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Creation of a large reference family with phenotype recording and genotype data generation in buffaloes

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ABSTRACT

Buffalo is an integral part of dairy industry in India. Improvement of buffalo productivity shall require usage of high genetic merit bulls evaluated on the basis of progeny testing. Marker assisted selection of bulls shall enhance the accuracy of selection but require large number of daughters of the sires to be produced for the identification of Quantitative Trait Loci. In this paper we present creation of a large reference family (members with known genotype and phenotype). We created 12 large half sib families each with approximately 1,000 daughters per sire. The economic traits of interest like body weights at different ages, age at first heat, age at first calving, milk yield, fat and protein percentage in milk along with somatic cell count during the first lactation were recorded. The conception rate of bulls under field conditions was recorded and found to be 48.64% for the 12 bulls. There was huge loss of data attributable to different reasons however sale being the most important reason for non-recording of performance data. Only 25% of the daughters born to the 12 sires could be recorded for first lactation milk yield. 8027 daughters with confirm paternity were genotyped for 79 microsatellite markers located on 8 chromosomes, thus 6.34 lakh genotypes were generated. The information of this reference family has been compiled into a Buffalo Reference Family Germplasm Catalogue and published by National Agricultural Innovation Project, ICAR.

Key words: Genotype, Microsatellite, Murrah buffaloes, Phenotype, Progeny testing, Reference family

The reference family is defined as the group of individuals of recorded pedigree whose phenotypes and genotypes have been recorded. Creation of a reference family is a prerequisite for development of markers for the economic traits for selection. The identification of QTLs require carefully designed experiment. In the absence of pedigree data at the field level there has not been any attempt to identify these QTLs in buffaloes. The design of such experiments are cost intensive and require large resources (Annual report, AICRP on buffaloes, 2013–14, 2014–2015 and 2015–16). As the number of records in institutional herds are quite low it requires the recording of data at the farmers doorstep. The generation of markers for economic traits require large reference family to be created and hence the project was conceived to be carried out at farmers doorstep. The project was funded by National Agricultural Innovation Project for a period of six years with an aim to identify the Quantitative Trait Loci for milk yield, fat and protein percentage in buffaloes. The field work, creation of reference family consisting of 12 half sib families, phenotype recording were assigned to BAIF and were carried out by BIRD (BAIF Institute of Rural Development with its headquarters at Allahabad). To the best of our knowledge, it is for the first time in India and perhaps in the world to create a large reference family of buffaloes for the purpose of identification of QTLs. To the best of the knowledge of authors, no progeny testing program at this scale has ever been carried out in buffaloes in India or abroad. National Dairy Development Board plans to execute progeny testing in buffaloes under National Dairy Plan I (http://www.nddb.org/ndpi/about/brief).

In this paper we present the work carried out at farmers doorstep and the traits recorded at farmers herd and genotypes created at the research institution. An analysis of the creation of large field based half sib families, conception rate of bulls, phenotypes generated are presented. The experiment took eight years in creating the families and their maintenance with an ultimate objective of recording phenotypes and identification of QTLs. The losses in collection of data during the period due to various reasons and total number of daughters required to record the first lactation phenotypes have been presented.

MATERIALS AND METHODS

A large buffalo reference population consisting of daughters belonging to 12 half sib families was created. This required the usage of semen of 12 sires over a large area using artificial insemination.

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Selection of sires: The 12 sires selected for this experiment belonged to Murrah breed and were purchased from the Central Herd Registration Scheme, Rohtak; Government Model Farm, Hisar and Murrah breeding tract of Haryana. The bulls were selected on the basis of mother's milk yield and 10 of these 12 sires belonged to farm or recorded animals. The name of the 12 bulls and the yield of their mother is in Table 1. The semen of these sires were collected at BAIF, Uruli Kanchan, Pune and the semen in liquid nitrogen containers were transported to Uttar Pradesh where it was utilised to inseminate thousand of the she buffaloes over a large tract consisting of 50 districts of Uttar Pradesh. The inseminators recorded the date of insemination, the sire's name and also confirmed the pregnancy and maintained the records.

