Abstracts

Oral presentations

O-1 New Autosomal and Y-Chromosome STR Loci to Aid Work with Degraded DNA and Male-Female Mixed Samples

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Background and Purpose: As national DNA databases grow in size and human identity DNA testing is applied to more challenging kinship scenarios, going beyond the current sets of core genetic loci will become a necessity. This presentation will emphasize the marker selection and characterization process applied at NIST in developing these new loci and assays. Method: New candidate STR markers were selected based on genomic position and allele frequencies in multiple populations. Nomenclature is defined using sequenced alleles and ISFG guidelines. Multiple primer sets were utilized to test for the presence of null alleles. Results: A total of 26 new autosomal STR loci were characterized across more than 600 samples and allele nomenclatures have been assigned for the NIST Standard Reference Material 2391b components enabling calibration of allele calls across laboratories world-wide. Primer sets have been developed to either amplify small amplicons (<140 bp) or simultaneously amplify more than a dozen loci to quickly type reference samples. In addition, more than 80 Y-STRs have also been examined and a megaplex developed to amplify optimal loci. Conclusion: Thus far, three of the 26 autosomal STR loci—D10S1248, D2S441, and D22S1045—have been recommended for extending the core European loci.

O-2 Simultaneous detection of length and nucleotide variability in STRs by High-Performance [Liquid Chromatography-Mass Spectrometry]

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Short Tandem Repeat (STR) fingerprinting looses its outstanding discrimination power when degraded DNA is investigated, as the higher molecular weight markers tend to fail in amplification. The resulting partial STR profiles lead to increased advantageous hits in DNA database searches, thus reducing the efficiency of the method. Conventional STR typing (electrophoresis) only differentiates distinct alleles based on their fragment size but does not take sequence variation into consideration, which has the potential to increase the information content of STR-loci. We applied the combination of ion-pair reversed-phase high-performance liquid chromatography and electrospray ionisation quadrupole time-of-flight mass spectrometry (ICEMS) for the simultaneous detection of length and sequence variation in STRs. We screened 21 forensically important STRs in an European population sample for the occurrence of nucleotide variability within or close to the repeat region. Eleven of the investigated loci (SE33, D2S1338, vWA, D21S11, D3S1358, D16S539, D8S1179, D7S820, D13S317, D5S818, D2S441) brought additional allele (sequence) variants that increased the information content of the loci significantly. A representative number of alleles were characterized by Sanger sequencing which confirmed the ICEMS results. The beauty of ICEMS-STR-analysis is an increased discrimination power with a system that allows direct comparison of newly generated data with existing data(bases).

O-3 Multiplex PCR electrospray-ionization mass spectrometry (ESI-MS): application to forensic mitochondrial DNA examinations

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Mitochondrial DNA (mtDNA) examinations play an important role in criminal investigations, identification of victims of mass disasters, and association of unidentified remains with family members. Typically, HV1 and HV2 are amplified via polymerase chain reaction (PCR) followed by fluorescent sequencing. While this method produces the highest level of resolution, it is labor intensive and unable to distinguish components of a mixture. Previously, an electrospray ionization mass spectrometry (ESI-MS) method was described to determine the base composition profile of enzymatically digested PCR amplified fragments derived from the HV1 and HV2 regions. Advantages of ESI-MS compared to sequencing include speed of analysis, automation, and increased sensitivity while retaining a high degree of resolution. Here we report the next generation of this method in which a base composition profile is determined from 24 overlapping PCR reactions. Because ESI-MS provides the relative abundance of each component present, this method allows for the quantitative typing of mixtures. This ESI-MS method does not rely on a priori knowledge of variable sites, allowing the capture of private mutations and individual-specific variation. Due to the multiplex design, automation, speed of analysis, and ability to interrogate mixtures, this method provides a powerful and rapid tool for forensic mtDNA examinations.

O-4 Simultaneous sequencing of multiple homologous templates using 5´ tagged primers and 454 parallel sequencing

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The degraded state of many pre-historic and forensic specimens has led to a focus on mitochondrial DNA analyses. Even though high copy number mtDNA is more likely to be recovered, it is labor-intensive and expensive to process a large quantity of samples, especially if cloning is required. We developed a method to retrieve pseudo-cloned sequences from homologous templates from multiple Neolithic individuals. The mitochondrial HV1 region was PCR amplified in 7 overlapping fragments. Primers were 5' tagged with a unique 4 base pair nucleotide sequence- 17 unique forward and 17 unique reverse primers per PCR fragment. When used in combination this allows 17*17=289 unique tagged combinations per fragment. Using this method we amplified DNA from 270 independent DNA extractions. The amplicons were purified, pooled together at equimolar concentration and sequenced on the 454 platform. Through emulsion-PCR followed by pyrosequencing, the 454 generates clonal-like sequences stemming from single molecules. Our samples are currently processed on the 454 and the results will be reported soon. We will identify each individual sample through the tagged primers. In theory, since the 454 generates about 200 000 reads, we could get approximately 100 clonal-like sequences from each individual for each fragment (200000 reads/7 fragements/270 samples).

O-5 SNP typing using molecular inversion probes, MIPS

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Background and purpose: SNP typing has a limitation in the multiplexing capacity of targets in PCR. In this study we have investigated the possibility to use molecular inversions probes, MIPS (Padlock probes), for forensic SNP typing. MIPS are linear oligonucleotides 70-100 nt in length, with end-segments that are complementary to a target sequence. The allele specific nucleotide is located at the 3' end of the probe and hybridization to target sequence leads to circularisation of the probe by DNA ligase. After hybridisation and ligation to perfectly matched targets the probe is cut and universal PCR-primers can be used to analyse a large number of targets in parallel. Methods: In total 20 SNPs were selected from http://www.cstl.nist.gov/div831/strbase/SNP.htm. SNP detection was done by Pyrosequencing was used for easy and fast SNP detection at first, but the assay will be transferred to an array platform in the future. Results and conclusions: SNP detection detected by Pyrosequencing gave correct genotyping. However, further optimization of the protocol is required as well as sensitivity tests. The high multiplexing capacity of MIPS make them a suitable for large scale SNP genotyping, but for use on forensic materials further evaluation is required.

O-6 Locked Nucleic Acids: Increased trace DNA amplification with improved primers

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Locked nucleic acids (LNAs) are a conformationally restricted DNA analog, which can be incorporated into DNA and RNA oligonucleotides. LNA bases show exceptionally high binding affinity towards complementary DNA sequences, with improved mismatch discrimination and considerably higher specificity than unmodified DNA bases. The incorporation of LNAs into oligonucleotide primers has been shown to increase specificity, decrease minimum template requirements and increase amplification efficiencies. To investigate if LNAs can increase amplification success for trace DNA samples in a forensic context, primer sequences for 4 routinely used STR loci (FGA, D7S820, D13S317 and D18S51) have been altered to include LNA bases. The LNA modified primers display a broader tolerance to a range of reaction conditions compared to unmodified DNA primers, with higher Tms giving increased specificity. Increased peak heights, improved peak height ratios and decreased template requirements were seen with LNA primers. The increased amplification success of LNA primers, and broader range of optimal reaction conditions, suggests that the use of LNA primers for multiplex STR genotyping assays could be beneficial for trace DNA genotyping.

