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 subcloned into BamHI digested SK Bluescript. Three microsatellite sequences for amphiregulin [SB64, SB65 and SB66 (1592 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-μl volume containing 15 ng genomic DNA, 1.5 mM MgCl\(_2\), 2 pmol of each primer, 100 μM 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 population\(^2\) 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 map\(^2\). 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 map\(^2\) confirming previous mapping by PCR length polymorphism in the PiGMaP reference families\(^2\).

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*References*


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\*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.

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

<table>
<thead>
<tr>
<th>Markers</th>
<th>Accession no.</th>
<th>Primers</th>
<th>Primer Sequences</th>
<th>Annealing temperature (°C)</th>
<th>Product size (bp)</th>
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</thead>
<tbody>
<tr>
<td>SB64</td>
<td>AF406986</td>
<td>Forward</td>
<td>CACCTCTCAGACACGTTTGC</td>
<td>62</td>
<td>105–125</td>
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<td></td>
<td></td>
<td>Reverse</td>
<td>TCTCTTTCTTGGCTGAGTTTCTCAG</td>
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<tr>
<td>SB65</td>
<td>AF406987</td>
<td>Forward</td>
<td>CCAGCAAGCAGTCCGCAAG</td>
<td>58</td>
<td>91–99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>GAGGGAGCAGGAGGTACAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SB66</td>
<td>AF406988</td>
<td>Forward</td>
<td>TGAGTAAATGAACTACAGACAGGATTG</td>
<td>58</td>
<td>113–127</td>
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<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>CAGTGATGAGTTTGATGAGTTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SB67</td>
<td>AF465465</td>
<td>Forward</td>
<td>AGGGATGAGTGAAAGTGCTAC</td>
<td>55</td>
<td>156–172</td>
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<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>TCTAAAGGGGAAAATGGG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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; EC. 6.3.1.2), catalyses the synthesis of glutamate from glutamine and has an important function in controlling body pH and removing ammonia from the circulation. In humans, the gene controlling body pH and removing ammonia from the circulation is localized by FISH to chromosome 1q25.1,2. The porcine cDNA sequence for glutamate-ammonia ligase, alias 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 sequences.3,4 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’-GGA CCT CAT CTC TTC ATG TA-3’
    Reverse: 5’-AAA CUC 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 μM 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 HaeIII (alleles A and B) in both the ~2 kb fragment (Pair 1 primers) and the ~1.2 kb fragment (Pair 2 primers). The HaeIII 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 HaeIII: allele A = 926 + 253 bp; allele B = ~700 + 233 + ~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 pedigree. Allele frequencies were determined in unrelated animals of several breeds. Pietrain (13), Hampshire (6), Duroc (12), Black Pied Prestige (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 HaeIII. The genotypes (AA, AB, or BB) are given at the top of each lane. M, 1000–100 bp marker; PCR, undigested PCR fragment.

References

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