%), followed by F(xK)-M213 (15.2%), E-B- SRY10831.1, R1(xR1a,b)-M173 and R1a-SRY10831.2 (each of them with a 8.7% frequency), K2-M70 (4.3%) and L-M22 (2.2%). When comparing our sample with other samples from Portugal, no significant differences were found.

P-178 Types and frequencies of variants in Amelogenin gene in Chinese population

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Objective: To investigate the types and frequencies of variants in Amelogenin gene in Chinese population and explore the mutations' influence to the sex identification. Methods: Amelogenin gene of 8850 unrelated Chinese individuals were typed with Powerplex® 16 system. The samples with abnormal typing results were calculated directly, validated with different primer sets, Y-STR typing and sequencing. Results: 2 samples with AMELX allelic drop-out and 2 samples with AMELY allelic drop-out were observed in male individuals, the total rate of variation is 0.045% and the rate in the male is 0.085%. 2 types of point mutation which may result in null allele were observed in the primer binding region of the "lost" AMELX alleles, and the variation rate in the male is 0.042%. The variation rate of AMELY allele is also 0.042%. 1 sample failed to amplify 10 Y-STR loci out of 12 loci, which could be speculated that large interstitial deletion of the Y chromosome encompassing the AMELY and other Y-STR loci was occurred. Conclusion: Mutations that result in a failure to amplify sex chromosome-specific products may lead to incorrect sex identification and should be alerted. [Key words] Amelogenin gene sex identification mutation

P-179 A meiosis study of the pentameric STR locus D10S2325

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Carsten Hohoff, Institute of Legal Medicine, University of Münster, Germany

We investigated 444 Germans from Westphalia and 337 Kurdish immigrants (mainly from northern Iraq) at the pentameric STR locus D10S2325. PCR-products were analysed on an ABI PRISM 310 Genetic Analyzer (ABI). Allele calling was done using a sequenced allelic ladder. D10S2325 was found to be highly informative in both populations with a power of discrimination (PD) of 0.967 and 0.961 and a power of exclusion (PE) of 0.736 and 0.727, respectively. No deviation from the Hardy-Weinberg equilibrium was observed. Furthermore, we have studied 132 maternal and 130 paternal meioses in the Westphalien population and two paternal mutations were observed. In the Kurdish population 163 maternal and 171 paternal meioses were investigated and no mutation was found. In addition to the alleles that have been described for Caucasoid populations (i.e., 6 - 17) we have detected long alleles 21, 22 and 23. The sequence structure, determined by direct sequencing after separation by native PAGE, revealed uninterrupted TCTTA repeats.

P-180 Study of the polymorphism of the blood groups, (ABO, Ss, Rhesus and Dufy) at the population Arabic-speaking person of the plate of Beni Mellal

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Prof. Hamid El Amri, Genetique Laboratory of the Royal Gendarmerie of Morocco, Morocco

The present study deals with anthropogenetical characterizations of the Arab speaking of Beni Mellal region which is located between barbers' of Moyen Atlas and arabs of meridional Morocco. The study of blood groups ABO, Rhesus, Ss, and Duffy was conducted on 131 individuals. The result shows that this population has the highest frequencies of the FyO allele (0.86) and s allele (0.524) in comparison to all Arab and Berber populations of North Africa and Middle East. However genetic distances estimated on the basis of these four marquers reveal that both populations of Beni Mellal and Beni Hlal are in the same cluster with populations from the Middle East< This height be explained by an oriental origin of these two Moroccan Arabic populations. The estimation of Reynolds diversity coefficients shows that within region diversity is more important than that between regions which leads that genetic drift is the principal factor of the micodifferentiation, Key words : Population, blood marquers ,gentic diversity, Polymorphism

P-181 Population genetic data of 5 X-chromosomal loci in Bauru (São Paulo, Brazil)

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This work analyzed the population genetic data of 5 X-STRs (DXS6854, DXS7424, DXS101, DXS6808, DXS7132) in sample of 90 unrelated men and women living in Bauru, central region of the state of São Paulo, Brazil. DNA samples were extracted using DNA IQ kit (Promega) and the PCR was realized with primer sequences from the Genome Database. Allelic ladders were made for all the markers and used for genotyping samples. Genotype profile from cell line K562 (Promega) was also analyzed. Allele frequencies between men and women samples were not significantly different in all markers. No haplotype in men and five combined genotypes in women were the same for this set of markers. Linkage disequilibrium analysis did not reveal association between X-STRs. No deviations from Hardy–Weinberg equilibrium were observed, with the exception of DXS101, and all markers, with the exception of DXS6808, present high genetic diversities (over 0.73). The combined powers of discrimination in men and women were 0.99897253 and 0.99999120, respectively. In conclusion, the 5 STRs analyzed constitute a powerful tool for forensic practice in our population. However, new markers will be studied and added to this system in order to obtain a better discrimination power.

P-182 Ancient DNA: Rare mtDNA haplogroups amongst Danish Iron-Age villagers

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The Roman Iron-Age (0-400 AD) in Southern Scandinavia was quite turbulent and the population composition may have changed in this period due to immigrants from Middle Scandinavia. We have analyzed mtDNA from multiple teeth from each of 22 individuals from two different Iron Age settlements in Southern Denmark. Bøgebjerggård (founded around 0 AD) represents the lowest level of free farmers, whereas Skovgaarde 8 Km to the east (founded around 200-270 AD) represents the highest level of the society. Reproducible results were obtained for 18 subjects har-boring 17 different haplogroups. This indicates that the South Scandinavian Iron Age population was as diverse as we are today. Several of the haplogroups (R0a, U2, I) observed in Bøgebjerggård are rare in present day Scandinavians. R0a, harbored by a male is frequent in East Africa and Arabia but virtually absent amongst modern Northern Europeans. We suggest that this subject was a soldier or a slave, or a descendant of a female slave, from Roman Legions stationed in Northern Germany. In contrast, Skovgaarde harbored haplogroups that are common in modern Scandinavians, and the Bøgebjerggård and Skovgaarde population samples differ significantly (P = 0.01). Skov-gaarde may represent a new upper-class formed by migrants from Middle Scandinavia.

22nd Congress of the International Society for Forensic Genetics

21–25 August 2007 - Copenhagen - Denmark

P-183 Evaluation of 12 Y-chromosome STR loci in Western Mediterranean populations

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The analysis of Y-chromosomal STRs has become a very useful tool in forensic casework. A practical approach is to combine a number of the most polymorphic markers in a Y-STR multiplex to increase the power of discrimination. The most common set is the Minimal Haplotype, consisting of nine STR loci well characterised in a multicenter study. Here we present a population study of 554 males belonging to seven Western Mediterranean populations (Valencia, Majorca, Ibiza (Spain), Sicily and three cities of Calabria (South of Italy)). We determined the 12 Y-STR loci (minimal haplotype extended by loci DYS437, DYS438 and DYS439) included in the Powerplex Y System (Promega), with the aim to establish a Y-STR haplotype database. Among the 554 males analyzed, 443 different haplotypes were obtained, of which 372 were unique. The other haplotypes were shared by two to seven men: 51 were present in one population, 17 in two, two in three and one was found in four populations. The two most frequent haplotypes were both found in seven Ibiza men. A remarkable haplotype diversity of 0.9988 ? 0.0002 was observed. The haplotypes were searched against the Y-Haplotype Reference Database (YHRD) and 151 haplotypes matched to at least one YHRD sample.

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P-184 Resolution of African versus non-African origin using a likelihood based method and 11 Y-chromosomal STRs

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1. Introduction Determining the ethnic origin of biological traces of a crime or unrecognizable human remains has forensic interest. While autosomal STRs have been applied to differentiate between major ethnic groups, Y-chromosomal genetic markers are better suited for this purpose. Y-chromosomal SNPs indeed permit prediction of ethnic affiliation. Since only few forensic DNA laboratories routinely apply SNPs, we have investigated the feasibility of metapopulation determination using a panel of widely used Y-chromosomal STRs. 2. Material and Methods Three Y-chromosome databases were selected, representing Caucasian, African and Asian origin. Allelic frequencies for the following Y-STRs were taken into account for the determinative strategy: DYS19, DYS385 a/b, DYS389 I, DYS389 II, DYS390, DYS391, DYS392, DYS393, DYS438 and DYS439. Statistical analysis was performed using a multistep approach. First, likelihood ratios (LR) were calculated by dividing - for each STR - the frequency of each allele of the sample's haplotype between the three metapopulations: Caucasian / African, African / Asian and Caucasian / Asian. Then, these individual ratios were multiplied across the 11 STRs, producing a combined LR. For each combined LR, a value above 1 means that the metapopulation in the nominator is more likely than the metapopulation in the denominator. The metapopulation which is more likely than the other one in 2 of the 3 calculations, is ultimately the most likely metapopulation. In order to examine the reliability of this strategy, it was performed on 488 males of known ethnic origin. We thus tested a representative sample of world populations: light (Flemish), and dark skinned Caucasians (Moroccans), diverse Africans (Afro-Belgians and Afro-Americans), North (Japanese) and South Eastern Asians (Indonesians). 3. Results and Discussion, Population N Metapopulation Correctly assigned Flemish 105 Caucasian 77 (73%) Moroccan 58 Caucasian 23 (40%) African Belgian 91 African 83 (91%) African American 92 African 66 (72%) Indonesian 86 Asian 76 (88%) Japanese 56 Asian 50 (89%) Total 488 375 (77%) As shown above, the overall accuracy of this strategy for estimation of ethnicity is 77%. Differententiating only Africans and non-Africans, the following binary classification square is obtained:

	True African origin (physical appearance)		
	yes	no	
African origin according to Y-STR test			
yes	149	18	167
no	34	287	321
	183	305	488

Thus, if only used to resolve African versus non-African, the positive predictive value of this method becomes 89%, while it correctly excludes African origin (negative predictive value) in also 89% of the cases.

P-185 Genetic Polymorphisms of four X-STR Loci: DXS6797, DXS6800, HPRTB and GATA172D05 in a Peruvian population sample

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MSc, Builes Juan José, GENES Ltda. Medellín – Colombia, Instituto de Biología, Universidad de Antioquia. Medellín-Colombia, The X linked short tandem repeats (STR) markers have proven to be very useful tools for paternity testing when the disputed child is female. The aim of this study was to describe the genetic polymorphism of four X-chromosomal STR loci (DXS6797, DXS6800, DXS6807, HPRTB and GATA172D05) in a Peruvian population sample. PCR products were separated in 4% acrylamide-bis-acrylamide denaturing gels followed by silver staining. Allele size determination and genotyping were performed according to recommendations of the DNA Commission of the International Society of Forensic Genetic using the allelic ladder manufactured at home and based on DNA controls including K562, 9947A (Promega Corporation). Gene frequencies were calculated using ARLEQUIN version 3.01. Population genetic data were obtained by analyzing 172 unrelated Peruvians (99 females and 73 males). (contact: genforense@genesltda.com).

P-186 Y-chromosome markers distribution in Northern Africa: high-resolution SNP and STR analysis in Tunisia and Morocco populations.

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Background and purpose: At the beginning of 2006 more than 301.000 immigrants resident in Italy resulted to come from Tunisia and Morocco, 66% of which are male subjects in addition, it is estimated that some other thousand are clandestine. Our data show that there is an increasing involvement of Tunisian and Moroccan individuals in paternity testing and in individual identification cases. For these reasons, the aim of this work was to enrich forensic Y chromosome databases with Northern Africa data to better know markers frequency and their distribution across these populations (in YHRD there are 246 Tunisian samples and 0 from Moroccan, access date to www.yhrd.org: 6 April 2007). Method: One hundred Tunisian and Moroccan healthy male donors were typed by 17 microsatellites extended haplotype and 38 Y-SNPs. Haplotype diversity, haplogroups frequencies and Y-STR haplotype diversity within each haplogroup were estimated. Results: A high resolution level database was created, including both haplotype and haplogroup for each sample. Conclusion: This study showed that precious informations may come both from Y-SNPs haplogroup distribution and microsatellite variability inside each SNP clade, besides Y-STRs data.

P-187 Population data for D10S1248, D14S1434, and D22S1045 miniSTRs loci from the Marches region (Central Italy).

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Background and purpose: The suitability of miniSTRs in degraded DNA typing has been well demonstrated. In order to collect allele frequencies of the "NC01" miniplex loci, a population study was performed. Method: D10S1248, D14S1434, and D22S1045 loci were investigated in a sample of 157 unrelated healthy individuals living in the Marches region (Central Italy). DNA was extracted from peripheral blood or buccal swabs. Allele frequencies were collected and forensic parameters were calculated. The population sample was also genotyped by Profiler Plus® kit (Applied Biosystems), in order to compare forensic parameters of miniSTR loci with nine of the most used STRs. Results: The amplicons observed in this population were between 67 and 119 bp in size. The frequency distribution showed no deviations from Hardy–Weinberg equilibrium expectations. The accumulated power of discrimination was 0.998587, and heterozygosity more than 0.7 was observed for all the three microsatellites. Conclusion: The accumulated power of discrimination and heterozygosity observed in this local database, make NCO1 miniplex a further tool to add in human identification for degraded specimens.

P-188 Egyptian population specific forensic and paternity parameters.

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for many years there were many studies to determine the specific forensic and paternity parameters which are very unique for each population group.So,we did our study to determine the specific forensic and paternity parameters for the Egyptian Population . The study was done for seven STR loci which are VWA, TPOX, CSFIPO,D75820,D3S1358, D13S317 and D21S11. Samples were collected from 197 Egyptien volunteers(100 unrelated individuals and 97 related individuals) The DNA was extracted using QIAampDNA blood Mini Kit (qiagen) The quality and quantity of extracted DNA were estimated by using minigel elecrtophoresis. All samples were amplified successfully as a co amplification of seven tetra nucleotide repeats STR loci plus amelogenin locus in single tube via PCR technique (Identifiler PCR amplification kit-AppliedBiosystems Lot n: 0506003 detection of all samples was done using AB3130 genetic analyzer Data was collected using data collection software version 3.0 and was analyzed using gene mapper version 3.2 Statistical analysis : the analysis was performed using GPA(lewis and zaykin,1999) TFPGA (miller, 1998) Power stats (Tereba 1999) software

P-189 Peopling of Central Yakutia inferred from ancient DNA analysis

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The Yakuts represent the northernmost Turkic-speaking pastoralists population in the world. Their cattle- and horse-breeding economy as well as their language contrast strikingly with the neighboring populations inhabiting Oriental Siberia. Considering these differences it has been widely assumed that the Yakuts migrated from a southern area under the pressure of the Mongol expansions. During their migration the ancestors of the Yakuts settled in the territory originally occupied by Tungusic-speaking reindeer-herders and hunters. Over the last three years, 64 ancient individuals dated from the 3rd century BC to the 18th century AD were analyzed by Y-chromosomal STR and SNP typing and by HV1 direct sequencing. Owing to the exceptional state of preservation of the bodies, we were able to test various methodological approaches for DNA extraction on different tissues to ensure the authenticity of the results. Our results bring new insights on early contacts between nomadic tribes from the southern steppes and peoples from Oriental Siberia. Moreover, the Yakuts seem to have experienced a important founder effect regarding the paternal lineages whereas the mtDNA data reveal admixture with autochthonous women. Finally, the Yakut gene pool appears as highly homogenous in spite of the important cultural modifications underwent by Yakuts throughout history.

P-190 Study of the genetic relationship and diversity patterns in Azores based on 15 STR markers

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The Azores, the largest Portuguese archipelago, is composed of nine islands unevenly distributed by three groups: the Eastern group (São Miguel and Santa Maria), the Central (Terceira, Pico, Faial, São Jorge and Graciosa), and the Western group (Flores and Corvo). Here, we describe the genetic diversity patterns for each Azorean island and their genetic relationship based on a total of 554 samples. Genotyping was carried out by the multiplex STR system PowerPlex® 16 (Promega). The results demonstrate that the average gene diversity values vary between 0.768 and 0.797 for Corvo and Terceira, respectively. The comparison of these data with mainland Portugal (0.765) reveals higher values for the Azorean islands. The dendrogram shows that the most distantly apart are Flores and Corvo however, there is no genetic differentiation between all islands. Moreover, we observe a clustering of the island's populations by their geographical and socio-cultural proximity. On the other hand, historical records mention a differential settlement between islands. The results show that, nowadays, there are no regional genetic differences in the archipelago due to internal migration. Taken together, the knowledge here obtained will provide insights about the allelic structure of health-related genetic variation in the Azorean population.

P-191 The Portuguese genetic background in analysis: São Miguel Island (Azores) versus mainland Portugal

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To study the genetic diversity of São Miguel's population we compared 21 microsatellite loci in 204 individuals from São Miguel island and 103 individuals from mainland Portugal. The results show that São Miguel and mainland Portugal populations have an average gene diversity of 0.767 and 0.765, respectively. TPOX and D17S976 markers have the lowest (~0.6) and highest (~0.9) values, respectively, of gene diversity for both populations. Allele frequencies of all markers are comparable to other European populations. This result is corroborated by the genetic relationships analysis based on the NJ tree and principal component, where São Miguel, and probably, Azores is closely related to mainland Portugal. The comparison of FIS values for the mainland (0.0326) and the São Miguel (0.0111) samples suggests higher inbreeding in the mainland. Overall, the data suggests that São Miguel population does not show population structure and is behaving as an outbred population with high genetic diversity. Taken together, the data complements the settlement history of the São Miguel island and of the Azorean population, and will be crucial to predict and explain genotypes implicated in genetic diseases in the Azorean population.

P-192 Analysis of the linkage disequilibrium extension in the Azores Islands (Portugal)

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The design of genetic studies of complex diseases is dependent on the extension and distribution of linkage disequilibrium (LD) across the genome and populations. Here, we characterize the LD extension in the Azores (Western, Central and Eastern islands groups) and mainland Portugal populations. LD was evaluated in three chromosomic regions: the Xq13.3, the Y chromosome and the HLA (6p21) for the Azorean population. The results obtained in the Western group for the Xq13.3 markers provide significant evidence of LD in 10 comparisons, after correction. In the São Miguel island population, the assessment of LD for HLA demonstrated a total of 13 out of 36 pairs with significant LD, with the largest genetic distance (2.5 Mb) between HLA DRB1 and D6S265. The pairwise association between Y markers in the Azorean population revealed 46% pairs with significant LD. In addition, D' analysis indicates that Western group presents higher values when compared with the Central and Eastern groups. Taken together, the data show that the Azorean population presents a lower D' (0.142) compared with mainland Portugal (0.226). Although, both populations do not show extensive LD, the easy reconstruction of large pedigrees in the Azorean population is a valuable resource for the fine mapping of disease genes.

P-193 Y chromosomal haplogroup distribution in the Hungarian population

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Based on the recommendations of YCC collaborative effort is necessary to study genetic variation on the human Y chromosome. Determining human Y-SNP haplogroups is the most useful tool in tracing human histories that have arisen a single time in human evolution. Testing of 50 Y-SNPs with TaqMan assays and 11 Y-STR loci with PowerPlex Y was performed in 119 independent Hungarian male samples. Genetic distances with 23 other populations were calculated based on haplogroup frequencies with AMOVA implemented in Arlequin2.0. Based on calculated distances tree was generated using neighbour joining method (Phylip 3.66). Haplotype and haplogroup diversity values were calculated according to Nei. Based on the data the major Hungarian Y-chromosomal haplogroups were R1a1-M198 (22%), R1b-P25 (16%), I1b-P37 (16%) and I1a-M253 (8%). Our previous data showed that the major Romani haplogroups in the Hungarian Romani population were J2f-M67 (19%), H1-M82 (16%) and E3b1-M78 (12%), while in the Hungarian population the same frequencies were significantly lower (J2f-M67 2%, H1-M82 7%, E3b1-M78 3%). According to the last census the presence of the Romanies, which is the biggest minority group in Hungary was 0.7% (2004), but because they often deny their Romani origin their real presence is estimated 3.5-10%.

