Dinucleotide repeat polymorphism (D16S285) on human chromosome 16

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Source/Description: An Alu PCR fragment (1) from an EMBL-3A human genomic phage library was found to hybridize to a poly dC-dA poly dG-dT probe. This fragment was subcloned into pBS SK and sequenced; sequences flanking a $(GT)_n$ repeat element were used to design PCR primers.

Primer Sequences:
CA strand: GCC-TAA-TTT-GAT-CTA-TAC
GT strand: AGT-ATC-TTT-AGA-GCC-CTT
Length of amplified fragment: 107 to 129 bp.

Frequency: Estimated from 138 chromosomes of 69 unrelated Caucasians

Allele	Frequency	Allele	Frequency
A12 107	0.072	A6 119	0.333
A11 109	0.065	A5 121	0.116
A10 111	0.007	A4 123	0.080
A9 113	0.007	A3 125	0.058
A8 115	0.145	A2 127	0.007
A7 117	0.101	A1 129	0.007

% Heterozygosity: 82.5.

Chromosomal Localization: Assigned to chromosome 16 using DNA templates from a panel of somatic cell hybrids obtained from Coriell Institute, Camden, N.J.

Mendelian Inheritance: Co-dominant inheritance was observed in 7 multi-generation families.

Other Comments: Conditions for the amplification reaction: 94°C, 1.5 min, then 25 cycles of 94°C/1 min, 55°C/1 min, 72°C/1 min, followed by a final extension period for 7 min at 72°C.

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Reference: 1) Nelson,D.I., Ledbetter,S.A., Corbo,L., Victoria,M.F., Ramirez-Solis,R., Webster,T.D., Ledbetter,D.H. and Caskey,C.T. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6686–6690.

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Dinucleotide repeat polymorphism of D15S10 in the Prader-Willi chromosome region (PWCR)

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Introduction: Interstitial deletions and uniparental disomy involving the PWCR at 15q11–13 have been demonstrated in individuals with the Prader-Willi syndrome (PWS) and Angelman syndrome. Determination of the parental source of the PWCR has been hampered by the limited number of informative polymorphisms.

Source/Description: A *Bam*HI-digested genomic library constructed in EMBL3 from the mother of an individual with PWS was screened with probe pTD3–21 (D15S10). A subclone of a positive phage clone which hybridised to poly(dC-dA).poly(dG-dT) was identified and sequenced. A dinucleotide repeat in the form $(CA)_{13}.TA.(CA)_5$ was found, and PCR oligonucleotide primers synthesised.

Polymerase Chain Reaction (PCR): Thirty cycles of amplification were performed, each consisting of denaturation at 94°C for 15s, annealing at 52°C for 15s, and extension at 72°C for 40s (10s for the final cycle). Fifty μl reactions contained ~250 ng genomic DNA, 1 U Amplitaq (Perkin Elmer-Cetus Corporation), 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM $MgCl_2$, 0.01% gelatin, primers 5'-GTA ACA CTA TGA ATT GTT AGT G-3' (CA strand) and 5'-GAC AGC TGA ACG TAG TTA AAG- 3' (GT strand) 20 pmoles, dCTP, dGTP and dTTP at 100 μM each, dATP at 2.5 μM, and 4 pmoles of ^{35}S-α-dATP. 2.5 μl of PCR products were electrophoresed on a 10% denaturing polyacrylamide gel.

Allele Frequency: Estimated from 80 chromosomes in unrelated normal Caucasians (PIC = 0.375);

Allele (bp)	Frequency
181	0.49
179	0.51

Mendelian Inheritance: Co-dominant inheritance has been demonstrated in one family.

Discussion: A deletion of the D15S10 locus in the PWCR was excluded by demonstrating heterozygosity in 7 of 20 individuals with classical PWS. In two individuals, a diagnosis of maternal heterodisomy was made using the dinucleotide repeat and an RFLP. In other cases the parental origin of allele loss at D15S10 was able to be determined.

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