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A framework radiation hybrid map of buffalo chromosome 1 ordering scaffolds from buffalo genome sequence assembly

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ABSTRACT. River buffalo chromosome 1 (BBU1) is a sub-metacentric chromosome homologous to bovine chromosomes 1 and 27. In this study, we constructed a new framework radiation hybrid (RH) map from BBU1 using $BBURH_{5000}$ panel adding nine new genes (*ADRB3*, *ATP2C1*, *COPB2*, *CRYGS*, *P2RY1*, *SLC5A3*, *SLC20A2*, *SST*, and *ZDHHC2*) and one microsatellite (CSSM043) to the set of markers previously mapped on BBU1. The new framework RH map of BBU1 contained 141 markers (55 genes, 2 ESTs, 10 microsatellites, and 74 SNPs) distributed within one linkage group spanning 2832.62 centirays. Comparison of the RH map

to sequences from bovine chromosomes 1 and 27 revealed an inversion close to the telomeric region. In addition, we ordered a set of 34 scaffolds from the buffalo genome assembly UMD_CASPUR_WB_2.0. The RH map could provide a valuable tool to order scaffolds from the buffalo genome sequence, contributing to its annotation.

Key words: *Bubalus bubalis*; Chromosome 1; Comparative map; Radiation hybrid mapping

INTRODUCTION

Buffalo (*Bubalus bubalis*) plays an important role in world economy, providing high-quality milk and meat, mainly in countries with less financial resources for livestock research. Its genome is organized in five pairs of bi-armed chromosomes, 19 pairs of acrocentric chromosomes, and a pair of X and Y sex chromosomes. The largest chromosome in the river buffalo karyotype, BBU1, is a sub-metacentric chromosome with reported homology between BBU1q and bovine chromosome 1 and between BBU1p and bovine chromosome 27 (Iannuzzi et al., 2003; Miziara et al., 2007).

Genome analysis of *B. bubalis* has advanced significantly in the last few years with the availability of genomic tools such as a radiation hybrid (RH) panel for whole genome mapping (Amaral et al., 2008; Stafuzza et al., 2009) and a genomic BAC library (Stafuzza et al., 2012) especially useful for region-specific re-sequencing and characterization of target genes (Stafuzza et al., 2014a,b,c).

In 2014, a buffalo whole genome sequence generated by next-generation sequencing platforms was publicly released in the NCBI database (http://www.ncbi.nlm.nih.gov/assembly/67671). Nevertheless, mapping of the buffalo genome remains important for identification of genes associated with complex economic traits and for evaluating chromosomal evolution among species of the Bovidae family. Considering that buffalo and cattle exhibit an evolutionary divergence estimated at 20 million years (Parma et al., 2004), investigations on chromosomal rearrangements and comparison of position and order of homologous genes are valuable to enhance our understanding of both the genomes.

In the present study, we constructed a new framework RH map from BBU1 using $BBURH_{5000}$ panel (Amaral et al., 2007), mapping ten new markers including nine coding genes and one microsatellite. The RH map was compared to the sequence from bovine chromosomes 1 and 27 and used to order scaffolds from the buffalo genome assembly UMD_CASPUR_WB_2.0 available in the NCBI database.

MATERIAL AND METHODS

Nine new genes (*ADRB3*, *ATP2C1*, *COPB2*, *CRYGS*, *P2RY1*, *SLC5A3*, *SLC20A2*, *SST*, and *ZDHHC2*) and one microsatellite (CSSM043) derived from bovine chromosomes 1 and 27

were typed by polymerase chain reaction (PCR) on the buffalo RH panel (Amaral et al., 2007). The reference for each marker is listed in Table 1.

PCR was performed in a *Veriti® Thermal Cycler* (Life Technologies™ , Carlsbad, CA,USA) with thermal gradient software. Bovine DNA samples were included as controls in each amplification experiment since the PCR primers were cattle-derived. The markers were scored after amplification of DNA from 90 RH cell lines and buffalo and hamster control DNA. The PCR mixtures included: 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM dNTPs, 0.2 mM each primer, 0.5 unit AmpliTaq Gold® DNA polymerase (Life Technologies™), and 50 ng DNA in a 10 µL reaction volume. The PCR conditions were as follows: initial denaturation at 94°C for 10 min, followed by 35 cycles at 94°C for 30 s (denaturation), 50-65°C for 30 s (annealing depending on the individual marker as described in Table 1), extension at 72°C for 30 s, and a final extension at 72°C for 7 min. The PCR products were electrophoresed on 2% agarose gels in 1X TBE buffer containing ethidium bromide and photographed under UV light. PCR products were scored as 0 for absent, 1 for present, and 2 for ambiguous amplification. All primer sets were typed twice with the RH panel DNA and scored independently to increase the accuracy of the results. Primer pairs that showed ambiguous results were typed a third time.

