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A. MITOCHONDRIAL DNA

MITOCHONDRIAL DNA VARIATION IN ANCIENT AND MODERN HUMANS

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Mitochondrial DNA (mtDNA) has been used widely in recent years as a valuable research tool to reconstruct human evolutionary history. The relative simplicity, rapid rate of evolution and maternal mode of inheritance of mtDNA make it suitable for phylogenetic studies over recent time scales, while its abundance in animal cells favours its survival in compromised and ancient biological samples.

Analysis of mtDNA polymorphisms has been shown to be ideally suited for investigating the migration patterns of ancient human populations. Using PCR amplification and direct sequencing, we have analysed informative mtDNA fragments in modern humans and archaeological bones from Polynesia, the last area of the world to have been settled by humans. The results indicate strikingly low levels of genetic diversity in Polynesia compared to the rest of the Pacific, suggesting that a stringent population bottleneck occurred during the recent settlement of remote Oceania. In contrast, the extremely high variability of mtDNA in European populations reflects the great depth and complexity of historical events in this continent.

MtDNA analysis of archaeological remains has proved particularly useful for answering questions that cannot be addressed by the study of modern human populations, for instance that of the ancestry of the original inhabitants of Easter Island. Our studies have shown that the prehistoric inhabitants of the island stem from the same mtDNA lineage as the Polynesians, rather than native Americans, although it is evident from the data that both groups originated from an ancestral population in island Southeast Asia.

A TWO STAGE STRATEGY FOR AUTOMATED ANALYSIS OF
MITOCHONDRIAL DNA

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DNA amplification and sequencing of the mitochondrial (mt) non-coding region provides a highly sensitive and discriminating test for individual identification. A major drawback with DNA sequencing as a forensic tool is the labour-intensive nature and therefore high cost of the technique, which presently limits its application to the investigation of serious crime only. This issue is being addressed by developing a two stage strategy for mtDNA typing which comprises a coarse screen using a rapid mini-sequencing technique followed by full automated DNA sequencing. The first stage entails multiplex PCR then solid phase mini-sequencing in which an electrophoretically-resolved array of 13 dye-labelled extension products are automatically analysed on a fluorescence-based sequencer with 36 samples run in less than 2 hours. This test discriminates between approximately 95% of unrelated samples and the remainder can then be analysed by two rounds of nested PCR followed by solid phase sequencing reactions using Sequenase plus dye-labelled primers in conjunction with an automated sequencer. This sequencing approach yields data with low background noise and minimal sequence context-specific variation, enabling subtle sequence characteristics such as heteroplasmy to be detected reliably.

Both mini-sequencing and full mtDNA sequencing have been successfully applied to a variety of discrete casework materials including ancient bones, hair shafts, hair roots, faeces and blood. Analysis of DNA from faeces has been greatly facilitated by utilising magnetic capture and washing of the template in solid phase prior to amplification, with results obtained from as little as 10mg wet weight of starting material.

In conclusion, this two stage strategy will enable mtDNA typing to be applied to large numbers of samples at low cost, making it more amenable than the conventional sequencing approach for the investigation of high volume crime such as burglary.

MITOCHONDRIAL DNA TYPING USING SEQUENCE-SPECIFIC OLIGONUCLEOTIDE PROBES. R. Reynolds, Ph.D., E. Clark, Ph.D., T. Marschak, B.A., J. Varlaro, B.S., S. Rubin, M.A. Roche Molecular Systems, Inc. Human Identity Group, Alameda, USA.

Mitochondrial DNA is a very valuable source of sequence variation for purposes of human identification because it has a high degree of sequence diversity between individuals, is present in high copy number, and is maternally inherited. While DNA sequence analysis provides the most definitive information about genetic variation, this method can be time-consuming and, particularly within the mtDNA control region, does not always yield complete, unambiguous sequence data. We have developed a mtDNA typing system involving PCR amplification of the hypervariable region of the mtDNA control region, followed by automated hybridization of the amplified DNA to a panel of immobilized sequence specific oligonucleotide probes. Automation of the hybridization and color development steps allow a single person to type ~500 samples per week. Population data and results obtained from casework samples and mixtures will be presented. Analysis of multiple mtDNA sequence databases showed that a typing system consisting of 23 oligonucleotide probes reveals 94-98% of the genetic diversity contained in these databases. Consequently, there is a very high likelihood of exclusion of randomly chosen individuals.

MIXING AND THERMIC TREATMENT OF MITOCHONDRIAL PCR FRAGMENTS REVEAL SEQUENCE DIFFERENCES BY HETERODUPLEX FORMATION- A RAPID METHOD FOR FORENSIC IDENTITY TESTING

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Within the 1200 bp D-loop region of human mitochondria (mt), there are two hypervariable segments designated HV1 and HV2. Since sequencing of D-loop regions is an expensive and time-consuming method, we tried a heteroduplex analysis (HDA) technique for rapid comparison of polymorphic mtDNA prior to sequencing. From 100 mother/child pairs and 100 father/child pairs, mtDNA was amplified out of the HV2 region between positions L28 and H 408. PCR fragments of different origin were mixed, thermicly melted and and chilled. Electrophorising the mixtures on native polyacrylamide gels followed by silver staining reveals sequence differences in 89/100 father/child mixes and 0/100 mother/child mixes by heteroduplex (HD) formation. If we mix the amplified mt-fragment of one proband with those of 100 not related persons, about 85% of the mixtures produce HDs. First experience show, that HDA in the HV 1 is similar useful. Usage of mild denaturing gels may enhance the discrimination power of this method. Five cases of forensic identification were performed using chromosomal marker testing, HV2 HDA and HV2 sequencing in parallel. No contradictions were seen between this methods.

Conclusions: HDA is a suitable technique to detect the majority of the nonidentities of sequences if mtDNA stemming from different sources is mixed. Thus, this method may help to focus the more expensive sequencing techniques on samples or stains of high identity probability.

- schnelle Sequenz-Methode
- interessant für Haare und isolierte Spure
- 85% Quote mit HV2
- bei System > 99%

GENETIC ANALYSIS OF SINGLE HAIR SHAFTS BY AUTOMATED SEQUENCE ANALYSIS OF THE MITOCHONDRIAL D-LOOP REGION

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Hair-samples are often found at the crime scene and are sometimes the only biological evidence sample for the identification of the offender. However, most hairs submitted for DNA analysis do not contain a hair root which will decrease the chance for generating a DNA profile with VNTR or STR markers. In contrast to nuclear DNA, sufficient amounts of mitochondrial DNA are present in these hairs to allow the analysis of the highly polymorphic mt d-loop region.

We have developed a procedure for the amplification of the two hypervariable regions in the d-loop followed by enzymatic sequence analysis and electrophoresis on the automated A.L.F. DNA sequencer. Depending on the source and the constitution of the biological sample either the two hypervariable regions or four overlapping segments were amplified. The primers complementary to the H-strand were biotinilated which allowed for the generation of single stranded sequencing templates after capture with Dynabeads (solid-phase-approach). The unlabelled PCR primers were chimeric with a universal sequence (5') and a specific sequence complementary to the L-strand (3'). This approach allowed us to read the sequence starting from the beginning of the PCR product. The sequencing procedure was recently further improved in terms of standardization and processing time by the implementation of the AutoLoad Sequencing Kit (Pharmacia-BioTech).

A database with 51 unrelated Caucasians of Belgian descent was generated. On average between 700 and 800 nucleotides were obtained for each individual and differences with the reference sequence were observed at 50 positions in the first hypervariable region (HVR1) and at 28 positions in the second (HVR2). In total, 49 different mtDNA types were identified and 47 were present only once. The mean pairwise sequence difference within the database was 3.52 for HVR1, 3.86 for HVR2 and 7.38 for the complete d-loop region.

The developed approach was evaluated on forensic hair samples which were typed before with VNTR or STR markers. Whereas with the nuclear markers a success rate was obtained of less than 50%, the success rate increased to 95-100% with the analysis of the mt d-loop region. Presently, the developed procedure has been used with success in several forensic cases where hair shafts were the only evidence sample. In addition, the current approach is used in several archaeological projects.

MITOCHONDRIAL HVRI POLYMORPHISM IN ITALY

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Sequence polymorphism of mitochondrial DNA (mtDNA) is a class of human diversity intensively studied in forensics. Considerable mtDNA sequence variation occurs at two hypervariable regions (HVRI and HVRII) encompassing a relative short area (800 base pairs) of the entire structure of mtDNA. Both domains have been used to study the structure and relationship of human populations.

There is a considerable advantage in the use of mtDNA for forensic applications, and the two hyperpolymorphic domains have been helpful to solve special problems of identification which would have not been possible to unravel with nuclear loci. However, to properly exploit the potential informativeness of mtDNA, an adequate database of the appropriate population should be made available as reference.

The present study intends to contribute a first map of the HVRI sequence polymorphism in Central and Southern Italy.

Amplification of the relevant fragment was achieved by two separate sets of primers, each one generating a 250 base pair length fragment. The two segments had an overlapping site of about 50 base pair allowed, ensuring consistency of the results.

The PCR products were sequenced by TAQ cycle sequencing procedure using either the forward or the reverse fluorescent-primer. The fluorescent sequence reaction products samples were processed using an ALF DNA sequencer (Pharmacia), then aligned over the original Anderson consensus sequence.

As a results, we report that the majority of sequences occur once in the database and that the most common genotype (the Anderson consensus sequence) was present in 13% of individuals.

Most of the polymorphic content was found in base transitions (45 polymorphic sites observed between 16002 and 16410 bp with a transition/transversion ratio of 31.6:1; pyrimidine resulted to be mostly involved in the transitional events) rather than in transversions. No insertion/deletion event was so far detected.

This first report on mtDNA polymorphism is intended as the basis for more extensive map of D-loop polymorphism in Italy.

Mitochondrial D-Loop sequence analysis from a single sperm:
A case reportW. Schmidt¹, J.Holtz², K.Olek¹¹Institut für Hormon-und Fertilitätsforschung, Hamburg, Germany²Institut für Rechtsmedizin, Universität Bonn, Germany

Human mitochondrial DNA (mtDNA) displays considerable sequence variation between individuals due to mtDNA evolving 5-10 times faster than nuclear DNA. A large amount of the variation can be observed in two hypervariable regions, approximately enclosing the non-coding D-loop region, which can be used for forensic purposes. In several cases amplification and sequencing of the two main regions was shown to have great forensic potential in identification of an individual.

Here we report about a double murder from February 1991 in Bonn and the use of the D-loop sequencing analysis in one case. A dead woman was found in a house near Bonn and her common-law husband was found dead the following day in a street. Several sperm could be identified in the swabs taken from the rectum of the male victim. It was assumed that the crime was committed by at least two persons considering the difficulties a single person would have had to face committing the double murder. Circumstantial evidence, particularly textile traces, led to the identification and arrest of one suspect. DNA analysis performed elsewhere on the anal swab of the male victim yielded no conclusive results. We obtained a slide of an anal swab stained with hematoxylin eosin. We could identify under the microscope five sperm lying between squamous epithelium cells. They were individually sucked into a micropipette and transferred into separate PCR-tubes. Each sperm was digested separately and underwent a nested PCR of its mitochondrial DNA. The D-loop sequence of each of the sperm turned out to be identical, leading to the assumption that they belonged to one person. The sequence was neither identical with that of the victim nor with that of the original suspect (nor the investigator). The sperm are presumably from another yet unidentified person involved in the crime. Should another suspect materialized, sequencing his mitochondrial DNA would answer the question whether the analyzed sperm belonged to him or not.

mtDNA SEQUENCES IN NORWEGIAN SAAMIS AND OTHER NORWEGIANS

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The mitochondrial DNA has several properties useful for the reconstruction of human population history. The origin of the Saami people is still not known; however, a recent report (Sajantila et al, submitted) shows that the Saami are distinct from other Europeans by a large amount of sequence difference.

The purpose of this study was to compare the mtDNA sequences of Saami populations in Norway with those of the main population.

The material consists of serum from 205 unrelated Norwegian Saami from the Karasjok and Kautokeino communities. The serum is spun in an Eppendorf-centrifuge, 12000 rpm for 30 min. The pellet is digested overnight by proteinase K, and the DNA extracted by phenol/chloroform and pelleted by 100% alcohol and resuspended in 50 ul TE-buffer. An equal number of samples from other non-Saami Norwegians (EDTA-blood) is extracted by the salt-method (modified after Miller et al, 1988, Nucl. Acid Res., 16;1215). The Perkin Elmer 9600 is used for the PCR reactions and the PCR products are analysed on the ABI 373A Sequencer.

In a preliminary study we have sequenced a hypervariable regions (15997-16401) in the mitochondrial control region from 30 each of unrelated Saamis and non-Saami Norwegians. Saamis ordinarily have one or the other of two distinct groups of sequence pattern. Within each group there is some, but limited variation. This might indicate two origins of the Saami people.

Non-Saami Norwegians showed great sequence variation and no particular pattern or very distinct groups of patterns were observed. The non-Saami sequences thus differed from those of the Saamis, both in the amount of individual variation and in the lack of restricted patterns.

MITOCHONDRIAL DNA QUANTIFICATION IN ANIMAL HAIR BY SLOT-BLOTTING

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More than 50% of the people have at least one pet at home, usually a cat or a dog. Thanks to the shapes of the scales of their cuticle, the hairs of these animals easily cling to various objects whose surfaces are not entirely smooth; their adherence is even quite considerable, with the result that hairs are constantly found on clothes, furnitures, carpets, etc. which are in contact with the everyday life of these animals. This type of evidence could become an extremely important evidential value in the investigations of certain crimes and offences. In forensic sciences, hairs are very similar to fibres regarding to their transfer process and to the situations where they can be encountered: if somebody breaks into an apartment where a cat or a dog lives, it is very probable that he will take with him some hairs of these animals.

Therefore it is always important to collect and to examine these hairs, and by their interesting unusual presence in certain places, they may give interesting informations on the circumstances of the crime.

The morphological examination of animal hairs is principally used for the identification of the species. However, with the arrival of DNA analysis, this kind of evidence could lead to an individual identification by identical methods to those used in human identification.

Hairs are potential sources of DNA. However, the samples which are analysed often have roots of bad quality or even no roots at all, this means that there is a great risk of not having enough genomic DNA to carry out an analysis, even by PCR. It is therefore interesting to analyse the mitochondrial DNA of the shaft of the hair. As for the analysis of genomic DNA, to know the amount of DNA available is a precious information.

A quantification of mitochondrial DNA by slot-blotting has therefore been developed by using a probe with a specific sequence for cats, respectively for dogs and conjugated to an enzyme. This quantification was able to evaluate the amount of DNA in various samples such as hairs and blood. A comparison of the amounts of genomic DNA and mitochondrial DNA has also been done. The results showed that the future analysis of this DNA in the shaft of the hair of these animals is conceivable.

DETECTION OF SEQUENCE VARIANTS IN HYPERVARIABLE SEGMENTS OF MITOCHONDRIAL DNA IN THE ASIAN POPULATION

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The analysis of highly polymorphic parts of mitochondrial DNA is most commonly used method for the personal identification. The recent advances of fluorescent detection method of nucleotide sequence has made it possible to rapid analysis of sequence variants without using isotopic labeling.

We chose to analyze the control region in mitochondrial DNA because it includes the most polymorphic region.

We collected saliva as samples from more than 200 individuals of Japan, China, Mongolia, Myanmar, Bangladesh, and Europe. DNA were extracted in 45 minutes by chelex based method.

The two hypervariable segments of control region were amplified separately by the method of semi-nested PCR. In the first PCR, the 982 bp segment was amplified with the primers L15996 and H408. In the second PCR, 406bp segment was amplified with primers of L15996 and H16401 (Segment I), and 380bp segment was amplified with primers of L29 and H408 (Segment II).

This method is extremely sensitive so that as low as 0.1pg concentration of the template DNA can be amplified. The sequence variants of the two hypervariable segments were examined by the one-lane-direct-sequencing labelled by dye-deoxy-terminator using DNA- sequencer (ABI; model 373A).

In our result, more than 12% of sequence variants were detected in each segment. Some of them were suspected to be Asian specific because they were rarely found in Caucasians. About 2% of sequence variants per individual were found in comparison with full sequence data by Anderson et al. (1981).

Forensic validation on the integrity of maternal inheritance of the mitochondrial DNA

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Analysis of the human mitochondrial DNA (mtDNA) was shown to have a great potential for the study of genealogical relationships of human subpopulations and also for the identification of individuals for forensic purposes. So far, limited research has been done on the integrity of maternal inheritance of the primary structure of mtDNA. Although generally accepted, further studies undertaken to validate maternal inheritance for several generations within different maternal lineages are valuable.

We analysed fifteen nonconsanguinous, three to four generation families from Germany by amplification and sequencing of the two hypervariable regions of the non-coding D-Loop. We obtained fifteen different D-Loop sequence pattern corresponding to the fifteen unrelated families. Sequence analysis with the 373 ABI Sequencer in conjunction with the SequenceEditor Software 675 showed no sequence variation between the family members of one maternal lineage. We confirm that analysis of mtDNA can be used to trace maternal inheritance and to test relatedness even if there are several generations between ancestor and living descendant.

ROUTINE MITOCHONDRIAL DNA IDENTIFICATION

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Genomic DNA typing is a useful analysis used everyday across the world and largely accepted by the courts.

Limits of the techniques have been brought down to a small amount of DNA (less than 1 ng) by PCR : cigarette butts, root hairs, stamps could be genotyped using STR. But some problems remain unsolved as for example bone identification.

Analysis of mitochondrial DNA is a new approach permitting a new "step" in DNA typing. A variable region of mitochondrial DNA (transmitted by the women) could be sequenced. Point mutations of the question DNA sample are defined and compared to the point mutations of reference DNA.

We are routinely performing mitochondrial sequencing in our laboratory for specific problems:

- Identification of human remains largely degraded
- Identification of rootless hair.

We describe in this paper the techniques used, the problems we have had and the answer proposed, the interpretation of the results and a general appreciation of cost in relation to the genomic DNA typing.

In conclusion we show applications to caseworks.

B. DNA SEQUENCING DATA

The STR approach

Possibilities and limitations

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STRs are a class of VNTR polymorphisms which offered a range of new perspectives for forensic applications. The most important one lies in the discrete distribution of alleles. Other advantages lie in the (short) fragment length which decreases to about 100 bp and which is extremely advantageous in many forensic samples. Also the applicability of PCR has led to a sensitivity increase by several orders of magnitude. - Experiences with a large number of polymorphic systems will be demonstrated. According to their structure they can be subdivided into: highly polymorphic systems with a multiplicity of length and sequence variants; intermediately polymorphic systems sometimes showing race-associated variants; regular system. - Different forensic criteria of usefulness do not always coincide with the aforementioned subdivision. Thus the laboratory has to make a selection depending on the problem to be solved. - The high sensitivity is a challenge on the one hand but a risk on the other. Criteria will be elaborated to avoid false conclusions. - Differences between major ethnic groups have led to new and efficient tools to attribute unknown samples with varying probabilities to certain populations. But again this includes the risk of significant mistakes. Strategies will be proposed to also avoid mistakes due to this type of unknown heterogeneity. The diversity of systems relative to questions to be solved makes new strategies for their selection and their application necessary.

SEQUENCE ANALYSIS OF POLYMORPHIC SHORT TANDEM REPEAT LOCI USED IN MULTIPLEX PCR DNA PROFILING SYSTEMS.

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A multiplex PCR DNA profiling system is currently in use in the casework section of this laboratory. The system is based on the co-amplification of 4 short tandem repeat (STR) loci using fluorescently labelled PCR primers. The products of the amplification are then sized using an ABI 373A DNA sequencer. A second multiplex system with a greater potential for discrimination has been developed. It is composed of 6 STR loci and a sex test which detects a deletion in the amelogenin gene on the X chromosome. This second system is intended for use in casework and will also form the basis of a National DNA Database.

Studies have shown that complex STR alleles, such as those at the SE33 and D11S554 loci, exhibit extensive variation in both their amplified fragment length and base sequence. Although the STR loci utilised in both the first and second multiplex systems are simpler, in that their alleles are of a smaller molecular size and have more regular repeat units, variation due to base insertions, deletions and substitutions have been reported. Therefore, a study has been carried out in order to examine the nucleotide sequences of the STR loci used in both multiplex systems in order to assess the extent of intra- and inter- population sequence variation.

Sequence data have been obtained for alleles of STR loci employed in both multiplex systems from the British Caucasian and British Afro-Caribbean populations. These studies have shown that for certain STR loci, alleles, which are of the same molecular size but are from different individuals, exhibit sequence variation in the repeat and flanking regions. These findings demonstrate that the designation of two alleles from these loci by the same repeat unit number cannot be taken to mean that both alleles have the same nucleotide sequence. Thus, samples with the same allele designation could be shown to differ if further analytical procedures are used.

ANALYSIS OF SEQUENCE VARIATIONS IN THE ALLELES FROM THREE STR LOCI.

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DNA polymorphic loci resulting from variations in the number of short tandem repeats (STR) are an abundant type of genetic markers that can be useful in human identification. The main advantage of these loci are the small size of their alleles and their ability to be detected by PCR based DNA amplification. We have developed a multiplex amplification system utilizing three loci. Although in theory the alleles from these loci differ by changes in the number of tetranucleotide repeats, additional variants may result from sequence variations between similar size alleles. Two of the loci examined, located in chromosomes 3 (D3) and 18 (D18), are composed of 9 size variants and the third, located in chromosome 12 contains 24 alleles. For each allele size several unrelated individuals, from the North American Black and Caucasoid populations, were selected and their alleles sequenced. DNA sequence analysis of alleles was performed by fractionating the amplified alleles by agarose gel electrophoresis and slicing the portion of gel containing the allele. The allele was re-amplified with one of the primers labeled at the 5' with biotin. The amplified product was purified with Streptavidin coated magnetic beads and sequenced using the Taq Dye-deoxy terminator cycle sequencing kit (Perkin Elmer, Foster City, CA) in an ABI Model 373A automated sequencer. The results of this study indicate that the most common alleles from the loci D3 and D18 differ only by the number of tetranucleotide repeats. The locus in chromosome 12 showed differences in several alleles resulting from changes in both the number of tetranucleotide repeats and sequence variations in both the repeat and flanking regions.

AMPFLP-TYPING FOR THE HUMCD4 STR POLYMORPHISM IN AN AUSTRIAN CAUCASOID POPULATION SAMPLE: SEQUENCE DATA AND ALLELE DISTRIBUTION

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We evaluated the (AAAAG)_n STR polymorphism in the CD4 gene (HUMCD4) by PCR-AMPFLP and automated laser fluorescence DNA-sequencing revealing a locus specific allelic ladder consisting of eight distinguishable alleles. The sequenced ladder was applied in allele determination of 300 healthy, unrelated Austrian Caucasoid individuals.

Sequencing of 35 alleles showed a regular repeat structure with only one polymorphic repeat motif. The shorter alleles 4 to 8 were designated according to the number of repeats. The longer alleles 8' to 11, which show a transition in one of the repeat units, were named in the same way including the modified repeat unit. Alleles 8 and 8' are of identical length and were indistinguishable on native polyacrylamide gels.

The following allele frequencies have been found:

A*4	0.363	A*7	0.002	A*10	0.025
A*5	0.316	A*8'+8	0.012	A*11	0.005
A*6	0.002	A*9	0.275		

The population investigated showed no deviation from Hardy-Weinberg equilibrium.

TYPING OF THE HUMVWA MICROSATELLITE POLYMORPHISM: ALLELE FREQUENCIES AND SEQUENCING DATA.

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Using a previously described method (PCR-AMPFLP[1]) we have determined allele frequencies for the (TCTA-TCTG) microsatellite repeat in intron 40 of the human vWF gene (HUMVWA,12p12-12pter,[2]) in unrelated individuals of our local (Austrian) population (n=354). In a first step, an allelic ladder composed of all alleles distinguishable by PAGE in screening experiments was constructed by pooling eluates of amplification fragments of different alleles and subsequent reamplification. This allelic cocktail was used as a reference in all subsequent population typings. 9 alleles were thus identified by their different migration, including two new alleles. Allele frequencies are given in Tab.1. No deviation from Hardy-Weinberg equilibrium was observed. Sequencing of the alleles that were included in the ladder and other eluted allele samples revealed several sequence variants not published so far[3]. Since typing of the VWA microsatellite polymorphism was unambiguous using the described protocol and the allelic cocktail and also the polymorphism is sufficient we conclude that typing for VWA may be included forensic casework, possibly replacing typing for loci that may not always give clear-cut results under certain conditions.

Tab 1. Allele frequencies

11*	0.28%	15f**	0.28%	19	19.21%
14	15.82%	16	36.44%	20	3.11%
14s**	0.28%	17	44.35%	21	0.28%
15	24.29%	18	38.70%		

* alleles were assigned according to the number of tandem repeats
** variant

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- 2.Kimpton, C. et al.: Hum.Mol.Genet 1:287 (1992)
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SEQUENCING AND SIZE DETERMINATION OF THE D1S80 INTERALLELE

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Many of VNTR and STR loci offer sufficient polymorphism to be suitable for genetic characterization. One such locus, DIS80, has proven useful in forensic identification and parental testing. Some variations (interalleles), which were not present in the allelic ladder, have been reported in various populations. In order to compare the fragment sizes of interalleles, we used different colored dye labels (FAM, HEX). Fluorescently labeled PCR products were electrophoresed in a 6% acrylamide gel with 8 M urea for 10 - 12 hours. The size of the PCR products was determined automatically and precisely by software using labelled size standards and the allelic ladder in each lane. To conduct sequencing, each band of the interallele was electroeluted, then cloned and sequenced. The sequencing data of the DIS80 interallele is described herein.

ANALYSIS OF THE SHORT TANDEM REPEAT POLYMORPHISM D18S51: ALLELE FREQUENCIES AND SEQUENCE STUDIES

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The analysis of Short Tandem Repeat polymorphisms is widely used for typing DNA samples. There is a demand for systems with high discrimination power and with the possibility to combine several STR loci for multiplex analysis. STR polymorphisms with small number of alleles (e.g.: TC11, F13A) do not have the discrimination power compared to STR-systems like SE33 (ACTBP2). SE33 has more than 30 alleles and thus the space for the combination with other STR loci in multiplex analysis is reduced. Therefore STR-polymorphisms like HUMFIBRA, D21S11 and D18S51 with allele numbers about 15 - 20 seem to be a good compromise.

To get data for allele frequencies in the German population we started to study the locus D18S51 which was first described by Straub et al. (1993) and was used for DNA typing in a heptaplex STR PCR system (Urquhart et al., 1995). PCR products of the D18S51 locus were separated with a direct blotting equipment, blotted onto a nylon membrane and the alleles were detected with a non-radioactive method. With this method we could easily type each allele and up to now we detected 13 alleles in the German population. We used these data for calculating allele frequencies. For a correct sizing of the PCR products we started to sequence some alleles.

Straub et al., *Genomics* 15:48-56, 1993.
Urquhart et al., *BioTechniques* 18:116-121, 1995.

Automated Sequence Based HLA Typing New Tools and Techniques in Forensic Sciences

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The HLA (Human Leucocyte Antigen) genes represent one of the most variable regions of the human genome. The combination of both the maternal and paternal allele determines the HLA type, which is unique for each individual. An exact identification of the specific HLA type is not only of essential interest in transplantation medicine or the analysis of disease susceptibility but gives valuable information for forensic purposes, either in paternity testing or for the identification of persons.

Sequencing data derived from HLA typing are usually very complex. A typical HLA sequence shows the superimposition of both alleles deriving from the chromosome pair. Conserved base positions are identical, while variable positions can deviate, leading to a heterozygous type of sequence information.

In addition to the heterozygocities at the variable base positions, background sequences deriving from coamplification of related sequences can appear. Therefore very high quality sequencing data are essential for sequence based typing (SBT). Actually the best results are obtained from solid phase sequencing with a T7 polymerase followed by automated sequence determination.

The classical solid phase approach uses streptavidine coated paramagnetic beads for binding the biotinylated PCR product and all following purification steps (1). We want to present a further development in solid phase sequencing where plastic combs, which fit into a sequencing gel are used as a solid support (2). This method drastically reduces pipetting steps and the possibility to mix samples.

To determine the HLA type the sequence data have to be matched to all possible combinations of HLA subtypes. A bottle-neck for high throughput HLA typing is this determination of the HLA type from sequence data. So a software dedicated to automated HLA typing is a very valuable help, taking over the time consuming and tedious part of the analysis, which is essential for any kind of routine diagnostics.

Pharmacia has developed a software for the automated determination of HLA types from ALF/ALFexpress sequence files.

The automated HLA typing with **HLA SBTyper** is done in two steps. First the ALF test sequence is aligned with the master template to a best fit. Then a comparison of all detected non-conserved bases with all possible combinations of pairs of master sequences is carried out. The result showing of the most probable candidates is displayed. It can, if necessary, be further investigated by using the dynamic link to the ALF raw data.

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INVESTIGATION OF THE STR HUMLIPOI IN AUSTRIAN CAUCASOID INDIVIDUALS: SEQUENCE DATA AND ALLELE FREQUENCIES

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The (TTTA)_n tandem repeat polymorphism HUMLIPOI, which is located in intron 6 of the lipoprotein lipase gene, was analysed by Amplification Fragment Length Polymorphism (AMPFLP) in an Austrian Caucasoid population sample of 550 healthy, unrelated individuals.

Precise allele assignment was achieved by means of an allelic ladder consisting of seven different alleles.

Automated laser fluorescence DNA-sequencing of the ladder alleles revealed a regular repeat structure of 7 and 9 to 14 repetitions of the core repeat with allele sizes ranging from 111 to 139 basepairs.

Allele designation was in accordance with the repeat number.

The resulting allele frequencies are shown in the following table:

A* 7	0.003	A*12	0.251
A* 9	0.053	A*13	0.026
A*10	0.385	A*14	0.004
A*11	0.278		

No deviations from Hardy-Weinberg equilibrium were observed.

D12S391: A HIGHLY USEFUL STR FOR FORENSIC PURPOSES

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The microsatellite in D12S395 (Genbank accession number 731) was amplified after PCR amplification in 130 unrelated individuals from Galicia (NW Spain). The optimization of different PCR parameters, including multiplex amplifications with STRs is demonstrated.

Precise allele assignment was achieved by means of an allelic ladder consisting of 12 common alleles.

Most of the alleles were sequenced revealing a regular (TAGA)_n structure of 9 to 20 repetitions with allele sizes ranging from 210 bp to 254 bp.

Allele frequencies of the more common alleles are: *9: 4.20%, *10: 2.29%, *11: 8.40%, *12: 22.52%, *13: 12.98%, *14: 9.92%, *15: 9.92%, *16: 13.74%, *17: 8.40%, *18: 5.34%, *19: 1.53%, *20: 0.76%.

The population was found to be in Hardy-Weinberg equilibrium. The heterocigosity value is 90.08%.

The characteristics of the system (including the high polymorphism, normal electrophoretic behaviour, easy amplification) makes this STR considerably interesting for forensic purposes.

DNA SEQUENCE ANALYSIS OF PCR AMPLIFIED PRODUCTS OF MCT118 LOCUS IN JAPANESE DNA SAMPLES

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Up to date, there has been no available reference on the whole DNA sequence of MCT118 locus. In this report the whole DNA sequence of MCT118 including both flanking regions and repeat region has been revealed and the difference between different alleles has been analyzed.

Cloned PCR amplified product of MCT118 locus have been sequenced. Then, the whole sequence of 10 alleles of MCT118 locus have been determined. The sequence obtained here showed a highly similarity to sequences reported by Kasai et al and Perkin Elmer's kit manual. However, one site in 5' flanking region which remained undetermined in the former reports could not be clarified under the conventional sequencing methods. Then, allele specific PCR procedure was specifically applied to the complete determination of sequence of the region and it was found that sequence of 5' flanking region is longer by one base than that in the previous report. It has been generally accepted that the sequence of repeat region at MCT118 locus consist of sixteen base pairs except the first repeat units. In this report an interesting finding was obtained that all the alleles have common sequences in the first six and the last four tandem repeat units as well as 5' and 3' flanking regions, and repeat units with variable sequence are inserted between such common sequence region. It was also found that a slight sequence difference is observed in the same allele from different persons with no appreciable mobility difference in 5% native polyacrylamide gel electrophoresis.

C. STATISTICS

STATISTICAL ANALYSIS OF STR DATA

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Many advances have been made over the last year in the statistical analysis of DNA data for forensic work. Because STR data are discrete and lack the ambiguity with regard to homozygotes which bedevils RFLP analysis, it is easier to focus on the essential issues. The author will review some of the most recent testing, arguing that the most important questions are simple though they have been unnecessarily clouded by complex population genetic considerations which are of negligible forensic importance.

- Between person comparison

1 in 50,000 cases $LR > 10,000$

- Within person comp.

65% of cases $LR > 10,000$

4 STR locus database $N = 1660!$

Tippett experiment $N(N-1)/2$ comparisons
between individuals

THE USE OF LIKELIHOOD RATIOS IN REPORTING DIFFICULT FORENSIC CASES

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Forensic scientists at ESR:Forensic in New Zealand use a likelihood ratio when reporting cases involving DNA profiling results.

This method of reporting has allowed relatively simple calculations to be performed in cases where it would have been difficult to report a number using a "frequentist" approach.

Three such cases will be discussed:

- 1 Police believed a missing woman had been murdered. Clothing was found which had a bullet hole and blood staining. DNA comparisons were made to the blood on the clothing using the victim's father and brother as references. A number was calculated for the likelihood that the blood on the clothing originated from the victim.
- 2 The accused in a rape case was the victim's natural father. She had been raped on a hay bale in a barn. The DNA profile from the semen on the hay matched that from the accused. The blood sample from the accused was ruled inadmissible due to a consent issue. We therefore calculated the likelihood that the semen on the hay originated from the victim's natural father rather than from another random male.
- 3 DNA profiling test on samples taken from a rape and homicide victim, showed that semen from two males was present. Likelihood ratios were calculated for each of the two suspects who could have contributed the semen.

IDENTIFICATION FROM BIOLOGICAL STAINS: PROBABILITY OF IDENTITY OR KINSHIP

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For the identification of stains, results based on genetically determined polymorphic markers including DNA are used for exclusion purposes only throughout the world. However, in forensic cases it is important to not only exclude but also, in the case of a non-exclusion to determine the probability of donorship of the individual under investigation. Hence the calculation of a probability of identity W_{id} (or of kinship) becomes mandatory. To illustrate the basic problem some specific cases and situations are discussed and their mathematical handling is reported. In analogy to a widespread custom in cases of disputed paternity, it is suggested to link the probability value with a verbal statement, e.g. $W_{id} \geq 99\%$ as "mostly probable" and $W_{id} \geq 99.73\%$ as "practically proven".

Keywords: Stains, Biometrics, Biostatistics, Probability of Identity, Kinship

DECISION-MAKING IN PATERNITY DIAGNOSTICS USING SLPs EVALUATION BASED ON A THREE-YEAR MATERIAL

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A number of factors represent a challenge to the establishment of a sound theoretical statistical basis for decision-making in paternity diagnostics with hyperpolymorphic minisatellite loci; for example the degree of variation itself, the "pseudo-continuous" allele distribution, the choice of matching criteria, the influence of kinship, the application of the "product rule", and the presence of considerable mutation rates. The need for comprehensive empirical data is therefore even more obvious than with traditional genetic markers.

We have studied consecutive Norwegian paternity cases with 3432 (mother)/child/man triplets or pairs using 5 SLPs (YNH24, g3, MS31, MS43A, and CMM101). A procedure with visual comparison between fragments in adjacent lanes has been employed, using fragment gel positions within 0.5mm as match criterion. Men with match (M) in each of the five SLPs were considered fathers, and those with non-match (NM) in four or five SLPs non-fathers. Together these cases constitute 97% of the total material. The paternity of the 103 men with any other results (4M,1NM; 3M,2NM; 2M,3NM) was established through the use of an additional battery of DNA markers (SLPs and STRs).

The paternity exclusion capacity experienced in the 921 non-fathers was highest in CMM101 with 96.7%; lowest in MS43A with 92.9%. The distribution of matches and non-matches fitted very well with expectancies employing the product rule. In the 2510 fathers, the great majority of non-matches was shown to represent mutations. There was no significant tendency towards multiple mutations.

Using these empirically obtained paternity exclusion capacities of non-fathers and non-match rates in fathers, we show that odds exceed 10^6 in favour of paternity in the 5M situation, and 10^7 of non-paternity in the 5NM and 4NM,1M situations. For the 3 % of cases left, further evidence is needed before firm conclusions are drawn.

PROBABLE RACE OF A STAIN DONOR

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Sometimes it would be useful to know the race of a stain donor. Any DNA typing provides some evidence, provided that population data of fragment sizes for the races in question is available. Here, we will estimate how good that evidence is likely to be, and present some ideas and calculations how best to use it.

DNA profiles obtained for a collection of people of known races are used as test data. Each person's profile frequency is then calculated under a variety of racial assumptions. Each test profile is favored to come from the race in which the profile frequency is greater, and is favored in proportion to the various frequency estimates. The frequency estimate as used for casework is not the only possibility, and if some other formula gives better predictive value no theoretical justification would be necessary in order to prefer it. In a practical application the prior probabilities also have to be taken into account in figuring the odds.

Calculations are made using many different probes and several races based on data and population frequencies from a variety of laboratories. For example, using 5 RFLP probes to distinguish African-American from Caucasian the typical frequency disparity is more than 100-fold. Moreover, there is a 70% chance to make a 95% confident decision, and a 90+% chance to guess correctly. For the purpose of distinguishing Whites from Blacks the best probe among those considered is D12S11 (MS43), which contributes a typical likelihood ratio or frequency disparity of more than 3. Least discriminating is D4S139 (pH30), worth only 1.3.

In some crime cases the methods discussed can be useful to aid the police in searching for a suspect. To some extent the likely value of such analysis can be predicted in advance.

SEDNA: A computer program for semiparametric estimation of densities and match probabilities in DNA forensic identification and paternity cases

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Several methods have been proposed for estimating allele frequencies and computing match probabilities in the context of SLP analysis in forensic identification and paternity cases. Among these methods, there is one which presents very satisfactory practical results, which is the semiparametric estimation of the density function on the fragment length (Valverde et al, 1993, Cao et al 1995). This method has been implemented in C language, resulting in the SEDNA program.

SEDNA uses an initial database containing information with measured fragment lengths and their correspondent true values to establish the experimental error. A variety of databases pertaining to different loci, different restriction enzymes or different populations can be handled by SEDNA. This allows computation of the semiparametric density estimation of the fragment length as well as match probabilities. The program produces graphical outputs and facilitates the use of information about relatives in paternity cases.

A practical demonstration of this program will be made at the time of the congress.

DNA PCR POLYMORPHISMS IN PATERNITY TEST PROTOCOLS A BIOSTATISTICAL APPROACH

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The introduction, in the last few years, of DNA PCR polymorphisms has resulted in substantial changes in parenthood tests protocols. Until 1991 the Forensic Haemogenetics Laboratory of University of Pisa used, in disputed parentage cases, six red cell blood groups (ABO, RH, MNS, K, FY, JK), nine serum protein systems (HP, GC, TF, PI, C3, BF, PLG, IGHG and IGK) and eight red cell enzyme polymorphisms (ACP, PGM, AK, ADA, 6PGD, GPT, ESD, GLO). Since then, we included PCR assay. Now, we use currently seven DNA polymorphisms: DQA1, D1S80, YNZ22, APOB, TH01, VWA and FES. Shortage of manpower resources and cost-benefit considerations discourage us, a little laboratory, from routinary typing of 30 different systems for every paternity case.

In this paper our criteria of choice of polymorphisms are discussed. The biostatistical evaluation of efficiency in paternity exclusion plays a predominant role. The average power of exclusion (A) for every locus has been calculated (Garber RA, Morris JW, 1983) as well as the cumulative power of exclusion both for traditionally used and PCR-based systems. In this regard, three parameters were taken in account:

$cumA \geq 1$ = cumulative chance of finding at least a single incompatibility;

$cumA \geq 2$ = cumulative chance of finding at least two incompatibilities;

$cumA \geq 3$ = cumulative chance of finding at least three incompatibilities.

The results are shown in the following table

# Systems	$cum A \geq 1$	$cum A \geq 2$	$cum A \geq 3$
23 (Traditional)	99.1%	94.2%	81.8%
7 (PCR-based)	99.9%	98.3%	90.8%
30 (Total)	99.999%	99.98%	99.82%
20 (13 Traditional + + 7 PCR based)	99.996%	99.93%	99.48%

With traditional markers, two incompatibilities are accepted as a solid proof of paternity exclusion, but when using DNA loci we feel that at least three incompatibilities should be obtained.

The protocol we adopted, including 13 traditional and 7 PCR-based polymorphisms (ABO, RH, MNS, HP, GC, TF, PI, C3, ACP, PGM, ESD, GLO, DQA1, D1S80, APOB, YNZ22, TH01, VWA, FES), assures a satisfying efficiency in paternity exclusion (see table, last row).

