GENOME METHODS

Xenoduplex Analysis—A Method for Comparative Gene Mapping Using Hybrid Panels

Lena Marklund, Jin-Tae Jeon, and Leif Andersson[1]

Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala Biomedical Centre, S-751 24 Uppsala, Sweden

Somatic cell hybrid (SCH) panels and radiation hybrid (RH) panels are powerful resources for comparative gene mapping because gene assignments are made without the detection of genetic polymorphism as needed for linkage mapping. A frequently encountered problem, however, is that the gene specific primers may amplify homologous PCR products of equal length from the donor and recipient species of the panel. Here, we describe a simple solution to this problem in which we utilize the formation of interspecies heteroduplexes that can be easily distinguished from the corresponding homoduplexes by native polyacrylamide gel electrophoresis. We denote these DNA–DNA interspecies hybrids, xenoduplexes (xeno = Gr. Xenos, foreigner). A merit of the method is that the formation of xenoduplexes strongly suggests that the PCR products from the two species represent homologous sequences. The method is thus particularly useful for comparative gene mapping when the PCR primers have been designed by use of sequence information from other species. In this study we have successfully used xenoduplex analysis and a pig-rodent SCH panel to map seven porcine genes (*ACADM, AT3, HOXD, IL8RB, LEPR, PAX8, PKLR*) for which no previous sequence information was available. The assignment of the leptin receptor gene (*LEPR*) to pig chromosome 6q32–35 excluded *LEPR* as a candidate gene for a QTL on pig chromosome 4 with a major effect on fatness.

A dense gene map of the human genome is currently being generated by mapping known genes and unknown expressed sequence tags (EST) by PCR screening of radiation hybrid (RH) panels and large insert contigs (Schuler et al. 1996); the PCR markers used for this purpose often correspond to the 3′-untranslated region. High-resolution comparative maps are needed to facilitate the exchange of mapping information among species and comparative positional candidate cloning (Johansson Moller et al. 1996). Comparative gene mapping will also shed light on the evolution of genome organization. Lyons et al. (1997) have recently proposed a strategy to systematically use available sequence information to generate comparative anchor-tagged sequences (CATS) that are likely to amplify the same coding sequence in a range of vertebrate species. In our experience, when testing the CATS primers in the pig, this approach is most successful for amplimers representing a single exon. However, those products are problematic because genetic polymorphism suitable for linkage analysis is difficult to find in short stretches of coding sequence and because somatic cell hybrid (SCH) mapping is often obstructed as the CATS primers are likely to amplify a homologous product of equal length from the rodent background. These comigrating PCR products may be resolved by digestion with restriction enzymes or by single strand conformation polymorphism (SSCP) analysis. However, these procedures are laborious, and complicated fragment patterns may be obtained. Here we describe a more convenient solution to the problem in which we allow xenoduplexes to form between homologous PCR products after denaturation and renaturation of double-stranded DNA.

We have assigned seven genes to the pig gene map by use of xenoduplex analysis (Table 1). The authenticity of all PCR products was confirmed by direct sequencing; only homeobox D (HOXD; Lahbib-Mansais et al. 1996) and leptin receptor gene (LEPR; Ernst et al. 1997) have been mapped previously in the pig. The xenoduplex results obtained for pyruvate kinase, liver, and red blood cells (*PKLR*), paired box homeoprotein 8 (*PAX8*), and *HOXD* are illustrated in Figures 1–3, respectively. A strong *PKLR* amplification product was obtained

[1]Corresponding author.
E-MAIL leif.andersson@bmc.uu.se; FAX 46-18-504461.

Table 1. Regional Localization of Seven Porcine Genes Using Xenoduplex Analysis and a Pig/Rodent SCH Panel

Gene	Product size (bp)	Map position		Error risk[a] (%)	Chromosome probabilities
		human	pig		
ACADM	188	1 p31	6 q31	5	1.00
LEPR	365	1 p31	6 q32–q35	5	1.00
PKLR	333	1 q21	4 q21–q23	<0.1	1.00
AT3	194	1 q23–q25.1	4 q15–q16	<1	0.99
PAX8	150	2 q12–q13	12 q11–q15	5	0.46
HOXD	157	2 q31	15 q23–q26	<0.5	1.00
IL8RB	445	2 q35	15 q23–q26	5	1.00

[a]The error risk provides the probability that the profile of positive and negative clones for the marker was generated by chance.

from both pig and rodent DNA and the products could not be distinguished by standard agarose electrophoresis. However, the two xenoduplexes ($+_{pig}/-_{rodent}$ and $+_{rodent}/-_{pig}$) were easily distinguished from the homoduplexes (Fig. 1) and the scoring of the presence of these xenoduplexes in the SCH panel firmly assigned *PKLR* to 4q21–q23.