Area of operation: The area of operation for the project was quite large. It consisted of 50 districts of Uttar Pradesh. The area of operation was divided into three zones, viz. Eastern, Central and Western zone. Each zone had several regions. The eastern zone had Sultanpur, Gorakhpur, Faizabad and Azamgarh regions. The central region had Allahabad, Etawah, Kanpur and Lucknow as the four regions. The Western zone had Bareilly and Meerut as the two regions. Most of the records were created at stockman centers in the field which were monitored and maintained at regional level. The conception rate of the bulls was assessed 60 days after the animals were inseminated and not repeated back in heat as per rectal examination for pregnancy.

Trainings for data collection, blood sample collection and maintenance of records at regional level, district level functionaries and at grass root level was carried out. Various meetings were arranged and conducted at Zonal, Regional and District headquarters; these included project launching workshop at the beginning of the project to acquaint district level BAIF staff regarding the project objectives, technical program and the expected outcome of the project. Awareness was created amongst farmers to explain them the importance and utility of the project, skilled /semiskilled persons engaged for undertaking generation and collection of project information and recording of phenotype data as envisaged under the project.

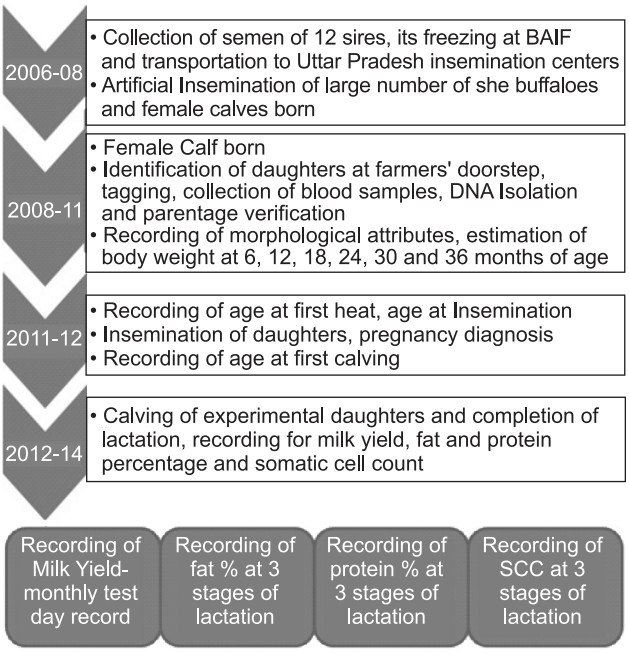

Fig. 1. Flow chart of the activities for the phenotype recording in the reference family.

The list of animals pregnant resulting from Artificial Insemination recorded at field centers were searched and the female calves born were listed. Region wise number of females identified from records. The identified progeny from center records were required to be verified, well trained manpower was engaged in physically verifying the availability of progeny. The region wise number of progeny was identified, verified and tagged. Before tagging, the verification of progeny was further crosschecked by deputing manpower from one district to another district as third party verification, this was to avoid any chance of dispute about the correctness of identification of the progeny born and the sire mentioned. After this process, the tagging of the progeny was undertaken. A total of 13,964 verified progeny were recorded. Sire wise number of daughters varied from 327 to 2,054 and the average number of daughters per bull were 1,163.7. Total of 11,972 progeny

Table 1. Name of bulls, mothers' milk yield and place of procurement

Bull name	Bull No.	Dam No.	Dam's milk yield (kg)	Sire	Source of procurement
SADA	E-09	Champa	4364	Mohan	CHRS, Rohtak
SATPAL	E-02	Chani	4572	Monta	CHRS, Rohtak
SHRIRANG	NA	NA	4246	NA	Murrah Breeding Tract
SHRINATH	E-05	Chameli	4325	Miracle	CHRS, Rohtak
SAKHA	RSM-3	Rupali	4240	Sheru	Murrah Tract
SUSHIL	FT385	F-61	5343	1132	Government Model Farm, Bhiwani
SALMAN	NA	NA	4040	NA	Government Model Farm, Bhiwani
SAHIL	B-374	B-634	4038	1124	Government Model Farm, Bhiwani
SAIMAN	JR-403	JJ-5	3591	1136	Government Model Farm, Bhivani
SANAM	FT-145	FT-145	4092	1148	Government Model Farm, Bhiwani
SHAGUN	NA	NA	4452	NA	Murrah Breeding Tract (Rohtak)
SAHIB	NA	6541	4242	RANA	Murrah Breeding Tract (Rohtak)

were tagged for blood collection which averaged 997.7 progeny per sire.