The RH map was constructed using the software rh_tsp_map (Schäffer et al., 2007) and CONCORDE (Applegate et al., 1998) linked to Qsopt (http://www.tsp.gatech.edu/concorde.html), adding the 10 new markers to the set of markers previously mapped on BBU1 (Amaral et al., 2008). We used the maximum likelihood estimation (MLE) criterion for creating framework MLE-consensus map (Agarwala et al., 2000). Considering the number of markers, the linkage group was made using a pairwise LOD score (logarithm of the odds) threshold of 8.0. The maps were drawn using the MapChart version 2.1 software (Voorrips, 2002).

The BBU1 RH map was compared to the sequence from bovine chromosomes 1 and 27 and used to order scaffolds from the buffalo genome assembly (UMD_CASPUR_WB_2.0).

RESULTS

The framework RH map of BBU1 constructed in this study contained 141 markers (55 genes, 2 ESTs, 10 microsatellites, and 74 SNPs) distributed within one linkage group spanning 2832.62 centirays (cR; Figure 1). The retention frequency of the mapped markers ranged from 20.0% for *MX1* and *SEC62* to 51.1% for *IFNGR2* gene. Additional information about all the mapped markers, including their retention frequency and cR position on the map, is compiled in Table 1.

To compare the BBU1 RH map to sequences from bovine chromosomes 1 and 27 (Btau_4.6.1), we constructed a map in mega bases (Mb) using the corresponding positions of the markers available in the NCBI database (Table 2).

A total of 34 scaffolds from the buffalo genome assembly (UMD_CASPUR_WB_2.0) were ordered on BBU1 following the order of the markers on the RH map (Table 3).

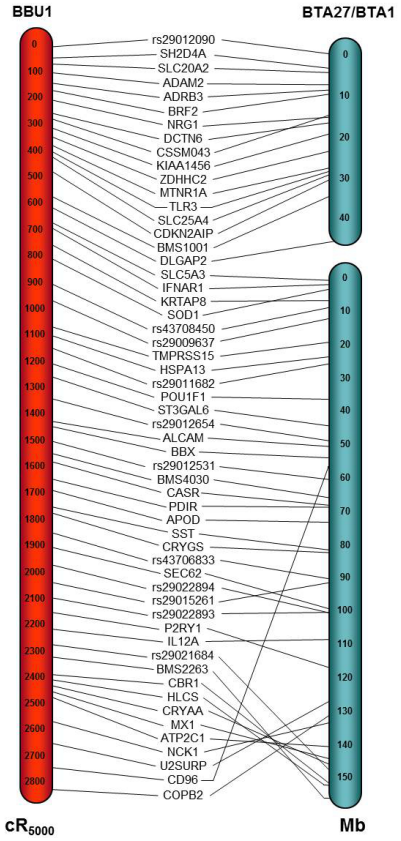

Figure 1. Comparison of BBU1 framework RH map (left) with the cattle chromosomes 1 and 27 from genome build Btau_4.6.1 (right). Positions in the RH map are in cR5000, while positions in the BTA maps are in megabases (Mb). For better illustration, the BBU RH map shows one marker per 50cR. Markers common on both BBU RH and cattle sequence maps are joined by a solid black line.

Table 1. New cattle-derived markers mapped on BBU1 with the BBURH5000 panel, along with their retention frequencies (RF %), PCR primer references and PCR annealing temperatures (Tm).

Marker		PCR primers reference	RF (%)	Tm (ºC)
Symbol	Name			
ADRB3	Adrenergic, beta-3, receptor	Everts-van der Wind et al., 2004	34.4	54
ATP2C1	ATPase, Ca++ transporting, type 2C, member 1	Ma et al., 1998	23.3	56
CSSM043	Microsatellite	Barendse et al., 1994	32.2	58
COPB2	Coatomer protein complex, subunit beta 2	Everts-van der Wind et al., 2004	41.1	56
CRYGS	Crystallin, gamma S	[1]F: AGTATCCTGAGTACCAGCACTG R: TGACTGAAGTCTCAGCAGCCAA	25.5	65
P2RY1	Purinergic receptor P2Y, G-protein coupled, 1	[1]F: CTTGTGAAGATGCAGGAATCCC R: CACGAGGTGTAGGCATTTCCAC	24.4	58
SLC5A3	Solute carrier family 5, member 3	Jann et al., 2006	43.3	50
SLC20A2	Solute carrier family 20, member 2	Everts-van der Wind et al., 2004	30.0	58
SST	Somatostatin	Stafuzza et al., 2014a	24.4	60
ZDHHC2	Zinc finger, DHHC-type containing 2	Everts-van der Wind et al., 2004	37.7	60

[1]Designed using Primer3plus (Untergasser et al., 2007), based on the gene sequence from *Bos taurus* UMD_3.1 reference assembly (GenBank accession No AC_000158.1).

