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Physical mapping of genes in the porcine ovarian transcriptome

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Summary

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 phenotypical 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 & 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.

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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 & 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

ovaries 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://www-genome.wi.mit.edu/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-μl reactions containing 25 ng of genomic DNA, 1.5 mM $MgCl_2$, 50 μM of each dNTP, 2 μM 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://imprh.toulouse.inra.fr/) 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://www.toulouse.inra.fr/lgc/pig/hybrid.htm) and regional assignments were obtained using the INRA database (http://www.toulouse.inra.fr/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://www.toulouse.inra.fr/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 for RH and/or SCHP mapping, primarily because of

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	TTTATCCTGCCATTCATCATTACCG	ATTATGCCTCGTTGTTTGGATGTGT
AW574403	Cytochrome B and tRNA-Thr and tRNA-Pro	TGGCCCTCCTTTTCTGGTTT	ACCCGTAGAACACCCGTTCA
AW584050	NADH dehydrogenase subunit 4, mRNA sequence	ATGGGGGTAGGGCTAGGTTT	GAAGTTTCATAGGGGCCACA
AW584057	Ferritin	TGGCTTTGAAGAACTTTGCCAAATA	GCTTAGGGCTTAGCTCTCACTGCTC
BI180977	Pro-1(I) collagen chain (Col1A1)	CTTCCCAGAACATCACCTACCACTG	CCAAAGTCCATGTGAAATTGTCTCC
BI180991	Actin-related protein 3, yeast (ARP3)	ATCCAAGATTTGATGGGATTTATCG	ACAGAACTGCAATACTGGTCCAACA
BI181040	Human chromosome 1q21 BAC 236c22	TTTCTTAGGCCATTCCAGTGCATAG	GTCAGGCTAGGGCAGAACGTTTAGT
BI181041	Low density lipoprotein-related protein 1 (LRP1)	AATGGGAAGCGACTGGACAA	GCTCACACTTGTCGCCTGTG
BI181067	Alpha-2-macroglobulin (A2M) i	ACGGCTTCCGGTGTAACTGA	TCCCATTTGCTTTGGAGGTG
BI181144	Pro-1(I) collagen chain (Col1A1)	CAAAGTCTTCTGCAACATGGAGACA	TCGTAGATCACGCTGTAGGTGAAGC
BI181194	60S ribosomal protein L4	CTGAAAGTGCTTTCCGCAAGTTAGA	CTTCTCTACCACGGGCTTCTTGTCT
BI181228	Selenium-binding protein 1 (SELENBP1)	ACTTGGTCGGTAGAGAAGGTGATCC	ATGAGATCGGGGTAAAACTGCTTGT
BI181240	Heat shock protein, DNAJ-like 2 (HSJ2)	GCAGCAAATAAAATGCCAGCAGTAA	CTTGACAACCGAACCATAGTCATCA
BI181249	Sus scrofa microsatellite, locus SO658	ACCCGAACTCCAACTAGGTCAACTC	GAGGTTTGAACACCAGCAATGTCTT
BI181263	Cytochrome P450 17-α-hydroxylase (CYP17)	AAGTACCCCAGGAGTCTCCCAGTC	GTAGTCTTGGAACCCAGACGAAAGG
BI181277	Cathepsin L	TGGAAGGATTCATGGCTTGC	TGCCAACGACACTGGTTACG
BI181293	No match	TTGAAGAAAATGCTTAGCTGCGAAA	GTGAGAAGTGTAAATCACCCCTTGG
BI181366	Cytosolic dihydrodiol dehydrogenase 3	TTTTTGACTTTGAATTGCCTCCAGA	TGCACCTTCTGTGTCTAATGGGACT
BI181384	Homo sapiens cDNA FLJ13813	CCACTGTGGATGTTGACCCTAAGTC	GGTGGATGACCTAAAACAGGAGAGG
BI181387	Tissue inhibitor of metalloproteinase 3 (TIMP3)	GGCCGCTCTGTATTTCATTCATATC	TTAGGAGAAATCATGGCTTCCCAGT
BI181421	3-β-hydroxysteroid dehydrogenase (3BHSD)	GGAGCTGCGTGAAGGGATACATACT	TCTGCTTCTCGCCACCAGAAC
BI181431	Sus scrofa scavenger receptor (SCARB1)	CCAGCCCCTGTAGAGATTTCAGAGA	TGGGGAGAGTCTTGACAACAAAGAG
