

Research article

Open Access

A robust linkage map of the porcine autosomes based on gene-associated SNPs

Rikke KK Vingborg[†], Vivi R Gregersen[†], Bujie Zhan, Frank Panitz, Anette Høj, Kirsten K Sørensen, Lone B Madsen, Knud Larsen, Henrik Hornshøj, Xuefei Wang and Christian Bendixen*

Address: Department of Genetics and Biotechnology, Faculty of Agricultural Sciences, Aarhus University, PO Box 50, 8830 Tjele, Denmark

Email: Rikke KK Vingborg - rikkek.vingborg@agrsci.dk; Vivi R Gregersen - vivi.gregersen@agrsci.dk; Bujie Zhan - zhan.bujie@agrsci.dk; Frank Panitz - frank.panitz@agrsci.dk; Anette Høj - Anette.hoj@agrsci.dk; Kirsten K Sørensen - kirstenk.sorensen@agrsci.dk; Lone B Madsen - loneb.madsen@agrsci.dk; Knud Larsen - knud.larsen@agrsci.dk; Henrik Hornshøj - henrikh.jensen@agrsci.dk; Xuefei Wang - xuefei1216@agrsci.dk; Christian Bendixen* - christian.bendixen@agrsci.dk

* Corresponding author †Equal contributors

Published: 27 March 2009

Received: 15 August 2008

BMC Genomics 2009, 10:134 doi:10.1186/1471-2164-10-134

Accepted: 27 March 2009

This article is available from: <http://www.biomedcentral.com/1471-2164/10/134>

© 2009 Vingborg et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: Genetic linkage maps are necessary for mapping of mendelian traits and quantitative trait loci (QTLs). To identify the actual genes, which control these traits, a map based on gene-associated single nucleotide polymorphism (SNP) markers is highly valuable. In this study, the SNPs were genotyped in a large family material comprising more than 5,000 piglets derived from 12 Duroc boars crossed with 236 Danish Landrace/Danish Large White sows. The SNPs were identified in sequence alignments of 4,600 different amplicons obtained from the 12 boars and containing coding regions of genes derived from expressed sequence tags (ESTs) and genomic shotgun sequences.

Results: Linkage maps of all 18 porcine autosomes were constructed based on 456 gene-associated and six porcine EST-based SNPs. The total length of the averaged-sex whole porcine autosome was estimated to 1,711.8 cM resulting in an average SNP spacing of 3.94 cM. The female and male maps were estimated to 2,336.1 and 1,441.5 cM, respectively. The gene order was validated through comparisons to the cytogenetic and/or physical location of 203 genes, linkage to evenly spaced microsatellite markers as well as previously reported conserved synteny. A total of 330 previously unmapped genes and ESTs were mapped to the porcine autosome while ten genes were mapped to unexpected locations.

Conclusion: The linkage map presented here shows high accuracy in gene order. The pedigree family network as well as the large amount of meiotic events provide good reliability and make this map suitable for QTL and association studies. In addition, the linkage to the RH-map of microsatellites makes it suitable for comparison to other QTL studies.

Background

Genetic linkage maps are essential tools for locating genes and quantitative trait loci (QTLs) that control important traits. The first linkage map covering all 18 autosomes of the pig was published in 1995 [1], followed by a large map containing approximately 1,200 markers [2]. These maps were primarily constructed on the basis of anonymous microsatellites and restriction fragment length polymorphism (RFLP) markers [1-3]. Other marker types including amplified fragment length polymorphisms (AFLP) and single nucleotide polymorphisms (SNPs) have been added to online versions of the maps [4].

SNP-based genetic variation is found with high density throughout the genome. Efficient technologies have been developed, which allow for highly parallel and cost efficient genotyping, SNPs have therefore become the markers of choice for genetic mapping. This makes SNP maps highly suitable for association studies, fine mapping of QTLs as well as haplotype determination. Moreover, to identify the genes underlying monogenic and quantitative traits, it is an advantage if the maps are based on gene-associated markers, such as genic SNPs. Genic SNPs, whether they are located in coding or in 5' and 3' untranslated regions are more likely to cause a functional change than those that occur outside genes [5]. Due to linkage disequilibrium intergenic SNPs closely linked to causative mutations in genes of interest can be of value [6]. The use of gene-associated SNPs implies an increased knowledge of the genomic region of interest and facilitates the possibility of identifying candidate genes and the actual genes that underlie the trait. At present, more than one million porcine expressed sequence tags (ESTs) are available [7], and tools to evaluate and select candidate SNPs in coding regions for application as genetic markers have been developed [8].

The pig genome has more conserved synteny with human than with mouse [9] and many of the porcine ESTs are orthologs of parts of human genes. Human-pig comparative maps based on ESTs exclusively [10] as well as ESTs in combination with bacterial artificial chromosome (BAC) end sequences [11] have been constructed using the INRA-Minnesota porcine Radiation Hybrid panel, IMpRH₇₀₀₀ [12]. This panel is a valuable tool for map refinement as it allows good precision in mapping. Moreover, a highly continuous BAC map of the pig genome has been developed [13]. Gene annotation, map positions and order of SNP markers developed from ESTs can be verified using the information derived from the BAC maps or from genes and sequences mapped in humans. In addition, comparison to human genes is a tool to link characteristics to causative genes in future QTL studies. Especially for insufficiently described regions, the comparative mapping provides the possibility of identifying central genes through programmes like GeneDistiller [14].

Previously, SNPs have been used for mapping only selected parts of the pig genome [15-17]. Here we present a linkage map covering all 18 porcine autosomes. The map is based on a large number of offspring and a high number of meioses that is suitable to establish gene order and genetic distances. In addition to application in gene-based genome-wide QTL and association studies, a map based on SNPs developed from EST sequence data is of value for the porcine genome project, providing information for validation of assembly and ordering of sequenced regions. Furthermore, this SNP map is useful as an anchoring map for future dense maps based on data from the PorcineSNP60 Genotyping BeadChip [18] (WG-410-1001-PRE, Illumina) due to marker overlap.

Results

SNP selection and genetic map

The 4,600 exons, from which the SNPs were identified, were distributed on all 22 human chromosomes. A total of 709 SNPs were initially detected in sequence alignments of the gene associated amplicons derived from EST and shotgun sequences. If a SNP segregated in at least one of the 12 Duroc boar families it was selected for further analysis. Of these, 506 SNPs were annotated prior to the mapping process. The remaining 203 SNPs were rejected either because of failure in assay design or low call rate in the genotyping assays. During the annotation six SNPs were discarded due to similarity and artefact problems. Six SNPs (Additional file 1) were not similar to any known gene or EST in the human genome and were mapped as porcine EST-based SNPs (designated as P followed by four digits).

After annotation of the SNP surrounding sequence the allelic structure of the SNPs were analysed. A between-family analysis showed differences that could only be accounted for by genotyping mistakes. Differences in how the resulting clusters were interpreted emerged because it was not always clear which cluster was heterozygote and which was homozygote if only two clusters were present. The data set was therefore reduced to 481 SNPs.

The two-point analysis resulted in 18 large linkage groups (LOD > 75) each assigned to one of the porcine chromosomes using the comparative map from INRA [19]. In addition, seven small linkage groups with an average of three SNPs in each were produced. Comparative mapping indicated that the largest of these, which comprised five SNPs located in the genes *USP24*, *EIF2B3*, *OMA1*, *CPT2*, and *GPX7*, was associated with the distal part of porcine chromosome 6 (*Sus scrofa* chromosomes, SSC 6). A few individual SNPs were assigned to linkage groups based on slightly lower LOD scores (LOD > 55) together with comparative association, whereas the remaining small linkage groups and singletons were rejected as a consequence of low linkage ability. Finally, a total of 462 SNPs, distrib-

uted on 440 different genes, and six EST sequences were mapped to the 18 porcine autosomes (SSC 1 to 18).

The sex-averaged map covered 1,711.8 cM with an average SNP distance of 3.94 cM, whereas the female map covered 2,336.1 cM and the male map 1,441.5 cM (Table 1). The chromosome length of the sex-averaged maps ranged from 15.7 cM for SSC 11 and 151.4 cM for SSC 1, and the number of SNPs on each chromosome map varied between six and 57 on SSC 11 and SSC 13, respectively (Table 1). A comparison of the sex-averaged, female and male maps is illustrated in Figure 1. The exact SNP position on these maps as well as information regarding MAF in the sows, number of meioses and heterozygous sires are indicated in Additional file 2.

The BAC assembly sequence of each chromosome is shown in relation to the genetic linkage maps as well as human reference sequences (Figures 2, 3, 4, 5 and 6). Microsatellites linked to evenly spaced markers using the IMPRH7000 on each SSC are also presented in the figures.

SNP localisation

A total of 97.8% of the sequences was matched to genes by Blast analysis against the refGene database. The remaining sequences were not matched to the available human exon information and as a consequence the genic status of these were classified as unknown (Table 2).

Validation through comparative mapping

Comparison of our linkage map and the RH-based comparative map from INRA indicated a high correlation with

the expected pattern of gene localisation. The marker gene order of the genetic map was compared to the order of previously physically and genetically mapped porcine genes. Only one of the genetically mapped genes *F13B* differed from previous results by being mapped to chromosome SSC 10 instead of SSC 4 (Additional file 1). Some genes or regions showed rearrangements on the porcine map compared to the expected order. To verify the linkage mapping the SNP-containing sequences in question were mapped onto the IMPRH₇₀₀₀ panel (Additional file 1).

For a better comparison to the microsatellite map evenly spaced SNP-containing sequences were mapped onto the IMPRH₇₀₀₀ panel (Additional file 1). A few rearrangements in the microsatellite order in relation to our SNP map were found.

Comparisons to the available chromosomes of the BAC assembly indicated a high similarity with the marker order. A total of 119 SNP sequences were found to match the chromosomes SSC 1, 4, 5, 7, 11, 13, 14, 15 and 17 by Blast analysis against the BAC assembly (Additional file 1). The relation between distance of markers on the assembly map and the genetic map was about 1:1 within each chromosome (Figures 2, 3, 4, 5 and 6). However, there was no obvious relation between the lengths of the BAC assembly and the genetic map between chromosomes indicating a difference in recombination rate between the individual chromosomes.

Markers were mapped to previously reported syntenic regions [19] except for three large segmental regions that

Table 1: Length of the sex-averaged, female and male linkage maps.

