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RFLP Report

A HIGHLY POLYMORPHIC DINUCLEOTIDE REPEAT AT THE D8S1222 LOCUS

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A highly polymorphic dinucleotide (CA) repeat clone was isolated from a CEPH mega-YAC clone (844E2), and was 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 ^{32}P-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 ^{32}P-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 ^{32}P-labeled 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. Thus a highly polymorphic dinucleotide repeat, M3131 (Genbank accession number: G00-389-817), was isolated from the YAC clone (844E2).

Primers for PCR

M3131-F = 5′-CGGGCTGGCCATAGACTG-3′
M3131-R = 5′-TCCTCAATGCTTGGTAT-3′

Polymorphism/frequency. Sixteen alleles were detected in 136 chromosomes of unrelated Japanese individuals. Observed heterozygosity = 0.91.

Allele	Size (bp)	Frequency
A1	116	0.01
A2	118	0.01
A3	120	0.01

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A4	122	0.02
A5	124	0.07
A6	126	0.04
A7	128	0.29
A8	130	0.04
A9	132	0.05
A10	134	0.01
A11	136	0.20
A12	138	0.13
A13	140	0.10
A14	142	0.02
A15	144	0.03
A16	146	0.01

Chromosomal localization. Both ends of the YAC clone (844E2) and the highly polymorphic dinucleotide (CA) repeat locus (D8S1222) 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 μl containing 20 ng of gemonic DNA, 2 pmol of rhodamine-labeled primers, 200 μM dNTP, 1% formamide, 2 mM $MgCl_2$, 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, 47°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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REFERENCES

Ishino Y, Mineno J, Inoue T, Fujimiya H, Yamamoto K, Tamura T, Homma M, Tanaka K, Kato I (1992): Practical application in molecular biology of sensitive fluorescence detection by a laser-excited fluorescence image analyzer. Biotechniques **13**: 936–943

Nagano K, Nakura J, Kihara K, Ye L, Kamino K, Mitsuda M, Ohta T, Jinno Y, Niikawa N, Miki T, Ogihara T (1993): Isolation and mapping of microsatellites from a library microdissected from the Werner's syndrome region, 8p11.2-p22. Jpn J Human Genet **38**: 391–397

Nakura J, Ye L, Mitsuda N, Takahashi-Fujii A, Ishino Y, Miki T, Ogihara T (1995): Dinucleotide repeat polymorphism at the D8S1053. Jpn J Human Genet **39**: 445–446

Semba K, Yamanashi Y, Nishizawa M, Sukegawa J, Yoshida M, Sasaki M, Yamamoto T. Toyoshima K (1985): Location of the c-*yes* gene on the human chromosome and its expression in various tissues. Science **227**: 1038–1040