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Chromosome and Gene Mapping Homology between River Buffalo, Cattle and Sheep using Molecular Markers

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Abstract: Chromosomal localization of sixteen bovine microsatellites in river buffalo has been assigned in this study, using polymerase chain reaction and buffalo-hamster somatic cell hybrids. These tested microsatellites were previously assigned to sheep chromosomes. This study also aimed to confirm that the chromosome band identity between river buffalo, cattle and sheep is a good indicator of genetic homology between these closely related species. The correlation coefficients between these tested microsatellites and other markers-representing syntenic groups and chromosomes in river buffalo- was calculated to assign these microsatellites to river buffalo chromosomes (BBU)). The results showed that BM719 is assigned to BBU5q, BM827 to BBU12, BM1818 to BBU2p, BM1824 to BBU1q, BM2113 to BBU2q, CSSM015 to BBU3p, CSSM034 to BBU4q, CSSM037 to BBU3q, CSSM043 to BBU1p, CSSM058 to BBU21, ILSTS011 to BBU15, ILSTS013 to BBU10, ILSTS019 to BBU5p, ILSTS029 to BBU6, ILSTS054 to BBU20 and ILSTS060 to BBU4p. The result also showed that 16 tested microsatellites are localized in river buffalo, cattle and sheep on the equivalent chromosomes, which have chromosome- band homology, in these closely related species belonging to Bovidae family, which has a high degree of chromosome conservation between its members and where the bi-armed autosomes are formed by centric fusions of acrocentric autosomes.

Key words: Microsatellites, chromosome homology, river buffalo, cattle, sheep

INTRODUCTION

River buffalo, cattle and sheep are three domestic species of the Bovidae family belong to two different subfamilies; *Bubalus bubalis* L. (river buffalo) and *Bos taurus* L. (cattle) are belonging to subfamily Bovinae while *Ovis aries* L. (sheep) belongs to subfamily Caprinea. Many workers, using various banding techniques including G- and R-banding, studied the karyotypes of these three species[1-5].

The diploid chromosome number (2n) of the Bovidae ranges from 30 to 60, but the autosomal arm number (NAA) is relatively constant at 56-58 for most karyotyped bovides. Wurster and Benirschke[6] speculated that the constancy in NAA was indicative of centric fusions and that bovides chromosomal evaluation has proceeded from a primitive karyotype of 58 acrocentric autosomes, a condition seen in the domestic cow, domestic goat and many other bovides.

Hediger[7] proposed that chromosome conservation of the Bovidae should allow the extrapolation of chromosomal localization of genes in one bovide species to chromosomes of other bovides and he provided support for his hypothesis by localizing the genes for the major histocompatibility complex, keratin alpha and keratin beta to sheep and cattle chromosomes with homologous banding. The cattle physical gene map is being rapidly developed, so the cattle gene map will be the template for gene mapping in other bovides[8].

In this study, chromosome and gene mapping homology between river buffalo, cattle and sheep was studied using molecular markers to confirm that the chromosome band identity between closely related species is a good indicator of genetic homology between these species. Here, the polymerase chain reaction and buffalo-hamster hybrids were used to assign 16 cattle microsatellite markers, previously mapped in sheep, to river buffalo chromosomes.

MATERIALS AND METHODS

Somatic cell hybridization: Somatic cell hybrids used in this study were produced by fusing river buffalo lymphocytes, in the presence of polyethylene glycol, with cells of hypoxanthine phosphoribosyl transferase deficient ($HPRT^-$) Chinese hamster cell line wg 3 h[9]. Fused buffalo-hamster cells were isolated from the parental cells, using hypoxanthine-aminopterine-thymidine (HAT) selective media[10].

