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# Rapid communication: Linkage mapping of the porcine Agouti gene

## Abstract

Genus and Species. *Sus scrofa*. Locus. Porcine agouti gene. Source and Description of Primers. The forward primer was designed from pig sequence (GenBank accession no. AF018166) and the reverse primer was developed from comparing the homologous regions of mouse and bovine agouti sequences (GenBank accession no. L06451 and X99691, respectively). The primers were used to amplify approximately 1.4 kb of the porcine agouti gene fragment spanning exons 2 and 3. Sequences of the PCR fragment revealed 83% and 89% exonic identities to the corresponding human and bovine agouti nucleotide sequences, respectively. The porcine agouti sequence has been submitted to GenBank, accession no. AF133261.

## Keywords

Agouti, Pigs, Gene Mapping

## Disciplines

Agriculture | Animal Sciences | Genetics and Genomics

## Comments

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# Rapid communication: Linkage mapping of the porcine *Agouti* gene<sup>1</sup>

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*Genus and Species.* *Sus scrofa*.

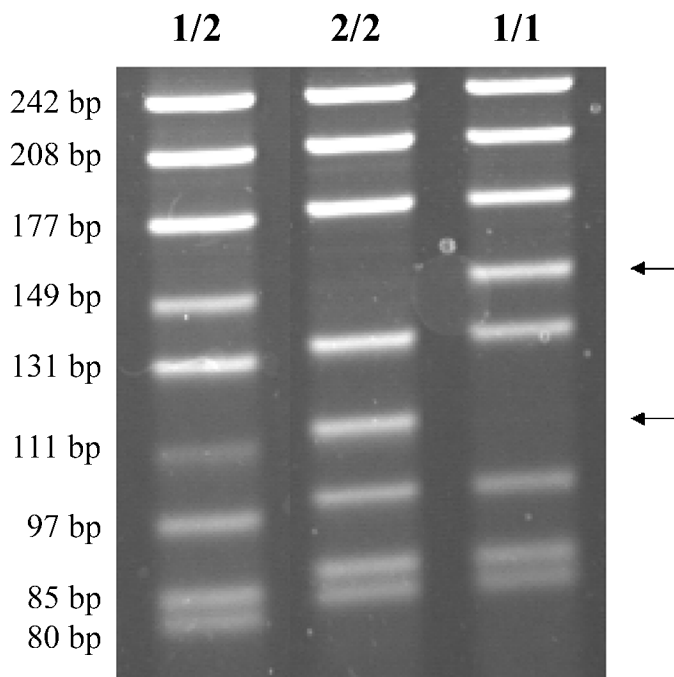
*Locus.* Porcine *agouti* gene.

*Source and Description of Primers.* The forward primer was designed from pig sequence (GenBank accession no. AF018166) and the reverse primer was developed from comparing the homologous regions of mouse and bovine *agouti* sequences (GenBank accession no. L06451 and X99691, respectively). The primers were used to amplify approximately 1.4 kb of the porcine *agouti* gene fragment spanning exons 2 and 3. Sequences of the PCR fragment revealed 83% and 89% exonic identities to the corresponding human and bovine *agouti* nucleotide sequences, respectively. The porcine *agouti* sequence has been submitted to GenBank, accession no. AF133261.

*Primer Sequences.* Forward primer: 5'-TCA TGG TAT GCC TGT GCT TCT TC-3'; reverse primer: 5'-CTT TTC CGC TTC ATT TCT GCT-3'.

*Method of Detection.* The PCR reaction was performed using 12.5 ng of porcine genomic DNA, 1.5 mM MgCl<sub>2</sub>, .125 mM dNTP, 3 pmol of each primer, .35 U *Taq* DNA polymerase (Promega, Madison, WI), and PCR buffer (10 mM Tris-HCl, 50 mM KCl, and .1% Triton X-100) in a final volume of 10 μL. The PCR profile included 2 min at 94°C; 35 cycles of 30 s at 94°C, 1 min at 54°C, 1.5 min at 72°C; and a final 15-min extension at 72°C in a Robocycler (Stratagene, La Jolla, CA). The PCR product was digested overnight with *Dde*I at 37°C. The digested fragments were separated by electrophoresis on a 3% agarose gel.

*Description of Polymorphism.* Sequence comparison of the PCR fragments of the pooled DNA from individual pigs of several different breeds revealed an intronic nucleotide difference situated within a *Dde*I restriction enzyme recognition site. The *Dde*I digestion of the PCR



**Figure 1.** *Dde*I digestion of the porcine *agouti* fragment, analyzed by agarose-gel electrophoresis. The arrows indicate polymorphic fragments. The *agouti* genotypes are indicated at the top of each lane. The 38-bp fragment is not shown on the gel.

product produced 149-bp (allele 1) and 111- and 38-bp (allele 2) polymorphic fragments. The monomorphic fragments were 242, 208, 177, 131, 97, 85, and 80 bp (Figure 1).

*Pattern of Inheritance.* Autosomal segregation following a pattern of Mendelian inheritance was observed in three families of the three-generation PiGMaP reference family (Archibald et al., 1995).

*Allele Frequencies.* Allele frequencies were determined by genotyping of 14 grandparental animals of the European PiGMaP families and 42 unrelated animals from several breeds in the Iowa State University herd. Allele 1 was observed with a frequency of .72 in Yorkshire (n = 9), .22 in Large White (n = 10), and .05 in Chester White (n = 9). Allele 1 was not observed in any Meishan (n = 8), Hampshire (n = 9), Duroc (n = 7), or Landrace (n = 4) pig.

*Chromosomal Location.* Two-point linkage analyses were performed using the genotypes of the three PiG-

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MaP families and the CRI-MAP program (Green et al., 1990). The *agouti* gene shared significant linkage to two markers on porcine chromosome 17 (SSC17). The linked markers (LOD score and sex-averaged centimorgan distances in parentheses) were *SO292* (7.22, 0) and *ENDO* (6.99, 15).

*Comments.* The porcine *agouti* gene had been physically mapped to SSC17q21 and the physical location corresponds to the comparative locations of the human (HSA 20) and mouse (MMU 2) *agouti* genes (Kijas et al., 1998). Our linkage results agree reasonably well with the physical data. The *agouti* protein antagonizes the binding of certain pro-opiomelanocortins to melanocortin receptors (MCR) involved in pigmentation and obesity (Fan et al., 1997). Further sequence analyses to find polymorphisms in addition to our intronic one are underway.

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Key Words: Agouti, Pigs, Gene Mapping