A comparative radiation hybrid map of bovine chromosome 18 and homologous chromosomes in human and mice

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A comprehensive radiation hybrid (RH) map and a high resolution comparative map of Bos taurus (BTA) chromosome 18 were constructed, composed of 103 markers and 76 markers, respectively, by using a cattle-hamster somatic hybrid cell panel and a 5,000 rad whole-genome radiation hybrid (WGRH) panel. These maps include 65 new assignments (56 genes, 3 expressed-sequence tags, 6 microsatellites) and integrate 38 markers from the first generation $WGRH_{5,000}$ map of BTA18. Fifty-nine assignments of coding sequences were supported by somatic hybrid cell mapping to markers on BTA18. The total length of the comprehensive map was 1666 $cR_{5,000}$. Break-point positions within the chromosome were refined and a new telomeric RH linkage group was established. Conserved synteny between cattle, human, and mouse was found for 76 genes of BTA18 and human chromosomes (HSA) 16 and 19 and for 34 cattle genes and mouse chromosomes (MMU) 7 and 8. The new RH map is potentially useful for the identification of candidate genes for economically important traits, contributes to the expansion of the existing BTA18 gene map, and provides new information about the chromosome evolution in cattle, humans, and mice.

Comparative genome analysis by interspecies transfer of information from well documented genomes like human and mouse to less analyzed genomes such as farm animals has become an established tool for increasing the knowledge of genome evolution (1–3). It also has become one of the most powerful approaches for the expansion of gene maps (4, 5). Radiation hybrid mapping (6) has been revived as an efficient technique for the generation of high-resolution gene maps in human (7–9). This technique has been integrated successfully into comparative mapping approaches to demonstrate conservation and rearrangements in gene order between different species (10–12). Likewise, comparative gene mapping and radiation hybrid (RH) mapping are increasingly powerful tools for the identification of candidate genes for economic traits as they provide precision in extrapolation of map position from one species to another (13).

The procedure of RH mapping became available for cattle genomics with the generation of a 5,000-rad cattle-hamster whole-genome RH cell panel (14). Since then, increasingly precise comparative RH maps, combining genes and polymorphic microsatellite markers have been constructed for different chromosomes (15–17). A new strategy of comparative mapping by annotation and sequence similarity (COMPASS; ref. 18) has accelerated the comparative gene-mapping process (19, 20). By using this approach, more than 1,000 markers were assigned and presented in a combined first generation cattle $WGRH_{5,000}$ and cattle/human whole-genome comparative map (21). New markers can now be placed effectively into this map (22).

Bos taurus (BTA) chromosome 18 contains a chromosome break point representing evolutionarily conserved fragments of human chromosomes 16 and 19 that are identified by three independent Zoo-fluorescence *in situ* hybridization (FISH) experiments (23–25). Somatic hybrid cell (SHC) mapping that identified a conserved fragment of HSA19 on BTA18 (26) supports these findings. However, the break point was assigned to different locations between BTA18q21 171 q22, BTA18q15 171 q21, and BTA18q14 171 q15. Physical and linkage mapping data weakly support all of the break-point positions suggested by Zoo-FISH (USArkDB database, http://bos.cvm.tamu.edu/bovarkdb.html; refs. 27 and 28). The $RH_{5,000}$ map for BTA18 narrowed the break point to a position between CDH3 and CAPN4 (21). These sequences were mapped in the human Genebridge4 (GB4) $WGRH_{3,000}$ panel (29) on HSA16 and on HSA19. Contrary to the Zoo-FISH data, the bovine $RH_{5,000}$ map has defined an additional chromosome break point at the telomere of BTA18 indicated by the gene LCAT.

A cattle-hamster SHC panel and a 5,000-rad WGRH panel were used in combination with a COMPASS predicted gene map *in silico* (30) for three purposes. First, the number of gene loci on BTA18 should be drastically increased to get a higher resolution gene map. Second, interchromosomal and intrachromosomal break points between cattle, human, and mouse genomes should be identified, analyzed, and their positions narrowed. Third, the conservation of the gene order within conserved segments should be analyzed. Therefore, we have ordered 65 new markers together with 38 existing markers in a comprehensive $RH_{5,000}$ map for BTA18 and have constructed a comparative gene map for BTA18 specific type I markers and the homologous chromosome fragments of HSA16, HSA19, MMU7, and MMU8. Assignments of sequences by SHC support the RH mapping data.

