# Evaluation of Genetic Merit of Buffaloes by Direct Sequencing

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**Abstract:** Sequencing the genome of the buffaloes enables scientists to more accurately identify the genetic markers that are responsible for economically important traits such as milk yield, milk fat and protein. Producers can use that information to breed healthier dairy animals that produce more and higher quality milk as well as beef animals. Consumers will benefit from more cost-effective and healthier products on store shelves. Understanding what genes contribute to specific buffalo animal traits will also have spinoff applications related to other fields like human health and disease. The animals used in the genome project have had a high impact on the breeding and commercial sectors in dairy and beef.

**Keywords:** Traits, chromosomes, selection, genetic marker, genotype.

## INTRODUCTION

The genetic improvement of animals is a fundamental, incessant, and complex process. In recent years many methods have been developed and tested. The genetic polymorphism at the DNA sequence level has provided a large number of markers and revealed potential utility of application in animal breeding. The invention of polymerase chain reaction (PCR) in accordance with the constantly increasing accuracy in DNA sequencing methods also represents a milestone in this endeavor. Selection of markers for different applications are influenced by certain factors - the degree of polymorphism, the automation of the analysis, radioisotopes used, reproducibility of the technique, and the cost involved. Presently, the huge development of molecular markers by DNA sequencing will continue in the near future. It is expected that molecular markers will serve as an underlying tool to geneticists and breeders to create animals as desired and needed by the society.

## MILK PRODUCTION

World milk production has doubled in the last few decades and it is noteworthy that in the last many years, buffaloes have been supplying about 12 per cent to the world's milk production. India has been producing 60 per cent of the world's buffalo milk, which is around 55 per cent of the total milk produced in the country [1].

India produces more than 84 million tons of milk, with 80 per cent of it being produced by small scale farms with herd size of 2 to 8 animals, by both buffaloes and cows. These micro enterprises are operated by an estimated 11 million farmers located in remote villages, who are members of 96,000 odd village dairy co-operatives. The producers, mainly landless laborers with underprivileged families, enjoy a relatively steady income through the sale of surplus milk. This income is vital for their well being and economic security [2, 3].

## BUFFALOES

The domestic water buffalo (*Bubalus bubalis*) belongs to the *Bovidae* family, sub-family *Bovinae*, genus *bubalis* and species *arni* or wild Indian buffalo. The animals are classified into two distinct classes as swamp and river buffalo. The water buffalo has many anatomical and physiological similarities with the other species in this family. River buffaloes have 50 chromosomes and swamp buffaloes have 48 chromosomes. While the two types of buffaloes can be mated to produce a fertile offspring having 49 pairs of chromosomes, buffaloes cannot be successfully mated with any other members in the *Bovidae* family [4].

## SWAMP BUFFALOES

The swamp buffaloes are found in the Indian subcontinent and throughout Southeast Asia and China. The name 'swamp' has probably arisen from the fact that they prefer to wallow in stagnant water pools and mud holes. These buffaloes produce relatively small quantities of milk, one to two kg per day and they are mainly used for meat and draught. These animals are very well adapted to hot and humid climates as well as marshy lands. With their large hooves they are able to wade through the swamps and reach a variety of fodder sources [4, 5].

## RIVERINE BUFFALOES

The Riverine breeds of the Indian subcontinent are mainly raised for milk production. Their milk yield is

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about 6 to 7 liters per day with an average content of fat, protein and lactose of 7.5, 4.2 and 5.0 per cent, respectively, which gives the buffalo milk higher energy content than milk from dairy cattle [6, 7]. Twelve of the 18 major Riverine breeds of buffaloes are kept primarily for milk production. The main millch breeds of India are Murrah, Nili-Ravi, Surti, Mehsana, Nagpuri, and Jafarabadi. The river buffalo prefers, as its name reveals, to wallow in clear, running water [4].

Buffaloes are known to be good grazers, but they graze a wider range of plants than cattle [8, 9]. They utilize low-grade roughage more efficiently compared to cattle [6, 10]. The unique ability of the buffaloes to survive under the most difficult conditions of nutrition and management has given them a competitive edge over other milk producers in the areas. In addition, the buffalo has a long productive life. A normal healthy female buffalo could have as many as 9 to 10 lactations [11] in her life time.

## LIMITATIONS IN BUFFALOES MILK PRODUCTION

Buffalo is still largely an animal of the village and many of its reported limitations are caused more by its environment than by the animal itself. Moreover, much of the animal's genetic potential is obscured by environmental influences. For example, for many breeds and types the genetic variations in milk yield and growth cannot be accurately determined because these are overwhelmed by the effects of inadequate nutrition and management.

