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ABSTRACTS

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RAPID DNA GENOTYPING ON CAPILLARY ARRAY ELECTROPHORESIS CHIPS

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Capillary array electrophoresis (CAE) allows the analysis of multiple samples in parallel by capillary electrophoresis. We have reported previously the potential of CAE with conventional capillaries for STR typing; multiplex genotyping can be done in 20-40 min. We demonstrate here that analytical throughput can be increased by at least an order of magnitude using microfabricated CAE chips. First generation CAE chips, microfabricated on 50x75 mm glass slides, have been designed to analyse 12 samples in parallel with run times under 3 minutes for DNA fragments up to 622 bp. Sample detection employs a laser-excited confocal fluorescence scanner; laser excitation is at 488 nm and two-color detection is achieved by splitting the fluorescence signal into a green channel (510-540 nm) and a red channel (645 nm). The value of the CAE chip for rapid, high-throughput analysis has been demonstrated with its use to genotype HFE, the candidate gene for hereditary hemochromatosis and a potential target for large scale genetic screening. The defective gene contains a mutation which introduces a restriction site into the HFE gene sequence; the CAE chip cleanly separates the restriction fragments associated with the variant and normal types in only a few minutes. A second generation CAE chip has been designed which enables the analysis of 48 or 96 samples on a single chip. These CAE chips should be valuable in the development of high-speed, high-throughput forensic identification and genetic screening methods.

DETECTION OF A DNA SEQUENCE BY SURFACE ENHANCED RESONANCE RAMAN SCATTERING OF A MODIFIED DNA PROBE

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Abstract

A new accurate and reliable method for DNA detection has been developed based on adsorption of DNA on colloidal silver and subsequent signal detection using surface enhanced resonance Raman scattering (SERRS). Improved surface and aggregation chemistry has resulted in acceptable reproducibility for biological applications. This involved the use of a novel aggregating agent and also chemically modified DNA. The increased sensitivity circumvents the need for an amplification step and the widespread fluorescence quenching inherent in SERRS greatly extends the range of effective DNA labels by enabling the use of both fluorescent and non-fluorescent chromophores. Further, compared to fluorescence, much greater selectivity is obtained due to the sharp vibrational spectra observed thus reducing the need for separation procedures. The presence of labelled single stranded DNA has been detected in solution down to a level of 8×10^{-13}M and the presence of double stranded DNA varying in length from 36 to 254 bases has been confirmed at concentrations estimated to be similar. A specific sequence, which is part of the DQα gene, has also been detected by using SERRS and a solid capture step. The existence of such sensitivity and selectivity could revolutionise current approaches for the detection of genetic material.

Cellmark Science

DETERMINATION OF THE MTDNA CONTENT IN A HUMAN HAIR BY USING A QUANTATIVE PCR ASSAY

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Sequence analysis of the d-loop region of mtDNA is a powerful method for genetic identification of biological samples with a very low or highly degraded nuclear DNA content. It is extremely useful for shed hair samples where the absence of a hair root does not allow to obtain a DNA profile. Up to now it is known that a typical hair root contains about 200 ng of nuclear DNA while nuclear DNA in the hair shaft can not be determined with present methods (D17Z1 quantification). However, the mtDNA content of hair shafts has not yet been determined although it contains sufficient material for mtDNA analysis.

In the present study, a quantitative PCR assay was developed to determine the number of mtDNA copies in a biological sample. The method is based on a competitive PCR where the same primers are used for the amplification of a fragment of 131 bp (16,290-16,420) in the first hypervariable region of the d-loop and an internal control of 121 bp. This control is a construct of the same mt-fragment containing a 10 bp deletion and the amount of input DNA of the internal construct is known. The resulting fluorescent-labelled PCR products are quantified on the ALF DNA sequencer. DNA was extracted from the root and from the shaft (each 1 cm) of hairs from different individuals. The results showed that a typical hair root contains about 24,000,000 copies of mtDNA and a hair shaft 600,000/cm. By using a differential lysis procedure on the hair root we were able to show that a substantial amount (7,000,000) of mtDNA is present in the hair bulb. No nuclear DNA profile could be obtained from this DNA extract. Therefore, if possible, a mtDNA analysis should be preferentially done on the bulb portion of the hair.

The method presented here is very sensitive and even allowed us to determine the mtDNA content in 140 year old hair (50 copies/cm). Therefore, it is not only useful in forensics but also for ancient DNA studies. Moreover, the assay is human (or primate) specific so that it can be used to determine the human origin of a hair sample.

bone 1g 1-2x 10⁵ copies

SEPARATION OF SPERM AND VAGINAL CELLS FROM POSTCOITAL SWAPS USING FLUORESCENCE ACTIVATED CELL SORTING (FACS) FOR FORENSIC PURPOSES

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The preferential lysis is a well established method to isolate sperm DNA from mixed stains. However, chances for successful typing are limited when only few spermatozoa and numerous vaginal cells are recovered from a swab. To overcome this problem a protocol involving fluorescence activated cell sorting (FACS) of vaginal cells and sperms was developed. To that end different quantities of sperm were added to vaginal swabs in a first step. Then the cells were extracted from the swabs, fixed with paraformaldehyde and saponin and labeled with monoclonal pancytokeratin antibodies followed by staining with FITC marked MHCI, Anti-CD45 antibodies and FITC marked secondary pancytokeratin antibodies. Finally the DNA was stained by incubation with RNAse and propidiumjodide. Using a flowcytometer with a piezosorter (FACSort, Beckton Dickinson) sperms and vaginal cells were sorted using the different quantity of the DNA, the different scattering of light, and differences in the expression of MHCI antigen, CD45 antigen and pancytokeratin as parameters. From both fractions DNA was extracted with chelex 100. Using this method it was possible to type DNA from minute quantities of sperms added to a vaginal swab and also from two postcoital swabs. The sensitivity of this method is compared to that of the preferential lysis.

min: 200 cells

SEQUENCING OF ALLELES IN THE AmpFlSTR PCR AMPLIFICATION KITS.

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Sequencing of short tandem repeat (STR) loci is an important aspect in the development of complete STR systems for forensic DNA typing. It is critical for good primer design that the primer binding regions are confirmed for individuals from different populations. A mutation in the primer region can lead to preferential amplification, resulting in poor balance between alleles or, in the more extreme case, allele dropout (when a mutation occurs at or near the 3' end of the primer sequence).

Each of the loci in the AmpFlSTR™ kits has been sequenced, including: D3S1358, vWA, FGA, amelogenin, THO1, TPOX, CSF1PO, D5S818, D13S317, D7S820, D8S1179, D21S11, and D18S51. The sequencing of a large number of D3S1358 alleles has confirmed the TCTA repeat structure. Microvariation has been found at the vWA locus not only in the repeat region but in the flanking primer region as well. The sequence variation in the primer region has led to the development of primers that can efficiently amplify all of the known variants. The FGA locus contains a small percentage of 2 bp length variants; many of these variants have been sequenced and they all share a common sequence motif. Some vWA and FGA alleles that exhibit reduced amounts of stutter compared to the norm have been sequenced and found to have mutations that result in an interruption of the pure tandem repeat sequence. The D8S1179, D21S11, and D18S51 loci were sequenced to resolve ambiguities (n's) in the GenBank sequences for the development of optimized PCR primers. Once optimal primers were selected for each of these loci alleles from a variety of interesting population samples, including length variants, were sequenced.

Amplification of some non-human (ex. dog, cow, horse) DNAs with the amelogenin primers results in a shorter (103 bp) PCR product. Sequencing of this product from a variety of species revealed a homologous sequence that has a common set of four base deletions relative to primates.

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HIGH PERFORMACE LIQUID CHROMATOGRAPHY (HPLC) DETECTION OF PCR INHIBITORS IN 18 SAMPLES OF ANCIENT DNA.

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High Performace Liquid chromatography (HPLC) can be used as a powerful technique of analysis of organic mixtures. Hereby we describe the HPLC detection of a PCR inhibitor in ancient DNA samples from different origin. X-Y chromosomes specific sequences (Sullivan et al., 1993) were screened in 18 ancient DNA samples. Despite several PCR trials were carried out, no succesful amplifications resulted. A mixture composed by a fixed quantity of fresh DNA solution and growing dilutions of an ancient DNA sample, showed the inhibition power of the latter, this being proportional to the concentration of ancient DNA sample in the amplification mixture. A 1:300 dilution of each sample was analyzed by HPLC. The peak pattern was similar in each of the samples and one of the peaks could be correlated to the inhibition power. Other ancient DNA samples from different origin showed a peak pattern close to the one analyzed. Due to the different site and age of some of the studied ancient DNAs, it is suggested the presence of a common endogenous inhibitor in ancient remains which is generated under certain circumstances. However, further studies will be now neccessary to clarify the bioquemistry of ancient bone and the possible presence of specific amplification inhibitors.

EVALUATION OF TWO GENEPRINT™ FLUORESCENT STR MULTIPLEX SYSTEMS IN PATERNITY CASE WORK USING THE ABI 310 GENETIC ANALYZER

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olymorphic Short Tandem Repeat (STR) loci can make a major contribution to parentage testing. The analysis of fluorescent dye labeled PCR products on the ABI Prism 310 analyzer, based on capillary electrophoresis, was performed for two Quadriplex systems.
PCR based analysis of STR loci has the advantage of being more sensitive and less time consuming than conventional systems.
The Quadriplex systems tested, are the GenePrint™ CTTv system, which co-amplifies the STR loci CSF1PO, TPOX, TH01 and vWA and the GenePrint™ FFFL system, which co-amplifies the STR loci F13A01, FESFPS, F13B and LPL (Promega Corp.).
Fragment analysis was performed using the short denatured POP4 polymer module (ABI Prism). The size of alleles was determined with reference to the GeneScan 500 internal lane standard and typed according to the number of repeat units.
Population studies were performed on unrelated Gambian Africans and unrelated samples from Dutch Caucasians involved in paternity cases. The allele distributions found showed no significant deviations from Hardy-Weinberg Eqilibrium. Typings of 69 family cases were analysed. Only CSF1PO showed one mutation. The performance of the STR systems varied. Especially for the F13B and vWA systems a major difference in performance of the two alleles in heterozygote samples was observed. The occasionally observed stutter peaks and artefacts did not cause interpretation problems. The analysis of the Paternity Index of the two Quadriplexes showed an major contribution to paternity testing. Therefore these STR-systems will be implemented in routine casework.

D1S80 TYPING IN CASEWORK: A SIMPLE STRATEGY TO DISTINGUISH NON-SPECIFIC MICROBIAL PCR PRODUCTS FROM HUMAN ALLELES

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It has recently been shown that some bacterial DNAs can act as non-specific templates for the amplification of D1S80, originating some extra-bands that could be misled with human alleles. Since casework samples are frequently exposed to environmental insults that can result in contamination by microbial DNA, these extra-bands can be observed from routine samples. Therefore, a protocol is needed to distinguish these products from human alleles. The purposes of this study are the following: 1) to increase the number of microbial species tested whose DNA could be a potential source of extra-bands, 2) to develop a simple strategy that allows us to distinguish non-specific products from human alleles, and 3) to establish the incidence of microbial extra-bands in casework. Microbial DNA templates from 47 standard strains and 19 strains isolated from forensic samples were tested by the AmpliFLP D1S80 System. Non-specific PCR products were observed by native PAGE from fourteen bacterial strains and one yeast strain. However, a second analysis by denaturing PAGE allowed us to distinguish these microbial products from human D1S80 alleles: while each human allele showed a clear double-band pattern (due to differences in the base content of each strand within the repeat array) and maintained their relative mobility (the same obtained by native PAGE) with respect to the alleles in the ladder, microbial PCR-products usually showed a single-band pattern (or a double-band pattern with a reduced inter-strand space) and they changed their relative mobility with respect to the human alleles in the D1S80 ladder. We demonstrated by this two-steps strategy of D1S80 analysis (native PAGE followed by denaturing PAGE) that the incidence of these artefactual PCR-products in casework is 2.5% in bloodstains (13 samples from a total of 517) and 3.6 % in semen stains (6 samples from a total of 165). In summary, we suggest that this two-steps strategy for D1S80 typing could be a good tool to distinguish microbial non-specific products from human alleles in casework specimens showing extra-bands by native PAGE.

D1S80 SUBTYPING WITH PCR - RFLP METHOD USING ECORII.

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Since the 16-bp repeat units of D1S80 contain an EcoRII restriction site, we tried to subtype 24 kinds of alleles of D1S80 ranging from allele 16 to allele 43 by using PCR-RFLP with EcoRII.

DNA was extracted from the blood of unrelated healthy Japanese. After electrophoresis on 6% PAG, all alleles were extracted from the gels and used as templates for re-PCR. The re-amplified products were treated with EcoRII at 37℃ overnight, and electrophoresed on a 12% PAG. The band patterns of the digested fragments were analysed after staining with ethidium bromide. The band pattern changes of the 24 alleles could be summarized into 11 types. All samples of allele 18 were of one type, while those of allele 24 were of two types. Thus, several types of each allele could be detected.

A SIMPLE FOLDABLE CARDBOARD BOX FOR THE DRYING AND STORAGE OF BIOLOGICAL MATERIAL RECOVERED ON COTTON SWABS

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Goal: A simple, foldable cardboard box was designed, which is suitable for the drying and storage of biological evidence recovered on cotton swabs.
Material and method: The box is made of 1 millimeter corrugated cardboard and can be sealed on both ends with a special non-removable security seal. Immediately after collection swabs are placed into the drying racks within the cardboard box, which is subsequently folded, labeled, sealed and initialed. The drying time of different biological materials in the box was evaluated at different temperature and humidity and compared to "air drying" on the bench and be means of a swab dryer.
Results: Swabs completely air dried within the sealed box in 3-5 hours, and PCR-based DNA analysis was successfully performed on all samples. On buccal swabs collected for a national DNA-database pilot project the PCR success rate was 100 % when swabs were air dried in this box and stored at room temperature for up to one year.
Conclusion: The ability to perform successful DNA analysis on biological evidence depends very much on the first step - how specimens are collected and preserved. Certain types of biological evidence, such as liquid body fluids and their wet or dry stains, are often recovered using dry cotton swabs or cotton swabs moistened with sterile water or saline. In order to prevent decomposition, it is recommended to either "air dry" or freeze these swabs as soon as possible after collection. This simple device can substitute either air drying of swabs by means of an air stream (Swab Dryer) or freezing of samples after collection, if the necessary equipment is not available. In this box the evidence is properly packed, labeled and sealed, thus preventing cross contamination, degradation and sample switch. It is a valuable device for the collection of biological evidence at a crime scene, during sexual assault examinations, and for PCR-based data basing and paternity testing.

OPTIMIZATION OF NESTED PCR OF THE HV1 REGION IN HUMAN MITOCHONDRIAL DNA USING THE AMPLITAQ GOLD™ ENZYME

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Our laboratory is currently preparing for routine analysis of mitochondrial DNA (mtDNA) in forensic casework. In this paper the optimization of the nested PCR for the HV1 region in human mtDNA of organic extracted hair (0.1-1.0 ng total DNA) using the enzyme AmpliTaq Gold™ is shown. The study was designed according to earlier experiences with AmpliTaq® DNA Polymerase. The exact region subjected to analysis was positions 15996-16399, numbered according to the reference sequence by Anderson et al. (1981).

Several parameters in the PCR were varied: the number of cycles (25-30), annealing temperature (54-72°C), concentration of KCl (10-50 mM), $MgCl_2$ (1.5-3 mM) and pH of Tris-HCl (7.5-8.5). The following parameters were kept constant: the concentration of each primer (0.1 µM), nucleotides (0.2 mM), and AmpliTaq Gold™ (1 U) as well as the predenaturation step (94°C, 10 min), denaturation (94°C, 15 sec), annealing time (30 sec) and extension (72°C, 1 min). All reactions were performed in 50 µl volumes.

In order to get reliable information of the quantity and quality of the PCR product, we performed both the first and the second round of amplification with fluorescently labelled left primers. The analysis of these products was done with polyacrylamide gel electrophoresis (PAGE) on an ABI PRISM™ 377 DNA sequencer. These results were compared with the corresponding results from the traditional agarose gel (2%) electrophoresis. The sequencing reactions were performed using Dye Primers and the sequences were determined on a ABI PRISM™ 377 DNA sequencer.

In conclusion, our results confirm the sensitivity of using PAGE to evaluate the quantity and quality of PCR products. Further, it is not possible to simply replace AmpliTaq® DNA Polymerase with AmpliTaq Gold™. The most important change in combination with adding a predenaturation step, was to lower the annealing temperature.

UNIVERSAL METHOD OF HYPERSENSITIVE NESTED PCR TOWARD FORENSIC DNA TYPING

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In forensic DNA case work, amplification from DNA obtained of old samples is sometimes found to be difficult. In rape cases, saliva and semen which could be transmitted to victims may sometimes need to be examined to determine the DNA type of the assailant. Forensic DNA typing is inevitable to distinguish between the accused, suspect and the victim to get the correct legal judgment.

For this purpose, Y-STR DNA typing is beneficial because male DNA alone is selectively amplified directly from mixed DNA of seminal/vaginal secretion stains. Though this method is extremely sensitive, some of old or trace element of samples could not be amplified by standard PCR procedure. So, we developed a new DNA extraction method using alkaline proteinase and nested PCR for extremely sensitive amplification.

For nested PCR, we must design new outer primer set correspond to up and downstream of the target locus, but it is not always easy to find such an appropriate set of primers. Therefore, we report nested primer pairs with extension of M13 and rM13 universal primer to be applicable to PCR for any locus.

In the first PCR, we used locus specific custom primers with M13 and rM13 extension, and in the second, forward universal primer of M13 and reverse custom primer or forward custom primer and reverse universal primer of rM13 were used. These primers were not annealed to unaimed sequences because human genome has not consensus sequence of M13.

This method was so sensitive that 10pg of male DNA could be amplified, in addition, amplified fragment could directly be sequenced by dye primer cycle sequencing reaction (ABI PRISM 377; PE Applied Biosystems).

We demonstrated our method applied for DYS19 typing from seminal/vaginal mixed stains preserved for 35 years after collection of autopsy.

USING D3S1358 FOR QUANTIFICATION OF DNA AMENABLE TO PCR AND FOR GENOTYPE SCREENING

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Before using a multiplex PCR, like the SGM from FSS, we need to know how amenable the DNA is to amplification due to the quantity and quality of the DNA. We have evaluated the suitability of D3S1358, a short, simple and informative STR loci, for quantification and rapid genotyping of large number of samples.

Fluorescent tagged primers and the polymerase Taq GOLD was used in the PCR. Genotyping was performed in a ABI 377 automated sequencer.
The electrophoresis was run on a 6% acrylamide gel with 36 lanes and a separation distance of 12 cm.

An allelic ladder was constructed and used as a size standard. By using this allelic ladder as a size standard the run time was reduced to about one hour.
The same acrylamide gel was reused for a second run of 36 samples.
This means that 72 samples can be analysed in three hours, including sample handling,

A Swedish population database of 317 individuals was constructed. The population was found to be in Hardy-Weinberg equilibrum. The heterozygosity value is 0.83 and the exclusion capacity is 0.91.

This indicates that the D3S1358 is a suitable STR locus for quantification and genotyping of large number of samples.

ANALYSIS OF STRs BY CAPILLARY ELECTROPHORESIS

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The introduction of fluorescent detection of STRs, and specially by capillary electrophoresis, has been a big step in the automation of analysis of forensic DNA samples.
We have analysed fifty paternity cases and forensic casework samples from three months with two fluorescent detection kits (AmpFlSTR Green I y Blue) using an ABI PRISM 310 Genetic Analyzer. This includes the analysis of six STR loci (CSF1PO, TPOX, TH01, D3S1358, VWA and FGA) plus sex determination. In some samples, the amplification and analysis were carried out in heptaplex (Blue+GreenI in the same tube).
With this samples we have tested the reproducibility of the system. The initial results are good enough:
-The standard deviation of internal standard (Rox 350) in every set of samples was under 0.20bp.
-The size deviation in both ladder and samples alleles respect the mean was under 0.5 bp.
Additionally, we have established preliminary frequencies for D3S1358 and FGA in a Spanish population:

D3S1358		FGA			
Allele	Frequency	Allele	Frequency	Allele	Frequency
14	0.08	19	0.06	23.2	0.01
15	0.28	20	0.15	24	0.15
16	0.30	21	0.15	25	0.08
17	0.16	21.2	0.01	26	0.05
18	0.18	22	0.15	27	0.01
		22.2	0.01	28	0.01
		23	0.17		

We conclude that these systems are very useful tolls in forensic and paternity analysis.

IMPROVEMENT IN MITOCHONDRIAL DNA ANALYSIS

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Mitochondrial DNA analysis is a powerful technique allowing identification of unknown individuals. Unfortunately, the method is time consuming (especially if the number of samples is high), and could fail depending on the sequence of the DNA. We describe two ways to improve the analysis.

a/ Rapid mutation screening

It is performed when we have numerous samples to analyse among which a known DNA is searched (victim's DNA among hundreds of rootless hair for example). After sequencing the DNA of the reference sample (for example victim's blood sample), a restriction enzyme site is designed targeting one of the observed mutations. If a mutation does not create or does not eliminate a restriction enzyme site, we modify the primer in order to create a new site. Two examples are shown.

b/ Primers for specific mutations

Some mutations in the DNA (especially mutations 16189 C in HV1 and 309.2 C in HV2, both creating long C stretches) dramatically decrease the sequencing efficiency due to heteroplasmy events and stuttering of the DNA polymerase. We have designed specific primers hybridizing on these C stretches (on both strands of the DNA fragments) in order to solve these sequencing problems.

VALIDATION OF FESFPS AND F13A01 TYPING USING CAPILLARY ELECTROPHORESIS

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Capillary electrophoresis is the most recently incorporated genotyping technique in forensic laboratories. Therefore validation parameters have to be updated to allow the use of usual DNA polymorphic loci with this novel technology.

FESFPS and F13A01 are two short tandem repeat loci broadly used in forensic casework and paternity testing. Up to this moment they were analysed by poliacrylamide gel electrophoresis (PAGE) under denaturing conditions, followed by silver staining or by different automated fluorescent detection systems.

In this paper we present some validation parameters in order to establish the reproducibility and reliability of the genotyping procedure within and between different runs, and the variability of the allelic sizing obtained for known alleles from samples previously analysed with conventional electrophoretic techniques

HIGH THROUGHPUT SILVER STAIN MULTIPLEX STR ANALYSES

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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. Nine STR loci have been incorporated into three multiplexes each containing three loci. The first triplex includes the loci

Multiplex Name	STR Loci
CTT	CSF1PO, TPOX, TH01
FFv	F13A01, FESFPS, vWA
SilverSTR™ III	D16S539, D7S820, D13S317

CSF1PO,TPOX, TH01 (CTT), the second contains F13A01, FESFPS, and vWA (FFv) and the third, still undergoing optimization, D16S539, D7S820, and D13S317. Allelic ladders for each locus (i.e. size standards containing many or all existing alleles) have been developed to allow rapid and accurate typing. This approach minimizes the amount of material required and increases efficiency.

We have collaborated with other laboratories to determine the allele frequencies of these nine STR loci in three or more population groups each containing at least 200 individuals.

STR System	Power of Discrimination		
	African-Amer.	Caucasian-Amer.	Hispanic-Amer.
CTT Multiplex	1 in 1639	1 in 424	1 in 547
FFv Multiplex	1 in 2785	1 in 912	1 in 1343
SilverSTR™ III Multiplex	1 in 1080	1 in 2722	1 in 2493
3 Multiplexes (9 STRs)	1 in 4.93×10^9	1 in 1.05×10^9	1 in 1.83×10^9

AUTOMATED FLUORESCENT DETECTION OF 8-LOCUS AND 4-LOCUS STR MULTIPLEXES.

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We have developed the following 3 fluorescein-labeled STR quadriplex systems:

Multiplex Name	STR Loci
CTTv	CSF1PO, TPOX, TH01, vWA
FFFL	F13A01, FESFPS, F13B, LPL
GammaSTR™	D16S539, D7S820, D13S317, D5S818

Each multiplex system has the following properties:

- Very few PCR artifacts are present (with vWA stutter being the most visible)
- Within each quadriplex, no alleles of different loci overlap.
- Fluorescent allelic ladders for each locus have been developed.
- All loci except vWA are simple STR systems. Microvariants have been observed with only two of the twelve loci (i.e. TH01 and F13A01).

The CTTv loci can be labeled with an alternative dye, a rhodamine derivative, and amplified simultaneously with the GammaSTR™ loci. This two-color multiplex, the PowerPlex™ System, allows simultaneous analysis of all eight loci using either the Hitachi FMBIO® Fluorescent Scanner or any of several fluorescent detection instruments from Applied Biosystems. We have determined that the discrimination power of this system exceeds 1 in 118.000.000 for all races tested. In combination with the remaining quadriplex system, FFFL, the discrimination power exceeds 1 in 178.000.000.000 for all races.

STR System	Power of Discrimination		
	African-Amer.	Caucasian-Amer.	Hispanic-Amer.
PowerPlex™ System	1 in 2.61×10^8	1 in 1.18×10^8	1 in 1.45×10^8
PowerPlex™ System plus FFFL (12 STRs)	1 in 2.91×10^{12}	1 in 1.78×10^{11}	1 in 2.37×10^{11}

CAPILLARY-GEL ELECTROPHORESIS (CGE). STUDIES ON ACCURACY AND REPRODUCIBILITY IN DNA SIZING AND SEQUENCING.

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Capillary-gel-electrophoresis with laser induced fluorescence (LIF) detection is a high-resolution analytical technique recently introduced in DNA analysis. Due to high voltages that can be applied to the thin-walled fused silica capillaries containing the separation medium, it is more efficient and faster than conventional slab gel electrophoresis. The instruments and the software now available are able to collect and analyze DNA data for both typing and sequencing applications.
The Applied Biosystems (ABI) Prism 310 Genetic Analyzer was employed for fragment sizing and sequencing applications of short tandem repeats using the software (GeneScan Analysis 2.0.2 and Sequence Analysis 3.0) supplied by the manufacturer.
DNA was extracted from blood and hair according to standard procedures, either by the phenol-chloroform method or Chelex 100, followed by slot blot quantitation using a higher primate specific probe.
For fragment analysis validation, the D21S11 locus was employed in this study. PCR amplification was carried out using 3 ng of genomic DNA in a final volume of 50 µl using the 6-FAM 5'-labeled reverse primer and unlabeled forward primer proposed by Sharma and Litt (Hum Mol Genet 1, 67, 1992). 1 µl of the PCR product and 0.5 µl of an internal size standard (TAMRA 500) were added to deionized formamide and, after denaturation, the sample was injected in the CE.
The accuracy and the run-to-run and day-to-day reproducibility of analysis were validated, sizing sequenced alleles against the molecular weight marker, comparing two different polymers.
Sequencing analysis was carried out by using Taq-Dye-Deoxy-Terminator Cycle Sequencing chemistry on a series of DNA fragments of known sequence, sizing 86-420 bp. Performance was evaluated by base calling accuracy.

SIMULTANEOUS DETECTION OF ABO GENOTYPES AND AN X-STR

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Personal identification is one of the first and important steps of criminal investigation in forensic practice. We report here simultaneous detection of ABO genotypes and an X-STR for identification in single tube. Until now, these two category of identification processes remain separate procedures by PCR because the former is based on the detection of single nucleotide deletion/substitution and the latter is detection of microsatellite polymorphism. We used an adapted PCR protocol to accomplished the above 2 processes in one reaction. With this, fluorescent labeled 6 primers were used for multiplex PCR in a PE 2400 thermal cycler. Amplification products were then digested with RE *Kpn*I and *Alu*I. The digested products were run in a 6% polyacrylamide sequencing gel in an ABI 373A DNA sequencer and analysis was performed by using 672 Genescan analysis software (PE Applied Biosystems).

For forensic purpose, DNA tests should be quick, sensitive, accurate and applicable to various samples usually encountered in actual practice. With the advent of ultra high sensitivity and highly precise laser scanning methods, 6 ABO genotypes and X-specific DNA fragments of HUMHPRT locus (5 alleles ranging from 279-294 bp) from various forensic samples were clearly identified on the gel image. Genescan analysis is possible after RE digestion, and simultaneous detection of the above mentioned loci because HUMHPRT locus contains no RE digestion site for *Kpn*I and *Alu*I.

INFRARED FLUORESCENCE DETECTION OF GENETIC MARKERS FROM FORENSIC SAMPLES

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This research was undertaken to determine if an automated real time detection system using infrared (IR) laser irradiation could be utilized to detect short tandem repeat (STR) loci, variable number of tandem repeat (VNTR) loci, alleles of the human blood group system (ABO), and the gender identifying loci, from forensic samples after amplification using the polymerase chain reaction (PCR).

Two different strategies for IR fluorescent detection of PCR products from polymorphic regions were used. In the first strategy a limited quantity of IR-labeled deoxynucleotide (dATP) was included in the amplification reaction. During DNA synthesis the polymerase will occasionally incorporate a labeled molecule into the growing DNA chain thus producing PCR products internally labeled with the IR fluorophore. The second strategy utilizes amplification primers flanking the repeat region of the loci with the forward primer labeled at its 5' end with an infrared fluorescent dye molecule. Amplification using this primer directly labels the PCR product. A LI-COR automated DNA sequencer was used for gel electrophoresis and allele detection. Image data was collected on-line in real time as autoradiogram-like images.

By using the above technology it was possible to detect PCR products from singleplex and multiplex reactions. This system combines IR-fluorescence chemistry and laser technology. Family studies, paternity analyses and various forensic case samples can be analyzed. Since the gels can be reloaded, at least 120 samples can be analyzed in one gel using the two dye system.

DETECTION OF AZOOSPERMIC SEMEN WITH A NEW RAPID STRIP-TEST FOR PSA AND DNA PROFILING OF THESE SAMPLES BY THE PCR TECHNOLOGY.

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We recently evaluated a commercial immunochemical strip-test for prostate specific antigen (PSA), Quickpac II OneStep PSA® test (SYNTRON BIORESEARCH Inc), on experimental human secretion stains and 51 casework samples, in parallel with a double sandwich ELISA test [1]. The new strip-test showed the same specificity and sensitivity as the ELISA, with the advantage of working faster.

The amount of DNA extracted from azoospermic semen, after differential lysis, was estimated on 13 liquid samples received from the urology clinic : average total amount 211 ng / 100 µl (range 25 ng to 500 ng) of which 90 % in the epithelial cells fraction.

Experimental mixtures in various proportions of azoospermic semen with semen free vaginal washing or with saliva were tested for STR amplification.

The PSA results, compared to the spermatozoa search and to the DNA results, allowed us to select 3 cases of probable azoo- or oligospermia in our casework. The DNA profile could be determined in 2 of the 3 cases by the PCR techniques, and in only one of these cases by the RFLP technique. In this last case, the suspect, after receiving the DNA results, admitted the rape and his vasectomy.

[1] B. HOSTE & D. LEONARD, submitted to Forens. Sci. Internat., 1997.

RAPID mtDNA TYPING USING IMMOBILIZED SEQUENCE-SPECIFIC OLIGONUCLEOTIDE PROBES: ADVANTAGES AND LIMITATIONS

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Mitochondrial DNA (mtDNA) has unique features that make it a potentially useful target for the analysis of telogen hairs, mass disaster and missing person remains, and other biological samples that are too small or too degraded to be analyzed by conventional DNA analysis. One approach to typing mtDNA is DNA sequence analysis of multiple amplified sections of the hypervariable region of the mitochondrial genome, but this method is very time-consuming and costly. To provide a faster, less expensive method for mtDNA analysis, we developed an immobilized sequence-specific oligonucleotide (SSO) probe-based typing system. Using an optimized reaction mix containing AmpliTaq Gold DNA polymerase, a 415 bp portion of hypervariable region II (HVII) is amplified. A panel of 16 SSO probes immobilized on a single strip is used to determine the HVII mitotype. It is possible to type and interpret the results of up to 40 samples in approximately 2 hours compared to 2 or more days for DNA sequencing results. This mtDNA typing system is highly specific: only human and gorilla DNA yielded PCR product. No other primate DNA samples were amplified under our standard conditions. This system is robust: typeable results were obtained from >95% of extracted telogen hairs (>200 hairs) and from severely degraded samples. We typed 689 unrelated individuals from 4 population groups. Pair-wise nucleotide difference comparisons of all observed HVII mitotypes indicated that the mitotypes in 3% of all pairs differ by zero nucleotides, while those in 14% of all pairs differ by one nucleotide change within the probe regions. We have typed and sequenced multiple shed and plucked hairs from over 10 people. We found that the majority of these individuals had one or more hairs with a different mitotype than the mitotype obtained from their blood, saliva, and plucked hairs. Most often the sequence differences were at a single nucleotide, but we found several examples of multiple base changes. These findings have a significant impact on how mtDNA typing results, whether performed with immobilized SSO probes or direct DNA sequence analysis, will be interpreted by scientists and the legal community.

