

## C to T polymorphism in exon 33 of the COL3A1 gene

Gerard Tromp, Caren Kleinert, Helena Kuivaniemi and Darwin J. Prockop

Department of Biochemistry and Molecular Biology, Jefferson Institute of Molecular Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107-6799, USA

**Source and Description of Sequence Polymorphism:** Nucleotide sequencing of type III procollagen (COL3A1) cDNAs from several individuals revealed a nucleotide polymorphism that converted the codon for amino acid 581 of the triple helix from -GGC- (C-allele) (1) to -GGT- (T-allele) in some alleles. The PCR was carried out with primers below; cDNA products were 226 bp and genomic products were 326 bp in length. Digestion of PCR products from cDNA with HaeIII yielded fragments of 9, 33, 36, 58 and 90 bp for the C-allele and fragments of 9, 36, 90 and 91 bp for the T-allele. Digestion of PCR products from genomic DNA yielded fragments of 33, 34, 35 and 224 bp for the C-allele and fragments of 34, 35 and 257 bp for the T-allele. Allele-specific oligomer (ASO) hybridization was used to study 50 unrelated individuals of the following origins: 23 US Americans, 11 Finnish, 6 Swedish, 4 Canadian, 2 British, 2 Dutch and 2 Japanese. Heterozygous individuals were present in all nationalities. All experiments were repeated at least three times with different PCR products.

**Oligomers Used to Amplify cDNA:**

5' GAG AAA GAG GAG GTC TT 3' (SP10)  
5' CCA GTT TCA CCT CTC TCA C 3' (SP31)

**Oligomers Used to Amplify Genomic DNA:**

5' CAA CAC TCC TGG AAA GTA ATC G 3' (in IVS32)  
5' AGT GCA GGA CTG TCC CAT ATG 3' (in IVS33)

**ASO Hybridization Probes:**

5' AAC CAG GCA GC CAG GTG C 3' (C-allele, sense)  
5' AAC CAG GCA GC CAG GTG C 3' (T-allele, sense)

**Frequency:** Estimated from 100 chromosomes:

C1	C-allele	0.29
C2	T-allele	0.71
	Expected	Observed
C/C	0.08	0.10
C/T	0.41	0.38
T/T	0.50	0.52

**Chromosomal Localization:** 2q31-q32.3

**Other Comments:** Conditions for amplification were:

- For cDNA, 40 cycles consisting of 1 min 30 sec at 94°C, 1 min at 46°C and 1 min at 74°C.
- For genomic DNA, 40 cycles consisting of 1 min 30 sec at 93°C, 1 min at 56°C and 1 min at 72°C.

Both ASO hybridization primers contain an additional identical mismatch two nucleotides before the allelic variant. This was necessary to distinguish the two alleles since ASOs without the additional mismatch exhibited dissociation kinetics that were not detectably different. The cDNA sequence data for COL3A1 (1) were submitted to EMBL/GenBank under accession no. X14420.

**Acknowledgements:** The work was supported in part by National Institutes of Health grant AR-38188.

**Reference:** 1) Ala-Kokko *et al.* (1989) *Biochem. J.* **260**, 509-516.

## A new polymorphic probe on chromosome 3p: λLIB13-49' (D3S723)

F. Latif, G.M. Glenn, H. Brauch, J. Delisio<sup>1</sup>, K. Hampsch<sup>1</sup>, M.L. Orcutt, B. Zbar and M.I. Lerman\*

Laboratory of Immunobiology, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD 21701 and <sup>1</sup>Program Resources Inc., Frederick Cancer Research and Development Center, Frederick, MD 21701, USA

**Source/Description:** λLIB13-49' is a 6.6 kb EcoRI fragment isolated from a Charon 21A human chromosome 3 library (1).

**Polymorphism:** MspI digestion of genomic DNA and hybridization with the probe detects two polymorphic systems: Upper system 9.5 kb (A1) and 8.0 kb (A2)  
Lower system 4.4 kb (B1) and 2.4 kb with 2.0 kb (B2)

**Frequency:** Estimated from 30 unrelated Caucasians.

M1: 0.44      M3: 0.84  
M2: 0.56      M4: 0.16

**Frequency of Heterozygosity:** Upper system, 0.49; lower system, 0.27.

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

**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 3p22 and 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) American Type Culture Collection No. 57717, National Lab. ID Code LA03NS01. 2) Lerman, M., Latif, F., Glen, G. *et al.* (1990) *Human Genetics* in press.

\*To whom correspondence should be addressed at Laboratory of Immunobiology, Frederick Cancer Research and Development Center, NCI-NIH, Fort Detrick, Building 560, Room 12-71, Frederick, MD 21701, USA