



## SNPRBb: economically important trait specific SNP resources of buffalo (*Bubalus bubalis*)

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### Abstract

Throughout Asian countries including India, water buffalo (*Bubalus bubalis*) plays a crucial role in socio-economic status of the farmers by providing nutritional security. The concept of genomic selection through genetic markers has been widely used in various livestock species and this is extended to buffalo species as well. Molecular markers have been extensively used in animal breeding for improvements of desirable animal traits. Single Nucleotide Polymorphism (SNP), one of the important molecular markers is widely used in animal breeding program. In this study, SNPs related to four important traits of buffalo i.e., milk volume, age at first calving, post-partum cyclicity and feed conversion efficiency have been identified based on genome sequence data generated using ddRAD (double digest Restriction-site Associated DNA) sequencing technology. These identified SNPs have been compiled as database accessible through Web and can be used in molecular breeding program of buffalo species. This database facilitates easy search of SNPs, Polymorphic Loci and Haplotypes along with their important features like minor and major allele frequencies, observed and expected heterozygosity, observed and expected homozygosity and nucleotide diversity. This database will help to accelerate the molecular breeding program for developing trait specific breeds of buffalo to meet the need of food and nutritional security of the world including India.

**Keywords** SNP · Database · DdRAD · Molecular breeding · Water buffalo

### Background

Asia comprise of 97% of total world buffalo (*Bubalus bubalis*) population (Faostat 2014) and 56.5% of Asian buffaloes are in India. Asian buffaloes contribute significantly to the agricultural economy and nutritional security of the country. India ranks highest in milk production with annual production of 216 million tonnes, and buffalo contributes 49% to India's milk production, which is higher than milk production by cattle (Hegde 2019). In addition, India has been world leader in meat exports till recently (Department of Animal Husbandry 2018–19) and out of total meat exports, buffalo meat contributes to 23% whereas 5% and 46% are contributed by cattle and poultry, respectively.

Cattle were domesticated around 8000–10,000 years ago (Lenstra and Felius 2015) whereas, buffaloes were around 1000–3000 years back i.e., later than cattle in *Bovidea* family (Bibi and Vrba 2010). Out of 16 registered breeds in India, Murrah is one the most important breed of buffalo (Borghese and Mazzi 2005) both in India and around the world. This breed's average milk production is as high as 5.76 kg/day as compared to average national milk production of 3.41 kg/day by cattle (Borghese and Mazzi 2005). Apart from this, it is an important resource to sustain livelihood of small and marginal farmers (Gogoi et al. 1985). This breed has been extensively used in India to upgrade the non-descript buffalo stock and improve the milk production (Dutt and Yadav 1988; Taneja and Bhat 1986). The home tract of Murrah buffaloes is a hot and dry climatic region of the north-western part of India (Thiruvenkadan 2011). The dairy industry in India has made significant progress in the last few decades (Seno et al. 2010). However, animal health and its wider adaptability to changing environment need attention, while planning breeding programs to retain India's contribution to world milk production.

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Genetic markers, mainly Single Nucleotide Polymorphism (SNP), Simple Sequence Repeat (SSR), Copy Number Variant (CNV) have been widely used for selection of livestock animal for desired traits (Matukumalli et al. 2009; Surya et al. 2018). Genetic variants underlying complex phenomic traits like animal reproduction, health and its productivity is an outcome of interactive polygenic (pedigree) and genomic (SNP/SNV) additive effects, which can be characterized using structure nucleotide variant (SNV) arrays. Also, the relationship between the SNPs and the functional feasibility of the identified genes over the genome has significant role to measure the feed efficiency in cattle (Duarte et al. 2019). Phenotypic superiority of animal has been determined through ‘breeding value’ which is assigned to mating individuals. This cumulative measure based on genomic information of an individual holds the key to the success of any genetic improvement program in livestock species (Barker 1994; Notter 1999).

Traits having high heritability ( $> 0.4$ ) have scope of genetic improvement (Kiplagat et al. 2012). Thus, the use of molecular markers, unraveling polymorphism at the DNA level has important role in identification of high merit germplasm. Markers developed from single nucleotide polymorphisms (SNPs) are considered to be potential markers (Group 2001; Stickney et al. 2002; Vignal et al. 2002) due to its high prevalence near the locus of interest and its occurrence in approximately every 1000 bases as reported in human genome (Landegren et al. 1998).