Up to 25% of hairs from an individual with variation

50-80% heteroplasmy for shed hair study

A STRATEGY FOR mt-DNA ANALYSIS OF HAIR SHAFTS IN PRACTICAL CASEWORK: RE-SSCP

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One of the main problems of mtDNA analysis of hair shafts in practical casework is the number of hairs involved in single cases which cannot be excluded after physical examinations.
Screening methods are therefore usually neccessary. A strategy prior to sequencing is described for detecting mtDNA variation which permits a rapid and straightforward screening. The method is based on the selection of fragments of adequate length for performing single strand conformation polymorphism (SSCP) analysis, selecting a set of restriction enzymes (RE) which yield fragments of prefixed lengths. After the PCR amplification of HV1 and HV2 regions and digestion of the appropriate enzyme or set of enzymes, SSCP analysis is performed in a semi-automatic electrophoretic system using a silver staining detection method. The conformational changes due to single mutations were therefore found not changing the electrophoretic protocol but the relative position of the mutations within the fragment. The discrimination power of this method is estimated to be 90% when two restriction enzymes (MspI and HinfI) are used, but almost all the variation is detected when other enzymes are added.
The proposed method implies an adequate selection of the restriction sites and can be applied to other genes of medical interest. A PC program was developed for automatic searching of restriction enzymes on the condition that the fragments are of prefixed length (usually 200 bp). For long PCR fragments this method and the program can also be used.

SHORT TETRAMERES FOR DEGRADED STAINS - A TEST OF THREE STRs

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Stains in forensic casework often consist of degraded DNA and even commonly used STRs as the Quadruplex and the Second Generation Multiplex (SGM) may fail to amplify in the PCR reaction. We have tested a triplex of very short tetrameric STRs in an effort to find robust markers for analysis of highly degraded stains. Such markers might be valuable supplements in stain analysis as well as for DNA intelligence databases. By searching through the tetrameric STRs in the Genome Data Base (Utah), we chose 12 AGAT and AAAG repeat tetrameres. Preliminary experiments were performed to test the markers for level of polymorphism, robustness and suitability in multiplex analysis. Three STRs were chosen for further studies. The three have heterozygosity level at 0.8-0.85 when studied in a population of 300 Norwegians. In preliminary studies in casework stains the present triplex has been compared with results from the Quadruplex and ACTBP2 analysis. Different kinds of stains have been studied, including highly degraded samples. Several degraded stains where some or all of the markers in Quadruplex/ACTBP2 failed, were typable with the triplex. This indicates that the triplex may be a valuable contribution in fragment length analysis of degraded stains.

SCREENING FOR MITOCHONDRIAL DNA SEQUENCE VARIATION BY SSCA AND HE: APPLICATIONS IN FORENSIC GENETICS

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We have recently described the use of the single strand conformation analysis (SSCA) as well as the heteroduplex analysis (HE) for the rapid detection of nucleotide sequence polymorphims in the mitochondrial DNA (mtDNA) control region (Alonso et. al. *Electrophoresis* 1996, 17, 1299-1301; Alonso et. al. *Electrophoresis* 1997, in press). These molecular scanning techniques have significant time and cost advantages over direct sequencing which is so far the most reliable and sensitive method for the detection of molecular genetic variation in the mtDNA control region. Here, we present the potential useful applications of this SSCA/HE typing method in forensic genetics. These applications include: (i) the primary screen of mtDNA types in cases involving large number of samples (e.g. hair shafts analysis), (ii) the rapid detection of intra-individual sequence variability (heteroplasmy), and (iii) the separation of mtDNA strands for the individualized sequence analysis of mtDNA types in DNA mixtures (e.g. body fluids mixtures and heteroplasmic tissues) .

RAPID MITOCHONDRIAL DNA TYPING BY SOLID-PHASE MINISEQUENCING: FORENSIC APPLICATIONS

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Shed hairs are often recovered from masks which have been found at a crime scene. The majority of these hairs do not have sufficient root material to enable successful analysis of genomic DNA polymorphisms, but sufficient mitochondrial DNA (mtDNA) can be extracted for analysis. However, sequencing of mtDNA is prohibitively expensive for all but the most serious cases. The present study describes the application of multiplex solid-phase fluorescent minisequencing of mtDNA to analysis of such evidential material and comparison against submitted control samples. Occurrence of heteroplasmy at these polymorphic nucleotides has also been investigated.

Three regions of mtDNA are amplified in a single round multiplex reaction, and the products are immobilised on a solid support. The immobilised DNA is then rendered single-stranded and 12 minisequence primers, each of which is adjacent to a polymorphic nucleotide, are annealed. In a simultaneous reaction, each of the minisequence primers is extended by a single, fluorescently labelled dideoxynucleotide. The fluorescent labels are detected during polyacrylamide gel electrophoresis of the extended primers in an automated sequencer, enabling the nucleotide present at each of the targeted polymorphisms to be determined.

The method has successfully been applied to both control samples of blood or saliva, and to forensic case samples including shed hair and faeces, enabling elimination of the majority of suspects in each case. Samples which matched by minisequencing have then been sequenced to confirm or eliminate a common maternal origin. The average probability of a random match between unrelated British Caucasians is approximately 0.05, so the proportion of samples which require sequence analysis is small. The reduced cost and processing time of this strategy have thus widened the applicability of mtDNA analysis to less serious criminal cases.

Minisequencing has also enabled accumulation of data on the frequency of occurrence of heteroplasmy at these polymorphic positions.

MITOCHONDRIAL DNA (mtDNA) ANALYSIS OF INDIVIDUALS FROM A MASS GRAVE

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In 1991 the remains of 127 individuals were recovered from a mass grave in Santiago as part of the process of Chilean national reconciliation, involving the identification of the victims of political killings. A number of individuals could not be identified by the analysis of ante- and post-mortem and dental records. mtDNA analysis was used in an attempt to identify 21 of the individuals that could not be identified using the more conventional methods.

The remains were 21 years old and therefore the DNA was highly degraded and present in very low quantities. mtDNA was utilised because of it s high genome copy number which increased the likelihood of successful analysis. In addition, the maternal mode of inheritance was a valuable feature as it made comparison to maternal reference sequences straight forward. Semi-nested PCR was used to generate sufficient template for direct sequencing. Extreme precautions had to be taken to minimise the possibility of PCR contamination.

mtDNA was successfully isolated and amplified from 20 of the 21 bone samples and a 170 bp region of hypervariable region I was sequenced. This revealed 14 distinct profiles, with the most common profile occurring in 3 individuals, and 9 profiles being found only once in the 20 samples.

Used in isolation, mtDNA would have been of limited use other than for exclusions. Based on the profiles of the 20 samples the probability of 2 random samples having the same profile was 8.5%, random matches would therefore be relatively frequent. However, when supported by other types of analysis, mtDNA analysis provided a powerful tool in human identification.

IDENTIFICATION OF HUMAN REMAINS: SHORT TANDEM REPEAT-SYSTEMS VERSUS MITOCHONDRIAL DNA

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The results of three cases of identification of human remains are presented in which both genomic-Short-Tandem-Repeat-systems (STR) as well as hypervariable regions HV I and HV II of the control region of mitochondrial DNA (mtDNA) were investigated.

All cases involved were highly decomposed bodies with severe putrefaction. For DNA-extraction different kinds of tissue were used, primarily bones but also muscle and hair. In a first step DNA-typing was performed using several STRs. Secondly mtDNA was analysed either in order to confirm results obtained with STRs or to clarify inconsistencies within STR-results. Analysis of mtDNA was performed both by formation of heteroduplices and by sequencing..

In the first case a victim of a murder had to be identified. According to police information there was no doubt about the identity of the man with respect to morphological findings and clothing. The comparison of DNA extracted from muscle tissue of the body and a dried blood sample of the assumed mother resulted in three exclusions of maternity. Analyses of mtDNA-regions HV I and HV II showed complete identity of sequences.

In the second case there were differences in STR-results between bone and muscle-tissue of a decomposed body, which could be confirmed by mtDNA-analysis. Apparently parts of two different corpses had been mixed.

In the third case three bones were recovered from a river. DNA-sequences of bone tissue of the deceased and of the assumed brother were compared. In this case STR-typing did not produce any useful results. In contrast analysis of mitochondrial DNA revealed complete identity of sequences.

These results once again demonstrate that analysis of mitochondrial DNA often is superior to analysis of genomic DNA in cases of identification of human remains.

IDENTIFICATION OF A MISSING ITALIAN SOLDIER OF WWII

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Analysis of the first higly polimorfic mtDNA D-loop region was carried out to identify the remains of a soldier of ARMIR (Armata Italiana in Russia, 1940-1944) missing since the World War II. In 1989, after disclosure of the Soviet Red Army archives, a series of war casualties graves have been discovered and the endevour of assigning the remains to relatives has begun. However, discrepancies emerged between official engraving reports and personal identification plates. We were therefore given the task to decide whether remains from two adiacent graves were correctly assigned. Bones and teeth from both questioned individuals were consequently analysed, and DNA was successfully extracted from only one body. A 250 bp mithocondrial HVRI fragment was amplified using specific primers and enzymatically sequenced. The sequence obtained showed a C >T transition at 16260 position (Anderson consensus sequence) corresponding to a mutation observed in one of the maternal lineages of relatives. These results led to correctly identify the grave. In this case, the genetic evidence also shed some light onto controversial historical events and helped their interpretation.

APPLICATION OF CONVENTIONAL AND DNA POLYMORPHISMS TO PATERNITY CASES

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In this paper we discuss our experience of the passage from the "pre-DNA era" to the "DNA-era" of paternity testing.
Up to 1990 the Forensic Haemogenetics Laboratory of University of Pisa used exclusively conventional markers for paternity testing. At the beginning of the 80's a series of 15 genetic polymorphisms was employed, whereas at the end of that decade the number of polymorphisms was increased up to 24. The relative power of exclusion was 91.2% and 98.7%, respectively.
In the 1991, we introduced the DNA-PCR polymorphisms into our routine. Initially we added them to the set of conventional markers, obtaining a cumulative power of exclusion of about 99.999%; subsequently we progressively substituted some of conventional systems with new DNA markers, in such a way that the cumulative power of exclusion remained unmodified.
We report a series of 60 cases of paternity testing, investigated using both conventional and DNA markers (series 2). This series is compared with 100 paternity cases, previously studied only by means of conventional polymorphisms (series 1).
In the case of series 1 additional typing was necessary in 5 out 42 excluded men (because of a isolated "opposite homozygosity"), whereas in series 2 we found at least 3 incompatibilities in all excluded man (n=25). In addition we observed, in series 1, 5% of non-excluded men with a probability of paternity <95%, against a value of 100% of non-excluded men from series 2 with a probability of paternity >99.9%.

HUMAN IDENTIFICATION USING MITOCHONDRIAL DNA EXTRACTED FROM A BEARD, HAIR AND LIVER

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Analysis of mitochondrial DNA (mtDNA) by amplification and subsequent sequencing of two hypervariable segments within the control region is an important tool for forensic human identification. Here, we present one of the first cases done in Croatia in which sequencing of mtDNA has been used for human identity testing.
A body of a young man was found in a lake in one neighboring country. This finding was linked to the disappearance of twenty-five years old man from Zagreb, three months before, in the same area. Besides the identification of a body, for the investigation purposes, it was necessary to answer the question whether the fragment of the liver (which was sent separately for the toxicological analysis) belonged to this body. Mitochondrial DNA was extracted from a beard, hair and liver that belonged to the body. Also, mtDNA was extracted from the fragment of liver received for the toxicological analysis. Both strands of both hypervariable mtDNA regions (HV1 and HV2) were sequenced for all samples. Identical sequences were generated from all examined samples. Since mtDNA is maternally inherited, sequence was compared to the sequence of mitochondrial DNA extracted from the putative mother's blood. An exact sequence match between samples from the body and putative mother was observed.

DNA ANALYSIS OF 1162 PARENTAGE CASES

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Parentage cases are investigated exclusively by means of DNA analysis at the Institute of Legal Medicine in Zürich since 1991. This study presents the evaluation of 1162 parentage cases.

The majority of the cases were simple trio cases of disputed paternity. In addition 60 deficiency cases, 8 maternity cases, 2 cases of reverse paternity and 1 case of incest were investigated.

DNA analysis was mainly performed by RFLP-typing of VNTR loci with the 4 single locus probes MS43A, MS31, G3 and yNH24. In difficult cases e. g. complicated deficiency cases or cases with mutational bands DNA analysis was extended on additional SLP systems as pH30, EFD52, TBQ7, MS205 and MS1 or on PCR based systems. Nine cases were examined with PCR systems only. 12 PCR systems were applied: SE33, TC11, D21S11, F13A1, VWA, FES, HLA DQα, LDLR, GYPA, HBGG, D7S8 and Gc.

75 % of the investigated cases showed nonexclusions and 25 % exclusions of paternity. In 20 cases a mutational band was observed. The biostatistical evaluation of nonexclusion cases was performed according to Essen-Möller. With the exception of 2 cases (examined with PCR systems only) all the resulting W-values were ≥ 99.8 %. The exclusion cases always showed multiple exclusions. The exclusion power for the 4 single locus systems MS43A, MS31, G3 and yNH24 was above 99.999 %.

The results demonstrate that 4 highly polymorphic DNA systems are capable to resolve all simple trio cases independent of their ethnic composition. Single exclusions indicate mutations; such cases can be resolved by extending the investigation on some additional VNTR systems. Also in complicated deficiency cases the application of multiple highly informative DNA systems provides essential evidence either for or against kinship.

EVALUATION OF PROSTATE - SPECIFIC ANTIGEN (PSA) MEMBRANE TESTS FOR THE FORENSIC IDENTIFICATION OF SEMEN

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Goal: The goal of this study was to evaluate prostate-specific antigen (PSA) rapid membrane tests for the forensic identification of semen from vasectomized individuals.
Material and Methods: Semen stains from vasectomized individuals, postcoital vaginal swabs resulting from intercourse with non vasectomized and vasectomized individuals and male urine were analyzed for the presence of PSA using PSA-specific rapid membrane tests (PSA-check-1, VED-LAB, France; Seratec® PSA Semiquant, Seratec, Germany; One Step ABA card PSA, Abacus, USA), and PSA-specific test sticks ("Onestep" Test Strip, FF Diagnostic, Cologne, Germany). Both methods use monoclonal antibodies directed against constant epitopes in free and complexed PSA as well as all its isoforms.
Results: Compared to time consuming ELISA-based measurements of PSA, rapid membrane tests offer the same sensitivity (4 ng PSA / ml) within 10 minutes using 200 µl of supernatant from the DNA extraction procedure. Although test sticks offer the same sensitivity, we found them not useful due for casework to the greater amount of liquid required. PSA was detected in all specimens, as well as in male urine. In the later, additional testing using the seminal vesicle specific antigen MHS-5 (SEMA test kit) can be useful.
Conclusions: Methods for the detection of PSA include Ouchterlony double diffusion, crossover electrophoresis, rocket immunoelectrophoresis, radial immunodiffusion, and ELISA. The disadvantage is that they are either not sensitive enough or cumbersome and time consuming. This study demonstrates that PSA-specific rapid membrane test assays offer the same sensitivity as ELISA-based tests and represent a rapid approach for the forensic identification of seminal fluid in vasectomized individuals.

FORENSIC DNA TYPING OF SINGLE HAIR: MITOCHONDRIAL DNA SEQUENCING AND HIGHLY DISCRIMINATING STR MULTIPLEXES

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DNA typing from single hair is often problematic in forensic casework due to the limited amounts of DNA found in a bad quality hair root. It particularly refers to nuclear DNA typing. The aim of our experimental study is to optimize PCR - based DNA typing of hair shafts by means of amplification efficiency obtained using highly discriminating multiplex STR systems compatible with two different detection platforms: a hexaplex PCR developed and validated in our laboratory (Amelogenin, D1S103, TH01, D21S11, D18S51, FGA; visualized using a single-wavelength A.L.F. DNA Sequencer) and the *GenePrint*™ Powerplex™ (Promega) recently introduced to our laboratory (visualized using the ABI Prism 377 DNA Sequencer). Additionally, DNA sequencing of both HV1 and HV2 segments of the mitochondrial non-coding region was performed using dye primer cycle sequencing chemistry (ABI Prism 377 DNA Sequencer). The study was carried out on single hair shafts from unrelated persons. 2 to 4cms of head hair shaft was used per DNA extraction. After the treatment with proteinase K and DTT at 56^0C overnight, the DNA was extracted with phenol:chloroform:isoamyl alcohol in combination with Microcon 100 concentration. For all hair shafts investigated, the amount of DNA extracted was sufficient enough to generate clean sequence data from both mtDNA hypervariable segments and to obtain full profiles using both multiplex STR systems. However, different amplification efficiencies were observed due to a wide variation of the DNA amount obtained from different individuals. Moreover, a hexaplex STR system was generally less sensitive than the *GenePrint*™ Powerplex™ system. From the above experiments, the optimal amount of nuclear DNA required for hexaplex amplification was within a range of 5-10 ng, whereas the *Powerplex* required no more than 5ng. Amplification conditions were optimized for single hair shafts to obtain balanced signals across all of the loci included in the hexaplex.

FINGERPRINTS FROM FINGERNAILS ?

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Unlike other cutaneous appendages, nails (either fingernails or toenails), are rich in genomic DNA, available by standard organic extraction procedures. The aim of this study is to evaluate quality and quantity of DNA from this tissue.
Distal cuts of nails (5 mm width on average; 100 μg grinded dry tissue) were incubated at 48°C in 500μl STB buffer solution (Tris base, Na_2EDTA, NaCl, DTT and SDS), supplemented with pK (1mg/ml) every 12 hrs, to a final concentration of 3 mg/ml. After a prolonged incubation period (40 hrs), the sample was completely dissolved, extracted by a standard phenol-chloroform and the DNA harvested by ethanol precipitation. The average amount of DNA from each sample, quantitated by two different methods, was considerable (up to 1 μg per/ 100 μg of tissue). Various nail regions did not return a different DNA/tissue (w/w) ratio. The quality of DNA was in all cases extremely degraded and had a distribution peak below 100bp. This phenomenon was observed in samples from ex vivo donors and from bodies A higly ordered phenomenon (apoptosys?) should presumably underlie such degradation, probably in connection with the keratinization process.
DNA samples were amplified by PCR for low molecular weight STRs (CD4, MBP, HumTH01), with inconstant amplification results.

COMPARATIVE ANALYSIS OF ABO TYPING BY BOTH DNA AND SEROLOGICAL ANALYSES FROM HIGHLY PUTREFIED SEMEN

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The ABO blood group system is a valuable tool for the forensic analysis of blood and physiological fluids in criminal investigation. To determine ABO types, molecular genetic techniques based on the detection of sequence defferences between A and B transferases, and serological methods including the detection of antigens and/or antibodies associated with the blood groups have been performed. We determined the ABO types of a highly putrefied semen specimen by both DNA and serological analyses and compared these results.
Materials and Methods: Sperm cells were observed in this semen specimen. This semen was in a condom and placed at room temperature for about 2 weeks. The semen had a putrid smell. Serological determination of ABO type was performed by the absorption/elution tests. The DNA samples were extracted from the supernatant and the sediment of the semen using the phenol-chloroform extraction method. DNA analysis for ABO typing was performed as described by Lee, J.C.-I. and Chang, J.-G., 1992).
Results: Microoganisms were observed in the semen specimen by microscopy. The ABO type of the semen was determined as AB by the serological analysis. In contrast, DNA analysis detected only the A gene in the DNA samples extracted from both the supernatant and the sediment. Also, HLA-DQα type of both the DNA samples was 3, 3.
Conclusions: 1) The ABO type of the semen determined by serological analysis was different from that determined by DNA analysis. The B antigen may have been aquired from microoganisms in this highly putrefied semen specimen. 2) These results indicate that tDNA analysis for ABO typing is a powerful tool for highly putrefied specimens.

FAMILY RELATIONS RECONSTRUCTION IN A MURDER CASE

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We studied a murder case involving a series of suspects related each other by familiar relationships. At the beginning the attention was focused on a man and a preliminary DNA analysis was carried out to determine the possible compatibility with some blood traces found on crime scene. Surprisingly the man was excluded by the genetic results that suggested anyway a probable brotherhood relation with the killer evidences. For this reason all the family of the first suspect was considered for a genetic screening; this analysis was particularly interesting because a member of the family, brother of the previous suspect, hidden himself from justice just after the crime was committed. No sample of the fugitive was available, so the only way-out was the reconstruction of his genotype, starting from the other members' ones, including a bone sample from the mother, dead some years earlier. The genetic screening based on typing of Amelogenin, D1S80, HUMvWA, HUMTH01, HUMTPOX, HUMCSF1P0, HUMF13B, LDLR, GYPA, HBGG, D7S8, GC and HLA-DQα loci permitted the reconstruction of missing genotype identifying the fugitive as the probable killer.

Family relations reconstruction based on genetic analyses became increasingly frequent in Italy not only for the particular family-based Mafia crimes but mostly because of the legal restrictions on suspect sample collection that must take place on a voluntary base. We are reporting here this work as a reference case.

DNA ANALYSIS ON SKELETAL REMAINS AFTER MONTHS OF PERMANENCE IN SEE-WATER

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Right tissue-made tennis shoe containing anatomical remains of a foot were found on a shore in southern Italy. After a preliminary view the specimen was dated back to several months and a police control on the previous year missing persons had the attention focused on a young man involved in organised crime. As a start out a genetic analysis was planned to assess the DNA profile of both the specimen and the criminal.

Lacking any kind of samples belonging to the missing man, an attempt to reconstruct his genotype was made starting from the genetic material of his parents. We encountered technical problems working on soft tissue portion of foot remains, probably due to a saponification process in act. Working on the bones however, we obtained results from 11 loci (D1S80, HUMvWA, HUMTH01, HUMF13B, LDLR, GYPA, HBGG, D7S8, GC and HLA-DQα), confirming the family relationship between the specimen and the two blood samples collected from the missing man parents.

Caseworks like the one reported here point out the importance of a genetic approach in murders in which there is an attempt to completely destroy the body of killed person. In some cases only little body evidences, generally bones, are available for analysis and case-oriented special variations to standard protocols must be developed.

NEWBORN GENETIC IDENTIFICATION: EXPANDING THE FIELDS OF FORENSIC HAEMOGENETICS.

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Classical newborn identification methods usually employed in nurseries and hospitals worldwide (brazalets, fingerprints, footprints, photographs) have been strongly criticized by pediatricians and gynecologists and found not reliable.
We have recently started a newborn genetic identification program to ensure a positive and definite mother/newborn identification regardless the circumstances of delivery (vaginal delivery, caesarean surgery), and the health status of the fetus (alive, dead).
After appropiate informed consent, over 250 samples of blood (5 - 500 microlitres) were taken at the time of delivery, deposited on FTA® papers and initially stored in the maternal medical files. Blood samples from mother were taken either from intravenous infussion devices or from bleeding surgical injuries; newborn blood samples were taken from the placental end of the umbilical cord.
Blood sample collection is easy in maternities, since based on medical procedures, and it is not influenced by the type of birth and the maternal and/or fetal status. Minimal amounts of blood (5 microlitres) yielded enough DNA to appropiately type the mother and the newborn, and so unequivocally establish the genetical relationship by using a minimum of 6 STRs loci.
The whole procedure, exclussively based on forensic genetic identification techniques, can be completed in less than 5 hours, and even less with the use of capillary-electrophoresis based technologies.

DOUBLE PATERNITY IN TWINS.

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Twins may originate from a single fertilized egg (monozygous, identical) or from two different fertilized eggs (dizygous, fraternal). If a woman has sexual intercourse with at least two men during a polyovulatory period, superfecundation could result and the twins could have different fathers.
Paternal identification was requested by the husband (considered at that time to be both the legal and biological father of the twins).
In this study, conventional marker blood typing included: hemagglutination assays for ABO, Rh, MNS, Fy, Jk, Kell-Cellano, and Lutheran; isoelectric focusing separation of the intraerythrocytic enzymes PGM1, and AcP, as well as serum proteins Tf, and Pi. Also 10 PCR based loci were studied: HLA-DQA1, D1S80, D17S5, HUMTH01, HUMVWA, LDLR, GYPA, HBGG, D7S8, and GC. Finally, 3 single locus loci were employed: D5S110 (LH1), D2S44 (YNH24), and D10S28 (TBQ7).
Based on the results of types on the loci Fy, Pi, HLA-DQA1, D1S80, D17S5, HBGG, D5S110, D2S44, and D10S28, the alleged father was excluded as the biological father of twin #2. On the other hand, the alleged father could not be excluded as the biological father of twin #1, with a probability of paternity of 99.9999998%.
Dizygous twins could have different biological fathers, and it could be thought that frequency of twins with different fathers is probabily underestimated, at least in small selected group of populations such as paternity suits.

MINIMAL AMOUNTS OF DNA: IMPROVING THE RESULTS OF THE ANALYSIS IN FORENSIC CASEWORK.

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In 1987 minimal amounts of DNA meant micrograms. Now, ten years later amounts of 1ng are usually used in forensic DNA analysis. The limit is not written and the technology is pushing it everyday a little further.
However, to refer to minimal amount in forensic haemogenetics means to talk about typing. In some cases we have theorically enough amount of DNA and we get no result. There are different factors involving the practical concept of minimal amount of DNA that we review and analyze to try to give some advice to be applied to distict situations in the forensic casework.
In the different protocols there must be a balance between the quality of the product obtained and the quantity. Minimal amount is a multifacetic concept where we have to consider the source of DNA, its degradation, the pressence of contaminants, the initial amount of DNA in the evidence, as well as its loss during the manipulation.
In forensic DNA analysis, 3 different steps must be considered: 1) Recovery of the cells from the evidence substrate. 2) Extraction of the DNA from the cells. 3) Amplify and type the DNA.
We show two procedures to handle evidences that can be considered as minimal amounts to improve problems due to the presence of contaminants, partially degrated evidences and loss of DNA during the Microcon purification.
The first one is Sequential Multiplex Amplification (SMA) for cases with minimal amount and/or partially degrated material; the second one is a procedure that allows to recover up to 25% more DNA from the Microcon membrane after the purfication step.

DANDRUFF AS A SOURCE OF DNA: VALIDATION STUDIES.

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Dandruff is a clinical alteration of the skin that consists histologically of orthokeratotic clumps with minute parakerathotic foci found in inflamatory pathologies as seborrheic dermatitis and psoriasis. Nevertheless in a number of people the physiologic process of keratinitation can be increased during some periods of time originating also dandruff-flakes. In all these cases, there is a number of nucleated cells (inflammatories and/or epithelials) that can be found mixed with the desquamation tissues, and hence there is a possibility to extract and type DNA from dandruff in forensic caseworks.
When comparing the two most common extraction procedures, the organic extraction one yielded better results than the chelex procedure. In this validation study, we have also analyzed the influence of different products oftenly employed in the daily care of hair (shampoo, hair-dyes, hairspray) in the quality of the amplification and typing of DNA.
The results show that it is possible to obtain DNA from dandruff in all cases, and that this DNA can be correctly typed. This results could be initially explained if it is considered that keratine serves as a shield that keeps the cells nuclei isolated from common external influences.
In conclussion, it can be deduced that dandruff is a robust and very useful biological evidence in those forensic cases where present, both as a criminal evidence and as a known control for missing persons.

RAPID MATERNITY EXCLUSION OF SKELETAL REMAINS BY mtDNA MINISEQUENCING

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Several human bones were found buried near a farm. The evidences consisted in fragments of femur, sacrum, humerus, radius, tibia, two different parts of ilium, four different vertebras plus several other small unrecognisable fragments. They were all severely degraded and partially burned. In order to make an identification, DNA profiling was attempted and the results matched to the profile of a woman, to establish if they belonged to her son. Amelogenin and STRs results showed the bones were from four different human male skeletals. Thirteen STRs were amplified and partially typed, three of the profiles compatible with the hypothesis of maternity, with a casual sharing from 7x10 to 2,5x10 . Mithocondrial DNA was tested using a novel method called minisequencing, described by Tully et all. This technique quickly defines an haplotype with the sequence in twelve different loci dispersed in HV1 and HV2, and rapidly excluded two out of the three compatible remains. The investigation was concluded by full sequencing of region HV1 and HV2 with dye primer cycle sequencing. Sequences showed to be identical in the woman and in the last skeletal remain, and not repeated in a database of 101 Caucasians.
This minisequencing Mt-DNA investigation quickly allows to exclude evidences without the time and labour expensive full sequencing. Matching results can be confirmed and further strongly supported by full sequencing.

Reference
1) Tully et al., Nature Genetics (1997)

STR ANALYSIS OF SEMEN CONTAINED IN VAGINAL SWABS AND POSTCOITAL INTERVAL

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Vaginal swabs were taken from eight women up to six days post coitum. The women in question had sexual intercourse (with ejaculation) with one partner only. Both partners had been abstinent from sexual intercourse five days beforehand.
Smears of these swabs were HE-stained for microscopical sperm detection. The activity of acid phosphatase was tested by means of PHOSPHATESMO-KM-strips (MACHERY-NAGEL, Germany). Parallel to this, examinations for seminal vesicle-specific antigen were performed with the MHS-5-kit (HUMAGEN, USA). The swabs were then submitted to STR analysis (373A-DNA-sequencer, ABI), including an amplification of the system DYS19.

Few isolated sperm heads could be detected microscopically up to three and in single cases up to five days post coitum. The acid phosphatase showed sperm-characteristic reactivity after two days, in single cases after up to four days. MHS-5-activity could be proven even longer than after five days. In contrast, tested STR systems of sperm DNA could only be seen up to the second postcoital day, with sufficient results also being possible for up to three days post coitum with the system DYS19.
In the postcoital interval, the evidence provided by sperm markers, including microscopical findings, was possible for a longer period of time than the demonstration of STR types was. STR typing from the sperm fraction of vaginal swabs without microscopical results is a rare exception.

FORENSIC APPLICATION OF HUMAN DNA QUANTITATION KIT: SPECIES IDENTIFICATION AND DNA QUANTITATION IN DENTAL PULP

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Species identification and human DNA quantitation for DNA extracted from forensic specimens were performed using QuantiBlot™ Human Quantitation Kit (PERKIN ELMER). The procedure is based on the hybridization of a biotinylated D17Z1 probe to DNA samples immobilized on a nylon membrane. Subsequent binding of enzyme conjugate allows colorimetric detection.

DNA samples were extracted from various animal bloodstains and various human tissues (body fluids, hard tissues, organs and dental pulp from 53 teeth). Not only human DNA but also monkey DNA showed positive reaction by this method. The detection limit of human DNA was 125 pg/μl.

The proportion of human DNA to spectroscopic DNA in the dental pulp DNA ranged from 9.8 % to 99.3 %, with a mean of 68.3 %. There was no distinct variation in the content according to the period of storage. DNA typing of 4 STR loci (CSF1PO, TPOX, TH01 and vWA) was possible in the samples containing above 23.8 % human DNA, the mean amount of human DNA template being 35 ng.

In a crime case we quantified the amount of human DNA extracted from pulpal wall without pulp, and typed a few kinds of STR loci.

DNA microsatellite polymorphisms and the Syringe Exchange Programme for AIDS Prevention

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More than two thirds of AIDS cases in the Basque Country are associated to intravenous drug addiction, mainly due to the sharing of syringes. Thus, it is of the highest priority to avoid the HIV infection in order to prevent AIDS. In this sense, the Syringe Exchange Programmes have probed to be highly effective.

Most of the previous studies have evaluated the impact of these Programmes based on the answers of drug addicts to questions about their risk habits. However, the reliability of these studies could be biased by some factors such as problems of memory or non-socially accepted practices. Thus, to verify the answers referring to the sharing of syringes, we have analyzed the blood traces in them.

One hundred and thirty seven syringes were collected. They were from people who have been consuming intravenous drugs for an average of 8,6 years. Fourteen syringes were not included in this study because they had no visible blood traces or because their users had already confirmed that they had shared the syringe. Then, 123 DNAs were extracted using phenol-cloroform procedure and CSF1PO-TPOX-TH01 and F13A-FES-VWA microsatellite loci were genotyped.

The results showed 2% mixed genotypes indicating tath these syringes had been used by more than one person. So, we detected only 2% false answers and the conclusion was that the DNA microsatellite polymorphisms are an invaluable tool to establish the reliability of the inquiry procedure in order to know the usefulness of the Syringe Exchange Programmes to avoid AIDS.

NEWBORN GENETIC IDENTIFICATION: MICROSATELLITE DNA AS AN ALTERNATIVE TO FOOTPRINTING

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Newborn identification systems based on foot or finger printing taken in the delivery room cannot guarantee a correct identification of the child (Butz et al. (1993) *Clin. Pediatr.* **32**, 111-113). In a study of full-term infants, 20 pairs of footprints were obtained; 89% were found to be technically inadequate for purposes of identification and only 1% had all the necessary points to be suitable for use in a court of law; footprints obtained from 20 low-birth-weight infants were compared with a second set of their prints obtained at 4 to 8 weeks of age. No sets of footprints were correctly matched (Thompson et al. (1981) *J. Pediatr.* **99**, 797-798).

Because of the inadequate quality of footprints we propose DNA genotyping using DNA microsatellite loci as a fast and reliable alternative for newborn identification.

