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Mapping of the porcine *AREG* and *EGF* genes to SSC8*

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Source/description of primers: Full coding regions of the porcine amphiregulin [*AREG* (GenBank accession no. AY028310)] and the epidermal growth factor (*EGF*) precursor cDNAs (GenBank accession no. AF336151) had been previously reported. Using these sequences, primers were designed to generate probes to screen a porcine bacterial artificial chromosome (BAC) library (RPCI-44)¹. The BAC clones for each gene were isolated (199P1 for *AREG* and 54C16 for *EGF*), digested with *Sau3AI* and

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subcloned into *Bam*HI digested SK Bluescript. Three microsatellite sequences for amphiregulin [*SB64*, *SB65* and *SB66* (3592 bp upstream from the translation start site of the amphiregulin gene)] and one microsatellite sequence for *EGF* (*SB67*) were identified. Numbers of repeats were 11, 17, 15 and 21 for *SB64*, *SB65*, *SB66* and *SB67*, respectively.

Primer sequences: Primers were designed to amplify the CA-repeat sequences (Table 1).

Polymerase chain reaction conditions: Primer pairs for each locus were optimized for PCR amplification by testing over a range of annealing temperatures (55–62°C). The PCR reactions were carried out in a 5- μ l volume containing 15 ng genomic DNA, 1.5 mM MgCl₂, 2 pmol of each primer, 100 μ M dNTP, and 0.35 U *Taq* polymerase. Amplification was performed under the following PCR conditions: 2 min at 92°C, 30 cycles of 30 s at 94°C, annealing for 1 min at marker specific temperatures (Table 1), 1 min at 72°C, and a final extension of 5 min at 72°C.

Polymorphism and allele size: The allele sizes at each locus were determined by examining 7 litters from two boars and seven sows from the US Meat Animal Research Center (MARC) Swine Reference Population. Amplification of genomic DNA from pigs in the reference population² revealed 6 (including one null allele), 5, 6 and 8 (including one null allele) alleles for *SB64*, *SB65*, *SB66* and *SB67*, respectively. Number of informative meioses were 135, 136, 154 and 57 for *SB64*, *SB65*, *SB66* and *SB67*, respectively.

Chromosomal location: Data were analysed using CRI-MAP version 2.4. Haplotyping *SB64*, *SB65* and *SB66* microsatellite markers provided 171 informative meioses for amphiregulin yielding a maximum two point LOD = 43.35 with *S0017* at 0 recombination. Multipoint analysis placed amphiregulin at position 65 cM on chromosome 8 in the MARC swine linkage map². *SB67* provided 57 informative meioses for *EGF* yielding a maximum LOD = 15.05 with *SW2160* at 0 recombination. *EGF* was mapped to position 84 cM on chromosome 8 in the MARC swine linkage map³ confirming previous mapping by PCR length polymorphism in the PiGMaP reference families⁴.

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Table 1 Primers used for microsatellite markers for the porcine *AREG* and *EGF*.

Markers	Accession no.	Primers	Primer Sequences	Annealing temperature (°C)	Product size (bp)
<i>SB64</i>	AF406986	Forward	CACTCTCTGAACACATTTGCC	62	105–125
		Reverse	TTTCTTTCTTTGCCTAGTTTCTCAG		
<i>SB65</i>	AF406987	Forward	CCAGCAGGACTCTACGCAG	58	91–99
		Reverse	GAGGCAGGCAGAGAATACATG		
<i>SB66</i>	AF406988	Forward	TGAGTAAATGAACTACAGACAGGATTG	58	113–127
		Reverse	CAGTGGATGATTTGATGCAGTG		
<i>SB67</i>	AF465465	Forward	AGGGATGAGTAAAAGTGTGCTAC	55	156–172
		Reverse	TCCTAAAGGTGAAAATGGGC		

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Assignment of the porcine *GLUL* gene to the distal end of chromosome 9q

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Source/description: Glutamate-ammonia ligase, alias glutamine synthetase (*GLUL*; E.C. 6.3.1.2), catalyses the synthesis of glutamine from glutamate and has an important function in controlling body pH and removing ammonia from the circulation. In humans, the gene *GLUL* was localized by FISH to chromosome 1q25^{1,2}. The porcine cDNA sequence for glutamine synthetase (EMBL accession number Z29636) was used to design polymerase chain reaction (PCR) primers (Pair 1). Exon boundaries were deduced on the basis of the rat exon sequences³ (EMBL M29595; M29596; M29597; M29598; M29599). The forward primer was from exon 3 and the reverse primer was from exon 4. The amplified fragment of porcine genomic DNA was cloned and sequenced and a new pair of primers (Pair 2) was designed from the sequence.

