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High-resolution comprehensive radiation hybrid maps of the porcine chromosomes 2p and 9p compared with the human chromosome 11

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Abstract. We are constructing high-resolution, chromosomal 'test' maps for the entire pig genome using a 12,000-rad WG-RH panel (IMNpRH2$_{12,000\text{-rad}}$) to provide a scaffold for the rapid assembly of the porcine genome sequence. Here we present an initial, comparative map of human chromosome (HSA) 11 with pig chromosomes (SSC) 2p and 9p. Two sets of RH mapping vectors were used to construct the RH framework (FW) maps for SSC2p and SSC9p. One set of 590 markers, including 131 microsatellites (MSs), 364 genes/ESTs, and 95 BAC end sequences (BESs) was typed on the IMNpRH2$_{12,000\text{-rad}}$ panel. A second set of 271 markers (28 MSs, 138 genes/ESTs, and 105 BESs) was typed on the IMpRH$_{7,000\text{-rad}}$ panel. The two data sets were merged into a single data-set of 655 markers of which 206 markers were typed on both panels. Two large linkage groups of 72 and 194 markers were assigned to SSC2p, and two linkage groups of 84 and 168 markers to SSC9p at a two-point LOD score of 10. A total of 126 and 114 FW markers were ordered with a likelihood ratio of 1000:1 to the SSC2p and SSC9p RH$_{12,000\text{-rad}}$ FW maps, respectively, with an accumulated map distance of 4046.5 cR$_{12,000}$ and 1355.2 cR$_{7,000}$ for SSC2p, and 4244.1 cR$_{12,000}$ and 1802.5 cR$_{7,000}$ for SSC9p. The kb/cR ratio in the IMNpRH2$_{12,000\text{-rad}}$ FW maps was 15.8 for SSC2p, and 15.4 for SSC9p, while the ratio in the IMpRH$_{7,000\text{-rad}}$ FW maps was 47.1 and 36.3, respectively, or an ~3.0-fold increase in map resolution in the IMNpRH$_{12,000\text{-rad}}$ panel over the IMpRH$_{7,000\text{-rad}}$ panel. The integrated IMNpRH$_{12,000\text{-rad}}$ and IMpRH$_{7,000\text{-rad}}$ maps as well as the genetic and BAC FPC maps provide an inclusive comparative map between SSC2p, SSC9p and HSA11 to close potential gaps between contigs prior to sequencing, and to identify regions where potential problems may arise in sequence assembly.

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Sequence assembly of the human, mouse and rat genomes required high resolution comprehensive maps of each genome. Genome maps for livestock have not reached the resolution available for the human (Olivier et al., 2001; Venter et al., 2001), mouse (Gregory et al., 2002) and rat genome (Kwitek et al., 2001; Moller et al., 2004). One efficient way to improve the resolution of the porcine physical map is to combine comparative and radiation hybrid (RH) mapping approaches to transfer the 'information-

rich' genomes of human and other sequenced and assembled organisms to the 'information-poor' maps of farm animals and to increase the number of gene loci (Burt, 2002; Liu et al., 2005) and reveal the degree of synteny conservation between species (Goldammer et al., 2002, 2004; Martins-Wess et al., 2003a; Liu et al., 2005).

