Rapid Communication: A PCR-RFLP Marker at the Porcine Complement Factor B Gene Locus Shows Between-Population Frequency Variation

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Name of Marker. Complement factor B (BF) gene locus.

Genus and Species. Sus scrofa.

Source and Description of Primers. The porcine genomic sequence for BF (Peelman et al., 1991) was used to develop primers in this study (see GenBank accession no. M59240). The expected PCR product was 390 bp.

Primer Sequences. Forward: 5’ ACT GCT ATG ACG GTT ACA CTC TCC G 3’; reverse: 5’ TCC AAG AGC CAC CTT CCT GG 3’.

Detection Method. Approximately 100 ng of genomic DNA was amplified in a final volume of 10 μL containing 30 ng of each primer, 200 nM dNTPs, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris HCl, .1% Triton X-100, and 1.5 U of Taq polymerase. After denaturation at 94°C for 4 min, 30 amplification cycles comprising denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s, followed by a further 5-min extension at 72°C, were performed and finally stored at 4°C until use. The amplified fragment was digested with restriction enzyme Smal in a total volume of 15μL containing 10μL of PCR products, 10 units of enzyme, and 1.5μL NEB buffer 4 (New England Biolabs, Beverly, MA) at 25°C for 3 h and then examined by electrophoresis on 1.6% agarose gel with 6× TBE buffer. The gels were stained with ethidium bromide and photographed.

Description of Polymorphism. The amplified DNA digested with Smal yielded a single 390-bp fragment (SmaI-allele) and two fragments of 237 and 153 bp (SmaI+ allele), respectively (Figure 1).

Frequency. Significant population differences in allele frequencies were found among 16 Erhualian, 30 Landrace, 26 Pietrain, 26 Duroc, and 27 Hampshire pigs ( = 94.6, P < .001). The Erhualian pigs were sampled from three farms in Jiangsu Province, China. The Landrace and Pietrain pigs came from the TUM-Thaulehausen research herd in Germany. The Duroc and Hampshire pigs came from 17 and 7 private breeding farms, respectively, taking part in a carcass study in Ontario, Canada. Pigs were chosen to be unrelated, but the Duroc and Hampshire samples included two and three full-sib pairs, respectively. The allele frequencies of SmaI+ in the five breeds were .53, .18, .06, .02, and .72, respectively.

Chromosome Location. Porcine chromosome number 7 (Lie et al., 1987; Wu et al., 1995; Peelman et al., 1996).

Comments. The BF gene is located in the SLA complex class III region. It has been confirmed that there is only one BF gene per haploid genome in pigs (Peelman et al., 1991). A restriction fragment length polymorphism was detected with TaqI (Lie et al., 1987; Peelman et al., 1991). The MHC class III gene organization is highly conserved across species (Wu et al., 1995).

Literature Cited


Figure 1. PCR-RFLP at porcine BF locus. Lane 1: PCR product, 390 bp. Lane 2: 100 bp ladder (New England Biolabs, Beverly, MA). Lanes 3, 4 and 7: homozygotes SmaI+/SmaI+, 237+153 bp. Lanes 5 and 6: heterozygotes SmaI+/SmaI-, 390+237+153 bp. Lanes 8 and 9: homozygotes SmaI-/SmaI-, 390 bp.