O-7 A new and novel technology for the in situ detection of body fluids and DNA using antibody-functionalised nanoparticles

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Title: A new and novel technology for the in situ detection of body fluids and DNA using antibody-functionalised nanoparticles The detection of potential sources of trace amounts of DNA at a crime scene or items submitted to a forensic laboratory is of utmost importance. Common sources of DNA include blood, semen and saliva hence forensic scientists test for the presence of these matrices in the hope of locating associated DNA for profiling purposes. Current tests for testing for these matrices are somewhat cumbersome and may fail to locate minute traces of these body fluids. We will report on methodologies we are developing for simultaneously detecting and spatially locating blood, semen and saliva in situ on items of forensic evidence. Our methodology is based on fluorescently-tagged antibody-functionalised nanoparticles, using antibodies and fluorophores selected to maximise specificity to human sources of body fluids and, most importantly, to simultaneously detect DNA directly in body fluids. The emphasis is on direct detection in situ and we will also report on strategies we are developing for this.

O-8 Evaluation of mtDNA coding region SNP typing assays for forensic analysis

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Background and purpose: For limited and degraded samples, sequencing of the hypervariable regions HVI/HVII in mitochondrial DNA (mtDNA) is commonly a solution. There is however a relatively high risk of mtDNA identity by chance due to lack of recombination. One approach to increase the discrimination power is to add information from the mtDNA coding region. To achieve this, several mtDNA coding region assays have been developed in recent years. In this study, eight recently developed coding region assays have been evaluated. Method: 495 similar Caucasian mtDNA sequences (38 % belong to haplogroup H) were trimmed to create eight datasets containing the HVI/HVII regions as well as the positions covered by each of the different mtDNA coding region assays. The discriminatory capacity among these samples was evaluated by pairwise analysis, with the requirement of at least two pairwise differences to be classified as a unique sequence. Results and conclusion: Most assays were able to discriminate between 20 and 30% of the sequences possible to resolve, compared to routine HVI/HVII analysis resolving 19%. The best performing coding region assay could resolve almost 50% of the sequences. Thus, sequences with identical HVI/HVII regions can be resolved by addition of coding region information.

O-9 Forensic validation of the Genplex SNP typing system - results of an interlaboratory study

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We present data from a multi-laboratory validation study of the Genplex typing system (Applied Biosystems), which interrogates a subset of 48 SNPs selected from the panel of 52 previously developed for forensic analysis by the SNPforID consortium (www.snpforid.org), plus amelogenin. The Genplex technology was developed through modification of the SNPlex™ system (also Applied Biosystems) and utilises oligo-ligation of PCR products followed by probe hybridisation to generate dye-labelled allele-specific oligos that are detected with capillary electrophoresis. We compare the success rate of Genplex in typing a common set of 55 samples in three laboratories with results obtained from STR typing of the same samples using Powerplex® 16 (Promega) and SGMPlus® (Applied Biosystems). The sample set was chosen to mimic extracts encountered in forensic situations and includes low concentration and degraded material. We demonstrate that the Genplex technique provides a significantly higher success rate than STR-based methods when typing degraded DNA. Mixture interpretation and variation between laboratories in typing success is also discussed.

O-10 Challenging DNA: assessment of a range of genotyping approaches for highly degraded forensic samples

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It is common in forensic casework to encounter highly degraded DNA samples from a variety of sources. In this category bone and teeth samples are often the principal source of evidential material for paternity analysis or identification of long-deceased individuals. In these circumstances standard STRs are prone to fail due to their long amplicon sizes (since DNA becomes progressively more fragmented as it degrades). To successfully resolve such cases alternative markers must be used and until recently the only other tool avaliable was mitochondrial DNA, which despite being more resistant to degradation is much less informative. A rapidly developing approach to analysing degraded DNA is the typing of polymorphisms in short amplicon PCR products based on markers such as mini-STRs and autosomal SNPs. We have performed an analysis of several cases with naturally degraded DNA using established STRs and mtDNA sequencing plus mini-STRs and autosomal SNPs in order to make an objective comparison of the performance of each method using challenging DNA. The main aim was to establish the benefits and drawbacks of each marker set to help the practitioner choose the DNA analysis method most suited to the circumstances of each case.

O-11 Genetic diversity in Aboriginal Australia

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Background: Aboriginal Australians have a unique evolutionary history and a complex system of inter- and intra-tribal relationships. Numerous studies have examined genetic diversity of Aboriginal Australia, but most using either a single sample or focusing on the global dispersal of modern humans, not the population genetics of the indigenous population itself. Method: In this study we examine the largest Aboriginal Australian sample yet analysed (N=8,868) at fifteen hypervariable autosomal microsatellite loci. Data were assigned to traditional Aboriginal regions and a comprehensive analysis of differentiation assessing allelic distribution, genetic distance, and various population genetic parameters was undertaken. Results: The most genetically differentiated populations inhabit the North of the country, in particular the Tiwi of Melville and Bathurst islands and Arnhem Land (itself divided into West and East Arnhem) regions. These tribal groups show marked heterogeneity from other Aboriginal Australian tribes and also from one another. Our genetic findings support observations on body measurements, skin colour, and dermatoglyphics which also distinguish tribes of the North and Central Australian regions and, more specifically, the Tiwi and West and East Arnhem tribes. Conclusion: This study provides the most comprehensive survey of the population genetics of Aboriginal Australia. This information is used to support DNA evidence interpretation in forensic casework.

O-12 Genetic structure of the present peoples in East Asia in the light of 105 STR markers

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We reported about phylogenetic relationship of the populations within and around Japan using 105 STR polymorphic loci (Hum Genet 118: 695, 2006). In this study, we genotyped the 105 loci with each 32 DNA samples collected from more three regional Japanese, and two regional Mongolian populations. Those genotype data were added into the data reported previously, and then all of the data were analyzed with a few software such as the Structure-Distruct program and the Genetix software. As a result, all of the five regional Japanese populations were extremely isolated from the continental East Asian populations, where Japanese will be potentially distinguished from Chinese at a probability using a statistical calculation. The Nagasaki population, which is the closest to the Korean Peninsula among the five regional Japanese populations, included more some continental genetic elements than the other four regional Japanese populations. However, Okinawa population (in the southeastern island) was distributed farer from the other regional Japanese populations. Among the continental Asian populations, the Mongolian populations were distributed far from the other ones, and possessed obviously different genetic elements. Therefore, STR genotype data for more than 100 loci will help know genetic relationships among very close genetic human populations.

O-13 Genome-wide SNP scans for finding markers to differentiate European populations

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Reliable ancestry-informative markers (AIMs) can identify the geographic origin of an individual which in principle provides a useful tool for tracing unknown suspects in forensics as well as to correct for population substructure in genome-wide association studies. We previously showed that it was possible to recover a substantial amount of continental population substructure in the worldwide CEPH-HGDP samples by means of only 10 autosomal SNPs carefully ascertained from genome-wide data (Lao et al. Am J Hum Genet 2006 78:680-690), but the study design did not allow detecting population substructure within continents. The European genetic landscape, rather than being completely homogeneous, shows clinal patterns in different genetic markers including many from the Y chromosome. Thus, if the number of selected AIMs was large enough, it could be possible to maximize European population differentiation. Together with our collaborators we obtained genome-wide data of >500.000 SNPs in ~2500 individuals from 23 widespread European populations and applied tests to detect population substructure in Europe and to deliver useful AIMs for European population differentiation. Our preliminary results show that it is possible to maximize the detection of population substructure within the European continent while minimizing the number of AIMs required.

