A radiation hybrid map of river buffalo (*Bubalus bubalis*) chromosome 7 and comparative mapping to the cattle and human genomes

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Abstract. A preliminary radiation hybrid (RH) map containing 50 loci on chromosome 7 of the domestic river buffalo *Bubalus bubalis* (BBU; 2n = 50) was constructed based on a comparative mapping approach. The RH map of BBU7 includes thirty-seven gene markers and thirteen microsatellites. All loci have been previously assigned to *Bos taurus* (BTA) chromosome BTA6, which is known for its association with several economically important milk production traits in cattle. The map consists of two linkage groups spanning a total length of 627.9 cR_{5,000}. Comparative analysis of the BBU7 RH_{5,000} map with BTA6 in cattle gave new evidence for strong similarity between the two chromo-

somes over their entire length and exposed minor differences in locus order. Comparison of the BBU7 RH_{5,000} map with the *Homo sapiens* (HSA) genome revealed similarity with a large chromosome segment of HSA4. Comparative analysis of loci in both species revealed more variability than previously known in gene order and several chromosome rearrangements including centromere relocation. The data obtained in our study define the evolutionarily conserved segment on BBU7 and HSA4 to be between 3.5 megabases (Mb) and 115.8 Mb in the HSA4 (genome build 36) DNA sequence.

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The economic importance of river buffalo as livestock species in many countries around the world is undisputed. More than 168 million domesticated river buffaloes (*Bubalus bubalis* (Linnaeus 1758) were counted worldwide, which is more than 11% of the Bovinae world livestock population

(Iannuzzi et al., 2003; FAO 2005, 2006, 2007). Construction of a high-resolution river buffalo gene map, linked with trait associated genome regions, will therefore be an important milestone for developing efficient breeding strategies and modern productive phenotypes. The map will be used as a tool for genome-wide analysis of functional diversity of trait associated candidate genes within the numerous described river buffalo breeds (e.g. FAO 2005, Kumar et al., 2007). Likewise, an elucidated genome of the river buffalo will contribute enormously to a better understanding of genome evolution in Ruminantia, specifically in domestic Bovidae. Various projects, including the definition of a river buffalo karyotype nomenclature (e.g. Iannuzzi, 1994; Popescu et al., 1996), and numerous synteny, fluorescence in situ hybridization (FISH), and Zoo-FISH mapping experiments (e.g. Iannuzzi et al., 1996, 1998, 2001, 2003; de Hondt et al., 1997;

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El Nahas et al., 2001; Di Meo et al., 2002) initiated the genome analysis in river buffalo and opened the gate for complex genome-wide studies such as RH mapping. A 5,000 rad whole genome radiation hybrid panel for river buffalo (BBURH_{5,000}) was recently generated by this mapping approach. The potential of this panel for genotyping RH markers by PCR and subsequent RH linkage map construction has been already confirmed (Amaral et al., 2007; Miziara et al., 2007; Stafuzza et al., 2007).

This study focused on comparative analysis of BBU7 by interspecies-specific transfer of cattle and human genome data to the river buffalo genome. Previous physical mapping has comparatively anchored five bovine genes and two microsatellite markers on BBU7, which made it possible to estimate the size and determine the orientation and order of conserved chromosome fragments between BBU7, BTA6, and the human reference chromosome HSA4 (Di Meo et al., 2000; Iannuzzi et al., 2001, 2003). A higher map resolution is required for a more detailed chromosome description that will allow trait association studies, and subsequently, the identification of positional candidate genes for functional studies. BBU7 received early attention in our buffalo mapping project because the homologous chromosome BTA6 harbors several quantitative trait loci for economically important traits, including milk production traits in dairy cattle as well as growth and body composition traits in meat cattle (e.g. Khatkar et al., 2004, 2005; Schnabel et al., 2005; Weikard et al., 2005; Polineni et al., 2006).

This study develops a first-generation RH map of the second largest acrocentric chromosome in river buffalo, BBU7, identifies chromosome rearrangements, analyses the comparative loci order, and determines evolutionarily conserved segments between BBU7, BTA6, and HSA4.