A CLASSICAL EXCLUSION IN A PATERNITY CASE WHERE THE W VALUE IS 99.999%: EVIDENCE FOR A MUTATION IN THE HAPTOGLOBINE SYSTEM

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The Haptoglobine system is one of the longest used plasma protein polymorphisms in the field of paternity testing. Up to now more than 3000 family data have been published. A classical exclusion in this system is regarded as "paternity obviously impossible". Nevertheless we found a classical exclusion of the alleged father in the haptoglobine system (starch gel electrophoresis), while a W-value of 99.80% after testing 24 conservative systems. To explain this obvious contradiction, we typed the short tandem repeat system ACTBP2, HumTH01, F13B, HumFES/FPS and HumVWA, which raised the W-value to 99.999%. The paternity of the alleged father is therefore highly probable despite the exclusion which is supposed to indicate "paternity obviously impossible". This case shows that the excessive exclusion chance offered by DNA-polymorphisms can help the expert in clarifying complicated paternity cases.

POPULATION AND PATERNITY CALCULATIONS - COMPARING THREE SOUTH AFRICAN POPULATIONS.

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The introduction of DNA technology in forensic work has re-awakened debate on populations and population substructure. In addition, in accordance with probabilistic reasoning, the multiplication rule of independent genetic events has led to very high Paternity Indexes (PI) and probabilities of paternity (W). The effect on the PI and W in different populations from the same geographic area was assessed using conventional genetic markers (blood groups, serum proteins, red cell enzymes and HLA-Class I).

Consecutive non-excluded paternity cases (n = 610) from white, black and mixed ancestry (MA) groups were reassigned to another population and those gene frequencies used to calculate PI and W values. The mixed ancestry group has been studied extensively and usually falls genetically midway between white and black but also has genes from Khoisan and Malay origin.

In casework, a W of greater than >99.00% was attained in 88.17% (149/169) of the white, 92.97% (240/258) of the MA and 77.64% (134/173) of the black populations respectively. The average PI was > 33374.40 (W = >99.99%) in all three groups. However, the median PI was 2357.19 (W = 99.96%) for white, 1908.53 (W = 99.95%) for the MA and 681.20 (W = 99.85%) for black cases.

The greatest contributor to the PI and W was HLA as expected, but the average PI for the HLA-Class I system was 139.5492 (W = 99.28%) for the white, 131.4613 (W = 99.24%) for the MA and only 77.2280 (W = 98.92%) for the black cases.

When the calculations were repeated using "obviously" inappropriate gene frequencies for the groups taking white as black and black as white, 97.64% (165/169) of white cases attained a W of greater than 99.00% similar to 96.6% (167/173) of blacks. The median PI was 108 878.83 (W = 99.999%) and 210 311.92 (W = 99.999%) respectively, but the average PI was now 26 million and 23,400 million for white and black cases.

Only 89.54% (231/258) or 87.99% (227/258) of cases had a W >99.00 for the MA when calculated with either white or black gene frequencies. The median PI and W were 8982.37 (W = 99.988) and 6430.4463 (W = 99.988) respectively. The average PIs were now 7million and 78 million respectively.

The use of inappropriate gene frequencies can lead to gross over-estimations of the PI and probability of paternity in conventional paternity testing, and probably also in DNA testing.

D. DNA POLYMORPHISMS

DNA POLYMORPHISMS AND THEIR APPLICATION TO HUMAN IDENTITY TESTING

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The ability to type DNA derived from biospecimens has changed the manner by which forensic scientists analyze forensic samples and perform paternity testing. Biological samples, such as blood, semen, saliva, hair, bone, and other tissues, can be characterized genetically and to a higher degree of certainty than ever before. The first widely used DNA typing technology in forensics was RFLP analysis of VNTR loci. These loci are highly polymorphic, and substantial data exist to support the utility of these loci for inferring the rarity of a multiple locus DNA profile.

RFLP typing yields reliable results for forensic purposes, yet some limitations to RFLP analysis may be encountered. These include: 1) a sufficient quantity of high molecular weight DNA (usually at least 50 ng) is required for RFLP analysis; 2) RFLP analysis is time-consuming, requiring, at times, four to eight weeks to obtain results on four VNTR loci; and 3) the RFLP technique cannot resolve unequivocally the alleles of most VNTR loci.

An alternative strategy for forensic testing at the DNA level is the use of polymerase chain reaction (PCR)-based assays. PCR-based assays improve upon the above mentioned RFLP limitations. Additionally, many degraded DNA samples can be amplified by PCR and subsequently typed because alleles amenable to PCR are much smaller in size compared with alleles detected by RFLP analysis. The PCR-based loci are sequence polymorphisms (such as HLA-DQA1); 2) VNTR and STR loci; 3) mitochondrial DNA sequences; and 4) a variety of sex typing markers. There are a variety of analytical approaches for typing the loci, ranging from immobilized ASO probes to capillary electrophoresis. Additionally, from a large number of compiled databases the frequency of departures from HWE and linkage equilibrium was no more than expected by chance. Furthermore, the population data for the loci from a number of ethnic and geographical groups are consistent with other polymorphic loci and human ethnohistory. The data demonstrate that estimates of multiple locus PCR-based DNA profile frequencies can be made using currently applied statistical methods and should not result in unfair bias.

95° }
67° } 30 sec. 28-30 cycles
70° } 0.4-5µg DNA
9600 TC

more stringent
annealing improves
amplification

6%T, 3%C (Pipeman digest) buffer
31 x 0.04 cm discant.

4%T, 5%C, 0.5X TBE, 7M urea
60W - 75 min

TH01 + HBG2 : Chr. 11 7cm
no evidence for
linkage
(paternity!)

SELECTION OF STR LOCI FOR FORENSIC IDENTIFICATION SYSTEMS

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The selection of Short Tandem Repeat (STR) loci for forensic identification systems presents many problems. Here we review the selection criteria used in the design of the fluorescent-labelled multiplex STR system used for the Forensic Science Service's National DNA Database. We sequenced STR loci which showed alleles differing by 1, 2, 3, 4 and 5 base pairs. Databases were prepared for each locus in 3 British populations and loci were tested for Hardy-Weinberg equilibrium and inter-locus independence. Null alleles were found at one locus (HUMCYAR04) and temperature-dependent null alleles at another (D19S253). Loci were also tested for their propensity to produce 'stutter' bands, to incorporate an extra 3' deoxyribonucleotide, and to give even peak-heights.

PCR TYPING OF ALU ELEMENTS - MOLECULAR GENETICS AND FORENSIC APPLICATION.

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Alu repeats belong to the family of short interspersed elements (SINEs) and are among the most abundant repetitive DNA sequences in the mammalian genome. They represent mobile genetic elements ancestrally derived from the 7SL RNA gene and have presumably spread within the genome by retroposition. A particular group of Alu repeats appears to be human-specific and has expanded only recently within the human genome as indicated by distinct dimorphisms at various loci due to the presence or absence of an Alu repeat. By comparison of the frequency distribution of Alu insertions at selected loci in various populations, the evolutionary origin as well as the phylogenetic relationship between racial groups can be determined (Batzer et al., PNAS 91:12288, 1994; J. Mol. Evol., in press). Thus the genetic typing of a selection of informative human-specific Alu elements might be useful to obtain clues on the race of an unknown stain donor in forensic casework.

Alu repeat loci can easily be typed using the polymerase chain reaction (PCR) with locus-specific primers flanking the potential Alu insertion site. After separation of the amplified DNA fragment, a size difference of approx. 300 bp indicates the presence or absence of the Alu repeat at a given locus. To assess the genetic stability of Alu repeat loci as well as the effects of racial admixture, six different Alu repeat loci (ACE, APO, D1, FXIIB, PV92, TPA25) are being analysed using family DNA samples with parents of German or ethnically mixed origin from routine paternity casework. In stain identification cases, the combined exclusion chance for these loci is >98% and >99% for Caucasians and Blacks, respectively. By comparing phenotype frequencies from different racial databases, significant differences were observed providing evidence for the discriminative power of Alu loci among human races.

AN INVESTIGATION OF VARIATION IN THE SIZING OF SHORT TANDEM REPEAT LOCI

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Short tandem repeat (STR) alleles used in forensic analyses are designated according to the number of base pair repeat units they contain, usually with reference to a allelic ladder, providing apparently discrete types. STR alleles display measurement variation, however, and knowledge of the extent of this variation provides important information to validate laboratory typing. In this study, measurement variation of HUMTH01 and HUMFES/FPS alleles has been examined after electrophoresis in an Applied Biosystems automated DNA scanner and allele sizing using Genescan 672 software. Within single gels we have examined variability within the same individual, within the same genetic material using mother-child pairs and within the same allelic type in unrelated individuals, both in adjacent lanes and across the gel. Between gel variation has also been examined using five individuals representing a broad range of HUMTH01 and HUMFES/FPS types run on separate gels in a Latin Square design. Bulk amplification and samples amplified individually for each run have also been used within the randomized design to examine amplification variation. Molecular size ranges have been applied to assign alleles to a series of 110 unrelated individuals typed for HUMFES/FPS. While one might expect the median size of each allelic group to be separated by four base pairs, analysis of this experimental data shows this difference to be larger as allele size increases, with nine base pairs separating the medians of alleles 10 and 12. In addition, there is an apparent increase in spread of the data, so extreme that a misclassification of alleles might occur. The designation of supposedly discrete allelic types thus depends on knowledge of the extent of the variation that is likely to occur within the experimental system being used in the relevant laboratory.

VARIABILITY AND SEGREGATION OF Y CHROMOSOME HAPLOTYPES DEFINED BY 8 POLYMORPHIC SHORT TANDEM REPEATS (STR)

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A selection of 8 polymorphic STR 's has been used to analyse the variability of a panel of 70 unrelated Caucasian Y chromosomes of German ancestry. Fluorescently labeled PCR products were run on an ALF™ DNA Sequencer (Pharmacia Biotech, Freiburg, Germany). The allele lengths were ascertained with the ALF™ Fragment Manager 1.1 Software. 69 of the 70 males can be distinguished using the proposed Y haplotyping. Because each compound haplotype defines a paternal lineage a mutation screening in up to 163 confirmed father/son pairs has been carried out. The estimated mutation rate of the Y haplotypes is low enough to provide a record of male-specific gene flow at least for 200 generations. The PCR based analysis of 8 different Y chromosomal STR provides a simple, reproducible and sensitive male identification system for forensic (stain analysis, paternity testing) as well as for anthropological applications.

DYS 385 duplicated locus
= 2 allele - haplotypes
→ natural duplex PCR

**CORRESPONDING REPEATS IN STRs AND INTERNAL
STANDARD IN FRAGMENT ANALYSIS
REPRODUCIBILITY WITH THREE HYPERPOLYMORPHIC STRs**

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Automated fragment analysis with ABI Sequencer using the internal standards GS2500 or GS500 gives sufficient reproducibility to allow typing of regular tetrameric STRs. It is, however, a common experience that it does not allow fool-proof typing of alleles differing in size by one base-pair (bp) only.

The main purpose of this study was to evaluate internal standards composed of the same repeats as the STRs to be measured. ACTBP2 (SE33), APOAI1, and D11S554 all have the main repetitive unit AAAG, and they all have some alleles differing by one bp. We constructed an internal standard composed of 21 alleles from ACTBP2 and 4 from D11S554. This internal standard was ROX-labelled and each fragment sized using GS2500. Some of the alleles have also been sequenced. ACTBP2, APOAI1, and D11S554 were labelled with TAMRA, FAM, and JOE, respectively, and the three STRs were run in one lane together with the ROX-labelled internal standard composed of alleles as described. Intergel measurements of identical alleles as well as measurements of unrelated alleles (300 unrelated individuals run on 15 gels) showed SDs generally well below 0.15 bp. The results obtained are considerably better than those achieved by commercially available standards, and yields safe discrimination at the one bp level.

The three STRs studied are each highly polymorphic. Forty ACTBP2 alleles were experienced, the discrimination power (DP) being 99.45%, the paternity exclusion power (EP) 89.19%. For APOAI1 the figures were: 33 alleles, DP: 98.81%, EP: 83.85%; for D11S554: 36 alleles, DP: 99.18%, EP: 86.72%. The probability that two unrelated individuals have the same type in all three STRs is 5×10^{-7} , and the combined paternity exclusion power is 99.77%. The triplex may therefore represent a very valuable test in forensic genetics, and in particular become a good choice for intelligence databases and paternity diagnostics.

**INTERPRETATION GUIDELINES FOR FLUORESCENT AUTOMATED
DETECTION OF STRS : DEFINING THE ALLELE AND THE LIMITS OF
DETECTION.**

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Human identity testing has rapidly evolved over the past few years. The quest for more automated and sensitive technologies based on PCR detection of short tandem repeat alleles promises to expand the scope of forensic DNA identification. The fluorescent detection of these alleles using laser-enhanced detection systems and internal molecular weight lane standards has enhanced the scientist's ability to quantitate and analyses amplified products. Analysis of DNA samples amplified from crime scene evidence has provided an excellent source for testing the limits of this new technology under typical casework conditions. Based on empirical data, interpretation guidelines have been drafted for the detection of true STR alleles and identification of potential PCR artifacts. Stutter "n-1", "n-4" and "n-10" PCR products, monomorphic bands and the matrix balance of fluorescent internal lane standards must be taken into account prior to the interpretation of STR allelic profiles. Fluorescently tagged amplified products from seven STR systems (FGA, D21S11, Amelogenin, F13A1, FES, THO1 and vWA) have been carefully evaluated for characteristic fluorescent product peaks. The major peaks represent "true alleles" and are easily distinguished from fluorescent noise or spurious PCR products. Specific casework examples with suggestions on interpretation of mixtures and low intensity signals are presented. Through empirical measurement and validation, comprehensive interpretation guidelines were derived for analysing STR profiles using fluorescent detection methods.

STR MATCH CRITERIA: AN EVALUATION OF THE PRECISION AND MEASUREMENT WINDOW FOR STR LOCI USING INTERNAL MOLECULAR WEIGHT STANDARDS AND AUTOMATED FLUORESCENT DETECTION.

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The anticipated change from conventional RFLP analysis and PCR dot blot format to detection of STR alleles based on size determination using various detection methods (i.e., silver staining or fluorescent labelling) requires careful determination of the limitations of instrument precision and evaluation of potential biological allele sequence variation. The measurements of instrument precision and reproducibility of representative alleles from seven STR systems (FGA, D21S11, TH01, vWA, FES/FPS, F13A1, Amelogenin and several prototype STR systems) were determined using different internal molecular weight standards (i.e., ABD GS 2500, ABD GS 500 and BRL prototype 50 base ladder). Allele sizes were calculated by the local Southern method and reproducibility assessed from replicate sample runs. Precision was plotted across the seven loci for each representative allele between individual lanes within the same gel (intragel) and compared to independent sample runs between gels (intergel). Intragel precision was determined to be ± 0.5 bases regardless of the STR locus and intergel precision ± 0.75 bases (taking into account a worst case scenario). Differences in precision of allele size estimation were noted using different internal lane standards. This is presumably due to characteristics of the marker (i.e., a dual label on both strands versus single strand tagging; the potential for split peaks after denaturing electrophoresis; strategic spacing of rungs in the molecular weight ladder standards). Acceptable standards of measurement precision for declaration of a match are discussed in context with internal lane standards.

Forensic Validation Of The STRs D8S320 And AR: Construction Of Allelic Ladders And Population Genetics

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Amplified fragments of STRs and AmpFlps are usually analysed after electrophoretic separation and staining with silver. Exact allele determination requires comparison with allelic ladders. We construct such ladders for the tetrameric STR D8S320 and the trimeric X-linked polymorphism on the Androgen receptor gene (AR). The Calibration of the ladders was performed by comparing the results of conventional separation on PAGE and subsequent silver staining with those of the ABI Sequencer in connection with the GeneScan Software 672. The optimization of different PCR parameters for amplifying the ladders with regard to the different sensitivity of both detection systems is demonstrated.

In addition population studies of both systems are presented. Agreement with Hardy-Weinberg equilibrium and forensic efficiency data are shown.

ESTABLISHING OF A HIGHLY DISCRIMINATING PENTAPLEX PCR SYSTEM FOR DETECTION OF PCR-FRAGMENTS IN SILVERSTAINED POLYACRYLAMIDE GELS

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Multiplex-PCR of STR-Systems can provide a highly discriminating single amplification DNA-profiling system suitable for use on small samples. However procedures are developed which are predominantly based on fluorescent detection. With this direct detection method different dyes are employed for each locus, so that it is not necessary to select primers that do not have the same fragment size and do not overlap after PAGE.

The intention of this work is to establish an amplification system of the five different STR-loci HUMTH01, HUMVWFA31, D18S51, D21S11 and HUMFIBRA. Each STR-system produces PCR-fragments which have a different size range for each locus and are detectable with silverstaining after polyacrylamide gel electrophoresis.

The influence of different primersets, different primer concentrations as well as different annealing temperatures is investigated. Dilution series of template DNA indicate the sensitivity of this system and therefore the possibility to use this system for routine work.

APPLICATION OF AUTOMATED FLUORESCENCE DETECTION OF A PCR STR QUADRUPLIX ANALYSIS SYSTEM TO FORENSIC CASEWORK

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In December 1994 we implemented, with minor modifications, Gill's multiplex amplification of HUMvWA, HUMTH01, HUMF13A01 and HUMFES/FPS followed by fluorescence detection in the ABD 373A sequencer with Genescan software. DNA has been successfully profiled from minute samples including bloodstains, cigarette butts, stamps, chewing gum, hair roots, semen mixtures, vomit, nasal secretion, compact bone and dental pulp.

Although some samples may fail to amplify at F13A1 and FES, in general the system is extremely robust and mixed profiles can often be easily resolved. We have now reported 35 cases. The evidence has been accepted in courts at all levels in Ontario.

EXTRACTION: organic with microcon filtration to 15 uL and slot blot quantitation with D17Z1. Adjacent unstained background control is routinely tested.

AMPLIFICATION: 1 ng target DNA in 15 uL total volume, [primer] balanced per lot, BSA, P.E. 9600, hot start.

TEST GEL: in the sequencer to verify amplification, balance case samples for analysis and assess unstained controls. 6 cm gel, 24 wells, no allelic ladders or size standards, 30 W, 50 mins. Calculate sample volume necessary to give heterozygotic peak heights of about 1000.

ANALYTICAL GEL: 6% denaturing acrylamide (premixed gel and TBE buffer-BRL), sample in formamide, boil and snap cool, 12 cm gel, Genescan 2500, allelic ladders(Gill), 24 well square-tooth, 30 W for 1hr 50 min, 35 min scan delay, collect scans 100 to 750.

INTERPRETATION: minimum reportable peak height 50 units; p^2 for homozygotes; in mixtures, no conclusion if can't distinguish allele from stutter; databases for Caucasians, Blacks, Orientals; no evidence of association between alleles or loci (Carmody); default frequency 1% or 99% confidence intervals. Interpretation by Genotyper from windows based on allelic size reproducibility from 13 gels. Windows are +/- 4 s.d. of mean. Smallest window is 1.03 bp, largest 3.16 bp. Only TH01 9.3 and 10 alleles are binned. Frequency of occurrence of the most common genotype is 1 in 1,000.

MULTIPLEX AMPLIFICATIONS AND AUTOMATED FLUORESCENT
TYPING OF SHORT TANDEM REPEAT (STR) LOCI :
THE FRENCH EXPERIENCE

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The great majority of the biological samples available in forensic investigations for DNA identification purposes are severely degraded or present in minute amounts and can not be analysed by conventional RFLP (Restriction Fragment Length Polymorphism) methods using single locus probing. The Polymerase Chain Reaction (PCR) amplification of Short Tandem Repeat (STR) loci appears to be an efficient, sensitive and rapid DNA identification system for such highly degraded forensic samples.

In a first step, we established the french caucasian data bases, for the 4 following STR loci : HUMTH01, HUMFESFPS, HUMVWA31A and HUMF13A1. The HUMTH01 and HUMFESFPS genotype frequencies were obtained through singleplex amplification and manual silver staining detection whereas the HUMVWA31A and HUMF13A1 genotype frequencies were determined by duplex amplification and automated fluorescent detection on an ABI 373 sequencer. The distribution of the genotype frequencies, for the 4 STR loci, satisfied the Hardy-Weinberg (H-W) equilibrium and our french population sample showed homogeneity with other already published caucasian populations.

In a second step, the quadruplex amplification of the 4 STR loci, using FAM or JOE fluorescent dyes for HUMTH01 and HUMFESFPS or HUMVWA31A and HUMF13A1 respectively, was developed and performed on biological samples of various origins : blood, tissue samples, blood and sperm stains, teeth, hairs, and cigarette butts. The quadruplex fluorescent detection of these 4 STR loci has been applied to forensic cases for one year : the multiplexing of STR loci proved to be a very interesting system and we only met minor technical problems concerning more specifically variations in the quality of some commercialized fluorescently labelled primers.

The multiplex amplification conditions of 4 other STR loci, HUMFIBRA, D21S11, D18S51 and D6S502, and of the X-Y homologous amelogenin gene for sex determination have also been determined, using the 6-FAM, TET and HEX fluorescent dyes. The french caucasian genotype frequencies of these loci agreed with those previously published.

KEYWORDS:

STR-Multiplex PCR.-Fluorescence-Population data.

A TETRAPLEX PCR SYSTEM FOR THE ANALYSIS OF
PATERNITY CASES.

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Short tandem repeat loci (STR) with repeat unit length between 2-6 bp represent highly polymorphic markers in the human genome, that are ideal markers for genomic mapping and genetic linkage analysis and can be used for forensic and paternity applications. In contrast to the highly polymorphic VNTR loci, STR loci exhibit a limited polymorphism resulting in a reduced discrimination power.

We have established a tetraplex PCR-STR system to combine the discrimination power of four STR Loci, HUMTH01 (locus: 11p15-15, repeat: AATG), CYP19 (locus: 15q21.1, repeat: TTTA), D8S639 (locus: 8, repeat: AGAT) and ACPP (locus: 3q21-qter, repeat: AAAT). Allele frequencies were collected from a minimum of 100 unrelated individuals for each STR Loci. Analysis was performed by multiplex PCR amplification with fluorescence labelled primers. Subsequently, allele assignment was conducted by fragment separation on polyacrylamide gels using an ABI 373A Sequencer (Genescan Software , Perkin Elmer - ABI). The following results were obtained:

STR loci	Alleles	HR	PIC	DI	pM
HUMTH01	6	0,746	0,727	0,91	0,09
CYP19	7	0,727	0,719	0,88	0,12
D8S639	14	0,857	0,819	0,95	0,05
ACPP	6	0,630	0,591	0,81	0,19

The combined discrimination index (DI) of all four STR loci is 0,99 and the probability of matching (pM) is $1,0 \times 10^{-4}$. This tetraplex STR-PCR systems can be used to increase the discrimination power of STR analysis for individual identification.

DEVELOPMENT AND APPLICATIONS OF HIGH THROUGHPUT MULTIPLEX STR SYSTEMS.

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We describe the development of a rapid, high throughput, nonisotopic, and inexpensive method for DNA analyses based upon the amplification of polymorphic short tandem repeat (STR) loci. We have achieved high throughput analysis with STR loci for forensic analysis and paternity determination using manual separation and silver stain detection to avoid the need for specialized or expensive equipment. Our approach includes the development of nine STR systems in combination with allelic ladders (i.e. size standards containing many or all of the existing alleles) for rapid typing of each locus. Six of the STR loci have been incorporated into two multiplexes each containing three loci. The first triplex includes the loci CSFIPO, TPOX, TH01, while the second contains F13A01, FESFPS, and VWF. This approach minimizes the amount of material required while increasing the efficiency of analysis.

We have collaborated with other laboratories to determine the frequency of alleles for each of the nine STR loci in three or more population groups each containing at least 200 individuals. In addition, the first triplex was validated for use in a study involving five laboratories. This system was tested for amplification quality with 0.1-500 ng of template, for sensitivity to variation of the annealing temperature, and for the ability to detect and interpret DNA mixtures. Each of the five contributing laboratories determined the triplex loci alleles for twenty DNA samples which had been prepared using four different DNA purification procedures. Results with monoplex systems were compared with the multiplex system. Use of the Perkin-Elmer model 480 and model 9600 thermal cyclers were also compared.

Finally, each of the nine systems were adapted for use with fluorescent detection with instrumentation which allows automation of the allele identification procedure (i.e. the Applied Biosystems DNA Sequencer and the Molecular Dynamics FluorImager). Two multiplex systems containing four loci each (i.e. (1) CSFIPO, TPOX, TH01, vWF, and (2) F13A01, FESFPS, F13B, LPL) were also developed for this purpose. It is possible to include amplification of the amelogenin locus for sex identification along with the first of the quadriplexes to generate a pentaplex. The development of these systems for multiple detection formats makes them especially appropriate for comparison of databases or individual samples among laboratories.

PROPERTIES OF AN STR MULTIPLEX MARKER SYSTEM SUITABLE FOR PATERNITY AND FORENSIC DETERMINATIONS.

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We have developed a combination of primers that can be used for the simultaneous PCR amplification of 3 polymorphic loci. The localization of these markers is on chromosomes 18, 3 and 12. The alleles for these loci are composed of variable number of tetranucleotide GATA repeats. The amplification was performed using 5 to 10 ng of DNA. Fractionation was performed in 4% acrylamide/8 M urea gels at 2000 volts for 1 hr and visualized by silver staining. The general properties of these loci are summarized below:

Chromosomal localization	18q12-21	3q23-24	12q11-12
Size range of alleles (bases)	94-134	154-182	212-306
# of tetra. nucleot. repeats	9-19	14-22	9-33
# of alleles	9	9	24
Heterozygosity (N.Amer.Cauc.)	75	83	95

An allelic ladder was created by selecting different size alleles from each locus. The frequency of the alleles of these loci was determined by the analysis of approximately 200 random individuals from North American Black and Caucasoid populations. For each locus, the frequency of the observed genotypes was in agreement with Hardy-Weinberg expectations. The use of these 3 genetic markers for paternity cases provides a combined power of exclusion of about 97% and an average power of identity of about 1 in 3100.

ANALYSIS OF D1S80 VNTR ALLELE POLYMORPHISM AND ASSOCIATION WITH A NEARBY FLANKING SEQUENCE POLYMORPHISM IN TWO SPANISH POPULATIONS
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We have recently described a sequence polymorphism in the 5' flanking region of the VNTR locus D1S80 characterized by the presence (*Hinf I*+) or the absence (*Hinf I*-) of a *Hinf I* restriction site.

In this study, we have determined the RFLP/VNTR D1S80 haplotype frequencies in two different spanish population samples: a cosmopolitan population sample of 200 unrelated individuals living in Madrid and a population sample of 200 autochthonous individuals from the Basque Country. The haplotype frequencies determined in both population samples show an extreme association between the *Hinf I* + allele and the VNTR allele of 18 repeats and between the *Hinf I* - allele and VNTR allele of 24 repeats, while the remaining VNTR alleles associate more randomly with the two flanking *Hinf I* alleles.

The linkage disequilibrium observed between the flanking polymorphism and the two high frequency modal VNTR alleles (18 and 24) that is shared by the two different populations analyzed, together with the fact that mutation at minisatellite occurs without exchange of flanking regions, suggest that the 18 and 24 alleles could be the original VNTR alleles at this locus and the rest of VNTR alleles associated with the *Hinf I* + mutation would arise from the 18 allele, while the VNTR allele associated with the *Hinf I* - mutation would arise from the allele of 24 repeats.

SOMATIC INSTABILITY IN CANCER AT SEVEN TETRAMERIC STR LOCI USED IN FORENSIC GENETICS.

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In an attempt to evaluate the impact that the microsatellite instability observed in human cancers could have in some forensic DNA studies (i.e. genetic analysis of fixed tumor specimens that were thought to have been mis-paired, paternity testing from fixed tumor biopsies in cases involving deceased parents,...) we have analyzed seven polymorphic STR loci (TH01, TPOX, CSF1PO, VWA, FES/FPS, F13A1 and F13B) in DNAs extracted from paired normal and tumor tissue samples corresponding to 21 individuals (13 colorectal adenocarcinomas and 8 gastric adenocarcinomas).

Instability at one or more loci was observed in 75% (6/8) of gastric tumors and 15% (2/13) of colorectal tumors. Instabilities were apparent for the majority of gastric tumors as partial allelic losses as well as extra-alleles of different size in the tumor that were not present in the normal DNA. The only instability observed in 2 out of 13 colorectal tumors analyzed was a partial allelic loss at CSF1PO locus (chromosome 5p). The loci that presented higher numbers of instabilities were CSF1PO (7 out of 21 cases) and VWA (5 out of 21 cases).

In conclusion, although the incidence of instability in tetrameric STR loci depends on the type of cancer and other factors, this kind of genetic alterations should be taken into account as a potential source of error when interpreting STR profiles obtained from neoplastic tissues in identity testing studies.

THE APPROACH OF USING RANDOM PRIMING FOR SMALL FORENSIC DNA-SAMPLES

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The sensitivity of the polymerase chain reaction (PCR) allows DNA-typing in single cells or small forensic DNA samples. In all these cases the single cell or small DNA sample can only be analyzed once and only for one locus. So independent confirmation and the investigation of several VNTR's of the genotype are not possible.

To circumvent this problem we used a method called *primer-extension-preamplification* (PEP; Zhang et al. 1992). In our study we have proved the applicability of this method to forensic samples. Using a random-primer mixture of 15-base oligonucleotides and 50 extension-cycles prior to the VNTR-typing we have been able to type quantities of DNA as low as 10 pg for VWA and 100 pg for Apo B.

Starting with 100 pg DNA for the PEP we have been able to receive reliable typing-results of several VNTR-loci. It has been noted that inputs of lower amounts of DNA can lead to allelic drop out, not only for the larger but also of the smaller allele.

USE OF A PCR TRIPLEX SYSTEM FOR DNA TYPING OF FORENSIC SAMPLES

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The use of DNA identification methods in forensic science dates from the application of RFLP analysis in the mid 1980's. The analysis of 4 - 8 independent VNTR loci produces a DNA print which provides strong evidence of the origin of a forensic biological sample. But limitations in the amount and quality of DNA recovered from forensic samples can compromise the RFLP approach.

An alternative approach to DNA testing utilizes PCR analysis which increases the chances of obtaining results from degraded samples, requires less DNA, and can be completed in a shorter time period than RFLP analysis. Since the early 1990's, PCR has been used to type DNA from forensic samples, the most commonly used locus is HLA DQalpha where base sequence changes in exon 2 can be identified colorimetrically using a reverse dot blot methodology. More recently, five (5) additional loci known commercially as polymarker (PM) have been applied to forensic PCR analysis of DNA.

In this report, PCR methodology has been applied to the analysis of forensic samples using a multiplex system of three (3) independent STR loci, each locus composed of fragments which differ by four (4) nucleotide repeats. Primers and PCR conditions are optimized to allow coamplification of the three loci simultaneously. The amplification mix contains 1-10 ng of DNA; 10 mM Tris HCl, pH 8.3; 50 mM KCl; 0.5 mM MgCl₂; 200 μM dNTPs; 0.5 μM each primer; and 2.5 units of Taq polymerase. The amplification was accomplished in 30 cycles at: 1' 95° C, 1' 65° C, and 1' 72° C. Alleles are detected by silver staining after fractionation in a 7.0% PAGE denaturing gel. Size determinations are made using allelic ladders.

EVALUATION OF NEW STR LOCI FOR FORENSIC DNA TYPING

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STR DNA typing has become the method of choice for the identification of the source of human biological material. The main reasons are its sensitivity and its ability to analyse degraded material. The main drawback of most of the STR loci presently in use in forensic science is their very limited polymorphism which contrasts with the extraordinary polymorphism of the VNTR loci used in RFLP typing. A few STRs are very polymorphic, but they are also rich in microheterogeneities (for example locus SE33/HUMACTBP2) which makes the identification of alleles difficult and the comparison of results between laboratories uncertain.

Recent advances in genetics have allowed the discovery of hundreds or even thousands of STR loci each year. All these new loci are stored in large databases which are then a potential rich source of STR loci. The Human Genome Data Base (GDB), at Johns Hopkins University in Baltimore, has been searched. Using the limited information available on the stored loci, a serie of STRs has been selected and tested for their degree of polymorphism. For the best of them, further evaluation has been made of their sensitivity, and the presence of microheterogeneity or irregular alleles. Among the tested loci some have displayed heterogeneities higher than 0.80, but none has yet brought the dramatic improvements expected.

AUTOMATED PROFILING OF MULTIPLEXED DNA MARKERS. AN ITALIAN DATABASE OF FOUR CO-AMPLIFIED STRs LOCI.

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A protocol for simultaneous amplification of four STRs loci (HUMvW31/A, HUMTH01, HUMF13A1 and HUMFES/FPS) detected by a fluorescence based technology is in use in our laboratory.

PCR products are obtained by fluorescent labelled primers. HUMTH01 and HUMFES/FPS products result 6-FAM labelled while HUMvWA31/A and HUMF13A1 are HEX labelled. Co-amplified products combined with an internal lane standard (GS2500) are detected by laser scanning during a 6 hours electrophoresis on a 6% denaturing acrylamide gel at constant power (42 W) on a 373 ABI Sequencer, analysed by 672 Genescan software and Local Southern method. Windows for the analysed loci designations were determined by several analysis of allelic ladders and genomic samples.

A total of 120 unrelated individuals has been typed for these co-amplified polymorphisms; resulting databases were compared to those obtained by manual silver stained methods and, as expected, no significative differences were found.

X
MICROBIAL DNA CHALLENGE STUDIES OF PCR-BASED SYSTEMS USED IN FORENSIC GENETICS

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Forensic biological samples presented for DNA analysis have often been subjected to contamination by microorganisms and, as a result of that, DNA isolated from such sources can potentially be a mixture of human and microbial DNA. The purpose of this study is to evaluate the influence of this source of foreign DNA on human PCR-based DNA typing by testing the specificity of 10 PCR-based systems widely used in forensic genetics (HLA-DQA1, LDLR, GYPA, HBGG, D7S8, GC, D1S80, HUMTH01, HUMTPOX and HUMCSF1PO) against microbial DNA templates obtained from 30 different microorganisms (10 Gram-negative bacterial strains, 16 Gram-positive bacterial strains and 4 yeasts) isolated from forensic casework or reference strains.

Although some inespecific amplification products were observed in the post-amplification yield gel when some bacterial DNA templates were used for the amplification of the HLA-DQA1 and PM systems, in no case false-positive results were found by the reverse dot-blot hybridization system used for the typing. No PCR products were observed with the HUMTH01/ HUMTPOX/ HUMCSF1PO multiplex STR system for none of the microbial DNA templates tested. On the other hand, D1S80 amplifications from six of the bacterial DNAs analysed showed some inespecific amplification products of different sizes that were located within the range of length variability of the human D1S80 alleles, as analyzed by native polyacrylamide gel electrophoresis and silver stain. In conclusion, our results validated the specificity of the majority of PCR-based systems analysed against microbial DNA templates, but also suggested that microbial DNA could be a potential source of extrabands when D1S80 typing is carried out from forensic biological samples subjected to contamination by microorganisms.

SOME CRITICAL COMMENTS AND EXPERIMENTAL CALCULATIONS USED AS VALIDATION STANDARDS IN THE IMPLEMENTATION OF RFLP ANALYSIS.

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In order to assess the validity of RFLP analysis and to overcome some interpretation difficulties in parentage testing, a critical review of our data was undertaken.

Samples from parentage determination and population studies had been digested using the Hinf I restriction enzyme and single locus analysed using probes corresponding to five loci: D2S44, D12S11, D7S21, D7S22, and D5S43.

Hybridization of membranes was accomplished using chemoluminescent labelled probes. For each probe allele sizing error, sigma, was calculated from the determination and comparison of multiple runs of the same DNA. K562 DNA, included on every gel was chosen for this purpose. We have estimated the ability of our gel system to resolve close bands (defined as delta, power of resolution) through a critical analysis of samples in which closely spaced bands occur in the same lane (heterozygous individuals or DNA mixtures from alleged father and child). Percent homozygosity and heterozygosity for each probe were calculated and a comparison of their average and "typical" Power of exclusion according to Brenner and Morris equations ($A = h^2 (1 - H^2)$ and $1/2 H$), was carried out.

Finally we present a conflicting paternity case with band sizing similarities between non-related mother and alleged father, in which matching decisions conducted either to father or to mother exclusion.

ANALYSIS OF SOMATIC MUTATIONS AT SHORT TANDEM REPEAT LOCI

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To further analyse the variability at tandem repetitive loci, we have studied mutations in a subset of such loci in human colorectal adenocarcinomas.

The existence of new tumour alleles in colorectal and other tumours has been extensively described. Most reports focus on dinucleotides and the mere registration of new tumour alleles. The somatic instability recorded is called replication error (RER), reflecting the mutation mechanism. We here report data on the nature and distribution of new tumour alleles at the four tetranucleotide short tandem repeats (STRs) HUMTHO1, HUMFES/FPS, HUMVWA31/A and HUMF13A1.

DNA was extracted from blood and carcinomas from 217 unselected patients. PCR amplification was performed with fluorescent dye labelled primers. Subsequent electrophoresis and analysis were carried out in an automated ABI 373A sequencer with Genescan 672 software.

Mutations are detected with all four markers. The somatic mutation frequencies range from 0.009 (HUMF13A1) to 0.122 (HUMVWA31/A). In the four STRs as a group, there is a significant excess of tumours displaying allele expansion at mutation (43 vs. 24 tumours showing allele contraction). The mutant alleles are consistently composed of perfect integers of the four base repeats. Three quarters of the tumours with mutant bands display only a single new band which differ with 1, 2 or 3 repeats from the assigned constitutional band. The remaining samples show either biallelic events or a "smear" of mutant bands.

Others have shown that colorectal adenomas always display a single mutant band at mutation, while carcinomas tend to show "smears". Our observations also indicate that there are two such different types of somatic mutations. We will perform further analyses to clarify any clinical significance of this observation.

SIMPLE AND RAPID DUPLEX PCR FOR FORENSIC AND PATERNITY TESTING

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The amplification of polymorphic short tandem repeat (STR) loci by PCR forms the basis of a sensitive and powerful technique for identification.

The aim of this study was to utilize the duplex PCR to improve the methodology for forensic medicine. Here we describe a duplex PCR of two STR loci: HumvWA (1,2) and HumFES/FPS (3) residing in chromosomes 12 and 15, respectively.

The sizes of the amplified fragments in the HumvWA and HumFES/FPS loci range between 134-170 and 211-231 base pairs, respectively. Because of the non-overlapping allele size ranges it is possible to amplify the loci simultaneously using non-labeled primers and perform the size separations and detection of the alleles using polyacrylamide gel electrophoresis (PAGE) (4) and silver staining without expensive automation.

In 100 mother-child-putative father combinations from paternity testing material the HumvWA and HumFES/FPS loci were successfully amplified with both duplex and single PCR. In this material no difference was found between the results of the single and the duplex PCR. Also no mother-child exclusion was found.

In Finnish population the combined exclusion propability (5) in paternity testing of HumvWA (64.6%) and HumFES/FPS (41.3%) loci is 79.2%. The duplex PCR method has also been successfully used to analyse semen and blood stains as well as postmortem tissue samples.

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**GENETIC STUDIES OF A STR AT THE UGB LOCUS.
PRELIMINARY RESULTS.**

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A new STR corresponding to a tetranucleotide repetitive motif, (ATTT)_n, in the first exon of the human uteroglobin (UGB) locus was analysed. After PCR amplification for 30 cycles, a semi-dry discontinuous polyacrylamide gel electrophoresis for molecular separation was accomplished, followed by Silver-Staining for DNA detection.

An initial survey in the population of Galicia demonstrates the existence of 6 alleles in polymorphic frequencies (allele sizes ranging between 387-407 bp) whose configure unbiased values of $h=0.80$ in this population. Formal genetic studies from family groups show a mendelian codominant way of inheritance. The potential usefulness of this system in individual characterization and population profiling is also discussed.

TUMOR INOCULATION BETWEEN TWO UNRELATED HUMAN INDIVIDUALS: STR ANALYSIS OF PARAFFIN-EMBEDDED TISSUE SECTION

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In this study we used the short tandem repeat (STR) technology to check the possibility of tumor inoculation between two unrelated individuals.

Case report: A surgeon injured his hand with a sharp instrument during a resection of an intestinal soft tissue tumor (tumor I). After one year a tumor (tumor II) was detected in his hurt hand.

Histological and morphological analysis revealed these two tumors (I and II) as identical.

Only paraffin-embedded tissue of these tumors was available to perform DNA-profiling. We succeed in DNA extracting from these tissues, but the material was unsuitable for molecular techniques which require high-molecular-weight genomic DNA. Although significant DNA degradation was observed, three STR loci could be detected: CYP (15q21.1), TH01 (11p15.5), SE 33 (ACTBP2).

The comparison of the surgeons blood sample with the tissue sections of tumor I and II gave exceptional findings: tumor II showed at each STR locus all alleles from tumor I. These results point at the chance of tumor inoculation between humans, first described in 1995 by Gärtner et al. (in press).

Thus we demonstrate the bridge between molecular genetics and classical histology meaning that the world-wide collection of archival paraffin-embedded tissues may be used to study rare cancers and other singular diseases.