The xenoduplexes formed between pig and rodent *PAX8* amplification products could not be reliably distinguished by standard PAGE (Fig. 2). We solved this problem by adding human amplification products that were slightly smaller (~20 bp) in size and the human:pig as well as the human:rodent xenoduplexes were easily separated from each other and all homoduplexes. Pig *PAX8* were assigned to 12q11–q15. Similarly, a human PCR product was utilized to facilitate the mapping of pig *HOXD* to 15q23–q26 (Fig. 3; Table 1). In this case, the human product was amplified with fluorescently labeled primers and mixed with unlabeled products, from pig, rodent, or SCH panel DNA, and separated with a DNA sequencing instrument. By this approach, only human homoduplexes as well as human-rodent and human-pig xenoduplexes were visualized. An advantage with xenoduplex analysis in this format is that nonspecific PCR products that cannot form stable xenoduplexes with the human product will not interfere with the scoring of correct products. The use of a DNA sequencing instrument should also facilitate high-throughput analysis as multiple products of different length may be loaded simultaneously and multiple loading on the same gel may be employed.

All seven chromosomal assignments, except

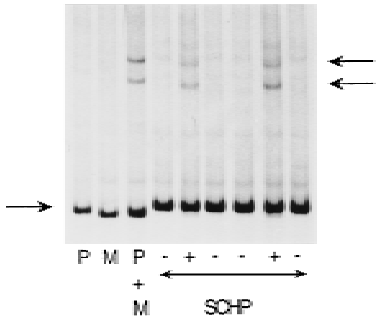

Figure 1 A silver-stained native polyacrylamide (8%) gel showing the separation of a 333-bp PCR product of the *PKLR* gene amplified from pig (P), mouse (M), and six clones from a pig/hamster SCH panel; P + M was mixed subsequent to the PCR amplification but prior to the denaturation/renaturation treatment. Homo- and xenoduplexes are marked by arrows to the *left* and *right,* respectively. SCH clones that were scored positive and negative for the pig *PKLR* gene are marked by + and −, respectively.

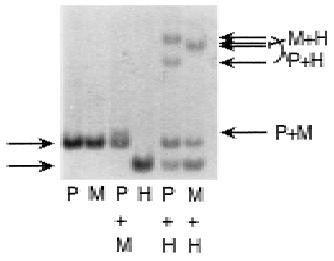

Figure 2 A silver-stained native polyacrylamide (8%) gel showing the separation of a 150-bp PCR product of the *PAX8* gene amplified from pig (P), mouse (M), and human (H) genomic DNA; P + M, P + H, and M + H were mixed subsequent to the PCR amplification but prior to the denaturation/renaturation treatment. Homo- and xeno-duplexes are marked by arrows to the *left* and *right,* respectively.

