Dinucleotide repeat polymorphism at the D6S89 locus

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Source and Description of Clone: Subclone XL25B of phage 25 from a flow-sorted human X-chromosome specific library contained a $(AC)_{14}$ repeat. Sequences flanking this repeat (EMBL accession number X52399) were used to design PCR primers.

PCR Primers: # 1717 5'-CTTGTTCATCTGCCTTGTGC- 3'
1718 5'-ACCTAAGCGACTGCCTAAAC-3'

Polymorphism: Using 5' [^{32}P] end labeled primer 1717 to label the GT containing strand, fragments of variable length were detected on DNA sequencing gels. Lengths of allelic fragments (nt) are expressed relative to Sau3A fragments of pBR322 used as size markers. A1 = 227, A2 = 225, A3 = 223, A4 = 221, A5 = 217, A6 = 215, A7 = 213, A8 = 211, A9 = 209, A10 = 207, A11 = 205, A12 = 203, A13 = 199.

Frequencies: Allele frequencies from 27 unrelated European Caucasians: A1 = .037, A2 = .204, A3 = .019, A4 = .111, A5 = .130, A6 = .092, A7 = .111, A8 = .019, A9 = 0.92, A10 = .037, A11 = .055, A12 = .055, A13 = .037. Heterozygosity = 92%; PIC = 0.88.

Chromosomal Localization and Mendelian Inheritance: Pairwise linkage analysis between D6S89 and HLA/A in CEPH families 13294, 1331, 1345, 1362 and 884 gave a maximum Lod score of 12.1 at theta = 0.1, indicating localization to chromosome 6p. Three-point analysis favored the order pter-F13-D6S89-HLA-cen over alternative orders by odds of at least 214:1. Mendelian inheritance was observed in all cases.

PCR Conditions: We carry out PCR in a total volume of 25 μl containing: 50 ng genomic DNA, 5 pmoles of each primer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 50 mM KCl, 10 mM Tris- Cl^-, pH 8.3, 0.6 units Taq polymerase (Perkin-Elmer/Cetus) and 0.01% gelatin. Amplification is for 30 cycles with denaturation at 94°C for 1 min, annealing at 54°C for 1 min and extension at 72°C for 30 sec.

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