MOLECULAR PHENOTYPING OF TWO TRINUCLEOTIDE REPEATS (D5S373 AND XT00444). EXPERIMENTAL CONDITIONS.

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PCR amplification of STRs constitutes today one of the most informative tools in Forensic Biology and Human Genetic studies. Within these STRs, special attention is currently being given to tetranucleotide repeats, the dinucleotide repeats being more problematic for an accurate phenotype diagnosis for technical reasons (mainly due to slippage effect).

In this paper two trinucleotide repeats are analysed. The D5S373 locus is a (TAA)₁₀ trinucleotide repeat sequence assigned to band 5q32. The XT00444 (D13S635E) sequence was identified as a (ATC)₁₅ trinucleotide repeat and is located at chromosome 13. In order to assess their usefulness in human genetic profiling, diverse technical conditions are applied both for amplification parameters and DNA amplified molecular separation and detection. As a result of this trials we conclude that suitable phenotyping can be accomplished under the following conditions. For D5S373, DNA samples were amplified for 35 cycles according this program: 92°C/30 sec, 60°C/30 sec, 72°C/30 sec and an additional extension at 72°C for 10 min. For XT00444 the amplification was initiated with a pre-denaturation step for 6 min at 94°C, 15 sec; 62°C, 23 sec; 72°C, 30 sec and a final extension at 72°C for 5 min.

The separation and detection of amplified fragments for both systems was carried out by means of discontinuous electrophoresis in polyacrylamide gels and subsequent silver staining. We have elaborated ladders with the alleles present in our population sample to facilitate the phenotype identification. In this way, up to 5 alleles in both systems, ranging between 88-106bp (PIC=0.69) for D5S373 and 132-144bp (PIC=0.67) for XT00444 were found. In our experience, phenotyping of these trinucleotide repeats is perfectly feasible, given that no slippage effects were ever found and amplification and separation results are similar to those obtained with tetranucleotide repeats.

DEVELOPMENT AND OPTIMISATION OF A HIGHLY DISCRIMINATING MULTIPLEX PCR SYSTEM SUITABLE FOR FORENSIC IDENTIFICATION
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Using fluorescence-based technology we have successfully developed a multiplex PCR system which combines the 6 tetranucleotide Short Tandem Repeat (STR) loci HUMVWFA31/A, HUMTH01, D6S502, HUMFIBRA, D21S11 and D18S51 with the X/Y homologous gene amelogenin enabling simultaneous individual and sex determination. We present a system which provides the ability to co-amplify loci with overlapping size ranges through the use of single lane, multiple dye chemistry and to consistently and reliably distinguish between alleles differing in size by 2 bp. The use of this system has been validated for use on the UK's National DNA Database and in forensic casework and gives a matching probability (P_m) of between 1.3×10^{-8} and 1.7×10^{-8} in three British populations. Profiles obtained are human-specific, consistent across a range of somatic samples and display similar signals within and between loci when amplified on 1-5 ng of template DNA. Minor components of mixtures can be detected in a 10:1 mixture with mock cases and cases of known outcome producing expected results.

ANALYSIS OF 12 SHORT TANDEM REPEATS IN BIOLOGICAL EVIDENCES

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Short tandem repeats (STRs) are highly informative polymorphic loci, occurring throughout the genome at very high frequency, so that they are attractive forensic genetic markers. We developed a panel of 12 STRs for personal identification applications, including di-, tri-, and tetranucleotide tandemly reiterated sequences. TNFa, D11S904, APC-LNSCA, D17S261, D2S123, D3S1359, D6S105 dinucleotide STRs, DM and DRPLA trinucleotide STRs are analyzed in our protocol by using radiolabelled PCR primers and denaturing polyacrylamide gel electrophoresis (PAGE). HUMTH01, HUMFES/FPS, HUMVWA tetranucleotide repeats are typed by non-denaturing PAGE and silver staining, after PCR amplification. This protocol enabled us to obtain an informative genetic profile in several biological specimens. The high sensitivity and the small size of amplified fragments make this analysis suitable also for typing forensic samples where minute amount of DNA, or degraded DNA is recovered.

MANUAL DNA TYPING VIA THE SHORT TANDEM REPEATS (STRs): KW 426, TH AND hTPO

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STRs represent a rich class of highly polymorphic markers in the human genome. They are usually composed of tandemly repeated sequences of 2 to 5 bp in length. Alleles range from 100 to 350 bp. STRs have gained increasing popularity for genetic mapping and linkage analysis, trimeric and tetrameric loci are especially suited for identity testing.

All the benefits of a STR monoplex system can be enhanced using a multiplex polymerase chain reaction (PCR).

We present a new combination of three unlinked tetrameric STR - loci (hTPO: 2p23 - 2pter, TH: 11p15.5, KW 426: 8q) performing the multiplex PCR technology. All fragments of these STR loci are separated in one lane of a polyacrylamidegele electrophoresis (PAGE) - system.

Thus we can do STR typing increasing throughput and discrimination power by use of manual techniques which can easily be carried out by most laboratories.

A MULTIPLEX AMPLIFICATION APPROACH FOR SIMULTANEOUS TYPING OF FIVE LOCI IN DNA OF ANCIENT BASQUE POPULATIONS

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The multiplex amplification of several genetic loci can be analyzed with the AmpliType PM PCR Amplification and Typing Kit (Perkin Elmer, USA) - also known as "Polymarker" or "PM" -, using a reverse dot blot approach and immobilized allele-specific oligonucleotide (ASO) probes. The following six loci were amplified in a sample of ancient basque populations: low density lipoprotein receptor (LDLR), glycoprotein A (GYPA), hemoglobin G gamma globulin (HBGG), D7S8, group-specific component (Gc) and HLA-DQA, the last one not being included in the typing strips. DNA was extracted from 18 teeth of three old basque populations: Urbiola (3700 BP), Atxuri (5000-3600 BP) and Garai (Middle ages). We hypothesized that optimum amplification protocols for very ancient DNA would be achieved using very little amount of template DNA. According to the manufacturer's protocol, DNA amounts between 2 and 40 ng can be successfully amplified with PM. This can also reduce polymerase inhibitors and perhaps jumping PCR effect too.

Double PCR of the samples was carried out in a sterile environment before typing. The resulting bands of the reaction products were checked in agarose electrophoresis together with a positive and a negative sample control. At least three out of the five considered genetic systems yielded a successful amplification. However, although some results changed its typing as repetition of the analysis was performed - probably due to the jumping PCR effect -, others showed a invariant pattern. In conclusion, AmpliType PM PCR Amplification and Typing kit can be a useful tool for typing sequence polymorphism of nuclear ancient DNA.

TRIPLEX PCR OF THREE STR LOCI WITH NON-OVERLAPPING ALLELE SIZES

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The PCR-based technique is a fast and sensitive tool in analyzing the very polymorphic VNTR/STR regions in human genome and enables the unambiguous sizing of the different alleles which is the prerequisite for all statistical computations.

One of the advantages in the PCR methodology is the possibility to amplify simultaneously several loci (multiplex PCR) which improves remarkably the information obtained from one analysis. Here we describe a triplex PCR of three STR loci: hTPO (1) in chromosome 2, HumTH01 (2) in chromosome 11 and D3S1359 (3) in chromosome 3.

The allele size ranges in hTPO, HumTH01 and D3S1359 loci are 106-130 base pairs (bp), 155-179 bp and 197-269 bp, respectively. Because of the non-overlapping allele size ranges it is possible to amplify the loci simultaneously using unlabeled primers and perform the size separation and allele detection using electrophoresis and silver staining (4) without expensive automation.

Utilizing the triplex PCR method the labour and costs needed are decreased. In Finnish population the mean exclusion probabilities (5) of hTPO, HumTH01 and D3S1359 loci in paternity testing are 33.4%, 58.5%, 76.6% respectively. The combined exclusion probability of these three loci is 93.5%.

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VISUALIZATION OF EPIGENETIC TOXICOLOGICAL DNA CHANGES

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This is an initial report on forensic DNA identification of victims of poisoning. Epigenetic DNA changes caused by poisons and their effect on well-known DNA analysis methods were examined. We tested for toxicological DNA changes in southern hybridization with multilocus probes with single locus probes, AmpFLP in a minisatellite locus and in a microsatellite locus and southern dot blotting after polymerase chain reaction (PCR) on HLADQa. As an example of acute poisoning, a case of human death by a fatal dose of CO from a fire was selected. The liver, spleen, kidneys, thymus, lungs, cerebellum, basal nucleus and pallidum were taken from the corpse and DNA was tested by both types of southern hybridization. As an example of chronic poisoning, tests were carried out for DNA changes in rabbits before and after methamphetamine intoxication (10mg/kg, every two days for two weeks, intravenous injection). With acute poisoning by CO, some bands in the pallidum caused by DNA fingerprinting using multilocus probe 33.15 became weaker and disappeared. With chronic poisoning by methamphetamine, some preintoxication bands caused by DNA fingerprinting using multilocus probe 33.15 and by AmpFLP on the D1S80 locus and on the TH01 locus became weaker and/or disappeared after intoxication. By PCR-dot hybridization testing for HLADQa, some preintoxication positive spots became negative after intoxication and preintoxication negative spots became positive after intoxication. In this experiment, clear epigenetic DNA changes by intoxication were confirmed.

THE 3' HYPERVARIABLE VNTR-LOCUS APO B :
THREE DIFFERENT ANALYSING METHODS REVEALING DIFFERENT
ALLELES AND LARGE FAMILY STUDIES.

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Genotyping hypervariable regions (HVR) is a widely used method for gene mapping as well as for forensic applications. One of these well-known tandemly repeated sequences is the apolipoprotein 3' hypervariable region on human chromosome 2p.

In this study we describe three electrophoretic methods detecting different alleles amplified at the APO B locus using identical PCR - parameters:

1. Agarosegel electrophoresis ; Ethidiumbromid staining.
2. Native polyacrylamidgel electrophoresis (PAGE); silver staining.
3. Denaturing polyacrylamidgel electrophoresis (PAGE); silver staining.

Differences between native and denaturing PAGE aren't observed until now. On the contrary the PAGE systems show several alleles which cannot be identified in the agarosegel system. This is especially important if the methods are used for forensic or other identification purposes.

Furthermore we present allele-frequencies and mutation rates from large family studies in NW-Portugal.

AUTOMATED FLUORESCENT PCR BASED ANALYSIS OF THE STR POLYMORPHISM AT LOCUS D8S639 AND AT THE CYP19 GENE.

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Short tandem repeat (STR) loci are polymorphic markers that can be used for discrimination between individuals in paternity and forensic testing. We have studied the allele distribution and sequence structure of the tetranucleotide repeat polymorphism at locus D8S639 and at the CYP19 gene, in a population sample of 80 German caucasians and 60 families. Solid phase DNA sequence analysis of genomic DNA samples was conducted on an ABD 373A DNA Sequencer. Allele and genotype frequencies were determined by PCR amplification using 6-FAM (6-carboxyfluorescein) labelled primers for locus D8S639 and HEX (hexa-chloro-6-carboxyfluorescein) labelled primers for locus CYP19. Amplified fragments were separated by electrophoresis on an 6% polyacrylamid gel including an internal size standard in each lane. Allele size determination was performed by measuring the laser-induced fluorescence emission of the DNA fragments (ABD 373A DNA Sequencer, Genescan-Software). The following results were obtained.

CYP19 HR = 0,727 PIC 0,719 ; DI 0,88		D8S639 HR = 0,857 PIC 0,819; DI 0,95			
RU (bp)	F	RU (bp)	F	RU (bp)	F
Allel 7-3*(168)	0,341	Allel 22 (317)	0,009	Allel 29 (345)	0,101
Allel 7 (171)	0,152	Allel 23 (321)	0,009	Allel 30 (348)	0,06
Allel 8 (175)	0,087	Allel 24 (325)	0,028	Allel 31 (352)	0,032
Allel 9 (179)	0,004	Allel 25 (329)	0,124	Allel 32 (356)	0,009
Allel 10 (183)	0,023	Allel 26 (333)	0,147	Allel 33 (360)	0,014
Allel 11 (187)	0,356	Allel 27 (337)	0,266	Allel 34 (364)	0,009
Allel 12 (191)	0,038	Allel 28 (341)	0,193	Allel 36 (373)	0,009

* Allele 7-3 has a 3bp deletion in the 5'flanking region
Hardy Weinberg Equilibrium was observed for both STR loci. The combined discrimination index (DI) was 0,99. These STR loci can be used for forensic or paternity case work.

MUTATIONS OF D2S44 AND D4S139 ALLELES AND PRESENCE OF TWO-FRAGMENT ALLELES FOR D4S139.

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We used probes YNH24 (D2S44), pH30 (D4S139), MS43A (D12S11) and g3 (D7S22) to study the transmission of VNTR alleles using Southern blotting technology. For a total of 536 meioses, 2 mutations for D2S44 and 4 for D4S139 were observed. No mutations for D12S11 (517 meioses) and for D7S22 (75 meioses) were found. When considering the parental allele most similar in size to the mutant allele as the mutated allele, all mutations concerned expansions of the repeat unit. Five were from paternal and one from maternal origin. The parental age seems not to be involved in the mutation mechanism. These observations confirm earlier reports on a majority of expansions and of the superiority of the paternal origin of these VNTR mutations (Vergnaud et al. Genomics 11 (1991) 135-144; Jeffreys et al. Cell 60 (1990) 473-485). To some extent, these results resemble the observations with STRs alleles and possibly a common mechanism is on the basis of this variation.

Using digestions with the restriction enzyme *HinfI* in combination with probe pH30, 10 unrelated persons from the 782 examined showed a third fragment. This additional fragment is due to an additional internal *HinfI* restriction site. An identical observation for pH30 on *HaeIII* digested DNA has been reported (Waye and Fourney, Hum Genet 64 (1990) 223-227). In one case, the transmission of a 3-fragment chromosome was seen. This complex allele pattern is probably due to the combination of the VNTR/RFLP profile and the presence of a methylable CpG sequence in one of the restriction sites for *HinfI*. This phenomenon was observed earlier by Budowle et al. (J Forensic Sci 35 (1990) 530-536) using probe YNH24 on *HinfI* digested DNA and making this restriction enzyme less suitable.

NON-AUTOMATIC MULTIPLEX ANALYSIS AND DETECTION OF STR- LOCI: HPRTB, FABP, CD4, F13A01, CYAR04, AND LIPOL

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Amplification by polymerase chain reaction (PCR) of polymorphic loci and subsequent typing by electrophoresis and silverstaining has become a useful tool for variety of applications. One group of highly polymorphic markers is represented by the short tandem repeats (STR), which are abundant throughout the human genome. STR loci consist of simple tandemly repeated sequences of 1-6 bp in length and are very useful for old or degraded DNA because of their small sizes (< 300 bp). We present a DNA-typing method using six unlinked STR loci (HPRTB, FABP, CD4, F13A01, CYAR04, LIPOL), which are amplified in two multiplex reactions.

Electrophoretic analysis was non-automatically performed in one gel system, followed by silverstaining. Thus we have established a highly informative system using six unlinked polymorphic STR loci not focusing on the automated systems.

DNA ANALYSIS OF POLYMORPHISM IN DRUG OR XENOBIOTIC METABOLISM

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Hereditary differences in activities of drug metabolizing enzymes among individuals have led to a phenotypic classification of humans as poor or extensive metabolizer. The metabolic genotype may be important in drug overdose with substances subjected to detoxication.

We determined the GSTM1 (glutathione S-transferase) gene deletion and the genotypes of NAT2 (N-acetyltransferase) and some other enzymes in healthy Japanese individuals' DNAs by allele specific PCR amplification and restriction fragment length polymorphism analysis. The influence of allelic differences at the gene loci on substance loading was also investigated.

Genotyping assays using DNA sample can be of great help at drug therapy and clinical or postmortem diagnosis.

Multiplex PCR with three short tandem repeats in forensic caseworks

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A multiplex PCR with three STRs is used as an easy and rapid method for individual identification with high probability. The PCR conditions were optimized especially by the temperature profile and by defined concentration of each primer pair to get comparable amounts of amplified fragments and clear cut banding pattern.

A Taq concentration of 0,35U/28 μ l was found to produce accurate results. The annealing temperature for the first 8 cycles was 60°C. During the following 17 cycles the annealing temperature was raised to 62°C with a prolongation of each extension time for 3 sec per cycle.

Locus	Chrom.loc.	No. of alleles	fragment size (bp)	conc. in PCR-mix (μ M each primer)
vWF	12	8	139-167	0,22
(THO1)	11	7	179-203	0,07)
D11S554	11	17	174-254	0,36
D18S51	18	14	267-319	0,14

Alternatively THO1 can be amplified in this triplex PCR instead of D11S554. The PCR products were simultaneously detected on a single PAA-gel and allele determination was carried out by comparison with allelic ladders.

E. FORENSIC APPLICATIONS

AUTOMATED SHORT TANDEM REPEAT (STR) ANALYSIS AND THE NATIONAL UK DNA DATABASE

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DNA profiling in forensic science in the UK is focussed on the analysis of short tandem repeat (STR) loci using PCR. It is the technique of choice for the national strategy to create a criminal intelligence databases which will ultimately consist of hundreds of thousands of individuals. Apart from the increased sensitivity inherent with any PCR technique, with STRs there is also the advantage of definitive allelic identification. This is a consequence of lower measurement errors associated with the use of polyacrylamide gel electrophoresis to detect DNA fragments ranging between 200-400bp in size. Because of their small sizes STRs are more likely to be successful on old or badly degraded material, an important consideration in forensic casework. The choice of STRs is critical. Whereas dimeric STRs are problematical because slippage during amplification results in the generation of spurious stutter bands, trimeric and tetrameric loci such as HUMTH01 are less prone to this problem. In addition, some recently discovered STR loci are hypervariable, consisting of 20-30 alleles - these loci are the most useful to generate the high discriminating powers required.

Several methods have been used to detect STR products, including silver staining, ^{32}P and automated fluorescence. We are using the latter method, utilising ABD 373 and 377 automated sequencers in conjunction with the use of dye labelled primers. We have developed two separate multiplex systems (where several loci are amplified in a single reaction). Automation of the process results in high throughput and reduced running costs, which compensates for the relatively high start-up capital costs. Databasing is automated, there is also the potential to automate further, particularly at the 'front-end', i.e. extraction and PCR preparation. A further advantage of automation is to reduce the number of manual sample manipulations required, improving quality and reducing the risk of laboratory error. The potential role of expert systems to aid interpretation is also discussed.

We have developed two separate multiplex systems. The first system introduced into routine casework in the UK was a quadruplex. This system has a probability of chance association (Pm) between 10^{-4} - 10^{-5} and has since been complemented by a second set consisting of 7 loci (including a sex - test), giving a Pm of 10^{-9} (equivalent to that of 4 single locus probes (SLPs)). A third set consisting of 6 loci is also undergoing investigation.

IDENTIFICATION THROUGH GENETIC TYPING OF THE VICTIMS OF THE SECT OF THE SOLAR TEMPLE (CHEIRY-SALVAN, SWITZERLAND)

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In October 5 1994, 23 corpses were discovered in a farm in Cheiry (Canton of Fribourg, Switzerland) and another 25 bodies were found in two chalets in Salvan (Canton of Valais, Switzerland). All of the victims belonged to the Sect of the Order of the Solar Temple.

In both locations, which are approximately 100 km apart, fire alarms were triggered on the same day approximately at 1:00 a.m. in Cheiry and at 3:30 a.m. in Salvan.

Our institute was commissioned to identify the victims, to determine the cause of death and to help elucidate the surrounding circumstances.

In the case of Cheiry, the bodies were well preserved and the methods of identification included visual recognition by relatives, dactiloscopic and/or odontostomatological examinations.

In Salvan, some of the corpses had been significantly damaged by fire and identification relied on genetic typing, odontograms, and/or medical data.

Positive identification of the victims was made possible by determining their genetic profiles. Here, we report the identification procedure and the interpretation of the results.

Determination of sex and kinship in mass grave skeletons by DNA (PCR) analysis on ancient bones

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In a 4000 year-old mass grave from Velké Pavlovice (CR) the remains of eight individuals, two adults and six children, were discovered. Agreements in anatomic variants suggested that the individuals all belonged to one family. First the individuals were submitted to a kinship analysis based on genetically determined odontologic traits. The results of this analysis indicated that the hypothesis is correct for at least seven of the eight individuals. To further verify the hypothesis that the group of individuals represent a proper family, i.e. parents and their children, as well as for the determination of the skeletons' sex, the bones of the suggested family were then submitted to aDNA analysis by PCR.

In the sexing of the individuals by PCR we used human X/Y-chromosome-specific primers (AMGL/AMG). We found two female individuals (an adult woman and a girl) and six male individuals (a man and five boys). In regard to the possible kinship we used the PCR-based loci HLA-DQ α , LDLR, GYPA, HBG, D7S8, GC, HUMVWA31, HUMFES, and HUMF13B. In agreement with the results of the morphological kinship investigation aDNA analysis verified the hypothesis that the individuals from the Early Bronze Age mass grave were genetically related and represent a family proper.

DESIGN OF NOVEL OLIGONUCLEOTIDE PROBES FOR SEX DETERMINATION AND ITS FORENSIC APPLICATION

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Sex determination of forensic samples is very important element in the analysis of biological evidence submitted to forensic science laboratories. Although Southern blotting and PCR have been used for this purpose, we sometimes observed that the results of PCR were different from those of Southern blotting. Since the contamination of the samples found in criminal spots is usually unknown, PCR may sometimes make wrong results because of its good sensitivity. In contrast, although Southern blotting for sex determination is not sensitive and takes a lot of time, the results are reliable.

Purpose: The goal of this study was to design the novel oligonucleotide probes for Southern blotting.

Materials and Methods: We generated 2 oligonucleotide probes (YJ1: TTC CAT TCC ATT CCA TT, Y1.3: TTC TAT TCC CTT CTA CTG CAT AC). 88.2% and over homologous region with YJ1 is found in 45.8% of DYZ1. In contrast, 80% and over homologous region with Y1.3 is located in only one part of DYZ1. These oligonucleotides were labeled with alkaline phosphatase by LIGHT SMITH II (Promega). After DNA from bloodstains picked up in criminal spots was digested with HaeIII, Southern blotting was performed using each probe. The DNA specimens were concluded to be male, when the 3.4-kb band was apparently observed.

Results: In Southern blotting, Y1.3 probe could detect 400 ng of DNA and the use of YJ1 as probe could lower the detection limit that was 25 ng of DNA. Sexes were determined in 103/120 (85.8%) of bloodstains by both probes. Of the remaining 17 specimens, we could not determine the sex, because the yield of DNA isolated from these bloodstains was very low. No mistake in sex determination results occurred.

Conclusions: 1) YJ1 and Y1.3 oligonucleotide probes designed for sex determination are to be an effective tool to shorten the working time in Southern blotting. 2) These probes also offered reliable and accurate results, although less sensitivity compared to PCR. 3) These data indicate that Southern blotting using YJ1 or Y1.3 oligonucleotide as a probe is the most suitable method for sex determination in forensic practical cases.

PCR CASEWORK EXPERIENCES WITH A MULTIPLEX STR SYSTEM.
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Since August 1994 a PCR DNA profiling system consisting of four Short Tandem Repeat (STR) loci has been used in routine casework at the Metropolitan Police Laboratory. The method uses a multiplex amplification of the HUMVWA31, HUMTHO1, HUMF13A1 and HUMFES/FPS loci. The fluorescently labelled products produced are detected using an ABI 373 Automatic Sequencer.

At present approximately 1500 hundred control samples (mainly blood) and 1800 hundred crime samples have been profiled. DNA is prepared from the majority of samples using the Chelex method. The results obtained demonstrate that this method is rapid and robust. However on occasions it has been necessary to further purify the DNA especially with samples of semen or saliva.

It has been necessary to quantitate the target DNA in all instances and a slot blot method is used. Starting quantities of DNA range from 1 to 3ng but on occasions full profiles have been obtained from as little as 0.15 ng. No evidence of "allelic dropout" has been detected though it is not unusual for both alleles at a locus to be lost when the starting DNA concentration is low.

The paper will contain tables detailing the relative success rates with different types of case stains.

The conclusion will be drawn that this multiplex PCR system is reliable and sensitive. Blood staining gives the best success rate though valuable results can still be obtained with other body fluids. This method has now completely replaced blood grouping at the MPFSL.

MULTIPLEXED DNA MARKERS FROM CIGARETTE BUTTS IN FORENSIC CASEWORK

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A forensic casework of DNA markers identification on 53 cigarette butts from crime scene and 10 suspected individuals is reported.

DNA from cigarette butts was Chelex extracted, while for fresh samples from suspects phenol/chloroform extraction was used; PCR amplification was carried out by tetraplex reactions for all the samples. Four DNA markers were amplified simultaneously (HUMF13A1, HUMTH01, HUMvWA31/A, HUMFES/FPS) and detected by a fluorescence based technology.

Electrophoresis on the Applied Biosystems automated DNA sequencer 373A Leon were run and fragment sizes were automatically determined by Genescan 672 software using an internal lane standard (GS2500). In every case gender identification of samples by using fluorescent Amelogenin primers was obtained.

High sensitivity of the method allowed the definition of identity values ($\leq 10^{-4}$) for 50 cigarette butts; four of the suspected people were indicated as smokers of the most of them (38), while 12 cigarettes were smoked by unknown men (7) or women (5) and three remained undetermined because of absence of results (1 case) or contamination (2 cases).

DNA POLYMORPHISMS IN DENTAL PULP: EFFECT OF ENVIRONMENTAL FACTORS

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This study was designed to determine the effect of various environmental factors on DNA typing from dental pulp.

Extracted teeth were subjected to different conditions and studied after 15 days, 1, 3, 6, 12, 24 and 36 months.

The following series were made:

-Interior series (4°C, 20°C, 40°C).

-Exterior series: Including an open air series, buried teeth (in soil and sand) and submerged teeth (sea and river).

In addition two series of high temperatures (75°C, 100°C, 200°C, 300°C, 400°C and 500°C) and another of old teeth (12-50 years) were made.

DQA1, D1S80, two STRs (HUMTH01 and HUMFES/FPS) and X and Y specific sequences were studied after PCR amplification of DNA extracted from teeth using different methods (crushing, slicing horizontally and endodontic access).

In general the best results were obtained using STRs and the poorest results using the D1S80 system. 100% of positive results were obtained for the STRs in the series of old teeth. Complete negative results for all the systems were obtained after heating the teeth 2 min at 500°C.

The teeth submerged in water (sea and river) gave the poorest results with an average of only 25% positive results in 6 months old teeth.

IDENTIFICATION OF HUMAN REMAINS USING DNA AMPLIFICATION
(PCR)

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DNA typing methods were used for the potential identification of unknown human remains. In different police cases, some bones and mandibles were available for the identification of missing persons.

DNA was isolated from bones tissue from the femur using descalcification and extraction with Phenol/Chloroform and from the dental pulp using DTT and Proteinase K and extraction with Phenol/Chloroform.

In all of the cases, DNA yield was too degraded or DNA amount was very low to obtain results in analysis of Restriction Fragment Length Polymorphisms (RFLPs). However, DNA extracted was successfully amplified using the Polymerase Chain Reaction (PCR) for HLADQA1, D1S80, Polimarker and four different STRs loci: TH01, FESFPS, F13A01 and vWF.

The combination of allelic frequencies of these genetic markers, allows get a high probability of identification in comparison with frequencies obtained from putative parents.

These results suggest that typing of DNA for PCR markers, extracted for human remains could be useful in the establishing the identity of a person.

THE SO-CALLED ALCOHOL-BLOOD-SAMPLE IDENTITY
EXPERTISE.

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From August 1986 to August 1994 the Institute of Forensic Medicine in Düsseldorf was engaged by courts to prove the identity of blood-ethanol samples in 157 cases. Non-identity was found in 11 cases. In last years the total number of expertises has declined but the number of expertises non-identity found has remained relatively constant. So - in the last years - we found non-identity in every fifth case. Further examinations on the content of blood ethanol resulted in a mean difference of BAC -0.09% . Only in one sample we found an small increase of ethanol. This paper reports on the population data of the collective, the changes in blood ethanol during storing and especially on the efficiency of serological markers used in serological identity examinations.

**APPLICATION OF THE STR ANDROGEN RECEPTOR
(HUMARA) POLYMORPHISM TO PATERNITY CASES**

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The human androgen-receptor gene (HUMARA) (Xcen-q13), contains an highly polymorphic trinucleotide repeat (ACG)_n in the coding region of the first exon.

We have studied the allele distribution in our reference population of at least 100 individuals and in 50 family case trios, divided into different group, with the aim to evaluate the efficiency of this STR system in paternity testing.

Genomic DNA samples were amplified according to the method published by Edwards et al. (Genomics 12, 241-253 1992) with minor modifications.

PCR amplified DNA was detected on vertical polyacrylamide gel, followed by silver staining. The various alleles were classified by comparison with a ladder obtained by mixing different alleles. Some amplified samples were sequenced to detect the right number of ACG repeat.

Furthermore this system was easily co-amplified with the STR HumTH01.

Our preliminary results suggest that the HUMARA system may represent an useful, additional genetic polymorphic marker for paternity testing, relatively the parental origin of the X-chromosome.

**MULTIPLEX ANALYSIS OF LOCI HUMTH01, TPOX AND
CSF1PO IN FORENSIC SAMPLES**

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Multiplex analysis of STR loci by PCR is a powerful tool for human identification purposes. Fluorescent automatized detection allows even the analysis of loci whose amplification products share the same size range. However, silver nitrate detection requires that alleles from different loci do not overlap their size ranges. In this sense, loci HUMTH01, TPOX and CSF1PO show allelic ranges between 179-203, 232-248 and 295-327 bp respectively, and can therefore be amplified simultaneously using their correspondent GenePrint STR Multiplex System.

The analysis of loci HUMTH01, TPOX and CSF1PO in the resident population of the Basque Country showed heterozygosities of 0.79, 0.63 and 0.72. The probability of two individuals sharing the same genotype with each of these loci is 0.07, 0.22 and 0.13 respectively, being their combined value 0.002.

To evaluate the applicability of these systems to forensic casework, we have examined several samples such as hair-roots, semen stains, vaginal swabs and blood stains, each of these with varying ages. We could type unambiguously 30 day old hair-roots, up to 6 month old phenol-chloroform or Chelex 30%-extracted semen-stains and up to 12 year old blood-stains, showing the high applicability of this Multiplex system.

A FIVE MINUTE PROCEDURE FOR EXTRACTION OF GENOMIC DNA FROM WHOLE BLOOD, SEMEN AND FORENSIC STAINS FOR PCR
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PCR does not require highly purified DNA and various methods have been devised to simplify the extraction of DNA for PCR. A widely used method for the extraction of human genomic DNA from various sources such as whole blood, semen and stains includes the use of Chelex 100[®] to stabilize DNA at a boiling treatment. But even the relatively simple Chelex method requires a number of manipulations and takes an hour or more to perform. Strong alkaline solutions have a powerful solubilizing effect on cell membranes as well as on biological stains and we have explored the possibility of using a simple alkaline extraction step for the liberation of DNA for PCR. We found that incubation of 5 μ l of whole blood or 1 μ l of semen (or the equivalents of stain material) with 0.1 M NaOH for 5 min at either room temperature (with whole blood) or 75^oC (with semen and stains) results in the release of high amounts of DNA, the yield being higher than with the Chelex method. After the addition of 200 μ l 0.02 M TRIS pH 7.5 the extract is ready for PCR; no washing, treatment with proteases, boiling or centrifugation are required. Five μ l of the 200 μ l extract are usually adequate as template in 50 μ l of PCR reaction. The extract is stable at 4^oC for months indicating that endogenous nucleases are effectively denatured by the extraction process.

FALSE RESULTS IN THE HLA-DQ α TYPING: TWO CASES REPORTED

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We report two forensic caseworks where the commercial HLA-DQ α kit failed, in spite of the positivity of the control "C", to type correctly the DNA samples.

Case 1: personal identification on 12 years old bloodstains: four STRs (HumTH01, HumVWA, MBP-B and SE33) systems gave repeatable and reliable results that identified the victim while the HLA-DQ α strips gave a different result whenever it was repeated.

Case 2: paternity test from the tissues of a 6 years buried male body: the paternity was confirmed by ABO grouping, two VNTRs systems (D1S80 and Coll2AI) and four STRs systems (HumTH01, HumWVA, MBP-B and SE33) with a paternity index > 99,99 %. Some of the HLA-DQ α tests confirmed the paternity while some others excluded it.

The DNA extracted from these forensic specimens was analyzed by HPLC. We found both a modified ratio of the nucleotides and the presence of several additional peaks of unknown origin.

We never obtained unreliable results when testing undegraded DNA and these observations suggest that a modified DNA template can lead, on single tests, to false results.

USE OF PCR FOR FORENSIC DNA ANALYSIS IN HAIRS, SALIVA
AND CIGARETTE ENDS

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In the last years, the number of limited specimen evidences (single hairs, cigarette ends, saliva in envelopes or stamps...) arrived to our laboratory has increased due to the successful results. Mostly these small evidences have a great significance to connect the suspect with the crime scene or to identify unidentified bodies and human remains, moreover, these samples have a particular resistance to environmental factors.

We present a casework review. DNA extraction has been made using different methods according to sample type. DNA obtained was amplified by the polymerase chain reaction (PCR), using, mainly, AmpliType DQ α (DQA1 locus), AmpliType PM (LDLR, GYPA, HBGG, D7S8, GC loci) and AmpliFLP D1S80 (all kits by Perkin Elmer Corporation). We conclude with an evaluation and comparison of the results obtained with minute amounts and other DNA sources related.

PCR-DNA TYPING FROM BEARD SAMPLES

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The analysis of DNA polymorphisms by means of PCR, has drastically increased the possibility of using some evidence samples that were not utilizable until a short while ago.

Moreover, the introduction of STR's and AmpliType PM analyses for forensic applications, has greatly improved the discriminating power of these techniques also in case of small amount of target DNA.

In this study we have investigated the feasibility of isolation, amplification and typing of DNA on beard samples collected from blades and electric razors belonging to several persons. From these same persons, blood samples were taken for a comparative study.

Genomic DNA was obtained from beard samples using a lysis buffer, followed by a phenol-chlorophorm extraction. The DNA was then amplified to investigate some of the AMPFLP's and STR's commonly used in our laboratory (D1S80, HUMTH01, HUMvWA, etc.), as well as HLA-DQ alpha and AmpliType PM, according to the literature. The separation and typing of the PCR products was achieved by horizontal polyacrylamide gel electrophoresis with silver staining for AMPFLP's and STR's and by means of reverse dot blot procedure as concern HLA-DQ alpha and AmpliType PM.

The results clearly indicated that beard samples can be considered a good and interesting specimen for identification purposes.

PCR-BASED DNA ANALYSES OF EPIDERMAL CELLS FOUND ON ADHESIVE TAPE

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Adhesive tape is widely employed by police forces to collect samples for forensic purposes, such as gunshot residues. Moreover, adhesive tape used in kidnapping cases or as a mean to wrap packages, frequently represents important evidences related to crimes.

In all these cases, cells from the outer epidermis layers are left on tape.

In this paper we investigated whether the cellular material stuck on the adhesive tape could be used for DNA analyses.

Many adhesive tape samples taken from various epidermal districts (face, finger, wrist) were collected from different individuals. From these same individuals, blood samples were taken for a comparative study. Part of the tapes were also used for a morphological control.

The DNA extraction was performed either by Chelex 100 and/or by phenol-chloroform procedures, modified in our laboratory, according to the problems due to inhibitors present in adhesive-tape matrix.

After quantification, DNA was amplified to investigate some of the AMPFLPs and STRs commonly used in our laboratory (D1S80, HUMTH01, HUMvWA, etc.), as well as HLA-DQ alpha and AmpliType PM, according to the literature. The separation and typing of PCR products was achieved by horizontal polyacrylamide gel electrophoresis with silver staining for AMPFLP's and STR's and by means of reverse dot blot procedure as concern HLA-DQ alpha and AmpliType PM.

The results showed that in most cases, the few nucleated cells that remain on adhesive tape contain enough DNA for PCR analyses.

DETERMINATION OF SEX IN DENTAL PULP USING PCR

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Dental pulp has proven to be a very good source of biological evidence for forensic casework. Its location in a especially protected cavity makes dental pulp very valuable.

In this work was analyzed 532 dental pieces to determination of sex. The study was carried out like it follow:

1. Study of the antiquity: dental pieces were analyzed of zero to 20 years of antiquity. They for their study formed a group in intervals of years: from 0 to 2, of 2 to 5, of 5 to 10, of 10 to 15 and of 15 to 20.

2. Studies of other parameters: the pieces teeth them they underwent to different means of conservation: to environmental temperature, to freezing, in immersion in saline serum, with interment and with incineration.

3. Study of the type of piece teeth: was proven with all the pieces (molars, incisive, etc.). The payees of the pieces teeth them they had ages understood between 11-88 years.

The extraction of DNA of dental pulp was carried out for the method of phenol/ chloroform with modifications. For the amplification the protocol of Psitzinger et al. (1993) was followed. X (181-199 bp) and Y (102 bp).

The amplified fragments were visualized by electrophoresis in agarosa minigels and stained with etidium bromure.

The result of this work was very satisfactory. Interesting outputs in all the studied cases were gotten. the employed technique could contribute data of utility in forensic casework.

USE OF PCR IN FORENSIC CASEWORK IN THE AREA OF BERLIN

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In the last few years a great variety of polymorphic DNA systems first discovered by means of molecular biology and human genetics were introduced into forensic science. In contrast to the great number of new DNA systems examined in the developmental laboratories the "normal lab" working on routine casework must concentrate on a few but distinctive DNA systems. In our laboratory (LKA Berlin) we are using the PCR systems D1S80, ApoB, VWA31A, F13A1, TH01, FESFPS and SE33 routinely and found them to be extremely useful in everyday forensic casework. As population data are essential for all forensic applications we studied the allele frequencies of these DNA systems in the Berlin population.

DNA was isolated according to standard methods: protein K digestion; phenol chloroform extraction and ethanol precipitation followed by a dialysis against TE buffer. Amplification of D1S80 was carried out with the commercial kit from Perkin Elmer (Kasai et al., *J. For. Sci.* 1990, 35, 1195), ApoB PCR was done according to Boerwinkle et al. (*Proc. Natl. Acad. Sci.*, 1989, 212). The Analysis of the four STR systems VWA31A, F13A1, TH01 and FES was done as described by Edwards et al. (*Am. J. Hum. Genet.* 1991, 49, 746), Sullivan et al. (*PCR Methods Appl.*, 1992, 2, 344) and Kimpton et al. (*PCR Methods Appl.*, 1993, 3; 13). Amplification of SE33 was done as described by Polymeropoulos et al. (*NAR*, 1992, 20, 1432) with modifications according to an optimized protocol developed by the LKA Hessen, Germany.

The two AMFLP systems D1S80 and ApoB can easily be separated by vertical or horizontal native PAGE (6%), whereas the STR systems require an improved and standardized separation on denaturing PAGE. In our hands we found that analysis of the above mentioned STRs were easiest and most reliable by working on the ABI system 373A in connection with the Genescan 350ROX or 500ROX fragment size standard.

By using the above mentioned 7 DNA systems we analysed more than 500 people of the Berlin population, mostly people who were involved in a crime, and over 2000 traces. In spite of the heterogeneity of the Berlin population which is composed of several national groups (turks, poles, vietnamese etc) the allele frequency distribution is in good accordance with the data in other european population studies.

The most useful system is SE33, but the non-standardized nomenclature presents a problem still to be solved.

THE NEVERENDING STORY: A NEW TSAREVITCH?

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In July 1991 five skeletons found in Ekaterinburg were identified as the remains of the last Tsar, Tsarina and three of their five children. Gill et al. confirmed this thesis by PCR- based typing and analysis of mitochondrial DNA. In 1994 four people from St. Petersburg approached us with the conjecture that their father could have been the still missed Tsarevitch. In spite of several cases with feigned relationship with the Russian royal family having been solved in the past the life-story of these people revealed a certain probability. Anyway in contrast to the other reported cases this family wanted to be checked up by scientific examinations. Blood samples from the four children and from the still living mother were individualized by typing five short tandem repeat systems. So it was possible to reconstruct the paternal characteristics as far as possible.

INFLUENCE OF MEDIAEVAL CLOTHES COLOUR PIGMENTS ON DNA EXTRACTION AND AMPLIFICATION

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Developments in molecular biology, in particular the PCR method, have provided new tools for analysing ancient DNA from archeological remains. The amplification of mitochondrial DNA sequences from human bones several hundred years old, has important implications for archaeology and palaeontology as bones are preserved under a wide range of environmental conditions.

Different factors are affecting DNA preservation and recovery such as depth of burial, soil pH, presence of humic acids or pigments of ancient clothes.

In this study we examined bone fragments (vertebra, calcaneus, astragal) from a mediaeval skeleton (1000 after J-C) exavated from a burial vault. This skeleton belonged to a duke dressed with mediaeval clothes stained with kermes vermilio binded to the wool by ellagic acid and alun [Al₂(SO₄)₃]. The vertebra was slightly brown colored and we showed in a previous study that cytochrom b mt DNA had been successfully amplified.

