

RFLP Report

**THREE DINUCLEOTIDE REPEAT POLYMORPHISMS
AT THE D8S1217, D8S1220, AND D8S1221 LOCI**

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

Key Words microsatellite, YAC, cosmid, chromosome 8

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-labeled total human DNA in order to select the cosmids with human insert. The selected cosmids were screened on the basis of hybridization to a ³²P-labeled poly(dA-dC)•poly(dG-dT) probe (Pharmacia) (Nagano *et al.*, 1993). The positive cosmids were digested completely with *Hae*III. *Hae*III-fragments were subcloned into *Sma*I site of pUC18 and hybridized with a ³²P-labeled poly(dA-dC)•poly(dG-dT) probe again. Each positive subclone was partially sequenced and the sequences flanking a (CA)_n repeat were used to design PCR primers. Thus three dinucleotide repeat polymorphisms, M4128, M4005, and M4079 (Genbank accession number: G00-389-817), were isolated from the YAC clone (936F7).

Primers for PCR

M4005-F = 5'-TTCCGTATACACATGCACCC-3'
M4005-R = 5'-CTAGCAGCCAGACACAGGAGC-3'
M4079-F = 5'-TGGTCTCTGATTCAAGGAGC-3'
M4079-R = 5'-GCACATCTCTGTTATGGTAAT-3'
M4128-F = 5'-TGATGTATGCAACTTACTTTT-3'
M4128-R = 5'-TGCCAGGTTTGC GTTATCTC-3'

Polymorphism/frequency

M4005 (D8S1220): Three alleles were detected in 128 chromosomes of unrelated Japanese individuals. Observed heterozygosity = 0.14.

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Allele	Size (bp)	Frequency
A1	90	0.08
A2	94	0.91
A3	98	0.01

M4079 (D8S1221): Four alleles were detected in 96 chromosomes of unrelated Japanese individuals. Observed heterozygosity = 0.44.

Allele	Size (bp)	Frequency
A1	114	0.01
A2	116	0.36
A3	122	0.40
A4	124	0.23

M4128 (D8S1217): Six alleles were detected in 50 chromosomes of unrelated Japanese individuals. Observed heterozygosity = 0.72.

Allele	Size (bp)	Frequency
A1	91	0.02
A2	93	0.18
A3	97	0.28
A4	99	0.06
A5	101	0.40
A6	103	0.06

Chromosomal localization. The YAC clone (936F7) and the three polymorphic dinucleotide (CA) repeat loci 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-labeled 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 Cycle for 30 cycles as follows: 94°C for 30 sec, annealing temperature (62°C for M4005, 56°C for M4079 and 52°C for M4128) 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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