University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Faculty Papers and Publications in Animal
Science

Animal Science Department

April 2005

Physical mapping of genes in the porcine ovarian transcriptome

A. R. Caetano

University of Nebraska-Lincoln

J. B. Edeal

University of Nebraska-Lincoln

K. Burns

University of Nebraska-Lincoln

R. K. Johnson

University of Nebraska-Lincoln, rjohnson5@unl.edu

C. K. Tuggle

Iowa State University, IA,

See next page for additional authors

Follow this and additional works at: <https://digitalcommons.unl.edu/animalscifacpub>

Part of the [Animal Sciences Commons](#)

Caetano, A. R.; Edeal, J. B.; Burns, K.; Johnson, R. K.; Tuggle, C. K.; and Pomp, Daniel, "Physical mapping of genes in the porcine ovarian transcriptome" (2005). *Faculty Papers and Publications in Animal Science*. 74.

<https://digitalcommons.unl.edu/animalscifacpub/74>

This Article is brought to you for free and open access by the Animal Science Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Papers and Publications in Animal Science by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

A. R. Caetano, J. B. Edeal, K. Burns, R. K. Johnson, C. K. Tuggle, and Daniel Pomp

Physical mapping of genes in the porcine ovarian transcriptome

A. R. Caetano*[§], J. B. Edeal*, K. Burns*, R. K. Johnson*, C. K. Tuggle[†], and D. Pomp*[‡]

* Department of Animal Science, University of Nebraska–Lincoln, Lincoln, NE, USA.

[†] Department of Animal Science, Iowa State University, Ames, IA, USA

[§] Present address: A. R. Caetano, Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Final Av. W/5 Norte, Brasília-DF, CP 02372, 70770-900, Brasil.

[‡] Corresponding author; Email: dpomp@unl.edu

Abstract: Reproductive efficiency and associated traits are of major economic importance to the swine industry and have been more difficult to improve genetically than other production traits. Integration of phenotypic data with gene mapping and expression studies provides a powerful approach for dissection of the genetic basis regulating complex traits. We developed a total of 101 polymerase chain reaction-based markers, representing 91 unique genes, for expressed sequence tags previously reported to be putatively differentially expressed in the porcine ovarian transcriptome of a swine line selected on an index of high ovulation rate and embryonic survival. These were subsequently used in physical mapping experiments with a porcine radiation hybrid and somatic cell hybrid panels. Our results increased the information content of the porcine physical map useful for comparative mapping by *c.* 10%. Moreover, the mapped genes are likely to be biologically relevant to the molecular mechanisms that control ovulation rate in the pig. A total of 12 differentially expressed genes were mapped to regions previously reported to contain quantitative trait loci affecting swine ovulation rate.

Keywords: expressed sequence tags, expression profiling, mapping, ovarian follicle, quantitative trait loci, radiation hybrid, swine.

Introduction

Reproduction is of central importance to the pork industry, and significant resources have been devoted to understand biological phenomena that could lead to further genetic improvement of reproductive efficiency in commercial swine lines. Although map-based approaches have been shown to be powerful at identifying genes that affect production traits in livestock (e.g. Fujii *et al.* 1991; McPherron and Lee 1997; Milan *et al.* 2000; Page *et al.* 2002; Grisart *et al.* 2004), quantitative trait loci (QTL) mapping methodology has not been very fruitful in the studies of reproductive traits, providing inconsistent results with low resolution, that have thus far not allowed for identification of the underlying genes.

Integrated approaches, merging mapping information with gene-expression data, have been proposed as a viable alternative to dissect the molecular basis of complex traits (Wayne and McIntyre 2002; Schadt *et al.* 2003; Pomp *et al.* 2004).

Such approaches require high-density maps, saturated with known genes and expressed sequence tags (ESTs), as well as reagents for high throughput gene expression analysis. In the pig, several studies have generated and/or used genomic tools to study the molecular basis of economically important traits (e.g. Rohrer *et al.* 1996; Fahrenkrug *et al.* 2002; Rink *et al.* 2002; Caetano *et al.* 2003, 2004; Tuggle *et al.* 2003, reviewed in Rothschild 2003). Although specific large-scale resources have been created to study swine reproductive traits (see Pomp *et al.* 2001; Tuggle *et al.* 2003; Rohrer 2004), these still require further development. In support of this, the information content of the swine radiation hybrid (RH) map has been significantly increased by the recent addition of a large number of ESTs (Rink *et al.* 2002; Tuggle *et al.* 2003), as well as large-scale comparative flanking sequence annotation of microsatellite loci (Robic *et al.* 2003).

Caetano *et al.* (2004) conducted an expression-profiling study to identify genes that are differentially expressed in ova-

ries and ovarian follicles of sows from swine lines selected for an index of high ovulation rate and embryo survival (Johnson *et al.* 1999), during the follicular phase of the estrous cycle. We now report on the physical mapping of 91 of the genes identified as differentially expressed in that study.

Materials and methods

Source of EST sequences

Caetano *et al.* (2003) generated 3479 unique ESTs by sequencing clones from a normalized ovarian follicle cDNA library. These clones, in addition to a few other ovarian follicle-derived ESTs, were used to build a 9216 feature cDNA microarray that was subsequently used in expression profiling experiments to compare mRNA levels from ovarian follicles and from ovaries between a swine line selected for enhanced reproduction and its randomly selected control line (Caetano *et al.* 2004). Using very strict statistical analyses, 131 probes representing 106 unique genes were found to be significantly differentially expressed (Caetano *et al.* 2004). Sequences of ESTs for these genes (Caetano *et al.* 2003) were used to design primers for RH and somatic cell hybrid (SCH) mapping.

PCR primer design and optimization of amplification

Design of polymerase chain reaction (PCR) primers was as previously described (Tuggle *et al.* 2003). Briefly, primers were designed with Primer3 (http://0-www-genome.wi.mit.edu/library.unl.edu:80/cgi-bin/primer/primer3_www.cgi) with some changes in the default parameters (primer size of 25 bp, melting temperature of 65 °C, and maximum difference between T_m of the left and right primers of 5 °C). Each primer set (Table 1) was tested against mouse, hamster and pig DNA in 10- μ l reactions containing 25 ng of genomic DNA, 1.5 mM MgCl₂, 50 μ M of each dNTP, 2 μ M each primer, 1 U *Taq* polymerase, 1X reaction buffer (Promega, Madison, WI, USA), and 1X Rediload (Invitrogen, Carlsbad, CA, USA). Cycling conditions used with each primer set are described in Table 2. Amplification products were visualized after electrophoresis in ethidium bromide-stained 4% high:low (3:1) melting agarose gels. Primers that did not amplify a pig-specific fragment were redesigned and retested at least once.

SCH panel and RH panel analysis

Primers that generated pig-specific PCR products were used to type the INRA-University of Minnesota porcine radiation hybrid panel (IMpRH) panel (Yerle *et al.* 1998) using optimized conditions (Table 2). Data were initially evaluated using the IMpRH database (<http://0-imprh.toulouse.inra.fr/library.unl.edu:80/>) to determine map positions. Those ESTs with LOD scores ≥ 6.0 were submitted to the IMpRH

database and those with scores < 6.0 were rescored and results were resubmitted. Primers for all ESTs with final LOD scores < 6.0 were subsequently used to type the INRA swine SCH panel (SCHP) (Yerle *et al.* 1996) with the same optimized PCR conditions. Data were submitted online (<http://0-www.toulouse.inra.fr/library.unl.edu/lgc/pig/hybrid.htm>) and regional assignments were obtained using the INRA database (<http://0-www.toulouse.inra.fr/library.unl.edu/lgc/pig/pcr/pcr.htm>).

