PCR detection of two RFLP's at the D21S13 locus

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We recently identified several new polymorphisms detected by probe pGSM21 at the locus D21S13 (1, 2). Two of these polymorphisms, a high frequency EcoRI polymorphism (1) and a less frequent TaqI polymorphism (2), are difficult to detect by standard Southern blot techniques. Here we report oligo primer sequences defining a 460 bp fragment overlapping both the EcoRI and TaqI polymorphic sites.

PCR Primers:
PSCR13-A: 5'-ATC CAT TCA TCC ATT CTC CC-3'
PSCR13-D: 5'-CAA CAT CAG GTC AAC CAG AG-3'

Polymorphisms: EcoRI digest of the amplified fragment identifies two alleles: E1: 390 bp, E2: 260 bp + 130 bp and a constant fragment of 70 bp (see figure). TaqI digest identifies two alleles: C1: 460 bp, C2: 360 bp + 100 bp.

Frequency: Allele frequencies were calculated from 36 individuals for the EcoRI polymorphism: E1: 0.34, E2: 0.66 and from 45 individuals for the TaqI polymorphism: C1: 0.90, C2: 0.10.

Chromosomal Location: 21q11.2 (3).

Mendelian Inheritance: Codominant inheritance of both polymorphisms was demonstrated in two extended families with Alzheimer's disease (106 individuals).

PCR Conditions: The PCR reaction is carried out in a total volume of 50 µl containing approximately 250 ng DNA, 2 u Taq DNA polymerase. 50 pmol of each primer, 200 µM dNTP's, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin. The amplification is performed for 30 cycles with an annealing temperature of 58°C. The amplified product is digested either with EcoRI or TaqI and the DNA fragments are analyzed on 1.5% agarose gels.

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RFLP for BglII at the human tyrosinase (TYR) locus

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Source/Description: 1.5-kb EcoRI-PstI fragment from complete mouse tyrosinase cDNA plasmid pTyrsJ (1) or complete 1.9-kb human tyrosinase cDNA plasmid pMel34 (2).

Polymorphism: BglII detects a two-allele polymorphism with fragment lengths of either 5.8 kb (A1) or 5.6 kb (A2). Constant bands of 17, 6.6, 4.3, 3.25, and 1.9 kb are also seen. A PCR fragment containing only the first exon of the human tyrosinase gene detects only the 5.6/5.8-kb polymorphic fragment.

Frequency: Estimated from 24 unrelated normal Caucasians of northern European ethnic origin.
A1 (5.8-kb fragment): 0.50
A2 (5.6-kb fragment): 0.50

Chromosomal Localization: The human tyrosinase gene has been mapped to chromosome 11q14→21 (3).

Mendelian Inheritance: Autosomal codominant segregation was observed in three large families.

Probe Availability: pTyrsJ was supplied by T.Takeuchi (1) and pMel34 by B.Kwon (2).

Acknowledgements: Aided by grant AR39892 from the National Institutes of Health and Clinical Research Grant No. 6–408 from the March of Dimes Birth Defects Foundation.

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