Comparative analyses of the porcine and human genomes have been carried out at different levels of resolution. Reverse or bi-directional Zoo-fluorescence in situ hybridization (Zoo-FISH) suggests that synteny between human chromosome 11 (HSA11) and the short arms of porcine chromosome 2 (SSC2p) and chromosome 9 (SSC9p) is highly conserved (Rettenberger et al., 1995; Goureau et al., 1996). This initial observation has been confirmed by numerous studies using FISH, somatic cell hybrid (SCH), RH and comparative mapping (Yerle et al., 1996, 1998; Fridolfsson et al., 1997; Lahbib-Mansais et al., 1999, 2006; Pinton et al., 2000; Rattink et al., 2001; Mikawa et al., 2004). The most recent SSC2p and SSC9p comparative maps to HSA11 are all based on a 7,000-rad pig-Chinese hamster whole genome (WG) RH panel (IMpRH) (Yerle et al., 1998). Rink et al. (2006) reported a second generation pig expressed sequence tag (EST) $RH_{7,000\text{-rad}}$ map (http://www.ag.unr.edu/beattie/research.htm) in which a total of 12 microsatellites (MSs) and 68 ESTs were mapped to SSC2p, and 16 MSs and 59 ESTs to SSC9p. The marker density for these two chromosome arms is roughly 800 kb per sequence tagged site (STS). Meyers et al. (2005) reported the integration of the bacterial artificial chromosome (BAC) end sequences (BESs) and BAC fingerprinted contigs (FPC) (http://www.sanger.ac.uk/Projects/S_scrofa/WebFPC/porcine/small.shtml) with a total of 119 BESs similar to the HSA11 genome sequence that mapped to SSC2p and SSC9p at an average marker spacing of ~1.1 Mb based on HSA11. Although the EST and the BES $RH_{7,000\text{-rad}}$ maps of SSC2p and SSC9p were integrated with the porcine genetic map (http://www.marc.usda.gov/genome/swine/swine.html) (Rohrer et al., 1996) and covered up to 90% of HSA11, several gaps still exist, and map resolution (~1 Mb/STS) remains lower than the target resolution of 94~381 kb/STS used in the human and mouse genome sequence assembly (Hudson et al., 2001; Olivier et al., 2001). The current SSC2p and SSC9p maps lack the resolution required for fine mapping, positional candidate cloning of the ~85 QTLs (quantitative trait loci) reported on SSC2p and SSC9p (http://www.animalgenome.org/QTLdb/) (Hu et al., 2005) and sequence assembly of these regions of the porcine genome. A 12,000-rad porcine WG-RH panel (IMNpRH2) (Yerle et al., 2002) has been used for high-resolution mapping of several chromosome regions in swine (Yerle et al., 2002; Martins-Wess et al., 2003a; Demars et al., 2006). We recently reported a detailed comparison of SSC12 with HSA17 using the IMNpRH2 panel at an overall resolution of ~252 kb/STS or ~15 kb/$cR_{12,000}$, and indicated that the IMNpRH2 panel provides a high-resolution scaffold for interval mapping and sequence assembly (Liu et al., 2005).

The primary goal of this study was to establish an accurate, high-resolution comprehensive $RH_{12,000\text{-rad}}$ map of SSC2p and SSC9p by integrating mapping vectors from the 7,000-rad IMpRH and 12,000-rad IMNpRH2 panels for all STS markers including MSs, genes/ESTs, BESs and SNPs (single nucleotide polymorphisms). A second goal was to refine existing evolutionary break points between the homologous chromosome segments of SSC2p, SSC9p and HSA11 at the molecular level.

Materials and methods

Genes, ESTs, MSs and BAC end markers

Primer pair sequences were obtained from normalized, essentially full-length cDNA libraries (Fujisaki et al., 2004) or from the literature. EST markers were derived from a panel of normalized porcine cDNA libraries as described earlier (Rink et al., 2006). BAC end primers were used essentially as reported (Meyers et al., 2005). Microsatellite primer sequences were acquired from the literature and public databases (Hawken et al., 1999; Krause et al., 2002; Fahrenkrug et al., 2005). All primers were optimized by determining the highest annealing temperature at which successful amplification of porcine genomic DNA took place. Primers were then tested with porcine and Chinese hamster genomic DNA at species specific temperatures (Liu et al., 2005).

RH typing

The $IMNpRH2_{12,000\text{-rad}}$ panel of 90 hybrids (Yerle et al., 2002) was used as well as data from the $IMpRH_{7,000\text{-rad}}$ panel (Meyers et al., 2005; Rink et al., 2006). RH typing was carried out in duplicate as described (Liu et al., 2005). Consensus vectors were established by two individuals using GelScore data (http://www.wesbarris.com/GelScore/) as a baseline. Markers with unusually high or low retention frequencies or with more than four discrepancies were eliminated. Bands (PCR products) were scored as present (1), absent (0) and ambiguous (2).