Material and methods

DNA sequences, primer design, genotyping by PCR, DNA sequencing

Initially, genotyping experiments were performed with published primer sequences. Seventy-five DNA sequences (genes and microsatellite markers) for mapping were chosen from the BTA6 RH_{12,000} map (Rexroad III et al., 2000; Weikard et al., 2006). When available bovine primer sequences did not generate any PCR product with river buffalo genomic DNA or displayed weak typing signals after PCR amplification within the RH panel, markers were completely excluded from further genotyping or new primer pairs were designed based on bovine and human reference DNA sequences by genome database search at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). The software program PRIMER3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) was used to design oligonucleotide primers for PCR (Rozen and Skaletsky, 2000). Genotyping by PCR of DNA sequences was performed in DNA from 90 selected RH cell lines of the BBURH_{5,000} panel (Amaral et al., 2007). The scoring of genotyping signals indicates presence (1) or absence (0) of RH markers within cell lines. Double typing of all DNA sequences within the BBURH_{5,000} panel increased the accuracy of retention data calculation for RH mapping; when the two typings gave discordant results, this was recorded as a 2 in the digitized input for map computation. PCR used in primer tests and hybrid cell lines followed a standard protocol (Goldammer et al., 2004). *Taq* cycle sequencing of bovine PCR products with the automated sequencer 310C (Applied Biosystems) identified corresponding

river buffalo DNA sequences. NCBI BLAST (Altschul et al., 1997) search with the river buffalo DNA sequences identified similarity with corresponding bovine and human genome data.

Construction of the BBU7 RH_{5,000} map

The software packages *rh_tsp_map* 3.0 (Agarwala et al., 2000; Schäffer et al., 2007) and CONCORDE (Applegate et al., 2006) were used for RH map construction, using the maximum likelihood (MLE) criterion. We followed the steps in the *rh_tsp_map* tutorial (ftp://ftp.ncbi.nlm.nih.gov/pub/agarwala/rhmapping/rh_tsp_map.tar.gz) through placement of markers with respect to framework maps, but we did not assign final cR positions to the placed markers. The same procedure was used for maps of BBU6 (Stafuzza et al., 2007) and BBU1 (Miziara et al., 2007), except that in this study, we increased the LOD score threshold for constructing linkage groups to 7.0 because the overall collection of markers has grown, so that higher inter-chromosomal LOD scores would be expected to occur by chance.

Results

A total of 75 bovine genes and microsatellites were included in the RH mapping study. Fifty-seven of the available or newly designed bovine primer sets produced PCR products with river buffalo genomic DNA and reliable genotyping results within the BBURH_{5,000} panel. DNA sequencing of PCR products confirmed sequence specificity of mapped RH markers. Seven markers were dropped because they could not be included in the framework maps and could not be reliably placed with respect to the framework maps. The RH maps include the remaining 50 RH markers (37 genes and 13 microsatellites) (Fig. 1). The first step of map construction resulted in the generation of two RH linkage groups. The map of the first group consists of eight RH markers (6 genes and 2 microsatellites) and has a length of 69.31 cR_{5,000}. The map of the second linkage group contains the remaining 42 RH markers (31 genes and 11 microsatellites). The calculated length for this group is 558.55 cR_{5,000}. The orientation of the large group was determined because several markers shown near the upper end have pairwise inter-group LOD score >5.0 with a marker in the small group, while markers near the lower end appear unlinked to the small group. The orientation of the small group was made consistent with BTA6 because inter-group scores do not strongly favor one possible orientation of the small group over the other. The cytogenetic anchor loci for *MTTP* and *BM1329* at position BBU7q21 and *CSN3* at BBU7q32 (Iannuzzi et al., 2003) enabled the chromosome orientation of the BBU7 RH_{5,000} map as presented in Fig. 1.

The BBU7 RH_{5,000} map is based on a framework map composed of 31 RH markers. Seventeen RH markers, which did not meet the statistical criteria for a frame marker were placed in the most likely framework interval on the map. The marker retention frequency averaged 27.8%, varying from 18.9% for *DMP1* to 51.1% for *NUP54* (Fig. 2). Table 1 presents additional information about RH markers, genotyping by PCR, and RH mapping results.