P-194 Refining the analysis of Y-chromosomal diversity in Alentejo (Portugal)

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Sickle cell anemia (HBB*S) and glucose-6-phosphatedehydrogenase (G6PD) deficiency, present a clinal distribution in Portugal, being more frequent in the South and showing foci of high prevalence in some places from Alentejo such as Coruche and Pias. Since the reconstruction of the evolutionary history of G6PD deficiency alleles and HBB*S lead to conclude that Sub-Saharan Africa was the place of origin of many of them, it is likely that at least some were introduced in Alentejo by Sub-Saharan individuals whose presence in the region is known to have had considerable demographic impact. To evaluate the male mediated Sub-Saharan influence in present-day populations, we have performed a high resolution analysis of 16 STRs and 23 SNPs of the Y-chromosome in 91 males from Coruche and 54 from Pias. The results showed the absence of any haplogroup of Sub-Saharan origin and a Y-chromosome composition not differing from those previously reported for other Portuguese mainland regions. Therefore, from the forensic point of view the studied populations can be dealt without special concerns. In the future, diversity in mtDNA and autosomal markers will be assessed in order to evaluate if absence of heterogeneity also holds true for those markers.

P-195 Y-chromosomal STR haplotypes in a Gypsy population from Portugal

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We have analyzed seventeen Y-chromosomal STR loci (DYS19, DYS389 I and II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS439, DYS438, DYS456, DYS458, DYS635, GATAH4.1, DYS437 and DYS448) in a sample of 126 unrelated Portuguese Gypsy males. Fifty three different haplotypes were detected out of which 39 were found only once. Three haplotypes were observed at the considerable high frequencies of 15.9%, 13.5% and 8.7%. The percentage of unique haplotypes (71.2%) was rather low, particularly given that the lineages were defined by 17 different Y-STRs. Accordingly, the haplotype diversity (0.9437) represented the smallest value up to now registered in any population for that set of loci. Comparisons with Gypsy groups from Bulgaria, Lithuania and Spain as well as with their host populations were performed based on the set of 7 Y-markers common to all studies. The Portuguese Gypsies showed significant differences from non-Gypsy populations from Portugal, Spain and Bulgaria as well as with Bulgarian Gypsies. Although the genetic distance with the Lithuanian group was larger than with the Spanish group, none of the values reached statistical significance. The Gypsy populations from Portugal, Spain and Bulgaria revealed to be more closely related to each other than with non-Gypsy populations from their own countries

P-196 A study of the Effectiveness of the New Zealand National DNA Databank

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Dr. John Buckleton, Institute of Environmental Science and Research Ltd, New Zealand

In 1996 the first DNA profiles were obtained from individuals and loaded to the New Zealand National DNA Database (NDD) following the introduction of the Criminal Investigations (Blood Samples) Act in 1995. Since that time more than 70,000 profiles from individuals and 16,000 profiles from crime scene samples have been added to the Databases generating hit rates of 34% (crime stain to crime stain) and 58% (crime stain to person) respectively. Following the highly successful implementation of the National DNA Databank our attention turned to maximising the effective use of the information held. Legislative changes introduced to enable more effective use of the data enabled a programme to "back capture" offenders already in prison at the time the original legislation was enacted to be undertaken. We also embarked upon research aimed at the effective use of familial searching in the solution of crime. In this presentation I will summarise the successful outcomes of the New Zealand National Databank, focusing on the results of the research we have done surrounding familial searching, positively demonstrating it's worth and on the outcomes of the "backcapture" of offenders. These results will be presented against an ethical and legislative framework and issues of public perception.

P-197 Investigation of a gamma model for mixture STR samples

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The behavior of PCR Amplification Kit when used for mixture STR samples is investigated. A model based on the Gamme distribution is fitted to the amplifier output for constructed mixtures and the assumptions of the model is evaluated via residual analysis.

P-198 Mutation rates of STR systems in Danes

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Assoc. Prof. E. Susanne Christensen, Aalborg University, Denmark
Assoc. Prof. Poul S. Eriksen, Aalborg University, Denmark
Forensic Geneticist Charlotte Hallenberg, University of Copenhagen, Denmark
Assos. Prof. Malene Højbjerre, Aalborg University, Denmark
Prof. Niels Morling, University of Copenhagen, Denmark
Forensic Geneticist Bo Simonsen, University of Copenhagen, Denmark

Danish paternity cases in the period 1999 to 2005 were investigated regarding to mutation rates in STR-systems. STR-typing was done with the Applied Biosystems Amplfstr Profiler Plus kit in the period 1999 to early 2005, and by Applied Biosystems Amplfstr Identifier for the rest of 2005. Test for Hardy Weinberg equilibrium revealed the presence of two subpopulations - one defined by paternity cases in courts in the geograhic area of Denmark and another originating from Greenland. Further investigations were performed without the data from Greenland. Sex and STR-system specific mutation rates were estimated for each kit and the 95% confidence limits were calculated using a fiducial argument (Clopper. C.J & Pearson, E. S . (1934). The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika, *26*, 404-413) [Mangler der noget? Nej der er bare smuttet en parentes for meget ind i referencen – se ovenstående blå deleted)] By Fisher's exact test, we found no significant interaction between sex and STR-system in the mutation rates, meaning that the relavtive difference in male and female mutationrates can be assumed constant over STR-systems. Male mutationrates are estimated to be approximately 3 times the corresponding female mutationrate. Also no significant interaction between the two kits and the STR systems common to them. By aggregating common STR systems over kit and sex, we found significant differences in mutation rates between STR-systems.

P-199 Heterogeneity in coding mtDNA mutation rates: implications in forensic genetics

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The high heterogeneity observed in mitochondrial DNA (mtDNA) hypervariable regions (HVI and, especially, HVII) renders match evaluation difficult in many forensic cases, especially with degraded and hair samples. It was expected that the slower evolving coding region would give vital additional information in the resolution of those cases. Accordingly, some SNapShot multiplex kits were developed based on mtDNA coding polymorphisms. However, recent phylogenetic analyses are indicating that there is also a high heterogeneity for coding region mutation rates, with some positions being hotspots, recurring in different and even in the same haplogroup. We will present which coding regions show higher heterogeneous mutation rates. These values were inferred from maximum likelihood phylogenetic reconstructions of two worldwide complete mtDNA published datasets, by using a model of site partition. The coding region hotspot polymorphisms will be compiled and checked against the available SNapShot multiplex kits. We will be able to advise if it is recommended to use a more reliable position for a certain haplogroup assignment aimed by the kit. This evaluation has a posterior application, by explaining some unexpected results between HVI-HVII sequence and SNaPshot typing, and a priori application, to an informed design of SNapShot multiplex kits.

P-200 A worldwide database of autosomal markers used by the forensic community

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Worldwide genetic databases are useful tools in forensic genetics, where any genetic individual profile must be compared against a population dataset. The success of the Y-chromosome database (http://www.yhrd.org/index.html) clearly shows it. Some recent catastrophes called the attention to the inexistence of an easy-available worldwide autosomal database, for evaluation of the multiethnic genetic profiles. In fact, the ENFSI DNA WG STR Database (http://www.str-base.org) registers only data for 24 European populations. We are compiling a huge dataset published in the main forensic international journals and developing a user-friendly web interface for its utilisation by the international forensic community. Most published data consist in allelic frequencies, which, given the broad geographical scale aimed in this database, will contribute new insights on general STRs informative power and on population genetics analyses. The database will have the obvious limitation of not performing a quality control of data, relying on the acceptance criteria used by the main forensic international journals. We aim to turn all the data freely available, so that people can perform their own tests.

P-201 SNPY – A community repository for Y-SNPs

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This poster describes an interactive website aimed to support the validation and application of Y-SNPs in forensic analysis. The classification of Y-Chromosome profiles is based on a stable phylogenetic tree, which was first published by the Y Chromosome Consortium (YCC) in 2002, but lacks curation in the last five years. For forensic application and databasing a reduction and evaluation of the tree and the branch-markers is necessary. The website should help to generate an agreeable and non-biased collection of markers and resulting "core" tree to allow the identification of major worldwide population clusters in a forensic context. The main concepts of this community repository are: -Three marker/branch quality sets: core, valid and public -All information is editable by registered users (core and valid markers/branches can only be modified by curators) -Open curator group -Online graphical representation of the current tree (core, valid or all branches) -Computer supported validation process for public markers/branches -High level of marker detail (e.g. primers and protocols) -Attachment of original publications (if not prohibited by copyright)

P-202 Genetic Profile from the DNA Databank of a Brazilian Missing Kids Program

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Since September 2004 a missing kids program called "Caminho de Volta" has been developed in Sao Paulo State, Brazil, to help the local authorities to face the problem. The program not only offers psychological support for these families but also provides a DNA databank to biological identification. The allele frequencies for 15 STR markers (AmpFISTR Identifiler kit - Applied Biosystems) were evaluated in 561 unrelated individuals from families that are under attendance by our group. The statistical tests were performed using the exact test of Fisher (GDA software v1.2) and the PowerStats v1.2 spreadsheet. No significant departure from Hardy-Weinberg equilibrium expectations was found. The most polymorphic loci were also the most discriminating: D2S1338 (PD: 97.6%), D18S51 (PD: 97.5%), FGA (PD: 97.1%) and D21S11 (PD: 96.3%). We observed one individual with the rare allele 45.2 for FGA marker allele 6 for D18S51, and allele 6 for D5S818 marker. There are not so many families included in our DNA databank of missing kids but it was already possible to identify three dead individuals (8, 14 and 17 years old) by DNA profile search. Recently the databank has been used also for confirmation of biological family's relationship of care placement children (FAPESP-LIM-40 –HC-FMUSP).

P-203 Applying the 16 Y-chromosome STRs in the population of central Poland

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Haplotype and allele frequencies for the panel of 16 Y-chromosome STR loci, namely DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385, DYS393, DYS391, DYS439, DYS635, DYS392, Y GATA-H4, DYS437, DYS438, DYS448 were determined in a population sample of 200 unrelated males from the central region of Poland. The 191 different haplotypes were identified, of which 182 haplotypes were unique and 9 were duplicated. None of observed haplotypes appears more than two times in the investigated population. The haplotype discrimination capacity was 0.955, and combined gene diversity was 0.9999. The analysed set of 16 Y-STRs is very useful in forensic practise to identify males and trace paternal lineages.

P-204 Population data of STR loci included in FFFL tetraplex in cental Poland.

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Allele distribution and statistical parameters for fluorescent quadruplex including following STR loci: LPL, F13B, FES/FPS and F13A01 were determined in a population sample of 200 unrelated individuals from central region of Poland. The applicability and usefulness of the investigated markers were evaluated in paternity testing and in personal identification. Combined values of power of exclusion and power of discrimination were 0.9004 and 0.9994, respectively. The allele frequency determination and forensic evaluation allow to use this system as an extension of commonly applied autosomal multiplexes in relevant population. This extension may be necessary to resolve most difficult cases in forensic practise.

P-205 Polymorphism of pentanucleotide STR markers in Polish population sample

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Since the application of genetic markers in forensic practise requires availability of suitable profile database, the allele frequency and forensic evaluation of four pentanucleotide markers: Penta B, C, D, E in central Poland population were established. Penta B, D and E show a high degree of polymorphism and heterozygosity i.e. 0.903, 0.859 and 0.908, respectively. While penta C is the least informative marker with heterozygosity of 0.548. Power of exclusion of Penta B, D, E and C markers in the investigated population were 0.802, 0.714, 0.812 and 0.233 respectively. Application of four investigated markers gives the total value of power of discrimination of 0.999989 and typical paternity index of 111.138. It makes the markers, especially Penta B, D and E, very useful genetic tool in complex cases of kinship analysis.

P-206 The most efficient STR loci in forensic genetics in population of central Poland

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Twenty eight tetranucleotide STR markers were analysed from a point of view of their applicability in forensic genetic practise. Polymorphism, discrimination and paternity exclusion indices for particular loci were compared based on allele distribution in central Poland population. The highest heterozygosity of 0.945 and polymorphism information content of 0.950 were determined for the SE33 locus. The 33 different alleles, with frequency bellow 0.1, were revealed in relevant population. With the highest power of discrimination of 0.991, and power of exclusion of 0.888, locus SE33 is the most useful marker in forensic identification as well as in kinship analysis. After SE33, the subsequent most informative markers, out of the 28 investigated in central Poland population, are D2S1338 and D12S391, which reach power of discrimination of 0.972 and power of exclusion of 0.761.

P-207 Analysis of the HVI, HVII and HVIII regions of mtDNA in Venezuelan population

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We analyzed the sequence polymorphisms of hypervariable regions HVI, HVII and HVIII of mitochondrial DNA in a sample of 100 unrelated Venezuelan individuals living in Caracas city (Venezuela). Polymorphisms were detected using dye terminator chemistry and capillary electrophoresis. Excluding those polymorphisms in the homopolymeric cytosine stretch (C-stretch) regions, we identified in HVI, HVII, and HVIII, 81, 48 and 18 polymorphic sites respectively. When sequences of HVI+HVII and HVI+HVII+HVIII were separately analyzed, we identified a total of 76 and 77 different mitochondrial haplotypes, respectively. Taking the three regions together, we found that 66 haplotypes (85.7%) were unique. The most frequent haplotype was 16111T, 16223T, 16290T, 16319A, 16362C, 73G, 146C, 153G, 235G, 263G, 309.1C, 315.1C, 522d, 523d found in ten individuals (10%). A statistical estimate of the results showed a random match probability of 2.48% (HVI+HVII) and 2.28% (HVI+HVII+HVIII). The genetic diversity was 0.9851 and 0.9871 for HVI+HVII and HVI+HVII+HVIII, respectively. Our results showed that the number of different haplotypes found when HVI+HVII and HVI+HVII+HVIII are separately analyzed differs by only one haplotype. In conclusion, for our database the inclusion of HVIII region added little to its discrimination capacity, but the information could be useful for comparative analysis with other population databases.

P-208 Comparing the growth and effectiveness of forensic DNA databases

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Background: Forensic DNA databases have altered the landscape of the criminal justice system and re-shaped the field of forensic science. They have provided new challenges to the mechanisms by which forensic evidence can be utilised and increased the onus of responsibility on those who administer their use. The growth of DNA databases internationally has been rapid with millions of STR profiles now held from convicted offenders, suspects and unsolved crimes. Links provided through DNA database searches have contributed valuable intelligence to thousands of police investigations, often for crimes which are notoriously difficult to resolve. There has been widespread review and commentary regarding the legal and socio-political basis of DNA databases, but there remains a lack of meaningful empirical assessment of database performance and effectiveness. Method: In this research DNA database growth and effectiveness has been assessed statistically from data collated from a number of Australian and international databases. Results: A rudimentary comparison of growth allows predictions to be made about future database expansion and drivers for effectiveness. Comparing database performance shows a correlation between the numbers of crime samples and database returns (in the form of investigations aided). Conclusion: Modelling DNA database performance is an important mechanism to assess correlates between sampling strategies and return from the database operations.

P-209 A likelihood ratio approach to familial searching of large DNA databases

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Familial searching is an investigative search technique which seeks to identify close relatives of an unknown suspect, in a DNA database. As close relatives are likely to share more alleles than do unrelated people, the database profiles can be ranked according to how likely they are to be relatives of the unknown stain donor. In the UK, this approach has been approved for use in serious cases and has been successfully applied in several high-profile crimes. The UK National DNA Database (NDNAD) is the world's largest intelligence database containing approximately 3.5 million SGM and SGM+ profiles. Familial searching of databases of this size presents a particular challenge, since huge lists of potential relatives are generated, all but very few of which are false hits. We report here the expected performance of a combined pre-filtering (on shared allele numbers) and likelihood ratio approach, using sets of randomly generated profiles with known relationships. Likelihood ratios for parent-child and sibling relationships are calculated for all potential relatives. A simulated DNA database of 5 million individuals with a similar composition to the NDNAD was constructed for this performance testing. Familial searching is found to be effective, with true relatives being identified near the top of ranked lists in a majority of cases. Keywords: Databases, Familial searching

P-210 The National DNA-database in Sweden: a survey about the new legislation passed in 2006 and it's effects on match reporting

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The Swedish legislation for the national DNA-database was first approved in 1999. However it was generally considered to be too restrictive. In 2003 the government initiated a legislative process which included a careful investigation and analysis of the consequences of the different suggestions for amendments. In 2006 a new law was passed. At the National Laboratory of Forensic Science it was proceeded by an intense preparation, both technically (i.e. robot usage, methods, LIMS improvements) and regarding staff recruiting and training. A vast increase in the amount of reference samples was expected along with a corresponding increase in the number of matches to be reported. CODIS and the LIMS manage loading, excluding, searching and matching of DNA-profiles. The database contains three sections with the following criteria for the registration of DNA-profiles (SGM-plus). Crime Scene Samples (DNA-typing during investigation). Suspects in ongoing cases if the crime may result in a prison sentence. Convicted Offenders if the person has been sampled as a suspect and is not only fined. This presentation includes a survey about the legislative changes. It also includes statistics showing the positive effects of the increased number of DNA-profiles and matches between crime scene samples and individuals.

P-211 Towards a Systematic Probabilistic Evaluation of Parentage Casework in Forensic Genetics: a Modest Attempt to Define a General Standardized Approach to Simple and Complex Cases

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In view of the increasing demand of ever complex parentage casework, our lab saw itself in the position to define a strategy the more standardized as possible to deal with the bulk of those cases. We search a coherent and exhaustive way to find answers, avoiding a multiplicity of different case complexity-dependent approaches. The major aim of this communication is to present the heuristical, mathematical and other aspects of a probabilistic evaluation procedure that has been developed since 2003. The mathematical aspects are based in general probability theory and Bayesian interpretation of probability. We also present casework examples to illustrate some of the problems that have to be faced.

P-212 Identifying factors that influence the STR DNA typing success for touched objects

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Samples coming from touched objects, or contact stains, are becoming the most frequent category of stain analyzed by the 6 official forensic DNA laboratories in Switzerland. For instance, in Lausanne, the proportion of contact stains received by the laboratory increased from 27% to 54% during the first semesters of 2002 (N = 565) and 2006 (N = 1094), respectively. In this study we were interested to identify the main factors that influence the STR DNA typing success for this kind of stain, which is generally characterized by the occurrence of minute amounts of DNA. Data (kind of contact, single or double swabs, DNA quantification, etc.) were gathered for more than 1'500 contact stains analyzed in routine from March 2006 to April 2007. DNA was extracted from these samples with the QIAshredder and QIAamp DNA Mini kit and amplified with the SGM Plus kit. A LCN strategy with 34 PCR cycles was used when necessary. Overall, DNA profiles were sent to Swiss DNA data base for about 25% of these samples. Detailed statistics are presented on the poster.

P-213 Genetic Population Data of 15 Autosomal Loci from Central Region of Venezuela

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Fifteen autosomal markers (CSF1PO, TH01, TPOX, F13A01, FESFPS, VWA, D16S539, D7S820, D13S317, D5S818, D3S1358, D8S1179, LPL, F13B, and D1S80) were evaluated in order to test their usefulness in forensic and filial relationship studies. 1031 non related individuals living in the Central Region of Venezuela were typed for at least 6 of the above markers Allele frequencies for each marker, as well as their statistical parameters of forensic interest are reported. All markers are in are in HW equilibrium (p>0,05), except FESFPS, TH01 and LPL due to Heterozygote deficiency (p<0,05). After sequential Bonferroni corrections LPL and FESFPS are in HW equilibrium at the p-0,05, and p-0,01 respectively whereas TH01 is still not in equilibrium at both nominal levels. The presence of population substructure and of presence of either null or dropout alleles in our population were discarded using the software STRUCTURE and MICRO-CHECKER, respectively. No linkage disequilibrium among each pair of loci (p>0,05).was observed when a sub sample of the 117 individual was used for which all 15 markers were typed. This battery of 15 markers presents a Combined Power of exclusion of 0,9999946 and a Typical combined paternity index of 139391, values that support its efficiency regarding paternity tests.