RH map construction

Consensus vectors of 271 markers from the $IMpRH_{7,000\text{-rad}}$ panel and of 590 markers from the $IMNpRH2_{12,000\text{-rad}}$ panel for SSC2p and SSC9p were loaded and merged into a single data-set using the data-set merging function of the CarthaGene software (http://www.inra.fr/mia/T/CarthaGene/) (Schiex and Gaspin, 1997). Markers were assigned to linkage groups at two-point (2pt) LOD 10 and a maximum distance of 100 cR between markers. A framework (FW) map for each linkage group was built with a likelihood difference threshold of 1000:1 to incorporate new markers. Non-FW markers were subsequently mapped onto the $IMNpRH2_{12,000\text{-rad}}$ panel with CarthaGene (Schiex and Gaspin, 1997). Maps were drawn using MapCreator (http://www.wesbarris.com/mapcreator/).

Comparative mapping

All sequences of the porcine genes, ESTs and BESs were BLAT (http://genome.ucsc.edu/cgi-bin/hgBlat?command=start) searched against the human genome sequence (Build 35/36). Once a sequence match was identified, the start position of the sequence in the human genome was collected. Chromosomal locations and start positions of their orthologs in the human genome were also established for all porcine genes analyzed (Figs. 1 and 2) using the NCBI human Map Viewer (Build 36) (http://www.ncbi.nlm.nih.gov/mapview/). Cartesian coordinates of 287 genes (ESTs) based on their map position in HSA11 and in SSC2p and SSC9p were also developed (Fig. 2). Map distances (cR) for SSC2p and SSC9p in Fig. 2 were the accumulated sum of the linkage groups from Fig. 1c and d.