Comparison of the calculated locus order for BBU7 with the RH_{12,000} reference map for cattle BTA6 (Weikard et al., 2006) and with the cattle genome sequence map for BTA6

Fig. 1. BBU7 RH_{5,000} map comparison to cytogenetic data of BBU7 (GTG-bands), cattle BTA6 RH_{12,000} map, and BTA DNA sequence (NCBI *Bos taurus* build 3.1). The cattle maps show only those loci that correspond to BBU7 markers. Black lines connect corresponding loci between displayed maps. Blue and black colors identify genes and microsatellite markers, respectively. Boldface type indicates assigned frame markers and frame identical markers. Italic type identifies placed markers and brackets determine intervals of placement in the map. Loci are in a rectangle, if RH markers are at the same position in the BBU7 RH_{5,000} map due to identical retention patterns.

Fig. 2. Retention frequencies of 50 RH markers on the BBU7 RH_{5,000} map. A solid line indicates the average retention frequency of 27.8% for all RH markers. The Y axis displays names of every other RH marker.

reveals complete synteny between analyzed chromosome segments and a similar locus order between both chromosomes. Contrary to the locus order on BTA6, mapping of *UGT8* on BBU7 resulted in a more distal locus position. Likewise, *GABRA2* and *UGDH* have assignments more distal in the presented map compared with cattle data. The telomere region of BBU7 displays the most conspicuous rearrangement in loci order compared with BTA6. Assignment of the chromosome segment harboring loci *PTPN13*, *MAPK10*, and *DMP1* to the telomere end of the BBU7 RH_{5,000} map suggests a chromosome rearrangement on BBU7 compared with BTA6.

Comparison of loci from orthologous genes between the BBU7 RH_{5,000} map and the human genome identified similarity of BBU7 exclusively with chromosome fragments of HSA4 build 36 (Fig. 3). The constructed comparative gene

map including 37 gene loci from BBU7 covers a syntenic region on HSA4 between 3.5 Mb for *LRPAP1* and 115.8 Mb for *UGT8*, which represents the chromosome fragment HSA4p16 to HSA4q26 at the cytogenetic level. According to newly apparent variations in gene order, we grouped comparative loci in five syntenic segments on BBU7, which correspond to five segments on HSA4. These segments identified several intrachromosomal breakpoints between BBU7 and HSA4. One major breakpoint on HSA4 is located between *DMP1* and *SPP1* (position 89 Mb). Whereas *DMP1* represents the telomere side of BBU7, *SPP1* is at the edge of the most proximal segment on BBU7. Another breakpoint was detected at the p-arm of HSA4. The telomere side of it corresponds to another distal segment on BBU7 harboring the genes *ACOX3*, *LRPAP1*, *LDB2*, and *QDPR*.

Table 1. Summarized information about BBU7 RH_{5,000} markers, PCR, and relevant RH_{5,000} mapping data based on the rh_tsp_map 3.0 analysis