BI181480	Plasminogen activator inhibitor-1	AACATTTTTCACTGAAGGGCATGGT	GGGTTGAAGGGAGACAGAGTTGTTT
BI181579	Ubiquitin carboxyl-terminal esterase L1 (UCHL1)	TTGCGAAGATGCAGCTCAAA	ACCGAGCCCAAAGACTCCTC
BI181663	Protective protein for β-galactosidase (PPGB)	ACTCTGGCCGTGTTGGTCAT	AGAGCCTGTTCCCCAGAAGG
BI181686	Connective tissue growth factor	GCTATTGGGTGTCATTGAGAAAGGA	TGGTGTTCAGAATGGAGGCTAACAT
BI181696	Regulator of G-protein signalling 2, 24 kDa (RGS2)	GAAGCCAGGAGCAGTCTAGGAGAAG	ACTCATCCTGTGACACACAGCAACT
BI181761	Collagen, type I, alpha-1 (COL1A1)	CTGAGCCAGCAGATCGAGAACAT	GGGAAGCCTCAGTGGACATCAG
BI181785	Homo sapiens clone 23785 mRNA	AACATTCATCTCCAAGAACTGCTTCA	CCAGAGAAACAAAATGGCAAACAGT
BI181787	Calpain I light subunit	GGGCTAAAGTCCAGGTTAGAGCAGA	GGGAGGACTCTGTTCAGGAAGGAAT
BI181791	Homo sapiens hypothetical protein MGC11256	GTGTCTGCACACAGGCATCC	GTCCCGAGGGGTGTAAGGAG
BI181803	Cytochrome P450 side chain cleavage	ATTTATTGCCTTCATGGGAGTGGAT	GAGCTCGAGATGACTCTCTTCCTCA
BI181842	Phospholipid transfer protein (PLTP)	GAGCAAGAACTGGAGACCATTACCA	CGAGGTGATGAAAGTGGACAGGAAT
BI181891	Serpentine receptor (CYT28)	CTGATGACCACGAGGGACTCTGTAG	CCCAGAACGTCTACACTAAGCCTGA
BI182004	Retinoic acid receptor responder 1	ACTAGTCGGATTCGTTTCTGCTGTG	CCACTGTCAAGAGCTACAGACACCA
BI182007	Putative lysophosphatidic acid acyltransferase	CGAGAGCTCCTGCATACGACAGT	ATTCAAGGAGGAGCAGCGGAAG
BI182045	Tissue inhibitor of metalloproteinase 3 (TIMP3)	GACAGGGTTTTCTCTGGTTGGTTTC	CCCTAGGAGACAAGACTCTCCCTGA
BI182047	No match	CTGATCAGAAAATCACCCCACACTC	GCTGCCCTGAAGTTACAGAAGGTG
BI182064	A kinase (PRKA) anchor protein 2 (AKAP2)	AGTGGTGTCTGACTGCAGTTGATTG	CTGCTGCCTCTTCACAAACGTTAAA
BI182164	Human cDNA FLJ11658 fis	GCTCACTCTCTGTCCCAGAACCTTA	TGTGATGGCCTGTGACTCTCCTATT
BI182214	Cathepsin U (CTSU)	AACTGCTGTGAAGAATCAGGGTCAC	GGATTCATGGCTTGCATCTATACCA
BI182232	Heat shock 10 kDa protein 1 (HSPE1)	GAAAGAAGTGCAGCTGAAACGGTAA	CTGGATGTCATCAGTTTGGATATCATT
BI182250	Annexin A2 (ANXA2)	GACACTAAGGCCGTCCTCCA	GTGGTGGGGATGACTGAAGC
BI182336	Cytochrome P450 17-α-hydroxylase (CYP17)	AGTCAGGAGGTACTCCCCTCAGTGT	CATTGGCGAATTTACCATTGACAAG
BI182401	No match	TTACCGCCTCTCTCCCCTTC	TGATGTGGGGTCGGGTTTAG
BI182422	Pilin-like transcription factor	TTCAAACCAAGGAAACACTGACCAT	CCTCTTCTCGTTTGACCTCTCACCT
BI182428	Homo sapiens cDNA FLJ22155	GTTTCCGCTTCCTGGTCTTGTAAA	TTTCCATCTCTAGAAAATGGCATGG
BI182474	Cysteine dioxygenase, type I (CDO1)	GGGCTCTACTGGTTTCACCCTCTAA	TTTGGTTTGTTGTGGGTTTGGATAC
BI182487	Ribosomal protein S4, X-linked (RPS4X)	TTACACCTGAGGAGGCCAAGTACAA	CTCTTGTCTCTCTCCTCAGCAATGG
BI182556	Thymosin, beta-4, X-chromosome (TMSB4X)	CTTCCTCCTTCACCAACATGCAA	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)	TCAGGGAACTCGTCCTTACTGAACA	CTAGGCCGGGTCTGCTACTCTAGTC
BI182872	Calpain I light subunit	TAGGTACACGGGGTCAGTCTAATGC	CAATTGGTCTGCTCTAACGTGGACT
BI182881	Growth factor receptor-bound protein 14 (GRB14)	TGAAGAGGCAGTGGGGCTAC	AATCCCACTGAGGCCCTTTC
BI183145	H2A histone family, member Z (H2AFZ)	TCCACCGAAGTGGAAATAATGTCTG	CATCCACAAATCTCTCATTGGGAAG
BI183240	Sus scrofa Ig gamma 2a (IGG2A)	AGACTTCTGGGTGTAGTGGTTGTGC	AAGGAGTTCAAGTGCAAGGTCAACA
BI183427	CD83 antigen	AGACTGTTGGCAACTACAAGCAAGC	ATAAGGAAACACAACAGCTGCCAAA
BI183514	Tropomyosin 2 (beta) (TPM2)	GTCTGGTGAATCTCCACGTTCTCCT	TGTACTGATGGCATGTGTCTGTTCC
