AUTOMATED ANALYSIS OF 5 STR LOCI: ALLELE FREQUENCIES AND FAMILY STUDIES IN THE GERMAN POPULATION

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Introduction

Analysis of polymorphic DNA loci by the polymerase chain reaction (PCR) technique is a very powerful tool not only in the field of criminal investigation but also in paternity testing. Compared to VNTR systems typed by Southern Blot hybridisation, STR elements offer considerable advantages concerning sensitivity, precision and reproducibility of fragment size estimates and biomathematical evaluation.

However, to reach the discrimination power of highly informative RFLP systems, at least the 2-fold number of STR loci has to be analyzed. Thus for both technical and economic reasons, an automated analysis / evaluation system is desirable for routine paternity testing. The Applied Biosystems 373A sequencer used in our laboratory allows the simultaneous electrophoretic separation and detection of the 5 STR loci HUMTH01, HUMFES/FPS, HUMvWF, D21S11 and ACTBP2 (also named SE33). We set up databases in order to establish reliable allele frequency distributions for the German population. Extensive family studies demonstrated autosomal codominant inheritance of segregating alleles at a very low rate of mutational events.

Material and Methods

Human genomic DNA was prepared from peripheral blood by a standard salting out procedure. [Miller, 1988]

PCR reactions were carried out in the the presence of 5'-fluorescence labelled primers. Mixtures contained: 5 ng template DNA, 2,5µl 10 x PCR buffer (GeneAmp, ABI), 200 µM dNTPs, 0,25 µM primer a and b, 1,5 mM $MgCl_2$ and 1U Taq polymerase (AmpliTaq, ABI).

Primers: primer sequences as described by Kimpton (1993) 1. HUMTH01, GTG-strand 6-FAM labelled 2. HUMFES/FPS, GGG-strand TAMRA labelled 3. HUMvWF, CCC-strand HEX labelled 4. D21S11, TGT-strand 6-FAM labelled 5. ACTBP2, AAT-strand HEX/TAMRA labelled.

Amplification: 94°C - 15 sec, 55°C - 30 sec, 72°C - 60 sec, 30 cycles (primers 1-4); 94°C - 15 sec, 60°C - 30 sec, 72°C - 60 sec, 30 cycles (primers 5); Thermocycler: Perkin Elmer 9600

Electrophoresis and detection: Pooled PCR products were analyzed by an ABI 373A DNA sequencer using ABI 672 GeneScan analysis and collection software. Separation was carried out on 6% denaturing polyacrylamide gels (2000 V, 20 mA, 30 W, 8 h). Internal standard: GS 2500 ROX (ABI); ACTBP2 allelic ladder (HEX labelled) was reamplified from a 1:10.000 dilution of pooled DNA samples.

Results

Automated analysis of fluorescent PCR products amplified from HUMTH01, HUMvWF, HUMFES/FPS and D21S11 loci is a straightforward and highly reproducible method. Standard deviation of fragment size estimates within gels ranged from 0,04 bp (HUMTH01, allele 9,3) to 0,09 bp (D21S11, allele 50). Similar results were obtained for between-gel reproducibility. For these systems typing of individual alleles can therefore solely rely on fragment size estimates without using an allelic ladder. In contrast, the complex ACTBP2

Power of discrimination

Of the 5 loci under consideration, ACTBP2 is the most polymorphic one displaying at least 38 different alleles. Heterozygosity rates, power of exclusion and average probability of paternity are summarized in Table 2. The combined power of exclusion is 99,7%. This value is equivalent to the discrimination power of 2 -3 highly informative single locus systems.

At present we are validating a set of 7 STR loci markers (FGA and D18S51 as additional loci) with a discrimination power corresponding to 4 SLS. We speculate that in the near future automated PCR analysis will completely replace RFLP methodology in routine paternity cases .