Phenotype recording: The phenotype data was generated on the daughters with correct paternity. Growth was recorded on the basis of morphological attributes for the estimation of body weights. The recording was done for age groups of 6, 12, 18, 24, 30 and 36 months. Age at first heat was recorded for grown up animals. The daughters born to the sires in the experiment were bred using Artificial Insemination. When the daughters calved they were recorded for milk yield, somatic cell score, fat and protein percentage. For milk yield, the recording was done on monthly test day milk records (morning and evening), the fat percentage, protein percentage and somatic cell count were recorded for three different stages of lactation (early lactation, mid lactation and late lactation). Figure 1 represents the sequence of phenotype recording and probable year (Overlapping activities during various years) when phenotypes were recorded.

Blood collection, DNA isolation, quality and quantity check: The blood samples collected from the field were brought to the district headquarters and then to regional headquarters and were immediately transported to Delhi from where they were taken in custody of NBAGR and then airlifted to Hyderabad for DNA isolation. A cold chain was maintained right from point of collection of blood sample till its reaching at Hyderabad where isolation of DNA was carried out. 10% of the all the genomic DNA samples were rerun in the lab for verification of the quality and quantity of the DNA samples. All the samples were found to be in order and could be used for further genotyping and other molecular analysis.

Selection of chromosomes and microsatellites: The QTL databases for cattle in public domain were searched for initiating work on identification of QTLs in buffaloes (http: //www.animalgenome.org and http: //bovinegenome.org/ bovineqtl_v2/searchViewer.html and http: //bovineqtl. tamu.edu.). Five chromosomes were selected (BTA1, BTA3, BTA6, BTA9, and BTA 14) for analysis. The selection of the chromosomes was carried out on the basis of the QTLs that had been reported for the milk yield, fat and protein percentage in cattle. Later 3 more chromosomes on which the QTLs had been reported for Somatic Cell score were added to the study. In the absence of genome sequence of buffalo, we utilised chromosomal synteny between cattle and buffalo chromosomes. Microsatellites from cattle database were selected which were equidistant from each other on the chromosome, amplification in buffaloes and were found to be polymorphic in buffalo species. The selection was also based on their being heterozygous in seven of the 12 sires. A total of 79 markers were selected on the 8 buffalo chromosomes (equivalent of cattle chromosomes). These chromosomes were q arm of BBU1 and BBU2, BBU6, BBU7, BBU8, BBU9, BBU10, BBU15 which are homologues to the cattle chromosomes BTA1, BTA2, BTA3, BTA4, BTA6, BTA7, BTA9, BTA 14 respectively (Amaral *et al.* 2008). Daughters with correct paternity were genotyped using 79 microsatellite markers. The list of markers are presented in Table 5.

RESULTS AND DISCUSSION

Conception rate: The conception rate provides information on the success of Artificial Insemination and determines the number of inseminations to be carried out for the pregnancy. It is a saying that the success rate of AI in buffaloes is very less leading the farmers not to adapt the practice for the improvement of buffalo germplasm. We carried out a study to know exactly the percentage that can be observed under field conditions. The average conception rate based on 26,468 inseminations (part number of AIs studied) was recorded as 48.64%. Bull wise conception rate varied from 42.06 to 54.03% The details of bull wise inseminations performed and pregnancies recorded is presented in Table 2.

A total of 13,964 daughters belonging to these sires were identified to be born to them and verified. These daughters were tagged on their ears for future identification. It is usually assumed that the conception rate under field conditions should be poor as a general belief. The present results reveal that the percent conception rate was almost similar to that of cattle under field or farm conditions. We compared the conception rate with the All India Co-ordinated Research Project on Buffaloes in operation at Central Institute for Research in buffaloes, National Dairy Research Institute, Karnal; LUVAS, Hisar; GADVASU, Ludhiana and at IVRI, Izatnagar. The conception rate (CR) was 51.8% at CIRB, Hissar on the basis of 276 services and 143 pregnancies obtained which is slightly higher than in field in our experiment. At GADVASU, the CR was 39.76% which is significantly less. At NDRI, the conception rate was lower than that obtained in our study. The overall conception rate at NDRI, CIRB and GADVASU was 40.54% which is much lower than obtained in our study. Results were very similar to our experiment at IVRI,

