

## A set of cattle microsatellite DNA markers for genome analysis of riverine buffalo (*Bubalus bubalis*)

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### Summary

One hundred and eight microsatellite primer pairs, originally identified from cattle, were evaluated for their applicability in buffalo. Eighty-one primer pairs (75%) amplified discrete products, and of these, 61 pairs (56%) gave polymorphic band patterns on a panel of 25 buffaloes. The mean number of alleles per polymorphic marker was  $4.50 \pm 0.20$ , and the mean heterozygosity per polymorphic marker was  $0.66 \pm 0.02$ . Successful genotyping of buffaloes using cattle specific primers suggests that the latter can be a valuable resource for genome analysis in bubaline species.

**Keywords** *Bubalus bubalis*, cattle, microsatellite, polymorphism, riverine buffalo.

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Deoxyribonucleic acid (DNA) polymorphisms are powerful tools for molecular dissection of traits of economic importance and for their potential applications in breeding more productive and efficient livestock. Deoxyribonucleic acid markers are also being used to understand the evolution and domestication of these species. At present, microsatellites are the markers of choice for genome mapping, genetic dissection of complex traits and genetic diversity studies because of their highly polymorphic nature, co-dominant mode of inheritance and ease of typing (e.g. Barendse *et al.* 1994; Buchanan *et al.* 1994; Crawford *et al.* 1995; Hudson *et al.* 1995; Rohrer *et al.* 1996; Takezaki & Nei 1996; Kappes *et al.* 1997). However, no genome mapping efforts have been devoted to riverine buffalo (*Bubalus bubalis*), the main dairy animal of the Indian subcontinent, and no systematic studies have been undertaken to develop polymorphic DNA markers in this species.

Comparative genomic studies have shown that microsatellite primer pair sequences are often conserved across related species and can be used for the development of markers in related species. More than a thousand microsatellite markers have been reported for cattle (Kappes *et al.* 1997; BOVMAP). To develop a microsatellite based linkage map of riverine buffalo, we have been evaluating cattle

microsatellite primer pairs, and here we report the identification of 61 polymorphic markers suitable for genome analysis of riverine buffaloes.

Genomic DNA was isolated from blood of 25 unrelated animals (Sambrook *et al.* 1989) representing three distinct and geographically defined buffalo breeds; viz. Murrah, Nili-Ravi and Mehsana. One cattle DNA sample was included as positive control. Polymerase chain reaction (PCR) was carried out on 100 ng of genomic DNA in a 50- $\mu$ l reaction volume. The reaction mixture consisted of 200  $\mu$ M each of dATP, dCTP, dGTP and dTTP, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM MgCl<sub>2</sub>, 1 unit of *Taq* DNA polymerase and 4 ng/ $\mu$ l of each primer using PTC-200 PCR machine (MJ Research, Watertown, USA). The PCR protocol involved an initial denaturation at 95 °C for 2 min, followed by 30 cycles of 92 °C (1 min), 55 °C (45 s) and 72 °C (1 min). A final elongation step of 10 min was carried out at 72 °C. The annealing conditions were similar to that reported for cattle (Kemp *et al.* 1995). At the end of the reaction, 5  $\mu$ l of stop dye (95% formamide, 0.25% bromophenol blue and 0.25% xylene cyanol) was added and 10  $\mu$ l of PCR products were loaded on to a 2% agarose gel, electrophoresed and visualized over UV light after ethidium bromide staining. A microsatellite primer pair was scored as positive by detection of a discrete band. To test for the presence of polymorphism, the PCR products were resolved on 6% denaturing polyacrylamide gels (Sequi-GT system, Bio-Rad, Richmond, USA). pGEM DNA marker and four base-pair allelic ladder (Promega, Madison, USA) were used as size standards for sizing PCR products. To visualize the

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resolved PCR products, gels were stained using silver staining kit (Promega) and dried between sheets of cellophane papers. The genotypes were scored manually.