O-14 Tracing back ancient south Siberian populations history using mitochondrial and Y-chromosome SNPs

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In a previous work, we determined the mitochondrial and Y-chromosomal haplogroups of 28 ancient specimens from the Krasnoyarsk area (southern Siberia), among which 13 were dated from the Bronze age and 15 from the Iron age. The goal of this preliminary study was to validate on ethnogenesis hypothesis inferred from anthropological and modern molecular data according to which West Eurasian populations predominated in south Siberia during the Bronze age whereas Asian component began to increase from the Iron age. The data we then obtained supported this hypothesis. In order to assign mtDNAs to particular haplogroups not clearly defined with the control region alone and to obtain a more precise information o, the geographical origin of the Siberian specimens under study, we secondly typed mtDNA coding region SNPs. Twenty five SNP markers were selected and combined in three multiplex systems based on SnaPshot minisequencing. Samples that were assigned to the H haplogroup by sequencing the control region were further screened for coding SNPs to identify H subclades. These three multiplex systems were successful on our ancient samples and allowed a further exploration of the H haplogroup that brought new insights about the origin of south Siberian populations.

O-15 Stable RNA markers for identification of blood and saliva stains revealed from whole genome expression analysis of time-wise degraded samples.

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Reliable tissue identification in forensic casework can reveal significant insights into crime scene reconstruction. We performed comprehensive whole-genome expression analysis on time-wise degraded blood and saliva stain samples using the Affymetrix GeneChip technology aimed to identify stable differential mRNA markers of body fluids. The most stable mRNA markers were selected after comparative microarray data analysis and subsequent in silico profiling of candidate markers across different human tissues using the GNF SymAtlas database comprising expression data upon >60 different normal human tissues. Validation of microarray data with RT-PCR, including additional forensically relevant tissue samples (i.e. semen and vaginal secret), resulted in the final identification of 9 blood-specific and 5 saliva-specific markers that were successfully amplified in samples of at least six months of age, expectedly older. However, we found considerable expression overlap of both saliva- and blood-targeted mRNA markers in vaginal secretion, explainable by the biological complexity of vaginal secret and its biological similarity with saliva. Additionally, all blood markers were successfully amplified from stain samples of 15 years of age. Thus, we propose a set of stable, differential body fluid mRNA markers, systematically selected from whole-genome analysis of time-wise degraded samples for tissue identification purposes in forensic casework.

O-16 mRNA profiling for body fluid identification

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Background and purpose: mRNA profiling is a promising new method for the identification of body fluids from biological stains. A number of studies have identified mRNA markers for the forensically most relevant body fluids blood, saliva, semen, vaginal secretions and menstrual blood. A multiplex reverse transcription-polymerase chain reaction (RT-PCR) method was adapted using the following mRNA markers: PBGD/SPTB for blood, STATH/HTN3 for saliva, HBD-1/MUC4 for vaginal secretions and MMP-7/MMP-11 for menstrual blood. The suitability and application to biological stains was tested. Method: The body fluids are identified by total RNA extraction, reverse transcription, amplification of gene-specific sequences and separation of the fluorescence-labeled PCR-products by capillary electrophoresis. For each body fluid two tissue-specific RNA species can be detected in a single multiplex RT-PCR assay. Results: Body fluid specificity and cross reactivity of the mRNA markers were verified by singleplex PCR. The sensitivity of the singleplex and multiplex assay was tested with different amounts of dried body fluids on cotton swabs. Up to one year old dried stains were shown to be suitable for mRNA profiling. The multiplex RT-PCR method was applied to casework samples and provided comparable results to conventional enzymatic or immunological tests. Conclusions: mRNA profiling is a promising alternative to conventional enzymatic or immunological methods for the identification of body fluids. Major advantages are high sensitivity, high specificity and the possibility for simultaneous DNA isolation without loss of material.

O-17 Gene expression analyses from forensic autopsy material

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For a long time RNA was believed to rapidly degrade after death due to the omnipresence of highly reactive RNases. Recently, several studies showed that RNA degradation is not necessarily correlated with the postmortem interval and that quantitative real time RT-PCR studies can be performed on human tissue even after postmortem intervals of several days. Analysis of RNA in postmortem material may provide a completely new field in forensic genetics. It may contribute to a better understanding of the molecular mechanism in different causes of death and to the emerging field of pharmacogenetics and -genomics. The present study addresses three questions: First, the stability of RNA in human forensic autopsy material with various postmortem intervals and various causes of death was analysed. Second, it was tested which RNA extraction method is best for different tissue types. Third, it was checked whether the transcripts of commonly used endogenous control genes are stable over various postmortem intervals.

O-18 Methods for Detection of Copy Number Variation: Real-Time and CE Based

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Copy number variations (CNVs) occur across the human genome and are now being recognized as an important type of polymorphism. These variations include whole gene copy number variations that directly impact phenotype. Also observed are insertions and deletions, many of which do not seem to have functional implications. It is estimated that about 6% of the genome in human's exhibits segmental duplication. Variation in copy number of genes involved in drug metabolism will, in addition to SNPs, contribute to phenotypic effects and maybe useful in post-mortem analysis of drug overdose victims. The allele frequency variation data for these insertion/deletions is emerging and CNVs can potentially serve as another marker for discrimination/ identification. We have developed two genotyping methods to estimate copy number variations in the human genome, one using real-time PCR and another using an oligonucleotide ligation (OLA) –PCR assay with detection on capillary electrophoresis. Representative data from analysis of copy number variations in the drug metabolism genes (CYP2D6, CYP2E1, CYP2A6, GSTM1 and GSTT1) using real-time methods and the MHC region using the OLA-PCR method will be presented. Acknowledgements: We thank Drs. Caifu Chen & Francisco De La Vega for sharing data and discussions.

O-19 New set of markers for individual geographic origin

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Inferring the geographic origin of an individual by analyzing DNA from a crimestain will help the investigation to find an unknown suspect and to save time in case of mass screening. Even thought the non recombining markers from the Y chromosome and the mtDNA are useful to determine an ethnic origin and tracing migration population, their analyzes could lead to conflicting results in case of admixed populations. Therefore, autosomal markers appear to be more suitable for the forensic field. We have selected 32 SNPs loci that exhibit a large difference in their allele frequency distribution between 3 major population groups (African, European ad East Asian). The most informative 9 markers (Fst higher than 0.70) are sufficient to have a correct population assignment. The use of the complete panel of 32 SNPs refine the percentage of membership, determine a genome admixture and allows a population group classification higher than 3.

