Rapid Communication: Restriction Fragment Length Polymorphism in Amplification Products of the Porcine Growth Hormone-Releasing Hormone Gene1

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Polymorphism. A restriction fragment length polymorphism was detected within PCR amplification products of the porcine growth hormone-releasing hormone (GHRH) gene using the restriction enzyme AluI.

Primer Description. The primer set used was designed based on homologous regions of human and mouse GHRH cDNA sequences (see Moody et al., 1995) to amplify a region of the bovine GHRH gene.

Primer Sequences. Forward primer: 5'-GTAAGGATGC(A/T)(A/G)CTCTGGGT-3'; reverse primer: 5'-TGCCTGCTCATGATGTCCTGGA-3'.

Method of Detection. PCR amplification (15 μL final volume) was performed using 100 ng of genomic DNA, 200 μM each dNTP, .2 units Taq polymerase, 400 nM each primer, 1.56 mM MgCl2 and PCR buffer (Tris-HCl, 10 mM; KCl, 50 mM; pH 8.3). Thermal cycling began with an initial denaturation at 95°C for 2 min followed by 40 cycles of 95°C for 30 s, 60°C for 45 s, and 72°C for 1 min, and concluded with a final extension at 72°C for 5 min. Digestion of the resulting 455-bp product with AluI revealed a polymorphism with two alleles (Figure 1) characterized by bands of 250 and 100 bp (A allele) or 230 and 100 bp (B allele). Additional smaller fragments representing the remaining 105 to 125 bp were likely generated by AluI digestion but were not detectable in agarose gels.

Inheritance Pattern. Mendelian inheritance of GHRH was confirmed in eight full-sib families (91 offspring).

Chromosome Location. Individuals from the three-generation PiGMAP reference families of European Wild Boar × Large White and Meishan × Large White pigs (Archibald et al., 1995) were genotyped for GHRH to determine linkage relationships between the GHRH gene and previously mapped loci. In a sex-averaged analysis, GHRH was linked to several loci on chromosome 17 (SSC17), including prodynorphin (Pdyn; recombination frequency = .02), S0204 (recombination frequency = .09), and S0296 (recombination frequency = .17) with lod scores of 11.1, 3.7, and 6.2, respectively.

Frequency. Frequency of the A allele was .36 in the 14 F1 parents of the PiGMAP reference families.

Comments. The porcine GHRH PCR amplification product was sequenced (Genbank accession no. U90275) and found to be 96 and 81% homologous to portions of exon 3 of human and rat GHRH, respectively. There was 87% homology to the homologous bovine GHRH PCR product described by Moody et al. (1995; GenBank Accession no. 029611).

Both GHRH and Pdyn have been mapped in mice to chromosome 2 and in humans to chromosome 20. Assignment of GHRH to SSC17 is therefore in agreement with comparative mapping predictions based on comparative mapping between bovine and porcine.

Literature Cited


Key Words: Pigs, Somatoliberin, PCR-RFLP


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