PCR assay for a polymorphic TaqI site in the human K-ras-2 gene on chromosome 12p (KRAS2)

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Source/Description: There is a TaqI site 175 bp downstream of the stop codon on Exon 4B of the human K-ras-2 gene (1A, 1B). With PCR primers E4b-A2 and E4b-B2 a 298 bp fragment is amplified; digestion with TaqI yields fragments of 151 and 147 bps.

Primers:

		5' end of primer	
		Ref 1A	Ref 1B
E4b-A2	AGGCATACTAGTACAAGTGG	299	37,301
E4b-B2	ACACCAACATTCACACTTGG	597	37,599

Frequency: 0.56 in 37 unrelated individuals.

Chromosomal Localization: 12p12.1 (2).

Mendelian Inheritance: Mendelian inheritance was demonstrated in 3 families (23 meioses).

Other Comments: PCR rxn: 67 mM Tris-HCl, pH 8.8; 17 mM (NH₄)₂SO₄; 1.5 mM MgCl₂; 10 mM 2-mercaptoethanol; 25 pmol of each primer; 100 µM each dNTP; 10 units of AmpliTaq; 20–100 ng genomic DNA; total volume of 100 microliters; 36 cycles (93°C 1 min; 60°C 2.5 min). The PCR products were fractionated on an 8% nondenaturing polyacrylamide gel. The TaqI polymorphism, and other polymorphisms in the K-ras-2 gene can be seen with denaturing gradient gel electrophoresis (E.S. Abrams, unpublished). For other RFLPs in the K-ras-2 gene, see (2).

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References: 1A) McGrath, J.P. *et al.* (1983) *Nature* **304**, 501. 1B) Gene Bank file HUMRASK 26. 2) *Cytogenetics and Cell Genetics* (1989), *Human Gene Mapping 10*, New Haven Conference.

Figure 1. Examples of individuals who are homozygous for presence (lane 5) or absence (lanes 1, 2) of the TaqI restriction site, or are heterozygous (lanes 3, 4).

PCR detection of a BstNI RSP in exon 6 of the human carbonic anhydrase II locus, CA2

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We have described a BstNI polymorphism in exon 6 of the human CA2 locus, (originally found in five sequences of genomic and cDNA clones) which can be typed by PCR. This polymorphism is silent at the amino acid level (189-Leu TTG to 189-Leu C-TG). It is located 12 kb downstream from the previously described TaqI RFLP in the 5' flanking region (1). This RFLP will be useful for linkage studies in carbonic anhydrase II deficiency patients as well as linkage to other CA isozyme genes (2).

Frequency: Estimated from 16 unrelated individuals.

B1 BstNI + allele: 0.50

B2 BstNI – allele: 0.50.

Chromosomal Location: 8q22 (2).

Mendelian Inheritance: Co-dominant segregation of the BstNI alleles was observed in one five member and one six member family.

PCR Primers:

P1, 5'-TTGTGTCTGCTGCTCTCCTA

P2, 5'-GGGTTCCCTTGAGCACAATCC

PCR Conditions: Standard conditions were used (3) with 0.5 µg genomic DNA, 0.1 µg of each primer, 1.5 mM MgCl₂ in a 25 µl reaction. The temperature was cycled in a Bellco thermocycler as follows: 94°C, 0.7 min; 59°C, 1.5 min; 71°C, 2.5 min for 40 cycles; 71°C, 10 min, final extension.

Other Comments: One µl of BstNI (5 U) was added directly to the cycled reaction and incubated at 60°C for 1 hr. Two common BstNI sites contained within exon 6 serve as internal controls for complete digestion. Products were run on 3% NuSieve (FMC Marine Colloids) plus 1% standard agarose.

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References: 1) Venta, P. and Tashian, R.E. (1990) *Nucl. Acids Res.* **18**, 5585. 2) Tashian, R.E. (1989) *BioEssays* **10**, 186–192. 3) Saiki *et al.* (1988) *Science* **239**, 487–491.