CSF1PO, TPOX and TH01 microsatellite loci were chosen to develop a fast and reliable protocol to be applied in cases where it is suspected that newborn children have been swapped. The adventage of these loci is that one can simultaneously amplify them by PCR (Polymerase Chain Reaction) multiplex reaction and determine their alleles, thereby reducing the time needed for identification tests. Moreover, these loci present a probability of genotype coincidence between non-related individuals of 0.002; their capacity for discrimination is therefore very high. Finally, the amplification products of these loci are very small (<350 pb) and so can be analyzed in samples with degraded DNA. We have been able to prove that it is possible to obtain results in blood-spots taken from newborns up to 13 years before and kept at room temperature. This means that the protocol proposed here can be applied in long-term post-natal identification cases.

HYPERVARIABLE MITOCHONDRIAL DNA SEQUENCES ANALYSIS OF VARIOUS SAMPLES IN A FORENSIC CASEWORK

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The high variability of non coding mitochondrial (mt) control region (CR) sequences permits utility in forensic identity investigations.
DNA amplification and sequencing of this mt region provides a sensitive and discriminating test for individual identification even from human biological traces. Although automated mt DNA sequencing is used on a routine basis in forensic casework within the laboratory, these analyses are specifically reserved for delicate and discrete samples such as hair shafts, degraded bones or saliva traces...
In this paper, we propose to describe a mt DNA analysis performed in a crime case involving the following evidential samples :

- a hair shaft
- a paper cup
- a plastic straw
- a metallic beer-can.

The hair shaft was found in the victim's hand whereas the other articles were part of a very dirty fast-food package found near the dead body on the roadside.
Hypervariable mt sequences 1 and 2 (HV1 and HV2) of these samples were determined and compared with HV1 and HV2 sequences of blood samples obtained from the victim and 4 suspects.
DNA extraction, amplification and sequencing techniques will be detailed. An additionnal purification step of the PCR product on a low-melting agarose gel drastically improved our sequencing results.
The sequence comparisons excluded the 4 suspects as far as the different evidential samples were concerned. New comparisons are foreseen with other individuals. The identity between the mt DNA sequences of the hair shaft and the victim was clearly established. However, the mt DNA being maternally inherited, this result could not exclude the maternal relatives as possible suspects.

HUMAN SEX DETERMINATION FOR FORENSIC PURPOSES: COMPARISON OF DIFFERENT METHODS.

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Sexing of human DNA in biological stains can be performed by amplifying different sequences of the Y chromosome or X-Y homologous genes. Gender identification can be problematic as forensic samples frequently contain a mixture of two or more types of biological material originating from more than one individual.
This study assesses the reliability and sensitivity of three markers for sex determination: the amelogenin gene (AME), the Zinc Finger Protein gene (ZFP) and a Y alpha satellite repeat region.
Marker reliability was tested by means of a short protocol and polymorphism sensitivity determined using a mixture of male and female cells in different concentrations.
Detection of the Y fragment was found to be the most sensitive method of identifying male cells in abundant female material.
The amplification of both AME and ZFP, can be considered a fast, sensitive and reliable technique for sex determination in a variety of specimens including those in which only small amounts of DNA are present.
Use of these polymorphisms provides an internal reaction control.
Other findings will be discussed in detail.

DRUG ABUSE DEATHS: DNA INDIVIDUALISATION OF TRACES OF BLOOD FROM HYPODERMAL SYRINGES.

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The aim of this work is the individual identification of traces of blood from hypodermic syringes involved in suspected overdose deaths. DNA was extracted from 3 mL of blood from the victim and from the methanolic residue left after toxicological analytical procedures applied to the syringe. DNA was quantified by a slot blot technique, amplified by the polymerase chain reaction (PCR) and typed. The number and type of genetic markers depended on the amount of DNA obtained from the syringe (DQA1, LDLR, GYPA, HBGG, D7S8, Gc, D1S80, and different STRs). An evaluation of our casework in the last 4 years is presented. Minimal amounts of methanol residues have proved to be a suitable sample in the resolution of forensic cases of death due to overdose of drugs of abuse.

RECOVERY OF HIGH QUALITY DNA FROM 500-YEAR-OLD SKELETAL REMAINS FOR FORENSIC DNA TYPING

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A simple and efficient method for extracting human genomic DNA from 500-year-old skeletal remains has been developed. This method used chelex 100 based extraction for deionizing bone fragments. Two skulls were discovered from 10 meters underground when digged in the grounds for construction. These bone fragments were well preserved with permeation of metal ions in accumulated layers. Both of them were relatively small and sutures had not been closed at all, so that these skulls were believed to be the remains of the children. Moreover, they were buried close to each other and thus seemed to be relatives. Before performing DNA typing, we measured oldness of skulls by carbon 14 measurement. In the results, they were proved to be buried last 460 years ago, at Muromachi-era in Japan.

To recover high yield of DNA, we used chelex 100 with constant shaking to decalcify and deionize the permeated other metals inside the bone fragments. By this simple procedure, we could extract high quality DNA, and with which successfully amplified XY homologous amelogenin, ABO blood group locus, some STR loci and mitochondrial hypervariable regions for direct sequencing. As a result, we could prove that the persons of these two skulls were not pertained to each other, and found unique polymorphisms rarely found in modern Japanese.

DNA TYPING FROM HUMAN DENTINE

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In recent years DNA-analysis from bone and teeth samples have become increasingly important in the identification of fire victims, skeletal finds, and disaster victims. It has been shown that dental pulp is a more reliable DNA source than bone since the DNA is better protected from environmental influences. The DNA in the dentine should be even more protected than in dental pulp.

For this reason the possibility of using odontoblasts, which participate in the formation of dentine, for DNA-analysis was studied.
It proved possible to amplify the extracted DNA and seperate and detect five different STR loci (TH01, VWA, CD4, FXIIIB, FGA) and one X-Y-specific length polymorphism through horizontal discontinous polyacrylamid gel electrophoresis. The data could be confirmed by 100% with comparative data obtained from blood samples from the respective individuals.

FORENSIC VIEW FOR GENETIC PROFILES AFTER BONE MARROW TRANSPLANTS

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After one year and a half of a collaborative work with the Centro de Histocompatibilidade do Centro on monitoring bone marrow transplantations, we can make a medico-legal point of view of the results.
DNA polymorphisms were identified on ABD Sequencers (373 or 377) and genescan software, after co-amplification of 4 STRs (HUMTH01, HUMFES, HUMVWA, HUMF13A1) plus Amelogenin gene in cases where the donor/recipient pair was of opposite sex.
In the majority of the cases, in spite of donors and recipients were brothers and with the same sex, we found different allele patterns at least in one of the 4 STRs identified.
It was also studied the chimerism with different amounts of mixed cells, which allow to evaluate the sensibility of the multiplex.
We still present some cases where blood samples as well as saliva, bone marrow and hair samples,were typed to evaluate what kind of material should be used in forensic identifications.

MONITORING PATIENTS AFTER BONE MARROW TRANSPLANTATION - A COMPARISON OF METHODS.

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Early detection of recurrent malign cell growth is essential for treatment of leukemias by bone marrow transplantation. We have monitored alleles in three different $(AAAG)_n$ polymorphisms to detect recipient derived cells in samples taken six months or more after transplantation. Comparisons were made between electrophoretograms of fluorescence tagged PCR products and autoradiograms of minisatellite analysis by Southern blot and single locus probe hybridisation (RFLP).

In addition compound samples were made experimentally of recipient and donor DNA to evaluate detection limits in selected samples.

In summary the data shows that the RFLP technique is the most sensitive in detecting minute amounts of inborn alleles in the post transplantation samples. Contributions at 10-15 % are easily detected at autoradiograms, while such levels of original cell growth are not always detectable by PCR. In contrast, at contributions slightly above this level, PCR monitoring tend to exaggerate the level of inborn compared to transplanted alleles. Therefore, RFLPs give better quantitative estimates.

The fact that chance allele sharing between donor and recipient is less at the minisatellite loci, also facilitates the evaluation of monitoring results.

SHORT TANDEM REPEAT ANALYSIS TO MONITOR CHIMERISM AFTER BONE-MARROW TRANSPLANTATION

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Bone marrow transplantation (BMT) is used to treat various haematological malignancies. The success, or otherwise, of the engraftment and the subsequent clinical progress is generally monitored through the regular review of peripheral blood and bone marrow morphology. In the case of allogeneic transplants, changes in red cell phenotype, HLA type, appearance of the Y chromosome and of some cell surface markers can also prove useful in some circumstances. The use of short tandem repeat (STR) loci to monitor chimerism adds a further and potentially more powerful tool to the diagnostic process. In a prospective study, twenty two patients eligible for BMT and their bone marrow donors were typed for up to four STR loci (TH01, VWA, F13A1 and FES/FPS) using the Pharmacia Alf Express as the analysis platform. Where donor material was available we aimed to identify useful splits between the individuals who were often sibling pairs. Six patients have been followed for up to 2 years post transplant or until death on a median of 7 occasions. Complete chimeric and mixed chimeric states have been detected along with both an initial failure to engraft and the subsequent relapse after achieving apparent successful engraftment. In samples with mixed chimeric status the percentage of donor or recipient type was assessed with reference to a standard curve, plotting peak area against a prepared series of mixtures of samples displaying differing STR types. The availability of a wide range of STR loci that can be used to discriminate between donor and recipient type in all cases of allogeneic BMT offers the haematologist a sensitive and rapid procedure to aid both measurement of the degree of engraftment and the early detection of relapse. Further prospective studies will examine whether this methodology provides earlier notice of changes in transplant status and whether this knowledge results in an improved outcome for these patients.

DNA TYPING OF EPITHELIAL CELLS

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PCR typing of epithelial cells which were transferred from the hands of the perpetrator to the handle of a weapon or the neck of the victim in cases of strangulation is crucial because usually only minute amounts of cells and / or unequal mixtures of cells from the victim and the perpetrator can be secured.
Method: Epithelial cells were removed with low pressure by using steril cotton swabs moistened with aqua bidest.
DNA extraction was carried out using 190 µl Chelex 100 (5%, Biorad) with the addition of 10 µl Proteinase K (10 mg/ml, Qiagen).
In the present investigation DNA typing was carried out on epithelial cells which could be removed from different substrates:
Strangulation: in three cases of strangulation epithelial cells which were transferred from the hands of the suspect to the neck of the victim could be successfully typed using the STR systems CD4, TH01, VWA and FGA.
Weapons: in stain cases in which different weapons were used (gun, knife, hammer) epithelial cells could be secured from the grip / handle and successfully typed using STRs.
Traffic accidents: in a traffic accident with a stolen car and a driver abscending from the scene of accident epithelial cells which were obtained from the steering-wheel of the identified car could be successfully typed and attributed to the suspect.

DETECTION AND QUANTITATION OF THE AGE-DEPENDENT 4977 BP DELETION OF HUMAN MITOCHONDRIAL DNA:

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In recent years the analysis of the mitochondrial genome has acquired increasing importance in legal medicine. It enables not only forensic identification of human trace material but also the investigation of specific deletions in mitochondrial DNA (mtDNA). The 4977 bp deletion is the most common deletion. An accumulation is observed with increasing age and in some degenerative diseases.
Quantification of the deleted mtDNA in skeletal muscle taken postmortem from individuals representing a broad age span enabled the calculation of normal values for the physiological aging process. We investigated whether our data allow inferences regarding biological age or the presence of degenerative diseases.

Applying two different PCRs we demonstrated the 533 bp fragment of undeleted and then the 677 bp fragment of deleted mtDNA, and their detection on silverstained polyacrylamide gels. We were able to obtain signals up to a detection threshold of 10 fg total DNA (nuclear and mtDNA) for the undeleted and 100 pg for the deleted mtDNA. Quantitation of the deleted mtDNA was done with a kinetic PCR.

No detection of the 4977 bp deletion would suggest of an individual less than 20 years of age. An amount between 0,00049 % and 0,14 % of deleted mtDNA was observed depending on the age of the individual. After evaluation of the kinetic PCR we achieved a correlation coefficient of 0,733 by investigation of 50 persons.

The data obtained can help to establish reference values for the increase in deleted mtDNA during physiological aging. Deviation from these norms could then provide supplemental information for clarifying diseases or the age of the individual.
The detection is also human specific and applicable after longer postmortem intervals.

INDIVIDUALIZATION OF URINE SAMPLES IN DOPING CONTROL BY STEROID PROFILING AND PCR BASED DNA ANALYSIS

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The individualization of urine samples plays an important role in doping control since: 1. In a positive case it is possible to prove that a urine sample belongs to an athlete; 2. It is possible to detect manipulation with heterologous urine; 3. In longitudinal studies for the establishing of subject based reference ranges, it can be proven that all urines are from the same athlete.
For individualization of urine samples we compared STR-polymorphisms of DNA and the profile of endogenous steroids. Urine samples of 10 individuals collected at two different times were used to type the samples and to find the correct pairs. STR polymorphisms were analysed by STR-PCR (D18S51, HUMFIBRA) followed by direct blotting of the PCR products. Steroid profiling was performed after hydrolysation of the steroidglucuronoides and trimethysilylation with gas-chromatography/mass-spectrometry.
Eight out of 10 pairs could be identified by PCR-based DNA analysis and all 10 pairs could be identified by the steroid profils. After storage of the urine samples for 4 weeks at +4°C no pair could be identified by STR-analysis. By steroid profiling again all 10 pairs could be identified.
In a second study six official dope control samples with identical steroid profile parameters were analysed for their DNA polymorphisms in two STR-loci. In four samples an identical allelic band pattern could be found. This result confirmed the suspicion of manipulation with the same heterologous urine for the athletes and they were declared positive. This is the first documented case in dope control were DNA-profiling method led to a positive case.
The combination of steroidprofiling and DNA-typing is a useful method for individualization of urine samples in dope control. The advantage of the steroidprofile method is the stability of the parameters, whereas the DNA-typing method allows the comparison of blood and urinary parameters.

FUNERARY RECRUITMENT IN A SEPULCHRAL CAVE DATED FROM THE BRONZE AGE.

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Four disorganised skeleton remains have been discovered in Elzarreko Karbia's cave (France), dating from 3700 BP by the 14C method. To determine if these four fellows had been gathered because of relationship, we performed paleogenetic analyses on the four sacrum and a collarbone.To test the potentialities for ancient DNA studies of our sample, we first performed a gender determination by amplifying the about 100 bp fragment length of the amelogenin gene, which permitted us to confirm the morphological data. Then we have amplified and sequenced the 2 hypervariable regions of the mt DNA control region. These paleogenetics analysis have first permitted us to gather the collarbone and the sacrum which belonged to the same corpse. Regarding to the relationship hypothesis,a high heteroplasmy rate in our sequences, perhaps due to damaged DNA, allowed us not to establish parental linkage between our four individuals.

ANALYSIS OF HIGHLY DEGRADED DNA BY SOLID FACE CYCLE SEQUENCING OF HLA CLASS II DRB1 AND DQB1 ALLELES USED IN PATERNITY AND IDENTIFICATION CASES

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We have used solid face cycle sequencing of HLA class II alleles to analyse highly degraded DNA. On this material amplification of STR (short tandem repeat) markers and the markers included in the DQA-PM identification kit (Perkin Elmer) usually failed or gave unclear results. DNA was extracted from paraffin embedded formalin fixed tissue sections from two individuals and from samples of marrow from femoral bones from two individuals that had been at the bottom of the sea for 23 years. For sequencing HLA DRB1 and DQB1 alleles were amplified using primers in the SB Typer™ DRB and DQB HLA Class II sequencing kits from Pharmacia Biotech. These kits utilize solid face sequencing on amplified DNA attached to Dynabeads. The sequencing primers included in these kits were used together with reagents from the Thermo Sequenase cycle sequencing kit from Amersham. Automated DNA sequencing was done on an A.L.F. DNA sequencer and the results were evaluated using the SB Typer™ program. The formalin fixed material was HLA DRB1 01,04; DQB1 05,06 and DRB1 01,04; DQB1 03,05. The bone marrow from one individual was DRB1 04,13; DQB1 06 and from the other DRB1 04,13 and DQB1 03,06. Amplification during both the PCR and the sequencing reactions permits very small amounts of template DNA for analysis. Only "full-length" templates are sequenced since the sequencing primers anneal to sites distal to the biotin labelled primer. To avoid disturbance from small DNA fragments, excessive washing was needed. However, if proper care is taken to evaluate only high quality sequencing results, this procedure can be used to solve cases where only highly degraded DNA is available.

EXPERIMENTAL EXERCISE ON MITOCHONDRIAL DNA SEQUENCING FOR FORENSIC CASEWORK ANALYSIS

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Hair analysis is sometimes part of forensic casework. We wanted to validate our working procedures of the handling of hair roots and shafts. We designed an experimental exercise intended to reveal weak points in our procedure.
Six hairs from six different persons were cut into three parts: the root, the inner shaft and the outher shaft. One blood sample was taken from each individual. Each of the samples was coded and the analyses were then performed blindly. In each case the inner shaft had been contaminated with blood from one of the other persons, wereas the root and the outher shaft were not contaminated.
DNA from the different parts of the hair was extracted. Phenol extraction was used for the hair roots and the outher shafts. The same procedure was used for the contaminated inner shafts but in this case each shaft was washed twice in 1ml H_2O and once in alcohol. The primary water, in which the shaft had been washed, was used for extraction of DNA of the contaminated blood. The DNA was extracted by phenol. The blood samples were extracted by Chelex.
The extractions and the PCR-work were performed in LAF-benches.
All extractions gave PCR-products which could be sequenced. All negative controls remained negative even in those cases were reamplification was nessesary.
In five of the individuals, the mtDNA sequences in blood, hair follicle and hair shaft were identical. In several of the sequences from the contaminated inner shafts, the mtDNA mutations of the hair itself as well as of the contaminating blood were encounted. In the sixth individual, a single mutation was experienced in the hair follicle and the inner shaft compared to the sequence of the blood and the outher shaft. The reason for this finding is still obscure.
The present experiment shows that the washing procedure is not very efficient. Typing of the washing water as well as the hair will, however, usually give indications of the presence of any contamination.

MITOCHONDRIAL DNA IN FORENSIC CASEWORK: SEQUENCING OF THE CYTOCHROME B GENE FOR SPECIES IDENTIFICATION

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Mitochondrial DNA (mtDNA) typing by direct sequencing of the PCR products has become a niche in forensic casework analysis. Amplification of the 2 hypervariable segments (HVI and HVII) within the control region proved to be a highly sensitive technique, leading to positive results, even when DNA is degraded or the quantity of DNA is very low. The technical improvements in the past few years for sequence analysis of PCR generated templates have provided automated procedures for routine laboratory work.
In casework analysis, the biological origin of a trace has been investigated by conventional methods (e.g. protein electrophoresis, immunological procedures), which have drawbacks: these techniques usually rely on the quality and the quantity of the evidence material and their analysis is restricted to a given assortment of species tested for. Therefore, amplification of the mitochondrial gene cytochrome b and direct cycle sequencing of the PCR product circumvents these problems. It has been demonstrated, that this system amplifies in a variety of different species. The mutation rate of the cytochrome b gene enables the differentiation of closely related species without substantial variation within a species. Further, cytochrome b sequences - for identification purposes - are available on databases for many diverse organisms.
In this study, homologous segments of the cytochrome b gene region were amplified from more than 50 vertebrate species, establishing the basis of a cytochrome b nucleotide sequence database for forensic relevant indigenous vertebrates. Further, different biological specimens (e.g. hair shafts, capes, and different tissues) were investigated comparing the nucleotide sequences to the blood sample of the same individual. No deviations in the nucleotide sequences between the specimens and the reference material have been observed.

VALIDATION OF SHORT TANDEM REPEAT POLYMORPHISMS AT LOCUS APOAI1, D11S554 AND ACTBP2 FOR PATERNITY TESTING.

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We presently use five single locus probes of minisatellite markers for paternity diagnostics, offering a probability of a false inclusion less than 1 in a million. Analysis of short tandem repeat polymorphisms has emerged as an alternative to the presently used laborious procedures. Such analysis have to offer a similar degree of reliability, as expected both by the public and the judicial system in our country. Eight independent microsatellite markers with exclusion capacities of approximately 0.85 will be necessary to reach a similar degree of reliability. Here we have evaluated the $(AAAG)_n$ repeat polymorphisms at ApoAI1, D11S554 and ACTBP2, which have an expected combined exclusion capacity of 0.998.

A total of 600 paternal and 1080 maternal meiosis were investigated by PCR and PAGE using automated fluorescent detection (ABD). The reaction protocols were optimized for unambigous identification of alleles. The ApoAI1 polymorphism displayed no mutations, whereas D11S554 showed three maternal and three paternal mutations and ACTBP2 three maternal and seven paternal mutations. Each mutated allele probably originated from a parental allele 4 bp larger or smaller in size, except for one maternal D11S554 mutation that was 8 bp larger than the possible parental origin. Only one of the mutating parental alleles were represented more than once in this material. There was no drift towards more frequent alleles among the observed mutations. It hence appears that the mutation frequencies are within acceptable levels.

After including five additional equivalent markers and developing further automation and hands off procedures, STR polymorphism analysis will represent an attractive alternative to the presently used paternity tests in terms of reliability, simplicity and speed.

FORENSIC ENTOMOGENETICS: MAY THE FLY BE THE «SPY» TO IDENTIFY THE PERPETRATOR?

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Encouraged by the discovery that human host DNA may be extracted from the gastrointestinal content of parasitic crab louse (*Pthirus pubis* (L.)), we wanted to explore the possibility of tracing human DNA from another natural exploiter of human tissue: The necrophagous larvae of the blowfly *Calliphora vomitoria* (L.) not seldom encountered when examining degraded human remains.

We designed a pilot study to give clues to two emerging questions: First, will it be possible to succesfully extract DNA of a deceased individual from the larvae inhabiting this individual? Second: If the deceased is a victim of a perpetrator leaving biological stains on the victims body, will it be possible to trace the DNA of the perpetrator from the same larvae?

Female skeletal muscle biopsies from two randomly selected autopsy cases were split into two pieces each: One piece was inoculated with living eggs of the blowfly *C. vomitoria* only, the other piece was inoculated with sperm from an anonymous donor and the blowfly eggs. All samples were stored in an environment suitable for the eggs to develop into mature larvae, which were subsequently harvested on succeeding days giving larvae of different maturing stages.

We were not able to succesfully extract human DNA from the larvae. We are currently performing analogue experiments from routine autopsy cases where the larvae are found on the body of the deceased at the post mortem examination. The results of these experiments will be reported.

CAPILLARY ELECTROPHORESIS IN A FORENSIC CASE

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A casework which occurred recently in the South of Italy is presented in details. In an armed robbery of a post office one of the robbers was hurt, but he fled together with the others. Small bloodstains on an envelope, a book and a telegram were found in the post office. The following day the car used in the robbery was found, and within it bloodstains on the dashboard, the windscreen and the front seats. The owner of the car was arrested, and he had a little wound on his left hand and a small bloodstain on his jeans.

DNA typing was performed on DNA extracted by the chelex method from the material mentioned as well as from a sigarette and a glass used by the suspect during the interrogation, and from four hairs from him. Amplification was carried out by the ABI PRISM Primer Set (British Home Office, Perkin Elmer) for the markers THO1, VWA31, FES/FPS, F13A1, TPOX, CSF1PO, FGA, D21S11, and Amelogenin. Amplified STRs were analyzed by the automatic capillary electrophoresis sequencer ABI PRISM 310, employing ABI software (Data Collection, GeneScan Analysis, Genotyper Fragment Analysis). The discrimination power (DP) of the STR loci analyzed was calculated using the local population frequencies.

DNA typing showed that the bloodstains at the post office, in the car and on the jeans had the same genotype, and that this genotype was different from that of the cigarette, the glass and the hairs. This analysis demonstrates that the suspect was not identical with the hurt robber. By comparing the bloodstain DNA profile with those in a file of previously examined persons, it was shown that the genotype was identical to that of a man involved in another armed robbery.

NEW PRIMERS OF ABO BLOOD GROUP SYSTEM FOR STAIN SAMPLES

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The ABO genotyping have been determined as the nucleotide types at nucleotide position 261 and 703 by means of the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. But some times ABO genotype from evidence samples was determined wrongly. Upper primer of PCR product including position 261 is divided by intron between 5th and 6th exon, because it was based on sequence of cDNA by Yamamoto et al.. Therefore, we designed new primer set using genomic structure of ABO locus by Bennett et al. and studied ABO genotype detection from stain samples using new primer set.

DNA was isolated from blood stains, hair, and vaginal swab from the rape case. Amplification was accomplished in 25 µl of reaction mixture, which contained 10 ng extracted DNA, 200 µM of dNTP, 2.5 units of Taq polymerase and 30 pmole of each primer. (O allele detection new primer 1 : 5'-cageteeatgtgaccgcacg c-3', 2 : 5'-tcgccactgcctgggtctctacc-3'; B allele detection Lidd's primer 3 : 5'-gtgcgtggacgtggacatggagttc-3', 4 : 5'-caggtagtagaaatcgccctcgtccttg-3') A total of 35 cycles of amplification were carried out according to the following reaction cycles: 2 min at 94℃, 2 min at 63℃ and 3 min at 72℃. Ten microliters of PCR products were digested with 5 units of Kpn I (products of primer 1,2) and Alu I (product of primer 3,4) for 2h. The digested amplified DNA were then run on a 3% Nusieve 3:1 agarose gel in TBE buffer at 100 V for 1 h. The gel was stained in ethidium bromide and the bands were visualized by UV light.

DNA analyses using this new primer sets is a more accurate and sensitive method to detect the ABO genotyping from evidence samples, especially, old blood stains and hair shaft samples.

STRUCTURE AND GENE FREQUENCY DISTRIBUTION OF LOCUS D16S543 (WG1F2) IN THE JAPANESE POPULATION.

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The tetrameric short tandem repeat (STR) locus (D16S543) was investigated to evaluate its usefulness in forensic practice in Japane.

Methods: Analysis was conducted by applying fluorescently labeled samples (PCR products) and a differently labeled allelic ladder within the same lanes in denaturing gels, followed by laser detection and automated analysis using Genescan software 672. Eighteen out of the 21 alleles detected in this study were sequenced using the Dye Terminator Cycle Sequencing Ready Reaction mix (Perkin-Elmer) using the purified alleles as templates.

Results and Conclusion: Twenty - one different alleles were identified in 122 healthy and unrelated donors. The estimated heterozygosity and the polymorphism information content (PIC) were 0.93 and 0.92, respectively. The power of discrimination was calculated to be 0.99, and no deviation from the Hardy-Weinberg equilibrium was observed. The D16S543 alleles were composed of the mixtures of five kinds of repeat units (a:AAGG, b:AGAA, c:AAAG, d:CAGG, e:TAGG, f:GAGG). D16S543 appears to be a useful STR locus in forensic practice in Japan.

THE DEVELOPMENT OF A THIRD GENERATION STR MULTIPLEX SYSTEM

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The Forensic Science Service (FSS) has made several advances in the field of short tandem repeat (STR) DNA profiling in recent years. A multiplex of 4 loci was developed (QUAD) followed by a second STR system (SGM) with a much higher discrimination power and a sex test. In order to meet the demand for greater discrimination a third STR system (TGM) is under development. It is envisaged that the TGM system will be used in casework, for paternity disputes and in conjunction with the National DNA database.
A range of loci were selected for the TGM system according to a strict criteria including chromosomal location and discrimination power. The loci were tested individually and in combination to establish their profile characteristics. A selection of 6 loci was made based on these profiles and these loci, together with HUMTHO1 from the SGM and QUAD systems, make up the TGM system. TGM has been optimised both for PCR conditions and multimix components. Allelic ladders are under construction and sequencing of some alleles has been completed. DNA samples from 3 major ethnic groups are being analysed.
The loci identified for the TGM system are: D1S518, GGAA3A09 (chr.2), D3S1358, D10S516, HUMTHO1, D14S306 and GATA4F03 (chr. 22).The PCR parameters include 29 cycles of 94°C (1 minute), 60°C (1 minute) and 72°C (45 seconds) followed by a 25 minute hold at 72°C. The multimix consists of dNTPs, PARR™ Excellence buffer, primers, HCl and TAQ GOLD™.
The discrimination power of the TGM system is approximately 1 in 30 million. To be used in casework comprehensive validation must be carried out. This validation includes analysis of mock and actual casework samples, , meioses studies, species, somatic and mixture studies.

MULTIPLEX ANALYSIS AND FORENSIC VALIDATION OF FOUR NEW TETRAMERIC STRs

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A search for new autosomal tetrameric STRs has been recently planned in our laboratory, with a view to enlarge the inventory of genetic loci of potential forensic use. We consulted the web site one of the largest collections of tetranucleotide-repeat microsatellites (available from the 'Utah Marker Development Group' and we selected a few dozens of systems (by their repeat composition, heterozygosity, average allele sizes and amplification conditions) as potential forensic candidate loci. Thirteen potential quadruplexes - with non-overlapping sizes - were eventually chosen. We here report the results obtained by experimenting our first STR combination.
Primers for two duplex combinations were designed at loci D8S1043/ D20S438, and at loci D6S972/ D3S1514. Optimisation of amplification conditions, primers ratio balancing, family segregation and allele distribution in a first Caucasian population sample were addressed. Amplification conditions were adjusted to the lowest annealing temperature in order to obtain a balanced harvest of both PCR products (54°C for D8/D20 and 58°C for D3/D6).
D20S438 locus showed an array of 8 common alleles ; 5 alleles were seen at D8S1043; D6S972 locus had 7 common alleles, D3S1514 had 5. No imperfect-repeat allele was observed in these duplexes. Alleles distribution, discrimination power and the relevant chance of exclusions were promising and led us conclude that these four STRs are excellent candidates for whatever purpose of human identification.

DEVELOPMENT AND VALIDATION OF THE AmpFlSTR PROFILER KIT

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A co-amplified system of ten loci has been developed for high discrimination in forensic casework and databank applications. The AmpFlSTR Profiler PCR Amplification kit co-amplifies nine STR loci and the amelogenin gender marker in a single PCR. The Probability of Identity (Pi) with this system is greater than 1 in 3 billion and 1 in 8 billion in U.S. Caucasian and African American population groups, respectively.

All ten PCR amplification products are detected in a single lane of an ABI Prism 377 DNA Sequencer, or in a single injection on an ABI PRISM 310 capillary electrophoresis instrument, thus providing high throughput. This kit utilizes a new fluorescent dye, NED, as a replacement for TAMRA. NED has both a higher excitation efficiency and a slightly blue-shifted emission spectrum as compared to TAMRA. The dye set FAM/JOE/NED/ROX provides similar sensitivity between colors and improved spectral resolution.

Many AmpFlSTR Profiler design features provide the goals of specificity, sensitivity and balance between loci. Primer sequences have been designed and optimized for multiplex amplification, to avoid primer-dimer and other conflicting PCR interactions. The primer sequences have been selected so that extreme primer concentration balance is not necessary to achieve similar peak heights between loci; all primers are present in the range of 12-16 pmoles. This allows for reproducibility in manufacturing and in the results obtained. Single-plex vs. ten-plex amplifications with hematin-inhibited and also degraded samples give equivalent results.

Forensic validation experiments recommended by the Technical Working Group on DNA Analysis Methods (TWGDAM) have been performed, including non-probative casework, mixture studies, environmental exposure, and non-human DNA specimens. The results clearly support the use of the AmpFlSTR Profiler kit for forensic casework.

Origin and dispersal of extraordinary alleles at the DYS390 microsatellite

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We have used PCR to investigate the frequency distributions of the male specific Y chromosomal microsatellite DYS390 in 8 population samples. Besides alleles 8-12 (203-219 bp) which were shared by all populations, extraordinary short alleles with only 5, 6 and 7 repeats (191, 195 and 199 bp) could be observed for Australian Aborigines and Papuans, respectively. Based on sequence analysis and the investigation of Y chromosomal haplotype diversity with additional Y-specific STRs, we propose a unique generation of the short alleles not following the single step mutation model. The dispersion of these alleles in populations of the pacific area reveals the usefullness of these markers to reconstruct male migration processes.

Kein T am 5'Ende!

FURTHER EXPLORING OF NEW STRs OF INTEREST FOR FORENSIC GENETIC ANALISYS

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A survey of genome databases (GDH, Genbank, CEPH) was carried out and 40 STRs (mostly tetranucleotide repeats) were chosen because of having heterozygosities of over 75%. After a preliminary screening, 12 STRs were selected: D1S549, D1S1656, D2S436, D3S1754, D6S477, D9S302, D12S391, D12S375, D18S535, D19S433 and D22S683.
A population genetic study of the Galician population (n:80-125 individuals) was carried out. A cocktail of samples containing all observed alleles was used as an allelic ladder. Separation was carried out with automated sequencers (Pharmacia ALF and ABI377 Applied Biosystem).
Observed heterozygosities ranged, in these systems, from 75% to 90%. Most of the systems used presented low stuttering characteristics and can be amplified in the same conditions making easy multiplexing.
4 systems D1S1656, D9S302, D12S391 AND D18S535 were selected for a more detailed study. The firts three have heterozygosities of 0.90% with sizes ranging from 123-163 bp (D1S1656), 250-314 bp (D9S302), 209-253 (D12S391). The D18S535 size is 129-157 bp with a heterozygosity of 0.80%. All the common alleles for each system and most of the rare alleles found were sequenced. Sequenced allelic ladders were constructed with sequenced individual alleles covering all common alleles of each system. Population genetic studies were carried out at greater depth.
The four systems selected are optimal for forensic purposes. In addition to their hypervariability, they have proved to be robust, have very low stuttering characteristics and are appropriate for inclusion in multiplexes.