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Table 1: The DNA sequence of the nine primers used

Primer	Sequence 5 ---------- 3	PCR product size (bp)	Anneal. Temp. (C°)	References
BM719	TTC TGC AAA TGG GCT AGA GG			
	CAC ACC CTA GTT TGT AAG CAG C	139-161	54	Bishop *et al.*[25]
BM827	GGG CTG GTC GTA TGC TGA G			
	GTT GGA CTT GCT GAA GTG ACC	206-216	58	Bishop *et al.*[25]
BM1818	AGC TGG GAA TAT AAC CAA AGG			
	AGT GCT TTC AAG GTC CAT GC	258-272	56	Bishop *et al.*[25]
BM1824	GAG CAA GGT GTT TTT CCA ATC			
	CAT TCT CCA ACT GCT TCC TTG	178-192	58	Bishop *et al.*[25]
BM2113	GCT GCC TTC TAC CAA ATA CCC			
	CTT CCT GAG AGA AGC AAC ACC	123-143	58	Sunden *et al.*[29]
CSSM015	ATC ACG TGA AAT TTG CCT CTG TCC			
	ATA ACA GGC CAG AAA GAT TTG ATC	175	55	Moore *et al.*[26]
CSSM034	CCA TAA CTC TGG GAC TTT TCC TCA			
	ATG TTC AGC CAT CTC TCC TTG TCC	174	55	Moore *et al.*[26]
CSSM037	CAG TCC CAT AGG TCA CAA AGA GTT			
	TCT CCC TTT AGG TGT GTT AAT ATC	172	55	Moore *et al.*[26]
CSSM043	AAA ACT CTG GGA ACT TGA AAA CTA			
	GTT ACA AAT TTA AGA GAC AGA GTT	253	55	Moore *et al.*[26]
CSSM058	TAT ATA AAA TCA AGG GCT TCC CAG			
	TGG CAC TGA GCA TTA TAG ATA GAT	173	55	Moore *et al.*[26]
ILSTS011	GCT TGC TAC ATG GAA AGT GC			
	CTA AAA TGC AGA GCC CTA CC	261 -271	58	Brezinsky *et al.*[33]
ILSTS013	CTT GAT CCT TAT AGA ACT GG			
	ACA CAA AAT CAG ATC AGT GG	120-126	58	Brezinsky *et al.*[33]
ILSTS019	AAG GGA CCT CAT GTA GAA GC			
	ACT TTT GGA CCC TGT AGT GC	152-174	58	Kemp *et al.*[31]
ILSTS029	TGT TTT GAT GGA ACA CAG CC			
	TGG ATT TAG ACC AGG GTT GG	156-164	58	Kemp *et al.*[30]
ILSTS054	GAG GAT CTT GAT TTT GAT GTC C			
	AGG GCC ACT ATG GTA CTT CC	132-150	56	Kemp *et al.*[30]
ILSTS060	TAG GCA AAA GTC GGC AGC			
	TTA AGG GGA CAC CAG CCC	196-206	60	Kemp *et al.*[30]

Genomic DNA extraction: Genomic DNA was extracted from buffalo whole blood, Chinese hamster cell line and buffalo-hamster hybrid cells according to established protocols[11-13]. Cells were incubated overnight in a shaking water-bath at 37°C in lysis buffer (10 mM Tris base, 400 mM NaCl and 2 mM sodium EDTA) pH 8.2, 20% sodium dodecyl sulfate (SDS) and proteinase K (10 mg ml^{-1}). Nucleic acids were extracted once with phenol, saturated with Tris-EDTA (TE) buffer (10 mM Tris, 10 mM NaCl and 1mM EDTA), followed by extraction with phenol-chloroform-isoamyl alcohol (25:24:1) until there was no protein at the interface. This was followed by extraction with chloroform-isoamyl alcohol (24:1).

To each extraction, equal volumes of the solvent were added, followed by thorough mixing and centrifugation for 10 min. at 2000 rpm. The top layer was carefully transferred to another Falcon tube for the next extraction. To the final aqueous phase, 0.1 volume of 2.5 M Na acetate and 2.5 volume of cold 95% ethanol were added. The tubes were agitated gently to mix the liquids and a fluffy white ball of DNA was formed. The DNA was picked up with a heat-sealed Pasteur pipette and washed briefly in 70% ethanol. The DNA was finally dissolved in an appropriate volume of 1XTE buffer. DNA concentrations were determined and diluted to the working concentration of 50 ng μl^{-1}, which is suitable for polymerase chain reaction (PCR).

Polymerase chain reaction (PCR): A PCR cocktail consists of 1.0 μM upper and lower primers and 0.2 mM dNTPs, 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin (w/v), 0.1% Triton X-100 and 1.25 units of Taq polymerase. The cocktail was aliquot into tubes with 100 ng DNA of buffalo, hamster or hybrid cells. The reaction mixture was overlaid with sterile mineral oil. The reaction was cycled for 1 min at 94°C, 2 min at an optimized annealing temperature that is determined for each primer (Table 1) and 2 min at 72°C for 30 cycles. The PCR reaction products were electrophoresed on 3% agarose gel containing ethidium bromide.