SSC ¹	Sex-averaged length (cM)	Female length (cM)	Male length (cM)	SNP ² count	Average distance (cM)
1	151.4	181.7	170.2	26	5.82
2	150.7	201.2	127.7	41	3.68
3	100.5	121.9	87.3	20	5.03
4	137.6	183.8	109.9	40	3.44
5	53.4	89.7	38.4	14	3.81
6	148	177.9	121	50	2.96
7	64.2	88.5	43.7	29	2.21
8	107.4	126.9	92.4	19	5.65
9	116.6	138.9	98.4	27	4.32
10	139.5	208.1	102.5	20	6.98
11	15.7	12.1	20	6	2.62
12	69.8	107.9	39.1	17	4.11
13	128	124.5	131.3	57	2.25
14	96.3	103.3	89.9	28	3.44
15	100.3	156.2	77.4	31	3.24
16	38.1	57.6	18.5	13	2.93
17	60.2	205.8	42.6	17	3.54
18	34.1	50.1	31.2	7	4.87
Total	1711.8	2336.1	1441.5	462	3.94

¹ *Sus scrofa* chromosome ² Single nucleotide polymorphisms

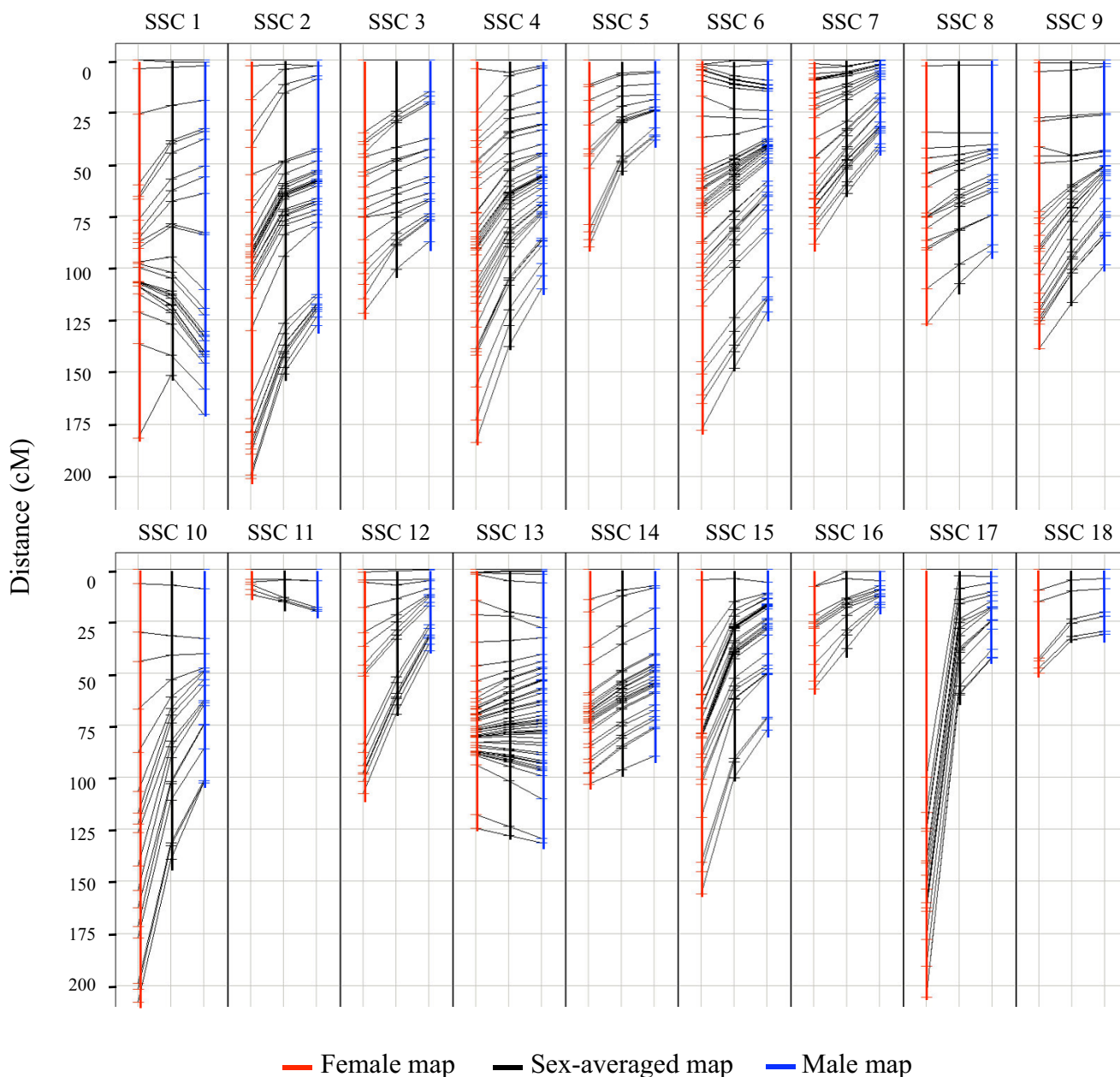


Figure 1
Female, sex-averaged and male linkage maps of the 18 porcine autosomes. Each chromosome is presented showing the relation in distance between the markers on the female, sex-averaged and male linkage maps.

was not identified in our study, two at SSC 1 from human chromosomes (*Homo sapiens*, HSA) 18 and 14, and one at SSC 5 equal to HSA 22q12-qter. None of the genes that were located in the linkage group matching SSC 12 have previously been mapped in pig and hence the verification and orientation of this chromosome relies on the comparative map and linkage to microsatellites located on SSC 12. All the genes showed homology to HSA 17p13.3-q24 indicating that these genes most likely should be located on SSC 12. This was further indicated by IMpRH₇₀₀₀ map-

ping of five SNP-containing sequences belonging to the SSC 12 linkage group (Additional file 1 and Figure 4).

Through linkage to the RH-mapped microsatellites [20] it was confirmed that the SNPs covered a large part of the autosome. However, by comparison to the linkage map [2] that relates to the RH-map, about 20% seems to be missing. The map presented here is shorter due to missing markers on parts of SSC5, SSC7, SSC11, SSC12, SSC16 and SSC18.

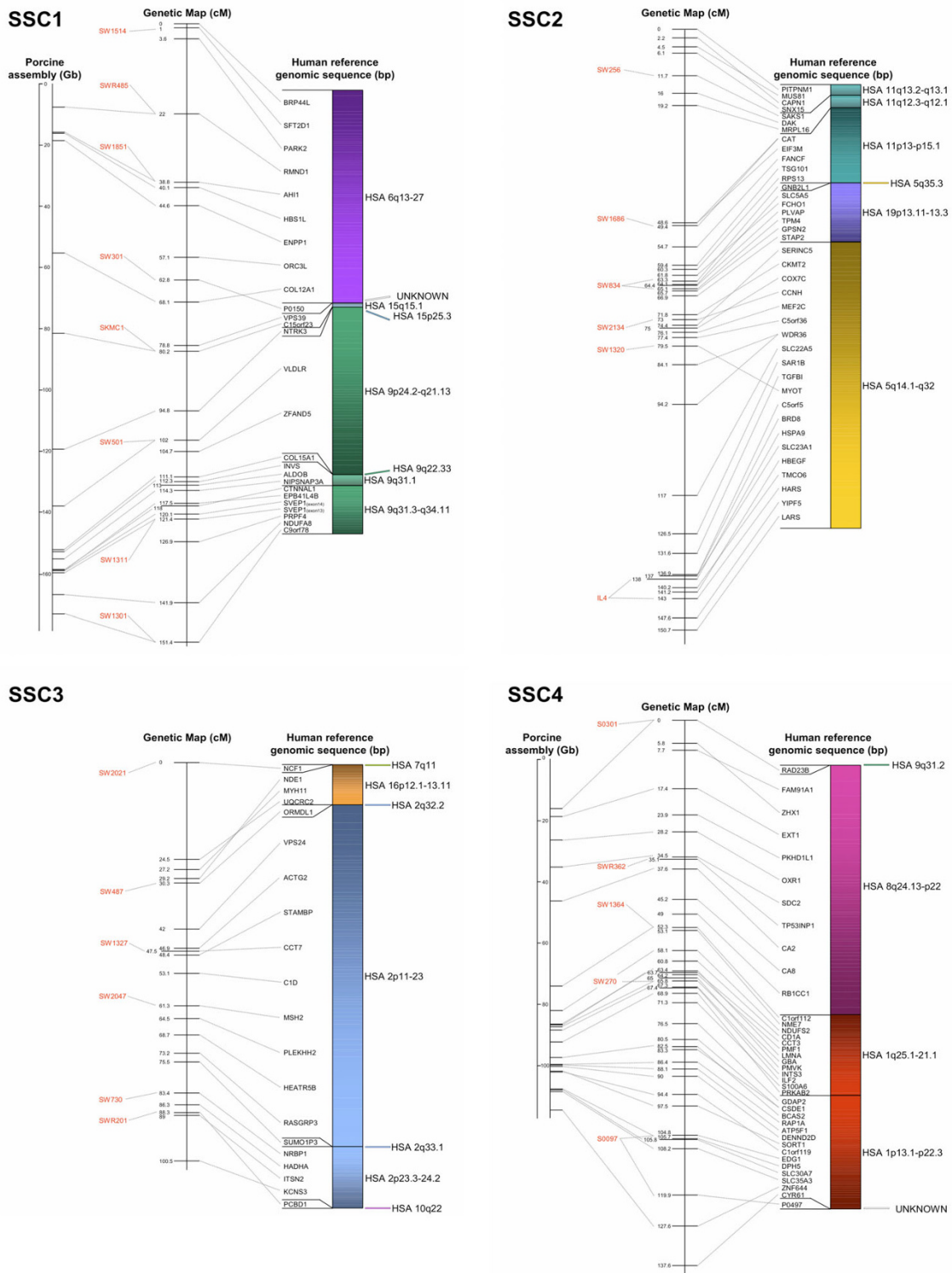


Figure 2
Averaged-sex linkage maps of porcine chromosome I to 4. Each linkage map (centre) is presented with linked microsatellite markers and if known the BAC assembly map positions (left), and the human reference sequence (right). The genes are presented in the order of the human genome and a link between position on the porcine genetic map and human reference and BAC assembly sequence is indicated by a grey line. The colour scheme for the human chromosomes is found on Figure 6.