Materials and Methods

Selection of Markers and Primer Design. The majority of cDNA markers were from the United States Department of Agriculture Meat Animal Research Center (USDA-MARC) library (31). Seven markers were from bovine placenta (BP) cDNA (32). Four markers were from differentially expressed hepatic or intestinal cDNA (22). A genome database search for all markers was performed by using BLAST v.2.0 software (available at National Center for Biotechnology Information, www.ncbi.nlm.nih.gov/blast). Sequences for genes MIA, PLAUR, and VASP were taken from the human genome database. The microsatellite MS936-FBN was isolated from the BTA18 specific BAC BBI B750-H03188 (33). The microsatellites IDVGA55, BMS2785, BMS929, BM6507, BM2078, and TGLA227 were chosen from the genetic map (28). Cattle-specific oligonucleotide primers were designed with the software program PRIMER3

Abbreviations: RH, radiation hybrid; WGRH, whole genome radiation hybrid; COMPASS, comparative mapping by annotation and sequence similarity; SHC, somatic hybrid cell; BTA, Bos Taurus; LG, linkage group; lod, logarithm of odds.

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(http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). Primer sequences for the microsatellites IDVGA55, BMS2785, BMS929, BM6507, BM2078, and TGLA227 were obtained from published data.

PCR and Agarose Gel Electrophoresis. Each PCR reaction contained 50 ng hybrid DNA, 1.5 mM $MgCl_2$, 15 mM Tris, 50 mM KCl, 200 μM each dNTP, 0.5–1.0 μM of each oligonucleotide primer, and 0.2 units AmpliTaq Gold DNA Polymerase (Applied Biosystems) in a total volume of 10 μl. The general PCR reaction was started with an initial hot start of 10 min at 95°C followed by 35 cycles for 30 s at 94°C, 30 s at the published annealing temperature, and 45 s at 72°C, followed by a final elongation step at 72°C for 7 min. PCR reactions were performed in PCR Systems 9700 thermocyclers (Applied Biosystems). The appropriate annealing temperature for each set of primers was established in preliminary experiments to yield specific amplification of a bovine PCR product in a murine/hamster background. The microsatellites were amplified according to the published PCR conditions. The PCR products were analyzed by electrophoresis on 2% agarose gels in 1.0× TBE buffer (90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3) and stained with ethidium bromide.

SHC/WGRH Typing. PCR was performed in 31 somatic and 90 selected radiation hybrid cell lines from the SHC and $WGRH_{5,000}$ panels, respectively (2, 14). Only primer sets that amplified bovine but not any of the hamster or mouse cell panel background DNA were used for mapping. PCR products were scored 1 for present, 0 for absent, or 2 for ambiguous. Syntenic assignments were made from correlations of marker retention (34). All WGRH typing experiments were performed twice and scored independently to increase the accuracy of the results. Only data concordant in both experiments were used for RH mapping.

Statistical Analysis of WGRH. To assign new markers to a chromosome, two-point linkage analysis was done with the software RHMAPPER (35). All markers assigned to BTA18, including the published markers of the cattle $WGRH_{5,000}$ gene map (21), were used for the construction of a comprehensive map by using the software RHMAP v.3.0 (36). Two-point linkage analysis (RH2PT) was performed to generate linkage groups (LGs) at different logarithm of odds (lod) scores. Framework maps were constructed for each LG at 1,000:1 odds. Markers with <1,000:1 odds were placed to get a comprehensive map.

Results

Comparative mapping combining COMPASS, SHC, and $WGRH_{5,000}$ mapping resulted in the construction of a comprehensive $RH_{5,000}$ map for cattle chromosome 18 (Fig. 1). The gene assignments were compared with human and mouse maps and a comparative map for BTA18, HSA16q, HSA19q, MMU7, and MMU8 was developed (Fig. 2). Table 3, which is published as supporting information on the PNAS web site, www.pnas.org, provides information about all new assigned markers, including GenBank accession numbers for markers and human orthologs, marker length, locus symbols, primer sequences, annealing temperatures, and PCR product size.

Sixty-three DNA sequences with a bovine specific PCR product were used for SHC typing. Twenty-three of the typed markers were predicted by COMPASS to map on BTA18 and twenty-five of the markers were predicted to map either to BTA7, 18, or 25. All 48 markers were assigned on BTA18. In total, 59 markers were assigned on BTA18 with significant concordant values between 94% and 100% to the reference marker UWCA5 (Table 1). No synteny was found for the markers TAX1, CBFB, COX6B, and AKT2.

