Polymerase chain reaction (PCR) for detection of MspI polymorphism at the D3S3 locus

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Source/Description: The insert from pMS1-37 detects an MspI polymorphism (1), and itself contains the polymorphic site. The DNA sequence which includes this site was determined (EMBL accession number X58033) and used to design PCR primers to amplify the region and generate a fragment of 828 bp.

Primer Sequences:

D3S3-1 5'-CAGAAGGACATATTTCCCATTTG-3'

D3S3-2 5'-GCAGTTTCCTCTAGCTTTTACT-3'

Polymorphism: MspI digestion of the PCR product of 828 bp (A1) yields fragments of 442 + 386 bp (A2) if the site is present.

Frequency: Allele frequencies differed from published estimates (1).

	Current work n = 76	Barker, <i>et al.</i> (1) n = 34
Allele A1	0.10	0.04
Allele A2	0.90	0.96

Chromosomal Location: D3S3 has been localised to 3p13-14.2 (2).

Other Comments: PCR amplification was performed in a volume of 15 μ l containing 0.3 μ g human DNA, 0.2 μ M each primer, 200 μ M each dNTP, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris pH 8.3, 0.01% gelatin, 1% Triton X100 and 0.4 units Taq polymerase (Promega). 30 cycles of PCR were carried out, each cycle consisting of 95°C for 0.5 minute, 55°C for 1 minute and 72°C for 1 minute. The entire PCR product was electrophoresed on a 4% NuSieve 3:1 gel after overnight digestion with MspI.

Acknowledgements: pMS1-37 was obtained from the Japanese Cancer Research Resources Bank. PSG is supported by an MRC Training Fellowship.

References: 1) Barker, D. *et al.* (1984) *Cell* **36**, 131-138. 2) Albertson, D.G. *et al.* (1989) *Hum. Genet.* **83**, 127-132.

Figure 1. MspI digestion of D3S3 polymorphic region amplified by PCR. Examples of two genotypes are indicated. (Third possible genotype not available.) DNA molecular weight marker was Φ x174 cut with HaeIII.

Polymerase chain reaction (PCR) for detection of MspI polymorphism at the D3S6 locus

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Source/Description: A clone spanning the MspI polymorphic site was isolated by using the insert from DR82 (1) to screen a human genomic library constructed from EBV transformed peripheral blood lymphocytes. The DNA sequence which includes this site was determined (EMBL accession number X58036) and used to design PCR primers to amplify the region and generate a fragment of 510 bp.

Primer Sequences:

D3S6-1 5'-CCTGTCCACATTGAACTCCT-3'

D3S6-2 5'-GAACCACTGCTTCTGAAATG-3'

Polymorphism: MspI digestion of the PCR product of 510 bp (A1) yields two fragments of 255 bp (A2) if the site is present.

Frequency: Allele frequencies differed from published estimates (1).

	Current work n = 77	Willard, <i>et al.</i> n = 9
Allele A1	0.92	0.78
Allele A2	0.08	0.22

Chromosomal Location: D3S6 has been localised to 3p14.2-14.5 (2).

Other Comments: PCR amplification was performed in a volume of 15 μ l containing 0.3 μ g human DNA, 0.2 μ M each primer, 200 μ M each dNTP, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris pH 8.3, 0.01% gelatin, 1% Triton X100 and 0.4 units Taq polymerase (Promega). 30 cycles of PCR were carried out, each cycle consisting of 95°C for 0.5 minute, 58°C for 1 minute and 72°C for 1 minute. The entire PCR product was electrophoresed on a 4% NuSieve 3:1 gel after overnight digestion with MspI.

Acknowledgements: DR82 was obtained from Dr A.Diesel. PSG is supported by an MRC Training Fellowship.

References: 1) Willard, H.F. *et al.* (1985) *Cytogenet. Cell Genet.* **40**, 391. 2) Albertson, D.G. *et al.* (1989) *Hum. Genet.* **83**, 127-132.

Figure 1. MspI digestion of D3S6 polymorphic region amplified by PCR. Examples of two genotypes are indicated. (Third possible genotype not available.) DNA molecular weight marker was Φ x174 cut with HaeIII.