Single-strand conformation polymorphism (SSCP) at the D8S86 locus

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Source/Description: pNCO907 contains a 500-bp *Pst*I fragment of pNC0901 inserted into pUC19 as described previously (1). The sequences for PCR primers were determined. A fragment of about 500-bp is amplified.

PCR Primers:
8.86-1 = TATCAGATAGAATGTGTGGG
8.86-2 = CTCTGAGGCCATGGAACAGA

Polymorphism: Two types of alleles (D1 and D2) were found by SSCP analysis.

Frequencies:
D1 = 0.64,
D2 = 0.36
Determined in 14 unrelated individuals. The observed heterozygosity is 0.50.

Chromosomal Localization: Chromosome 8, assigned with a panel of human-mouse somatic cell hybrids (1).

Mendelian Inheritance: Codominant segregation, observed in one informative family (Figure).

PCR and SSCP Conditions: 5'-end-labelled primers (0.5 pmol each) were added to a 5 μl mixture of 50 ng genomic DNA, 1×PCR buffer specified by Cetus Corporation, 125 μM concentrations of deoxynucleotide triphosphates (dATP, dCTP, dTTP and dGTP), and 0.125U *Taq* DNA polymerase. Thermal conditions were 32 cycles at 94°C for 20 sec and at 65°C for 2 min (2). Denatured PCR products were separated at 30 W at room temperature in 5% polyacrylamide gel containing 5% glycerol with vigorous cooling.

Other Comments: The probe pNCO907 also detects three RFLPs (*Eco*RI, *Sph*I and *Taq*I) (3).

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References: 1) Iizuka,M. *et al.* (1990) *Cancer Res.* **50**, 3345-3350. 2) Mashiyama,S. *et al.* (1990) *Technique* **2**, 304-306. 3) Iizuka,M. *et al.* (1991) *Nucl. Acids Res.* **19**, 680.

HindIII-polymorphism in the LPL-gene detected by PCR

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Source/Description: We designed two oligonucleotides derived from the sequences in exon 8 and 9 in the LPL-gene (1) to amplify the sequence around a polymorphic HindIII-site. This polymorphism is located in intron 8 (2), in contrast to the HindIII-polymorphism in the 3' untranslated region as described by Oka *et al.* (3). The amplified fragment has a size of 1200 bp. The polymorphism in intron 8 was detected upon digestion of the PCR-product with HindIII.

Primer Sequences:
Hind5: 5'-AGTGATTCATACTTTAGCTG-3'
Hind3: 5'-TGAGACACTTTCTCCCTAGA-3'

Frequency:

	Heinzmann *et al.* (5) (n = 131)	Funke *et al.* (4) (n = 50)	PCR-method (n = 20)
Allele H1 (1200 bp)	0.33	0.26	0.25
Allele H2 (600 bp)	0.67	0.74	0.75

Chromosomal Location: The LPL-gene is located on chromosome 8p22 (6).

Reaction Conditions and Analysis: We used genomic DNA in the PCR-reaction. The primer-concentration is 160 ng/ml, the Mg^{++}-concentration is 2 mM and the dNTP-concentration is 200 μM. The temperature profile is a) denaturation for 1 min at 95°C, b) annealing for 1 min at 50°C and c) extension for 1 min at 72°C. We used 30 cycles. The reaction products were analysed on 2% agarose in TBE after HindIII-digestion.

References: 1) Wion,K.L. *et al.* (1987) *Science* **235**, 1638-1641. 2) Kirchgessner,T.G. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9647-9651. 3) Oka,K. *et al.* (1990) *Biochem. Biophys. Acta* **1049**, 21-26. 4) Funke,H. *et al.* (1987) *Nucl. Acids Res.* **15**, 9102. 5) Heinzmann,C. *et al.* (1987) *Nucl. Acids Res.* **15**, 6763. 6) Sparkes,R.S. *et al.* (1987) *Genomics* **1**, 138-144.

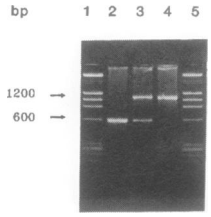

Figure 1. Individuals who are homozygous for presence (lane 2) or absence (lane 4), or who are heterozygous for the HindIII- restriction site (lane 3). The DNA-molecular weight marker (lane 1 + 5) is a combination of λ-DNA cut with HindIII and φX-174-RF DNA cut with HaeIII.