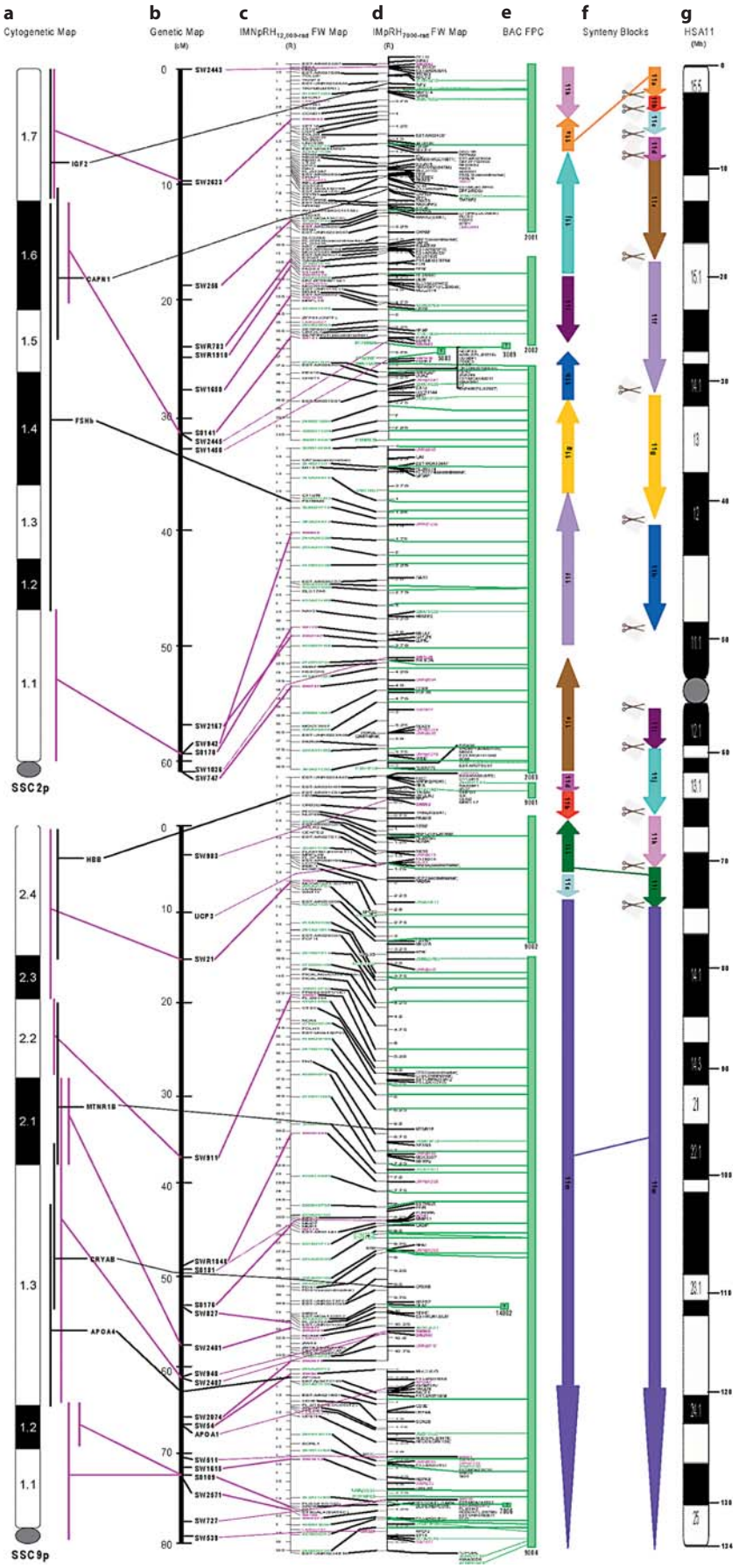

Fig. 1. High-resolution RH comprehensive and comparative maps of SSC2p and SSC9p and HSA11. (**a**) Cytogenetic maps of the pig chromosomes (SSC) 2p and 9p; (**b**) Genetic maps of SSC2p and 9p; (**c**) The 12,000-rad IMNpRH2 framework (FW) map; (**d**) The 7,000-rad IMpRH FW map. Genes/ESTs appear in black, BESs in green, MSs in magenta, and MSs found in the genetic map in bold magenta. Markers listed between the 12,000- and 7,000-rad FW maps are framework markers; those on the right of the IMpRH FW map are non-framework markers; (**e**) BAC fingerprint contigs (FPC). Contig number, e.g. 2001, corresponds to the FPC maps at http://www.sanger.ac.uk/Projects/S_scrofa/; (**f**) Synteny blocks between pig and human. The colored arrows to the left of HSA11 indicate the orientation of synteny blocks of genes on HSA11 and their rearrangements in SSC2p and 9p. Scissors indicate potential break points. (**g**) Cytogenetic map of human chromosome 11. The size (in Mb) is listed on the right side of the chromosome. MSs in the RH FW maps are linked to the corresponding genetic map with solid magenta lines. Similarly, BES markers in the FW RH maps are linked to the corresponding FPC maps with green lines. MSs and BESs that are linked to non-framework markers are indicated with dashed lines in the respective colors.

