A synthetic probe STR 14C19, detects a new polymorphic locus at 16pter (D16S282)

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Description: STR 14C19 is the double stranded DNA probe of sequence (GGGTGCTCGGGTAC)_n obtained by annealing of the oligonucleotide 5'GGGTGCTCGGGTAC3' with the overlapping complementary 5'AGCACCCGTACCCG3' followed by ligation and size selection of fragments above 400 bp, as described previously (1, 2).

Polymorphism: HaeIII identifies seven alleles ranging from 4 kb to 10 kb.

Size (kb)	4	5	5.5	6	6.5	7	10
Frequency (%)	1	3	47	16	25	7	1

Frequency: The heterozygosity rate in 86 unrelated individuals (grandparents or parents when no grandparent is available) from the CEPH panel is 73%.

Not Polymorphic For: Unknown.

Chromosomal Localisation: The locus detected by STR 14C19 has been localised to the extremity of the short arm of chromosome 16 by linkage analysis using the CEPH pedigree panel and CEPH database version 3 (1 cM from probe pBRZ, Lod score 19).

Mendelian Inheritance: Codominant segregation of the HaeIII RFLPs was observed in 40 families from the CEPH panel.

Other Comments: The STR probe is labelled by random priming, hybridised at 50°C overnight in a buffer containing 2% SDS; 0.45 M Na₂PO₄ pH 7.2; 1 mM EDTA; 0.5% dried milk and washed at 50°C in 1×SSC and 0.1% SDS for 2×45 min. (1×SSC is 0.15 M NaCl – 15 mM sodium citrate).

References: 1) Vergnaud, G. (1989) *Nucl. Acids Res.* **17**, 7623–7630. 2) Vergnaud, G., Mariat, D., Zoroastro, M. and Lauthier, V. (1991) *Electrophoresis* **12**, 134–140.

Detection by PCR of the VNTR polymorphism at D4S95

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Source and Description: The anonymous human DNA probe BS674E-D reveals a variety of polymorphisms within the D4S95 locus (1), including a VNTR RFLP detected with AccI. To assay this VNTR by a PCR format, we analyzed a 6.0 kb HindIII fragment from this region. An A/T-rich 39 bp repeated segment was found approximately 220 bp from one end of this fragment, while a segment flanking the other other end of this repeat was found by sequencing from an internal PstI site.

PCR Amplification: We used 0.4 μM of each of the primers 5'-GCATAAAATGGGGATAACAGTAC-3' and 5'-GACATTGCTTTATAGCTGTGCCTCAGTTT-3', 'standard' PCR buffer and dNTP concentrations (2), 1 μg genomic DNA, and 30 cycles of PCR using 1 min at 94°C, 2 min at 60°C, and 3 min at 72°C. The sizes of the PCR products were found to correspond with those seen in Southern blot RFLP analysis, and co-dominant segregation of the alleles was verified in two- and three-generation families.

Frequency: Genomic DNA from 41 unrelated Caucasian individuals were analyzed using these PCR primers. The allele sizes and frequencies were: 1600 bp (1%), 1560 (1%), 1520 (3%), 1500 (4%), 1480 (9%), 1440 (3%), 1330 (3%), 1240 (4%), 1180 (1%), 1150 (20%), 1090 (29%), 1030 (12%), and 990 (5%). This bimodal distribution of 13 alleles represents a PIC value of 0.82, versus 0.67 for the five alleles seen in Southern blot analysis (1).

Chromosomal Location: The D4S95 locus maps to 4p16.3, very close to the locus responsible for Huntington's disease (HD). The D4S95 locus also shows linkage disequilibrium with HD (3, 4).

Comments: Amplification with annealing at 55°C leads to many non-specific products. The products from seven unrelated individuals are shown below, compared to the HaeIII fragments of φX174.

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References: 1) Wasmuth *et al.* (1988) *Nature* **332**, 734–736. 2) Saiki *et al.* (1988) *Science* **239**, 487–491. 3) Theilmann *et al.* (1989) *J. Med. Genet.* **26**, 676–681. 4) Snell *et al.* (1989) *J. Med. Genet.* **26**, 673–679.

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