O-20 SNPs associated with physical traits: a valuable tool for the inference of biogeographical ancestry

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The ability to infer an individual's biogeographical ancestry can be instrumental in reducing a pool of potential suspects in the primary stages of a criminal investigation. Single nucleotide polymorphisms (SNPs) have been widely investigated as candidate markers for DNA-based assessment of biogeographical ancestry. Currently available commercial systems for the SNP-based inference of biogeographical ancestry and physical traits are useful to forensic investigators who are unable to match crime scene samples with database profiles. However, such systems are not directly relevant to the Australian population. Based on an extensive literature survey, sixteen SNPs, including membrane-associated transporter protein (MATP), were specifically selected for their potential to distinguish major sub-populations in Australia. Allele and genotype frequency data were used to assess the usefulness of this multiplex PCR assay as a predictor of biogeographical ancestry for investigative purposes. Preliminary analysis of over 200 individuals indicates a high degree of accuracy for the inference of Asian, African and Caucasian sub-populations. MATP was also investigated for its association to pigmentation. Results were cross-compared to genealogical information self-declared by participants. The development of rapid and robust tests suitable for inference of biogeographical ancestry in the Australian population will provide a valuable intelligence tool for forensic investigators

O-21 Human Pigmentation Genes

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Current methods for forensic DNA analysis require identification of a suspect for comparison purposes. The ability to determine the physical characteristics of the source of a crime scene sample would provide important probative information. There are potentially useful human physical biometric indicators, namely height, colouring and facial features, that are amenable to molecular analysis since they are all highly heritable. The considerable understanding of the genetics of mouse coat colour determination has assisted the understanding of normal human pigment variation. Over 100 pigmentation genes have been identified in the mouse. SNPs in a handful of these genes have been associated with various human hair, skin and eye colour phenotypes. We (AvD) have previously identified a strong association of the MATP gene with skin and hair pigmentation as well as a functional association of an ASIP SNP with pigmentation. A population database of approximately 700 samples with corresponding phenotypic information relating to pigmentation, height, weight and facial morphology has been collected. These samples have been used to screen 58 SNPs in 26 different candidate human pigmentation genes. Associations with pigmentation phenotypes have been identified in greater than half of these genes. Associations with different gene SNP combinations was also investigated.

O-22 The OCA2 gene as a marker for eye colour prediction

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A recent whole genome scan indicated that more than 70% of eye colour variation is due to QTL localised on chromosome 15q. This observation is in good concordance with some other results suggesting that the OCA2 gene located in this area may be involved in normal variation in eye colour among humans. We further addressed this issue and conducted a population association study examining the relationship of eleven SNPs in the OCA2 with different pigment traits. All but one polymorphism were in Hardy-Weinberg equilibrium. The study revealed significant correlation between eight analysed SNP positions and eye colour. Statistical tests also detected an association with other pigment traits for three positions, thus the effect of OCA2 may not be limited to iris colour. In order to eliminate the problems of statistical dependence among analysed SNPs which, as we found, reveal extensive linkage disequilibrium, the data were subjected to haplotype reconstruction. Using a Bayesian procedure implemented in the PHASE program, twenty three different haplotypes were inferred. We found that three of them are significantly associated with non-blue eye colour and another is associated with blue irises. The OCA2 gene should therefore be considered a valuable marker for future forensic phenotype prediction. The study was financially supported by the Ministry of Education and Science in Poland, Grant no. 0 T00C 018 29.

O-23 Towards Genetic Prediction of Human Externally Visible Characteristics: The Case of Iris Color

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Reliable genetic prediction of human externally visible characteristics would offer a new way of tracing unknown suspects in forensics. However, the current knowledge on the genetic basis of such traits does not yet allow genetic prediction in a reliable way (except perhaps red hair color). Such future forensic application also requires a legal basis and societal acceptance as already established in the Netherlands. To investigate the genetic basis of human iris color variation and to provide predictive markers we performed genome-wide association studies in an inbred isolated and an outbred population sample from the Netherlands. A SNP close to the OCA2 gene at 15q13.1 reached genome-wide significance (P-value<0.01) in both studies, providing confirmed evidence for association with iris color. We next expanded the total sample sizes towards ~10.000 samples and genotyped our candidate SNP as well as 3 SNPs from the OCA2 gene recently reported to be in association with iris color (Duffy et al. 2007). Our candidate showed the strongest association with iris color (P-value = 4.5×10-122) as well as significant linkage for blue iris color (LOD=22.0). Our association studies also revealed other genomic regions that are likely to contribute additionally to iris color variation.

O-24 DNA-based prediction of physical traits – a new dimension for forensic genetics, or a first step towards violation of privacy leading to genetic discrimination?

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The possibility to investigate large numbers of single nucleotide polymorphisms (SNPs) from minute amounts of DNA, e.g. from a blood stain left behind at a crime scene by an unknown perpetrator, has led to increased research efforts to identify genetic markers for physical characteristics. Examples are SNPs relevant for pigmentation genes influencing the colour of hair, skin, and eyes, as well as markers which allow to make inferences about the ethnic or geographic origin of a person. Furthermore, some countries such as the Netherlands have already passed legislation allowing to use this type of genetic information to predict the physical appearance, and thus enabling the creation of a genetic "photo-fit" or "phantom" picture which should aid the police with their investigations to identify unknown stain donors. As most of these markers are derived from coding genes, there may be concerns that this approach will ultimately lead to an unrestricted use of all types of genetic information for police investigations thus violating the basic privacy rights of all citizens. Therefore, a balanced exchange of arguments about risks and advantages is required. The current presentation is intended to provide facts and guidance in this difficult discussion.

O-25 MiniMito: Multiplex-PCR of size reduced amplicons for compound sequence analysis of the entire mtDNA control region in highly degraded samples

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Mitochondrial DNA (mtDNA) analysis is nowadays routinely used in the forensic field. The traditional protocol involves the amplification and sequencing of the two hypervariable segments HVS-I and HVS-II of the mtDNA control region. The PCR spans approx. 300 bp each region, depending on the choice of primers, which may result in weak or failed amplification in highly degraded samples. Here we introduce an improved and more stable approach using shortened amplicons in the fragment range of 141 to 236 bp. Ten such amplicons are required to cover the entire human mtDNA control region. They are co-amplified in 2 multiplex reactions and sequenced with the individual amplification primers. The primers were carefully selected to possibly avoid binding on highly polymorphic or haplogroup specific-sites that would otherwise result in loss of amplification due to mis-priming. The multiplexes have successfully been applied to forensic samples such as hair, bones and teeth that did not give results with the conventional HVS-I/HVS-II protocol. We will present casework applications that demonstrate the efficiency of the new method.

O-26 A modified mini-primer set for the mtDNA control region sequence analysis from highly degraded forensic remains

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To facilitate mitochondrial DNA analysis on highly degraded skeletal remains, the Armed Forces DNA Identification Laboratory (AFDIL) developed a mini-primer set strategy which consists of four overlapping PCR products that span each of the hypervariable regions. Validation study showed a dramatic increase in amplification success on highly degraded specimens when compared with larger PCR amplicons. However, use of the mini-primer set can be problematic on samples with HV1 length heteroplasmy and can produce reduced or inhibited amplification yields by some frequent mutations. In the present study, a modified mini-primer set was designed to overcome the limitations of the AFDIL mini-primer set. The modified mini-primer set consists of four and three overlapping PCR amplicons that span the HV1 and HV2 regions, respectively. It showed improved amplification efficiency and successful sequencing alignment results even for samples with length heteroplasmy. Unlike the AFDIL mini-primer set which often shows amplification failure with Mps4a, the three HV2 region PCR amplicons of the modified mini-primer set allowed successful sequence analysis with a reduction in overlaps. In addition, PCR amplification was less affected by mutations due to the primers whose 3’ end were placed at the sites with low mutation frequency in various populations.