P-214 STR data for 15 AmpFLSTR Identifiler loci in the Moroccan population

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For forensic applications, allele frequency data for 15 short tandem repeat (STR) loci, D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA, were determined in 500 unrelated individuals representing all ethnic groups in Morocco using AmpFlSTR Identifiler kit (Applied Biosystems). DNA was extracted from blood samples, by the DNA IQ System (Promega) and the PCR products were typed using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The results were analyzed using GeneMapper ID v3.2 software (Applied Biosystems) and The values of heterozygosity, polymorphic information content (PIC), power of discrimination (PD), power of exclusion (PE), paternity index (PI) and matching probability (pM) were calculated.

P-215 How many Markers are enough for Motherless Cases of Parentage Testing

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In motherless cases of parentage testing, only the alleged father and child pairs are tested. The absence of mother sample increases the probability of false paternity inclusion. How to avoid false inclusion of paternity should be considered. This work presents simple criterions needed with this consideration, to analyze motherless cases of parentage testing. Using STR markers, motherless cases have been investigated. We focus on the relationship between the probability of exclusion and the value of likelihood. It is emphasized that using the correct formulae for calculating the average probability of exclusion is necessary, because the false formulae generally overstate the power of a test battery. We suggest two criterions for paternity inclusion. If both criterions have been reached in the motherless cases of parentage testing, paternity is practically proven.

P-216 The New Genetic Database of Argentina

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A new Criminal Database, proposed by our Latin American Society For Forensic Genetics, is ready at the maximum level of the Argentinean National Government, in order to promulgate a law that represents a positive contribution to the pressing subject of the security, fighting against the crime, and that at the same time contemplates and care the human rights of guilty persons and their victims. We have been presenting several proposals from 2003, in some cases with small successes. Ephemeral, by the way, like the resolution of the Ministry of Justice 415/2004, that created the Registry, but never it was carried out. From there, a dozen of projects has been presented by other organizations, but no one has prospered, although all the papers say almost the same. In fact, we did not invent anything either: we based our proposals on the successful European and American experiences (see notes in spanish in www.slagf.org). This work describes the antecedents and characteristics of the Argentinean criminal database, which is similar to the American CODIS, but with some differences because of the local law and procedures of the Justice (see "Organizing The Argentinean Combined DNA Index System", Penacino G, Progress in Forensic Genetics 11, Elsevier Science, 2006)

P-217 Examples of combining genetic evidence – Bayesian network approach

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Bayesian networks provide a valuable tool in addressing a range of issues that affect the coherent evaluation of evidence. The aim of the study was to implement existing ways of probabilistic evaluation of DNA evidence in building appropriate network structures. We present some cases of identification of human remains using different genetic markers, which have a different inheritance mode. As a result, several different pieces of evidence could be put together, providing a single combined likelihood ratio. Autosomal DNA estimate is formed by the product rule, but mtDNA or ChrY-STR and ChrX-STR estimates are the frequencies in the appropriate databases, so multiplying them may provide the first estimate of the joint probability. But in general cases, these estimates may be conditionally non-independent items of evidence, given some alternative hypotheses. Hence we combined some local networks that separately represented existing likelihood ratios using Hugin software. In conclusion, applying a Bayesian network approach to some routine cases of human identification provides a coherent evaluation of different items of evidence, which would otherwise be difficult to achieve.

P-218 SNPs for the analysis of human pigmentation genes - a comparative study

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In specific forensic cases, a genetic marker set allowing to deduce information about phenotypic features of the individual in question may be helpful to investigators. Candidates for such markers include pigmentation genes relevant for eye, hair and skin colour. The project presented here aims at the development of a marker set which may be able to derive phenotypic information regarding the pigmentation pattern of an individual from DNA. A set of eleven single nucleotide polymorphisms (SNPs) for which linkage to such phenotypic information has been published in the recent literature, have been selected to develop an initial assay based on the SNaPshot technology. A multiplex-PCR to amplify the 11 SNPs has been developed and appropriate single-base extesnsion primers have been designed. After optimisation of reaction conditions to allow efficient amplification and extension of all products in a single 2-step procedure (Multiplex-PCR and Multiplex-SBE), followed by automated allele calling using the GeneMapper 4.0 software, a first study has been performed to gather information about allele distribution in the general population. In a comparative study, two populations with strongly varying phenotypes (i.e. northern Europeans vs. sub-saharan Africans) is currently being tested for significant differences regarding the selected SNPs.

P-219 The time-course analysis of gene expression during wound healing on mouse skin

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Introduction The determination of wound age is indispensable in forensic practice. Conventional histological examination is useful for the examination of wound age. However, the age can only be assessed to some degree, because of the lack of markers in the initial state of healing process. Recently, Cooper et al. reported a portfolio of over 1000 genes expressed across repair response in a neonate mouse using microarray. The aim of our study is to establish a correlation between wound age on adult mouse and the expressional patterns of the six mRNAs that Cooper et al. reported. Materials and Methods Full-thickness dermal wounds were punched using 4mm biopsy needles. The mice were killed after various intervals of punching. Quantitative mRNA detection was performed with ABI PRISM 7900HT. Results and Discussion The level of c-fos, fos B and MKP-1 increased drastically, peaked within 1 hour and decreased to base line until 3 hours. CD14 and Ccl9 peaked from 12 to 24 hours. The increase of Mcpt5 mRNA showed on 5 days. Thus, each gene had the differentially expressional patterns with time-course. These results indicated that the comparison of the genes might be useful in evaluating wound age, especially early stage of healing skin.

P-220 Rapid prenatal diagnosis of common chromosome aneuploidies by QF-PCR: evaluation of two in vitro diagnostic test kits (Chromoquant and Aneufast).

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Prenatal diagnosis for common chromosomes aneuploidies (21, 18, 13 trisomy and sex chromosomes) in high risk pregnancies is performed by cytogenetic analyses of metaphase chromosomes (karyotype analysis). The chromosomes are obtained from fetal samples (amniotic fluid or chorionic villous) after several days of culture in vitro. This method is very accurate and it allows the detection of minor abnormalities. Nevertheless several days (two weeks) are required to obtain results. So parental anxiety becomes bigger and eventual therapeutic interventions are not possible anymore. In recent years, rapid prenatal diagnosis of common aneuploidies has been achieved by the Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) of short tandem repeat markers. This method is based on the quantitative analysis of the allele peaks on a automated DNA sequencer to determine chromosome copy number and/or imbalances between peaks. The main advantages of the QF-PCR are speed (24-48 h), accuracy and automation. Since one year, some different multiplex QF-PCR kits are commercially available for the diagnosis in vitro of common chromosomes aneuploidies. We tested two kits: Chromoquant Version 2 (Cybergene AB) and Aneufast (Genomed Ltd) .

P-221 Haplotypes and haplotype stability within a 126.6 kb region at Xq28

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The three polymorphic X-chromosomal STR markers DXS10146, DXS10134, DXS10147 are already known as linkage group in Xq28. They are located within a 79.1 kb region. By testing this cluster in a German population 1512 different haplotypes can be generated, theoretical. In fact genotyping of 233 men revealed the presence of 141 haplotypes. Recombination analysis for testing haplotype stability was performed in 115 meiosis by checking father-daughter-grandson trios. In two cases we observed crossing over, which concerned the markers DXS10146 and DXS10134 although their physical distance is only 13.3 kb. This confirms the knowledge of higher recombination activity in the telomere neighbourhood of ChrX. Further genetic analyses are required to use this STR cluster for haplotyping in kinship testing. In order to proof haplotype stability in this region we added the marker DXS7423 located only 47.5 kb distal. The new cluster spans an area of 126.6 kb at Xq28. We present haplotype data and results of recombination analysis to verify that kinship testing can also involve marker clusters with low tendency to recombine.

P-222 Somatic hypermutability of microsatellite sequences in Turcot syndrome: implications for forensic genetics

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Turcot syndrome (TS) is a genetic condition characterized by the association of colorectal and brain tumors. A subtype of TS (TS1) is caused by biallelic constitutional mutations in genes implicated in the Mismatch Repair (MMR) system, which is involved in the repair of insertions/deletions consequent to replication slippage at microsatellite sequences. TS1 patients have constitutional deficiency of MMR, which affects all cell types. We investigated allelic profiles at microsatellite loci included in the AMPFISTR Identifiler kit (Applied Biosystems) as well as at additional microsatellite sequences (di- and monucleotide repeats) in a family with 3 siblings affected by CNS (2 siblings) or colorectal cancer (1 sibling). Normal DNA isolated from intestinal mucosa of the patient with colorectal cancer was characterized by the presence of additional peaks that were not present in parental DNA. For markers included in the AMPFISTR Identifiler kit, peak sizes were usually larger by 1-2 repeat units than those of parentally derived alleles. The same peaks were observed when the loci were amplified in singleplex PCR. It is important that professionals involved in forensic genetics be aware of the existence of a genetic condition that can cause a peculiar pattern of microsatellite alterations in constitutional DNA.

P-223 Discovery of three related females who type XY at the amelogenin locus

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Discovery of three related females who type XY at the amelogenin locus We have identified a woman and her daughter who have the male variant at the sex specific locus, amelogenin. The father of the woman types male as expected. The mother of the woman is deceased and cannot be tested. The sister of the woman also has the amelogenin male variant. We have not been able to tell when this male variant has become associated with a female X chromosome, although identification of these three females with the male variant shows its Mendelian inheritance within this family. The woman was investigated for 16 genetic markers, other than amelogenin, that are known to be Y chromosome specific. She did not have any of this male specific DNA. DNA from the woman containing the male specific variant was cloned and sequenced and the sequence was found to be identical to the published male Y chromosome sequence at amelogenin. The data are novel and have implications for those performing sex determination assays using the amelogenin locus, as well as for geneticists interested in the extent of gene conversion events involving the X and Y chromosome.

P-224 Estimating human age in forensic samples by analysis of telomere repeats

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Background and purpose: Within the area of forensics it would be of great help if an age estimate on humans, dead or alive, could be done simply on the basis of DNA. The aim of this work was to optimize a molecular method based on telomere shortening to estimate the age of an individual from small amounts of biological material. Method: In humans, telomeres are tandem repeats of the hexanucleotide sequence TTAGGG. Due to the "end replication problem" telomeres, in most somatic cells, will shorten progressively thru life. With real-time PCR, the relationship between telomere and a single copy gene content in 96 blood samples were measured on small amounts of template DNA. Also, 19 samples taken 20 years apart from the same individuals were analyzed in order to study the loss of telomeres at an individual level. Results: There is a significant loss of telomeres with age. However, the individual correlation between chronologic age and the amount of telomeric repeats showed to have a standard error of prediction of 22.5 years. Conclusion: Our results showed that age estimation based on telomere shortening has too much variation to be appropriate for age estimation at the individual level for forensic purposes.

P-225 Molecular analysis of the PGM phenotype

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Historically, protein variants have been investigated for the purposes of human identification using electrophoresis and immunological techniques. These methods require considerable operator expertise and are often time consuming and labour intensive. Many of the reagents employed carry potential health risks and are becoming more difficult to obtain from suppliers. We have developed an assay for DNA analysis of protein polymorphisms and red blood cell markers, using classical typing methodology to validate the results. The SNPs underlying the polymorphic forms of selected classical systems were identified from published literature and the NCBI SNP database. Samples were genotyped using the ABI PRISM® SNaPshot™ Multiplex System (Applied Biosystems). A total of 93 samples were analysed in duplicate for a number of systems, but we describe only the results for PGM here. Concordance between the two techniques was observed in almost all cases. Analysis of non-concordant data highlighted the subjectivity of classical methods and pointed to errors associated with interpretation and run-to-run variability. Classical markers can be easily incorporated into routine laboratory testing thereby enabling reviews of 'cold cases' to be conducted. Indeed, we have successfully used this technique in two cold cases at this laboratory and these are discussed.

P-226 Association of genetic variations in alcohol dehydrogenase 4 with alcohol dependence in Italian population sample

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Alcoholic abuse has a great interest in forensic medicine, for the evaluation of driving and working ability, for permanent invalidity to alcohol-related pathologies and for the high incidence of alcohol-related deaths. It is a quantitative disorder, with a combined incidence of environmental and genetic factors. Since it was observed that variations in ADH4 gene are associated with alcoholism, SNPs mapping in different regions of ADH4 gene were selected. A new approach was performed in this study: mutations that could modify the splicing sites were selected using "Splice site prediction" available at http://www.fruitfly.orgseq_tools/sp/lice.htmlin addition, since the correct splicing is fine regulated by RNA binding proteins, SNPs that could affect splicing, destroying or creating protein target sequences and changing RNA secondary structure, were predicted by a tool available at www.introni.it. 250 unrelated volunteers, diagnosed for alcohol dependence (DSM-IV), were included in this study. SNPs typing was performed setting up 3 multiplex PCRs and minisequencing reactions. 12 SNPs were selected from literature data and 26 SNPs, among 233 polymorphisms screened, were chosen by computational analyses. The pairwise LD between any two markers was analysed through the program Haploview, to define the haplotypes block structure and identify tag SNPs associated with alcoholism.

P-227 Analysis of mtDNA Point and Length Heteroplasmy in Single Cells

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The nature of mitochondrial heteroplasmy, which is not affected by natural selection, is still open to discussion. It might be the consequence of both normal and mutant mtDNA molecules coexisting within a single cell, or alternatively, of an admixture of homoplasmic cells, which contain only one sort of mtDNA molecules. To address this question, an accurate and efficient method was designed including flow cytometry assisted cell sorting and PCR based mtDNA typing of single blood lymphocytes by cycle sequencing, minisequencing and RFLP analysis. To attain the required PCR sensitivity, the reactions were carried out on glass slides with chemically structured surfaces in a reaction volume of 1 or 2 µL. To study mitochondrial point heteroplasmy in single cells, blood samples from two donors showing a mitochondrial point heteroplasmy in direct sequencing (195Y and 234R, respectively) were analyzed. In the vast majority both the major and the minor haplotypes of the test persons were found in a homoplasmic state on single cell level. These findings clearly show that most individual blood cells are not heteroplasmic, indicating that heteroplasmy to a large degree arises from admixture of homoplasmic cells. In order to analyze mitochondrial length heteroplasmy, single lymphocytes from donors showing mitochondrial length heteroplasmy at positions surrounding position 16189 and between positions 303-309 in direct sequencing and RFLP analysis were studied. Occurrence and frequency of both point and length heteroplasmy are presented.

P-228 Evaluation of mitochondrial DNA mutations to predict age

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Ageing is a predictable, universal and detrimental process in humans. The ‘mitochondrial theory of ageing’ implies that the accumulation of mitochondrial DNA mutations, and the subsequent cytoplasmic segregation of these mutations during life, is an important contributor to the ageing process. Mitochondria have a separate autonomously replicating DNA genome and are maternally inherited. Previous research has identified accumulation of mutations in the ‘hypervariable regions’ (I and II) of the mitochondrial genome with age. In this study we analysed hypervariable region sequence variations within twelve separate families, eleven of three unbroken maternal lineages and one with four maternal lineages (ages 5 to 96). Since mitochondria are maternally inherited, any sequence difference between the youngest member of the family compared with the others was considered to be age related. No age related mitochondrial mutation/s observed in the twelve families analysed. Therefore this methodology of direct sequencing of mitochondrial hypervariable regions does not appear to be a useful tool to assist in the prediction of the age of the person from whom a biological sample was collected at a crime scene.

P-229 Forensic Detection of Marijuana Trace

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Marijuana (Cannabis sativa L.) was reported as the most seized drug during 2000-2005. In Thailand, the number of marijuana seizure cases is second to amphetamine. Chemical examinations are routinely used to detect the presence of the hallucinogenic substance called "tetrahydrocannabinol" (THC), which is specific to Cannabis sativa in alleged materials. Examination of Cannabis by DNA is not widely used in the Thai forensic community. In this study, we aimed to compare the use of both chemical and biological tests for precise screening. Marijuana leaves had been treated in simulated conditions according to the way they are consumed leaves materials were boiled in water for 5 min to 8 hr, dried in hot-air oven, shade and sunlight, and burned to black and white ashes. The THC band was detected in all samples analysed by TLC. It was found that the solvent system consisted of hexane: dioxane: methanol (7:2:1) gave the best resolution for cannabinoid separation in our hands. In contrast, the 197-bp mitochondrial trnL-F PCR-amplified fragment was detected in only one sample, i.e. the DNA extracted from fresh marijuana leaves that were boiled for 5 min. The results suggested that TLC is a robust method for the detection of THC in marijuana. However, DNA analysis seems to be limited when DNA from heat-treated materials were analysed.

P-230 Forensic application of a multiplex PCR system for the typing of pig STRs

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The Animaltype Pig PCR Amplification kit (Biotype AG), a multiplex PCR system that allows the simultaneous amplification of 11 tetrameric STRs and an Amelogenin-like locus for individual identification and sexing of pig (sus scrofa), was employed in the investigation of an alleged case of veterinary malpractice. A veterinarian responsible for collecting random blood samples from Landrace x Large White crossbred sows from a pig farm located in Piedmont (north-west Italy), as part of the program for the serological surveillance of swine vesicular disease, was accused of having drawn multiple samples from a single animal. STR typing of sera derived from the five questioned blood tubes revealed that two samples shared the same DNA profile. Moreover, alleles of this profile where constantly present in mixed profiles obtained from the remaining three samples. Random match probabilities under the distinct hypotheses of unrelatedness and relatedness, as well as likelihood ratios for mixtures interpretation, were calculated based on the allele distribution of the 11 STRs observed in a local population sample of Landrace x Large White pigs from Piedmont. The obtained results strongly supported the conclusion that all the samples included serum originating from a single sow.

P-231 A new autosomal STR multiplex for canine genotyping

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Background and purpose: Canine genotyping is an increasing need in a vast range of situations that more commonly include pedigree verification, parentage and forensic investigations, and population structure, genetic origin and diversity studies. In this work, we aim at the development of a robust PCR multiplex that performs consistently up to the standards required for forensic analysis and population studies. Method: Primers were designed and fluorescently labelled for amplification of 9 tetra-nucleotide STR loci in an optimized single PCR reaction for fragment size detection in automated capillary electrophoresis. The system includes previously described FH3210, FH3241, FH2004, FH2658, FH4012, REN214L11, FH2010, FH2361 loci (on chromosomes 2, 8, 11, 14, 15, 16, 24 and 33, respectively) and a newly described tetra-repeat on chromosome 38. Results: Balanced amplification of loci was obtained and sequencing of alleles was performed aiming at the proposal of a standardized nomenclature and the construction of an allelic ladder. Genotyping results and discrimination power of this system in a sample population are also herein presented. Conclusion: This PCR multiplex for canine genotyping presented robustness and reproducibility of results in a variety of samples. To validate its power and applicability as a forensic tool, further population studies will be performed.

P-232 Genetic Identification of Red Deer Using Autosomal STR Markers

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P-233 Species identification of botanical trace evidence using molecular markers

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Species identification of botanical trace evidence may provide links between crime scenes and individuals, or help verify alibis. Historically, identification has been performed using morphological techniques, preventing small or damaged fragments to be identified at the species level. DNA based techniques can aid in identification of this type of evidence, and are a first step in individual identification, generating high value evidence. Several DNA regions were evaluated for their use as plant-identification marker in a forensic setting. Characteristics include robustness when using bad quality material, reference sequence availability and 'universal' use throughout the plant kingdom. Following a theoretical examination of markers described in literature, three chosen DNA regions were amplified and sequenced from several Dutch plants. Comparison of these sequences to each other and to public databases enabled us to determine advantages and drawbacks of the markers. All markers had several positive and negative features, but when combined the correct species could be identified for most samples, provided that data about this species was present in the used databases. To combine the different markers, a scheme has been devised to weigh the available data, determine the level of identification (i.e. species, genus or family) and identify the species of origin.