In recent times, thousands of SNPs, distributed across the genome were identified using restriction site associated DNA sequencing (RAD-Seq)—Reduced Representational Sequencing approach (Hohenlohe et al. 2010; Malik et al. 2018). This technique reduces the complexity of the genome by sub-sampling only at the sites identified by restriction enzymes (Davey et al. 2011). RAD-Seq can be used to study the population genetics of a species with availability of very less or no sequence data contributing to the added advantage over other methods for SNP discovery.

Currently SNP database (dbSNP) of National Center for Biotechnology Information (NCBI) contains over 9.5 million bovine SNPs; however, number of SNPs identified in buffalo species is limited (Amaral et al. 2008; Michelizzi et al. 2010, 2011). As buffalo and cattle are closely related species, therefore, it was expected that large quantity of genomic resources developed for cattle might be helpful in characterizing the buffalo genome, and in the genomic selection of these species. However, transference of genomic information contained in the Illumina Bovine HD Bead Chip from cattle to buffaloes revealed only 16,580 polymorphic markers ( $MAF > 0.05$ ) out of 688,593 bovine SNPs which were successfully genotyped from the 384 buffalo samples (Borquis et al. 2014). Hence, identification of SNP markers for high throughput genetic analysis has great scope in

buffaloes as well. Recently, Axiom buffalo genotyping array has been used to estimate molecular diversity in Swamp buffalo, describing population structure and its historic relationship with other population (Iamartino et al. 2013), confirming some genomic regions equivalent to BTA 3 and BTA 14 of *Bos taurus* in African buffaloes (*Syncerus caffer*) (Liu et al. 2018; Mokhber et al. 2019).

However, reports on trait based SNP information for Indian buffalo genome are scarce. SNPs from whole exome sequencing related to milk production performance level from buffalo breeds of Banni/Jaffrabadi and Mehsana were obtained (Han et al. 2018; Li et al. 2019; Menon et al. 2016). In this study, SNPs and Polymorphic Loci associated with ddRAD genomic data for four different traits (milk volume, age at first calving, post-partum cyclicity, and feed conversion efficiency) in Murrah buffalo have been identified. These SNPs are documented as a web based database for its wider access and application. This database aims to understand the influence of SNP markers related to four economically important traits of Murrah buffaloes.

## Materials and methods

### Animals, phenotypic resource and genotyping data generation

Murrah buffaloes used in the present study were from the progeny testing programme at ICAR-Central Institute for Research on Buffaloes, Hisar (Government of India) for genetic improvement of the buffalo breed. All the pedigree and performance records are maintained starting from birth to death/culling of every calf at farm. Selection of individual animals for implementing mating plans is based on specific performance criteria of mates for genetic improvement and productivity enhancement. These specific criteria are phenotype determinants for selective genotyping. Selective genotyping based on discrete levels of performance to harness true genetic variants as source of mapping nucleotide based markers to achieve higher gain in selection programmes have been followed.

Individual animals were selected from unrelated pedigree and extreme performance levels for traits as: milk volume (milk yield), age at first calving, post-partum cyclicity and feed conversion efficiency [Institutional Animal Ethics Committee (IAEC); Reg. No. 406/GO/RBI/L/01/CPCSEA]. Data search on phenotype records (for almost 25 years) were made while considering animals selection for genotyping, ensuring wide variation over sire, parity of animals, best milk yield in whole production span, milk constituents, lactation length subject to performance criteria is shown in Table 1.