Results

As expected, the mapped genes are distributed across all pig chromosomes. Results for six of the ESTs mapped with the RH and SCHPs were discordant. Estimated positions of human orthologues, based on the UCSC Genome Browser (<http://genome.ucsc.edu/>; assembly of July 2003), are listed in Table 2, when a link was available in the TIGR Pig Gene Index [SsGI (v8.0); Quackenbush *et al.* 2000] for the tentative consensus sequence containing the corresponding EST. The expected position of human orthologues was not available for 17 of the mapped ESTs. Predicted positions of human orthologues, based on the human-pig chromosomal painting data (Goureau *et al.* 1996) and results of single locus physical mapping (<http://0-www.toulouse.inra.fr/library.unl.edu/lgc/pig/cyto/cyto.htm>), are provided in Table 2. The expected cytogenetic positions of the human orthologues were in agreement with 85% of the mapped ESTs, based on comparisons of both of these databases. The human physical position of three additional ESTs was in agreement when we considered the expected human cytogenetic position based on the pig SCHP results.

Discussion

Development of RH mapping panels has proven to be an extremely useful tool for rapidly constructing high-density physical maps of mammalian genomes. This method is more amenable to high throughput mapping relative to efforts based on linkage, especially for species with limited SNP resources. The latest version of the published porcine RH map was based on 1058 EST-derived markers (Rink *et al.* 2002). The current effort resulted in addition of 101 ESTs, representing 91 unique genes, to the porcine RH map, improving its density and coverage, and increasing the information content useful for comparative mapping. Moreover, the ESTs mapped in this study represent genes found to be putatively differentially expressed in ovaries and ovarian follicles of pigs selected for enhanced female reproduction (Caetano *et al.* 2004), and are thus likely to play important roles in the biological processes that control ovulation rate in swine.

The systematic approach adopted to design primers and optimize PCR conditions was successful. Less than a quarter of the primer sets designed failed to produce amplicons useful

Table 1 Primer pairs for mapping swine expressed sequence tags.

Genbank accession no.	BLAST hit ¹	Forward primer (5' → 3')	Reverse primer (5' → 3')
AW507066	Cytochrome P450 side chain cleavage (CP450SSC)	GATGTGGCCCTTCTCTTTAAGTTCG	TGGAACATCTGGTAGACAGCATCAA
AW507072	Cytochrome c oxidase subunit 3	ATAGTAAACCCAAGCCCATGACCAC	TGATGGGCTCAGGTAATGGATACTC
AW574381	Cytochrome B	TTTATCTGCCATTTCATCATTACCG	ATTATGCCTCGTTGTTGGATGTGT
AW574403	Cytochrome B and tRNA-Thr and tRNA-Pro	TGGCCCTCCTTTTCTGGTTT	ACCCGTAGAACCACCGTTCA
AW584050	NADH dehydrogenase subunit 4, mRNA sequence	ATGGGGGTAGGGCTAGGTTT	GAAGTTTCATAGGGGCCACA
AW584057	Ferritin	TGGCTTTGAAGAAGCTTTGCCAATA	GCTTAGGGCTTAGCTCTCACTGCTC
BI180977	Pro-1(I) collagen chain (Col1A1)	CTTCCCAGAACATCACCTACCACTG	CCAAAGTCCATGTGAAATTGTCTCC
BI180991	Actin-related protein 3, yeast (ARP3)	ATCCAAGATTGTATGGGATTTATCG	ACAGAACTGCAATACTGGTCCAACA
BI181040	Human chromosome 1q21 BAC 236c22	TTTCTTAGGCCATTCCAGTGCATAG	GTCAGGCTAGGGCAGAACGTTTAGT
BI181041	Low density lipoprotein-related protein 1 (LRP1)	AATGGGAAGCGACTGGACAA	GCTCACACTTGTGCGCTGTG
BI181067	Alpha-2-macroglobulin (A2M) i	ACGGCTTCCGGTGTAAGTGA	TCCCATTGTCTTTGGAGGTG
BI181144	Pro-1(I) collagen chain (Col1A1)	CAAAGTCTTCTGCAACATGGAGACA	TCGTAGATCACGCTGTAGGTGAAGC
BI181194	60S ribosomal protein L4	CTGAAAGTGCTTTCCGCAAGTTAGA	CTTCTCTACCACGGGCTTCTTGCT
BI181228	Selenium-binding protein 1 (SELENBP1)	ACTTGGTCGGTAGAGAAGGTGATCC	ATGAGATCGGGGTAAAAGTCTTGT
