DOI: 10.53992/njns.v10i2.261 NUST Journal of Natural Sciences, Vol. 10, Issue 2, 2025

Research Article

Comparative genetic profiling of BMP4 gene in three native goat breeds from Khairpur district Sindh, Pakistan

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Abstract

Bone morphogenetic protein 4 (BMP4) is a protein found in goats, cattle, and humans, encoded by the BMP4 gene. BMP4 is found on chromosome 10q in goats. BMP4 gene is the subfamily of the superfamily transforming growth factor beta. It is an evolutionary conserved member of BMPs family. BMP-4 is a protein-coding gene that is concerned with the connective and soft tissues of the body structure. It plays main role in goats, cattle, humans and other animal's bodies for maintaining the body activities such as cell proliferation, differentiation, apoptosis, and migrations. The present study aimed to investigate mutational variations in the same genomic region of three indigenous goat breeds. Genomic DNA was extracted from the blood sample of three different indigenous goat breeds including Bari, Kamori, and Baddi. The samples were stored (4°C) at Molecular Genetic Lab. Department of Zoology for further process. The targeted region of BMP-4 gene was amplified by the specific set of primers. The amplified products were sequenced by the ABI Genetic Analyzer 3500 and sequencing data was analyzed by Bio Edit v 7.2 and blasting was performed on ensemble.org. Results revealed (19) Mutations in Bari Goat Breed including (16) Missense mutations, (2) Nonsense mutations, (1) Deletion mutation with the help of PCR-Gel electrophoresis and DNA sequencing. Similarly (8) Missense mutations were identified in Kamori goat breed. The results of Baddi goat breed showed (4) Missense mutations. It was concluded that essential mutations in the Bari goat breed may influence characteristics such as meat and milk production. Obtained results revealed a great heterogeneity in genetic makeup of Bari goat and hence could be used in marker assisted selection of Bari goat breed than the Kamori and Baddi goat breed.

Keywords: DNA extraction, Electrophoresis, PCR, Polymorphism, Mutations.

Article History: **Received**: 15 Feb 2025, **Revised**: 24 Jul 2025, **Accepted**: 25 Jul 2025, **Published:** 19 Sep 2025.

Introduction

Single nucleotide polymorphisms (SNPs) continue to be one of the most common genetic mutations in the genomes of organisms and it is now understood that the methods used to classify SNPs have a significant impact on the results of population genetics. The SNPs identified in the Bovine HapMap project (BHP) were expected to influence detection accuracy due to the use of a taurine reference sequence. It is because the taurine reference sequence was used to compare SNPs when

they were first being characterized. SNPs are the starting to be used in genetic studies of traits that are relevant to biology and commercially relevant traits, it is important to analyze the effects of systematic distortion in them. BMP-4 means Bone Morphogenetic Protein is an evolutionary conserved associate of the BMP's family, it belongs to superfamily of transforming growth factor beta [1]. It is detected in the eyes, ventral peripheral zone, cardiac and circulatory fluids, and otic vesicles during the early embryonic development. Some other BMP members performed a role in the formation of bones and cartilages, especially limb and tooth development. Target genes are also involved in the development of adipose tissues [2]. BMP-4 is a protein (polypeptide) which is encoded in humans in the form of BMP4 gene [3]. In human BMP-4 gene remains on chromosome number 14q22-q23. Associated with the superfamily that contain huge members of human and animal growth and development factors. The potentially demineralized extract bone involved in stimulating endochondral osteogenesis in vivo extra skeletal site. BMP4 genes are considered a Master gene that controls many aspects of development in all species, including palates production, sight development and various others. The encoded protein also performed main part in pathophysiology with a number of cardiovascular illnesses and malignancies in humans [4]. BMPs are the members of TGF-beta as secreting signal molecules. About 20 members of BMPs are found in mammals. All these members express throughout limbs development, initial limb modeling and development of skeletogenic. But BMP4 play main role in human as well as animal's body for maintaining many body activities such as cell propagation, diversity, and caspase-mediated cell death, stem cells including embryonic, hematopoietic, mesenchyme, neural stem cells. Target gene also performed main role in stem cell therapy [5]. During the mammalian developmental process, the deregulation of the BMP signaling system can have serious implications. Mutation in BMP receptors are linked with different vascular diseases like pulmonic artery hypertension (PAH), skeletal anomalies such as brachydactylic, and polyp production in colon [6]. Wozney purified and cloned the BMP4 for the first time in 1988 [7]. Apart from its high phenotypic expression and crucial role in embryogenesis and their development, it was found on chromosome number 14 and is substantially conserved across humans and mice [8]. Many adult tissues rely on BMP4 for their preservation and performance [9]. By assuming a beige or brown phenotype during development, it promotes metabolically helpful in hyperplastic adipose tissue expansion and enhances intravenous adipose cell oxidative capacity in a paracrine way [10].