Statistical analysis: The segregation profile (presence or absence) of buffalo-specific PCR product for each primer was studied in the 45 somatic cell hybrids. Synteny between each two markers (presence of them on the same chromosome) was tested by calculating the correlation coefficient (φ) at an error Q = 0.025 with a probability of a correct decision P=0.96[14].

$$\text{correlation coefficient } (\varphi) = \frac{ad-bc}{\sqrt{(a+b)\,(a+c)\,(d+b)\,(d+c)}}$$

Where n = number of tested hybrids
a = number of hybrids which are positive for both markers
b = number of hybrids which are positive for the first marker and negative for the second marker
c = number of hybrids which are negative for the first marker and positive for the second marker
d = number of hybrids which are negative for both markers

The two markers are considered syntenic (are located on the same chromosome) when (φ) exceed 0.69 and asyntenic (are located on the different chromosomes) if it is less than 0.69.

RESULTS

The assignment of the 16 tested microsatellites to river buffalo chromosomes was done by calculating the correlation coefficient (φ) between the segregation of these tested markers and markers representing syntenic groups and chromosomes of river buffalo (Table 2).

The two markers BM719 and ILSTS019 are segregated dependently with the HUJ614, the marker of syntenic group U1 and buffalo chromosome 5q[15] with φ values 1.00 and 0.94, respectively. Also, these two tested markers are segregated with the marker of syntenic group U7 and buffalo chromosomes 5p (OBCAM)[15] with φ values 0.94 and 1.00, respectively. So, BM719 and ILSTS019 are assigned to the bi-armed river buffalo chromosome 5 (BBU5).

From Table 2, BM827 is segregated dependently (φ=1.00) with LGB, the marker of U16 and river buffalo chromosome 12[16]. This tested marker was segregated independently from the other markers where the highest φ value did not exceed 0.55, which was reported between BM827 and G10P1, the marker of U26 and buffalo chromosome 23. Due to the syntenic relationship between BM827 and LGB, BM827 is assigned to the river buffalo chromosome 12.

BM1818 and BM2113 are segregated dependently with the PRL, the marker of syntenic group U20 and buffalo chromosome 2p[16] with φ values 1.00 and 0.95, respectively (Table 2). Also, BM1818 and BM2113 are segregated with the marker of syntenic group U17 and buffalo chromosomes 2q (INHA)[17] with φ values 0.95 and 1.00, respectively. So, BM1818 and BM2113 are assigned to the bi-armed river buffalo chromosome 2.

The two markers BM1824 and CSSM043 are segregated dependently with the CD18, the marker of syntenic group U10 and buffalo chromosome 1q[18] with φ values 0.95 and 0.90, respectively. Also, these two tested markers are segregated with the marker of syntenic group U25 and buffalo chromosomes 1p (ANT1)[19] with φ values 0.90 and 0.95, respectively. So, BM1824 and CSSM043 are assigned to the bi-armed river buffalo chromosome 1.

CSSM015 and CSSM037 are segregated dependently with CSSM047, the marker of syntenic group U18 and buffalo chromosome 3q and also segregated dependently with MAP2C, the marker of syntenic group U21 and buffalo chromosomes 3p[17] with a φ value 1.00. So, CSSM015 and CSSM037 are assigned to the bi-armed river buffalo chromosome 3.

The two markers CSSM034 and ILSTS060 are segregated dependently with the IGF1, the marker of syntenic group U3 and buffalo chromosome 4q[15] with φ values 1.00 and 0.95, respectively. Also, these two tested markers are segregated with the marker of syntenic group U29 and buffalo chromosomes 4p (ETH1112)[15] with φ values 0.95 and 1.00, respectively. Due to this syntenic relation, CSSM034 and ILSTS060 are assigned to the bi-armed river buffalo chromosome 4.

