A new polymorphic probe on chromosome 3p:λLIB4A-52''(D3S732)

F.Latif, G.M.Glenn, L.Geil¹, L.Schmidt¹, J.Delisio¹, K.Hampsch¹, M.L.Orcutt, B.Zbar and M.I.Lerman*
Laboratory of Immunology, National Cancer Institute-Frederick Cancer Research Facility, Frederick, MD 21701 and ¹Program Resources Inc., Frederick Cancer Research Facility, Frederick, MD 21701, USA

Source/Description: λLIB4A-52''(D3S732) is a 4.0 kb EcoRI fragment isolated from a Charon 4A total human chromosome 3 library.

Polymorphism: HindIII digestion of genomic DNA and hybridization with the probe detects a two-allele polymorphism: 9.0 kb (H1) and 7.5 + 1.5 kb (H2).

Frequency: Estimated from 30 unrelated Caucasians.
H1:0.78
H2:0.22

Frequency of heterozygosity: 0.34

Not Polymorphic For: BamHI, BglII, DraI, EcoRI, HinfI, MspI, PvuII, RsaI and TaqI.

Chromosomal Localization: Using a somatic cell hybrid panel (2) which was based on linkage groups anchored to physically localized markers, the probe was assigned to the short arm of chromosome 3 between 3p24-3p26.

Mendelian Inheritance: Mendelian inheritance has been demonstrated in a two generation von Hippel-Lindau disease (VHL) family.

Probe Availability: Available for collaboration.

References: 1)Lerman,M., Latif,F., Glen,G. *et al.*: Isolation and regional localization of a large collection (2,000) of single copy DNA fragments of human chromosome 3 for mapping and cloning tumor suppressor genes. *Human Genetics* (In press, 1991).

Dinucleotide repeat polymorphism at the debrisoquine 4-hydroxylase (CYP2D) locus

J.A.Trofatter, J.F.Gusella and J.L.Haines
Molecular Neurogenetics Laboratory, Massachusetts General Hospital, Charlestown, MA 02129, USA

Source and Description: dG-dT dinucleotide repeats represent a potential source of highly polymorphic genetic markers that can be used to generate fine-structure genetic linkage maps. The polymerase chain reaction can be used to amplify these repeats which can then be size-fractionated by polyacrylamide gel electrophoresis to distinguish the alleles from each other (1). This dG-dT repeat was found by a search of the CYP2DP8) sequence in Genbank (EMBL accession number M33387).

Chromosomal Localization: 1.3 kb downstream from exon 9 of the debrisoquine 4-hydroxylase gene in 22q13 (2).

Primer Sequences:

5'-CCCTCTTGGCTCTAACAGC-3' (GT strand);
5'-CTCGGTGACAGAGCAAGAC-3' (AC strand).

PCR Conditions: PCR was carried out in a total of 10 μl containing 10 ng genomic DNA; 1.6 pmoles of each primer; 200 μM of dATP, dCTP and dTTP; 2 μM dGTP; 1 μCi α³²dGTP at 3000 Ci/mmol (Amersham); 50 mM KCl, 10 mM Tris-HCl pH 8.5, 1.5 mM MgCl₂, 0.01% w/v gelatin; 0.25 units Taq polymerase (Cetus). Each reaction was heated for 4 minutes prior to 25-30 cycles of amplification, 1 minute at 94°C, 20 seconds at 57°C and 1 minute at 72°C.

Polymorphism and Frequency: Eleven alleles have been detected, though a twelfth allele (B8) is expected by its location in the allele size distribution. Sequencing of an individual homozygous for B11 showed a repeat length of 17. All other alleles differed by increments of 2 bases pairs. Allele frequencies were calculated from 70 parents of the CEPH pedigrees.

Allele	Allele (bp)	Frequency
B12	108	0.01
B11	110	0.43
B10	112	0.32
B9	114	0.05
B8	116	0.00
B7	118	0.01
B6	120	0.01
B5	122	0.01
B4	124	0.01
B3	126	0.13
B2	128	0.01
B1	130	0.01

Observed heterozygosity in our sample = 70%

Mendelian Inheritance: Codominant segregation was shown in 30 informative two and three generation families.

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References: 1)Weber,J.L. and May,P.E. (1989) *Am. J. Hum. Genet.* **44**, 388-396. 2)Kimura,S., Umeno,M., Skoda,R.C., Meyer,U.A. and Gonzalez,F.J. (1989) *Am. J. Hum. Genet.* **45**, 889-904.

*To whom correspondence should be addressed: Laboratory of Immunology, Frederick Cancer Research Facility, NCI-NIH, Ft. Detrick, Building 560, Room 12-71, Frederick, MD 21701, USA