Simultaneous analysis of STR and VNTR polymorphisms.

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One Variable Number of Tandem Repeat (VNTR) polymorphism, D1S80(1), and two Short Tandem Repeat (STR) systems, ACTBP2 (2,3) and HUMTH01 (4) were simultaneously analysed on an Automatic Laser Fluorescent (A.L.F.) DNA Sequencer.
Allelic ladders were used as external standards and two internal size markers were added into each lane. For D1S80 and HUMTH01 21 and 8 distinct alleles were found respectively. ACTBP2 contained 35 alleles including alleles with intermediate sizes. These intermediate alleles are distributed all over the size range. The difference between two alleles can be as small as 1 basepair. Frequencies were established for the three loci and no mutations were found by analysing mother-child combinations. All three loci are in genetic equilibrium and give a combined exclusion capacity of 0.98.

MATERIALS AND METHODS

Amplification

DNA from randomly selected blood donors was amplified separately for D1S80, ACTBP2 and HUMTH01 (tab. 2). Additionally DNA from 212 mother-child combinations was amplified for all the systems.
For all amplifications the 50 ul reaction volume contained 0.5 uM of each primer, of which one was labelled with fluorescein, 200 uM of each dNTP and 1.5 uM of $MgCl_2$. For the D1S80 locus 2U of Taq poymerase was added and for the two STR loci 1U each. Amplification conditions are presented in table 1.

Table 1.

Locus	Temperatures (°C)	Times (sec.)	No of cycles
D1S80	94, 66, 70	42, 42, 470	25
ACTBP2	94, 60, 72 *	15, 30, 1+1+1.....	30
HUMTH01	94, 61, 72	45, 30, 1+1+1.....	34

* Prior to the original program a hot start at 94^0 C for 15 sec. was used.

A.L.F. analysis.

The amplified products were analysed on an Automatic Laser Fluorescent, A.L.F. DNA sequencer (Pharmacia LKB). Up to 1ul from the three different amplification products were mixed with approximately 5 fmol of two internal sizemarkers, 109 bp and 380 bp

respectively. This mixture was added into one lane of a 4 % hydrolink gel. In this way 36 samples and 4 external allelic ladders for each marker could be analysed in one run (fig. 1). The gel was used two or three times. The total running time was 4.5 hours at 1500 V. The fragment sizes were automatically determined by the software in basepairs or in number of repeats.

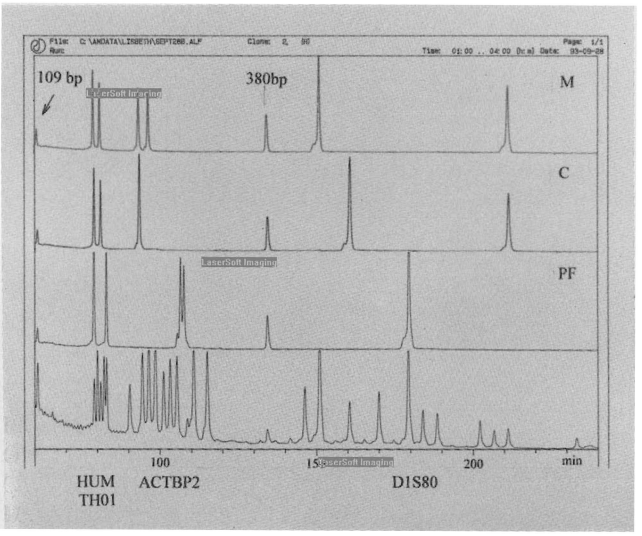

Fig. 1. Simultaneously analysed PCR fragments from mother (M), child (C) and putative father (PF) for HUMTH01 (79–83 min), ACTBP2 (90–115 min) and D1S80 (145–234 min.). Two internal size markers at 109 bp and 380 bp are marked with arrows.

RESULTS

For D1S80 21 alleles were found ranging from approximately 400 to 765 bp. The core sequence is 16 bp and the alleles were named after their number of repeats. The alleles number 18 and 24 are the most common ones with frequency values of 0.25 and 0.37 respectively (fig. 2).

HUMTH01 contained 8 alleles named in allele numbers 1–8. This locus ranged from 178 to 202 bp and the repetitive unit is AATG. Allele number 5 was the most common one with a frequency of 0.35 (fig. 2).

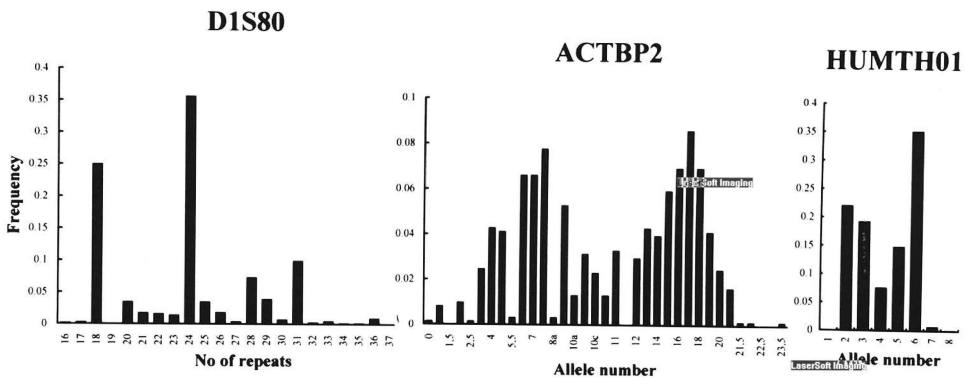

Fig. 2. Frequency diagrams for D1S80, ACTBP2 and HUMTH01.

The basepair difference between alleles 5 and 6 is 3 basepairs and there is only 1 basepair between alleles 6 and 7.
The ACTBP2 system is more complex and was more complicated to analyse due to the alleles of intermediate sizes. The difference between two close alleles can be as small as 1 basepair.
After having classified the alleles according to their basepair lengths, they were given allele numbers. We have found 35 alleles including the intermediates distributed all over the size range. The alleles range from 223 to 316 bp containing an AAAG repeat unit (fig. 2). For ACTBP2 the most common allele was number 17 (289 bp) with a frequency of 0.086.
No mutations were found for the loci after having analysed mother–child combinations. All markers were in genetic equilibrium according to Hardy Weinberg. The combined exclusion capacity is 0.98 for these loci.

Table 2.

LOCUS	NO. OF SAMPLES	EXCL. CAPACITY
D1S80	455	0.62
ACTBP2	302	0.88
HUMTH01	249	0.54
		0.98

CONCLUSION

The alleles of the D1S80 and HUMTH01 loci are distinct and are readily identified as number of repeats and as allele numbers. For D1S80 the allele frequencies are similar to those found in a Finnish and a North American population (5). For ACTBP2 the identification of alleles is difficult due to the close intermediate allele sizes. Under the present conditions we have had some difficulties with the reproducibility for these alleles. This problem may be solved by using internal size markers of accurate sizes close to the ACTBP2 size range.

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