A total of 108 cattle microsatellite primer pairs, taken from the literature were tested for amplification of genomic DNA from a panel of 25 buffaloes (Table 1 and references therein). Of these, 81 primer pairs gave discrete amplification products, and these were tested for polymorphism by resolving on polyacrylamide gel. Of these, 61 loci were polymorphic. Given the genetic diversity in the test panel, it is likely that loci detected by the remaining 20 loci lack polymorphism in riverine buffalo. The heterozygosity per polymorphic marker ranged from 0.31 to 0.89, with an average value of  $0.66 \pm 0.02$ . The mean number of alleles per polymorphic locus was  $4.5 \pm 0.20$ . To compare mean numbers of alleles per marker between buffalo and cattle, we recalculated this value for a set of 39 markers (ILSTS series) from our data on buffalo and from the corresponding cattle data available at the site <http://cagst.animal.uiuc.edu/genemap/loci.list>. In cattle mean number of alleles per marker was 5.62 and in buffalo it was 4.87. A similar comparison was made for mean allele size per marker. Allele size was estimated for each locus by averaging the smallest and largest allele. Mean allele size in buffalo was 160.8 (SE = 6.5) and this was 164.1 (SE = 6.9) in cattle. The difference in mean allele size in buffalo and cattle was not statistically significant but the trend is consistent with conclusions of earlier studies. In cross species amplification of microsatellite loci the experimental species had smaller mean allele size than that of source species (Rubinsztein *et al.* 1995; de Gortari *et al.* 1997; van Hooft *et al.* 1999).

To ascertain that the loci amplified by us from buffalo genome are orthologous to those of cattle, we sequenced PCR products from loci of cattle and buffalo. Like their cattle counterparts all the six buffalo sequences have simple repeats in them (data not shown). The sequencing data for each locus was aligned using AUTOASSEMBLER software (Perkin Elmer, Applied Biosystems, Foster City, USA). Significant sequence identity (78–95%) between cattle and buffalo sequences strongly suggests that cattle primers detect orthologous loci in buffalo in our study (e.g. see Fig. 1). However, the final proof of orthology will have to await the establishment of linkage map of microsatellites for river buffaloes and comparative analysis of cattle and buffalo maps.

The cattle primer sets can produce discrete amplification products from buffalo (*B. bubalis*-present study), African wild buffalo (*Syncerus caffer*- van Hooft *et al.* 1999), sheep (*Ovis aris*- de Gortari *et al.* 1997) and goat genome (*Capra hircus*- Kemp *et al.* 1995; Pepin *et al.* 1995). This indicates conservation of DNA sequences flanking microsatellites within the Bovidae family. In sheep and goats 63% of cattle

primer sequences were conserved (Kemp *et al.* 1995) in contrast to 75% conservation in bubaline genome. These results are in agreement with the taxonomic classification of the Bovidae family based upon morphometric characteristics and paleontological data. In the light of these observations, the conclusions drawn by Mattapallil & Ali (1999) that *Bubalus* and *Ovis* have closer evolutionary relationship than that of *Bubalus* and *Bos* cannot be sustained. These workers used simple repeat DNA oligos as Southern hybridization probes for comparative genomic analysis of *Bubalus*, *Bos* and *Ovis* species. Given the relatively fast mutation rates of these repeats, it is unlikely that any significant signature of evolutionary history would have been left on these repeat sequences to enable us now to draw conclusions across various genera. On the other hand, comparative genome analysis using heterologous microsatellite primer pairs across different genera is likely to provide more meaningful reflection of evolutionary relationships because the rate of mutation in primer sequences is likely to be much lower than that of repeat sequences analysed by Mattapallil & Ali (1999).

The present study identifies 61 polymorphic microsatellite markers for riverine buffaloes in addition to 24 documented by Moore *et al.* (1995). These markers should find immediate use for analysis of the bubaline genome, including construction of a linkage map, analyses of biodiversity and gene flow, parentage determination, etc. It may be noted that Barker *et al.* (1997) have studied genetic diversity in water and swamp buffaloes using 21 bovine microsatellites. Thus, it is likely that polymorphic microsatellite markers reported by us in riverine buffalo may also be useful in swamp buffalo.

Extensive conservation of cattle microsatellite loci in the buffalo genome, as shown by our results, means that cattle primer pairs can be a very cost effective and time saving resource for construction of a framework linkage map of the buffalo genome by using polymorphic microsatellite markers. In addition, all the conserved microsatellite loci, including the monomorphic ones, can be used in the development of comparative radiation hybrid maps of buffalo and cattle. Several regions harbouring genes controlling traits of economic importance have been mapped in cattle through linkage analysis using microsatellite markers (Georges *et al.* 1995; Ashwell & Van Tassell 1999; Beuzen *et al.* 2000; BOVMAP; Casas *et al.* 2000; Nadesalingam *et al.* 2001). If it turns out that synteny and gene order over these regions are conserved between the cattle and buffalo genomes, it is fair to expect that cattle microsatellite markers linked with some of these quantitative trait loci (QTL) may also be useful in defining genetic variability in quantitative traits in buffaloes. In that case, such microsatellite markers could provide quick entry points to buffalo QTL without