O-27 mtDNAmanager: A forensic mitochondrial DNA database aimed at supporting data quality control and generating reliable frequency estimates

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mtDNAmanager is a web-based forensic mitochondrial bioinformatics resource aimed at supporting data quality control and generating reliable frequency estimates, using a new approach based on haplogroup estimation and data comparison with the contents of a given database. mtDNAmanager consists of previously reported world-wide forensic mtDNA sequences, and a set of bioinformatics tools, able to automatically characterize newly submitted data by estimating its haplogroup according to the haplogroup-specific control region mutation motif. Upon registration, the data can be imported and stored with batch mode. A query system enables us to search sequences which show a match or include the queried nucleotide polymorphisms having options to select analysis regions (HV1, HV2, HV3 and control region), ignore insertions at poly C-stretches and permit mismatch. A match system not only permits retrieval of matched sequences for a sample of a data group but also enables cross match of all samples between two selected data groups. In both systems, mtDNAmanager also calculates frequency estimates for random matches in a selected group or the database.

O-28 Haplogroup affiliation of mtDNA control region sequences using a maximum-likelihood approach

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Mitochondrial haplogroups are clusters of evolutionary closely related lineages, defined by the presence of specific polymorphisms in the mitochondrial genome. They have been defined in selected population genetic and anthropological investigations which discovered associated diagnostic polymorphisms by sequence analysis of the mitochondrial genome. We assembled a collection of >3000 published mtDNA sequences defining >500 subhaplogroups to generate a datafile that serves as basis for the estimation of the most probable haplogroup of a given mtDNA profile. We discuss the application of a maximum-likelihood approach to estimate the haplogroup affiliation of an mtDNA control region profile. Assuming that almost all mutations on the mtDNA genome occur independently, the likelihood that the polymorphisms observed in the haplotype in question match a certain haplogroup-lineage is the sum or difference of the log-likelihoods of noise-induced base changes, which are estimated individually for each site using the EMPOP database. The score-based haplogroup estimation offers a valuable tool to identify the mtDNA lineage of an unknown sample, which is an important feature for quality control and alignment of forensic mtDNA sequences.

22nd Congress of the International Society for Forensic Genetics

21–25 August 2007 - Copenhagen - Denmark

O-29 Standardizing the Nomenclature for mtDNA Haplotypes with an Intuitive Hierarchal Execution Software Program

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Typing mtDNA is particularly advantageous, compared with nuclear DNA, for analyzing highly degraded or limited quantity samples. While the technology is robust and reliable, use of consistent nomenclature has been more refractory. Displaying more than six hundred bases to describe results from HV1 and HV2 would be cumbersome and unwieldy for communication. Thus, a published sequence is used as a reference standard (termed the revised Cambridge Reference Sequence, or rCRS). The mtDNA sequences are described by referring only to the differences from the rCRS. This would seemingly provide a common language and an easy tool for describing the mtDNA variation observed in human populations. However, there are some situations where different alignments may be proffered. If these are not standardized within an operational system, identical sequences could be (and have been) aligned and listed differently and inconsistently. Contrary to some assertions, inconsistency in nomenclature does not typically lead to mistyping, either false inclusions or exclusions. Most comparisons in forensic casework are one to one in which the entire string of nucleotides in a sequence is available for comparison. However, when determining the number of times the evidentiary sequence is observed in a reference population database(s), the count may be underestimated. Although the impacts on estimates of the rarity of an mtDNA haplotype are nominal, one should attempt to minimize the impact by developing a standard, stable nomenclature. Most ambiguities in alignment (and thus nomenclature) arise with the placement of insertions and deletions. There are two approaches advocated: a phylogenetic one and an arbitrary one. Neither to date is entirely stable. Phylogenetic interpretations have limitations because such treatment may use information from other samples in a data set, and the process may be somewhat arcane to many practitioners. Phylogenetic comparisons are also subject to substantial variation when the existing data set is changed or extended. Arbitrary nomenclatures are simple, but are not followed well because they may be discordant with traditional practices or seem counterintuitive. Wilson et al proposed an arbitrary approach to attempt to standardize alignments using differential weighting of transitions, transversions, insertions and deletions. There are four hierarchical rules (called 1, 2A, 2B, and 3) that address most situations. However, they do conflict with traditional approaches and thus have not been strictly followed. Rule 2A specifies that alignments with indels are preferred to those with substitutions. Reversing the prioritization of Rule 2A and preferring substitutions to indels is very consistent with most practitioners use and is more consistent with traditional practices. Enabling software is under development to stabilize the hierarchical rules for nomenclature. From 4839 mtDNA haplotypes, 91720 regions, of which 41025 are polymorphic, were data mined. Only 2 of the regions did not resolve to only one possible nomenclature and 38 results that were inconsistent with the historical results by following the hierarchical 1, 2A Reverse, 2B, and 3 process. These few can be handled by adding another rule(s) to the execution process. Thus, a software tool will be available that is intuitive, consistent with traditional schemes, and enables stability of nomenclature (regardless if new haplotypes are identified). Lastly, use of different nomenclatures need not hinder reference database searches, if the search criterion is based on the entire string of nucleotides.

O-30 A Novel 24-plex STR Genotyping Method to Identify Racehorses and Sample Contamination by Human Fluid

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Besides paper trail chain of custody, evidence at molecular level to identify racehorses has not been widely employed. The purpose of this study was to develop a reliable multiplex polymerase chain reaction (PCR) method for identifying the donor horse and sample contamination by short tandem repeat (STR) loci. Twenty-one STRs (VHL20, UMNe156, HTG4, AHT4, HMS6, HMS7, ASB9, ASB2, COR045, HMS3, UMNe222, LEX074, COR008, HMS2, UM015, COR018, UMNe116, UMNe479, HMS1, UMNe191, and LEX073) were employed based on variability and ease of amplification. Genomic DNA was isolated from blood and urine samples of racehorses using PureGene DNA Purification System (Gentra, USA). All loci used were successfully amplified using HotStar Taq DNA Polymerase (Qiagen, USA). Samples were genotyped by fluorescent-labeled capillary electrophoresis on CEQ8800 Genetic Analysis System (Beckman Coulter, USA). Results of the twenty-one STRs in a population of 200 horses indicated that this method was highly discriminating and reproducible. To identify human contamination, three human specific STRs (TH01, TPOX, and D18S51) were successfully incorporated in the method. To our knowledge, this method is the first in the racehorse industry to identify the horse and possible human contamination of the sample.

O-31 DNA chips for forensic animal species identification

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Due to its high sequence diversity the mitochondrial cytochrome b (Cyt b) gene has been applied for species identification, e.g. in food. While previous methods have utilised DNA sequencing or RFLP pattern analysis we employed modern DNA chip technology that combines high specificity and sensitivity. In a consensus PCR that included a fluorescence-labelled (Cy5) primer a short region of the mitochondrial Cyt b gene was amplified. These labelled PCR products hybridised to a subset of animal species -specific oligonucleotide probes tethered to the surface of a DNA chip. After stringent washing the resulting fluorescence was detected with a laser chip scanner. Up to 12 different species of domestic and wild animals could be analysed in parallel without cross talking. The applicability of this method to the forensic field is demonstrated by routine casework samples such as blood, tissue, hair and saliva samples that had been characterised serologically or by Cyt b sequencing before.