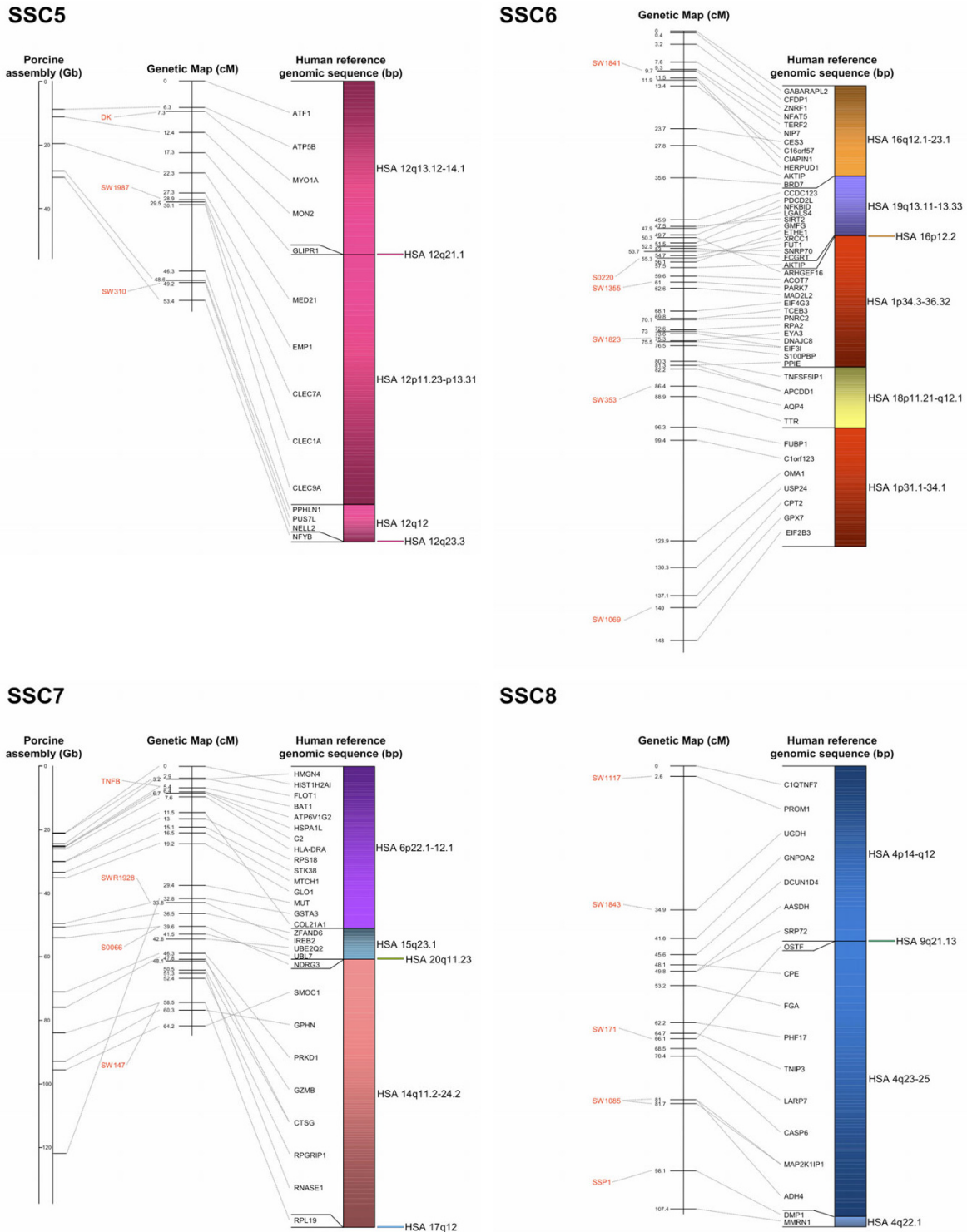
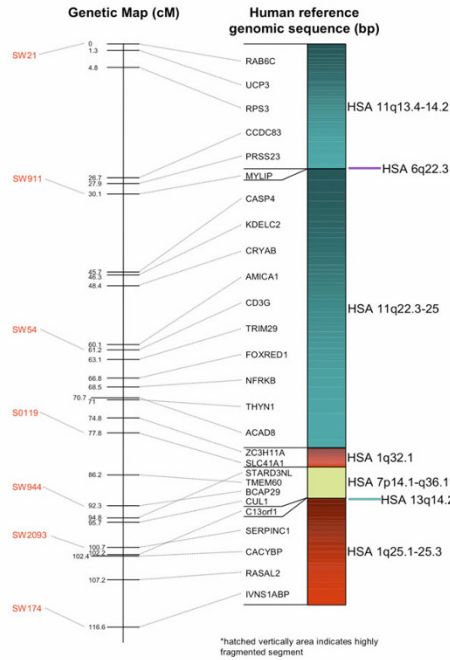
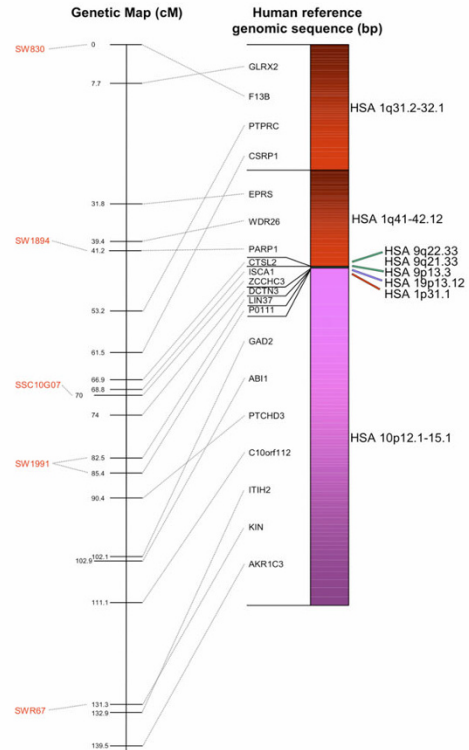


Figure 3
Averaged-sex linkage maps of porcine chromosome 5 to 8. Each linkage map (centre) is presented with linked microsatellite markers and if known the BAC assembly map positions (left), and the human reference sequence (right). The genes are presented in the order of the human genome and a link between position on the porcine genetic map and human reference and BAC assembly sequence is indicated by a grey line. The colour scheme for the human chromosomes is found on Figure 6.

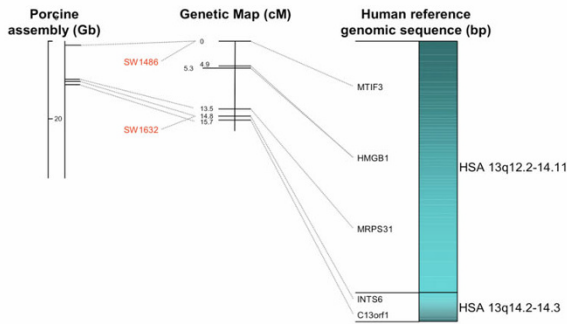
SSC9



SSC10



SSC11



SSC12

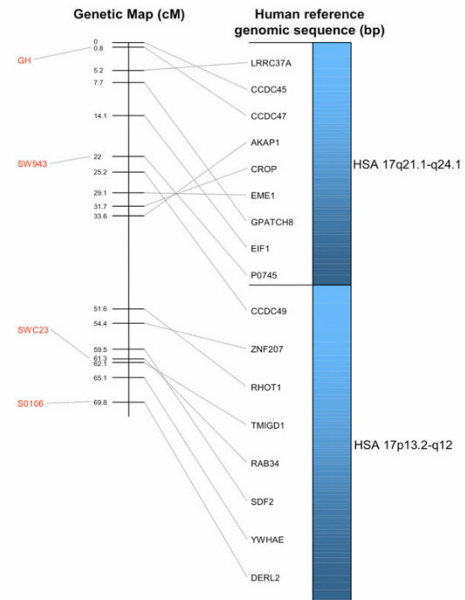
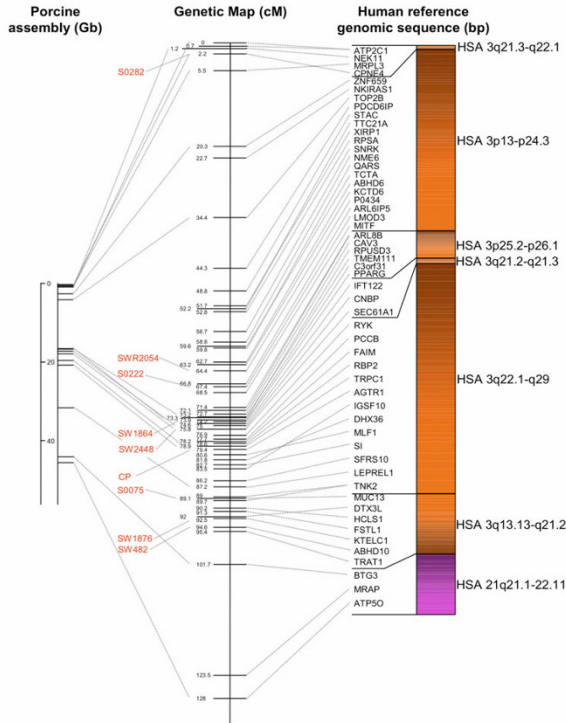


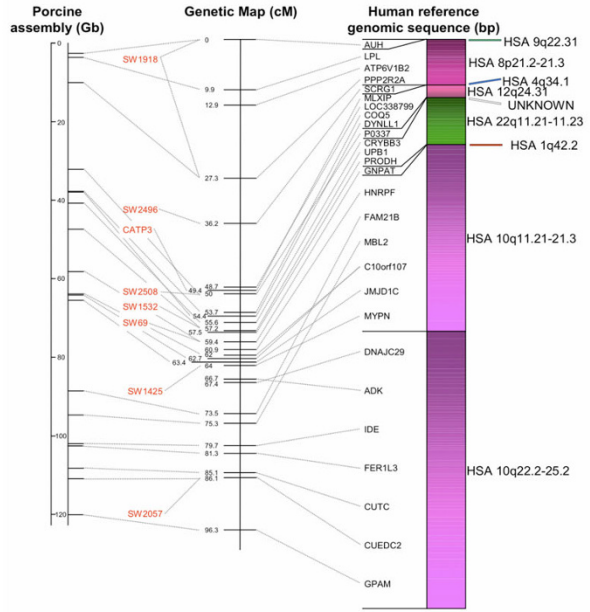
Figure 4

Averaged-sex linkage maps of porcine chromosome 9 to 12. Each linkage map (centre) is presented with linked microsatellite markers and if known the BAC assembly map positions (left), and the human reference sequence (right). The genes are presented in the order of the human genome and a link between position on the porcine genetic map and human reference and BAC assembly sequence is indicated by a grey line. The colour scheme for the human chromosomes is found on Figure 6.

