PCR detection of a COL1A1 *RsaI* RFLP

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We have designed PCR primers, based on published DNA sequence (1), which allow the amplification of a 685 bp fragment containing exon 6 of the $\alpha 1$ (I) collagen gene (COL1A1). This allows the detection of a previously described *RsaI* RFLP (2) (allele system B-HGM10) in intron 5 of the gene.

PCR Primers:

Sense oligo-5'AGCAAGTTGCTAACATCAGG 3'

Antisense oligo-5'TCACCGGTGAATCCCAAGCTCTCTAT-ACCAG 3'

The 5' most 12 bases of the antisense oligo are an extension added to allow the incorporation of a site for *EcoRI* to aid in the cloning of amplified DNA. These 12 bases need not be incorporated into the primer.

Polymorphism: The PCR amplification product reveals the two alleles, B1 (fragments of 562 bp and 123 bp) and B2 (a single 685 bp fragment) following digestion with *RsaI*. In persons homozygous for the B1 allele, a small amount of amplification product often appears refractory to digestion even after extensive incubation with the enzyme. We have noted this same phenomenon when the site is tested in Southern blot analyses of genomic DNA. By direct sequencing of the PCR products, we have determined that the variant site results from a C to T transition 388 bp 5' to the start of exon 6 (data not shown).

Allele Frequencies: for 102 British individuals: B1 = 0.86, B2 = 0.14 (2).

Chromosomal Location: 17q21.3-q22 (3).

PCR Conditions: PCR amplifications were carried out, using a modification of a published method (4), in a volume of 50 μ l containing: 0.2 μ g human DNA, 0.5 μ M each primer, 1 mM each dNTP, 4.5 mM MgCl₂, 11.1 mM (NH₄)SO₄, 45 mM Tris-HCl pH 8.8 (at room temperature), 4.5 μ M EDTA, 110 μ g ml⁻¹ BSA, 2 units Taq polymerase (Amersham) for 30 cycles. Each cycle consisted of 94°C for 1.4 min, 60°C for 1.2 min and 70°C for 1.2 min. The products were digested with *RsaI* and analysed by electrophoresis through a 2.0% agarose gel in 1×TAE.

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