**Table 1** Characteristics of cattle microsatellite markers tested on a panel of 25 riverine buffaloes.

Marker	Amplification	No. of alleles	Allelic range	Heterozygosity	Chromosome no. in cattle <sup>a</sup>
<i>BM1818</i> <sup>10</sup>	+	4	241–253	0.66	23
<i>BM1824</i> <sup>10</sup>	+	3	160–196	0.79	1
<i>BM2113</i> <sup>10</sup>	–	–	–	–	2
<i>CSRM60</i> <sup>6</sup>	+	6	160–188	0.84	10
<i>CSSM66</i> <sup>7</sup>	+	7	168–202	0.84	14
<i>ETH10</i> <sup>9</sup>	+	3	178–182	0.44	5
<i>ETH152</i> <sup>1</sup>	+	3	181–189	0.44	5
<i>ETH225</i> <sup>1</sup>	+	1	140	0	9
<i>ETH3</i> <sup>9</sup>	+	4	106–118	0.88	19
<i>HAUT24</i> <sup>8</sup>	+	3	114–150	0.52	22
<i>HAUT27</i> <sup>8</sup>	+	5	133–151	0.63	26
<i>HEL13</i> <sup>2</sup>	+	5	165–191	0.66	11
<i>HEL9</i> <sup>2</sup>	–	–	–	–	8
<i>ILSTS001</i> <sup>12</sup>	+	1	91	0	7
<i>ILSTS004</i> <sup>12</sup>	+	1	102	0	1
<i>ILSTS005</i> <sup>12</sup>	+	5	173–183	0.62	10
<i>ILSTS006</i> <sup>11</sup>	–	–	–	–	7
<i>ILSTS008</i> <sup>12</sup>	+	4	168–176	0.89	14
<i>ILSTS010</i> <sup>12</sup>	–	–	–	–	12
<i>ILSTS011</i> <sup>12</sup>	+	2	140–166	0.78	14
<i>ILSTS012</i> <sup>12</sup>	+	4	102–110	0.84	11
<i>ILSTS013</i> <sup>12</sup>	+	1	104	0	9
<i>ILSTS014</i> <sup>12</sup>	+	2	130–136	0.47	19
<i>ILSTS015</i> <sup>12</sup>	–	–	–	–	25
<i>ILSTS016</i> <sup>12</sup>	–	–	–	–	21
<i>ILSTS017</i> <sup>12</sup>	+	4	104–124	0.89	X
<i>ILSTS019</i> <sup>12</sup>	+	5	160–180	0.57	29
<i>ILSTS020</i> <sup>12</sup>	+	4	143–155	0.36	10
<i>ILSTS021</i> <sup>12</sup>	+	5	119–133	0.47	18
<i>ILSTS022</i> <sup>12</sup>	+	3	118–126	0.57	5
<i>ILSTS023</i> <sup>12</sup>	+	4	179–187	0.63	17
<i>ILSTS024</i> <sup>12</sup>	–	–	–	–	11
<i>ILSTS025</i> <sup>12</sup>	+	7	115–135	0.57	2
<i>ILSTS026</i> <sup>12</sup>	+	5	140–158	0.31	2
<i>ILSTS027</i> <sup>12</sup>	+	1	179	0	15
<i>ILSTS028</i> <sup>12</sup>	+	7	140–168	0.73	11
<i>ILSTS029</i> <sup>12</sup>	+	6	160–168	0.63	3
<i>ILSTS030</i> <sup>12</sup>	+	5	146–158	0.84	2
<i>ILSTS031</i> <sup>12</sup>	+	6	266–276	0.55	24
<i>ILSTS033</i> <sup>12</sup>	+	6	126–138	0.89	12
<i>ILSTS034</i> <sup>12</sup>	+	5	130–144	0.66	5
<i>ILSTS035</i> <sup>12</sup>	+	1	145	0	6
<i>ILSTS036</i> <sup>12</sup>	+	5	143–157	0.47	11
<i>ILSTS037</i> <sup>12</sup>	–	–	–	–	9
<i>ILSTS038</i> <sup>12</sup>	+	5	151–163	0.84	29
<i>ILSTS039</i> <sup>12</sup>	+	3	219–223	0.63	14
<i>ILSTS040</i> <sup>12</sup>	–	–	–	–	Unknown
<i>ILSTS041</i> <sup>12</sup>	+	3	126–130	0.31	9
<i>ILSTS042</i> <sup>12</sup>	+	1	139	0	5
<i>ILSTS043</i> <sup>12</sup>	+	1	158	0	29
<i>ILSTS044</i> <sup>12</sup>	+	1	166	0	3
<i>ILSTS045</i> <sup>12</sup>	+	4	169–177	0.31	11

Table 1 (Continued).

