PCR detection of the PvuII (Ea) 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 2, intron 2 and exon 3 of the PAH gene. Subsequently we isolated the 4 kb BglII-fragment harbouring exon 2 and flanking intronic sequences. Using this plasmid clone we were able to map the precise location of the PvuII polymorphic site which resides within this fragment.

Polymorphism: PvuII (CAG/CTG) identifies a two allele polymorphism with fragments of either 19 kb (–) (E1) or 6 kb (+) (E2) in length.

Frequency: Studied in 202 unrelated Europeans:

Non-PKU:	E1 0.271	PKU:	E1 0.087
	E2 0.729		E2 0.913 (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'-GGCATGACTGGATACGATTAG-3' and 5'-CTAGACT-CAGAATGCCTGGG-3'. PCR was performed using 200 ng genomic DNA and 50 pmoles of each primer. Initial denaturation for 240 sec was 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 374 bp fragment. If the polymorphic site is present this amplification product can be cleaved by PvuII into a 225 bp and a 149 bp long fragment (fig). Our analyses revealed that this polymorphic site is located in the second intron of the PAH gene 1.4 kb 3' to exon 2.

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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.

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PCR detection of the BglII 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 lambda clones covering the genomic 5' region of the gene and subsequently to isolate a 3.6 kb BglII-fragment harbouring exon 1 and flanking intronic sequences. Using this fragment which represents the larger band of the BglII polymorphism (1) we were able to map the precise location of the polymorphic site.

Polymorphism: BglII (A/GATCT) identifies a two allele polymorphism with fragments of either 3.6 kb (–) (D1) or 1.7 kb (+) (D2) in length.

Frequency: Studied in 202 unrelated Europeans:

Non-PKU:	D1 0.729	D2 0.271	PKU:	D1 0.891	D2 0.109 (2)
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Chromosomal Location: The human PAH gene has been localised to 12q22-q24.2 (3).

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

Other Comments: Oligonucleotides used for amplification are 5'-GCAGGAACTCTCTGACTTTGG-3' and 5'-TGGCAGTT-CTGGAGGCCAGA-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 290 bp fragment. If the polymorphic site is present this amplification product can be cleaved by BglII into a 209 bp and a 81 bp long fragment (fig). Sequence analyses revealed that the polymorphic site is located 55 bp 3' to exon 1.

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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.

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