Table 2. Name of sire, inseminations carried out and the number of buffaloes found pregnant by rectal palpation method

Sires name	Number of inseminations done	No. of buffaloes observed pregnant	Conception rate (%)
Sada	5889	2870	48.73
Sahil	756	396	52.38
Sahib	2210	1194	54.03 **
Salman	2794	1286	46.03
Sakha	2197	1105	50.30
Sanam	1310	551	42.06 *
Satpal	4148	1991	48.00
Shrinath	2311	1139	49.29
Sushil	1962	1001	51.02
Shrirang	1995	959	48.07
Sagun	419	180	42.96
Saiman	477	201	42.14
Total	26468	12873	48.64

*Lowest conception rate in field. **Highest conception rate in field.

Izatnagar which were 48.80% (Project Reports AICRP on Buffaloes, 2015–16; http: //www.cirb.res.in/attachments/289_nwp.pdf). The literature reveals that the number of services per conception was 1.31±0.23 in Nagpuri buffaloes (Kadu *et al.* 1978) while it was reported to be 1.49 during first parity of Murrah buffaloes (Gupta *et al.*1994). The least square means for the number of services per conception in Murrah buffaloes ranged from 1.73 (57%) to 3.74 (26.73%) as reported by Yadav and Rathi (1983). Several of the scientific studies have attributed the large difference in conception rates to parity and season (Basu *et al.*1977, Luktuke and Roy 1964, Cady *et al.* 1983).

The sire wise distribution of daughters in these ten regional stations is in Table 3. The daughters were tagged in the ears. This helped in future follow up of phenotype recording.

Recording of phenotypes: We recorded growth parameters at different age groups, age at first heat, age at first calving, test day milk yield (monthly), fat and protein percentage and somatic cell count on three stages of lactation.

The average estimated body weight (kg) for 6, 12, 18, 24, 30 and 36 months was 76.98±2.6, 100.58±0.20, 137.57±1.9, 166.73±1.5, 194.24±1.5 and 209.11±1.4 kg respectively (Table 4). The estimated body weights were calculated using body biometry. The maximum number of records were obtained from 24 and 30 months of age.

The mean age at first heat of the progeny was 34.00±0.70 months. Mean age at first calving was 43.77±0.37 months.

Recording for milk yield and milk composition: A total of 2,321 completed first lactations of the daughters of 12 sires were recorded and the average milk yield per lactation was 1,682.5 kg. Milk fat was recorded for 2,177 daughters and the average fat percentage was 7.85%. Similarly, on the same set of records, the protein percentage was 3.53%. The somatic cell score records of 1,418 daughters revealed mean somatic cell score of 3.07.

The information given in Table 4 revealed that out of 8,027 daughters selected for recording, we could obtain only 2,177 records which is about 27.12% of the total daughters born in half sib families. We had provided mineral mixtures, veterinary care and insemination services to the farmers free of cost but did not provide any cash incentive to the farmers to keep the animals under recording, however we motivated them from time to time so that they cooperate for the success of the experiment. The sale and purchase of the animals was reported to be maximum when the animal was pregnant. This was the major reason for loss of phenotype data, the other reason being death and disease or infertility. Thus it is expected that under field conditions the success rate of obtaining first lactation records is roughly 25%.

All the daughters with correct paternity were genotyped for 79 markers. PCR conditions were standardized and the primers labeled with fluorescent dyes were utilised. In all for the identification of QTLs for traits of interest we generated 6.34 lakh genotypes.

