Automation of DNA Profiling by Fluorescent Labelling of PCR Products

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INTRODUCTION

Variable number tandem repeat (VNTR) and short tandem repeat (STR) loci display considerable variation within human populations and are useful markers for both the construction of human linkage maps (Nakamura et al. 1987) and for individual identification in forensic investigations (Hagelberg et al. 1991). PCR amplification of these loci has greatly improved the sensitivity of DNA profiling, and the amplified alleles from small VNTR loci such as D17S5, D1S80 and ApoB can be directly visualised and resolved on ethidium bromide-stained agarose or acrylamide gels (Horn et al. 1989, Kasai et al. 1990, Boerwinkle et al. 1989), whilst STRs are characterised on denaturing sequencing gels using sequence extension products as a size standard (Litt and Luty 1989; Weber and May 1989).

We describe here the use of fluorescent tagging of the PCR products coupled with their detection by laser scanning during electrophoresis to increase the precision and automation of VNTR/STR characterisation. Such technology is routinely used in sequence analysis and has been recently applied to the quantitative determination of Duchenne Muscular Dystrophy status and amplification of VNTR/STR loci (Mayrand et al. in press; A Edwards unpublished results). The utility of this approach is demonstrated in the analysis of incorrectly labelled fixed surgical specimens.

AMPLIFICATION OF VNTR LOCI

Locus D1S80 was amplified in the following reaction mix: 200µM each dNTP, 4U Taq polymerase 1X Amplification buffer, and 0.1µM each primer labelled with fluorescent dye 'JOE' (Applied Biosystems). Samples were denatured at 94°C for 4 mins, followed by 27 cycles of 94°C for 1 min, 56°C for 1 min and 70°C for 1.5 mins, with a final extension at 70°C for 5 mins. For locus D17S5 the reaction mix included 0.4µg each primer labelled with dye 'TAMRA'. Samples were denatured at 94°C for 5 mins, followed by 27 cycles of 94°C for 0.5 mins, 53°C for 0.5mins and 65°C for 4 mins, with a final extension at 65°C for 7 mins. The hypervariable region 3' to the ApoB locus was amplified using 0.1µM each primer labelled with dye 'FAM'. Samples were amplified through 26 cycles at 94°C for 1 min, followed by 58°C for 6 mins. Amplification products were analysed on an Applied Biosystems 362A Genescanner by combining 1µl of each PCR product with 6fmol internal size standard labelled with dye 'ROX', then loaded in a 2% SeaPlaque agarose gel with a well-to-read distance of 4cm. Electrophoresis was for 5.5 hours at 100V. The fragment sizes were automatically determined by the software generating a curve of best fit from the internal standard in each lane.

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More than 100 British Caucasians have been analysed at loci D17S5, D1S80 and ApoB by this method. Alleles differing by a single 15bp repeat (ApoB) were readily resolved. By using 3 different dyes for the three different loci it was possible to combine the amplification products and analyse them simultaneously in the same lane thereby providing a rapid and highly discriminating test. The automatically called band sizes fall in discrete groups which define the alleles and the results are summarised as follows:

Table 1. Discrimination power of three VNTR loci

Locus	No. Observed Allele Sizes	Heterozygosity	Prob. of random Match (pM)	Commonest Genotype
D17S5	15	87%	0.035	0.10
D1S80	20	81%	0.065	0.16
ApoB	15	64%	0.081	0.16
		Combined:	2×10^{-4}	2.8×10^{-3}

All 3 VNTR loci show broadly similar levels of amplification efficiency, with results obtainable from less than 1ng genomic DNA. The absolute limit for reliable VNTR amplification and analysis is between 100pg and 1ng. At low initial copy numbers of template DNA, there is a danger of only amplifying one allele in heterozygotes. To date we have had preliminary success with a duplex MCT118/ApoB reaction and a triplex MCT118/ApoB/Col 2A1 (Wu et al. 1990) reaction. The choice of compatible loci will be widened as more systems become available.

STR AMPLIFICATION

STR HUMTH01 was amplified using primers cited by Edwards (unpublished results) one of which was dye-labelled: 29 cycles of 94°C for 45 seconds, 60°C for 30 seconds and 72°C for 30 seconds. Amplification products were electrophoresed with an internal standard in a 6% denaturing acrylamide gel using an Applied Biosystems 373A Automated Sequencer, and fragment sizes were determined automatically with ABI 675 software. In a limited survey of 69 unrelated British Caucasians 5 different alleles were detected with an overall heterozygosity of 81%. Amplification products were accurately sized to the nearest 1bp. In addition, sub-banding problems normally associated with dimeric microsatellites were minimal with the trimeric and tetrameric microsatellites utilised.

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Table 2. Allele frequency distribution for 69 individuals at locus HUMTH01

Allele size (bp)	Frequency
191	0.22
195	0.20
199	0.11
203	0.12
207	0.34

ANALYSIS OF FIXED SURGICAL SPECIMENS

Four DNA samples were extracted from paraffin-embedded surgical tissue specimens, which were thought to have been mis-paired, using essentially the protocol of Wright and Manos (1990). Attempts were made to amplify the samples at loci ApoB, D1S80, D17S5, HUMTH01[AATG]n and HUMFABP[AAT]n (Edwards unpublished results), broadly using the conditions described previously. No amplification products were generated at loci ApoB and D1S80, but 2 of the samples generated a single band of 304bp at locus D17S5. Failure to amplify bands within the size range of ApoB and D1S80 (381-932bp) is likely to be due to the DNA being predominantly of very low molecular weight. In contrast, all 4 samples yielded strong results from HUMTH01 and HUMFABP (size range 191-238bp), which correlated with the D17S5 match result and confirmed that the samples had been incorrectly paired. These results may indicate that the use of microsatellites for forensic purposes may prove to be preferable to VNTRs.

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