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Identification of superior Nili Ravi buffalo bulls (*Bubalus bubalis*) for increasing milk production through selection by estimation of breeding values and genome analyses

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Buffalo is considered as the best dairy animal in livestock as it contributes about 61% to the total milk production in Pakistan. Novel genetic tools are being developed to discover variants with better milk quality and yield, and uncovering of inherited markers is vital to map and characterize quantitative traits for superior bull selection. Genotyping by sequencing (GBS) is flexible and novel approach to provide standard markers for genome analyses. This study aimed to find the mutual effect of milk production associated genes on its yield and quality by GBS analysis in order to identify superior buffalo bulls that ultimately enhanced the milk production. DNA was extracted from 150 blood samples of healthy bulls with respect to their physical and reproductive parameters, and restriction digestion of the whole genome was done. Furthermore, overhangs of restricted fragments were ligated with adapters. Then all samples were pooled and amplified with primers complementary to adapter sequences. These amplified products were further purified and quantified for sequencing by adding 3'sequences compatible to flow-cell oligonucleotides. Then single nucleotide polymorphism (SNP) genotype profile was created to process GBS raw profiles to make it as data. The results of the GBS analyses provided evidence for the mutual effect of milk production associated genes on its yield and quality. Selection of bulls by this approach helped to identify real superior breed in terms of high milk production rate so that they could be used in Artificial Insemination (AI) programs to improve the genetic value of buffaloes in the country.

Keywords: Buffalo milk, genotyping by sequencing, milk production associated genes, single nucleotide polymorphism

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