Dinucleotide repeat polymorphism at D16S287

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Source/Description: Lambda clone 16XE81 was isolated as previously described (1). DNA from this clone was positive when hybridized with poly(dC-dA)·poly(dG-dT). *Sau*3AI fragments were subcloned into the *Bam*HI site of M13mp18 and positive clones were sequenced. The dinucleotide repeat sequence was of the form $(GT)_{23}$. The length of the amplified fragment using primer set 1 was 215 bp (allele A6).

Primer Sequences (Set 1):
GT strand: 5′ GCT TGT ATT AGT CAG CAT TCT CCA G3′
CA strand: 5′ TAC AGA CCA TAG ACT TGA CAG TCT C3′

Primer Sequences (Set 2):
GT strand: 5′ CAC CTC CAT TTT AGC ACT ATT TGG 3′
CA strand: 5′ GAG CCA AGT CCT TAT AGT GGA TAC 3′

Frequency: Estimated from 79 CEPH parents using primer set 1. Heterozygosity was 78%.

Allele (bp)	Frequency	Allele (bp)	Frequency
A1 (225)	0.01	A6 (213)	0.38
A2 (221)	0.14	A7 (211)	0.12
A3 (219)	0.13	A8 (209)	0.01
A4 (217)	0.07	A9 (207)	0.01
A5 (215)	0.12	A10 (201)	0.01

Chromosomal Localisation: Localised by PCR to 16p13.11 using a panel of somatic cell hybrids.

Mendelian Inheritance: Co-dominant segregation was observed in 39 of the 40 CEPH families. The remaining family, CEPH pedigree 1333, had 4 out of 8 offspring who did not inherit an apparently homozygous allele from their father, who in turn appeared not to inherit an allele from his mother. One explanation was mis-match between one of the primer and template DNA sequences resulting in segregation of a null allele. A second primer set (set 2 above) was designed from different locations (giving an amplified fragment length of 208 bp). Mendelian segregation was now observed, confirming the hypothesis of a null allele in CEPH pedigree 1333 using primer set 1.

PCR Conditions: The PCR reaction was performed on 100 ng of genomic DNA with 150 ng of each oligodeoxyribonucleotide primer and 10 μCi alpha ^{32}P dCTP. Conditions for the PCR amplification were as follows: 10 cycles of 1 minute at 94°C, 1.5 minutes at 60°C, and 1.5 minutes at 72°C, then 25 cycles of 1 minute at 94°C, 1.5 minutes at 55°C, and 1.5 minutes at 72°C. The final elongation cycle was 10 minutes at 72°C.

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Reference: 1) Hyland,V.J. *et al.* (1989) *Hum. Genet.* **83**, 61–66.

Dinucleotide repeat polymorphism at the D8S135 locus on chromosome 8p

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Source/Description: A $(GT)_n$ microsatellite was detected within the LA08NC01 library (1) cosmid 171B10 that contains a NotI site. Using a poly(dG-dA)·(dC-dT) hybridization probe (Pharmacia). A subclones containing the repeat was sequenced and flanking PCR primers synthesized.

PCR Primers:
D8S137-GT: 5′GCTAATCAGGGAATCACCCAA3′
D8S137-CA: 5′AAATACCGAGACTCACACTATA3′

Allele Frequencies: Estimated from 160 chromosomes of CEPH parents.

D8S137

allele	size (bp)	frequency
1	162	0.00625
2	160	0.01875
3	158	0.03125
4	156	0.31875
5	154*	0.44375
6	152	0.18125

Heterozygosity: 0.67 PIC: 0.61
Asterisk indicates size of cloned allele.

Chromosomal Localization: Located on 8p using somatic cell hybrids.

Mendelian Inheritance: Codominant segregation was observed in 37 CEPH families.
D8S137 reference genotypes: 133101 = 4,6; 133102 = 5,6.

PCR Conditions: 30 cycles of 1 min at 94°C, 30 sec at 58°C, and 2 min at 72°C. 40 ng genomic DNA was used with 10 pmole each primer in 25 μl reactions. The buffer was 1.5 mM $MgCl_2$ (D8S137), 50 mM Tris-HCl pH 8.3, 0.02% NP 40, 0.02% Tween 20, 200 μM each dNTP. The D8S137-CA primer was end labelled and 1 pmole (0.5 μC) was added to each reaction. Allele sizes were determined relative to the sequenced allele. Reactions were electrophoresed on 5% denaturing Hydrolink (AT Biochemicals) gels.

Other Comments: The repeat sequence is $(TG)_5CG(TG)_9T$. The GT repeat region has been deposited under the Accession Number X61694 and the associated NotI site region 171B10N1 under the Accession Number X61693 (GenBank).

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Reference: 1) Wood,S. *et al.* (1991) *Cytogenet. Cell Genet.*. In Press.