Table 2. Framework markers mapped on BBU1 and their respective position on BBU1 (cR5000) and on bovine sequence from chromosomes 1 and 27 (Btau_4.6.1).

Marker	Position on BBU1 (cR5000)	Position on Bos taurus genome (bp)
BBU1p		BTA27
rs29012090	0.00	43633072
rs29025654	7.96	43629497
rs29020649	31.33	41093242
SH2D4A	42.52	40495081
THAP1	53.53	39760978
SLC20A2	64.54	39466243
rs29013339	79.14	39532022
ADAM2	97.38	36663247
ADRB3	140.00	35215574
CSSM036	153.51	35129771
BRF2	167.14	35109839
rs29012498	184.44	32961800
rs29012293	203.18	33393165
rs29024459	229.71	32281344
NRG1	241.32	29416336
DCTN6	255.61	28223036
CSSM043	266.70	29861771
WRN	283.26	29024009
KIAA1456	309.04	25830826
rs29016185	325.99	24178540
ZDHHC2	342.32	21578329
CNOT7	355.23	21558961
MTNR1A	374.29	17584825
rs29013544	385.30	17250896
TLR3	399.63	17342873
SLC25A4	416.35	16657012
BM1856	432.15	17420230
ODZ3	450.96	14505500
CDKN2AIP	475.07	15423000
rs29010442	494.33	15461470
rs29012623	512.83	12210114
DEFB1	539.80	7415644
BMS1001	571.60	11835596
DLGAP2	611.03	556266
CLN8	620.61	1111548
BBU1q		BTA1
SLC5A3	669.05	40417
IFNAR1	693.38	1268604
AW267109	714.60	1299641
IFNGR2	724.07	1176873
KRTAP8	755.03	3828967
rs29013376	772.49	3548655
TGLA49	782.14	3267630
SOD1	808.70	2902719
rs29012842	850.24	3073723
rs29010139	857.88	3489251
rs29012844	870.64	3073460
rs29025993	891.77	5297323
rs43708450	904.92	6306960
rs29013360	915.37	8023064
rs29013440	925.90	7923870
AJ496763-027.SP6-429	939.15	Unknown
rs29009637	968.29	11228029
rs29013338	1016.33	14723290
rs29013314	1039.31	16827182
rs29019581	1050.92	16917608
TMPRSS15	1068.22	18220248
RM095	1086.93	19538641
HSPA13	1102.32	22524113

Continued on next page

Table 2. Continued.

Marker	Position on BBU1 (cR5000)	Position on Bos taurus genome (bp)
rs29026806	1124.27	22002538
rs29011682	1147.65	24884732
rs43709788	1161.97	24238388
rs29013247	1178.97	26816631
rs29012747	1201.58	35117150
POU1F1	1209.49	35723143
rs43710099	1226.08	34312971
PROS1	1243.08	38728912
ST3GAL6	1257.68	43994350
rs29013985	1266.17	44609625
FILIP1L	1274.25	45119183
rs29013984	1291.25	44609344
rs29010923	1319.30	47123631
rs29012653	1327.80	48804954
rs29012654	1341.75	48805054
rs29017614	1400.14	52511890
ALCAM	1430.18	50417917
BBX	1444.02	52670743
LOC151584	1454.86	52966936
rs29013666	1463.36	54341564
rs29026512	1482.24	59000296
rs43709748	1494.98	59799461
rs29012531	1504.34	59725040
BMS527	1516.78	62536761
BM1312	1534.75	61174083
BMS4030	1552.05	65413568
rs29013617	1572.74	68201840
CASR	1581.83	67574173
rs43705567	1607.14	68447239
PDIR	1648.84	68394647
KALRN	1670.37	69451283
rs43710098	1678.71	71574739
APOD	1690.32	73019925
rs43709791	1709.58	76197996
rs43708475	1725.92	78106961
rs29009958	1735.39	78856792
SST	1762.02	81184020
CRYGS	1770.67	82232306
rs29015647	1779.40	82593594
rs29022387	1792.18	88075394
rs43705570	1802.41	85828502
rs29009859	1835.02	89329402
rs43706833	1851.31	89898926
rs29010311	1860.75	92071361
rs29019397	1873.10	93781792
rs29025711	1888.63	97462173
SEC62	1908.87	99158891
rs29025486	1925.21	99613024
rs29020368	1933.71	101031521
rs29022894	1975.25	100428314
rs29015261	2040.34	91411837
rs29022893	2099.84	100428167
rs29012901	2131.51	109599237
rs43702504	2143.87	115024260
P2RY1	2156.23	116995048
rs42712516	2172.12	121129184
rs29012024	2188.02	118649544
IL12A	2220.19	108584853
rs29021684	2279.49	146683590
C1H8orf42	2303.82	149961374
BMS2263	2327.50	156871277
rs29010039	2336.93	157546306
AF440368-538	2381.62	Unknown
CBR1	2407.46	151929713
HLCS	2422.99	152889925
CRYAA	2440.96	146540499
MX1	2456.15	144732426
ATP2C1	2465.51	141332311
rs29017591	2478.05	137963609
rs29024361	2490.13	136296259
rs29026838	2513.20	144906700

Continued on next page

Table 2. Continued.

