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Rapid Communication: A Restriction Fragment Length Polymorphism in the Porcine Leptin Receptor (LEPR) Gene

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Abstract

Polymorphism. A HinfI PCR-RFLP was identified in the porcine leptin receptor (LEPR) gene. Source and Description of Primers. Human cDNA (Gen- Bank accession no. U43168) sequence was used to design primers to amplify porcine genomic DNA. Primer Sequences. Forward primer: 5¢-GCATCCCATATCTGAACCC- 3¢; reverse primer: 5¢-CCACTTAAACCATAGCGAATC- 3¢.

Keywords

Pigs, PCR, RFLP

Disciplines

Agriculture | Animal Sciences | Genetics and Genomics

Comments

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Rapid Communication: A Restriction Fragment Length Polymorphism in the *Porcine Leptin Receptor* (*LEPR*) Gene[1]

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Polymorphism. A *Hin*fI PCR-RFLP was identified in the *porcine leptin receptor* (*LEPR*) gene.

Source and Description of Primers. Human cDNA (GenBank accession no. U43168) sequence was used to design primers to amplify porcine genomic DNA.

Primer Sequences. Forward primer: 5′-GCATCC-CATATCTGAACCC-3′; reverse primer: 5′-CCACTTAAAC-CATAGCGAATC-3′.

Method of Detection. The PCR amplification (25 μL final volume) was performed using 37.5 ng of genomic DNA, 350 *μM* each dNTP, .3 *μM* each primer, 1× Expand Buffer 1, and .7 unit Expand Polymerase (Boehringer Mannheim). The thermal cycler profile was 92°C for 2 min; 10 cycles of 92°C for 30 s, 53°C for 30 s, and 68°C for 2 min; then 25 cycles of 92°C for 30 s, 53°C for 30 s, and 68°C for 2 min plus 20 s per cycle; followed by a final extension at 68°C for 7 min. Twenty microliters of the 3.8-kb product was digested with *Hin*fI and separated on a 3% NuSieve gel (FMC).

Description of Polymorphism. Bands of approximately 2,100, 700, 395, 350, 240, 140, and 110 bp were produced. The 350-bp fragment was designated as the A allele. When this fragment was cut with *Hin*fI, 240- and 110-bp fragments were generated and were designated as the B allele.

Inheritance Pattern. The *LEPR Hin*fI polymorphism was observed to have a Mendelian inheritance pattern in six three-generation families of the PiGMaP reference family (Archibald et al., 1995).

Frequency. Frequencies for the A allele were .18 for Hampshire (n = 14), .11 for Landrace (n = 13), .17 for Duroc (n = 12), .09 for Large White (n = 11), .75 for Meishan (n = 14), .38 for Berkshire (n = 4), and .56 for Chester White (n = 8).

Chromosomal Location. The *LEPR* was significantly linked to seven markers on the published PiGMaP chromosome 6 map (recombination fraction and LOD score in parentheses): *S0059* (.24, 6.41), *S0228* (.15, 12.86), *S0003* (.13, 9.25), *S0299* (.05, 11.03), *S0121* (.07, 19.33), *S0146* (.11, 4.29), and *S0031* (.24, 4.16). A multiple-point analysis produced the best map order of these markers and *LEPR* (with distance in Kosambi cM): *S0059*-13.3-*S0228*-1.0-*S0003*-4.4-*S0299*-4.5-*S0121*-7.9-*LEPR*-22.1-*S0146*-3.3-*S0031*.

Comments. The ends of the pig PCR product were sequenced to confirm the product was *LEPR*. The coding portion of the 5′ and 3′ ends had 93% and 90% identities at the amino acid level, respectively, to the corresponding regions of the human sequence. The *LEPR* is the high-affinity receptor for leptin, a hormone secreted by adipose tissue that regulates fat deposition and satiety. Mutations in *LEPR* have been associated with obesity in mice (*db*/*db*) and rats (*fa*/*fa*) (Chen et al., 1996; Phillips et al., 1996).

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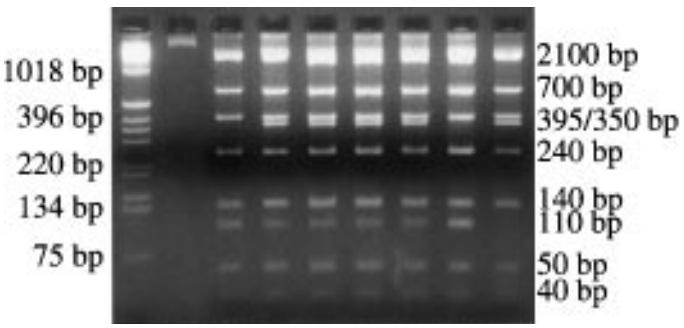

Figure 1. *Hin*fI PCR-RFLP in the porcine leptin receptor PCR fragments. Lane 1: 1-kb ladder (Promega); lane 2: uncut PCR product; lane 3: BB grandsire; lane 4: AB granddam; lane 5: AB sire; lane 6: AB dam; lane 7: AB offspring; lane 8: BB offspring; lane 9: AA offspring.

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