RFLP Report

**DINUCLEOTIDE REPEAT POLYMORPHISM
AT THE D8S1054**

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A polymorphic CA repeat was isolated from a chromosome microdissection library and was mapped to chromosome 8q11.23-q21.3 using human-mouse cell hybrids and linkage analysis.

Key Words chromosome microdissection, microsatellite, linkage map

A microdissected library of human chromosome 8 region 8p11.2-p22, was prepared and clones were screened for long dinucleotide repeats (Nagano *et al.*, 1993; Kamino *et al.*, 1993; Ye *et al.*, 1994; Nakura *et al.*, 1994). A clone, pMS8-156, was sequenced and complex dinucleotide repeats (CA)₁₃ was detected (GDB accession No. G00-330-827).

PCR primers. PCR primers were designed from sequences flanking the repeat:

MS8-156-F = 5'-ACCAAGACCTTCCATATAATA-3'

MS8-156-R = 5'-TGTTACAATTTAATAGGTGTG-3'

Polymorphism. Allele frequencies were calculated from the genotypes of 50 unrelated CEPH parents. Heterozygosity was 0.48. Mendelian inheritance was observed in all cases.

Allele sizes and frequencies.

A1	121 bp	.01
A2	119 bp	.03
A3	117 bp	.02
A4	115 bp	.01
A5	105 bp	.71
A6	103 bp	.22

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Reference CEPH genotypes. 1331-01 121/105; 1331-02 105/103.

PCR conditions. PCR was performed in a volume of 10 μ l containing 20 ng genomic DNA, 2 pmol of each rhodamine-labeled primers, 200 μ M dNTP, 10 mM Tris-HCl pH 8.3 (25°C), 0.001% (w/v) gelatin, 1% deionized formamide, 1.25 mM MgCl₂, 50 mM KCl and 0.25 U Taq polymerase (Perkin-Elmer) for 35 cycles in a Gene Amp PCR system 9600 (Perkin-Elmer) as follows: 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min. The amplified product was fractionated in 6% polyacrylamide gel. Gel images were obtained by scanning the gels with a fluorescent image analyzer FMBIO (Ishino *et al.*, 1992). Allele sizes were determined by comparison of each amplified band with DNA sequencing ladders of M13mp18 DNA.

Chromosomal localization. The clone was mapped on chromosome 8 using human-mouse cell hybrids provided by Yoshida *et al.* (Semba *et al.*, 1985). Three microsatellites such as D8S360, D8S1053, D8S1055 were previously mapped to chromosome 8 by these hybrids (Kamino *et al.*, 1993; Nakura *et al.*, 1994; Ye *et al.*, 1994). Linkage analysis with 5 CEPH families using data from the CEPH database ver.5 gave a maximum LOD score of 19.48 at theta=0.000 with D8S260, 10.20 at theta=0.104 with D8S279, and 8.50 at theta=0.092 with D8S285, which indicated the location of D8S1054 at 8q11.23-q21.3. Although there was no obligate recombination between D8S1054 and D8S260, flanking sequences were different from each other. This suggests that D8S1054 is a new microsatellite.

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