8 alleles

SEQUENCE STRUCTURE OF the D8S320 locus: A NEW STR IN FORENSIC SCIENCE

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The STR D8S320 (Riley et al.1993) (386-426bp) has been shown to be a robust and reliable system in forensic application (Huber et al.1995).
The aim of this study was , to characterize the repeat structure of the D8S320 locus and to get more information about the extent of complexity of the repeat region.
A total of 33 alleles from a Western German (Rhine region) were sequenced from both sides on an ALFexpress Sequencer (Pharmacia Biotech, Freiburg) using Auto load ©solid phase combs and an M13 24mer fluorescent primer.
20 different alleles differing by sequence, and 6 alleles differing by size were found.
As result, we found out, that D8S320 is not, as published by Riley et al., a simple (AAAg) repeat, but shows a complex repeat structure:
5 different types of tetrarepeats (AAAg)n=26-31,(AAAC)n=8-11, (AgAA)n=7, (AAgg)n=2, (AgAg)n=2-3, one tri- (AAg)-repeat and one penta (AAAAg)-repeat were found.
A sequenced allelic ladder has been constructed to standardize the routine case work. Using native PAGE the alleles differing in sequence but not in fragment lengths showed significant differences in electrophoretic mobility. According to the recommendations of the International Scociety of Forensic Haemogenetics a sequence related nomenclature for D8S320 is described..
In conclusion the D8S320 locus shows as far as we investigated no allele subtypes differing in size less than 4 bp. This sequence data are the basis for the standardisation of a powerful new STR System in forensic applications.

DNA ANALYSES OF ABO BLOOD GROUP VARIANTS, A3, Aend AND Bm.

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The ABH antigen polymorphism at the allelic cDNA level is used in forensic science investigation. The O allele is identical to the A allele apart from a single-base deletion at nucleotide position 261. Another O allele without the nucleotide 261 deletion characterized by typing as B at position 526 and A at positions 703, 796, and 803 has been reported. Accordingly, analyses at nucleotide positions 261, 526 and 703 or 796 or 803 are essential for ABO genotyping. The purpose of this study was to analyze ABO blood group variants, A3, Aend and Bm at the genomic DNA level.

Materials and method: 1) Serum, saliva, blood stains and saliva stains obtained from ABO variant individuals were used for DNA preparation. Genomic DNA was extracted by the phenol-chloroform method. 2) ABO genotyping was performed at nucleotide positions 261, 526 and 703 by PCR-RFLP and at nucleotide position 803 by PCR-SSP. 3) PCR-SSP products were sequenced (from nucleotide 665 to 821) using Taq DyeDeoxy Terminator Cycle Sequencing Kit.

Results and Discussion: 1) Phenotypes A3(two cases), Aend(three cases) and Bm(three cases) were found to be identical to the genotypes *AO*, *AO* and *BO* respectively. Phenotypes AendB(three cases) and ABm(three cases) were also found to be identical to genotype *AB*. No differences were observed using genomic DNA obtained from bloodstains and saliva. 2) The nucleotide sequences from position 665 to 821 were demonstrated to be identical in the products of Bm and B. 3) We also used a primer extension preamplification (PEP) method for subsequent PCR analyses of small DNA samples. Our results indicated that PEP is an effective tool for ABO genotyping from small quantities of genomic DNA.

Conclusion: ABO blood group varinats, A3, Aend and Bm were genotyped as common alleles at ABO locus.

AHSG PHENOTYPING IN DENTAL PULPS

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AHSG phenotyping was attempted on 80 dental pulps using isoelectric focusing and immunoblotting. The pulp tissue weighing 10 to 20 mg was macreated in 20 µl 1% Triton X-100. The amount of AHSG present in the dental pulp lysates was quantitated by rocket immunoelectrophoresis. The values ranged from 55.0 to 160.0 µg/ml and the mean value was 93.0±7.1 µg/ml. Nine µl of the lysates was treated with 1 µl 50 U/ml neuraminidase. Isoelectric focusing was performed on polyacrylamide gel containing carrier ampholytes pH 4.2-4.9 and pH 4.5-5.4. After run, the focused proteins were transferred onto nitrocellulose membrane. The membrane was incubated with rabbit anti-human AHSG serum and goat anti-rabbit IgG serum conjugated with alkaline phosphatase, and then stained with β-naphthyl phosphate and Fast Blue BB salt. The AHSG patterns in fresh dental pulps were identified as clearly and intensely as those in serum samples, and the types observed in dental pulps completely agreed with those in the corresponding serum samples. All the dental pulp samples examined were correctly phenotyped after storage for up to 5 weeks. AHSG phenotyping by isoelectric focusing and immunoblotting is of potential use for medicolegal individualization of teeth.

A NOVEL APPROACH FOR ABO BLOOD GROUP GENETIC SUBTYPING AND THE ALLELE FREQUENCY DISTRIBUTION IN A HUNGARIAN ROMANY POPULATION GROUP

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The ABO genotyping is a routine procedure in numerous laboratories over the World. The recently used methods are time-consuming and cumbersome procedures. These require analyses (e.g.individual PCRs and restriction endonuclease (RE) enzym digestions) for as much as seven nucleotide positions of the A glycosyltransferase enzyme gene for the complete ABO genetic subtyping for all possible alleles. In this work a new strategy is reported based on the use of different flowgrams during the genotyping including the subtyping of the newly described 0^2 allelevariant. Using such a flowgrams we can avoid any unnecessary PCR amplification and restriction endonuclease digestion and we can save time and materials as well.
We were able to discriminate all of the 21 possible genotypes using maximum only three sets of primers and two different PCR amplification conditions followed by a rapid so called *in situ restriction endonuclease digestion (ISRED)*. We analysed mutations at only five nucleotide positions (261 nps, 526 nps, 467 nps, 646 nps, 802 nps) which are responsible for some fundamental differences among the A(Pro), A(Leu), B, O(A), O(T), and 0^2 alleles.
Our method was tested in a sample of a Romany population group from Baranya County of Hungary (n: 132 individuals). Six alleles (including the 0^2 allele with a frequency of 3 %) and 19 genotypes out of the possible 21 could be distinguished. No deviation from Hardy-Weinberg expectation was found ($0.9>P>0.8$).
Our approach provides an accurate and rapid way for ABO genetic subtyping.

THE EXPRESSION OF LEWIS ANTIGENS AND CARBOHYDRATE CHAINS IN HUMAN BRAIN

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We have been studying the distribution of ABH and related antigens in human organs, such as reproductive tissues, salivary glands, taste bud cells, and Hassall's corpuscles in the thymus, inorder to resolve the biological significance of the antigens. The neurobiological interest in the expression of the carbohydrates antigens in human brain is still limited, although the occurrence of Lewis x antigen in this organ has long been noted. The expression of glycoconjugates in human brain was examined using formalin-fixed, paraffin-emmbedded tissues and lectin- and immuno-histochemical methods.

Anti Lewis X antibody stained astrocytes and medial fibers in the cerebrum, glanular cell layer of the cerebellum, and basophil cells in anterior lobe of the hypophysis. Anti Le a, Le b and Le y antibodies stained basophil cells in anterior lobe of the pituitary. Anti A, B or H antibody stained the colloid material in the hypophysis. PSA lectin which is specific to methyl-D-mannopyranoside stained the cytoplasm of neurons in the cereberal cortex including the Hippocampus. GSA-IB4 lectin specific to galactose stained the cytoplasm of neurons in the brain stem. The β-amyloid-bodies recognized in aged brains werestained with UEA-I specific to fucose and GSA-IB4 lectin.

These results suggest that the glycoconjugates might be related to a biological function of human brain and the activation of Fuc-transferases in the brain is complex.

TWO GENETIC VARIANTS, ISEHARA-1 AND 2, OF α1-ANTICHYMOTRYPSIN

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Alpha 1-antichymotrypsin (ACT) is a human 64 kDa plasma protein. In addition to its protease inhibitory activities, ACT is involved in many physiological functions. In particular, it is revealed that this protein is present in senile plaque of Alzheimer's disease. ACT presents genetic polymorphism originating from several allelic products. Tsuda *et al.* reported two variants of Isehara-1 and 2 for this protein. ACT Isehara-1 is characterized by a nucleotide substitution of GTG (Val) from ATG (Met) at codon 389. On the other hand, ACT Isehara-2 is characterized by a deletion of two nucleotides (AA) from AAA (Lys) at codon 391, which shifts the reading frame and disturbs the secretion of the transcript from the producing cells. In this time, we analyzed the substitutions by PCR-SSCP (single strand comformation polymorphism). Methods: The following primers (5'-TTACTGAGAGCCCCACTGCATGAT-3' and 5'-CATAAGGCTGTGCTTGATGTA-3') were labeled by ^{32}P at the 5'-end. PCR was performed using the labeled primers by *Taq* polymerase for 30 rounds of temperature cycling. The denatured products were electrophoresed in 6% polyacrylamide gel and exposed onto X-ray film. Results and Discussion: The frequencies of Isehara-1 and 2 alleles were determined as 0.05 and 0.001, respectively, in a Japanese population. Furthermore, we are comparing the frequencies with those in the cases of cardiac sudden death in our department to sreach for their relationship to the disease.

Pi SUBTYPING WITH CAPILLARY ELECTROPHORESIS

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The capillary electrophoresis (CE) was developed for different separation applications. The CE involves a separation of charged and uncharged molecules (i.e. Proteins, Peptides, DNA) in a buffer filled capillary by the application of high voltage (30 kv). The aim was to find a method which is good for automatisation (you can applicate your samples with an autosampler), reproducible and fast.
Isoelectric focusing has been commonly performed using slap and tube gel electrophoresis. However, gel methods generally require tendious and time consuming gel preparation and staining procedures. In the capillary format, IEF can be run with or without supporting gel. For an isoelectric focusing in CE the substances to be separated are applied on to the capillary together with the ampholytes. The ampholytes will create the pH gradient within a short time on application of electric current. At the same time the substances are forced to move towards their isoelectric points within the pH gradient. The focused zones will then be mobilized electrokinetically by replacing one electrolyte at one end of the tube.
For our parternity testing we adapted the subtyping of Pi variants done by isoelectric focusing to the CE-system. As an additional step for the accurate determination of the Pi variants we enrich the Pi Protein by immunaffinity chromatography. For the evaluation of our Pi system we tested serum which Pi protein was previous determined by slapgel isoelectric focusing. We found a reproducible characteristic bandpattern for the Pi proteins. Data were collected on a PC workstation which allow comparison with unknown samples.
With the CE isoelectric focusing of the α-1 antitrypsin protein we have a reproducible method under defined conditions. The CE is a step forward to the standardization and automatisation of the protein analysis. We believe that CE-technology will be used for other forensic parameters in the future.

ABO GENOTYPING WITH A FLOURESCENSDETECTION SYSTEM

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The nucleotide sequences of the ABO gene were reported in 1990 (1), since then the direct genotyping of ABO bloodgroup system has been explored using the polymerase chain reaction (PCR) in conbination with digest of the PCR product with restriction enzymes (2) or with the use of sequence specific primers (SSP) based on several single base substitutions between A and B allels and the single nucleotide deletion in O allels (3).
We used the TaqMan™ System for the detection of the sequence specific products. The principal of the TaqMan™ System relays on the 5` nuclease activity of the Taq polymerase. An additional probe was added into the PCR reaction mix, which hybridisates within the amplified DNA. If there is an amplification the probe hybridisate to the amplified DNA. This partly doublestranded DNA will be degraded by the Taq polymerase. The probe is an oligonucleotide with both a reporter fluorescent dye and a quencher dye attached. While the probe is intact, the quencher greatly reduces the fluorescensce emited by the reporter dye by Förster resonance energy transfer through space. If the probe is degraded by the Taq Polymerase the reporter dye emits its characteristic fluorescence.
We developed a SSP PCR which consists of 5 single reactions with the corresponding probes for the ABO genotyping (including A_1 and A_2). The reaction conditions were the same for all 5 reactions, so we can test 18 samples on a 96 well plate at a time. The detection was done with an ABI 7200 PCR detection system.
The advantage of this system is that no gel is needed anymore, the samples are measured in the closed reaction tube. The danger of contamination by amplified DNA which causes false positive reactions is left out. The ABO type can be determined by computer out of the fluorescence data of the ABI 7200 PCR. In our opinion the fluorescense detection technology is a step forward to an automatisation and standartisation and in the safety of the PCR technology.

1 Yamamoto et.al, Nature 1990;229-33.
2 Lee et. al. J Forensic Sci 1992;37:1269-75

ANALYSIS OF ABO TYPES USING A PANEL OF SEQUENCE-SPECIFIC OLIGONUCLEOTIDE PROBES

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The ABO blood group was discovered at the beginning of this century by Landsteiner. Shortly thereafter, agglutination assays were developed that divided populations into 4 ABO phenotypes (A, B, O, and AB). Once it was understood that different forms of a glycosyltransferase were responsible for modifying the "H" antigen to form the "A" and "B" antigens, the gene for this enzyme was cloned and sequenced. Using this DNA sequence information, a number of groups developed PCR-based tests to distinguish the three common alleles. The most common approach to determine ABO type is to amplify regions in exons 6 and 7 of the glycosyltransferase gene and use restriction enzymes (typically KpnI and AluI) to digest the PCR products; distinct patterns are reported for the 6 ABO genotypes. This method is valuable for human identification purposes but we have found that the sequences at these two restriction enzyme sites are not sufficient to determine ABO blood type. We developed sequence-specific oligonucleotide (SSO) probes to these polymorphic restriction enzyme recognition sequences and to other polymorphic sites we discovered by DNA sequence analysis. We used this panel of SSO probes to type 689 unrelated individuals from 4 population groups. We were able to resolve most of the ambiguous and incorrect ABO types obtained by restriction enzyme analysis, but ~4.5% of the samples still had ambiguous types. We have performed DNA sequence analysis on over 250 samples, including all of the ambiguous types and numerous samples of each common ABO genotype. Based on these sequences, we have identified 25 ABO alleles.

EVALUATION AND APPLICATION OF HIGHLY INFORMATIVE AND SENSITIVE HLA-DRB1 - AND -DPB1- INNO-LIPA TYPING SYSTEMS IN FORENSIC PRACTICE

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Highly polymorphic HLA regions - DRB1 and DPB1 - have been analyzed by enzymatic amplification with biotinylated primers compared with reverse hybridization on membrane-based strips. This method is technically simple and reproducible by high sensitivity. It allows discrimination of 124 DRB1 and 61 DPB1 alleles. However, some heterozygous combinations might still yield an ambiguous reactivity pattern. The analytical sensitivity of this test was determined using a DNA titration series. In this study we report the usefulness of this polymorphic and sensitive system to solve forensic problems. We have investigated a wide range of case types including rape, incest or body identification, stain analysis of blood, hair and saliva samples, fingernails and identification of old dental pulpes. A selected series of cases will be demonstrated. The limited validity of this method in stain mixtures will be shown.

MVR ANALYSIS OF THE HRAS1 MINISATELLITE: A NEW POLYMORPHISM OF MEDICAL, ANTHROPOLOGICAL AND FORENSIC INTEREST

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The HRAS1 minisatellite locus presents two polymorphic sites within the 28 bp repeat units. Minisatellite variant repeat mapping by PCR (MVR-PCR) has been used to study internal variation among alleles. This has been complemented by an in-depth analysis of the PCR fragment lengths of these alleles. 13 different fragments ranging from 27 to 80 repeats were found in a sample of 80 healthy caucasian individuals from NW Spain. 18 MVR sequences could be observed, and all fragments were perfectly identifiable by their MVR code in the first 20 repeats. We present the allele frequencies and a population genetic analysis of the data. The extremely conservative arrays of repeats allow us to infer the theoretical origin of rare alleles from a major group of specific alleles. The HRAS1 minisatellite has been extensively studied due to its association with cancer. Our study also shows that it could be a useful model for evolutionary studies of minisatellites. The forensic usefulness of this new MVR system is also discussed.

SEPARATION OF MVRPCR LOCUS D1S8 BY CAPILLARY ELECTROPHORESIS

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MVR-PCR analysis showed great advantages for forensic application and human identification. However, the technique is based on conventional electrophoresis is tedious and time consuming. Almost twice the amount of labor is required to process one sample since two sets of sample is required. Several approaches such as silver staining and automated fluorescent detection have been attempted. The ultimate goal of this study is to separate D1S8 MVR-PCR by capillary electrophoresis. Beckman P/ACE 5510 coupled with Laser Induced Fluorescence (LIF) Detector and analysis conducted on Gold soft ware (Beckman). Data base for 102 unrelated Arab individuals were generated using conventional technique. Ten samples of the data base were randomly selected and annualized by CE showed identical mapping for the first thirteen repeats. Peak area used to determine homosigosity at particular code marker. Capillary electrophoresis show a great advantages in sensitivity and speed however further optimization is required to determine more code numbers for MVRPCR mapping.

AUTOMATED FLUORESCENT DETECTION OF FORWARD & REVERSE 4-STATE MVR-PCR: EXTENDED DISCRIMINATORY POWER OF DIGITAL DNA TYPING BY SIMULTANEOUS ASSAYS WITHIN MINISATELLITE VARIANT REPEATS AT D1S8 LOCUS.

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Digital minisatellite variant repeat (MVR) mapping by PCR has already been successfully performed on 3 hypervariable minisatellites including D1S8 and the degree of allelic variation is potentially unlimited. Tamaki et al used conventional methods on forward and reverse 4-state mapping at D1S8. Here we present the analysis of D1S8 using automated fluorescent detection techniques.

Methods: Blood samples and case stains were from casework. DNA was extracted using the chelex method and the MVR-PCR reaction was performed in 25µl volume in a PE 480 thermal cycler. The PCR products generated were fluorescently detected on a 6% denaturing polyacrylamide gel in 1X TBE with 7M urea (2500V, 38W power limiting for 8 hours). Product sizes were determined with the GS500 or GS2500 size standards using the 672 Genescan software.

Results: 4-state mapping at D1S8 detected 4 repeat types (E,e,Y and y), the repeats analysed were doubled, thereby doubling the discriminatory power. 4-state mapping was applied to both separated alleles and genomic DNA (here the diploid codes of both alleles superimposed were one of 10 possible coding states.Forward and reverse reactions were co-amplified in a single tube for each repeat type by labelling each of the flanking DNA primers with a different fluorescent dye and the products were simultaneously detected in a single lane. Results of 2 forensic cases previously analysed by STR typing corresponded with those analysed by the 4-state forward and/or reverse MVR-PCR.

Conclusions: We have shown that both the 4-state forward and reverse MVR-PCR works extremely well and complements other DNA typing techniques. Although the MVR-PCR method is not routinely used by the forensic community it may be extremely useful in the analysis of badly degraded DNA where STR or other currently used systems failed. The simultaneous co-amplification of 4-state forward and reverse MVR-PCR would remarkably increase the discriminatory power for the purpose of forensic DNA typing, human identification and kinship testing.

A MODEL SYSTEM IN YEAST FOR THE STUDY OF MUTATIONAL MECHANISMS OPERATING AT HUMAN MINISATELLITES

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Minisatellites are tandem repeat loci that frequently show allele-length hypervariability. New allele-lengths are generated by germline-specific recombination-based mechanisms which alter the repeat copy number. Internal structures of *de novo* mutant alleles show that mutation frequently involves complex rearrangements of repeat unit blocks between or within progenitor alleles. Although a wealth of data exists on allele length variability at human minisatellites as well as on the complexity of the structures generated by the mutational processes involved, the mechanistic details are still obscure.

To further investigate the recombination-based mechanisms responsible for changes in the internal structures of minisatellite alleles we have developed a model system in the yeast *Saccharomyces cerevisiae*. Transminisatellitic diploid yeast strains have been constructed which are heterozygous for different alleles of the human minisatellites MS1, MS32 or MS205 integrated in the vicinity of a meiotic recombination hot spot upstream of the *LEU2* locus in chromosome III.

Striking similarities between minisatellite mutation in yeast and human were observed at all minisatellites, including high mutation rates in meiosis resulting in an ascospore mutant frequency of up to 10% and complex rearrangements of repeat units. Tetrad analysis of MS1 mutations has shown that, with exceedingly few exceptions, mutant alleles occur in one of the four spores. Internal structures and flanking marker combinations confirmed that the mutants resulted from conversion.

REPEAT ARRAY VARIATION IN D7S22 ALLELES REVEALED BY ALLELE SPESIFIC MVR-PCR

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MVR-PCR technique have been used to study repeat variation in several hypervariable minisatellites. These studies have revealed polymorphic patterns of variant repeats giving information about evolution and mutation events in these minisatellites. The aim of this study was to apply MVR-PCR technique on D7S22 alleles to study repeat array structure in this minisatellite.

A C/G basepair substitution 54 bp upstream of the repeat array allowed an allele spesific MVR-PCR to be developed. Fluorochrome labelled Tag primers and typing on an ABI 373 permitted typing of several repeat variants in the same lane as well as length measurement of the fragments.

Selected alleles from different size groups and 54 C/G haplotype and six heterozygote parent-child samples with de novo mutations were analyzed. In small alleles (<2 Kb), all common 14 repeat alleles (14R) were similar, while other rare alleles showed differences in MVR-pattern within same repeat group. The MVR-pattern in the rare alleles suggests that they originate from a 14 R. Larger alleles revealed a different MVR-pattern than found in small alleles, but in alleles similar in size and with the same flanking haplotype the MVR-patterns were similar. In one of the de novo mutations the breakpoint was revealed, while the other mutants showed no change in the part of the repeat array investigated.

The results support earlier findings indicating a closer evolutionary relationship between alleles close in size and 54 C/G haplotype. In some rare small alleles repeat variants not present in the 14R allele were found, indicating an inter-allelic mechanism for the expansion. In several other minisatellite loci a polarity has been revealed with one end of the repeat array being stable and the other hypervariable. The low degree of MVR-variation revealed in larger D7S22 alleles together with a low detection of repeat array change in the D7S22 mutants investigated, indicate that this end of the repeat array is stable.

DETECTION OF A DE NOVO MUTATION IN THE GC SYSTEM BY DNA SEQUENCE ANALYSIS

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In the course of paternity testing an apparent father-child incompatibility was observed in the group-specific component (GC) system. Isoelectric focusing (IEF) of sera on polyacrylamide gel (pH gradient 4.5-5.4) followed by print immunofixation with a specific GC-antiserum revealed the following phenotypes: mother GC 2-1S, child GC 2-1A(variant), and alleged father GC 2-1S. In the child we observed an anodal GC variant. It was found neither in the mother nor in the putative father. The double band pattern of the variant was located only slightly cathodal to GC 1A7. A comparison with several reference samples showed no identity. Testing the 3 individuals in 18 further genetic marker systems (11 serological and 7 DNA marker) gave no exclusion in the fatherhood. Without the GC system a paternity probability of W = 99,9997% was calculated for this disputed case.

To elucidate the origin of the unknown GC variant we performed a DNA analysis. Genomic DNA was prepared from EDTA blood of the 3 persons. DNA sequence analysis of amplified PCR products obtained from exon 1 (GC-23), exon 8 (GC-283) and intron 8 (GC-I8) by single-strand conformation polymorphism (SSCP) showed no differences in the genotypes of the mother, child, and putative father. Also in exon 11, which contains two polymorphic sites at the codons for the positions 416 and 420, the sequence of the amplified DNA samples was identical by SSCP. Analysis of the coding region GC-103, a polymorphic site in exon 4, presented a point mutation in the child. A G-A transition resulting in a substitution of Gln (CAA) for Arg (CGA) at amino acid position 103 explains the pI shift of GC 1S to the anodal position detected by IEF. This mutation was not found in the parental DNA's, thus confirming a de novo mutation in the child's gene. According to the birth place of the child we designated this variant GC 1A7 berlin.

ALLELE FREQUENCY DISTRIBUTION OF D3S1358 AND FGA LOCI IN A SOUTHERN SPAIN POPULATION

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D3S1358 -TCTA $(TCTG)_{1-3}$ $(TCA)_n$ - and FGA - $(TTTC)_3$ TTTT TTCT $(CTTT)_n$ CTCC $(TTCC)_2$ - were analysed using an Applied Biosystems, ABI 310, equipment. Polpulation genetic data from Southern Spain, including Andalucia, Extremadura and Canary Islands inhabitants, are presented. DNA was obtained from non related individuals of routine casework, paternity testing and staff. Some validation parameters of the analytical technique have been calculated in order to establish the reproducibility and reliability of the genotyping procedure within and between runs.

FORENSIC VALIDATION, POPULATION DATA, AND AUTOMATED ALLELE DETECTION OF THE CO-AMPLIFIED STR LOCI D3S1358, VWA AND FGA

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To introduce the commercial triplex STR kit (AmpFlSTR blue) for identity testing we performed a population study (200 unrelated Germans) complemented by analysis of stains and mixtures using the A.L.F. DNA sequencer, and followed by automated allele detection.

D3S1358	frequency	VWA	frequency	FGA	frequency
11.2	0.3	14	7.8	18	1.8
13	0.5	15	10.0	19	6.8
14	14.3	16	23.3	20	12.0
15	25.0	17	27.8	21	16.3
16	23.3	18	20.8	22	22.0
17	18.5	19	9.5	22.2	1.3
18	17.5	20	1.0	23	17.5
19	0.5			23.2	0.5
20	0.3			24	11.5
				25	7.8
				25.2	0.3
				26	2.3
				28	0.3

No other alleles were detected. All alleles at the three loci were independent ($p = 0.07$, exact test). Combined exclusion chance was 0.9998 for random suspects and 0.954 for paternity cases. Successful typing was possible using a) DNA amounts down to 50 pg, b) mixtures (minor component was visible down to a ratio of 1:10), c) urine from doping controls, d) saliva, and e) hair roots. FGA clearly tended to produce lower peaks than the other systems.

PCR-NUCLEAR DNA POLYMORPHISMS IN AN ALPIN SWISS VILLAGE. COMPARAISON WITH EUROPEAN POPULATIONS.

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The chain of mountains of the Alps determine the presence of valleys with a geological configuration that allowed the isolation of populations established in its. The village of Isérables -about 1000 inhabitants- remained isolated till these recent years because of its particular geographical situation.

Blood samples from about one hundred people in the village (all descendants of eleven original families) were analysed in order to establish the DNA-PCR profil for the following systems : HLA-DQalpha, LDLR, GYPA, HBGG, D7S8, GC, D1S80, HUMTH01, HUMFES/FPS, HUMVWA31A, SE33, HUMCD4 and HUMF13B.

No significant deviation from Hardy-Weinberg equilibrium could be detected excepted for D7S8 system.

Data from Isérables were compared with those from Lausanne region (South-western part of Switzerland), German and Andalousian (Southern Spain) populations. Heterozygosity in the Isérables sample was lower than in the three others. Genetic distance between the four populations and statistical differences in allele frequency were evaluated.

ALLELE FREQUENCIES OF THE STR LOCUS HUMFGA IN AN ITALIAN POPULATION.

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The HumFGA short tandem repeat locus (GenBank M64982), located in the third intron of the human alpha fibrinogen gene at the chromosomal position 4q28, was first investigated by Mills et al. (1992).
A collaborative research on the polymorphism of this system was carried out by the Instituts of Legal Medicine in four Italian regions (Marches, Veneto, Tuscany and Lombardy). The aim was to establish a database in view of HumFGA application in forensic identification and paternity testing. The goal for each participating laboratory was to study at least 100 genotypes of unrelated, locally residing individuals.
After DNA extraction, PCR was carried out using the amplification conditions proposed by Barber et al. (1996); the alleles were identified by two separation protocols (vertical or horizontal polyacrylamide gel electrophoresis), followed by silver staining. The alleles were typed by side-to-side comparison with a ladder consisting of a mix of sequenced amplified products.
The most frequent alleles found were FGA*20, FGA*21, FGA*22, FGA*23, FGA*24; the remaining ones showed intermediate or very low frequencies (< 1%). 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.
The allele variants were sequenced by Taq-cycle-sequencing using dye terminator labeling and capillary gel electrophoresis (CGE) on an ABI Prism 310 (Applied Biosystem).

Barber M.D., McKeown B.J., Parkin B.H. (1996), Int J Legal Med, 108:180-185
Mills K.A., Even D., Murray J.C. (1992), Int J Legal Med, 106:319-323

GENETIC STUDIES OF THE NUCLEOTIDE REPEAT CYP19

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The genetic polymorphism of a tetranucleotide repeat $(TTTA)_n$ at the human Aromatase Citochrome P-450 gene (CYP19) was analysed via PCR. Separation of the molecular products was carried out by means of thin-layer polyacrylamide gel electrophoresis, in native and denaturing gels. The results were concordant in both methods.

Genetic studies in family groups were in agreement with a codominant way of inheritance for the alleles of this STR. A population study in 260 individuals from the Galician population displayed 8 allele variants, ranging from 154 bp to 182 bp, exhibiting a value of H= 0.713 and PIC= 0.662. The distribution of observed and expected phenotype frequencies fits with Hardy-Weinberg equilibrium, after both the conventional χ^2 and exact tests.

TWO STRs IN A POPULATION OF NORTHERN ITALY: PHENOTYPES AND ALLELE FREQUENCIES

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PCR typing of STRs is considered a powerful tool in forensic medicine; however, before routine application of these genetic markers in identification and parentage testing, it is necessary to have some data-base about different populations.
To this end, in order to promote knowledge of the allele frequencies distribution in the Italian population and to realize an own data-base, we have analyzed the STR systems CSF1PO (5q33.5 - p34) and HPRTB (Xq26) by using Gene print STR kits by Promega Corporation.
DNA was obtained from whole blood samples (about 120 unrelated individuals for each marker) according to standard methods (proteinase K digestion , pCIA extraction and ethanol precipitation). Amplification was performed following the manufacturer's recommended protocol; electrophoresis was carried out on 6% denaturing polyacryamide gel and the bands were visualized by silver staining.
The observed allele distribution had been compared with the expected one according to Hardy-Weinberg equilibrium, by means of the traditional X^2 statistic.and with data obtained from other population.

FRENCH POPULATION DATA OF FIVE VNTR LOCI

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Allele sizes and allele frequencies were studied in a population sample of 150 unrelated individuals living in the area of Paris. DNA was restricted with HinfI, hybridized to five alkaline phosphatase labeled probes: MS1 (locus D1S7), MS31 (locus D7S21), MS43a (locus D12S11), g3 (locus D7S22) and MS205 (locus D16S309) and VNTR alleles detected according to the NICE (Non Isotopic Chemiluminescent Enhanced) protocol (Cellmark). Lumigraphs were analyzed using Bioimage equipment.
Results are sumarized as follows:

probe	Hetero-zygosity %	no.of resolved alleles	common allele size (kb)	common allele frequency	Probability of match*
MS1	98.3	101	3.1	0.030	5.5^{-4}
MS31	95.9	71	6.6	0.044	3.2^{-3}
MS43a	97.96	90	8.9	0.044	8.2^{-4}
g3	95.86	94	3.2	0.058	3.3^{-3}
MS205	98.00	35	2.8	0.120	7.9^{-4}

*probability of 2 unrelated individuals possessing the same genotype

The expected combined chance association of the five loci is 3.937^{-15}
Minor differences were observed when comparing our results to frequency histograms of neighbourhood populations.

POPULATION GENETICS OF 14 STRS: vWA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820, D16S539, F13A01, FESFPS, F13B, LPL, D3S1358 AND FGA IN THE POMERANIA-KUJAWY REGION OF POLAND.

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We have examined the allele distribution at fourteen tetranucleotide STR loci: vWA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820, D16S539, F13A01, FESFPS, F13B, LPL, D3S1358 and FGA in a population sample of over 200 Polish Caucasians.

Blood samples were obtained from healthy unrelated individuals from the Pomerania-Kujawy Region of Poland. DNA was extracted from blood according to standard "salting out" procedure. For DNA amplification and typing we have used three higly discriminating multiplex systems: *GenePrint* PowerPlex Fluorescent STR System which allows co-amplification and two-color detection of eight STR loci: vWA, TH01, TPOX, CSF1PO, and D5S818, D13S317, D7S820, D16S539; *GenePrint* FFFL multiplex from Promega (F13A01, FESFPS, F13B, LPL) and AmpF*l*STR Blue from Perkin Elmer (D3S1358, vWA and FGA . Amplified fragments were separated by electrophoresis in a 5% denaturing polyacrylamide gel using the ABI Prism 377 DNA Sequencer. Fluorescent Ladder CXR 60-400 Bases (Promega) was included in each lane as an internal standard. Allele size determination and genotyping were performed using ABI Prism GeneScan Analysis 2.1 and ABI Prism Genotyper 2.0 software by comparison of amplified fragments with internal size standards and allelic ladders.

