TaqI RFLP polymorphism of the ovine complement component C4 gene

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Source/Description: The probe pCUT2 is a 470 bp clone derived from a liver cDNA λ library subcloned into the EcoRI site of pUC 13. It encodes the N-terminus of the γ chain of bovine complement component C4.

Polymorphisms: TaqI identifies two allelic restriction fragments C4*A1 of 2.2 kbp and C4*A2 of 2.0 kbp with invariant bands of 2.1 kbp, 0.5 kbp and 0.15 kbp.

Frequency: Ovine C4*A1: 0.63, Ovine C4*A2: 0.36. Estimated from 31 merino animals.

Not Polymorphic For: HindIII, BamHI, EcoRI, PstI and ClaI.

Other Polymorphisms: Other polymorphisms detected with KpnI.

Chromosomal Location: pCUT 2 hybridises with the 3' end of the portion of the C4 gene encoding the γ chain.

Mendelian Inheritance: Codominant segregation was observed in 3 half-sib families.

Probe Availability: Requests should be sent to J.D.Wetherall.

Other Comments: Lowered stringency conditions were employed 0.5 $\times$ SSC/0.2% SDS at 60°C.

Acknowledgement: Work was partially funded by the Australian Wool Corporation.

Human collagen, type II, alpha 1, (COL2A1) gene: VNTR polymorphism detected by gene amplification

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Source/Description: Stoker *et al.* (1) have described an AT-rich VNTR in the 3'-flanking region of the human Type II collagen gene. Using the published sequence (1) as a guide, upstream (5'-CCAGGTTAAGGTTGACAGCT-3') and downstream (5'-GTCATGAACTAGCTCTGGTG-3') oligonucleotides were selected for the PCR.

Protocol: Reactions were performed as described (2) using 1 μ g of DNA and 100 pmoles of each primer. DNA was amplified for 20 cycles using a Perkin Elmer Cetus DNA Thermal Cycler: denaturation, 94°C, 1 min; annealing, 60°C, 1 min; and extension, 70°C, 1.5 min. Ten μ l of the reaction was analyzed directly on a 2.5% NuSieve GTG agarose gel run in Tris-borate buffer. The amplified DNA fragments varied in size from ~600 to 700 bp (Fig. 1).

Heterozygosity: 81% was observed in 16 unrelated Caucasians.

Chromosomal Localisation: 12q14.3.

Mendelian Inheritance: Co-dominant segregation was observed in one four generation family in which 29 individuals were typed; four alleles of the COL2A1 VNTR segregated in this family.

References: 1) Stoker, N.G. *et al.* (1985) *Nucl. Acids Res.* **13**, 4613-4622. 2) Saiki, R.K. *et al.* (1988) *Science* **239**, 487-491.

Fig. 1. PCR amplification of the COL2A1 VNTR. The fragments seen in twelve members of one family are shown.