The calcaneus and astragale showed red-brown color due to the kermes vermilio and the extracts were also brown with aggregated materiel that precipitated from solution, presumably from the previous contaminants. These extracts could not be amplified. To investigate the inhibition power of these 3 contaminants (Alun, Kermes vermilio, ellagic acid), DNA was incubated with those pigments and then extracted and subjected to cytochrome b gene mt DNA amplification.

The authors showed the extraction and/or amplification inhibitions of these 3 components used at mediaeval age as colour and colour fixative.

The inhibition was particularly induced by ellagic acid. The other contaminants showed a weaker inhibition power.

Individualization of a dismembered body by a bomb

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Introduction: DNA fingerprinting and PCR methods have been already applied in forensic practice. The authors report an interesting case in which a dismembered body by a bomb was successfully individualized using DNA fingerprinting.

Case profile: On September 15, 1994, a blind shell suddenly and unexpectedly exploded, and three workers died due to the explosion. Two of them were able to be identified. The remaining one was, however, severely dismembered. On that day, 15 parts (Sample No. 1-15) of the dismembered body were found at the scene of the explosion. Five days after the explosion, another five parts (Sample No. 16-20) of a dismembered body including the face, which could be identified as the dead worker, were also discovered at the same scene. Therefore, DNA fingerprinting was performed in order to decide whether every part of the dismembered body was derived from the identical person or not.

Materials and methods: Each sample (ca.10 g) was homogenized in liquid nitrogen, and, thereafter, DNA was isolated by the method of Pöche et al.¹⁾. DNA was completely digested with Hae III. The digested DNA was electrophorezed on 0.7 % agarose gels. DNA fragments were blotted onto a nylon membrane, and the membrane was hybridized with the multilocus (GTG)₅ probe.

Results and discussion: From every sample, high molecular weight DNA was obtained, and 7-9 bands ranging from 6.0 to 23.1 kb were clearly observed in DNA fingerprinting (Table 1). Especially, five bands (12.7, 8.8, 7.0, 6.7 and 6.0 kb) were completely identical in all of the samples tested, and the probability of these identical band patterns with the (GTG)₅ probe in two unrelated German individuals is 10⁻⁹. Therefore, these results indicate that the 20 different body parts have the same origin of one person.

1) Pöche et al., Pathologie, 14, 193-198, 1993.

Table 1.

No. Sample	The number of bands detected	No. Sample	The number of bands detected
1. Left arm	7	11. Muscle (II)**	7
2. Right hand	7	12. Rib (III)*	7
3. Right leg	7	13. Spinal column (I)*	9
4. Muscle of the thorax	7	14. Penis	9
5. Corpus of the sternum*	7	15. Abdominal skdn	9
6. Rib (I)*	7	16. Spinal column (II)*	9
7. Muscle (I)**	7	17. Right clavicle*	7
8. Manubrium of the sternum*	7	18. Muscle (III)**	9
9. Rib (II)*	7	19. Muscle (IV)**	9
10. Lung tissue	7	20. Face	9

* Each sample had the soft tissue including muscle.

** The origin of each muscle was unknown.

FORENSIC INVESTIGATIONS AFTER SEXUAL ABUSE OF SEVERAL INFANTS.

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Within the first week of June, 1994, four foreign girls, between 9 and 12 years of age, were sexually abused in Vienna. The four crimes had many features in common, which led to the assumption of a single offender. We investigated whether this assumption could be correct by DNA analysis of the secured stains. Sperm cells were found in vaginal or anal swabs and in the underwear from two of these victims. HLA-DQ α and D1S80 analysis of DNA from the sperm containing mixed stains (male fractions) were performed and the results confirmed the hypothesis of a single offender but excluded several suspects from the crimes. Three months later three other girls were abused under similar circumstances, from one of these a mixed sperm stain was available. At the same time it became clear that already in January 1994 from a further victim a jacket with a pure sperm stain had been secured but the stain had not yet been investigated with serological or DNA methods. In all three mixed stains HLA-DQ α alleles (2 and 3) and D1S80 alleles (T24 and T25) were found in addition to the corresponding alleles from the victims. The pure sperm stain was again of HLA-DQ α type (2 3) and D1S80 type (T24 T25).

In December 1994 from approximately 3000 known sexual offenders 6 potential offenders were selected by computer search following several criteria. Photographs of these men were shown to the victims and on one of them the offender was recognized by one of the victims. He was taken into custody and, after having heard that DNA analysis would possibly charge him with the crimes, he made a partial confession.

The results of the DNA analyses (HLA-DQ α , D1S80, Polymarker) confirmed the hypothesis that the accused was involved in the sexual abuse of four of the abused girls (probabilities range from 99.7 % to 99.98 %). Although from the five other victims no informative stains were available, he admitted that within a one year's period he had abused nine young girls between 6 and 12 years old and described and showed the various locations. He was convicted to 12 years sentence on May, 9th, 1995.

SEX DETERMINATION ON ANCIENT BONES BY DNA(PCR) ANALYSIS.

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Summary. In the past sex determination on ancient bones was dependent on the stage of preservation. It was often impossible to examine fragments because anthropometric techniques could not be used. PCR based methods are a powerful tool for sex determination on smallest fragments. For this study we used amplification of the amelogenin gene (AMGL Y-specific fragment and AMG X-specific fragment). The paper reports on specificity, sensibility and reliability of the method.

IDENTIFICATION OF THE SKELETAL REMAINS OF TWO 12-YEARS-OLD BODIES BY NUCLEAR DNA POLYMORPHISMS ANALYSIS.

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Two skeletons were found in a grave near Alicante (a eastern province of Spain) in 1985. Autopsy findings revealed that both skulls had bullet wounds and other signs of violence and mistreatment before death, but the cadavers were kept unidentified in the morgue.

On 1995, the police obtained some kind of evidence which indicated that the two bodies would corresponded with those of two individuals, disappeared in 1983, that presumably collaborated with a terrorist group from the Basque country. The anthropological analysis carried out by forensic experts allowed to perform stature and age estimation and sexing of the remains, but no clinical or odontology records were available for comparison.

In this study we report the successful identification of the two skeletal remains by comparative typing of nuclear sequence polymorphisms (HLA-DQA1, LDLR, GYPA, HBGG, D7S8 and GC) and STR polymorphisms (HUMTHO1, HUMTPOX, HUMCSF1PO, HUMVWA, HUMFES/FPS, HUMF13A1, and HUMF13B) in the compact bone of the remains and in their presumptive parents.

IDENTIFICATION IN VESTIGES FROM DIVERSE BIOLOGICAL SOURCES USING DUPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI.

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The amplification of polymorphic short tandem repeat (STR) loci by PCR provides the basis of a rapid and sensitive technique for individual identification.

We have studied DNA of vestiges biological humans employing the amplification by duplex PCR with STRs.

The two loci analyzed there were HUMTHO1 and HUMFES/FPS. The samples were: hair, saliva, sperm, bloodstain and dental pulp.

The extraction of DNA was carried out for the method of the phenol/ chloroform. The amplified fragments were visualized by electrophoresis in a 6% denaturing polyacrylamide gel and silver stain. The time for assay was 45 minutes.

The results were satisfactory included in the cases in those that there was little quantity of DNA or that the sample was degraded.

The amplification of polymorphic STRs by PCR is specially useful when we have a very small amount of biological material.

A RARE PATERNITY CASE, EXHIBITING MULTIPLE MUTATIONS BY ANALYSIS OF VNTR LOCI.

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The mutation rates (MR) of VNTR loci used for paternity testing are below 1% and in general the analysis of three different VNTR loci with combined changes of paternity exclusion greater than 99,9% results in probability of paternity (PP) values that are sufficient for the determination of paternity. In a survey of 80 paternity cases that have been analysed in our laboratory we observed one paternity case exhibiting potential mutations regarding three single locus probes (SLP), G3 (MR 0,007), MS1 (MR 0,043) and MS205 (MR 0,004), whereas five SLP's, YNH24, MS43A, MS31, LH1 and PH30 did give concordant results. All potential mutations were affecting paternal fragments resulting in size differences of 100-300bp's between the observed and expected fragments and were reproducible with new DNA samples. In contrast to the analysis of VNTR loci, the analysis of five STR-PCR loci (HUMTH01, D8S639, CYP19, ACPP and ACTBP2), 21 conventional serum protein and erythrocyte membrane systems including HLA-Class I (ABC) analysis did not reveal any exclusion. Biostatistical calculation using the results obtained by the conventional, HLA and STR analysis resulted in an PP value of 99,99%. In addition, calculation of the results obtained from all analysed polymorphisms including the analysis of VNTR loci resulted in an PP value of 99,97% giving evidence for paternity. These results represent a rare case of non-exclusion paternity where several mutations of VNTR loci are observed.

THREE INTRIGUING BODY IDENTIFICATION CASES.

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The use of DNA analysis has proved to be a powerful tool in cases where identification by ordinary means is impossible. The 3 cases presented here, involved highly decomposed bodies.

I. A decapitated body was found in the sea March-94 in northern Norway -the police related the finding to a missing person, a man born in 1916 who was last seen December-93. The only relative was a nephew.

II. A young Norwegian sailor disappeared at Crete in November-93. A headless body found at the seaside of Crete in January-94 was supposed to be the missing man. Relatives: parents.

III. A body with a high degree of adipocere was found in a lake in September-94. The only missing person in the area was a man (born 1896) who disappeared in 1972. His only relatives were two sons living in the USA.

Different kinds of samples were taken from all three bodies, including soft tissue, organs and cartilage. DNA was phenol/chloroform extracted, dialysed and analysed by using HLA DQ A1 and up to 8 STR's (analysed in an ABI 373 sequencer using Genescan 672 software). In case I and II there were also a problem with inhibitors, most likely related to the sea water. Comparing the different kinds of samples, cartilage proved to be the best source of DNA for PCR.

The odds in favour of the missing persons were 22, 46454 and 5339, respectively.

Even though the bodies were heavily decomposed, HLA DQ A1 and the STR's thus gave sufficient information for the identification purpose.

FORENSIC DNA-TYPING IN THE SPANISH POLICE USING VNTRs
SINGLE LOCUS PROBES

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More than 230 stains and other biological specimens - deriving from 125 Spanish crime cases, since 1994, were - subjected to DNA PROFILING. DNA-investigations of VNTRs - system with the Single Locus Probes RFLPs technique of Hinf I digested DNA include four independent DNA VNTRs System: D1S7 (probe MS1); D2S44 (probe YNH24); D12S11 (probe MS43a) and D7S21 (probe MS31).

All cases examined have been sexual assaults and consist of vaginal swabs or washings, anal swabs, condoms, knickers and other victim and suspect's clothing.

In all cases the preferential extraction procedure for - separating spermatoc DNA from the DNA of epithelial cells - is normally used.

The results obtained seem very interesting and represent a valid aid which can certainly contribute to the solution of a crime if the quality and quantity of DNA extracted is available for VNTRs Analysis, because they allow us to exclude or determine probable identities of suspects in rape cases.

PCR typing of DNA extracted from epidermal particles won by scratching.

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Conventional serological methods are of limited value for the identification of scratched epidermal particles. The aim of our experimental studies was to evaluate the evidence of PCR typing for such status.

In a pilot study 4 volunteers scratched each other. The attached epidermal material was removed and analysed.

Based on positive PCR typing results in these first experiments, the necessary force to obtain adequate amounts of tissue by scratching procedures was investigated. A special mechanical scratching apparatus was constructed. DNA was isolated from the scratched epithelial material, quantified by slot-blot analysis and typed using the STR systems HUM ACTBP2 (SE33), HUMTH01 (TC11) and HUMVWFA31 (VWA). A statistical correlation between the employed force and the quantity of extracted DNA could be proved.

Results

- 1) PCR typing of scratched epidermal particles was successful in about 80% of the cases.
- 2) A correlation between the quantities of DNA and the employed scratch force (200g-2000g) could be demonstrated.
- 3) Forces of over 1000 g did not lead to a higher amount of DNA.
- 4) This phenomenon can be explained by the structure of the skin; after removal of the epidermis only a small amount of DNA could be extracted from the dermis.

EFFECT OF VARIOUS SUBSTRATA AND AMBIENT TEMPERATURE ON MCT118 AND HLADQ α TYPINGS OF BLOODSTAIN SAMPLES

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Many studies have been published reporting that it becomes impossible to determine the DNA types of aged bloodstain samples. Regarding such unfavorable result several factors will be raised. Among such factors the substrata adhering blood and ambient temperature were investigated with regard to the effect on DNA typings of aged bloodstain samples.

The substrata used here were wool, blue denim and rush floor (tatami), and they have exerted their influence on the amount of DNA with lower recoverability. The substrata such as colored printing paper, rush floor, and wood have significantly inhibited PCR amplification of extracted DNA.

The determination of MCT118 types has proved to be impossible in various experimental conditions of ambient temperature and stored time such as 200°C and 10 min., 150°C and one hr, and 100°C and two weeks. HLADQ α type could not be determined from the samples stored in such conditions as 250°C and 10 min., 150°C and 6 hrs, but in the condition of 100°C the determination of the typing was possible even after one month storage.

PCR GENOTYPING IN DENTAL PULP FROM OLD HUMAN SKELETAL REMAINS AND FIRE VICTIMS.

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There is an on-growing forensic interest in the possibility of DNA typing of skeletal remains, thus solving the identification of recent or ancient victims, and specially difficult parentage testing problems. Dental pulp has proved to be a rich source of adequately preserved DNA which can be successfully amplified and typed. We present here the results of PCR genotyping of dental pulp samples obtained from dental surgeons and from casework: two paternity testings where alleged fathers, dead several years ago had been exhumated, and an identification of a fire victim. Dental pieces were cleaned, frozen with liquid nitrogen, crushed to fine powder, completely decalcified with 0.5 M EDTA, and incubated in the presence of protein kinase. DNA was extracted with phenol-chloroform-isoamylalcohol and concentrated in a Centricon device. Before amplification, DNA extracts were quantified by a slot blot technique. Dental pulp from the burnt body yielded the best DNA recovery. DQA1, D1S80, PM and different STRs were genotyped.

ABO GENOTYPING OF THE SUSPECTS USING THEIR SPERM DNA.

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⟨PURPOSE⟩ In sexual crimes, the first step to identify a suspect is ABO phenotyping of material available at the criminal spot. However, mixture of fluids cannot be separated for conventional ABO phenotyping by detecting antibody or antigen material. We therefore studied the ABO blood group genotyping of isolated sperm DNAs from contaminated vaginal fluid by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

⟨METHODS⟩ 4 male spermiatic fluids and 4 female vaginal fluids (OO, AO, BO, AB) were collected. Sperm was experimentally mixed with the same amount of each vaginal fluid. 10 μ l post-coital vaginal fluids in 4 sexual assaults were also obtained after the crimes. The two-step extraction procedure for purification of sperm DNA or vaginal epithelial DNA in contaminated specimens by a modification of the NaI and DTT method. Two PCR fragments spanning positions 261 and 700 of the cDNA sequence of the ABO locus were amplified. Then, the PCR products were digested with a restriction enzyme, Kpn-1 or Msp-1, for each site. The digested PCR products were analyzed by electrophoresis on polyacrylamide gels.

⟨RESULTS⟩ Each of samples were successfully separated and genotyped by this method, even mixed with vaginal fluid of any ABO phenotype. Sperm DNAs of suspects were also separated and genotyped from contaminated fluids obtained in 4 sexual assaults.

⟨CONCLUSION⟩ This reliable ABO genotyping method by the PCR-RFLP using separated sperm DNA should be applicable in forensic identification in sexual crimes.

PCR-BASED ANALYSIS FOR THE IDENTIFICATION OF VICTIMS OF ALLEGED "MUTI" MURDERS.

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The use of animal tissues/organs/body parts in the making of "muti" or medicine by witch-doctors/inyangas in primitive communities has long been known. However the use of human sacrifices to this end, although suspected, has been difficult to prove, and the Government has now appointed a Commission of Enquiry into witchcraft. The advent of DNA testing and in particular the polymerase chain reaction, has made it possible to link various body parts to each other in two cases we recently encountered.

First case: The mutilated torso of a 5 year old black male was found in a metal trunk. Later two small arms were found on a rubbish dump. The charred remains of a skull was found in the fireplace and sections of cervical spine being wind-dried at the shack of the suspected "muti"-doctor.

Second case: The body of an adult man was found with both hands and external genitalia removed. The two hands and genitalia were later found by the police in the possession of the alleged murderer.

We used the AmpliType^R HLA-DQ α and the PM Forensic DNA PCR Amplification and Typing kits (Perkin Elmer) in parallel. The two kits use the same reverse dot blot technique. The markers which were amplified were HLA DQA1, LDLR (low density lipoprotein receptor), GYPA (glycophorin A), HBBG (hemoglobin G gamma globulin), D7S8 and GC (group specific component).

The results obtained from the DQA1 tests showed a match in both cases. This evidence alone was not conclusive due to the limited number of alleles. The increased discriminatory power of the additional AmpliType PM markers confirmed that in both cases the tissue samples had been taken from a number of parts of the same body.

THE MC KEMY CASE :
A UNIOUE RAPE AND MURDER CASE

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In this unusual case the victim, the two-year-old daughter of a U.S. Army sergeant stationed in Babenhausen (Germany) vanished from her family's apartment during the night of September 14th, 1993. Her brutally abused body was found shortly after dawn near a gravel quarry. The girl had been raped and battered to death.

On the victims clothings a minute amount of semen mixed with large quantities of blood was found. By analyzing the stain with a total of six PCR-based VNTR systems we were able to determine the possible genotype of the semen. Given the nature of the abduction, the german police and the U.S. CID had been almost certain that the murderer was someone who knew the family. But the police failed to find a suspect. When compared to DNA extracted from blood samples donated by the victims father, family acquaintances and several other likely suspects, no match was found.

With no witnesses and virtually no dues despite a \$ 20.000 reward we suggested a massive DNA screening of every male who had been near the crime scene.

From approx. 1900 blood samples we were able to exclude all except one by using a new high-resolution vertical PAGE-system (SCHNEIDER & RAND,1995) in less than 3 months. The person, who could not be excluded was charged in May 1994. On December, 1994 he pleaded guilty in exchange for life imprisonment.

Schneider, H.R. and Rand, S. (1995)
Int.J.Leg. Med. , in press

FORENSIC IDENTIFICATION USING DNA RECOVERED FROM SALIVA ON HUMAN SKIN

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Bitemarks and suckmarks are common in sexual assaults, homicides and cases of child sexual abuse. Since saliva is presumed to be deposited on the victim's skin in these situations, we have developed a experimental protocol to study the amount of saliva that could be recovered and successfully typed.

Aliquots of saliva (40 ul) from a single donor were deposited on the intact skin of several cadavers. Samples were collected over a two days period (t= 5 min., 24 h and 48 h). Known controls (blood, tissue or oral swabs) and negative controls (swabs from undisturbed skin) were also collected from the cadaver in each case.

DNA was extracted using a modification of the Chelex-100 extraction protocol (Walsh et al, 1991), quantified using a slot-blot apparatus, amplified using short tandem repeats (HUMTH01 & HUMVWA) and separated by polyacrylamide gel electrophoresis.

For the HUMTH01 locus, positive amplifications were obtained for 78.8% of the saliva samples recovered after a 5 m period, 75.8% for those recovered after 24 h, and 69.7% for those recovered after 48 h.

For the HUMVWA locus, results were similar using the same samples and periods (78.8%, 66.7% and 57.6%, respectively).

These results indicate that the forensic analysis of DNA from saliva recovered from human skin may be a valuable identification tool in cases involving bitemarks or suckmarks or in any case where saliva trace evidence is recovered.

ANALYSIS OF STR LOCI IN OLD BLOOD STAINS USING AUTOMATED AND MANUAL GENOTYPING SYSTEMS

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Polymorphic short tandem repeat (STR) loci are increasingly being used in forensic identity testing. The most usual method of analysis is by PCR amplification followed by separation of the alleles by polyacrylamide, agarose or capillary electrophoresis.

This study investigates the suitability and stability of aged bloodstains, dating from 1968 to 1994, from four individuals, for STR typing. Two loci, humTH01 and humVWa, have been analysed. Various sizes of stained material, from a number of time points were used as starting material. PCR products were genotyped using both the ABI automated fluorescent detection system and a manual high sieving Metaphor agarose techniques.

The results show that blood stains up to 26 years old can be successfully typed for these STR loci. They also confirm that the automated fluorescent detection system is considerably more sensitive than agarose electrophoresis and ethidium bromide staining. However, the results obtained using the manual technique indicate that, in some circumstances, this is a valid alternative to the more expensive and complex automated system.

MICROSATELLITE DNA POLYMORPHISM ANALYSIS IN A CASE OF ILLEGAL CATTLE PURCHASE

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Traditionally, illegal animal purchasing has been difficult to prove, especially in two cases: Young animals not-registered yet in the official genealogical book, and those animals not registered because they don't belong to any pure breed. Microsatellite Short Tandem Repeat (STR) DNA polymorphism is currently used in human forensic medicine as a powerful tool to solve criminal cases with a high degree of accuracy in a short period of time. However, this practice is not common in criminal cases affecting animals of different species. Polymorphic STR loci have been described for cattle, horses, swine, sheep or dogs. Specifically, more than 300 polymorphic loci have been described in cattle with the aim of constructing the Bovine genomic map. These loci are currently used in the detection of Quantitative Trait Loci (QTL) or pedigree analysis. In the present paper a case of illegal cattle purchase has been proven through maternal testing by using polymorphic DNA microsatellites.

DISASTER VICTIMS IDENTIFICATION BY THE DNA TECHNOLOGY ON DENTAL PULP

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On March 31st, 1995 a Roumanian Airbus A310 flying to Brussels crashed in Bucarest. The identification team identified 52 of the 60 victims. We were requested to identify by DNA typing eight victims for which no other morphological data was available.

The dental pulp of the unidentified jaw fragments (stored for two months and a half at 4°C) was sampled after selection of 53 teeth by radiographic examination.

About 1 to 5 micrograms amounts of partially undegraded human DNA were obtained from 17 of these teeth. The amount ranged from 2 to 500 nanograms for the remaining teeth.

Blood samples of the victims' relatives were collected.

The genetic investigations consisted of comparing the genotypes determined by VNTRs analyses (pH30 and pLH1 monolocus probes) and by PCR amplification of the D1S80 and THO1 loci.

Until now, one victim has been formally identified by both profiling methods. The other results are in progress and will be discussed.

F. METHODOLOGY

LONG PCR: EXPANDING APPLICATIONS WITH A NOVEL TECHNIQUE

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The PCR technique has been expanded recently to achieve long products of up to 50000 bases. The key parameters are an optimal combination of *two* heat-stable DNA polymerases: first, the forward reaction of DNA polymerization (by a DNA polymerase lacking a 3'->5' exonuclease) and second, the backward reaction called the 3'->5' exonuclease (lacking a DNA polymerase). The latter activity proofreads for the DNA polymerase thus minimizing mistakes and allowing the DNA polymerase to synthesize long products after corrections had been carried out. Further key parameters for long PCR include integrity of the DNA isolated, optimized denaturation time and temperature for complete strand separation with minimal DNA damage, optimal temperature and times for accurate primer annealing and complete strand synthesis, primer design and concentration and finally reaction conditions such as cosolvents, pH and concentration of ions. Application possibilities will be given for forensic medicine as well as for other disciplines in medicine and biology.

VNTR AND STR CHARACTERIZATION BY CAPILLARY ELECTROPHORESIS

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The characterization of VNTR and STR size is rapidly becoming an invaluable tool for the accurate typing of individuals' genome. Typically, after the initial PCR amplification, the resulting products are separated by slab gel electrophoresis. Depending on the size of the repeat unit (i.e., VNTR vs. STR), denaturing electrophoresis conditions may be required to obtain an adequate resolution level.

The present paper will demonstrate how automated capillary electrophoresis can be successfully applied to the issue of the accurate characterization of repeated sequences. Examples will be presented dealing with the identification of individuals based upon their genetic makeup for loci such as TH01, TPOX, etc. In addition, by using various combinations of loci, it is possible to expand the use of STR analysis to some clinical applications, such as the detection of residual mixed chimerism, following bone marrow transplantation.

The exquisite sensitivity provided by the capillary electrophoresis system in fluorescence mode allows to detect as little as 0.1 % of the recipient's DNA in the presence of 100 % of the donor's DNA. This type of performance is not currently easily attainable with traditional slab gel methodology; it is possible that capillary electrophoresis combined with fluorescence detection will become the method of choice for the unambiguous characterization of mixed and/or contaminated DNA samples.

HETERODUPLEX ANALYSIS IS A RAPID METHOD FOR DETECTION OF SUBALLELES CAUSED BY MIXED LENGTH AND SEQUENCE VARIABILITY IN STR SYSTEMS

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The allelic polymorphism of STR systems under forensic application is usually caused by a variable number of simple motif repeats. However, additionally mixed length and sequence polymorphisms are known in some STR systems e.g. vWA (Möller et al.) D12S64 (Kaiser et al.) and others. Forensic evaluation of STR-Systems requires knowledge about the possible existence of sequence variability within alleles of equal length beside the establishment of several population genetic data. Heteroduplex (HD) analysis (HDA) is a well-established technique in clinical genetics for mutation detection but only sporadic reports have been found in the literature on investigations in systems of forensic interest.

By help of two well-established STR Systems we demonstrate that HDA is an appropriate tool for detecting sequence variability (e.g. in vWA) or a high degree of sequence homogeneity (e.g. in CD 4) within alleles of equal length. In special cases for the purpose of HDA, the construction of artificial heterozygote genotypes is possible by band dissection from PA gels, reamplification, PCR product mixing and thermal treatment. This technique has been used for the study of the Y chromosomal STR Y27H39 (DYS 19).

Furthermore, using choiced examples from routinely vWA testing, we can demonstrate that taking into consideration HD shapes provide an increase of useful informations for paternity testing and forensic stain case work. STR system analysis using native PAGE enables the employment of HD observation without additional expenditure of work and material.

USE OF CDP-STAR IN A FAST AND HIGHLY SENSITIVE CHEMILUMINESCENT DETECTION PROCEDURE FOR VNTR LOCI WITH NEUTRAL OR CHARGED MEMBRANES.

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The use of alkaline phosphatase labeled oligonucleotides (AP-probes) and chemiluminescent substrates, for the analysis of VNTR loci, has proven to be a simple and convenient format for DNA typing. We have now developed a protocol for use with AP-probes, the chemiluminescent substrate CDP-Star™ (Tropix) and different types of nylon membranes. This chemiluminescent substrate has characteristics that makes it highly desirable for DNA typing of VNTR loci: it is highly sensitive and reaches maximum light output very quickly. The objective of this study was to establish a protocol that would allow the detection of 25 to 50 ng of DNA, bound to neutral or charged nylon membranes, in an exposure time of a few hours or less.

Different amounts of alkaline phosphatase labeled probes, for the loci D1S7, D1S339, D2S44, D4S163, D5S110, D6S132, D7S467, D10S28, D17S26 and D17S79, were individually hybridized to various amounts of HaeIII-digested human DNA. The final protocol consisted of a 20 to 30 minute hybridization at 55 °C, followed by four 10 minute washes at the same temperature. Finally, after a rinse with an alkaline buffer the membranes were dipped in the substrate and exposed to X-ray film for 3 hours or less at room temperature.

The results indicate that, based upon their sensitivity, the probes fell into four groups: D1S7, D1S339, D2S44 > D6S132, D7S467, D17S79 > D4S163, D5S110 > D10S28, D17S26. The first group detected about 10 ng of DNA and the last had a sensitivity of about 50 ng. The protocol can be used with both types of nylon membrane. Although there was slightly higher background with the charged membrane, it had better sensitivity than the neutral membranes, especially with small size alleles.

Repeated stripping and rehybridization showed only a small loss in sensitivity that could be corrected by longer exposure times.

EVALUATION OF PRIMER EXTENSION PREAMPLIFICATION (PEP) IN FORENSIC STUDIES

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One PCR experiment is conducted in an analysis of DNA from single hair, because of isolating very small DNA. Primer extension preamplification (PEP) is reported to be a method of whole genome amplification in single cells. We have attempted to use a PEP method for subsequent PCR analyses as much as possible. The purpose of this study is to evaluate the PEP-PCR analysis in forensic studies.

Method: 1) PEP-PCR: Template DNA samples were first subjected to PEP reaction, and then the small aliquots were further subjected to PCR using VNTR, STR and sex determining primers. A method of PEP was essentially used as reported by Zhang et al. (Proc Natl Acad Sci USA 89, 5847-5851, 1992). 2) Sequencing; Taq DyeDeoxy Terminator Cycle Sequencing Kit was used for determining nucleotide sequence.

Results: 1) Minimum detection levels were compared in PEP-PCR and PCR alone. We could not detect the quantities under 5 ng in our PCR alone. The quantities of DNA were calculated before PEP reaction. In COL2A1 locus, 100pg of template DNA was detected by PEP-PCR analysis. These amplified sizes were under 660 base long. Although similar results were obtained in YNZ22 and MCT 118 loci, several samples could not be amplified. In ACTBP2 locus, 10pg of template DNA was detected by PEP-PCR. Similar data were also observed in TH01, APOC2G, D9S52 and D19S49 loci and sex determining regions. They were under 310 base long. The Effect of PEP for subsequent PCR was closely related to the PCR primers. The STR and sex determining primers were more effective than the VNTR primers. 2) Both products of PEP-PCR and PCR alone showed identical mobility of the allelic state. In our ACTBP2 bands, both of them have identical nucleotide sequences and 12 base deletion compared to data base (V00481).

Conclusion: 1) The PEP method was possible to offer more copied template for subsequent locus-specific amplification by PCR. 2) The nucleotide sequences in the products of PEP-PCR and PCR alone are demonstrated that PEP-PCR is an effective tool in forensic science investigation.

SEQUENTIAL MULTIPLEX AMPLIFICATION (SMA) UTILITY IN CASES WITH MINIMAL AMOUNTS OF DNA

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One of the main problems in forensic casework results from the minimum amounts of biological materials (DNA) that can be extracted from certain evidences. In these cases, PCR is the only approach, but even with this technique the number of loci that can be studied is strongly limited by the quantity of template.

We have developed a method called Sequential Multiplex Amplification (SMA) [Lorente et al, 1994] in which a limited amount of DNA extracted from a sample can be reutilized for several single PCR amplifications.

The DNA is amplified for a particular target sequence; after typing the locus the remaining PCR sample is washed using a Microcon-100 filtration device; the recovered DNA is amplified for another locus, and the process is repeated until there is a high degree of discrimination.

Using this method, at least 8 loci (5 included in the Polymarker kit, plus HUMTH01, D1S80 and HLA-DQA1) have been amplified from minute casework samples where, otherwise, only 1 or 2 sets of amplifications could have been attempted. Probabilities of discrimination have been significantly increased from 1 in 2.500 to 1 in 3.000.000, depending on the allele frequencies in each case.

Therefore, we propose this approach for those cases where only a very few nanograms of DNA are available. With this procedure it is also possible to recover template DNA from PCR tubes that yielded no amplification product (e.g., DNA from evidentiary samples that might be degraded for a given locus, but could be amplified for some other loci).

EVALUATION OF HEREDITARY DISTANCE BY RESTRICTION LANDMARK GENOMIC SCANNING (RLGS)

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"Genomic Scanning" is defined as a high-speed scan to simultaneously detect signals from many loci throughout a genome by one process. Restriction Landmark Genomic Scanning (RLGS) was developed as the powerful type of "Genomic Scanning". RLGS employs direct-end labelling of a genome digested by a restriction enzyme and then subjected to high resolution two-dimensional electrophoresis. In this report, we have used the RLGS method to calculate the DNA polymorphic rates as a means of evaluating the intraspecies hereditary distance of animals.

Two species were examined for the detection of polymorphisms: mice (B6, D2, M.Spretus); hamsters (ACN, Bio, CBN, APGN, BN, GN, AN, CN). All tissues came from the liver and genomic DNA extraction was performed. The extracted DNA was treated following the 8 steps of the RLGS method, that is (1) blocking, (2) landmark cleavage by NotI, (3) labelling, (4) fragmentation of the labelled DNA with PvuII, (5) first fractionation by agarose gel electrophoresis, (6) fragmentation of labelled DNA with PstI, (7) second fractionation by polyacrylamide gel and (8) autoradiography. The polymorphic ratio between a domesticated laboratory strain and a wild strain is bigger than that between purely domesticated laboratory strains. This result shows that the polymorphic rates determined by the RLGS method seem to correlate to the hereditary distance of the intersubspecies.

DETECTION OF SINGLE BASES CHANGES IN PCR-AMPLIFIED DNA USING DOUBLE STRAND CONFORMATIONAL POLYMORPHISMS (DSCP)

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DSCP (also known as heteroduplex analysis) is one of the most widely used method (together with SSCP) for detecting single base changes in PCR amplified DNA. Changes in a single base are distinguished by means of the analysis of heteroduplexes and changes in more than two bases can be distinguished in both homoduplexes and heteroduplexes. PCR conditions can be designed not only to amplify homoduplexes, heteroduplexes and single strand DNA at the same time, but also to focus the analysis on either SSCP or DSCP.

In general DSCP seems to be advantageous in typing DNA polymorphisms or mutations in loci with few variants, but, because it is necessary to have a simple pattern of all possible combinations of the alleles, it is not as advantageous in typing systems with many variants. The application of DSCP in forensic genetics is illustrated through the study of two HLA-class II loci (HLA DQA1 and DPB1), the analysis of hidden variation in STRs (due to sequence structure or single base changes) and the analysis of mitochondrial DNA.

High accurate automated fragment analysis

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Mapping as well as allele determination using STR markers is a fast growing field - concerning the applications (linkage analysis, forensics, prenatal diagnostics) and capacities in huge mapping projects. The success of gaining fast highly accurate results is limited by sample preparation, but even more by the electrophoresis and evaluation. We want to introduce an integrated system for fragment analysis securing high throughput as well as absolutely reliable and secure data showing two examples- one paternity case and one forensic investigation.

For the analysis we use common microsatellite markers. The PCR-primers pair used always consists of one primer labelled with biotin, the other with Cy5™. The use of these modified primers enables us to capture a defined amount of the PCR product on streptavidin coated AutoLoad™ combs (Pharmacia Biotech), preventing the loading of too small or big amounts of sample. The defined position of the sample on the solid support minimizes the risk of mixing the samples by misloading. The combs also greatly facilitate the loading process. They are simply inserted in the wells of an ALFexpress (Pharmacia Biotech) gel. Then the labelled strand is released and electrophoresis is performed.

Detection of the amplified fragments is recorded to a computer system where an implemented pre-linkage software automatically performs the genotyping.

EVALUATION OF HYBRIDISATION EQUIPMENT FOR USE WITH NON-ISOTOPICALLY LABELLED PROBES

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Various systems are available for the hybridisation of membranes produced for DNA fingerprinting and DNA profiling applications. In recent years non-isotopic probe labelling systems have been widely adopted eg, NICE™ probes (Cellmark). One of the advantages of these alkaline phosphatase labelled oligonucleotide probes is that the hybridisation is very rapid, typically 20 minutes at 50°C compared with 16 hours when using ³²P labelled probes. However, such short incubation times may not allow full temperature equilibration, which can result in incorrect stringencies, sometimes leading to inconsistent quality. Strict temperature control and thorough agitation of membranes are essential for high quality results. A wide variety of hybridisation equipment is now available, and several systems were assessed for use with non-isotopically labelled probes.

The following equipment was evaluated, Hybritube™ (Life Technologies), rotary ovens (Stratagene), NICE™ chamber (Cellmark Diagnostics), sandwich boxes and perspex hybridisation chambers (in either waterbaths or dry incubators). Standard membranes (K562 genomic DNA) were hybridised to NICE™ probes using standard procedures.

Hybritube™ and rotary ovens: These systems are advantageous for small scale users as reasonable quality results can be obtained using small volumes of probe. However, significant problems are often encountered where membranes overlap (due to the small diameter of the tube). These problems include secondary bands, high background, and poorly hybridising areas and are particularly prevalent with hybritubes due to the lack of agitation. Artifacts can be reduced by increasing agitation, solution volume and tube diameter.

Sandwich boxes and perspex hybridisation chambers: These systems produce superior quality results when used in waterbaths. In dry incubators significant problems can be encountered due to poor heat exchange and poor maintenance of correct temperatures. The most precise temperature control was achieved using the NICE™ chamber.

CDP-STAR™ AS A CHEMILUMINESCENT SUBSTRATE FOR USE WITH ALKALINE PHOSPHATASE LABELLED PROBES

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Non-isotopically labelled probes have been widely adopted for use in DNA fingerprinting and DNA profiling eg, NICE™ probes (Cellmark). The short operational time of the procedure is one of the main advantages, typically the whole hybridisation procedure is complete within seven hours. Although these procedures are rapid, exposure of the hybridised membranes to photographic film can be lengthy (typically 3-4 hours). Traditionally Lumiphos® 530 (Lumigen Inc) has been used as the chemiluminescent substrate. In order to reduce exposure times we compared the performance of CDP-Star™ (Tropix Inc) with Lumiphos® 530.

Standard membranes (K562 genomic DNA) were hybridised by the standard NICE™ procedure using the probes MS31 (D7S21), MS43A (D12S11), G3 (D7S22) and MS205 (D16S309). Membranes were either rinsed in a diethanolamine buffer and incubated in CDP-Star™ for 5 minutes at room temperature or sprayed evenly with Lumiphos® 530. All membranes were exposed to X-ray film at 30°C.

On CDP-Star™ treated membranes 200 ng of DNA could be detected after 30 minutes exposure and 50 ng of DNA after 1 hour exposure. Immediate exposure is not required as light emission continues for several days, although sensitivity of detection decreases with time, eg, 500 ng of DNA was detected after a 30 minute exposure, 3 days after CDP-Star™ application. On membranes treated with Lumiphos® 530, 200 ng of DNA could be detected after a 3 hour exposure. CDP-Star™ can be re-used, although sensitivity of detection does decrease. 100 ng of DNA was detected after a 1 hour exposure using CDP-Star for the third time.

Our findings indicate that the use of CDP-Star™ has advantages for those users with high throughput and for applications where speed is important. It allows overall process times to be reduced by several hours.

SIMPLE AND RAPID TYPING OF STRS ON AN AUTOMATED DNA SEQUENCER

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Short Tandem Repeats (STRs) have become the key markers in forensic DNA analysis. The higher sensitivity for small amounts of degraded DNA has also led to an increased success rate in the analysis of bone and hair shaft specimens. Analysis of the PCR products can be accomplished in a non-radioactive manner either by silver staining or by electrophoresis of fluorescent PCR products on an automated DNA sequencer.

We have evaluated the A.L.F. DNA sequencer (Pharmacia-Biotech) for separating 4 STR loci at high speed and with a high throughput. The STR loci D21S11, HUMvWF, HUMTHO1 and HPRT were selected on the basis of non-overlapping allele size distributions and identical PCR conditions (D21S11 and HUMvWF; HUMTHO1 and HPRT). One of the two PCR primers was labelled with fluorescein and the PCR products were run on a denaturing gel (8% Hydrolink, 7M Urea) with a well-to-laser distance of 19 cm. By the inclusion of two internal markers (123 and 375 bp), we were able to size the different alleles in 120 min. with a high accuracy (average 99.89%). The short electrophoresis time also allowed for re-loading of the same gel without a significant decrease in accuracy. This way, we obtained a throughput of at least 76 samples in one day.

A population study was done on 100 unrelated Caucasian individuals for the four loci. Seven alleles were observed for HUMvWF, HUMTHO1 and HPRT, and 13 alleles for D21S11. For HUMTHO1, two alleles were observed with a difference of 1 bp which could be differentiated on a gel with a well-to-laser distance of 19 cm. However, on shorter gels with a well-to-laser distance of 10 cm differentiation between these two alleles was problematic.

The expected heterozygosity for the four STR loci ranged between 85.7% (D21S11) and 72.5% (HPRT). The genotype distributions for all the four loci were in Hardy-Weinberg equilibrium. The combined power of discrimination (PD) for forensic identity testing was .999977 while the power of exclusion (PE) in paternity determinations was 0.9988.

A MODIFICATION TO THE "CHELEX" DNA EXTRACTION METHOD FOR CASEWORK SAMPLES.

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The use of Chelex resin in the extraction of DNA prior to PCR is a well established method. It is simple, quick and relatively inexpensive. However when dealing with forensic casework samples such as semen or saliva staining the DNA obtained is often too impure for successful amplification. In order to avoid time consuming organic extractions, an additional purification step using a "Microcon microconcentrator" has been introduced for certain categories of sample at this laboratory.

The membrane in the "Microcon" device enables the DNA to be retained whilst the buffer and any contaminants pass through during centrifugation. Following a wash with fresh buffer the DNA is eluted by inverting the column into a clean tube and centrifuging briefly.

Full details of the method together with the results of casework trials and validation will be presented. The conclusion will be drawn that this additional step is a rapid way to increase the success rate of casework PCR analysis.