The buffalo has long been considered a poor breeder-slow to mature sexually, and slow to rebreed after calving. Accumulated experience now shows, however, that this is mainly a result of poor management and nutrition. Buffaloes are not sluggish breeders. Nevertheless, their gestation period is about a month longer than that of cows, buffalo estrus is difficult to detect, and many matting’s occur at night such that farmers are likely to encounter more problems in breeding buffaloes than cattle [1].

## CHARACTERISTICS OF BUFFALO TRAITS

Although, the river buffalo is the main dairy animal in some countries, it is a primitive animal when compared to the developed dairy cattle breeds like Holstein-Friesian and Jersey. Many generations of selective breeding has produced cattle with almost predictable productive and reproductive traits. This has not been the case among the buffalo breeds as the majority of these animals are reared by land less and marginal farmers where they breed naturally [12].

In general the age of buffaloes at first calving is usually around 40 to 60 months [11]. However, there are indications that the productive traits can be improved. As an example, the Mediterranean breeds and the swamp buffaloes calved earlier than those of Indian subcontinent [13]. Average calving intervals for Indian and Pakistani buffaloes ranged from 15 to 18 months. The dry period has been reported to be 90 to 150 days for the Nili-Ravi breed of Pakistan while for the Murrah, it ranged from 60 to 200 days. Average lactation length ranged from 252 to 270 days. As a result of these factors the productive life of a buffalo is only 39 per cent of its total life when compared to 52 per cent in developed dairy breeds [5, 11].

In most of the buffalo milk-producing countries of Asia, it is observed that there are large seasonal variations in breeding and calving in buffaloes [11]. In India and Pakistan, 80 per cent of the buffaloes calve during June and December causing a decline in milk production in the summer months. However, others have suggested this summer decline in milk production could be due to heat stress and shortage of greens. Dark body, lesser density of sweat glands and thick epidermis makes it difficult for the buffaloes to thrive in extreme hot and dry conditions. Buffaloes have developed survival mechanisms to seek water for immersion in these conditions. When exposed to extreme hot or cold conditions, the buffaloes’ milk production and reproductive efficiency are strongly affected [5]. In addition to climatic influences, poor nutrition and management also affect breeding and production.

## BUFFALO BREEDS IN INDIA

Well defined breeds of buffaloes with standard qualities are found mainly in India and Pakistan. There are 18 River buffalo breeds in South Asia, which are further classified into five major groups designated as the Murrah, Gujarat, Uttar Pradesh, Central Indian and South Indian breeds. The best known breeds are Murrah, Nili Ravi, Jafarabadi, Surti, Mehsana, Kundi and Nagpuri. Most of the remaining buffaloes of the Indian subcontinent belong to a nondescript group known as the Desi buffaloes. Karnataka state possesses mainly nondescript buffalo populations. Animals of Surti and Murrah breeds are widely distributed in northern parts of Karnataka, along with the local animals reared by the Gowli tribe. Local breeds of buffalo like South Kanara are present in and around Dakshina Kannada districts of Karnataka state, India.

## EVALUATION OF GENETIC MERIT OF ANIMALS

Many of the traits of interest in animal production are quantitative traits. Evaluation of genetic merit of animals is still essentially based on the application of the theory of quantitative genetics. The conceptual basis of this theory is the polygenic model, which assumes that quantitative traits result from the action (and interaction) of a large number of minor genes, each with small effect. The resulting effects are then predicted using powerful statistical methods (animal model), based on pedigree and performance recording of traits from the individual animal and its relatives [14].

The advances in molecular genetics technology in the past two decades, particularly Nucleic acid-based markers, has had a great impact on gene mapping, allowing identification of the underlying genes that control part of the variability of these multigenic traits. Broadly, two experimental strategies have been developed for this purpose: linkage studies and candidate gene approach [15].

## LINKAGE STUDIES

Linkage studies rely on the knowledge of the genetic map and search for quantitative trait loci (QTL) by using pedigree materials and comparing segregation patterns of genetic markers (generally micro satellites) and the trait being analyzed. Markers that tend to co-segregate with the analyzed trait provide approximate chromosomal location of the underlying gene (or genes) involved in part of the trait variability determinism [14].

