

## A polymorphic dinucleotide repeat at the DXS7 locus

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**Source/Description:** A 0.8 kb (CA)-positive TaqI sub-fragment of a cosmid hybridizing with the probe L1.28 for locus DXS7 (Xp11.4-Xp11.3, see Mandel *et al.*, 1989), was cloned into pBluescript and sequenced. The fragment was found to contain the compound perfect repeat (Weber, 1990): (CAC)<sub>5</sub>(CA)<sub>18</sub>. PCR primers were derived from the sequence as follows:  
CA strand: 5'CCACCTCACTCCAGCCTGAG 3'  
GT strand: 5'ACTTTGACCTTATCAAACAGGTG 3'

### Polymorphism and Frequency:

Alleles	BP	Frequency*
D1	167	0.03
D2	165	0.28
D3	159	0.03
D4	157	0.66

\*Studied in 52 unrelated Caucasian females. 46% of these females were heterozygous. PIC = 0.52.

**Mendelian Inheritance:** Co-dominant X-linked inheritance was demonstrated using four families (19 meioses).

**Other Comments:** PCR was carried out using a Perkin-Elmer Cetus DNA Thermal Cycler. Each 20  $\mu$ l reaction contained 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.0 mM MgCl<sub>2</sub>, 170  $\mu$ g/ml BSA, 0.05% Tween 20, 0.05% Nonidet P-40, 50 ng genomic DNA, 0.25  $\mu$ M of each primer, 20  $\mu$ M of each of dATP, dGTP, dCTP and dTTP, 10  $\mu$ Ci [ $\alpha$ -<sup>32</sup>P] dCTP at 3000 Ci/mmol (Amersham), and 1U Taq DNA polymerase (Pharmacia). Amplification conditions were 94°C/7 min., then 30 cycles of 94°C/30 sec; 62°C/30 sec; 72°C/2 sec with a final elongation step of 72°C/7 min. A 2  $\mu$ l aliquot was electrophoresed on a 8% polyacrylamide sequencing gel which was dried and exposed to Cronex 4 Daylight film overnight.

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**References:** 1) Mandel, J.-L., Willard, H.F., Nussbaum, R.L., Romeo, G., Puck, J.M. and Davies, K.E. (1989). *Cytogenet. Cell Genet.* **51**, 384-437. 2) Weber, J.L. (1990) *Genomics* **7**, 524-530.

## Two CA-dinucleotide polymorphisms at the COL4A5 (Alport syndrome) gene in Xq22

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**Source/Description:** Two CA-positive Sau3A fragments were identified from clone MG-2 by Southern hybridisation.

### Primer Sequences:

2B6-1	TAT AAT GGA AGT TAT TCA TGT AGA C
2B6-3	GTG ATT CAG ATG TTA CTT AAG GAC
2B20-2	GGT GCC CTT TTT AAT ACA TTT TCC
2B20-4	TTA GAC AAG CTT TTA CTC CCA AGA

**Polymorphism/Frequencies:** In 33 CEPH mothers are:

Allele	bp	Frequency	Allele	bp	Frequency
2B6: A1	164	.075	2B20: B1	122	.02
A2	162	.44	B2	120	.80
A3	160	.18	B3	118	.18
A4	158	.09			
A5	156	.15			
A6	154	.015			
A7	150	.045			

Observed heterozygosities are: 2B6, 25/33 = .76; 2B20, 13/33 = .39; combined, 26/33 = .79.

**Mendelian Inheritance:** Both markers show complete linkage to X-linked Alport phenotype in families with an X-linked defect, with a combined LOD of 5.97 at  $\theta = 0$  in two such families with 2B6.

**Chromosomal Location:** Xq22 is the location of the COL4A5 gene as confirmed in (3) and references therein. Genetic mapping of the 2B6 polymorphism with respect to key recombinant individuals in the CEPH families indicated a location proximal to DXS287 and distal to (DXS328, DXS265), consistent with physical localization data (3).

**PCR Conditions:** Amplifications in 10  $\mu$ l volumes were initiated with 25 ng of genomic DNA in reactions containing 0.5-1  $\mu$ M each primer, 1 unit of Taq polymerase, 10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200  $\mu$ M dNTPs except 5  $\mu$ M dCTP plus 2.5  $\mu$ Ci alpha-<sup>32</sup>P-dCTP at 3000 Ci/mmol. The thermal profile was 3 minutes at 94°C, followed by 15 cycles of 90 seconds at 94°C, 120 seconds at 55°C, 120 seconds at 72°C; then 15 more similar cycles with extension of the 72°C portion by 3 seconds per cycle.

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