We have estimated allele frequency for each STR locus, its heterozygosity, accordance with Hardy Weinberg Equilibrium and both single locus and combined power of exclusion. In conclusion, higly dscriminating multiplex STR systems we have used in our population study turn out to be very efficient tools in both paternity determination and forensic identification

SEQUENCING AND POPULATION DATA OF A SHORT TANDEM REPEAT LOCUS IN THE HUMAN ALPHA FIBRINOGEN GENE (HUMFGA) IN AN AUSTRIAN POPULATION SAMPLE

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The alleles of the short tandem repeat polymorphism in the third intron of the human alpha fibrinogen locus (HumFGA, 4q28, GenBank accession number M64982)[1] were amplified by PCR. Until now 16 different alleles have been distinguished in a sample of 213 unrelated individuals of the Vienna region by their different electrophoretic mobility in native polyacrylamide gels and subsequent silver staining. The alleles were assigned by comparison to an allelic ladder, which contains the most common alleles. The following frequencies have been observed:

A* 16	0,002	A* 22.2	0,005
A* 17	0,002	A* 23	0,120
A* 18	0,007	A* 23.2	0,005
A* 19	0,066	A* 24	0,131
A* 20	0,127	A* 24.2	0,002
A* 21	0,225	A* 25	0,075
A* 21.2	0,005	A* 26	0,023
A* 22	0,200	A* 27	0,005

The several alleles sequenced up to now were found to be in absolute agreement with previously published data[2].

References:
1. Mills KA, Even D, Murray JC (1992) Hum Mol Genet 1:779
2. Barber MD, McKeown BJ, Parkin BH (1996) Int J Legal Med 108: 180-185

TPOX, VWA31/A, HUMTH01, CYP19, D5S373, D8S323, D8S344, D8S345: an STR database for a West African population.

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Given the scarce number of studies carried out of genetics markers in African populations, an analysis of 8 STRs was carried out in a West African population (Cabo Verde), in order to establish a database for population and forensic studies.

Following phenol/chloroform extraction, the molecular phenotypes were analysed by thin-layer polyacrylamide gel electrophoresis and then by non-radiactive detection (silver staining).

The PIC values obtained were as follows: 0.7274 for TPOX, 0.7815 for VWA31/A, 0.7588 for HUMTH01, 0.6649 for CYP19, 0.7040 for D5S373, 0.3818 for D8S323, 0.6202 for D8S344 and 0.5422 for D8S345.

The distribution of observed phenotypes fit with those expected under the Hardy-Weinberg hypothesis for each of the STRs.

Phenotype expression of the 8 STRs was performed after estimating pairwise linkage disequilibrium by means of an exact test (based on Markov's chains). No statistical evidence of association between the loci was observed in any of the combinations.

Population comparisons were carried out in order to assess the biological significance of this database. These were based on the distribution of allele frequencies for these STRs, especially with data from other African and Afro-American populations so far reported up to now.

DNA-FINGERPRINTING WITH MULTI-LOCI PROBES MZ1.3, 33.15 AND CAC/GTG5 , THE FREQUENCIES OF POLYMORPHIC FRAGMENTS IN THE SAMPLE OF POLISH POPULATION

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The results of DNA-fingerprinting using MLP probe MZ1.3 with restriction en -zyme haeIII /325 cases/,hinfI /511 cases/ and rsaI /785 cases/were presented and compared with results obtained by other polish authors. The distribution of the bands were similar in all tested samples of Polish population /Central Poland, Southeast and Southwest Poland/. Also results of DNA finger-printing with MLP CAC/GTG5 probe with restriction enzyme haeIII /73 cases/ and hinf I /342 cases/and 33.15 with restriction enzyme haeIII /133 cases/ and hinfI /214 cases/ were presented. The band frequencies were culated and compared with obtained by some other authors. The results are similar - in all population samples and in all multi - loci probes frequency of heavy fragments are low and frequencies of light fragments increased, with maximum about 5.5 KB. This is an example of obtained results: /MLP probe MZ1.3 with restriction enzyme rsaI/

/Bp./	Number	/%/	/Bp./	Number	/%/
4000-5000	35	4.46	15001-16000	10	1.27
5001-6000	221	28.15	16001-17000	4	0.51
6001-7000	101	12.87	17001-18000	8	1.02
7001-8000	18	2.29	18001-19000	13	1.66
8001-9000	70	8.92	19001-20000	6	0.76
9001-10000	47	5.99	20001-21000	4	0.51
10001-11000	106	13.50	21001-22000	3	0.38
11001-12000	79	10.06	22001-23000	0	0.00
12001-13000	32	4.08	23001-24000	6	0.76
13001-14000	6	0.76	Over 24001	0	0.00
14001-15000	16	2.04	TOTAL:	785	100.00

Key terms: DNA-fingerprinting,MLP,MZ1.3,33.15,CAC/GTG5

ALLELE FREQUENCIES OF FOUR STR POLYMORPHISMS IN AN ITALIAN POPULATION SAMPLE

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Four polymorphic microsatellites (STRs) have been recently introduced in the routine of our laboratory, with a view to use them in forensic casework. The loci (CD4, D3S1358, TPOX, CSF1PO) map at various autosomal chromosomes and are suitable candidates as first-choice systems for paternity investigations and criminal evidentiary analysis. We here report data on the allele distribution at these systems from a sample of over 100 unrelated volunteer donors from Italy. Standard PCR protocols were adopted, with minor modifications. Alleles were detected in polyacrylamide denaturing gels using a automatic laser fluorescent DNA sequencer. Two of the systems (TPOX and CSF1PO) were coamplified and typed simultaneously. No mutant nor a significant deviation from Hardy-Weinberg equilibrium were found.

CD4	allele	5	6	7	9	10	11	12
	frequency	.33	.31	.01	.01	.30	.20	.01
D3S1558	allele	1	2	3	4	5	6	7
	frequency	.06	.22	.24	.26	.17	.01	-
TPOX	allele	8	9	10	11	12	13	14
	frequency	.51	.11	.09	.25	.02	-	-
CSF1PO	allele	7	8	9	10	11	12	13
	frequency	.01	.03	.23	.31	.29	.09	.01

ALLELE FREQUENCIES DISTRIBUTION OF THREE STRs LOCI (F13B, CSF1PO, CD4) IN TUSCANY (ITALY)

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We present the allele frequency distributions of three STRs loci, calculated from a sample of 100 unrelated healthy individuals born in Tuscany.
DNA was extracted using phenol-chloroform technique. After PCR, the amplified alleles were separated in vertical native (CD4) or denaturating (F13B, CSF1PO) polyacrilamide gels and visualized by silver staining.
The allele frequencies, showed in the table, were consistent with those previously reported in other Caucasian populations. No significant deviation from HW equilibrium was found in our sample.

CD4	n=100	F13B	n=100	CSF1PO	n=100
5	.355	6	.060	9	.025
6	.310	7	.015	10	.260
10	.275	8	.315	11	.275
11	.030	9	.150	12	.330
12	.020	10	.460	13	.105
Rare	.010			Rare	.005

NEW ALLELES OF D12S391 STR LOCUS

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Short tandem repeat (STR) loci analysis is commonly used for DNA profiling in individual identification. D12S391 PCR products were detected by an ALF Pharmacia automated sequencer. Allele sizes and allele frequencies were determined in a Portuguese population sample of 109 unrelated individuals from paternity investigation. Allele frequencies were compared with other published populations.
New alleles of D12S391 locus were detected and caractherized. The discriminating power obtained with this system make it a considerably interesting one for forensic purposes.

ALLELE FREQUENCIES (HUMvWA; HUMTHO1 AND HUMFES/FPS) IN A POPULATION SAMPLE OF SOUTH-WEST-GERMANY

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Short Tandem Repeat (STR) polymorphisms provide an important tool for forensic casework, human identification, paternity analysis and genetic mapping. STR loci consist of tandemly repeated short core units (2-6 base pairs) and exhibit a high degree of length polymorphism due to variation in the number of repeat units.
In the present study we describe the frequency distribution of the STR loci vWA, THO1 and FES/FPS within a population sample of more than 500 unrelated individuals living in south-west Germany.
DNA was isolated by standard methods: proteinase K digestion, phenol-chloroform extraction and ethanol precipitation or by the Chelex extraction method. All loci were amplified according to standard protocols. Polymerase chain reaction (PCR) fragments were separated by high resolution native polyacrylamide vertical gel electrophoresis and silver stained. The genotype frequencies we found are in accordance with Hardy-Weinberg expectations.

ALLELE FREQUENCY DISTRIBUTION OF 7 STR LOCI IN THE BASQUE COUNTRY AUTOCHTHONOUS POPULATION

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Short tandem repeat (STR) loci are highly polymorphic markers composed of repetitive sequence elements of 2-7 base pairs in lenght which are amenable to analysis by the polymerase chain reaction (PCR). These polymorphisms provide an important tool in forensic casework for human identification, paternity analysis.

Before a new marker system can be introduced in routine casework, a population database for the relevant population must be established for statistical evaluation of forensic evidence. In conformity with this guideline, we analyzed the STR systems HUMTH01, HUMTPOX, HUMCSF1PO, HUMVWA, HUMFES/FPS, HUMF13A01 and HUMF13B in a Basque Country autochthonous population sample (n=202-208).

DNA was extracted by the standard phenol/chloroform extraction procedure. PCR amplification was performed according to the manufacturer's recommendations using *GenePrint*™ STR Systems (Promega Corporation). The electrophoresis was carried out on denaturing polyacrylamide vertical gels and the bands were visualized by silver staining.

This study provides allele frequency data, heterozygosity rates and other parameters of forensic importance (mean exclusion probability, polymorphism information content and discrimination power). In all loci, the genotype frequencies are in accordance with Hardy-Weinberg expectations (e.g. χ^2 test, likelihood ratio test and exact test).

The 7 investigated polymorphisms systems are reliable and sensitive marker systems that are well suited for use in forensic casework and paternity testing.

DUPLEX STR ANALYSIS OF D19S253 AND D18S51 IN A PORTUGUESE POPULATION

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DNA profiling in paternity investigation in our laboratory is based on the analysis for short tandem repeat (STR) loci using PCR. Selection for use in a forensic analysis depends on discriminating power and the ability to coamplify with other loci. Recently, we have introduced a duplex STR system for routine paternity investigation using D19S253 and D18S51. Detection of PCR products was undertaken on an ALF Pharmacia automated sequencer.
A population database of these two loci D19S253 and D18S51 was established in unrelated individuals (n=112) from a Portuguese population. Allele frequencies were studied for each of these loci. New alleles in D19S253 locus were caractherized.
Due to discriminating power this duplex analysis is very useful in paternity investigation and individual identification.

SARDINIAN POPULATION DATA ON STR LOCUS HUMTHO1

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A population study on the STR locus HUMTHO1 was carried out in a sample of 103 unrelated Sardinian individuals. The locus was amplified according to the method of promega STR HUMTH01 system.
Electrophoresis of the amplified products was carried out in 6% denaturing polyacrylamide gels at costant power of 37W at ambient temperature.
The allelic size were detected by silver stain method and genotypes determined by comparison with the allelic ladder of the Promega STR HUMTHO1 system.
A total of 6 alleles could be found to be identified in this study. Allele 9 was found to be the most common with a frequency of 0.305. The most common genotype found was 6-9 with a frequency of 0.20. The observed and expected heterozigosities were 0.7759 and 0.8135. No significant deviation from Hardy- Weinberg equilibrium could be found in this Sardinian population sample.
This study demonstrate that HUMTH01 locus can be used for paternity testing and individual identification in the Sardinian population.

AUSTRIAN POPULATION DATA ON THE POLYMORPHIC STR LOCI DYS19 AND HUMF13B

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The STR polymorphisms DYS19 (GATA)n and HumF13B (ATTT)n were analysed in unrelated, healthy individuals of the Vienna region by PCR amplification and detection on 6% native polyacrylamide gels employing silver staining. Sequencing of 25 alleles (DYS19) and 13 alleles (HumF13B) revealed results which were in absolut agreement with the previously published data[1,2]. Alleles 10C and 11 of HumF13B could not be discriminated unambiguously and were therefore grouped together.
The resulting allele frequencies are listed in the following table.

	HumF13B	DYS19
A*6	0.089	
A*7	0.013	
A*8	0.242	
A*9	0.195	
A*10	0.457	0.081
A*11	0.004	0.421
A*12	0.000	0.222
A*13		0.213
A*14		0.063

No deviations from Hardy-Weinberg equilibrium could be detected for DYS19 (n= 221 individuals) and HumF13B (n=549 individuals).

References:
[1] Roewer et al. 1992, Hum.Genet. 89: 389-394
[2] Alper et al. 1995, Int.J.Legal.Med. 108: 93-95

GENETIC PROFILING OF S.MIGUEL ISLAND (AZORES)

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In this work we present data on three STR polymorphisms (VWA31/A, CD4 and TH01) in the major island (S. Miguel) of the Azores archipelago. The Azores archipelago was discovered in the XV century and it was populated since then by a mixture of settlers, of which Portuguese were the most important.
DNA was extracted from blood samples by the Chelex method and amplified by PCR. Genotyping was performed in non-denaturing PAGE. Data obtained up to now can be summarised as follows (N: nr. individuals):

allele frequencies (%)

	5	6	7	8	9	10	11	12	N
CD4	40.0	23.1	0.0	0.6	0.0	31.9	3.1	1.3	80

allele frequencies (%)

	6	7	8	9	9.3 + 10	N
TH01	21.6	18.1	13.7	16.2	30.4	102

allele frequencies (%)

	14	15	16	17	18	19	20	N
VWA	10.3	11.9	26.6	26.6	17.9	6.3	0.4	126

All systems are in Hardy Weinberg equilibrium and estimated gene frequencies are not significantly different from those reported for mainland Portugal.

DISTRIBUTION OF FXIIIA SUBTYPES IN JAPANESE AND IN CHINESE

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FXIIIA polymorphism was investigated by isoelectric focusing using 3 different carrier ampholytes. EDTA plasma samples were collected from 620 unrelated Japanese and 157 unrelated Chinese individuals. Isoelectric focusing was performed on polyacrylamide gel containing ampholytes pH 4-6.5, pH 5-6 and pH 5-8 (2:2:1). After run, the focused proteins were transferred onto nitrocellulose membrane. The membrane was incubated with rabbit anti-human FXIIIA serum and goat anti-rabbit IgG serum conjugated with alkaline phosphatase, and then stained with β-naphthyl phosphate and Fast Blue BB salt. With this technique, 10 common subtypes with FXIIIA*1A, FXIIIA*1B, FXIIIA*2A and FXIIIA*2B and a variant type 4-1B were clearly and reliably identified. There was no significant difference of the phenotypic distribution between Japanese and Chinese. In both populations, the data fitted the Hardy-Weinberg equilibrium. It is anthropologically remarkable that the FXIIIA*1A frequencies in Japanese and in Chinese are higher than those in other racial groups whereas the FXIIIA*2B frequencies in Japanese and in Chinese are lower.

A STUDY ON FIVE SHORT TANDEM REPEAT SYSTEMS IN A YEMENIAN POPULATION SAMPLE

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The STRs HumVWA, Hum F13B, HumTH01, HumCD4 and FGA were amplified by the polymerase chain reaction on blood samples from 100 unrelated Yemenians. The samples were analyzed by native horizontal discontinual electrophoresis. The mean exclusion chances were 0.636, (VWA), 0.490 (FXIIIB), 0.587 (Th01), 0.528 (CD4), and 0.733 (FGA), the discriminating powers were 0.939, 0.904, 0.922, 0.905, and 0.964,and the heterozygosity rates were 0.84, 0.81, 0.77, 0.65, and 0.83. No deviations from Hardy Weinberg expectations were found, but significant differences between the Arab population studied herein and an European population from Austria were found for all STRs.

VWA	
14	0.060
15	0.080
16	0.195
17	0.335
18	0.180
19	0.100
20	0.030
21	0.010

TH01	
6	0.290
7	0.185
8	0.125
9	0.235
9.3	0.160
10	0.005

FXIIIB	
6	0.140
7	0.045
8	0.210
9	0.320
10	0.280
11	0.005

CD4	
5	0.300
6	0.280
7	0.010
8	0.025
9	0.000
10	0.270
11	0.105
12	0.010

FGA	
17	0.005
18	0.025
19	0.020
20	0.075
21	0.135
21.2	0.005
22	0.200
22.2	0.005
23	0.150
24	0.225
25	0.085
26	0.055
27	0.010
28	0.005

ALLELE FREQUENCIES AND SEQUENCE STUDIES OF A HIGHLY VARIABLE SHORT TANDEM REPEAT AT THE D17S976 LOCUS

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The STR locus in D17S976 was amplified and typed in 158 unrelated Austrian Caucasians. PCR conditions were optimized at 94°-1 min, 66°-1 min, 72°-2 min for 6 cycles followed by 93°-1 min, 65°-1 min, and 72°-2 min for another 6 cycles and 93°-1 min-63°-1 min, and 72°-2 min for 18 cycles. Using this protocol it was possible to type 100 pg of K562 cell line DNA. Resolution was achieved using 5% gels in horizontal native polyacrylamide gel electrophoresis. The mean exclusion chance was 0.792, the discriminating power was 0.980, and the observed heterozygosity rate was 0.873. No deviations from Hardy-Weinberg equilibrium were observed. A sequenced allelic ladder consisting of 14 alleles (236-288 bp) was constructed. Sequence analysis revealed that the locus comprises 3 different repeat motifs: ATCA, ATCT, and ACCT, all of which vary in number between alleles. The aggregate number of the 3 tetrameric repeat types was used for allele designation. Since a repeat with a singlebase deletion (ATC) was found in both the smallest and the largest alleles, a ".3" was added to the allele designation in those cases. Therefore the smallest allele is designated 19.3, and the largest allele is designated 32.3. In conclusion the locus D17S976 proved to be valuable for forensic purposes due to its high polymorphicity and sensitivity, and its satisfactory electrophoretic resolution even on norizontal gels.

POPULATION GENETIC STUDY FOR THE STR LOCI D21S11 AND HUMFIBRA ANALYSED BY USING A FLUORESCENT BASED CO-AMPLIFICATION AND AUTOMATED DETECTION

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Up to now, numerous Hungarian Caucasian databases have already been generated regarding different conventional and DNA polymorphisms. To the best of our knowledge no Hungarian data concerning the STR loci HUMFIBRA and D21S11 were available so far. Therefore, in this work population genetic data were generated for the STR systems HUMFIBRA and D21S11 in a Hungarian Caucasian population sample, residing in Baranya County, Hungary (127 unrelated individuals).
The loci were co-amplified (duplex PCR) using a fluorescenct based PCR method and were typed automatically in the SQ-5500-S DNA Sequencer (Hitachi Electronics Engineering, Japan).
Twelve different alleles for both loci could be found including some " .2" types. Those alleles (22.2, 23.2 in HUMFIBRA and 30.2; 31.2; 32.2; 33.2; 34.2 in D21S11) differ by two base pairs compared with the regular four bp length tandem repeats. The heterozygosity rates were found to be very high (0.905 and 0.819 for HUMFIBRA and D21S11, respectively). No deviations from Hardy-Weinberg expectations were observed using the homozygosity, the likelihood ratio, and the exact tests.
Both loci proved to be highly discriminating, valuable polymorphisms for forensic analyses (PD scores were over 0.95 and PIC values over 0.70). The fluorescent based coamplification followed by an automated typing provides an accurate way for simultaneous analysis of HUMFIBRA and D21S11 DNA STR polymorphisms. The allele frequency distributions for these STR systems can be used to estimate DNA profiles in Hungarian Caucasian population.

ALLELE FREQUENCIES OF D3S1358 AND FGA IN A CENTRAL SPANISH POPULATION

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Population genetics studies were carried out on 214 Caucasians from Central Spain using the commercial Perkin-Elmer-AmpFlSTR blue PCR Amplification Kit (D3S1358, vWA AND FGA) followed by an automated analysis of fluorescent amplified DNA fragments with an Applied Biosystems 377 sequencer and Genescan 2.1 analysis software. Fragment size determination is easily accomplished by using an internal size standard (GS 350 Rox) and two allelic ladders by gel which contains the most common alleles.

This study provides allele and genotype frequencies for two tetrameric short tandem repeat (STR) loci: D3S1358 and FGA, and allowed to confirm frequencies for vWA that were previously analyzed by manual typing. For D3S1358, 7 alleles were found with frequencies in the range 0.0070 (allele 13) - 0.2664 (allele 15). The observed heterocygosity, discrimination power and polymorphism information content were 0.7944, 0.9229 and 0.7627, respectively. For FGA, 11 alleles were found with frequencies in the range 0.0023 (alleles 22.2 and 27) - 0.1869 (allele 22). The observed heterocygosity, discrimination power and polymorphism information content were 0.8458, 0.9599 and 0.8368, respectively. Two loci meet Hardy-Weinberg expectations (e.g. likelihood ratio test and exact test). Allele frequency distributions, heterozygosity rates and other parameters of forensic importance are similar to those found in other Caucasians populations. Distribution of observed allele frequencies for the two PCR systems indicated that these STR loci may be very useful in routine paternity testing and forensic identification.

PYRENEAN POPULATION DATA ON 3 TETRAMERIC SHORT TANDEM REPEAT LOCI- HUMTH01, TPOX AND CSF1PO-DERIVED USING A STR MULTIPLEX SYSTEM

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PURPOSE : In order to use genetic loci in forensic identity testing some population data are needed. This paper presents a report of allele/genotype frequency data for the loci HUMTH01, TPOX and CSF1PO in a Pyrenean population from Spain (n=103).

METHODS : Allele and genotype frequencies for the 3 tetrameric short tandem repeat loci were determined using GenePrint STR Multiplex System, electrophoresis of the PCR products in denaturing polyacrilamide gels and subsequent detection of allelic fragments by silver staining.

RESULTS : All the loci met Hardy-Weinberg expectations. Futhermore, there was little evidence for departures from expectation of independence between loci within the sample population. There were also obtained some statistics of medico-legal interest such as the allelic diversity value (80% for HUMTH01; 64% for TPOX ; 73% for CSF1P0) and the chance of exclusion in paternity cases (95% for HUMTH01; 87% for TPOX ; 92% for CSF1P0). An additional aim was to compare the results obtained with other population data. Tests for homogeneity were carried out between the Pyrenean population and other Spanish and Caucasian populations.

CONCLUSIONS : Thus the allele frequency data can be used in identity testing to estimate the frequency of a multiple PCR-based profile in this Spanish population.

D3S1358 AND D8S1179: ANALYSIS AND ALLEL FREQUENCIES IN A SOUTH GERMAN POPULATION

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STRs are used in forensic identification as well as in parentage testing due to their high grade of polymorphism.
We point out two powerful STR-systems, D3S1358 and D8S1179.
Analysis was realized both with electrophoretical separation of the PCR-products on polyacrylamid gels followed by silverstaining and automatically with the ABI 310 Genetic Analyzer.
Additionally we describe the preparation of the allelic ladders and present allele frequencies revealed in SW-Germany.
A comparison with other Caucasian populations showed no significant differences.

ALLELE FREQUENCIES AND SEQUENCE DATA OF THE STR LOCUS D11S554 IN A JAPANESE POPULATION

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A population study on the short tandem repeat (STR) locus D11S554 was carried out in a sample of 360 unrelated Japanese individuals living in Gifu Prefecture (central region of Japan).
DNA was extracted from whole blood by the phenol-chloroform method. PCR amplification of the locus was carried out according to the method of Phromchotikul et al. (1992). The amplified products were separated in 6% denaturing polyacrylamide gels and visualized by silver staining. Alleles were determined by comparison with the allelic ladders composed of D11S554 alleles sequenced in our laboratory. Sequencing was carried out using Thermo Sequenase core sequencing kit with 7-deaza-dGTP (Amersham). The PCR primer labelled with Texas Red was used as sequencing primer. Sequence data were analyzed on a SQ-5500-S DNA Sequencer (Hitachi Electronics Engineering).
A total of 39 alleles could be identified in the present study. No significant deviation from Hardy-Weinberg equilibrium was observed. The sequence data revealed that the repeat region was compound in structure, as already reported by Adams et al. (1993). Sequence variation was found at several alleles.
The results of the present study demonstrate that D11S554 is a useful genetic marker for paternity testing and individual identification in the Japanese population.

DETECTION OF VNTR AND SEQUENCE VARIANTS OF THE HUMAN DOPAMINE D4 RECEPTOR GENE (DRD4) IN THE JAPANESE AND MONGOLIAN POPULATION

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Amplifiable length polymorphism (AmpFLP) genotyping and direct sequencing of a hypervariable segment in the human dopamine D4 receptor (DRD4) gene were performed from 100 each unrelated Japanese and Mongolian donors. The 4-repeat allele (314 bp) was the most prevalent and appeared in each population with a frequency ranging from 0.82 to 0.88, respectively. Chi-square test for Hardy-Weinberg equilibrium at the DRD4 locus showed no significant deviation between expected and observed values, and also the homogeneity test of distribution between the data from Japanese and Mongolian subjects showed no significant deviation by the observed frequencies of genotypes, but sequence variants were found according to the result of sequence analysis of 48-bp unit of allele with 4 imperfect tandem repeats. These results suggest that there could be racial differences in the specific nucleotide change within repeat sequence because some alleles with same number of repeat units have different sequence variation.

COMPLEX STRS (ACTBP2, D21S11 AND FIBRA/FGA) IN TWO SPANISH POPULATIONS: NEW INTERMEDIATE ALLELES AND POPULATION GENETIC PECULIARITIES IN ACTBP2

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Population genetic data from two different Spanish populations was obtained for the three hypervariable STRs ACTBP2 (SE33), D21S11 and FIBRA/FGA. No significative differences were observed for D21S11 and FIBRA/FGA with other caucasians. A nomenclature based on the real bp number is proposed for typing the ACTBP2 locus in which a total of 9 new alleles (types-242, 246, 250, 272, 282, 292, 300 and 307) were reported. In addition other alleles found in the Catalonian population and not detected in other European populations were found. Application of interpretative guidelines (±0.5bp rule and the shift calculation) for detecting intermediate alleles isuseful but it is has problems in detecting intermediate alleles in the gaps of the already existing ladders. The ladder was therefore improved by including sequenced alleles (found in our population study) in the gaps.
In addition to the polymorphism and the corresponding discrimination power of complex STRs, ACTBP seems to be of particular interest for population genetic purposes, due to the population differences observed, in spite of the relatively high mutation rates expected for this system.

HumTH01, HumvWA31A, and 3'ApoB VNTR-PCR LOCI FREQUENCIES STUDIED IN SAHARAOUI SAMPLE POPULATION (WEST SAHARA NATIVE POPULATION).

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The purposes of this study were to report allele and genotype frequencies data in Saharaoui (Aium and Dadla population sample n=103) for a three VNTR polymorphisms (3'ApoB, HumTH01 and HumvWA31A). DNA were extracted from root hair samples by chelex method. PCR singleplex amplifications were accomplished with fluorescein labelled primers, and genotypes were analysed in denaturing 6% polyacrilamide gel electrophoresis, using automated laser fluorescence for STR loci (HumTH01, and HumvWA31A). 3'ApoB minisatellite locus was carried out routinely by PCR amplification and ethidium bromide 3% agarose gel electrophoresis. Allele and genotype frequencies have been determined. The following allele frequency ranges were found:

3'ApoB 13 alleles with frequencies ranging between 0.006 and 0.32. Discrimination power (DP): 0.90
HumTH01 6 alleles with frequencies ranging between 0.03 and 0.28. DP: 0.92
HumvWA31A 6 alleles with frequencies ranging between 0.08 and 0.31. DP: 0.93

For all markers the observed genotype frequencies are in good agreement with the expected distribution under the Hardy-Weimberg law. (Exact-test).

The allelic frequencies, compared to other populations shows a high degree of variation between Saharaoui sample and other populations studied.

ANNOBON ISLAND POPULATION (EQUATORIAL GUINEA) CHARACTERISED BY FIVE VNTR-PCR POLYMORPHISMS.

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The purposes of this study were to report allele and genotype frequencies data in Annobon Island population sample (n=86) for a five VNTR polymorphisms (3'ApoB, HumTH01, HumvWA31A, HumTPOX and HumCSF1PO). DNA were extracted from blood samples by chelex method. PCR singleplex amplifications were accomplished with fluorescein labelled primers, and genotypes were analysed in denaturing 6% polyacrilamide gel electrophoresis, using automated laser fluorescence for STR loci (HumTH01, HumvWA31A, HumTPOX and HumCSF1PO). 3'ApoB minisatellite locus was carried out routinely by PCR amplification and ethidium bromide 3% agarose gel electrophoresis. Allele and genotype frequencies have been determined. The following allele frequency ranges were found for the five systems:

3'ApoB: 19 alleles with frequencies ranging between 0.005 and 0.21. Discrimination power (DP): 0.97
HumTH01: 5 alleles with frequencies ranging between 0.08 and 0.45. DP: 0.87
HumvWA31A: 8 alleles with frequencies ranging between 0.03 and 0.21. DP: 0.96
HumTPOX: 7 alleles with frequencies ranging between 0.006 and 0.34. DP: 0.92
HumCSF1PO: 7 alleles with frequencies ranging between 0.03 and 0.36. DP: 0.91

For all markers the observed genotype frequencies are in good agreement with the expected distribution under the Hardy-Weimberg law. (Exact-test).
The allelic frequencies, compared to other populations shows a high degree of variation between Annobon sample and other populations studied.

VALIDATION OF A FREQUENCY DATABASE FOR TWO STR LOCI (CD4 AND D12S391)

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The main purposes of this study were to obtain allele and genotype frequencies of the two STRs, CD4 and D12S391, for a North of Portugal population sample and to calculate some of the statistical parameters of medico-legal interest. Family data from paternity cases from our routine work were also analysed in order to provide additional information about mutations.
The population data was obtained using PCR and the alleles were determined after denaturing PAG electrophoresis followed by fluorescent detection in an automated sequencer. We found 8 alleles for the CD4 and 12 alleles for the D12S391. Both systems were in accordance with the Hardy-Weinberg equilibrium.
The characteristics of these systems, including easy amplification, high heterozygosity and sequence simplicity, make them useful for forensic analysis.

AUTOMATED TYPING OF 4 TETRAMERIC STR: A NORTH OF PORTUGAL DATABASE

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In this study we report allele/genotype frequency data in a North of Portugal population sample for 4 tetrameric systems (TPOX, CSF1PO, LPL and F13B) analysed by using recent commercially available kits for PCR (Promega).
After extraction DNA was amplified according to the manufacturer recommmendations and fluorescent detection was made in an ALF sequencer (Pharmacia).
These loci were tested for departures from Hardy-Weinberg expectations. Other statistic parameters were calculated to evaluate the forensic utility of the systems.
In conclusion, a North of Portugal population database has been established for these 4 tetrameric systems and it was demonstrated that they are a valuable tool for forensic identity testing.

POPULATION GENETICS OF THE F13A1 STR POLYMORPHISM IN NORTH PORTUGAL AND S. TOMÉ E PRÍNCIPE

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The F13A1 intronic STR polymorphism was studied by PCR and denaturing PAGE in North Portugal and S. Tomé e Príncipe (a small archipelago in the Gulf of Guinea, W Africa).
Results obtained up to now can be summarised as follows (sample sizes are, respectively, 239 and 279):

Allele frequencies (%)

	3.2	4	5	6	7	8	9	11	12	13	14	15	16	17
Portugal	10,2	2,9	21,5	25,8	34,0	0,8	0,0	0,0	0,2	0,4	1,0	2,1	0,6	0,4
S.Tomé	10,9	5,4	38,9	12,0	17,0	5,2	0,2	0,5	0,7	5,9	2,3	0,5	0,4	0,0

Agreement with Hardy-Weinberg expectations is good in North Portugal (exact test P=0,43±0,01), but poor in S. Tomé e Príncipe (exact test P=0,05±0,01).
Gene frequencies from North Portugal are compared with other Caucasian populations and those from S. Tomé e Príncipe with Afro-American data.
Relevant forensic parameters are also presented.

ANALYSIS OF THREE STRs (HUMCD4, HUMCSF1PO, HUMTPOX) IN FORENSIC SAMPLES AND PATERNITY TESTS

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The study of the STRs by means of the genetic amplification method *via PCR* allows to obtain important information for the identification of a person. The versatility of these systems sometimes allow the amplifications in multiplex and consequently we save time and material. In any case, in our experience the manual revelation method with silver staining allows for a good resolution with the identification of also small quantities of DNA. In order to use these particularly informative systems, we studied allele distribution in our reference population of Central Italy (Tuscany) for the following three STR systems:

system	localization	fragment range	n° of examined subjects
HUMCSF1PO	5q33.3-34	299-323	158
HUMTPOX	2p23-2pter	232-248	151
HUMCD4	12p12-pter	125-175	159

The analysis of these loci shows heterozygosity of respectively 68, 71 and 73%.
In 22 paternity tests no mutations were found for these loci. To verify the validity of these STR systems and the possibility to detect very small quantities of material, we also carried out a sensibility study with progressive dilutions of DNA samples.