P-234 STR typing of hairs from domestic cats

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Cats are popular domestic animals. Because of the constant hair loss a lot of cat hairs are found in the household. These hairs could be used by a forensic laboratory to link a suspect with a crime scene, since the hairs, attached to clothes, are left at crime scenes or are taken from there. Therefore, we have developed a STR (Short Tandem Repeat) concept for typing cat DNA. Saliva and hair from one hundred cats (British Shorthair) were investigated. PCR was carried out with a multiplex comprising five high polymorphic STR loci (F41, F141, F85, FCA733, FCA749). The primers are labelled with the sensitive fluorescent dyes FAM, JOE and TET. The power of discrimination (PD) values for the five STR loci range from 0.89 to 0.98 and the combined PD value for all five STRs is 0.999999. Over 90% of the saliva samples could be analyzed with our multiplex kit. The typing of hair samples was less successful because of DNA degradation. But after all 38% of the hair samples gave a complete profile and 49% a partial profile due to the short amplicon lenghts.

P-235 Food Forensics: Analysis of food, raw and processed materials in food and pharmaceutical products with molecular biological methods

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High quality products of the food and pharma industry may be blended with products of minor quality during processing. This may happen for several reasons including unintended contamination or intended fraud for commercial reasons. To test for such non-authentic contributions in products, requires both, forensic-like knowledge in DNA testing for difficult samples like processed food products, and a broad portfolio of DNA testing for animal and plant species. For supplier, importer and trader of food, raw materials for food products and pharmaceutical products we optimized and developed new methods for animal, plant, bacteria and funghi species determination. These methods also can be used in forensic casework, e.g. when plant or animal traces found at a suspect has to be compared with traces found on scene of crime. For analysis of a wide range of raw material or processed material methods for DNA extraction with commercially available kits needs thorough optimization. We demonstrate examples for qualitative and quantitative detection of blended food products from animals (e.g. caviar), plants (e.g. Basmati rice), as well as blended or wrong declared raw material for pharmaceutical products from animals (e.g. heparin) or plants (e.g. ginseng) by microsatellite analysis, sequencing analysis and Real-Time PCR.

P-236 STR and SNP loci in Atlantic salmon: tools for tracing large scale escapees from salmon farms.

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Atlantic Salmon (Salmo salar) is the most important aquaculture species in Norway and salmon farms are spread along the coastal bays, the salmon being kept in openwater netpens. About 5-700 000 salmons escaped from these netpens in 2006. The number of escapees is higher than the total number of wild salmon that annually returns to their native rivers to reproduce. Large scale escapes are referred to as serious environmental crime by the Norwegian Director General of Public Prosecution. To take such crimes to court a mean of tracing the origin of the escapees is needed. The use of data from typing of a combination of DNA polymorphisms has been put forward as such a tool. However, this approach requires the availability of a set of validated polymorphic markers. In this study we report allele frequencies of six microsatellite loci in samples from broodstock as well as wild strains (N=984). In addition we have identified 50 SNP loci, and as part of a validation of these loci we have typed 24 individuals selected from both wild strains and broodstock in all SNP loci. Locus diversity, allele distributions and other relevant forensic genetic parameters will be presented in detail for all loci.

P-237 Land Plants Identification In Forensic Botany: Multigene Barcoding Approach

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Background and purpose: Botanical trace evidence can provide useful leads during criminal investigations, however many plant materials cannot be identified to the species level only by morphological features. Taking advantage of DNA sequencing and other biomolecular techniques, exact identification of plants becomes invaluable in selected cases in court producing successful results. We propose a new barcoding markers for plant molecular systematic investigations at the species level by universal primers amplifications. Method: DNA of 30 different plants were extracted by QIAGEN DNeasy plant mini kit and amplified by universal primers of plastid loci trnL-F and psbAf-trnHr. Sequencing products were analyzed on the ABI Prism 3130. The gene sequences were aligned with the program ClustalW. Statistical analysis was performed on Arlequin software ver. 3.01. Results: The selected regions were successfully amplified and sequenced, confirming the robustness of universal primers. The genera and the species of local plants were entirely established. Intergenic spacer psbAf-trnHr showed a higher gene variability, but also a higher rate of length-heteroplasmy. Conclusion: The psbAf-trnHr intergenic spacer is the best plastid option for species identification since it has good priming sites and higher interspecific variation. The search results in GenBank and EMBL databases showed that forensic botanical identification can be hampered by the lack of plant sequences in DNA databases. This report showed that it may be necessary to employ more than one locus to attain species-level discrimination and that new sequences of selected standard genes are needed.

P-238 Supporting sustainable management of the world's fish populations: Towards forensic population genetic identification in the fisheries sector.

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Background and purpose: Captures of marine fish have reached a record high of 86 million tonnes a year and more than 60 percent of fish stocks rank as either overexploited or depleted. In this context a central and global problem is Illegal Unreported and Unregulated (IUU) fishing, identified as one of the main impediments to the achievement of sustainable world fisheries. While forensic genetic species identification methods exist, inspection and enforcement throughout the industry are hampered by the lack of validated population genetic identification techniques capable of demonstrating the geographic origin of traded fish. Methods: An EU research programme into the development of tools for traceability in the fish supply chain is set to generate an array of SNP-based assays for population assignment. The application of these assays to forensic analysis will require extensive validation of individual markers and the associated statistical methods used to generate evidential data. This presentation discusses recent developments in forensic population identification and raises some of the technical challenges facing the project. We propose potential methods and are seeking opinion in response to questions concerning the transfer of forensic genetic protocols from human casework to fisheries enforcement.

P-239 Molecular genetic identification of forensically important diptera in the UK.

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Insects (in particular blow fly (Calliphoridae) and flesh fly (Sarcophagidae) species) can be important indicators in a forensic examination, as the stages and species of insect identified on a body can provide evidence of time since death, location of death and even cause of death or evidence of abuse. Rates of development are highly species specific thus accurate species identification is essential. Molecular genetic identification could provide a quick and accurate way to identify species. We present a pilot study investigating molecular genetic identification of UK blowflies.

P-240 DNA Typing from Non-Human Samples

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The use of non-human DNA in a forensic context has increased greatly in the last few years. The purpose of the analysis is either for the identification of a particular species, such as protected species, or for the identification of a particular individual organism. The trade in species protected by the Convention on the International Trade in Endangered Species (CITES) is second only to that of prohibited drugs. There is an increasing requirement for forensic science laboratories to be able to assist those involved with the investigation of this trade. The science behind species identification uses species specific polymorphisms on the mitochondrial genome. Identification is based upon homology between the unknown sample and DNA sequence held on a DNA database such as GenBank or EMBL. The identification of a particular organism requires the isolation and characterisation of genetic loci, such as STRs. Match probabilities are only as accurate as the frequency database used when combined with knowledge of the breeding habits and ancestry of the species. This presentation aims to illustrate the issues of the use of non-human DNA in forensic casework and the problems that may be associated with the evaluation of the evidence. (please note that this abstract is for the invited speaker presentation)

P-241 Dog mitochondrial DNA and forensics in the UK

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Mr. Pat Heath, Queen Mary, University of London, UK
Mr. David Ballard, Queen Mary, University of London, UK
Ms. Cheryl Harrison, Queen Mary, University of London, UK
Ms. Catherine Thacker, Queen Mary, University of London, UK
Dr. Denise Syndercombe Court, Queen Mary, University of London, UK

Pet hairs are easily transferred during contact between individuals or between individuals and a crime scene and this, combined with high levels of dog ownership in the UK, means that dog hair is frequently recovered as forensic evidence. Until recently, forensic investigators were limited to microscopic analyses in order to examine such material, a technique that provides relatively little information other than an indication of the species of origin. However, it is now possible to extract and analyse mitochondrial DNA from dog hairs, thus increasing their evidential value. We present data obtained from sequencing part of the mtDNA control region of several randomly selected dog breeds commonly found in the UK and compare these results with other published databases. While this data was obtained by sequencing DNA from blood samples, results from extracted dog hairs are also presented. The evidential value of this data and opportunities for breed predictions are discussed.

P-242 Use of SNPs for the study of ancient mycobacterium genome

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Dr. Sylvain Amory, Institute of Legal Medicine, France
Dr. Christine Keyser, Institute of Legal Medicine, France
Prof. Eric Crubezy, Anthropobiology laboratory, France
Prof. Bertrand Ludes, Institute of Legal Medicine, France

In the last years, the use of single nucleotide polymorphisms (SNPs) has increased in numerous areas (i.e. medicinal genetics, forensic and human population genetics). These genetic markers interest today an emerging field termed molecular palaeomicrobiology. Our team currently develops a three-step assay based on SNPs for the detection of mycobacterial DNA and differentiation of the various members of the Mycobacterium tuberculosis complex (MTC). This distinction is important because the MTC gathers all mycobacteria that can cause human tuberculosis (M. tuberculosis, M. canettii, M. africanum, M. microti, M. pinnipedii, M. caprae and M. bovis). First, the conventional method for the detection of mycobacterial DNA, i.e. amplification by nested-PCR of the insertion sequence IS6110, will be used. The isolate will then be screened using a SNP species specific based assay. If M. tuberculosis is found to be the causative agent of the infection, it will further be characterized regarding to the phylogenetically distinct "SNP cluster group" described recently in the literature. Screening of ancient human samples showing typical skeletal lesions of tuberculosis using this assay should provide a direct insight into the evolution of the MTC and help us to better understand the development of the relation between M. tuberculosis and humans.

P-243 A SNaPshot multiplex typing strategy for mtDNA identification of mouse inbred strains

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Mouse inbred strains have been continuously used for research in numerous fields in laboratories throughout the world. With the widespread establishment and maintenance of strains, and the accumulation of mouse tissue and cell collections, it becomes more and more important to certify the source of these materials, in order to ensure authenticity of the results, and avoid mistaking one material by other as it has been recently reported. Quick genotyping methods are also needed to identify mtDNA origin when new strains are generated. Taking advantage of the homogeneity of mitochondrial DNA (mtDNA) sequences inside each inbred strain, and of the few mtDNA polymorphisms that differentiate inbred strains, we are reporting a strategy for identification of mtDNA haplotypes of a selected group of priority strains. We present a preliminary version of a SNaPshot multiplex typing strategy that, with only a pair of reactions, allows the distinction between common inbred and wild-derived mice strains, and provides the identification of 10 different common inbred and 6 wild-derived mice mtDNA haplotypes. We believe that this SNaPshot typing strategy will be of use for researchers that work regularly with mice strains and/or mouse tissues or cell lines. Moreover, it may also prove valuable in forensic identification of materials collected in laboratory accidents.

P-244 Assessing the frequency of somatic mutation from single dog hairs - Forensic testing of StockMarks® Canine I Ver3 kit

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As dogs are widespread and popular pets they have overriding importance from criminal point of view. The relevancy exist not only in cases of dog attacks or in traffic accidents, but the scattered, shaded hairs can often provide indirect evidence in crime scene - person relations as well. We tested the probability revealing the true profile from single dog hairs. 393 hairs were collected by pulling from a bullmastiff donor, and 0,3-0,5 cm pieces were cut from the root end for DNA extraction. The samples were amplified by multiplex PCR of 10 loci, electrophoresed on ABI 310, and analyzed using Genescan 3.1 software and Genotyper 2.5 software. 44 aberrant - triallelic, drop-out or imbalanced - patterns were found in 290 full profiles. All aberrant loci were re-checked by monoplex and/or a new multiplex amplification and only one sample left mutant. As one hair sample presented different genotype after repeated analysis the individual in the study has been proved to be a somatic mosaic. Altogether 15,2% of the full profiles presented divergent genotypes from the original, which phenomenon suggests a careful use of this canine multiplex PCR kit especially in DNA samples of low copy number.

P-245 Species identification from hair remains isolated from food served in a Basque psychiatric hospital: a case report

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Public Health is not usually the target of Forensic Genetics however, herein we present a case in which DNA analyses helped to solve the origin of a possible contamination of food destined for hospitals. A psychiatric hospital from the Basque Country sent to our laboratory a sample of puréed vegetables showing a great amount of hair remains of about 1 cm in length. The aim of our study was determining the origin of these hairs, in order to take legal action against the company who provided the catering. Several hairs were isolated from the purée and those showing root remains were chosen for DNA extraction to increase the chance of obtaining DNA amplicons. They were repeatedly washed with distillated water. DNA was extracted following the standard phenol: clorophorm procedure and purified by ultrafiltration using Microcon YM-100 (Millipore). A fragment of 358bp of cytochrome b gene was PCR amplified. Sequences obtained from different hairs were launched in a BLAST search. The hairs contained in the food were identified as cow hairs (Bos taurus), thus unveiling a possible negligence from the catering company during the preparation of the purée.

P-246 Quantification of Trace Amounts of Human and Non-Human Mitochondrial DNA (mtDNA) Using SYBR Green and Real Time PCR

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There are currently no tests available to quantify non-human mammalian mtDNA. Standard universal DNA quantification tests such as EtBr are unsuitable due to the large size difference between nuclear and mitochondrial genomes and the ubiquitousness of human mtDNA. A method has therefore been developed to quantify total mammalian mtDNA and total human mtDNA present in a sample using SYBR Green. Mammalian primers designed to react with all mammals was designed on the 12S gene and human specific primers were designed on the cytochrome b gene. Each primer set was reacted separately with sample DNA and SYBR Green and detected using RT-PCR. A standard curve was created using dilutions ranging from 1 billion copies to 100 copies of mtDNA. Twenty four human samples were analysed and an average log (copy number) human/universal ratio of 1.00 was obtained. Samples falling below this ratio will contain some non-human mtDNA. Samples falling above this ratio contain human mtDNA only. Twenty nine animal samples were tested of which 96.6 % showed human contamination to some extent. This test is able to quantify mtDNA down to the femtogramme (10-15g) level.

P-247 A Novel Method to Identify Mammalian Species From Trace Samples and Mixtures

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There are relatively few tests available to identify the species of origin of a sample. A general method is to amplify part of the mitochondrial genome, commonly the 12S, 16S or cytochrome b gene, and sequence the PCR product for comparison with known sequences on GenBank. Highly degraded samples and mixtures make this technique unsuitable. As a functioning protein, cytochrome b cannot mutate unconditionally. Detrimental changes may result in cell death and would not be passed on to offspring. By examining the cytochrome b sequences non-detrimental variation can be found which can be used for specific species identification. Areas of high homology can also be identified for universal amplification sites. Species-specific primers have been developed based on these single base differences in the cytochrome b gene such that they will only react for a particular species. By combining universal priming sites with species-specific sites, a simple yet effect test has been developed for the identification of species. This test will produce a product of a particular size in the presence of DNA from a particular species. The test can differentiate species, work on mixtures and has a sensitivity to the femtogramme (10-15g) level.

P-248 Monte Carlo Bayesian (MCB) identification using SNP profiles

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Background and purpose: The MCB method enables an investigator to analyse individual SNP profiles quickly, without using reference groups. It facilitates criminal and "unknown remains" investigations, and also legal proceedings. Method: The MCB method, in the form of a computer program, iteratively applies Bayes's theorem to stratified random sample arrays comprising 10 discrete, equal-sized SNP allele frequency ranges. An 11th array element, representing a known subject with a matching profile, may be added. The method: (a) evaluates and adjusts for substructure (b) accommodates variation in prior probabilities according to the investigator's judgment (c) produces both identity probabilities and court-ready match likelihoods (d) does not use reference group data (e) takes 40 seconds for a 1000-sample calculation. Results: Results can vary widely. For example, a 50-SNP profile having 42 homozygous SNPs and a prior probability of 10-10 yields a posterior probability of 0.85 however, the same profile with a prior probability of 0.5 from an MCB STR analysis [PFG11 Elsevier ICS 1288 (2006) 471-473], yields a posterior probability of 1.000... to 10 decimal places. Conclusion: MCB methods, applied both to SNPs and to STRs, yield a pair of independent, synergistic forensic tools.

P-249 Limits of autosomal and sexual chromosome STRs in defining parental relationship: a case report

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Sometime ago the Prosecutor asked for a genetic analysis on some evidences found in a murder scene in order to find a match with a suspect. First the suspect was typed for 15 STRs loci using the commercial kit "Identifiler". The profile obtained from the suspect and that obtained from the evidence were different, but the analysis showed that for 10 loci the presence of a common allele between the suspect and the evidence, and for 2 loci the same genotype. So the hypothesis of the involvement of a relative of the suspect was very suggestive. The analysis of Y-Chromosome STRs excluded a paternal relationship and the X-Chromosome STRs did not give enough information for a maternal relationship. A genetic evaluation through the software "Familias" was performed. Three different hypothesis were tested: 1) the suspect is an half brother of the subject wanted (probability 62,15%), 2) the suspect is a cousin of the subject wanted (probability 52,06%), 3) the suspect and the subject wanted are sons of a brother and sister (probability 59,32%). This statistical analyis was inconclusive. From this case it has to be stressed the importance of the analysis through both autosomal and sexual chromosome STR, but to reach a good degree of probability other analysis have to be performed, such as the mt-DNA.

P-250 A Bayesian approach to a case of double infanticide

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The facts. The body of a newborn infant was found in the rubbish dump of the town of Pontedera (Tuscany, Italy). Nothing could lead to its mother. The investigators requested the genetic profile. About a year later, a woman from Pontedera presented to the hospital of another town more than 200 miles away with massive genital bleeding. She denied pregnancy, though the genetic profile of the child was obtained from the retained placenta. Three mutually exclusive hypotheses. 1) The two infants were unrelated, 2) The two infants were half sibs by mother, 3) The two infants were full sibs. Assuming equal priors, the posterior probabilities were 1.0%, 20.3%, and 78.7%, respectively

P-251 Use of "Anydirect PCR Buffer" for PCR Amplification of Washed Bloodstains: A Case Report

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AnyDirect PCR buffer (BioQuest) permits to perform direct PCR from different kinds of forensic samples (blood, saliva,sperm, etc.) without any DNA purification step. This is very useful in particular when working with small quantity of sample since it avoid the the risk of loosing sample step by step as often happens with traditional DNA extraction procedures. In the present casework we analyzed some bloodstains found by luminol test onto washed clothes and linen : in particular stains were subjected either to a traditional extraction and amplification procedure than to direct PCR amplification. To improve more the chance of obtaining a result from degraded DNA, all samples were analysed etheir using the AmpFlSTRs Identifiler kit (Applied Biosystems) than the Mentype®Nonaplex II PCR Amplification Kit (Biotype) that amplify simultaneously 8 polymorphic mini STRs. STRs amplification has been performed preparing a Master mix containing, AnyDirect™ PCR buffer, dNTP mix, Identifiler and Nonaplex Primers mix (from commercial kits) and adding directly an aliquot of each sample. We found that the direct PCR buffer system gives reliable results and could replace conventional complex and time consuming procedures for DNA analysis.

P-252 Zip lock poly bags in drug cases – a valuable source for identifying DNA results?