**Table 1** The performance criteria for measurement of phenotypic records

S.no	Traits	Phenotype determinant		
		Higher limit	Lower	Pre-determined/Experimented
1	Milk volume (milk yield) in kg per lactation (measured in 305 days lactation length)	> 3000 kg	< 1800 kg	Phenotype records available at Farm
2	Age at first calving (Records are kept for every heifer at farm under the Genetic Improvement Programme)	< 40 months	> 55 months	Phenotype records available at Farm
3	Post-partum cyclicity (It is taken as gestation period i.e., period between two subsequent calvings)	< 70 days	> 200 days	Phenotype records available at Farm
4	Feed conversion efficiency trials	-0.437	0.359	Based on feed trials

For generation of feed conversion efficiency, feeding trials for feed conversion efficiency were conducted for 90 days feed trial on 42 growing animals of age 10 to 12 months. Individual feeding of animals was conducted for 3 months period to identify efficient energy utilizing animals for growth. Data on feed (dry matter) intake and body weight gain was recorded respectively, daily, before and after trial for 90 days to determine high and low residual feed Intake [RFI] per unit daily weight gain, to estimate Feed Conversion Efficiency (FCE). RFI is determined as difference in actual and predicted dry matter intake (DMI) of individual heifer (Sikka et al. 2020). Average daily weight (g/day) gain was recorded for each of the animals under trial. Under this trial, eleven high and eleven low feed convergent animals out of 42 animals were selected for genotyping based on residual feed intake /daily intake. Actual dry matter intake per unit body weight gain was determined. High and low feed convergent were identified by determining residual feed intake variation for genotyping.

Selective genotypes were bled for isolation of nucleic acid (DNA and RNA) by Phenol-Chloroform method (Sambrook and Russell 2001) and further used for SNP genotyping. Study comprised of 85 buffaloes, out of these, 8 to 14 buffaloes were selected for each trait based on their contrasting phenotypic performance i.e., low and high performance as per records maintained at farm. However, in the current study, 25 animals for milk volume, 19 animals for age at first calving, 19 animals for post-partum cyclicity and 22 animals for feed conversion efficiency were selected. Individual sample-wise ddRADdata were generated by Hi-Seq Illumina sequencing platform (Peterson et al. 2012).

## Data quality check, processing and analysis

The ddRAD sequencing of genome data generated over 85 animals were checked for quality parameters followed by trimming of these quality sequences using Trimmomatic tool (Bolger et al. 2014). These sequences were further aligned to the available reference genome i.e., *Bubalus bubalis* (UOA\_WB\_1) (Low et al. 2019) and indexing has been done

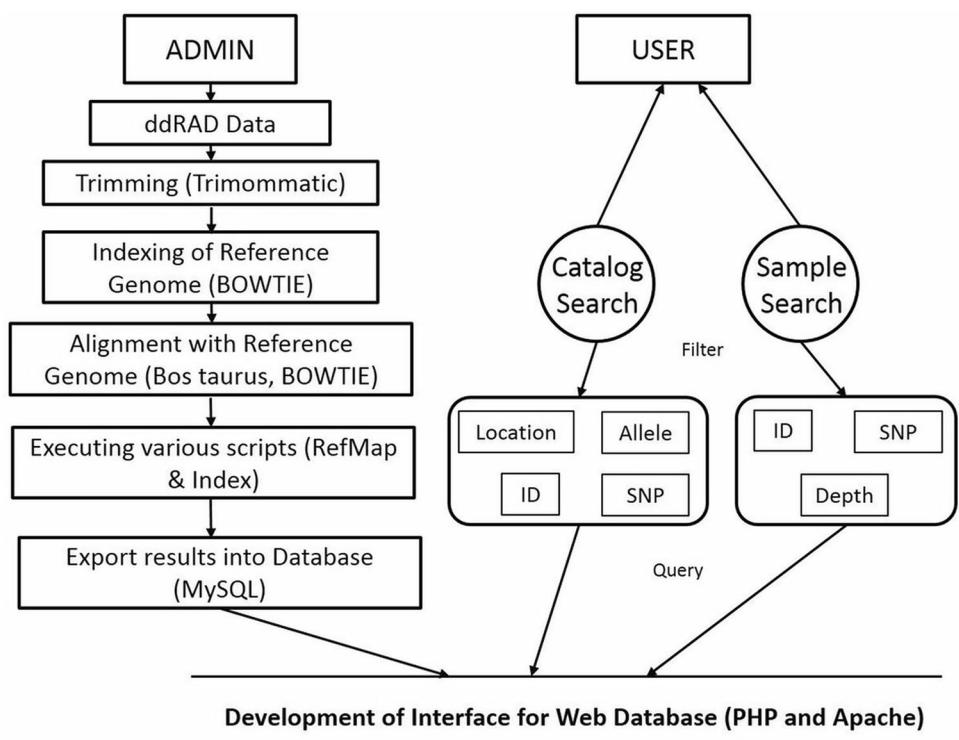
by using Bowtie2 tool (Langmead and Salzberg 2012) with default parameter settings. Further, the resultant sequences were aligned and produced as Sequence Alignment Map (SAM) formatted files and then passed to STACKS pipeline (Catchen et al. 2013). The wrapper program *ref\_map.pl* available in STACKS pipeline was used to create the catalog of loci for SNP identification using the standard parameters. First, the sequences were aligned to the same genomic location were stacked together and merged to form loci. These loci with a sequencing depth of three or more reads per individual were retained and catalog has been created. SNPs at each locus were selected using a maximum likelihood framework. Population program of STACKS pipeline (*populations*) was used to process all the SNP data across individuals to generate summary statistics. Genetic diversity was estimated using important genetic parameters such as major and minor allele frequency, observed and expected heterozygosity, observed and expected homozygosity and nucleotide diversity ( $\pi$ ) (Fig. 1). Further, annotation of these SNPs was carried out with the help of SnpEff tool and related gene Ids were converted into UniProt gene Id using DAVID software. Moreover, the functional classification of the genes was carried out by using agriGo tool with FDR value  $\leq 0.05$  based on uniProt gene Ids. The agriGo tool maps genes to function according to GO categories: molecular function, cellular component and biological process (Young et al. 2019). The location of the uniProt gene Ids was retrieved from Ensembl genome browser.