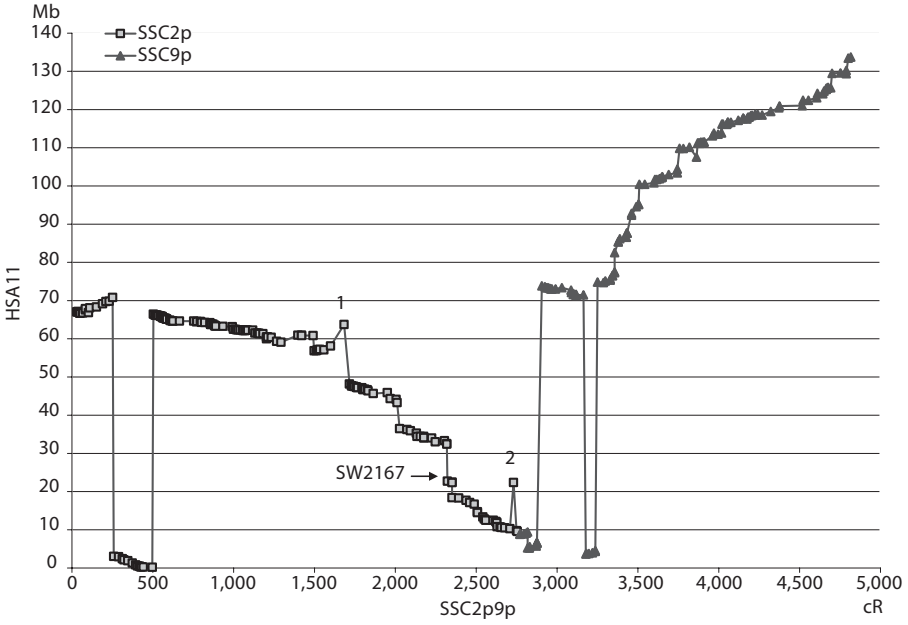

Fig. 2. Comparison of gene order between HSA11 and SSC2p and SSC9p. A total of 287 genes were compared based on their positions in HSA11 and in SSC2p and SSC9p. The positions of genes in HSA11 (Build 36) are compared with corresponding genes in the SSC2p and 9p $RH_{12,000\text{-rad}}$ map (Fig. 1). Map distances (cR) for SSC2p and SSC9p are the accumulated sum of the linkage groups from Fig. 1c. Each square represents the Cartesian coordinates for a gene in SSC2p, while triangles represent genes in SSC9p. Any significant change in coordinates between adjacent genes indicates a rearrangement. The map position of SW2167 and two other markers (number 1 and 2) are indicated and discussed in the text.

Table 1. Comparison between the 12,000- and 7,000-rad RH framework (FW) maps

Chromosome region	Linkage group (No. of markers)[a]	12,000-rad FW map			7,000-rad FW map		Fold changes between 12,000- and 7,000-rad FW maps[b]
		FW marker	Non-FW marker	Map distance (cR)	FW marker	Map distance (cR)	
SSC2p	1 (194)	91	102	2181.6	52	750.0	2.9
	2 (72)	35	35	1864.9	31	605.2	3.1
SSC9p	1 (168)	86	81	3179.9	65	1251.8	2.5
	2 (84)	28	52	1064.2	22	550.7	1.9

[a] A total number of markers in a linkage group includes markers typed in both the 12,000- and 7,000-rad RH panels, which is usually bigger than the sum of FW and non-FW markers in the 12,000-rad RH map because some of those markers mapped on the 7000-rad map were not typed in the 12,000-rad RH panel.
[b] Fold changes were calculated by dividing the map distance in the 12,000-rad FW map by the map distance in the 7,000-rad FW map.

Results and discussion

SSC2p and SSC9p $RH_{12,000}$ and $RH_{7,000}$ FW maps and resolution

Two sets of RH mapping vectors were used to construct the RH FW maps for SSC2p and SSC9p. One set was generated from the $IMNpRH2_{12,000\text{-rad}}$ panel, the other from the $IMpRH_{7,000\text{-rad}}$ panel. A total of 590 markers, including 131 MSs, 364 genes/ESTs, and 95 BESs were typed on the $IMNpRH2_{12,000\text{-rad}}$ panel in this work. A second set of 271 markers (28 MSs, 138 genes/ESTs, and 105 BESs) was previously typed on the $IMpRH_{7,000\text{-rad}}$ panel (Meyers et al., 2005; Rink et al., 2006). The two data-sets were merged under the 'dsmergor' command in CarthaGene (Schiex and Gaspin, 1997) in a single (consensus) data-set of 655 markers, of which 206 markers were typed on both panels. The merged data-set was analyzed using a maximum multipoint likelihood linkage strategy. This strategy yielded four large linkage groups with 72, 84, 168 and 194 markers, and three small linkage groups with four markers each assigned at a 2pt LOD score of 10 and a threshold distance $\leq$100 cR between markers. We did not assign the remaining 125 singletons or pairs to the RH maps at this time. The four large linkage groups and FW maps (Table 1 and Fig. 1) were simultaneously built from consensus vectors on both RH panels at a likelihood ratio of 1000:1. Figures 1c and d show the $RH_{12,000\text{-rad}}$ and $RH_{7,000\text{-rad}}$ FW maps with the framework markers listed in between. The order of the linkage groups along SSC2p and SSC9p was determined based on common markers mapped to the porcine cytogenetic (Fig. 1a) and genetic maps (Fig. 1b). A total of 126 and 114 FW markers (Table 1) were ordered to SSC2p and SSC9p $RH_{12,000\text{-rad}}$ FW maps, respectively, with an accumulated map distance of 4046.5 $cR_{12,000}$ and 1355.2 $cR_{7,000}$ for SSC2p, and 4244.1