The information on the buffaloes, viz. date of birth of

Table 3. Sire wise distribution of daughters in ten regional stations

Name of bull	Allahabad	Azamgarh	Bareilly	Etawah	Faizabad	Gorakhpur	Kanpur	Lucknow	Meerut	Sultanpur	Total number of daughters born
Sada	337	489	363	67	184	110	83	168	95	158	2054
Sahil	94	103	10	6	115	74	4	13	0	12	431
Sahib	100	227	126	32	187	153	2	76	0	47	950
Salman	163	251	166	50	122	119	80	119	14	135	1219
Sakha	158	154	318	56	67	74	62	268	140	205	1502
Sanam	114	105	22	34	51	13	42	9	24	43	457
Satpal	234	294	263	41	226	304	8	125	74	194	1763
Shrinath	220	143	275	149	115	86	183	66	101	196	1534
Shusil	330	233	272	106	157	2	203	234	42	139	1718
Shrirang	157	88	305	153	30	81	137	56	68	75	1150
Sagun	132	99	252	30	16	0	59	83	51	137	859
Saiman	38	50	61	36	13	10	24	51	44	0	327
Total	2077	2236	2433	760	1283	1026	887	1268	653	1341	13964

Table 4. Sire wise number of records for various traits

Sire name	Fat %	Protein %	Milk yield	Somatic cell count (in thousand)
Sada	292	292	325	191
Sagun	163	163	179	104
Sahib	87	87	103	51
Sahil	207	207	221	134
Saiman	264	264	280	184
Salman	71	71	78	35
Sanam	327	327	343	224
Satpal	264	264	278	146
Shrirang	219	219	224	146
Shrinath	159	159	159	90
Sushil	77	77	81	73
Sakha	47	47	50	40
Total	2177	2177	2321	1418

Table 5. List of markers, their chromosomal location and primers used for genotyping

