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RFLP Report

SIX DINUCLEOTIDE REPEAT POLYMORPHISMS ON CHROMOSOME 7

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Six polymorphic dinucleotide (CA) repeat clones isolated from a chromosome microdissection library were mapped to chromosome 7 using human-mouse cell hybrids and linkage analysis of 5 CEPH families.
Key Words microsatellite, chromosome microdissection, linkage analysis

A microdissected library of human chromosome 8, region 8p11.2-p22, was prepared (Nagano *et al.*, 1993) and clones were screened for long dinuceotide repeats as previously described (Kamino *et al.*, 1993; Ye *et al.*, 1994; Nakura *et al.*, 1995). Six out of 24 polymorphic dinucleotide repeat clones were mapped on chromosome 7 using human-mouse cell hybrids (GDB accession No. G00-340-978).

Primers for PCR

	primer-F	primer-R	Ta (℃)
D7S1679 (MS8-93)	5' TACCAATTCACATATGCATGCA	5' TCCTCTGCTTGGCAGCCC	55
D7S1680 (MS8-98)	5' AAGGATTCCATTTAGCATCTC	5' TCAATATAATTCCTAATACAT	47
D7S1681 (MS8-135)	5' GGCTTGCCCATTGCTACAC	5' AAACGCATGGGTCTCAGTATG	60
D7S1682 (MS8-148)	5' GACAGAGCAAGACTCGACACA	5' AAACCATCCTGAGGAAAGTCA	57
D7S1683 (MS8-161)	5' CAGGATATGGTTTTAATGAAAT	5' GGAGTCTCTGAAGCGGTTAT	50
D7S1684 (MS8-173)	5' AGAAAACCCTAAGGACTACA	5' GTATCCTGCAACTTTACAGT	55

Allele size and frequencies. Allele fragments were resolved on DNA sequencing gels. Allele frequencies were calculated from the genotypes of 47-53 unrelated CEPH parents (Table 1).

PCR condition. The reaction was carried out in a volume of 10 μl containing

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Table 1. Characteristics of six microsatellites on chromosome 7.

D7S1679		θ max	Zmax
AFM200vc7	D7S506	0.038	10.47
AFM199vh8	D7S502	0.000	10.84
AFM070yc1	D7S482	0.000	10.84
AFM136xe3	D7S489	0.056	2.78
AFM248ta5	D7S524	0.107	3.99

allele	bp	frequency
A	163	0.77
B	167	0.23

H=21/49=0.43

D7S1680		θ max	Zmax
AFM032xa1	D7S478	0.033	7.13
AFM200wc7	D7S506	0.019	13.51
AFM191xh6	D7S499	0.071	7.95
AFM165zf4	D7S494	0.024	10.00
AFM240ve9	D7S520	0.029	8.56
AFM070yc1	D7S482	0.071	4.89

allele	bp	frequency
A	98	0.10
B	100	0.03
C	102	0.06
D	104	0.03
E	106	0.55
F	108	0.03
G	110	0.10
H	112	0.10

H=33/47=0.70

D7S1681		θ max	Zmax
AFM113xc1	D7S488	0.052	3.42
AFM248zd1	D7S529	0.031	6.80
AFM224xg5	D7S516	0.050	3.69
AFM248vc9	D7S526	0.129	3.25
AFM087yd11	D7S484	0.156	2.71
AFM177xf10	D7S497	0.129	3.25

allele	bp	frequency
A	88	0.40
B	90	0.00
C	92	0.58
D	94	0.01
E	96	0.01

H=26/51=0.51

H=heterozygosity

D7S1682		θ max	Zmax
AFM158xa1	D7S492	0.063	9.18
AFM248vd9	D7S527	0.015	17.26
AFM036xg5	D7S479	0.021	12.10
AFM151xf10	D7S491	0.018	14.80
AFM248te5	D7S554	0.000	3.01
AFM225xg9	D7S518	0.021	12.33
AFM220xc11	D7S515	0.024	9.69
AFM199vb2	D7S501	0.088	5.53

allele	bp	frequency
A	115	0.24
B	117	0.15
C	119	0.55
D	121	0.04
E	123	0.02

H=33/50=0.66

D7S1683		θ max	Zmax
AFM113xc11	D7S488	0.000	19.27
AFM248zd1	D7S529	0.111	8.07
AFM224xg5	D7S516	0.093	9.99

allele	bp	frequency
A	73	0.01
B	81	0.08
C	89	0.07
D	95	0.28
E	97	0.28
F	99	0.10
G	101	0.03
H	103	0.09
I	105	0.04
J	107	0.02
K	115	0.01

H=43/53=0.81

D7S1684		θ max	Zmax
AFM248ve5	D7S528	0.045	4.43
AFM095xe9	D7S485	0.030	7.53
AFM240yh4	D7S521	0.000	13.15

allele	bp	frequency
A	81	0.04
B	83	0.01
C	85	0.39
D	87	0.52
E	89	0.04

H=31/51=0.61

20 ng genomic DNA, 2 pmol of rhodamine-labeled primers, 200 μM dNTP, 10 mM 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: denaturation at 94°C for 30 sec, annealing at the Ta specific for each clone for 30 sec, and extension at 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 and Mendelian inheritance. Localized to chromosome 7 using human-rodent cell hybrids. Linkage analyses with 5 CEPH families using data from the CEPH database v5 gave more precise localization information (Table 1). Co-dominant segregations were observed in all informative CEPH pedigrees.

Comments. The reason why six out of twenty-four polymorphic dinucleotide repeat clones from a chromosome 8 microdissection library were mapped to chromosome 7 seemed to be contamination of a part of chromosome 7 during microdissection. Some clones showed no obligate recombinations with the loci reported by CEPH database v5, however flanking sequences of both repeats were different from each other. These results suggest that six microsatellites are new clones.

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

Kamino K, Nakura J, Kihara K, Ye L, Nagano K, Ohta T, Jinno Y, Niikawa N, Miki T, Ogihara T (1993): Population variation in the dinucleotide repeat polymorphism at the D8S360 locus. Hum Mol Genet **2**: 1751

Nagano K, Nakura J, Kihara K, Ye L, Kamino K, Mitsuda N, Ohta T, Jinno Y, Niikawa N, Miki T, Ogihara T (1993): Isolation and mapping of microsatellites from a library microdissected from the Werner 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 (1994): Dinucleotide repeat polymorphism at the D8S1053. Jpn J Human Genet **39**: 445–446

Ye L, Kihara K, Nakura J, Kamino K, Mitsuda H, Takahashi-Fujii A, Ishino Y, Miki T, Ogihara T (1994): Dinucleotide repeat polymorphism at the D8S1055. Jpn J Human Genet **39**: 441–443