CSSM058 is segregated dependently (φ=0.90) with CSSM006, the marker of U12 and river buffalo chromosome 21[17]. CSSM058 was segregated independently from the other markers where the highest φ value did not exceed 0.54, which was reported between this marker and BSPN, the marker of U24 and buffalo chromosome 15. Due to the syntenic relation between CSSM058 and CSSM006, CSSM058 is assigned to the river buffalo chromosome 21.

ILSTS011 is segregated dependently (φ=0.90) with BSPN, the marker of U24 and river buffalo chromosome 15[18]. This marker was segregated independently from other markers where the highest φ value did not exceed 0.57, which was reported between ILSTS011 and DU23S1 (the marker of U23 and buffalo chromosome 17. Due to the syntenic relation between ILSTS011 and BSPN, ILSTS011 is assigned to river buffalo chromosome 15.

ILSTS013 is segregated dependently (φ=1.00) with CGA, the marker of U2 and river buffalo chromosome 10[18]. This marker was segregated independently from the other markers where the highest φ value did not exceed 0.46, which was reported between this marker and INHBA, the marker of U13 and buffalo chromosome 7. Due to the syntenic relationship between ILSTS013 and CGA, ILSTS013 is assigned to the river buffalo chromosome 10.

From Table 2, ILSTS029 is segregated dependently (φ=0.95) with CYM, the marker of U6 and river buffalo chromosome 6[17]. ILSTS029 was segregated independently from the other markers where the highest

Table 2: The correlation coefficient (φ) of segregation of tested markers and markers representing buffalo syntenic groups

