Sequencing and Four-State MVR-PCR Mapping of the Highly Polymorphic D1S7 Locus (MS1) by Fluorescence Detection

Peter Hau[1], Nigel Watson[2]
[1] Forensic Science Laboratory, Lothian & Borders Police, 11 Howden Hall Road, Edinburgh, EH16 6TF, Scotland, UK.
[2] Forensic Science Unit, Department of Pure and Applied Chemistry, Royal College,
204 George Street, Glasgow, G1 1XW, Scotland, UK

The cloning of highly repetitive DNA sequences in human minisatellites has been shown to be highly problematic. At least some of the most variable DNA fragments detected in a DNA fingerprint, MS1, are amenable to cloning provided that the inserts are stabilized by propagation of the vector in a specialized E. coli host. The D1S7 locus (MS1), is the most variable locus studied, with greater than 99% allele length heterozygosity and consists of 9bp tandem repeat units containing many sequence variations. The classic technique described by Jeffreys *et al* (1991) is known as Minisatellite Variant Repeat Polymerase Chain Reaction (MVR-PCR). MVR-PCR generates PCR products of lengths that reflect the positions of the two major repeat types ‘ A’ and ‘ T’ . In addition to D1S8, D7S21 (MS31) and D16S309 (MS205) have been mapped successfully by Neil and Armour respectively.

This paper reports a fourth locus, D1S7, that is amenable to cloning and sequencing as well as MVR-PCR mapping. The purified plasmid DNA was sequenced using the fluorescent dye terminator sequencing kit on a 373A sequencer (ABD). The same plasmid DNA preps prepared for sequencing were used for MVR-PCR mapping. The sequenced cloned PCR fragments revealed 44 MS1 repeats containing 6 repeat types, plus the flanking DNA sequence. The 4-State MVR-PCR mapping of the same clones gave identical haploid profiles containing the same order of repeat types as those sequenced; furthermore, up to 61 repeats were mapped.

Diploid MVR-PCR maps were generated using genomic DNA from 3 unrelated individuals. Up to 49 repeats were reliably mapped from these individuals and each had a unique MVR-PCR map. Only the first 10 repeats were required to be read in order to distinguish between the 3 individuals. The D1S7 locus is very useful in discriminating between unrelated individuals. Perhaps it would be a useful tool for forensic applications