## MARKER ASSISTED SELECTION

Most economically important traits in dairy animal production are influenced by many genes as well as environmental factors. Breeding programs aim at selecting animals with the most favorable set of genes to produce animals for the next generation. Selection in most of these breeding programs is for a combination of production, conformation, and functional traits. Evaluation procedures like BLUP have been developed to estimate breeding values of animals. The nature of the underlying genes (quantitative trait loci, QTL) affecting traits, however, is still largely unknown. Recently, efforts have been undertaken to locate genes affecting economically important traits in dairy animals. Genetic markers associated with these genes can be used in marker-assisted selection (MAS) to increase genetic progress. For dairy cattle, the focus was initially on milk production traits. The granddaughter design was used to locate genes involved in milk, fat, and protein production. Implementation of MAS in breeding strategies for production traits has been started. However, MAS is expected to be especially beneficial for traits that cannot be improved very efficiently by current breeding programs, for example, due to low heritability. Important traits in dairy cattle breeding with low heritability include functional traits like fertility and health traits. Although some markers associated with functional traits were reported, these studies were limited to only a few traits or used a marker map that was not very dense [16].

## CANDIDATE GENE APPROACH

The second approach focuses on the study of the genetic polymorphism of a few genes (candidate genes) suspected, on the basis of the biological and physiological information of the trait, to be implicated for part of the trait variability. Hence, association analysis (QTL) is carried in order to test whether a particular genotype or haplotype (a series of alleles along a stretch of DNA) are stably associated with the analyzed trait, such as the rate of the synthesis of the protein or milk yield. Introduction of such additional molecular information in selection procedures would be definitely beneficial to assess the true genetic merit of animals. That is, it allows selection to occur among individuals that do not exhibit the trait in question e.g. milk protein genotypes in males. This approach is already being employed with regard to bovine leukocyte adhesion deficiency (BLAD) [17] and genes with major effects, such as the halothane locus in swine [18] and alpha $_{S1}$-casein in goat [19].

The candidate gene approach studies the relationship between the traits and known genes that may be associated with the physiological pathways underlying the trait. In other words, this approach assumes that a gene involved in the physiology of the trait could harbor a mutation causing variation in the trait. The gene or part of gene, are sequenced in a number of different animals, and any variation found in the DNA sequences, is tested for association with variation in the phenotypic trait [20]. Candidate gene approach is performed in 5 steps: 1) collection of resource population. 2) Phenotyping of the traits. 3) Selection of gene or functional polymorphism that potentially could affect the traits. 4) Genotyping of the resource population for genes or functional polymorphism. Lastly, one is statistical analysis of phenotypic and genotypic data [21]. This is an effective way to find the genes associated with the trait. So far a number of genes have been investigated. Candidate gene approach has been ubiquitously applied for gene disease research, genetic association studies, biomarker and drug target selection in many organisms from animals [22].

## CANDIDATE GENE POLYMORPHISM AND DNA SEQUENCING TECHNIQUE

Recent developments in DNA technologies have made it possible to uncover a large number of genetic polymorphisms at the DNA sequence level and to use them as markers for evaluation of the genetic basis for observed phenotypic variability. These markers posses unique genetic properties and methodological advantages that make them more useful and amendable for genetic analysis and other genetic markers. The possible applications of molecular markers in livestock industry are by conventional breeding programme to transgenic breeding technologies. In conventional breeding strategies molecular markers have several short range or immediate applications viz., parentage determination, genetic distance estimation, determination of twin zygosity and free martinism, sexing preimplation embryo and identification of genetic disease carrier and long range applications viz., Genome mapping and marker assisted selection. In transgenic breeding molecular markers can be used as reference points for identification of the animals carrying the trasgenes. The progress in the genetic markers suggests their potential use for genetic improvement in the livestock species.

## DNA SEQUENCING

The revolution in molecular genetics, and particularly genome sequencing, has already provided benefits for animal breeding, but in comparison with what the future holds, our present tools will undoubtedly be seen as primitive. Complete genome sequences are available for a number of species, genome sequences for the chicken, cow, horse, mouse and chimpanzee are either completed or nearing completion, and single nucleotide polymorphism (SNP) libraries for these species are growing rapidly. This information will underpin most of the developments in livestock breeding and breed management during the coming two decades. The invention and commercial introduction of several revolutionary approaches to DNA sequencing, the so-called next-generation sequencing technologies. Although these instruments only began to become commercially available in 2004, they already are having a major impact on our ability to explore and answer genome-wide biological questions. These technologies are not only changing our genome sequencing approaches and the associated timelines and costs, but also accelerating and altering a wide variety of types of biological inquiry that have historically used a sequencing-based readout. Furthermore, next-generation platforms are helping to open entirely new areas of biological inquiry, including the investigation of ancient genomes, the characterization of ecological diversity, and the identification of unknown etiologic agents.