YNZ22 POPULATION DATA IN THE TUSCAN POPULATION (CENTRAL ITALY)

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The use of the VNTRs and STRs by means of enzymatic amplification with PCR allows to solve cases of paternity tests, on condition that the genetic frequencies of that marker in the population is well known.
In the present study, allele frequency data were obtained at D17S5 VNTR locus (YNZ22) in samples of 312 unrelated individuals from a well-defined part of Central Italy. The main objective of the study was to analyse allele frequencies in an area of Tuscany defined by the surroundings of the cities of Florence, Prato and Pistoia and to compare these frequencies with data from a previous study of the Central Italian population. After amplification via PCR and gel electrophoresis, 13 alleles and 54 genotypes were identified. To simplify the individualisation of alleles, an allelic ladder was created by amplifying a mixture of DNA from six heterozygote individuals. The system was tested for Hardy-Weinberg equilibrium and comparison was carried out with a two-way RxC contingency table. The heterozygosity index (HI) was calculated to be 0.885. The power of discrimination (PD) was 0.963 and the chance exclusion (CE) was 0.68.

STR DATABASE FROM ARGENTINA: STATISTICAL COMPARISON WITH OTHER CAUCASIAN DATABASES.

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STR typing is an important tool in forensic identification. The weight of the typing profile in incrimination index calculation will depend on the alleles frequencies distribution in the population from which the evidence was obtained. Over 400 unrelated individuals from the metropolitan area of Buenos Aires were typed by means of 12 STR markers (THO-1, FABP, HPRTB, RENA4, FES/FPS, vWA, F13A1, D6S366, CSF1PO, TPOX, MBP A and MBP B). The ethnic composition of this population allows us to consider it as a caucasoid group, with a very reduce aboriginal and a relevant caucasian components. Since no significative differences were detected when six minisatellite VNTRs markers were compared with the FBI Caucasian database (Sala et. al,1997), a comparison of the above mentioned STRs with other Caucasian populations previously described (Edwards et al., 1992; Hammond et al., 1994; Lorente et al., 1994; Möller et al., 1994) was carried out. Statistical comparison of allele frequencies distribution was performed by means of two different methods: G-test (Sokal and Rohlf, 1981) and Fst-test with correction for unequal sample sizes according to Weir (Weir, 1996). Significant differences were found between our metropolitan population and other Caucasian or Mex-american database, e.g in FABP, D6S366, F13A, THO-1, vWA. The difference was higher with Caucasian than Mex-amer group. Our results underscore the importance of the generation of local databases for STRs when these markers are being currently used in forensic casework.

STR SYSTEM D8S1132: GENETIC DATA OF TWO GERMAN POPULATION SAMPLES

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The short tandem repeat (STR) locus D8S1132 (also known as G08685, CHLC. GATA26E03) was examined in two populations of German Caucasians from the area around (1) Frankfurt and (2) Halle/Saale.
Methods: PCR cycling conditions: 94°C - 1 min, 61°C - 1 min, 72°C - 1 min using 30 cycles in a PC cycler (Biometra, Germany).
Electrophoresis was carried out using non-denaturing conditions in a vertical system according to Schneider and Rand (1996) and in a horizontal system according to Wiegand et al. (1993).
The STR D8S1132 is a tetranucleotide repeat system with TCTA as basic repeat motiv. Ten different alleles could be typed in the present study ranging between 135 - 171 bp (according to Katsuura 1996). In the Japanese study of Katsuura (1996) the heterozygosity value was 85%, while in the two German population samples the value was 83%. No interalleles could be determined in a sample size of approximately N = 200 individuals.

References:
1) Katsuura Y (1996), DNA-Polymorphism 4: 53-54
2) Schneider HR, Rand S (1996) Int J Legal Med 108: 276-279
3) Wiegand P, Budowle B, Rand S, Brinkmann B (1993) Int J Legal Med 105: 315-320

FLUORESCENCE BASED MULTIPLEX ANALYSIS OF THE STR POLYMORPHISM FIBRA, VWFA31 AND D18S51 IN GERMAN CAUCASOID INDIVIDUALS

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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 of the tetranucleotide repeat polymorphism at locus FIBRA, VWFA31 and D18S51 in a population sample of 280 German caucasians. Alleles were defined by solid phase DNA sequence analysis of genomic DNA samples on an 373A automated DNA Sequencer (Perkin-Elmer, ABD). Allele and genotype frequencies were determined by PCR amplification with fluorescently labelled primers in multiplex analysis (D18S51 and VWFA31: HEX (hexa-chloro-6-carboxyfluorescein); FIBRA: TAMRA (*N,N,N',N'*-tetramethyl-6-carboxyrhodamine) (Genescan™-Software, ABD). The following results were obtained.

	FIBRA	VWFA31/A	D18S51
Alleles (N)	10	8	12
Range RU(bp)	26(180) -42(212)	13(134) -20(162)	10(278) -21(322)
HR	0.90	0.77	0.91
GeneD	0.839	0.807	0.874
PIC	0.820	0.780	0.860
pM	0.046	0.057	0.035

pM=probability of match; PIC=polymorphic information content;
HR=heterozygosity rate; GeneD= gene diversity; RU=repeat unit

The phenotypic distribution showed no significant deviation from Hardy Weinberg expectations. The combined pM of all four STR loci is $< 1x10^{-4}$. Sensitivity in mixed DNA samples was at 0.01% (100ng total DNA). In conclusion, amplification profils of these STR loci are suitable for rapid analysis of large numbers of samples in forensic or paternity case work.

SEQUENCE ANALYSIS AND ALLELE FREQUENCIES OF STR LOCI D19S246 AND D11S488 IN GERMAN CAUCASIANS.

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This study was designed to determine sequence variations and population genetics of the STR loci D19S246 and D11S488 in German caucasoid individuals (N=100). Solid phase sequence analysis was performed on an 373A automated DNA sequencer (PrismT7 Sequencing kit, Perkin-Elmer, ABD). Allele and genotype frequencies were determined by PCR amplification with fluorescently labelled primers, D19S246: TAMRA (*N,N,N',N'*-tetramethyl-6-carboxyrhodamine) and D11S488: 6-FAM (6-carboxyfluorescein) using the Genescan™-Software (ABD). The following results were obtained.

Locus	Alleles(N)	Range RU(bp)	HR	GeneD	PIC	pM
D19S246	11	28(182) - 40(230)	0.87	0.814	0.784	0.059
D11S488	15	26(242) - 41(302)	0.85	0.88	0.868	0.021

pM=probability of match; PIC=polymorphic information content;
HR=heterozygosity rate; GeneD= gene diversity; RU=repeat unit

Sequence data did reveal a considerable polymorphism of the individual alleles in our population sample. Locus D11S488 (AAAG)n (GAAG)n GAAAG (GAAG)n GAAAGAAAGGAAAG (GAAG)n and D19S246 (CTAT)n CTAAT (CTAT)2 CTGT (CTAT)3 (GTAT)1 CTGT (GTAT)n(CTAT)n are characterized by 'complex' repeat structures. At both loci we observed alleles of identical fragment lenght that are characterized by structural differences in the repeat unit (RU) pattern. Locus D11S488 has two variable regions (VR). VRI is highly polymorphic due to A/G mutations or AAAG/GAAG insertion/deletions. In contrast, VRII has a low variability and in our population sample we did observe only 4 bp insertion/deletions. Locus D19S246 is characterized by a core repeat pattern (GTAT)n(CTAT)n with an $(CTAT)_{1-2}$ repeat in the 5' flanking region. Deletions and/or insertions of 'CTAT' or 'GTAT' repeats are the primary cause of the structural differences at this locus. In conclusion, the repeat structures at these loci give an example for the complexity of STR loci and demonstrate the importance to determine sequence variations of STR loci for use in forensic or paternity case work.

GENETIC VARIATION AT 6 STR LOCI IN THE JAPANESE POPULATION

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Genetic typing has been done on 80 individuals from the metropolitan Tokyo area and on 107 individuals from five other geographic regions of Japan, from Hokkaido in the north to Okinawa in the south. The loci typed were CSF1PO, TPO, THO1, D3S1358, vWFA31, and FGA. No previously undescribed alleles were noted. Considering first the data at the level of the total population, allele frequencies at the TPO, THO1, D3S1358, vWFA31, and FGA loci were in good agreement with Hardy-Weinberg equilibrium expectations; with the Bonferroni correction for multiple tests, the deviation of CSF1PO was not significant. Pairwise comparison of loci using Fisher's Exact Tests showed no evidence of linkage disequilibrium; a multi-way independence test on all six loci also showed no departure from independence expectations.

Statistical analysis on the allele frequencies in the regional subpopulations showed only two deviations from HW equilibrium expectations at the $p<0.05$ level and none at the $p<0.01$ level. Comparison of the subpopulation frequencies to the total population averages showed only a few instances of significant deviation ($p>0.99$ or $p<0.01$), the preponderance of which ($n=3$) being associated with the Okinawa population. Fst analysis showed only small differences between the regional populations, the largest being between Okinawa and the others. The estimated value for Fst in the total entire population was 0.009. These data are comparable to prior Fst analyses based on the classical genetic markers.

POPULATION AND SEGREGATION DATA ON THE MULTIPLEX SYSTEM (THO1, VWA, FES, F13A1) FROM CENTRAL PORTUGAL

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New data on the genetic profiling of the population of Central Portugal, based upon a large sample (N=811) of unrelated individuals are presented.
Multiplex amplification of "chelex" extracted samples, including the STRs: HUMTHO1, HUMVWA, HUMFES/FPS and HUMF13A1, followed EDNAP (European DNA Profiling Group) exercise protocols.
Detection of fluorescent labelled PCR products was made in 373-A and 377 ABD Sequencers, with Genescan Software.
All systems are in H-W equilibrium, except for HUMF13A1, which shows a significant excess of homozygotes, in accordance with previous results on other populations.
Mother/child pair analysis (N=233) has not evidenced any exclusions or segregation distortions, except for HUMVWA, where a null allele was detected (estimated frequency: 0.018).
A new allele, with estimated size of 240 bp, was detected in the STR HUMF13A1.
Sequencing is under progress.

POPULATION STUDY OF THE HUMVWA, HUMTH01, HUMFES AND HUMF13A1 STR POLYMORPHISMS IN THE SOUTH-WEST OF SPAIN.

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Allele and genotype frequencies for 4 tetrameric short tandem repeat loci HUMVWA, HUMTH01, HUMFES and HUMF13A1 were determined in a population sample (n=201) from the South-west of Spain using multiplex PCR. Detection of PCR products was carried out in a ABI 373 DNA sequencer. After detection of PCR products, 9 alleles were identified for HUMVWA, 5 alleles for HUMTH01 and HUMFES and 11 alleles for HUMF13A1. No deviation from Hardy-Weinberg equilibrium was found.

AN ITALIAN COLLABORATIVE STUDY ON HumFES/FPS LOCUS (GEFI PROJECT ANCONA 2). ALLELE AND PHENOTYPE FREQUENCIES.

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The Italian working group for Forensic Haemogenetics (GEFI) promoted in 1994 a collaborative study on the HumFES/FPS short tandem repeat polymorphism (Polymeropoulos et al, 1991). Twenty-two laboratories collaborated to the exercise, each one characterizing at least 50 unrelated samples from locally resident subjects, and two blind controls. Common amplification protocols were suggested and each laboratory was supplied by a ladder to run in the gel. Database included 2,376 individuals from 13 Italian regions. In a single laboratory were generated results which showed significant deviation from Hardy-Weinberg equilibrium and diversity of genotype frequency, so they were excluded from the database. Allele frequencies were computed weighting the regional frequencies by the resident population. A high level of gene frequency heterogeneity among regions (P=.00025) was observed. Northern regions showed an excess of low molecular weight alleles (8 and 9 repeats), while in southern regions an excess of larger alleles (13 and 14 repeats) was observed. Four alleles (10, 11, 12 and 13) were present in 96% of the analyzed sample and contributed to 88.3% of observations by their 7 phenotypes. The observeded heterozygosity at the locus was 70%. All laboratories typed correctly blind samples.

Reference
Polymeropoulos et al. (1991), Nucleic Acids Research 19:4018

ALLELE FREQUENCY OF THE HUMVWA31 LOCUS IN ITALY. REPORT OF THE GEFI COLLABORATIVE STUDY "ANCONA 1"

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A collaborative study on the HumVWA31 locus (a tetranucleotide marker located in an intron of the von Willebrand factor gene on the short arm of chromosome n. 12) was organized by the Italian Group of Forensic Haemogenetics (GEFI). Twenty-two laboratories partecipated, on the basis of a protocol involving the characterization of at least 50 unrelated, locally residing subjects, and two common blind controls. A specific amplification procedure was recommended and allele typing was performed by a side-to-side comparison with an allelic ladder.
The final database included 2,465 individuals from 13 regions, scattered throughout Italy. A single sample showed significant deviation from Hardy-Weinberg equilibrium and diversity of genotype frequency and was excluded from the database. There was a modest level of gene frequency heterogeneity among regions (chi square = 94.59, 72 d.f., P = .038). Italian allele frequencies (Sardinia excluded) were computed weighting the regional frequencies by total resident populations. Seven alleles (14, 15, 16, 17, 18, 19 and 20) were common to all subsamples and showed an overall frequency higher than 1%; another three rare alleles (11, 13 and 21) were identified in some regions. The expected heterozygosity of the locus was 80.4%.
This work validates the use of the HumVWA31 marker in forensic and population analyses and contributes to harmonizing analytical methods in our forensic haemogenetic laboratories.

GENETICS OF THE D12S391 STR LOCUS IN THE CHINESE, THAI AND GERMAN POPULATIONS

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To investigate the population genetics of the the short tandem repeat (STR) locus D12S391, genomic DNA samples from 222 individuals from Southern China, 154 individuals from Thailand as well as from 124 German individuals were analysed. Typing was carried out by polymerase chain reaction (PCR) amplification and subsequent polyacrylamide gel electrophoresis and silver staining.
In total, 12 alleles were observed in the three populations. Among Chinese, allele 19 was the most common with a frequency of 0.225, and among Germans, allele 18 with a frequency of 0.186. In the Thai population only 11 alleles could be distinguished, and allele 19 was the most common with a frequency of 0.198. The expected exclusion chances for Chinese, Thai and Germans in paternity cases is 0.67, 0.71, and 0.75, respectively, and the discrimination power in identification cases is 0.95, 0.964 and 0.97, respectively. Testing of the observed genotype distributions for Hardy-Weinberg equilibrium did not reveal any significant deviations. Allele frequency distributions were significantly different between the Chinese and German populations, but not in other comparisons. Segregation studies of 124 meioses among German families did not reveal any mutations at the D12S391 locus. Thus D12S391 appears to be a robust and efficient STR system both for paternity and stain analysis.
(#Supported by a fellowship from the Dept. of Forensic Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.)

CD4, CYP19, FABP2 AND LPL: ANALYSIS, SEQUENCING AND FREQUENCY DATA

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This study was carried out in order to obtain the nucleotide sequences of the STR-loci CD4, CYP19, FABP2 and LPL as well as to examine their allele distributions.
To reveal the sequencing data the ABI PRISM 310 Genetic Analyzer was used.
Genotyping was performed both manually on PAGE and automatically on the ABI PRISM 310 Genetic Analyzer.
We investigated a population sample of two little villages in South-West Germany where inbreeding exists apparently until now.
The sequencing data and a comparison of the allele frequencies of this study and other German population samples are presented.

POPULATION DATA ON THE LOCI HLA-DQA1, LDLR, GYPA, HBGG, D7S8, GC, AND D1S80 IN A HUNGARIAN ROMANY POPULATION

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Allele and genotype frequencies for 7 frequently applied PCR-based genetic markers - HLA-DQA1, LDLR, GYPA, HBGG, D7S8, GC, D1S80 - were determined in a Hungarian Romany population sample.

The study was performed on a sample of 135 unrelated Romanies living in Baranya County (south-western Hungary). Typing was carried out by using AmpliType™ HLA-DQ∝, AmpliType™ PM, and AmpliFLP™ D1S80 kits from Perkin Elmer.

All seven loci meet Hardy-Weinberg expectations and there is little evidence for association of alleles between and across loci. To the best of our knowledge the observed values for alleles D1S80*24 (f=0.552) and D7S8*A (f=0.785) in this Hungarian Romany population are the highest that have been reported. The D1S80 and D7S8 markers are slightly less informative in the Romany population than in the general Hungarian Caucasian population. However, the combined forensic efficiency values for all seven markers in the Romany population ($pM = 8.9x10^{-5}$; MEC = 0.921) are similar to that reported previously for general Hungarians. Using a test for homogeneity, all loci except LDLR and GC were statistically different between Hungarian Romanies and Hungarians, as well as other Caucasians.

Although there are a number of statistical differences observed between Hungarian Romany and general Hungarian Caucasian databases, in most cases, there still would not be considerably different estimates of multiple locus profile frequencies for the seven PCR-based loci using either database.

A STUDY OF SEQUENCE POLYMORPHISM IN HUMAN ALPHA FIBRINOGEN GENE IN POMERANIA-KUJAWY REGION OF POLAND. IDENTIFICATION OF TWO ALLELES PREVIOUSLY REPORTED TO BE ABSENT IN CAUCASIANS.

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Human alpha fibrinogen gene (HumFGA) contains a microsatellite sequence belonging to the group of highly polimorphic complex repeat STRs. More than twenty alleles were described for this locus in general population. However, some of these alleles were reported to be characteristic for Afro-Caribeean population only while other alleles are common in all investigated populations. There are 14 alleles of HumFGA reported to be common in Caucasians including 4 interalleles differing by 2 bp from full repeat alleles. We have investigated 150 unrelated individuals from the Pomerania-Kujawy population (Northwestern Poland) for the sequence variation in HumFGA locus. The aim of our work was to establish the number and sequence structure of HumFGA alleles present in this population.

Primer sequences for FGA locus were designed using Oligo ™ software. DNA samples were amplified on an GenAmp 9600 thermal cycler. PCR products were electrophoresed, detected and sized using an A.L.F. automated DNA sequencer with A.L.F. Manager and Fragment Manager software. Individual HumFGA alleles were resolved on agarose, purified, reamplified and sequenced with -21M13 primer using Taq FS Cycle Sequencing Kit and an ABI 377 DNA sequencer.

We were able to identify 12 full repeat alleles as well as 1 interallele. All sequenced alleles were used for construction of an fluorescein-labeled allelic ladder. Among the sequenced alleles we have found two alleles (17 and 18) which were previously reported to be absent in Caucasians. Additionally, population studies allowed us to estimate the allele frequencies for HumFGA locus in the Pomerania-Kujawy population and to estimate the power of discrimination for this locus in this population.

POPULATION GENETIC COMPARISON OF DNA POLYMORPHISM IN DRUG METABOLIZING ENZYME

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Polymorphic N-acetyltransferase (NAT2) catalyses N-acetylation of various arylamine-containing chemicals in the liver and causes N-acetylation polymorphism. Acetylator phenotype in humans has been assessed by various methods with a test drug, such as INH etc, and ethnic variation in acetylation capacity has been reported.

We compared the NAT2 genotype distribution among some populations. DNAs were prepared from Japanese, German and Hungarian subjects and the NAT2 genotype was determined based on the fact that four alleles in the NAT2 gene differ at single base substitutions which modify restriction enzyme cleavage sites.

The distribution of NAT2*1/*1 genotype in the Japanese population was 45% and less than 10% in Germans and in Hungarians. NAT2*4 frequency in German and in Hungarian population was almost 0.4 and Japanese had a lower frequency of 0.02. The NAT2*1 is considered to correspond to high N-acetylation activity and the *2, *3 and *4 give rise to low acetylation activity. In the samples we have studied, 10% of Japanese and almost 60% of Germans and of Hungarians were slow-acetylator. The Romany people in Hungary showed different genotype distribution and allele frequency from other mixed Hungarian people, Germans or Japanese.

Analysing the ethnic difference of drug metabolism might be used in evaluation of the genetic relationship among populations.

POLYMORPHISM ANALYSIS OF NINE STR LOCI IN JAPANESE USING MULTIPLEX PCR AND CAPILLARY ELECTROPHORESIS

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Short tandem repeat (STR) loci has been becoming widely used in forensic caseworks. Recently a kit for STRs typing, named the AmpFℓSTR Profiler PCR Amplification kit, has been newly developed. This kit is an epoch making STR system to type nine STR loci (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317 and D7S820) simultaneously using both size markers and allelic ladder markers. By capillary electrophoresis, it takes only about 30 min to type one sample and up to 45 samples a day can be typed automatically. In the present study, we analyzed allelic distribution of nine STR loci in the Japanese population using tis typing system. DNA was collected from blood of 113 unrelated healthy Japanese and extracted by organic DNA extraction method. Amplification of nine loci was carried out according to the protocol with the kit. Amplified alleles were sized by the Genetic Analyzer 310. Since samples were labeled by the same fluorescent dye as allelic ladder, size variations between each allele and the corresponding allelic standard were extremely small. Genotype was then assigned automatically using the software "Genotyper 2.0". We estimated the observed heterozygosity (Hz) and the power of discrimination (PD); at each loci. The each values are as follows: D3S1358, 0.68, 0.85; vWA, 0.79, 0.92; FGA, 0.86, 0.96; TH01, 0.67, 0.84; TPOX, 0.66, 0.82; CSF1PO, 0.70, 0.85; D5S818, 0.79, 0.92; D13S317, 0.81, 0.93; D7S820, 0.76, 0.90. Data are expressed as name of locus, Hz and PD. The combined heterozygosity and PD was 1.00, 0.999999999, respectively. Our results suggest that typing system is also a very powerful tool for personal identification because of its high discriminating power in the Japanese population.

Population genetic Study using the AmpFℓSTR Blue™ Typing-Kit

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The AmpFℓSTR Blue™ - Kit is a PCR based typing kit, recently introduced by Perkin Elmer/Applied Biosystems, which allows the simultaneous STR typing at the loci D3 S1358, VWA and FGA.

DNA of 104 unrelated Caucasians from central Germany was amplified with the test kit according to the manufacturers recommendations and analyzed with an ABI Prism 310 Genetic Analyzer.

The data obtained are in Hardy-Weinberg Equilibrium (exact test). The Triplex-System has a combined power of discrimination of 0.99987 and a mean exclusion chance in paternity-cases of 0.96. The present study demonstrates the usefulness of the AmpFℓSTR Blue™ Kit in forensic casework.

COMPARISONS OF ALLELE FREQUENCIES IN THREE HYPERPOLYMORPHIC STRs BETWEEN NORWEGIAN SAAMIS AND THE MAIN POPULATION

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People have lived in Norway during 10 000 years and those who lives here today may descend from several founder populations up to the latest of quantative significance, the battle-ax / boat-ax people arriving about 2500 BC.

The Saamis constitute a special subgroup whose origin is much discussed. Great efforts in the analysis of mtDNA sequences and nuclear markers have shown that Saamis are distinct from other Europeans by caracteristic patterns in mtDNA and nuclear marker loci (Sajantila, 1995, Lahermo,1996)

In this study we have used three highly polymorpfic STRs for comparison of allele frequencies in samples from the two populations of Norwegian Saamis (from Karasjok and Kautokeino respectively) and the main Norwegian population. The STRs studied are ACTBP2 (SE33), APOAI1 and D11S554. The method used has been described elsewhere (Dupuy and Olaisen,1997).

Number of alleles rarer than 0.02 were 22, 17 and 19 for non-Saaamis and 11, 5 and 7 for Saamis in ACTBP2, APOAI1 and D11S554 respectively. Since the frequency of homozygotes is $I=p^2+p\alpha$ where p is the gene frequency and α is the general inbreeding coefficient, rare alleles provide more information on population structure than the common ones. Using previously inbreeding coefficients (Gedde-Dahl,1973), and assuming a long term $\alpha=0.02$, we found a nonsignificant increase of ACTBP2-homozygosity over panmixia for rare alleles, but no obvious inbreeding effect on the other two systems. An explanation to this may be simultaneous outcrossing over the last generations.

UK CAUCASIAN DATABASES FOR THE SHORT TANDEM REPEAT LOCI D12S11, HUMFIBRA AND D8S1179

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300 unrelated Caucasians from various parts of the UK have been typed for the short tandem repeat (STR) loci D12S11 and humFIBRA (FGA). A subset of the above sample consisting of 200 individuals has also been typed for the STR locus D8S1179. All three STRs were amplified in a 5µl singleplex PCR reaction with identical amplification conditions using the dye-labels (*) D21S11/6-FAM, FGA/HEX and D8S1179/TET. The primer sequences were:

D21S11	forward primer	ATA TGT GAG TCA ATT CCC CAA G
D21S11	reverse primer*	TGT ATT AGT CAA TGT TCT CCA G
FGA	forward primer	GCC CCA TAG GTT TTG AAC TCA
FGA	reverse primer*	TGA TTT GTC TGT AAT TGC CAG C
D8S1179	forward primer	TTT TTG TAT TTC ATG TGT ACA TTC G
D8S1179	reverse primer *	CGT AGC TAT AAT TAG TTC ATT TTC

Amplified fragments were detected using an ABD 373A automated sequencer with TAMRA 350 molecular weight marker and allelic ladders for each STR on every gel run. Allele assignment was made when the difference in molecular weight between the unknown fragments and the nearest alleles in the allelic ladder (δ_1 and δ_2)[1], were less than or equal to 0.5 base pairs (BP). Furthermore, the band shift observed - being the absolute value of δ_1 and δ_2, was required to be less than or equal to 0.5BP. At the FGA locus in the sampled population a single example was found of an allele which consistently differed in molecular weight from the closest allelic ladder marker (FGA 22) by 1BP. This was designated as an FGA 23.3 allele. All 3 databases were analysed for indications of linkage disequilibrium.

Ref: 1. Gill P, Urquhart A, Millican E, Oldroyd N, Watson S, Sparkes R, Kimpton CP. A new method of STR interpretation using inferential logic - development of a criminal intelligence database. Int. J. Legal Med. (1996) 109: 14-22

MVR ANALYSIS OF THE MSY1 MINISATELLITE

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The analysis of Y chromosome polymorphisms is becoming increasingly interesting for forensic and anthropological purposes.
Here we describe the variation of the MSY1 (DYF155S1) minisatellite, the first Y specific minisatellite described. This variation can be accessed by Minisatellite Variant Repeat-PCR (MVR-PCR) which maps the digital DNA typing system and has revealed an enormously high degree of variability at autosomal loci.
In this study, different methodologies were used for analyzing the internal variation of this locus after MVR-PCR, including conventional agarose gels and non radioactive hybridization, polyacrylamide electrophoresis in miniaturized gels followed by silver staining and automated sequencer with fluorescent technology. Advantages and disadvantages of each methodology are discussed.
A population study was carried out in a sample form Galicia (NW Spain). Preliminary analysis of data confirms the extraordinary interest of this system for anthropological purposes and a peculiar pattern distribution in the Galician population.

ALLELE FREQUENCIES AND HAPLOTYPES OF EIGHT HUMAN Y-STRS: A COMPARISON BETWEEN SARDINIAN AND CONTINENTAL ITALIAN SAMPLES

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Eight polymorphic STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385) mapping at the male-specific part of human Y-chromosome were investigated in 125 unrelated Italian individuals and 80 Sardinians. The aim was to evaluate the efficacy of the resulting Y-haplotype for forensic purposes. DNA sample were amplified by PCR; the resulting fragments were run on a semi-automatic sequencer (A.L.F. Pharmacia) and classified against a ladder built from known fragments. Significant differences emerged between these two samples at most of the systems considered. Most Y-haplotypes (117 out of 125) of continental Italians were unique, whereas unique sequences were rarer in Sardinians (51/80). By the predominant representation of some particular genotype at six STRs, a pecular Y-haplotype was found in Sardinians, which could possibly be what presently remains from the ancestral islander-specific haplotype. The overall results confirm the extreme polymorphisms at the selected haplotype, which is therefore a very promising tool for forensic identification.

Cooper et al 1996

Y-SPECIFIC STR IN FORENSIC CASEWORK: VALIDATION STUDIES.

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The smallest element in the human karyotype is the male specific Y chromosome. Some of its features makes it valuable for tracing male lineage ascent and descent. Among them, its inability to recombine except in the so called pseudoautosomal region, homologous to the X chromosome, and the presence of a high number of polymorphic sequences. On this basis, a novel and powerful tool has been developed to be applied in forensic casework. Nine well characterized and informative Y-chromosome specific STRs are available for paternity testing and forensic casework including biological fluid stains, human remains or rape perpetrator identification. In the Y-chromosome linkage group there are located highly polymorphic single band (DYS19, DYS390, DYS391, DYS392, DYS393) and double band (DYS385, DYS389, YCAII) profile generating loci. As a prerequisite for validation studies a haplotype database was created including unrelated Caucasian (Buenos Aires and Berlin metropolitan) and aboriginal (Mapuche, Tehuelche and Wichi) male individuals. A Fox Pro-based software was developed that allows a rapid search of haplotypes in the database and the calculation of its frequencies providing an statistical tool for including this values in incrimination or paternity indexes calculations. Althought the database was constructed with nine STRs, however, the single band loci were chosen for casework. The program allows to evaluate the frequency of a given haplotype, including 1 to 9 loci, in different populations. A set of over 20 forensic cases were tested as part of validation studies.

Y-CHROMOSOME STR HAPLOTYPES: CONSTRUCTION OF ALLELIC LADDERS, SEQUENCING DATA AND POPULATION DATA FROM GALICIA (NW SPAIN)

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Y chromosome polymorphisms are increasingly being used for anthropological and forensic purposes. The non-pseudoautosomal part of the Y chromosome is particularly interesting due to its peculiarities such as its lack of recombination and mode of inheritance. Forensic usefulness of recently described Y polymorphisms (DYS19, DYS385, DYS389-I, DYS389-II, DYS390 and DYS393) was analysed in order to introduce these systems in forensic routine. Tetrameric STRs polymorphisms can be used to construct highly discriminative Y haplotypes useful for some particular forensic cases.
Our study includes population data from Galicia (NW Spain), sequencing of alleles to study sequence variability and the construction of allelic ladders. This is particularly important for forensic and anthropological application of Y haplotypes, since the availability of sequenced allelic ladders is crucial for intercomparison purposes. Some examples of the aplication of Y polymorphisms in forensic cases are also given, including data from father-son combinations.

Geographic clustering of haplotypes of the human DYS389 tetranucleotide repeat locus

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The tetranucleotide repeat polymorphism at the DYS389 locus consists of two repetitive stretches with different numbers of $(TCTA)_n(TCTG)_m$ repeat units. Comparison of the alleles found at this locus in males from populations of 8 different geographic regions revealed, besides to the known length polymorphism, several sequence variants for a defined fragment length resulting from differences in the number and arrangement of repeats in the two repetitive stretches. Based on these data, 45 haplotypes were defined which exhibited remarkable geographic clustering. We propose a model, in which both founder effects and genetic drift together with a high rate of new mutation explain the picture of haplotype diversity observed

SOMATIC INSTABILITY IN GASTRIC TUMORS AT STRs USED IN FORENSIC GENETICS

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We have studied four polymorphic STRs (FES, TH01, TPO and VWA31/A) in gastric tumors with paired normal tissue. DNA was extracted from frozen samples by the phenol/chloroform method, amplified by PCR and submitted to non-denaturing PAGE. Results obtained up to now are summarised in the following table:

	Instability type (%)		
locus	loss of heterozygosity	extra bands	total of alterations
FES	8.7 (2/23)	30.4 (7/23)	39.1 (9/23)
TH01	3.8 (1/26)	7.6 (2/26)	11.5 (3/26)
TPO	7.1 (2/28)	10.7 (3/28)	17.9 (5/28)
VWA	7.1 (2/28)	21.4 (6/28)	28.6 (8/28)

In this cancer type, loss of heterozygosity is consistently less frequent than the appearance of new alleles over the four loci which suggests distinct instability pathways or differential regional susceptibility. On the other hand, FES is the most unstable locus among those studied which is not surprising since the corresponding gene is a proto-oncogene.
Furthermore we are now studying: (a) if there are genotypes prone to instability and (b) if instability is age-related irrespectively of tumorigenesis.

DURING A RAPE INVESTIGATION THE SUSPECT WAS FOUND TO BE HETEROPLASMIC IN HUMVWA31/A.

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A man was charged for sexually assaulting a female business owner. The woman had been forced to oral sex, after which she had wiped her face using a towel. The towel was accordingly sent to the laboratory for analysis, subsequently stains of seminalfluid were detected.

DNA extracted from the sperm was amplified by PCR for the STR markers routinely used in the laboratory: HUMTH01, HUMVWA31/A, HUMF13A1, HUMFES/FPS and HUMACTBP2, and analysed using a ABI 373 Sequencer. In addition PolyMarker™ was used. A match, in all marker analysed, was found between DNA extracted from the suspects blood and that of sperm-stain(s). However, in HUMVWA31/A a discrepancy between the blood sample and the sperm stain(s) was observed. In the DNA of the sperm the two alleles 17 and 19 were observed, whereas in the reference blood sample, in addition to 17 and 19, a third allel appeared, being 20. Using increased and decreased concentration of DNA for genotyping the additional allele proved to be ubiquitous, representing 5-8% of the total peak area.

