A VNTR isolated by size selection of human DNA fragments detects RFLPs at the extremity of 1p and 4q

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Source/Description: Human genomic DNA fragments above 1.5 kb after an *Sau*3A total digestion were used to construct a library (using a Puc 18 vector and a TG1 bacterial strain). Fifty clones were isolated, grown and plasmid miniprepations (1) were digested with *Hae*III and *Hinf*I.

The largest DNA insert called CEB8 was 3 kb in size after digestion with both enzymes. It was used as a probe (2) on the panel of CEPH families.

At a high stringency CEB8 detects two loci which can be discriminated on the basis of band intensities (Fig. 1) (at a lower stringency many polymorphic bands are observed).

Polymorphism: CEB8 used on *Hae*III digestions identifies two polymorphic loci, distinguishable by their relative intensities (Fig. 1).

D4F35S1 (strong signal): 17 alleles ranging <1 kb to 10 kb.

Size (kb)	<1	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	8	9	10
Frequency (%)	21	1	0.5	6	21	13	10	10	4	7.5	1	2	0.5	0.5	1	0.5	0.5

D1F35S2 (weaker signal): 11 alleles ranging <1 kb to 8 kb.

Size (kb)	<1	1.5	2	2.5	3	3.5	4	4.5	5	6	8
Frequency (%)	1	4	5	4	55	12	12	2.5	3	1	0.5

Frequency: D4F35S1: The heterozygosity rate in 120 unrelated individuals (grandparents, or parents when no grandparent is available) from the CEPH panel is 70%. D1F35S2: 118 unrelated individuals and 78%. (This is an underestimation since individuals with a small non-visible allele will be classified as homozygotes.)

Chromosomal Localisation: The two loci have been localised by linkage analysis using the CEPH pedigree panel and CEPH database version 3.

D4F35S1: to the extremity of the long arm of chromosome 4 (7 cM from L518, Lodscore 40).

D1F35S2: to the extremity of the short arm of chromosome 1 (3 cM from CMM121, Lodscore 43).

Mendelian Inheritance: Co-dominant segregation of the *Hae*III RFLPs was observed in 40 families from the CEPH panel.

Other Comments: The probe is labelled by random priming, hybridised at 65°C overnight in a buffer containing 2% SDS; 0.45 M Na₂HPO₄ pH 7.2; 1 mM EDTA; 0.5% dried milk and washed at 70°C in 0.1 SSC for 2×45 min.

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Mononucleotide repeat polymorphism in the APP gene

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Source/Description: The polymorphic (A)_n repeat resides within intron 13 of the Amyloid Precursor Protein gene (APP) on human chromosome 21. The polymorphism can be typed following amplification with PCR as described previously (1). The predicted length of the amplified sequence was 171 bp.

Primer Sequences:

GGAGTCTCTTGCATTACAAC (A strand)

TGTTGTTTAAAACCTCATTAAC (T strand)

Frequency: Estimated from 56 chromosomes of unrelated individuals.

Allele (bp)	Frequency
M1 173	0.02
M2 172	0.39
M3 171	0.34
M4 170	0.04
M5 168	0.05
M6 167	0.13
M7 165	0.04

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

Chromosomal Localisation: APP has been assigned to chromosome 21q21.2. Localization of the reported amplimer to chromosome 21 was confirmed by amplification of DNA from a somatic cell hybrid WA17 that contains 3 copies of human chromosome 21 (2).

Other Comments: The PCR was performed on 20 ng of genomic DNA using 50 pmoles of each oligonucleotide primer. Samples were processed through 27 cycles consisting of 20s at 94°C, 20s at 56°C and 30s at 72°C. Reaction buffer contained 1.8 mM MgCl₂, 10 mM Tris-HCl pH 9, 50 mM KCl, 0.01% w/v gelatin, 0.1% Triton X 100. Each allelic fragment appears on the autoradiograph as three prominent bands. The central (also darkest) band was used to define the allele size.

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References: 1) Weber and May (1989) *Am. J. Human Genet.* **44**, 388–396. 2) Raziuddin et al. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5504–5508.

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