Dinucleotide repeat polymorphism adjacent to the ANT1 gene on 4q35

Cisca Wijmenga, Larry Deaven[1] and Rune R.Frants*
MGC-Department of Human Genetics, Leiden University, PO Box 9503, 2300 RA Leiden, The Netherlands and [1]Los Alamos National Libraries, Los Alamos, USA

Source/Description: The polymorphic $(TG)_{16}$ repeat resides within cosmid 168D11 (Los Alamos chromosome 4 library), containing the ANT1 gene. The polymorphism can be typed following amplification with PCR as described previously (Weber and May, 1989). The predicted length of the amplified fragment was 157 bp.

Primer Sequences:
5' CCTTGTTCTAGCAAGCACAT 3' (TG strand)
5' CGGCTTCATAAGCCAATTTC 3' (CA strand)

Frequency: Estimated from 80 chromosomes of 40 unrelated Caucasian individuals.

allele	(bp)	frequency
A1	163	0.01
A2	161	0.05
A3	159	0.04
A4	157	0.57
A5	155	0.03
A6	143	0.30

The observed heterozygosity in our sample is 57%.

Mendelian Inheritance: Co-dominant segregation was observed in 13 informative families.

Chromosomal Localisation: Assigned to 4q35 by *in situ* hybridisation of cosmid 168D11 (Wijmenga et al., manuscript in preparation).

Other Comments: The PCR reaction (15 μl) contains 30 ng genomic DNA, 30 ng of each oligonucleotide primer, 200 μM each dATP, dGTP and dDTP, 2.5 μM dCTP, and 0.75 μCiα^{32}P-dCTP at 800 Ci/mmol, plus 10 mM Tris-Cl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% (w/v) gelatin, 0.1% Triton X-100, and 0.06 unit Super Taq (HT Biotechnology Ltd.). Samples were processed through 27 cycles consisting of 1 minute at 94°C, 2 minutes at 55°C and 1 minute at 72°C.

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Reference: Weber,J.L. and May,P.E. (1989) *Am. J. Hum. Genet.* **44**, 388-396.

* To whom correspondence should be addressed

Dinucleotide repeat polymorphism at the D11S860 locus

L.A.McNoe, M.R.Eccles and A.E.Reeve*
Molecular Carcinogenesis Laboratory, Biochemistry Department, University of Otago Medical School, Dunedin, New Zealand

Source and Description of Clone: Phage clone BS48 from a human/CHO hybrid J-43A library containing human sequences from 11p15 (1). DNA sequence from BS48 (a subclone of BS48 containing a compound imperfect dinucleotide repeat) was used to design flanking PCR primers.

PCR Primers:
(1) 5'-GCAACACGTACACACTGAGACA-3'
(2) 5'-TAGTATTGCCATAGAAGAAGC-3'
PCR products were analysed by electrophoresis through a 6% polyacrylamide-urea gel. GenBank accession nos; (1) M86259, (2) M86260.

Polymorphism: Allele frequencies in 40 unrelated individuals.

Allele	Allele size (bp)	Frequency
A1	196	0.038
A2	182	0.038
A3	180	0.063
A4	178	0.25
A5	176	0.012
A6	174	0.012
A7	168	0.012
A8	166	0.10
A9	164	0.075
A10	162	0.075
A11	160	0.33
A12	154	0.013

Heterozygosity in 40 unrelated individuals was 80%.

Chromosomal Location: The probe was localized by PCR analysis to band 11p15 between D11S12 and INS using DNA from chromosome 11 deletion hybrids (1).

Mendelian Inheritance: Co-dominant segregation was observed in 3 families with a total of seven informative meioses.

PCR Conditions: PCR reactions were in a total volume of 20 μl containing 100 ng of genomic DNA, 40 pmol of unlabelled primers, 1.5 pmol of 5'-^{32}P labelled primers, 4.5 mM Tris pH 8.8, 1.1 mM $(NH4)_2SO4$, 4.5 mM $MgCl_2$, 0.67 mM β-mercaptoethanol, 0.45 mM EDTA, 0.025 mM spermidine, 200 mM DNTPs and 0.3 units of Taq polymerase (Perkin-Elmer/Cetus). Amplification was for 31 cycles. The first 7 cycles were between 96°C and 60°C for 1 min each. The remaining 24 cycles were between 90°C and 60°C for 1 min each.

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References: 1) Glaser,T., Housman,D., Lewis,W.H., Gerhard,D. and Jones,C. (1989) *Somat. Cell. Molec. Genet.* **15**, 477-501.

* To whom correspondence should be addressed