Dinucleotide repeat polymorphism at the D5S260 locus on chromosome 5q

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Source/Description: Phage λ Alu32 (D5S260) was isolated from LA05NL03, a partial Sau3A library obtained from Los Alamos National Laboratory, using Alu PCR differential hybridization (1). (GT)_n sequence was detected within this phage with poly(dC-dA)·(dG-dT) (Pharmacia). A Subclone containing the repeat was sequenced and flanking PCR primers synthesized.

PCR Primers:

D5S260-GT: 5'AGTTTTCCTGAGAGTCATTAGC 3'

D5S260-CA: 5'AATTCAACTGTGACATATGCAAG 3'

Allele Frequencies:

D5S260: calculated from 138 chromosomes of CEPH parents.

D5S260:	allele	size (bp)	frequency
	A1	154	0.065
	A2	152	0.38
	A3	150	0.14
	A4	148	0.27
	A5	146*	0.15

PIC: 0.69

An asterisk indicates the size of cloned alleles.

Chromosomal Localization: Located on 5q11.2-q13.3 by hybridization to somatic cell hybrid DNA.

Mendelian Inheritance: Co-dominant segregation for D5S260 was observed in 8 CEPH families.

D5S260 reference genotypes: 133101 = A2, A4; 133102 = A3, A4

PCR Conditions: The (GT)_n tract was amplified using the following conditions: 40 ng genomic DNA, 50 mM Tris pH 8.0, 0.05% Tween 20, 0.05% NP40, 2 mM MgCl₂, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 0.4 μ Ci (0.8 pmoles) endlabeled D5S260-GT primer, 10 pmoles of D5S260-CA and D5S260-GT primers, and 0.625 units of Taq DNA polymerase in a total reaction volume of 25 μ l. Thirty cycles of 1 min denaturation at 94°C, 30 sec annealing at 58°C, and 1 min extension at 72°C were performed. After the PCR reaction, 8 μ l of sequencing stop mix was added. 8 μ l from each reaction was run on a 6.0% Hydrolink (AT Biochemicals) gel. Sizes of alleles were determined relative to the sequenced allele using an M13 sequencing reaction as a secondary size standard.

Other Comments: The repeat sequence of the cloned product for D5S260 is of the form (TG)₆CG(TG)₁₁.

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References: 1) Bernard, L.E. *et al.* (1991) *Genomics* **9**, 241–246.

Dinucleotide repeat polymorphism in D20S17 (CRI-L127)

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Primers/Description: Two primers (L127–2A, 5'-CTAAGTATGCAGCAGTTAGA-3', and L127–2B, 5'-GGATGAGTAAATGTATCTCCC-3') were used to amplify a CA-repeat rich region in D20S17.

Frequency: Six alleles were observed in 35 unrelated Caucasians. The heterozygosity was 71%.

Allele	bp	Frequency	Allele	bp	Frequency
B1	140	0.01	B2	138	0.23
B3	136	0.27	B4	134	0.04
B5	132	0.40	B6	130	0.04

Chromosomal Localization: D20S17 was assigned to chromosome 20q12-q13.1 (1).

Mendelian Inheritance: Co-dominant inheritance was observed in a family with maturity onset diabetes of the young in which 99 members were typed (2).

Other Comments: The PCR was performed using ³²P-labeled L127–2A and unlabeled L127–2B for 30 cycles: denaturation at 94°C for 1 min; annealing at 56°C for 1 min; and extension at 72°C for 2 min. The PCR products were analyzed on a 5% denaturing polyacrylamide gel (Figure 1). The dinucleotide repeat sequence in CRI-L127 was of the form (CA)₆TG(CA)₄CG(CA)₈C(CA)₅; the sequence of this region has been submitted to GenBank.

References: 1) Grzeschik, K.H. and Skolnick, M.H. (1990) *Cytogenet. Cell Genet.* **55**, 229. 2) Bell, G.I. *et al.* (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1484.

Figure 1. PCR amplification of CA-repeat in D20S17. The genotypes are noted at the bottom of the figure.