## NEXT-GENERATION DNA SEQUENCING

Three platforms for massively parallel DNA sequencing read production are in reasonably widespread use at present: Roche/454 FLX, Illumina/ Solexa Genome Analyzer and Applied Biosystems SOLiDTM System. Recently, another two massively parallel systems were announced: the Helicos HeliscopeTM and Pacific Biosciences SMRT instruments. Roche/454 FLX Pyrosequencer. This next-generation sequencer was the first to achieve commercial introduction (in 2004) and uses an alternative sequencing technology known as pyrosequencing.

The impact of massive parallel sequencing or next generation sequencing (NGS) in Biology, and hence in Animal Genetics, is difficult to overstate. It is a revolution comparable with the one that followed Sanger sequencing forty years ago. It is not just a dramatic increase in sequencing speed: it means a change in paradigm that obliges researchers, institutions and funding agencies alike. It is also a tremendous and passionate technological race worth millions. Although it is uncertain which, if any, of the extant technologies will prevail in the future, and whether they may be replaced by new technologies, one thing is certain: next generation sequencing is this generation sequencing. Current NGS technologies provide a throughput which is at least 100 times that of classical Sanger sequencing and the technologies are quickly improving. Compared with standard sequencing, these technologies do not need cloning, i.e. they are basically shotgun sequencing and result in shorter sequences, currently from 50 to 400 bp. Further, it is promised that new, third generation single molecule sequencing will soon be available and should deliver complete genome sequence that is in time and pricing affordable for every research group [13].

## SEQUENCING THE BUFFALOES

The water buffalo is vital to the lives of small farmers and to the economy of many countries worldwide. Not only are they draught animals, but water buffalo also produce meat, horns, skin and particularly the rich and precious milk that gives creams, butter, yogurt and many cheeses. Just recently, Jiang *et al.,* [23], sequenced a male water buffalo animal using the Illumina Genome Analyzer II with a paired end of 101 bp. A full run generated more than 230 million reads, which resulted in approximately 46 Gb of high quality sequences. In this pilot study, Jiang *et al.,* [23] tested 210 pairs of water buffalo sequences for their bovine orthologs using a cross species mega BLAST approach developed at NCBI. Among these 210 pairs of sequences, only 7 pairs (3.3%) hit absolutely nothing in the bovine genome. One hundred twenty pairs of sequences (57%) had sole unique hits with one or both ends. For the remaining 83 pairs that had multiple hits in the bovine genome, 31 unique hits can be identified manually based on the aligned length and sequence similarity to the bovine orthologs. The alignment size between both species ranged from 42 -122 bp, but with 93% alignments having more than 85 bp in length. This pilot study provides initial evidence that de novo water buffalo genome sequences can be comparatively assembled based on the cattle genome assembly [23].

The advent and widespread availability of next-generation sequencing instruments has ushered in an era in which DNA sequencing will become a more universal readout for an increasingly wide variety of front-end assays. However, more applications of next-generation sequencing, beyond those covered here, are yet to come. Practical advantages Animal breeding by DNA sequencing technology: Advances in genomic technology in recent years were driven primarily by human DNA sequencing and genotyping projects. The human sequence was completed in 2001, the cattle sequence in 2004. Design of the cattle SNP chip required obtaining equally spaced SNP that are highly polymorphic across many dairy and beef breeds [24] Breeders now use thousands of genetic markers to select and improve animals. Previously only phenotypes and pedigrees were used in selection, but performance and parentage information was collected, stored, and evaluated affordably and routinely for many traits and many millions of animals. Genetic markers had limited use during the century after Mendel's principles of genetic inheritance were rediscovered because few major QTL were identified and because marker genotypes were expensive to obtain before 2008. Genomic evaluations implemented in the last two years for dairy cattle have greatly improved reliability of selection, especially for younger animals, by using many markers to trace the inheritance of many QTL with small effects. More genetic markers can increase both reliability and cost of genomic selection. Genotypes for 50,000 markers now cost <US$200 per animal for cattle, pigs, chickens, and sheep. Lower cost chips containing fewer (2,900) markers and higher cost chips with more (777,000) markers are already available for cattle, and additional genotyping tools will become available for cattle and other species [25]. Livestock selection has used estimated breeding values (EBV) based on phenotypic data and pedigree records for more than 40 years. More recently, advances in molecular genetic techniques, in particular DNA sequencing, have led to the discovery of regions of the genome that influence traits in livestock. However utilizing both sources of data in genetic evaluation schemes, such as BREEDPLAN, has been a challenge due to the heterogeneity of data sources, the multi-trait nature of the evaluations, and unknown effects of the marker information on all traits in the evaluation. The SmartGene for Beef project identified significant effects of the Catapult Genetics GeneSTAR tenderness markers on meat tenderness as recorded by the objective measure of shear force These results have been used to further develop methods for combining EBVs (i.e. phenotypic and pedigree data) and gene marker information into a single marker-assisted EBV called an EBVM. Flight time is an objective measure of an animal's temperament which has been shown to be heritable and moderately genetically correlated with SF, thus representing a potential genetic indicator trait for meat tenderness [26].

