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Abstract

Source and Description of Primers. Primers (Set A) were designed based on a porcine glutathione-peroxidase- 5 (GPX5) genecDNAsequence available in the Gen- Bank (#D37916). Using porcine genomic DNA as a template in the PCR, these primers amplified either a 2.9- kb or a 2.4-kb fragment. Set B and Set C primers were designed based on the pig DNA sequence generated using Set A primers.

Keywords

Pigs, Epididymis, Genes, Polymorphism, Gene Mapping

Disciplines

Agriculture | Animal Sciences | Genetics and Genomics

Comments

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Rapid Communication: Mapping of the *Glutathione-peroxidase-5 (GPX5)* Gene to Pig Chromosome 7[1]

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Source and Description of Primers. Primers (Set A) were designed based on a porcine glutathione-peroxidase-5 (*GPX5*) gene cDNA sequence available in the GenBank (#D37916). Using porcine genomic DNA as a template in the PCR, these primers amplified either a 2.9-kb or a 2.4-kb fragment. Set B and Set C primers were designed based on the pig DNA sequence generated using Set A primers.

Primer Sequences. Set A primers were as follows: forward 5′ GGC CTT CTA TCT TTT CCC TCT T 3′ and reverse 5′ TGG TGC CTG TCA CGT CTT 3′. Set B primers were as follows: forward 5′ TTC ATG TAG AAC TTA TTT CTG 3′ and reverse 5′ TGA CTT ACC CAT TCT TCA G 3′. Set C primers were as follows: forward 5′ TAG ACA CGT CAC TTC ACC TCC 3′ and reverse 5′ GGG AGT AAG CAT TTT CTC TGT G 3′.

Method of Detection. The PCR reactions with Set A PCR primers were performed in 25-μL reactions including .9 U *Platinum Taq* DNA polymerase (Gibco BRL, Grand Island, NY), 1× PCR buffer, 1.5 m*M* $MgCl_2$, .24 m*M* of each dNTP, 4 pmol of each primer, and 25 ng of genomic DNA. Thermocycling was carried out in a MJ Research, PTC-100 instrument (Watertown, MA) and included initial denaturation at 94°C for 3 min, followed by 40 cycles at 94°C for 40 s, 63°C for 45 s, 72°C for 2.5 min, and a final extension step at 72°C for 5 min. Products from different breeds were directly sequenced using dye terminator chemistry and an ABI 377 sequencer (Perkin-Elmer, Foster City, CA). Set B primers amplified a .5-kb fragment that was used for physical and genetic mapping, whereas the Set C primers amplified two alleles differing by length (due to a 523-bp deletion/insertion), which were used for genotyping in a population study. Set B and C PCR were performed in 10-μL reactions including .35 U of *Taq* DNA polymerase (Promega, Madison, WI), 1× PCR buffer, 2.0 m*M* $MgCl_2$, .2 m*M* of each dNTP, 5 pmol of each primer, and 12.5 ng of porcine genomic DNA. The temperature conditions were as above except that the annealing temperature was 51 and 61°C for Set B and Set C, respectively.

Sequencing and Polymorphisms. A pig sequence tagged site (**STS**) spanning 2,849 bp was generated from the Set A amplicon and has been submitted to GenBank (#AF124818). The first 38 bp of this sequence showed 100% identity with the porcine *GPX5* cDNA sequence, from which the Set A primers were designed (#D37916), and thereby confirmed *GPX5* specificity. That the rest of this STS did not match up is most likely because it is intronic sequence. Thus, the reverse primer of Set A is probably located in an exon very close to the upstream intron border. This STS was completely sequenced from pigs representing the Meishan and Hampshire breeds, and a Yorkshire pig was partially sequenced. By comparing these sequences, as many as 16 polymorphic sites were revealed, one of which was a 523-bp deletion/insertion (sequence position 790 to 1,312). The Hampshire sequence represented the shorter of these length variants and also differed from the Meishan sequence at 13 other sites. Thus, these two sequences must be considered unusually divergent. The BLAST program was used to search for sequences similar to the deleted/inserted sequence, but this did not result in any noteworthy matches. The Set C primers, flanking the deletion, amplified two allele-specific PCR products with lengths .63 kb and .11 kb, and they were designated as alleles 1C and 2C, respectively (Figure 1).

