

2002

## Rapid communication: Physical and linkage mapping of the porcine connexin 37 (CX37) gene

L. Grapes

*Iowa State University*

Y. Zhang

*Iowa State University*

Max F. Rothschild

*Iowa State University, mfrothsc@iastate.edu*

Follow this and additional works at: [http://lib.dr.iastate.edu/ans\\_pubs](http://lib.dr.iastate.edu/ans_pubs)

 Part of the [Agriculture Commons](#), [Animal Sciences Commons](#), and the [Genetics and Genomics Commons](#)

The complete bibliographic information for this item can be found at [http://lib.dr.iastate.edu/ans\\_pubs/280](http://lib.dr.iastate.edu/ans_pubs/280). For information on how to cite this item, please visit <http://lib.dr.iastate.edu/howtocite.html>.

---

This Article is brought to you for free and open access by the Animal Science at Iowa State University Digital Repository. It has been accepted for inclusion in Animal Science Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact [digirep@iastate.edu](mailto:digirep@iastate.edu).

---

# Rapid communication: Physical and linkage mapping of the porcine connexin 37 (CX37) gene

## **Abstract**

Genus and Species. *Sus scrofa*. Locus. Porcine connexin 37 (CX37) gene. Source and Description of Primers. A set of primers (F1, R1) was designed from the porcine CX37 mRNA sequence (GenBank Accession no. X86024) as well as an additional reverse primer (R2) from human (GenBank Accession no. 6093424) and murine (GenBank Accession no. NM\_008120) CX37 consensus sequence. The F1 and R2 primers were used to amplify porcine CX37 from genomic DNA. Using sequence obtained from the amplified product, an additional pig-specific forward primer (F2) was designed.

## **Keywords**

Gap Junctions, Gene Mapping, Ovulation Rate, Pigs

## **Disciplines**

Agriculture | Animal Sciences | Genetics and Genomics

## **Comments**

This is an article from *Journal of Animal Science* 80 (2002): 1375, doi:/2002.8051375x. Posted with permission.

# Rapid communication: Physical and linkage mapping of the porcine *connexin 37* (CX37) gene<sup>1</sup>

L. Grapes, Y. Zhang, and M. F. Rothschild<sup>2</sup>

Department of Animal Science, Iowa State University, Ames 50011

*Genus and Species.* *Sus scrofa*.

*Locus.* Porcine connexin 37 (CX37) gene.

*Source and Description of Primers.* A set of primers (F1, R1) was designed from the porcine CX37 mRNA sequence (GenBank Accession no. X86024) as well as an additional reverse primer (R2) from human (GenBank Accession no. 6093424) and murine (GenBank Accession no. NM\_008120) CX37 consensus sequence. The F1 and R2 primers were used to amplify porcine CX37 from genomic DNA. Using sequence obtained from the amplified product, an additional pig-specific forward primer (F2) was designed.

*Primer Sequences.* F1: 5'-TTC CTG GAG AAG CTG CTG GA-3'; R1: 5'-CGA GAT CTT GGC CAT CTG TC-3'; F2: 5'-ACT CGA CCG TGG TGG GCA A-3'; R2: 5'-GTG GTC AGG TTG GCC CAG TT-3'.

*Method of Detection.* A PCR of 10  $\mu$ L volume containing 1  $\mu$ L PCR buffer, 1  $\mu$ L MgCl<sub>2</sub> (15 mM), 1  $\mu$ L dNTPs (2 mM), 0.25  $\mu$ L of each PCR primer (F1 and R2) (10 pM), 0.07  $\mu$ L Promega *Taq* Polymerase (Madison, WI), and 5.43  $\mu$ L H<sub>2</sub>O was used to assay 12.5 ng of genomic DNA from four individuals for each of five swine breeds (Landrace, Hampshire, Yorkshire, Berkshire, and Meishan). An 872-bp fragment from within the single CX37 exon was amplified using primers F1 and R2 in a Robocycler (Stratagene, La Jolla, CA) under the following thermocycling conditions: initial denaturation at 94°C for 4 min, 35 cycles of 94°C for 45 s, 62°C for 1 min, 72°C for 1 min 20 s, and a final extension time of 9 min at 72°C. For each breed, the PCR products from the four individuals were pooled. These pools were then directly sequenced using dye terminators and an ABI 377 sequencer (Perkin-Elmer, Foster City, CA) at the Iowa State University DNA Sequencing and Synthesis Facility. The F1 and R1 primers produced a 399-

