

NcoI RFLP in the pseudogene (CYP2D8P) of the human debrisoquine 4-hydroxylase locus

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Source/Description: A 1567 bp human cytochrome P450IID1 cDNA probe (1) or the following couple of PCR-primers selected from the recently published sequence (2) : 5'-CTCGCATGCCCTGCACCACT-3' (upstream) and 5'-AGGTGGTGAAGAGCATCATC-3' (downstream).

Polymorphism: On Southern blots of genomic DNA, NcoI identifies two allelic bands at 6 kb (allele 1) and 4.3 kb (allele 2), respectively. They correspond to fragments of 595 bp (allele 1) and 415 bp + 180 bp (allele 2) in the NcoI digest of the PCR-product.

Protocol: Using 100 pmoles of each primer and 1 µg of genomic DNA the target sequence was amplified through 30 cycles of denaturation (94°C, 60 sec), annealing (55°C, 30 sec) and extension (72°C, 30 sec). One tenth of the PCR-product was digested with NcoI, size-separated on 6% polyacrylamide gel, stained with ethidium bromide and UV-visualized.

Frequency: As determined from 37 unrelated French Caucasians
Allele 1 0.55
Allele 2 0.45

Chromosomal Localization: The human CYP2D loci have been localized on the long arm of chromosome 22 (1) and CYP2D8P between immunoglobulin (q11.1 and q11.2) and mitochondrial aconitase (q11.2–q11.31)

Mendelian Inheritance: Codominant segregation of the alleles was observed in 3 Caucasian families.

Probe Availability: For the cDNA probe, request to U.A. Meyer (1).

Other Comments: On Southern blots, constant bands at 9.2 kb and 7.5 kb are observed which correspond to the CYP2D6 and CYP2D7 genes.

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References: 1) Gonzalez,F.J., Vilbois,F., Hardwick,J.P., McBride,O.W., Nebert,D.W., Gelboin,H.V., Meyer,U.A. (1988) *Genomics* 2, 174–179. 2) Kimura,S., Umeno,M., Skoda,R.C., Meyer,U.A. and Gonzalez,F.J. (1989) *Am. J. Hum. Genet.* 45, 889–904.

FnuD II RFLP at the human dopamine-β-hydroxylase (DβH) locus

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Source/Description: A 142 bp fragment of exon 11 of the DβH gene was amplified by the Polymerase Chain Reaction.

Polymorphism: FnuD II digestion gives a two allele polymorphism. In the absence of the polymorphic FnuD II site, 95 (A1) and 47 bp fragments are seen. In the presence of the polymorphic site, the 95 bp fragment yields 66 bp and 29 bp fragments (A2) and the 47 bp constant fragment remains.

Frequencies: Studied in 26 unrelated Black Americans
A1 : 0.04
A2 : 0.96

Studied in 48 unrelated Caucasians
A1 : 0.12
A2 : 0.88

Studied in 37 unrelated Centre d'Étude du Polymorphisme Humain (CEPH; Human Polymorphism Study Center) panel members
A1 : 0.07
A2 : 0.93

Chromosomal Location: 9q34

Mendelian Inheritance: Codominant inheritance was demonstrated in 6 families (69 individuals).

Other Comments: The sense primer is derived from nucleotides 1539–1563 (CTG CAC CTG CCC TCA GGC GTC CGT) and the antisense primer from nucleotides 1657–1681 (CTG GAA GCG GAC GGC TGA GGA CTT) of the published cDNA sequence (Kobayashi *et al.*, 1988). Amplification conditions were (a) denaturing temperature = 94° for 0.5 mins; (b) annealing temperature = 72° for 1.0 min.

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Reference: Kobayashi *et al.* (1989) *Nucl. Acids Res.* 17, 1089–1102.