O-32 Development of STR profiling systems for individual identification in wildlife species

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Dr. Rob Ogden, Wildlife DNA Services
Dr. Ross McEwing, Wildlife DNA Services

Background and Purpose: The use of STR profiling systems for the individual identification of wildlife is a developing field. Genetic evidence is now used in investigations of illegal trade, animal poaching and persecution, however protocols for the validation of such techniques are not established. Focussing on the Eurasian badger, this study aimed to provide a framework for the application of genetic validation guidelines and match probability statistics to wildlife DNA forensics. Method: A 10-locus STR profiling system was used to generate 1083 genotypes from 20 UK populations. Markers were individually validated as extensively as possible following SWGDAM guidelines. Estimates of population substructure (FST) and inbreeding (FIS) were calculated and their impact on subsequent match probabilities was examined. Results: Validated limits for the application of the STR loci were obtained, although not all SWGDAM criteria could be included. Values for FST(0.12) and FIS(0.11) indicated high levels of substructure and inbreeding that strongly affect the power of individual discrimination. Conclusion: The transfer of wildlife STR markers from research to forensic applications is problematic due to the limited ability to perform validation studies. Population genetic parameters due to breeding behaviour in wildlife species must be accounted for in statistical interpretation of profile matches.

O-33 Tracking Tiger in Traditional East Asian Medicines

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Products termed Traditional East Asian Medicines (TEAM) are available throughout the world. A number of TEAM products advertise that they include animal species that are protected under international trade legislation such as the Convention on the International Trade in Endangered Species (CITES). This presentation highlights the use of DNA based tests to identify trace levels of CITES listed species from TEAM samples. Such samples contain trace amounts of human DNA and DNA from non-protected species. The DNA test is based upon the identification of species specific polymorphisms on the mitochondrial genome that can differentiate between protected and closely related non-protected species. In the case of products alleged to contain tiger a test has been developed that will identify Panthera tigris (including the 5 extant sub-species) and a range of other Panthera species. Specific known PCR products are produced for each of the species. Direct sequencing of the PCR products can be used to confirm the identification. The test was used in the positive identification of tiger within a number of TEAM products bought in Scotland and illustrates the trade in CITES listed species and the need for these type of tests.

O-34 MtDNA analysis for genetic identification of forensically important insects

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Background and purpose: Unequivocal identification of insect specimens is a crucial step in forensic entomology and could be difficult using only morphological features, especially for immature stages. The majority of published studies consider the cytochrome oxidase subunits one (COI) analysis and the obtained sequences are representative for insect population of specific geographic regions. This work describes a different approach for genetic identification of insect specimens. Method: The immature specimens found in human corpses recovered in Italy were bred to obtain adult flies. DNA was extracted and analyzed by a 900 bp fragment of COI and COII genes, using four degenerate primers. The sequences were aligned and compared with the Diptera sequences in GenBank for species-specific identification. Results: The sequenced fragment allowed an insect species-specific identification intra-specif and geographic variations were found. COI and COII sequences were compared with GenBank sequences indipendently and sometimes species-specific identification was ambiguous. Conclusion: The combined analysis of COI and COII fragments is more accurate approach for Diptera species identification than single COI fragment analysis. The geographic and intraspecific variations are important for insect identification and local databases set up are strongly recommended.

O-35 Interpretation of complex DNA profiles using Tippett plots to measure robustness

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A new methodology is presented in order to report complex DNA profiles. We have devised a new protocol to interpret complex cases using likelihood ratios. We have brought together a number of different theories in order to report a four-person mixture as an example. The calculations are designed to be highly conservative and are widely applicable. We apply a low copy number (LCN) interpretation framework, which includes the probabilities of dropout and contamination, to 'conventional' DNA cases. Stutters often compromise calculations when observed to be the same height as a minor contributor to a mixture. Stutters cannot be distinguished from minor alleles. We compensate by treating them as real alleles and including them in the calculation. By increasing the number of potential contributors to the DNA profile, we can account for the extra alleles that result. We propose that the likelihood ratio is qualified with additional robustness parameters to indicate the probability of misleading evidence in favour of the prosecution, under the assumption that a random man was a contributor instead of the suspect. To do this we apply a new kind of case-specific 'Tippett' test. Although the method is complex, we suggest a 'user-friendly' way to explain the results to a court.

O-36 Probabilistic modelling of DNA mixtures.

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Professor Steffen Lauritzen, Oxford University, UK
Professor Julia Mortera, Universita' Roma Tre, Italy

Background and purpose: Taking peak area information into account when analysing STR DNA mixtures is a difficult task. There have been a number of non-probabilistic methods proposed in the literature, and some have been incorporated into computer systems, but little has been done from a probabilistic perspective. Here we propose a simple probabilistic model that is amenable to efficient computational analysis. Method: The underlying representation of, and algorithms for carrying out, the complex calculations are based on Bayesian networks, for which both object-oriented networks (using Hugin) and a purpose built computer Program (MAIES), have been developed. Results: We describe MAIES and illustrate its use on several real examples of DNA mixtures. We show how both evidential calculations and mixture separation calculations may both be carried out using MAIES. For problems of mixture separation we show how the most likely combinations of genotypes of the contributors may be efficiently calculated. Conclusion: Bayesian networks provide a probabilistic framework within which the problem of mixture analysis may be expressed and solved. In future work we intend to model real-life complications such as stutter and drop-out that we currently ignore.

O-37 Amplification of DNA mixtures - Missing data approach

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This paper presents a model for the interpretation of results of STR typing of DNA mixtures based on a multivariate normal distribution of peak areas. From previous analyses of controlled experiments with mixed DNA samples, we exploit the linear relationship between peak heights and peak areas, and the linear relations of the means and variances of the measurements. We furthermore the contribution from one individual allele to the mean area of this allele, is assumed proportional to the average of height measurements on alleles where the individual is the only contributor. For shared alleles in mixed DNA samples, it is only possible to observe the cumulative peak heights and areas. Complying with this latent structure, we use the EM-algorithm to impute the missing variables based on a compound symmetry model. This allows intra- and intersystem correlations on the measurements and does not depend on the alleles of the DNA profiles. Due to factorization of the likelihood and properties of the normal distribution, an ordinary implementation of the EM-algorithm solves the missing data problem. We estimate the parameters in the model based on a training data set. In order to asses the weight of evidence provided by the model, we use the model with the estimated parameters on STR data from real crime cases with DNA mixtures.

O-38 Informativeness of Genetic Markers for Forensic Inference

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Background and Purpose: Forensic inference from genetic markers uses highly polymorphic multi-locus genotypes. Measures of informativeness can aid in selecting efficient genetic markers for forensic inference. Existing measures are based on heterozygosity (H) and polymorphism (PIC), they do not account for multiple sources of genetic variation (i.e. mutation, silent alleles, etc.) and they are not directly amenable to more complex identification problems. Methods: Using decision-theoretic principles within a probabilistic expert system we define a general measure of informativeness, Iq, of a marker for answering a forensic query. Properties of Iq are compared with existing measures. Results: Neglecting mutation and other anomalies, Iq gives a similar ranking of most genetic markers as its comparable measures. Accounting for sources of variation, such as mutation, silent and null alleles, reduces Iq and may affect ranking. Conclusion: This new criterion has a solid theoretical basis and can account for multiple sources of genetic variation and other anomalies. It can be directly applied to a variety of planning issues concerning the type, quantity and specific choice of markers for use in paternity testing and more general forensic problems.

O-39 STR-profiling for differentiation between related and unrelated individuals in cases of citizen rights.