Marker	Amplification	No. of alleles	Allelic range	Heterozygosity	Chromosome no. in cattle <sup>a</sup>
<i>ILSTS046</i> <sup>12</sup>	–	–	–	–	29
<i>ILSTS049</i> <sup>12</sup>	+	1	164	0	11
<i>ILSTS050</i> <sup>12</sup>	–	–	–	–	2
<i>ILSTS051</i> <sup>12</sup>	+	3	158–174	0.61	Unknown
<i>ILSTS052</i> <sup>12</sup>	+	7	147–179	0.63	21
<i>ILSTS053</i> <sup>12</sup>	+	2	131–135	0.52	10
<i>ILSTS054</i> <sup>12</sup>	+	1	144	0	21
<i>ILSTS055</i> <sup>12</sup>	–	–	–	–	Unknown
<i>ILSTS056</i> <sup>12</sup>	+	7	140–172	0.42	12
<i>ILSTS057</i> <sup>12</sup>	+	1	232	0	25
<i>ILSTS058</i> <sup>12</sup>	+	7	118–170	0.89	17
<i>ILSTS059</i> <sup>12</sup>	+	1	145	0	13
<i>ILSTS060</i> <sup>12</sup>	+	5	188–198	0.31	28
<i>ILSTS061</i> <sup>12</sup>	+	11	105–133	0.84	15
<i>ILSTS062</i> <sup>12</sup>	+	1	196	0	4
<i>ILSTS063</i> <sup>12</sup>	+	4	137–145	0.84	29
<i>ILSTS064</i> <sup>12</sup>	+	1	155	0	3
<i>ILSTS065</i> <sup>12</sup>	–	–	–	–	24
<i>ILSTS066</i> <sup>12</sup>	–	–	–	–	5
<i>ILSTS067</i> <sup>12</sup>	–	–	–	–	Unknown
<i>ILSTS068</i> <sup>12</sup>	+	7	147–161	0.68	20
<i>ILSTS070</i> <sup>12</sup>	+	3	100–108	0.73	10
<i>ILSTS071</i> <sup>12</sup>	+	2	121–125	0.63	11
<i>ILSTS072</i> <sup>12</sup>	–	–	–	–	20
<i>ILSTS073</i> <sup>12</sup>	+	6	156–176	0.89	19
<i>ILSTS081</i> <sup>12</sup>	+	1	110	0	29
<i>ILSTS084</i> <sup>12</sup>	–	–	–	–	9
<i>ILSTS085</i> <sup>12</sup>	+	3	89–97	0.61	20
<i>ILSTS086</i> <sup>12</sup>	+	5	163–191	0.73	13
<i>ILSTS087</i> <sup>12</sup>	+	1	116	0	6
<i>ILSTS088</i> <sup>12</sup>	–	–	–	–	9
<i>ILSTS089</i> <sup>12</sup>	+	6	118–126	0.89	29
<i>ILSTS090</i> <sup>12</sup>	+	1	148	0	6
<i>ILSTS092</i> <sup>12</sup>	+	5	173–225	0.78	21
<i>ILSTS093</i> <sup>12</sup>	–	–	–	–	6
<i>ILSTS094</i> <sup>12</sup>	+	5	188–196	0.78	10
<i>ILSTS095</i> <sup>12</sup>	+	5	197–205	0.63	21
<i>ILSTS096</i> <sup>12</sup>	–	–	–	–	3
<i>ILSTS097</i> <sup>12</sup>	+	6	222–252	0.84	6
<i>ILSTS098</i> <sup>12</sup>	–	–	–	–	2
<i>ILSTS099</i> <sup>12</sup>	–	–	–	–	28
<i>ILSTS101</i> <sup>12</sup>	+	5	150–172	0.31	24
<i>ILSTS102</i> <sup>12</sup>	–	–	–	–	29
<i>ILSTS103</i> <sup>12</sup>	–	–	–	–	21
<i>ILSTS104</i> <sup>12</sup>	+	4	175–183	0.57	1
<i>ILSTS105</i> <sup>12</sup>	+	5	109–117	0.73	Unknown
<i>INRA005</i> <sup>3</sup>	+	4	119–133	0.73	12
<i>INRA032</i> <sup>4</sup>	–	–	–	–	11
<i>INRA035</i> <sup>4</sup>	+	1	135	0	16
<i>MILSTS076</i> <sup>12</sup>	–	–	–	–	9
<i>MILSTS077</i> <sup>12</sup>	+	2	193–197	0.52	13
<i>MILSTS079</i> <sup>12</sup>	–	–	–	–	28



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