RH marker				PCR ^a			RH _{5,000} mapping data ^b		
<i>HGNC locus symbol</i>	Alternate cattle locus symbol	Locus type	Cattle reference acc. number	Primer sequences (5'-3') or primer source reference	AT °C	L bp	Position of RH marker cR _{5,000}		MP
							accum.	next	
RH-LG 1									
<i>PPP3CA</i>		gene	NM_174787	GAACCACCTGCTTATGGACCT ACACCCCTGACTGTGTTGT	58	111	0.0	6.0	F
	BM1329	MS	G18422	Bishop et al., 1994	58	119	6.0	0.0	P
<i>MTTP</i>	MTP	gene	XM_592505	TTGGGAGGACTTGAAAAAGC AGGGACATCATATCTCTGGAGTG	58	171	6.0	3.4	P
	DIK5359	MS	AB166346	Ihara et al., 2004	60	129	9.4	17.1	F
<i>UBE2D3</i>		gene	NM_001075135	Weikard et al., 2006	55	124	26.5	18.4	F
<i>UNC5C</i>	LOC533256	gene	XM_612601	Weikard et al., 2006	55	220	44.9	7.3	P
<i>SMARCAD1</i>	LOC530506	gene	XM_608980	Weikard et al., 2006	60	153	52.2	17.1	F
<i>SNCA</i>		gene	NM_001034041	Weikard et al., 2006	60	171	69.3		F
RH-LG 2									
<i>UGT8</i>		gene	NM_001083635	Weikard et al., 2006	55	108	0.0	45.5	F
<i>ABCG2</i>		gene	NM_001037478	GGAACTCAGTTTATCCGTGGTG CAGGAGCAAAAAGGACAGCAT	60	150	45.5	0.0	F
<i>SPP1</i>		gene	NM_174187	GACCTAGATCATAAGAGTGAAGAAGAC GTATTTTCTCCTTTCAATTGACCTC	58	135	45.5	6.0	Fi
	DIK5407	MS	AB166386	Ihara et al., 2004	60	186	51.5	0.0	P
<i>LAP3</i>	LOC781648	gene	BC105385	TACAGCTGCAGCATTCCTGA TCTTGACTGAACCGAAACAGG	60	196	51.5	8.0	P
<i>NCAPG</i>	LOC531234	gene	XM_609721	Weikard et al., 2006	60	117	59.5	8.4	F
<i>SLIT2</i>		gene	XM_613831	Weikard et al., 2006	58	161	67.9	8.0	F
	BMS382	MS	G18842	Stone et al., 1995	55	150	75.9	3.3	P
	FBN12	MS	Z83209	Weikard et al., 2006	58	164	79.2	11.4	F
<i>PPARGC1A</i>		gene	NM_177945	Weikard et al., 2006	58	244	90.6	0.0	F
	BM143	MS	G18387	Bishop et al., 1994	60	90	90.6	12.6	Fi
<i>CCKAR</i>	LOC506207	gene	XM_582625	Weikard et al., 2006	58	223	103.2	0.0	P
<i>PI4K2B</i>	LOC521790	gene	XM_600059	Weikard et al., 2006	58	128	103.2	2.3	P
<i>SOD3</i>	ECSOD	gene	XM_611006	Weikard et al., 2006	60	244	105.5	22.0	P
	FBN13	MS	Z83210	Weikard et al., 1997	58	151	127.5	6.6	P
	BMS483	MS	G18848	Stone et al., 1995	58	109	134.1	13.5	F
<i>PHOX2B</i>	LOC783703	gene	XM_001252230	Weikard et al., 2006	55	206	147.6	2.4	F
<i>ATP8A1</i>		gene	NM_174838	Weikard et al., 2006	55	114	150.0	6.9	P
<i>CENTD1</i>	LOC512010	gene	XM_589445	TCCTCAGCTAGTGAAGTGAATGTT TATTCCAACTTTGAGGAGCACAA	55	150	156.9	12.5	F
	FBN21	MS	AJ416566	Weikard et al., 2002	55	223	169.4	13.4	F
	BM4621	MS	G18529	Bishop et al., 1994	55	137	182.8	13.5	F
<i>CHIC2</i>	LOC781477	gene	NM_001078048	Weikard et al., 2006	55	156	196.3	8.0	P
<i>KDR</i>	FLK-1	gene	XM_611785	Weikard et al., 2002	55	225	204.3	16.9	F
<i>IGFBP7</i>	LOC616368	gene	XM_868373	Weikard et al., 2006	55	114	221.2	3.6	F
	BM415	MS	G18413	Bishop et al., 1994	62	141	224.8	1.4	P
<i>PPAT</i>	MGC148403	gene	XM_586663	Weikard et al., 2006	55	106	226.2	7.4	P
	FBN14	MS	Z83211	AGATCCCTTGAGGAGGAAA GAATTTGCATCACTGTTCTCCA	55	221	233.6	11.9	F
<i>LPHN3</i>		gene	NM_181033	GGTGGCAAAACACAGTGAGC CGTGCTGCTGAAGGAGATGT	60	106	245.5	27.1	F
<i>GABRA2</i>		gene	NM_174541	TTGGACATACAATGCGTCTGA AATTGTCTCCTTCCCAATTGAT	55	146	272.6	13.9	F
<i>CENPC1</i>		gene	NM_001045881	AGGCCACGAGATACATACCAA TGCTGCTTTCCCTTTTCTTG	58	128	286.5	7.3	F
<i>EPHA5</i>	LOC784561	gene	XM_001252832	GGGAAGAGATTGGTGAAGTTG CCGCAGAGTAAATTGAGTTCTATG	58	158	293.8	16.2	P
<i>CSN3</i>	CSN10	gene	NM_174294	CGCTGTGAGAAAGATGAAAGATT TTTGGGCAGGTGACCTAACT	55	190	310.0	35.1	F
<i>UGDH</i>		gene	NM_174211	Weikard et al., 2006	55	86	345.1	34.8	F
<i>NUP54</i>	LOC513286	gene	NM_001075285	Weikard et al., 2002	55	127	379.9	48.9	F
<i>ACOX3</i>	LOC510065	gene	XR_028866	TGCGCTGTAAGAGGGTCTTC CATGCAGGAAGAAGCTTGACG	55	135	428.8	12.7	P
<i>LRPAP1</i>	LOC504957	gene	NM_001080225	GAGGAGCTCAAGCACTTTGAA AGCTCCTTGGTCCTCTCCTC	55	167	441.5	20.3	F
<i>LDB2</i>		gene	NM_001046611	Weikard et al., 2006	62	140	461.8	14.2	F