## Results and discussion

### Data statistics

The total identified SNP by using group wise computational approach in all low and high performance groups of genotypes (animals) belonging to four traits and the trait wise minimum and maximum number of identified unique stacks, polymorphic loci and SNPs for all samples is shown in Tables 2, 3 and Fig. 2.

**Fig. 1** The data and process flow architecture



**Table 2** The total identified SNP by using group wise computational approach in all low and high performance groups of genotypes (Animals) belonging to four traits

S.no	Traits	Genotypes/ Samples (n)		SNPs (n)	
		High	Low	High	Low
1	Milk volume	11	14	119,755	127,227
2	Age at first calving	08	11	97,030	86,461
3	Post-partum cyclicity	11	08	42,144	44,232
4	Feed conversion efficiency	11	11	98,753	98,454

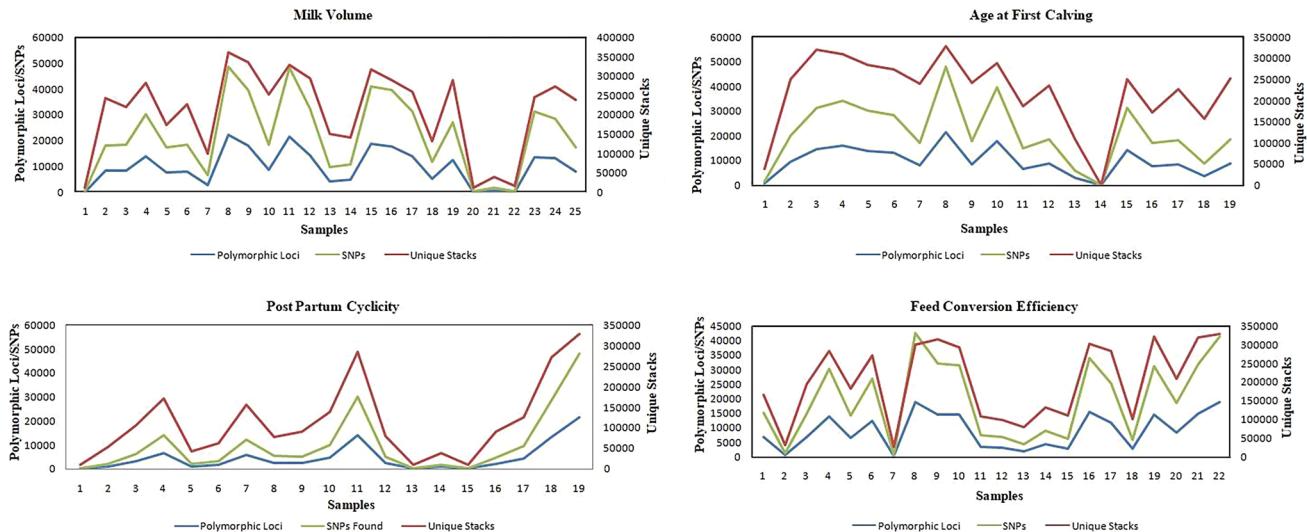
### SNPRBb: design and implementation

The structure of the database has been designed by applying the normalization procedure of Relational Database Management System (RDBMS) to avoid the duplicate values

