Polymerase chain reaction (PCR) for detection of MspI polymorphism at the D3S30 locus

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Source/Description: The insert from pYNZ86.1 detects an Msp polymorphism (1), and itself spans the polymorphic site. The DNA sequence which includes this site was determined (EMBL accession number, X58034) and used to design PCR primers to amplify the region and generate a fragment of 293 bp.

Primer Sequences:
D3S30-1 5'-AAAGACTTCCTTCTGAGATGGG-3'
D3S30-2 5'-CAGTCTGCTGTGGTTTCAGTCT-3'

Polymorphism: MspI digestion of the PCR product of 293 bp (A1) yields fragments of 224 + 69 bp (A2) if the site is present.

Frequency: Allele frequencies agreed with published estimates (1).

Chromosomal Location: D3S30 has been localised to 3p (2), and to 3p13−14 (Dr A.C.Heppell-Parton, personal communication).

Other Comments: PCR amplification was performed in a volume of 15 μl containing 0.3 μg human DNA, 0.2 μM each primer, 200 μM each dNTP, 1.5 mM $MgCl_2$, 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: pYNZ86.1 was obtained from the Japanese Cancer Research Resources Bank. PSG is supported by an MRC Training Fellowship.

References: 1) Nakamura,Y. *et al.* (1987) *Nucl. Acids Res.* **15**, 10079. 2) Leppert,M. *et al.* (1987) *Cytogenet. Cell Genet.* **46**, 648.

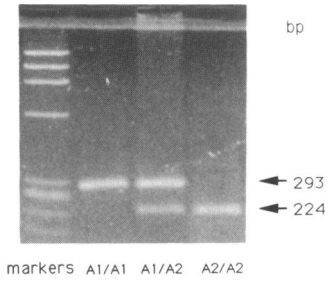

Figure 1. MspI digestion of D3S30 polymorphic region amplified by PCR. Example of the three genotypes are indicated. DNA molecular weight marker was Φx174 cut with HaeIII.

Polymerase chain reaction (PCR) for detection of BamHI polymorphism at the THRB gene

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Source/Description: A clone which contained the BamHI polymorphic site was isolated using the insert from pHeA2 (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 X58039) and used to design PCR primers to amplify the region and generate a fragment of 364 bp.

Primer Sequences:
EA2B-1 5'-AACATCCAAGATGGCTGGAGTT-3'
EA2B-2 5'-CCCTGAAAATGCAGAGGACTCT-3'

Polymorphism: BamHI digestion of the PCR product of 364 bp (A1) yields two fragments each of 182 bp (A2) if the site is present.

Frequency: Allele frequencies agreed with published estimates (1).

Chromosomal Location: THRB has been localised to 3p24 (2).

Other Comments: PCR amplification was performed in a volume of 15 μl containing 0.3 μg human DNA, 0.2 μM each primer, 200 μM each dNTP, 1.5 mM $MgCl_2$, 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 BamHI. Although digestion was often incomplete (see homozygous A2/A2 in figure), genotype assignment was always unequivocal.

Acknowledgements: pHeA2 was obtained from Dr N.Spurr. PSG is supported by an MRC Training Fellowship.

References: 1) Middleton,P.G. *et al.* (1986) *Nucl. Acids Res.* **14**, 1925. 2) Albertson,D.G. *et al.* (1989) *Hum. Genet.* **83**, 127−132.

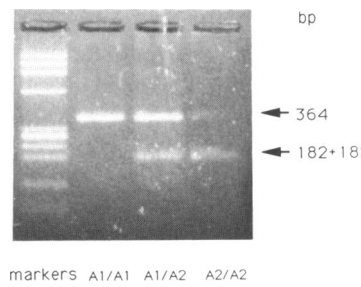

Figure 1. BamHI digestion of a THRB polymorphic region amplified by PCR. Examples of the three genotypes are indicated. DNA molecular weight marker was Φx174 cut with HaeIII.