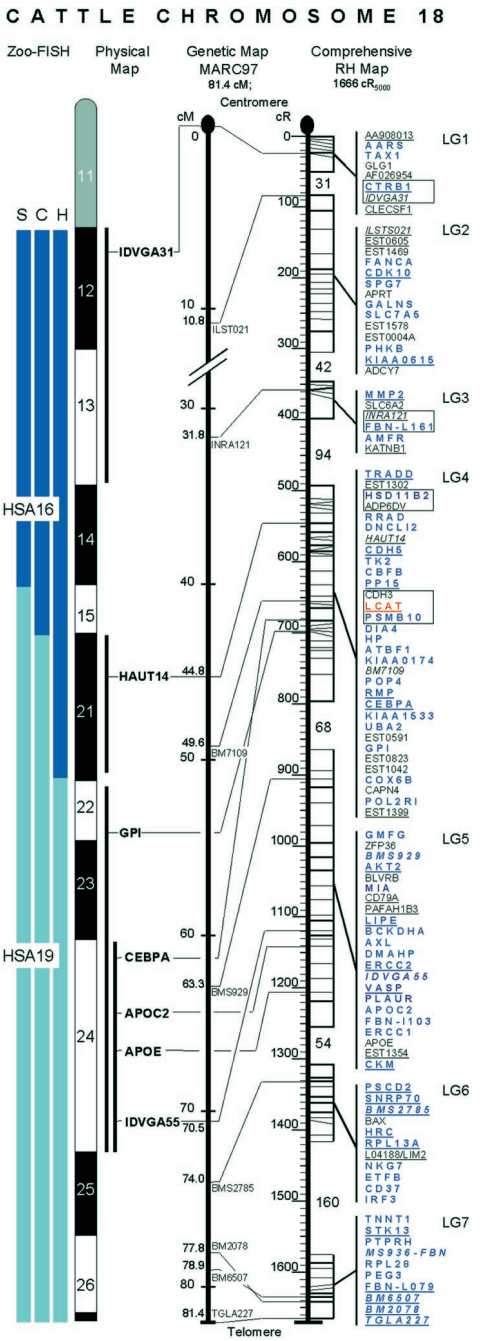

Fig. 1. Comprehensive $WGRH_{5,000}$ map of BTA18 in comparison with Zoo-FISH [S (24); C (25); H (23)], cytogenetic, and linkage maps of BTA18. The map is scaled in $cR_{5,000}$ and drawn proportionally. Loci in the map are represented by solid lines connected to the markers (bold for framework markers). Markers are ordered in seven RH LGs. The distances between LG's are shown. Framework markers are underlined. New assigned markers are displayed in blue. Previously mapped markers are shown in black. Markers assigned for the second time are shown in marine blue. Markers with the same loci are framed. Microsatellites are presented in italic type. The new locus of LCAT is shown in red.

Seventy-two markers were typed in the $WGRH_{5,000}$ panel. LCAT, HSD11B2, MIA, PLAUR, VASP, and the microsatellite IDVGA55 were previously assigned (21). However, except for IDVGA55 different sequences were used for typing in our experiments. RHMAPPER was used to identify BTA18 specific markers and to exclude unlinked markers from map construc-

