PCR detection of the MspI (Aa) RFLP at the human phenylalanine hydroxylase (PAH) locus

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Source/Description: phPAH 247, a human phenylalanine hydroxylase cDNA clone 2448 bp in length, was previously reported to detect at least 10 polymorphic sites at the PAH locus (1). We have used this cDNA to identify a lambda clone which has inserted genomic DNA covering exon 5 up to exon 12 of the PAH gene. Subsequently we isolated a 4.5 BamHI-fragment harbouring intron 7, exon 8 and 3.5 kb flanking sequences of intron 8. Using this plasmid clone we were able to map the precise location of the MspI polymorphic site which resides within this fragment.

Polymorphism: MspI (C/CGG) identifies a two allele polymorphism with fragments of either 23 kb (−) (A1) or 19 kb and 4 kb (+) (A2) in length.

Frequency: Studied in 202 unrelated Europeans:

Non-PKU:	A1 0.393	PKU:	A1 0.355
	A2 0.607		A2 0.645 (2)

Chromosomal Location: The human PAH gene has been localised to 12q22-q24 (3).

Mendelian Inheritance: Mendelian inheritance was demonstrated in all two-generation families tested.

Other Comments: Oligonucleotides used for amplification are 5′-TGAGCATATTGTATCTGCCC-3′ and 5′-CACATGTCCC-AACAGCTCAT-3′. PCR was performed using 200 ng genomic DNA and 50 pmoles of each primer. Initial denaturation for 240 sec followed by 30 cycles of 40 sec at 93°C, 40 sec at 72°C and 70 sec at 50°C. The final extension was at 72°C for 300 sec. PCR amplification yields a 425 bp fragment. If the polymorphic site is present this amplification product can be cleaved by MspI into a 300 bp and a 125 bp long fragment (fig). Our analyses revealed that this polymorphic site is located 268 bp 5′ to exon 8.

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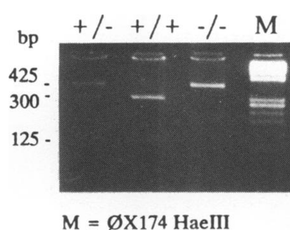
References: 1)Lidsky *et al.* (1985) *Am. J. Hum. Gen.* **37**, 619−634. 2)Daiger *et al.* (1989) *Am. J. Hum. Gen.* **45**, 310−318. 3)Lidsky *et al.* (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6221−6225.



M = ØX174 HaeIII

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Dinucleotide repeat polymorphism at the T cell receptor δ locus (TCRD)

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Source/Description: A polymorphic $(GT)_n$ repeat is located in the third intron of the T cell receptor δ gene (TCRD). The Polymerase Chain Reaction (PCR) was used to selectively amplify the sequence from genomic DNA using two oligonucleotides flanking the repeat. The predicted length of the amplified region was 126 bp.

Primer Sequences:
GTTAGTGGAAGAGCAGAGCA−GT strand GCTGAGAC-TAAACCTACCAC−CA strand

Frequency: Estimated from 104 chromosomes of unrelated CEPH family parents (Caucasians) and 26 chromosomes of unrelated Irish individuals. PIC = 0.74.

Allele (bp)	Frequency	Allele (bp)	Frequency
E1 128	0.0616	E4 122	0.2615
E2 126	0.3077	E5 120	0.1615
E3 124	0.1769	E6 118	0.0308

Chromosomal Location: The TCRD locus has been assigned to chromosome 14q11.2 (1).

Mendelian Inheritance: Co-dominant segregation was observed in one large two generation Irish family TCDG and in 4 CEPH families.

Other Comments: The PCR reactions were carried out as described in the reference (2) except that 50 mM KCl was replaced by 5mM KCl, 10 mM NH_4Cl. The amplification conditions were: 94°C; 7 min, followed by 30 cycles of 94°C; 1 min, 55°C; 2 min, 72°C; 3 min. The PCR product was fractionated on 8% denaturing polyacrylamide gels. The allele sizes were calculated by comparison to pUC19 DNA sequencing ladder. The dinucleotide repeat sequence at the TCRD locus was of the form: TT $(GT)_{18}$ AC.

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