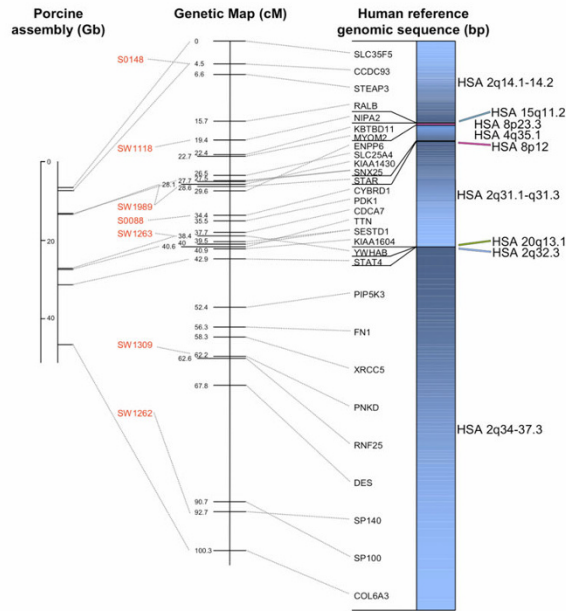
SSC13



SSC14



SSC15



SSC16

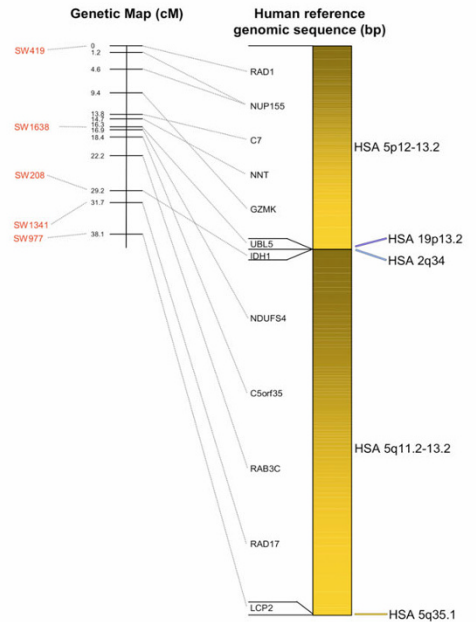
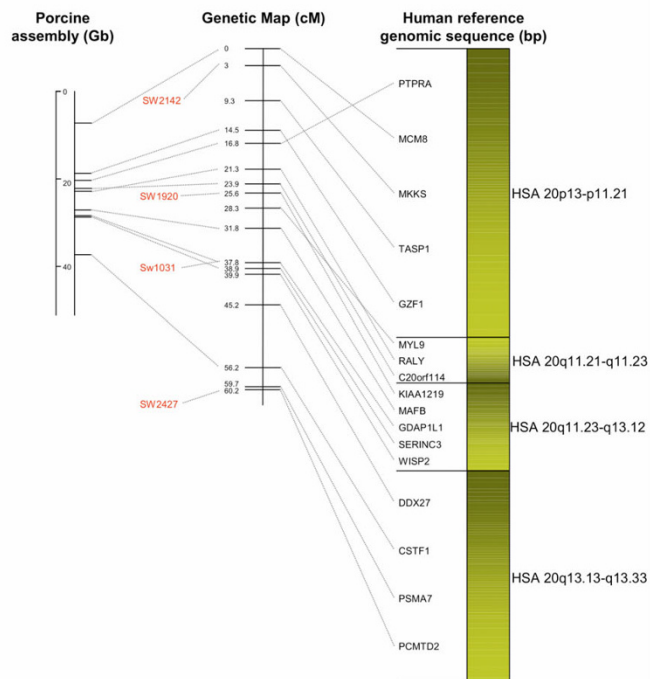


Figure 5

Averaged-sex linkage maps of porcine chromosome 13 to 16. Each linkage map (centre) is presented with linked microsatellite markers and if known the BAC assembly map positions (left), and the human reference sequence (right). The genes are presented in the order of the human genome and a link between position on the porcine genetic map and human reference and BAC assembly sequence is indicated by a grey line. The colour scheme for the human chromosomes is found on Figure 6.

SSC17



SSC18

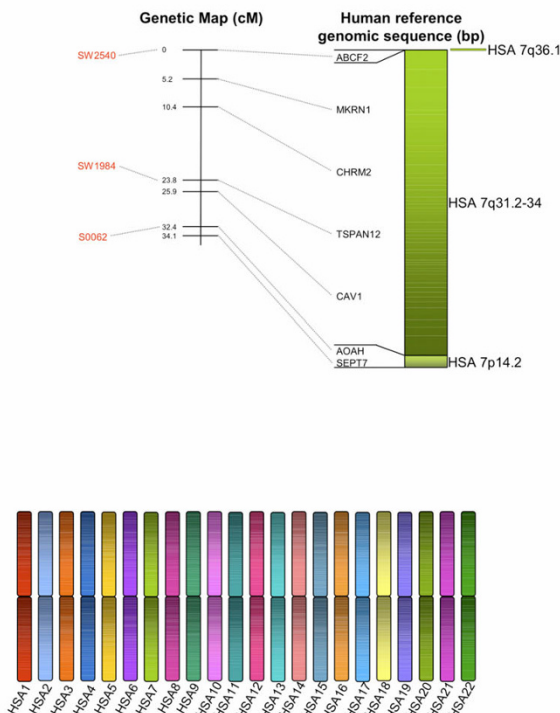


Figure 6

Averaged-sex linkage maps of porcine chromosome 17 and 18. Each linkage map (centre) is presented with linked microsatellite markers and if known the BAC assembly map positions (left), and the human reference sequence (right). The genes are presented in the order of the human genome and a link between position on the porcine genetic map and human reference and BAC assembly sequence is indicated by a grey line. The colour scheme for the human chromosomes is found on Figure 6.

Discussion

The map presented here is the first map of all 18 porcine autosomes based on gene-associated SNPs and it contains 330 not previously located genes. The positions of 81 SNP-containing sequences representing these genes were confirmed by Blast analysis against the newly assembled BAC porcine sequence (Additional file 1).

A total of 456 gene-associated and six porcine EST-based SNPs identified from re-sequenced exons were mapped to

the 18 porcine autosomes. The main selection of the SNPs was unbiased as they were found randomly in the amplicons containing the EST sequences, which were distributed across the 22 human autosomes. Almost all SNPs showed heterozygosity in the sires except 14 SNPs that were included because of interest to other projects. As the SNPs were selected on linkage ability with a very high two-point LOD score (>55) the number of informative meioses is expected to be high. In the present study it ranges from 401 to 6,898 allowing us to calculate a very robust map. The family comprised Duroc sires crossed with Danish Landrace/Danish Large White sows and as the SNPs were selected from alignments of re-sequenced exons in the 12 Duroc sires this implies that most of the informative meioses arose from this population. For the sow population we considered instead the minor allele frequency (MAF) of each SNP which gave us an idea of the distribution between male and female meiosis. The analysis did not indicate that the difference in meiosis numbers affected the results as the number generally was very high (Additional file 2).

Table 2: Single nucleotide polymorphism (SNP) status.

SNP information	# SNP
3'-UTR	52
5'-UTR	3
Synonymous	121
Non-synonymous	53
Intragenic	223
Unknown	10
Total	462

The length of the averaged-sex linkage map was calculated to be 1,711.8 cM, the female map was 2,336.1 cM and the male map was 1,441.5 cM. As described in the result section when comparing to the map of Rohrer and colleagues [2] our map is about 20% shorter. The animal material they used to create their linkage map is much smaller than the material used here, which might influence the recombination rates at the ends of the chromosomes and thereby overestimate the length. We see this phenomenon on the female map when the number of meioses is low like on SSC 17p where the MCM8 marker is positioned 100 cM from MKKS (Figure 1). The first whole-genome map estimated the female map to be about 21 Morgan (M) and the male map around 16.5 M [1]. When these female and male maps are compared a recombination ratio of 1.3:1 is found. Another estimation of the ratio suggests the recombination ratio to be 1.55:1 [3]. In our case the ratio was even higher, approximately 1.65:1, resembling the human recombination ratio of 1.7:1 [21].

When considering each chromosome map of the female and male only three chromosomes separate from the rest (Figure 1). The SSC 11 and the SSC 13 differ from the rest by having a longer male map. Regarding SSC 11, this is in accordance with previous work where a longer p-arm of the SSC 11 male map was found [22]. However, these authors also reported that the complete male map of SSC 11 was shorter than the female map, which could be the case in the present study too if the entire map of SSC 11 was available. On SSC 1 there is a clear difference in how the distance of the markers on the female and male maps varies across the entire chromosome as reported previously [23]. However, the female map presented here is longer than the male map, which is due to a single marker (C9orf78) positioned at the telomeric end of SSC 1q. The MAF found in the sow population of this marker is low leading to few female informative meioses, which can explain the high recombination rate between the markers NDUFA8 and C9orf78 on the female map.

The linkage analysis showed that most synteny groups were present and only few single gene and micro rearrangements were found. Three synteny regions at SSC 1 were not represented on the linkage map, i.e. the HSA 14q21 region and both extremes of HSA 18. The HSA 22q12-qter, which mapped to SSC 5 [19], was also not present on our map. In addition to the absence of these regions, segments from four human chromosomes were missing on SSC 2 (HSA 1), SSC3 (HSA 9) and SSC 17 (HSA 4 and 8) when comparing to the comparative segments identified by Meyers and colleagues [11]. These regions were missing due to the fact that no SNPs were identified in exons representing these genomic areas. However, on SSC 3, 7, 8, 9, 10, 15 and 16 the map presented here contains nine additional regions. In addition,

the telomeric ends of SSC 3 and 4 were divided in more segments than the previous comparative map [11], though in general the present map is divided in fewer segments, probably due to fewer markers. Apart from this, the similarity concerning conserved synteny between these two maps is very high.

All single gene rearrangements and SNPs denoting porcine ESTs were analysed to verify the linkage order. A total of ten genes and six ESTs were mapped onto the IMpRH₇₀₀₀ panel and in all cases the location was confirmed (Figures 2, 3, 4, 5 and 6). The six ESTs were all mapped to the human genomic sequence. In three cases the location matched the expected synteny group but for P0497, P0150 and P0337 the human homologous region could not be determined.

The nine new regions discussed above were mapped onto the IMpRH₇₀₀₀ panel to confirm their locations. These regions refer to the following genes: *PCBD1* from HSA 10q22.1 on SSC 3; *NDRG3* and *RPL19* from HSA 20q11.23 and HSA 17q12, respectively, on SSC 7; *OSTF1* from HSA 9q21.3 on SSC 8; *MYLIP* from 6p22.3 on SSC 9; *LIN37* from HSA 19p13.12 on SSC 10; *YWHAB* from HSA 20q13.12 on SSC 15 and finally *IDH1* and *UBL5* from HSA 2q34 and HSA 19p13.2, respectively, on SSC 16. None of these genes/human regions have previously been mapped to these porcine chromosomal locations [11], but *NDRG3*, *RPL19* and *YWHAB* were confirmed by Blast of the SNP-containing sequence against the recently assembled porcine map [13]. Moreover, all gene locations and orders in relation to other markers were confirmed by linkage to the microsatellite RH-map [20]. Most of the single gene rearrangements were located in regions between larger synteny groups, which also support the calculated marker order on this genetic map.

