Rapid Communication: A Restriction Fragment Length Polymorphism in the Ovine Prolactin Gene

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Abstract
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Keywords
Sheep, PCR-RLFP, Prolactin Gene

Disciplines
Agriculture | Animal Sciences | Genetics and Genomics

Comments
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Rapid Communication: A Restriction Fragment Length Polymorphism in the Ovine Prolactin Gene

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Source and Description of Primers. Human genomic (Truong et al., 1984) and pig cDNA (GenBank accession no. X14068) sequences were compared to design primers to span the second intron of the prolactin gene.

Primer Sequences. Forward primer: 5′-ACCTCTCTTCCCGGAAATGGTTCA-3′; reverse primer: 5′-CTGGTTGGCTTGCTTTGTC-3′.

Method of Detection. PCR amplification (25 µL final volume) was performed using 25 ng of genomic DNA, 350 µM each dNTP, 3 µM each primer, 1.1 mM MgCl2, 1 x Taq Extender Buffer, 1 unit Taq Extender, and 1 unit Taq polymerase. The thermal cycler profile was 92°C for 2 min; 35 cycles of 92°C for 45 s, 56°C for 45 s, and 72°C for 3 min, followed by a final extension at 72°C for 7 min.

Description of Polymorphism. Twenty microliters of the 2.5-kb product was digested with HaeIII to produce bands of approximately 1,400, 530, 360, and 150 bp for the A allele and bands of approximately 1,400, 510, 360, and 150 bp for the B allele (Figure 1).

Inheritance Pattern. The PRL HaeIII polymorphism was observed to have a Mendelian inheritance pattern in nine two-generation sheep families of the AgResearch International Mapping Flock (Crawford et al., 1995).

Frequency. Frequencies for the A allele were .30 for crossbred whitefaced (n = 10), .25 for Suffolk (n = 6), .375 for Coopworth (n = 4), and .50 for Texel (n = 1).

Chromosomal Location. Unknown.

Comments. The ends of the sheep PCR product were sequenced to confirm that the product was PRL. The coding portion was 100% homologous to the corresponding region of the ovine PRL cDNA (GenBank accession no. M27057). Two Suffolk animals had a differing restriction pattern with HaeIII, but this was not observed in any other animals. A polymorphism was also identified with the restriction enzyme MseI. This second polymorphism was observed to be in complete phase with the HaeIII polymorphism in the families typed. Prolactin is an anterior pituitary hormone involved in many reproductive pathways.

Literature Cited


Key Words: Sheep, PCR-RFLP, Prolactin Gene

Figure 1. HaeIII RFLP in the ovine prolactin PCR fragments separated on a 3% NuSieve gel (FMC). Lane 1: 1-kb ladder (Promega); lane 2: AA genotype; lane 3: AB genotype; lane 4: BB genotype; lane 5: uncut PCR product.

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