Dinucleotide repeat polymorphism at the D21S198 locus

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Source and Description: Cosmid ICRFc102D1122 was from a flow sorted chromosome 21 cosmid library. DNA sequences flanking a (TG)₂₃ repeat within a HaeIII subclone (VS1) of this cosmid (EMBL accession no. X58124) were used to design PCR primers.

PCR primers:

VS1 #1: 5'-TGTCACATAAGGGAAGAG-3'

VS1 #2: 5'-GATGTTAGCAGCTCTGC-3'

Polymorphism: Nine allelic fragments were resolved on DNA sequencing gels. Lengths of the allelic fragments (nt) were: A1 = 128, A2 = 126, A3 = 124, A4 = 122, A5 = 120, A6 = 118, A7 = 116, A8 = 114, A9 = 112. Alleles found in four CEPH parents were: 140801 = A1, A4; 140802 = A6, A8; 142301 = A1, A6; 142302 = A5, A9.

Frequencies: Allele frequencies in 44 unrelated European Caucasians were:

A1 = .045 A2 = .068 A3 = .068 A4 = .023
A5 = .113 A6 = .205 A7 = .068 A8 = .295
A9 = .113 PIC = .81

Chromosomal Localization and Mendelian Inheritance: PCR of genomic DNAs from a somatic cell hybrid panel (1) indicated localization to 21q22.3. Linkage analysis with 3 CEPH families showed no recombination in 24 informative meioses with PFKL probe pG-PFKL3.3 (localized to q22.3 (2)), giving a maximum LOD score of 6.4 at theta = 0. Mendelian inheritance was observed in all cases.

PCR Conditions: PCR reactions are carried out in a total volume of 25 µl containing: 25 ng genomic DNA, 15 pmole of each primer, 1.5 mM MgCl₂, 200 µM dNTPs, 50 mM Tris-Cl, 20 mM (NH₄)₂SO₄, pH 9 and 0.6 units of ReplinasTM (NEN/Dupont). Amplification is for 35 cycles with denaturation at 95°C for 60 seconds, annealing at 54°C for 60 seconds and extension at 72°C for 30 seconds. PCR products were resolved on DNA sequencing gels, capillary-blotted onto Hybond N+TM nylon membranes (Amersham) and revealed by probing with 5' [³²P] labeled (TG)₇ oligomer.

References: 1) K.Gardiner, M.Horisberger, J.Kraus, U.Tantravahi, J.Korenberg, V.Rao, S.Reddy and D.Patterson (1990) *EMBO J.* 9, 25–34. 2) B.Carritt and M.Litt (1989) *Cytogenet Cell Genet* 51, 358–371.

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Dinucleotide repeat polymorphism at the D9S55 locus

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Source and Description: Cosmid ICRFc102F0621 was from a flow sorted chromosome 21 cosmid library. DNA sequences flanking a (TG)₂₄ repeat within an EcoRI subclone (1F6) of this cosmid (EMBL accession no. X58125) were used to design PCR primers.

PCR primers:

1F6 #2: 5'-GAGAAAATTCCAGGCA-3'

1F6 #3: 5'-GGTTGAGTCGTTCTTA-3'

Polymorphism: Nine allelic fragments were resolved on DNA sequencing gels. Lengths of the allelic fragments (nt) were: A1 = 197, A2 = 191, A3 = 189, A4 = 187, A5 = 185, A6 = 183, A7 = 181, A8 = 179, A9 = 165. Alleles found in four CEPH parents were: 134901 = A2, A3; 134902 = A5, A7; 141601 = A6, A7; 141602 = A3, A4.

Frequencies: Allele frequencies in 57 unrelated European Caucasians were:

A1 = .01 A2 = .01
A3 = .170 A4 = .315
A5 = .245 A6 = .130
A7 = .105 A8 = .01
A9 = .01
PIC = .75

Chromosomal Localization and Mendelian Inheritance: Fluorescence in situ hybridization localized cosmid ICRFc102F0621 to 9p12. Linkage analysis with 4 CEPH families showed no recombination in 40 informative meioses with D9S18 probe pHHH220 (localized to 9p13 (1)), giving a maximum LOD score of 11.5 at theta = 0. Mendelian inheritance was observed in all cases.

PCR Conditions: PCR reactions are carried out in a total volume of 25 µl containing: 25 ng genomic DNA, 15 pmole of each primer, 1.5 mM MgCl₂, 200 µM dNTPs, 50 mM tris-Cl, 20 mM (NH₄)₂SO₄, pH 9 and 0.6 units of ReplinasTM (NEN/Dupont). Amplification is for 35 cycles with denaturation at 95°C for 60 seconds, annealing at 52°C for 60 seconds and extension at 72°C for 30 seconds. PCR products were resolved on DNA sequencing gels, capillary-blotted onto Hybond N+TM nylon membranes (Amersham) and revealed by probing with 5' [³²P] labeled (TG)₇ oligomer.

Reference: 1) M.Lathrop, Y.Nakamura, P.O'Connell, M.Leppert, S.Woodward, J.M.Lalouel and R.White (1988) *Genomics* 3, 361–366.

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