

**Table 1** Number of alleles of *SJ108* within porcine *IL8* gene in different breeds.

Breeds	Number of animals	Allele sizes (bp)	No. of alleles
Landrace	26	164–178	5
Large White	13	164–182	6
Duroc	5	164–176	5
Meishan	6	164–166	2
Wild pig	3	164–176	4

**Table 2** The sex averaged linkage mapping of the *SJ108* within porcine *IL8* gene using Map Manager ver. 2.6<sup>3</sup> and two-point analysis.

Locus 1	Locus 2	Recombination fraction	LOD score
<i>SW29</i>	<i>KIT</i>	0.114	21.5
<i>KIT</i>	<i>SJ108</i>	0.217	8.2
<i>SJ108</i>	<i>S0086</i>	0.201	6.5

94 °C, 30 s at 55 °C, 30 s at 72 °C and final extension of 5 min at 72 °C.

**Allele frequency:** Allele frequencies of *SJ108* were determined for 53 unrelated pigs from five breeds. Polymorphisms consisting of eight alleles between 164 and 182 bp were being widely distributed in the breeds (Table 1).

**Mendelian inheritance:** Codominant Mendelian segregation of *SJ108* was observed in a Meishan × Göttingen miniature pig experimental family<sup>7</sup>.

**Chromosomal location:** Using genotype data from the experimental family the microsatellite marker, *SJ108*, within the *IL8* gene was mapped on porcine chromosome 8 by linkage analysis (Table 2). These results confirmed the previous mapping study<sup>3</sup>.

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## Mapping of the porcine alpha-fetoprotein (*AFP*) gene to swine chromosome 8<sup>1,2,3</sup>

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**Source/description of primers:** A cDNA clone containing the full coding region of the porcine alpha-fetoprotein (*AFP*) was isolated (GenBank accession no. AF517770) from the 'Meat Animal Research Center (MARC) 2PIG' expressed sequence tag primary library by iterative screening<sup>1</sup> using a forward (*AFP* F1) and a reverse (*AFP* R2) primer for polymerase chain reaction (PCR) amplification. *AFP* F1 was designed from the horse *AFP* sequence (GenBank accession no. U28947), and *AFP* R2 corresponds to bases 841–822 of the porcine cDNA sequence. After alignment of the sequence with the human *AFP* gene sequence, primers to amplify across putative intron 10 were designed based on the porcine cDNA sequence. The forward (*AFP* exon 10 F1) and reverse (*AFP* exon 11 R1) primers correspond to bases 1246–1264 and 1442–1424 of the porcine *AFP* cDNA (GenBank accession no. AF517770). Based on the human genomic sequence, this intron was expected to be approximately 482 bp. Agarose gel electrophoresis and sequencing of the PCR amplicons of porcine genomic DNA indicated that the corresponding region of the pig gene was 565 bp. The PCR product was confirmed by sequencing (GenBank accession no. AY120900).

**PCR primer sequences and a flanking sequence for a single nucleotide polymorphism:**

*AFP* F1: CTAGCAACTATGAAAGTGGGTGGTATC

*AFP* R2: CTCTGCAGCATTCTCTGTGG

*AFP* exon 10 F1: CAGGAGAGCCAAGCACTGG

*AFP* exon 11 R1: GCCAACTGCCTGTCTTCAC

Flanking sequence: ATCATGTCTT(T/C)TGATGGCAAG

*AFP* probe primer: GAGCTGACATCATGTCTT

**PCR conditions:** Polymerase chain reactions were carried out in a 25- $\mu$ l volume containing 100 ng genomic DNA, 1.5 mM MgCl<sub>2</sub>, 20 pmol of each primer, 100  $\mu$ M dNTP, and 0.35 U

<sup>1</sup>Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

<sup>2</sup>Authors thank Dr Gary Rohrer for help in the mapping of the *AFP* gene.

<sup>3</sup>This article is a material of the US Government, and can be produced by the public at will.