bp fragment that was used for physical mapping. The F2 and R1 primers produced a 385-bp fragment that was used for linkage mapping. All mapping was completed using the thermocycling conditions described above. A PCR-RFLP marker was confirmed using the *Mlu*I enzyme, and this marker was used for linkage mapping and determining allele frequencies in a commercial population of pigs (see below). For the PCR-RFLP assays, 3  $\mu$ L of PCR products was digested with 4 U *Mlu*I in 1 $\times$  buffer solution with 1 $\times$  BSA added. Total volume for the digestion reaction was 10  $\mu$ L. Following digestion for 3 h at 37°C, digested products were loaded into a 4% agarose gel containing ethidium bromide, electrophoresed, and photographed.

*Sequencing and Polymorphisms.* The 872-bp exonic fragment amplified using F1 and R2 primers was sequenced and showed 97% homology to the 413 bp of pig CX37 mRNA sequence available from GenBank (GenBank Accession no. 6093424). Comparison of this fragment from the five breed pools revealed two single-nucleotide polymorphisms, neither of which changed an amino acid. A single T to C base substitution was identified at position 366 from the ATG site based on human CX37 sequence (GenBank Accession no. AF132674) and resulted in the formation of an *Mlu*I restriction site. A PCR-RFLP test was designed using this restriction site and products from the F2 and R1 primers. Resulting allelic fragment sizes from this test were 385 bp (allele 1) and 317 bp and 68 bp (allele 2) (Figure 1). The smaller fragment for allele 2 (68 bp) is not visible in this figure. The second polymorphism, a G to A base change, was located at position 345 from the human CX37 ATG site listed above but was not used for any mapping purposes.

*Inheritance Pattern.* In the five PiGMaP families (Archibald et al., 1995) the *Mlu*I PCR-RFLP segregated in accordance with autosomal Mendelian inheritance.

*Allele Frequencies.* Individuals (n = 844) from four commercial populations consisting of Landrace, Large White, Duroc, and Pietrain backgrounds were genotyped using the *Mlu*I PCR-RFLP assay. Allele 2 was the rarer allele, with an average frequency of 0.14 (range 0.07 to 0.20).

*Chromosomal Location.* Physical mapping of CX37 was completed using the French pig/rodent somatic cell hybrid panel (Yerle et al., 1996) and products resulting from PCR using primers F1 and R1. Analysis of the PCR results was completed as previously described (<http://>

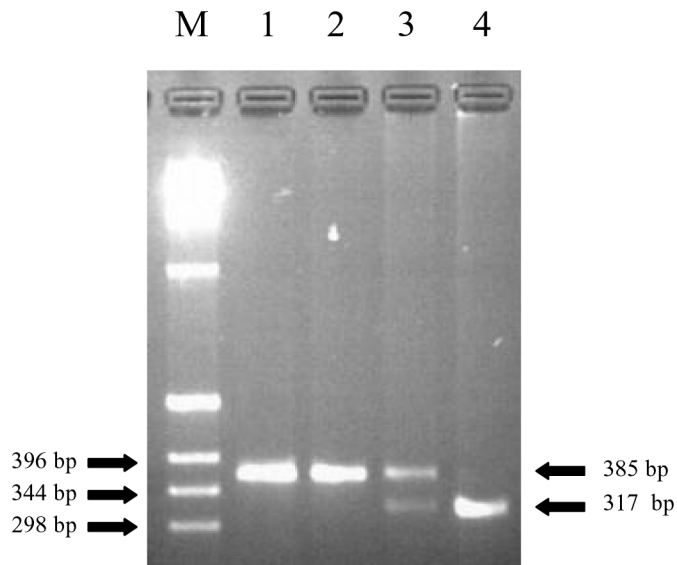
<sup>1</sup>We thank Jeannine Helm and Daniel Ciobanu for their advice and suggestions, Alan Archibald for use of the PiGMaP DNA and the ResPig database, and Martine Yerle for use of the French SCH panel. Support was provided by a USDA National Needs Fellowship in Animal Biotechnology and Quantitative Genetics, PIC International Group, and the Iowa Agric. and Home Econ. Exp. Stn., Ames, paper no. J-19292, project no. 3600, as well as by Hatch Act and State of Iowa funds.