Primer sequences:

Pair 1: Forward: 5'-GGA ATT TCG ATG GCT CTA GT-3'

Reverse: 5'-CCA TGT CCA TTA TCC GTT TA-3'

Pair 2: Forward: 5'-GGG CCT CAT CTC TCC ATC TA-3'

Reverse: 5'-AAA CCC CAG TAA AAT GCA ATG-3'

PCR conditions/cloning/sequencing: Using Pair 1 primers, PCR was performed in 25 µl reactions containing 100 ng genomic DNA, reaction buffer, 2.0 mM MgCl₂, 2% dimethyl sulphoxide (DMSO), 200 µM each dNTP, 10 pmol each primer, and 1 U LA polymerase (Top Bio, Prague, Czech Republic). A 2-min denaturation at 95 °C was followed by 35 cycles (45 s at 94 °C, 45 s at 58 °C, 2 min at 68 °C) and a final extension at 68 °C for 7 min. A major ~2 kb fragment was amplified, with a weak

non-specific band of ~1 kb. The major fragment was subcloned (pUC18; *Escherichia coli* DH5α) and sequenced (ALFexpress Sequencing System, Pharmacia Biotech, Uppsala, Sweden). Comparison of the deduced coding sequence from the amplified fragment with porcine cDNA (EMBL Z29636) confirmed that porcine *GLUL* was amplified. The sequence we obtained (parts of exons 3 and 4 and all of intron 3) has been deposited in the EMBL database under accession number AJ430415. In the intron a SINE (PRE-1) is present (positions 395–653). Flanking the 5' end of the PRE-1 there is a repeat region (microsatellite) containing 11 CT repeats (positions 373–394). In another clone from the same pig 10 CT repeats were present.

The PCR conditions with Pair 2 primers were slightly modified (concentration of MgCl₂ was 1.0 mM, time of amplification was 90 s, and 30 cycles were performed). Also, *Taq* polymerase (Top Bio) was used. A fragment of ~1.2 kb was amplified.

Polymorphism/Mendelian inheritance/allele frequencies: A polymorphism was observed after digestion with *Hae*III (alleles A and B) in both the ~2 kb fragment (Pair 1 primers) and the ~1.2 kb fragment (Pair 2 primers). The *Hae*III polymorphism in the ~2 kb fragment is shown in Fig. 1. The following fragments were observed after restriction of the ~1.2 kb fragment with *Hae*III: allele A – 926 + 253 bp; allele B – ~700 + 253 + ~230 bp. According to the sequence, two additional short fragments (46 and 3 bp) are present in both alleles, but these were not seen on the gel. The polymorphic restriction site is within the intron (deduced from the sequence information), but its exact position has not been determined. For routine testing Pair 2 primers may be preferable.

Codominant inheritance was confirmed in the Hohenheim Meishan × Pietrain pedigree⁴. Allele frequencies were determined in unrelated animals of several breeds. Piétrain (13), Hampshire (6), Duroc (12), Black Pied Prestice (7) and Czech Meat Pig (15) were monomorphic for allele A. In Large White (25), Landrace (13) and Meishan (8), the frequencies of allele A were 0.76, 0.88 and 0.25, respectively.

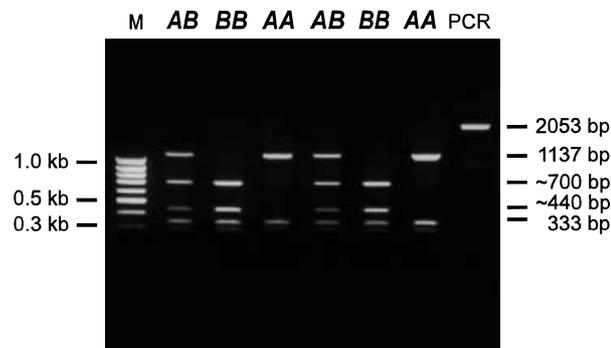


Figure 1 Agarose gel electrophoresis (1.5%) showing genotypes of the porcine *GLUL* gene after digestion of the 2053 bp PCR fragment (Pair 1 primers) with *Hae*III. The genotypes (AA, AB, or BB) are given at the top of each lane. M, 1000–100 bp marker; PCR, undigested PCR fragment.