Table 1 (continued)

RH marker				PCR ^a	AT °C	L bp	RH _{5,000} mapping data ^b		
<i>HGNC locus symbol</i>	Alternate cattle locus symbol	Locus type	Cattle reference acc. number	Primer sequences (5'–3') or primer source reference			Position of RH marker cR _{5,000}	MP	
							accum.	next	
QDPR	LOC509926 BMC4203	gene MS	NM_001076492 G19110	Weikard et al., 2006	60	146	476.0	27.0	F
				GGCCCGAGGTGTAGTGTAAA	55	152	503.0	16.6	F
PTPN13		gene	NM_174590	GGTGGGCTACAGTCCATAGG	52	400	519.6	4.2	P
				CAGCTGCTGCAACCTGTGATAA					
MAPK10	LOC509652	gene	XM_878132	GCGGATGTATAGCATGTATGGAG	55	110	523.8	34.8	F
				CCAAGCCAGAGACTTGTCTGT					
DMP1		gene	NM_174038	CAGCAGGGTCATACCAGACA	55	180	558.6		F
				GACGATGATGAAGACGAGAGC					
				CCTTGTGGGGTGTCTGTCT					

^a AT: annealing temperature; L: length of PCR product.

^b F: framework marker; Fi: marker with identical locus as framework marker; HGNC: HUGO Gene Nomenclature Committee; MP: marker placement; P: placed markers (CentiRay positions of placed markers were estimated by interpolation).

Fig. 3. Comparative map between gene loci in river buffalo BBU7 RH_{5,000} map and corresponding orthologous gene loci in human chromosome HSA4. Continuous blocks connected by lines identify related conserved syntenic chromosome segments between species. Breaks between blocks determine evolutionary breakpoints. A scale of the HSA4 DNA sequence (NCBI *Homo sapiens* build 36.2) and a cytogenetic drawing allow a detailed comparison of similarity between BBU7 and HSA4. A bold solid line represents a summary of single cytogenetic loci on HSA4 (precise loci order available at NCBI *Homo sapiens* build 36.2). Arrows indicate a suggested inversion in gene order of BBU7 segments compared with loci order of HSA4. Dotted lines identify genes of continuous segments of BBU7 separated on HSA4.

Discussion

We used a comparative mapping approach, based on available human and cattle genome data to construct an RH map for river buffalo chromosome BBU7. The RH_{5,000} map provides sufficient information for identification of syntenic blocks between related chromosomes of the three species and uncovers evolutionary breakpoint regions. Additionally, the map contributes new physical anchors, which enable improved *in silico* gene assignment to BBU7.

We mapped 50 of the 57 typed markers on river buffalo chromosome BBU7. Surprisingly, only 36 (48%) of the initially tested 75 cattle primer sequences from the RH_{12,000} map of BTA6 (Weikard et al., 2006) amplified corresponding DNA sequences in river buffalo genomic DNA. Another twenty-one were developed by designing new sets of primers primarily in genome regions conserved between cattle and human. However, the drop out of numerous RH markers decreased density in some regions of the map. Thirty-seven gene loci allowed a comparative analysis with the human genome, and 13 nearly uniformly distributed microsatellite markers represent putative informative markers potentially useful in quantitative trait studies in river buffalo. Except for a few discrepancies and small rearrangements, we found that the BBU7 RH_{5,000} map has a similar marker order to BTA6. We found a locus for *NUP54* on BBU7; *NUP54* has been assigned by RH mapping on BTA6 (Weikard et al., 2006) and likewise on HSA4 (NCBI *Homo sapiens* build 36.2), but is not identified on the annotated *Bos taurus* genome build 3.1.

