A CA repeat 30 – 70 KB downstream from the adenomatous polyposis coli (APC) gene

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Source/Description: A cDNA clone (G31) was isolated from a fetal brain cDNA library that contained sequences from the DP1 gene which is located 30 – 70 KB downstream of the APC gene (1, 2). A sequence was identified that contained 13 CA repeats. DNA sequences flanking the CA repeat were used to design PCR primers to reveal any polymorphism present. A highly polymorphic system was identified.

PCR Primers:
LNS-CA1: 5′-ACTCACTCTAGTGATAAATCGGG-3′
LNS-CA2: 5′-AGCAGATAAGACAAGTATTACTAGTT-3′

Polymorphism: Thirteen alleles were observed in 162 unrelated individuals. The sizes and allele frequencies are listed below.

Allele	Base Pairs	Frequency	Allele	Base Pairs	Frequency
A1	122	.003	A8	106	.006
A2	118	.009	A9	104	.071
A3	116	.009	A10	102	.247
A4	114	.019	A11	100	.269
A5	112	.071	A12	98	.093
A6	110	.145	A13	96	.012
A7	108	.046			

The observed heterozygosity is 0.83 and the HGM locus designation number is D5S346.

Chromosomal Localization: This CA repeat marker is located 30 – 70 KB downstream from the APC gene within the 3′ untranslated message of a nearby gene, DP1. This highly polymorphic system has been mapped genetically to the region of the APC gene using three CEPH reference families in which Mendelian inheritance was observed. The marker was also run on a previously ascertained polyposis research family and showed complete linkage to the disease phenotype (3).

PCR Conditions: 200 ng of genomic DNA was used with an end-labelled primer and 2.0 mM Mg PCR buffer in a total volume of 50 µl. The PCR amplification protocol is as follows: One initial denaturation cycle of 94°C for 3 minutes, 35 cycles of 94°C for 1 minute (denaturation), 58°C for 1 minute (annealing) and 72°C for 1 minute (extension) followed by cooling to room temperature. The radioactively amplified PCR products were then run on a formamide denaturing acrylamide gel electrophoretic system and analyzed by autoradiography.

Comments: This marker will be extremely useful for clinical diagnosis in families segregating an allele for Adenomatous Polyposis Coli (APC).

References: 1) Joslyn *et al.* (1991) *Cell* **66**, 601 – 613. 2) Groden *et al.* (1991) *Cell* **66**, 589 – 600. 3) Spirio *et al.*. unpublished data.

PCR of a VNTR linked to mucopolysaccharidosis type I and Huntington disease

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Source/Description: Oligonucleotide primers were used to amplify a region of intron II of the human α-L-iduronidase (HGM locus symbol, *IDUA*) gene. The VNTR-like polymorphism can be used diagnostically in families with IDUA deficiency (mucopolysaccharidosis type I) and Huntington Disease, which is closely linked (about 1 Mb) to the *IDUA* locus (1). It is probably the same VNTR detected by p157.9 at *D4S111* (1, 3).

PCR Primers:
ID-53, 5′-TAGGTGTCTCCTCAGAGAGG-3′
ID-54, 5′-AGGACCTGGTGGACACCTCA-3′

Polymorphism: Three different alleles are detected varying in size by about 85 bp (800, 715 and 630 bp).

Frequency: Studied in 136 chromosomes from 68 unrelated healthy individuals.

allele 1	(800 bp)	0.28
allele 2	(715 bp)	0.55
allele 3	(630 bp)	0.17

Observed heterozygosity 60%

PCR Conditions: 250 ng of genomic DNA was amplified using 500 ng of each primer, 2 units of Taq polymerase (Cetus) in the recommended buffer, except that the reactions contained 400 µM dNTPs, 2.5 mM $MgCl_2$ and 0.1% v/v Triton X-100. After 7 min at 96°C the reactions were subjected to 20 cycles of 95°C for 30 sec, 65°C for 45 sec and 72°C for 90 sec.

Chromosomal Localization: *D4S111* is within the *IDUA* gene and they have both been mapped to chromosome 4p16.3 about 1100 kb from the telomere (1, 2).

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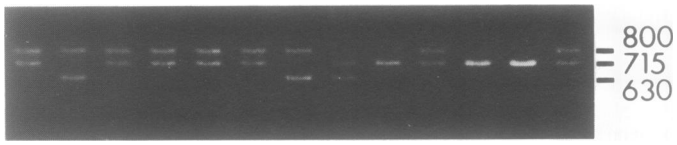
References: 1) MacDonald,M.E. *et al.* (1991) *Somatic Cell Mol. Genet.* **17**, 421 – 425. 2) Scott,H.S. *et al.* (1990) *Am. J. Hum. Genet.* **47**, 802 – 807. 3) Skraastad,M.I. *et al.* (1991) *Am. J. Med. Genet.* **39**, 217 – 222.



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