Rapid communication: Mapping of the Myeloperoxidase (MPO) gene to pig chromosome 12

L. Marklund, S. Kaul, and C. K. Tuggle

Department of Animal Science, Iowa State University, Ames 50011

Name of Gene Marker. MPO, myeloperoxidase.

Source and Description of Primers. Primers (set A) were designed based on human (Genbank Accession no. D14466) and mouse (Genbank Accession no. X15313) MPO exon 9 and 10 sequences available in the Genbank database. Using porcine genomic DNA as template in the PCR, these primers amplified a 1-kb fragment. Set B primers were designed based on pig DNA sequence data generated using set A primers. This 893-bp pig sequence has been deposited into Genbank as a sequence tagged site (STS), Accession no. AF208527. Because the predicted exon 9 and 10 sequences total 172 bp, the predicted pig intron 9 is 721 bp, slightly longer than the human MPO intron 9 length of 495 bp.

Primer Sequences. Set A primers were as follows: forward 5′ CCA ACC CCC GTG TCC CCC TCA GC 3′ and reverse 5′ CGT GGT CCC TGC TGC GCT GCA TG 3′. Set B primers were as follows: forward 5′ GAG GGT AGT GCT GGA AGG 3′ and reverse 5′ GCT CCC GAA TCT CAT CTA C 3′.

Method of Detection. The PCR with set A primers was performed in 10-μL PCR reactions including 0.38 U Taq Gold DNA polymerase (Perkin-Elmer, Branchburg, NJ), 1× PCR buffer, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 3 pmol of each primer, and 12.5 ng genomic DNA. Thermocycling was carried out in a Robocycler (Stratagene, La Jolla, CA) or a MJ Research, PTC-100 instrument (Watertown, MA) and included initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 45 s, 63°C for 1 min, 72°C for 1.5 min, and a final extension step at 72°C for 5 min. Products from Yorkshire, Landrace, and Meishan breeds were directly sequenced using dye terminators and an ABI 377 sequencer (Perkin-Elmer, Foster City, CA). The set B primers were used to amplify an 828-bp fragment. This product was used for physical and linkage mapping. Set B-PCR was performed using the MJ Research, PTC-100 instrument and conditions as for set A except that Taq DNA polymerase (Promega, Madison, WI) was used and that the first denaturation step in the PCR was 2 min for 95°C. All PCR products were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining.

Description of Polymorphisms. A comparison between the 172-bp predicted coding sequence (parts of exon 9 and 10) of AF208527 and the human MPO sequence showed 88 and 89% identity at the nucleotide and predicted amino acid level, respectively. Based on this homology and comparative mapping results (see below), we propose the STS represents the porcine MPO gene sequence. To identify possible polymorphisms within this region of the pig MPO gene, the sequence of individual pigs representing three different breeds, Meishan, Yorkshire, and Landrace, was determined for this STS. In total, among these three breeds nine single nucleotide substitutions were revealed. Four of these were confirmed by enzyme digestions in PCR-RFLP analysis. A single nucleotide polymorphism (SNP) (G to A substitution at position 254 in AF208527) affecting a TaqI restriction site was used for linkage mapping and a population study. The PCR-RFLP (TaqI) with set B products included fragments of sizes 745 bp (allele 1) or 492 and 253 bp (allele 2) as well as an 83-bp fragment generated from a monomorphic TaqI site within the exon 10 sequence (Figure 1).

![Figure 1. Agarose gel image showing the three different PCR-RFLP (TaqI) genotypes.](image-url)
**Table 1.** Frequency of MPO genotypes and alleles in different breeds, based on set B primer genotyping of the PCR-RFLP (TaqI)

<table>
<thead>
<tr>
<th>Breed</th>
<th>n</th>
<th>1/1</th>
<th>1/2</th>
<th>2/2</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large White</td>
<td>11</td>
<td>0.27</td>
<td>0.18</td>
<td>0.55</td>
<td>0.36</td>
</tr>
<tr>
<td>Meishan</td>
<td>9</td>
<td>0.111</td>
<td>0.444</td>
<td>0.444</td>
<td>0.33</td>
</tr>
<tr>
<td>Wild boar</td>
<td>2</td>
<td>0.5</td>
<td>0.0</td>
<td>0.5</td>
<td>0.50</td>
</tr>
<tr>
<td>Commercial population</td>
<td>215</td>
<td>0.24</td>
<td>0.40</td>
<td>0.36</td>
<td>0.44</td>
</tr>
<tr>
<td>Total (n)</td>
<td>237</td>
<td>57</td>
<td>92</td>
<td>88</td>
<td>206</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>0.24</td>
<td>0.39</td>
<td>0.37</td>
<td>0.43</td>
</tr>
</tbody>
</table>

*Landrace- and Large White-based.

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

**Allele Frequency.** The allele 1 and 2 frequencies, as well as genotype frequencies, were estimated through collecting data from the parental PiGMaP animals and animals in a commercial population from the Pig Improvement Company (PIC, USA, Franklin, KY) as shown in Table 1.

**Chromosomal Location.** A pig/rodent somatic cell hybrid panel (SCHP) comprising 27 cell lines (Yerle et al., 1996) was used for physical mapping. The PCR data were submitted and analyzed as described on the Web site http://www.toulouse.inra.fr/lgc/pig/hybrid.htm. Analysis of the SCHP results showed that MPO is located on pig chromosome 12q11–q15. Linkage mapping was performed by CRI-MAP (Green et al., 1990) analysis using PCR-RFLP (TaqI) genotypes of the three-generation PiGMaP families (Archibald et al., 1995). A total of 86 informative meiosis (40 with known phase) were available for MPO linkage analysis. The linkage results confirmed the physical mapping localization. The linked markers with two-point recombination frequencies and LOD scores, respectively, in parentheses were as follows: GH (0.22, 4.74), S0090 (0.04, 9.06), and Sw957 (0.20, 4.48). Multipoint analysis indicates that MPO is most likely located between Sw957 and S0090.

**Comments.** Myeloperoxidase is a lysosomal heme-protein in the azurophilic granules of polymorphonuclear leukocytes and monocytes. In humans, it participates in the defense system of polymorphonuclear leukocytes, responsible for microbicidal activity against many different organisms (Murao et al., 1988). In this study, the MPO gene was mapped to provide information on a possible candidate gene for immunogenetic traits in the pig.

The human MPO gene maps to Hsap17q23.1. The entire Hsap17 and Sscp12 chromosomes have conserved synteny at both the bidirectional chromosome painting (Goureau et al., 1996) and at the single gene-level (http://www.toulouse.inra.fr/lgc/pig/compare/SSCHTML/SSC12S.HTM). The mapping of MPO to Sscp12q11–q15 thus agrees with these results across species and adds another locus to this conserved synteny group.

**Literature Cited**


Green, P., K. Falls, and S. Crooks. 1990. Documentation for CRI-MAP, version 2.4. Washington Univ. School of Medicine, St. Louis, MO.


**Key Words:** Gene Mapping, Myeloperoxidase, Pigs, Polymorphism