BI183574	Interferon-stimulated protein (ISG15)	GGTGCACATAGGCTTGAGGTCATAC	TAGGGAACTGAAGGTGAAGATGCTG
BI183661	Homo sapiens RGC32 protein (RGC32)	TGCTACGTTTTAAGAGAAAATACCA	AAAAATGCCTTTGGGCTCCT
BI183694	Homo sapiens BORG2 protein (BORG2)	TCATGTTGCCCTTATTGTCACCAGT	TTTATCCATGACATTCAGCACCTCA
BI183769	Apolipoprotein E receptor 2 (ApoER2)	CAAAGCAGTAAACCAAATCCCAGTG	TTAGGACCCTTGGGTTGAAGTCATT
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)	CTTAAAGGAGACCAGGGGGAGTCTG	AAGAGTCCCTTGCTGTTGAAGTCAC
BI184390	Integrin-linked kinase (ILK)	CCTACTTGTCCTGCATCTTCTCCAG	ACTCAACAGTCGCAGTGTCATGATT
BI184392	No match	CCTGGCTCTCCTCAGGGTTT	AACCCCCAGCATCCAAGAAT
BI184415	Cytolytic trigger molecule G7	TCCATGTGACTTTGCCATTCC	TTCTGCCTGGTGATGGGATT
BI184594	Collagenase inhibitor	TTTCATCATCTTGGAGAAAGACAGGT	ATGGGAGGGACTGTTCTTGAGTCAC
BI184598	Dihydrodiol dehydrogenase-2 (DD2)	ATTCCACCTGGTTGCAGACG	GGCGGCTCTGGAGAAGTGTA
BI184629	Homo sapiens mitogen-inducible gene 6 (MIG-6)	GCTCAAATAACATCACTCAGCAGTGTC	CCTCTTCAGTATTTCCTGGGGATTG
BI184678	Homo sapiens cDNA FLJ22353	GCTGGAGCTGCTCTTTGATTATGAG	CTCATCGGCCCTTGAGTAGAGGTAG
BI184717	Homo sapies syndecan 4 (SDC4)	ATGGCTGGAGGGAAGGGACAT	ACACTAACCACATTTCAAGGGCTCA
BI184862	Lysosomal associated protein transmembrane 4-beta (LAPTM4B)	GCTGTGGCTTTTGAACACAGG	CTCTCCTGCAAGGCACGTCT
BI184876	Cytochrome P450 aromatase (CYP19)	AGTGGCTGAGGCATAAATTGAGAGA	TCCACAAAACGTGCTTTGAGAAAAA
BI184877	Glutathione S-transferase (GSTP)	AAGCAACCGTTATCTTCCTGCATTT	AATAGTTTCTTGGTCCCACAACACC
BI185002	GTP-binding protein RAB22A	AGAGCTCCTCTGCCTTCCCTTG	CACCAACTTCACTTCAGCGCTTTAT
BI185003	H.sapiens (TL27) mRNA from (PC3) cell line	GCCTGTTTCTTTTTAGATCCCCGATA	ACAGGGACAAATATCACATCCCAAT
BI185028	H1 histone family, member 4 (H1F4)	CCGCTGTACTTTTTGCTTTTCCTG	CTTTCTTAGCTTTGGGCTTGGCTTC
BI185061	Homo sapiens cDNA FLJ23553	AAAAGAATCAAGAAGTGAAGCTGGG	TGGAGGTACTTTATTGGGGATAGGA
BI185105	MHC class I antigen PD1 (SLA1)	AGGCTGCAGGCAGTGACAGT	TGCTGCTGCAGAAACAGGAG
BI185137	Serine carboxypeptidase 1 precursor (HSCP1)	GGTGTTCAGGACATAGGGATGAGTG	CCTCTGTGGCAACTTGGAAATCAT
BI185217	Sus scrofa CC chemokine receptor genes	CCCCAGTCTGTTTTTATTTGGCAGT	TTGGGGCATGTCATTGGTAATGTAT
BI185259	No match	ATGGAGTCCCAGAAAGCGTAACTGT	AGCAAGGATTTGATCCTGCCTAGAG
BI185343	ATP synthase gamma-subunit (L-type)	ACAGCCTGGCCAACATCATC	GGCACCGGAGATGATTTCAA
BI185380	Selenium binding protein 1 (SELENBP1)	TGAGGTTTTGACATTGTTAGCAGCA	CTCCATGTTCTGAGCCATCCTTTC
BI185480	Porcine endogenous retrovirus PERV	GCAAACAGCAAGAGGATTTTTATTTCA	GGCCTGTTTTACGTCCTTTCACCTA
BI185578	No match	CCTCATACCAAGTGCCGGAATTTAT	ATGATCATCAAAGTGGACCCTTGAA
BI185839	Fibronectin (FN precursor)	TATTGCGGGCCAGACAGTTAAGTAG	TCCCGAGAGTAAAATTTCCAAGCTG
BI185976	Sus scrofa CC chemokine receptor genes	CACAGCTGTAGCAACACCAGATCTTT	CCTGGTGAACTCAGGAGGGAATATG
BI186028	Peroxiredoxin 3 (PRDX3)	ACATTGAGCACGTCAAGAAAGAAGA	CTTCTGATCAGGGGTCCTGAAGATT
BI186221	Protein p8 (candidate of metastasis 1)	AGCCTCAGTCACGTGGGGTA	GAGACAGGACTGCGGAGGAA
BI186223	Human chromosome 1q21 BAC 236c22	CACCCTCCCTCCCGTTATTC	CTCTCCTGCAAGGCACGTCT
BI186431	Calpain I light subunit	CTTGGCCTGAATGCACGAA	GTGTCAGGGGTGGCATCTTC
M36513	Follistatin	GTCTGTGGGCTGGATGGGAAA	ATCAAGGAAATCATGAATGGAAGCA
U53020	Steroidogenic acute regulatory protein (STAR)	CAGACCCAGGTGGACTTTGC	CGACACACACCACTGCAACA