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In our experience the police authorities question the success rate of recovering identifiable DNA profiles from zip lock poly bags ("poly bags") in drug cases. Hence, we conducted a retrospective study with 183 swabs sampled from reclosable zip lock poly bags from drug related casework in Norway. The success rate of recovering DNA results that could be linked to an offender was estimated. DNA was extracted from the swabs using Chelex®. The samples were analysed using the AmpFISTR SGM plus system kit running 28 cycles. In 26 (14%) of the cases DNA results that could connect a person to the cellular material (possibly epithelial cells) recovered from the poly bags were retrieved. Seventeen (65%) of these samples were found to match a casework reference sample or a national DNA database profile. In the remaining 157 swabs (86%) either no DNA results were obtained, or the DNA results did not contain enough information to identify the person who had left the cellular material on the poly bag. The study demonstrates that DNA analysis may be a valuable method to identify the person who has left cellular material on a poly bag. Keywords: crime casework, epithelial cells, poly bags, DNA analysis

P-253 Hair Shaft Typing in a Cold Case

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A young prostitute disappeared in 1988 and her decomposed body was found in dumped 1991 in a front garden. A small clump of hairs was recovered from tapings taken from the body. In a first step the victim was identified by an anthropomorphic analysis and than her identity was confirmed by DNA test., but unfortunately traditional FSS DNA typing procedure of hairs gave no satisfactory results. As reported in our previous papers whenever hairs are not too short and there's a sufficient quantity of medulla cells inside the hair stem, a multiplex STRs typing of hair-shaft can be performed. Since hairs were between 3 and 8 cm long and many of them appeared to be medullated, with the medulla being present along most of each hair, it was decided to try our procedure. Hairs were cut in small fragments and than incubated at 56°C in a special lysis solution untill they were completely dissolved. DNA was than purified by magnetic bead-based treatment. Extracts were quantified by the Quantifiler Human DNA Quantification kit using a 7300 Real-Time PCR System and amplified by AmpFlSTR Identifiler kit (Applied Biosystems). Amplified samples were than analysed on an ABI PRISM 3130 multicapillary sequencer. Since the quantity of DNA extracted was very low, samples were treated as LCN, so the number of PCR cycles increased till to 42 cycles using a reduced reaction volume. Some hairs gave a result only for Amelogenin as belonging to a male person. Nothwistanding different temptatives (even with mini STRs) no other loci were determined. On the contrary, for other hairs we obtained the same male partial profile incuding 9 loci plus Amelogenin (XY). The profile obtained was run against the UK National DNA Database that contains over 3,000,000 DNA profiles but, to date, no match has been obtained.

P-254 A more efficient extraction method of human bone resulting in improved DNA profiling

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Recently two articles about the efficiency of bone DNA extraction have been published. Loreille (et al., 2007) describes the significant enhanced quantity of extracted DNA after total demineralization of the bone material. In a study on a wide range of methods, the most promising method uses a buffer without any additive (Rohland and Hofreiter, 2007). The findings of both publications were tested on forensic casework samples of human origin. Eight human bone samples were extracted in parallel using our standard protocol with and without PTB in the buffer. PTB decreases the DNA yield resulting in none or incomplete STR profiling. The standard method was sometimes also inadequate for (complete) STR profiling. The complete decalcification in the presence of SDS of these bone extraction residues recovered sufficient amounts of DNA, which resulted in complete STR profiling for all samples. Complete decalcification without SDS in the buffer yielded even higher amounts of DNA and also complete STR profiling for all samples. The pulp of a human canine was recovered via an endodontic incision, while the remaining tooth was split by a vertical section. From one half treated by the standard lab procedure no DNA was recovered, while the other decalcified half yielded sufficient DNA for proper STR profiling.

P-255 Skeletal Reassociation in an Illegal Common Grave by Using STR, miniSTR, and mtDNA ANALYSIS.

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In 2004, the investigation of an illegal, 30 year old common grave in Avellaneda Cemetery, Buenos Aires, Argentina was resumed. There were found skeletal remains of "missing" people due to political reasons during the military dictatorship in Argentina (1976-1983). Bone remains were found mixed up, without anatomical order and it was necessary to determine the minimum number of individuals represented. Bones were grouped in 10 skeletons according to anthropological criteria. As there was no certainty that the reassembling was correct, genetic analysis was carried out on different bone samples to ascertain the correct association. Commercial kits and miniSTR systems were used to analyze autosomic STR markers. Mitochondrial DNA analysis was performed in those severely degraded bone samples. From each skeleton, teeth and a piece of femur diaphysis were analyzed, and it was also necessary to examine a vertebra in two of them. From the results of the genetic analysis, it was determined that the minimum number of people represented in the grave was more than expected, and the gender was confirmed for each one by Amelogenin. It was proven that six skeletons had been correctly reassociated by their morphological characteristics, while the assembly of the remaining four had been erroneous.

P-256 Analysis of LCN DNA Recovered from Synthetic Ropes Tissues: A Practical Approach Used in a Real Homicide Investigation

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An old woman was found dead in her apartment in the south of Italy. Her arms were tied behind the back with a nylon rope. Due to the absence of any biological or fingerprints evidence, investigations were immediately focused on the rope. Few centimetres of the knot and of the portions not in direct contact with the victim were separately collected. Samples were firstly washed with sterile bidistilled water. The obtained solutions were then submitted to several concentration/filtration steps by means of CentriconTM filters. Hence the pellets were processed through Low Copy Number DNA adjusted protocols (low reaction volume and "low bind" materials) which included a preliminary quantification of the total DNA by means of Real Time PCR. A complete male 15 STR loci profile was obtained from some of the above reported samples. Thanks to the collaboration with the local law enforcement, reference samples from the relatives of some alleged offenders were collected and submitted to the author's lab. Kinship testing revealed a high degree of parentage with the genotype of the father and the sister of one of the suspects. The efforts of the investigators were then addressed to the latter one, eventually leading to a prosecution for second degree murder.

P-257 A new multiplex-PCR comprising autosomal and y-specific STRs and mitochondrial DNA to analyse highly degraded material

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Analysis of short tandem repeat markers (STRs) has become the most powerful tool for DNA typing in forensic casework. It also allows a reliable identification of individuals. In identification cases of severely putrefied or burned bodies, the DNA is highly degraded and STR typing might be difficult. Despite the application of so called mini-STR PCRs that amplify fragments smaller than 200 bp, the DNA typing of those materials might not be successful, leaving the sequencing of mitochondrial DNA as an alternative method for identification. Furthermore, in those cases it is often unclear whether a negative PCR result is caused by PCR inhibitors or simply too little amounts of DNA. We developed a PCR that amplifies very small fragments from the gender determining amelogenin plus DYS390 and DYS391, TH01 and vWA. Two fragments from the mitochondrial HVI region are co-amplified to determine whether a sample contains enough mitochondrial DNA for sequencing analysis. Additionally, a quality sensor was developed to check PCR efficiency. This new PCR will be presented as well as its use in several forensic cases including analysis of hairs, bone fragments, and putrefied bodies.

P-258 Identification of several profiles in a sexual assault case

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In forensic examinations concerning victims of sexual assault, sometimes the physical and genital injuries identified by the medico-legal examination are not sufficient to confirm the presumption of the sexual assault. In these cases the results obtained from the study of the STRs markers are the main possibility to provide forensic evidence. Here we report a case of a female victim sexually assaulted by a known individual. During the examination (with no signs of injuries) three swabs were taken (vaginal, anal and oral). The victim's bed clothing, where the presumed assault occurred, was recovered from her house and the condom used by the suspect was provided by the victim. Two swabs were made by us from the external and internal parts of the condom. The samples were examined for semen (Phosphatesmo KM). DNA was extracted using the organic method. The STR analysis was made using the AmpFlSTR® Identifiler Kit (Applied Biosystems) and the PowerPlex® Y System (Promega). A total of four masculine profiles were obtained, two of them in bed clothing, one in the swab of the internal part of the condom and the last one in the swab of the external part of the condom and in the anal swab.

P-259 The use of the Y-chromosome not only for identification purpose but for investigation also.

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Background and purpose: The use of Y-chromosome in forensic field is well known during the last ten years. In a criminal case the male specific Y-haplotype is used to solve mixture stains (male/female), particularly in rapper cases and murders with sexual assault. We report here a case of murder of a young woman: the solution of the case was obtained through the specific information of male lineage given by the study of Y-chromosome. Method: The DNA was purified from saliva (reference samples) and from vaginal swabs using the BioRobot EZ1 workstation with his "Forensic Card". The samples were amplified using Identifiler Kit (Applera) and the PowerPlex Y system (Promega) and the amplification products were detected using the ABIPRISM 3100 Genetic Analyzer (Applera). Results: We found that the nuclear STR's profile extracted from the male fraction of victim's vaginal swab was different from her father's one, but they were identical in the Y-specific haplotype. Conclusion: This important information suggested us to extend inquires about all male components of her father's family, and allowed us the identification of the murderer (a victim's cousin). Here we suggest that the use of Y-haplotipe, in particular cases, could give further help for the investigations.

P-260 Infants kidnapping in Central Brazil solved by genetic analysis

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This is a report involving two apparently isolates hospital baby kidnapping cases in Central Brazil. The first, involving a girl, occurred in Goiânia (GO) in 1979, and another, a boy, 350 km apart, in Brasília (DF) in 1986. In 2002, morphological similarities between the parents of the missing child from Brasília and a teenager boy in Goiânia were observed. Based in this suspect, DNA samples were collected and tested for genetic kinship using Powerplex 2.1 and 1.1. Results showed a clear paternity and maternity inclusion and the maternity exclusion of the legal mother, whose were accused of kidnapping. The 1979 kidnapped girl´ biologic mother suspected that the accused woman was the same who kidnapped her daughter. DNA samples were collected from tips of cigarettes from two accused daughters, which were discarded in a trash of the Goiás State Police Station (warrant of 15/01/2003), and from the 1979´s kidnapped girl mother blood. Results indicated that one of the daughters matches the genetic profile of the suspect mother sample and excluded the accused. In the absence of the father, the maternity index was estimated in 27,532,050 and the maternity probability was 99.999996%. The investigation concluded that the same woman committed both crimes.

P-261 Difficult identification of two brothers among arson victims due to a Y-STR mutation

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Difficult identification of two brothers among arson victims due to a Y-STR mutation Forensic identification and parentage testing with male children are often based on Y-chromosomal STR evidence. In few cases, Y-haplotypes of related persons can differ at a single locus due to a mutation. In this work we report an arson case with three male victims, two brothers and one unrelated person. The 15 Y-STRs typing for the victims revealed two identical haplotypes for all loci except for DYS19. Y-profiles were further compared with the haplotypes belonging to the putative victims' fathers. The genetic data were completed with the odontological investigation data and the single-step DYS19 mutation (one repeat loss) was finally assigned to the younger brother. Our case thus conclude that knowledge of the mutational process and mutation rates of Y-chromosomal STRs is important for the correct interpretation of haplotypes in forensic and population genetic studies.

P-262 Validation of automated DNA-extraction from various Forensic Casework Samples using the BioRobot Universal System

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High Volume crime cases, like burglaries, car thefs, robberies etc. demands highly efficient and fast methods in order to type large series of forensic case work samples in parallel in a short time. Recently a new platform for the extraction of genomic DNA from trace material was released based on silica gel membrane technology by QIAGEN. A fully automated system was developed for the extraction of genomic DNA from buccal swabs or forensic case work samples in a 96-well format. We report on the results of an internal validation study of the BioRobot Universal system for the fully automated isolation. Experiment were performed to test the reproducibility, crosscontamination, sensitivity and overall performance of DNA typing by measuring peak intensities of different DNA marker (AmpFlSTR® SGM Plus®). DNA typing from various forensic case work samples show a high succesrate of complete DNA profiles. DNA analysis for blood, saliva, cigarette buts and chewing gum resulted in 97%, 69%, 92% and 100% complete DNA profiles, respectively. Highly reproducible results were found and no crosscontamination was observed. Results show that this new system could be used as medium- to high throughput system for the extraction of DNA from various forensic casework samples.

P-263 Condom as Trace Evidence: A Case Report

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The evidentiary potential of the condom is enormous. When a condom is found in the crime scene we can individualize both the victim and the offender. This is what was asked from the Institute of Forensic Sciences in a case where a condom was found and 5 suspects were accused for raping a 15 year old boy. The buccal swabs of the 5 suspects and the victim were sent to the laboratory together with the condom taken from the crime scene. Unfortunately the condom was in a terrible condition when arrived to the laboratory. We were intended to analyse both the inner and outer surface of the material to prove that it was used from one of the suspects to rape the specific victim. It was difficult to separate spermatozoa from epithelial cells and therefore it was not easy to type the victim DNA profile. However the concentrated DNA samples revealed a clear phenotype of the victim for 9 STR loci which completely matched. The DNA type of the perpetrator was typed for all 15 loci and amelogenin and the comparison showed that none of the suspects were the actual perpetrator. These results matched the testimony of the victim.

P-264 The rule of mixed DNA profiles in sexual crimes, both from swabs and fingernails

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After sexual raping both vaginal,or rectal swabs contain mixed DNA profile from both the victim and the assailent.also in these violent crimes ,multiple actions of resistant,aggressionand defence often take place.The fingernail hyponychium is an isolated area where biological material may accumulate and can provide a source of evidential material. DNA transfer between victim and sspect- Comparing these mixed profiles both from swabs& finger nail mixtures could be the biological clue. The DNA was extracted using QIAamp DNA blood minikit (Qiuagen) The quality and quantity of extracted DNA were estimated by using minigel electrophoresis. All samples were amplified successfully through (Identifiler PCR amplification kit- Applied Biosystems- Detection of all samples was detected using AB 3130 genetyic analyzer.

P-265 D16S539 microvariant or D2S1338 off-ladder allele? A case report about a range overlapping between two loci.

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Background and purpose: All forensic laboratories routinely use commercial kits and softwares for automated typing in rare cases genotyping misinterpretations or mislabellings occur. This study refers to the investigation on a D2S1338 off-ladder allele mislabelling observed in DNA profile of murdered woman. Method: DNA samples were typed by Identifiler® (AB) and PowerPlex®16 (Promega) kits. All the evidences were re-extracted and re-amplified. Singleplex amplification and sequencing analysis of D16S539 and D2S1338 loci were performed. Results: The Identifiler revealed heterozygosity in the range of D16S539, with a presumptive microvariant allele "14.2", based on assigned size, while PowerPlex®16 resulted in a homozygosity of allele "11". Singleplex amplification of D16S539 locus confirmed homozigosity. D2S1338 locus, the closest to D16S1338 in Identifiler®, genotyped as homozigote "19", was singleplex amplified. The off-ladder peak was gel-isolated, sequenced and designed as a rare "11" allele variant [(TGCC)6(TTCC)5]. Genotype was finally designed as D16S539 "11,11" and D2S1338 "11,19". Conclusion: To avoid genotyping misinterpretations or mislabelling, ambiguous genotypes should be established by two commercial kits at least. Furthermore, off ladder alleles as well as allele microvariants should be assigned by direct sequencing. This issue should be considered in Criminal DNA database requirements, that is still under debate in Italy.

P-266 Molecular characterization of a null allele at locus DXS8378

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Two multiplex PCR systems for the combined analysis of 12 X-chromosomal STRs (Robino et al. Int J Legal Med 2006 120:315-318) were used to type a female child and her putative paternal grandmother in a deficiency paternity case. A single Mendelian incompatibility was observed at locus DXS8378, consisting of an opposite homozygosity between the child (genotype 10/10) and the woman (12/12). The presence of either a primer binding site mutation or a microdeletion at locus DX8378 on the child's paternally inherited X chromosome was confirmed by the absence of a DXS8378-specific PCR product in the deceased putative father's brother. Amplification with newly designed DXS8378 external primers generated heterozygous genotypes in the child (10/11) and putative grandmother (11/12), and an hemizygous genotype (11) in the putative uncle. Sequencing of the man's allele showed a point mutation (C>T transition at position 168, according to GenBank accession G08098) in the binding site of the original DXS8378 reverse primer. No allele dropout was detected when the tested subjects were re-typed by means of a commercial kit (Mentype Argus X-8, Biotype AG) including DXS8378-specific primers (amplicon size 154-178 bp) potentially overlapping those that were employed in our in-house multiplex PCR assay (amplicon size 163-187 bp).

P-267 Effects of Changes in Reference Population and Number of Markers on Paternity Index

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In recent years a growing number of disputed paternities have involved parents from different ethnic backgrounds. We examined, considering three sets of commonly used autosomal short tandem repeat (STR) markers (containing 15, 10 and 9 markers) and 14 population databases, how the selection of the population database and the number of considered markers would influence the statistical evidence that is usually produced to favour paternity. The study was based on a sample collected during paternity testing case work and contained 100 randomly chosen Finnish paternity trios, without any exclusion after use of 15 STR markers. Paternity Index, Power of Evidence, Typical Paternity Index and Random Men Excluded were computed and descriptive statistics were provided separately for trios (mother, child, alleged father) and duos (obtained from trios but not considering the genetic information of the mother). This was done for all combinations of markers and databases. In all trio cases considered, Power of Evidence and Random Men excluded have the same distribution but this does not happen in the duos. Only in trio cases, the Power of Evidence is always greater than 0.99 independently from the set of markers and database used.

P-268 Heteropaternal Superfecundation in a Pair of Danish Twins

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In a case of disputed paternity to a pair of male twins, the results suggested that the twins had different biological fathers. The twins, the mother, and two alleged fathers were investigated using 15 STR-markers, 7 RFLP-based VNTR-markers and 11 Y-chromosomal STR-markers. Two samples from each of the five tested persons were obtained at different occasions, and each of the samples were tested in duplicate Alleged father #1 was excluded as the father of Twin #1 because 13 genetic inconsistencies were observed among the autosomal loci, and 6 genetic inconsistencies were observed among the Y-chromosomal loci. The paternity index of Alleged Father #1 to Twin #2 was in favour of paternity, with a weight exceeding 1010 compared to an unrelated man of Danish origin. Alleged father #2 was excluded as the father of Twin 2, because 13 genetic inconsistencies were observed among the autosomal loci, and 6 genetic inconsistencies were observed among the Y-chromosomal loci. The paternity index of Alleged Father #2 to Twin #1 was in favour of paternity with a weight exceeding 107 compared to an unrelated man of Danish origin. A single genetic inconsistency between Man#2 and Twin #1 was observed in the locus D18S51, which could possibly be due to a paternal mutation. The frequency of mutations among Danes in the system D18S51 is approximately 0.003. Heteropaternal superfecundation is rarely observed among humans, although it has been reported to appear as often as in 2.4% of dizygotic twin births (1). The present case is the first one observed among Danish cases of disputed paternity in cases involving twins since 1975. References. 1. R.E. Wenk, T. Houtz, M. Brooks, F.A. Chiafari (1992): How frequent is heteropaternal superfecundation? Acta Genet. Med. Gemellol (Roma). 41:43-47.

P-269 Autosomal SNPs in paternity investigation

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Interest in SNPs has increased in the last years concerning forensic casework. We have studied ten of the most polymorphic SNPs (~50% heterozygoty) in European populations, especially NW Spain, selected from a 52 SNP panel reported by Sanchez et al.(Electrophoresis,2006). These 10 autosomal SNPs - #1(rs1490413), #6(rs1029047), #8(rs763869), #10(rs735155), #12(rs2107612), #14(rs1454361), #27(rs2111980), #49rs1005533, #50rs8037429, #51rs891700 - were amplified in one PCR reaction followed by detection of the SNPs by single base extension reaction (SNaPshot multiplex) analyzed using capillary electrophoresis. A total of 80 individuals from a South Portuguese population were typed and the allele frequencies were estimated. More than twenty paternity investigation cases, already performed routinely with STRs were also studied. Allele frequencies are in good agreement with published population genetic studies. Exclusion, non-exclusion cases and two alleged father cases have been performed. SNP profiles are in the majority of cases in agreement with STR profiles. In exclusion cases, we have detected one, two or three exclusions, mainly in #8(rs763869) and #12(rs2107612) loci. Even performing few SNP loci, but selecting SNPs with allele frequencies near 0.5, the SNP methodology can also be a useful tool in paternity investigation.