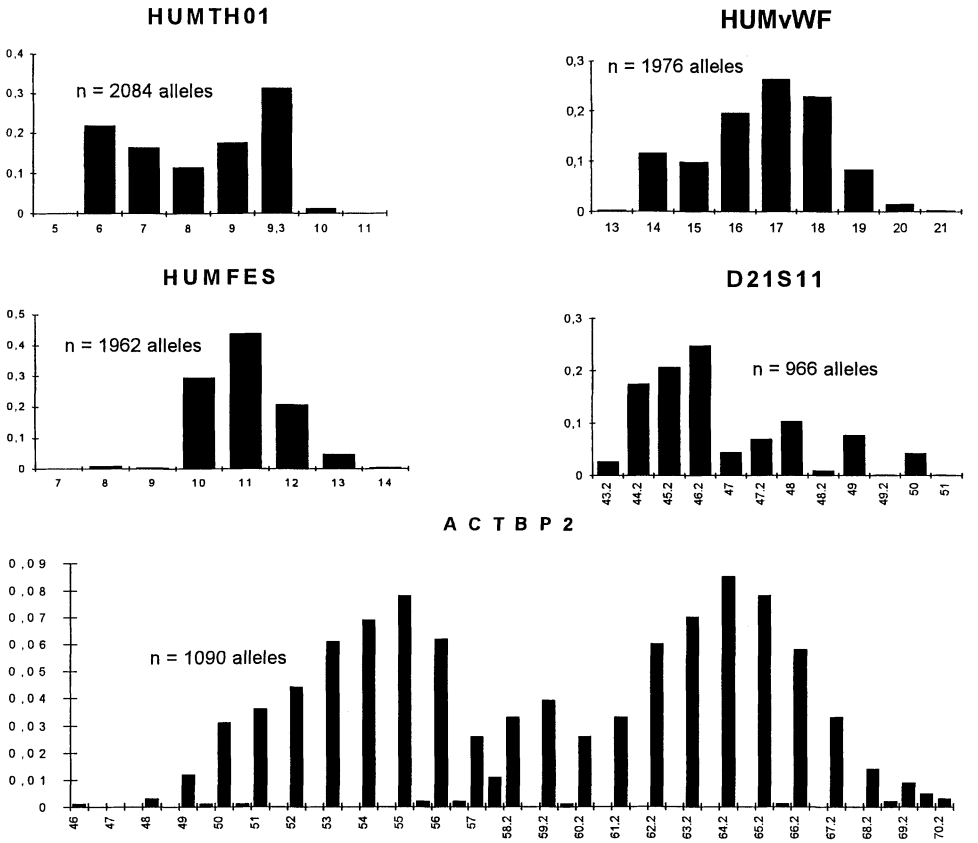

Figure 2: Allelic distribution at 5 STR loci. Nomenclature for HUMTH01, HUMFES/FPS and HUMvWF is based on sequencing data; nomenclature for D21S11 and ACTBP2 refers to Schmitter (1995) / personal communication.

References

Kimpton C. P., Gill P., Walton A., Urquhart A., Millican E. S. and Adams M. (1993). PCR Methods and Applications. **3**: 13 - 22

Miller S. A., Dykes D. D. and Polesky H. F. (1988). A salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. **16**: 1215

Schmitter H., Sonntag M.-L. (1995). Proposal for general rules for the denominations of alleles of STR-loci based on the electrophoretic mobilities of their fragments. Klin. Lab. **41**: 173 - 176

locus demonstrates a consistently higher standard deviation (up to 0,19 bp within gels and 0,61 bp between gels), so that identification of alleles differing by just 1 bp was not possible in each case. We therefore decided to use an allelic ladder as additional internal standard. DNA samples were labelled with TAMRA, the ladder labelled with HEX. Difference in electrophoretic mobility due to different dyes was in the range of 0,1 - 0,2 bp for a given fragment. As sizing errors within a single lane affect both ladder and sample to the same extent, this method allows identification of ACTBP2 alleles with very high precision (Figure 1). Very rare events of overlapping ACTBP2/FES alleles can be simply clarified by separate analysis of the respective fragments.

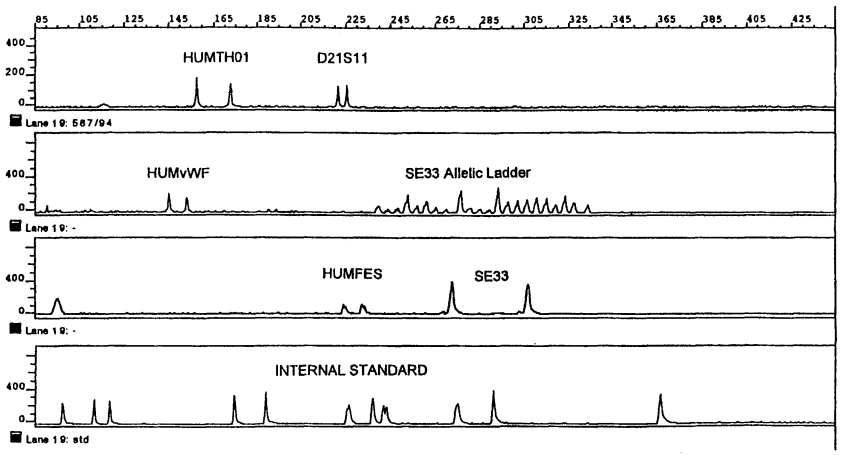

Figure 1: Electrophoretogram display of PCR-products of 5 STR systems run in the same gel lane

For all STR systems we set up data bases with a large population sample originating from southern and middle Germany. Allelic distributions (n = 966 - 2084 fragments) are shown in Figure 2. Chi square tests performed with all systems indicated that there is no significant deviation from the assumed Hardy-Weinberg equilibrium (data not shown).

Family studies

With regard to paternity testing, genetic stability of polymorphic DNA loci is of major interest. As a guideline, de novo mutation rates should not exceed 1%. We carried out extensive family studies consisting mainly of paternity cases investigated parallel to 12 conventional bloodgroup markers. Altogether 435 - 938 parent / child combinations were investigated for each system. Table 1 shows the individual mutation rates associated with the 5 loci. We conclude that these 5 STR loci are genetically stable and do not give rise to false exclusions as seen with some single locus systems.

Table 1: Genetic stability of STR loci

System	meiosis	mutat. events	rate
HUMTH01	938	0	< 0,11
HUMFES	870	0	< 0,12
HUMvWF	862	0	< 0,12
D21S11	435	0	< 0,23
ACTBP2	481	1	0,21

Table 2: Discrimination power of 5 STR systems

System	Hetero-zygosity	Power of exclusion	Mean EM value
HUMTH01	78,3%	57,0%	9,6335
HUMFES	67,8%	46,7%	9,7267
HUMvWF	81,1%	61,8%	9,5821
D21S11	83,9%	67,5%	9,5119
ACTBP2	94,7%	89,0%	9,0414
Combined		99,7%	7,4956