PCR-based detection of polymorphic *DdeI* and *KpnI* sites in intron 5 of the adenylate kinase (AK1) gene

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Description/Primers: AK7401 and AK8401 [(5'-TCCCCAGG-ATGGCACCCACACACG-3') and (5'-GCGGCACCTACAA-CAGCCCTGGAAG-3')] were constructed from the published sequence (1) and used to amplify a 1025 bp genomic DNA fragment in intron 5 of the adenylate kinase gene. Two polymorphic sites are detected by digestion of the amplified product with *DdeI* and *KpnI*.

Polymorphism: Digestion of the amplified product with *DdeI* followed by electrophoresis in 3% NuSieve, 1% agarose gels for 5–6 hours at 100 volts produces four allelic bands between 450 bp and 600 bp (see Fig. 1A). Digestion with *KpnI* yields bands of approximately 640 bp and 335 bp when the polymorphic *KpnI* site is present. When the site is absent, *KpnI* does not cleave the product (see Fig. 1B). *RsaI* also detects this polymorphic site.

Frequency: For *DdeI*: allele B1, 5%; allele B2, 53%; allele B3, 2%; and allele B4, 40%. For *KpnI*: allele C– (site absent), 10%; allele C+ (site present), 90%. Allele frequencies computed from 30 unrelated Caucasian individuals.

Not Polymorphic For: *AccI*, *AluI*, *BglI*, *BglII*, *EcoRI*, *HaeIII*, *TaqI*, *PstI*, *MspI*, *HinfI*, *NciI*, *NdeI*, *PvuII*, *XbaI*, *BamHI*, *HpaII*, *StuI*, *BalI*, *HindIII*, *CfoI*, *HhaI*, *MboI*, *ThaI*.

Chromosomal Localization: AK1 has been mapped to 9q34.1-q34.2 (2).

Mendelian Inheritance: Codominant inheritance has been demonstrated in a 4-generation, 82-member family.

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References: 1) Matsuura, S. *et al.* (1989) *J. Biol. Chem.* **264**, 10148–10155. 2) Allderdice, P.W. *et al.* (1986) *Am. J. Hum. Genet.* **39**, 612–617.

Figure 1. Digestion of amplified product with *DdeI* (A) and *KpnI* (B).

Dinucleotide repeat polymorphism at the DXS538 locus

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Source and Description of Clone: Lambda XL27 was isolated from a flow-sorted X chromosome genomic library (LAOXNL01). pXL27B, a subclone of lambda XL27, was partially sequenced, and the sequences (EMBL accession number X55145) flanking the dinucleotide repeat (AT)₈(AC)₁₅(AT)₄ were used to design PCR primers.

PCR Primers:

XL27b-A = 5'- CTGATTCACTGTACAATGGT-3'

XL27b-B = 5'- ATGGATAATAAACAGACAGGA-3'

Polymorphism: Allelic fragments were detected on DNA sequencing gels. Lengths (nt) are: A1 = 184, A2 = 180, A3 = 178, A4 = 176, A5 = 174, A6 = 156, A7 = 154. pXL27b contains allele A7.

Frequencies: From 47 unrelated females and 17 unrelated males: A1 = 0.018, A2 = 0.126, A3 = 0.234, A4 = 0.252, A5 = 0.054, A6 = 0.045, A7 = 0.270. The expected heterozygosity in females calculated from these frequencies is 79%. We observed 34/47 = 72% heterozygosity.

Mendelian Inheritance: Mendelian inheritance was observed in 7 informative CEPH families with a total of 66 children.

Chromosomal Location: Amplification of DNA from a panel of somatic cell hybrids containing cytologically defined portions of the human X chromosome (1) suggested a location to Xp11.21-Xp21.1. Linkage analysis of 5 informative CEPH families indicates linkage to DXS7 (LOD = 7.09, theta = 0.125) and DXS164 (LOD = 10.87, theta = 0.023). Three-point linkage analysis suggests that the most likely order is cen-DXS7-XL27-DXS164-pter. Odds against inversion of DXS7 and XL27 are 2 × 10⁶:1. However, odds against inversion of XL27 and DXS164 are only 7:1.

PCR Conditions: PCR was performed according to (1) except that the products were labelled internally with α-³²P-dCTP. Annealing temperature was 60°. Amplified alleles were resolved on denaturing polyacrylamide gels and detected by autoradiography.

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References: 1) J.A.Luty, Z.Guo, H.F.Willad, D.H.Ledbetter, S.Ledbetter and M.Litt (1990) *Am. J. Hum. Genet.* **46**, 776–783.

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