P-270 Eight X-STRs in paternity casework

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The aim of our study was to perform a reliable X-chromosomal PCR multiplex system for forensic casework, especially for use in deficiency paternity cases with daughters. We used a new commercially available kit, Mentype® Argus X-8, which contains eight X-chromosomal STR markers - DXS7132, DXS7423, DXS8378, DXS10074, DXS10101, DXS10134, DXS10135 and HPRTB. These markers belong to four linkage groups of the X-chromosome and must be handled as haplotypes. A hundred and twenty South Portuguese Caucasian individuals and sixty two paternity investigation cases, previously performed with routine STRs markers, were studied. DNA was extracted with Chelex method followed by Wizard purification. Amplification was done according to manufacture's recommendations and PCR products were analysed in an ABI PRISM® 3130 Genetic Analyzer. Allele and haplotype frequencies were estimated and correlated with other population studies. More than five exclusions between the alleged father and the female child were observed in almost all exclusion cases, especially in DXS10101, DXS10134 and DXS10135 loci. Deficiency cases without mother and complex paternity investigation cases were also performed. This set of eight X-chromosomal STRs appears to be quite useful for father-daughter studies and in some kinship investigations.

P-271 Autosomal and Y-STR mutations in Lithuanian population

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Background and purpose:The mutational features for STRs have important consequences for forensic application such as for exclusion in paternity testing and the interpretation in identification analysis. Method:Data were collected from 683 paternity cases (probability =99.99%) for autosomal STR analysis and 130 father/son pairs (confirmed by autosomal STR analysis) for Y-STR analysis from different regions of Lithuania. Amplification was carried out with the AmpFlSTR®Identifiler and AmpFlSTR®Yfiler kits (AppliedBiosystems). Results:In autosomal STR investigations identified 23 mutations in 12 microsatellite loci. No mutations were observed in three of these fifteen researched loci. In two loci was found mutation three times, in six loci-two times and in two loci-once. Mutation cases in the male germ line were five times more frequent than in the female germ line. On investigated samples for Y-STR analysis 8 mutations were found and all of them in different loci. The event was always repeat related: thirty- a single-step and one a double-step mutation. At report we present paternity case: is here exclusion or mutations? Conclusion:Mutation rates, observed in Lithuanian population, were compared with NIST, YHRD databases and no significant differences were found. More detailed mutations characteristics on investigated 15 STRs and 17 Y-STRs will be displayed on poster section.

P-272 "False positive" or true paternity: Investigating one or two STR mismatches by detailed SNP analyses

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Paternity and kinship testing are performed in e.g. paternity disputes and immigration cases. In paternity testing, one or two mismatches, between alleged parent-offspring pairs are usually still regarded as representing true paternity, because they might be due to one-step mutations or null alleles. If such an inconsistency is observed, the possibility of inclusion is calculated with a mismatch correction factor. The criteria concerning exclusions and consequences of mutations have been evaluated in 131 presumed parent-child combinations with mismatches from immigration families, using 22 STR loci. However, in non-paternity cases one or two mismatches can also occur, e.g. when a close relative is the true father. Reversely, in case of a close relative of the father, with limited numbers of STRs, the outcome can be zero mismatches and subsequently result in a false decision of paternity. We investigated two multi-generation reference families by genotyping thousands of SNPs. By unraveling the kinships through various analysis programs a comprehensive overview of the different familial relationships was obtained. Furthermore, the informative SNP genotypes revealed the true kinship in paternity investigations with one or two mismatches. The criteria used for exclusion have to be considered critically in immigration casework to prevent false inclusions.

P-273 Evaluation of Incest Indices Determined From Microsatellite Genotypes of Mother and Child

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INTRODUCTION: Suspected incestuous paternity is encountered infrequently. Investigation may be complicated by absence of the alleged father. Incest indices (I.I.s) calculated from microsatellite (STR) typing of only mother and child were evaluated for accuracy. METHODS: Combined I.I.s (CI.I.s) of 50 randomly mated (RM) mothers and their children were compared with CI.I.s of 50 simulated incestuous (SI) mothers and their children. Each CI.I. was calculated from the same 18 STR loci and categorized as 'diagnostic', 'indicative' and 'misleading'. 'Diagnostic' likelihood thresholds were <0.010 and >100 for RM and SI cases respectively. Variables affecting CI.I.s were evaluated. RESULTS: CI.I.s were 'diagnostic' in 41 cases and 'indicative' (directionally correct) in 52. 'Misleading', non-diagnostic likelihoods occurred in 3 RM cases (CI.I.>1.0<100) and 4 SI cases (CI.I.<1.0>0.010). The number of mother-child genotype similarities/18 loci was the most important determinant of CI.I. Infrequent alleles were important in raising CI.I. in SI cases, but class of mother-child similarity (identical homozygotes, identical heterozygotes, etc.) was of minor import. CONCLUSIONS: Eighteen STRs produce diagnostic CI.I.s in only ~40% of suspected 'fatherless' incest cases. About 29 independent STRs would assure that >95% of these cases would achieve 'diagnostic' CI.I.s. Study of loci more informative than typical STRs would be advantageous.

P-274 Two examples of null alleles at the D19S433 locus due to the same 4 bp deletion in the presumptive primer binding site of the AmpFlSTR Identfiler kit

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This study reports two cases of allelic loss at the D19S433 locus after multiplex PCR with the AmpFlSTR Identifiler kit (Applied Biosystems). In both cases the failure of PCR resulted in apparent homozygosity and false exclusions: in the first case the mother appeared to be homozygous for an allele *13 and her child for an allele *15, in the other case the child seemed to be homozygous for an allele *13 and the biological father for an allele *13.2. After singleplex PCR with published primers additional alleles were observed and Mendelian inheritance was restored. These PCR products were sequenced and in both cases the same 4 bp deletion near the 3’ end of the repeat region was detected in two alleles of different length. The frequency of these null alleles (2 events in 1026 allelic transfers) mathematically amounts to 0.0019 (95% confidence limits: 0.0002-0.0070). Additionally, it must be considered, that such a null allele remains undisclosed in case the parent/child pair shares the other allele. Such a genetic incompatibility is referred to as an indirect exclusion, which has a much lower conclusive force than a direct exclusion. These situations have to be treated with caution.

P-275 Unusual FGA and D19S433 off-ladder alleles and other allelic variants at the STR-loci D8S1132, vWA, D18S51 and ACTBP2 (SE33)

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In the course of population genetic studies and other investigations a series of new and rare variant alleles have been found and sequenced. A very short off-ladder FGA allele was difficult to be assigned to a locus in the Powerplex 16 (Promega) system as its amplicon size was between the expected allelic size ranges for the TPOX and the FGA locus. Sequencing exhibited a FGA allele *14 which was 8 bp shorter than the common allele *16. An analogous finding was observed at the D19S433 locus, where an allele *19.2, 8 bp longer than the common allele *17.2, was found. At the D8S1132 locus the new alleles, *12.1, *14 and *15.1, have been found and sequenced. Further sequence data (vWA allele *18.3 and the D18S51 allele *11.2) will be presented. Most of the new variant alleles, either in sequence or in length, have been identified at the HumACTBP2 locus including the alleles *14.2, *15.2, *15.3, *24.2, *31.1, *32, *33.2, *34, *34.2, *35, *37. Additional rare variant alleles have been sequenced. These alleles add further data to the enormous polymorphism at this locus.

P-276 The use of some genetic markers in paternity problems

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This work was a project financially supported by the university of Alexandria to form a nucleus for genetic markers laboratory. The study was carried out on 10 biological families.Each family was composed of a father,a mother & one of their children (5 sons & 5 daughters).Blood grouping was done for each one. This included 22 red cell antigens of 10 blood group systems in addition to HLA typing class 1 (HLA-A,B). As regards the frequency of different blood groups & the different HLA-A-B types,no 2 similar HLA-A & B were found in the sample studied.On comparing these results with those of the different blood groups,a significant statistical relation was found,denoting that HLA typing is extremly individual.The relation between blood groups of parents & children in different systems were correlated.This study is needed to be repeated on a wider scale involving a larger number of the egyptian population,to find out the gene frequencies of thefferent blood groups. this will enable a correct interpretation of the probability of paternity results.

P-277 False Inclusion in a Deficient Paternity Case with Two Presumptive Fathers

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Abstract We present a case of deficient paternity with two presumptive fathers analysed with 19 Autosomic STRs and resolved by means of the study of 12 Y-chromosome STRs. This is case PA-GYQ-62-06, in which two presumptive fathers disputed the paternity of a male child and the mother was not available. The presumptive fathers are not genetically related to one another and the three individuals studied are males of Mestizo origin, born and residing in Ecuador. 15 autosomic STRs consensued from the commercial kit PowerPlex-16® (Promega) were analysed and a Combined Paternity Index (PIcom) of 13,811.215 and a probability of paternity (W) of 99.9999928% were obtained for presumptive father 1 and a PIcom of 35,332.241 with a W of 99.9999971% for presumptive father 2, which meant that inclusion was found for both fathers. We amplified the study with the FFFL® (Promega) system, whereby an exclusion was found in the HUMLPL marker between presumptive father 1 and the son, possibly involving a first-order mutation. These results did not enable us to exclude either of the two fathers. Due to the importance of the case, 12 Y-chromosome STRs were analysed with the commercial PowerPlex-Y® kit, which led to the exclusion of presumptive father 1 due to the fact that he displayed a different haplotype in this pair, exclusion of the HUMLPL was also found. Keywords: autosomic STRs, Forensic Genetics, Paternity testing, Y-Chromosome STR.

P-278 10 Years of Forensic Genetics in Ecuador

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Abstract We present a brief analysis of the advances and perspectives of Forensic Genetics (FG) in a developing country such as ours over the last 10 years, during which time this area of Legal Medicine has been partially consolidated. To speak of FG in our country is to speak of a change in the behaviour of the legal system. Both the criminal prosecution system and the National Legal Medicine System are very recent in Ecuador. The first DNA test was carried out in 1997 for a case of migration in which a paternity test was requested. So far around 5000 paternity tests have been carried out, around 60% in the judicial sphere and 40% privately. Over 200 forensic studies have been carried out in criminal cases since the introduction of the new Criminal Procedure Code, a figure which could be insufficient but which, due to the existing legal system, is a significant number. There are currently three unofficial laboratories which carry out forensic studies and the test is now consolidated in court cases.At the same time there has been an evolution in legal regulations with the passing of the recent Criminal Procedure Code, the Childhood and Adolescence Code and the National Legal Medicine System Regulation. Keywords: Forensic Genetics, Paternity testing, Legal medicine, Ecuador.

P-279 Molecular HLA typing is an invaluable tool in some complex paternity cases analyses

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Background and Purpose: HLA is known to be the most polymorphic genetic system in humans. Molecular typing of HLA A, B and DRB loci by means of Luminex technology provides an extremely high power of discrimination, allowing standardization, and high throughput analyses. Methods: We present two paternity cases in which the analysis of 15 STRs was inconclusive: a) a paternity trio with 2 STRs showing potential germ-line mutations b) a case with deceased AF with incompatibilities in 2 STRs. HLA was supplementary typed in all the individuals using the LifeMatch® fluoroanalyzer and the Lifecodes HLA-A, B and DRB typing kits. Blood samples (e.g. ~25-50 ul) were used for the analyses. Results: HLA results yielded a combined paternity index of 6009.59 for the a) trio, and allowed to conclude a paternity exclusion in case b). Conclusions: DNA typing of HLA using LifeMatch® fluoroanalyzer and the Lifecodes HLA-A, B and DRB typing kits is a not time-consuming method that allows high throughput analyses of small samples (e.g. bloodstains, bucal swabs), providing a highly informative system that could help to solve some complex paternity cases when STRs or other currently used markers, such as SNPs, are not enough to achieve an informative result.

P-280 Utility of X-chromosome SNPs in relation testing

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X-chromosome markers may complement the results obtained from other genetic markers in complex cases of kinship. Until now, reports on relationship testing using X-chromosome markers have only included data of Short Tandem Repeats (STRs) while no data on Single Nucleotide Polymorphisms (SNPs) in relationship testing have been published. We selected 25 highly polymorphic biallelic SNPs distributed through the human X-chromosome. One 25-plex PCR reaction and one 25-plex single base extension (SNaPshot) reaction were developed. The maximum size of the PCR products was 120 bp and the size of the SBE primers varied between 18 and 85 nucleotides. We analyzed the allele and haplotype frequencies in 1,078 unrelated males. All the SNPs were polymorphic and the lowest minor allele frequency was 0.103. All the haplotypes were unique. The forensic parameters were calculated on Danish and Somali populations. In the Danish population (N=93), the Power of Discrimination (PD) in females was one in 4.4 x 109 individuals and the PD in males was one in 2.6 x 106. The PD in Somalis (N=91) was one in 2.7 x 109 in females and one in 1.7 x 106 in males. Finally, we present an example of how the 25 X-chromosome SNP-plex may help to solve a complex immigration case.

P-281 Considerations on a motherless paternity case with two related fathers: possible pitfalls.

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In the course of a surveying of identification from burnt a particular case of paternity has been introduced. In a locality near Spoleto (Umbria), an oil mill has burned determining the death of four men. One of them was identified comparing the genetic profile with those of his relatives: a daughter and a brother. The wife was not available for the analysis. Data obtained with AmpFlSTR Identifiler (AB) showed a genetic compatibility between the man and his daughter. By chance we observed that a genetic compatibility was also evident between the daughter and the other man analyzed, the uncle. To clarify this situation we implemented the panel of markers analyzed, adding PowerPlex 16 (Promega), 6 miniSTRs (NCO1, NCO2) and 11 STRs of X chromosome.

P-282 X chromossome STR typing in deficiency paternity cases

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Many cases of deficiency paternity testing need more genetic information to complement the autossomal information. The sexual STR markers are a good approach to accomplish the task. Here we describe a case where the parents of a deceased man claimed that their son could not be the father of a girl, as alleged by the mother. We received biological material from the mother and the child and from the putative grandmother and grandfather. Only three genetic incompatibilities were detected within 21 autossomal STR markers. X chromosome (ChrX) STR markers could clarify this kind of results. Saliva and blood DNA obtained from people involved, was extracted by the Chelex 100 method. Twelve ChrX STRs plus the gender marker Amelogenin were studied, using the Mentype®Argus X-8 (Biotype AG) and a Decaplex X protocol from a GEP-ISFG proficiency study. The results obtained, clearly indicated that the putative grandmother could not be linked with the disputed child, as the X haplotypes didn't match. The conclusion was undoubtedly for the exclusion of the putative father. These results reinforce the importance of ChrX STR in deficiency paternity cases, particularly when there is genetic information from the child and from the putative paternal grandmother.

P-283 Complex Family Relationship Reconstruction by Y-STRs Test

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Y-STRs are common used by forensic laboratories to analyze male DNA from evidence containing mixtures of male and female DNA (for example in case of sexual assault) or in difficult paternity analysis and for reconstruction of male linage or application in kinship analysis. In fact since fathers pass their Y chromosome onto their sons unchanged (except for an occasional mutation),all males in a paternal lineage will possess a common Y chromosome haplotype. In the present casework we reconstruct the family relationship (father, brother, paternal uncle, paternal grandfather) of male individuals belonging to different generations till beginning of XXth century, who supposed to come from a unique common ancestor even if they showed different surnames. DNA was extracted from questioned blood samples by Instant Gene Matrix (Biorad) treatment and than quantified by the Quantifiler™ Y Human Male DNA Quantification Kit using a 7300 Real Time System. Samples were amplified by the AmpFlSTRs Yfiler™ that amplify 16 Y-chromosome STRs (DYS456, DYS389I, DYS390, DYS389II, DYS458 DYS19 DYS385 DYS393 DYS391 DYS439 DYS635 DYS392 YGATAH4 DYS437 DYS438 DYS635 DYS448) according to kit protocol and using a GeneAmp PCR Systems 2720 thermal cycler (Applied Biosystems). Female and Male Positive controls and negative controls were used during all amplification steps. Amplified products were analyzed by capillary electrophoresis on an ABI PRISM 3130 Genetic Analyzers (Applied Biosystems) employing GeneMapper 3.2 software.

P-284 The Use of X-STRs in the Investigation of Complex Kinship Cases

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Human identification analysis is a highly standardized routine procedure, performed by autosomal STR typing, occasionally complemented by Y-STRs and mtDNA studies. In spite of the high discrimination power and sensitivity of STRs, there are some instances whose results are inconclusive. Recent update of X-STRs provide a complementary tool, if the disputed child is a girl. The aim of this work is to underscore the applicability of the X-STRs for clarifying unsolved cases. Two sample sets were selected. Case 1: a maternity case involving a girl, her alleged father and two putative, full sibs, mothers. Originally tested with 21 STRs (PP16, Indentifiler, F13AO1 F13B, LPL, FES/FPS,) and HVRI/II mtDNA sequencing to confirm matrilineage. The father was excluded and the maternity test inconclusive. Case 2: a deficient incest case including three full sibs, a man and two women, and a daughter of one of them. The man who fathered the three offsprings was accused to fathered the daughter, but his sample was unavailable. STR results were inconclusive. Both cases were further investigated by 10 X-STRs: DXS8378, DXS9898, DXS7133, GATA31E08, GATA172DO5, DXS7423, DXS6809, DXS7132, DXS9902 and DXS6789. X-chromosome ability to recombine in females is the clue for investigating the first case. In the other case, the male unrecombined X chromosome transmission to all their daughters, allowed to exclude the supposed incest case.

P-285 Haplotype-assisted characterization of germline mutations at short tandem repeat loci

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In our routine case work for parentage testing we have observed > 300 cases in which a de novo mutation had occurred. The paternity was highly validated with W = 99.99% including the mutation. Germline mutations occur in STRs as deletion or expansion of repeat units. The widely accepted mechanism for these mutations is slipped strand mispairing (SSM), whereas also the possibility of unequal crossing over (UEC) exists. The following example shows the difficulty to assess the mutation's origin: child: 14/17, mother 14/18, father 14/16. Thus, the child's allele 17 could be caused by a maternal expansion or a paternal deletion of a repeat unit. With routinely used STRs it was not possible to determine the mutation's origin. We here report our results from 112 families in which we have observed a mutation at one of four loci (i.e., ACTBP2, D3S1358, FGA, VWA). We chose five to seven polymorphic flanking markers in each case. These were typed by amplicon sizing on an ABI PRISM 310 Genetic Analyzer to generate family-specific haplotypes. This allows the characterization of the present mutation with regard to origin, effect, degree, and mechanism. The results of our study will be presented and consequences for the analysis of STR mutations in parentage testing will be discussed.

P-286 Meiosis study in a population sample from Nigeria: allele frequencies and mutation rates of 16 STR loci

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The 16 short tandem repeat systems ACTBP2, CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, and VWA were amplified in a population sample composed of 341 immigrants from Nigeria. The 16 loci met Hardy-Weinberg expectations and the most informative loci were FGA, D18S51 and ACTBP2with a power of discrimination from 0.968 to 0.981. More than 30 mutations were observed in immigration cases from West Africa (mainly from Nigeria, Ghana, Togo). Mutation rates for the above loci will be presented and the consequences for paternity testing will be discussed.

P-287 Y Chromosome Polymorphisms in Argentine population

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Short tandem repeats (STRs) loci are the most informative PCR based genetic markers available to date for attempting to individualize biological material. The full use of DNA typing technology in forensic science has grown up by the development of National DNA databases. That is the reason why today, many efforts are made to build up Y STRs databases for forensic purposes. Knowledge about mutation rates and mutational process of short tandem repeats (STRs), microsatellite loci used in paternity testing and forensic analysis, is crucial for the correct interpretation of genetic profiles. In our study, we analyzed Y Chromosome Polymorphisms for the loci: DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS439, DYS438, DYS458, DYS635, Y GATA H4, DYS437, DYS456 and DYS448. Multiplex PCR amplification of 16 loci was performed using AmpFlSTR® Yfiler™ kit (Applied Biosystems) in unrelated Argentine individuals, most of them from Buenos Aires. Statistical interpretation of the results let us create a database of our own population. Keywords: Paternity Testing Y Chromosome STRs Mutation * Corresponding author. E-mail address: amdl@fibertel.com.ar (A.M. Di Lonardo).