BI181240	Heat shock protein, DNAJ-like 2 (HSJ2)	GCAGCAAATAAAATGCCAGCAGTAA	CTTGACAACCGAACCATAGTCATCA
BI181249	Sus scrofa microsatellite, locus SO658	ACCCGAAGTCCAACTAGGTCAACTC	GAGGTTTGAACACCAGCAATGTCTT
BI181263	Cytochrome P450 17- α -hydroxylase (CYP17)	AAGTACCCAGGAGTCTCCAGTC	GTAGTCTTGGAAACCAGACGAAAGG
BI181277	Cathepsin L	TGGAAGGATTTCATGGCTTGC	TGCCAACGACACTGGTTACG
BI181293	No match	TTGAAGAAAATGCTTAGCTGCGAAA	GTGAGAAGTGTAATACCCCTTGG
BI181366	Cytosolic dihydrodiol dehydrogenase 3	TTTTGACTTTGAATTCGCTCCAGA	TGCACCTTCTGTGTCTAATGGGACT
BI181384	Homo sapiens cDNA FLJ13813	CCACTGTGGATGTTGACCCTAAGTC	GGTGGATGACCTAAAACAGGAGAGG
BI181387	Tissue inhibitor of metalloproteinase 3 (TIMP3)	GGCCGCTCTGTATTTTCATTCATATC	TTAGGAGAAATCATGGCTTCCAGT
BI181421	3- β -hydroxysteroid dehydrogenase (3BHSD)	GGAGCTGCGTGAAGGGATACATACT	TCTGCTTCTCGCCACCAGAAC
BI181431	Sus scrofa scavenger receptor (SCARB1)	CCAGCCCCTGTAGAGATTTAGAGA	TGGGGAGAGTCTTGACAACAAAGAG
BI181480	Plasminogen activator inhibitor-1	AACATTTTTCCTGAAGGCGATGGT	GGGTTGAAGGGAGACAGAGTTGTTT
BI181579	Ubiquitin carboxyl-terminal esterase L1 (UCHL1)	TTGCGAAGATGCAGCTCAA	ACCGAGCCCAAAGACTCCTC
BI181663	Protective protein for β -galactosidase (PPGB)	ACTCTGGCCGTGTTGGTCAT	AGAGCCTGTTCCCCAGAAGG
BI181686	Connective tissue growth factor	GCTATTGGGTGTCATTGAGAAAGGA	TGGTGTTCAGAAATGGAGGCTAACAT
BI181696	Regulator of G-protein signalling 2, 24 kDa (RGS2)	GAAAGCCAGGAGCAGTCTAGGAGAAG	ACTCATCCTGTGACACACAGCAACT
BI181761	Collagen, type I, alpha-1 (COL1A1)	CTGAGCCAGCAGATCGAGAACAT	GGGAAGCCTCAGTGGACATCAG
BI181785	Homo sapiens clone 23785 mRNA	AACATTTCATCTCCAAGAACTGCTTCA	CCAGAGAAAACAAAATGGCAAACAGT
BI181787	Calpain I light subunit	GGGCTAAAGTCCAGGTTAGAGCAGA	GGGAGGACTCTGTTTCAAGGAAT
BI181791	Homo sapiens hypothetical protein MGC11256	GTGTCTGCACACAGGCATCC	GTCCCGAGGGGTGTAAGGAG
BI181803	Cytochrome P450 side chain cleavage	ATTTATTGCCTTCATGGGAGTGGAT	GAGCTCGAGATGACTCTCTTCTCA
BI181842	Phospholipid transfer protein (PLTP)	GAGCAAGAACTGGAGACCATTACCA	CGAGGTGATGAAAGTGGACAGGAAT
BI181891	Serpentine receptor (CYT28)	CTGATGACCACGAGGGACTCTGTAG	CCCAGAACGTCTACACTAAGCCTGA
BI182004	Retinoic acid receptor responder 1	ACTAGTCCGATTCGTTTCTGCTGTG	CCACTGTCAAGAGCTACAGACACCA
BI182007	Putative lysophosphatidic acid acyltransferase	CGAGAGCTCTCTGATACGACAGT	ATTCAAGGAGGAGACGCGGAAG
BI182045	Tissue inhibitor of metalloproteinase 3 (TIMP3)	GACAGGGTTTTCTCTGGTTGGTTTC	CCCTAGGAGACAAGACTCTCCCTGA
BI182047	No match	CTGATCAGAAAATCACCCCACTC	GCTGCCCTGAAGTTACAGAAGGTG
BI182064	A kinase (PRKA) anchor protein 2 (AKAP2)	AGTGGTGTCTGACTGCAGTTGATTG	CTGCTGCCTCTTCAAAACGTTAAA
BI182164	Human cDNA FLJ11658 fis	GCTCACTCTCTGTCCAGAACCTTA	TGTGATGGCCTGTGACTCTCTATT
BI182214	Cathepsin U (CTSU)	AACTGCTGTGAAGAATCAGGGTCAC	GGATTTCATGGCTTGCATCTATACCA
BI182232	Heat shock 10 kDa protein 1 (HSPE1)	GAAAGAAGTGCAGCTGAAACGGTAA	CTGGATGTCATCAGTTTGGATATCATT
BI182250	Annexin A2 (ANXA2)	GACACTAAGGCCGCTCTCCA	GTGGTGGGATGACTGAAGC
BI182336	Cytochrome P450 17- α -hydroxylase (CYP17)	AGTCAGGAGTACTCCCTCAGTGT	CATTGGCGAATTTACCATTGACAAG
BI182401	No match	TTACCGCTCTCTCCCTTC	TGATGTGGGGTCCGGTTTAG
BI182422	Pilin-like transcription factor	TTCAAACCAAGGAAACACTGACCAT	CCTCTTCTCGTTTGACCTCTCACCT
BI182428	Homo sapiens cDNA FLJ22155	GTTTCCGCTTCTGGTCTTGATAA	TTTCCATCTCTAGAAAATGGCATGG
BI182474	Cysteine dioxygenase, type I (CDO1)	GGGCTCTACTGGTTTCACCTCTAA	TTTGGTTTGTGTGGGTTTGATAC
BI182487	Ribosomal protein S4, X-linked (RPS4X)	TTACACCTGAGGAGGCCAAGTACAA	CTCTGTCTCTCTCTCAGCAATGG
BI182556	Thymosin, beta-4, X-chromosome (TMSB4X)	CTTCTCTCTTACCAACATGCAA	AACCATGTCTGACAAACCCGATATG
BI182567	NDR1-related protein NDR2 (NDR2)	AAGCTGATAGGGCTTCTCATGAACC	TTACAGCAGACACTGTGGGAGACAG
BI182727	Porcine endogenous retrovirus PERV-MSL	GAGTGTGGAGTCGGGACAGC	TCAAATTGGTTGCGCCATAA