Of the 440 genes and ESTs located on the map, a total of 110 genes have previously been mapped either by linkage or physically and all of these except one were mapped to the expected chromosome. The *F13B* gene located at HSA 1q31.3 deviated as it was formerly mapped by linkage analysis to SSC 4 [24], but as this fragment of the human genome is known to correspond to a region on SSC 10 [10] the difference is highly likely. The linkage result of *F13B* to SSC 10 was confirmed by mapping the gene onto the IMpRH₇₀₀₀ panel.

Only two previously physically mapped genes were ordered differently than expected. The first was the *TPM4*, which was mapped to SSC 2q24-q29 [25]. In the present study it was positioned further up on the linkage map than *MEF2C* on SSC 2q21-q22 (Figure 2). On the comparative map of this region, only *TPM4* from HSA 19 is located among genes from HSA 5 [19] which indicates that our positioning of the *TPM4* gene among other genes from HSA 19 might

be correct. The other gene that was located at a different position than expected was *FUT1* from SSC 6q11 (Figure 3). This gene was previously cytogenetically mapped closer to the centromeric region than the genes from SSC 6q12 [26]. In our analysis the gene was located more distal, close to the *XRCC1* gene from the same human chromosomal region on HSA 19q13.31. The *XRCC1* gene has previously been mapped to SSC 6q12-22 [27] which might be the actual position of the *FUT1* gene as well.

A total of 81 genes were matched by Blast analysis of SNP-containing sequences against the PreEnsembl porcine BAC sequence. For nine short regions containing one to three markers on the chromosomes, minor rearrangements of the gene order occurred (marked in bold in Additional file 1). Within these regions of minor rearrangements the distance between the markers was small both on the linkage map and on the BAC assembly. The discrepancies could be caused by rearrangements in the BAC assembly of the porcine chromosomes or deviations on the genetic map, due to missing segregations in some boar families despite the SNP network resulting from sows having litters with more than one boar. However, these areas should be subject to further investigation.

This SNP map forms the basis for ongoing studies on QTLs for meat quality, growth, osteochondrosis, lung diseases and other traits. An advantage of the map is the ability to compare to previously reported QTL studies made on microsatellite markers as well as the fact that 138 of the SNPs is represented on the new Illumina PorcineSNP60 Genotyping BeadChip [18]. The actual SNP overlap can be identified through the PorcineSNP60 name (Additional File 2). Therefore, the map can function as an anchoring map for future maps created by use of Bead Chip technology. Furthermore, new SNPs can be added to this version of the map for fine-mapping.

Conclusion

Of the 462 genetically mapped SNPs, a total of 330 genes were located for the first time on the porcine chromosomes. The linkage map of the porcine genome reported here is based on a large number of meioses providing high accuracy in the relative ordering of genes and in the estimate of genetic distances. Only few discrepancies were observed between the present and previous studies. As this map was calculated on gene-associated SNPs the generated SNP map will be valuable for further QTL and association studies of porcine traits with importance for health and production as well as for verifying the genome assembly. In addition, it will be useful as a framework map.

Methods

The family material

A two-generation pedigree was produced by crossing 12 Duroc boars from the Danish breeding program (DanAvl)

with 703 Danish Landrace/Danish Large White sows from three different Danish pig production herds. Boars unrelated to at least the great-grandparent level were chosen based on selection index. Some of the sows were related as aunts or sisters. A total of 1,079 litters with an average of ten piglets per litter were produced and offspring from all boars were represented in all three stable environments. A total of 150 disease and phenotypic traits were registered for each pig. The calculations for this genetic map were performed on 236 sow families since these sows had litters with more than one boar. This provided the possibility for generating accurate genetic maps, even though a close network of SNPs segregating in some of the boar families was missing. Blood samples from the sows were collected in the stables. Tissue samples from tenderloin (*psaos major*) were collected from the piglets and boars at processing. Liver samples were collected from piglets that died before slaughtering. Genomic DNA was isolated from all specimens by treatment with proteinase K followed by sodium chloride precipitation [28].

Exon re-sequencing and primer design

A total of 4,600 candidate exons for SNP detection were selected from alignment of porcine EST sequences from the Sino-Danish Pig Genome Sequencing Project [9]. The human reading frame information was used to capture the exons and the surrounding sequence [29]. PCR primer pairs were designed using Primer3 [30] and purchased from DNA Technology (Aarhus, Denmark). The exons were PCR-amplified in a reaction mixture with a total volume of 10 μ l containing 0.25 mM dNTP, 2.5 mM MgCl₂, 10 \times Buffer II Applied Biosystems, 0.5 U AmpliTaq Gold DNA polymerase (PE, Applied Biosystems, Foster City, California), 5 pmol primer and 20–100 ng of genomic DNA. The cycling conditions were: 94°C for 12 minutes; 10 cycles at 94°C for 15 seconds, 65°C for 15 seconds with a touchdown of 0.5°C per cycle, and 72°C for 15 seconds, followed by 30 cycles with an annealing temperature reaching 60°C, and finally an extension step at 72°C for 7 minutes and storage at 4°C.

DNA sequencing of the 12 Duroc boars was performed using BigDye Terminator v.3.1 Cycle Sequencing with AmpliTaq DNA polymerase FS (ABI PRISM™ Genetic Analyzer Model 3730xl, PE, Applied Biosystems). Automated SNP detection was performed using PolyPhred v4.05 [31] and candidate SNPs were selected after visual inspection of the respective chromatograms. For mapped SNPs one sequence for each allele were submitted to GenBank. Information regarding the ID, primers and sequences surrounding the SNP, sequence ID and GenBank Accession numbers are listed in Additional file 2.

Genotyping

The SNPs were genotyped in the family material either by TaqMan oligo-displacement assay (Assay-by-Design) or

SNPlex Genotyping System (PE, Applied Biosystems). Reactions were carried out according to manufacturer's protocols, using dried DNA in optical 384-well plates. The TaqMan assay fluorescent signals were detected on an ABI PRISM SDS 7900 HT Sequence Detection System. Results were analysed in the SDS 2.1 software for allelic discrimination. The SNPlex assay signals were detected on an automated DNA sequencer (ABI PRISM™ Genetic Analyzer Model 3730xl) and the results were analysed using the GeneMapper software v3.7. Most SNPs that were selected for the map were heterozygous in at least one sire. A total of 14 SNPs were only heterozygous in the sow population but included in the analyses because they were expected to hold interesting characteristics in relation to other projects. The number of informative meioses, the MAFs of the SNPs in the sow population and the number of heterozygous sires for each SNP was calculated and for each SNP the applied assay is given (Additional file 2).

Annotation of the SNP-containing genes

Sequences surrounding the SNPs were subjected to blast analysis on a DeCypher FPGA computer using the accelerated BLASTNH algorithm (Timelogic/Active Motif). To get the human homologous genes the refGene database retrieved from the UCSC Genome Browser database was used [29,32]. The hg18 (NCBI build36.1) exon FASTA sequences were retrieved from the UCSC Table Browser [33,34] and used for SNP annotation. For SNP-containing sequences with no match to any known genes the representative EST sequence was used. For all sequences the cytogenetic location and physical position of the human homologue were obtained from Ensembl [35]. Information on SNP-containing genes is available in Additional file 2.

Linkage analysis

The averaged-sex, female and male linkage maps were calculated using CRIMAP v. 2.50, which is a revised version of CRIMAP v. 2.4 [36] modified by Jill Maddox and Ian Evans (Jill Maddox, University of Melbourne, pers. comm.). Pair-wise linkage analysis was performed with the TWOPOINT function. The SNPs were initially divided into linkage groups based on the LOD threshold (LOD > 75). However, a few SNPs were added to the linkage groups with a LOD > 55. The BUILD option was used to determine the best order of the SNPs in each linkage group. Subsequently, multipoint linkage analysis was performed to determine the most significant position of the SNPs in each linkage group by sequential insertion of SNPs using the ALL option. The FLIPS option was used to ensure correctness of the order. The order of markers in each linkage group was confirmed using the flips4 option. The comparative map between human and porcine segments from INRA [19] was used as an initial guide to link each linkage group to the 18 porcine autosomes.

Literature study of gene position

A literature search was conducted to find the positions of the SNP-containing genes previously located on the porcine autosomes. This was done to validate the marker order that was calculated on our map. The previously mapped genes (listed according to the map order) are: *PARK2* [37], *ENPP1* [38], *NTRK3* [39], *VLDLR* [40], *COL15A1* [41], *ALDOB* [42], *C9orf78* [43], *CAPN1* [25], *CAT* [44], *RPS13* [45], *GNB2L1* [46], *TPM4* [25], *CKMT2* [47], *MEF2C* [48], *SLC22A5* [16], *SAR1B* [49], *HBEGF* [50], *HARS* [51], *NCF1* [52], *UQCRC2* [53], *ACTG2* [54], *KCNS3* [42], *ZHX1* [55], *EXT1* [56], *OXR1* [55], *NDUFS2* [55], *LMNA* [57], *CCT3* [58], *PMF1* [59], *GBA* [60], *PRKAB2* [61], *S100A6* [40], *ATP5F1* [53], *EDG1* [62], *SLC35A3* [63], *ATP5B* [44], *EMP1* [64], *NELL2* [64], *NFYB* [65], *NFAT5* [66], *TERF2* [53], *SIRT2* [67], *LGALS4* [67], *GMFG* [67], *FCGRT* [68], *XRCC1* [27], *FUT1* [26], *PARK7* [69], *MAD2L2* [69], *EIF4G3* [69], *RPA2* [69], *TTR* [70], *FUBP1* [71], *FLOT1* [72], *BAT1* [19], *HSPA1L* [19], *ATP6V1G2* [73], *COL21A1* [74], *RPS18* [75], *STK38* [74], *MTCH1* [74], *GLO1* [74], *MUT* [76], *GSTA3* [74], *GZMH* [19], *CPE* [77], *FGA* [1], *UCP3* [44], *CRYAB* [44], *SERPINC1* [78], *F13B* [24], *CTSL2* [79], *DCTN3* [80], *PTCHD3* [81], *GAD2* [82], *ITIH2* [83], *AKR1C3* [84], *HMGB1* [19], *MRPS31* [17], *INTS6* [85], *TOP2B* [86], *MITF* [87], *ARL8B* [19], *CAV3* [88], *PPARG* [89], *RYK* [90], *PCCB* [86], *RBP2* [19], *AGTR1* [40], *SI* [86], *MUC13* [91], *HCLS1* [92], *BTG3* [93], *LPL* [94], *HNRPF* [95], *MYPN* [96], *MBL2* [97], *MYOM2* [47], *SLC25A4* [19], *SNX25* [10], *STAR* [98], *TTN* [47], *FN1* [99], *DES* [99], *NNT* [100], *RALY* [101] and *CHRM2* [102]. The actual positions are given in Additional file 1.

