AUTOMATED DNA PROFILING EMPLOYING MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI.

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INTRODUCTION

Short tandem repeat (STR) loci are a class of polymorphic markers which occur throughout the human genome and which consist of simple tandemly repeated sequences 1-6bp in length. Their abundance, hypervariability and amenability to amplification by the polymerase chain reaction (PCR) make them ideal markers for use in the identification of individuals.

DNA profiling based on PCR amplification of STRs has the advantage of being more sensitive than conventional techniques. Furthermore, typing of material containing highly degraded DNA is more likely to be successful when using STR analysis (Hagelberg et al. 1991; Gill et al. 1992; Jeffreys et al. 1992).

Tri-, tetra- and pentameric STR loci appear to be less prone than dimeric STRs to artifactual "stutter" banding caused by enzyme slippage during amplification and such loci are therefore more suitable for routine forensic applications (Edwards et al. 1991; Fregeau and Fourney 1993; Kimpton et al 1993; Wiegand et al; 1993).

We have evaluated the use of automated fluorescence-based technology to automatically detect tri-, tetra- and pentameric STR loci electophoresed on denaturing polyacrylamide gels.

AUTOMATED STR DETECTION.

STR loci were fluorescently tagged during amplification by employment of a dye-labelled PCR primer. Amplified products electrophoresed on 6% denaturing polyacrylamide gels were detected by laser scanning on an Applied Biosystems automated DNA sequencer. An internal size standard consisting of fluorescently labelled Lambda *Pst*I restriction fragments was co-electrophoresd with each sample. This eliminated differences in electrophoretic mobility between gel lanes and allowed the automatic sizing of STR-PCR products with Genescan 672 analysis software.

The consistency of the automatic size calling was evaluated for each locus studied by examination of the distribution of computer-generated bands sizes for a number of samples run on different gels (Figure 1). All the STRs evaluated which contained straight-forward repeat regions were shown to be sized to a high degree of precision allowing unambiguous allele designation.

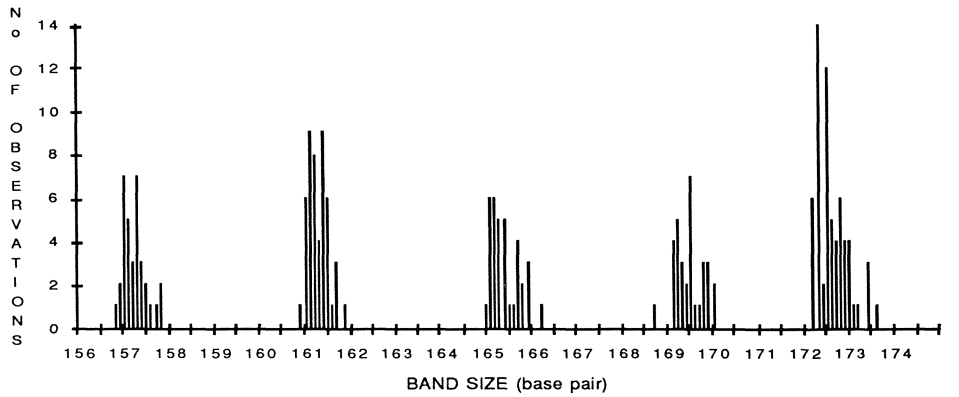

Figure 1: Frequency distribution of automatically generated TH01 PCR product sizes.

MULTIPLEX SYSTEMS

The availability of 4 distinguishable fluorescent dyes facilitates the development of STR multiplex PCR systems by the allowing the labelling of loci which have overlapping allele size ranges with different fluorescent dyes. By combining loci which have similar optimal amplification parameters and adjusting individual primer concentrations we have constructed 3 STR multiplex systems containing a total of 14 different loci. These multiplex systems have been employed to rapidly generate population databases and to assess the usefulness of the loci for the identification of individuals.

A further multiplex system was developed (Table 1, Fig 2) containing the four loci which we felt were most likely to be suitable for routine forensic identification, based on the information generated from the above database construction and the individual STRs amplification and sizing efficiency. All four loci within this quadruplex system have relatively simple repeat regions and display regularly spaced alleles differing by 4 bases - with the exception of one 2 base allele in the HUMF13A1 locus. This quadruplex has undergone detailed evaluation for use in routine forensic case work and found to be both robust and reproducible.

Table 1: Matching Probability (pM) of STR loci in multiplex 1

STR LOCUS	MATCHING PROBABILITY Caucasian	Afro-Carib.	Asian	SIZE RANGE (bp)	DYE LABEL
HUMVWA31/A	0.05	0.06	0.07	135-167	ABI "JOE"
HUMTH01	0.09	0.12	0.08	154 -178	ABI "FAM"
HUMF13A1	0.13	0.05	0.08	182-236	ABI "JOE"
HUMFES/FPS	0.16	0.09	0.14	214-238	ABI "FAM"
COMBINED pM	$1.1x10^{-4}$	$3.0x10^{-5}$	$5.9x10^{-5}$		
MOST COMMON	$8.0x10^{-4}$	$9.0x10^{-5}$	$4.4x10^{-5}$		

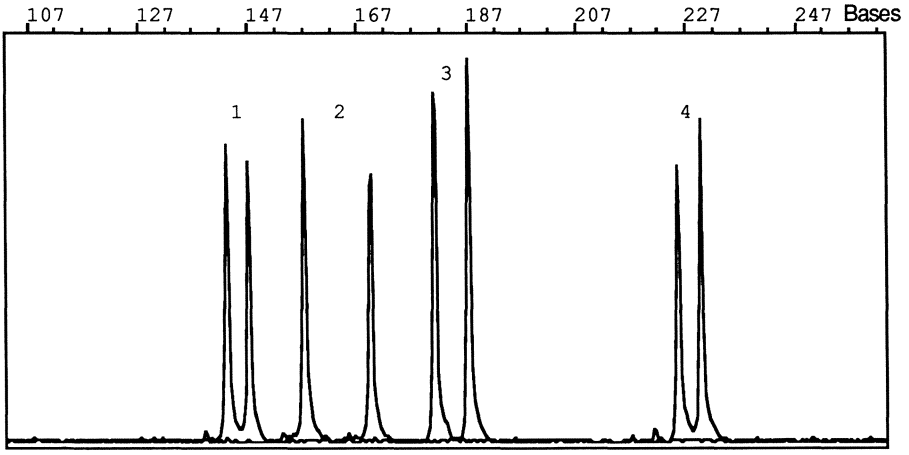

Figure 2: Electrophoretogram of a quadruplex amplification. Locus 1 HUMVWA31/A, locus 2 HUMTH01, locus 3 HUMF13A1, Locus 4 HUMFES/FPS.

HYPERVARIABLE LOCI

Certain highly polymorphic STR loci (eg. ACTBP2, APOAI1 and D11S554) contain complex compound repeat regions and may display alleles which differ by just 1bp (Adams et al 1993; Urquhart et al 1993). Although single base differences can be resolved on denaturing polyacrylamide gels, the consistency of automatic sizing employing the Lambda *Pst*I sizing standard is not sufficient to allow accurate allele designation. We have therefore constructed allelic ladder sizing standards for these hypervariable loci. By labelling these allelic ladder standards with a different dye marker to the individual samples we are able to co-electrophorese the ladder and sample in the same gel lane. The 672 Genescan analysis software then allows us to programme the sizes of the ladder bands into the computer thus allowing the automatic sizing of sample products to a high degree of precision. Using this approach we have developed a highly informative duplex amplification system containing the hypervariable loci ACTBP2 and D11S554. This system has considerable potential for routine forensic identification.

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