To evaluate these results further, new reference samples were collected. These samples, being hair roots and buccal swabs, were genotyped in HUMVWA31A only. The result of the hair root correlated to that of the semen, whereas the result of the buccal swab correlated to that of the blood.

The results obtained did not interfere with the legal process and the man was sentensed for rape.

WHY NOT USE MVR IN FORENSIC CASEWORK? FORENSIC USEFULNESS OF MVR VARIATION AT THE MS32 AND MS31 LOCI

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Minisatellite Variant Repeat Mapping by PCR (MVR-PCR) is a new approach to analyzing individual variation in human DNA. This technique uses repeat unit sequence variation within minisatellite alleles rather than size variation between them. This approach has clear potential for individual identification because the resulting MVR code varies greatly among individuals. These codes can be translated into digital information which can be recorded in computer databases. Also, MVR-PCR offers additional advantages which make it a a very reliable method for forensic analysis. Despite its potential forensic usefulness, this methodology was not implemented in practical casework in forensic labs, in part due to the lack of forensic validation studies.

This study was designed to observe the results of MVR-PCR typing of the MS32 and MS31 loci on bloodstains subjected to aging, semen and saliva stains, and teeth subjected to high temperatures. In addition, different electrophoretic methods (agarose and polyacrylamide gel electrophoresis, automated fluorescent detection) have compared and a population genetic study was carried out in the Galician population. Advantages and disadvantages of this methodology and its application in forensic routine are discussed.

TWO DISCRIMINATORY MULTIPLEX STR SYSTEMS FOR FORENSIC IDENTIFICATION: VALIDATION AND CANADIAN CASEWORK EXPERIENCE WITH AUTOMATED FLUORESCENT TECHNOLOGIES

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Multiplex DNA profiling systems comprised of three polymorphic short tandem repeat (STR) loci HumD21S11, HumFGA and HumD3S1358 and the gender determination system amelogenin have been evaluated for their potential use in forensic identification. The automated fluorescence detection with the ABD Models 373A/377 DNA Sequencers were used in this study. Sensitivity studies revealed that complete STR profiles are detected with as little as 0.08 ng of target DNA. Examination of mixtures of DNA and mixtures of body fluids indicated that the optimal DNA ratio allowing discrimination of heterozygous individuals contributing distinct sets of alleles was 1:12. In the case of individuals whose profiles show potential stutter alleles overlapping with true alleles, this ratio was 1:8. The robustness of each locus in the multiplexes was evaluated using various harsh conditions. No anomalous STR profiles or artefacts were observed in samples subjected to the insults. The HumD21S11, HumFGA and HumD3S1358 primer sets generated detectable fluorescence signals mainly in Higher Primates. The multiplex systems were also tested on miscellaneous samples such as cigarette butts, chewing gum, fingernails and envelope flaps, processed using both a standard organic extraction protocol and a recent QIAamp protocol. Using the QIAamp extraction protocol, complete STR profiles were obtained from all sources, including samples over two years old. The organic extraction procedure resulted in successful typing of all samples with the exception of envelope flaps. The validation of the multiplexes was then extended to include 142 casework specimens representing 24 cases previously processed using RFLP analysis. The validation of one of the multiplex systems also included 19 additional cases comprising a total of 232 miscellaneous exhibits strictly processed using STR analysis. Of the three STR systems incorporated in the multiplexes, the HumD21S11 locus appeared to be the most sensitive to PCR inhibitors. Collectively, these studies established the robustness of both multiplex STR DNA profiling systems in actual casework situations and identified them as suitable DNA markers for routine forensic investigations.

Analysis of Manual and Automated Allele-Typing of the STR-Locus SE 33

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The STR-locus SE 33 with its basic repeat AAAG shows great variability, not only in length but also in sequence structure.

Analysis was carried out both by manual typing, via horizontal native and denaturating PAGE, and by automated typing, via capillary electrophoresis using the ABI Prism™ 310 Genetic Analyzer, wherein 2 different polymers were compared.

We present first the results obtained by these two different methods and demonstrate secondly the comparison between the Sequencing polymer and the new polymer Pop 6.

INCREASING OF INFORMATIVENESS OF THE HIGH MICROVARIATION STR SYSTEMS WITHOUT SEQUENCING

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The human beta-actin related pseudogene (ACTBP2) is one of the most informative but also one of the most complex tetranucleotide markers tested so far. Our sequence data revealed the presence of significant sequence variation between alleles of the same size which has very important implications for the forensic use of ACTBP2 locus. In the alleles ranging in size from 275 to 323 bp, hexamer units AAAAAG or AGAAAG occurred in the repeat region in addition to AAAG repeats. Two alleles (317 and 321 bp) contained two hexamers in the repeat region. There was a considerable polymorphism of the hexamer position leading to three different allelic variants of 275 bp, two of 279 bp, two of 287 bp and four of 307 bp. In the first place, the presence of microheterogeneity can make calling of discrete alleles difficult. Thus, methods distinguishing alleles solely by size are not sufficient in ACTBP2 typing. On the other hand, however, length and sequence polymorphism leads to a greater discrimination in human identification. Increasing of informativeness by direct sequencing the alleles from the upper size range would be problematical in casework. Thus, our research effort focuses on the methods allowing maximum utilizing the ACTBP2 sequence variation without direct sequencing. This paper describes the results of automated fluorescent PCR-SSCP analysis performed on 11 allelic variants of the ACTBP2 locus. All allelic variants of the same size have been easily resolved on the basis of their secondary conformation. An interesting application of the method is presented when an individual was homozygous for two distinct alleles of the same size. SSCP analysis combined with automated fluorescence detection provides a rapid and sensitive means of screening small sequence changes in DNA amplified from high microvariation microsatellite loci. Possibilities of the routine implementation of the method in forensic casework are discussed.

INVESTIGATION OF MICROVARIANTS FROM THE REGION D1S80

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In a population study consisting of 500 Caucasian from Giessen (Germany) we detected alleles that migrated anodicly or cathodicly with regard to a common double stranded allele on a repeat leader.

These AmpFLP microvariants were detectable by horizontal PAGE for 13 from 25 alleles. Microvariants were detected for the alleles 16, 20, 21, 22, 23, 24, 25, 26, 27, 29, 31, 36 and 37.

When sequencing some repeats of the alleles, we could observe deviations from modified KASAI sequence (Accession No.: D28507; see SEKIGUCHI et al. 1994).

For some of this microvariants we can show that they are inherited through generations without change in mobility and conformed to single-allele Mendelian expectations.

Literature:

Sekiguchi,K., Sakai,I., Mizuno,N., Yoshida,K., Kasai,K., Sato,H. and Seta,S. : DNA Sequence Analysis of PCR Amplified Product of MCT118 locus in Japanese DNA Sample. Thesis (1994) National Research Institute of Police Science

A NOVEL GENETIC POLYMORPHISM OF HUMAN CHOLECYSTOKININ GENE AND RELATIONSHIP TO ALCOHOL DEPENDENCY.

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Neuroactive peptide cholecystokinin (CCK) mediates actions increasing neuronal firing, anxiety and nociception in central nervous system, and exhibits a variety of functions in gut such as gall-bladder contraction, secretion of pancreatic enzymes. Multiple molecular forms of CCK peptides result from post-transcriptional processing of the preproCCK gene.
In our study, genetic variation in the promoter and the coding region of the preproCCK gene were analyzed among 66 Japanese, 66 American Whites, 54 Chinese, and 41Colombian Natives.
One μl of PCR product amplified with primers labeled by 5'-Dye Amidide-667 (Pharmacia) was mixed with 7 μl of the denaturing reaction solution, and the mixed solution was denatured for 3 min at 94℃, followed by cooling on ice. Single-strand PCR products were loaded on a 10% nondenaturing polyacrylamide gel containing 0.5x Tris-Borate-EDTA buffer (TBE). Electrophoresis and detection for the banding patterns were performed at 700 V, 30 mA, and 30W for 3 hours at 18℃ by using automated fragment analyzer (Pharmacia, ALF express). The purified template DNA was directly sequenced by using Genetic Analyzer (Perkin Elmer, ABI-Prism™ 310) after termination-dideoxy cycle sequencing reaction with forward or reverse primer.
A highly polymorphic variant was found in the different ethnic groups; a frequent mutation at nucleotide position -45 C to T involved in core sequence (GGGCGG) of Sp1 binding cis-element of the promoter region. Analysis for the segregation study in 10 families of twins confirmed co-dominant heredity of the two alleles. Distribution of genotypes and gene frequencies of 66 controls and 108 alcoholics in Japan presented that allelic variant T type in alcoholics was found in higher frequencies than those of controls, and distribution of these genotypes was significantly different between the both groups, The data indicated that the variant in the promoter of CCK gene might be a possible genetic risk factor for alcoholism.

SOMATIC MUTATION SPECTRA OF THE HYPERPOLYMORPHIC MICROSATELLITES HUMACTBP2, D11S554 AND HUMAPOAI1

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To investigate somatic mutations in the three hyperpolymorphic microsatellites HUMACTBP2, D11S554 and HUMAPOAI1, we have employed corresponding blood leukocyte and tumour DNA as a somatic model system. This study focus on mutation rate, the magnitude of change at each event and allelic expansion vs contraction at mutation.

70 unselected fresh frozen colorectal adenocarcinomas and their corresponding blood samples were investigated with PCR amplification of the selected fragments using fluorescent dye labelled primers. Electrophoresis and analysis were carried out in an automated ABD 373A sequencer using a dedicated internal ladder composed of optimally spaced sequenced alleles.

RER, defined as new microsatellite alleles in tumour DNA compared to blood leukocyte DNA, is detected with all three markers. HUMACTBP2 is the most efficient RER detector of these three markers, with a somatic mutation frequency of 0.50. There is marker heterogeneity concerning gain vs loss at mutation, with HUMACTBP2 and HUMAPOAI1 displaying more losses than gains while D11S554 mutations are nearly evenly distributed. All markers show a wide spectrum of changes at mutation with all new alleles corresponding to known population database alleles.

This study adds knowledge to the biology of hyperpolymorphic short tandem repeats in general and may be useful when applying these markers on fixed tissue samples in paternity investigation or human identification.

A NEW " .3" TYPE VARIANT ALLELE IN THE STR LOCUS HUMTH01. ALLELE *6.3* FOUND IN A HUNGARIAN CAUCASIAN INDIVIDUAL

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The HUMTH01 STR locus is one of the most intensively used STR polymorphism in the routine forensic DNA practice . It may be characterized as an STR with low microvariations. Up to now, only three " .3" type allelevariants were published for this locus (8.3, 9.3, 10.3). These are fundamentally similar anomalies consisting one base pair (bp) deletion in one of the four bp long (AATG)n tandem repeat motifs. In this work a new allele variant, an allele 6.3 is reported with a corresponding mutation event verified by sequencing analysis. This allele was found in a blood sample of a Hungarian Caucasian individual.

We observed an allele with its migration in between the allele 6 and 7 after a high-resolution polyacrylamide gel (PAG) electrophoresis followed by a manual typing. The sequencing was performed in the SQ-5500-S DNA Sequencer (Hitachi Electronics Engineering, Tokyo, Japan) automatically as follows: The PCR products of three independent isolates were separated in a PAG (5%T, 4%C, 30 cm long and 1 mm thick).The 6.3 alleles were excised immediately from the gel and allowed to dissolve in 10 µl sterile water overnight. Two and a half microliter aliquots were used in the reamplification of the allele. The sequencing reactions were performed using the Thermo Sequenase core sequencing kit with 7-deaza-dGTP. A PCR primer labeled at the 5' end with Texas Red was used as a sequencing primer. Sequence data were analyzed automatically using the SQ-5500 Ver. 2.00 software.

The allele variant 6.3 could not be detected in a large scale Hungarian Caucasian population study for HUMTH01 including allele frequency data of 734 unrelated individuals. Therefore, presently the frequency of this variant allele can be estimated as low as 0.07 % among Hungarian Caucasians and this might even be an overvalue.

MOLECULAR ANALYSIS OF A MUTATION GENERATED AT THE HUMHPRTB[AGAT]n LOCUS

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We currently use a set of nine STR polymorphic loci (Alford et al., Am J Hum Genet 55:190-195, 1994) for paternity identification purposes. Results are obtained with three triplex PCR reactions followed by silver staining of the fragments separated on 6% polyacrylamide gels. To date, 236 tests have been performed resulting in 161 exclusions and 78 inclusions. Only one mutation was observed involving the chromosome X-linked HUMHPRTB[AGAT]n locus. In this case, a daughter inherited a mutated allele from the presumed father. Inclusion was obtained for the 8 other STRs and additional evidence was gathered by HLA DRB1, DPB1 and DQB1 typing, and with VNTRs D17S5 and D1S80. A combined probability of inclusion W=99.998% was calculated. The mutant daughter allele and the father allele were cloned and sequenced. The mutation consists in the addition of a full-length repeat unit to the paternal chromosome. As often observed, mutations in tandem repeats, including STRs and VNTRs, are in majority from paternal origin and involve expansions. In our series, we found 39 informative families for locus HUMHPRTB[AGAT]n with an inclusion pattern. As a consequence, the mutation frequency is 1.28% of overall meiosis or 2.56% of paternal meiosis in our study. These numbers seem elevated, but are probably overestimated and data should be accumulated from different laboratories to obtain adequate estimates of the mutation frequency.

Expansions of di- and tri-nucleotide sequence motifs are also involved in a growing number of pathologies and understanding the mechanism of mutation has a common interest in legal and pathological medicine.

SCREENING CYSTIC FIBROSIS DELETION ΔF508 IN TWO ANCIENT BASQUE POPULATIONS

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We have developed a rapid protocol for ΔF508 detection in ancient DNA samples. This protocol was used in a number of samples from two different Basque sites: a total of eighteen individuals from Atxuri, Vizcaya (5000-3600 B.P.) and Garai, Vizcaya (XI-XIII A.D.). DNA was extracted from teeth and quantified through gel electrophoresis and "Human DNA quantitation" Kit (Perkin Elmer). A 97 bp DNA fragment, encompasing the ΔF508 region, was amplified with primers described by Kerem et al. (1989). Though 16 out of 18 samples were strong or slightly positive to the quantiblot probe (DYZ1), only 8 samples were conclusively typed as 97-97, after at least three rounds of amplification/PAGE. Despite the low percentage of successful results, the method is efficient to test ancient samples. Further studies have to be carried out to search sequence polymorphism among ΔF508 mutants and, in the case of the Basque population, a larger sample is needed to test the controversial hypothesis of the high frequency of ΔF508 mutation among the ancient basques.

THE STR-MARKER D8S347: SEQUENCING DATA, SEPARATION WITH DIFFERENT DYE-COLOURS, ALLELE FREQUENCIES

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The purpose of this study is to elaborate on the STR-locus D8S347.
This marker, located on the long arm of chromosome 8, has a tetranucleotide repeat sequence (AGAT) with fragment lengths ranging from 344 to 380 bp.
We describe the detection of this locus via capillary electrophoresis with the ABI Prism™ 310 Genetic Analyzer (Perkin Elmer) and present the separation results of fragments labeled with different dye-colours. Furthermore we provide sequencing data, an allelic ladder (11 alleles) and frequency data of this new and highly informative marker.

THE STR-MARKER FGA: SEQUENCING DATA, ALLELE FREQUENCIES

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The STR-locus FGA (human alpha fibrinogen locus) is located on chromosome 4 (4q28). Its tetranucleotide repeat sequence is (TCTT). Alleles range from 176 to 224 bp.
We describe the detection of this locus via manual typing on a PAA-Gel (7% acrylamide, 7 M urea) and via capillary electrophoresis with the ABI Prism™ 310 Genetic Analyzer (Perkin Elmer). We provide sequencing data, an allelic ladder and frequency data of this STR-system.

SEQUENCING DATA OF THE MITOCHONDRIAL DNA CONTROL REGION FROM MOTHER AND CHILD SAMPLES

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Sequence analysis of the mitochondrial DNA (mt-DNA) became a powerful tool in population studies and forensic identity testing. The aim of this study was to analyze the first hypervariable region (HV1) of the mitochondrial D-loop in mother and child samples from white caucasian origin and to develope a database for further forensic application.
A 403 bp fragment of the control region (positions 15997-16401) from 84 blood samples (42 mother-child-pairs) obtained from paternity cases was therefore amplified and sequenced.
Sequence comparison with the Cambridge reference sequence defined a high number of variable poisitions. The results are in accordance with caucasoid specific sequencing data.

DNA MUTATIONS IN D-LOOP OF SIDS VICTIMS

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Mitochondrial DNA (mtDNA) codes for 13 polypeptides, tRNAs and rRNAs essential to the enzymes involved in energy production in the cells, and mutations might induce ATP depletion which in turn induces accumulation of hypoxanthine (Hx). The combination of elevated vitreous humor Hx levels in SIDS victims and indications of hypotonia in infants who later succumb to SIDS might indicate a death mechanism due to energy deficiency. There is a clustering of SIDS in families, but no Mendelian trait. Since mtDNA is maternally inherited it seems interesting to look for inherited (or somatic) mutations in mtDNA. The D-loop in mtDNA is a hypervariable region, and substitutions, when compared to the most commonly Cambridge sequence, might be indicative of mutations elsewhere in the mtDNA. The T8993G mtDNA mutation within the ATPase6 gene is associated with Leigh syndrome, inducing oxidative defects in the developing brain, but might also be the basis for the occurence of SIDS through a defect energy metabolism in for instance the myocardium, leading to heart failure, or in the diaphragm leading to respiratory deficiency. ***Patients and methods:*** We have recorded substitutions in the range 16055-16500 in the mtDNA sequence in blood from 82 cases of SIDS and 56 controls. The sequencing is done using a ABI373A sequencer and the sequencing reactions PRISM-TM Solid Phase Sequenase Dye Terminator DNA Sequencing Kit (Perkin Elmer). Bp 8993 is investigated in SIDS cases: 142 blood samples, 14 samples from heart muscle, 45 from both vastus lateralis and diaphragm. The method used is PCR and restriction fragments analysis. ***Results:*** In the D-loop the SIDS cases had significantly higher substitution rate than the controls ($p=0.04$). None of the SIDS cases had the T8993G mutation, either in blood, heart, diaphragm or vastus lateralis.
Conclusion: The finding of a higher substitution level in the D-loop of the mtDNA in SIDS indicates involvement of mtDNA mutations in SIDS. It does not seem that the T8993G mutation is the source of this involvement.

SEVERAL STR MARKERS AND THEIR GENETIC INSTABILITIES IN SOME RELATED DISEASES.

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The origin and growth of endometrial cancer are presumed to be under the influence of a variety of peptide and steroid hormones. Androgen receptors are found in the proliferative phase and in the secretory phase in normal human endometrium. Low estrogen and relatively high androgen serum levels characterize the post-menopausal status, and it has been speculated that androgens might be involved in the development and progression of endometrial cancer. This transactivation activity resides in the N-terminal domain 1 of the protein, which is encoded in exon I and contains polymorphic GCA and GGC repeats (STRs).

In the present study, we tested the involvement of the AR in endometrial cancer by analyzing the allele frequency, distribution and the heterozygosity of the two STRs at this locus in women diagnosed with endometrial cancer and in healthy normal woman.

The genotypes were determined in the white blood cell DNA of 200 Japanese and 177 German normal subjects and 15 patients with endometrial cancer. For each subject, we measured the number of repeats in the polymorphic GCA and GGC STRs of exon 1 of the androgen receptor gene.

The number of alleles ranged from 15 to 29, and from 10 to 17 in these STRs, respectively. We observed no association between the two microsatellites among normal subjects. And, DNA obtained from cancer tissue and normal uterine tissues of 15 patients of the endometrial cancer, the number of repeats in these loci were measured. We revealed that the genetic instability existed in 6 cases of 15 patients at GCA or GGC STRs of exon 1 of the androgen receptor gene.

In conclusion, Somatic mutations resulting in extension in these STRs have some influences on the development of that tumor.

POPULATION AND FAMILY STUDIES OF THE SEQUENCE POLYMORPHISM AT LOCUS D8S639 REVEALS COMPLEX REPEAT PATTERNS

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The allele distribution and sequence polymorphism of the tetranucleotide polymorphism at locus D8S639 shows considerable variations. In a population sample of 464 individuals (110 unrelated blood donors and 354 family members) a total of 25 alleles could be characterized. The basic tetranucleotide repeat unit structure contains a core repeat pattern (AGAT)n GAT (AGAT)2 AGGT (AGAT)n. Variations in the number of tri- and tetranucleotide repeats however, lead to alleles differing by only one base pair in lenght or to alleles of identical lenght. Deletions and/or insertions of single nucleotides and tetranucleotide repeats in the flanking region of the core repeat are the prevalent cause of these structural differences. Family analysis revealed one maternal mutation giving an estimate for the mutation rate of 3.97×10^{-3}. Furthermore, sequence variations between particular alleles demonstrate a 'branching effect' in the generation of the allelic polymorphism at this STR locus. In this respext, the repeat structure at locus D8S639 gives an example for the evolution of short tandem repeat polymorphisms. The detailed analysis of other polymorphic STR loci could be useful to determine the extend to which the sequence structure of individual loci affects the evolution of STR polymorphism.

STR ALLELE SIZE VARIATION DUE TO DYE LABELLING STRATEGIES

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The information on STR systems available to the scientific community includes usually: PCR, cycling conditions, reaction mix components, primer descriptions or references and gel conditions.
In this work we put on evidence the effect that different labelling strategies has on allele size determinations.
Allelic ladders and reference samples were amplified for some STRs namely, HUMFIBRA/FGA and APOAI1, and detected in the same conditions except for fluorescent labelling strategies (that is, forward and reverse primer labelling).
Detection of fluorescent labelled PCR products was made in a 373-A and 377 ABD Sequencers.
Significative differences in allele sizes were found, from 3 pb to 6 pb.
Strand migration account for such variation when labelling each strand.
The assignment of STR alleles by reference to sequenced ladders must take in account the forward /reverse original primer labelling .
This work corroborates the "DNA Commision of the ISFH" recomendation which states that precise primer labelling conditions should be mentioned in every study.

ANALYSIS OF BAND SHIFT IN THE TYPING OF THREE BASE-PAIR REPEAT ALLELES IN THE SHORT TANDEM REPEAT LOCUS D12S391

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It has been suggested that it is possible to distinguish single base pair differences between short tandem repeat (STR) alleles analysed on an automated sequencer by an assessment of band shift, relative to allelic ladders run on the same gel. Many of the STR loci used in forensic analysis have a regular four base pair repeat sequence; some also have rarer intermediate alleles which differ from the common alleles by one or two bases. The STR, D12S391 is one such locus. We have assessed the efficacy of band shift analysis in the differentiation of three base-pair repeat alleles from alleles just one base-pair larger. DNA was extracted from blood or tissue samples originating from 260 individuals using a chelating resin and amplified in singleplex oil free PCR reaction containing 0.2 units Taq polymerase, 200µM dNTP, 0.25µM primers and 1ng DNA extract in a total volume of 5µl under the following conditions: 94°C - 45s; 54°C - 60s; 72°C - 60s. Primers used were 5'AAC/ AGG/ ATC/ AAT/ GGA/ TGC/ AT3' [forward] and 5'TGG/ CTT/ TTA/ GAC/ CTG/ GAC/ TG3' [reverse] with the latter being 5' labelled with the fluorescent dye TET (Applied Biosystems Inc). Amplified fragments were detected on an ABI373A automated sequencer in a 6%, 12cm denaturing polyacrylamide gel employing GS-350 TAMRA internal sizing standard and two allelic ladders per 36 lane gel. Alleles were assigned according to the nearest allelic ladder fragment providing they lay within 0.5bp of the ladder fragment and providing the absolute shift of the alleles in each sample was also within 0.5bp. Alleles that gave a reproducible absolute shift >0.5bp were assigned to the appropriate imtermediate allele and accounted for 25 (4.8%) of the allelic designations. The mean absolute shift (95% confidence interval) of this group was 1.10bp (1.04-1.16bp) and the minimum mean observed shift was 0.85bp, providing a mean difference of over 0.35bp between the groups. Analysis of absolute band shift measurement thus appears to allow the differentiation of 4bp and 3bp alleles of the D12S391 locus, thus improving the system's discriminatory potential.

DNA-SEQUENCING OF AN ALLELIC LADDER COMPRISING AAAG REPEATS

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Our purpose was to construct a common allelic ladder comprising some of the alleles from three different STR markers, ACTBP2, D18S51 and D17S906, all exhibiting the AAAG repeat unit. The allelic ladder is to be used as an internal size standard when determining the fragment lengths of the alleles on an ABI 377.
GS 500, a commersially available size standard, was previously used to determine the allele sizes of the above markers. However, these analyses showed that the reproducibility of the ACTBP2 and D17S906 fragment sizes was not sufficient since they contain complex hyperpolymorphic repeats giving rise to fragment lengths differing by as little as 1 bp.
Therefore, to improve the sizing of the fragment lengths our strategy was to construct a ladder of sequenced alleles and compare the precision of the size measurement when using this ladder compared to using the GS 500 standard.
The exact fragment lengths of all the alleles were determined by sequencing using the TTTC strand as template.
An improvement in reproducibility when using this allelic ladder compared to GS 500 was achieved.
The results also revealed a large polymorphism within the repetitive sequence of the ACTBP2 and the D17S906 markers.
Even though all three markers contain repeats of the AAAG unit the large intrarepeat variation affects the migration. Alleles with exactly the same number of bases migrate differently depending on which marker the allele belongs to. This result speaks in favour of using sequenced, locus specific allelic ladders when highly polymorphic markers are to be analysed.

REGIONAL ASSIGNMENT OF THE ACTBP2 LOCUS TO THE LONG ARM OF CHROMOSOME 5, AND CONFIRMATION OF D5S110 TO THE SHORT ARM OF CHROMOSOME 5.

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The $(AAAG)_n$ polymorphism at the ACTBP2 locus (SE33) is one of the microsatellite markers in current use at our institute, for instance as an additional marker when analysing putative family members other than the putative father, or in other cases where an expanded battery of markers is necessary. According to The Genome Database, ACTBP2 is assigned to chromosome 5 by somatic cell hybrids (Accession ID 118966), but has not been included in any genetic linkage map.

D5S110 (probe MS621) is one out of five markers routinely in use for paternity testing - this locus is located at the short arm of chromosome 5 , but genetic linkage has not been established.

The purpose of this study was to narrow the regional assignments of ACTBP2 and D5S110.

The ACTBP2 polymorphism is analysed by PCR and PAGE using automated fluorescent detection (ABD). D5S110 is analysed by Southern blot technique, hybridisation with probe MS621 and chemoluminescent detection (Cellmark Diagnostics).

More precise physical assignments were obtained by typing imbalanced chromosome 5 abberations; a family with a balanced translocation mother and trisomic 5p child, and a monosomy 5q21-q31 de novo deletion case.
So far the translocation family confirms the D5S110 localisation to 5p, and excludes the localisation of ACTBP2 from 5p and the 5q21 - q31 region.
Further attempts to finer mapping will be performed by linkage analyses of a few large families segregating for C5;C9 at 5p and for APC flanking markers at 5q21.

FORENSIC CASEWORK EXPERIENCE WITH AmpF1STR PROFILER™

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Since 1994, we have successfully processed and reported more than 500 cases using the Forensic Science Service Quadruplex (vWA, TH01, F13A01, FES/FPS) in an automated fluorescence detection format. Multiplex Short Tandem Repeat (STR) analysis of mixed and degraded samples has yielded interpretable and significant results. Integral to the successful interpretation of mixtures is the potential for quantitative assessment of the components. However, the frequency of common alleles at STR loci may preclude the unequivocal allocation of genotypes usually possible in RFLP interpretation of multi-source mixtures and thus necessitate STR analysis at additional loci. Successful amplification and analysis of 1 nanogram or less of target DNA with the 10 locus multiplex, AmpF1STR PROFILER™ from Applied Biosystems Division not only conserves case material but also substantially reduces the time required to generate extremely informative results. Results from several cases, including severely challenged samples such as spermatozoa from a sink trap and commonly encountered epithelial mixtures such as fingernail scrapings, amplified with a multiplex of Amelogenin, D33S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820 (AmpF1STR PROFILER™) will be presented and interpreted.

Pääbo : insertion of control region fragment into human chromosome 11p15

Elefant: mt DNA from liver different from blood!

old DNA: oxidative damage inhibits PCR

L. Roewer: T/C Y-Alleles

T/C only – Mongolians, Siberia + North. Europ.

Zerjal et al (1997):

Am J Hum Genet 60: 1174-1183

M. Prinz: Y Multiplex

FSI 85, 1997

REPEAT STRUCTURE OF THE Y-CHROMOSOMAL STR LOCUS DYS385 AND FREQUENCY STUDIES IN THE GERMAN, CHINESE, AND THAI POPULATIONS

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The short tandem repeat (STR) locus DYS385 can be typed using PCR amplification and separation of the resulting polymorphic fragments by non-denaturing high resolution polyacrylamide gel electrophoresis followed by silver staining. The PCR primers amplify a duplicated repeat sequence on the Y chromosome revealing a two-band pattern in male individuals. The present study was performed to determine the internal repeat structure of DYS385 as a basis for allele designation, and to investigate the genotype frequencies in three different populations. DNA sequence analysis was carried out after subcloning of PCR-amplified fragments, and revealed the uniform 4-bp repeat structure "GAAA". The shortest allele observed in our study consisted of ten repeat units thus providing the basis for the designation "allele 10" with a length of 364 bp. Except for isolated point mutations, no systematic differences could be observed either in the repeat sequence or in the flanking regions between the two fragments of a given individual. Thus it is not yet possible to discriminate between the two loci of the DYS385 system.

Three population samples of German (n=146), Chinese (n=95), and Thai (n=100) origin were studied. In the three groups, alleles 10 to 24 could be observed. Genotype frequencies differed significantly. Whereas in Germans one common genotype was present (11-14; 33.8% frequency), the frequencies were more evenly distributed among Chinese with the 13-13 (9%) and Thai with the 14-18 genotype (7%) being the most common. Overall, 63 different genotypes were found. Of these, 36 were observed in Germans, 36 in Chinese, and 44 in Thai. Thus DYS385 is a highly polymorphic STR system with population-specific genotype distributions.

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POPULATION GENETIC STUDY OF NINE Y-CHROMOSOME SPECIFIC STR-LOCI IN DUTCH CAUCASIANS

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A set of 9 Y-specific microsatellites was tested on a Dutch Caucasian population sample. Two different triplex and one monoplex PCR-reaction systems enabled us to reliably amplify the 9 different Y-specific STR-loci. The fluorescent labelled PCR-products were detected on the ABI 373A automatic DNA sequencing system. The table summarises the results from our population genetic study on a sample of 100 unrelated male Dutch Caucasians.

Y-STR LOCUS	PCR-reaction system	Genetic variation
DYS-19	Multiplex I	0.44
DYS-389a/389b		0.82 (haplotype)
DYS-390		0.71
DYS-391	Multiplex II	0.54
DYS-392		0.56
DYS-393		0.33
DYS-385a/385b	Monoplex	0.87 (haplotype)

The PCR-products from the loci DYS389 and DYS385 display two polymorphic loci that are amplified with the same primerset.
The genetic variation was calculated according to the formula $1-\sum i^2$, where i is the allele- respectively the haplotype (DYS389a/b, DYS385a/b) frequency.

It is concluded that this set of Y-specific STR-loci reveals a high degree of polymorphism in the Dutch population. The Y-specific STR-loci will be further evaluated in forensic case studies, particularly in cases with mixtures of body fluids and where traditional STR-typing systems failed to reveal the DNA-profile from the perpetrator(s).

CHROMOSOME Y ANALYSIS IN PATERNITY TESTING: EXPERIENCES AND RECOMMENDATIONS

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Y-chromosomal DNA polymorphisms characterize paternal lineages because of uniparental inheritance and lack of recombination. Thus, if the paternity of a male offspring is in question the analysis of a missing putative father can be replaced by the Y analysis of only one consanguineous male relative. Based on a multicenter study to evaluate all known Y-chromosomal short-tandem-repeat-(STR) systems (Kayser et al. Int J.Legal Med. 1997, 110(3), 125-133) an application of these systems in forensic practice, e.g. paternity testing is now feasible. A retrospective analysis of all deficiency cases with a male offspring investigated in our institute during the last five years has been carried out. Instead of the putative father his brother or his nephew were analysed. We use the 10 most informative STR systems DYS19, DYS390, DYS391, DYS392, DYS393, DYS389I, DYS389II, DYS385, YCAII, YCAIII in single or multiplex PCR approaches and analyse the fragment lengths on an automated sequencer (ALF, Pharmacia) or by autoradiography using ^{32}P as a label for STR fragments. A difference in the Y-STR haplotype clearly demonstrates non-paternity for all members of a paternal lineage including the alleged father. Because of the possibility of a spontaneous mutational event such an exclusion should always been confirmed in additional Y-STR systems. The problem to calculate realistically the probability of paternity in case of Y chromosome haplotype sharing between the offsprings and the putative fathers lineage has been discussed already (Jobling et al. Int. J. Legal Med. 1997, 110(3), 118-124). In our study we have calculated paternity probabilities (i) using additional evidence from autosomal systems including only the most informative Y-STR system and (ii) on the basis of full Y-STR haplotype frequencies. For the latter approach the Y-STR databases have still to be expanded.