VALIDATION OF THE APPLIED BIOSYSTEMS PRISM 377 AUTOMATED SEQUENCER FOR
FORENSIC ANALYSIS

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The validation of the Applied Biosystems Prism 377 Automated Sequencer was carried out in an attempt to increase the throughput for forensic STR casework and the National DNA Database. Gels for the 377 are 4% acrylamide with 6M urea. A 36 cm well-to-read run takes 1 hour 40 minutes; this is approximately 4 times as fast as a 24 cm well-to-read run on a 373A sequencer and gives improved resolution and the sensitivity is slightly improved. The sequencers and software have been validated for use on forensic samples. The precision of the results obtained from the 377 are comparable to that of results obtained from the 373A.

APPLICATION OF HLA-DR TYPING BY PCR-SSP TO
FORENSIC SAMPLES

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HLA genes show very high polymorphisms. These genes are one of useful human genetic markers in forensic purposes. To date one commercial kit for HLA-DQA1 typing has been validated for forensic investigations, and is being used routinely by many laboratories. The HLA-DRB1 gene is more useful in forensic practice than the DQA1 gene because it has a greater number of alleles. In this study we applied HLA-DR typing by PCR-SSP (sequence specific primers) to forensic samples and discussed the applicability of this simple and easy technique in forensic practice.

To define HLA-DR1 to DR10 by PCR-SSP, we designed 9 forward primers and 9 reverse primers, which were created by a little modification in previous publications (1, 2). For typing from extremely small amounts of DNA, we applied the nested PCR method to increase amplification sensitivity. Generic primers were generated from outside sequences to the nested primers used for PCR-SSP.

The PCR-SSP method could type DR1 to DR10 specifically (DR1, DR2, DR3, DR4, DR11, DR12, DR13, DR14, DR7, DR8, DR9, DR10). Furthermore this method could determine the suballelic types of DR2, DR4 and DR8, which have relatively high gene frequencies in Japanese (0.182, 0.228 and 0.133), when combined with the PCR-RFLP method (2). We could determine DR types from at least a total of 10ng DNA. Moreover, when we used the nested PCR, it was possible to determine the type using only 10pg of DNA. This nested PCR method made it possible to conduct typing using only 0.2ng of DNA extracted from hair (DR2, DR13). This system yielded a power of discrimination value of 0.90 and a chance of exclusion value of 0.71. This method may be a useful tool in forensic investigations.

References

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RAPID, NON-RADIOACTIVE DNA TYPING OF HLA-DRB AND -DPB LOCI FROM SINGLE HAIRS

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HLA genes exhibit a very high polymorphism level and provide a set of markers suitable for individual forensic identification. DNA typing from single hairs is possible with PCR techniques, also in shed hairs, where there is an atrophic root. We present here two rapid non-radioactive methods to simultaneously type HLA- DRB and -DPB loci on single shed hairs.

After DNA extraction, the exon 2 of HLA-DRB1 gene is amplified by consensus primers. The amplified DNA is subjected to a second round of amplification by a set of 19 nested allele- or group- specific primer pairs (PCR-SSP). The results are easily readable after agarose gel electrophoresis.

For HLA-DPB typing, 30 cycles of PCR are followed by additional 30 cycles, the latter performed by biotinilated nested primer pair. The amplified DNA is simultaneously hybridized to a set of 21 sequence-specific probes, immobilized on a membrane strip (INNO-LiPA, Innogenetics, Zwijndrecht, Belgium). Our results indicate that this approach provides a rapid, non-radioactive, highly discriminating test that may be of great utility in the field of human identification.

THE MVR-PCR APPROACH FOR THE TYPING OF THE MS32 LOCUS: USEFULNESS AND TECHNICAL PROBLEMS.

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Minisatellite variant repeat mapping by PCR (MVR-PCR) is a new approach to study variation in human DNA which analyzes interspersion patterns of variant repeats within minisatellite arrays.

MVR-PCR has been applied to the hypervariable human minisatellite MS32 (locus D1S8) which contains two major classes of variant 29 bp repeat units designated a-type and t-type, which differ by a single base substitution, and show highly diverse dispersion patterns within alleles.

In this study we describe our experiences in the analysis of the D1S8 locus using conventional agarose electrophoresis and non-radioactive hybridation as well as using automatic detection of fluorescent alleles (automatic sequencer). The technical problems encountered in the analysis are discussed. The usefulness of this method for forensic practice is also shown.

INTRODUCTION OF TWO NEW ELECTROPHORESIS GEL SYSTEMS FOR
SCREENING AND HIGH RESOLUTION IDENTIFICATION OF STRS UNDER
NATIVE CONDITIONS

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Two new types of polyacrylamide gels have been developed for optimized separations of short tandem repeats (STR) under nondenaturing conditions. They are polymerized on film support, measure 11 x 25 cm, and can easily be silver stained. The gels are run on a standard horizontal electrophoresis apparatus on a cooling plate. The alleles are identified with the help of sequenced allelic ladders of the respective systems.

The "screening gel" can be loaded two times with 48 samples, still exhibiting a resolution of Δ 4 bp. The total running time for 96 samples is 3 hours.

The "high-resolution gel" has the same size, however it is run along the long distance for 4 hours. In this gel a resolution of down to Δ 1 bp is obtained, even a base-line separation between allele 9.3 and 10 of the HumTH01 allelic ladder.

Separations of the HumVWA, HumTH01, HumFes, and the HumD21S11 system are shown on separation examples with both gel types.

With these gels, high sample throughput, good reproducibility, and high resolution for all of these STR systems can be achieved.

OLIGO-AP PROBES AND CHEMILUMINESCENCE :
SENSIBILITY FOR STAINS ANALYSIS

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The sensibility of the oligo-AP probes MS1 (Cellmark) and YNH24 (Promega) is studied on stains with respect to white cells count for blood stains, sperm count for sperm stains, and for volumes varying from 1 ul to 50 ul.

DETECTION OF STR LOCI USING AUTOMATED DNA ANALYZERS

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The use of PCR to amplify Short Tandem Repeat (STR) loci is being pursued by the forensic community as a method to analyze biological evidence. The ABI PRISM™ 377 DNA Sequencer and ABI PRISM 310 Genetic Analyzer can be used to automatically detect and analyze amplified STR loci. Loci are co-amplified with primers labeled with fluorescent dyes, run through a gel matrix, and automatically sized using an internal standard with either instrument. The throughput, resolution, and ease-of-use of both instruments were compared by investigating different STR loci. The results showed that protocols can be developed for both instruments to address the needs of laboratories requiring either high throughput or increased automation, while yielding reproducible results. Additionally, a panel of STR loci were identified that provide a high discrimination power when coanalyzed in an automated format.

AUTOMATION OF DNA SAMPLE PREPARATION FOR RFLP AND PCR USING THE FTA DNA COLLECTION SYSTEM, ORAL SWABS, AND THE ROSYS LABORATORY WORKSTATION

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Current Protocols for the sample preparation of DNA for Restriction Fragment Length Polymorphism (RFLP) and Polymerase Chain Reaction (PCR) require that the DNA be removed from the Matrix that is in (whole blood, blood stains, and/or tissue) and purified before the restriction or amplification of the DNA can be performed. The FTA™ DNA collection system, used in conjunction with the Rosys laboratory workstation, allows the direct and automated sample clean-up of DNA and the set-up of RFLP and PCR procedures. Samples of FTA™ Blood Stain Cards and Oral Swabs were processed on the Rosys workstation using traditional and experimental protocols prior to restriction or amplification. Results demonstrate that the FTA™ DNA collection system, and oral swabs, when used in conjunction with the Rosys Laboratory Workstation, provides the capability to process hundreds of DNA samples/day in an automated format.

STR TYPING WITHOUT DNA EXTRACTION USING AN INFRARED-BASED NON-RADIOACTIVE AUTOMATED DNA SEQUENCER

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The discovery of highly polymorphic short tandem repeats (STR's) has proven very useful for medical diagnostics, genome mapping and forensic analysis. The purpose of this research was to use an automated detection system using laser irradiation along with PCR primers labeled with an infrared (IR) fluorophore for detection of PCR amplified STR's from minute quantities of dried bloodstains. A mixture consisting of *Tth* enzyme buffer plus the forward and reverse primers was prepared for each bloodstain. This mixture was aliquoted into tubes containing a single bloodstained thread approximately 1mm in length. The forward primer pair of each contained a 5' end tail identical to a universal M13 forward primer sequence facilitating fluorescent labeling of the PCR products during amplification. The tubes were incubated at 92°C for 20 minutes in a Perkin-Elmer GeneAmp™ PCR System 9600. A second mixture consisting of *Tth* DNA polymerase, dNTP's and IR-labeled M13 forward primer was prepared and added to the tubes at the end of the high temperature incubation while tubes were still at 92°C. The reactions were cycled at 94°C for 20 seconds and 62°C for 1 minute for 27 cycles. Seven percent denaturing Long-Ranger gels run at 2000V (constant) were used to resolve the STR bands.

DNA was also extracted from bloodstains, saliva and hair samples and subjected to amplification. STR alleles were detected and displayed the same banding patterns from purified DNA as from bloodstains using either *Tth* or *Taq* polymerase. Multiplexing of three primer pairs in a single PCR amplification mixture was accomplished using *Taq* polymerase.

By using the infrared fluorescence automated DNA sequencer and *Tth* DNA polymerase, the polymorphic STR alleles were detected rapidly and efficiently from bloodstains without prior DNA extraction. This system combines infrared fluorescence chemistry and laser technology and thus eliminates the necessity of using radioactivity. Allelic bands are detected by incorporation of the M13 primer-fluorescent dye conjugate into the PCR products which eliminates the need for direct conjugation of fluorescent dye to STR primers. The STR alleles are displayed as familiar autoradiogram-like bands. It is also possible to determine gender and perform human 'ABO' grouping analysis using the automatic sequencer. By using multiplexing strategies it is possible to generate STR images of at least 6 loci for up to 120 individuals in less than a day.

G. CONVENTIONAL MARKERS

THE PI SYSTEM: GENETIC VARIATION, FORENSIC APPLICATION AND CLINICAL ASPECTS

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Alpha-1-antitrypsin (α 1AT), a major plasma protease inhibitor (PI), is one of the most polymorphic genetic marker in the human genome. On the basis of the protein sequence homology it has been recognized as one member of the large serpin gene family. The highly glycosylated protein is coded by a gene located on chromosome 14q32.1. More than 100 different variants have now been identified by either isoelectric focusing (IEF) of serum and/or sequence analysis. The most common phenotype is PI M which can be differentiated in 10 M-subtypes and some rare M-variants. At least 5 of the PI alleles (M1, M2, M3, S, and Z) occur at polymorphic frequencies in most populations. In addition to the extensive variation found in the protein by electrophoretic methods, further variation can be obtained at the DNA level, where a number of restriction enzymes reveal polymorphisms (RFLPs).

The considerable number of alleles, together with the highly reproducible techniques available for phenotyping and genotyping, make the PI polymorphism valuable for forensic investigations. In paternity testing α 1AT is a very useful serological marker which has a theoretical exclusion rate of 25%. Only in cases of low producing alleles errors may arise in precise subtyping by IEF if there are heterozygous carriers with one highly deficient and one normal allele. In such cases rare deficiency alleles can be differentiated by sequencing of the PCR amplified genomic DNA.

Genetic deficiency of α 1AT predisposes for the development of liver cirrhosis in early childhood, and chronic degenerative lung disease in early adult life. The most common α 1AT gene defect of clinical significance is the PI Z-mutation. PI Z individuals have only 15% of the normal α 1AT serum level and a 20-30 fold increased risk of developing chronic obstructive pulmonary disease. Heterozygotes of type MZ are thought to have a moderately increased risk but only if they smoke. In addition, there are a number of rare deficient PI M and PI null variants which are associated with diseases in the homozygous or compound heterozygous state.

A PARENTAGE TESTING STUDY REVEALING A POSSIBLE
DELETION AT THE PLG LOCUS

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Extended testing including parents and a sib of an alleged father was carried out when a single indirect exclusion (reverse homozygosity) of PLG (Locus 6q26-q27) was observed. A residual paternity index of greater than 2.7 billion was calculated based on tests of ABO, RH, MNSs, Kell, Duffy, Kidd, ESD, ACP, PGM1i, GCi, BF, F13B, F13A, D1S47, D2S44, D7S104, D7S467, D12S11, D17S79, and D18S27. The parents of the alleged father, who typed as PLG 2, were both PLG 1,2. This makes it unlikely that a "null" allele is present in the alleged father and the child in question, who typed as PLG 1. Testing of this family with an additional RFLP locus, probe/enzyme combination D6S132, SL1090/PstI appears to exclude the child who has a single band (1.92 kb) while the alleged father has two bands (1.89, 3.47). The mixture of the alleged father/child has three distinct bands (1.89, 1.92, 3.47). Based on data from Human Gene Mapping 11 (Cytogenet Cell Genet 58:295-336, 1991) locus D6S132 has been assigned to region 6q27. The finding of two apparent discrepancies in expected inheritance in a single individual at the same chromosomal region can best be explained by a deletion involving a single locus or two closely linked loci.

DETECTION OF THE ABO, GC, ACP and HLA-DQ α
POLYMORPHISMS AT THE DNA LEVEL USING PCR AND SSCP

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The formal genetics of the classical polymorphisms such as the ABO marker are well established via extensive World wide population studies, and gene frequencies are known for vast numbers of populations and subpopulation. The use of these polymorphisms as evidence in paternity testing and criminal case work has been accepted for decades by courts throughout the World. In step with the attainment of sequence information on the genes encoding classical markers it has become possible to design methods for genotyping based on DNA technology. We have developed methods for the detection of three of these markers, ABO, GC and ACP1, as well as for HLA-DQ α . The strategy is based on PCR amplification of gene segments containing allele specific mutation sites followed by an electrophoretic characterization of the separated DNA strands at low temperature (4-20°C) allowing the strands to attain an allele specific conformation (SSCP). The technique allows multiplexing, is simple and fast and is much less labour-intensive than classical phenotyping. Using the Pharmacia Phast System automatic electrophoretic separation and staining can be accomplished in 2-3 hours. In addition to the well known alleles (ABO*A and *B; GC*1F, *1S and *2; ACP1*A, *B and *C; HLA-DQ α *0101, *0102, *0103, *0201, *0301, *0401, *0501 and *0601) three new common ABO*O alleles (*O1, *O2 and *O3) were detected. Combined with the ability to detect heterozygosity with respect to the ABO*O alleles this increases greatly the informative value of the ABO polymorphism. The cumulative power of discrimination of these four markers is 99.93% and the theoretical chance of exclusion of non-fathers in paternity testing is 90%. Thus genotyping of "classical" structural loci by PCR and SSCP offers a valuable supplement or alternative to mutation prone VNTR and STR loci.

NUCLEOTIDE CHANGES IN VARIOUS VARIANTS OF THE COAGULATION FACTOR XIII A SUBUNIT GENE.

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We have examined nucleotide changes responsible for various variant alleles at the coagulation factor XIII a subunit (F13A) locus. Genomic DNAs were obtained from German and Japanese individuals with F13A variant bands in isoelectric focusing (IEF) gels. Each coding exon of 15 variant alleles was amplified by the polymerase chain reaction (PCR). The products showing heterozygous patterns in single strand conformational polymorphism (SSCP) analyses were then direct-sequenced or cloned into pGEM-T vector for sequencing. Thirteen variant alleles were found to result from single nucleotide changes and one allele was due to deletion of a single codon. The predicted amino acid changes were consistent with the mobility shifts of the variants in IEF. A silent allele seemed to be involved in the remaining variant because of discrepancy in the F13A genotypes between a mother and her child. In these samples a G to A transition was observed at the splice acceptor site of intron V, thus probably resulting in aberrant splicing of mRNA. Among the 14 variants with a single nucleotide change, a half of the mutation events was found to occur in the CpG sequences. Furthermore, mutations occurred at the four F13A sequence haplotypes (presented elsewhere in this abstract) in frequent order. An identical nucleotide change at the same site was observed in one German and one Japanese variant samples. The silent allele would result in the F13A deficiency if it occurs at the homozygous state. Further investigation will be required for outcome of the splicing mutation.

NOVEL POLYMORPHISMS IN THE CODING SEQUENCE OF THE COAGULATION FACTOR XIII A SUBUNIT GENE

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We have disclosed novel point mutations in the coding sequence of the coagulation factor XIII a (F13A) subunit gene. We identified nucleotide differences between the four alleles, *F13A*1A*, **1B*, **2A*, and **2B*, defined by isoelectric focusing. A T↔C change in codon 564 determines the A/B difference and a couple of changes in codons 650 and 651 result in the 1/2 difference. When we examined the allelic nucleotide changes for Caucasian DNA samples, we found novel sequence polymorphisms in exon 2, 5, 8, 12 and 14 in addition to the nucleotide changes mentioned above. Furthermore, an allele with a nucleotide change only in codon 651 was identified, the findings which demonstrate the allelic evolution from 650GTT·651GAG to 650ATT·651CAG through 650GTT·651CAG. In Caucasian samples (Finn, Russian and German) investigated in this study, two kinds of sequences of exon 14 was thus found to be involved in *F13A*2*. Sequence polymorphisms were thus defined in a total of 7 sites of the F13A coding regions. Each allelic sequence of the five polymorphic exons was named numerically to represent haplotype sequences. Of the predicted haplotype sequences (a total of 72 haplotypes), 18 haplotypes were identified in 64 Caucasian individuals. Interestingly, there is no disequilibrium between the combinations of the polymorphic sites, the findings implying that there are hot spots for recombinations in the F13A gene regions and that frequent recombinations equilibrate deviations of the haplotypic combinations.

ABO GENOTYPING WITH PCR

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Since Landsteiner discovered the bloodgroup antigens in 1902 more than 20 independent blood group systems have been identified by serological means. All together more than 250 different antigens are known to be present on human red cells. Their characterization has made rapid progress due to the developments in molecular biology. The application of sequence analysis has allowed the identification of gene products.

The molecular basis of ABO polymorphism has been known for about 30 years to be mediated via carbohydrate determinants and variant glycosyl transferases encoded by the ABO locus on the long arm of chromosome 9. A individuals express an N-acetyl-D-galactosaminyl transferase and the B encoded enzyme is a D galactosyl transferase. AB individuals express both and O individuals none of the two enzymatic activities.

The molecular cloning of the ABO specific mRNAs was carried out by Yamamoto et al. in 1990. Sequence analysis revealed that the A gene differs from B at 7 nucleotides which results in 4 amino acid substitutions. The O gene was found to be identical to the A coding sequence except for a single base deletion at nucleotide position 258 which leads to a frame shift mutation resulting in the synthesis of a functionally inactive transferase.

The minor subtype of A, the A2 transferase, differs from A1 by a single base substitution at nucleotide 464.

Based on the sequence data a PCR assay was developed. Single mutations can be detected either via allele specific PCR (ASP) or restriction enzyme digests of the PCR product.

The differentiation of A1, A2 and B gene products was carried out with two allele specific PCR reactions. For the first PCR amplification a 407 bp fragment was chosen which contained the variable nucleotide positions responsible for A1, A2 and A,B differentiation. The nested PCR was carried out with the respective allele specific oligonucleotide for the A2 and the B locus.

The more interesting DNA analysis is the genotyping of A and B individuals in order to demonstrate the possible presence of a O allele. In this case a new restriction site arises due to the deletion of a nucleotide. This can be demonstrated via enzymatic digestion, resulting in a PCR fragment shortage of 30 base pairs. The smaller fragment is clearly visible after agarosegel electrophoresis and silver staining. All A or B positive samples which show this additional smaller fragment after PCR and following restriction analysis are heterozygote null allele carriers.

The ability of PCR analysis to identify ABO genotypes should be useful for paternity testing and stain analysis.

INDICATION FOR A SILENT ALLELE OF PROPERDIN FACTOR B POLYMORPHISM (BF*Q0) IN A PATERNITY CASE

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The null allele BF*Q0 was first described by Weidinger *et al* (1979). Its frequency ranges close to 0.001 (Polesky *et al*, 1983).

In this paper we present further indication for a silent BF allele, in a case of disputed paternity.

Blood samples were drawn from four apparently healthy individuals (mother [M], child [C] and two alleged fathers [AF1, AF2]) and were investigated - according to the protocol used in 1992 in our laboratory - in the following genetic systems: ABO, RH, MNS, HP, IGHG, IGK, GC, TF, PI, BF, ACP, PGM1, AK, ADA, GPT, GLO, ESD, DQA1, D1S80.

AF1 (the husband of M) was excluded as the father of the child in MNS, HP, PI and GLO systems. Only a single indirect exclusion in the BF polymorphism was found in the case of the AF2 (AF2 was S, C was F): the man could not be excluded in the remaining 18 genetic systems. Biostatistical evaluation of combined data (BF + other systems) yielded a paternity probability of W = 96.73%.

A request of new blood samples - necessary for the second step of paternity analysis (including the HLA system) and for the assessment of factor B protein concentration and functional levels - was rejected by mother and AF2 (the lover of M), because the probability of paternity was "high enough" for them.

As new DNA polymorphisms were added to our protocol, in the years following 1992, we decided to test the hypothesis of the occurrence of a null BF allele. YNZ22, APOB, TH01, VWA and FES systems were determined, on previously extracted and stored DNA from M, C, and AF2. The final figure of probability of paternity was W = 99.995%: the existence of a BF null allele could be reasonably assumed.

When a single indirect exclusion occurs in a polymorphic protein system, in case of disputed parentage, more extensive studies are needed: in our opinion biostatistical evaluation plays an important role, especially if quantitation and pedigree analyses are not possible.

MOLECULAR GENETIC DIFFERENTIATION OF DUARTE-1 AND
DUARTE-2 VARIANTS OF GALACTOSE-1-PHOSPHATE
URIDYLTRANSFERASE

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Duarte (D) and Los Angeles (LA), which we call D2 and D1 respectively, are the most common variants of the polymorphic galactose-1-phosphate uridyltransferase (GALT). These variants are identified by faster electrophoretic mobility and different isoelectric focussing points. Isoelectric focussing of GALT has been used for the examination of galactosemic patients and in forensic hematology. An A to G transition at nucleotide 2744 leading to the N314D polymorphism has been detected in D1 and D2 variants (Podskarbi et al., *J Inherit Met Dis*, 17: 149, 1994) as well as in D and LA variants (Elsas et al., *Am J Hum Genet* 54: 130, 1994). However D1 and LA alleles exhibit about 110-130% of the normal GALT activity in erythrocytes, whereas D and D2 show only about 40-50%. In order to find out the difference between the D1 and D2 variants we investigated the GALT gene further by direct sequencing of PCR amplified segments of genomic DNA modified according to Gathof et al. (*Hum Genet* 1995, in press).

In all exons of the GALT gene no other mutation than N314D was found. In intron E we detected a G to A transition of nucleotide 1391, which was observed exclusively in D2 alleles. The presence of the mutation was also confirmed by family studies and a restriction enzyme. The mutation was not detected in D1 and normal alleles.

This study suggests that the decreased GALT activity in D2 variants is most probably related to the intron E mutation. This mutation may lead to an alternative splicing of exon 6 and possibly to a truncated protein.

PRELIMINARY STUDIES ON THE POPULATION SUBSTRUCTURE
FOR C1R PROTEIN IN CHINESE POPULATIONS

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For the purpose of revealing the genetic microdifferentiation among Chinese populations, genetic polymorphism of C1R in five Chinese populations (2 from northern China and 3 from southern China) have been investigated using IEF in polyacrylamide gels followed by immunoblotting. Two common alleles, C1R*1 and C1R*2, have been observed in all studied populations with allele frequencies of 0.4588-0.5676 for C1R*1 and 0.2619-0.4023 for C1R*2. Several other alleles, designated C1R*5, C1R*6 and C1R*7 have also been encountered. The distributions of allele frequencies for C1R in these 5 Chinese population samples suggested that there was no obvious population substructure for C1R protein within Chinese populations. Moreover, the allele frequencies exhibited bimodal distribution, much different from those in Caucasoid and Negroid populations in which the distributions were unimodal. Together, these results indicated that the C1R is one of the useful marker for the studies of the population and anthropological genetics.

This project was supported by the grant 39300072 from the National Natural Science Research Foundation of PR China and grant from the Chinese Medical Board of New York

A STUDY OF POLYMORPHISM OF ANTITHROMBIN III AT THE LEVEL OF BOTH PROTEIN AND DNA IN A CHINESE POPULATION

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The protein and DNA polymorphisms of human antithrombin III (ATIII) were studied by IEF and PCR techniques from a Chinese population. Products of two alleles within coding region of ATIII locus were observed by IEF and allele frequencies were: ATIII*1=0.9608, ATIII*2=0.0392. The PCR analysis of the fragment length polymorphism for ATIII 5' locus disclosed two alleles and their frequencies were: ATIII 5'*1=0.4118, ATIII 5'*2=0.5882. The results of estimation of pairwise haplotype frequencies and the linkage disequilibrium coefficient between ATIII and ATIII 5' loci indicated that two haplotypes (ATIII*1/ATIII 5'*2 and ATIII*2/ATIII 5'*1) were preferred. However, the D linkage value was not significantly different from 0 ($\chi^2 = 0.4178$, $P > 0.05$). This analysis between two ATIII polymorphisms revealed the incompatibility between the ATIII 5' FLP and the ATIII IEF variants, indicating that the ATIII 5'FLP is not the primary factor in the determination of ATIII IEF variants

This project was supported by the grant 39300072 from the National Natural Science Research Foundation of PR China and grant from the Ministry of Public Health of PR China.

Genetic polymorphisms of Alpha-1-Antitrypsin and Group-Specific Component in Spanish Gypsy population.

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Blood samples from 165 individuals of gypsy population in Spain, were investigated for genetic polymorphisms of Alpha-1-Antitrypsin (Pi) and Group-Specific Component (Gc), in order to apply them to paternity diagnosis and to genetic-population studies.

The analytical method used, was isoelectric focusing by means of widely known conventional techniques employed for these purposes. The statistical method was the usual for studies of paternity and comparison of populations, using the genetic frequencies of such markers.

These genetic markers were not studied before in Spain in gypsy population. The phenotypic distribution at all the loci was at Hardy-Weinberg equilibrium.

The allele frequencies were: $Pi^*M1=0.7727273$, $Pi^*M2=0.1151515$, $Pi^*M3=0.0333333$, $Pi^*S=0.0636364$, $Pi^*Z=0.0030303$, $Pi^*F=0.0121212$, $Gc^*1S=0.5$, $Gc^*1F=0.1575758$, $Gc^*2=0.3424242$.

In this population a high proportion of homozygosis stood out for the phenotype M1M1 (Pi). We also found a very high frequency for the allele F (Pi), the highest known among the frequencies studied up to the present time (1.2%).

These data may be used for paternity diagnosis and may contribute to studies about the gypsies in the world as an ethnic group, since it is a widely extended population, in all countries in all continents.

STUDIES ON BLOOD GENETIC MARKERS IN SOME MONGOLOID
POPULATIONS OF EASTERN SIBERIA

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To analyze the distribution of genetic markers and to examine the affinities with Japanese, we collected blood samples from indigenous populations of Mongoloid group in eastern Siberia and Russian Far East. They comprised 194 Evenkis (Ust-Nukja and other 2 villages), 110 Udeghe (Agzu and Krasnii Yar), 180 Buryats (Ulan Ude) and other about 100 admixtures with Russian whites. The field-work was undertaken in summer in 1991 and 1993.

Blood group typing except ABO was omitted due to the storage condition of the samples, and 10 or more serum proteins and erythrocyte enzymes were phenotyped.

Compared with the figures of Japanese, in general, the Evenki was characterized by having high HP*1, AHS*2, C1R*5, PGM*1A and low GC*2, TF*2, the Udeghe was characterized by high HP*1, AHS*2, ACP*A and low GC*1S, TF*2, PI*2, and the Buryat by high AHS*2, ACP*A, C1R*1 and low PI*2. A variant allele, PLG*M5, so far observed only in Japanese, was detected in the Buryats. A high GM*ST frequency was also disclosed in the present Buryats, as previously reported.

Together with the data derived from the literatures, Nei's genetic distance was calculated for each pair of northern populations. When a UPGMA phenogram was depicted, our Evenki was linked first to Even, and the Buryat to Mongol. Our Udeghe made a compact cluster not with the Evenki and Even but with the Buryat and Mongol, although they belong to a Tungus-speaking group.

PI SUBTYPING IN DENTAL PULPS

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PI subtyping was attempted on dental pulp tissues using isoelectric focusing and immunoblotting. The dental pulp tissue weighing 10-20 mg was macerated and mashed with 30 μ l distilled water. The amount of PI present in the extract was determined by rocket immunoelectrophoresis. The mean value of PI was 0.86 ± 0.12 mg/ml. 8 μ l of the extract was treated with 1 μ l 0.1M dithiothreitol and 1 μ l 0.1M iodoacetamide and subjected to polyacrylamide gel isoelectric focusing at pH 4.2-4.9. After run, focused proteins were transferred onto nitrocellulose membrane and reacted with rabbit anti-human PI serum and goat anti-rabbit IgG serum conjugated with alkaline phosphatase. The membrane was stained with β -naphthyl phosphate and Fast Blue BB salt. The results of PI typing coincided with the types obtained from serum samples of the same individuals. Subtyping was also possible in dental pulp tissues obtained from the teeth extracted and stored for 4 weeks at room temperature. PI subtyping by isoelectric focusing and immunoblotting is of practical use for medico-legal individualization of teeth.

GC SUBTYPING IN SERUM AND SEMEN AFTER NEURAMINI-
DASE TREATMENT

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GC subtyping in serum and semen was carried out using isoelectric focusing and immunoblotting after neuraminidase treatment. Serum was desialylated in 1/10 volume of 50 U/ml neuraminidase and diluted 1:150 with distilled water. Semen was diluted 1:10 with 6 M urea containing 0.5% BSA and incubated in 1/10 volume of 50 U/ml neuraminidase. The samples were subjected to polyacrylamide gel isoelectric focusing at pH 4.5-5.4. After run, focused proteins were transferred onto nitrocellulose membrane and reacted with goat anti-human GC serum and rabbit anti-goat IgG serum conjugated with alkaline phosphatase. The membrane was stained with β -naphthyl phosphate and Fast Blue BB salt. By neuraminidase treatment, the anodal bands of GC 1F and 1S in serum disappeared, but these bands in semen remained unchanged. The GC 2 type in semen exhibited 2 bands: the main GC 2 band and another fast band which focused at the position of the cathodal band of GC 1F. These seminal GC bands were unaffected by the enzyme digestion. It seems that the seminal GC is devoid of sialic acid.

SIMULTANEOUS FOCUSING OF PGM AND ACP PHENOTYPES
USING MINIATURIZED GELS AND 3-ELECTRODE TECHNIQUE

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A method for simultaneous focusing of Phospho-glucomutase₁-subtypes (PGM₁) and phenotypes of Acid Erythrocyte Phosphatase (ACP) is described.

Ulathin-layer self-prepared and precasted commercial gels were used for 3-electrode-technique. Using this method the gels are horizontally divided by placing a third electrode in the middle resulting in 2 gel halves with 5 cm interelectrode distance each.

Detection of the protein bands takes place for PGM₁ and ACP in the upper, and the lower gel half, respectively. In this way, disturbances caused by the considerable difference of the pH-optimum of the enzymes are avoided.

Despite the reduced interelectrode distance there are no doubts with regard to the differentiation of the phenotypes.

The conditions for gel handling, focusing and detection are described.

The method allows the focusing of about 40 samples per run for each marker. Consumption of chemicals and time are reduced considerably. The method has turned out to be an economical and reliable procedure for routine work.

ABO GENOTYPING BY MS-PCR

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ABO blood groups were determined by mutagenically separated polymerase chain reaction (MS-PCR). The products of two PCR reactions for the 258th and 523rd nucleotides of cDNA from ABO locus were used to distinguish A, B and O alleles. Two forward mutagenic allele-specific primers with different length for polymorphic site were paired with the same reverse primer in each PCR reaction. For the 258th nucleotide, the 196 bp fragment of PCR products was O allele specific and the 228 bp fragment was A or B allele specific. For the 523rd nucleotide, the 132 bp fragment of PCR products was B allele specific and the 112 bp fragment was A or O allele specific. The ABO genotypes were determined by the intersection of predicted alleles from these two PCR reactions. The PCR products were obtained using 10 ng of DNA in 50 μ L of PCR reaction, and electrophoresed in 3 % agarose gel. Results in this study were confirmed by PCR-RFLP ABO genotyping method. This technique provides a simple and reliable method in ABO genotyping.

Genotyping of ABO blood group system by polymerase chain reaction in mummies discovered at Taklamakan desert in 1912

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Introduction: The authors have already performed sex determination by polymerase chain reaction (PCR) in eight adult mummies and one child mummy which were discovered at Taklamakan desert in 1912 (Forensic Sci. Int. in press). Archaeologically, these mummies were human being in the seventh century, that is, more than 1,300 years before. In the present study, genotyping of ABO blood group system was performed by PCR using the nine mummies' samples.

Materials and methods: DNA was isolated from hair, muscle, bone (rib) or skin samples of every mummy. Forty PCR cycles were performed by the use of two different pairs of primer (Sasaki et al. Jpn. J. Legal Med., 1994, 48, 428); the one is 1f, 1r (1f: 5'-TGACACCGT-GGAGGATGTCCTGGT-3'; 1r: 5'-TGAAGTCTCGTTGAGGATGTCGA-3'), and the other is 3f, 3r (3f: 5'-CGCATGGAGATGATCAGTGACTTC-3'; 3r: 5'-GCTCGTAGGTGAAGGCCTC-CC-3') as follows: denaturation at 96 °C for 24 sec., annealing at 62 °C for 30 sec. and extension at 72 °C for 1.5 min. Primer 1f+1r amplified a 96-bp DNA fragment including nucleotide 258 (a single-base deletion site of the O allele) cDNA at the ABO gene locus, and primer 3f+3r amplified a 181-bp DNA fragment including nucleotide 700 (a single-base substitution site in the A and B alleles) of the cDNA. PCR products by primer 1f+1r were digested with Kpn-I, and those by primer 3f+3r were digested with Msp-I. After the digestion, the DNA fragments were electrophoresed on 12% polyacrylamide gel, and the bands on the gel were visualized by silver staining. The criterion of ABO genotyping are shown in Table. In the eight except for the child mummy, ABO phenotype was examined by absorption-elution method using hair samples.

Results and discussion: In all the nine mummies, ABO genotype was determined to be BO type, and in the eight adult mummies, ABO phenotypes were determined as B type. Judging from these results, in the eight adult mummies, ABO phenotypes estimated from ABO genotypes by PCR were consistent with those by absorption-elution method. In all of the hair and bone samples tested, ABO genotype as well could be determined. Therefore, the present study indicates that in archaeological study using DNA technology, hair, muscle, bone or skin samples can be rather available than expected.

Table: The criterion for ABO genotyping

Product	Restriction enzyme	bp	BB	BO	AB	AA	AO	OO
Primer 1f+1r	Kpn-I	96	+	+	+	+	+	
		69		+			+	+
		27		+			+	+
Primer 3f+3r	Msp-I	159	+	+	+			
		140		+	+	+	+	+
		22	+	+	+	+	+	+
		19		+	+	+	+	+

**PLATELET & NEUTROPHIL ALLOANTIGEN TYPING
USING SEQUENCE-SPECIFIC PRIMER PCR
AMPLIFICATION**

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A Polymerase Chain Reaction amplification technique has been investigated for use in phenotyping three medically important polymorphic loci. The technique uses sequence-specific primers to distinguish single base substitutions in the sequence of each diallelic locus. Two platelet alloantigen markers; HPA I & HPA III and one neutrophil alloantigen marker; NA, were studied.

The PCR typing technique provides an effective alternative to immunophenotyping for screening patients and predicting the development of alloimmune thrombocytopenia. The results obtained from this technique are in agreement with those obtained from serologically based tests. Investigations of mixed samples have provided evidence of the sensitivity of the technique in such situations. The use of mother-child pairs and paternity trios has also produced evidence of the reliability of the technique and its usefulness in problems of identity. The allele distributions of the three loci in Caucasians produce a combined DP of 92.5%, providing a potentially useful supplement to established polymorphic PCR systems.

**FREQUENCY DATA OF 11 CONVENTIONAL PROTEIN GENETIC
MARKERS IN THE RESIDENT POPULATION OF MADRID (SPAIN).**

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Between 1986 and 1994, six serum protein systems (Hp, PLG, ORM, Gc, Pi and Tf) and five red cell enzymes (GLO I, AcP, EsD, AHSG and PGM1) were studied in our laboratory mainly for paternity testing and also for other medico-legal purposes. The size of the sample ranges from 36 to 321 for each marker, the average size being 202 individuals per marker. No departure from Hardy-Weinberg equilibrium was observed except for ORM system ($X^2= 3.949$, 1 d.f.). Since just a few populations are typed for the whole marker set, ranges of frequency for each allele, according to the literature, were established. In general, our frequency data range within the normal variation interval of the Spanish population.

PLASMA PROTEIN MARKERS IN S.TOMÉ E PRÍNCIPE

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S.Tomé e Príncipe, once a Portuguese colony, is an archipelago mainly composed by two inhabited islands in the Gulf of Guinea, near the Equator. In spite of being poorly documented, the colonisation of these previously deserted islands begun in the XV century. The main demographic contributions to the nowadays population were slaves from the African nearby coast and some Portuguese.

Cord blood from unrelated newborns was sampled; phenotyping was performed by agarose gel electrophoresis (BF and TF types) or isoelectric focusing (GC and TF subtypes).

Results obtained up to now can be summarised as follows

BF (n=50; Fv and Sv stand for fast and slow variants)

F	S	Fv	Sv
0.520	0.410	0.060	0.010

GC (n=77; 1C stands for type 1 cathodal variant)

1F	1S	2	1C
0.708	0.156	0.110	0.026

For TF, 122 samples were typed, D frequency is 3.7%; among 54 individuals already subtyped, no C3 gene product was found.

DETECTION OF A SILENT GC ALLELE IN A DANISH MOTHER AND CHILD.

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In a case of disputed paternity a mother-child incompatibility was recently observed for the GC system. Phenotyping was performed by isoelectric focusing (IEF) followed by an immunoblotting procedure using specific anti-GC as the first antibody and an alkaline phosphatase conjugated second antibody. The inverse homozygosity (mother GC 2, child GC 1S) was verified on new samples. No indication of decreased protein levels were observed by inspection of the electrophoretic pattern. By conventional bloodgrouping and DNA examinations (ABO, MNS, RH, HP, PGM1, ACP1, GPT, ESD, GLO, D2S44, D4S139, D5S43, D7S21, D7S22, D12S11, D16S309, D1S80) a probability of more than 10.000 to 1 for maternity was found. The mother-child pair was further investigated by PCR/SSCP-analysis of a DNA fragment (186 bp) comprising two mutation sites specific for the alleles GC 1F, GC 1S and GC 2 in exon 11 of the GC gene. The amplified DNA from the sample of the mother was of type GC 1S,2 while that of the child was of type GC 1S. This result complied with the expected mode of inheritance and showed that the mutation responsible for the silent allele probably is located outside exon 11.

ABH-RELATED ANTIGENS PARTICIPATE IN THE SPERMATOGENESIS OF CAT AND RAT

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Although the ABO-related antigens have been constituting a major blood group system in the forensic science and are known to be present in secretory cells of the vertebrate species, the functional significance of the antigens is not yet elucidated. In the previous studies we have reported that the expression of anti-gens in the organs and tissues is closely related to the evolutionary stage of vertebrate species and the antigens affect the chemoreceptor systems in the taste buds of mammals.

In this study we examined the distribution of the antigens in the urogenital organs and salivary glands of cats and rats. The A antigen is expressed in the caput and cauda of the epididymis of both mammals, and LeX and Y antigens are recognized only in the caput epididymis of cats. These antigens are also expressed in the collecting tubule of the kidney and secretory cells of the salivary glands.

The results suggest that the ABH related antigens might involve the spermatogenesis of mammals and the species identification prior to ABO typing in forensic stain analysis might be indispensable, since the stains might be contaminated with the fluid from indoor pets.

SPECIES IDENTIFICATION BY ANALYSIS OF THE GENES FOR ABO BLOOD GROUP

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Since ABH substances are detected in various species, it is considered that some animals possess the genes homologous to those for human ABO blood group. We compared the nucleotide sequences among some mammals.

Three pairs of primers were designed according to the sequence of human ABO genes and the PCR amplification was performed using genomic DNA of human, Japanese monkey, pig, rabbit, hamster, rat, mastomys, mouse and gold fish. Each species showed different band pattern electrophoretically. In Japanese monkey three amplified fragments hybridized with corresponding PCR products from human DNA and the other animals gave the results differing among both the species and the region amplified.

Combination of the electrophoretic and hybridization analyses of the PCR products might be utilized for species identification.

H. POPULATION GENETICS

STATISTICAL ISSUES IN DNA PROFILING

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Estimation of the frequencies of DNA profiles at several loci is based on the product of the frequencies of the constituents of the profiles. Early challenges were based on suggestion of dependence of these constituent frequencies, and on the possible effects of population substructuring. Methods are now in place to meet these challenges. Attention in this talk will be given to more recent challenges having to do with the interpretation of mixed stains, and the provision of confidence limits on profile frequencies. The work to be reported has been performed by the author and by John S. Buckleton. Methods will be illustrated with results from current casework.