[1]BLASTN results on the NR database as of 20 September 2004.

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

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

			SCHP[4]		RH[5]						
GenBank accession no.[1]	PCR programme[2]	Fragment size (bp)[3]	Map location	R	Closest marker	LOD	SSC	Reported linkage group position (SSC) (cM)[6]	Reported cytogenic position (SSC)[7]	Position of human orthologue (HSA)[8]	Expected cytogenic position (HSA)[9]
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] Map location	SCHP[4] R	RH[5] Closest marker	RH[5] LOD	RH[5] SSC	Reported linkage group position (SSC) (cM)[6]	Reported cytogenic position (SSC)[7]	Position of human orthologue (HSA)[8]	Expected cytogenic position (HSA)[9]
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] Map location	SCHP[4] R	RH[5] Closest marker	RH[5] LOD	RH[5] SSC	Reported linkage group position (SSC) (cM)[6]	Reported cytogenic position (SSC)[7]	Position of human orthologue (HSA)[8]	Expected cytogenic position (HSA)[9]
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

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

[2]PCR 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.

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

[4]Results 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).

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

[6]Linkage 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'*'.

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

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

[9]Expected 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).

sequences. Our empirical mapping results agreed in 85% of the cases 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.

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 *et al.* (1999)	BI186221 BI181228	*SW1443*	40.1
4	80 (32)	Rathje *et al.* (1997)	BI181384 BI185380	*SW512*	80.5
7	156 (150)	Wilkie *et al.* (1999)	–	–	–
8	5 [–10:20]	Rohrer *et al.* (1999)	–	–	–
8	50 (29)	Wilkie *et al.* (1996)	AW507072 BI181579	*SW206*	55.4
8	50 (101)	Wilkie *et al.* (1999)	AW507072 BI181579	*SW206*	55.4
8	105	Rathje *et al.* (1997)	–	–	–
9	11 (1)	Cassady *et al.* (2001)	BI184390	*SWR68*	4.0
9	67 [57:122]	Rohrer *et al.* (1999)	BI182164	*SW511*	70.4
10	89 [44:118]	Rohrer *et al.* (1999)	BI182422	*SW1829*	90.2
15	62 (51)	Rathje *et al.* (1997)	U53020 BI182881	*SW1989*	57.9
15	79 [53:101]	Rohrer *et al.* (1999)	BI185839	*SW1683*	79.3
15	100 (107)	Wilkie *et al.* (1999)	AW574403	*SW2608*	95.0

[1]Porcine chromosome.

[2]Relative 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.

[3]Genbank accession no. for the EST sequence used for primer design.

[4]RH-linked marker closest to the EST-derived marker.

[5]Linkage position of the closest RH-linked marker also present in the porcine linkage map Rohrer *et al.* (1996).

Concurrently, map positions of the ESTs evaluated in this study 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.

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