The most interesting loci in our map are *UGT8* and *NUP54*. In cattle, *UGT8* is located in a very proximal position of 6.1 Mb on BTA6, which is about 13 Mb away from *UBE2D3*, the next distal locus with a comparative placement in the BBU7 RH_{5,000} map (NCBI *Bos taurus* build 3.1; Fig. 1). According to our data, *UGT8* represents the distal boundary of conserved segments on HSA4 compared with BBU7. Although no corresponding position for *UGT8* was identified in the BTA6 RH_{12,000} map (Weikard et al., 2006) and *UGT8* shows only weak linkage with *SNCA* (RH linkage group 1) and is furthermore located in a relative high distance of 45.5 cR_{5,000} to its next marker *ABCG2* in RH linkage group 2, we calculated its locus as proximal frame marker of RH linkage group 2 on BBU7. Mapping of more RH markers and some cytogenetic anchors in this chromosome region is required to define the exact position of *UGT8* and to form a single linkage group with the remaining markers of the first linkage group. The high retention frequency of 51% for *NUP54*, differs substantially from mean value of the other mapped RH markers and caused distances of 34.8 cR_{5,000} and 61.6 cR_{5,000} to its neighboring frame markers *UGDH* and *LRPAP1*, respectively. Perhaps the cattle primers for *NUP54* amplified sequences of other genes in the *NUP* multigene family in some cell lines of the BBURH_{5,000} panel. Nevertheless, loci for *NUP54* in BTA6 and HSA4 are consistent with its relative position on BBU7. Further mapping of region-specific markers should clarify these findings.

A first view at the telomere of BBU7 would suggest an inversion of eight loci in this area compared with BTA6. However, the region illustrates inconsistency in the locus order, which makes it crucial to assign more markers in this region. The BBU7 RH_{5,000} map is cytogenetically linked by *MTTP* and *BM1329* to BBU7q21 and by *CSN3* to BBU7q32 (Iannuzzi et al., 2003). No other cytogenetic anchors link the BBU7 RH_{5,000} map to the chromosome yet. Therefore, we suggest that some of the new markers on the RH map be mapped cytogenetically. This is a useful approach in comparative mapping studies for ordering complex RH linkage groups, as illustrated in the recent construction of an RH_{5,000} map for sheep chromosome 23 (Tetens et al., 2007).

As expected, the evolutionary conservation of chromosome segments between BBU7 and HSA4 follows principally the known comparative order of segments between BTA6 and HSA4 (Weikard et al., 2006, 2007). Small discrepancies in locus order within conserved segments might indicate small chromosome rearrangements, but could also be due to the insufficient resolution of the BBU7 RH_{5,000} map in specific regions of the chromosome. Segments in the middle part of BBU7 correspond with HSA4 p-arm and q-arm fragments covering the HSA4 centromere. This suggests that a centromere translocation in Bovidae or in Hominidae occurred during evolution. The suggestion of rearrangements between the two segments in the telomere region of BBU7 and HSA4 q-arm and p-arm are based on seven loci. The findings indicate noticeable rearrangements, which need further evaluation with more markers. The proximal syntenic block on BBU7, composed of nine loci, was located at the q-arm of HSA4, which indicates another chromosome rearrangement between BBU7 and HSA4. Based on the identified gene order, we suggest an inversion of loci between both chromosomes. However, on BBU7 this block includes genes of two distinct RH linkage groups. Consequently, a more precise characterization of this region on BBU7 is necessary.

In sum, the constructed RH_{5,000} map for BBU7 allows the description of variation and similarity between corresponding chromosomes of different species as cattle and human. The future assignment of numerous orthologous loci to the new identified breakpoints and to the start and end points of the BBU7 RH_{5,000} map will improve the presented map and contribute to the generation of a high-resolution gene map for the river buffalo genome.