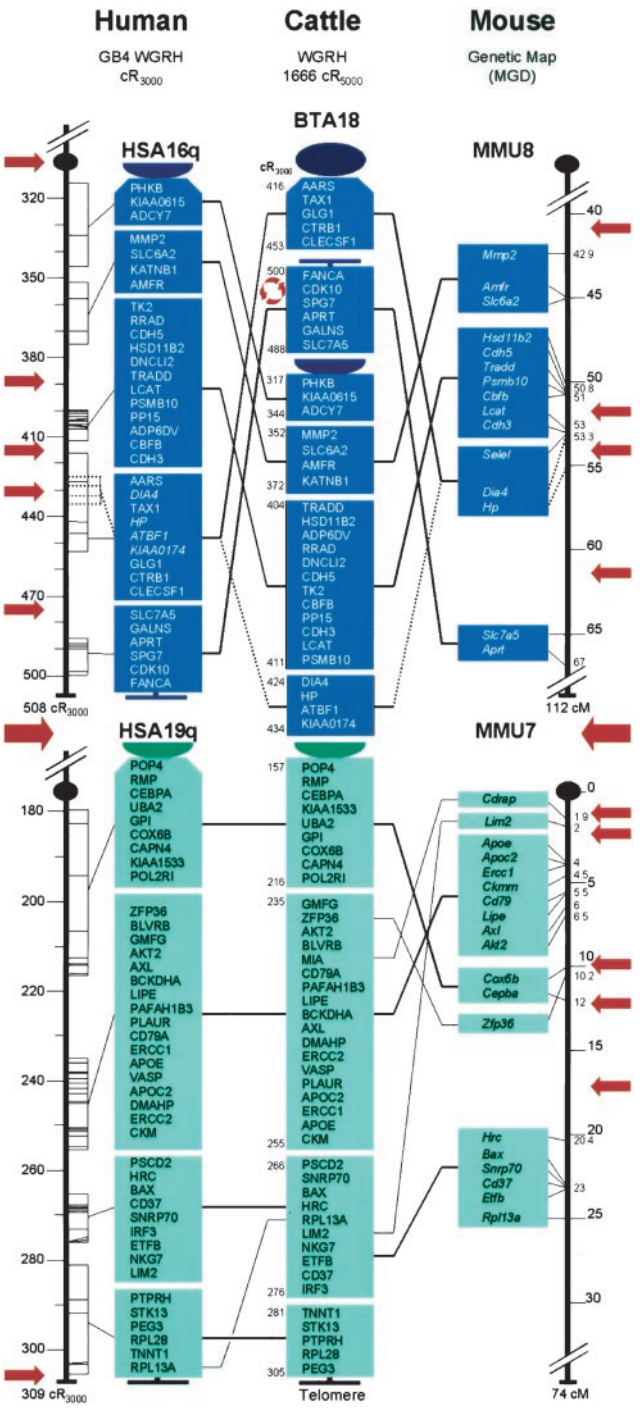

Fig. 2. Comparative map of cattle chromosome BTA18 and chromosomes HSA16q, HSA19q, MMU7, and MMU8. Conserved chromosome segments between human, cattle, and mice were displayed in the same color and were connected by bold lines. Thinner lines identify the connection of single genes. Space between chromosome segments indicates large or unanalyzed distances. Horizontal red arrows indicate probable locations of evolutionary break points. Dotted lines identify genes of human and mouse continuous chromosome segments that are separated in cattle. Half ellipses and very bold horizontal lines give approximate positions of human centromeres and telomeres in cattle, respectively. The human locus order in chromosome segments was taken from the GB4 $WGRH_{3,000}$ maps of HSA16q and HSA19q as shown in $cR_{3,000}$ scaled chromosome drawings on the left. The locus order in mice was taken from the linkage maps of MMU7 and MMU8 as shown in cM-scaled chromosome drawings on the right. A human RH $cR_{3,000}$ scale on the left of cattle chromosome segments represents size and origin of covered conserved human segments and indicates the size of gaps.

tion. No linkage was found for SIAH1. All remaining 71 markers were assigned to BTA18 with lod scores higher than 10. Except for LCAT, the remapped markers were linked to orthologous loci in the published cattle $WGRH_{5,000}$ gene map. No linkage was found between the new and the first identified LCAT loci. Two different sequences were used to assign DNCLI2. Displaying identical retention patterns, both were assigned to the same location.

Finally, 65 new markers that were confirmed by SHC and RHMAPPER analysis and 38 published markers were analyzed for the generation of a comprehensive and a comparative map using the RHMAP3.0 program. There were seven LGs at lod score of six. Framework maps were established separately for each LG. Thirty seven framework markers with odds of 1000:1 were found. The marker pairs CTRB1-IDVGA31, INRA121-FBNL161, ADP6DV-HSD11B2, CDH3-LCAT, and LCAT-PSMB10 were totally linked. A comprehensive map (Fig. 1) was constructed by placing markers at <1000:1 odds. Table 2 summarizes all markers used for the comprehensive map and provides information about the retention frequency of each locus, the average retention frequency for each LG, the distance between the linked markers, and the probability value for placed markers. The average retention frequency for all markers was 0.24. The total length of the comprehensive map was 1665.8 $cR_{5,000}$. The comprehensive map includes seven LG's consisting of 76 genes, 15 EST's, and 12 microsatellites.