$cR_{12,000}$ and 1802.5 $cR_{7,000}$ for SSC9p (Table 1). The DNA content of SSC2p is estimated at 63.8 Mb (168 × 0.38); and the size of SSC9p is calculated to be 65.4 Mb (145 × 0.451) based on chromosome size (Schmitz et al., 1992) and metaphase chromosome arm ratio (Gustavsson, 1988). Therefore, the kb/cR ratio in the IMNpRH2$_{12,000\text{-rad}}$ FW maps was 15.8 for SSC2p, and 15.4 for SSC9p, while the ratio in the IMpRH$_{7,000\text{-rad}}$ FW maps was 47.1 and 36.3, respectively. The kb/$cR_{12,000}$ ratio is consistent with the observation of 15.3 kb/cR for the entire SSC12 (Liu et al., 2005), suggesting that a realized average map resolution is ~15 kb/cR for the IMNpRH2$_{12,000\text{-rad}}$ panel. This represents a 3-fold increase in resolution over that of the 7,000-rad panel on SSC2p, and a 2.4-fold increase on SSC9p (Table 1). This increase is within the range of the 2.2–3.0 folds previously reported over SSC6q1.2 (Martins-Wess et al., 2003b), SSC7q11→q14 (Demeure et al., 2003), SSC12 (Liu et al., 2005), and SSC15q25 (Yerle et al., 2002).

Once FW markers were ordered (FW markers are listed between the 12,000- and 7,000-rad RH maps, Fig. 1c, d), non-FW markers (Table 1) were added to the RH$_{12,000\text{-rad}}$ FW maps with their most likely position in relation to the FW markers using CarthaGene software (Schiex and Gaspin, 1997). A total of 137 and 133 non-FW markers (Table 1) were mapped to SSC2p and SSC9p, respectively, and are listed to the right of the IMpRH$_{7,000\text{-rad}}$ FW maps (Fig. 1d). A ratio of ~1:1 between the number of FW and non-FW markers was observed for all linkage groups except for SSC9p linkage group 2, in which a 1:1.9 ratio was found (Table 1). If we consider FW and non-FW markers together, map resolution is ~15.2 $cR_{12,000}$/marker, or ~239 kb/marker on SSC2p, and 17.2 $cR_{12,000}$/marker, or ~265 kb/marker on SSC9p. This is a significant increase in resolution over the ~800 kb/marker on the latest IMpRH$_{7,000\text{-rad}}$ EST map (Rink et al., 2006).

Map accuracy and inflation

Map quality and accuracy was improved by 1) typing all markers in duplicate and retyping a third time if the discrepancy rate between duplicates was ≥5% of the 90 hybrids in the panel, 2) independently scoring individual gels and generating consensus vectors by two experienced investigators to reduce human error, 3) independently mapping two primer sets from different regions of 12 genes *(NAT10 (FLJ10774), CAT, PARVA, UCP2, SERPING1, CTSC, RAB6A, NXF1, WDR74 (FLJ10439), FBXL11, PICALM,* and *FDX1).* Except for ESTs representing *SERPING1* and *RAB6A*, all paired markers mapped either to the same position or next to each other in a small interval (Fig. 1c, d). Both *SERPING1* markers mapped to a 17-cR interval on the FW map, containing the genes *UBE2L6* and *CLP1 (HEAB)* (Fig. 1c, d). As *SERPING1*, *UBE2L6* and *CLP1 (HEAB)* are located within 100 kb in HSA11 (Build 36.2), these genes may exceed the resolution of the IMNpRH2$_{12,000\text{-rad}}$ panel. The two *RAB6A* markers mapped to an interval of 29.3 cR containing the gene *UCP2* (Fig. 1d) at 2pt LOD scores between 12.2 and 17.3 for the pairs of *RAB6A* and *UCP2* markers. HSA2, 3, 11 and 17 bear duplicated-copies of *RAB6A* suggesting there may be multiple copies of *RAB6A* in the porcine genome confounding the mapping result.