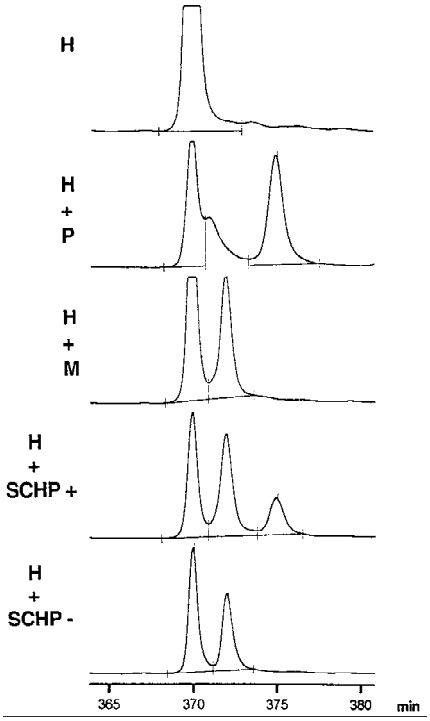

Figure 3 Electropherograph showing the separation of a 157-bp PCR fragment of the *HOXD* gene in a 8% native polyacrylamide gel by use of a DNA sequencing instrument (ALF Express, Pharmacia Biotech). *HOXD* was amplified from human (H), pig (P), mouse (M), one pig/mouse SCH clone containing the pig *HOXD* gene (SCHP+), and one clone negative for the pig *HOXD* gene (SCHP−). Only the human product was amplified by use of fluorescently labeled primers and this product was added to the other amplification products prior to the denaturation/renaturation treatment. As a consequence, only human homoduplexes and human/mouse and human/pig xenoduplexes were visualized.

PAX8, are consistent with the current pig comparative map (Rettenberger et al. 1995; Frönicke et al. 1996; Goureau et al. 1996). It is well documented that a part of human 1p shares homology with a part of pig 6q, whereas the next segment on human 1p–1q shares homology with a part of pig 4q (Table 1). *LEPR* was included in this study as a potential candidate gene for a quantitative trait locus (QTL) with a major effect on fatness located on pig chromosome 4 (Andersson et al. 1994). Previous data suggested that the border of conserved synteny between human chromosome 1 on the one hand and pig chromosomes 4 and 6 on the other is located close to human 1p31, and pig *LEPR* may thus reside on any of these chromosomes. The assignment of pig *LEPR* to 6q32–35 obviously excludes *LEPR* as a candidate gene for the fatness QTL on pig chromosome 4.

Human chromosome 2 shares homology with pig chromosomes 3 and 15. Thus, the assignments of *HOXD* and interleukin 8 receptor*B* (*IL8RB*) to 15q23–q26 were thus expected, whereas the assignment of *PAX8* to 12q11–12q15 was unexpected. The regions of conserved synteny between the human and pig genome have primarily been established by ZOO–FISH analysis in which human chromosome-specific libraries are used to paint pig metaphase spreads. It is known that the region of conserved synteny needs to be of a certain size (+5 Mbp) to give a detectable signal. Thus, available ZOO–FISH data do not exclude the possibility that a segment of human chromosome 2 is homologous to a small segment on pig 12q including *PAX8*. However, the assignment must be considered preliminary as the probability for the chromosomal assignment was quite low (Table 1). The reason for this low chromosome probability was that there was some clones that were supposed to contain the region 12q11–q15 which was negative for *PAX8*. This may be due to the classical problem with SCH mapping, namely, that SCH clones are heterogenous and the proportion of cells within each clone carrying a specific chromosome segment is variable. The consequence may be discordant results for markers that are more difficult to amplify. This problem may be particularly severe with CATS primers that amplify both the donor and recipient loci. A solution to the problem is to sequence the PCR product and design donor-specific PCR primers.

Xenoduplex analysis should be a useful method for mapping genes in all species for which extensive sequence information is not available, but for which PCR primers may be designed on the basis of information from related species. The majority of genes are sufficiently well conserved (at least among mammals) that stable xenoduplexes will form when PCR products from two species are denaturated and renaturated. Moreover, even highly conserved genes will show nucleotide substitutions between species that will be revealed by xenoduplex analysis; if the homoduplex and xenoduplex fragments tend to comigrate, PCR products from a third species may be added to resolve the problem as shown in this study. Both xenoduplex and SSCP analysis are expected to reveal species-specific banding patterns for most amplicons and the work load involved is similar for the two methods. However, an advantage with xenoduplex analysis is that the banding patterns are more simple and robust, facilitating high-throughput analysis. Another major advantage is

that the formation of stable xenoduplexes is a strong indication of true homology that resembles one of the classical criteria used in SCH mapping, namely, the formation of functional heteropolymers (Comparative genome organization 1996). This feature may also circumvent the need to sequence all PCR products; it may be sufficient to sequence some of the PCR products where the chromosomal assignments are consistent with known synteny relationships and all that are in disagreement. The PCR amplimers used for this purpose preferably should be intraexonic fragments and computer scripts may be developed to identify such regions sequenced in two or more species as described by Lyons et al. (1997). We believe that this method, combined with the use of RH panels will become a very powerful method for developing high-resolution ordered comparative maps in any mammalian species.