Validation of the linkage map

After ordering the annotated SNP-containing genes, the homologous genes were used to analyse the known conserved synteny between human and pig of each porcine chromosome [19]. Rearrangements in segments within and between the chromosomes were registered. Single SNP rearrangements were verified on the IMpRH₇₀₀₀ panel [12,103]. Localisation of the genes was considered according to genes mapped previously. Sequences were analysed by blast [104] against the porcine BAC assembly sequence (pig PreEnsembl, version 20071221142932) [13] to identify potential discrepancies and finally validate marker order.

Linkage to microsatellite markers

Since this is the first porcine SNP-map, a total of 104 evenly distributed markers from the 18 porcine linkage groups were linked to microsatellites or genes using the whole-genome radiation hybrid IMpRH₇₀₀₀ panel. Markers were mapped to the panel comprising 118 hybrid clones (90 clones plus 28 complementary clones) using the IMpRH database [103]. In the two-point analysis the

markers were linked to a chromosome by use of the LOD option. The position on the RH map was determined by use of the "linkage of markers to chromosome" option [20]. This mapping facilitates the comparisons of the positions of future QTLs identified by the use of our gene-associated SNP map with results from the whole-genome porcine radiation hybrid map [20].

Authors' contributions

CB conceived the research and coordinated the project. FP, HH and BZ contributed to annotation of exons, KL to primer design and XW, RKKV, AH and LBM to SNP selection. FP, HH and VRG developed the data pipeline. AH, RKKV and VRG performed the genotyping and VRG validated the data. RKKV and VRG made the linkage groups and RKKV constructed and outlined the linkage maps. BZ, VRG and RKKV annotated the sequences. VRG retrieved reference information for the gene position study, and VRG, RKKV and KKS drafted the manuscript. All authors have read and approved the final manuscript.

Additional material

Additional file 1

Gene location. Genes linked to the microsatellites on the IMPRH₇₀₀₀ mapped previously by linkage and physically either by RH panels, somatic-cell hybrids or by Blast analysis of the reference sequence against the pig BAC assembly. The genes are listed in chromosomal (SSC) order. Rearrangements according to the microsatellite map or BAC assembly positions are indicated in bold.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2164-10-134-S1.xls>]

Additional file 2

Data about the SNPs. The data file contains the following information on each SNP used in the study: SSC number, SNP ID, SNP assays, gene name, sex-averaged distance, female distance, male distance, number of meioses, sow minor allele frequency, number of heterozygous sires, forward and reverse primer sequences, human cytogenetic position, human accession number, physical human location, SNP-containing sequences, SNP status, accession number and name on the PorcineSNP60.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2164-10-134-S2.xls>]

Acknowledgements

This work was funded by the Danish Meat Association and Danish Ministry of Food, Agriculture and Fisheries. The authors thank Mette Jeppesen, Hanne Jørgensen, Helle Jensen, Søren Svendsen, Susanne Østerbye Lousdal and Rikke Barsdal for excellent technical assistance.

References

- Archibald AL, Haley CS, Brown JF, Couperwhite S, McQueen HA, Nicholson D, Coppieters W, Weghe A Van de, Stratil A, Wintero AK: **The PiGMaP consortium linkage map of the pig (*Sus scrofa*)**. *Mamm Genome* 1995, **6**:157-175.
- Rohrer GA, Alexander LJ, Hu Z, Smith TP, Keele JW, Beattie CW: **A comprehensive map of the porcine genome**. *Genome Res* 1996, **6**:371-391.
- Mikawa S, Akita T, Hisamatsu N, Inage Y, Ito Y, Kobayashi E, Kusumoto H, Matsumoto T, Mikami H, Minezawa M, Miyake M, Shimanuki S, Sugiyama C, Uchida Y, Wada Y, Yanai S, Yasue H: **A linkage map of 243 DNA markers in an intercross of Gottingen miniature and Meishan pigs**. *Anim Genet* 1999, **30**:407-417.
- ArkDB [<http://www.thearkdb.org>]
- Jorgenson E, Witte JS: **A gene-centric approach to genome-wide association studies**. *Nat Rev Genet* 2006, **7**:885-891.
- Brookes AJ: **The essence of SNPs**. *Gene* 1999, **234**:177-186.
- Gorodkin J, Cirera S, Hedegaard J, Gilchrist MJ, Panitz F, Jorgensen C, Scheibye-Knudsen K, Arvin T, Lumholdt S, Sawera M, Green T, Nielsen BJ, Havgaard JH, Rosenkilde C, Wang J, Li H, Li R, Liu B, Hu S, Dong W, Li W, Yu J, Wang J, Staefeldt HH, Wernersson R, Madsen LB, Thomsen B, Hornshoj H, Bujie Z, Wang X, Wang X, Bolund L, Brunak S, Yang H, Bendixen C, Fredholm M: **Porcine transcriptome analysis based on 97 non-normalized cDNA libraries and assembly of 1,021,891 expressed sequence tags**. *Genome Biol* 2007, **8**:R45.
- Panitz F, Stengaard H, Hornshoj H, Gorodkin J, Hedegaard J, Cirera S, Thomsen B, Madsen LB, Hoj A, Vingborg RK, Zahn B, Wang X, Wang X, Wernersson R, Jorgensen CB, Scheibye-Knudsen K, Arvin T, Lumholdt S, Sawera M, Green T, Nielsen BJ, Havgaard JH, Brunak S, Fredholm M, Bendixen C: **SNP mining porcine ESTs with MAVIANT, a novel tool for SNP evaluation and annotation**. *Bioinformatics* 2007, **23**:i387-i391.
- Wernersson R, Schierup MH, Jorgensen FG, Gorodkin J, Panitz F, Staefeldt HH, Christensen OF, Mailund T, Hornshoj H, Klein A, Wang J, Liu B, Hu S, Dong W, Li W, Wong GK, Yu J, Wang J, Bendixen C, Fredholm M, Brunak S, Yang H, Bolund L: **Pigs in sequence space: a 0.66X coverage pig genome survey based on shotgun sequencing**. *BMC Genomics* 2005, **6**:70.
- Rink A, Santschi EM, Eyer KM, Roelofs B, Hess M, Godfrey M, Karajusuf EK, Yerle M, Milan D, Beattie CW: **A first-generation EST RH comparative map of the porcine and human genome**. *Mamm Genome* 2002, **13**:578-587.
- Meyers SN, Rogatcheva MB, Larkin DM, Yerle M, Milan D, Hawken RJ, Schook LB, Beever JE: **Piggy-BACing the human genome II. A high-resolution, physically anchored, comparative map of the porcine autosomes**. *Genomics* 2005, **86**:739-752.
- Yerle M, Pinton P, Robic A, Alfonso A, Palvadeau Y, Delcros C, Hawken R, Alexander L, Beattie C, Schook L, Milan D, Gellin J: **Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs**. *Cytogenet Cell Genet* 1998, **82**:182-188.
- Humphray SJ, Scott CE, Clark R, Marron B, Bender C, Camm N, Davis J, Jenks A, Noon A, Patel M, Sehra H, Yang F, Rogatcheva MB, Milan D, Chardon P, Rohrer G, Nonneman D, de J, Meyers SN, Archibald A, Beever JE, Schook LB, Rogers J: **A high utility integrated map of the pig genome**. *Genome Biol* 2007, **8**:R139.
- Seelow D, Schwarz JM, Schuelke M: **GeneDistiller – distilling candidate genes from linkage intervals**. *PLoS ONE* 2008, **3**:e3874.
- Jungierus BJ, Rattink AP, Crooijmans RP, Poel JJ van der, van Oost BA, te Pas MF, Groenen MA: **Development of a single nucleotide polymorphism map of porcine chromosome 2**. *Anim Genet* 2003, **34**:429-437.
- Mote BE, Rothschild MF: **SNP detection and linkage mapping for pig genes involved in growth**. *Anim Genet* 2006, **37**:295-296.
- Sawera M, Cirera S, Jorgensen CB, Gorodkin J, Fredholm M: **Linkage mapping of gene-associated SNPs to pig chromosome 11**. *Anim Genet* 2006, **37**:199-204.
- Ramos PL, Crooijmans RP, Amaral AJ, Bendixen C, Hedegaard J, Rohrer G, Smith C, Tassel van J, Taylor M, Groenen MAM: **High throughput SNP discovery and validation in the pig: towards the development of a high density swine SNP chip [abstract]**. *Pig Genome II, Ljubljana, Slovenia, 4 – 5 June, 2008* 2008 [<http://library.wur.nl/WebQuery/wurpubs/lang/372472>].
- Hayes H, Elduque C, Gautier M, Schibler L, Criubu E, Eggen A: **Mapping of 195 genes in cattle and updated comparative map with man, mouse, rat and pig**. *Cytogenet Genome Res* 2003, **102**:16-24.
- Hawken RJ, Murtaugh J, Flickinger GH, Yerle M, Robic A, Milan D, Gellin J, Beattie CW, Schook LB, Alexander LJ: **A first-generation porcine whole-genome radiation hybrid map**. *Mamm Genome* 1999, **10**:824-830.

