Trinucleotide repeat polymorphism at the D19S190 locus

David E.Iles, Bart Segers, Pieter de Jong[1], Jennifer Alleman[1] and Bé Wieringa
Department of Cell Biology and Histology, School of Medical Sciences, University of Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands and [1]Human Genome Center, Biomedical Sciences Division L-452, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA

Source/Description: A cosmid clone f20102 was isolated from a cosmid library constructed from sorted human chromosomes 19 (de Jong et al., 1989), on the basis of its hybridization to the single-copy human DNA probe pOL5 (DNF 11). Sau3AI fragments from f20102 were subcloned into pGEM4 and clone pOL5.12a2 was selected by hybridization to an end-labeled $(C\text{-}AC)_5$ oligonucleotide. Sequencing pOL5.12a2 provided the information required for the synthesis of polymerase chain reaction primers. The clone insert length was 469 bp and the predicted length of the amplified fragment was 114 bp.

Primer Sequences: CCCATTGGTTGATTTTGCTGC (CAC) strand; TTCTACTTGGAGGAAGAGGAGG (GTG) strand.

Frequency: Estimated from 70 chromosomes of unrelated individuals (Caucasians). PIC = 0.55.

Allele(bp)	Frequency
A1 117	0.17
A2 114	0.44
A3 111	0.39

Chromosomal Localization: Localized at chromosome 19q13.1 using a somatic cell hybrid mapping panel.

Mendelian Inheritance: Co-dominant inheritance has been observed in 6 three generation families (57 meioses).

Other Comments: Amplification reactions were performed on 100 ng samples of genomic DNA in 16.5 mM $(NH_4)_4SO_4$, 65 mM Tris-HCl pH 8.8, 1.5 mM $MgCl_2$, 0.065 mM EDTA, 10 mM 2-mercaptoethanol, 10% DMSO, 0.17 mg/ml BSA, 0.2 mM dNTP, 100 ng forward (CAC strand) primer and 100 ng ^{32}P-labeled reverse (GTG strand) primer in a 50 μl final volume. Samples were incubated at 95°C for 5 minutes, at 80°C for the addition of 1 U Taq DNA polymerase (Ampli-Taq, Perkin Elmer Cetus) per reaction, and processed through 30 temperature cycles consisting of 94°C for 45 seconds, 55°C for 60 seconds and 72°C for 45 seconds. Sizes of the alleles were determined by comparison to amplified plasmid control (20 pg) and DNA sequence ladders on 8% denaturing polyacrylamide gels. The trinucleotide repeat sequence in pOL5.12a2 has the structure $(CAC)_{14}C$. The sequence of OL5.12a2 has been submitted to the EMBL data bank, accession number X62851 Homo sapiens satellite DNA.

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Reference: de Jong,P.J., Yokobata,K., Chen,C., Lohman,F., Pederson,L., McNinch,J. and van Dilla,M. (1989) *Cytogen. Cell Genet.* **51**, 985.

Dinucleotide repeat polymorphism at the D19S191 locus

David E.Iles, Bart Segers, Pieter de Jong[1], Jennifer Alleman[1] and Bé Wieringa
Department of Cell Biology and Histology, School of Medical Sciences, University of Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands and [1]Human Genome Center, Biomedical Sciences Division L-452, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA

Source/Description: A cosmid clone f18277 was islated from a cosmid library constructed from sorted human chromosomes 19 (de Jong et al., 1989), on the basis of its hybridization to the single-copy human DNA probe p5B18 (D19S28). Sau3AI fragments from f18277 were subcloned into pGEM4 and clone p5B18.10b2 was selected by hybridization to an end-labeled $(TG)_6G$ oligonucleotide. Sequencing 5B18.10b2 provided the information required for the synthesis of polymerase chain reaction primers. The clone insert length was 579 bp and the predicted length of the amplified fragment was 103 bp.

Primer Sequences: AGTAAAGAGGTTGAATTAATGACC (AC) strand; TGCCAGCGAAGCTATCTGG (TG) strand.

Frequency: Estimated from 70 chromosomes of unrelated individuals (Caucasians). PIC = 0.79.

Allele (bp)	Frequency	Allele (bp)	Frequency
A1 125	0.014	A7 111	0.0285
A2 123	0.57	A8 109	0.0285
A3 121	0.0285	A9 107	0.3
A4 119	0.057	A10 105	0.143
A5 117	0.0285	A11 103	0.257
A6 115	0.057		

Chromosomal Localization: Localized at chromosome 19q13.1 using a somatic cell hybrid mapping panel.

Mendelian Inheritance: Co-dominant inheritance has been observed in 6 three generation families (57 meioses).

Other Comments: Amplification reactions were performed on 100 ng samples of genomic DNA in 1× GeneAMP buffer (Perkin Elmer Cetus), 0.2 mM dNTP, 100 ng reverse (TG strand) primer and 100 ng ^{32}P-labeled forward (AC strand) primer in a 50 μl final volume. Samples were incubated at 95°C for 5 minutes, at 80°C for the addition of 1U Taq DNA polymerase (AmpliTaq, Perkin Elmer Cetus) per reaction, and processed through 30 temperature cycles consisting of 94°C for 45 seconds, 45°C for 90 seconds and 72°C for 45 seconds. Sizes of the alleles were determined by comparison to amplified plasmid control (20 pg) and DNA sequence ladders on 8% denaturing polyacrylamide gels. The dinucleotide repeat sequence in 5B18.10b2 has the structure $(AC)_6AT(AC)_{18}C$. The sequence of 5B18.10b2 has been submitted to the EMBL data bank, accession number X62850 Homo sapiens satellite DNA.

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Reference: de Jong,P.J., *et al.* (1989) *Cytogen. Cell Genet.* **51**, 985.