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Short Communication

DINUCLEOTIDE REPEAT POLYMORPHISM ON CHROMOSOME 9q32

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Summary A new microsatellite was developed from a cosmid clone (cCI9-246) assigned to human chromosome 9q32.
Key Words microsatellite, human chromosome 9q32

Source/description. A cosmid clone, cCI9-246, was isolated from a cosmid library (cCI9) from a mouse-human somatic cell hybrid carrying only an intact human chromosome 9 (Takahashi *et al.*, 1994). An *Eco*RI subclone (p246-37) of cCI9-246 was isolated by hybridization to a $(CA)_{20}$ oligonucleotide and was partially sequenced. The sequences flanking a $(CA)_{16}$ repeat were used to design PCR primers (DDBJ accession No. D63668).

Primer sequences.

CA strand 5′-TTAGGATGAGATCCATGTCAGC-3′
GT strand 5′-AACCTATCTTGCCACAGAGACA-3′

Frequency. Allele frequencies were estimated from the genomic DNA of 60 chromosomes from unrelated Japanese individuals. The observed heterozygosity was 0.77.

Allele	Size (bp)	Frequency
A1	93	0.03
A2	87	0.03
A3	83	0.28
A4	81	0.17
A5	79	0.42
A6	77	0.05
A7	73	0.02

Chromosomal localization. cCI9-246 has been assigned to human chromosome

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9q32 by fluorescence *in situ* hybridization (Takahashi *et al.*, 1994).

Mendelian inheritance. Co-dominant segregation was observed in 12 two-generation families.

PCR conditions. PCR was performed in 25 μl reaction volumes containing 20 ng of genomic DNA; 20 pmol of one unlabeled primer and 20 pmol of one primer end-labeled with 1.0 μCi [γ-^{32}P]ATP using T4 polynucleotide kinase; 1 × PCR buffer (16.6 mM NH_4SO_4, 67 mM Tris-HCl pH 8.8, 10 mM β-mercaptoethanol, 6.7 μM EDTA); 10% (v/v) dimethyl sulfoxide; 1.5 mM each of dNTP; 5 mM $MgCl_2$ and 1.25 units Taq DNA polymerase. Samples were incubated in a DNA thermocycler (Nippon Genetics, Tokyo) for 36 cycles under the following conditions: 94°C for 2 min, 55°C for 3 min, and 72°C for 2 min. The first denaturation and final elongation steps were extended to 5 min and 10 min, respectively. The PCR products were resolved on 6% polyacrylamide gels containing 7 M urea and 32% formamide.

Comments. A polymorphic dinucleotide repeat subclone, p246-37, was isolated from a cosmid library containing an human chromosome 9. cCI9-246 lies within the Fukuyama-type congenital muscular dystrophy gene candidate region on 9q31-33 (Toda *et al.*, 1993, 1994). Since there was no sequence homology with known markers, this is a new microsatellite and may be useful for fine mapping and prenatal diagnosis.

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