

RFLP Report

A NOVEL (CA)_n POLYMORPHISM ON 6p21.1-21.2

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Summary A novel highly polymorphic CA repeat locus D6S2213 was identified on human chromosome 6p21.1-21.2. It should be a useful marker for linkage studies on chromosome 6 and also in forensic use.

Key Words microsatellite, polymorphism, D6S2213, 6p21

Source/description. Cosmid 55Y-38H was isolated from a human cosmid library by screening with a (CA)₂₀ oligonucleotide. The flanking sequences of microsatellite were determined by direct sequencing of cosmid DNA using the idea of motif-specific primers proposed by Yuille *et al.* (1991).

Polymorphism. Eight allelic fragments ranging in size from 140 to 160 bp were observed in 40 unrelated Japanese individuals using DNA sequencing gels. Allele frequencies and their upper and lower confidence limits according to Goodman (1965) are presented in Table 1. Heterozygosity of 0.675 was observed. Polymorphism information content (PIC) was calculated as 0.63 (Krawczak and Schmidtke, 1994).

PCR conditions. Two primers: 55Y-38H1, 5'-AATAGAGCTCCAGCTTC-AGTG-3'; and 55Y-38H2, 5'-AGACTGAGACCCTGTCTCTGA-3' were developed. One of them was 5' labeled with T4 polynucleotide kinase and [γ -³²P]-dATP (Amersham). Reaction mixture contained 20 ng of genomic DNA, 1.5 units of Taq-polymerase in 25 μ l of: 67 mM Tris-HCl, pH 8.7; 16.6 mM (NH₄)₂SO₄; 4 mM MgCl₂; 10 mM 2-mercaptoethanol; 1 mM dNTPs and 0.4 μ M each primer. Thirty cycles were performed as follows: 1 min 94°C; 1 min 60°C and 1 min 72°C. Initially incubation at 94°C was performed for 4 min. Finally incubation at 72°C for 10 min was fulfilled. PCR products were analyzed in 6% polyacrylamide gel (PAAG) with 8 M urea and 32% formamide (Boehringer Mannheim). The electrophoresis was

Received June 4, 1996; Revised version accepted September 13, 1996.

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Table 1. Allele frequencies estimated at the D6S2213 locus from 40 unrelated Japanese individuals.

Allele (size of PCR product, bp)	Frequency	95% LCL/95% UCL*
140	0.025	0.0045/0.1269
144	0.0125	0.0013/0.1072
150	0.0375	0.0088/0.1454
152	0.625	0.4721/0.7564
154	0.1	0.04/0.2286
156	0.1125	0.0474/0.244
158	0.075	0.0261/0.1967
160	0.0125	0.0013/0.1072

* LCL, lower confidence limit; UCL, upper confidence limit.

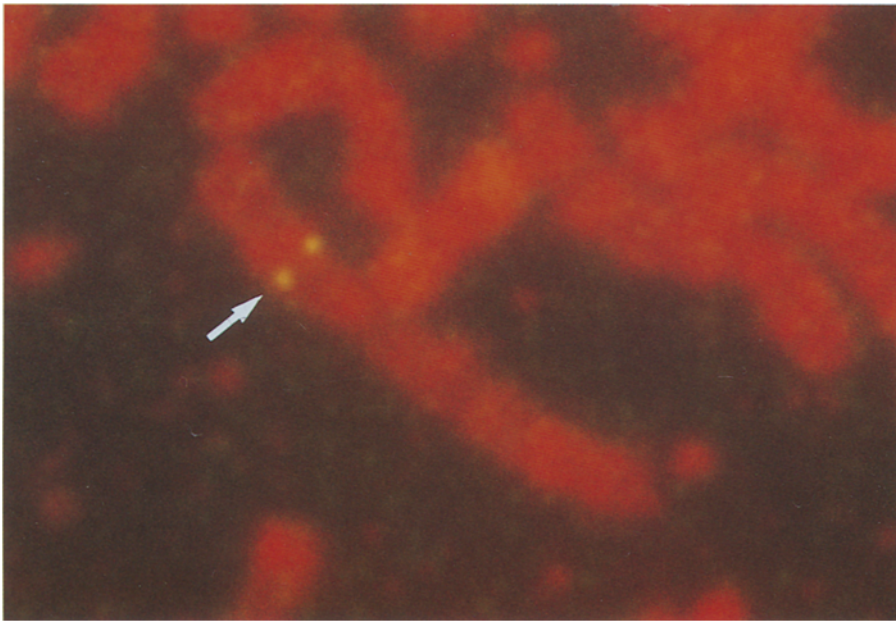


Fig. 1. Sublocalization of new $(CA)_n$ repeat marker D6S2213 on human chromosome 6 by FISH. The 55Y-38H cosmid containing the microsatellite hybridized to the 6p21.1-21.2.

carried out at 2,500 V for 3 hr. After soaking for 20 min in 5% of acetic acid and 5% methanol, the gel was dried and exposed in direct contact with a Fuji X-ray film overnight without intensifying screen.

Chromosome localization. The marker was sublocalized to 6p21.1-21.2 by *in situ* hybridization (Fig. 1) using the protocol of Hirai *et al.* (1994).

Pattern of inheritance. Codominant segregation of the different alleles was

revealed in 20 two-generation families.

Comment. The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases with the following accession number, D78267. Accession numbers for the presented locus D6S2213 in GDB are: 1238479 and 1238480.

Acknowledgments S.E.K. was supported by JSPS postdoctoral fellowship #P95114. This work was supported by the Grants-in-Aid for General Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

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