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DNA profiling of individuals STR markers is commonly used in forensic genetics and paternity testing. This technique can be used for the establishment of family relation as well as determining genetic relationships for immigration purposes. In cases of family based immigration, a legal permanent resident or citizen and their relatives must provide proof of their relationship. In addition, immigration of partners (spouses and husbands) can require the proof of nonSiblingship. In these cases genetic evidence is based solely on biostatistics. We have therefore analysed the validity of STR markers and biostatistics using empiric and simulated data. Based on pair-analysis of related and unrelated individuals, the four hypothesis were tested: (A) unrelated, (B) siblings, (C) cousin, (D) uncle/aunt/partial-siblings. 200 empirical and 10.000 simulated data-pairs were analysed with a maximum of 26 STR markers/pair. Our results demonstrate, that unrelated individuals can be separated from siblings using the results of 15 STR markers by following the maximum likelihood principle. The differentiation between 2nd- or 3rd related individuals (group C and D) are limited. In these cases extended analysis with 26 STR markers improved the biostatistics.

O-40 Matching odds for rare haplotypes

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Mitochondrial and Y-chromosomal haplotypes offer special advantages to the forensic scientist for identifying criminals. For different reasons, each of them is sometimes detectable in a crime stain for which autosomal typing fails. But they also present special problems, including a simple mathematical one: When a rare haplotype is shared between suspect and crime scene, how strong is the evidence linking the two? The traditional methods of evaluating the strength of DNA evidence include fundamental misconceptions. Contrary to received wisdom (1) sample frequency is not a plausible estimate for population frequency — not for rare types, (2) the pertinent question is not even one of frequency but of probability, and the distinction matters. A simple analysis shows the surprising fact that the matching odds of a rare haplotype match are usually more than the size of the population sample. For example, the "95% confidence interval from zero" approach for a new haplotype that is sometimes suggested not only makes no sense, but is easily tenfold too conservative.

O-41 Incorporating population structure into forensic Bayesian networks

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Background and purpose: Bayesian networks are gaining popularity as a graphical tool to communicate the complex probabilistic reasoning required in the evaluation of DNA evidence. Incorporating allelic dependencies that result from population structure within these networks is a relatively new endeavour and this study provides some initial thoughts on how to approach the construction of these networks. Method: We introduce object-oriented Bayesian networks designed to model forensic identification cases while accounting for population structure. A blocking Gibbs sampling method is used, via HUGIN's API interface, to model the unknown subpopulation frequencies. Results: Averaging over the unknown subpopulation frequencies provides a robust estimate of the weight of evidence by accounting for this uncertainty. Comparisons are made with previous approaches that have appeared in [1] and [2]. We explore several forensic examples, including complex paternity cases arising due to missing data. Conclusion: Accounting for population structure within the Bayesian network framework is an important step forward, and illustrates the flexibility this technology provides as a formal tool for handling complex forensic calculations. References: 1. F. Taroni, C. Aitken, P. Garbolino, and A. Biedermann (2006). Bayesian Networks and Probabilistic Inference in Forensic Science. 2. A. Hepler (2005). PhD Thesis, North Carolina State University.

O-42 The impact of null alleles in kinship probability calculations

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Prof, Antonio Amorim, IPATIMUP and Faculty of Sciences, University of Porto, Portugal

A null allele (or silent gene) formally designates a rare (non-polymorphic, with a frequency below 1%) recessive allele. Therefore, its presence can be unnoticed, even in large samples. The currently used routines in kinship expertise are based on the analysis of autosomal Short Tandem Repeat (STR) loci, which are treated as harbouring just codominant alleles. Only when incompatible results are obtained (like mother-child opposite homozygosity), the silent allele is invoked. The main purpose of this work is to quantify the impact of silent alleles in paternity testing in "non-exclusion" situations. Using PYTHON, an object-oriented programming language, we created an algorithm that simulates paternity cases, calculating paternity indexes with different silent and codominant allele frequencies. Taking advantage of the robustness and flexibility of PYTHON algorithms and WXWIDGETS GUI Toolkit we have also created a graphical interface software that provides graphic outputs along with paternity calculations. We demonstrate that silent alleles can non-negligibly lower the paternity index in trios where opposite homozygosity is observed and we provide some examples in real cases.

O-43 Evaluation of DNA evidence in mass identification projects: examples and considerations from the ICMP.

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In its large scale DNA identification casework on skeletal remains from mass graves in the former Yugoslavia, the ICMP conducts comparisons of DNA autosomal STR profiles from bones to a large database of profiles from family members of the missing. DNA match reports are issued when posterior probabilities of named identifications (or, more properly, stated relationships) exceed 99.95%. This posterior probability threshold considers only the DNA evidence from kinship analysis, and a prior probability based on the number of missing individuals in a particular region/event. In a great majority of instances this is straightforward in concept and practice. However, in large events such as mass graves from the former Yugoslavia, many cases present additional complexities that must be navigated. Among other things, complications arise from the presence of related individuals among the missing, individuals among the missing who are not reported missing, and genetic inconsistencies with stated family reference relationships. In many cases, multiple family members are missing together. Identified victims can then serve as references for further identifications, but this can complicate a formal evaluation of the evidence. Lastly, we will discuss integration of less-than-conclusive DNA evidence with non-DNA in the identification process.

O-44 Resolution of paternity investigations showing ambiguous STR results using autosomal SNPs as supplementary markers

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In a small number of cases a paternity test will produce an ambiguous result - where the claimed relationship cannot be confirmed by a high enough paternity index or excluded with a consistent set of incompatible genotypes. The majority of such cases arise from testing an individual closely related to the true father, so the exclusion rate is markedly reduced, while comparison to a random man to calculate the paternity probability no longer applies. Less frequently ambiguous results occur from the observation of multiple second-order exclusions. While adding extra STRs can help to resolve cases with ambiguous results, using SNPs in large multiplexes is more convenient and, as this report shows, more informative in both the analysis of close relatives of the true father and in cases with two or more second order exclusions. We detail eight cases that illustrate the ability of SNPs to provide sufficient additional evidence to resolve ambiguous results in disputed paternities or the identification of remains. In all cases SNPs were genotyped using the standard 52plex identity set developed by SNPforID (1). Results also suggest that modeling with extended families, while informative, cannot always accurately gauge how novel markers will behave in real-world analyses. (1) J.J. Sanchez et al. 2006, Electrophoresis 27, 1713-1724

O-45 Multiplex PCR detection of GSTM1, GSTT1 and GSTP1 gene variants

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The glutathione S-transferase (GST) genes code for a super family of enzymes that are involved in phase II drug-metabolism by conjugation of electrophilic substances with glutathione. The GSTs detoxify a broad range of substances including carcinogens, environmental toxins and drugs. Genetic polymorphisms in the GSTM1, GSTT1 and GSTP1 genes have been studied intensively for their potential role in cancer susceptibility and drug response. In Caucasians, the enzyme activity of GSTM1 and GSTT1 is absent in approximately 50% and 15% of the population, respectively, due to deletions of both chromosomal copies of the genes. A trimodal phenotype pattern exists in which individuals with two, one or no functional genes are fast, intermediate or slow "conjugators", respectively. However, most studies investigating the effect of genetic polymorphisms in GSTM1 and GSTT1 did not distinguish between individuals with one or two copies of the genes because the applied genotyping assays only detect if at least one copy of either gene is present. We have developed a PCR multiplex method that in a fast, inexpensive and reliable manner can detect if a person has two, one or no GSTM1 and GSTT1 genes and which at the same time can detect the allelic status of the GSTP1 Ile105Val genetic variant. A total of 200 Danes, 100 Somalis and 100 Greenlanders were genotyped. This multiplex PCR assay enables future large-scale studies to investigate the role of GSTs.