METHODS

Markers

CATS primers (Lyons et al. 1997) for the amplification of the following genes were employed: acyl-CoA dehydrogenase (*ACADM*), antithrombin III (*AT3*), *HOXD, PAX8, PKLR.* Primer pairs for the *LEPR* (exon 20), F-5′-TCTAATAGCTCATGGGAGATAG, R-5′-TGGAATTGAGGCATGTAAGATG, and for the *IL8RB,* F-5′-GATGTCTACCTGCTGAACCTAG, R-5′-CCGTAGCAGAACAGCATGAT, were designed on the basis of alignments of human and mouse sequences present in the GenBank database (accession nos. U59263, U42467, and U11869, L13239, respectively). Some of the forward primers were fluorescently labeled with 5′-Cy5 (Pharmacia Biotech) as a prerequisite for detection of the PCR fragments by use of the ALF Express instrument (Pharmacia Biotech).

PCR Amplification

PCR was performed in 10 µl containing 120 µM dNTP, 1–2 mM $MgCl_2$, 4 pmoles of each primer, 0.25 units of ampliTaq Gold (Perkin-Elmer) with a PE9600 (Perkin Elmer) or a PTC100 (M.J. Research) thermocycler. A touch-down program was used that included an initial denaturation step at 94°C for 5 min, followed by 45 cycles with denaturation at 94°C for 30 sec, annealing at 59–49°C for 30 sec (the first 10 cycles touch down 1°C per cycle), extension at 72°C for 45 sec, and a final extension at 72°C for 5 min. After column purification (Qiagen), the pig PCR products were sequenced directly (without cloning) by use of DyePrimer chemistry with an ABI377 instrument (Perkin Elmer). The sequences were tested for homology against sequences in the GenBank database by use of the BEAUTY program (Worley et al. 1995).

Xenoduplex Analyses

All products were first checked on agarose gels so that the amount of product used in the xenoduplex analysis could be equalized as much as possible. PCR products (0.5–5 µl representing individual samples or pooled samples) were covered with a drop of mineral oil, heated to 95°C for 10 min, and incubated at 37°C for 2 hr to allow the formation of homo- and xenoduplexes or, alternatively, at 95°C for 3 min followed by 55°C for 5 min and 15°C for 5 min in a PTC100 thermocycler (M.J. Research). Loading buffer (40% sucrose and 100 mg/ml Dextran blue for ALF Express analysis or 10% sucrose, 0.01% bromphenol blue, and 0.01% cyanol FF for Hoefer SE600 analysis) was added to the samples in a 3:1 ratio. The samples were electrophoresed in 8% native polyacrylamide gels (acrylamide/bisacrylamide; 37.5:1; Protogel, National Diagnostics) on a Hoefer SE600 unit (Pharmacia Biotech) with 0.75 mm × 16 cm × 18 cm gels for 600–1100 V · hr overnight at 20°C. DNA fragments were visualized by silver staining according to Marklund et al. (1995). Alernatively, fluorescently labeled products were separated on 8% native polyacrylamide gels with an ALF Express instrument (Pharmacia Biotech) at 300 V, 150 mA, 28 W, and 30°C for 680 min using 30-cm plates and 0.5-mm spacers. Lanes with xenoduplex fragments from the SCH panel were compared with lanes containing PCR products from individual species so that nonspecific products could be disregarded.

SCH Mapping

A pig/rodent SCH panel composed of 19 pig/chinese hamster and 8 pig/mouse hybrid clones was used (Yerle et al. 1996). The panel is informative for all 18 pig autosomes and the X chromosome, in most cases allowing a regional localization (Robic et al. 1996; Yerle et al. 1996). Twenty-five nanograms of DNA from each hybrid was used. Regional gene assignments were achieved through the concordant segregation of pig gene-specific PCR amplifications and chromosome fragments retained in the hybrid cells as described by Robic et al. (1996). The statistical evaluation of data followed Chevalet et al. (1997; see http://www.toulouse.inra.fr/lgc/lgc.htlm).

ACKNOWLEDGMENTS

We thank Drs. Leslie Lyons and Stephen J. O'Brien (National Cancer Institute, Fredrick, MD) for providing CATS primers and Maritha Mendel-Hartvig, and Elsy Johnsen (Department of Medical Genetics, Uppsala, Sweden) for their trustful help when using their ALF Express instrument. The study was supported by grants from the Swedish Research Council for Forestry and Agriculture.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked ''advertisement'' in accordance with 18 USC section 1734 solely to indicate this fact.