Seventy-six of the mapped genes having human orthologs and 34 genes displaying similarities with mouse genes and were used for the construction of a comparative map (Fig. 2). The BTA18 homologs were identified as HSA16q, HSA19q, MMU7, and MMU8. Chromosome segments in BTA18 with conserved synteny to homologous human or mouse chromosome segments were grouped together based on the order and distance of the LG's in the new BTA18 $RH_{5,000}$ map and the estimated high distances between homologous groups of loci in the human GB4 $WGRH_{3,000}$ map. The analysis of the comprehensive and comparative maps identified several break points on BTA18. The interchromosomal break point representing HSA16q and HSA19q was narrowed down between the genes KIAA0174 and POP4 in LG3 to a distance of about 10 $cR_{5,000}$. Comparing conserved cattle, human, and mouse chromosome segments, six intrachromosomal break points were identified between human and cattle and nine between cattle and mouse, respectively.

Discussion

We have constructed a comprehensive $RH_{5,000}$ map for BTA18 consisting of 103 markers ordered in seven LG's. The map was produced with a comparative approach using the information of COMPASS predictions in combination with SHC and $WGRH_{5,000}$ mapping. A comparative map was developed containing 76 markers homologous to human or mouse genes. The results correspond in general to the bovine chromosome standard nomenclature (37), the genetic map (28, 38), and the cattle $RH_{5,000}$ map (21) for BTA18. COMPASS (30) provided a very simple and fast identification of mapped human genes homologous to cattle. The synteny assignments initially verified COMPASS predictions. This result demonstrates the usefulness of this approach for comparative mapping in cattle and for the targeted analysis of chromosomes or chromosome regions. SHC results and COMPASS predictions support the assumption that BTA18 is linked evolutionarily to HSA16q and HSA19q.

The PCR amplification with three primer pairs designed from cDNA sequences for TAX1, POLR2I, and PSCD2 resulted in longer products than expected, suggesting the amplification of intron DNA in the genomic DNA of the cell hybrids. The markers TAX1, CBFB, COX6B, and AKT2 failed in the SHC mapping. Hamster control genomic DNA of the RH panel was used as negative control for the primer tests to simplify the

Table 1. Mapping information for new assigned markers, predictions, results of SHC and WGRH$_{5,000}$ mapping

Locus symbol	Compass prediction	Cattle mapping results: SHC CV	SHC Chr	WGRH (cR$_{5,000}$) Chr
AARS	18	1.00	18	18
TAX1	18			18
CTRB1	18	0.97	18	18
FANCA	18	0.97	18	18
CDK10	18	0.97	18	18
SPG7	18	0.97	18	18
GALNS	18	0.97	18	18
SLC7A5	18	0.97	18	18
PHKB	18\25	0.97	18	18
KIAA0615	18\25	0.97	18	18
MMP2	18\25	0.97	18	18
FBN-L161		0.97	18	18
AMFR	18	0.97	18	18
TRADD	18	1.00	18	18
HSD11B2	18	0.97	18	18
RRAD		0.97	18	18
DNCL12	18	0.97	18	18
DNCL12	18	0.97	18	18
CDH5	18	0.97	18	18
TK2	18	0.97	18	18
CBFB	18			18
PP15	18	0.97	18	18
LCAT	18	0.97	18	18
PSMB10	18	0.97	18	18
DIA4	18	0.97	18	18
HP	18	0.97	18	18
ATBF1	18	0.97	18	18
KIAA0174	18	0.97	18	18
SIAH1	18\25	0.97	18	
POP4	7\18	0.97	18	18
RMP	7\18	0.97	18	18
CEBPA	7\18	0.94	18	18
KIAA1533	7\18	0.97	18	18
UBA2	7\18	0.97	18	18
GPI	7\18	0.97	18	18
COX6B	7\18			18
POLR2I		0.97	18	18
GMFG	7\18	0.97	18	18
BMS929				18
AKT2	7\18			18
MIA				18
LIPE	7\18	0.97	18	18
BCKDHA	7\18	0.97	18	18
AXL	7\18	0.97	18	18
DMAHP	7\18	0.97	18	18
ERCC2	7\18	0.97	18	18
IDVGA55				18
VASP				18
PLAUR				18
APOC2		0.97	18	18
FBN-I103		0.94	18	18
ERCC1	7\18	0.97	18	18
CKM	7\18	0.97	18	18
PSCD2	7\18	0.97	18	18
SNRP70	7\18	0.97	18	18
BMS2785				18
HRC	7\18	0.94	18	18
RPL13A		0.97	18	18
NKG7	7\18	0.97	18	18
ETFB	7\18	0.97	18	18
CD37	7\18	0.97	18	18
IRF3	7\18	0.97	18	18
TNNT1	7\18	0.97	18	18
STK13		0.94	18	18
PTPRH	7\18	0.97	18	18
FBN-L079		1.00	18	18
MS936-FBN		0.97	18	18
RPL28	7\18	0.97	18	18
PEG3	7\18	0.97	18	18
BM6507				18
BM2078				18
TGLA227				18

Chr, chromosome; CV, concordant value.

procedure. However, SHC mapping was a helpful tool in the identification of 59 BTA18 specific markers. The syntenic assignments strongly support the RH mapping results.

