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Performance of Illumina® BovineHD BeadChip in genotyping Indian riverine buffalo breeds

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About 20.5% of the total livestock population in India are buffaloes with total buffalo population in the country at 109.9 million during 2019 (Anonymous 2019). Riverine buffalo and cattle are phylogenetically very close and have a high chromosomal homology between species, and share gene order and chromosome banding homology. Genomic tools developed for cattle are used for applications like parentage verification and SNP based tests like genetic disorders screening in buffaloes also. The Illumina® BovineHD Beadchip (Illumina®, USA) with 777k SNPs was developed and utilized for genotyping studies in various breeds of cattle, but has also been used in other members of the bovidae, including buffalo. The present study was designed to assess the performance of Illumina® BovineHD BeadChip in three Indian riverine buffalo namely Jaffarabadi, Mehsana and Murrah breeds and to examine if SNPs on BovineHD beadchip are informative enough to study the variability among breeds using Principal Component Analysis (PCA).

The present study was performed on 96 buffaloes belonging to three different breeds viz. Jaffarabadi (29), Mehsana (26) and Murrah (41). Blood samples (3 ml) from buffaloes in farmers herds were collected in EDTA vacutainers as per institutional ethical procedures. DNA was isolated from 200 µl blood using Macherey Nagel Nucleospin® blood kits as per manufacturer's instructions. Four animals were genotyped in duplicate for checking data quality.

The animal samples were genotyped using 777k BovineHD BeadChip (Illumina®, Inc., USA) at M/s Sandor LifeSciences Ltd., Hyderabad, India as per manufacturer's instructions. Animals genotyped in duplicate had a SNP concordance of > 99.5% indicating good quality genotyping. Data for animals with a call rate >80% were kept for further analysis. During subsequent quality control, SNPs with genotyping rate <90% and SNPs deviating from Hardy Weinberg equilibrium ($p<10^{-4}$) were excluded; Minor Allele Frequency (MAF) for alleles were calculated using PLINK software version 1.9 (Purcell *et al.* 2007). Markers on autosomal chromosomes with MAF greater than 0.05 were retained for further analysis. To examine the variability in buffalo breeds, PCA was carried out using PLINK software. Eigenvectors obtained using PCA were plotted using R Library (plot3D) (Soetaert *et al.* 2019) to visualize breed clustering.

An efficient SNP array for genotyping a particular population, requires SNPs to be distributed evenly covering the entire genome, polymorphic and in Hardy-Weinberg equilibrium. The Illumina® BovineHD BeadChip contains 777962 SNP markers spread throughout the bovine genome with average distance between markers being 3.43 kb (www.Illumina®.com/Documents/products/datasheets/datasheet_bovineHD.pdf). Illumina® Bovine HDchip had polymorphic SNPs that captured sufficient variation in *Bos indicus* and *Bos taurus* × *Bos indicus* cattle to develop INDUSCHIP (Nayee *et al.* 2018), a medium density SNPchip for genomic selection in *Bos indicus* and *Bos taurus* × *Bos indicus*. Similar strategy of using BovineHD BeadChip was considered for genotyping riverine buffaloes. Genotyping rate for 100 buffalo samples was in range of 83.9% to 86.6% with an average of 86%. MAF distribution after QC with SNP Genotyping rate and HWE is reported in Table 1. SNPs removed in each step of Quality control filters are shown in Table 2. Extent of polymorphism is an important criteria to determine informativeness of SNPs for a particular population. Number of polymorphic

Table 1. MAF distribution after QC with SNP genotyping rate and HWE

MAF	Jaffarabadi No. of SNP	Mehsana No. of SNP	Murrah No.of SNP
0	587523	589095	586346
0.000001-0.05	3618	2792	5081
0.050001-0.1	2564	3683	2710
0.100001-0.25	4973	4841	4653
>0.25	5379	5317	5514
Grand Total	604057	605728	604304

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Table 2. SNPs available after genotyping, HWE and MAF 0.05

Total autosomal SNPs = 735293	Number of SNPs filtered		
SNP filtering criteria	Jaffarabadi	Mehsana	Murrah
Genotyping > 90%	130193	128449	130012
HWE (0.0001)	1043	1116	977
MAF 0.05	591141	592778	591427

SNPs (MAF>0.05) in Jaffarabadi, Mehsana and Murrah, were 12916, 12950 and 12877, respectively. Polymorphic SNPs in the studied Indian river buffalo breeds were lower than in Chinese water buffaloes (Pérez-Pardal *et al.* 2018) where 20,479 polymorphic SNPs with a call rate of 95% were identified while Borquis *et al*. (2014), reported 16,580 polymorphic SNPs with call rate of 80% using Illumina® BovineHD BeadChip for 384 water buffaloes in Brazil.