of attributes and corresponding entities. The database was developed in MySQL. MySQL feature-rich database software that provides speedy data access, ease of use, portable etc. (Bowman et al. 1996). The identified SNPs and their characteristics from the different samples across the four traits of water buffalo has been integrated and assembled in this relational open source database i.e., MySQL. The accessibility of this data is provided to the end users by writing server side scripts in PHP language and hosted at Apache Web Server (Lee and Ware 2002). The Web based end user interface has been developed by using HTML5 and Java script language and dynamic Web interface has been created using PHP for quick and easy retrieval of SNPs and their associated information from the database. This web application has been hosted using an open source software Apache web Server to provide multi-user access of the developed interface. This database is named as SNP resources on *Bubalus bubalis* (SNPRBb) (Fig. 3).

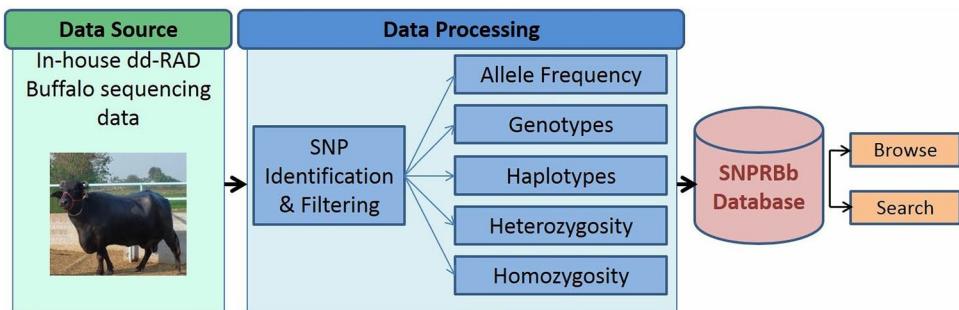
**Table 3** Trait wise minimum (Min) and maximum (Max) number of identified unique stacks, polymorphic loci and SNPs for all samples

S. no	Traits	Unique Stacks		SNPs (n)		Polymorphic loci	
		Min	Max	Min	Max	Min	Max
1	Milk volume	10,382	362,429	203	26,465	144	22,120
2	Age at first calving	38,440	329,562	771	26,778	673	21,415
3	Post-partum cyclicity	11,021	329,562	165	26,778	132	21,415
4	Feed conversion efficiency	25,873	329,262	434	22,505	373	18,832



**Fig. 2** Sample wise identified unique stacks, polymorphic loci and SNPs for all the selected traits

**Fig. 3** Shows the Data Flow Diagram (DFD) of the SNPRBb, starting from data collection to upload the results in database including data processing and analysis



The Web access has been developed to provide seamless integration with the underlying database. The database is accessible from <http://snprbb.icar.gov.in> and home page (Fig. 4a). End users access the available options to click on any of the item i.e., Browse, Gene Ontology, Team, Disclaimer and Help to view the required information (Fig. 4b).

If any user wants to revert back to home page, the “Home” link is made available at every page. The identified SNP resources can be browsed or explored by clicking on the link “Browse”. This option allows the users to search and explore SNP resources based on catalog and samples for selected trait. The current database contains the identified SNP resources for four traits of *Bubalus bubalis* i.e., milk volume, age at first carving, post-partum cyclicity and feed conversion efficiency.