Table 1 Continued.

Genbank accession no.	BLAST hit ¹	Forward primer (5' → 3')	Reverse primer (5' → 3')
BI182751	Ras homologue gene family, member B (ARHB)	TCAGGGAACCTCGTCTTACTGAACA	CTAGGCCCGGTCTGCTACTCTAGTC
BI182872	Calpain I light subunit	TAGGTACACGGGGTCAGTCTAATGC	CAATTGGTCTGCTCTAACGTGGACT
BI182881	Growth factor receptor-bound protein 14 (GRB14)	TGAAGAGGCACTGGGGCTAC	AATCCCACTGAGGCCCTTTC
BI183145	H2A histone family, member Z (H2AFZ)	TCCACCGAAGTGGAAATAATGTCTG	CATCCACAAATCTCTCATTGGGAAG
BI183240	Sus scrofa Ig gamma 2a (IGG2A)	AGACTTCTGGGTGTAGTGGTTGTGC	AAGGAGTTCAAGTCAAGGTCAACA
BI183427	CD83 antigen	AGACTGTTGGCAACTACAAGCAAGC	ATAAGGAAACACAACAGCTGCCAAA
BI183514	Tropomyosin 2 (beta) (TPM2)	GTCTGGTGAATCTCCACGTTCTCCT	TGTACTGATGGCATGTGTCTGTTC
BI183574	Interferon-stimulated protein (ISG15)	GGTGCACATAGGCTTGAGGTCATAC	TAGGGAAGTGAAGGTGAAGATGCTG
BI183661	Homo sapiens RGC32 protein (RGC32)	TGCTACGTTTTAAGAGAAAATACCA	AAAAATGCCTTTGGGCTCCT
BI183694	Homo sapiens BORG2 protein (BORG2)	TCATGTTGCCCTTATTGTACCAGT	TTTATCCATGACATTACGACCTCA
BI183769	Apolipoprotein E receptor 2 (ApoER2)	CAAAGCAGTAAACCAATCCCAGTG	TTAGGACCCCTGGGTGAAGTCATT
BI183910	BTG family, member 2 (BTG2)	GCACTGACAGAGCACTACAAACACC	ACGGCAGGCAGAATGAGTACAGTAG
BI183974	Tissue inhibitor of metalloproteinase-3 (TIMP3)	TGAAGTTTCCTTGCTGAAAAGTTGG	CTGGCTGCCTGCACAGTAGAAGTAT
BI184102	Heat shock 70 kDa protein 8 (HSPA8)	TGTGGATAAGAGCACAGGAAAGGAG	CCTGCACTCTGGTAGAGCTTGGTAA
BI184141	TNF, alpha-induced protein 1 (TNFAIP1)	GTGGTTAGCAGGCTTCTGGTTTTCT	CTCCCCGTTGAGGTATTTTAATGCT
BI184356	Complement component 1, q, alpha (C1QA)	CTTAAAGGAGACCAGGGGAGTCTG	AAGAGTCCCTTGCTGTTGAAGTCAC
BI184390	Integrin-linked kinase (ILK)	CCTACTTGTCTGCATCTTCTCCAG	ACTCAACAGTCGCAGTGTCTATGATT
BI184392	No match	CCTGGCTCTCCTCAGGGTTT	AACCCCAAGCATCCAAGAAT
BI184415	Cytolytic trigger molecule G7	TCCATGTGACTTTGCCATTCC	TTCTGCCTGGTGATGGGATT
BI184594	Collagenase inhibitor	TTTCACATCTTTGGAGAAAGACAGGT	ATGGGAGGGAGCTGTTCTTGAGTCAC
BI184598	Dihydrodiol dehydrogenase-2 (DD2)	ATTCCACCTGGTTGCAGACG	GGCGGCTCTGGAGAAGTGTA
BI184629	Homo sapiens mitogen-inducible gene 6 (MIG-6)	GCTCAAATAACATCACTCAGCAGTGTG	CCTCTTCAGTATTTCTGGGGATTG
BI184678	Homo sapiens cDNA FLJ22353	GCTGGAGCTGCTCTTTGATTATGAG	CTCATCGGCCCTTGAGTAGAGGTAG
BI184717	Homo sapiens syndecan 4 (SDC4)	ATGGCTGGAGGGAAGGGACAT	ACACTAACCACATTTCAAGGGCTCA
BI184862	Lysosomal associated protein transmembrane 4-beta (LAPTM4B)	GCTGTGGCTTTGAACACAGG	CTCTCTGCAAGGCACGTCT
BI184876	Cytochrome P450 aromatase (CYP19)	AGTGGCTGAGGCATAAATTGAGAGA	TCCACAAAACGTGCTTTGAGAAAAA
BI184877	Glutathione S-transferase (GSTP)	AAGCAACCGTTATCTTCTGCATT	AATAGTTTCTTGGTCCCAACACC
BI185002	GTP-binding protein RAB22A	AGAGCTCCTCTGCCTTCCCTTG	CACCAACTTCACTTCAGCGCTTAT
BI185003	H.sapiens (TL27) mRNA from (PC3) cell line	GCCTGTTCTTTTTAGATCCCGGATA	ACAGGGACAAATATCACATCCCAAT
BI185028	H1 histone family, member 4 (H1F4)	CCGCTGTACTTTTTGCTTTTCTG	CTTCTTAGCTTTGGGCTTGGCTTC
BI185061	Homo sapiens cDNA FLJ23553	AAAAGAATCAAGAAGTGAAGCTGGG	TGGAGGTACTTTATTGGGGATAGGA
BI185105	MHC class I antigen PD1 (SLA1)	AGGCTGCAGGCAGTGACAGT	TGCTGTGTCAGAAACAGGAG
BI185137	Serine carboxypeptidase 1 precursor (HSCP1)	GGTGTTTCAGGACATAGGGATGAGTG	CCTCTGTGGCAACTTGGAAATCAT
BI185217	Sus scrofa CC chemokine receptor genes	CCCCAGTCTGTTTTATTTGGCAGT	TTGGGGCATGTCATTGGTAATGTAT
BI185259	No match	ATGGAGTCCCAGAAAGCGTAACTGT	AGCAAGGATTGTATCCTGCCTAGAG
BI185343	ATP synthase gamma-subunit (L-type)	ACAGCCTGGCCAAACATCATC	GGCACCAGGAGATGATTTCAA
BI185380	Selenium binding protein 1 (SELENBP1)	TGAGGTTTTGACATTGTTAGCAGCA	CTCCATGTTCTGAGCCATCCTTC
BI185480	Porcine endogenous retrovirus PERV	GCAAACAGCAAGAGGATTTTATTTC	GGCCTGTTTACGTCTTTACCTA
BI185578	No match	CCTCATACCAAGTGCCGGAATTTAT	ATGATCATCAAAGTGGACCCTTGAA
BI185839	Fibronectin (FN precursor)	TATTGCGGGCCAGACAGTTAAGTAG	TCCCGAGAGTAAATTTCCAAGCTG
BI185976	Sus scrofa CC chemokine receptor genes	CACAGCTGTAGCAACACCAGATCTTT	CCTGGTGAAGTCAAGGAGGAATATG
BI186028	Peroxisome oxidoreductin 3 (PRDX3)	ACATTGAGCACGTCAAGAAAGAAGA	CTTCTGATCAGGGGCTCTGAAGATT
BI186221	Protein p8 (candidate of metastasis 1)	AGCCTCAGTCACGTGGGGTA	GAGACAGGACTGCGGAGGAA
BI186223	Human chromosome 1q21 BAC 236c22	CACCCTCCCTCCCGTTATTC	CTCTCTGCAAGGCACGTCT
BI186431	Calpain I light subunit	CTTGGCCTGAATGCACGAA	GTGTACAGGGGTGGCATCTTC
M36513	Follistatin	GTCTGTGGGCTGGATGGGAAA	ATCAAGGAAATCATGAATGGAAGCA
U53020	Steroidogenic acute regulatory protein (STAR)	CAGACCCAGGTGGACTTTGC	CGACACACACCACTGCAACA

for RH and/or SCHP mapping, primarily because of amplification of PCR fragments of similar size from pig and mouse/hamster, and secondarily because of the presence of large introns in the swine genomic sequences.

The UCSC Human Genome Browser (<http://genome.ucsc.edu/>) represents a useful tool for predicting the physical position of human orthologues to swine expressed sequences. Our empirical mapping results agreed in 85% of the cases

Table 2 PCR conditions for generating pig-specific amplicons, mapping results and comparative mapping with the human genome.

