Polymerase chain reaction (PCR) for detection of MspI and DraI polymorphism at the THRB gene

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Source/Description: The insert from pBH302 detects MspI and DraI polymorphisms at the THRB gene (1) and itself contains both of these polymorphic sites. The DNA sequence which includes these sites was determined (EMBL accession number X58042) and used to design PCR primers to amplify the region and generate a fragment of 683 bp.

Primer Sequences:

EA β MD-1 5'-AACGTTGGACCTCAAGCCCAT-3'
EA β MD-2 5'-CAGGGTTCCTTCTATAAACATG-3'

Polymorphism: MspI digestion of the PCR product of 683 bp (M1) yields two fragments of 560 + 123 bp (M2) if the MspI site is present. DraI digestion of the PCR product yields a fragment of 657 bp (D1), or two fragments of 405 + 252 bp (D2) if the polymorphic DraI 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 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 or DraI.

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

References: 1) Brauch, H. *et al.* (1990) *Nucl. Acids Res.* **18**, 2833. 2) Albertson, D.G. *et al.* (1989) *Hum. Genet.* **83**, 127–132.

Figure 1. MspI digestion (lanes left of markers) or DraI digestion (lanes right of markers) of a THRB polymorphic region amplified by PCR. Examples of the three genotypes are indicated for each of the two polymorphisms. DNA molecular weight marker was Φ x174 cut with HaeIII.

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

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Source/Description: A clone which spanned the EcoRI 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 X58040) and used to design PCR primers to amplify the region and generate a fragment of 367 bp.

Primer Sequences:

EA2R-1 5'-GCTAATCTAGAAATGTATTTACTATAGG-3'
EA2R-2 5'-TTTACTTCATGAAGCTGGCACG-3'

Polymorphism: EcoRI digestion of the PCR product of 367 bp (A1) yields two fragments of 298 + 69 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 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 EcoRI.

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. EcoRI digestion of a THRB polymorphic region amplified by PCR. Examples of the three genotypes are indicated. DNA molecular weight marker was Φ x174 cut with HaeIII.