RFLP Report

TWO DINUCLEOTIDE REPEAT POLYMORPHISMS AT THE D8S1444 AND D8S1445 LOCI

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Summary Two polymorphic dinucleotide (CA) repeat clones were isolated from two P1 phage clones, 1239F3 and 8H11, and were localized to chromosome 8 using a panel of 13 mouse/human somatic cell hybrids.

Key Words microsatellite, P1 phage, chromosome 8

Two P1 phage clones, 1239F3 (Genome System Inc.) and 8H11 (kindly provided by Dr. Fumihiko Matsuda and Dr. Tasuku Honjo), were digested completely with *Hae*III. *Hae*III-fragments were subcloned into the *Sma*I site of pUC18 and screened on the basis of hybridization to a ³²P-labelled poly(dA-dC)·poly(dG-dT) probe (Pharmacia) (Nagano *et al.*, 1993). Each positive subclone was partially sequenced and the sequences flanking a (CA)_n repeat were used to design PCR primers. Thus two polymorphic dinucleotide (CA) repeat polymorphisms, 58CA and 134IS1CA (Genbank accession number: G00-450-243), were isolated from the two P1 phage clones, 1239F3 and 8H11.

Primers for PCR

58CA-F=5' TTCTTCTAGATTTCCTACTA 3'

58CA-R=5' CATTTGTTAAAAGTACAACC 3'

134IS1CA-F=5' GCAACAGAGCGAGACTCCGTC 3'

134IS1CA-R=5' AAGCTTACATTCTGGGTGAC 3'

Polymorphism/frequency

58CA (D8S1444): Four alleles were detected in 88 chromosomes of unrelated Japanese individuals. Observed heterozygosity=0.32.

Received January 24, 1996 ; Revised version accepted March 28, 1996.

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Allele	Size (bp)	Frequency
A1	91	0.16
A2	115	0.77
A3	117	0.03
A4	119	0.03

1341S1CA (D8S1445): Eight alleles were detected in 124 chromosomes of unrelated Japanese individuals. Observed heterozygosity=0.74.

Allele	Size (bp)	Frequency
A1	117	0.07
A2	127	0.02
A3	129	0.08
A4	131	0.26
A5	133	0.31
A6	135	0.14
A7	137	0.08
A8	139	0.04

Chromosomal localization. The two dinucleotide (CA) repeat loci (D8S1444 and D8S1445) 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 (52°C for 58CA and 62°C for 1341S1CA) 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.

Acknowledgments This work was partly supported by a Grant-in-Aid for Creative Basic Research (Human Genome Program) from the Ministry of Education, Science and Culture of Japan, and a grant of the Research Project on Health and Aging.

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