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RFLP Report

TWO DINUCLEOTIDE REPEAT POLYMORPHISMS AT THE D8S1442 AND D8S1443 LOCI

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Two polymorphic dinucleotide (CA) repeat clones were isolated from cosmids, cCI8-1121 and cCI8-1199, mapped to chromosome 8p11.2-p12.
Key Words microsatellite, cosmid, chromosome 8

Two cosmids, cCI8-1121 and cCI8-1199, were digested completely with *Sau*-3AI. *Sau*3AI-fragments were subcloned into *Bam*HI site of pUC18 and screened on the basis of hybridization to a ^{32}P-labeled poly(dA-dC)•poly(dG-dT) probe (Pharmacia) (Nagano *et al.*, 1993). Positive subclones were partially sequenced and the sequences flanking a $(CA)_n$ repeat were used to design PCR primers. Thus two dinucleotide repeat polymorphisms, M215 and M231 (Genbank accession number: G00-450-243), were isolated from the cosmids, cCI8-1121 and cCI8-1199, respectively.

Primers for PCR

M215-F = 5′-TGGTACTAGGTTGTGATGGTTACA-3′
M215-R = 5′-ACAGGGCAGTTGTGAGATGTACT-3′
M231-F = 5′-ACATTCAGCAGCGTTTTTCAG-3′
M231-R = 5′-GAGGAGCAACGTCTACTTCTG-3′

Polymorphism/frequency

M215 (D8S1443): Four alleles were detected in 30 chromosomes of unrelated Japanese individuals. Observed heterozygosity = 0.93.

Allele	Size (bp)	Frequency
A1	169	0.30
A2	171	0.30
A3	173	0.37
A4	175	0.03

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M231 (D8S1442): Six alleles were detected in 100 chromosomes of unrelated Japanese individuals. Observed heterozygosity=0.52.

Allele	Size (bp)	Frequency
A1	111	0.01
A2	117	0.16
A3	119	0.01
A4	121	0.20
A5	123	0.60
A6	125	0.02

Chromosomal localization. The cosmids, cCI8-1121 and cCI8-1199, have been localized to chromosome 8p11.2 and 8p12, respectively, by fluorescent *in situ* hybridization (Emi *et al.*, 1992, 1993).

Mendelian inheritance. Mendelian inheritance was observed.

Amplification conditions. PCR reaction was carried out in a total volume of 10 μl containing 50 ng of genomic DNA, 4 pmol of one unlabeled primer, 4 pmol of a ^{32}P-ATP end-labeled primer (0.2 μCi), 200 μM dNTP, 1% deionized formamide, 0.001% gelatin, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl at pH 8.4 and 0.25 U Taq polymerase, using a Perkin Elmer Cetus Thermal Cycler for 35 cycles as follows: 94°C for 45 sec, annealing temperature (47°C for M215 and 57°C for M231) for 30 sec, and 72°C for 30 sec for each cycle. The amplified product was fractionated in a 6% polyacrylamide gel containing 30% formamide and visualized by autoradiography. The size of the alleles was determined by comparison to M12mp18 DNA sequencing ladders.

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