Syntnic grroup	Markers	BM 719 φ	BM 827 φ	BM 1818 φ	BM 1824 φ	BM 2113 φ	CSSM 015 φ	CSSM 034 φ	CSSM 037 φ	CSSM 043 φ	CSSM 058 φ	ILSTS 011 φ	ILSTS 013 φ	ILSTS 019 φ	ILSTS 029 φ	ILSTS 054 φ	ILSTS 060 φ
U1	HUJ614	**1.00**	0.23	0.38	0.03	0.18	0.13	0.54	0.13	0.17	0.00	-0.03	0.03	**0.94**	-0.09	0.27	0.23
U2	CGA	0.24	-0.04	0.22	0.31	0.22	0.17	0.44	0.17	0.41	0.03	0.20	**1.00**	0.15	0.27	0.15	0.20
U3	IGFI	0.54	-0.12	0.53	0.24	0.48	0.22	**1.00**	0.22	0.28	0.26	0.45	0.42	0.60	0.37	0.18	**0.95**
U4	CSSM18	0.25	0.07	0.42	0.18	0.17	0.14	0.37	0.14	0.05	0.08	0.32	0.18	0.40	0.30	**1.00**	0.19
U5	NP	0.25	0.39	0.02	-0.01	0.17	0.32	0.42	0.32	0.21	0.39	0.56	0.14	0.12	-0.16	0.11	0.16
U6	CYM	0.20	-0.09	0.34	0.27	0.34	-0.10	0.42	-0.10	0.29	0.47	0.21	0.27	-0.07	**0.95**	0.44	0.42
U7	OBCAM	**0.94**	0.09	0.50	0.03	0.53	0.34	0.54	0.34	-0.05	-0.02	0.17	0.15	**1.00**	0.31	0.49	0.13
U8	ELN	0.63	-0.16	0.61	0.15	0.58	-0.05	0.62	-0.05	0.17	0.12	0.26	0.30	0.33	0.48	0.43	0.43
U9	GPI	0.09	0.14	0.13	-0.20	0.00	0.31	0.48	0.31	-0.16	0.38	0.10	0.00	0.20	-0.09	0.31	0.00
U10	CD18	0.37	-0.04	0.01	**0.95**	-0.01	0.24	0.31	0.24	**0.90**	0.09	0.00	0.38	0.03	0.27	0.22	0.32
U11	PRNP	0.18	-0.13	0.32	0.09	0.32	-0.09	0.44	-0.09	0.41	0.26	0.33	0.29	0.22	0.31	0.12	0.35
U12	CSSM6	-0.15	0.33	0.22	-0.07	0.27	-0.07	0.34	-0.07	0.03	**0.90**	0.56	0.14	-0.02	0.38	0.30	0.44
U13	INHBA	0.40	0.30	0.03	0.13	0.42	0.33	0.44	0.33	0.00	0.14	0.36	0.46	-0.03	-0.10	0.49	0.60
U14	MAP1B	-0.17	0.01	0.29	0.29	0.12	0.42	-0.02	0.42	-0.11	0.29	0.32	0.24	-0.01	0.38	0.31	-0.01
U15	ADH2	0.02	0.03	0.02	0.16	0.46	0.00	-0.07	0.00	0.16	0.00	0.02	0.29	0.43	0.00	0.19	-0.07
U16	LGB	0.44	**1.00**	0.19	-0.04	0.19	-0.08	0.44	-0.08	0.18	0.04	0.02	-0.04	0.09	-0.07	0.18	0.41
U17	INHA	0.43	0.19	**0.95**	-0.10	**1.00**	-0.08	0.50	-0.08	0.33	0.18	0.27	0.22	0.53	0.34	0.25	0.53
U18	CSSM47	0.13	-0.10	-0.09	0.24	-0.08	**1.00**	0.10	**1.00**	0.18	0.00	-0.11	0.22	0.34	-0.03	0.14	0.08
U19	HBB	0.20	-0.10	0.14	-0.10	0.35	-0.06	0.10	-0.06	-0.08	0.18	0.22	-0.10	0.27	-0.05	0.14	0.16
U20	PRL	0.55	0.19	1.00	0.01	**0.95**	-0.06	0.55	-0.06	0.22	0.08	0.27	0.22	0.50	0.34	0.35	0.54
U21	MAP2C	0.13	0.00	0.00	0.00	0.00	**1.00**	0.23	**1.00**	-0.11	0.00	0.00	0.00	0.34	-0.03	-0.09	0.00
U22	LDLR	0.18	-0.15	0.13	0.28	0.04	-0.09	0.39	-0.09	0.33	0.08	-0.03	0.14	0.06	0.38	-0.05	0.35
U23	DU23S1	0.09	-0.13	0.13	0.19	0.12	0.09	0.28	0.09	0.17	0.16	0.57	0.07	0.20	0.27	0.25	0.14
U24	BSPN	0.43	0.02	0.27	0.14	0.16	-0.11	0.17	-0.11	0.24	0.54	**0.90**	0.10	0.17	0.21	0.19	0.31
U25	ANT1	0.17	0.18	0.22	0.90	0.33	-0.11	0.50	-0.11	**0.95**	0.03	0.24	0.41	-0.05	0.29	0.05	0.44
U26	G10P1	0.45	0.55	0.00	0.14	-0.14	-0.06	0.40	-0.06	0.09	0.00	0.09	0.37	-0.11	0.00	-0.13	0.45
U27	F10	0.09	0.13	0.36	0.03	0.34	-0.11	0.31	-0.11	0.04	0.52	0.46	-0.10	0.22	0.32	0.38	0.40
U28	YES1	0.13	-0.02	0.33	0.12	0.47	0.38	0.24	0.38	0.03	0.07	0.41	0.08	0.30	0.00	0.60	0.30
U29	ETH1112	0.43	-0.16	0.49	0.21	0.49	0.24	**0.95**	0.24	0.17	0.30	0.28	0.08	0.51	0.38	0.18	**1.00**
X	G6PD	0.06	0.19	0.21	0.03	0.05	0.12	0.05	0.12	0.22	0.29	0.28	0.19	0.23	0.28	0.09	0.05
Y	ZFY	0.19	-0.11	0.27	0.03	0.26	-0.10	0.03	-0.10	-0.14	0.22	0.18	0.18	0.24	0.42	0.21	0.03

Two primers are syntenic when φ > 0.69 at an error rate Q = 0.025 and probability for correct decision P = 0.96

Table 3: Localization of 16 microsatellite markers on river buffalo chromosomes and their cattle and sheep equivalent chromosomes

Marker	Syntenic group	River buffalo	Cattle	Sheep
BM719	U1	5q	16	12
BM827	U16	12	11	3p
BM1818	U20	2p	23	20
BM1824	U10	1q	1	1q
BM2113	U17	2q	2	2q
CSSM015	U21	3p	19	11
CSSM034	U3	4q	5	3q
CSSM037	U18	3q	8	2p
CSSM043	U25	1p	27	26
CSSM058	U12	21	22	19
ILSTS011	U24	15	14	9
ILSTS013	U2	10	9	8
ILSTS019	U7	5p	29	21
ILSTS029	U6	6	3	1p
ILSTS054	U4	20	21	18
ILSTS060	U29	4p	28	25

φ value did not exceed 0.48, which was reported between this marker and ELN, the marker of U8 and buffalo chromosome 24. Due to the syntenic relationship between ILSTS029 and CYM, ILSTS029 is assigned to the river buffalo chromosome 6.