Marker	Position on BBU1 (cR5000)	Position on Bos taurus genome (bp)
rs29026456	2536.27	142785760
rs29024604	2558.27	139456223
NCK1	2573.81	134262058
BP230018B20G10	2590.57	132497697
FOXL2	2600.04	132085550
rs29022779	2609.32	130916681
RNF7	2621.86	129473169
rs29027942	2630.88	128394472
U2SURP	2655.21	128037601
CD96	2750.20	56951704
rs43708463	2791.31	57086245
COPB2	2832.62	131999543

Table 3. Buffalo sequence scaffolds ordered according to the positions of the markers mapped on BBU1.

Marker	Position on BBU1 (cR_{5000})	Buffalo scaffold UMD_CASPUR_WB_2.0
SH2D4A	42.52	NW_005784720.1
THAP1	53.53	NW_005785655.1
SLC20A2	64.54	NW_005785655.1
ADRB3	140.00	NW_005784334.1
BRF2	167.14	NW_005784334.1
NRG1	241.32	NW_005785682.1
DCTN6	255.61	NW_005784975.1
WRN	283.26	NW_005785682.1
KIAA1456	309.04	NW_005785635.1
ZDHHC2	342.32	NW_005785878.1
CNOT7	355.23	NW_005785878.1
MTNR1A	374.29	NW_005785680.1
TLR3	399.63	NW_005784957.1
SLC25A4	416.35	NW_005785619.1
CDKN2AIP	475.07	NW_005785619.1
DLGAP2	611.03	NW_005784735.1
SLC5A3	669.05	NW_005784098.1
IFNAR1	693.38	NW_005784098.1
IFNGR2	724.07	NW_005784098.1
KRTAP8	755.03	NW_005784558.1
SOD1	808.70	NW_005784534.1
TMPRSS15	1068.22	NW_005784906.1
HSPA13	1102.32	NW_005785817.1
POU1F1	1209.49	NW_005785338.1
ST3GAL6	1257.68	NW_005784065.1
FILIP1L	1274.25	NW_005784065.1
ALCAM	1430.18	NW_005784632.1
BBX	1444.02	NW_005784504.1
CASR	1581.83	NW_005785537.1
PDIR	1648.84	NW_005785826.1
KALRN	1670.37	NW_005785826.1
APOD	1690.32	NW_005784025.1
SST	1762.02	NW_005785332.1
CRYGS	1770.67	NW_005784864.1
SEC62	1908.87	NW_005784729.1
IL12A	2220.19	NW_005785508.1
HLCS	2422.99	NW_005783537.1
CRYAA	2440.96	NW_005785117.1
MX1	2456.15	NW_005785734.1
ATP2C1	2465.51	NW_005784738.1
NCK1	2573.81	NW_005785398.1
FOXL2	2600.04	NW_005785379.1
RNF7	2621.86	NW_005785806.1
U2SURP	2655.21	NW_005785806.1
CD96	2750.20	NW_005785563.1
COPB2	2832.62	NW_005785379.1

DISCUSSION

In the present study, we obtained a framework RH map of buffalo chromosome 1 based on cattle-derived markers. The BBU1 framework map presented, herein, provided a comparison of the gene order from buffalo chromosome 1 and bovine chromosomes 1 and 27. The alignment of this RH map to the current bovine genome sequence assembly (Btau_4.6.1) indicated regions of possible rearrangements between the chromosomes of both the species. A set of linked markers on the telomeric region of BBU1q showed a rearrangement when compared to the sequence from bovine chromosome 1, revealing an inversion in the region flanked by the markers rs29021684 and COPB2 (Figure 1). In the previous RH maps constructed for this buffalo chromosome (Miziara et al., 2007; Amaral et al., 2008), an inversion in the same region was also observed when compared to the bovine chromosome 1.

The RH map constructed in this study could provide a valuable tool to order scaffolds from the buffalo genome sequence, thereby contributing to the annotation of the buffalo genome, an important agricultural species whose genetic improvement has lagged behind other species because of limited genomic characterization previously.

Conflicts of interest

The authors declare no conflict of interest.

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