PCR detection of the TAQ1 polymorphism at the CA2 locus

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In humans, at least seven isozymes of carbonic anhydrase (CA1-CA7) are known, five of which are encoded by genes on chromosomes 1 (CA6), 8 (CA1-3) and 16 (CA7) (1). We have previously described a *TaqI* RFLP with a 50% allele frequency near the CA2 gene (2). We have now bracketed the site with PCR (3) primers to develop an STS site. The original DNA samples (2) produced the same typings (+ = *TaqI* site present, - = not present). This STS and *TaqI* site should be useful for following inheritance of the mutant CA2 genes responsible for the CA2 deficiency syndrome (1) as well as for other mapping and linkage studies involving human chromosome 8.

PCR Primers:

P1, 5'-GGAATTCAAAAAGGGACCCACCAAGGTGA-3'

P2, 5'-GAATTCGTTAGGAGCTATGACCTTGGG-3'

Underlined regions are not present in genomic DNA.

PCR Conditions: Standard conditions were used (3) with 2 μ g genomic DNA and 0.1 μ g of each primer per 100 μ l reaction. The temperature was cycled in a Techne Thermal Cycler as follows: 96°C, 0.8 min; 60°C, 0.7 min; and 72°C, 1.7 min for 32 cycles; 72°C, 10 min, final extension.

Other Comments: The reaction was boiled 5 min to ensure denaturation of the GC-rich island next to the STS prior to addition of *Taq* DNA polymerase. Five units of *TaqI* were added directly to 25 μ l of the finished reaction and the products were run on a 5% polyacrylamide gel.

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References: 1) Tashian,R.E. (1989) *BioEssays* **10**, 186-192. 2) Lee,B.L. *et al.* (1985) *Hum. Genet.* **69**, 337-339. 3) Saiki *et al.* (1988) *Science* **239**, 487-491.