*Taq* polymerase. Amplification was performed under the following PCR conditions; 2 min at 94 °C; 45 cycles of 30 s at 94 °C, annealing for 1 min at 65 °C, 1.5 min at 72 °C; and a final extension of 5 min at 72 °C. The amplified genomic DNA of eight parents (seven F1 sows and one white composite boar) from the MARC swine reference population<sup>2</sup> were bidirectionally sequenced and evaluated for polymorphisms<sup>3</sup>.

**Polymorphism and chromosomal location:** A C/T single nucleotide polymorphism was detected in intron 10 (GenBank accession no. AY120900), position 476 from the exon/intron boundary. This polymorphism was heterozygous in six of the seven F1 sows. An assay was designed to genotype this polymorphism using primer extension with the *AFP* probe primer and analyte detection on a MALDI-TOF mass spectrometer<sup>4</sup> (Sequenom Inc., San Diego, CA, USA). This marker generated 68 informative meioses in the MARC swine reference population. The *AFP* gene was mapped to chromosome 8 position 60.4 cM, which is the same position as marker *S0017* on the current MARC swine chromosome 8 linkage map (<http://www.marc.usda.gov/>) using CRI-MAP. The most significant two-point linkage detected was with *S0017* (LOD = 18.36) at 0 recombination. The location of the porcine *AFP* gene is within the 95% confidence interval of the uterine capacity quantitative trait locus (QTL) on chromosome 8<sup>5</sup>. The *AFP* gene in human is located on chromosome 4q11-q13, which shares homology with swine chromosome 8. *AFP* is the predominant protein in foetal plasma during early gestation (days 15–30) in the pig<sup>6</sup>, which may be important for foetal development.

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## Linkage mapping of porcine *DGAT1* to a region of chromosome 4 that contains QTL for growth and fatness<sup>1,2,3</sup>

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**Source/description:** Diacylglycerol acyltransferase (DGAT1) is a microsomal enzyme that catalyses the final and only committed step in formation of triglycerides, which are the major form of stored energy in eukaryotes<sup>1</sup>. *DGAT1*-deficient mice are viable, lean, able to synthesize triglycerides, and resistant to diet-induced obesity<sup>2</sup>. *DGAT1*-deficient females also have a complete absence of milk production and a substitution in the bovine *DGAT1* gene has a major effect on milk yield and fat content<sup>3</sup>.

A full-length porcine *DGAT1* complementary DNA (cDNA) was identified from the MARC 2PIG normalized library<sup>4</sup> and sequenced (GenBank accession number AY093657). The 1935 bp cDNA contained 198 bp of 5'-UTR, 1470 bp of coding sequence and 261 bp of 3'-UTR. Porcine *DGAT1* cDNA has 83, 88 and 91% nucleotide identity in the coding region with mouse, human and bovine cDNAs, respectively. Pig *DGAT1* cDNA codes for a protein of 489 amino acids with 85, 86 and 92% identity to mouse, human and bovine proteins, respectively.

The porcine *DGAT1* gene was cloned by iterative polymerase chain reaction (PCR) screening of a cosmid library<sup>5</sup> using PCR primers DGAT-F5 and DGAT-R8 (Table 1). Primers were designed from porcine *DGAT1* cDNA and used to sequence the cosmid and amplify genomic DNA.

**Gene organization:** The genomic structure was identical to that of the human and bovine genes with respect to intron size and organization of the 17 exons<sup>6,3</sup>. A GT/poly(dG) repeat was found immediately following (66 bp) the polyadenylation signal and cleavage site. The complete *DGAT1* gene was sequenced except for about 700 bp of intron 1 (GenBank accession number AY116586); however, enough flanking sequence was obtained in order to amplify coding sequence of exons 1 and 2 from genomic DNA (Table 1).

**PCR conditions:** The PCR reactions were performed in 25 µl using 100 ng of genomic DNA, 1XPCR buffer, 1.5 mM MgCl<sub>2</sub>,

<sup>1</sup>The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession numbers AY093657 and AY116586.

<sup>2</sup>Mention of trade names or commercial products is solely for the purpose of providing information and does not imply recommendation, endorsement or exclusion of other suitable products by the U.S. Department of Agriculture.

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