POPULATION GENETICS OF D1S80, HUMVWA31A AND HUMF13A1 FROM PORTUGAL AND GOA (INDIA)

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Short tandem repeat (STR) and VNTR polymorphisms provide a potential tool to forensic casework in paternity and criminal investigation. These polymorphisms are studied by polymerase chain reaction (PCR) and databases from reference populations are needed for forensic analysis.

We have studied 150 Portuguese samples and 132 samples from Goa (India), which had Portuguese presence during four centuries.

D1S80 and two STR loci - HUMVWA31A and HUMF13A1 were amplified by standard PCR protocols. Amplified products were simultaneously analysed by automated laser fluorescent DNA analyser. The genotype distribution and allele frequencies obtained from both populations were discussed. Comparison with other studied populations were also analysed.

This is the initial study for a better understanding of the genetic contribution of the Portuguese presence in Goa during four centuries.

GENETIC SUBSTRUCTURE AT THE STR LOCI HUMTH01 AND HUMVWA IN HAN POPULATIONS, CHINA

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In order to assess the genetic substructure in Chinese populations and analyse human evolution on the basis of DNA sequences, three populations of the Chinese Han nationality from northern and southern as well as western China, respectively, were directly sampled. The distributions of genotypes and allele frequencies at two STR loci HUMTH01 and HUMVWA in these population samples were investigated by amplified fragment length polymorphism technology. PCR products were analysed by horizontal electrophoresis in polyacrylamide gels and developed with silver staining. Allele determination in each STR locus was carried out by comparison with a well-defined allele ladder based on the number of core repeat units. The data obtained have been compared with a number of populations already investigated. Based on the allele frequencies of HUMTH01 and HUMVWA, genetic distances among populations were analysed and different phylogenetic trees were constructed accordingly. The distributions of allele frequencies for HUMTH01 and HUMVWA loci in the investigated populations suggest that there is an obvious population substructure. Some new informations concerning the analysis of genetic variability in man were obtained from these two new genetic markers.

Work supported by grants from the Alexander von Humboldt Foundation, Germany.

COLLABORATIVE STUDY ON THE POLYMORPHISM OF THE D1S80
LOCUS IN THE ITALIAN POPULATION.

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The "Gruppo Ematologi Forensi Italiano" organised a collaborative study on the polymorphism of the D1S80 locus to identify different alleles, evaluate the allele frequencies and possible allele frequency variations in the Italian population. Twenty Laboratories were involved and a total of 1720 blood samples of unrelated people of 19 towns were analysed. DNA extraction, primers, reference allelic ladder and amplification conditions were those of the Perkin-Elmer commercial kit.

Alleles. A total of 33 different alleles were observed, ranging from 14 to over 41 repeats, notably four of these alleles dropped in between the rungs of the allelic ladder. Five alleles were larger than 41 repeats and they were all grouped in the 42 repeat class. The most frequent alleles were the 18 (20.4%) and the 24 (37.8%). Following grouping of the 33 alleles into 7 allelic classes an homogeneity test among laboratories was performed. Only one laboratory showed allelic frequencies significantly different from those of the other laboratories. Actually this laboratory changed allelic ladder during the analyses and moreover, because of a specific purpose, selected a population sample with 4 autochthonous grandparents. Because of a possible bias, the data of this laboratory were excluded from further analyses.

Phenotypes. Of the 561 possible phenotypes only 146 were found. The most frequent phenotypes were: 18/24 (15.2%) and 24/24 (14.1%). No significant excess of homozygotes was observed. Phenotypic classes with expected frequency >4 were tested for Hardy-Weinberg equilibrium: the 51 classes did not differ from the expected frequencies.

Conclusion. Within the limits of this study, the 19 Italian cities population appeared homogeneous for the D1S80 locus and in Hardy-Weinberg equilibrium.

DNA-FINGERPRINTING IN BALEARIC POPULATIONS
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Previous genetic polymorphism studies with classical markers and with mtDNA have shown the genetic differentiation of the Balearic population in relation to those of the Iberian Peninsula.

Individual-specific DNA-fingerprintings obtained with the Jeffrey's 33.15 multilocus probe were studied in a sample composed of 40 autochthonous individuals from Majorca Island, 20 individuals from the Chueta population (descendants of Majorcan Jews) and 23 individuals from the Iberian Peninsula resident in Majorca.

We determined the number of bands (> 5 Kb) exhibited in the individual lanes, in order to establish the average number of bands in each population and the level of band-sharing in DNA fingerprints among unrelated individuals for each population. In the Chueta population, the figures were in the order of 9.9 bands, in the Majorcan population they were of 12.6 and in the people from the Iberian Peninsula of 15.6. The similarity index (Lynch M.,1990) was also calculated for each population and the results were in the order of 0.07 in Chuetas, 0.06 in the Majorcan group and 0.03 from the Iberian Peninsula. This is in accordance with the level of homogeneity that each population had shown in previous studies.

The average level of band-sharing may provide valuable information about the population structure, especially in inbred populations such as those from the Balearic Islands.

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HLA-DQA1 AND DIS80 SYSTEMS IN THE POPULATION OF VALENCIA (SE SPAIN)

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HLADQA1 and DIS80 systems were studied in a sample from the Valencia area (S.E. Spain).

DNA was extracted from blood using a phenol-chloroform procedures. PCR amplification of HLADQA1 and DIS80 was performed using comercial kits (Perkin Elmer). HLADQA1 Alleles were analyzed using dot-blot (HLADQA1 Amplytype -Perkin-Elmer) and the protocol provided by the manufacturer. The resolution of the allele HLADQA1*04 was achieved using DSCP (Barros et al. 1994) and SSCP (Orita et al. 1991).

For DIS80 typing was performed using discontinuous electrophoresis in miniaturized polyacrylamide gels (PhastGels, Pharmacia).

Both systems meet Hardy-Weinberg expectations. We have no found significative differences between our population and other spanish populations.

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GENE FREQUENCIES OF HUMAN POPULATIONS ALONG PYRENEAN CHAIN

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We have analized the relationship between different Pyrenean populations based on their hemotypological characteristics in order to stablish the differences among populations caused by migration, isolation phenomena or other populational events.

We intend to make a genetic map of the Pyrenean area taking into account the Pyrenean populations we have studied, the data of other Pyrenean population valleys and Basque populations.

We have selected those autochtonous Pyrenean populations on both sides of this mountainous range and Basque Country populations that have been studied for a set of blood groups (ABO, Rhesus, Duffy, Kell and MN).

The data have been submitted to the principal component analysis and has been expressed with two types of graphic representations. We have obtained a good correlation between the results on the two first principal components and the geographical situation of the populations we have considered.

**MULTIPLEX PCR AND AUTOMATED FLUORESCENCE DETECTION
OF FOUR TETRAMERIC STRS IN AN WEST-AUSTRIAN
POPULATION**

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The application of short tandem repeats (STRs) in forensic caseworks becomes more and more important, especially in cases where only a limited quantity or partly degraded DNA is available. We investigated four tetrameric STR loci simultaneously (HUMTHO1, HUMvWFA31/A, HUMFES/FPS and HUMF13A1) in the Western Austrian population. The polymorphism of the four systems was studied on a population of more than 350 unrelated individuals to establish large databases of the relevant population for routine individual identification and parentage testing. DNA was extracted from blood of more than 350 unrelated persons. Small amounts of template DNA (1-3ng) were typed by using fluorescently labelled primer, electrophoresed on denaturing polyacrylamid sequencing gels and detected on an automated DNA 373A Sequencer (Applied Biosystems). Unambiguous allele assignement was possible by comparison with a home-made ladder of 270 individuals (unpublished data). In all cases no deviation from Hardy-Weinberg equilibrium was observed. The results were compared to those reported for other caucasian populations. The four investigated polymorphic systems are reliable and sensitive markers to use in forensic casework.

**POPULATION AND FORMAL GENETICS OF THE STRs TPO, TH01
AND VWFA31/A IN NORTH PORTUGAL**

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Genotyping of a sample of unrelated individuals from N.Portugal was performed using manual systems and separate PCR amplifications for the following STRs:

TPO, repeat (AATG), intron 10 of thyroid peroxidase gene (2p23-pter)

TH01,repeat (TCAT),intron 1 of tyrosine hydroxylase gene (11p15.5-p15)

VWFA31/A,repeat (TCTR), intron 40 of von Willebrand factor gene (12p12-pter).

Allele frequencies (%) estimated up to now are:

TPO N=191

6	7	8	9	10	11	12	13
0.52	0.0	48.69	8.12	7.85	30.10	4.45	0.26

TH01 N=132 (alleles 10 and 9.3 were pooled)

6	7	8	9	9.3+10
18.18	20.08	16.67	19.32	25.76

VWFA31/A N=185

12	13	14	15	16	17	18	19	20	21
0.27	0.27	11.62	10.54	20.00	31.35	17.84	6.76	1.08	0.27

Genotype distributions are in accordance with Hardy-Weinberg expectations and mother-child pair analyses (nr. of pairs: 137, 102 and 136 for each system, respectively) show no deviations to mendelian rules.

POPULATION GENETICS OF THREE STRs: TH01, CSF1PO AND TPOX IN SOUTHERN SPAIN.

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A Southern Spain population has been investigated for three human short tandem repeat loci: tyrosine hydroxylase (TH01), c-fms protooncogene for CSF1 receptor gene (CSF1PO) and thyroid peroxidase (TPOX), using a polymerase chain reaction amplification (Promega®) followed by simultaneous gel electrophoresis and silver staining. Genotype frequencies were obtained from non related individuals of routine casework, paternity testing and staff. A statistical comparison with other Caucasian populations has been included. The usefulness of these STRs in conflictive paternity cases was analysed.

FREQUENCY MULTIVARIATE ANALYSIS OF LDLR, GYPA, HBGG, D7S8 AND GC LOCI IN 12 DIFFERENT POPULATIONS.

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The studied loci (LDLR, GYPA, HBGG, D7S8 and GC) are all of them comprised in the multiplex PCR comercial kit "Amplitype Polymarker Amplification and Typing Kit" (PM). We tried to compare frequency data of a Madrid population tested by us in the context of a set of several populations studied in the bibliography. A cosine genetic distance matrix and cluster UPGMA analysis, together with a plot of Principal Component Analysis was carried out in order to evaluate graphically the relationship among populations. The results classify the populations as could be expected on the basis of bio-historical reasons with the exception of a US hispanic sample located within the caucasoid group.

**WORLDWIDE DISTRIBUTION OF D1S80 POLYMORPHISM.
COMPARISON OF GENETIC DISTANCES AND CLUSTER ANALYSIS.**

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D1S80 system is one of the most extended polymorphisms in the field of legal medicine, due to its extreme variability. We have analyzed a worldwide sample of 48 populations - all of them typed for at least 28 alleles -, including a sample from Barcelona studied by us. A genetic distance matrix was calculated through a cosine algorithm and the results were clustered with the UPGMA procedure. In general, the samples are classified within three main groups corresponding to the main three racial groups (caucasoids, negroids and mongoloids). Oriental and black samples can be clearly distinguished from the caucasoid cluster. US hispanics are located in a intermediate position between the caucasoid and non-caucasoid cluster.

**ALLELE FREQUENCY DISTRIBUTIONS OF 3 STR-LOCI IN A
POPULATION SAMPLE FROM NORTHERN GERMANY**

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We studied the allele and genotype frequency distributions of the three tetrameric Short-Tandem-Repeat-(STR)-polymorphisms HUMVWA31, HUMFES/FPS and HUMF13B in a population sample of ca. 300 unrelated individuals from northern Germany. The aim of this study was to obtain population frequency data bases for these STR-loci. This is necessary for their use in forensic serology. Blood samples were obtained from routine paternity and stain analysis cases. The three STR-loci were amplified separately by polymerase chain reaction (PCR) and fragments were analyzed by high resolution native polyacrylamide gel electrophoresis and subsequent silver staining. The genotype distributions of all three loci were in accordance with Hardy-Weinberg expectations. Allele frequency distributions, heterozygosity rates and other parameters of forensic importance (e.g. discrimination index, mean exclusion chance) are similar to those found in other Caucasian populations.

ALLELE FREQUENCY DISTRIBUTION OF FIVE STR's LOCI: HUMARA, HUMPLA2A, VS17T, vWF AND F13B IN THE SPANISH POPULATION

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The use of short tandem repeat loci for population genetic studies, genetic analysis of inherited diseases and individual identification purposes requires the establishment of databases for each reference population. Some groups have reported a number of STR's loci that are amenable to polymerase chain reaction (PCR) analysis, as Edwards et al (HUMTH01, HUMARA), Polymeropoulos et al (HUMPLA2A), Kimpton (vWF) or Sharma (D21S11 or VS17T).

In the present study we have analysed variability at five STRs loci (HUMARA, HUMPLA2A, VS17T, F13B and vWF) in a representative sample of the Spanish population (200 unrelated individuals). Two distinct multiplex-PCR reactions were performed using the same amplification conditions (HUMARA/VS17T/HUMPLA2A and F13B/vWF). Both amplification reactions were separated in different lanes of the same polyacrylamide gel under denaturing conditions.

Statistical analysis demonstrate consistency with Hardy-Weinberg equilibrium for all these loci, except for HUMARA, which seems to exhibit an excess of homozygotes. In addition, using HUMARA in our conditions we found equivocal results regarding paternity analysis.

The results obtained are compared with other population data.

DETERMINATION OF THE ALLELE AND GENOTYPE FREQUENCIES OF LOCI HLA-DQA1, LDLR, GYPA, HBGG, D7S8 AND GC IN BOGOTA-COLOMBIA.

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The analysis of the allele and genotype frequencies for the reference population is necessary for studies of forensic identification. In Colombia, this work began with the determination of the allele and genotype frequencies of the loci HLA-DQA1, LDLR, GYPA, HBGG, D7S8 and GC of the whole blood samples obtained from 104 volunteer donors of blood banks, randomly selected and unrelated, who live in Bogotá.

DNA was extracted using the Chelex procedure. The Amplitype HLA DQA and Amplitype Polymarker PCR Amplification and Typing kits were used to detect the alleles in each system. HLA DQA allele frequencies for 1.1, 1.2, 1.3, 2, 3 and 4 were 0.16, 0.13, 0.05, 0.07, 0.28 and 0.31 respectively. In this sample 18 of the 21 possible genotypes were observed; The 1.2, 2., 1.3, 1.3 and 1.3, 2. were not represented. The allele and genotype frequencies of HBGG, LDLR, GYPA, D7S8 and GC are shown in the table 1. The distribution of alleles in our sample is very similar to those reported for hispanic populations.

Table 1. Allele and genotype frequencies of the HBGG, GC, LDLR, GYPA and D7S8 loci in the population of Bogotá-Colombia

LOCUS	ALLELE FREQUENCIES			GENOTYPES FREQUENCIES					
	A	B	C	AA	AB	AC	BB	BC	CC
HBGG	0.42	0.56	0.02	0.23	0.37	NR	0.35	0.05	NR
GC	0.26	0.25	0.49	0.06	0.14	0.27	0.06	0.25	0.23
	A	B		AA	AB	BB			
LDLR	0.6	0.4		0.38	0.44	0.18			
GYPA	0.7	0.3		0.52	0.33	0.15			
D7S8	0.63	0.37		0.41	0.45	0.14			

NR: not represented

ALLELE DISTRIBUTION OF THE AMPLIYPE PM COAMPLIFICATION SYSTEM IN A POPULATION OF NORTHERN ITALY

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The application of any genetic marker in Forensic casework requires a study to carry out a data base of the relevant population to have an accurate interpretation of the analysis results.

According to this guideline, in order to promote knowledge of the allele frequencies distribution in the Italian population and to realize an own data base for practical application in paternity testing and identification of stains, 5 different PCR-polymorphisms were investigated in a population sample from the Brescia area (Lombardy Northern Italy).

Particularly the system LDLR, GYPA, HBGG, D7S8 and GC were studied by using the Ampliype PM - PCR Amplification and Typing kit as described by the Perkin Elmer protocol. The preliminary results in a sample of about 80 unrelated, healthy individuals of the genotype frequencies shows a good accordance between observed and expected values for all the 5 systems.

The allele comparison with previous studies on Caucasians no significant difference.

CHARACTERIZATION OF YNZ22 LOCUS FOR FORENSIC PURPOSES. ALLELE AND GENOTYPE FREQUENCIES IN A POPULATION OF NORTHERN ITALY

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Highly polymorphic segments of the human genome containing VNTRs have been widely used to establish DNA profiles of individuals for use in forensics. One of the most studied of these system is YNZ 22 (HGM locus D17S30) This genomic region contains 70 base pair sequence tandemly repeated a variable number of times.

The Authors have investigated a sample group of unrelated subjects living in Brescia (Lombardy-North Italy) to verify the distribution of the YNZ 22 gene. The genomic DNA was amplified with Polymerase Chain Reaction; the amplified products were separated in agarose gel electrophoresis and visualized by ethidium bromide staining.

The phenotypes were identified by comparison with a molecular weight marker. The allelic frequencies found were compared with previous studies on Italian populations and the preliminary results confirmed the superimposed distribution. The most frequent allele were: allele 2 (0,34), allele 3 (0,15) and allele 4 (0,28).

D1S80 POPULATION DATA IN NORTH-EAST OF SPAIN

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Population data studies were carried out on 183 Caucasian individuals living in North-East of Spain. Allele and genotype frequencies for the D1S80 locus were determined by polymerase chain reaction (PCR) amplification, and subsequent electrophoresis in PAGE native and silver staining.

The determination of alleles was by comparison side by side with a 27 alleles ladder (Roche Molecular Systems). Twenty-two different alleles were detected. The most frequent alleles were 24 (0.3333) and 18 (0.2267), as in others compared populations. The genotype distribution in our population is in agreed with Hardy-Weimberg expectations.

Due to the high polymorphism degree, is a robust and suitable system for forensic applications and paternity testing.

POPULATION STUDY FOR THE HLA-DQA1, LDLR, GYPA, HBGG, D7S8 AND GC LOCI IN NORTH-EAST OF SPAIN

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A population database of the loci HLA-DQA1, LDLR (low density lipoprotein receptor), GYPA (glycophorin A), HBGG (hemoglobin G gammaglobin), D7S8, and GC (group-specific component) has been established for forensic purposes.

This polymorphism has been analysed in a population sample of 210 Caucasian living in North-East of Spain (Catalonia). 195 individuals for HLA-DQA1 and 146 for LDLR, GYPA, HBGG, D7S8 and GC loci.

The typing was carried out with two commercial kits developed by Roche Molecular Systems- Amplitype HLA DQ α [®] and Amplitype PM[®]- supplied by Perkin-Elmer. The amplification was carried out in a Linus Dualcyler thermocycler and the detection was by reverse dot-blot on a nylon membrane strip.

All the 21 possible genotypes with this system for the HLA-DQA1 locus have been observed. The most common allele was HLA-DQA1-A4, and the least frequent allele was HLA-DQA1-A1.3. About the PM loci, all genotypes and alleles were observed, excepting the CC genotype in the HBGG locus.

All loci meet Hardy-Weimberg expectations. We have not found significative differences between our population and others caucasian populations.

We are using these systems in our laboratory for biological evidences typing and paternity testing.

ALLELE FREQUENCIES OF VWA31A, FESFPS, FXIII A1 AND D21S11 IN AN ITALIAN POPULATION SAMPLE

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Four highly polymorphic Short Tandem Repeats were recently chosen in our laboratory as suitable candidates to our forensic routine. Due to their small size of alleles (100-250 bp), these markers are very useful in this context. However, prior to their practical employ in identification tests and parenthood analysis, a database of enough individuals from reference populations is needed. To comply with this general recommendation, we collected blood samples from over 100 unrelated individuals (Central and Southern Italy) and analysed them following standard PCR protocols with minor modification. The amplified products were separated in denaturing polyacrylamide gels using an automated laser fluorescent apparatus (A.L.F.). The following allele frequencies were calculated:

System						
vWA31	Allele	14	15	16	17	18
	Frequency	0.10	0.14	0.20	0.26	0.20
FESFPS	Allele	8	10	11	12	13
	Frequency	0.01	0.24	0.41	0.28	0.05
FXIII A1	Allele	3.2	4	5	6	7
	Frequency	0.08	0.07	0.25	0.25	0.27
D21S11	Allele	29	30	31	32	33.2
	Frequency	0.17	0.21	0.30	0.05	0.12

ANALYSIS OF THE APO B AND F8VWF ALLELE FREQUENCIES IN THE CROATIAN POPULATION

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A polymerase chain reaction based DNA typing method has become increasingly important in the field of forensic science and forensic medicine.

We reported allele frequencies in Croats for the loci APO B and F8VWF. The allele frequencies were determined using PCR for 50 unrelated Croatian Caucasians. The observed heterozygosity was 70 % for the APO B locus and 56% for the F8VWF locus.

These results were compared with findings observed in another Caucasian (Italian) population sample.

Relative to the frequencies previously described for these two loci in the neighboring Italian population, only for the F8VWF locus a highly significant difference was found.

These results may be useful for population and individual identification studies.

Key words: PCR, individual identification, DNA polymorphism, APO B, F8VWF

STR ANALYSIS - HUMTHO1 AND HUMFESFPS FOR FORENSIC APPLICATION

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Dimeric, trimeric and tetrameric short tandem repeats (STRs) are highly polymorphic markers investigated by PCR and introduced into forensic laboratories.

Before their routine application in identification and parentage testing, a database from various population samples must be established.

Allele and genotype frequencies for two of these polymorphic markers - HUMTHO1 (TC 11) and HUMFESFPS (FES), were determined in a Portuguese population sample. We also investigated these two loci on human hairs and forensic stains. Samples were co-amplified using PCR, and detected by denaturing polyacrilamide gel electrophoresis in DNA sequencer.

The forensic efficiency of the two STRs was estimated by the combined discrimination index and the chance of exclusion. The allele frequencies profiles obtained were consistent with those previously reported in Caucasian. Our data meet the Hardy-Weinberg criteria.

This two STR loci are useful genetic markers for discrimination between individuals to forensic casework.

PROFILING THE NORTH-EAST ITALIAN POPULATION BY FOUR HIGHLY POLYMORPHIC PROBES

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A sample of the population living in the North-East Italy was analysed by Southern blot analysis to generate a *HinfI* database of the loci D1S7, D7S21, D12S22 and D7S22. The K562 cell line DNA was used as reference standard and 1 Kb ladder as molecular weight marker. The alleles frequency distribution was estimated adopting the "fixed bin" method (with bin of 0,1 Kb).

The results are the following:

locus	gametes	heterozygosity	range of the alleles
D1S7	664	0,9879	0,4-27,3 Kb
D7S21	680	0,9529	2,5-19,5 Kb
D12S11	678	0,9471	1,7-13,3 Kb
D7S22	664	0,9518	1,3-17,1 Kb

No discrepancy from the Hardy-Weinberg law was observed. Our data basically do not differ significantly from those reported for the caucasian population.

ALLELE FREQUENCIES OF HLA-DQ α , LDLR, GYPA, HBGG, D7S8 AND GC IN THE RESIDENT AND AUTOCHTHONOUS POPULATIONS OF THE BASQUE COUNTRY

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Before a new marker system can be introduced in forensic casework, a population database for the relevant population must be established for statistical evaluation of forensic evidence. In conformity with this guideline, the allele and genotype frequencies of 6 different PCR-systems (HLA-DQ α , LDLR, GYPA, HBGG, D7S8 and GC) were determined for a Basque Country resident and autochthonous populations.

DNA was extracted by the chelex method. The amplification by PCR and the typing by reverse dot-blot methodology using allele specific oligonucleotide (ASO) probes were performed according to the AmpliType HLA DQ α and AmpliType PM PCR Amplification and Typing kit protocol provided by the manufacturer (Perkin-Elmer Cetus). All samples carrying the allele 4 in the HLA-DQ α system were digested using 2 restriction enzymes (Rsa I and Fok I). The digested fragments were separated by vertical discontinuous polyacrylamide gel electrophoresis using the Tris-chloride/Tris-glycine buffer system and detected by silver stain.

In all 6 systems, the genotype frequencies are in accordance with Hardy-Weinberg expectations. The 6 investigated polymorphism systems are reliable and sensitive marker systems that are well suited for use in forensic casework.

STUDY OF HUMACTBP2 STR POLYMORPHISM, PERFORMED BY PCR AND AUTOMATED LASER FLUORESCENCE (ALF) SEQUENCER.

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In the last years the distribution of Polymerase Chain Reaction (PCR) polymorphisms has been analyzed in different populations. Here we show the results of a survey of the tetra-hexa-nucleotide repeat locus (Short Tandem Repeat) HUMACTBP2 (SE33) in samples from people living in Catalonia Country (n=153). It is a polemic length and sequence polymorphism because a high number of alleles have been described, some of which may vary by as little as 1 base, moreover its AT-rich sequence may have anomalous migration rates in different electrophoretic systems. Nevertheless as it is one of the most powerful PCR markers, we describe the experience in our laboratory.

The study was carried out with blood sample donors from the "Hospital Clínic" Blood Bank, and with samples (blood or hairs) of unrelated paternity cases and university employees. PCR amplification of ACTBP2 was accomplished by the method and primers described by Polymeropoulos M.H. et al. with fluorescein labeled primers. Genotypes were analyzed in denaturing 6% polyacrylamide gel electrophoresis, using Automated Laser Fluorescence (ALF. Pharmacia). Cocktail allelic ladders of sequenced known alleles were used.

The phenotype distribution and gene frequencies obtained are discussed. Thirty two alleles, ranging in size between 210 and 350 bp. were found. Calculation of the phenotype distribution indicates no deviation from the Hardy-Weinberg equilibrium. Theoretical values of forensic suitability were calculated (IH= 92; PD= 0.99; CE= 0.89).

HUMACTBP2 is an interesting polymorphism that needs standardisation of the experimental condition, in order to obtain reproduced allele identification. Its high degree of variability could makes it an extremely useful marker in forensic genetics diagnosis. The use of sequenced allelic ladders is very important. At the moment, it may be used as an internal marker in individual laboratories.

AYMARA AND QUECHUA AMERINDIAN POPULATIONS CHARACTERIZED BY HUMTH01 AND vWA STR POLYMORPHISMS.

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Microsatellites, defined by inherited short runs of tandemly repeated nucleotide sequences (STR's) are a rich source of highly polymorphic markers in human population, easily detected by PCR. We report DNA typing of two tetrameric repeats: HUMTH01, with the AATG core sequence repeat, mapped to chromosomal locus 11p15.5., and vWA with the (TATA) repeat, localized in chromosomal 12 (12p12-12pter).

Quechuas and Aymaras are two ancient precolumbian ethnic groups in South-America. Quechua samples (n= 68) were collected in Dalence (Oruro), and Aymara samples (n=80) in Pacajes and Murillo provinces (La Paz). DNA samples were obtained from single hairs.

PCR amplifications were accomplished with fluorescein labeled primers. Genotypes were analyzed in denaturing 6% polyacrilamide gel electrophoresis, using Automated Laser Fluorescence.

Allele and genotype frequencies have been determined. The analysis demonstrated 3 alleles and 6 genotypes in Quechua population and 4 alleles and 8 genotypes in Aymara population for the HUMTH01 locus. For the vWA polymorphism we observed 5 alleles and 12 genotypes for Quechuas, and 6 alleles and 10 genotypes for Aymaras.

Calculation of the phenotype distribution indicates no deviation from the Hardy-Weimberg equilibrium.

The allelic frequencies, compared to other populations, shows a high degree of variation between amerindian and caucasoids.

SUITABILITY OF THE HUMTH01, CD4, AND vWA STR POLYMORPHISMS FOR LEGAL MEDICINE INVESTIGATIONS IN THE POPULATION OF CATALONIA (NORTH-EAST SPAIN)

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The polymerase chain reaction (PCR) method has been applied to amplify three short tandem repeat (STR) loci: HUMTH01 (TC11, 11p15.5), CD4 (12,p) and vWA (12p12-12pter). The study was conducted on a Catalonian population sample (TC11 n=161; CD4 n= 117; and vWA n=122).

PCR amplifications were accomplished with fluorescein labeled primers. Genotypes were analyzed in denaturing 6% polyacrilamide gel electrophoresis, using Automated Laser Fluorescence (ALF. Pharmacia).

Allele and genotype frequencies have been determined. The analysis demonstrated 8 alleles (5 common alleles and 3 rare variants) and 18 genotypes for the HUMTH01 locus, 6 alleles and 13 genotypes for CD4 locus and 7 alleles (6 common alleles and 1 rare variant) and 20 genotypes for the vWA locus.

Calculation of the phenotype distribution indicates no deviation from the Hardy-Weimberg equilibrium. Theoretical values of the discrimination power of each locus on criminalistics and paternity testing were calculated (IH= 79.5; 63.2; 77.8; PD= 0.92; 0.87; 0.93; CE= 0.58; 0.47; 0.61).

Comparison of allele distribution between catalans and other populations are shown.

The high degree of variability of each PCR polymorphism makes these markers very useful in forensic genetics diagnosis.

POPULATION AND FORMAL GENETICS OF THE STR SYSTEM MBP -
LOCUS B - IN NORTH PORTUGAL

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In human myelin basic protein gene (18q22-qter) two intron STR polymorphisms have been described: locus A and locus B. In this work we present population and forensic validation data for B locus (repeat: ATGG).

Genotyping of a sample of unrelated individuals from N.Portugal was performed using a manual system after PCR amplification. Allele frequencies (%) estimated up to now are (n=51):

7	8	9	10	11	12	13
35.29	0.0	2.94	10.78	40.20	9.80	0.98

Genotype distribution conforms Hardy-Weinberg expectations and the results of mother-child pairs analysis (n=37) do not show deviations to mendelian rules.

ALLELE FREQUENCY DISTRIBUTIONS OF FIVE LOCI (LDLR, GYPA, HBGG, D7S8 AND GC) IN A JAPANESE POPULATION

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The distribution of allele frequencies of 5 genetic markers (LDLR, GYPA, HBGG, D7S8, GC) were simultaneously investigated on DNA samples from 257 Japanese individuals using the Amplitype PM-PCR Application and Typing Kit (Perkin Elmer). The allele frequencies were calculated at: LDLR A = 0.146, LDLR B = 0.854; GYPA A = 0.582, GYPA B = 0.418; HBGG A = 0.372, HBGG B = 0.628; D7S8 A = 0.621, D7S8B = 0.379; GC A = 0.245, GC B = 0.502, GC C = 0.253. The population data at each locus fitted the Hardy-Weinberg law. The cumulative probability of paternity exclusion at the 5 loci was 0.690 and the cumulative probability of discrimination at the 5 loci was as high as 0.992. The present PM-PCR technique permits combined genotyping of LDLR, GYPA, HBGG, D7S8 and GC with one application of DNA samples. The method is simple, rapid and therefore recommendable to be routinely used for individual identification as well as paternity testing.

FREQUENCY DATA ON THE LOCI LDLR, GYPA, HBGG, D7S8 AND GC IN A POPULATION RESIDENT IN MADRID CITY (SPAIN).

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The allele and genotype frequency distribution of five loci amplified by PCR have been studied in a population of 217 individuals from Madrid city (Spain). The following loci were studied: low density lipoprotein receptor (LDLR), glycophorin A (GYPA), hemoglobin G gammaglobin (HBGG), D7S8, and group specific component (GC). No departure from Hardy-Weinberg equilibrium was observed in any of the systems and some statistical parameters of forensic interest were also calculated, e. g. heterocigosity index, power of discrimination and chance of exclusion.

According to the results obtained, the loci studied can be successfully used in individual identification and paternity testing.

GERMAN DATA ON THE PCR BASED LOCI HUMVWA, HUMFES, HUMTH01, HUMF13B AND D1S80

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A population study was carried out on Caucasians from Northrhine-Westphalia using the short tandem repeat (STR) systems HumVWA (n=548), HumFES (n=523), HumTH01(n=575), HumF13B (n=301) and D1S80 (n=378). No deviations from Hardy-Weinberg equilibrium could be observed. These results were compared with other population studies from Germany

D1S80 Alleles In Wielkopolska Population (Poland)

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Allele frequencies for the VNTR locus D1S80 were determined in the population of Wielkopolska (Poland) using PCR reaction and PAGE with silver staining. In the population of 177 unrelated individuals 18 nominal alleles were detected and the allele distribution met Hardy-Weinberg expectations. Frequencies of the D1S80 alleles were compared with the corresponding data for other populations.

A STUDY ON THE SHORT TANDEM REPEAT SYSTEM ACTBP2 (SE33) IN AN AUSTRIAN POPULATION SAMPLE USING NON-DENATURING ELECTROPHORESIS AND A SEQUENCED ALLELIC LADDER

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Although ACTBP2 (Se33) is one of the most widely used short tandem repeat systems, comparison between different laboratories was difficult as, due to the complexity of the system, no standardized allelic ladder was at hand. In the present study a sequenced allelic ladder which might help overcome this problem was used. It was recently made available by the Institut for Rechtsmedizin, Münster, Germany (A. Möller, B. Brinkmann 1994, Int J Leg Med 106: 262-267). In 100 unrelated Austrians analyzed by horizontal, non-denaturing polyacrylamide gel electrophoresis 23 alleles were found. The heterozygosity was 0.95, the mean exclusion chance 0.87, and the discriminating power 0.98. The allelic distribution was in accordance with Hardy Weinberg expectations.

HLADQA1 ALLELE FREQUENCIES IN THE WORLD USING A BILOT TO VISUALISE ALLELES AND POPULATIONS SIMULTANEOUSLY

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Population genetic studies show differences within and between racial groups for DNA-polymorphism's. Many statistical tests are available to test for population homogeneity when comparing different population samples for a particular polymorphic locus. We compared the allele frequencies of the HLADQA1-locus in a Dutch Caucasian population sample to the published frequency data from many other populations. The populations and alleles are visualised in one single plot: a biplot. This type of plot is related to principal components analysis and has many variants which are increasingly used in anthropometric and genetic analysis. A nice feature of the biplot is that it visualises both alleles and populations in the same plot and it allows a quick overview of the distances between populations and deviant allele frequencies.

EFFICIENCY OF 6 STR-SYSTEMS, HLADQ α AND THE POLYMARKER SYSTEM (PM) IN PATERNITY TESTING

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In order to test the efficiency of PCR based DNA systems in paternity testing the following 12 PCR systems were selected: the 6 STR systems SE33, THO1, D21S11, F13A1, VWA and FES, HLA DQ α and the 5 PM systems LDLR, GYPA, HBGG, D7S8 and Gc. 32 cases of disputed paternity (16 nonexclusion and 16 exclusion cases) were examined with the 12 PCR systems so far and the results were compared with the efficiency of the 4 single locus VNTR-systems MS43A, MS31, G3 and yNH24 routinely used in paternity cases since 1991.

DNA-Amplification and typing of HLA DQ α and the PM systems were performed according to protocol of the manufacturer. The fluorescently labeled PCR products of the STR systems were analyzed and detected on an automated DNA sequencer (ABI GeneScan 672).

The PCR systems showed considerable differences in their power of exclusion as well as in their positive evidence for paternity. With the exception of the STR system SE33 all PCR systems studied have a smaller efficiency than each of the 4 single locus VNTR-systems. The PCR systems are useful and reliable marker systems for paternity testing but they cannot replace the high polymorphic single locus systems. Only the STR system SE33 is comparable with a the single locus VNTR-system. For paternity testing at least 12 PCR systems must be applied to obtain a comparable efficiency as with a battery of 4 single locus VNTR-systems.

SWISS POPULATION DATA FOR THE STR SYSTEMS HumVWA, HumF13A1 AND HumFES

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STR loci are ideal marker systems for genetic linkage analysis, genetic mapping as well as for human identification and paternity analysis. The forensic application of any genetic marker system requires a sufficiently large data base of the relevant population. This study presents allele frequency data of the 3 STR systems HumVWA, HumF13A1 and HumFES for a Swiss population sample of at least 300 unrelated individuals.

DNA samples were amplified using fluorescence-labeled primers. F13A1 and VWA were coamplified. Analysis and detection were performed on an automated DNA sequencer (ABI GeneScan 672). The following allele frequency ranges were found for the 3 STR systems:

- HumF13A1: 13 alleles could be identified with a allele frequency range between 0.1% and 34 %.
- HumVWA: 9 alleles could be identified with a allele frequency range between 0.2% and 27.1%.
- HumFES: 6 alleles could be identified with a allele frequency range between 0.6% to 37.8 %.

The allele frequencies are similar to those observed in other Caucasian population samples. All 3 investigated STR systems are reliable and sensitive marker systems that are well suited for use in forensic casework.

STUDIES ON THE HUMTH01 AND HUMVWA POLYMORPHISMS IN A SOUTH WEST GERMAN POPULATION

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The genetic variation at loci containing short tandem repeats (STR's) have been shown to be highly informative for purposes of paternity testing and criminal investigations. The application of any new genetic marker system requires a sufficiently large data base of the relevant population for reliable calculations of probabilities.

For this reason we have determined the genotype and allele frequencies of TH01 and VWA in a population sample of more than 250 unrelated German nationals residing in the Heidelberg area. Furthermore a sample of 40 true family trios were analysed.

We have employed electrophoretic separation of the TH01- and VWA-PCR products on horizontal discontinuous polyacrylamid gels with subsequent silver staining. The various alleles were identified by side-to-side comparison with an allelic ladder.

7 alleles (5,6,7,8,9,9.3,10) were observed for TH01 with calculated frequencies in the range 0.0057-0.3240. The VWA system showed 8 alleles (13-20) with frequencies range between 0.006 and 0.244. For both markers the observed genotype frequencies are in good agreement with the expected distribution under the Hardy-Weinberg law. The heterozygosity and the discrimination power were calculated. Allele frequencies in Heidelberg, South West Germany are consistent with those reported for other German and European populations.

In addition, the genetic stability of TH01 and VWA was tested. In the small sample of families no new mutations could be found.

SPANISH POPULATION DATA ON SEVEN LOCI (D1S80, D17S5, HUMTH01, HUMVWA, ACTBP2, D21S11 and DQA1): EQUILIBRIUM AND INDEPENDENCE.

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Amplification by the polymerase chain reaction (PCR) and subsequent electrophoresis of the amplified products have become useful approaches for typing variable number of tandem repeat (VNTR) loci and others loci studied by inverse hybridation by dot blot. These markers are useful for paternity and forensic identity testing.

For the genetic markers, such as STRs, in identity testing it is desirable to collect allele/genotype data from relevant populations, so that the forensic scientist can provide estimate of the rarity of a genetic profile.

This study provides frequency data for four STR loci, D1S80, D17S5 and the HLA DQA1 in a Spanish population sample (n=120) of caucasians from the south of Spain (Andalucía). For Hardy-Weinberg equilibrium we used: 1) A goodness-of-fit chi-square test based on total number of heterozygotes and homozygotes. 2) A likelihood ratio test comparing the frequencies of each specific genotype with their expectations under the Hardy-Weinberg assumptions; and 3) The Guo-Thompson's (1992) exact test with 1000 replications. For linkage equilibrium we did: 1) Inter-class correlation test (Karlin et al.1981), and 2) Test of the observed variance (Sk_2) of the number of heterozygous classes.

The results show that all loci but one (ACTBP2) do not depart from equilibrium, and that there is an independence between all of them. We discuss these results, the plausible reasons for the departure from HWE for the ACTBP2 locus. The data demonstrate that these markers can be useful for identity testing.

TWO HIGHLY POLYMORPHIC VNTR-LOCI D5S110 (LH1) AND D4S139 (PH30): ANALYSIS, FORMAL AND POPULATION GENETIC DATA.

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The most informative genetic markers for discrimination among individuals are the highly polymorphic variable number of tandem repeats (VNTR) loci. The technology to use these markers is the well-characterized restriction fragment length polymorphism (RFLP) analysis. Both loci D5S110 and D4S139 are compatible with the restriction enzyme HaeIII which can be used with other known VNTR loci (D2S44, D10S28, D17S26). In this study we present:

1. Experimental technique
 - DNA-isolation, -digest, electrophoresis, detection
2. Formal and population data
 - from 500 individuals involved in paternity cases from SW-Germany
 - from 30 big families (three generations) from NW-Portugal
 - from 500 unrelated persons from SW-Germany
 - allele frequencies are analysed by the classical rounding method. All data are available in tabulated and graphical form.
 - both loci reveal more than 40 alleles ranging generally between 1000 and 12000 base pairs. Heterozygosity is greater than 90% for each of the two loci.
3. Descriptive statistics
 - common statistical parameters, arithmetic mean (AM), standard deviation (SD) and coefficient of variation (CV) were checked using serial intergel measurements of the two K562 DNA fragments for both loci.

Summarizing all results we conclude that the single-locus probes LH1 and ph30 are valuable complements to the well-established probe-systems.

ALLELE FREQUENCY DISTRIBUTION OF FIVE VNTR LOCUS AND PATERNITY TESTING IN NORTH-EAST OF SPAIN

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In paternity testing and in forensic analysis with single locus probes (SLPs), a reference population database must be established before their use in casework. The reproducibility of the system must be too established in order to assure the adequate match window.

We have study Hinf I restricted DNA from 210 unrelated Caucasian individuals living in North-East of Spain (Catalonia) with five single locus probes: MS1, MS31, MS43, MS8 and YNH24. The allele frequency distributions are similar to others Caucasian populations.

