Polymerase chain reaction (PCR) for detection of HindIII polymorphism at the D3F15S2 locus

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Source/Description: pH3H2 detects a diallelic polymorphism on chromosome 3, and 2 intense constant bands from chromosome 1 where the locus is transposed and amplified (1). The inserts from pH3H2 and p3A flank the HindIII polymorphic site and sequences from them were determined and used to design primers which amplified a 340 bp product from genomic DNA. This product was derived both from chromosome 3, including the polymorphic site, and also from the transposed regions on chromosome 1. Their sequences were determined (EMBL accession numbers X58031, X58037 and X58038) and primer H3H2-destab. was designed which created an A:G mismatch at its 3' terminal end on each of the chromosome 1 sequences. In addition it was further mismatched at its 3'-2 position for both chromosome 3 and 1 sequences. Using this primer and primer H3H2-5, chromosome 1 sequences were refractory to amplification and the product of 535 bp was derived entirely from chromosome 3.

Primer Sequences:

H3H2-destab. 5'-TAGATCTGAGCCCGGTGCCTGGGCCAC-GAA-3'
H3H2-5 5'-GAGTCCATGGCAGCAGTTGGCAACCA-TGGC-3'

Polymorphism: HindIII digestion of the PCR product of 535 bp (A1) yields fragments of 383 + 152 bp (A2) if the site is present.

Frequency: Allele frequencies agreed with published estimates (2).

Chromosomal Location: D3F15S2 has been localised to 3p21 (3).

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 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, 65°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.

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References: 1) Carritt,B. *et al.* (1986) *Amer. J. Hum. Genet.* **38**, 428-436. 2) Goode,M.E. *et al.* (1986) *Am. J. Hum. Genet.* **38**, 437-446. 3) Kok,K. *et al.* (1987) *Nature* **330**, 578-581.

Polymerase chain reaction (PCR) for detection of RsaI polymorphism at the D3S32 locus

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Source/Description: The insert from pEFD145.1 detects an RsaI polymorphism (1), and itself contains the polymorphic site. The DNA sequence which includes this site was determined (EMBL accession number X58035) and used to design PCR primers to amplify the region and generate a fragment of 421 bp.

Primer Sequences:

D3S32-1 5'-TTGGCGGTGGGAGCCTGAAGTGT-3'
D3S32-2 5'-GGCTGGAAGTGGAGGAGTGCATG-3'

Polymorphism: RsaI digestion of the PCR product of 421 bp (A1) yields fragments of 211+210 bp (A2) if the site is present.

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

Chromosomal Location: D3S32 has been localised to 3p (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 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. After amplification and before digestion a nonspecific band of approximately 140 bp was often seen (see heterozygous sample in figure). However genotype assignment was unequivocal.

Acknowledgements: pEFD145.1 was obtained from the Japanese Cancer Research Resources Bank. PSG is supported by an MRC Training Fellowship.

References: 1) Fujimoto,E. *et al.* (1988) *Nucl. Acids Res.* **16**, 9357. 2) Leppert,M. *et al.* (1987) *Cytogenet. Cell Genet.* **46**, 648.

Figure 1. RsaI digestion of D3S32 polymorphic region amplified by PCR. Examples of the three genotypes are indicated. DNA molecular weight marker was Φ x174 cut with HaeIII.