P-288 Interpretation of Paternity Test Data with Two Possible Exclusions

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A co-habiting couple whose commercial paternity test was reported as negative requested a second opinion. The disputed result was based on two apparent exclusions between the putative father and child at D8S1179 and CSF1PO (PowerPlex 16®). Our laboratory repeated the test using PowerPlex 16®, FFFL System® and SGM Plus® kits (total 21 loci). Apparent exclusions at D8S1179 and CSF1PO were observed. All Y-Chromosome haplotypes of the putative father and male child matched (PowerPlex Y® kit). From the 13 matching autosomal loci, PI >10 million. The reproducibility of the two exclusions, high PI, matching Y-Chromosome haplotypes and the absence of further exclusions indicate that it is possible that the exclusions were due to mutations at the D8S1179 and CSF1PO loci in the putative father. However, it is also possible that the observed alleles at CSF1PO: Man (11,12), Child (10,11), Mother (11,12) have arisen as a result of a mutation from either the mother or father. It was incorrect to report this case as a negative paternity as three exclusions were not found and indeed it is possible that a single exclusion exists between the tested man and child. This highlights the necessity for caution when interpreting paternity data. The possibility of a mutation having arisen from the mother must be taken into consideration.

P-289 A DECADE FOR SEARCHING THE FATHER

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She was a baby when her father went to USA from Turkey. When her mother got old, one of her close friends asked her to go as a trio to give blood for paternity test. The results using the conventional systems showed 91.6% paternity for that "friend". The evidence was considered to be adequate to accept her as his daughter, although the required percentage by the Turkish Legal System for paternity proof is 99.73%. Following the death of the father in 1996, his family rejected the parentage. They claimed that her real father is in the USA and they asked for DNA test. The person was excluded by 4 loci with the RFLP technology. The family still rejected the proof of paternity and they asked for DNA test from bones of the father in Turkey. The Council of Legal Medicine in Turkey which is the official expert witness institution was unable to extract DNA from the bones. The daughter (57 years old by now) applied finally to the International Forensic Science Services, a private DNA testing laboratory for an opinion. DNA test of the exhumation material included a very good amount of hair proved the paternity by 99.95%.

P-290 The Use of Supplemental STR Loci for Resolving Difficult Family Relationship Cases

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The combination of PCR and short tandem repeats (STR's) is by far the most common technology used for parentage and relationship testing. The multi-locus Identifiler (ABI) and Powerplex 16 (Promega) STR kits are adequate for resolving the majority of cases, but a small proportion require additional testing for resolution. We routinely employ secondary panels of STR loci in conjunction with Identifiler to help resolve problematic biological relationship tests such as those involving related alleged fathers and genetic inconsistencies. One multiplex (DDCPlex) contains commercially available STR markers for Penta B, Penta C, Penta D, Penta E, F13A1, F13B, FES/FPS, LPL and SE33. In addition to the DDCPlex loci we have developed a 4-locus multiplex made up of "mini-STRs". These markers were initially designed to allow degraded DNA to be more readily typed due to their reduced amplicon size (Coble and Butler, JFS 2005). This multiplex provides a robust set of markers (D1S1677, D2S441, D10S1248 and D22S1045) that can also be used to supplement our battery of test systems. We present examples whereby DDCPlex and mini-STR loci have proven useful in resolving complex kinship and parentage cases.

P-291 Increased Discrimination Using a Panel of 83 Immobilized SSO Probes Targeting 48 Informative Sites within the Mitochondrial Genome

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Mitochondrial DNA is an informative and useful target for the forensic genetic analysis of limited and/or highly degraded samples. However, there are some inherent limitations to targeting only the hypervariable regions I/II independent of the method of analysis. Notably, the power of discrimination is limited for all population groups as a result of a few common HVI/II sequences. Therefore, additional sequence polymorphisms outside the HVI/II regions need to be targeted to increase the power of discrimination of mtDNA analysis. To address this need, we have developed a multiplex PCR and immobilized probe assay which targets 48 polymorphic sites (24 HVI/II and 24 VR/CR). This linear array, expanded from our initial HVI/II assay, allows for simultaneous genotyping of informative sites distributed throughout the mitochondrial genome using 83 immobilized probes. By targeting sites that help subdivide common HV sequences as well as rapidly evolving sites, we have greatly increased the discrimination power beyond that of the HVI/II assay for all populations. Moreover, the expanded array is more informative than sequencing the HVI/II regions for the Caucasian population, h= 0.996 compared to 0.995. We present here population data to illustrate the increased informativeness as well as the ease of use of this assay.

P-292 DNA obtained from decomposed corpses cartilage: a comparison with red muscle source

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The outcome of typing DNA obtained from human remains is, in great part, a matter of the choice of the correct tissue to be collected. According to the literature, when the decision falls into soft tissue, red muscle has been often the first choice. Nevertheless, decomposition runs in different speed for different tissues. It is known that hyaline cartilage from joints decomposes slower than red muscle, leading to hypothesize that DNA degrades in the same way. To test this hypothesis, DNA was extracted from red muscle and hyaline cartilage from knee, elbow and shoulder joints, sampled 48 to 120 hours after death from 22 decomposed corpses of victims of GOL Linhas Aéreas flight 1907 aircraft crash (september 29, 2006) on an inhospitable place of Brazilian Amazon rain forest, collected as part of the efforts to identify them. To test the quantity and quality of obtained DNA, DNA samples was quantified, employing real-time PCR, and quality was verified by amplification results for multiplex system of DNA Amplification Identifiler (Applied Biosystem). The number of cartilage samples whose amount of DNA overcame 0,5 nanograms was remarkable higher, compared to red muscle samples. A second extraction confirmed the outcome. The quality of cartilage DNA was confirmed by the obtained genetic profiles. According to theses data, cartilage may be the first choice among soft tissue, in cases of decomposed bodies, at least in tropical countries like Brazil.

P-293 BCR Amplification of DNA extracted from old human bones

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The DNA polymorphisms are detected either by (RFLPs) or by PCR that allows the detection of genetic markers in samples containing as little as 2ng of DNA.DNA are stable in postmortem tissues (blood) as well as in bones & teeth. The aim of this work was to verify the medicolegal importance of amplification of DNA extracted fronm the human bones as a tool of identification of unidentifiable human remains.It was conducted on 35 bone specimens & 5 complete teeth (at least 20 years old).It was extracted by the organic phenol chloroforme method & extracted DNA was assessed by runing yield gels.DNA amplification was done using Perkin Elmer 480 DNA thermal cycler.Typing for 6 loci(DQAI,LDLR,GYPA,HBGG,D7S8 & GC)was performed.Significant amount of degraded DNA was detected.DNA yields were higher in spongy than in compact bone.Most of the samples were succefully amplified & typed for the 6 mentioned loci.

P-294 Genetic investigation of highly decomposed bodies: bone or soft tissue analysis?

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The identification of putrefied bodies is a common task in forensic routine work. The deceased are usually identified by dental records, fingerprinting, or DNA analysis. However, with progressive putrefaction, DNA integrity is rapidly decreasing. The aim of our study was - based on an optimised extraction method - to determine, whether the identification of decomposed bodies should be done by soft tissue investigation or bone analysis. Different tissues from 19 bodies in different stages of decomposition were taken during forensic autopsies. DNA was extracted, and the quality and quantity were controlled by agarose gel electrophoresis and real time PCR, respectively. STR analysis was done using the AmpFlSTR Identifiler Kit. Additionally, mtDNA sequencing was performed. The results show, that the quality and quantity of extracted DNA was very different from individual to individual and - as expected - got worse with advancing decomposition of the bodies. Soft tissue investigation alone yielded sufficient results for the identification of 21 out of 23 individuals. The additional analysis of DNA extracted from bones did not increase the number of successful individualisations. Thus, the identification of decomposed bodies by time consuming bone investigations seems not to be advantageous compared to soft tissue analysis.

P-295 The Italian Forensic science police service DVI team.

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Background and purpose: Mass disaster can involve natural or non-natural catastrophes: each incident has its own charateristics and will involve a different approach. Following our experience in Thailand and in Sharm el Sheikh (Egypt), in Italy it has been formally instituted a DVI group in April 2006. Method: The Italian police DVI group uses the Interpol AM and PM forms. In case of a national disaster it is assumed that 1 or more units work on the place of the disaster to obtain PM data and 1 unit works to collect AM data (DNA, fingerprints and dental records): all the AM and PM datas are collected in the central headquarter based in Rome that coordinate all the activities and is in charge for the identifications using ADVIS software (like Plassdata). Results and Conclusion: The Italian police DVI group is constituted of 12 sub-groups. Every sub-group is composed of: one pathologist, one DNA expert, one forensic odontologist, two fingerprints, two technical personnel (photographer and computer scientist), one psychologist and two interpreters: all these figures come from the Italian national police so the DVI group is almost independent and could work either by itself or in collaboration with other groups.

P-296 The use of DNA analysis in the identification of victims in mass disasters occurred in Brazil and Paraguay

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The Institute of Forensic DNA of Polícia Civil do Distrito Federal (Brazil) participated in the identification of victims in mass disasters taken place in the last years. In 2003, a slaughter of 26 gold miners prospectors took place in an aboriginal reserve in Rondônia (Brazil). Fifteen bodies in advanced decomposition state were identified by DNA analysis. In 2004, an explosion in a supermarket in Asunción (Paraguay) killed 356 people, most of them carbonized. Amongst the 356 bodies, 270 were identified by relatives, 14 by fingerprints, 14 by anthropology studies, 33 by dental structure, 28 by belongings and 38 by DNA. It took the analysis of 197 families, 115 body fragments and 11 exhumed skeletons to solve problems regarding the initial release of the bodies. In 2006, a Brazilian Boeing collided with an American jet, killing 154 people. The rescue of the bodies in the forests of Mato Grosso (Brazil) involved a group of forensic technicians, including experts in DNA. 129 bodies were identified by fingerprints, 12 by anthropology studies and 13 by DNA, including four children. 60 body fragments were examined, 18 of which consisted of bones used in the identification of nine passengers founded under the airplane wing.

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P-298 Spanish Civil War and the Recovery of Historic Memory: a view from Forensic Genetics

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During the Spanish Civil War, around 70 years ago, Spanish history met one of its darkest periods. From 1936 to 1939, many people were killed and buried into graves all over Spain. The slaughter of Valdedios town (Asturias, Northern Spain) is among the most remembered of these episodes. In October 1937, 17 people, most of them workers of a psychiatric hospital, were forced to excavate their own tomb and once inside they were assassinated by national troops. This is the grave featuring the greatest amount of nurse women ever executed in Spain. Currently, some projects addressed to the "Recovering of Historic Memory" and, in some cases, forensic genetics, are helping to clarify events like this. Unfortunately, the degree of degradation often prevents the anthropological identification of the bone remains, including sex determination. To overcome this difficulty, DNA was extracted from bone remains and the amelogenine locus analyzed in order to determine the sex. When a putative matrilineal relative was available, mitochondrial DNA was analyzed to determine their belonging to the same maternal lineage. Up to the moment, these analyses have allowed sex determination of several bone remains and mitochondrial DNA lineage assignment of some others.

P-299 May a speaking individual contaminate the routine DNA laboratory?

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There is a risk of contamination from non-casework DNA in a forensic laboratory producing DNA profiles. Port et al. (2005) demonstrated that a speaking person can contaminate bench papers deposited in front of him. They carried out PCR of 34 cycles. These results call for further analyses to be carried out using routine forensic DNA laboratory methods. Hence, we here report a pilot study using PCR of both 28 and 30 cycles. Three test persons wearing protective clothing without face mask repeated a sentence in sitting and standing positions for different time intervals. They were placed under controlled conditions in front of sheets of new bench papers. After the speaking procedure, the bench papers were swabbed. DNA was extracted from the swabs using Chelex-100®, and STR profiling was carried out using the AmpFISTR SGMPlus® PCR Amplification kit. Complete DNA profiles were detected from two of the test persons after speaking in the standing position. No DNA profile was detected from the third person. The results indicate that a detectable contamination may take place as a result of unprotected speaking in a routine forensic DNA laboratory. We plan further investigations in a crime scene setting.

P-300 A forensic laboratory information management system for daily case work

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The number of forensic samples is rapidly increasing nowadays and it is necessary to speed up their analysis by concurrent increase of the quality. The way to meet this challenge is the integration of a laboratory information management system (LIMS) in a forensic lab. The system supports the whole process of forensic case work from the registration of forensic materials and samples, the analysis of the samples by pre tests, PCR and fragment analysis, up to the automatic creation of surveys. The laboratory procedures (SOP) could be easily implemented by definition of specific workflows for the sample analysis and the data collection by supporting lab devices and robots. All critical steps could be monitored by the use of the integrated quality assurance systems like contamination controls and audit trail. The creation of surveys is supported by validated bio statistical analysis methods and integrated population databases. Thus a LIMS can shorten the turnaround time of a sample in a lab and improve the quality assurance by supporting all necessary guidelines for forensic labs.

P-301 IGNA's original LIMS : a complete traceability of administrative and analytic process for forensic cases

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IGNA is the first French laboratory for forensic DNA analysis. IGNA LIMS was developed from the software Microsoft Dynamics NAV which is an ERP (Enterprise Resource Planning) with a real opened solution for specific development and compatible with a lot of Microsoft applications. Thanks to the LIMS, we assure the traceability of analysis and also administrative process (traceability of letters, quotes or phone calls for each case and registration of actions to be done in a case (call for results…)). For one penal case, we can associate one or more sealed items. The DNA expert indicates the analysis to do on each piece (biological fluid detection, type of extraction, nuclear or mitochondrial DNA analysis…). During the technical process, samples automatically pass from a step to the next. Technicians indicate all the information needed for traceability (consumables lot number, used robots …). At the end of the process, the DNA expert validates his results, which are then directly transferred to the final report. Development of the IGNA LIMS from Microsoft Dynamics NAV allows us to use all specific applications of an ERP such as purchases, marketing, sales, etc. and to assure computerization of all our process, from quote to analysis report.

P-302 New Resources for the Forensic Genetics Community Available on the NIST STRBase Website

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For more than a decade, NIST has maintained the Short Tandem Repeat DNA Internet Database (STRBase), which is located at http://www.cstl.nist.gov/biotech/strbase/. The purpose of STRBase has been and continues to be an attempt to bring together the abundant literature and information in the forensic genetics field in a cohesive fashion to make future work easier. New resources that are regularly added to the website can now be quickly found at http://www.cstl.nist.gov/biotech/strbase/updates.htm. For example, a page was recently created to track null alleles detected through DNA testing with different PCR primer sets (see http://www.cstl.nist.gov/biotech/strbase/NullAlleles.htm). In addition, a new software program enabling concordance checking with multiple data sets can be downloaded from http://www.cstl.nist.gov/biotech/strbase/software.htm. Hundreds of new pages of information have been added in the past few years and this presentation hopes to introduce conference participants to the new information freely available on-line at the STRBase website.

P-303 Development and Usage of a NIST Standard Reference Material for Real Time PCR Quantitation of Human DNA

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Background and purpose: SRM 2372 Human DNA Quantitation Standard has been produced to support the need for a human-specific DNA quantitation standard in forensic casework and calibration of new quantitative polymerase chain reaction (qPCR) assays. Method: The conventional DNA concentration has been assigned with the U.S. National Reference UV/Visible Spectrophotometer, assuming an absorbance of 1.0 at 260 nm equals 50 ng/µL of double stranded DNA. In addition, an interlaboratory study has been conducted, to verify that the SRM 2372 materials perform well in currently used DNA quantitation assays by the forensic DNA community. Results: Each unit of SRM 2372 consists of three well-characterized DNA extracts. Component A is a single-source human male material derived from blood. Component B is a multiple-source human female material derived from blood. Component C is a multiple-source male and female material derived from freeze-dried human placentas. Conclusion: SRM 2372 is intended to enable the comparison of DNA concentration measurements across time and place. Manufacturers can use SRM 2372 to validate the values assigned to their own reference materials. Individual forensic laboratories can use SRM 2372 to validate DNA quantitation methods and to verify the assigned concentration of in-house or commercial DNA calibration standards.

P-304 INTERNAL VALIDATION OF THE 7500 REAL TIME PCR SYSTEM FOR USE AT FORENSIC CASEWORK AT THE DEPARTMENT OF ANALYSIS OF BIOLOGICAL MATERIALS, HELLENIC POLICE

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According to the guidelines of the quality assurance standards for the forensic DNA testing laboratories, prior to introduce an existing DNA analysis procedure, the forensic laboratory has to demonstrate reliability of the procedure by carrying out internal validation. In order to use a sensitive and accurate DNA quantification method in our laboratory, a validation study of the 7500 Real-Time-PCR System (Applied Biosystems) using the QUANTIFILER Human DNA Quantification Kit (Applied Biosystems) was performed. Forty (40) case samples were used in this study and four (4) elements which comprise reproducibility, mixture studies, sensitivity and accuracy were studied. Following the Real Time-PCR Quantification, 1ng DNA of each sample was amplified in a GeneAmp PCR System 9700 thermal cycler using the PowerPlex 16 System (Promega) and was run on a 3100 Genetic Analyzer (Applied Biosystems). Mixed stains were also amplified using the PowerPlex Y system. Here we report the results and the experience we have gained during this internal validation study.

P-305 DNA Isolation from Stored Blood and Urine for Forensic Identity Testing in Racehorses: Effects of Storage, Freeze-Thaw Cycles, Drugs, and Contaminants

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In the horse racing industry, blood and urine samples are employed for drug screening. DNA typing for identification of the horse and the origin of the sample is uncommon. Little is known of the recovery and amplification of DNA for short tandem repeat (STR) detection from stored samples. In this study, DNA was isolated from blood and urine using PureGene DNA Purification System (Gentra, USA). Quality of DNA isolate was tested by polymerase chain reaction (PCR) amplification of five STR loci. Factors that may influence DNA recovery and PCR were investigated. High molecular weight DNA can be recovered from refrigerated blood samples but its recovery from urine varies. Following centrifugation, urine supernatant is usually discarded during DNA isolation. However, amplifiable DNA was recovered from both urine supernatant and sediment. Repeated freeze-thaw cycles significantly reduced DNA yield and induced DNA degradation resulting in failure to detect STR. The presence of select drugs in the samples had no effect on PCR amplification. Contaminants in DNA isolate inhibited PCR. We employed multiple centrifugations to deplete protein contaminants before DNA precipitation. Results indicated that properly stored samples of total urine and blood could be successfully genotyped.

P-306 Identification of human urine and DNA-typing from urine stains

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Forensic investigation of urine stains in murder cases is necessary in order to ascertain the exact location of the crime and the precise manner of death. We have previously reported one method for identifying human urine stains with the analysis of 5-major 17-ketosteroid conjugates and second one for DNA typing from urine stains. In this report, we attempt to combine the LC-MS analysis of 17-ketosteroid conjugates and genotyping for practical forensic investigation of small urine stains. A urine stain was extracted with PBS, and the extract was filtrate using a Centricon-100 device. The filtrate was subjected to electrospray ionization liquid chromatography-mass spectrometry (ESI-LC-MS) for the identification of human urine and a DNA-typing sample was obtained by dialfiltration of the residue using a DNA purification kit. The extracted DNA was amplified with the AmpflSTR® Profiler™ PCR amplification kit, the DNA-types were analyzed by capillary electrophoresis using a Genetic Analizer ABI310. It was possible to identify human urine and DNA profiles could be obtained from urine stain at the same time. This method can be recommended for identification and genotyping of urine stain.

