RFLP Report

**DINUCLEOTIDE REPEAT POLYMORPHISM
AT THE D8S1223 LOCUS**

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Summary A polymorphic dinucleotide (CA) repeat clone was isolated from a CEPH mega-YAC clone (936F7), and was localized to chromosome 8 using a panel of 13 mouse/human somatic cell hybrids.

Key Words microsatellite, YAC, cosmid

A YAC clone (936F7) was subcloned into cosmids which were prepared without previous separation of cloned DNA from host DNA. The cosmids were hybridized with ³²P-labelled total human DNA in order to select the cosmids with human inserts. The selected cosmids were screened on the basis of hybridization to a ³²P-labelled poly(dA-dC)·poly(dG-dT) probe (Pharmacia) (Nagano *et al.*, 1993). The positive cosmid was digested completely with *Hae*III. *Hae*III-fragments were subcloned into the *Sma*I site of pUC18 and hybridized with a ³²P-labelled poly(dA-dC)·poly(dG-dT) probe again. A positive subclone was partially sequenced and the sequences flanking a (CA)_n repeat were used to design PCR primers. A polymorphic dinucleotide repeat polymorphisms, M4087 (Genbank accession number: G00-389-817), was isolated from one of the YAC clone (936F7).

Primers for PCR

M4087-F=5' CTTTCTACTGATTCCATAGT 3'

M4087-R=5' GGATACGAGTGGAGATGACG 3'

Polymorphism/frequency

Eight alleles were detected in 70 chromosomes of unrelated Japanese individuals. Observed heterozygosity=0.67.

Allele	Size (bp)	Frequency
A1	125	0.20
A2	127	0.06
A3	129	0.46
A4	131	0.17

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A5	133	0.02
A6	135	0.02
A7	137	0.29
A8	139	0.04

Chromosomal localization. Both ends of the YAC clone (936F7) and the polymorphic dinucleotide (CA) repeat loci (D8S1223) were localized to chromosome 8 using a panel of 13 mouse/human somatic cell hybrids (Semba *et al.*, 1985).

Mendelian inheritance. Mendelian inheritance was observed.

Amplification conditions. PCR reaction was carried out in a total volume of 10 μ l containing 20 ng of genomic DNA, 2 pmol of rhodamine-labelled primers, 200 μ M dNTP, 1% formamide, 2 mM MgCl₂, 50 mM KCl, 0.001% gelatin, 10 mM Tris-HCl at pH 8.4 and 0.25 U *Taq* polymerase, using a Perkin Elmer Cetus Thermal Cycler for 30 cycles as follows: 94°C for 30 sec, 53°C for 30 sec, and 72°C for 30 sec for each cycle. The amplified product was fractionated on a 6% polyacrylamide gel and images were obtained by scanning the gels with a fluorescent image analyzer FMBIO (Ishino *et al.*, 1992; Nakura *et al.*, 1995). The size of the alleles was determined by comparison to M13mp18 DNA sequencing ladders.

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