SIMPLE AND RAPID TYPING OF STRs ON AN AUTOMATED DNA SEQUENCER

R. Decorte and J.-J. Cassiman

Center for Human Genetics, K.U.Leuven, Herestraat 49, B-3000 Leuven, Belgium

INTRODUCTION

Short Tandem Repeat (STR) loci are very abundant in the human genome: di-nucleotide repeats occur approximately every 6 kb while tri- and tetra-nucleotide repeats every 300 to 500 kb (Edwards 1991). The lower size of the polymorphic region increases the sensitivity for typing of small amounts of degraded DNA which is in contrast to VNTRs. It is therefore not surprising that STR loci have become the principal polymorphic markers in forensic DNA typing. Whereas, initially the analysis of the PCR products relied on radioactive procedures, it is now possible to type STR alleles in a non-radioactive manner either by silver staining or by electrophoresis of fluorescent PCR products on an automated DNA sequencer.

We have previously reported about the accuracy for sizing fluorescent-labelled VNTR alleles (APOB) on the A.L.F. DNA sequencer (Decorte 1993). In this paper, we evaluated the A.L.F. DNA sequencer for separating 4 STR loci simultaneously at high speed and with a high throughput. Furthermore, a population study was performed on 122 unrelated Caucasians of Belgian descent.

MATERIALS AND METHODS

Genomic DNA was extracted from venous blood samples according to standard procedures. Amplification of the STR loci HUMvWF, D21S11, HUMTHO1 and HPRT was done as described by Frégeau and Fourney (1993). The PCR primers have been reported by Frégeau and Fourney (1993) and the forward primers were labelled with fluorescein. In total 28 cycles were done on a GeneAmp 2400 or 9600 Cycler (Perkin-Elmer) in a reaction volume of 25 µl. PCR products were diluted five fold after quality control on 6% polyacrylamide gels. The diluted PCR products for the 4 STR loci were mixed together and 4 µl was applied to 4 µl of stop-mix (95% formamide, 5 mg/ml dextran blue and fluorescein-labelled PCR products of 123 and 375 bp). The PCR products were loaded on gel after 3 min. denaturation at 85°C. A.L.F. gels with a well-to-laser distance of 19 cm and spacers of 0.35 mm contained 6% Hydrolink Long Ranger (J.T. Baker), 7M Urea (BRL) and a running buffer of 0.6xTBE (53.4 mM Tris, 64.2 mM boric acid and 1.2 mM EDTA). Running conditions were 2000 V, 70 mA, 45 W and 50°C, 1.5 sec. sampling interval for 140 min. The same gel was reloaded with other samples without a pre-run of 10 min. Each run included two lanes with a combination of allele markers for each locus (Fig.1).

RESULTS AND DISCUSSION

The STR loci D21S11, HUMvWF, HUMTHO1 and HPRT were selected on the basis of non-overlapping allele size distributions (Table 1) and identical annealing conditions in the PCR (D21S11 and HUMvWF: 64°C; HUMTHO1 and HPRT: 60°C). Initially, we evaluated an A.L.F. gel with a laser-to-well distance of 10 cm and a 8% Hydrolink Long Ranger - 7M Urea gel. This way, it was possible to separate the STR alleles in a run of 75 min. The accuracy of the size

estimates was high (average of 99.92%) when two internal markers (123 and 375 bp) were included in each lane to compensate for any lane-to-lane differences. An average accuracy of 99.16% was obtained when no internal markers were used.

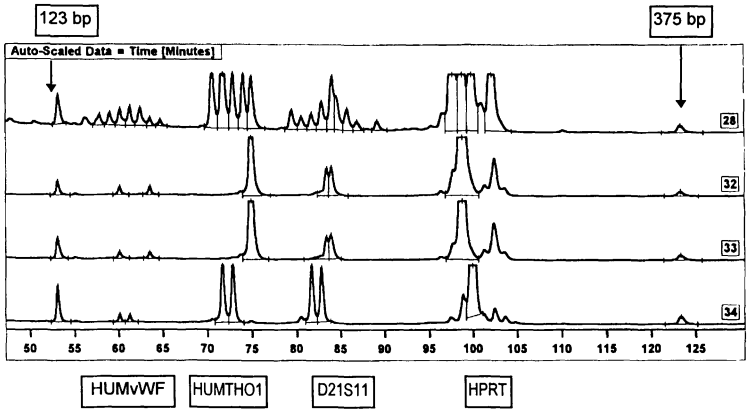

Figure 1: Multiplex analysis of 4 STR loci on the A.L.F. DNA sequencer.
Lane 28 contains the 4 allelic markers, Lane 32 and 33 are DNA samples from a twin and lane 34 is a positive control. The results are displayed in function of the running time.

