Ornithine transcarbamylase polymorphism detected by PCR introduction of DraI site

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Description: Primers O 46 (5′ GTGACCTTCTCACTTTAA) and T 46 (5′ AGAGAAAATGTTACATACC) were designed to amplify a 116 bp segment of ornithine transcarbamylase (OTC) exon 2. Both AAA and AGA sequences have been reported in normal individuals at nucleotide positions 138–140 corresponding to amino acid position 46 (1, 2). Although this variation does not alter a restriction site, introduction into the PCR product of a T instead of the native sequence G at nucleotide position *135* by use of primer O 46 creates a DraI site when the AAA but not AGA codon is present.

Protocol: 100 μl volume reactions of .5 μg–10 μg genomic DNA, .3 U Promega TAQ DNA polymerase, 200 μM of each dNTP, 1 μM of each primer, .5 μM of spermidine, and 1×Promega PCR buffer underwent 35 cycles of PCR (95°C×1 min., 50°C×1 min., 72°C×2 min.) followed by 72°C×5 min. final extension. 25 μl of PCR product was digested with 20 U DraI (37°C×2 hours) and analyzed by agarose gel electrophoresis (3% NuSieve GTG and 1% Seakem LE).

Polymorphism: DraI digestion of PCR products distinguished a two allele polymorphism with 100 bp and 16 bp fragments when the AAA codon was present and a 116 bp fragment when AGA was present.

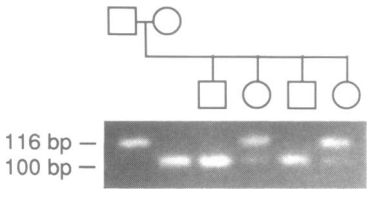
Frequency: Estimation is based on the study of 56 chromosomes from unrelated individuals.
AAA codon (110 bp): 0.68
AGA codon (116 bp): 0.32

Gene Localization: The OTC gene was mapped to Xp21.1 by Lindgren *et al.* (3). The PCR polymorphism co-segregates with the BamHI OTC RFLPs in one kindred and DMD RFLPs in a second kindred.

Mendelian Inheritance: X-linked segregation was demonstrated in individuals from 8 2- and 3-generation families.

Other Comments: There is no evidence for linkage disequilibrium with the OTC MspI RFLPs detected with pH0731.

References: 1) Hata,A. *et al.* (1988) *J. Biochem.* **103**, 302–308. 2) Horwich,A.L. *et al.* (1984) *Science* **224**, 1068–1074. 3) Lindgren,V. *et al.* (1984) *Science* **226**, 698–700.



Bgl-I and Kpn-I RFLPs at the human liver/islet glucose transporter (GLUT2) gene locus

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Source/Description: pSPGT 2 contains a cDNA corresponding to the human liver/islet glucose transporter entire cDNA and 10 bp of the 5′ and 900 bp of the 3′ region (1).

Polymorphisms: Bgl-I detects invariant bands at 12.6 and 7.9 kb and a two allele polymorphism with bands at 4.2 and 4.0 kb. This RFLP can also be detected with Hind-III. Kpn-I detects invariant bands at 9.0 and 4.7 kb and a dimorphism with the presence or absence of a band at 2.8 kb.

Frequency: Bgl-I: studied in 36 Caucasians, C1: 4.2 kb band = 0.1; C2: 4.0 kb band = 0.90. Kpn-I studied in 30 Caucasians, presence of D1: 2.8 kb band = 0.25; absence of D2: 2.8 kb band = 0.75.

Not Polymorphic For: Ava-II, Bgl-II, Bst-I, Msp-I, Pst-I, Pvu-II, Rsa-I, Sst-I, Stu-I, Xba-I, Xmn-I.

Chromosomal Localization: 3q26.1-q 26.3 (1).

Mendelian Inheritance: Bgl-I: co-dominant segregation observed in 3 families with 13 members. Kpn-I: co-dominant segregation observed in 2 families with 9 individuals.

Probe Availability: Request for probe to G.I. Bell at Howard Hughes Medical Institute and Departments of Biochemistry and Molecular Biology, the University of Chicago, IL 60637, USA.

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Reference: 1) Fukumoto,H., Seino,S., Imura,H., Seino,Y., Eddy,R.L., Fukoshima,Y., Byers,M.G. and Shows,T.B. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5434–5438.

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