Marker name	Chromo-some	Position in cM	Forward Primer	Reverse Primer	Fluorescent dye used to tag
TGLA49	1	5.113	GGCAGGACTTCACTCTTTTTCA	AGAAAAGGAATAATGAGACAGATTA	PET
BMS2321	1	15.428	TCACTTCACAAAATACACAATGC	CCAAACTCCATAATCACCACTT	PET
BMS711	1	23.94	AGCTTCTTATGGCAACACCTG	TGAAATCGCAGAGTTGTACATG	FAM
BMS4037	1	45.252	CCCCCATAATGCTACATATTGC	TTAAGACATGAATCCTCAGGGC	NED
BMS4048	1	84.471	AGGTCCCTCACAAAATGGC	AGTCCCAGGTTGCTGAAATG	HEX
BMS119	1	100.083	TCTGTGTTCAGGAAGCAGTTG	AGGTGTCACTTCCTTGACGC	NED
DIK5127	1	110.547	AATGGTGAGACATGGGATGC	TCCAAGAGAACCCAACTTTGA	HEX
BMS4011	1	115.052	TGAAGCTGACATTTCCACATG	CTTCCAGGCAACTAAATCAACC	PET
DIK2189	1	122.094	ATAGTGGGGAGGATCTGTGCT	GCAAGGAGGCATCAGGAAT	NED
DIK4443	1	154.672	TCTTGAATCTCTAGCTCTGCTCA	TTGCAGGCGGATCATACATA	FAM
URB006	3	9.342	CCATAGCTCTGGCAAAGACC	CCATCATGTGGCTACACGTC	VIC
DIK1057	3	17.368	GGTCAACTACTCCAGTTTCCAG	TGCACTTTACTGCTTGAGTCAT	VIC
CSSM054	3	35.76	AAACACATGGAGAATCAGACCTCC	TTCCAACAACCGTAGCACCTCCTG	NED
DIK4353	3	52.549	TGAACTTTAGGGCAGCATGA	AAGACTGAGATGTGGGGAAAA	PET
ILSTS029	3	64.906	TGTTTTGATGGAACACAGCC	TGGATTTAGACCAGGGTTGG	HEX
DIK4116	3	74.883	TCCTGAATTGCAGTGGTCAA	TGTAAGCAACCTGAGGACTGA	NED
BM3020	3	84.824	TATATAAAAGGCCCTGATTCCA	GATCTGGAGCAGTCTGGCTA	FAM
DIK2511	3	114.921	TGGTAGACCCCTTGGATTTG	ACACACGGGCACACACATAC	FAM
DIK5085	3	123.366	CACCCCATCATCCTTCAAAT	GCTCCCTTGGAGGACACTTT	FAM
ILSTS093	6	0	TGAAATATACCTGAGTAGCAGC	TTGTTTTAACTCCCCACCCC	NED
DIK5153	6	8.053	ACGTTTGAAGCTGGGAGATT	CATGTGGTTGCAGAGATTTGA	FAM
DIK5285	6	15.362	AGCAATTCCTCACACACTGCT	GAAGCCAATGACAACCCACT	PET
MNB-66	6	29.374	CTGCATGTTTGTCATAATGCTC	TTAGACTGGGTGACCTTGTACC	VIC
BM1329	6	35.398	TTGTTTAGGCAAGTCCAAAGTC	AACACCGCAGCTTCATCC	NED
BM143	6	53.724	ACCTGGGAAGCCTCCATATC	CTGCAGGCAGATTCTTTATCG	FAM
BMS690	6	56.441	CATAGGGATATGTTGTGCATCC	TCAAAGAAACTTCAAGCCAGC	VIC
DIK3024	6	71.561	TTTTCTCACCCGGATTTTAGTTGT	TCTCTTCTCCGATTGCTTCTCTTG	HEX
ILSTS035	6	87.265	TTGACCATAACAGCTACTCC	TAGGTCCATGAATCACAGGG	HEX
BM4311	6	97.728	TCCACTTCTTCCCTCATCTCC	GAAGTATATGTGTGCCTGGCC	FAM
BM6449	9	10.208	TATGTCCAGCAGCCCCTAAC	ACTACAGAGCAACCATGCTGG	VIC
DIK2433	9	15.121	TGTGGGGCTCCTTTGTAACT	CAGAGCGGCTACAGTTTGTG	FAM
BMS817	9	42.489	TGGGAAAGTTGGCAAATG	TTGTGATACCTGAAATGGTCAA	VIC
DIK2810	9	45.739	TCTGAAACCTGGAGGAGGAG	GAAACTTCCACCACCCTCAA	FAM
TGLA261	9	49.659	TCAAATCTCATTCTCTCCAGAAGGC	CCAACTTATATTAGGCACAATGTCC	PET
BMS1909	9	59.516	ACTTGTTAGGAGGGCTATTGTTAA	CCACATACACCACCAACATTAA	NED
DIK2816	9	68.072	ACCTTGGGAATCAAGGTCAT	CCCAGTAGTCCAGTGGCTCA	VIC
TGLA73	9	77.554	GAGAATCACCTAGAGAGGCA	CTTTCTCTTTAAATTCTATATGGT	NED
BMS2063	9	95.38	AAGGGGAGGAGCTTAAGTAGG	GAGAATCAGACATGAATGAGTACG	PET
BMS2295	9	98.646	GCTCTGGTGACCCAGGTG	CTGGCAGGAGATGAGAGGAG	FAM
ILSTS026	2	10.772	CTGAATTGGCTCCAAAGGCC	AAACAGAAGTCCAGGGCTGC	VIC
TEXAN-2	2	25.974	ACATTGTCATGTGGTTGCTAAC	ACTCTGGGTATGTATATGTGCAAG	PET
BMS803	2	44.514	GAGGTAGGGAATCAGGTAAGGC	AGCTGCATGGCTGAACAAG	PET
TGLA226	2	85.848	AGTGGAATCCAGATAAGATGTATCA	ACATGAAAAGAAGCAATATCGTAAC	PET
BM1223	2	100.176	AGGCAAATTGTGTTTCCAGC	TCATAAGGGTTTGGAGGCTG	VIC
BMS1987	2	107.475	TGATGCAGAGAACGTTTTAATTT	CTTGGGGTAGGCAGAGATTT	FAM
BMS356	2	109.009	ACCTCAGAGATGACGCAAGG	TTGAAGTTTTTGTGCTGTTTGG	PET
BMS2519	2	110.255	CATGGTTCTCATCTGGTGTG	AGTGAAGACCTACTGCAGCC	NED
DIK4364	4	0	TCTAAGGGCAGTGTGTGTGTG	GCCTACCCCCAGAATCTTTC	VIC
BMS1788	4	12.544	ACGTCCAGATTCAGATTTCTTG	GGAGAGGAATCTTGCAAAGG	FAM
BMS827	4	28.447	GGACCAACTGCACAAATTCT	AAGTGAAATTACTAGTGGGGTGTG	PET
BMS1237	4	34.379	GTTTTCACTAGCACCCTGTGG	CCCAGTTAACCCTAGAGTCGG	FAM
DIK4816	4	39.038	TGGTTTGCATCTGCTAATCTC	AAGACACAAATGAGCGACT	FAM
DIK4373	4	44.549	GGCTGGCTGGCTTTTATTCT	GAATAGCCTGGTGGGCTACA	PET
BM1260	4	48.873	AAGTACATGCATGCTGCTGC	TCCTAAGTTCCATCAACAGGTG	VIC
TGLA116	4	52.490	GCACAGTAATAAGAGTGATGGCAGA	TGGAGAAGATTTGGCTGTGTACCCA	VIC
BMS2571	4	69.726	CCCCAGTGATGTTCACACAG	CAGCTGTCCAGCATCTGAAG	NED
BMS1074	4	79.924	CAGTAGCCAAGATATGGAAGCA	AGCTCCTTGCTGCTACAAATG	NED