The generation of the comprehensive and comparative maps was based on BTA18 specific markers. We used SHC mapping and RHMAPPER two-point linkage analysis to make chromosomal assignments. The program RHMAP3.0 was used to construct a comprehensive and a comparative map. RH2PT was performed to obtain retention frequencies and LGs at different lod scores. At lod score 6 and higher, there were seven LG's. It was not possible to get reliable framework markers grouped at lod scores <6. This finding is based primarily on the retention patterns between markers of different LG's and on large physical distances between the groups of mapped markers. The large distances found between homologous chromosome segments in human support this assumption. Additionally, because the idea was to analyze evolutionary break points on BTA18, markers chosen were not equally distributed in human but were selected to be as close as possible to human centromeres and telomeres of HSA16q and HSA19q and in positions with probable intrachromosomal breaks in cattle. It can be concluded that the large gaps between grouped markers in cattle and their RH mapping positions in human underlie the useful establishment of several LG's. We closed the gaps between LG's to establish a continuous map by accepting large distances between the end framework markers of the neighboring LG's. However, the distant connections between the LG's were useful for their orientation.

Our results strongly suggest a mapping position of the framework marker LCAT within LG4. We analyzed nine genes within a distance of 6 cR$_{3,000}$ in human including LCAT. All genes have shown very similar retention patterns, and after RH mapping, CDH3, LCAT, and PSMP10 were assigned with odds of 1,000:1 to the same location. Because of the importance of this mapping for candidate gene approaches, we particularly analyzed the telomere region of BTA18 using several genes and six microsatellite markers with published location in the distal 20 cM of the chromosome. As a result, a new LG7 was added to the telomere end of the RH map, including six coding sequences and four microsatellites. Linkage was found between framework markers RPL13A (LG6) and STK13 (LG7). This finding and the known order of the microsatellites BM6507, BM2078, and TGLA227 in the genetic map suggested an orientation of LG7 with TNNT1 proximal and TGLA227 on the telomere side.

An interchromosomal evolutionary break point was identified between the KIAA0174 and POP4. The comparison of these

Table 2. Marker names of comprehensive map, retention frequencies, placements, and references