CHECKING OF LARGE FEMALE LINEAGE PEDIGREES FOR MITOCHONDRIAL D-LOOP MUTATIONS

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Mitochondrial (mt) DNA sequencing has become the method of choice in forensic identification of unknown corpses and skeletons (or stains) of historical importance when DNA samples of close relatives are not available.
In such cases mitochondrial DNA testing seems to be superior to the STR examination. However, we feel that the problem of stability or instability of D-loop sequences within the inherited mt population has not been sufficiently investigated in the context of forensic examination.
We checked five large female lineage pedigrees for mitochondrial D-loop mutations. Each pedigree was characterised by not less than six maternal transmissions of the familial mt population.
In one family three brothers exhibited an AC repeat heteroplasmon in the blood, hair roots and mucoid cell samples (coexistence of 4 and 5 AC repeats). The mt population characterised by the 4 - AC allele was hardly detectable in any of the investigated tissues of their cousin.
In another case we found a (1:1) T-C heteroplasmon related to nt 16147. All investigated tissues of a male member of the same pedigree across six mitochondrial transmissions did not exhibit any heteroplasmon.
In the third pedigree once more a number of members also exhibited major differences in the proportions of the heteroplasmic mt population components.
In two other pedigrees exhibiting a chain of nine mitochondrial transmissions each, no D-loop sequence divergencies were detected.
These findings demonstrate that the forensic exclusion of a relationship in mt tests requires that D-loop deviations be detectable in more than one position.

3' D-loop AC repeat

mtDNA ANALYSIS OF THE GALICIAN POPULATION: CORRELATION OF HISTORICAL AND GENETIC DATA

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mtDNA hypervariable I and II regions were analyzed in a sample from individuals from the Galician population (NW Spain). The aim of this work was to shed some light on the general European genetic variation by studying the most westerly European geographical edge, the Galician population, through the analysis of mtDNA hypervariable regions HV1 and HV2. In addition to its geographical position, its simple history and its migratory pattern (with high emigration rates and almost no immigration for centuries) makes this population very attractive for these type of studies.
mtDNA analysis of HV1 and HV2 was performed in the Pharmacia ALF automated sequencer using a seminested PCR method and cycle sequencing.
Data from 11 other European populations was used for comparison.
The present results are compatible with an population expansion model in Europe during the Upper Paleolithic Age. Our results show that Galicians present a very low genetic diversity compared with the rest of European populations. This is shown by the low nucleotide diversity, low frequency diversity and low Shannon index value. Moreover, the mean of the pairwise difference distribution is one of the lowest in Europe. Futhermore, the frequency of the reference sequence reaches its maximum value in the present population. The most probable scenario shows the Galician population at the edge of an expansion into Europe from the Middle East.

MITOCHONDRIAL DNA TYPING OF THE HYPERVARIABLE REGIONS I AND II (HVRI AND HVRII) IN A WESTERN GERMAN POPULATION (RHINE AREA)

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The occurence of sequence polymorphism within the two hypervariable regions (HVRI and HVRII) in the non-coding region of the mitochondrial DNA (mtDNA) provides a highly sensitive and discriminating tool for individual identification. Mitochondrial DNA is maternally inherited, shows a rapid sequence evolution and is present in high copy number in each human cell. These characteristics makes mitochondrial DNA valuable in forensic application.
In order to exploit the extent of sequence polymorphism and the potential informativeness of mtDNA in casework an adequate population study must be performed to be used as reference. A valuable resource for maternally inheritance has been the pedigrees available through the Centre d'Etude du Polymorphisme Humain (CEPH). The CEPH pedigrees include three generations and permit scoring of as many as 600 meiotic events (CEPH families were kindly provided by the Institute of Human Genetics, Bonn).
MtDNA was extracted from whole blood samples from 30 unrelated individuals. Amplification of the HVRI and HVRII was achieved by four seperate sets of primers generating a 404 basepair (HVRI) and 379 basepair (HVRII) length fragment. The PCR products were sequenced by solid phase sequencing procedure using M13 fluorescent primer. The fluorescent sequence reaction were analyzed using an ALF*express* DNA sequencer (Pharmacia). Sequencing results were compared to the Anderson consensus sequence.
A total of 30 different haplotypes were identified. Most of the polymorphic content was found in base transitions rather than in transversions. Some individuals showed a 1 bp and 2 bp insertion within the HVRII region. No deletion could be observed so far, except of a 7 basepair deletion in HVRII for the cell line K562.
In conclusion mtDNA provides a powerful tool for the study of the genetic relationships as well as for forensic identification.

HVRII MT DNA POLYMORPHISM ANALYSIS IN ITALIANS

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Mitochondrial polymorphism of the D-Loop region has been extensively used in the last years for identification purposes and to study the evolution of human populations. Attention has been paid to the analysis of the two hypervariable region (HVRI, HVRII) for which a wealth of information is today available. In a previous paper, we reported data concerning the polymorphisms of the first hypervariable region in 70 Italians. In this paper we supply further information on the polymorphism of HVRII in the same sample of individuals.
A double-stranded HVRII fragment was amplified using specific primers (L34 and H370), then the specific 380-bp-product was purified and directly subjected to cycle sequencing reaction. Both strands were sequenced, using fluorescent primers and an automatic sequencer. We observed 32 transition/transversion sites and three insertions. At position 73 (Cambridge consensus sequence), a highly variable site was present. An A>G transtion at site 263 was conversely present in nearly all individuals. Only 3 sites presented a mutation in more then 20% of our population, other mutation sites occured just once/twice. Less then 40 % of the whole sequences were found to be unique.

ANALYSIS OF MITOCHONDRIAL DNA : EXPERIENCE WITH A FAMILY

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We have introduced recently in the routine work of our laboratory the analysis of mitochondrial DNA (mtDNA) by amplification of two hypervariable regions HV1 and HV2 of the highly polymorphic mt d-loop region and automated sequencing of the PCR products using a genetic analyzer ABI PRISM 310. The aim of this paper is to check the method employed for the amplification, sequencing analysis of mtDNA and detection of eventual contaminations, and to study the polymorphism found in the analysis of mtDNA from a Catalonian family.

We amplified in different turns mtDNA from 16 maternal related individuals of four generations, and the results of the sequencing analysis shown the same sequence for the regions HV1 and HV2. We observed a stability of the regions HV1 and HV2 during the four generations. Negative results were obtained for the sequencing analysis of reagent blank controls and negative amplification controls.

In conclusion we consider that the method employed to amplify and sequencing mtDNA could be used to solve routine casework.

COMPARATIVE INVESTIGATION OF THE STR POLYMORPHISM AT LOCUS D12S391 IN AN AUSTRIAN POPULATION SAMPLE: ADDITIONAL SEQUENCE DATA AND ALLELE DISTRIBUTION

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The compound STR polymorphism D12S391 (AGAR)n[1] was investigated in an Austrian population sample of 150 healthy, unrelated individuals (Vienna region) employing PCR amplification and detection by laser induced fluorescence on an ALF sequencer and PAGE on 6% native gels with subsequent silver staining. In automated detection on the ALF sequencer (size windows +/- 0.5 basepairs) a total of 15 alleles, including three interalleles, which were one bp shorter than the next larger allele, could be distinguished. The resulting allele frequencies – no deviations from Hardy-Weinberg equilibrium could be detected - are shown in the following table:

A* 15	0.023	A* 18.3	0.013	A* 22	0.137
A* 16	0.027	A* 19	0.113	A* 23	0.074
A* 17	0.110	A* 19.3	0.003	A* 24	0.020
A* 17.3	0.001	A* 20	0.133	A* 25	0.007
A* 18	0.200	A* 21	0.127	A* 26	0.003

Sequence analysis of the interalleles 17.3, 18.3 and 19.3 proved the provisional allele designation revealing an incomplete (GAT) repeat.

Unambiguous allele designation of the various allele classes and interalleles on native PAG was not possible due to the varying electrophoretic behaviours of different alleles of one length class.

[1] M.V.Lareu, C.Pestoni, M.Schürenkamp, S.Rand, B.Brinkmann and A.Carracedo (1996) A highly variable STR at the D12S391 locus. Int.J.Legal.Med. 109: 134-138

Y-CHROMOSOMAL STR SYSTEMS: APPLICATION OF A TRIPLEX-PCR IN FORENSIC STAIN ANALYSIS

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The analysis of polymorphic short tandem repeats (STR) provides the opportunity of rapid identification of forensic stains. In cases of mixed stains of male and female DNA, Y-chromosomal STR-Systems can be used to analyse male DNA without having to cope with high background of the victim´s female DNA. This is important when working with small amounts of DNA. Our Triplex-PCR includes the three Y-chromosomal STR-Systems DYS 393, DYS 19 and DYS 392. We tested its applicability in forensic stain analysis. Allele frequency distributions of the systems in the population of Cologne were determined and used for biostatistical calculation. Blood samples were taken from 110 unrelated male individuals living in Cologne to determine the genotype in the three Systems. DNA was extracted from stains by differential lysis and phenol-chloroform extraction. Different materials were analysed: defined mixtures: female and male DNA; two and three male DNAs; stains: vaginal, oral and anal swabs, victim´s fingernails with skinstains of the murderer. PCR-products were labeled with fluorescent dye and detected with the ALF-Sequencer for fragment analysis. The distributions of allele frequencies of the STR-Systems were moderate polymorphic. The sensitivity of the PCR was tested down to 250 pg DNA. Male DNA in the mixtures was always amplifiable, female DNA in the sample (1:3000) did not disturb the reaction. In all cases the genotype of the stains matched with the one of the stain-producer, however, some stains did not give reliable results, e.g. we had several stutterbands in the system DYS 393, or did not get signals at all. It was possible to type the supernatant of the differential lysis, that means small sperm fractions could be used elsewhere.The technique is appliable when few male DNA is to be examined in a mixture of male and female DNA, including stains without sperms but should be used in context with other STR systems.

POPULATION STUDIES OF THE Y-CHROMOSOME SPECIFIC POLYMORPHISMS DYS19, DYS389 I + II AND DYS390 IN A WESTERN GERMAN POPULATION (RHINE AREA)

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Y-chromosome specific microsatellites - as a counterpart of the maternal inherited mitochondrial DNA - became of increasing interest in forensic medicine and anthropological applications in the past. Extensive studies were carried out to get more detailed information about these paternal inherited polymorphisms e.g. allele distribution in different populations, their evolutionary function and their applicability in forensic identification.

The purpose of this study was the determination of allele and haplotype frequencies of the loci Dys19, Dys389 I + II and Dys390 to constitute the basis for further forensic investigations. Population studies of the y-chromosome specific polymorphisms Dys19, Dys389 I + II and Dys390 were carried out on 100 unrelated male individuals from the Rhine area. In order to get more information about the paternal inheritance of these loci, families including up to 3 generations - available from the Centre d'Etude du Polymorphisme Humain (CEPH) - were investigated (CEPH families were kindly provided by the Institute of Human Genetics, University Bonn).

Genomic DNA was extracted from 100 unrelated male individuals using the salting out procedure. Amplification products were analyzed either on a horizontal, dicontinuous (Dys19, Dys390) or a denaturing (Dys389 I + II) gel system (ALFexpress, Pharmacia Biotech, Freiburg).

Allele frequencies of each polymorphism were determined and compared to those of other published data. Based on the four loci a haplotype was constructed for each individual. The haplotype frequencies range from 1% up to 10% indicating that the combination of these loci is a powerful tool for forensic identification of male DNA.

POPULATION GENETICS OF THE TWO Y-STRs DYS 19 AND DYS 390 IN WEST SAXONIA (GERMANY)

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Y-chromosomal STRs have been established recently for routine casework in paternity testing and stain analysis.
We studied the Y-STRs - DYS 19 and DYS 390 - in a population sample (n = 140) from the Leipzig area (West Saxonia) using conventional as well as capillary electrophoresis.
DNA was extracted from blood using KCl-ethanol procedures. PCR amplification of DYS 19 was performed using a commercial kit (InViTek). PCR fragments were electrophoresed in polyacrylamide gels and detected by silverstaining. PCR amplification of DYS 390 and additionally of DYS 19 was also performed using fluorescently labelled primers, followed by capillary-electrophoresis in denaturing polymers (POP4, PE) and detection in the automated ABI 310 DNA Sequencer (Applied Biosystems / Perkin Elmer). Allele assignment was possible by comparison with a self-established allelic ladder.
We found no differences regarding STR DYS 19 between results obtained by silverstaining and fluorescent detection using the ABI 310.
Results of STR DYS 19:

Allel	13 (186 bp)	14 (190 bp)	15 (194 bp)	16 (198 bp)	17 (202 bp)
abs.	9	68	34	26	13
%	6,4	48,6	24,3	11,4	9,3

Results of STR DYS 390:

Allel	22 (207 bp)	23 (211 bp)	24 (215 bp)	25 (219 bp)	26 (223 bp)
abs.	16	32	44	44	4
%	11,4	22,9	31,4	31,4	2,9

The results were compared to those reported for other caucasian populations. The investigated Y-STRs are useful in forensic casework especially for paternity testing in cases of deficiency and sex-related crime cases.

NORTH ITALIAN POPULATION GENETIC DATA FOR THE Y STR SYSTEMS DYS19, DYS390, DYS392 AND THEIR POSSIBLE APPLICATION TO FORENSIC ROUTINE CASEWORK.

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In order to introduce Y microsatellites into our routine casework, an internal validation study for the systems DYS19, DYS390 and DYS392 was initiated with the aim of verifying their behaviour under our laboratory conditions.
We began initially with a population study on a North Italian population sample (Milano residents).
This is a preliminary study on the method of collecting data from at least 7 Y microsatellites as suggested in a recent wide collaborative experiment (*Kayser M et al. Int J Legal Med 1997 - in press*).
The frequency profile comparison between our data and those from most European populations shows no significant differences for all the systems investigated.
In our lab Y STR systems are applied in forensic routine with the sole aim of collecting data for validation purposes, but their results, to date, have not been included in written statements for criminal cases submitted in court.

STUDIES ON 7 AUTOSOMAL AND 5 Y-CHROMOSOMAL STR LOCI IN A SOUTH-WEST GERMAN POPULATION

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Due to their polymorphic nature, short tandem-repeat loci are a powerful tool in forensic haemogenetics. The application of these marker systems in stain analysis and for paternity testing requires databases of the relevant population to obtain reliable calculations of probabilities.
In order to establish genotype and allele frequencies of TH01, VWA, CD4, FXIIIB, FGA, FES/FPS, and TPO, we have determined these markers in population samples of 173 - 484 unrelated German nationals residing in the Heidelberg area. The y-chromosomal STR's DYS19, DYS390, DYS391,DYS392, and DYS393 were analysed in 104 - 174 unrelated men. We employed electrophoretic separation of the PCR products on horizontal discontinous polyacrylamid gels with subsequent silver staining. The different allels were identified by side-to-side comparison with an allelic ladder.
The observed genotype frequencies of the autosomal markers are in good agreement with the expected distribution under the Hardy-Weinberg law. The discrimination power was calculated in the range 0.85 (FGA) - 0.50 (TPO). The genetic stability of the autosomal STR's was tested in a sample of 85 true families. Apart from one exception in the FGA-System, all other STR's showed normal segregation according to Mendelian inheritance.
Allele frequencies of the different DYS markers in Heidelberg, South-West Germany, are consistent with those reported for other German populations. In a small sample of 27 father/son pairs, whose paternity had been positively confirmed by conventional and DNA analysis, no mutation could be found in DYS19 locus. The combined paternity exclusion capacity of the five DYS loci is 99,07%.

ANALYSIS OF THE Y-CHROMOSOME: THE Y-27H39 POLYMORPHISM IN A SAMPLE OF TUSCANY (CENTRAL ITALY)

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Due to the absence of informations on Y-linked genes, for many years the Y chromosome was not studied as X or autosomal chromosomes. The most recent DNA researches, allowed to design a new map of Y chromosome which contains the TDF candidate gene SRY and also other important genes (fertility, stature, etc). Another important step in the Y chromosome research is the identification of several STRs. By means of the analysis of the Y-chromosome polymorphisms with the PCR method, it is possible to obtain important information for various purposes, for example during forensic investigation in case of rape, for anthropological studies or for paternity tests. One of the most interesting STR markers of the Y-chromosome is the tetranucleotide Y-27H39/DYS19, located on the short arm of the Y-chromosome (Roewer 1992).
In order to obtain frequency-data for this marker, we carried out a frequency-study on a male population in Central Italy, including the provinces of Florence, Pistoia and Prato (Tuscany). We obtained 102 samples from donors who are living in those areas included in our DNA registry; the samples have been amplified and analysed with vertical electrophoresis, followed by silver staining.

POPULATION GENETICS OF THE Y-CHROMOSOMAL ALU REPEAT INSERTION POLYMORPHISM DYS287 (YAP)

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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. In population studies, significant differences in frequency distribution between human populations were observed which can be used to determine the evolutionary origin as well as the phylogenetic relationship between ethnically diverse groups. The locus DYS287 describes a male-specific Y-chromosomal Alu polymorphic (YAP) insertion. Due to the lack of meiotic recombination, the distribution pattern of this polymorphism appears to be strongly conserved within ethnic groups and my serve as a population-specific marker (M.F. Hammer, *Mol. Biol. Evol.* 11:749;1994).

In the present study, we have investigated the frequency of the YAP element in the German, Chinese and Thai populations. Typing was carried out by PCR amplification using YAP-flanking primers followed by agarose gel electrophoresis. Presence of the YAP element was established by detection of a 455 bp fragment, and absence by dectection of a 150 bp fragment. It was found that the YAP frequency was less than 2% in the two Asian populations, but significantly higher in Germans with 7.8% thus confirming the population-specific distribution of this marker.

([#]Supported by a fellowship from the Dept. of Forensic Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.)

POPULATION DATABASES OF DYS19 AND DYS385 IN 150 NORWEGIANS

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Two polymorphic microsatellite systems (DYS19 and DYS385) located on the male-specific portion of the human Y-chromosome were studied in 150 unrelated Norwegian males involved in consecutive paternity cases.

Amplification was performed in a Perkin Elmer System 9600 and fragment lengths were analyzed in a ABD Prism™377 Sequencer.

One haplotype in DYS385 occurs at a relative high frequency in Norwegian males. Otherwise preliminary results show that allele frequencies and haplotypes are reasonably similar to those found in other white Caucasian population samples.

METHAMPHETAMINE AND DNA TOPOLOGY

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The unwinding of the double strands of the DNA helix is a feature of intercalating drugs. This can easily be tested by examining the effect of an agent on the average number of linking topoisomers. The influence of a compound on the distribution of topoisomers will reveal the presence of a DNA intercalator.Intercalators induce a change in the average number of DNA links with a population of topoisomers generated by topoisomerase I.

The purpose of this report is to determine if stimulants may induce the DNA unwinding characteristic of DNA intercalators. Methamphetamine was selected as the most genera stimulant.

Testing to detect the influence of methamphetamine on relaxed DNA was carried out with a DNA Unwinding Kit (TopoGEN, Ohio) using topoisomerase I in agarose gel electrophoresis.

First, supercoiled DNA was relaxed with topoisomerase I for 30 min and then with methamphetamine added for an additional 30 min. Second, the same procedure was carried out without methamphetamine at the same time as the topoisomerase I. Third, the procedure was carried out with methamphetamine and without topoisomerase I. Lastly, these three procedures were repeated using m-AMSA, which is a well known DNA intercalator in place of methamphetamine. The dilutions of each drug were as follows: 100 microM, 200 microM, 500 microM and 1000 microM.

After incubation, proteinase K digestion , DNA extraction by CIA(Chloroform:Isoamyl alcohol=24:1) and electrophoresis using 1% agarose gel were carried out in TPE buffer(36mM Tris-HCl, pH7.8, 1mM EDTA, 30mM NaH_2PO_4 at room temperature at 15 volts for 15 hrs. After that, the electrophoresis gel was stained in 0.5microg/ml ethidium bromide.

Methamphetamine and m-AMSA shifted the gaussian distribution of topoisomers in each dilution. Only relaxed DNA was observed in the condition without drugs.

Results show that methamphetamine acts as a DNA intercalator in inducing DNA unwinding.

FSS: 3 sites

33 37's

200+ staff

216.000 analysed

53.000 removed

6000 stain matches

1800 scene matches / links

↳ burglary !!

SGM, confirm with TGM

1 in 10^{15}

Automation!

Expert system

New phenomena more commonly observed:

- gene duplication / translocation (021!!)
- somatic mutations (1:1000!)

Single cell PCR → som. mutation

NEW DEVELOPMENTS IN THE UK NATIONAL DNA DATABASE - IMPLICATIONS FOR THE LABORATORY AND THE CRIMINAL JUSTICE SYSTEM

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Since the introduction of STRs into criminal casework by the FSS in 1994, their use in routine casework has become well established in the majority of European countries. The STR database of England and Wales is the largest of its kind in the world. More than 150,000 samples are stored on the database. The database operates using 6 different short tandem repeat loci and the Amelogenin sex test (second generation multiplex - SGM). The analysis of more than 160,000 samples has provided an opportunity to study the population genetics of rare alleles. We have found some which are as rare as 1 in 100,000 individuals. The discovery of new alleles has enabled us to construct new allelic ladders to encompass wider ranges than has hitherto been possible. To reduce the match probability further, we will shortly introduce a third generation multiplex (TGM) system with a discrimating power similar to the SGM. The two systems combined give a match probability of 10^{-15}. The implications of reporting very low match probabilities to the courts are examined - in particular the question of "uniqueness" in relation to the size of the target population is explored. Current research efforts are directed towards reducing costs by increasing throughput by introduction of automation at every level of the technique. For example, the use of robotic systems to manipulate samples and expert systems to interpret results is having a significant and fundamental impact on the way in which laboratories organise work.

FTA cards (paper)
- blood collection
- easy DNA extraction

NATIONAL CASEWORK AND NATIONAL DNA DATA BASE: THE ROYAL CANADIAN MOUNTED POLICE PERSPECTIVE

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The use of the polymerase chain reaction with short tandem repeats (STRs) in a multiplex fashion combined with the highly sensitive four-color fluorescence-based detection technology has revolutionized strategies for casework processing. A careful selection of STR multiplexes combining highly polymorphic STR loci can offer discrimination powers better than those of the highly informative restriction fragment length polymorphism (RFLP) systems. Currently, the Royal Canadian Mounted Police (RCMP) processes its casework samples using three discriminatory multiplex STR systems 1) HumD3S1358, HumD21S11 and HumFGA, 2) HumvWA, HumTHO1, HumF13A1 and Humfes/fps, 3) amelogenin, HumD5S818, HumD13S317 and HumD7S820. These STR systems have proven to be robust under forensic conditions and provide excellent sensitivity down to less than 1 ng of targeted DNA. The estimated frequency in the Canadian population of the most common DNA profile across these 10 STR loci is one in 1.9×10^8 and the estimated frequency of the most rare genotype is one in 7.7×10^{42}. The RCMP strategy involves processing casework samples first with the multiplex #3 (capable of gender determination and found to be the most robust of all three multiplexes), followed by multiplex #2 (more discriminatory than multiplex #1) and finally by multiplex #1. Since its implementation a year ago in two of the six RCMP forensic laboratories, several hundred cases have been processed using this approach. Approximately 41% of the cases processed using the multiplex #3, representing inclusions, had sufficient DNA to be further typed using multiplex #2 and multiplex #1. Another 28% had sufficient DNA to be analysed using multiplex #2 only. The need for enhanced efficiency and decreased operational cost based on one multiplex reaction versus three separate STR reactions, along with the anticipation of the development of a Canadian National DNA Data Base prompted the RCMP to examine some of the new and powerful STR multiplexes such as the nineplexes I and II, recently developed by ABD. It is likely that the RCMP will adopt one of these systems for national casework and the national DNA Data Base following completion of their on-going validation.

PCR SYSTEMS USED BY LABORATORIES PARTICIPATING IN THE AABB/CAP PARENTAGE TESTING PROGRAM

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The availability of genetic systems used for determining disputed parentage can be monitored through the results reported by laboratories participating in the proficiency testing survey program of the American Association of Blood Banks (AABB) and the College of American Pathologists (CAP). This report will evaluate trends for PCR testing based on the results from whole blood samples from a total of twelve trios sent to subscribers since 1993.

Ten (10) PCR results were reported on five loci by the 60 laboratories participating in the initial survey (PI-A 1993). With each subsequent mailing the number of PCR results submitted has increased. The most recent results (PI-C 1996) from 105 participants included 373 PCR results on 49 loci. In 1993 no system had five or more results, the minimum number needed to determine a consensus answer. On PI-C 1996 more than 5 participant results were reported for 16 loci (6 dot blot, 2 LTR, 8 STR). The largest number of reports was for HUMTHO1 (34). The phenotype reported was considered a nonconsensus answer on 45 (4.9%) of 921 phenotypes. In some cases failure to achieve a consensus answer occurs because participants use arbitrary designations rather than repeat numbers for the allele. Reported paternity index values for PCR systems also show wider ranges than expected based on published frequencies.

Continued monitoring of proficiency survey results provides a tool to monitor current practices and to identify areas where there is need for standardization.

SCORING SYSTEMS FOR DNA TEST RESULTS FROM A PARENTAGE TESTING PROFICIENCY PROGRAM

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Results from over 100 participants in the AABB/CAP Parentage Testing Program have shown that laboratories using RFLP and PCR marker systems report similar band sizes and allele designations using various methods. To facilitate evaluation of laboratory proficiency, a system for scoring results reported during the 1997 survey has been implemented. Qualitative results for each system will be scored based on consensus when there is 90% agreement between ten or more participants. Target values for systems with quantitative results (i.e. mean band size for RFLP locus/enzyme results) will be determined based on 90% agreement between ten or more participants after removal of outliers (bands +/- 3 SD from the group mean). In addition all the bands reported by each participant for a system are compared to the mean size for all results after removal of outliers. The spread of the normalized band sizes is determined by dividing the difference between each band size and the mean by the standard deviation. A plot of these results displays the variability in band measurements and the bias of the participants results. The consequences of using these scoring methods will be discussed.

THE EVOLUTION AND IMPACT OF THE UNITED STATES DNA ADVISORY BOARD FORENSIC DNA TESTING STANDARDS

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Since its formation and first meetings during 1995, one of the first goals of the DNA Advisory Board (DAB) was to create a set of national guidelines for forensic DNA testing in the United States. An ad hoc committee composed of DAB, Technical Working Group on DNA Analysis Methods (TWGDAM) and the American Society of Crime Laboratory Directors-Laboratory Accreditation Board (ASCLD-LAB) members helped in putting together the initial working drafts that were presented and discussed at the first few DAB meetings. Comments were then solicited from various groups of the forensic DNA testing community attending American Academy of Forensic Science, Promega International Symposium on Human Identification, TWGDAM, and regional forensic science association meetings. These comments plus DAB meeting comments were continually incorporated into the document. The American Society of Crime Laboratory Directors (ASCLD) formed an ad hoc group to suggest criteria for a technical leader or manager in a forensic DNA testing laboratory. ASCLD also proposed a waiver process for technical leader or manager educational criteria. Final comments on the standards were solicited and the final draft was voted on in February 1997. The DAB approved version has been submitted to the Director of the FBI for approval. The final approved standards are very similar to the already existing TWGDAM guidelines, but their are some differences, and there are still some areas which will need to be addressed in future DAB meetings. Additional resolutions regarding statistical issues, CODIS (Combined DNA Indexing System) database applications, and the enforcement of DAB promulgated standards by accreditation and/or certification bodies remain as potential areas for DAB consideration.

TOWARDS THE ESTABLISHMENT OF A FRATERNITY INDEX IN FORENSIC IDENTIFICATION CASES.

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The increasing possibilities provided by DNA technology have originated an on-growing social demand for identification of human remains of diverse origin. Frequently the only living relative is a single sibling wich causes a controversial forensic problem. Due to the fact that no genetic exclusions can be demonstrated between two individuals, the aim of this work was to analyse different statistical strategies in order to establish a meaningful fraternity index wich could help the interpretation of such a conflictive question.
Two differently calculated coefficients based on S/Y and likelihood ratio considerations, have been compared in pairs of individuals from our casework; full brothers and/or sisters, half brothers and/or sisters, and non related individuals.

CREATION OF A PCR-BASED POLYMORPHISMS DATABANK OF MEDICO-LEGAL INTEREST.

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A general description of a worldwide updated database of PCR-based polymorphism was constructed for 34 genetic systems found in the medico-legal or genetic literature references. In determining the number of different populations analyzed for each system, D1S80, PM, DQA1, HUMFESFPS, HUMTH01 and HUMVWFA31 were the most studied genetic systems per population, but only in 8 cases were the 6 studied at the same time.
Given that the availability of highly-organized scattered data is of the uttermost importance for the population genetics, a computerized copy of the database is available upon request and further versions can be easily updated.

MIXED STAIN CALCULATOR

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A computer program calculates likelihood ratios (LR) for the infinite class of mixed stain problems described by Weir et al (Interpreting DNA Mixtures, J Forensic Sci 1997;42(2)213-222).

A feature of especial interest is that the appropriate algebraic expression for the LR is derived and displayed, not just the numeric value. As an example of the benefit, it can thereby be seen that in some cases so-called "conservative" allele frequencies would have an unfortunately anti-conservative effect in evaluating the evidential value of the genetic data.

The algorithm is well-adapted to systems with discrete alleles; RFLP systems can present special difficulties. If bands are close to one another or, even worse, the number of bands is not certain, then no program can substitute for human judgement and case-by-case analysis. However, the spreadsheet-like presentation of the program makes it a useful tool even in these ambiguous cases because various interpretations can conveniently be computed and compared.

A STATISTICAL ANALYSIS BY MEANS LINEAR MODEL ON ITALIAN STRs POPULATION DATA

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In the last few years the analysis of STR has been routinely introduced into the forensic haemogenetics protocols.
However, forensic application of any new genetic marker requires a study to carry out a sufficiently large data-base of the relevant population for a correct use of the analysis results.
According to this guideline, the allele distribution of many STR based systems (FESFPS, vWA, THO1, F13B, F13A, LPL, TPOX) was performed using the Gene Print STR kits by Promega Corporation.
The observed allele distribution had been compared with the expected one according to Hardy-Weinberg equilibrium, by means of the traditional X^2 statistic.
A more comprehensive analysis based on statistical model will be performed. This approach permitts to overall evaluate the contribution to X^2 to each single allele.
Different models will be tested and discussed.

IMPROVING EFFICIENCY AND RELIABILITY BY MEANS OF A COMBINED LABORATORY/OFFICE MANAGEMENT SYSTEM

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The vast majority of our casework focuses on parentage testing. We are employing both conventional marker systems and locus-specific DNA-polymorphisms (Southern-blot technique and PCR).

A Laboratory and Office Management System (LOMS) was designed in order to improve efficiency and reliability by directing and monitoring all major activities in both departments. LOMS was materialized by means of a client-server technology (NOVEL network and SQL Base). LOMS is directing and supervising all activities like

- acknowledgeing receptions of Court records
- Determination of analyses to be done
- Organisation of phlebotomy
- Approval of blood/saliva samples
- Bar-code aided identification of samples
- Set-up of typing programs for different laboratory stations
- Import and storing of typing results
- Compilation of typing results
- Statistical evaluation of typing results
- Compilation of population genetic data

LOMS is a central unit that is able to give information about the actual statuses and progress of analyses at any time.

LOMS is effective since August 1996. Actually, we are aiming at combining the system with a PCR robot.

SCREENING FOR SERIAL RAPE CASES AMONG UNSOLVED CRIMES IN SWEDEN.

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The aim of this study was to investigate if any of 195 unsolved rape cases submitted during the period 1990-1995 had a perpetrator in common.

The stains were extracted with differential lysis and organic extraction followed by purification using Centricon 100. The amount of sperms and epithelial cells in the extracts were estimated by microscopic examination.

DNA was amplified in a multiplex PCR including the loci HUMTH01, HUMVWA, HUMFES and HUMF13A1 using PE 9600 followed by electrophoresis on a ABI 373 sequencer. Results were evaluated with Genescan 2.0.2 and and Genotyper 2.0 softwares. In addition, matching stains were typed at HUMACTB2 (SE33) and D21S11.

In 80 % of the cases the semen was succesfully typed, despite the fact that no control sample from the victim was available. We found five STR-profiles that each occured in two cases. The chance of a random match was lower than 1 in 1 million.

The rape cases with matching profiles were comitted fairly close in time and in the same geographical area and some but not all matching cases had similar modus operandi. This study contribute to show the usefulness of a national databases in criminal investigation.

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