GenBank accession no. ¹	PCR programme ²	Fragment size (bp) ³	SCHP ⁴		RH ⁵			Reported linkage group position (SSC) (cM) ⁶	Reported cytogenic position (SSC) ⁷	Position of human orthologue (HSA) ⁸	Expected cytogenic position (HSA) ⁹
			Map location	R	Closest marker	LOD	SSC				
BI181686	OPT65	382	–	–	SW1417	9.25	1	(1) 44.1	1p2.2–2.3	6q23.2	6q
BI184876	OPT65	378	–	–	SWR702	8.92	1	(1) 67.6	1q1.6–1.7	15q21.2	15q22
BI183514	OPT65	210	–	–	SW2551	13.16	1	(1) 95.8	1q2.8	–	9q22–31
BI182064	OPT65	410	–	–	SW1311	7.72	1	(1) 100.8	1q2.9–2.11	9q31.3	9q22–31
AW584057	OPT65	416	–	–	SWR783	6.52	2	(2) 23.7	2p1.6–1.7	11q12.3	11
BI181791	OPT62	192	5p11–15	1.00	SW747	3.62	2	(2) 60.6	2q2.1	22q13.33	5q/19p (22q)
AW584050	OPT60	199	–	–	S0010	14.83	2	(2) 77.9	2q2.1–2.2	2q33.1	5q/19p
BI182474	OPT65	376	–	–	SW1695	13.16	2	(2) 80.6	2q2.1–2.8	5q22.3	5q/19p
BI181480	OPT65	381	–	–	S0335	6.40	3	(3) 12.4	3p1.7	7q22.1	7p15–q22
BI185976	OPT65	307	–	–	SW833	6.63	3	(3) 17.3	3p1.7	–	16p
BI186221	OPT65	100	–	–	SW1443	6.25	3	(3) 40.1	3p1.4	16p12.1	16p
BI183694	OPT65	401	3q21–27	0.83	IL1B	4.30	3	(3) 53.7	3q1.1–2.4	2p22.2	2p–q21
BI182751	OPT65	399	–	–	S0002	8.75	3	(3) 102.2	3q2.5–2.6	2p24.1	2p23–24
BI181040	OPT65	387	–	–	SWR362	10.32	4	(4) 55.9	4q1.1–1.2	8q22.1	8q
BI186223	OPT65	102	–	–	SWR362	8.44	4	(4) 55.9	4q1.1–1.2	8q22.1	8q
BI184392	OPT65	137	–	–	SWR362	9.45	4	(4) 55.9	4q1.1–1.2	8q22.1	8q
BI184862	OPT65	182	–	–	SWR362	8.76	4	(4) 55.9	4q1.1–1.2	8q22.1	8q
BI184415	OPT62	112	–	–	SW589	19.48	4	(4) 74.4	4q1.6–2.1	–	1p21–q25
AW574381	OPT65	401	1p21/ q23–27	0.74	SW1996	3.71	4	(4) 77.0	4q1.6–2.1	5p15.31	1p21–q25 (7q/9/14q/18)
BI181228	OPT65	403	–	–	SW512	14.49	4	(4) 80.5	4q1.6–2.1	1q21.3	1p21–q25
BI185380	OPT65	394	–	–	SW512	15.10	4	(4) 80.5	4q1.6–2.1	1q21.3	1p21–q25
BI181384	OPT65	330	–	–	SW512	10.26	4	(4) 80.5	4q1.6–2.1	–	1p21–q25
BI181041	OPT65	181	–	–	DK	6.15	5	(5) 72.3	5p1.1–1.2	12q13.3	12/22q
BI182045	OPT65	370	5p11–15	1.00	DK	4.64	5	(5) 72.3	5p1.1–1.2	22q12.3	12/22q
BI183974	TD1	389	–	–	DK	6.51	5	(5) 72.3	5p1.1–1.2	22q12.3	12/22q
BI182727	OPT62	161	–	–	SW1134	9.87	5	(5) 80.8	5p1.1–1.2	–	12/22q
BI181067	OPT60	101	–	–	SW963	9.45	5	(5) 95.5	5q1.2	12p13.31	12
BI181785	OPT65	207	–	–	SW1383	12.11	5	(5) 125.0	5q2.3	12q22	12
BI181891	OPT65	378	–	–	SW1057	13.76	6	(6) 47.1	6p1.4–1.5	16q13	16q
BI184629	OPT65	306	–	–	SW1355	6.01	6	(6) 83.3	6q2.2–2.3	1p36.23	1p22–36
BI184356	OPT65	397	–	–	SW709	17.39	6	(6) 89.3	6q2.5–2.6	1p36.12	1p22–36
BI183769	OPT65	416	–	–	SW322	21.13	6	(6) 149.8	6q3.1–3.5	1p32.3	1p22–36
BI185578	OPT65	398	6q32–35	0.75	SW1069	3.26	6	(6) 155.2	6q3.5	–	1p22–36
BI184678	OPT65	388	–	–	SW2466	11.25	6	(6) 164.6	6q3.5	–	1p22–36
BI183427	OPT65	406	–	–	SW2155	16.04	7	(7) 32.9	7p1.1–1.2	6p22.1	14q/15q
BI181293	OPT65	393	7p11/13	0.84	SSC11F02	6.33	7	(7) 57.7	7p1.1–q1.1	–	6p/15q
BI185028	OPT65	406	–	–	SSC11F02	14.55	7	(7) 57.7	7p1.1–q1.1	6p22.2	6p/15q
BI185105	OPT62	156	–	–	SSC25E05	7.68	7	(7) 57.7	7p1.1–q1.1	6p22.1	6p/15q
BI181249	OPT65	414	7q12–23/q26	0.92	SW859	5.07	7	(7) 75.3	7q1.3–1.4	–	6p/15q
BI184877	OPT65	399	–	–	SW859	6.00	7	(7) 75.3	7q1.3–1.4	6p12.2	6p/15q
AW507066	OPT65	410	–	–	SWR1210	20.67	7	(7) 82.8	7q1.3–1.4	15q23.1	6p/15q
BI181803	OPT65	401	–	–	SWR1210	14.68	7	(7) 82.8	7q1.3–1.4	15q24.1	6p/15q
BI182567	OPT65	396	–	–	SW255	12.96	7	(7) 85.6	7q2.1	14q11.2	14q/15q
BI181194	OPT65	371	1q23–27	0.78	SW2537	3.03	7	(7) 139.5	7q2.5–2.6	3q27.2	14q/15q (7q/9/14q/18)
BI183240	OPT65	377	7q12–23/26	0.91	SSC12B09	3.37	7	(7) 156	7q2.5–2.6	14q32.33	14q/15q

Table 2 Continued.