Two significant features of the common FW map are that the marker order on both FW RH maps is identical (Fig. 1c, d), which provides an accurate platform for mapping additional non-framework markers, and that the map length (distance) in a given linkage group (map) is fixed, so that adding additional non-FW markers to the group (map) will not change map distance, preventing significant map inflation (Lincoln and Lander, 1992; King et al., 2002; Gaile et al., 2007). The first generation porcine EST map (Rink et al., 2002), where only 10 of 1,058 markers (0.94%) were forced into alignment, conserved MS order between the RH and genetic maps (Rink et al., 2002). However, the addition of over 2000 ESTs to the MS vectors generated during construction of the initial IMpRH$_{7,000\text{-rad}}$ map (Hawken et al., 1999), excluded a significant number of MS from the comprehensive map (Milan et al., 2002), due to differences in marker typing between investigator groups. Additional confounding arose when ~10,000 markers, reported to the IMpRH$_{7,000\text{-rad}}$ Web Server (http://imprh.toulouse.inra.fr/) (Milan et al., 2000) by members of the international community, were merged. Only ~30% (~3000) of markers were able to be assigned to an FW map at a 2pt LOD 6 (Milan et al., 2006). Typing errors from all sources are the major cause of map inflation. We minimized map inflation for SSC2p and SSC9p by using only those vectors typed in our laboratories. As a result, 63.1% (171/271) of vectors assigned on the IMpRH panel were mapped at 2pt LOD 10 to the SSC2p and SSC9p RH$_{7,000}$ FW maps (Table 1, Fig. 1c, d).

Integration of RH maps with the corresponding genetic maps and BAC FPC maps

Linkage group marker order and orientation were based on the genetic maps of SSC2p and SSC9p (http://www.marc.usda.gov/genome/swine/swine.html) (Fig. 1b) which resulted in an identical order for all common MS markers, except SW2167 which flipped with SW942/S0170, possibly due to the proximity of the synteny break and rearrangement on SSC2p (Fig. 2). The genetic maps of SSC2p and SSC9p have seven bins, each with two MSs (Fig. 1b). These binned markers were all separated on the RH$_{12,000}$ maps (Fig. 1c and 1d), indicating significantly increasing in map resolution.

We integrated the SSC2p and SSC9p RH maps and corresponding BAC FPC map on the basis of the common BES markers (Fig. 1c–e) which aligned BAC contigs to the RH, genetic and cytogenetic maps and provided a scaffold for sequence assembly of these chromosomes. In addition to major FPCs for the corresponding porcine chromosomes, i.e. contigs 2001, 9001, etc., we also found a few BES markers that mapped to chromosomes/regions other than SSC2p and SSC9p in the FPC database. These chromosomes/regions were SSC3 (contig 3009), SSC5 (contig 5003), SSC7 (contig 7006), and SSC14 (contig 14002) (Fig. 1e). The BES markers 311760638 (http://rhdev.toulouse.inra.fr/Do=R&M=IMpRH09079) and 311629091 (http://rhdev.toulouse.inra.fr/Do=R&M=IMpRH09081&Choix=default) were mapped to SSC2p by Meyers et al. (2005) in the RH$_{7,000}$ map. How-

ever, the original BAC (PigE-48I1) with end sequence 311760638 was placed in the center of the large contig 3009 (http://www.sanger.ac.uk/cgi-bin/Projects/S_scrofa/WebFPCdirect.cgi?&contig=3009) that maps to SSC3, while the original BAC (CH242-44H3) with end sequence 311629091 was placed in the center of another large contig 5003 (http://www.sanger.ac.uk/cgi-bin/Projects/S_scrofa/WebFPCdirect.cgi?&contig=5003) on SSC5 in the pig FPC database. We assigned these two FW markers to the gap between contig 2002 and 2003 on the SSC2p $RH_{12,000}$ FW map where no FW markers were previously mapped (between marker S0141 and 256A11B09, ~150 cR), although over a dozen non-FW markers were assigned to this region (Fig. 1c). This region on SSC2p corresponds to the centromeric region on HSA11, which may help to explain the conflicting results, although we cannot exclude the possibility that two copies of these fragments (one in SSC2, and the other in SSC3/SSC5) are present in the porcine genome, and/or that either the BAC FPC maps or the RH maps were incorrect. Each of these cases argues for a denser RH map to resolve this type of issue and close this and other gaps prior to sequence assembly. Similarly, BACs RPCI44-410K1 and RPCI44-313O9 were placed in FPC contigs 14002 and 7006 (http://www.sanger.ac.uk/Projects/S_scrofa/WebFPC/porcine/small.shtml), respectively, but the map positions for BES 410A10F01 and 313A11H05 in the current RH map fit well with the conserved synteny group 11m between HSA11 and SSC9p (Fig. 1f) providing an additional example of integrating FPC data prior to sequencing and assembly.