REFERENCES

Andersson, L., C.S. Haley, H. Ellegren, S.A. Knott, M. Johansson, K. Andersson, L. Andersson-Eklund, I. Edfors-Lilja, M. Fredholm I. Hansson, J. Håkansson, and K. Lundström. 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science* 263: 1771–1774.

Chevalet, C., J. Gouzy, and M. San Cristobal-Gaudy. 1997. Regional assignment of genetic markers using a somatic cell

hybrid panel: A WWW interactive program available for the pig genome. *Comput. Appl. Brosci.* 13: 69–73.

Comparative genome organization; First International Workshop. 1996. *Mamm. Genome* 7: 717–734.

Ernst, C.W., P.A. Kapke, M. Yerle, and M. Rotschild. 1997. The leptin receptor gene (*LEPR*) maps to porcine chromosome 6. *Mamm. Genome* 8: 226.

Frönicke, L., B.P. Chowdhary, H. Scherthan, and I. Gustavsson. 1996. A comparative map of the porcine and human genomes demonstrates ZOO-FISH and gene mapping-based chromosomal homologies. *Mamm. Genome* 7: 285–290.

Goureau, A., M. Yerle, A. Schmitz, J. Riquet, D. Milan, P. Pinton, G. Frelat, and J. Gellin. 1996. Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. *Genomics* 36: 252–262.

Johansson Moller, M., R. Chaudhary, E. Hellmén, B. Höyheim, B. Chowdhary, and L. Andersson. 1996. Pigs with the dominant white coat color phenotype carry a duplication of the *KIT* gene encoding the mast/stem cell growth factor receptor. *Mamm. Genome* 7: 822–830.

Lahbib-Mansais, Y., M. Yerle, P. Pinton, and J. Gellin. 1996. Chromosomal localization of homeobox genes and associated markers on porcine chromosomes 3,5,12,15,16 and 18: comparative mapping study with human and mouse. *Mamm. Genome* 7: 174–179.

Lyons, L.A., T.F. Laughlin, N.G. Copeland, N.A. Jenkins, J.E. Womack, and S.J. O'Brien. 1997. Comparative anchor tagged sequences (CATS) for integrative mapping of mammalian genomes. *Nature Genet.* 15: 47–56.

Marklund, S., R. Chaudhary, L. Marklund, K. Sandberg, and L. Andersson. 1995. Extensive mtDNA diversity in horses revealed by PCR-SSCP analysis. *Anim. Genet.* 26: 193–196.

Rettenberger, G., C. Klett, U. Zechner, J. Kunz, W. Vogel, and H. Hameister. 1995. Visualization of the conservation of synteny between humans and pigs by heterologous chromosomal painting. *Genetics* 26: 372–378.

Robic, A., J. Riquet, M. Yerle, D. Milan, Y. Lahbib-Mansais, C. Dubut-Fontana, and J. Gellin. 1996. Porcine linkage and cytogenetic maps integrated by regional mapping of 100 microsatellites on somatic cell hybrid panel. *Mamm. Genome* 7: 438–445.

Schuler, G.D., M.S. Boguski, E.A. Stewart, L.D. Stein, G. Gyapay, K. Rice, R.E. White, P. Rodriguez-Tomé, A. Aggarwal, E. Bajorek et al. 1996. A map of the human genome. *Science* 274: 540–546.

Worley, K.C., B.A. Wiese, and R.F. Smith. 1995. BEAUTY: An enhanced BLAST-based search tool that integrates multiple biological information resources into sequence similarity search results. *Genome Res.* 5: 173–184.

Yerle, M., G. Echard, A. Robic, A. Mairal, C. Dubut-Fontana, J. Riguet, 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.

Received November 20, 1997; accepted in revised form February 18, 1998.

Xenoduplex Analysis—A Method for Comparative Gene Mapping Using Hybrid Panels

Lena Marklund, Jin-Tae Jeon and Leif Andersson

Genome Res. 1998 8: 399-403
Access the most recent version at doi:10.1101/gr.8.4.399