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References

- Agarwala R, Applegate DL, Maglott D, Schuler GD, Schäffer AA: A fast and scalable radiation hybrid map construction and integration strategy. *Genome Res* 10:350–364 (2000).
- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, et al: Gapped BLAST and PSI-BLAST – A new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402 (1997).
- Amaral MEJ, Owens KE, Elliott JS, Fickey C, Schäffer AA, et al: Construction of a river buffalo (*Bubalus bubalis*) whole-genome radiation hybrid panel and preliminary RH mapping of chromosomes 3 and 10. *Anim Genet* 38:311–314 (2007).
- Applegate D, Bixby R, Chvátal V, Cook W: The Traveling Salesman Problem: A Computational Study (Princeton University Press, Princeton, NJ, USA 2006).
- Bishop MD, Kappes SM, Keele JW, Stone RT, Sundén SLF, et al: A genetic linkage map for cattle. *Genetics* 136:619–639 (1994).
- De Hondt, Gallagher D, Oraby H, Othman OE, Bosma AA, et al: Gene mapping in the river buffalo (*Bubalus bubalis* L.): five syntenic groups. *J Anim Breed Genet* 114:79–85 (1997).
- Di Meo GP, Perucatti A, Schibler L, Incarnato D, Ferrara L, et al: Thirteen type I loci from HSA4q, HSA6p, HSA7q and HSA12q were comparatively FISH-mapped in four river buffalo and sheep chromosomes. *Cytogenet Cell Genet* 90:102–105 (2000).
- Di Meo GP, Perucatti A, Incarnato D, Ferretti L, Di Berardino D, et al: Comparative mapping of twenty-eight bovine loci in sheep (*Ovis aries*, 2n = 54) and river buffalo (*Bubalus bubalis*, 2n = 50) by FISH. *Cytogenet Genome Res* 98:262–264 (2002).
- El Nahas SM, de Hondt HA, Womack JE: Current status of the river buffalo (*Bubalus bubalis* L.) gene map. *J Hered* 92:221–225 (2001).
- FAO: Buffalo Production and Research. Borghese A (ed), Rome, Italy: 321 p (2005).
- FAO: Livestock's Long Shadow: Environmental Issues and Options. Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M, de Haan C (eds), Rome, Italy: 390 p (2006).
- FAO: The State of the World's Animal Genetic Resources for Food and Agriculture. Rischkowsky B, Pilling D (eds) Rome, Italy: 523 p (2007).
- Goldammer T, Kata SR, Brunner RM, Kuhn C, Weikard R, Womack JE, Schwerin M: High-resolution comparative mapping between human chromosomes 4 and 8 and bovine chromosome 27 provides genes and segments serving as positional candidates for udder health in cattle. *Genomics* 84:696–706 (2004).
- Iannuzzi L: Standard karyotype of the river buffalo (*Bubalus bubalis* L., 2n = 50). Report of the committee for the standardization of banded karyotypes of the river buffalo. *Cytogenet Cell Genet* 67:102–113 (1994).
- Iannuzzi L, Gallagher DS, Womack JE, Meo GP, Shelling CP, et al: FISH mapping of the alpha-S2 casein gene on river buffalo and cattle chromosomes identifies a nomenclature discrepancy in the bovine karyotype. *Chromosome Res* 4:159–162 (1996).
- Iannuzzi L, Di Meo GP, Perucatti A, Bardaro T: ZOO-FISH and R-banding reveal extensive conservation of human chromosome regions in euchromatic regions of river buffalo chromosomes. *Cytogenet Cell Genet* 82:210–214 (1998).
- Iannuzzi L, Di Meo GP, Hayes H, Perucatti A, Incarnato D, et al: FISH-mapping of 31 type I loci (Texas markers) to river buffalo chromosomes. *Chromosome Res* 9:339–342 (2001).
- Iannuzzi L, Di Meo GP, Perucatti A, Schibler L, Incarnato D, et al: The river buffalo (*Bubalus bubalis*, 2n = 50) cytogenetic map: assignment of 64 loci by fluorescence in situ hybridization and R-banding. *Cytogenet Genome Res* 102:65–75 (2003).
- Ihara N, Takasuga A, Mizoshita K, Takeda H, Sugimoto M, et al: A comprehensive genetic map of the cattle genome based on 3802 microsatellites. *Genome Res* 14:1987–1998 (2004).