21. Morton NE: **Parameters of the human genome.** *Proc Natl Acad Sci USA* 1991, **88**:7474-7476.
22. Riquet J, Milan D, Woloszyn N, Schmitz A, Pitel F, Frelat G, Gellin J: **A linkage map with microsatellites isolated from swine flow-sorted chromosome 11.** *Mamm Genome* 1995, **6**:623-628.
23. Beeckmann P, Schroffel J, Moser G, Bratenschlager H, Reiner G, Geldermann H: **Linkage and QTL mapping for *sus scrofa* chromosome 1.** *Journal of Animal Breeding Genetics* 2003, **120(Suppl 1)**:1-10.
24. Marklund L, Johansson MM, Hoyheim B, Davies W, Fredholm M, Juneja RK, Mariani P, Coppeters W, Ellegren H, Andersson L: **A comprehensive linkage map of the pig based on a wild pig-Large White intercross.** *Anim Genet* 1996, **27**:255-269.
25. Fridolfsson AK, Hori T, Wintero AK, Fredholm M, Yerle M, Robic A, Andersson L, Ellegren H: **Expansion of the pig comparative map by expressed sequence tags (EST) mapping.** *Mamm Genome* 1997, **8**:907-912.
26. Meijerink E, Fries R, Vogeli P, Masabanda J, Wigger G, Stricker C, Neuenschwander S, Bertschinger HU, Stranzinger G: **Two alpha(1,2) fucosyltransferase genes on porcine chromosome 6q11 are closely linked to the blood group inhibitor (S) and Escherichia coli F18 receptor (ECF18R) loci.** *Mamm Genome* 1997, **8**:736-741.
27. Bosak N, Faraut T, Mikawa S, Uenishi H, Kiuchi S, Hiraiwa H, Hayashi T, Yasue H: **Construction of a high-resolution comparative gene map between swine chromosome region 6q11-->q21 and human chromosome 19 q-arm by RH mapping of 51 genes.** *Cytogenet Genome Res* 2003, **102**:109-115.
28. Sambrook J, Fritsch EF, Maniatis T: **Molecular Cloning: A Laboratory Manual.** Edited by: Ford N, Nolan C, Ferguson M. New York: Cold Spring Harbor Laboratory Press; 1989:16-19.
29. Pruitt KD, Tatusova T, Maglott DR: **NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins.** *Nucleic Acids Res* 2007, **35**:D61-D65.
30. Rozen S, Skaletsky H: **Primer3 on the WWW for general users and for biologist programmers.** *Methods Mol Biol* 2000, **132**:365-386.
31. Nickerson DA, Tobe VO, Taylor SL: **PolyPhred: automating the detection and genotyping of single nucleotide substitutions using fluorescence-based resequencing.** *Nucleic Acids Res* 1997, **25**:2745-2751.
32. Karolchik D, Baertsch R, Diekhans M, Furey TS, Hinrichs A, Lu YT, Roskin KM, Schwartz M, Sugnet CW, Thomas DJ, Weber RJ, Haussler D, Kent WJ: **The UCSC Genome Browser Database.** *Nucleic Acids Res* 2003, **31**:51-54.
33. Karolchik D, Hinrichs AS, Furey TS, Roskin KM, Sugnet CW, Haussler D, Kent WJ: **The UCSC Table Browser data retrieval tool.** *Nucleic Acids Res* 2004, **32**:D493-D496.
34. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, Levine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendt MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubinfeld M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Raymond C, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramsay J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blocker H, Hornischer K, Nordsieck G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kasprzyk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de JP, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ: **Initial sequencing and analysis of the human genome.** *Nature* 2001, **409**:860-921.
35. Hubbard T, Andrews D, Caccamo M, Cameron G, Chen Y, Clamp M, Clarke L, Coates G, Cox T, Cunningham F, Curwen V, Cutts T, Down T, Durbin R, Fernandez-Suarez XM, Gilbert J, Hammond M, Herrero J, Hotz H, Howe K, Iyer V, Jekosch K, Kahari A, Kasprzyk A, Keefe D, Keenan S, Kokocinski F, London D, Longden I, McVicker G, Melsopp C, Meidl P, Potter S, Proctor G, Rae M, Rios D, Schuster M, Searle S, Severin J, Slater G, Smedley D, Smith J, Spooner W, Stabenau A, Stalker J, Storey R, Trevanion S, Ureta-Vidal A, Vogel J, White S, Woodward C, Birney E: **Ensembl 2005.** *Nucleic Acids Res* 2005, **33**:D447-D453.
36. Green P, Falls K, Crooks S: **Documentation for CRIMAP. (version 2.4).** St Louis, Missouri, Washington University School of Medicine; 1990.
37. Bjerre D, Madsen LB, Bendixen C, Larsen K: **Porcine Parkin: molecular cloning of PARK2 cDNA, expression analysis, and identification of a splicing variant.** *Biochem Biophys Res Commun* 2006, **347**:803-813.
38. Grapes L, Rothschild MF: **Investigation of a QTL region for loin eye area and fatness on pig chromosome 1.** *Mamm Genome* 2006, **17**:657-668.
39. Nonneman D, Smith TP, Freking BA, Rohrer GA: **Assignment of 12 genes to porcine chromosome 1 by linkage and radiation hybrid mapping.** *Anim Genet* 2005, **36**:532-533.
40. Pinton P, Schibler L, Cribiu E, Gellin J, Yerle M: **Localization of 113 anchor loci in pigs: improvement of the comparative map for humans, pigs, and goats.** *Mamm Genome* 2000, **11**:306-315.
41. Jorgensen CB, Wintero AK, Yerle M, Fredholm M: **Mapping of 22 expressed sequence tags isolated from a porcine small intestine cDNA library.** *Mamm Genome* 1997, **8**:423-427.
42. Thomsen PD, Wintero AK, Fredholm M: **Chromosomal assignments of 19 porcine cDNA sequences by FISH.** *Mamm Genome* 1998, **9**:394-396.
43. Kuehn LA, Rohrer GA, Nonneman DJ, Thallman RM, Leymaster KA: **Detection of single nucleotide polymorphisms associated with ultrasonic backfat depth in a segregating Meishan x White Composite population.** *J Anim Sci* 2007, **85**:1111-1119.
44. Biltueva LS, Yang F, Vorobieva NV, Graphodatsky AS: **Comparative map between the domestic pig and dog.** *Mamm Genome* 2004, **15**:809-818.
45. Rattink AP, Favre M, Jungerius BJ, Groenen MA, Harlizius B: **A high-resolution comparative RH map of porcine chromosome (SSC) 2.** *Mamm Genome* 2001, **12**:366-370.
46. Lahbib-Mansais Y, Mompert F, Milan D, Leroux S, Faraut T, Delcros C, Yerle M: **Evolutionary breakpoints through a high-resolution comparative map between porcine chromosomes 2 and 16 and human chromosomes.** *Genomics* 2006, **88**:504-512.
47. Davoli R, Fontanesi L, Zambonelli P, Bigi D, Gellin J, Yerle M, Milc J, Braglia S, Cenci V, Cagnazzo M, Russo V: **Isolation of porcine expressed sequence tags for the construction of a first genomic transcript map of the skeletal muscle in pig.** *Anim Genet* 2002, **33**:3-18.
48. Bertani GR, Johnson RK, Robic A, Pomp D: **Mapping of porcine ESTs obtained from the anterior pituitary.** *Anim Genet* 2003, **34**:132-134.
49. Wang XM, Liu B, Zhao SH, Fan B, Zhu MJ, Yu M, Xiong TA, Li K: **Sequence identification, tissue distribution, mapping and polymorphism of the porcine sar1b gene.** *Anim Biotechnol* 2006, **17**:99-107.