To evaluate the differences in the measurement of the same sample in two different gels, three kinds of comparisons have been made: intergel comparison of the genomic control (K562 DNA) bands, intergel comparison of duplicated sample analyses and inter/intragel comparison between mother/child shared bands. Evaluation of paternity cases results with SLPs is presented.

POPULATION GENETICS OF TWO AMP-FLPs (D1S80 AND D17S30) IN NORTH INDIAN POPULATIONS.

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Allele and genotype frequencies for two highly polymorphic amplifiable VNTRs, MCT118 (D1S80) and YNZ22 (D17S30) were determined in five Northern Indian populations (Brahmins, Khatri, Jat Sikh, Lobanas and Rajputs) by amplified-fragment-length polymorphism (AMP-FLP) technique. The Lobanas until recently lived as gypsies and had highly conserved mating patterns. Other populations are endogamous groups of varying social and economic status. For MCT118, in all populations, 23 alleles ranging from 340 bp to 780 bp in size and from 0.005 to 0.3 in frequency were detected in 488 unrelated individuals of these caste groups. The expected heterozygosity varied from 72% (Lobanas) to 78% (Jat Sikhs). The discrimination power of this locus showed interesting variation which ranges from 0.86 (Lobanas), 0.88 (Brahmins), 0.90 (Jat Sikhs and Rajputs) and 0.93 (Khatri). For YNZ22 a total of 14 alleles of sizes from 168 bp to 1078 bp were detected in a sample of 479 unrelated individuals. The small sized alleles (1 to 5) were found to be more frequent than the larger alleles. The combined frequency of these small alleles is greater than 50% in the populations investigated. The comparison of MCT118 data with other Caucasian, Japanese and African Blacks showed interesting variation, indicating that Asiatic Indian population genetic structure is significantly influenced by endogamy. Overall genetic affinity analysis confirms population relationships observed with conventional genetic markers. The findings demonstrate the potential utility of highly informative hypervariable loci such as MCT118 and YNZ22 in population genetic research as well as in forensic and paternity determination.

SPANISH POPULATION DATA ON 13 PCR-BASED SYSTEMS

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Allele and genotype frequencies for 7 tetrameric short tandem repeat loci and 6 sequence polymorphism loci were determined in a Spanish sample population (n = 186-244) using PCR and subsequent analysis of the PCR products by denaturing polyacrylamide gel electrophoresis in the case of STR systems or by the reverse dot-blot hybridization system in the case of sequence polymorphism systems. The loci are HUMFES/FPS, HUMVWA, HUMTH01, HUMF13B, HUMCSF1PO, HUMF13A1, HUMTPOX, HLA-DQA1, LDLR, GYPA, HBGG, D7S8 and GC. All loci meet Hardy-Weinberg expectations, except for the LDLR locus whose departure is not highly significant. In addition, there is little evidence for association of alleles among these loci. Thus, the allele frequency data can be used in identity testing to estimate the frequency of a multiple PCR-based DNA profile in the Spanish population.

ALLELE FREQUENCIES OF D1S80, LDLR, GYPA, D7S8, GC, HBGG AND SE 33 IN POLISH POPULATION SAMPLE

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The application of any new genetic system in forensic case-work requires a sufficiently large data base of population. Approximately 200 unrelated individuals of Pomerania-Kujawy Region from Poland were examined. D1S80, Polymarker: LDLR, GYPA, HBGG, D7S8, GC and SE 33 were analysed and compared with other population samples. DNA was isolated from blood by Proteinase K/Phenol/Chloroform extraction. PCR reactions for D1S80 and Polymarker (PM) were done on Gene Amp 9600 Perkin Elmer thermocycler with commercial kits and procedure. Primers for SE 33 were used according to Polymeropoulos. Amplification conditions were: 1) 94°C-10s, 66°C-10s, 72°C-10s:10 cycles; 2) 94°C-10s, 64°C-10s, 72°C-10s:20 cycles. The analysis of PM alleles were based on reverse dot blot reactions with oligonucleotide probes. D1S80 alleles were separated by electrophoresis on ultra-thin layer horizontal polyacrylamide gels 12%T, 3%C and Multiphor II (LKB). SE 33 alleles were diagnosed by vertical electrophoresis on Gibco-BRL Apparatus. Bands were visualised by silver staining method and recorded by computer analysis. Twenty alleles of D1S80 were observed. The most common were 18 (f= 0.187) and 24 (f= 0.339). Interalleles 20^m and 24^m were found too. Heterozygosity Index (HI) was 0.80 and power of discrimination (PD) was 0.94. Seventeen alleles of SE 33 were determined. The most common were 19 (f=0.145), 26 and 28 (f=0.113); HI= 0.94. Allele frequencies of Amplitype PM systems were: LDLR: a=0.38, B=0.62; GYPA: A=0.54 B=0.46; HBGG: A=0.51 B=0.49; D7S8 A= 0.59 B=0.41; GC A=0.45 B=0.07 C=0.48. HI for this markers was 43.75 - 84.37. PD was found to be about 0.99. No significant deviations from Hardy-Weinberg equilibrium could be demonstrated. The allele frequencies found were compared with those reported in literature and showed minor differences.

HAPLOTYPE FREQUENCIES OF TWO STRs OF THE CHROMOSOME 8q (D8S344 AND D8S323).

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Two STRs found in the chromosome 8q (Region D), D8S344 and D8S323, corresponding to two tetranucleotide repeats, $(GT)_n$ and $(A_3G)_n$ respectively, were analysed. DNA extracted after Chelex-100 procedure was amplified by PCR and electrophoresed in discontinuous polyacrylamide gels (6% T and 5% C). A population study enabled us to detect 5 alleles for D8S344, ranging between 300 and 324 bp, and 2 alleles (192 and 200 bp) for D8S323.

Given that the molecular sizes of the respective alleles of both systems do not overlap, a multiplex amplification for simultaneous analysis was carried out. Bearing in mind that these loci are chromosomally linked, both allele and haplotype frequencies were analysed, with a view towards individual and population genetic characterization.

JAPANESE POPULATION DATA ON SIX STR LOCI

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Population studies on six short tandem repeat (STR) loci - F13A01, F13B, LPL, TH01, TPOX, and vWF - were carried out in samples of unrelated Japanese individuals living in Gifu Prefecture (central region of Japan).

Amplification of each locus was achieved using the *GenePrint*TM STR Systems (Promega). Electrophoretical separation of the amplified fragments was performed in 5% denaturing polyacrylamide gels (30 cm long and 1 mm thick). After electrophoresis, the gels were silver-stained.

For F13A01, 6 alleles were found with frequencies in the range 0.004 (alleles 7 and 12) - 0.525 (allele 6) in 140 individuals. The observed heterozygosity was 0.671 and the power of discrimination (PD) and the polymorphism information content (PIC) were 0.756 and 0.535, respectively. For F13B, 5 alleles were found with frequencies in the range 0.003 (allele 7) - 0.725 (allele 10) in 357 individuals. The observed heterozygosity, PD and PIC were 0.429, 0.624 and 0.380, respectively. For LPL, 6 alleles were found with frequencies in the range 0.002 (allele 9) - 0.707 (allele 10) in 276 individuals. The observed heterozygosity, PD and PIC were 0.453, 0.660 and 0.412, respectively. For TH01, 6 alleles were found with frequencies in the range 0.011 (allele 10) - 0.377 (allele 9) in 531 individuals. The observed heterozygosity, PD and PIC were 0.704, 0.861 and 0.660, respectively. For TPOX, 6 alleles were found with frequencies in the range 0.005 (allele 14) - 0.463 (allele 8) in 314 individuals. The observed heterozygosity, PD and PIC were 0.643, 0.819 and 0.590, respectively. For vWF, 8 alleles were found with frequencies in the range 0.004 (allele 13) - 0.275 (allele 17) in 362 individuals. The observed heterozygosity, PD and PIC were 0.796, 0.928 and 0.770, respectively. No significant deviations from Hardy-Weinberg equilibrium could be found in these Japanese population samples.

The data demonstrate that TH01, TPOX and vWF are more useful than F13A01, F13B and LPL for forensic identification in Japanese population.

FORENSIC APPLICATION OF STR POLYMORPHIC MARKERS

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STR(short tandem repeat) polymorphisms are powerful tools for human identification, paternity analysis and genetic mapping. STR loci consist of short, repetitive sequence elements of 3 to 7 bases pairs in length, and may be amplified using the polymerase chain reaction (PCR).

In the present study, we analysed 6 STR systems CSF1PO, FESFPS, F13B, TH01, TPOX and vWF to obtain allele frequency data for a Japanese population living in Tokyo.

Blood samples were obtained from 150 healthy Japanese population living in Tokyo. DNA was isolated from EDTA-treated blood samples by proteinase K/phenol/chloroform extraction. PCR amplification was performed using the *GenePrint*TM STR Systems (Promega Corporation, USA) and technical manual #TMD004 provided by the manufacturer. The electrophoresis was carried out on 4% denaturing polyacrylamide gels and the bands were visualized by silver staining with Silver Stain Plus Kit (BIO-RAD, USA). The alleles were determined with the allelic ladder provided in the *GenePrint*TM STR Systems from Promega Corporation.

In 150 Japanese subjects, a total of 8 alleles for CSF1PO, 6 alleles for FESFPS, 5 alleles for F13B, 6 alleles for TH01, 6 alleles for TOPX and 8 alleles for vWF was observed. The most common alleles were 12(35.4%), 11(33.3%), 10(74.3%), 9(41.1%), 8(48.6%) and 17(28.8%), and heterozygosities were calculated as 76.0%, 74.3%, 40.4%, 69.8%, 62.7% and 79.3% for CSF1PO, FESFPS, F13B, TH01, TPOX and vWF, respectively. The combined matching probability of these 6 STR systems was estimated as 9.2×10^{-6} and the combined power of discrimination was therefore 99.99908%. The efficiency of these STR systems in paternity testing including postmortem paternity cases was also analysed. In each case an unequivocal conclusion was obtained.

These results suggested that these STR systems are useful genetic markers for paternity tests and individual identification in forensic analysis.

POPULATION STUDIES OF TWO AMPFLP'S AND TWO STRs SYSTEMS IN A NORTH POLISH POPULATION.

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The polymorphism of two AMPFLPs systems (D1S80, D17S5), and two STRs systems (HUMTH01, HUMVWA31) were investigated in population samples from North Poland. DNA was isolated using non-organic and non-enzymatic method, and then amplified using described primers. PCR products were separated on non-denaturing polyacrylamide gels in discontinuous buffer systems. Gels were stained with silver.

In the analysed population we observed 19 phenotypes and 7 HUMTH01 alleles (n=203). The most frequent alleles were: 9.3 (30.5%), 6 (24.9%), 9(18.5%), 7(12.6%) and 8(12.1%). The observed heterozygosity (H) and power of discrimination (PD) were 0.78 and 0.92 respectively.

Number of observed alleles and phenotypes for HUMVWA system were 8 and 27 respectively (n=185). The most frequent alleles were: 17(8.6%), 18(23.2%) and 16(19.45%). Observed heterozygosity was 0.79 and PD was 0.92.

Analysis of 204 persons in a D17S5 showed 53 phenotypes and 13 alleles. The most frequent alleles were 4 (26.7%), 3 (19.6%) and 2 (13.2%). H and PD were 0.80 and 0.95 respectively.

In a population of 207 persons we observed 59 phenotypes and 19 alleles of D1S80 system. The most frequent alleles were 18 (20.0%) and 24 (36.2%) and DP and H were 0.94 and 0.80 respectively.

We have found no significant deviations from Hardy-Weinberg equilibrium for all analysed systems.

Distribution of observed frequencies of alleles are similar to the most reported populations in Europe.

ALLELE FREQUENCY DISTRIBUTION OF 15 PCR-BASED DNA POLYMORPHISMS IN THE POPULATION OF GALICIA (NW SPAIN)

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The allele frequency distribution of 15 PCR-based DNA polymorphisms is reported. The systems studied include HLADQA1, Polymarker (LDLR, GYPA, HBGG, D7S8 and GC), 4 AmpFLPs (D1S80, YNZ22, COL2A1 and 3'ApoB) and 5 STRs (HUMTHO1, HUMVWA31/A, HUMF13A1, HUMFES/FPS and HUMLPL). The population data size ranged from 150 to 250 unrelated Galicians. HLADQA1 and Polymarker were studied using commercial dot-blot with ASO probes. D1S80 and YNZ22 were studied using discontinuous electrophoresis in miniaturized polyacrylamide gels. All the others systems were studied using the ALF sequencer with denaturing polyacrylamide gels. Sequenced alleles ladders were used for all the systems. Compliance with Hardy-Weinberg equilibrium was tested using an exact test for AMFLPs and STRs. All the systems were found to be in H-W equilibrium. Population comparisons with data from other populations are reported. Some statistical parameters of forensic interest (PD, CE, h) are also given. No mutations were found for any of the systems in a total of 95 paternal meiosis and 120 maternal meiosis. Electrophoretic anomalous mobility was observed under non-denaturing conditions for F13A1 and 3'ApoB systems. Selection criteria and the usefulness of these systems for forensic purposes are also discussed.

THE ALLELIC DISTRIBUTION OF 5 STRs SYSTEMS IN A NORTH ITALIAN POPULATION.

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The short tandem repeat (STR) systems HumFES/FPS, HumF13B, HumACTBP2, HumvWa and HumD21S11 were investigated in a North Italian population sample of 120 Caucasian unrelated individuals. A complete statistical analysis was carried out for each system. No deviation from Hardy Weinberg equilibrium was observed. A comparison with Turkish and German population studies showed a certain degree of similarity for these systems. A further comparison with other Italian population studies with the system HumvWA showed no significant differences.

POPULATION STUDY OF 3 STR LOCI IN THE NORTH OF PORTUGAL

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Short tandem repeat (STR) loci are a group of polymorphic markers which occur in the human genome, consisting of simple tandemly repeated sequences 1-6 bp in length. Their abundance, hypervariability and amenability to amplification by the polymerase chain reaction (PCR) make them ideal systems for use in the individual identification.

In this study we have used the automated fluorescence-based technology with an Automatic Laser Fluorescent (A.L.F. - Pharmacia) DNA Sequencer and the Fragment Manager on Hydrolink Long Ranger gels.

The system described incorporates an internal size standard in each sample, allowing the STR products to be automatically sized with a high degree of precision.

We have examined 3 STR loci, HUMVWA31A, HUMTH01 and HUMFES/FPS and constructed population databases in the North of Portugal.

These systems did not deviated from the Hardy-Weinberg equilibrium and their allele frequency distribution in our data (North of Portugal) are similar to other Caucasian populations.

The study of several STR-PCR with automated fluorescent detection are a rapid and powerful DNA profiling technique for routine forensic casework.

POPULATION GENETICS OF THE STRs TPO, TH01 and VWFA31/A IN S.TOMÉ E PRÍNCIPE

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S.Tomé e Príncipe, a former Portuguese colony, is an archipelago comprising two inhabited islands in the Gulf of Guinea, near the Equator. Although poorly documented, colonisation begun in the XV century (the islands were not inhabited) and main demographic contributions to the present population were slaves from African facing coast and a few Portuguese.

Cord blood from unrelated newborns was sampled; genotyping was performed using manual systems and separate amplifications for the STRs:

TPO, repeat (AATG), intron 10 of thyroid peroxidase gene (2p23-pter)

TH01, repeat (TCAT), intron 1 of tyrosine hydroxylase gene (11p15.5-p15)

VWFA31/A, repeat (TCTR), intron 40 of von Willebrand factor gene (12p12-pter)

From the results obtained up to now the most important features are:

1. **TPO** (n=78) - a new allele (7), relatively common (1.3%) and a very high frequency for allele 6 (6.4%), which is rare in Caucasians; no other data on Blacks are available for comparisons.
2. **TH01** (n=38) - very low frequency (5.3%) for the pooled alleles 10 and 9.3 (still lower than in USA Blacks) and a high frequency for allele 8 (32.9%).
3. **VWFA31/A** (n=41) - high frequency of allele 13 (2.4%), a rare allele both in Caucasians and USA Blacks (0.5%)

AUTOMATED ANALYSIS OF 5 STR LOCI: ALLELE FREQUENCIES AND FAMILY STUDIES IN THE GERMAN POPULATION

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Automated analysis of fluorescent amplified DNA fragments represents a very powerful and economic tool for evaluation of inheritable STR polymorphisms. We have established a method, that allows simultaneous detection of STR systems HUMTH01, HUMvWF, HUMFES/FPS, D21S11 and ACTBP2 using an Applied Biosystems 373A sequencer and GeneScan 672 analysis software. Fragment size determination of the first 4 loci is easily accomplished by using an internal size standard (GS 2500). Maximum precision of ACTBP2 allele sizing, however, requires additional internal standardisation by an appropriate allelic ladder.

Distributions of allele frequencies ($n = 700-2000$) are in close agreement with published data and observed genotypes conformed to Hardy-Weinberg equilibrium.

Extensive family studies (> 300 meioses per locus) demonstrated Mendelian inheritance of segregating alleles and very low mutation rates were detected, thus indicating that these systems are genetically stable.

Forensic efficiency data of the 5 PCR systems suggest, that automated analysis of STR loci may be very useful in routine paternity testing.

SOUTHERN SPAIN POPULATION FREQUENCIES OF THE LOCI LDLR, GYPA, HBGG, D7S8 AND Gc. A COMPARISON BETWEEN ANDALUSIAN AND CANARY ISLANDS FREQUENCIES.

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The aim of this paper is to present population data for the following loci: low density lipoprotein receptor (LDLR), glycophorin A (GYPA), hemoglobin G gammaglobin (HBGG), D7S8, and group specific component (Gc). They were simultaneously amplified and typed using the AmpliType® PM PCR Amplification and Typing Kit from Perkin Elmer Corporation. DNA was obtained from non related individuals of routine casework, paternity testing and staff. Our Institute, located in Southern Spain, covers Andalusia and the Canary Islands; a comparison between both populations was carried out in order to assess the applicability of common or separate reference data bases.

Additionally we report here the correspondence between PCR genotyping and IEF phenotyping results for Gc, including individuals which showed rare migrating protein patterns.

ALLELE FREQUENCY DISTRIBUTION OF D2S44, D12S11, D7S21, D7S22 AND D5S43 LOCI IN SOUTHERN SPAIN.

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Before a marker system is used in forensic casework, a sufficiently large database of the relevant population must be established. For this reason the allele sizes and the allele frequency distributions were determined for the five hypervariable loci D2S44, D12S11, D7S21, D7S22 and D5S43 in the population of Southern Spain.

DNA extraction was carried out with phenol chloroform and separation of Hinf I restricted DNA fragments was carried out by standard methods. Membrane hybridization was performed sequentially with alkaline phosphatase conjugated single locus probes YNH24, MS43a, MS31, G3 and MS8. VNTR fragment sizes were determined by computerized digital image analysis.

Our findings show that allele frequency distribution for the five hypervariable loci in Southern Spain presents good correlation with previously reported caucasian populations.

HLA-DQA1 POLYMORPHISM IN TWO PORTUGUESE POPULATION SAMPLES FROM LISBON AND THE SOUTH OF PORTUGAL.

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The purpose of this study was to analyse the HLA-DQA1 polymorphism in view of its application in forensic casework, as recommended by the DNA Commission of International Society of Forensic Haemogenetics.

Two portuguese population samples, one from Lisbon (120 unrelated individuals) and one from the South of Portugal (104 unrelated individuals) have been investigated for HLA-DQA1 polymorphism by the polymerase chain reaction (PCR), using allele specific oligonucleotide probes and reverse dot-blot methodology (Cetus).

Phenotype and allele frequencies of both population samples were similar to other Caucasian populations and no deviation from Hardy-Weinberg equilibrium was observed.

D1S80 LOCUS POLYMORPHISM IN A POPULATION SAMPLE FROM LISBON

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The objective of this work was to analyse D1S80 polymorphism in order to apply the data in forensic cases, as recommended by the DNA Commission of the International Society of Forensic Haemogenetics.

The study was performed on a population sample of 110 unrelated individuals living in Lisbon.

D1S80 polymorphism was studied by the polymerase chain reaction (PCR) using Forensic DNA Amplification Reagent Set (Cetus) and the analysis of amplified alleles was performed using the Phast System and Phast Gels 10-15 were silver stained.

We observed 20 alleles and the most frequent were T24, T18 and T29. The frequency curve for the Lisbon population sample was similar to those obtained in Caucasian populations by other workers.

PCR-AMPFLP TYPING OF THE D21S11 MICROSATELLITE POLYMORPHISM: ALLELE FREQUENCIES IN THE AUSTRIAN POPULATION.

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Using PCR-Amplification Fragment Length Polymorphism (AMPFLP) as described[1] we have determined allele frequencies for the complex microsatellite repeat at the D21S11 locus[2,3] in healthy unrelated Caucasians of our local (Austrian) population (n=200). 12 alleles were identified by their distinct migration on native polyacrylamide gels. In Tab.1 a provisional nomenclature based on side-to-side comparison to the cocktail provided by Brinkmann[3] is used since sequencing is so far not completed. The electrophoretic migration pattern suggests the occurrence of interalleles (incomplete repeats) equivalent to those described by Moeller [3]. No deviation from Hardy-Weinberg equilibrium was observed in the population sample tested.

An allelic ladder composed of all alleles distinguishable by PAGE was constructed by pooling eluates of amplification fragments of different alleles and subsequent reamplification. This allelic cocktail can be used as a reference in all typing for forensic casework.

Tab.1 Allele frequencies

V25	0.5%	V29	35.5%	V31s*	1.5%
V26	0.5%	V30	40.0%	V32	11.0%
V27	5.5%	V30s*	16.5%	V33	8.5%
V28	40.0%	V31	20.5%	V34	1.0%

* electrophoretic mobility suggests a (incomplete repeat) variant

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TYPING FOR THE HUMFES/FPS SHORT TANDEM REPEAT
POLYMORPHISM IN AN AUSTRIAN CAUCASOID POPULATION
SAMPLE

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The tetranucleotide STR polymorphism HUMFES/FPS was examined by PCR and native polyacrylamide gel electrophoresis (AMPFLP) in an Austrian Caucasoid population sample of 451 healthy, unrelated individuals.

An allelic ladder consisting of seven sequenced alleles in regular intervals of four basepairs was used as a standard size marker.

Sequencing of 9 different alleles occurring in this population sample revealed identical results as published by Möller et al. 1994 (1).

Allele frequencies recieved in this study are listed below:

A* 8	0.017	A*11	0.435
A* 9	0.003	A*12	0.225
A*10a	0.234	A*13	0.029
A*10	0.033	A*14	0.001
A*11a	0.023		

The population investigated showed no deviation from Hardy-Weinberg equilibrium.

1. Möller A et al (1994) Int J Legal Med 106(6): 319-323

ANALYSIS OF THE STR POLYMORPHISM vWA AND FES:
ALLELE FREQUENCY AND FAMILY STUDIES IN AN
ITALIAN POPULATION SAMPLE.

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To evaluate the allelic distribution of the two STR systems vWA and FES we have investigated at least 100 individuals in our reference populations (Veneto, Italy).

The alleles were identified using PCR in according to standard protocols. Amplified products were separated in vertical polyacrylamide gels followed by silver staining. The various alleles were identified by a comparison side to side with an allelic ladder. All the common alleles were found. The allelic frequencies were analyzed under the Hardy-Weinberg law.

Furthermore, the efficiency of this two STR systems in paternity testing was also analysed by a retrospective analysis of paternity cases (trios) in which the exclusion had already been established with other genetic markers.

ANALYSIS OF THE SHORT TANDEM REPEAT POLYMORPHISM D21S11 IN GERMAN CAUCASIANS.

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Short tandem repeat loci (STR) are polymorphic markers that can be used for genetic analysis of individuals in forensic or paternity casework. We have investigated the polymorphism of the STR locus D21S11 in a population sample of 77 german caucasians. Analysis of genomic DNA samples was performed by PCR amplification using fluorescence (HEX - hexa-chloro-6-carboxyfluorescein) labelled primers. Allele sizes were determined by measuring the laser light induced fluorescence emission of the PCR fragments on an ABD 373A automated DNA sequencer. The following results were obtained:

D21S11		Individuals: 77		Heterozygosity : 0,818	
Allele (bp)	F (%)	Allele(bp)	F (%)	Allele(bp)	F (%)
56 (209)	0,007	63 (223)	0,007	67 (231)	0,097
58 (213)	0,013	64 (225)	0,266	69 (235)	0,117
60 (217)	0,13	65 (227)	0,071	71 (239)	0,02
62 (221)	0,201	66 (229)	0,071		

No deviation from Hardy-Weinberg-Equilibrium was observed (Chi-square value 0,368; df 65; $p > 0.99$). The gene diversity was 0,838, the resulting polymorphic information content (PIC) 0,819, and the probability of a match (pM) 0,012. Locus D21S11 exhibited a number of allelic bands differing in size by only 2bp. Proper allele assignment was easily performed using the Local Southern sizing algorithm (Genescan-software, ABD) and an internal size standard. Allelic size variations in 6% polyacrylamid gels was $\pm 0,24$ bp. The high polymorphism of this STR locus observed in our population sample can be used for paternity and forensic casework.

POPULATION DATA OF THE VNTR LOCI D10S28, D4S139, D16S309 AND D5S110 IN GERMAN CAUCASIANS.

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Variable number of tandem repeat (VNTR) loci are highly polymorphic markers that are commonly used for paternity and forensic testing. We have studied the allele distribution of four VNTR loci, D10S28 (TBQ7), D4S139 (PH30), D16S309 (MS205) and D5S110 (LH1) in a population sample of german caucasians. Analysis of VNTR (SLP) polymorphism was performed using HAEIII or HINFI digested genomic DNA samples. Fragments were separated by agarose gelelectrophoresis (24-26 hours at 0.8-1.0 V/cm). Electrophoresis conditions were chosen depending on the range of fragment sizes detected by the various DNA probes. Hybridisation, southern blot transfer to nylon membrans (Qiabrane from Qiagen Inc, Chatsworth, CA, USA) and chemiluminescence detection of probes was performed as recommended by the manufacturers (NICETTM MS205 ICI/CELLMARK, GenePrint LightTM TBQ7 Promega and ACESTM LH1/pH30 Gibco BRL). Fragments were analysed by an semiautomatic computerized system (DNA-Auswertungssystem Version 2.40 from Muche M. Immucor Medizinische Diagnostik GmbH, Rödermark, Germany). The following results were obtained:

VNTR loci	Individuals	Allele size range	HR
D10S28 (TBQ7) - HAEIII	201	0,94 - 9,18 kb	0,90
D4S139 (PH30) - HAEIII	133	2,21 - 22,62 kb	0,97
D16S309(MS205) - HINFI	221	0,80 - 4,36 kb	0,98
D5S110 (LH1) - HAEIII	147	1,12 - 8,03 kb	0,96

The allele distribution observed with the SLP's TBQ7, LH1 and MS205 did reveal frequent fragments lying close together in the lower kb region, whereas the SLP PH30 exhibited a more irregular distribution with 19,8% of fragments lying above 10 kb.

A POPULATION STUDY OF 5 PCR GENETIC MARKERS LDLR, GYPA, HBGG, D7S8 AND Gc IN ITALY.

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Allele and genotype frequencies for 5 genetic loci: LDLR (Low Density Lipoprotein Receptor), GYPA (Glycophorin A), HBGG haemoglobin G-gamma Globin), D7S8 and GC (Group-specific Component), were investigated in an Italian population sample.

The study was performed on a sample of 100 unrelated donors. In addition, 5 mother-child pairs were examined under the same conditions using the AmpliType® PM PCR Amplification and Typing Kit (Polymarker Kit, supplied by Cetus Corporations). This PM kit allows the simultaneous typing of the 5 loci using a reverse dot blot technology.

The aims of the present study were: 1) to analyse the polymorphism of these 5 loci, 2) to establish a database of allelic frequencies, in view of its application in forensic investigations, and 3) to examine the performance of this method and the resultant problems arising from its use.

The distribution of PM allele frequencies were found to be similar to those described for Caucasians (Americans, Swiss and North Bavarians).

Extreme care must be exercised when interpreting the typing of the GC locus, because the B allele could be misinterpreted due to signal imbalance under particular conditions.

ALLELE FREQUENCIES OF THE HUMFES/FPS SYSTEM NORTHERN AND CENTRAL ITALY.

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Domenici R., Fornaciari S., Spinetti I., Nardone M., Bargagna M. (°°)

Universities of Ancona (*), Parma (**), Pavia (°), Pisa (°°)

The HUMFES/FPS system is a tetrameric short tandem repeat located on chromosome 15 (15q25-qter) whose polymorphism was recently investigated by the Polymerase Chain Reaction (PCR) technique.

The polymorphism of this STR system was studied in population samples from four Italian regions (Lombardy, Emilia, Tuscany and Marches) with the aim to set up a database in view of its application in forensic identification and paternity testing.

After amplification, the alleles were identified by high-resolution horizontal PAGE followed by silver staining. The alleles were typed by side-to-side comparison with a ladder obtained by coamplification of different alleles.

All the common alleles were found. The distribution of the observed genotypes did not deviate from the Hardy-Weinberg equilibrium. The discrimination power (PD), the heterozygosity rate, the allelic diversity and the chance of exclusion were calculated and the Italian sample frequencies were compared with previous studies on Caucasians.

The results of this study demonstrated that this STR system may be a useful marker in forensic case studies.

ANALYSIS OF THE Y-LINKED LOCUS Y27H39: FREQUENCY DISTRIBUTION IN SOUTH BAVARIAN AND APPLICATION TO PATERNITY TESTING

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The tetranucleotide repeat polymorphism Y-27H39 (Roewer L., Epplen J.T., 1992, *Forensic. Sci. Int.*, 53, 163-171) was analysed in a sample of 213 unrelated males by the polymerase chain reaction. Electrophoresis was performed on 6% denaturing polyacrylamid gels on an ABI 373A DNA sequencer. Five alleles with sizes varying from 186 to 202 were distinguished with frequencies in ranging from 1% to 48%. From 35 father-son exclusions, previously tested with different autosomal SLS and STR-loci, the Y-27H39 polymorphism confirmed 80% of the attribution.

Because of the male inheritance the Y-linked polymorphism is extremely useful in paternity testing, especially in some deficiency cases. We present a paternity case lacking the two suggested putative fathers. Analysis of half-brothers and one grandson of the deceased putative fathers with 4 SLS, 4 STR and the Y-27H39 loci shows clearly, that only one of the suggested men is the right father and the other not.

STUDIES ON THE HUMACTBP/SE33 POLYMORPHISM IN A SOUTH BAVARIAN POPULATION

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Population genetic studies were carried out on 303 caucasians from south Bavarian using the short tandem repeat (STR) system HumACTBP2/SE33. Using our own allelic ladder as internal standard in each lane, a total number of 32 different alleles could be observed. The allelic frequencies range from 0,2% to 9,8%. The power of discrimination is 0,98.

Additional one hundred paternity cases (trios) with a probability of paternity $\geq 99,9\%$ have been investigated. In two cases isolated father/child mismatches were observed. A mutation could be assumed. In both cases a deletion of one repeat unit could be verified by sequencing.

Detection of the PCR-Products and the sequencing-reaction was carried out on 6% denaturing polyacrylamid gels using a 373A DNA Sequencer from ABI. Sequencing was done with the Taq Cycle sequencing reaction.

HUNGARIAN POPULATION DATA FOR 11 PCR-BASED POLYMORPHISMS

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We carried out a Hungarian population study for 11 PCR-systems: HLA DQ α , LDLR, GYPA, HBG, D7S8, GC, D1S80, ApoB, HUMVWA, HUMTH01, and HUMFES/FPS. In this survey a minimum of 200 unrelated individuals were typed for each system.

Amplification and typing of HLA DQ α and PM loci were performed according to the Cetus protocol. The rest of these systems were analysed on horizontal non-denaturing/high-resolucional PAGE followed by silver staining except ApoB, for which agarose gel electrophoresis and ethidium bromide staining was used.

For all markers the genotype and allele frequencies found are similar to those reported in the literature for European caucasians and are in good agreement with Hardy-Weinberg equilibrium.

ALLELE FREQUENCY DISTRIBUTION OF THE STR SYSTEM ACTBP2 (SE33) IN A POPULATION OF PORTUGAL (CENTRAL AREA)

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In order to apply the highly polymorphic STR system ACTBP2 (SE33) to our forensic casework, we develop preliminary studies both on inter-laboratory comparisons (by participating in a EDNAP Exercise, results to be published) and in generating a significant database for our population of reference (Portugal - Central Area).

EXTRACTION:

DNA was obtained by CHELEX extraction of bloodstains from unrelated individuals.

AMPLIFICATION:

Primers (Oswell Ltd.) :

SE33 /1: AATCTGGGCGACAAGAGTGA (5'FAM)
SE33/2: ACATCTCCCCTACCGCTATA

Cycling Conditions:

94 °C, 45''
60 °C, 30''
72 °C, 30 ''

DETECTION:

Alleles were determined by automatic fragment analysis, on a ABI 373A DNA Sequencer, using the internal standard GeneScan Rox 2500 and a allelic ladder of 23 sequenced alleles.

Genotype distributions are in accordance with Hardy-Weinberg equilibrium. Allele frequency distribution as well as statistical parameters show no significant differences with other populations.

ALLELE FREQUENCIES IN 4 STR'S FOR A POPULATION OF PORTUGAL
(CENTRAL AREA)

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Short Tandem Repeat (STR) loci are tandemly repeat units (1-7bp) which show a remarkable ubiquity throughout human genome.

The usefulness of the STR's :HUMTHO1; HUMVWA31A; HUMF13A1 and HUMFES/FPS in forensic studies, namely in parentage testing, has been validated by a ever growing number of authors, as well as by the EDNAP (European DNA Profiling Group) .

The results so far published, show that fluorescent labelling coupled with automated detection and sizing by means of internal lane size standards represents a robust and reproducible system in assessing STR allele frequencies and in forensic casework analysis.

Amplifications followed the 2nd. EDNAP Collaboration STR Exercise and detection employed an ABI 373A DNA Sequencer with Genescan Software. In this study we present the results of samples of, at least, 200 unrelated individuals, showing allele and genotype frequencies for the population of Portugal (Central Area).

Family studies involving 100 families have also been accomplished.

All of the STR's show good accordance with Hardy-Weinberg equilibrium and the allele frequencies are similar to other population studies.

DNA PROFILING : A GENETIC STUDY OF TWO VNTR LOCI IN THE
EAST MIDLANDS

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The objectives of this investigation were to establish the Federal Bureau of Investigation(FBI) fixed bin allele frequencies of MS1 and MS43a minisatellite loci in the Caucasian East Midlands population. To determine if Caucasian sub-population heterogeneity exists within the United Kingdom(UK) at the MS1 and MS43a loci. To discover the efficiency of MS1 and MS43a single-locus probes for forensic and paternity purposes in the East Midlands populations

Blood samples were taken at random from the Caucasian East Midlands population. Using standard Southern blotting technique, DNA was hybridised with the NICE single-locus probes MS1 and MS43a. The log molecular weights of the DNA bands were determined and the alleles placed into FBI fixed bins. The frequency of the alleles in each bin were calculated along with standard errors.

Significant heterogeneity chi-square values for MS43a were found in a large number of FBI bins in UK Caucasian, world Caucasian and UK racial population comparisons. Heterozygosity of MS1 was 92.17% and MS43a 87.93% in the Caucasian East Midlands database. MS1 probability of match(PM), discrimination probability(DM) and probability of exclusion(PE) were 0.0322, 0.9678 and 0.8096 respectively. MS43a PM, DM and PE were 0.0499, 0.9501 and 0.7692 respectively. Combination of the probes produced a PM of 0.0016, DM of 0.9984 and PE of 0.9559.

The findings suggest that heterogeneity may exist for the MS43a locus in the Caucasian UK population. In addition, MS1 and MS43a were found to be efficient markers for forensic and paternity work.

I. STANDARDIZATION. ETHICS

ETHICAL AND LEGAL ASPECTS

SANTIAGO GRISOLIA

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Any major Scientific advance or program often generates new dilemmas. The notable advances in the Human Genome Project have without doubt presented new problems and brought back to light older ones. There is no doubt that public enthusiasm is great, but it is mixed with the fear of how the new knowledge will be used. The discussions about how new genetic knowledge is going to concern us all will increase in the coming years. Principles of bioethics elaborated recently in relation to people, benefits, autonomy and justice, must be included in legal protection of the people as well as of society. These principles will not be adequate to solve many of the complex questions and controversies resulting the advances in genetics. Certainly, ethical dilemmas will arise because of the conflicts between collective and individual interests.

So-called eugenics and racial hygiene were based on an erroneous belief in the improvement of the inheritance pool, leading to negation of individual decisions about having children, choosing mates, and, under nazi genocide, the right to life. Eugenics in terms of coercive social politics has not disappeared; it is alive in some countries in which there are still sterilization codes, and as ideology it is taken into consideration in some countries and is trying to appear in certain groups, e.g. American right-wing extremists. Investigations related to behavioral genetics and their results are easily misused, as we have often seen in the controversies about IQ tests, affective disorders and other mental diseases. The problems and dilemmas related to the use of eugenic information will not need special draconian measures, but we must be ready. For example, employers, personnel directors and insurance companies could discriminate against those whose genetic characteristics make them susceptible to certain diseases, premature death or disability. Without doubt there is a potential conflict between individual interests and those of society. However, no one chooses his or her genes, and the information given by them must be kept confidential. No one should be analyzed genetically without his knowledge, and the information obtained must not be released without the consent of its owner, unless it is necessary to avoid damage to other people.

THE DEVELOPMENT OF QUALITY ASSURANCE MEASURES IN FORENSIC DNA TYPING.

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The purpose of this paper is to discuss the development of quality assurance measures used in forensic DNA typing. The use of RFLP and PCR analyses have revolutionized the impact of biological stain evidence in criminal investigations. Throughout its development, quality assurance (QA) and quality control (QC) measures have been incorporated into forensic DNA typing procedures. QA and QC measures were incorporated to provide evidence to all concerned, of the quality and reliability of the DNA typing results.

In the early application of forensic DNA technology, the need for QA and QC measures became evident. In 1989, the Technical Working Group on DNA Analysis Methods (TWGDAM) published the first in a series of QA guidelines for forensic DNA typing. In 1995, TWGDAM published the latest revision to these guidelines which covers both RFLP and PCR technologies. The TWGDAM guidelines have become de facto benchmark for forensic laboratories throughout the United States for establishing QA programs. Additionally, the TWGDAM guidelines have been adopted by the American Society of Crime Laboratory Directors (ASCLD)/Laboratory Accreditation Board (LAB) and portions incorporated into the accreditation standards for forensic DNA laboratories.

In recognition of the need for quality DNA standards, The National Institute of Standards and Technology (NIST) developed a standard reference material (SRM 2390) for forensic DNA typing. SRM 2390 was developed for use in the standardization of forensic and paternity QA procedures for RFLP testing using the HaeIII restriction enzymes.

In the United States, Congress recently passed the DNA Identification Act of 1994 which established the DNA Advisory Board. This board has begun deliberations regarding quality assurance standards for forensic DNA analysis used by the U.S. forensic community.

In the European community, the DNA Commission of the International Society of Forensic Haemogenetics (ISFH) has also generated quality assurance protocols for forensic DNA analysis. Together with the TWGDAM guidelines, these voluntary quality assurance standards have further insured the reliability and comparability of DNA profiles.

THE AMERICAN ASSOCIATION OF BLOOD BANKS INSPECTION AND ACCREDITATION PROGRAM FOR PARENTAGE TESTING LABORATORIES.

R. H. Walker, American Association of Blood Banks, Inspection and Accreditation Program, Bethesda, MD

Following the Airlie Conference in 1982, the American Association of Blood Banks (AABB) established guidelines for use by Parentage Testing Laboratories in the United States which related to the mathematical method used in expressing the probability of paternity when there is a failure of exclusion. The guidelines were followed in 1984 by the AABB publication of Standards for Parentage Testing Laboratories which addressed all phases in the process and all methods used in laboratory testing. A voluntary inspection and accreditation program was also established in 1984 in which accreditation was based upon compliance with the published standards. Initially, accreditation was dependent upon a review of required documents and case records. In 1991, an on-site inspection program was initiated in addition to the document review. Accredited laboratories are inspected on-site every two years. Accredited laboratories must participate in a national proficiency testing program or a specimen exchange program with another laboratory to monitor their performance in every genetic system which they use in case work. Most laboratories participate in the College of American Pathologists (CAP)/AABB-PI Program which offers blood samples in a voluntary survey and compiles results in all genetic systems except HLA. An HLA Class I antigen typing survey is also available from the CAP/ASHI. The second edition of Standards for Parentage Testing Laboratories was published in 1994; it addressed PCR based methods. This year a newly revised Inspection Report Form (IRF) and an Accreditation Requirements Manual (ARM) have been released. The IRF details the requirements for accreditation and it is based upon the Standards. The ARM assists laboratories in preparing for the inspection by explaining the basis for the items in the IRF. Currently approximately 53 laboratories hold AABB accreditation. These laboratories process about 99% of the total case work in the United States. AABB accreditation is regarded as a necessary requirement for many states which award contracts following competitive bidding.

