RFLP Report

**TWO DINUCLEOTIDE REPEAT POLYMORPHISMS
AT THE D8S1218 AND D8S1219 LOCI**

Lin YE,¹ Jun NAKURA,^{1,*} Noriaki MITSUDA,¹
Asako TAKAHASHI-FUJII,² Yoshizumi ISHINO,²
Ikunoshin KATO,² Tetsuro MIKI,¹
and Toshio OGIHARA¹

¹*Department of Geriatric Medicine, Osaka University Medical School,
2-2 Yamadaoka, Suita, Osaka 565, Japan*

²*Biotechnology Research Laboratories, Takara Shuzo Co., Ltd.,
3-4-1 Seta, Ohtsu, Shiga 520-21, Japan*

Summary Two polymorphic dinucleotide (CA) repeat clones were isolated from a CEPH mega-YAC clone (844E2), 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 (844E2) 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 cosmids were 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. Each positive subclone was partially sequenced and the sequences flanking a (CA)_n repeat were used to design PCR primers. Thus two dinucleotide repeat polymorphisms, M3073 and M3136 (Genbank accession number: G00-389-817), were isolated from the YAC clone (844E2).

Primers for PCR

M3073-F=5' GGCTGGTCTCTGATTCA 3'

M3073-R=5' CTGTTATGGCAATTATCT 3'

M3136-F=5' GAATAGTTATTTAGCTTGA 3'

M3136-R=5' GAGGATATTAAGATGCGC 3'

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* To whom correspondence should be addressed.

Polymorphism/frequency

M3073 (D8S1218): Five alleles were detected in 96 chromosomes of unrelated Japanese individuals. Observed heterozygosity=0.69.

Allele	Size (bp)	Frequency
A1	109	0.01
A2	111	0.41
A3	117	0.28
A4	119	0.29
A5	121	0.01

M3136 (D8S1219): Seven alleles were detected in 92 chromosomes of unrelated Japanese individuals. Observed heterozygosity=0.48.

Allele	Size (bp)	Frequency
A1	104	0.68
A2	106	0.01
A3	110	0.13
A4	112	0.01
A5	116	0.14
A6	118	0.01
A7	122	0.01

Chromosomal localization. Both ends of the YAC clone (844E2) and the two polymorphic dinucleotide (CA) repeat clones (D8S1218 and D8S1219) 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, annealing temperature (45°C for M3073 and 47°C for M3136) 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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