50. Aldenhoven J, Spotter A, Distl O: **Radiation hybrid mapping of 10 genes to porcine chromosomes.** *Anim Genet* 2005, **36**:540-542.
51. Chaudhary R, Wintero AK, Fredholm M, Chowdhary BP: **FISH mapping of seven cDNA sequences in the pig.** *Chromosome Res* 1997, **5**:545-549.
52. Jiang Z, Michal JJ, Melville JS, Baltzer HL: **Multi-alignment of orthologous genome regions in five species provides new insights into the evolutionary make-up of mammalian genomes.** *Chromosome Res* 2005, **13**:707-715.
53. Karnuah AB, Uenishi H, Kiuchi S, Kojima M, Onishi A, Yasue H, Mitsuhashi T: **Assignment of 64 genes expressed in 28-day-old pig embryo to radiation hybrid map.** *Mamm Genome* 2001, **12**:518-523.
54. Wimmers K, Lin CL, Tholen E, Jennen DG, Schellander K, Ponsuksili S: **Polymorphisms in candidate genes as markers for sperm quality and boar fertility.** *Anim Genet* 2005, **36**:152-155.
55. Moller M, Berg F, Riquet J, Pomp D, Archibald A, Anderson S, Fave K, Zhang Y, Rothschild M, Milan D, Andersson L, Tuggle CK: **High-resolution comparative mapping of pig Chromosome 4, emphasizing the FAT1 region.** *Mamm Genome* 2004, **15**:717-731.
56. Nonneman D, Rohrer GA: **Linkage mapping of porcine DGAT1 to a region of chromosome 4 that contains QTL for growth and fatness.** *Anim Genet* 2002, **33**:472-473.
57. Wagenknecht D, Stratil A, Bartenschlager H, Van PM, Peelman LJ, Majzlik I, Geldermann H: **SNP identification, linkage and radiation hybrid mapping of the porcine lamin A/C (LMNA) gene to chromosome 4q.** *J Anim Breed Genet* 2006, **123**:280-283.
58. Hiraiwa H, Sawazaki T, Suzuki K, Fujishima-Kanaya N, Toki D, Ito Y, Uenishi H, Hayashi T, Awata T, Yasue H: **Elucidation of correspondence between swine chromosome 4 and human chromosome 1 by assigning 27 genes to the ImpRH map, and development of microsatellites in the proximity of 14 genes.** *Cytogenet Genome Res* 2003, **101**:84-89.
59. Dvorak CM, Flickinger GH, Hendrickson JA, Fahrenkrug SC, Murtaugh MP, Rutherford MS: **Mapping of genes expressed in activated porcine Peyer's patch.** *Anim Genet* 2006, **37**:62-65.
60. Stratil A, Wagenknecht D, Van PM, Kubickova S, Bartenschlager H, Musilova P, Rubes J, Geldermann H, Peelman LJ: **Comparative and genetic analysis of the porcine glucocerebrosidase (GBA) gene.** *Comp Biochem Physiol B Biochem Mol Biol* 2004, **138**:377-383.
61. Jung KC, Yu SL, Chung HJ, Kim TH, Jeon JT, Choi KD, Sang BC, Park CS, Lee JH: **Assignment of protein kinase, AMP-activated, beta 2 non-catalytic subunit (PRKAB2) gene to porcine chromosome 4q21.23 by somatic cell and radiation hybrid panel mapping.** *Cytogenet Genome Res* 2003, **103**:202C.
62. Murphy WJ, Fronicke L, O'Brien SJ, Stanyon R: **The origin of human chromosome 1 and its homologs in placental mammals.** *Genome Res* 2003, **13**:1880-1888.
63. Andersen PK, Veng L, Juul-Madsen HR, Vingborg RK, Bendixen C, Thomsen B: **Gene expression profiling, chromosome assignment and mutational analysis of the porcine Golgi-resident UDP-N-acetylglucosamine transporter SLC35A3.** *Mol Membr Biol* 2007, **24**:519-530.
64. Tuggle CK, Green JA, Fitzsimmons C, Woods R, Prather RS, Malchenko S, Soares BM, Kucaba T, Crouch K, Smith C, Tack D, Robinson N, O'Leary B, Scheetz T, Casavant T, Pomp D, Edeal BJ, Zhang Y, Rothschild MF, Garwood K, Beavis W: **EST-based gene discovery in pig: virtual expression patterns and comparative mapping to human.** *Mamm Genome* 2003, **14**:565-579.
65. Boettcher D, Paul S, Bennowitz J, Swalve HH, Thaller G, Maak S: **Exclusion of NFYB as candidate gene for congenital splay leg in piglets and radiation hybrid mapping of further five homologous porcine genes from human chromosome 12 (HSA12).** *Cytogenet Genome Res* 2007, **118**:67-71.
66. Hebinck A, Dalski A, Engel H, Mattei M, Hawken R, Schwinger E, Zuhlike C: **Assignment of transcription factor NFAT5 to human chromosome 16q22.1, murine chromosome 8D and porcine chromosome 6p1.4 and comparison of the polyglutamine domains.** *Cytogenet Cell Genet* 2000, **90**:68-70.
67. Martins-Wess F, Rohrer G, Voss-Nemitz R, Drogemuller C, Brenig B, Robic A, Yerle M, Milan D, Leeb T: **Generation of a 5.5-Mb BAC/PAC contig of pig chromosome 6q1.2 and its integration with existing RH, genetic and comparative maps.** *Cytogenet Genome Res* 2003, **102**:116-120.
68. Bosak N, Yamamoto R, Fujisaki S, Faraut T, Kiuchi S, Hiraiwa H, Hayashi T, Yasue H: **A dense comparative gene map between human chromosome 19q13.3-->q13.4 and a homologous segment of swine chromosome 6.** *Cytogenet Genome Res* 2005, **108**:317-321.
69. Larsen K, Madsen LB, Hoj A, Bendixen C: **Porcine DJ-1: cloning of PARK7 cDNA, sequence comparison, expression analysis and chromosomal localization.** *Cytogenet Genome Res* 2007, **116**:93-99.
70. Archibald AL, Couperwhite S, Jiang ZH: **The porcine TTR locus maps to chromosome 6q.** *Anim Genet* 1996, **27**:351-353.
71. Lahbib-Mansais Y, Tosser-Klopp G, Leroux S, Cabau C, Karsenty E, Milan D, Barillot E, Yerle M, Hatey F, Gellin J: **Contribution to high-resolution mapping in pigs with 101 type I markers and progress in comparative map between humans and pigs.** *Mamm Genome* 2003, **14**:275-288.
72. Shigenari A, Ando A, Renard C, Chardon P, Shiina T, Kulski JK, Yasue H, Inoko H: **Nucleotide sequencing analysis of the swine 433-kb genomic segment located between the non-classical and classical SLA class I gene clusters.** *Immunogenetics* 2004, **55**:695-705.
73. Renard C, Hart E, Sehra H, Beasley H, Coggill P, Howe K, Harrow J, Gilbert J, Sims S, Rogers J, Ando A, Shigenari A, Shiina T, Inoko H, Chardon P, Beck S: **The genomic sequence and analysis of the swine major histocompatibility complex.** *Genomics* 2006, **88**:96-110.
74. Barbosa A, Demeure O, Urien C, Milan D, Chardon P, Renard C: **A physical map of large segments of pig chromosome 7q11-q14: comparative analysis with human chromosome 6p21.** *Mamm Genome* 2004, **15**:982-995.
75. Tanaka M, Suzuki K, Morozumi T, Kobayashi E, Matsumoto T, Domukai M, Eguchi-Ogawa T, Shinkai H, Awata T, Uenishi H: **Genomic structure and gene order of swine chromosome 7q1.1-->q1.2.** *Anim Genet* 2006, **37**:10-16.
76. Kierstein S, Peters U, Habermann FA, Fries R, Brenig B: **Assignment of the methylmalonyl-CoA mutase gene (MUT) to porcine chromosome 7q13-->q14 by in situ hybridization and analysis of radiation hybrid panels.** *Cytogenet Genome Res* 2003, **101**:92F.
77. Cargill EJ, Baskin LC, Pomp D: **Rapid communication: localization of the porcine carboxypeptidase-E gene by linkage analysis further extends the region of synteny between human chromosome 4 and porcine chromosome 8.** *J Anim Sci* 1998, **76**:2211-2212.
78. Sun HS, Tuggle CK, Goureau A, Fitzsimmons CJ, Pinton P, Chardon P, Yerle M: **Precise mapping of breakpoints in conserved synteny between human chromosome 1 and pig chromosomes 4, 6 and 9.** *Anim Genet* 2002, **33**:91-96.
79. Spotter A, Drogemuller C, Kuiper H, Brenig B, Leeb T, Distl O: **Characterization and comparative mapping of the porcine CTSL gene indicates a novel synteny between HSA9q21-->q22 and SSC10q11-->q12.** *Cytogenet Cell Genet* 2001, **95**:92-96.
80. Aldenhoven J, Chen Y, Backofen B, Moran C: **Improving the comparative map of porcine chromosome 10 with respect to human chromosomes 1, 9 and 10.** *Cytogenet Genome Res* 2003, **102**:121-127.
81. Nonneman DJ, Kuehn LA: **Segmental duplication and copy number variation of the patched domain containing 3 (PTCHD3) locus on pig chromosome 10 [abstract].** *Animal Genome XVI Conference Proceedings January 12-16, San Diego 2008*:578.
82. Nonneman D, Rohrer GA: **Comparative mapping of human chromosome 10 to pig chromosomes 10 and 14.** *Anim Genet* 2004, **35**:338-343.
83. Yasue H, Kiuchi S, Hiraiwa H, Ozawa A, Hayashi T: **Assignment of 101 genes localized in HSA10 to a swine RH (IMpRH) map to generate a dense human-swine comparative map.** *Cytogenet Genome Res* 2006, **112**:121-125.
84. Nonneman DJ, Wise TH, Ford JJ, Kuehn LA, Rohrer GA: **Characterization of the aldo-keto reductase 1C gene cluster on pig chromosome 10: possible associations with reproductive traits.** *BMC Vet Res* 2006, **2**:28.
85. Liu XG, Tamada Y, Shimogiri T, Nishibori M, Hiraiwa H, Yasue H: **Assignment of 56 genes from HSA13q to the porcine IMpRH map.** *Anim Genet* 2007, **38**:184-186.
86. Van PM, Yerle M, Tuggle C, Piumi F, Genet C, Van ZA, Peelman LJ: **Integration of porcine chromosome 13 maps.** *Cytogenet Cell Genet* 2001, **93**:297-303.

87. Okumura N, Hayashi T, Sekikawa H, Matsumoto T, Mikawa A, Hamasima N, Awata T: **Sequencing, mapping and nucleotide variation of porcine coat colour genes EDNRB, MYO5A, KITLG, SLC45A2, RAB27A, SILV and MITF.** *Anim Genet* 2006, **37**:80-82.
88. Zhu Z, Li Y, Mo D, Li K, Zhao S: **Molecular characterization and expression analysis of the porcine caveolin-3 gene.** *Biochem Biophys Res Commun* 2006, **346**:7-13.
89. Grindfleke E, Sundvold H, Lien S, Rothschild MF: **Rapid communication: physical and genetic mapping of the Peroxisome Proliferator Activated Receptor gamma (PPAR-gamma) gene to porcine chromosome 13.** *J Anim Sci* 2000, **78**:1391-1392.
90. Van PM, Yerle M, Chardon P, Jacobs K, Genet C, Mattheeuws M, Van ZA, Peelman LJ: **A refined comparative map between porcine chromosome 13 and human chromosome 3.** *Cytogenet Genome Res* 2003, **102**:133-138.
91. Zhang B, Ren J, Yan X, Huang X, Ji H, Peng Q, Zhang Z, Huang L: **Investigation of the porcine MUC13 gene: isolation, expression, polymorphisms and strong association with susceptibility to enterotoxigenic Escherichia coli F4ab/ac.** *Anim Genet* 2008, **39**:258-266.
92. Wang H, Zhu ZM, Yerle M, Wu X, Wang HL, Gu MS, Li K: **Assignment of three novel genes to porcine chromosome 13 by a radiation hybrid panel.** *Cytogenet Genome Res* 2005, **108**:363.
93. Feng Z, Tang ZL, Li K, Liu B, Yu M, Zhao SH: **Molecular characterization of the BTG2 and BTG3 genes in fetal muscle development of pigs.** *Gene* 2007, **403**:170-177.
94. Gu F, Harbitz I, Chowdhary BP, Davies W, Gustavsson I: **Mapping of the porcine lipoprotein lipase (LPL) gene to chromosome 14q12-q14 by in situ hybridization.** *Cytogenet Cell Genet* 1992, **59**:63-64.
95. Lahbib-Mansais Y, Dalias G, Milan D, Yerle M, Robic A, Gyapay G, Gellin J: **A successful strategy for comparative mapping with human ESTs: 65 new regional assignments in the pig.** *Mamm Genome* 1999, **10**:145-153.
96. Wimmers K, Murani E, te Pas MF, Chang KC, Davoli R, Merks JW, Henne H, Muraniova M, da CN, Harlizius B, Schellander K, Boll I, Braglia S, de Wit AA, Cagnazzo M, Fontanesi L, Prins D, Ponsuksili S: **Associations of functional candidate genes derived from gene-expression profiles of prenatal porcine muscle tissue with meat quality and muscle deposition.** *Anim Genet* 2007, **38**:474-484.
97. Marklund L, Shi X, Tuggle CK: **Rapid communication: mapping of the Mannose-Binding Lectin 2 (MBL2) gene to pig chromosome 14.** *J Anim Sci* 2000, **78**:2992-2993.
98. Splan RK, Pilcik BR, Tomsen UJ, Arango JA, Ruiz-Flores A, Hallock JW, Allan MF, Karnati SK, Vega V, Lindsey BR, Moody DE, Pomp D: **Rapid communication: mapping of the Steroidogenic Acute Regulatory Protein (StAR) gene to porcine chromosome 15 by linkage analysis using a novel PCR-RFLP.** *J Anim Sci* 1998, **76**:658-659.
99. Robic A, Jeon JT, Rey V, Amarger V, Chardon P, Looft C, Andersson L, Gellin J, Milan D: **Construction of a high-resolution RH map of the human 2q35 region on TNG panel and comparison with a physical map of the porcine homologous region 15q25.** *Mamm Genome* 2001, **12**:380-386.
100. Shimogiri T, Kiuchi S, Hiraiwa H, Hayashi T, Takano Y, Maeda Y, Yasue H: **Assignment of 117 genes from HSA5 to the porcine IMpRH map and generation of a dense human-pig comparative map.** *Anim Genet* 2006, **37**:503-508.
101. Drogemuller C, Giese A, Martins-Wess F, Wiedemann S, Andersson L, Brenig B, Fries R, Leeb T: **The mutation causing the black-and-tan pigmentation phenotype of Mangalitzta pigs maps to the porcine ASIP locus but does not affect its coding sequence.** *Mamm Genome* 2006, **17**:58-66.
102. Lahbib-Mansais Y, Leroux S, Milan D, Yerle M, Robic A, Jiang Z, Andre C, Gellin J: **Comparative mapping between humans and pigs: localization of 58 anchorage markers (TOASTs) by use of porcine somatic cell and radiation hybrid panels.** *Mamm Genome* 2000, **11**:1098-1106.
103. Milan D, Hawken R, Cabau C, Leroux S, Genet C, Lahbib Y, Tossier G, Robic A, Hately F, Alexander L, Beattie C, Schook L, Yerle M, Gellin J: **IMpRH server: an RH mapping server available on the Web.** *Bioinformatics* 2000, **16**:558-559.
104. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: **Basic local alignment search tool.** *J Mol Biol* 1990, **215**:403-410.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