THE PARENTAGE TESTING PROFICIENCY SURVEY PROGRAM OF THE COLLEGE OF AMERICAN PATHOLOGISTS

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In collaboration with the American Association of Blood Banks (AABB), the College of American Pathologists (CAP) initiated a new survey in 1993 to replace the PSP program. Three shipments (March, July and October) containing 3 samples of about 3ml of whole blood collected in ACD-A are sent to participants. Questionnaires are designed to allow participants to report their testing results in all genetic systems used. Due to sample acquisition and preservation restrictions, HLA testing by serological methods cannot be performed on these specimens. Participants also report PI and RMNE calculations for each locus tested and an overall interpretation of results. Methodology information is collected from participating laboratories.

Enrollment increased from 62 participants for the 1993 PI-A survey to 113 for the 1994 PI-C survey. Participation is voluntary but is required for laboratories accredited for parentage testing by AABB. Of those laboratories responding an average of 81.5% performed parentage testing routinely. The remaining laboratories reported performing either forensic serology or monitoring of bone marrow transplantation.

Of the 6 trios tested in the two year span, 5 consisted of true biological trios and one (PI-A 1994) included an alleged father who was unrelated to the mother and child. In this latter case none of the red cell antigen systems tested and only 3 (PLG, PGM1i, ACP) of the serum proteins and red cell enzymes systems excluded the man. Only 9 out of 18 polymorphisms tested by PCR revealed an exclusion. Of the 26 RFLP loci tested, 5 did not demonstrate an exclusion.

This survey program provides participating laboratories a comprehensive report that can be used as a management tool to compare their testing results with those of others in the field and to assess current techniques and methodologies used in parentage testing.

PARENTAGE TESTING SURVEY PROGRAM OF THE COLLEGE OF AMERICAN PATHOLOGISTS: RED CELL ANTIGENS, SERUM PROTEINS AND RED CELL ENZYMES TESTING RESULTS.
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Since its inception in 1993 the College of American Pathologists (CAP) parentage testing survey program has received results from participating laboratories on 6 mailings. Results were reported in 6 red cell antigens (RCA), 14 serum proteins (SP), and 11 red cell enzymes (RCE) genetic systems. Results in ABO, Rh, MNSs, Kell, Duffy, Kidd, Gc, HP, PGM1 and ACP were reported by more than 10 laboratories.

The total number of laboratories reporting results, the number of results reported, and the number of non consensus answers are tabulated below.

	1993 PI-A	1993 PI-B	1993 PI-C	1994 PI-A	1994 PI-B	1994 PI-C
Total labs reporting	51	58	60	85	93	89
No. of Labs	33	39	36	49	54	50
RCA Total results	534	537	561	702	678	726
RCA Non consensus	4	1	5	1	2	1
No. of Labs	8	14	12	22	20	17
SP Total results	102	114	105	171	138	165
SP Non consensus	1	1	1	4	2	2
No. of Labs	11	20	8	32	36	29
RCE Total results	108	216	171	321	303	339
RCE Non consensus	0	0	2	11	3	8

These data showed good consistency between laboratories doing genetic testing with conventional systems.

DNA LEGISLATION IN THE NETHERLANDS

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In December 1991 the Dutch Minister of Justice has proposed a bill regarding the use of DNA investigation in crime. The bill was the response to a report of a commission which studied the renewal of the penal code. The new DNA legislation became effective in September 1994.

The new law gives the Investigating Judge or Public Prosecutor an exclusive position in the process of a DNA investigation in criminal cases. To perform a DNA comparison the suspect can be obliged by the judge to give a blood sample, but only if it concerns a serious crime with a penalty of eight years or more of imprisonment. Until September 1994 it was possible for a suspect to refuse to give any body sample, such as blood or saliva, needed for the identification of biological trace evidence. Because the integrity of the body is protected in the Dutch Constitution it was necessary to make this new legislation. Furthermore the legislation provides defendants with a right to a second test by an independent laboratory.

The legislator has imbedded the new law in a range of very detailed procedures and the construction of a DNA database to trace suspects has been provided.

The new DNA legislation and the consequences for the working procedures of the laboratories involved will be presented.

SCIENCE AND CONSCIENCE: REGULATION OR GUIDELINES FOR FORENSIC HAEMOGENETICS?

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Throughout the history of mankind, there has always been some misunderstanding between science and society. Most of the time, this misunderstanding has been translated as *fear of something* in the community, and not always without reason.

Forensic Science, and more-specifically Forensic Haemogenetics, increasingly deals with the analysis of a number of loci dispersed along the human genome to identify individuals. But genetic analysis, perceived by the people as "study and/or manipulation of the human genome" is actually the main fear of people worldwide.

We believe that more than the imposition of detailed social, civil or criminal laws and legal regulations, a better approach would be the definition of limits or guidelines that must be followed by forensic scientists. **Laws and regulations** are specific to a given country or group of countries (i.e., EEC); however, **guidelines** proposed by societies (such as the ISFH) would be followed by any scientist in any circumstance.

To increase the social conscience, three different considerations should be clearly differentiated in forensic science:

1. Guidelines for the collection of biological evidence since there are issues regarding self-incrimination, right to counsel, search and seizure, due process, etc.

2. Guidelines about which specific technology and DNA loci can and cannot be considered in this field, how to manipulate and store the biological evidence, laboratory accreditation, etc.

3. Guidelines regarding databases and their management, information exchange (national, international), what kind of data should be stored and for how long, who has access to the data (from the general population, offenders, or convicted criminals), etc.

Therefore we recommend that the ISFH be actively involved in its member countries and provide scientific counsel and guidelines to achieve basic harmony in this field.

REPORT ON THE THIRD EDNAP STR EXERCISE

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This report describes an inter-laboratory exercise completed on behalf of the European DNA Profiling (EDNAP) group. The exercise is one in a series designed to identify STR loci which could be used for harmonisation between participating European forensic science laboratories.

Participants were asked to identify the alleles present in five bloodstains at the STR loci HUMTH01 and HUMVWFA31/A. Two of the stains were prepared from mixtures of two different blood samples. There were no special instructions and each laboratory was requested to use the methodology normally employed for crime case investigations. All participating laboratories achieved the same results for both loci.

In addition, the laboratories were also requested to report the results obtained from any other loci which would normally be used in crime case investigations. A comparison of the results obtained showed some inter-laboratory variation.

HUMTH01 ALLELE FREQUENCIES IN ITALY - REPORT OF THE GEFI COLLABORATIVE STUDY

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In the lastest years Short Tandem Repeat (STR) polymorphism analysis has become an essential tool in forensic casework. The vast number of STRs now available led the Italian Group for Forensic Hematology (GEFI) to launch a program aimed at studying their genetics in the Italian peninsula. Recently, 15 laboratories have been committed in the project of producing at least 50 HumTH01 (11p15.5) genotypes from their own district (and to type two bloodstains, as controls).

DNA was extracted from whole blood according to standard procedures, either by Chelex or by phenol-chloroform method. PCR was carried out with the primers described by Edwards (1991), at the final concentration of 0.2 μ M; the amplification conditions suggested were as followed: 94°C for 60 seconds, 64°C for 75 seconds and 72°C for 120 seconds for 26 cycles.

The participating laboratories employed several separation protocols (mostly native or denaturing polyacrilamide gel electrophoresis) followed by either silver staining, radioactive detection or automated laser-fluorescent detection. Allele designation was achieved by side to side comparison with an allelic ladder containing all the alleles described in literature but 9.3. 1560 individuals were typed in the end, covering most of Italian peninsula and a considerable number of families were investigated.

Eight alleles, ranging from allele number five to the one containing eleven repeats, were detected but not all laboratories showed the same ability to clearly identify 9.3 and 10, due to the choice of native separation conditions employed. Slight internal differences were observed in the distribution of frequencies. The 9.3 allele showed the highest frequency (24.5%), allele 5 and 11 were only observed twice. No mutation were observed in over 200 meioses.

LEGAL AND DEONTOLOGICAL ASPECTS OF INCIDENTAL PATERNITY
EXCLUSION IN GENETIC COUNSELLING

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Paternity exclusions may incidentally occur during family analyses for genetic diagnosis of inherited diseases. In such cases deontological and legal problems could arise in order to assess the intergenerational transmission of the disease and inform patients and relatives about the complete outcome of tests.

In many cases consultants are not previously informed about the possibility to detect a non-paternity within the sibship through linkage studies and the disclosure of this unrequested result would determine critical situations.

When the legal father is affected, detection of non-paternity can be considered an implicit diagnosis of non transmission; while in cases of mother affliction different perspectives can occur.

Four cases are analysed where paternity exclusion was incidentally diagnosed by molecular analysis for adult dominant polycystic kidney disease (ADPKD) and confirmed by STR polymorphisms analyses.

In one family 2 out of 4 children were excluded from paternity; only one child was involved in other cases. For one of the latter, it was impossible to assess diagnosis because patient's mother was excluded from paternity and she was the only affected relative still alive, being the affected grandmother dead.

In these cases a conflict between professional secrecy and the right to be informed about one's own diagnosis may not find simple practical solutions. Moreover, legal fall-out of the "unrequested" paternity exclusion may induce the counsellor to grant a privilege to professional secrecy. In any case, previous information could contribute to resolve such situations even if some patient would renounce to predictive tests in view of such a perspective.

A REVIEW OF THE COLLABORATIVE EXERCISES OF THE SPANISH AND
PORTUGUESE ISFH WORKING GROUP

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The Spanish and Portuguese working group of the ISFH comprises a total of 22 laboratories from Spain, Portugal and some South American countries. Practically all the casework in forensic genetics in Spain (15 labs) and Portugal (5 labs) is carried out in these laboratories. Since 1990 the Group have organized collaborative exercises in DNA polymorphisms with the idea of progressing in standardization and for the discussion of technical and statistical problems as a first step towards a quality control program in Spain and Portugal. Three exercises have been carried out up to now. In all these exercises 6 bloodstains were sent to the different laboratories to be studied using DNA polymorphisms. The systems used in the first exercise (1991-1992) were two SLPs (YNH24 and MS43a) and two PCR systems (HLADQA1 and D1S80). HUMTH01 was added in the second exercise (1993-1994). In the last exercise (1994-1995) Polymarker, VWA31, F13A1 and FES were added to the above mentioned systems. In this exercise 15 labs have participated with very satisfactory results. The main aim of the Spanish and Portuguese labs is now to organize a quality control program for both countries.

These collaborative exercises together with other activities of the group (statistics, legal regulation in Spain and Portugal) have proved to be extremely valuable and have clearly improved the quality of the medico-legal work in forensic genetics in Spain and Portugal.

COMPATIBILITY OF RFLP RESULTS BETWEEN LABORATORIES: AABB/CAP SURVEY DATA

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RFLP results were reported by over 60 participants on the three mailings in the AABB/CAP Parentage Testing Survey in 1994. Over 200 RFLP analysis were reported on each of the three whole blood samples included with every mailing. To determine variability between laboratories of reported band size (kb) a comparison was done on selected probe/enzyme combinations for loci with multiple participant results. The following table shows band sizes reported in multiple laboratories.

D10S28/pTBQ7 (HaeIII)	A(ch) n=21	B(mo) n=17	C(af) n=21
Range (kb)	5.63 to 6.0	3.68 to 4.05	0.62 to 0.73
Mean +/- SD	5.85 +/- 0.105	3.74 +/- 0.084	0.68 +/- 0.021
CV	1.79%	2.2%	3.1%
D12S11/SL1737 (PstI)	A(mo) n=7	B(mo) n=9	C(mo) n=7
Range (kb)	9.14 to 10.16	13.03 to 13.72	8.41 to 8.83
Mean +/- SD	9.89 +/- 0.32	13.42 +/- 0.22	8.55 +/- 0.13
CV	3.2%	1.7%	1.6%

Paternity indices reported for these same RFLP marker systems had coefficients of variation greater than 40% and a 3 to 5 fold range between low and high values.

AABB/CAP survey results show that although laboratories using different test systems reports comparable band sizes for numerous RFLP markers, calculation of the paternity index from similar test data is highly variable.

PROFICIENCY TESTING IN FORENSIC DNA ANALYSIS

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The aim of proficiency testing is to provide a mechanism by which laboratories can assess the accuracy of their systems. In the forensic setting, proficiency testing allows each laboratory to compare the results of their analysis on mock crime samples, with results produced by other laboratories around the world, whilst retaining their anonymity.

Since April 1991, when Cellmark launched the International Quality Assessment Scheme (IQAS), 78 participating laboratories have received 17 sets of mock crime samples, returning 551 sets of results. Every year, four distributions of the proficiency test are sent to each laboratory. Each distribution consists of five samples, one mock scene of crime sample, three control blood stains and a blank control for the crime sample. Participants are asked to analyse the samples, using their usual laboratory procedures and return data for each sample, identifying which of the control samples matches the mock crime sample.

A range of different methods has been used to analyse samples, including single locus probes (both using Hinf I and Hae III), STR's, HLA-DQ α and Polymarker. Although results on more than 2,000 samples have been reported to date, no incorrect matches have ever been made. Where laboratories have used both SLP testing and PCR-based systems on the same sample, no inconsistent results have ever been reported. Comparison of SLP data from all the different laboratories reveals a high level of reproducibility. For example, the band sizes calculated by 40 laboratories for both alleles in the mock crime sample and the genomic control K562 from the latest IQAS distribution, all fell within a range of ± 2.5 standard deviations.

PARENTAGE TESTING SURVEY PROGRAM OF THE COLLEGE OF AMERICAN PATHOLOGISTS: DNA POLYMORPHISMS BY PCR TECHNIQUE.

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Since its inception in 1993 the College of American Pathologists (CAP) parentage testing survey program has received results from participating laboratories on 7 mailings. A marked increase in the number of laboratories reporting PCR results and loci tested by PCR occurred between 1993 and 1994.

Six of these mailings occurred in 1993 and 1994. The data from the seventh mailing in March of 1995 will be available in June 1995 and will be presented along with the data summarized below.

In 1993, 7 laboratories reported results at the HLA DQA1 locus and 4 at the D1S80 locus. In contrast in 1994 results were reported in up to 18 different loci, with more than 10 laboratories reporting results in 7 loci for the 1994 PI-C mailing. At these loci, HLA-DQA1, D1S80 and Amplitype PM (LDLR, GYPA, HBG, D7S8, GC), consensus could be determined. There were 3 non consensus results out of 240 results reported in these systems. Standardization of nomenclature used for some of these systems would make interlaboratory comparison easier.

It is interesting to note that 2 genetic systems tested by PCR, GYPA and GC correspond to the MN locus (red cell antigen) and Gc locus (serum protein by isoelectric focusing) respectively. Results in all 1994 mailings showed consistency between the types reported for all 3 individuals tested by both techniques.

THE FORENSIC IDENTITY PROFICIENCY SURVEY PROGRAM OF THE COLLEGE OF AMERICAN PATHOLOGISTS

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The College of American Pathologists (CAP) introduced a new survey in 1993: the Forensic Identity (FID) Proficiency Testing Program. The FID Program is designed to provide proficiency testing for laboratories that perform forensic casework analysis using current DNA technology. Through this program, a laboratory can compare its results with those produced by other DNA testing laboratories. Two survey test kits consisting of 3 to 7 specimens (bloodstain references, bloodstain evidentiary material, semen stains, and mock vaginal swabs) are sent to participants each year.

Laboratory methodology and genetic results are compiled and tabulated. This information consists of band sizing data from restriction fragment length polymorphism analysis and results from *in vitro* nucleic acid amplification. Results show that approximately 93 percent of the respondents use *HaeIII* restriction enzyme; while the other seven percent of laboratories use either *HinfI* or *PstI* restriction enzymes. Polymerase chain reaction-based results consist of reverse dot blot assays and amplified fragment length polymorphisms (both long tandem repeats and short tandem repeats).

The data from the cell line K562, a National Institute of Standards and Technology (NIST) certified standard reference material (SRM 2390) for DNA profiling, reported in the FID Surveys will be presented and compared to the NIST certified value. The accuracy and precision of these results are outstanding.

International enrollment has continued to increase. Four of the 44 laboratories enrolled in the first pilot Survey (March 1993) were from other countries. Twelve of the 90 enrollees in the most recent Survey (March 1995) were from abroad. Surveys are being shipped to Australia, Canada, Germany, Japan, Singapore, Switzerland, the United Kingdom and the United States.

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Marcotte A.	EP33	124		HP24	206
Marcher H.	EP 6	98		HP25	207
Marschak T.	AO 2	5	Morris J.	IO 3	256
Martín P.	DP 1	58		IO 4	257
	DP 2	59		IO 5	258
	DP 9	66		IP 5	266

	IP 7	268	Oliva H.	DP 2	59
Moscetti A.	AO 5	8	Oste C.C.	FO 3	130
	HP18	200	Ota M.	BO 5	22
Mount M.	GO 1	154		FP 8	143
Mourello S.	HP42	224	Oya M.	GP 7	165
Mudd J.	IO 1	254		GP 8	166
Mudd J.L.	IP 8	269	Pádár Z.	HP64	246
Muñoz I.	BP 4	26	Paoli M.	HP61	243
	EP 1	93	Pappalardo G.	DP17	74
	FP 1	136		FP 9	144
	FP10	145	Paredes M.	HP11	193
	HP46	228	Parker M.	IP 6	267
Murai T.	HP27	209	Parkin B.H.	BO 1	18
Murray W.	DO10	53	Parson W.	HP 4	186
Muylle L.	DP24	81	Pascal O.	AP 5	14
Nagai A.	HP43	225	Pascali V.L.	AO 5	8
Nagy M.	DO 4	47		HP18	200
Nakamura S.	FO 8	135	Paszkowska R.	HP45	227
	HP44	226	Patsoura A.	GP 2	160
Nakatome M.	AP 3	12	Pawlowski R.	HP45	227
Nardone M.	CP 2	37	Peeters I.	DP24	81
	GP 1	159	Peloso G.	HP61	243
	HP61	243	Pelotti S.	DP17	74
Narveson S.	IP 8	269		FP 9	144
Neuweiler J.	DO14	57	Penaud A.	AP 5	14
Newall P.	DO10	53	Peñuela C.	HP11	193
Niederstätter H.	HP 4	186	Pérez-Pérez A.	DP19	76
Nishi K.	DP26	83	Perlee L.	BO 2	19
	GP16	174		DO14	57
	GP17	175	Pescarmona M.	HP18	200
Nishimura A.	DP26	83	Pestoni C.	BP 4	26
	GP16	174		EP 1	93
	GP17	175		FP 1	136
Nogués R.M.	HP 3	185		FP10	145
Ogura Y.	AP 3	12		HP 2	184
Ohkubo I.	GP17	175		HP46	228
Ohshima T.	EP15	107	Pfitzinger H.	DO11	54
	GP11	169	Phillips C.	DO 3	46
Ohue O.	FO 8	135		EP31	122
Ohya I.	HP43	225		GP12	170
Okazaki Y.	FO 8	135	Piccinini A.	HP47	229
Olaisen B.	AP 1	10	Picornell A.	HP 1	183
	CO 3	34	Pichler S.	EO 2	89
	DO 5	48	Pierucci G.	HP61	243
	DP 4	61	Pignatti P.F.	HP19	201
	DP11	68	Pinheiro M.F.	HP48	230
	EP21	113	Pinto da Costa J.	HP48	230
Oldroyd N.J.	DO 1	44	Pivetti S.	HP13	195
	DP16	73	Plate I.	AO 3	6
	Lecture	87		FO 4	131
Olek K.	AO 6	9	Pöche H.	EP15	107
	AP 4	13	Podskarbi T.	GP 2	160

Polesky H.	FP14	149	Ritter H.	DP14	71
	GO 1	154		DP18	75
	IO 3	256		DP22	79
	IO 4	257		DP25	82
	IO 5	258		HP37	219
	IP 5	266	Rittner C.	DO 2	45
	IP 7	268	Rittner G.	DO 2	45
Pörtl R.	DP18	75	Riva A.	HP61	243
	DP22	79	Rivas E.	EP22	114
Pontes M.L.	HP48	230	Robertson J.M.	FO 1	128
Ponzano E.	EP 4	96	Roby R.	IO 3	256
	HP57	239		IO 4	257
Prata M.J.	HP 5	187		IO 5	258
	HP26	208		IP 5	266
	HP49	231		IP 7	268
Presley L.A.	IO 1	254	Roby R.K.	IP 8	269
Previderè C.	HP61	243	Rocchi A.	CP 2	37
Prieto L.	DP19	76		GP 1	159
	EP 2	94	Rodriguez D.	HP61	243
	GP13	171	Rodriguez-Calvo M.S.	BP 4	26
	HP 7	189		EP 1	93
	HP 8	190		FP 1	136
	HP28	210		FP10	145
Prieto V.	EP25	117		HP46	228
	HP 6	188	Roewer L.	DO 4	47
	HP51	233	Rojas A.	EP19	111
Primorac D.	HP19	201		GP13	171
Prinz M.	DP 5	62	Rostedt I.	DP20	77
Püschel K.	EP23	115	Rousselet F.	DO11	54
	HP 9	191	Roussy P.D.	DO 6	49
Rabold U.	EP20	112	Roy R.	FP15	150
	HP59	241	Rubin S.	AO 2	5
Ramírez E.	EP 8	100	Rudbeck L.	EP 6	98
	HP14	196	Ruíz B.	EP32	123
	HP15	197	Ruiz de la Cuesta J.M.	DP19	76
	HP16	198		GP 5	163
	HP38	220		GP13	171
Ramon M.M.	HP 1	183		HP 7	189
Rand S.	HP30	212		HP 8	190
	HP47	229		HP28	210
Reeder D.J.	IP 8	269	Rundgren A.	FP 2	137
Reichert W.	HP35	217	Rysiecki G.	FP 3	138
Reinhold J.	BP 1	23		FP 4	139
	HP50	232		IP 6	267
Repetto G.	DP10	67	Saito K.	HP27	209
	HP52	234	Sakai I.	BP 5	27
Reynolds R.	AO 2	5		EP24	116
Reys L.	HO 1	180	Salas A.	BP 4	26
	HP20	202	San José S.	EP19	111
Ribeiro T.	HO 1	180		GP13	171
	HP20	202	Sánchez C.	HP25	207
Riordan A.S.	IP 6	267	Sanchez J.	EP11	103

Sanchez-Hanke M.	EP23	115	Seidl C.	DO12	55
	HP 9	191		DP14	71
Sancho M.	DP 1	58		DP23	80
	DP 2	59		EP20	112
	DP 9	66		HP58	240
	EP18	110	Seifried E.	HP59	241
	HP40	222		DO12	55
Santos M.F.	GP14	172		DP23	80
Santos S.M.	HP53	235		EP20	112
	HP54	236		HP58	240
Sanz P.	DP10	67		HP59	241
	EP25	117	Seim S.	FP14	149
	HP 6	188		GO 1	154
	HP51	233	Sekiguchi K.	BP 5	27
	HP52	234	Sekiguti K.	EP24	116
Sasaki M.	EP26	118	Seta S.	BP 5	27
Sato H.	BP 5	27		EP24	116
Sato M.	DP26	83	Sevin A.	HP 3	185
	GP17	175	Shin Y.S.	GP 2	160
Sawaguchi A.	DP21	78	Shiono H.	EP26	118
	FO 8	135	Shutler G.G.	DO 6	49
	HP44	226		DO 7	50
Sawaguchi T.	DP21	78	Singer R.L.	IP 8	269
	FO 8	135	Sinões F.	HP53	235
	HP44	226		HP54	236
Scoltz H.J.	EP27	119	Sjerps M.	HP32	214
Schacker U.	GO 5	158	Sliwka K.	HP41	223
Schalsphoff T.	EP27	119	Souto L.	HP65	247
Schans van der M.J.	FO 2	129		HP66	248
Scheil H.G.	HP17	199	Sparkes R.L.	DP16	73
	HP29	211	Spinetti I.	CP 2	37
Schickle H.P.	FP11	146		GP 1	159
Schmidt W.	AO 6	9		HP61	243
	AP 4	13	Sprecher C.	DO13	56
	DO 8	51	Stancu V.	HP29	211
Schmitt C.	DP 5	62	Steffens D.L.	FP15	150
Schneider H.R.	EP28	120	Stenersen M.	CO 3	34
Schneider P.M.	DO 2	45	Stinton V.J.	HP67	249
Schneider V.	EP15	107	Stover P.	FP14	149
Scholten D.	HP29	211	Sullivan K.M.	AO 1	4
Schumm J.	DO13	56	Summerfield O.	EP31	122
Schurenkamp M.	HP30	212	Sun R.	GP11	169
Schütte U.	HP30	212	Suzuki K.	GO 3	156
Schwartz D.W.M.	BO 3	20		GO 4	157
	BO 4	21		GP 6	164
	BP 3	25	Sweet D.J.	EP30	121
	HP55	237	Syndercombe Court D.	DO 3	46
	HP56	238		IO 6	259
Schwartz-Jungl E.M.	BO 3	20	Syroczyńska A.	HP41	223
	BP 3	25	Szekelyi M.	GO 3	156
	HP56	238	Szibor R.	AO 3	6
Sedlmayr S.	DP22	79		FO 4	131

Tafuro S.	HP21	203	Vide M.C.	HP65	247
Tagliabracci A.	HP60	242		HP66	248
	HP61	243	Vieira D.N.	HP66	248
Takada A.	HP27	209	Villanueva E.	EP30	121
Tamayo G.	DP 3	60		FO 7	134
	EP 5	97		HP36	218
Tauscher O.	EP16	108		IO 8	261
Teixidor D.	EP20	112	Virgili A.	EP10	102
	HP59	241	Wakasugi C.	AP 3	12
Terada M.	AP 3	12	Walker R.	IO 3	256
Tesson C.	AP 5	14		IO 4	257
Teyssier A.	FP12	147		IO 5	258
Thomson J.	DO 3	46		IP 5	266
	EP31	122		IP 7	268
Thymann M.	GP15	173	Walker R.H.	IO 2	255
Torre C.	EP 9	101		IP 8	269
	EP10	102	Walsh P.S.	FP13	148
Trovoada M.J.	GP14	172	Walter H.	HO 2	181
	HP49	231	Walton Rogers P.	EP14	106
Tsai L.C.	GP10	168	Wang X.	DP21	78
Tsuji H.	GO 3	156		FO 8	135
Tully G.	AO 1	4	Watanabe H.	HP27	209
Turbón D.	DP19	76	Watanabe Y.	HP43	225
Uchida A.	GO 4	157	Watson S.K.	DP16	73
Ueyama H.	GP17	175		Lecture	87
Urquhart A.J.	DO 1	44	Weedn V.W.	IP 8	269
	DP16	73	Weichhold G.M.	HP62	244
	Lecture	87		HP63	245
Ushiyama I.	DP26	83	Weidinger S.	Lecture	153
	GP16	174	Weiler G.	DO 9	52
	GP17	175	Weir B.S.	Lecture	179
Valentina V.	GP 6	164	Weispfenning R.	DP25	82
Valenzuela A.	EP30	121	Welz A.	HP45	227
Valverde E.	CP 1	36	Wenz M.	FO 1	128
	EP32	123	West S.	HP29	211
	HP10	192	Westermeier R.	FP11	146
Valverde J.L.	EP 8	100	Wiebe V.P.	GP 6	164
	HP14	196	Wiegand P.	EP23	115
	HP15	197	Wiessner L.	FP14	149
	HP16	198		GO 1	154
	HP38	220	Williams P.E.	FP14	149
Vandenbergh A.	DP24	81	Woller J.	HP64	246
Vandenbroeck M.	DP24	81	Wouters J.	EP14	106
Varlaro J.	AO 2	5	Wu J.	GP 3	161
Venturi M.	CP 2	37	Wu M.	GP 3	161
	GP 1	159		GP 4	162
Venturini J.	DO14	57	Xiao F.-X.	AO 4	7
Verdú F.	HP 2	184	Xu Y.	GP 3	161
Verheesen M.	DP27	84	Yabe K.	HP27	209
Vespi G.	EP10	102	Yamada M.	DP26	83
Vicente M.T.	EP22	114		GP16	174
Victor J.	FO 5	132		GP17	175

Yamada S.	HP43	225
Yamada Y.	DP26	83
Yamamoto Y.	GP16	174
Yamazaki M.	AP 3	12
Yoshida K.	BP 5	27
	EP24	116
Zang K.D.	EP20	112
Zanon C.	EP 9	101
	EP10	102
Zapata M.	GO 5	158
ZawTun	AP 3	12
Zehethofer K.	HP 4	186
Zhang Y.	GP 4	162
Zignale G.	EP 9	101

INTERNATIONAL SOCIETY FOR FORENSIC
HAEMOGENETICS

16th INTERNATIONAL CONGRESS

LATE ENTRIES

(Abstracts received after the deadline)

Santiago de Compostela. Spain
September 12-16, 1995

AUTOMATED FLUORESCENT SEQUENCING OF MITOCHONDRIAL
DNA FOR ITALIAN POPULATION FREQUENCY DATA.

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Servizio Polizia Scientifica; Sez. Indagini Biologiche, Rome-Italy

Analysis of mitochondrial DNA sequence is an method to established mtDNA type. In the context of forensic science a determination of the frequency of the different mtDNA types in the population must be done.

This study presents population databases from 100 unrelated individuals representative of all Italian regions. The mtDNA regions subjected to analysis was HVI (16.024-16.365) and HVII (73-340). In both HVI and HVII, two sets of primers was used to amplify the regions of the mtDNA control region. Mitochondrial DNA sequencing was performed with fluorescence-based automated sequencer (373, ABD of P.E. CA) from PCR products, purified by filtration in a Microcon-100. Two different types of chemistries was employed with fluorescent sequencing, Primer chemistry and Terminator chemistry, in order to confirmed each sequence. Replication of sequencing runs was used to overcoming sequence ambiguities. Sequence comparisons was made by using the SeqEd sotware (ABD, P.E. CA).

A calculation of the frequency of a mtDNA type is the number of times the specific sequence has been observed, divided by the number of samples in the databases. This counting method was adopted only with DNA sequence information that was free from ambiguities within the region of comparison.

HARDY-WEINBERG EQUILIBRIUM IN RFLP DATABASES

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Assessment of HW Equilibrium in RFLP systems is complicated by measurement error and the apparent continuous nature of the allele spectrum. Attempts to assess it by binning usually result in an apparent excess of homozygotes. Jeff Morris and I explained in the 1990 Promega proceedings how an artifact would always inflate the homozygote count, but we were not able to surmount the problem at that time. Devlin, Risch, and Roeder confirmed that the effect was artefactual for some particular databases by a complicated method of statistical analysis. Other papers have claimed that the explanation is bands running off the gel.

I have discovered a new and simple approach that gets around the artifact. Analysis of 200+ databases shows that there are many for which the impression of disequilibrium disappears by using the new method. On the other hand, there are also many databases where the disequilibrium remains. Many of these involve the probes g3 and YNH24, for which running off the gel is confirmed.

FORENSIC EFFICIENCY AND GERMAN POPULATION DATA FOR THE TETRAMERIC STR POLYMORPHISM DHFRP2 (HUMFOLP23)

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guertel 60-62, D-50823 Koeln

Hypervariability in short tandem repeated sequences of genomic DNA (STRs) is one of the most reliable ways of personal identification from biological samples. We examined the properties of the human dihydrofolate reductase psi-2 pseudogene (Polymeropoulos et al. 1991) in a German population. Analyzing the allelic distribution of 102 unrelated Germans we detected six alleles; minor differences to the allelic distribution given in the original paper were found. A new allele was found.

allele	our results	Polymeropoulos et al.
A1	4%	4%
A2	36%	44%
A3	30%	15%
A4	22%	25%
A5	5%	12%
A6	4%	---
Heterozygosity	72%	70%

Amplification of samples containing less than 50 pg DNA led to clear signals on the ALF sequencer. Due to the simple repeat structure (Urquhart et al. 1994), the short fragment length and the low detection limit, this locus should be a valuable forensic marker.

Kimpton et al. (1993) PCR Meth Appl 3, 13-22
Urquhart et al. (1994) Int J Leg Med 107, 13-20
Polymeropoulos et al. (1991) NAR 19, 4792

Typing for *Alu* insertions in paternity disputes in Colombia.
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 Rene J. Herrera (2) and Emilio Yunis T. (1) Instituto de
 Genética, Universidad Nacional de Colombia, Santa Fé de
 Bogotá, D.C. Colombia South America (1) and Department of
 Biological Sciences, Florida International University,
 Miami, FL. (2)

We have analyzed 2111 Colombian individuals from 606 families in paternity disputes in Colombia after red cell antigen typing was performed and no exclusions were demonstrated. These 606 families were further analyzed for MHC class II alleles, (DRB, DQA1 and DQB1) and a paternity exclusion was demonstrated in 116 of them (19.14%). Out of the 116 families, 82 families were typed for five different *Alu* insertion markers, named TPA, ACE, PV, APO, and F13. Typing was performed by PCR amplification followed by electrophoresis analysis to determine the presence of the insertion and/or the lack of insertion alleles. This methodology was used to determine the power of exclusion of these markers in paternity studies and to establish a database to be used in similar studies. One or more *Alu* polymorphic loci confirmed the paternity exclusion in 47/82 (57.32%). Table 1 shows the percent of exclusion for each *Alu* insertion markers analyzed.

	TPA	ACE	APO	PV	F13
# of cases typed	82	82	82	82	82
Lack exclusions	70	68	75	69	61
Exclusion	12	14	7	13	21
% Exclusion	14.63	17.00	8.53	15.85	25.60

According to our results, although 42.68% of the MHC class II excluded samples failed to be excluded by the polymorphic *Alu* insertion loci, there were innumerable paternity cases in which polymorphic *Alu* insertion markers were critical in proving exclusion when red cell antigen and MHC class II typing were non-conclusive.

MOLECULAR INVESTIGATION OF THE HUMAN REMAINS FROM AMIA EXPLOSION IN BUENOS AIRES CITY BY COMBINED STRs, VNTRs, MVR-PCR TYPING AND mt-DNA SEQUENCING. Corach, D.; Sala, A.; Penacino, G. and Sotelo, A. Serv. de Huellas Digitales Genéticas, Fac. de Farm. y Bioq. UBA. Junín 956, 1113 Buenos Aires, ARGENTINA.

On July 18th, 1994 an explosion destroyed the building of a Jewish Association (AMIA) in Buenos Aires City. About 300 injured and 90 fatal victims resulted. Our national authorities required us to characterize hypervariable DNA markers of human remains, (circa 80) which displayed variable degrees of putrefaction. The goal of this investigation was: a- to determine the real number of victims whose fragmentary remains were received, b- to identify remains by comparison with conventionally identified victims and c-to establish the identity of the remains by comparison with potential relatives of the victims. DNA-based molecular typing included STRs *loci* (HUMTHO-1, HUMFABP, HUMRENA4, HUMFES/FPS, D6S366, HUMHPRTB, HUMVWA and Y27H39), VNTRs *loci* (D1S7, D2S44, D4S139 and D5S110) and PCR-MVR (*locus* MS-32). Our methodological strategy was designed in order to speed up the characterization procedure. For this purpose multiplex STR typing was performed; one of the reactions included two sex chromosome specific microsatellites (HUMHPRTB and Y27H39) and a monomorphic Y-specific sequence, that allowed gender determination and efficiently contributed to identification purposes. Samples displaying identical STR genotypes were then confirmed by means of VNTR typing. Band shift or absence of signal due to high degradation of DNA from cadaveric samples demanded additional typing. MVR-PCR and mitochondrial DNA sequencing approaches were used; these systems denoted a high sensitivity and efficiency for identifying remains especially in those cases in which severe degradation affects forensic DNA samples.

BIOSTATISTICAL STUDY INTO THE EFFICIENCY OF
INDIVIDUALISATION USING NON-ISOTOPIC CHEMILUMINESCENT
ENHANCED NICE™ MULTI-LOCUS DNA PROBES

Mr Peter P.C. HAU, Miss ELIZABETH H. Watt.
DUNDEE, SCOTLAND

The efficiency of individualisation using non-isotopic chemiluminescent enhanced NICE™ DNA multi-locus (MLP) probes was investigated by the analysis of DNA fingerprints produced from 191 unrelated Caucasians. Novel analysis of the scoring procedure allowed the inclusion of 585 pairs of the possible comparisons of these samples for each of two probes. When the results of NICE™ probes 33.6 and 33.15 were combined, the mean percentage of band sharing between two unrelated individuals was found to be $16.75\% \pm 1.12\%$. The mean number of bands identified by 33.6 and 33.15 in an individual DNA fingerprint was 54.8 ± 6.8 .

Pilot study results from both isotopically-labelled and NICE™ MLP probes showed that the two labelling systems were very similar indicating comparable efficiencies for differentiating between individuals. Analysis of DNA fingerprints produced from 37 showed a mutation rate of 0.71% per band when using the NICE™ probes and that the two labelling systems were equally efficient in establishing family relationships.

COMPARISON OF TURKISH SUBPOPULATIONS
USING TWO STR's

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Institute of Legal Medicine, University of Münster 48149 Münster-Germany

The short tandem repeat (STR) systems HumTHO1 and HumVWA were used for PCR typing of two Caucasian subpopulations, one living in the Black sea area (northern Turkey), the other being a subpopulation from eastern Anatolia (eastern Turkey). The allele frequency data were compared with a Turkish subpopulation from Adana area (southern Turkey) and a group of Turks living in Brussels (Belgium). The HumTHO1 data showed a significant difference to the Black sea region compared with the 3 other groups, while the subpopulation from the eastern Anatolia showed significant differences in the HumVWA system.

COMPARISON OF THE GENETIC STRUCTURE AMONG BUENOS AIRES METROPOLITAN POPULATION AND THREE ARGENTINIAN ABORIGINAL GROUPS BY MEANS OF TEN STRs ANALYSES. Sala, A.; Penacino, G.; Goycochea, A.; Carnese, F.; Tomeo, A. and Corach, D. Serv. de Huellas Dig. Genéticas, Fac. Farm. y Bioq. UBA. Junín 956, 1113-Buenos Aires, **Argentina**.

D1S80 AMP-FLP ATTRIBUTES IN TWO DIFFERENT ETHNIC GROUPS OF ARGENTINIAN POPULATIONS. Penacino, G.; Sala, A.; Smerick, J.; Perez Calvo, J.; Baechtel, S.; Budowle, B. and Corach, D. Serv. de Huellas Dig. Genéticas, Fac. de Farm. y Bioquímica UBA. Junín 956, 1113-Buenos Aires, ARGENTINA.

STR is becoming the most popular DNA typing approach, of frequent use in forensic casework and, more recently, in paternity testing. The availability of a high number of different STRs systems may overcome the intrinsic restrictions of these genetic markers such as reduced number and predominant frequencies of some alleles. Nevertheless its applicability in forensic identification should be underscored especially due to sample economy, sensitivity even with severely degraded evidentiary DNA samples, and rapidity of analysis. Besides its applications in the identification field, the microsatellites offer a rapid survey for population genetics studies. The aim of this work was the characterization of two different ethnic groups: caucasian (Buenos Aires Metropolitan Area habitants) and three aboriginal tribes (Mapuche and Tehuelche from South-West Argentina and Wichi from Northern Argentina)- by means of the following STRs: HUMTHO-1, HUMFABP, HUMHPRTB, HUMRENA4, HUMVWA, HUMFES/FPS, D6S366, HUMCSF1PO, HUMF13A01 and Y27H39. Allelic and genotypic frequencies distributions were determined, and heterocigosity and discriminative power were then estimated. The overall comparison of our Metropolitan population with other North American caucasian and Mexican-American data denoted similar attributes. In contrast, some STRs depicted differences among Metropolitan and aborigin populations, the latter exhibiting some distinct attributes that may be summarized as: different genotype distributions for HUMTHO-1 and HUMVWA, a sharply distinct distribution in the Y-specific alleles of Y27H39, and increased values of homocigosity. The overall data may represent a contribution to the knowledge of the genetic attributes of our autoctonous and aloctonous populations and allow the construction of database to be used in forensic casework.

D1S80 is a highly polymorphic, variable number tandem repeat locus that is used widely in forensic analysis of biological materials. In order to gain a further appreciation of the population genetics of the D1S80 locus two Argentinian populations of different ethnic origin were analyzed. One sample consisted 191 unrelated donors inhabiting the metropolitan area of Buenos Aires; this sample was composed essentially of Caucasians. The other sample population consisted of 62 unrelated Mapuche aborigines from three geographically close comunites of the Rio Negro Province. Blood samples were amplified at the D1S80 locus by PCR, the amplicons were separated by PAGE and the products were detected by silver staining. There were 60 and 28 different genotypes observed in the Buenos Aires and Rio Negro Province communities, respectively. The aboriginal sample exhibited some genotypes, not observed in the metropolitan sample, such as: 31/37; 18/34; 21/25, 21/34, 23/24 and 25/25. These different observed genotypes may be the result of sample variance. There were some allele frequency differences between the two samples. Observed heterozygosity in both groups displayed the following estimates: Metropolitan 82.7%; Mapuche 87.1%. The expected heterozygosity was 87.1% and 86.7%, respectively. These data are contributing to the development of a local database and the search for candidate genetic markers for anthropological studies.