ILSTS054 is segregated dependently (φ=1.00) with CSSM18, the marker of U4 and river buffalo chromosome 20[17]. This marker was segregated independently from the other markers where the highest φ value did not exceed 0.60, which was reported between this marker and YES1, the marker of U28 and buffalo chromosome 22. Due to the syntenic relationship between ILSTS054 and CSSM18, ILSTS054 is assigned to the river buffalo chromosme 20.

DISCUSSION

The family Bovidae is the most diverse of the nine Artiodactyla families with 45 extant genera and 124 extant species[20]. River buffalo, cattle and sheep belong to the Bovidae family, which has a high degree of chromosome conservation between its members and where evolution occurs mainly by means of centric fusion between acrocentric chromosomes[6]. Although the diploid chromosome numbers of river buffalo, cattle and sheep are different; 50, 60 and 54, respectively, the autosomal arm number (NAA) of these three species is 58; river buffalo has 10 bi-armed and 38 acrocentric chromosomes, cattle has 58 acrocentric chromosomes and sheep has 6 bi-armed and 46 acrocentric chromosomes.

Many workers reported great similarities between banding patterns of cattle and river buffalo chromosomes, emphasizing chromosome conservation and genetic homology between buffalo and cattle[21-23]. Extensive chromosome arm homology between cattle and river buffalo has been established[4]. Based on banding homology, the five bi-armed autosomes of the river

buffalo from 1 to 5 are formed by centric fusions of ten acrocentric autosomes of cattle; 1 and 27, 2 and 23, 8 and 19, 5 and 28 and 16 and 29, respectively.

Also, Cattle and sheep karyotypes show a high degree of similarity in regard both to their fundamental numbers (number of autosomal arms, NAA = 58) and the band patterns on most of their chromosome arms[5,24]. Comparison of banded karotypes of cattle and sheep showed that the three sheep metacentric chromosomes from 1 to 3 are the result of Robertsonian fusions between cattle chromosomes; 1 and 3, 2 and 8 and 5 and 11, respectively.

Chromosome and gene mapping homology between river buffalo, cattle and sheep was studied in this study using molecular markers to confirm that the chromosome band identity between closely related species is a good indicator of genetic homology between these species. The polymerase chain reaction and buffalo-hamster somatic cell hybrids were used to assign 16 cattle microsatellite markers, previously mapped in sheep, to river buffalo chromosomes.

BM1824 and CSSM043 are located on the bi-armed river buffalo chromosome 1. The two markers were assigned to 2 separated cattle chromosomes. BM1824 was assigned to cattle chromosome 1 (BTA1)[25], which is homologous to river buffalo chromosome 1q (BBU1q). Also, CSSM043 was assigned to BTA 27[26], which is homologous to BBU1p. In sheep, BM1824 was assigned to sheep chromosome 1q (OAR1q)[27], which is homologous to BTA1 and CSSM043 was assigned to OAR26[28], which is homologous to BTA 27. Chromosome bands and gene mapping homology between BBU1q, BTA1 and OAR1q and also between BBU1p, BTA27 and OAR26 is presented in Table 3.

BM1818 and BM2113 are located on the bi-armed river buffalo chromosome 2. The two markers were assigned to 2 separated cattle chromosomes. BM1818 was assigned to cattle chromosome 23 (BTA23)[25], which is homologous to river buffalo chromosome 2p (BBU2p). Also, BM2113 was assigned to BTA 2[29], which is homologous to BBU2q. In sheep, BM1818 was assigned to chromosome 20 (OAR20)[28], which is homologous to BTA23 and BM2113 was assigned to OAR2q[27], which is homologous to BTA 2. This result showed chromosome bands and gene mapping homology between BBU2p, BTA23 and OAR20 and also between BBU2q, BTA2 and OAR2q.