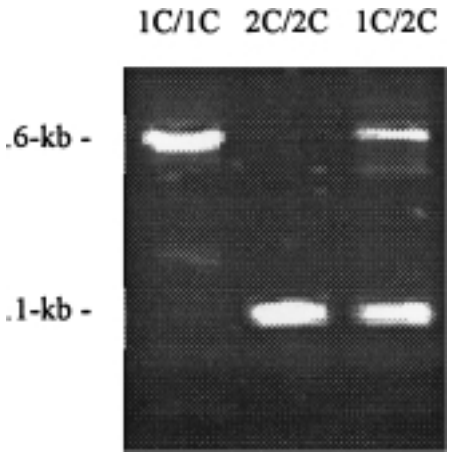

Figure 1. Agarose gel image showing three genotypes of the .5-kb length polymorphism.

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Table 1. Frequency of *GPX5* genotypes in different breeds, based on Set C primer genotyping of the .5-kb length polymorphism

		Genotypes			Allele[a]	
Breed	n	1C/1C	1C/2C	2C/2C	1C	2C
Duroc	6	1.0	0	0	1.0	0
Fengjing	5	1.0	0	0	1.0	0
Hampshire	14	.11	.46	.43	.43	.57
Landrace	12	.49	.23	.28	.71	.29
Meishan	6	1.0	0	0	1.0	0
Mini Pig	4	1.0	0	0	1.0	0
Minzhu	5	1.0	0	0	1.0	0
Yorkshire	13	1.0	0	0	1.0	0
Total	65	48	11	6	107	23
Total freq.		.74	.17	.09	.82	.18

[a]1C = .6 kb; 2C = .1 kb.

Even though the deletion was interesting, it was not polymorphic in the mapping families. Therefore, Set B primers were used and a single nucleotide polymorphism (A to G substitution) affecting a *Hin*fI restriction site was used for linkage mapping. The PCR-RFLP (*Hin*fI) produced a .53-kb product, which included two major allelic fragments of sizes .3 kb and .23 kb for alleles 1B and 2B, respectively.

Inheritance Pattern. The segregation of the PCR-RFLP (*Hin*fI) on the PiGMaP families (Archibald et al., 1995) was consistent with autosomal Mendelian inheritance.

Allele Frequencies. The allele 1C and 2C frequencies were estimated in different breeds as shown in Table 1. It is noteworthy that the shorter (2C) allele was found only in the Hampshire (.57) and Landrace (.29) breeds.

Chromosomal Location. A pig/rodent somatic cell hybrid panel (**SCHP**) comprising 27 cell lines (Yerle et al., 1996) was used for physical mapping. The SCHP results were compared to the established regional assignment (http://www.toulouse.inra.fr/lgc/pig/hybrid/chromo7/chromo7.htm). The results showed that *GPX5* is located on pig chromosome 7(1/2)p12–p11. Linkage mapping was performed with CRI-MAP (Green et al., 1990) analysis using PCR-RFLP (*Hin*fI) genotypes of three three-generation PiGMaP families (Archibald et al., 1995). The linkage results confirmed the physical mapping. The linked markers with two-point centimorgan distances and LOD scores in parentheses were as follows, respectively: S0013 (4.0, 9.97), SLA-DQA (6.0, 7.18), SLA (0.0, 5.72), TNFB (1.0, 16.11), RXRB (0.0, 11.44), S0102 (14.0, 6.38), and S0078 (9.0, 5.31). These results show that *GPX5* is most likely located within the SLA complex.

Comments. The number of accessory sperm in the zona pellucida is related to early embryo viability in pigs and is a result of sperm-egg interaction in the female genital tract (Bertani et al., 1997). The *GPX5* gene is one of several genes that are expressed in the epididymis and interact with sperm cells (Okamura et al., 1997). Therefore, it might play a role in sperm-egg interaction, influencing the number of accessory sperm in the zona pellucida, and have a pleiotropic effect on early embryo viability. These facts make *GPX5* a candidate gene for boar fertility, which is supported by the fact that the allele 2C (observed using the Set C primers) was found mainly in the Hampshire breed, which is known to have boars with low fertility (Jones, 1998). In addition, the linkage analysis of *GPX5* showed that it is closely linked to the SLA, which has been suggested to have an effect not only on reproduction, but also on other performance traits in swine (Vaiman et al., 1998). The results presented here are part of a project that has as its major goal the investigation of the role of *GPX5* on boar fertility and fertility in females.

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