- Khatkar MS, Thomson PC, Tammen I, Raadsma HW: Quantitative trait loci mapping in dairy cattle: review and meta-analysis. *Genet Sel Evol* 36:163–190 (2004).
- Khatkar MS, Thomson PC, Tammen I, Costa F, Raadsma HW: Combined QTL map of dairy cattle traits. http://www.vetsci.usyd.edu.au/reprogen/QTL_Map (2005).
- Kumar S, Nagarajan M, Sandhu JS, Kumar N, Behl V, Nishanth G: Mitochondrial DNA analyses of Indian water buffalo support a distinct genetic origin of river and swamp buffalo. *Anim Genet* 38:227–232 (2007).
- Miziara MN, Goldammer T, Stafuzza NB, Agarwala R, Schäffer AA, et al: Generation of a radiation hybrid map of river buffalo (*Bubalus bubalis*) chromosome one (BBU1). *Cytogenet Genome Res* 119:100–104 (2007).
- NCBI *Bos taurus* (cow) build 3.1, www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9913.
- NCBI *Homo sapiens* (human) build 36.2, www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9606.
- NCBI – National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/>.
- Polineni P, Aragonda P, Xavier SR, Furuta R, Adelson DL: The bovine QTL viewer: a web accessible database of bovine Quantitative Trait Loci. *BMC Bioinformatics* 7:283 (2006).
- Popescu CP, Long S, Riggs P, Womack J, Schmutz S, Fries R, Gallagher D, eds: Texas nomenclature, 1996 – Standardization of cattle karyotype nomenclature: report of the committee for the standardization of the cattle karyotype. *Cytogenet Cell Genet* 74:259–261 (1996).
- Rexroad CE III, Owens EK, Johnson JS, Womack JE: A 12,000 rad whole genome radiation hybrid panel for high resolution mapping in cattle: characterization of the centromeric end of chromosome 1. *Anim Genet* 31:262–265 (2000).
- Rozen S, Skaletsky H: Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol* 132:365–386 (2000).
- Schäffer AA, Rice ES, Cook W, Agarwala R: rh_tsp_map 3.0: end-to-end radiation hybrid mapping with improved speed and quality control. *Bioinformatics* 23:1156–1158 (2007).
- Schnabel RD, Kim J-J, Ashwell MS, Sonstegard TS, Van Tassel CP, et al: Fine-mapping milk production quantitative trait loci on BTA6: analysis of the bovine osteopontin gene. *Proc Natl Acad Sci USA* 102:6896–6901 (2005).
- Stafuzza NB, Ianella P, Miziara MN, Agarwala R, Schäffer AA: Preliminary radiation hybrid map for river buffalo chromosome 6 and comparison to bovine chromosome 3. *Anim Genet* 38:406–409 (2007).
- Stone RT, Pulido JC, Duyk GM, Kappes SM, Keele JW, Beattie CW: A small-insert bovine genomic library highly enriched for microsatellite repeat sequences. *Mamm Genome* 6:714–724 (1995).
- Tetens J, Goldammer T, Maddox JF, Cockett NE, Leeb T, Drögemüller C: A radiation hybrid map of sheep chromosome 23 based on ovine BAC-end sequences. *Anim Genet* 38:132–140 (2007).
- Weikard R, Goldammer T, Kühn C, Barendse W, Schwerin M: Targeted development of microsatellite markers from the defined region of bovine chromosome 6q21–31. *Mamm Genome* 8:836–840 (1997).
- Weikard R, Kühn C, Goldammer T, Laurent P, Womack JE, Schwerin M: Targeted construction of a high-resolution, integrated, comprehensive, and comparative map for a region specific to bovine chromosome 6 based on radiation hybrid mapping. *Genomics* 79:768–776 (2002).
- Weikard R, Kühn C, Goldammer T, Freyer G, Schwerin M: The bovine *PPARGC1A* gene: molecular characterization and association of an SNP with variation of milk fat synthesis. *Physiol Genomics* 21:1–13 (2005).
- Weikard R, Goldammer T, Laurent P, Womack JE, Kuehn C: A gene-based high-resolution comparative radiation hybrid map as a framework for genome sequence assembly of a bovine chromosome 6 region associated with QTL for growth, body composition, and milk performance traits. *BMC Genomics* 7:53 (2006).
- Weikard R, Goldammer T, Womack JE, Kuehn C: Forward to a detailed sequence map of bovine chromosome 6. *Cytogenet Genome Res* 116:72–79 (2007).