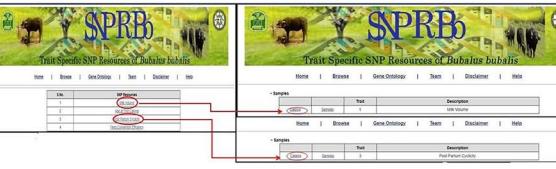
Furthermore, the users have two choices to browse these SNPs i.e., either by catalog or samples for the selected trait. Catalog will provide identified Tags, SNPs, consensus sequence based on samples corresponding to chromosome number, position of base pair, matches and allele ratio of the selected trait. The user can even explore more details by choosing a specific Id to get the information related to the

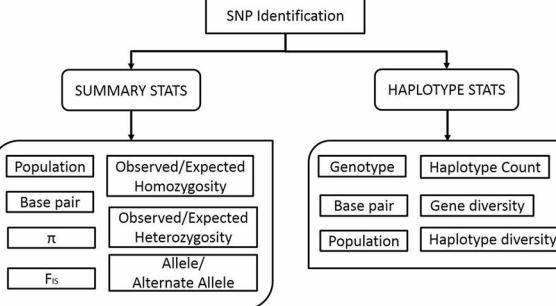
SNP position, haplotypes, genotypes and their count, allele depth and LnLs (Log Likelihood filter). In case of more SNPs, the summary statistics can be viewed by clicking on the SUMMARY STATISTICS. This will display population, base pair, column (position), allele, alternate allele, and other useful information related to the identified SNPs. Similarly, the information is also accessible for HAPLOTYPES STATS in a separate window. The features of the SNPs, step-wise procedure for selection of mentioned parameters and statistics are described in Fig. 4c–f.

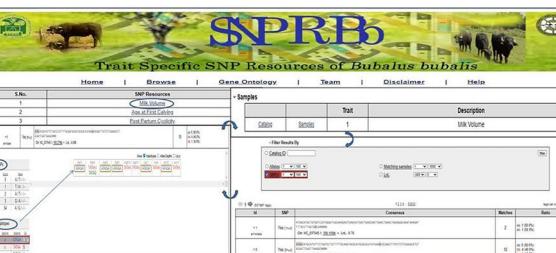
## Conclusion

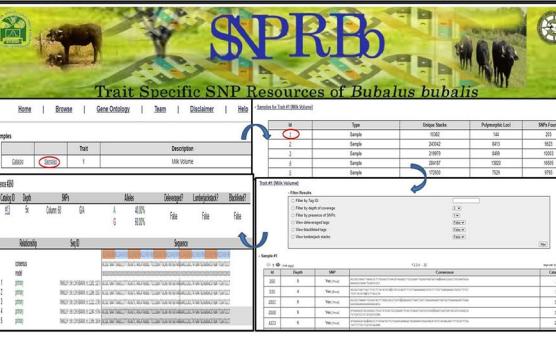
SNPRBb documents the identified SNPs using ddRAD data for four important traits in *Bubalus bubalis* i.e., milk volume, age at first carving, post-partum cyclicity and feed conversion efficiency. These identified SNPs specific to these traits are assembled as database and available to end users through web browser. Detailed information of each SNP, including allele frequencies in buffalo populations, heterozygosity and homozygosity value, haplotypes statistics,  $F_{IS}$  and

**a** 

**b** 

**c** 

**d** 

**e** 

**f** 

◀ **Fig. 4** **a** Database is accessible source; **b** End users options (Browse, Gene Ontology, Team, Disclaimer and Help to view the required information); **c** Features of the SNPs; **d** & **e** Step-wise procedure for selection of mentioned parameters; and **f**: Gene Ontology

their position on reference chromosome are available in the database. SPRBb mainly provides the information associated to SNP in two ways viz. Catalog and sample which will enable the researchers in the comparative analysis for quick comparison of SNPs in different samples. This developed database will be a useful resource for planning breeding program for genetic gain by studying the genetic variants associated with economically important traits buffalo.

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**Data availability** Database URL:<http://snprbb.icar.gov.in/>

## Declarations

**Conflict of interest** The authors declare that they have no conflict interest.

**Ethical approval** The data and samples of buffaloes used in this study were provided by buffalo herd of ICAR-Central Institute for Research on Buffaloes Hisar (Haryana), India. Experiment design and animal treatments were approved by the Institutional Animal Ethics committee (IAEC) of the Institute.

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