Unusual variability of the complex dinucleotide repeat block at the SPN locus

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Source/Description: A GenBank data screening for (CG)_n repeats revealed one (CG)_n>7 tract in the complex dinucleotide repeat block (TC)₁₁(AC)₁₃(GC)₁₂ within human sialophorin gene (SPN). Complex repeat block was identified in the 3'-transcribed region from exon 2 at position 4303 bp of EMBL accession no. X52075.

PCR Primers:

SI1 = 5'-4273 bp TCCATTCTTCGAGTACACATGCA 4295 bp-3'
SI2 = 5'-4426 bp AGTCCCGAGACCAGGGCAAAC 4405 bp-3'

Polymorphism/Frequency: Estimated from 56 chromosomes of unrelated individuals.

Observed heterozygosity = 0.96.

Allele	Length (bp)	Frequency	Allele	Length (bp)	Frequency
A1	185	.02	A10	155	.16
A2	169	.02	A11	154	.07
A3	163	.04	A12	153	.04
A4	161	.05	A13	152	.02
A5	160	.05	A14	151	.02
A6	159	.04	A15	149	.07
A7	158	.05	A16	148	.07
A8	157	.10	A17	146	.04
A9	156	.14	A18	145	.04

Chromosomal Location: the sialophorin gene has been assigned to 16q11.2 (1).

Mendelian Inheritance: Codominant segregation was demonstrated in a 9 member pedigree.

PCR Conditions: PCR amplification were carried out in a 25 µl vol., containing 200 ng human DNA template, 10 pmol of each primer, 1 mM of each dNTP and 5 mCi ³²P-dCTR, 20 mM Tris-HCl, 10 mM (NH₄)₂SO₄, 8 mM MgCl₂, 0.0017% BSA, 2 units Taq polymerase. Samples were processed through 34 cycles consisting of 0.3 min at 94°C, 0.5 min at 55°C, 1 min at 72°C. Aliquots of the amplified DNA were electrophoresed on the denaturing 6% polyacrylamide DNA sequencing gel. DNA fragments were revealed by autoradiography exposure of polyacrylamide gel.

Probe Primers Availability: E.I.Rogaev.

Other Comments: Unusual odd-nucleotide polymorphism of the dinucleotide repeat block can be explained by peculiarities of the structural variations or mobility in gel of twofold symmetric (CG)_n tract. Owing to high PCR polymorphism and low 'extra-bands' background, this marker can be useful for forensic identifications and linkage analysis of sialoprotein gene which are important for immune function

Reference: 1) Pallant, A. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86, 1328-1332.

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Dinucleotide repeat polymorphism in the human DCC gene at chromosome 18q21

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Source/Description: A 170-bp *Xba*I-*Eco*0109 restriction fragment from an intron of the candidate tumor suppressor gene DCC was reported to contain a polymorphic 130-bp region of alternating purine-pyrimidine base pairs including two (TA)_n repeats (1). This restriction fragment is located 165 bp downstream from the exon ending at nucleotide 1267 (GenBank accession number M32292). Primers based on the published sequence were designed to amplify the polymorphic region, with a predicted PCR product size of 158 bp.

Primer Sequences:

DCC-L (TA strand): 5'-TCCCTCTAGAAATTGTGTG-3'
DCC-R (AT strand): 5'-TGACTTTATCTCATTGGAG-3'

Polymorphism/Frequency: Estimated from 136 chromosomes of unrelated individuals. Observed heterozygosity = 0.82.

Allele	(bp)	Frequency	Allele	(bp)	Frequency
D1	160	0.01	D11	138	0.07
D2	158	0.01	D12	136	0.02
D3	154	0.01	D13	134	0.02
D4	152	0.01	D14	132	0.01
D5	150	0.05	D15	128	0.02
D6	148	0.02	D16	126	0.01
D7	146	0.11	D17	124	0.01
D8	144	0.11	D18	118	0.01
D9	142	0.19	D19	116	0.25
D10	140	0.02	D20	106	0.02

Chromosomal Location: The DCC gene has been assigned to chromosome 18q21 (1).

Mendelian Inheritance: Co-dominant segregation was observed in a 13 member, three generation family.

PCR Conditions: PCR amplifications were carried out in a total volume of 20 µl containing: 50 ng of genomic DNA, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 200 µM each of dATP, dGTP, dTTP, 20 µM dCTP, 1 µCi of [³²P]dCTP (3000 Ci/mmol), 1 µM of each primer, and 1 U of *Taq*I polymerase (Perkin-Elmer/Cetus). Amplification was for 30 cycles consisting of 1 min at 94°C, 1.5 min at 50°C, and 1.5 min at 72°C. For the initial cycle, the block was preheated to 94°C before adding the sample tubes. The products were separated on a 5% denaturing polyacrylamide gel, using a radiolabeled M13mp18 sequencing ladder for reference.

Other Comments: This assay will facilitate the assessment of allelic loss of the candidate tumor suppressor gene DCC from human tumors.

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Reference: 1) Fearon, E.R. *et al.* (1990) *Science* 247, 49-56.

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