CSSM015 and CSSM037 are located on the bi-armed river buffalo chromosome 3. The two markers were assigned to 2 separated cattle chromosomes. CSSM015 was assigned to cattle chromosome 19 (BTA19)[26], which is homologous to river buffalo chromosome 3p (BBU3p). Also, CSSM037 was assigned to BTA 8[26], which is homologous to BBU3q. In sheep, CSSM015 was assigned to chromosome 11 (OAR11)[28], which is homologous to BTA19 and CSSM037 was assigned to OAR2p[28], which is homologous to BTA 8. This result showed chromosome bands and gene mapping homology between BBU3p, BTA19 and OAR11 and also between BBU3q, BTA8 and OAR2p.

CSSM034 and ILSTS060 are located on the bi-armed river buffalo chromosome 4. In Cattle, CSSM034 was assigned to cattle chromosome 5 (BTA5)[26], which is homologous to river buffalo chromosome 4q (BBU4q). Also, ILSTS060 was assigned to BTA 28[30], which is homologous to BBU4p. In sheep, CSSM034 was assigned to OAR3q[28], which is homologous to BTA5 and ILSTS060 was assigned to OAR25[30], which is homologous to BTA 28. This result showed chromosome bands and gene mapping homology between BBU4q, BTA5 and OAR3q and also between BBU4p, BTA28 and OAR25.

BM719 and ILSTS019 are located on the bi-armed river buffalo chromosome 5. The two markers were assigned to 2 separated cattle chromosomes. BM719 was assigned to cattle chromosome 16 (BTA16)[25], which is homologous to river buffalo chromosome 5q (BBU5q). Also, ILSTS019 was assigned to BTA 29[31], which is homologous to BBU5p. In sheep, BM719 was assigned to chromosome 12 (OAR12)[27], which is homologous to BTA16 and ILSTS019 was assigned to OAR21[31], which is homologous to BTA 29. This result showed chromosome bands and gene mapping homology between BBU5q, BTA16 and OAR12 and also between BBU5p, BTA29 and OAR21.

BM827 is assigned to river buffalo chromosome 12 (BBU12). In cattle, this marker was assigned to cattle chromosome 11 (BTA11)[25], which is homologous to BBU12. In sheep, BM827 was assigned to sheep chromosome 3p (OAR3p)[32], which is homologous to BTA 11. This result showed chromosome bands and gene mapping homology between BBU12, BTA11 and OAR3p.

CSSM058 is assigned to river buffalo chromosome 21 (BBU21). In cattle, this marker was assigned to cattle chromosome 22 (BTA22)[26], which is homologous to BBU21. In sheep, CSSM058 was assigned to sheep chromosome 19 (OAR19)[28], which is homologous to BTA 22. This result showed chromosome bands and gene mapping homology between BBU21, BTA22 and OAR19.

ILSTS011 is assigned to river buffalo chromosome 15 (BBU15). In cattle, this marker was assigned to cattle chromosome 14 (BTA14)[33], which is homologous to BBU15. In sheep, ILSTS011 was assigned to sheep chromosome 9 (OAR9)[27], which is homologous to

BTA 14. This result showed chromosome bands and gene mapping homology between BBU15, BTA14 and OAR9.

ILSTS013 is assigned to river buffalo chromosome 10 (BBU10). In cattle, this marker was assigned to cattle chromosome 9 (BTA9)[33], which is homologous to BBU10. In sheep, ILSTS013 was assigned to sheep chromosome 8 (OAR8)[27], which is homologous to BTA 9. This result showed chromosome bands and gene mapping homology between BBU10, BTA9 and OAR8.

ILSTS029 is assigned to river buffalo chromosome 6 (BBU6). In cattle, this marker was assigned to cattle chromosome 3 (BTA3)[30], which is homologous to BBU6. In sheep, ILSTS029 was assigned to sheep chromosome 1p (OAR1p)[27], which is homologous to BTA 3. This result showed chromosome bands and gene mapping homology between BBU6, BTA3 and OAR1p.

ILSTS054 is assigned to river buffalo chromosome 20 (BBU20). In cattle, this marker was assigned to cattle chromosome 21 (BTA21)[30], which is homologous to BBU20. In sheep, ILSTS054 was assigned to sheep chromosome 18 (OAR18)[28], which is homologous to BTA 21. This result showed chromosome bands and gene mapping homology between BBU20, BTA21 and OAR18.

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