Marker name	Type	RF*	Marker placement distance: Accum. cR_{5000}	Marker placement distance: Next† cR_{5000}	P‡	Reference
LG 1		0.30				
AA908013	EST	0.28	0.0	2.7	F	(21)
AARS	Gene	0.30	2.7	2.7	1.5	Table 3§
TAX1	Gene	0.28	5.4	5.4	1.5	Table 3
GLG1	Gene	0.32	10.8	5.4	1.5	(21)
AF026954	EST	0.30	16.2	8.2	1.5	(21)
CTRB1	Gene	0.28	24.4	0.0	F	Table 3
IDVGA31	MS	0.30	24.4	29.5	3	(21)
CLECSF1	Gene	0.34	53.9	30.9	F	(21)
LG 2		0.21				
ILSTS021	MS	0.28	84.8	21.4	F	(21)
EST0605	EST	0.22	106.2	25.8	F	(21)
EST1469	EST	0.28	132.0	33.7	2	(21)
FANCA	Gene	0.27	165.7	21.9	2	Table 3
CDK10	Gene	0.21	187.6	6.5	F	Table 3
SPG7	Gene	0.21	194.1	13.3	1.5	Table 3
APRT	Gene	0.18	207.4	6.8	1.5	(21)
GALNS	Gene	0.19	214.2	6.6	1.5	Table 3
SLC7A5	Gene	0.21	220.8	15.4	1.5	Table 3
EST0004A	EST	0.18	236.2	11.7	1.5	(21)
EST1578	EST	0.17	247.9	10.1	1.5	(21)
PHKB	Gene	0.20	258.0	16.8	1.5	Table 3
KIAA0615	Gene	0.19	274.8	29.2	F	Table 3
ADCY7	Gene	0.18	304.0	41.8	1	(21)
LG 3		0.15				
MMP2	Gene	0.16	345.8	4.3	F	Table 3
SLC6A2	Gene	0.17	350.1	8.7	1	(21)
INRA121	MS	0.14	358.8	0.0	F	(21)
FBN-L161	EST	0.15	358.8	4.4	3	Table 3
AMFR	Gene	0.16	363.2	36.1	1.5	Table 3
KATNB1	Gene	0.13	399.3	93.5	F	(21)
LG 4		0.23				
TRADD	Gene	0.20	492.8	18.9	F	Table 3
EST1302	EST	0.23	511.7	6.8	1.7	(21)
HSD11B2	Gene	0.25	518.5	3.2	1.7	(21); Table 3
ADP6DV	Gene	0.26	518.5	0.0	1.7	(21)
RRAD	Gene	0.27	521.7	10.0	0.1	Table 3
DNCLI2	Gene	0.25	531.7	15.6	1.7	Table 3
HAUT14	MS	0.26	547.3	11.3	F	(21)
CDH5	Gene	0.28	558.6	6.2	F	Table 3
TK2	Gene	0.30	564.8	3.0	2.5	Table 3
CBFB	Gene	0.29	567.8	9.8	2.5	Table 3
PP15	Gene	0.26	577.6	6.8	F	Table 3
CDH3	Gene	0.23	584.4	0.0	3	(21)
LCAT	Gene	0.23	584.4	0.0	F	Table 3
PSMB10	Gene	0.23	584.4	6.8	3	Table 3
DIA4	Gene	0.26	591.2	22.2	2.5	Table 3
HP	Gene	0.21	613.4	18.0	0.3	Table 3
ATBF1	Gene	0.27	631.4	16.7	0.3	Table 3
KIAA0174	Gene	0.28	648.1	6.5	0.1	Table 3
BM7109	MS	0.26	654.6	3.3	0.3	(21)
POP4	Gene	0.24	657.9	6.8	2.5	Table 3
RMP	Gene	0.24	664.7	18.4	F	Table 3
CEBPA	Gene	0.20	683.1	7.3	F	Table 3
KIAA1533	Gene	0.21	690.4	3.6	0.5	Table 3
UBA2	Gene	0.20	694.0	1.8	0.3	Table 3
EST0591	EST	0.20	695.8	1.8	0.3	(21)
GPI	Gene	0.21	697.6	13.5	0.3	Table 3
EST0823	EST	0.23	711.1	8.9	0.7	(21)
EST1042	EST	0.19	720.0	3.8	2	(21)
COX6B	Gene	0.18	723.8	11.5	0.3	Table 3
CAPN4	Gene	0.21	735.3	23.6	2	(21)
POLR21	Gene	0.12	758.9	37.2	0.1	Table 3
EST1399	EST	0.22	796.1	67.7	F	(21)
LG 5		0.19				
GMFG	Gene	0.19	863.8	29.8	2	Table 3
ZFP36	Gene	0.15	893.6	12.2	2	(21)
BMS929	MS	0.16	905.8	12.2	0.1	(39)
AKT2	Gene	0.14	918.0	21.9	F	Table 3
BLVRB	Gene	0.13	939.9	30.7	1.5	(21)
MIA	Gene	0.19	970.6	25.3	2	(21); Table 3
CD79	Gene	0.14	995.9	20.2	F	(21)
PAFAH1B3	Gene	0.21	1016.1	18.3	F	(21)
LIPE	Gene	0.23	1034.4	22.0	F	Table 3
BCKDHA	Gene	0.21	1056.4	18.2	0.1	Table 3
AXL	Gene	0.22	1074.6	22.6	1.5	Table 3
DMAHP	Gene	0.20	1097.2	7.1	2.5	Table 3
ERCC2	Gene	0.20	1104.3	15.2	F	Table 3
IDVGA55	MS	0.18	1119.5	7.5	1.5	(40)
VASP	Gene	0.18	1127.0	3.6	F	(21); Table 3
PLAUR	Gene	0.19	1130.6	11.0	0.1	(21); Table 3
APOC2	Gene	0.20	1141.6	24.5	0.1	Table 3
FBN-I103	EST	0.14	1166.1	20.8	1.5	Table 3
ERCC1	Gene	0.17	1186.9	18.4	1.5	Table 3
APOE	Gene	0.16	1205.3	12.8	2.5	(21)
EST1354	EST	0.21	1218.1	36.1	F	(21)
CKM	Gene	0.33	1254.2	53.8	F	Table 3
LG 6		0.23				
PSCD2	Gene	0.24	1308.0	18.3	F	Table 3
SNRP70	Gene	0.22	1326.3	5.8	F	Table 3
BMS2785	MS	0.20	1332.1	6.9	F	(41)
BAX	Gene	0.21	1339.0	14.4	0.3	(21)
HRC	Gene	0.19	1353.4	9.0	F	Table 3
RPL13A	Gene	0.20	1362.4	12.2	F	Table 3
LIM2	Gene	0.19	1374.6	8.8	F	(21)
NKG7	Gene	0.23	1383.4	2.8	2	Table 3
ETFB	Gene	0.24	1386.2	20.8	2	Table 3
CD37	Gene	0.30	1407.0	8.1	2	Table 3
IRF3	Gene	0.31	1415.1	159.8	2	Table 3
LG 7		0.41				
TNNT1	Gene	0.48	1574.9	11.8	1.5	Table 3
STK13	Gene	0.49	1586.7	7.0	F	Table 3
PTPRH	Gene	0.46	1593.7	14.7	2	Table 3
FBN-L079	EST	0.41	1608.4	12.2	2	Table 3
MS936-FBN	MS	0.42	1620.6	7.1	1.5	Table 3
RPL28	Gene	0.41	1627.7	2.3	1.5	Table 3
PEG3	Gene	0.40	1630.0	4.7	1.5	Table 3
BM6507	MS	0.40	1634.7	7.9	F	(39)
BM2078	MS	0.38	1642.6	23.2	F	(42)
TGLA227	MS	0.31	1665.8		F	(43)
Total average	RF →	**0.24**	**1665.8**		← Total map length	

