Dinucleotide repeat polymorphism at D16S533

A.M.Cleton-Jansen[1,*], H.W.Moerland[1], R.H.A.M.Vossen[2], D.F.Callen[3], M.H.Breuning[2] and P.Devilee[1,2]
[1]Department of Pathology and [2]Human Genetics, University of Leiden, 2300 RC Leiden, The Netherlands and [3]Department of Cytogenetics and Molecular Genetics, Adelaide Children's Hospital, Adelaide, Australia

Source/Description: The cosmid C82 was isolated from a flow-sorted chromosome 16 library (1). The polymorphic $(CA)_n$ repeat was identified in an M13mp18 subclone of the cosmid. The predicted length of the amplified product is 207 bp. The length of the repeat in 154 chromosomes of unrelated individuals ranged from 15 to 29 CA/GT blocks.

Primer Sequences:

D16S533F 5'-GGGTCATATTCCTGCTCATGGTCC-3'
D16S533R 5'-CTGTGTAGCTAGAATGCATGTACC-3'

Frequency: Estimated from 154 chromosomes of unrelated female Dutch individuals. Heterozygosity = 78%.

Allele	(bp)	Frequency	Allele	(bp)	Frequency
A1	199	.01	A6	209	.03
A2	201	.05	A7	211	.05
A3	203	.46	A8	213	.01
A4	205	.11	A9	215	.01
A5	207	.27			

CEPH: 1331 01: 203, 203/1331 02: 207, 207/1333 01: 207, 203/1333 02: 203, 199/1340 01: 207, 203/1340 02: 203, 203/1341 01: 205, 203/1341 02: 207, 205/1345 01: 213, 207/1345 02: 205, 203.

Chromosomal Localization: The location of cosmid C82 has been determined by using the human X mouse hybrid cell line panel of chromosome 16 (2). C82 has been assigned to band 16q21 in the region bordered by the breakpoints of hybrid cell line CY130 and by the proximal breakpoint of CY125.

PCR Conditions: The PCR reaction was carried out in 12 μl volumes containing 100 ng of genomic DNA, 6 pmoles of each primer, 1×SuperTaq buffer (HT Biotechnology Ltd), 200 μM each of dGTP, dATP and dTTP, 2.5 μM dCTP, 1 μCi α-^{32}P-dCTP and 0.06 units of SuperTaq polymerase (HT Biotechnology Ltd). The amplification is for 27 cycles with 1 min 94°C, 2 min 55°C and 1.5 min 72°C. Products are detected on a 6.5% denaturing polyacrylamide gel.

Mendelian Inheritance: Co-dominant segregation of D16S533 was observed in 5 two generation families and in 3 three generation families.

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References: 1) Breuning *et al.* (1987) In: *Advances in the Pathogenesis of Polycystic Kidney Disease*. Baxter Healthcare Corporation, pp. 17–21. 2) Callen *et al.* (1992) *Genomics* **13**, 1178–1185.

* To whom correspondence should be addressed

Dinucleotide repeat polymorphism in the human RFX1 gene

Ilse Kern*, Catherine Ucla and Bernard Mach
Jeantet Laboratory of Molecular Genetics, Department of Genetics and Microbiology, University of Geneva Medical School, Geneva, Switzerland

Source/Description: The (TG)n dinucleotide repeat sequence is located in an intron in the 5' region of the human RFX1 gene (1). The expected length of the amplified fragment was 186 bp.

Primer Sequences:
Forward 5' ATTGCACCACTGTACTC 3' (TG strand)
Reverse 5' CATGTGTACATATATGTATACAGTG 3' (AC strand)

Frequency: Estimated from 120 chromosomes of unrelated Caucasian individuals.

Allele	Size (bp)	Frequency
A1	194	0.01
A2	192	0.17
A3	190	0.28
A4	188	0.28
A5	186	0.26

Heterozygosity: 0.72.

CEPH:
13291 01 (A2/A5), 13291 02 (A4/A4)
13292 01 (A5/A5), 13292 02 (A3/A4)
1331 01 (A4/A4), 1331 02 (A4/A4)

Chromosomal Localization: 19p13.1 (2).

Mendelian Inheritance: Co-dominant segregation was observed in 3 three-generation families.

PCR Conditions: PCR amplification was performed on 200 ng of genomic DNA in a 20 μl reaction volume containing 10 μM Tris pH 8.3, 50 μM KCl, 1.5 μM $MgCl_2$, 1 U AmpliTaq, (Cetus), 250 μM dNTPs, 250 μM of each unlabelled primer and 5 μM end-labelled forward primer. 11 cycles of denaturing at 92°C for 30 sec, annealing at 52°C shifting to 42°C for 40 sec, extension at 72°C for 30 sec, followed by 35 cycles of denaturing at 92°C for 30 sec, annealing at 42°C for 40 sec and extension at 72°C for 30 sec were carried out with an initial denaturation step of 5 mins at 94°C.

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* To whom correspondence should be addressed