GenBank accession no. ¹	PCR programme ²	Fragment size (bp) ³	SCHP ⁴		RH ⁵			Reported linkage group position (SSC) (cM) ⁶	Reported cytogenic position (SSC) ⁷	Position of human orthologue (HSA) ⁸	Expected cytogenic position (HSA) ⁹
			Map location	R	Closest marker	LOD	SSC				
BI183574	OPT65	434	–	–	SW2521	8.13	8	(8) 23.1	8p2.3	–	4p-q31
AW507072	OPT65	419	8p12–21	0.78	SW206	4.00	8	(8) 55.4	8p2.1	17p11.2	4p-q31
BI181579	OPT60	133	8p21–23	0.92	SW206	5.85	8	(8) 55.4	8p2.1	4p14	4p-q31
BI182250	TD1	135	1q11–17	0.92	SW1924	3.91	8	(8) 59.3	8p1.1–1.2	4q31.3	4p-q31 (15q/18)
BI183145	OPT65	396	–	–	SW1671	13.13	8	(8) 99.0	8q2.5	4q23	4p-q31
BI184390	OPT65	401	–	–	SWR68	6.86	9	(9) 4.0	9p2.4	11p15.4	11
BI182164	OPT65	378	–	–	SW511	13.38	9	(9) 70.4	9p1.1–1.2	11q23.3	11
BI184102	OPT65	375	–	–	SW1615	8.39	9	(9) 72.6	9p1.1–1.2	11q24.1	11
BI183910	OPT65	396	–	–	S0119	9.40	9	(9) 83.3	9q1.1–1.2	1q32.1	1q31–41/11
BI181696	OPT65	397	–	–	SW830	10.93	10	(10) 0.0	10p1.6	1q31.2	1q
BI181240	OPT65	401	–	–	SSC25A02	6.79	10	(10) 67.5	10q1.3	9p21.1	9/10
BI181277	TD1	113	10q11–12	0.75	SSC10G07	5.11	10	(10) 67.5	10q1.3	10q23.31	9/10
BI182214	OPT65	388	–	–	SSC10G07	6.39	10	(10) 67.5	10q1.3	10q23.31	9/10
BI182422	OPT65	391	–	–	SW1829	8.33	10	(10) 90.2	10q1.3–1.4	10p12.2	9/10
BI181366	OPT65	399	10q14–16	1.00	SW305	5.99	10	(10) 94.5	10q1.3–1.4	–	9/10
BI185217	OPT65	408	10q17	1.00	SW305	4.35	10	(10) 94.5	10q1.3–1.4	–	9/10
BI184598	TD1	133	10q14–16	0.72	SWR67	4.82	10	(10) 122.0	10q1.7	10p15.1	10p
BI185343	OPT62	185	–	–	SWR67	6.24	10	(10) 122.0	10q1.7	14q22.2	9/10
BI183661	OPT62	112	–	–	SSC6E09	9.35	11	(11) 16.3	11p1.3	13q14.11	13q
BI185480	OPT65	394	12p11–13	0.92	SW943	13.76	12	(12) 62.8	12p1.1–1.4	–	17
BI185061	OPT65	140	12p11–13	0.92	SW874	15.81	12	(12) 64.7	12p1.1–1.4	17q21.32	17
BI180977	OPT65	441	–	–	SWR390	13.81	12	(12) 70.5	12p1.1	17q21.33	17
BI181144	OPT65	378	–	–	SWR390	21.72	12	(12) 70.5	12p1.1	17q21.33	17
BI181761	OPT65	380	–	–	SWR390	12.22	12	(12) 70.5	12p1.1	17q21.33	17
BI181387	OPT65	412	5p11–15	0.93	S0090	2.23	12	(12) 80.2	12p1.1–q1.3	22q12.3	17 (12/22q)
BI184141	OPT65	392	12p11–13	0.92	SWC23	15.93	12	(12) 95.8	12p1.1–q1.3	17q11.2	17
BI181842	OPT65	395	–	–	S0288	7.43	13	(13) 35.4	13q2.1	20q13.12	3
BI182004	OPT65	411	13q23–41	1.00	SWR926	4.28	13	(13) 56.4	13q2.3–2.4	3q25.32	3
BI185003	OPT65	401	13q23–41	1.00	CP	13.21	13	(13) 90.9*	13q4.6–4.8	3q25.1	3
BI185137	OPT65	351	–	–	CP	16.79	13	(13) 90.9*	13q4.6–4.8	17q23.2	3
BI181431	OPT65	409	–	–	SW245	16.97	14	(14) 32.0	14q2.1	12q24.31	12q/22q
BI182401	OPT62	157	–	–	SW1109	11.14	14	(14) 53.2	14q2.6–2.7	–	1p22/10q
BI181263	OPT65	143	–	–	SW1333	11.68	14	(14) 62.4	14q2.6–2.7	10q24.32	1q22/10q
BI182336	OPT65	393	–	–	SW2057	11.61	14	(14) 62.4	14q2.6–2.7	10q24.32	1p22/10q
BI186028	OPT65	374	–	–	SW55	13.89	14	(14) 79.0	14q2.6–2.9	10q26.11	1p22/10q
BI182047	OPT65	168	–	–	SWC27	8.22	14	(14) 111.5	14q2.9	10q26.3	1p22/10q
BI180991	OPT65	414	–	–	SW1562	8.50	15	(15) 29.5	15q1.1–1.5	2q14.1	2q/4q
U53020	OPT62	200	–	–	SW1989	6.61	15	(15) 57.9	15q1.5	8p12	2q/4q
BI182881	OPT62	102	–	–	S0088	6.32	15	(15) 65.1	15q2.2	2q24.3	2q/4q
BI182556	OPT65	379	–	–	SW1263	9.29	15	(15) 67.5	15q2.2	4q22.1	2q/4q
BI182232	OPT65	390	–	–	SW1316	6.03	15	(15) 73.1	15q2.2	2q33.1	2q/4q
BI181421	OPT65	239	4q21–23	0.92	SWR1002	3.56	15	(15) 76.0	15q2.2	1p12	2q/4q (1/8q)
BI185839	OPT65	409	–	–	SW1683	15.41	15	(15) 79.3	15q2.3–2.4	2q35	2q/4q
AW574403	TD1	171	–	–	SW2608	5.56	15	(15) 95.0	15q2.5	5p15.31	2q/4q
BI182428	OPT65	392	–	–	SW742	9.92	16	(16) 9.3	16q1.1–1.2	5p15.1	5
M36513	OPT60	340	16q14/22–23	0.92	S0077	4.24	16	(16) 33.2	16q1.4	5q11.2	5

Table 2 Continued.

GenBank accession no. ¹	PCR programme ²	Fragment size (bp) ³	SCHP ⁴		RH ⁵			Reported linkage group position (SSC) (cM) ⁶	Reported cytogenic position (SSC) ⁷	Position of human orthologue (HSA) ⁸	Expected cytogenic position (HSA) ⁹
			Map location	R	Closest marker	LOD	SSC				
BI181787	OPT65	385	–	–	SW1897	7.18	16	(16) 86.2	16q2.3	5q33.1	5
BI182872	OPT65	425	–	–	SW1897	6.66	16	(16) 86.2	16q2.3	5q33.1	5
BI186431	OPT65	103	–	–	SW1897	8.70	16	(16) 86.2	16q2.3	5q33.1	5
BI182007	OPT65	421	–	–	SW24	14.73	17	(17) 23.3	17q1.1–1.2	–	20
BI184717	OPT65	405	17q21–23	1.00	SW1031	4.46	17	(17) 63.4	17q2.1	20q13.12	20
BI185002	OPT65	386	–	–	S0332	13.99	17	(17) 88.7	17q2.3	20q13.32	20
BI181663	OPT62	142	17q21–23	0.80	SW2431	5.08	17	(17) 94.0	17q2.3	20q13.12	20
BI185259	OPT65	401	–	–	S0177	15.16	18	(18) 55.3	17q2.1	–	7
BI182487	OPT65	424	Xq11–12	0.86	SW1549	2.22	X	(X) 11.9	Xp2.4	–	X
BI184594	OPT65	402	–	–	SSC13B11	7.18	X	(X) 74.4	Xp2.1–2.2	Xp11.3	X

PCR, polymerase chain reaction; SCHP, somatic cell hybrid panel; RH, radiation hybrid

¹Accession no. for the EST sequence used for primer design.

²PCR programmes used for amplification were as follows: OPT60, 40 cycles of 95 °C/30 s, 60 °C/45 s and 72 °C/90 s; OPT62, 40 cycles of 95 °C/30 s, 62 °C/45 s and 72 °C/90 s; OPT65, 40 cycles of 95 °C/30 s, 65 °C/45 s and 72 °C/90 s; TD1, 10 cycles of 94 °C/60 s, 64 °C (–1 °C/cycle)/60 s, 72 °C/60 s, plus 30 cycles of 93 °C/30 s, 55 °C/45 s, 72 °C/45 s.

³Base-pair size of the porcine PCR fragment amplified with the corresponding primers listed in Table 1.

⁴Results obtained after submitting the data to the INRA database-R refers to the correlation coefficients between each marker and the chromosomal region(s) listed, estimated according to Chevalet & Corpet (1986).

