PCR detection of the HindIII polymorphism at the thyroid hormone receptor, beta, gene (THRB)

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Source/Description: A clone (pλEAβ3, sub-clone of λEAβ) which contained the HindIII polymorphic site was isolated by using the insert from pBH302 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 X58041) and used to design PCR primers to amplify the region and generate a fragment of 432 bp.

Primer Sequences:
EAβH-1 5'-ACGTTAGTGGCTCATATGAG-3'
EAβH-2 5'-TCATTCGAGTTAGTGCAAAG-3'

Polymorphism: HindIII digestion of the PCR product of 432 bp (A1) yields two fragments of 221 + 211 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 HindIII.

Acknowledgements: pBH302 was obtained from Dr. W.Bradley. PSG is supported by an MRC Training Fellowship.

References: 1) Gareau,J.-L.P. *et al.* (1988) *Nucl. Acids Res.* **16**, 1223. 2) Albertson,D.G. *et al.* (1989) *Hum. Genet.* **83**, 127–132.

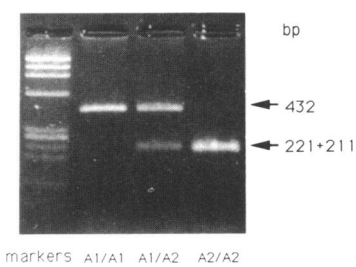

Figure 1. HindIII digestion of a THRB polymorphic region amplified by PCR. Examples of the three genotypes are indicated. DNA molecular weight marker was Φx174 cut with HaeIII.

Polymerase chain reaction (PCR) for detection of MspI polymorphism at the D3S2 locus

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Source/Description: The insert from pHF12-32 detects an Msp polymorphism (1), and itself contains the polymorphic site. The DNA sequence which includes this site was determined (EMBL accession number X58032) and used to design PCR primers to amplify the region and generate a fragment of 473 bp.

Primer Sequences:
D3S2-1 5'-CCAAGTGGCAGAGCTACTTA-3'
D3S2-2 5'-ACTATCTTGCCCTAGAGCAA-3'

Polymorphism: MspI digestion of the PCR product of 473 bp (A1) yields fragments of 237 + 236 bp (A2) if the site is present.

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

Chromosomal Location: D3S2 has been localised to 3p21.1 (2) and to 3p14 (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: pHF12-32 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) Drabkin,H. *et al.* (1989) *Genomics* **4**, 518–529.

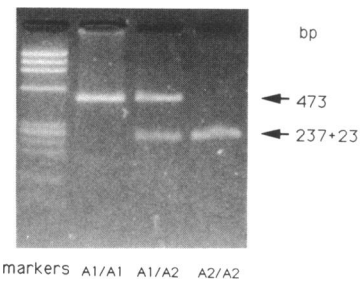

Figure 1. MspI digestion of D3S2 polymorphic region amplified by PCR. Examples of the three genotypes are indicated. DNA molecular weight marker was Φx174 cut with HaeIII.