RF, retention frequency; LG, RH linkage group; MS, microsatellite marker; EST, expressed sequence tag; F, framework map marker.

*Average RF for LG's at the top of each LG.

†Distance in $cR_{5,000}$ between immediately following markers beginning from the centromere.

‡Probability of placement: $P > 3$ for F; $P > 3$, totally linked markers; $P < 3$, placed markers.

§Table 3 is published as supporting information on the PNAS web site, www.pnas.org.

data and known physical loci suggested a physical break-point position distal from HAUT14 and proximal from GPI and CEBPA. The bovine microsatellite BM7109 located between KIAA0174 and POP4 marked a genetic break-point position about 50 cM from the centromere (Fig. 1). These mapping data support the assumption of a more distally located evolutionary chromosome break point on BTA18 (23). The assumed second interchromosomal break point at the telomere (21) could not be confirmed.

In addition to the interchromosomal break point, several evolutionary intrachromosomal rearrangements were found. The chromosome segments proximal to the chromosome break point between HSA16q and HSA19q showed a different order in cattle but a similar order of segments in mice (44). However, the order of the chromosome segments between the distal part of BTA18 and HSA19q was highly conserved, whereas the order in mice differed and indicated rearrangements in the proximal part of MMU7. A conserved order of loci was found within the cattle and human chromosome segments containing PHKB, KIAA0615, ADCY7 and POP, RMP, CEBPA, respectively. The locus order in the chromosome segments including the genes FANCA, CDK10, SPG7, APRT, GALNS, and SLC7A5 also was conserved but inverted between cattle and human. No conserved gene order was detected in mice. The genes AARS, TAX1, GLG1, CTRB1, CLECSF1 and the genes DIA4, HP, ATBF1, KIAA0174 have their origin in the same conserved chromosome segment in human. In mouse, this segment also is conserved. In cattle, the rearrangement of these genes to LG1 and LG4 indicates a break point in this chromosome region.

This higher resolution $RH_{5,000}$ map for BTA18 provides new putative candidate genes for economic trait mapping approaches. The results contribute to a better understanding of chromosome evolution by the demonstration of genome conservation between cattle, human, and mouse. The observed high proportion of intrachromosomal rearrangements within homologous chromosome fragments underlines the impact of species-specific gene-mapping approaches. In addition, the new mapping data closed gaps in the published BTA18 $RH_{5,000}$ map. The BTA18 map is an example for the next generation of higher resolution cattle chromosome $RH_{5,000}$ maps and is a prerequisite for the use of the recently developed $WGRH_{12,000}$ panel (45) to construct a high resolution BTA18 gene map.

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