O-46 A collaborative assessment of the use of Applied Biosystems MiniFiler STR multiplex system for degraded DNA samples

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Under the auspices of EDNAP and the ENFSI DNA working groups, two collaborative exercises have been carried out to compare the efficacy of different STR (and SNP) multiplex systems to analyse degraded samples. We demonstrated that mini-STRs systems with amplicon sizes between approximately 60-150bp were more efficient. Subsequently, it was recommended that new mini-STR markers should be incorporated into commercially available multiplex systems. The AB MiniFiler STR system consists of eight mini-STRs plus amelogenin. In the second study eleven different laboratories compared the use of MiniFiler with their usual casework method (e.g. SGM plus, SEFiler). Each laboratory was supplied with sets of blood and saliva stains that had been subjected to varying levels of degradation. The stains were processed using the standard DNA profiling protocols normally used within each laboratory. Since the MiniFiler procedure uses 30 PCR cycles, participants also carried out an additional exercise to compare PCR amplification results of their standard multiplexes using an equivalent 30 cycles.

O-47 Results of the 2006-2007 EDNAP Collaborative Exercise on mtDNA SNP Screening

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The EDNAP (European DNA Profiling) Group undertook a collaborative mtDNA SNP screening exercise on hair shafts and saliva samples of 5 volunteers. The aim of the study was to test the usefulness of mtDNA screening analysis taking haplogroup information into consideration. For this purpose the "West-Eurasia-Plex" [1] including 16 loci from the mtDNA coding region was typed by the participating laboratories on the on-site extracted stains. The overall performance of the multiplex and the success rate in obtaining the correct result were surprisingly high given that some of the participating laboratories had no previous experience with the technology and/or mtDNA analysis. The results of this collaborative exercise stimulate the expansion of screening methods in forensic laboratories to increase efficiency and performance of mtDNA typing and thus add a powerful tool to forensic casework analysis.
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[1] A. Brandstätter, T.J. Parsons, W. Parson, Rapid screening of mtDNA coding region SNPs for the identification of west European Caucasian haplogroups. Int.J.Legal Med. 117 (2003) 291-298.

O-48 Results of the 2007 Paternity Testing Workshop of the English Speaking Working Group

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this evidence was sufficient for the prosecutor to request DNA analysis of both the woman and her current companion. A further spectrum of hypotheses. Given the genetic profiles of four individuals and one certain relationship (the woman and the second child), six mutually exclusive hypotheses were possible about their biological relationships. Conclusions. Assuming equal priors, the probability that the two newborns were both children of the two suspects was 0.999968, whereas all other hypotheses had probabilities <0.0001, this result remained substantially unchanged after applying different priors. 6:683-685. [2] Bjerre A, Syndercombe Court D, Lincoln P, Morling N. A report of the 1995 and 1996 Paternity Testing Workshops of the English Speaking Working Group of the International Society of Forensic Haemogenetics. Forensic Sci Int 1997, 90(1-2):41-55. [3] Hallenberg C, Morling N. A report of the 1997, 1998 and 1999 Paternity Testing Workshops of the English Speaking Working Group of the International Society of Forensic Genetics. Forensic Sci Int 2001, 116(1):23-33. [4] Hallenberg C, Morling N. A report of the 2000 and 2001 Paternity Testing Workshops of the English Speaking Working Group of the International Society of Forensic Genetics. Forensic Sci Int 2002, 129(1): 43-50.

O-49 Accredited Relationship Testing and Current Practices in the United States

Dr. David W. Gjertson for the Relationship Standards Program Unit of the AABB, USA

The AABB has accredited laboratories in parentage testing since 1984, and our 8th edition of Standards for Relationship Testing Laboratories will become effective January 2008. Revised standards include several new sections ensuring that laboratories 1) test emergency procedures, 2) control systems when no formal proficiency testing is available, and 3) report mitochondrial DNA testing consistently. Guidance is provided in an accompanying document containing new material on HLA molecular typing, kinship analyses and mutations. The AABB collects and disseminates survey information regarding the practice of parentage testing, also. Last year, surveys were received from 42 laboratories testing >390,000 relationships (>1 million subjects) on a broad range of issues (e.g., standard paternity, immigration, prenatal and postmortem evaluations). The survey showed a continued trend toward the dominant use of PCR-STR technology (98% of cases, up from 93% in the previous year) with a decrease in the use of other methods. Y chromosome analysis increased from 0.02% to 0.06% of cases. The report provided commentary for the lay public on common misconceptions, tracked mutations and discussed PI calculations. During this presentation, changes in AABB Standards and current practices of relationship testing in the United States will be highlighted.

O-50 The GEDNAP Proficiency Tests. Recent Trends and developments

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This paper presents an overview of the developments in the GEDNAP (German DNA Profiling group, organized by our institute under the auspices of the Stain Commission, a joint commission of German institutes for Legal Medicine and Forensic Sciences, http://www.gednap.org/) Proficiency Testing program in the last three years, demonstrating the changing approach to DNA investigations in the European forensic community. The present GEDNAP format includes certification of 18 autosomal, 11 Y-chromosomal STRs, Amelogenin and –for the first time in this year- mtDNA (HVI and HVII). The recent trends cover the following aspects: a still increasing number of participating laboratories (171 in the year 2004) from more than 30 (mostly European) countries that type all certifiable DNA systems and not only a single commercially available kit (e.g., SGM plus). The evaluation of the submitted original data also identifies the most common types of error encountered (e.g., transcriptional errors, drop out-related errors, contamination, false typing of variant alleles) which can also occur during routine DNA typing and ways of recognising and preventing such errors are suggested.

O-51 GEP-ISFG proficiency testing programs: 2007 update.

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This year the annual GEP-ISFG proficiency test consisted on two practical and three theoretical exercises. The practical exercise included a deficiency maternity test for which blood samples from a woman and two alleged children were analyzed and a forensic case that included a cigarette butt for STR typing and two hairs shafts sent specifically for mitochondrial DNA analysis, both to be compared with a reference blood sample from a suspect. In the theoretical approach of the exercise, three different proposals were offered (one included in the certificate of participation, and two optional)the most relevant was a paper challenge on mixtures prepared with the idea to review some interesting aspect of the recent ISFG recommendations on mixtures interpretation and specially the likelihood ratio method calculation under both the unrestricted and the restricted combinational approaches. A total of 122 laboratories participated in the exercise belonging to 16 different countries. More than 50% of participating labs performed both the paternity and the forensic trials, while 52 laboratories performed only the paternity test. Analysis of the data is currently underway and a summary of the results will be presented during the Congress. Other working groups of the GEP-ISFG have also organized other collaborative exercises during this year with a relevant interest for the forensic scientific community. These included a collaborative exercise with a battery of STR from X chromosome to improve standardization and the first collaborative exercise on non-human (dog) mtDNA sequencing coordinated by the Sexual Chromosomes Working Group and the Non-Human Forensic Genetics Working Group of the GEP-ISFG, respectively.