MAF indicates extent of polymorphism for a SNP in a given population. Autosome-wise distribution of MAF in 100 buffalo samples was estimated using PLINK. MAF was classified into 5 different categories as shown in Fig. 1 before and after quality control. Results revealed that the average MAF of the SNPs distributed across the autosomes after quality control was 0.23. Polymorphic SNPs (41.12%) had MAF > 0.25. The 13150 polymorphic SNPs among the three breeds are uniformly distributed among the autosomes. SNPs monomorphic in Jaffarabadi, Mehsana and Murrah breeds were 234, 200 and 273, respectively. The observed heterozygosity obtained was 0.3273, 0.330, 0.3286 and expected heterozygosity was 0.3208, 0.3252, 0.363, respectively in Jaffarabadi, Mehsana and Murrah breeds. The average MAF observed was 0.23 for Jaffarabadi, 0.235 for Mehsana and 0.236 for Murrah breed, respectively. The results indicated that though majority of SNPs on BovineHD BeadChip are not informative for buffalo breeds under the study, still there are around 13,150 SNPs that can be used. Utility of Illumina® BovineHD BeadChip had been already demonstrated for parentage verification in buffaloes (Perez-Pardal *et al.* 2018).

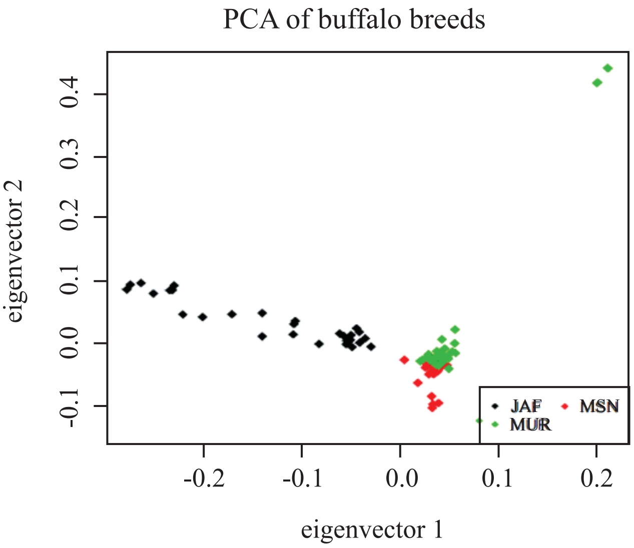

Fig. 1. PCA of buffalo breeds.

Total variability explained by first 20 principal components was 33.20%, out of which first and second component explained 2.91% and 2.51% variability, respectively. PCA based on the polymorphic SNPs revealed that animals of Jaffarabadi breed showed larger variation, whereas Murrah and Mehsana animals formed tight clusters or were found to be overlapping (Fig. 1). The results indicated that the selected SNPs may not be informative enough to reveal differences in Indian buffalo breeds. In a similar work, Thakor *et al.* (2021) could differentiate phenotypically distinct breeds like Surti, Pandharpuri, and Jaffarabadi using SNPs in Axiom™ Buffalo Genotyping Array (90 K) but not others like Banni, Mehsana, Murrah and Niliravi. In a related development, Sudhakar *et al.* (2021) studied false positive results of Deficiency of Uridine Monophosphate Synthase (DUMPS) in buffaloes which were tested using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) tools developed for cattle and reported that using SNP detection genomic tools developed for a species requires genome sequencing for validation before using in a new species/population. These results indicates the need for identifying SNPs that are polymorphic in buffaloes and developing a buffalo specific genotyping SNP chip for applications like genomic breeding value estimation, breed purity analysis etc.

From this study, it can be concluded that out of total 777k SNPs in Illumina® BovineHD BeadChip, only 86% SNPs were called for buffalo samples under the study. Out of 670,955 successfully genotyped SNPs, only 13,150 SNPs were polymorphic in Jaffarabadi, Murrah and Mehsana. The SNPs that are polymorphic in three breeds under the study, were having similar behaviour in all the three breeds and the polymorphic SNPs were not enough to effectively capture the variation between Jaffarabadi, Murrah and Mehsana buffalo breeds. A specialized buffalo SNP chip may be required to study population structure and variability in riverine buffalo breeds available in Indian subcontinent.

SUMMARY

The current study tested the suitability of Illumina® BovineHD BeadChip in genotyping three riverine breeds of buffalo viz. Jaffarabadi, Murrah and Mehsana. Of the 777962 SNPs, 670955 SNPs were called in all animals and only 13150 SNPs were found to be polymorphic at a call rate of 90%, and had Minor Allele Frequency (MAF) greater than 0.05. The average Minor Allele Frequency (MAF) of polymorphic SNPs was 0.23 and average expected heterozygosity (HE) of 0.321. Principal Component Analysis (PCA) revealed 2 different clusters where Murrah and Mehsana breeds clustered together, while Jaffarabadi breed formed a distinct cluster. Result obtained in this study indicated that the SNPs available in Illumina BovineHD BeadChip may not be good enough for studying population structure and genetic analyses in Indian buffalo breeds. Hence it might be necessary to develop a custom SNP marker panel for Indian riverine buffaloes.

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