<sup>2</sup>Correspondence: 2255 Kildee Hall (E-mail: mfrothsc@iastate.edu).

Received April 25, 2001.

Accepted January 16, 2002.

©2002 American Society of Animal Science. All rights reserved.



**Figure 1.** *MluI* PCR-RFLP of the *connexin 37* gene. Lane M includes the 1-kb ladder with predicted sizes indicated on the left. Lane 1 includes undigested PCR product using the F2 and R1 primers. Lane 2 indicates the 1,1 genotype, lane 3 indicates the 1, 2 genotype, and lane 4 indicates the 2, 2 genotype. Fragment sizes of alleles 1 and 2 are listed on the right.

[www.toulouse.inra.fr/lgc/pig/pcr/pcr.htm](http://www.toulouse.inra.fr/lgc/pig/pcr/pcr.htm)) and placed *CX37* on SSC6 q24-31 with probability of 0.87. Linkage mapping was performed using CRI-MAP (Green et al., 1990) analysis of genotypes for the PiGMAP families. Using two-point linkage analysis, four markers were found to be significantly linked to *CX37*. The markers were (LOD score and recombination frequencies in parentheses) S0031 (6.21, 0.10), S0059 (6.66, 0.09), S0228 (3.52, 0.08), and SW71 (7.50, 0.02). Using CRI-MAP, a multipoint map of SSC6 including *CX37* was constructed and placed its location centromeric of S0059 by 6.3 cM. These results confirmed the position obtained from physical mapping.

**Comments.** Gap junctions, channels formed between adjacent cell membranes, facilitate cellular communi-

cation by allowing ions and small molecules to transfer from cell to cell. Oocyte maturation and ovulation are thought to be regulated by metabolic cooperation between the oocyte and surrounding granulosa cells. Gap junctions form between the oocyte and granulosa cell processes (Anderson and Albertini, 1976) as well as between granulosa cells of the follicle (Gilula et al., 1978). It has been shown that connexin 37 is expressed in these gap junctions, and mice lacking *CX37* were unable to produce mature follicles and developed false corpora lutea (Simon et al., 1997). Thus, proper function of *CX37* appears to be critical for oocyte maturation and ovulation. Previously, *CX37* was mapped to HSA1 p35.1 in humans (Camp et al., 1995). Comparative mapping aligns this region to SSC6 q22-26 and q31-35. Our results are in agreement with this location.

#### Literature Cited

- Anderson, E., and D. F. Albertini. 1976. Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. *J. Cell Biol.* 71:680-686.
- Archibald, A. L., and C. S. Haley, et al. 1995. The PiGMAP consortium linkage map of the pig (*Sus scrofa*). *Mamm. Genome* 6:157-175.
- Camp, G. V., P. Coucke, F. Speleman, N. Van Roy, E. C. Beyer, B. A. Oostra, and P. J. Willems. 1995. The gene for human gap junction protein connexin37 (GJA4) maps to chromosome 1p35.1, in the vicinity of D1S195. *Genomics* 30:402-403.
- Gilula, N. B., M. L. Epstein, and W. H. Beers. 1978. Cell-to-cell communication and ovulation. *J. Cell Biol.* 78:58-75.
- Green, P., K. Falls, and S. Crooks. 1990. Documentation for CRI-MAP, version 2.4. Washington Univ. School of Medicine, St. Louis, MO.
- Simon, A. M., D. A. Goodenough, E. Li, and D. L. Paul. 1997. Female infertility in mice lacking connexin 37. *Nature (Lond.)* 385:525-529.
- Yerle, M., G. Echard, A. Robic, A. Mairal, C. Dubut-Fontana, J. Riquet, P. Pinton, D. Milan, Y. Lahbib-Mansais, and J. Gellin. 1996. A somatic cell hybrid panel for pig regional gene mapping characterized by molecular cytogenetics. *Cytogenet. Cell Genet.* 73:194-202.

**Key Words:** Gap Junctions, Gene Mapping, Ovulation Rate, Pigs