⁵Closest RH-linked marker, the LOD score for the estimate and the porcine chromosome where the respective RH group is located.

⁶Linkage position of the closest RH-linked marker also present in the porcine linkage map Rohrer *et al.* (1996) or according to Marklund *et al.* (1996), where marked by a**.

⁷Location of the closest RH-linked marker also present in the porcine cytogenetic map.

⁸Position of the corresponding human orthologous sequence according to the UCSC Genome Browser (<http://genome.ucsc.edu/>), assembly of July 2003.

⁹Expected cytogenic position (HSA) according to Goureau *et al.* (1996) (see <http://www.toulouse.inra.fr/lgc/pig/compare/compare.htm>) based on the RH and SCHP map-location (data in parentheses refers to the expected position based on the SCHP results that were discordant of the RH-mapping results).

with the chromosomal locations predicted (Table 2). In the remaining cases, the observed disagreements may be the result of shuffling of small terminal regions of ancestral chromosomes, which cannot be detected by chromosome painting techniques, and result in disruptions of the many large synteny blocks conserved between human and pig chromosomes, as previously reported in other studies (Messer *et al.* 1997; Larsen *et al.* 1999). In addition, discrepancies may be caused by mapped loci belonging to gene families and/or being duplicated loci that were physically separated during the independent evolution of the human and porcine genomes. Further characterization of the amplicons used for mapping these respective ESTs will be necessary to clarify these issues.

Identifying the underlying genes and respective polymorphisms regulating complex traits such as ovulation rate in swine has been a major challenge (see Pomp *et al.* 2001; Rohrer 2004). Integrating phenotypic data with mapping and gene expression experiments provides a powerful approach to dissect the nature of mechanisms controlling complex traits

(Pomp *et al.* 2004). All of the genes mapped in this study were found to be putatively differentially expressed in the ovaries and ovarian follicles between a swine line selected for high ovulation rate and its control line (Caetano *et al.* 2004). Quantitative and/or qualitative changes in mRNA expression may be the result of cis-acting allelic variations at the specific gene (i.e. a QTL) or conversely, may result from trans-acting mutations at QTL that control the particular gene (Yvert *et al.* 2003; Pomp *et al.* 2004). Several porcine genomic regions have been found to contain QTL affecting ovulation rate, and twelve of the genes we mapped in this study are located in those regions (Table 3). These differentially expressed genes found to map to regions containing QTL that are associated with differences in ovulation rate in swine become immediate positional candidates. Of particular interest are the genes of yet unknown identity and/or function (i.e. BI182164). This approach is somewhat limited because of the very broad confidence intervals attributed to most QTL localizations. Concurrently, map positions of the ESTs evaluated in this study

Table 3 Previously reported QTL affecting ovulation rate in swine and associated EST-derived markers.

SSC ¹	Position (cM) ²	Reference	GenBank accession no. ³	Closest RH marker ⁴	Reported linkage group position (cM) ⁵
3	36 [3:70]	Rohrer <i>et al.</i> (1999)	BI186221	SW1443	40.1
			BI181228		
4	80 (32)	Rathje <i>et al.</i> (1997)	BI181384	SW512	80.5
			BI185380		
7	156 (150)	Wilkie <i>et al.</i> (1999)	—	—	—
8	5 [–10:20]	Rohrer <i>et al.</i> (1999)	—	—	—
8	50 (29)	Wilkie <i>et al.</i> (1996)	AW507072	SW206	55.4
			BI181579		
8	50 (101)	Wilkie <i>et al.</i> (1999)	AW507072	SW206	55.4
			BI181579		
8	105	Rathje <i>et al.</i> (1997)	—	—	—
9	11 (1)	Cassady <i>et al.</i> (2001)	BI184390	SWR68	4.0
9	67 [57:122]	Rohrer <i>et al.</i> (1999)	BI182164	SW511	70.4
10	89 [44:118]	Rohrer <i>et al.</i> (1999)	BI182422	SW1829	90.2
15	62 (51)	Rathje <i>et al.</i> (1997)	U53020	SW1989	57.9
			BI182881		
15	79 [53:101]	Rohrer <i>et al.</i> (1999)	BI185839	SW1683	79.3
15	100 (107)	Wilkie <i>et al.</i> (1999)	AW574403	SW2608	95.0

¹Porcine chromosome.²Relative position in Kosambi centimorgans as reported based on maps developed by Rohrer *et al.* (1996). Numbers in parentheses are relative position as reported by the authors. Numbers in brackets indicate the mapping range of the detected QTL as reported by the authors.³Genbank accession no. for the EST sequence used for primer design.⁴RH-linked marker closest to the EST-derived marker.⁵Linkage position of the closest RH-linked marker also present in the porcine linkage map Rohrer *et al.* (1996).

will likely become more refined as the number of loci in the RH database, against which two-point analyses can be conducted with the current set of ESTs, continues to grow.

Disagreements between mapping results using the RH and SCH panels were observed with six markers (Table 2). This was also observed in other experiments that involved mapping markers with these two methodologies (Lahbib-Mansais *et al.* 2000, 2003; Tuggle *et al.* 2003). The underlying cause of the discrepancy was most likely a low LOD score for the RH panel (RHP), and thus the SCHP should be used as the best predicted map position, especially if it matches predictions based on human data. Amplification of multiple fragments from paralogous gene families and/or duplicated loci has been reported in other SCHP mapping studies (Caetano *et al.* 1999) and may also be the cause of the observed discrepancies between the RH and SCH mapping results. Seven of the mapped genes are represented by more than one EST/primer-set (i.e. BI181787, BI182872 and BI186431). This redundancy was used retrospectively to check the robustness of the results. In these cases, mapping results based on different primer sets were in complete agreement, except for *TIMP3* (BI181387, BI183974 and BI182045) where one of the markers mapped to SSC12 with the RHP with a low LOD score (2.23). This EST mapped to SSC5 using SCHP, in agreement with results from the other EST representing this gene.

Acknowledgements

The authors are grateful to Sara Olberding and Jackie Potts for laboratory assistance in this project. This work was partially supported by USDA-NRICGP 99-35205-8370 and by the National Science Foundation under Grant no. 0091900 (Nebraska EPSCOR infrastructure improvement grant). This work is published as paper number 14728 of the Journal Series, Nebraska Agricultural Experiment Station.

References

- Caetano A.R., Shiue Y.L., Lyons L.A., Laughlin T.F., O'Brien S.J., Bowling A.T., and Murray J.D. (1999) A comparative gene map of the horse (*Equus caballus*). *Genome Research* **9**, 1239–49.
- Caetano A.R., Johnson R.K., and Pomp D. (2003) Generation and sequence characterization of a normalized cDNA library from swine ovarian follicles. *Mammalian Genome* **14**, 65–70.
- Caetano A.R., Johnson R.K., Ford J.J., and Pomp D. (2004) Microarray profiling for differential gene expression in ovaries and ovarian follicles of pigs selected for increased ovulation rate. *Genetics* **168**, 1529–37.
- Cassady J.P., Johnson R.K., Pomp D., Rohrer G.A., Van Vleck L.D., Spiegel E.K., and Gilson K.M. (2001) Identification of quantitative trait loci affecting reproduction in pigs. *Journal of Animal Science* **79**, 623–33.

