Dinucleotide repeat polymorphism at the D16S288 locus

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Source and Description of Clone: A cosmid library (1) of flow-sorted human chromosome 16 from the somatic hybrid cell line CY18 was digested with *Sau3AI*, subcloned into *SalI* cut M13 mp 19 after partial end-filling of both restriction sites and screened by hybridization to poly(dC-dA)·poly(dG-dT). One of the positive cloned fragments was sequenced and designated 16A-C7.1. The length polymorphism of the AC repeat contained in this subclone was typed using the polymerase chain reaction (PCR). The length of the amplified fragment (A3 allele) was 160 bp.

PCR Primers:

ATGAGGAGCAGAGGTGTCTCTT (CA strand)

TAAATCACAGACTCCCTTGGCT (GT strand)

Frequencies: Estimated from 146 chromosomes of unrelated CEPH parents.

Het = 0.73 PIC = 0.69

Allele (bp)	Frequencies	Allele (bp)	Frequencies
A6 (154)	0.034	A3 (160)	0.226
A5 (156)	0.158	A2 (162)	0.411
A4 (158)	0.164	A1 (166)	0.007

Chromosomal Localisation: Assigned to chromosome 16p11.2–12.1 by PCR using DNA templates isolated from a panel of somatic cell hybrids (2).

Mendelian Inheritance: Co-dominant segregation was observed in CEPH families 45 and 1334.

PCR Conditions and Other Comments: The PCR reaction was performed on 100 ng of genomic DNA with 150 ng of each oligodeoxyribonucleotide primer and 10 μ Ci alpha 32 PdCTP using a previously described reaction mixture (3). Conditions of the PCR amplification are as follows: 10 cycles of 1 minute at 94°C, 1.5 minutes at 60°C, and 1.5 minutes at 72°C. 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. The amplified alleles were resolved on denaturing polyacrylamide gels and detected by autoradiography. The dinucleotide repeat was based on a AACAGG(GT)₂₂ACATCT sequence.

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Mbol RFLP at the D4S43 (C4H) locus

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Source/Description: Probe PKP 1.65, 1.65 kb *PstI* fragment subcloned into pGEM 3 (2.7 kb) (MacDonald *et al.*, 1989).

Polymorphism: Probe PKP 1.65 identifies a two allele polymorphism with the restriction endonuclease MBO I.

Frequency: Studied in 141 normal chromosomes

MBO I	(L1) 1.25 kb	0.43
	(L2) 0.66 kb	0.57

Chromosomal Localisation: The probe is found within the D4S43 (C4H) locus and maps to 4q16.3 (Gilliam *et al.*, 1987). The polymorphism is contained within hybrids JS4 A9.1 (4 only) and HHW 693 (pter-15.1).

Mendelian Inheritance: Co-dominant inheritance shown in 68 meioses.

Probe Availability: Available upon request from M.E. MacDonald.

Other Comments: The polymorphism will increase the informativeness of the D4S43 locus in HD presymptomatic testing.

References: 1) Gilliam *et al.* (1987) *Science* **238**, 950–952. 2) MacDonald *et al.* (1989) *J. Clin. Inv.* **84**, 1013–1016.