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RFLP Report

DINUCLEOTIDE REPEAT POLYMORPHISM AT THE D8S1053

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A polymorphic dinucleotide (CA) repeat clone isolated from a chromosome microdissection library was mapped to chromosome 8p22-p23.1 using human-mouse cell hybrids and linkage analysis of 5 CEPH families.
Key Words microsatellite, chromosome microdissection, 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). A clone, pMS8-109, was sequenced and mapped on chromosome 8 using human-mouse cell hybrids. Complex dinucleotide repeats $(CA)_{15}$ was detected (GDB accession No. G00-330-798).

Primers for PCR. A primer set designed from sequences flanking repeat as MS8-109-F, 5′-AGTCCCTTCCCTGCTTCTTTC-3′, and MS8-109-R, 5′-GAACCCACAAAGCATTTGATG-3′.

Polymorphism. Allele fragments were resolved on DNA sequencing gels. Allele frequencies were calculated from the genotypes of 49 unrelated CEPH parents. Heterozygosity was 0.47. Mendelian inheritance was observed in all cases.

Allele sizes and frequencies.

A1	83 bp	0.29
A2	81 bp	0.45
A3	79 bp	0.24
A4	77 bp	0.02

Reference CEPH genotypes. 1413-01 83/79; 1413-02 81/81.

PCR condition. The reaction was carried out in a volume of 10 μl containing 20 ng genomic DNA, 2 pmol of rhodamine-labeled primers, 200 μM dNTP, 10 mM

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Tris-HCl pH 8.3 (25°C), 0.001% (w/v) gelatin, 1% deionized formamide, 1.0 mM $MgCl_2$, 50 mM KCl, and 0.25 U Taq polymerase (Perkin-Elmer) for 30 cycles as follows: 94°C for 30 sec, 57°C for 30 sec, and 72°C for 30 sec in a Gene Amp PCR system 9600 (Perkin-Elmer) and the images were obtained by scanning the gels with a fluorescent image analyzer FMBIO (Ishino *et al.*, 1992). The amplified product was fractionated in 6% polyacrylamide gel. Allele sizes were determined by comparison of each amplified band with DNA sequencing ladders of M13mp18 DNA.

Chromosomal localization. Localized to chromosome 8 using human-mouse cell hybrids. Linkage analysis with 5 CEPH families using data from the CEPH database v5 gave a maximum LOD score of 10.47 at theta=0.000 with D8S261, 5.97 at theta=0.038 with D8S280, 9.63 at theta=0.024 with D8S258, and 4.45 at theta=0.182 with D8S265, which indicated the location of D8S1053 at 8p22-p23.1.

Comments. A polymorphic dinucleotide repeats clone, pMS8-156, was isolated from a library that was developed by a chromosome microdissection and enzymatic amplification method. Although there was no obligate recombination between D8S1053 and D8S261 (AFM123xg5), flanking sequences of D8S1053 (CA) repeat were different from those of D8S2621. This result suggest that D8S1053 is a new microsatellite and may be useful to make a fine map.

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