Farm animals are quite valuable as resources, often notable as models for pathology and physiology studies. The reproductive physiology of farm animals is more similar to humans than that of rodents because farm animals have longer gestation and pre-pubertal periods than mice. Specific farm animal physiology, such as the digestive system of the pig is similar to that of humans. These attributes of farm animals reveal that they are an unparalleled resource for research replicating human physiological function. For decades, breeders have altered the genomes of farm animals by first searching for desired phenotypic traits and then selecting for superior animals to continue their lineage into the next generation. This genomic work has already facilitated a reduction in genetic disorders in farm animals, as many disease carriers are removed from breeding populations by purifying selection. By studying diverse phenotype over time, researchers can now monitor mutations that occur as wild species become domesticated [27].

Genome sequencing in farm and other animals has advanced significantly in recent years At there are 2,509,850 cattle, 3,237,358 pigs, 2,195,532 chicken, 6,259,791 sheep, 470,489 horse, 2,886,083 cat and 2,599,789 dog sequences available in the GenBank nucleotide databases (http://www.ncbi.nlm.nih.gov). Whole genome sequencing has been completed in cattle, horse, chicken and dog and sequencing of the porcine genome is almost completed [28]. Present, more than 400 loci were mapped in river buffalo, noticeably extending the physical map of this species. Iannuzzi *et al*., [29] and Peructti *et al*., [30] cytogenetically mapped about 300 loci, most of them are homologs mapped in other species in turn contributed significantly to comparative mapping. A total of 66,935 nucleotide sequences for the water buffalo have been deposited in the GenBank database and are mainly 64,212 whole genome shotgun sequences, while the rest includes 974 mitochondrial genomic sequences and 1,748 nuclear gene/genomic DNA sequences.

India, after gaining laurels in sequencing rice genome in a global partnership, has now decided to sequence buffalo genome. A multi-crore project has been cleared and the Karnal-based National Bureau of Animal Genetic Resources (NBAGR) and the Hisar-based Central Institute for research on Buffaloes (CIRB) would work on a network mode for the project, Buffalo genome sequencing would pave the way for improved breeding of the animal having higher lactation. Buffalo genome sequencing will helps to develop buffaloes having more meat and lactation. Using Roche 454 GS-FLX Titanium technology, a group of researchers at the Anand Agricultural University, India completed <1x genome sequencing of water buffalo in 2009 with a total of 64,212 sequences submitted to the GenBank database (ACZF01000001-CZF01064212). The submission information from NCBI shows that a mature buffalo bull of Jaffrabadi breed was used to provide DNA materials for the project.

Buffmap database published by Institut National De La Recherche Agronomique, Laboratoire de Génétique Biochimique et de Cytogénétique de Jouy-en-Josas, Offers genome data on the buffalo. Includes gene listing, sequences, submission data, and related links. INRA buffaloe map data base acknowledged 136 loci in different chromosomes, identified 67 genes, 66 microsatellite markers.

## CONCLUSIONS

Sequencing the genome of the buffaloes enables scientists to more accurately identify the genetic markers that are responsible for economically important traits such as milk yield, milk fat and protein. Producers can use that information to breed healthier dairy animals that produce more and higher quality milk as well as beef animals. Consumers will benefit from more cost-effective and healthier products on store shelves. Understanding what genes contribute to specific buffalo animal traits will also have spinoff applications related to other fields like human health and disease. The animals used in the genome project have had a high impact on the breeding and commercial sectors in dairy and beef. This sequencing is significant to the dairy industry because the bull's genes are likely to make an important contribution to the genetic makeup of future generations. There will also be benefits for the beef industry. Better knowledge of the genetic variation across the breeds will, through better breeding decisions, improve production efficiency, product quality and animal health, and reduce the environmental footprint of milk and beef production.

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