During a population study for HUMTHO1, one allele was observed which showed a dif-ference of 1 bp (allele 10: 199 bp) with the preceding allele (9.3: 198 bp). However, exact sizing of this allele on the short gels was problematic: one of the 4 times that the sample was loaded on a short gel, resulted in a classification of the allele as a 9.3 allele. This could be overcome when the distance between the two alleles in a heterozygous DNA sample was taken into account. To avoid this complication, we developed a high speed sizing protocol on a regular A.L.F. gel (well-to-laser distance of 19 cm) as described in the materials and methods section. The run time increased from 75 min. to 125 min. without a significant decrease of the average accuracy (99.91%) compared to a 10 cm gel, although a 6% Hydrolink gel was used instead of a 8% (Fig. 1). The gels could be reloaded again and there was no difference in the accuracy of the size

Table 1: Summary of the results for the 4 STR loci in the Belgian population

Locus	Number of chromosomes	Size range (bp)	Heterozygo-sity (%)	Probability of paternity exclusion	Match probability
HUMvWF	244	138-162	80.9	0.621	0.064
HUMTHO1	204	179-199	78.9	0.582	0.077
D21S11	236	209-249	85.3	0.709	0.038
HPRT	149	275-299	73.2	0.345	0.114
			Cumulative:	**0.970**	**0.000021**

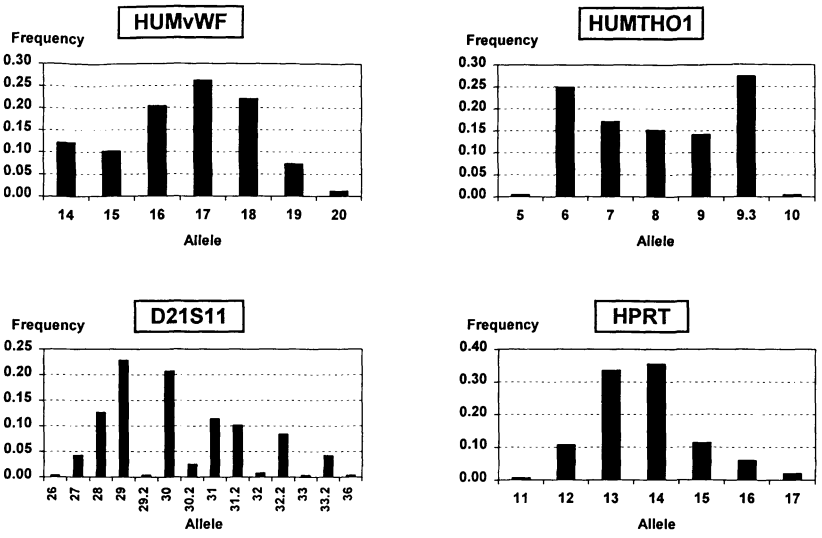

Figure 2: Frequency distribution of the STR alleles in the Belgian population.
Alleles indicate the number of repeats present in the PCR product according to the nomenclature recommended by the DNA commission of the ISFH.

estimates between the first (99.93%) and the second run (99.88%). This way, a throughput was obtained of 76 DNA samples for 4 loci in one working day.

The 4 STR loci were studied in a population of 122 unrelated Caucasian individuals. Seven alleles were observed for HUMvWF, HUMTHO1 and HPRT, and 14 alleles for D21S11 (Fig. 2). The expected heterozygosity for the four STR loci ranged between 85.3% (D21S11) and 73.2% (HPRT). The genotype distributions for all the four loci were in Hardy-Weinberg equilibrium. The combined match probability for forensic identity testing was 2.1×10^5 while the combined probability of paternity exclusion was 0.97 (Table 1). In combination with 3 highly polymorphic AMP-FLPs (D1S111, D17S5 and DXYS17), the match probability increased to 2.8×10^9 and the probability of paternity exclusion to 0.9988. Therefore, this highly discri-minating system can be used as a rapid and efficient method in the analysis of forensic evidence samples and for paternity determinations.

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