P-307 Paternity Testing in Italy via Internet: who protects the minors?

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Paternity analyses carried out by laboratories via Internet often involve sending the requesting parties a kit for collecting samples followed by sending back the samples to the laboratory. Information are given via the laboratory's web site or by the mailing of written notification. The repercussions of the result have a great emotional impact that could, when unexpected, cause turmoil among the people involved, the greatest repercussions will inevitably be felt by the children. Recently easy-to-use kits have also been introduced in Italy. The Italian Privacy's Garante is assessing the ethical and legal implications but regulations are not yet in place. In this work, we want to consider some legal and ethical issues: we will analyze problems relating to information, consent and certifying the origin of samples by way of outlining the relevant Italian deontological laws and codes, and the Oviedo Convention's. Finally, we will analyze issues regarding the position of the personnel who carry out the analysis. In our opinion, the complexity of the situations and expectations linked to paternity investigations require a special sensitivity in dealing with each case, each with its own specific legal and ethical-deontological issues, while taking into account the emotional stability of the subjects involved.

P-308 The Application of mtDNA SNPs to Forensic Casework

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The Armed Forces DNA Identification Laboratory (AFDIL) routinely uses mitochondrial DNA (mtDNA) sequence data for sample sorting and individuation in Vietnam, Korea, and WWII-era cases where the DNA is expected to be highly degraded. A significant limitation to the use of mtDNA is the low power of discrimination when common mtDNA hypervariable region types (HV types) are encountered. We have previously identified mtDNA SNPs to target several of the most common W. European HV types. We will report on the use of a SNP multiplex to assist in the resolution of four cases in which samples matched or closely matched the most common HV type in W. Europeans. In two of the cases, the SNP typing successfully excluded one of two reference families that could not be excluded on the basis of HV1/HV2 sequencing. In a third case, SNP typing confirmed the sorting and re-association of multiple commingled skeletal elements. In a fourth case, the addition of the SNP data more than doubled the discriminatory power of the mtDNA evidence. The application of a specific mtDNA SNP assay in these cases demonstrates its utility in sample individuation when the most common W. European HV type is encountered in forensic casework.

P-309 Assessing trace DNA evidence from a residential burglary: Abundance, Transfer and Persistence

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The considerable identifying power of DNA can see it treated as an absolute form of evidence, rather than as any other trace evidence. This study aimed to provide data into the abundance, transfer and persistence of trace DNA, in a particular crime scenario – residential burglary. Windows and doorframes in 30 residences were swabbed to determine the background levels of DNA present. The persistence of DNA on exterior surfaces was examined by depositing set quantities of DNA, left for up to 6 weeks. The deposits were quantitated using real-time PCR, to estimate the chance of recovering DNA at certain time periods after an event. The likelihood of DNA transferring during a break-in was tested. Negligible DNA was found to be present during the abundance study, indicating that if a profile is recovered at a break-in point, it is unlikely to be the result of pre-existing DNA traces. The persistence study indicated that profiles recovered on exterior locations are likely to be from a recent contact. The effect of shedder status was noted during the transfer experiments. The findings demonstrate that research to provide background data on the trace evidence characteristics of DNA will aid its interpretation and presentation in criminal trials.

P-310 Transcriptome of mouse lung tissue in postmortem stage after hanging

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We investigated a transcriptome profile of mouse lung in postmortem stage after hanging by serial analysis of gene expression (SAGE) method. RNA was extracted from the lung tissue left for 0, 30 or 60min after hanging previously reported. Control groups were killed by decapitation without hanging of the neck and left for the same time. First, we investigated the level of immediate early genes (IEGs) mRNA such as c-fos, fos B and c-jun that induced another genes. As the results, the all IEGs drastically increased at 30min and rapidly decreased at 60min. These results indicated that transcriptome profiles might change at 60min. Therefore, we subjected 60min samples for SAGE. We have been able to obtain the transcriptome profiles after analyzing 11717 tags in hanging and 10335 tags in control. 7551 different genes in hanging and 7166 in control were observed. Compared with the two groups, 58 genes, including Glutathione reductase 1, and Heat shock protein 40, were increased in hanging. On the other hand, 79 genes such as Selenoprotein P and Calmodulin 1 were decreased. These results may contribute to clarify the pathophysiology of asphyxia in the lung and aid in the diagnosis of suffocation.

P-311 Gene responses to prevent neuronal apoptosis following cocaine administration in mouse brain

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Cocaine abuse represents a worldwide significant issue because of one of the most dangerous illicit drugs in common use. Psychiatric and neurological symptoms are usual manifestations of cocaine toxicity. A few reports mentioned some mRNA changes of CNS by cocaine administration. We analysed the transcriptome in mouse brain following cocaine administration, using a fluorescence differential display. 17 differentially expressed genes were found, and confirmed by nucleotide sequence and real time PCR. Of these genes, 4 genes including silencer of death domain (SODD) and Mpv 17-like protein (M-LP) increased. On the other hand, 13 genes containing A20 binding inhibitor of NF-kappa B activation-2 (ABIN-2) decreased. The inhibition of TNF cascade by SODD binding to TNF receptor, and the decreasing reactive oxygen species production by M-LP via SOD, lead to apoptosis suppression. In addition, decreased ABIN-2 suppresses apoptosis via NF-kappa B reduction. Therefore, our results indicated that the expressional change of the genes following cocaine administration might prevent apoptosis. However, Nassogne et al. reported that cocaine induced neuronal apoptosis, conversely. There is discrepancy between their perspective and our results. As regard this, although neuronal apoptosis actually occurred as phenomenon, we assumed that brain tissue might change gene expression to survive neuron.

P-312 Sequence analysis of two de novo mutation alleles at the Y-STR locus

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We have performed haplotyping of 16 Y-STRs (giving 17 PCR products) in 47 biological paternity father and son cases in Japan. We encountered two paternity cases where unusual alleles at the DYS439 and DYS 448 loci were detected in the son. In case 1, allele 15 at the DYS439 locus of the son was found not to derive from the father (allele 14). In case 2, the father (allele 18) and the son (allele 17) shared no allele at DYS448. In this study we characterized the mutant alleles at the two loci by sequence analysis. The repeat region of DYS439 consists of simple sequence (GATA). Sequencing showed that the son gained a single unit (GATA) at DYS439 in case 1. Allele 15 of the son seemed to result from gain of a single unit from allele 14 of the father in this case. On the other hand, DYS448 shows complex structure in the repeat region, containing two variable regions (AGAGAT) and one constant region. Direct sequencing showed that the son lost one unit (AGAGAT) at DYS448 in case 2. In this case, allele 17 of the son must mutate from the father?s allele 18 by loss of a single repeat unit.

P-313 Correlation of surnames and Y-chromosome in Central-Brazil

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In patrilineal societies, surnames and Y-specific haplotypes and haplogroups are expected to be correlated. This characteristic could help defining an initial pool of suspects in forensic genetics analysis. Here we evaluated this correlation in a sample of Central-Brazilian men. Surnames and Y-SNP haplogroup and Y-STR haplotype were analyzed in 55 pairs of Central-Brazilian men sharing surnames (n = 110). Seven haplogroups and 32 haplotypes have been observed, none correlated solely to any of the 28 surnames represented here. In this sample, two men with the same surname showed a chance of 0.41 of sharing a Y-specific haplogroup. This chance is higher for surnames of intermediate frequencies, whereas rare surnames show distinct chances as zero and one. The results observed may be overestimated due to a predominance of a specific haplogroup (P92R7 = 49%) in the sample, which makes it possible for two men with no coancestry to share this haplogroup. Considering STR, only three pairs of men shared haplotypes. The average difference between the haplotypes in each pair was 2.45 mutational steps. This relatively low correlation is due to some historical and cultural peculiarities of the Country, making it improper for forensic purposes in Brazil.

P-314 Concordance Study between the AmpFLSTR® MiniFiler PCR Amplification Kit and the AmpFLSTR® Identifiler® PCR Amplification Kit

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The AmpFLSTR® MiniFiler PCR Amplification kit was developed to recover information from compromised DNA samples which could be degraded and /or inhibited. Eight of the largest STR loci included in the Identifiler® and SGM Plus® kits were reduced in size to yield smaller amplicons. The MiniFiler™ Kit amplifies CSF1PO, FGA, D2S1338, D7S820, D13S317, D16S539, D18S51, and D21S11 as well as the sex-typing locus Amelogenin. Population studies were conducted with both the MiniFiler™ and Identifiler® STR kits and a total of 1,308 samples were evaluated including: 449 African American, 445 Caucasian, 207 Hispanic, and 207 Asian individuals. Full concordance between the Identifiler® and MiniFiler™ Kits was observed in 99.7% of STR allele calls compared (10,437 out of 10,464). The 27 differences were observed in the loci D13S317 (n=14) and D16S539 (n=10) as well as D18S51 (n=1), D7S820 (n=1), and CSF1PO (n=1). Samples containing genotyping discrepancies between the Identifiler® and MiniFiler™ kits were sequenced in order to determine the nature of the genetic variations causing the allelic dropout or apparent shifts in the repeat unit. The possibility of discordant results should be taken into consideration during interpretation when utilizing kits which contain different primer sequences for the same loci.

P-315 Analysis of DNA from degraded tissue

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Background and Purpose: To investigate the PCR-amplified fragments sizes generated from DNA templates obtained from degraded tissue samples at various stages, we simulated conditions leaving 2-inch cubic chunks of pork to decompose for one week in water, sea water, and air-dried. Method: Tissue samples were collected everyday and submitted for PCR analysis using two sets of PCR primers designed from the nuclear beta-actin gene and one from the mitochondrial cytochrome b gene loci. Results: It was shown that the 211-bp, 289-bp and 366-bp nuclear DNA fragments were amplified from all samples immersed in water and sea water up to the 8th day. The 289-bp fragment was amplified from samples which were left to air-dry up to the 4th day. The 323-bp mitochondrial cytochrome b fragment was amplified from all samples up to the 8th day. In addition, the experiment was extended up to the 14th day for the air-dried samples and we found that the 323-bp fragment was PCR-amplified from all samples. Conclusion: The results suggested that there is a good chance to obtain a full nuclear DNA profile from tissue samples immersed in the water and sea water for a week. However, if tissue samples were left air-dried in open environment over 4 days, there would be a high chance to obtain an incomplete/ no DNA profile.

P-316 Optimisation of recovery and profiling of DNA from toothbrushes

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In human identification, the toothbrush is an invaluable personal item from which DNA can be gathered to produce a reference profile. The profile obtained allows direct comparison to be made with the profile from an unidentified body or a victim of a mass disaster. This study was undertaken to determine the minimum number of bristle bundles that would generate a complete DNA profile. The minimum period of usage for a toothbrush to retain enough cells for genotyping was also investigated. In addition, two commonly used DNA extraction methods: QIAamp® DNA Mini Kit and Chelex® 100 were tested to explore the efficiency of these protocols in recovering DNA from toothbrushes. In this experiment, volunteers brushed their teeth for 1, 7, 14, or 30 days. DNA was extracted from five and ten bundles of bristles cut from the collected toothbrushes. The amount of DNA recovered was quantified by quantitative real-time PCR, and DNA genotyping was performed for each sample. Data revealed that QIAamp® DNA Mini Kit performed better at yielding DNA in terms of purity, quantity, and quality than Chelex® 100. It was also found that, with a suitable method of recovery, DNA from 5-bundle samples of all periods could be used to generate complete profiles. Based on the experimental results, a general guideline concerning the appropriate extraction method and the quantity of the starting material for the analysis of DNA from toothbrushes could be suggested.

P-317 GENOTYPING OF HUMAN NUCLEAR DNA RECOVERED FROM THE ALIMENTARY CANAL OF FLY LARVAE

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Fly larvae recovered from dead bodies are often examined in medico legal investigation for Post Mortem Interval (P.M.I.) estimations. Some authors also suggested that the gut's content of such insects can be submitted to DNA typing analysis in order to identify the source they have fed on. In this study we aimed to recover suitable human DNA form the crop of third instar maggots (larvae of Calliphoridae) recovered from a cadaver in the first stage of decomposition. Different collection, storage and extraction methods were compared as to DNA yield and quality, for this purpose Real Time PCR was performed on the extracted samples. Finally autosomal and Y-STR profiles matched positively with a reference sample of the dead man. Our results showed the suitability of DNA gut's content of fly larvae for forensic purpose, moreover, they confirmed the importance of strict collection and storage guidelines to guarantee successful downstream analysis.

P-318 Identification of CYP2D6 and CYP2C19 alleles and CYP2D6 gene copy number in forensics using Pyrosequencing

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Background: Two important cytochrome P450 enzymes, debrisoquine 4-hydroxylase (CYP2D6) and S-mephenytoin 4-hydroxylase (CYP2C19), are polymorphically distributed in the population. They metabolise many commonly used drugs. About 7-10% of Caucasians lack CYP2D6 and approximately 3% lack CYP2C19, known as poor metabolisers (PMs). They may have an increased risk for adverse reactions and in some cases unexpected intoxications even with fatal outcome, upon administration of drugs in normal therapeutic doses. In contrast, 1-7% of the Caucasian population carry a gene multiplication of CYP2D6 leading to increased activity. These so called ultrarapid metabolisers (UMs) do not reach therapeutically plasma levels of the drugs and the treatment fails. The aim of this work was to study different CYP2D6 and CYP2C19 alleles associated with poor metabolism, and to identify CYP2D6 gene multiplication in different forensic cases compared to blood donors. Method: Pyrosequencing was used to identify CYP2D6*1-*6, CYP2D6 multiple gene copy number and CYP2C19*1-*4. Results and Conclusion: Preliminary results indicate differences in the structural polymorphisms in CYP2D6 between 242 individuals who committed suicide by intoxication, 262 individuals who committed suicide by hanging or shooting and 212 individuals who had died but not by intoxication, shooting or hanging have been genotyped and 282 Swedish blood donors.

P-319 Genetic Identification in Endodontic Treated Tooth Root

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During endodontic treatment the pulp tissue and the inner layer of dentine are removed. The treated root may be a significant sample to study in forensic situations in the field of genetic analysis, when all other tissues are degraded and when it is the only remaining tooth structure. In this kind of sample the remaining cells are odontoblasts and cementocytes, retained in the hard tissue. Treated root fragments and blood on a compress as a reference sample were studied. During cleaning procedure the external layer of the root and the filling were removed in order to reduce contaminants. DNA extraction from hard tissue powder: Puregene® DNA Purification System kit (PE Gentra), DNA quantification in an ABI Prism 7000, nuclear DNA: AmpFlSTR®Identifiler (Applied Biosystems), mitochondrial DNA: HVRI and HVRII, results obtained by ABI Prism 310 and 3130 sequencers. Skeletal remains and mass disaster victims found in severe adverse conditions, as well as the low standard of oral hygiene in the general population prevent us from finding many integral teeth. This study showed that even in roots with endodontic treatment, were obtained full STR profiles, giving the possibility of individual genetic identification.

P-320 AmpFlSTR® MiniFiler™ PCR Amplification Kit: the new MiniSTR multiplex

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Multiplex STR typing of forensic samples is a powerful tool for genetic identification, that allows quickly to achieve a high combined discrimination power with low DNA consumption. If full profiles are obtained at the majority of high quality DNA samples, in degraded samples the higher molecular weight markers fail partial or completely their amplification. In order to minimize this problem several miniSTR (with redesigned primers that generate reduced amplicon fragments) have been developed. The AmpFlSTR® MiniFiler™ PCR Amplification Kit (AB), a new available 8-miniSTR multiplex, includes the most common problematic loci (above 200bp) of the Identifiler™ kit: D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO and FGA. The MiniFiler™ sensitivity was tested as well as several casework samples with different DNA contents. DNA extraction was made with Chelex (stains and swabs) or commercial kits (bones and hairs) and quantified with an ABI Prism® 7000 amplification was performed in accordance with the manufacturer´s instructions samples were genotyped using an ABI Prism® 310 Analyser and the GeneMapper ID 3.2 software. Results allowed to complete several partial Identifiler™ profiles and additional information was achieved in a 15 pg DNA sample, revealing that this miniSTR kit can improve identification of degraded or LCN samples.

P-321 Identifying the origin of cells

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In many cases, it can be impossible to attribute a DNA profile recovered from a sample to a specific body fluid or cell type, limiting the evidential value of a match. The introduction of highly sensitive 34 cycle SGMPlus™ amplification technology has allowed DNA profiles to be obtained from samples so minute that the examiner is unable to determine the body fluid from which the DNA originated. Whilst the sensitivity of 34 cycles is an incredible asset in cases where biological material is scant, it brings with it the problem of determining whether the presence of an individual's profile is a truly significant event in the context of the case, or merely the consequence of innocent contact. Ballantyne et. al. (2005) identified specific messenger RNA markers for detection of the most forensically relevant body fluids – saliva, semen, blood, vaginal secretions and menstrual blood. The Forensic Science Service has developed a test based on these markers to allow detection of various body fluids in a multiplex reaction.

P-322 SNPs for Individual Identification

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We have previously published population genetics criteria for SNPs for individual identification (IISNPs)--nearly maximum informativeness in populations from all parts of the world--as well as a panel of 40 candidate SNPs meeting those criteria.* This panel gave 40-SNP genotype probabilities of <10-16 in almost all populations. Those studies included several small, isolated groups. Therefore, we have re-evaluated our data, as well as other data, after excluding the most isolated populations from consideration, reducing the screening panel from 40 to 31 populations, those most likely to be forensically relevant. A much larger panel of 108 candidate SNPs meets our operationalized criteria of an Fst <0.06 and average heterozygosity >0.40. In addition to the previously published 40 SNPs we are now able to include some of the markers proposed by the SNPforID consortium.** Many of these candidate SNPs are molecularly close and/or genetically linked making them unsuitable for studies involving relationships. However, because additional criteria may be relevant for any subset of these, it is appropriate to keep all these markers among the candidates until the laboratory and other criteria can be evaluated. We still advocate screening more SNPs to assure identifying a sufficient number meeting broad forensic criteria. We also believe that all of the near-final candidates should be evaluated on multiple, additional populations so that reasonably small (e.g. <10-12) genotype frequencies can be demonstrated to occur even more broadly. *Kidd et al. 2006. Forensic Science International 164:20-32 Pakstis et al. 2007. Human Genetics 121:304-317. PDF files of these papers can be downloaded at: http://info.med.yale.edu/genetics/kkidd/pubs.html. (Publications #449 & #461 respectively) **Sanchez et al. 2006. Electrophoresis 27:1713-1724.

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P-323 Results of the GEP-ISFG collaborative study on a X-STR Decaplex

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A collaborative work was carried out by the Spanish and Portuguese ISFG Working Group (GEP-ISFG) with a PCR multiplex for X chromosome STRs. Markers were selected among those described as polymorphic in humans and that have been used by some laboratories in forensics. The selection took into consideration the potential for multiplexing and preference was given to simple rather than complex STRs, following the ISFG recommendations concerning locus selection for forensic applications. In order to reduce the potential PCR-generated slippage artefacts, trinucleotide repeats were avoided. Primers and various technical methods were investigated with the aim of optimizing a multiplex for the ten selected X-STRs: DXS8378, DXS9898, DXS7133, GATA31E08, GATA172D05, DXS7423, DXS6809, DXS7132, DXS9902 and DXS6789. Primer mix stock solutions (all primers at same concentration) were sent to the laboratories that were asked to analyse the multiplex in two female bloodstains, taking as reference the profiles from 9947A, 9948 and NA3657 samples. In this work, we report the results obtained by 29 GEP-ISFG working group laboratories, using this X-STR Decaplex, as well as alternative technical conditions that also produced good results. This newly constructed decaplex has proved to be technically very robust since most laboratories correctly typed the distributed samples.