Comparative maps between SSC2p, SSC9p and HSA11

All genes, ESTs and BESs were BLAT (UCSC Genome Browser, http://genome.ucsc.edu/) and/or BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) searched against human genome sequence builds 35 and/or 36 to identify regions of synteny between the human and porcine genomes. Regions of altered synteny to HSA11 (Fig. 1g) were also present as rearranged blocks in SSC2p and SSC9p (11a to 11m, Fig. 1f). The comparative map shows that HSA11 is entirely homologous to SSC2p and SSC9p, in spite of complicating rearrangements that occurred during the evolution of the two species. Micro-rearrangements within a synteny block were analyzed using the coordinates of 287 genes/ESTs based on their sequence locations in HSA11 and RH map position in SSC2p and SSC9p (Fig. 2). It appears that SSC2p represents an inversion of the region 0–70 Mb on HSA11, while SSC9p is a homolog to the region 71–134 Mb on HSA11, with the exception of three small blocks (11b, 11c and 11d in Fig. 1f, Fig. 2). In addition, two markers, AY211494 (MGC11134) at coordinate 1700.5 cR/63.75 Mb (No. 1 in Fig. 2, a FW marker at position 8.5 R in the SSC2p linkage group 1, Fig. 1c) and EST-AR078C07 at 2748.4 cR/22.39 Mb (No. 2 in Fig. 2, a non-FW marker at position 5.9 R in the SSC2p linkage group 2, Fig. 1d) were not aligned with their synteny blocks. Finally, we identified three regions on SSC2p (Fig. 2) corresponding to regions 23–32, 38–43, and 49–56 Mb on HSA11 (Fig. 1c, d and f), where we were only able to assign a limited number of genes/ESTs (Fig. 1c, d). As all genes/ESTs were randomly selected from cDNA libraries, it is possible that chance alone precluded our selection of cDNAs to develop primers as markers for regions 23–32 and 38–43 Mb. However, region 49–56 Mb corresponds to the centromere on HSA11 (Fig. 1g), which may have affected identification and selection. In any event, the comparative map between SSC2p, SSC9p and HSA11 appears more complex than previously appreciated. Two blocks on SSC2p (Meyers et al., 2005; Lahbib-Mansais et al., 2006) and four blocks on SSC9p (Meyers et al., 2005) were reported to be conserved on HSA11. Here, SSC2p is organized into at least five blocks (11k, 11a, 11j, 11i and 11e–h), assuming no break(s) between 11e, 11f, 11g and 11h (Fig. 1f). Another five blocks (11d, 11b, 11l, 11c and 11m) were also identified on SSC9p (Fig. 1f). Meyers et al. (2005) described block 11m as divided into three blocks with a very small region in the middle of 11m corresponding to the sequence 107.1–108.8 Mb on HSA11, that was mapped close to the centromeric region on SSC9p. In the present study, we mapped one gene, *NPAT*, which is located at 107.6 Mb in HSA11, in the middle of the block 11m in SSC9p, suggesting that the synteny block 11m is not likely rearranged in SSC9p.

In conclusion, we were able to rapidly and accurately construct a high-resolution RH map for SSC2p and SSC9p by taking advantage of the data set merging function of the CarthaGene software. This provided a platform to integrate the $IMNpRH2_{12,000\text{-rad}}$ and $IMpRH_{7,000\text{-rad}}$ maps as well as the genetic and BAC FPC maps. An inclusive map of SSC2p, SSC9p and HSA11 offers one example of how a comparative approach may be used to close potential gaps between contigs prior to sequencing, and identify regions that complicate if not outright hinder the assembly of genome sequence.

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