BL1121	4	101.314	CCAGGCTAGGAAGGCAGTAG	AGGGTACAAAAATCCCACACC	VIC
DIK4854	4	117.161	AGGTCAGCGTGTCCACGAGT	GAGGCTGCTCGGTTCTCTG	NED
BM7160	7	0	TGGATTTTTAAACACAGAATGTGG	TCAGCTTCTCTTTAAATTTCTCTGG	PET
MB053	7	17.56	GGTGCTGTTATCTAGAATTTGG	GGAGTCATACACAACTGAGC	PET
DIK4204	7	24.392	CGTGGAACAACAACCAAAAA	TGCTCTTACCCAGGGAGCTA	FAM
BM6105	7	36.949	ACTAATAAGAAATTCTGCATGTGTG	CCACCATGACTCAGAAGTAGTTC	NED
DIK2819	7	47.908	TTACTTTTCGTGGGCCAGAG	GGAACTGTGCCACATAGCAA	NED
BM6117	7	62.246	GTTCTGAGGTTTGTAAAGCCC	GGTGAGCTACAATCCATAGGG	FAM
BMS2258	7	77.194	CCAGCAGAAGAGAAAGATACTGA	AGTGGTAGAACTTCCATCTCACA	FAM
BB719	7	83.466	AAATGCCAGGACCTCACAG	GCTAGGAGATGTTGCTGCTG	NED
BMS522	7	120.509	CTTGCTTACTGCTTGCTATGAA	CCCAACAAAATTTCTGATTCTC	VIC
BMS1979	7	126.245	TTCTTTTTATATCTCCCCCTTCA	GTTTTCTGGAAATCTGTTTAATGC	VIC
BL1029	14	59.439	CAAATCAGCCTCTCCTCTTCC	CAAATCAGCCTCTCCTCTTCC	PET
BM8215	14	48.225	CCAAAGAAGCTGAAGTTGACTG	CCAAAGAAGCTGAAGTTGACTG	6-FAM
BMS2055	14	93.696	ATGCTAAGTGAAGAACAAATCATTC	ATGCTAAGTGAAGAACAAATCATTC	VIC
DIK4015	14	10.029	TTCTAAGCTCCTTGAAGACAGG	TTCTAAGCTCCTTGAAGACAGG	6-FAM
DIK4314	14	28.594	GGCCCTAAAACTCATTGCAC	GGCCCTAAAACTCATTGCAC	NED
DIK5082	14	21.298	TTAAGGCCAAAGCCACATCT	TTAAGGCCAAAGCCACATCT	PET
CSSM066	14	5.125	ACACAAATCCTTTCTGCCAGCTGA	ACACAAATCCTTTCTGCCAGCTGA	6-FAM
BM6425	14	95.139	AGTTGAACCTGGGTCTCCTG	AGTTGAACCTGGGTCTCCTG	NED
ILSTS011	14	25.708	GCTTGCTACATGGAAAGTGC	GCTTGCTACATGGAAAGTGC	VIC

the buffalo calf, name of sire, paternity check, their tag numbers, name of district, center, village and the owners name and the phenotype generated on 1st lactation has been compiled and published by authors under National Agricultural Innovation Project of Indian Council of Agricultural Research entitled "Identification of Quantitative Trait Loci for Milk Yield, Fat and Protein Percentage in Buffaloes" - Buffalo Reference Family Germplasm Catalogue. The catalogue has entries of 8027 buffaloes reared in the project and had been found to have correct paternity using molecular markers.

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