- Chevalet C. and Corpet F. (1986) Statistical decision rules concerning syntenic or independence between markers. *Cytogenetics and Cell Genetics* **43**, 132–9.
- Fahrenkrug S.C., Smith T.P., Freking B.A., et al. (2002) Porcine gene discovery by normalized cDNA-library sequencing and EST cluster assembly. *Mammalian Genome* **13**, 475–8.
- Fujii J., Otsu K., Zorzato F., de Leon S., Khanna V.K., Weiler J.E., O'Brien P.J., and MacLennan D.H. (1991) Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* **253**, 448–51.
- Goureau A., Yerle M., Schmitz A., Riquet J., Milan D., Pinton P., Frelat G., and Gellin J. (1996) Human and porcine correspondence of chromosome segments using bi-directional chromosome painting. *Genomics* **36**, 252–62.
- Grisart B., Farnir F., Karim L., et al. (2004) Genetic and functional confirmation of the causality of the DGAT1 K232A quantitative trait nucleotide in affecting milk yield and composition. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 2398–403.
- Johnson R.K., Nielsen M.K., and Casey D.S. (1999) Responses in ovulation rate, embryonal survival, and litter traits in swine to 14 generations of selection to increase litter size. *Journal of Animal Science* **77**, 541–57.
- Lahbib-Mansais Y., Leroux S., Milan D., Yerle M., Robic A., Jiang Z., Andre C., and Gellin J. (2000) Comparative mapping between humans and pigs: localization of 58 anchorage markers (TOASTs) by use of porcine somatic cell and radiation hybrid panels. *Mammalian Genome* **11**, 1098–106.
- Lahbib-Mansais Y., Tosser-Klopp G., Leroux S., Cabau C., Karsenty E., Milan D., Barillot E., Yerle M., Hatey F., and Gellin J. (2003) Contribution to high-resolution mapping in pigs with 101 type I markers and progress in comparative map between humans and pigs. *Mammalian Genome* **14**, 275–88.
- Larsen N.J., Marklund S., Kelly K.A., Malek M., Tuggle C.K., Yerle M., and Rothschild M.F. (1999) New insights into porcine-human synteny conservation. *Mammalian Genome* **10**, 488–91.
- Marklund L., Johansson Moller M., Hoyheim B., Davies W., Fredholm M., Juneja R.K., Mariani P., Coppieters W., Ellegren H., and Andersson L. (1996) A comprehensive linkage map of the pig based on a wild pig-Large White intercross. *Animal Genetics* **27**, 255–69.
- McPherron A.C. and Lee S.J. (1997) Double muscling in cattle due to mutations in the myostatin gene. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 12457–61.
- Messer L.A., Wang L., Tuggle C.K., et al. (1997) Mapping of the melatonin receptor 1a (MTNR1A) gene in pigs, sheep, and cattle. *Mammalian Genome* **8**, 368–70.
- Milan D., Jeon J.T., Looft C., et al. (2000) A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. *Science* **288**, 1248–51.
- Page B.T., Casas E., Heaton M.P., et al. (2002) Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. *Journal of Animal Science* **80**, 3077–85.
- Pomp D., Caetano A.R., Bertani G.R., Gladney C.D., and Johnson R.K. (2001) Applying functional genomics research to the study of pig reproduction. *Reproduction Supplement* **58**, 277–92.
- Pomp D., Allan M.F., and Wesolowski S.R. (2004) Quantitative genomics: exploring the genetic architecture of complex trait predisposition. *Journal of Animal Science* **82**, 300–12.
- Quackenbush J., Liang F., Holt I., Pertea G., and Upton J. (2000) The TIGR Gene Indices: reconstruction and representation of expressed gene sequences. *Nucleic Acids Research* **28**, 141–5.
- Rathje T.A., Rohrer G.A., and Johnson R.K. (1997) Evidence for quantitative trait loci affecting ovulation rate in pigs. *Journal of Animal Science* **75**, 1486–94.
- Rink A., Santschi E.M., Eyer K.M., Roelofs B., Hess M., Godfrey M., Karajusuf E.K., Yerle M., Milan D., and Beattie C.W. (2002) A first-generation EST RH comparative map of the porcine and human genome. *Mammalian Genome* **13**, 578–87.
- Robic A., Faraut T., Iannuccelli N., Lahbib-Mansais Y., Cantegrel V., Alexander L., and Milan D. (2003) A new contribution to the integration of human and porcine genome maps: 623 new points of homology. *Cytogenetics and Genome Research* **102**, 100–8.
- Rohrer G.A. (2004) An overview of genomics research and its impact on livestock reproduction. *Reproduction Fertility and Development* **16**, 47–54.
- Rohrer G.A., Alexander L.J., Hu Z., Smith T.P., Keele J.W., and Beattie C.W. (1996) A comprehensive map of the porcine genome. *Genome Research* **6**, 371–91.
- Rohrer G.A., Ford J.J., Wise T.H., Vallet J.L., and Christenson R.K. (1999) Identification of quantitative trait loci affecting female reproductive traits in a multigeneration Meishan-White composite swine population. *Journal of Animal Science* **77**, 1385–91.
- Rothschild M.F. (2003) From a sow's ear to a silk purse: real progress in porcine genomics. *Cytogenetic and Genome Research* **102**, 95–9.
- Schadt E.E., Monks S.A., Drake T.A., et al. (2003) Genetics of gene expression surveyed in maize, mouse and man. *Nature* **422**, 397–402.
- Tuggle C.K., Green J.A., Fitzsimmons C., et al. (2003) EST-based gene discovery in pig: virtual expression patterns and comparative mapping to human. *Mammalian Genome* **14**, 565–79.
- Wayne M.L. and McIntyre L.M. (2002) Combining mapping and arraying: An approach to candidate gene identification. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 14903–6.
- Wilkie P.J., Paszek A.A., Flickinger G.H., Rohrer G.A., Alexander L.J., Beattie C.W., and Schook L.B. (1996) Scan of eight porcine chromosomes for growth, carcass and reproductive traits reveals two likely quantitative trait loci. *Animal Genetics* **27** (Suppl. 2), 117–8 (Abstract).
- Wilkie P.J., Paszek A.A., Beattie C.W., Alexander L.J., Wheeler M.B., and Schook L.B. (1999) A genomic scan of porcine reproductive traits reveals possible quantitative trait loci (QTLs) for number of corpora lutea. *Mammalian Genome* **10**, 573–8.
- Yerle M., Echard G., Robic A., Mairal A., Dubut-Fontana C., Riquet J., Pinton P., Milan D., Lahbib-Mansais Y., and Gellin J. (1996) A somatic cell hybrid panel for pig regional gene mapping characterized by molecular cytogenetics. *Cytogenetics and Cell Genetics* **73**, 194–202.
- Yerle M., Pinton P., Robic A., et al. (1998) Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs. *Cytogenetics and Cell Genetics* **82**, 182–8.
- Yvert G., Brem R.B., Whittle J., Akey J.M., Foss E., Smith E.N., Mackelprang R., and Kruglyak L. (2003) Trans-acting regulatory variation in *Saccharomyces cerevisiae* and the role of transcription factors. *Nature Genetics* **35**, 57–64.