Material and Methods

Collection and transport of blood samples

Approximately, thirty samples of blood were collected from three different indigenous goat breeds named as Kamori goat breed, Baddi goat breed, Bari goat breed from the animal's hospital of district Khairpur Mir's by applying careful sample techniques. The breeds having an age of 1 to 2 years old. About (5ml) of sterilized disposable syringe had been used for taking the blood from goat's jugular vein of each animal. Subsequently, blood was transferred into EDTA tubes (250 µL, 0.5M) of the EDTA (ethylenediamine tetra acetic acid) tubes that protect the blood samples from anticoagulation, after that EDTA tubes transported into cool box which contain dry ice, then preserved the blood samples into the fridge at -4°C for further process of DNA extraction at Molecular Genetic lab in Zoology Department of Shah Abdul Latif University Khairpur, Sindh Pakistan.

DNA extraction

DNA extraction has been done through collected samples of whole blood, and it has been proceeded by using MQ Blood Genomic DNA Extraction Kit (MOLEQULE-ON® (MQ-Kit) followed by producer's protocol in which DNA extraction kit contains components, materials and protocols.

DNA quantification and purification by Nano drop

Extracted DNA was used to quantified by using the Nano drop™1000 Spectrophotometer (Thermo Fisher Scientific) at the "Jamil-Ur-Rahman Center for Genome Research at Karachi University, Sindh Pakistan. Quantification of DNA has been done to ensure the presence of DNA into extracted samples from blood samples which could be used for further process of amplification in next step. Furthermore, purity of DNA was dignified through the 260/280 nm ratio on absorbance (absorbance calculated by taken 260nm distributed by absorbance at 280nm). Purity of DNA is regarded if it had rating among 1.7-1.8 [11 - 13].

Primers' design and synthesizing

Initially, BMP-4 gene sequences were used to retrieve for the formation of primers designing on the website of National Center for Biotechnology Information (NCBI). Primers (Forward primers and Reverse primers) obtained from particular website and blast was again through NCBI website for checkered specificity. Primers which were handpicked for amplification of PCR and their application commercially synthesized from Bionics Company Islamabad, Pakistan. The lengths of primers designed by NCBI are given in Table 1.

PCR amplification of BMP-4 gene

A reaction of mixture has been prepared for the PCR amplification of BMP-4 gene into PCR tube about (200ul). All reagents have optimization of concentration with total volume of 200ul were transferred into PCR tubes. The reagents included into mixture were genomic DNA template (5ul), absolute red PCR Master Mix (7ul) from MOLEQULE-ON® Company, forward primer (2ul; 10pmol/ul), reverse primer (2ul; 10pmol/ul). The reagents included genomic DNA template (5ul), Absolute red Master Mix of PCR (7ul) from MOLEQULE-ON® Company, PCR grade water or molecular DDH2O (Double Distal water) (4ul). Consequently, specific tubes of PCR were retained in Thermal Cycler Machine (Bio Rad T-100) for Amplification of PCR. PCR reactions were accomplished through already issued procedure defined by [14].

Table 1: Details of primers used for PCR Amplification of BMP-4 gene.

S/No	Name of Primers	Sequences	No. of Bases
1	BMP F1	CTACCGTACTCCCCAGACCC	20
2	BMP R1	GCACTACGGAATGGCTCCTAA	21
3	BMP F2	ACCACGAAGGTCAGTCCCTA	20
4	BMP R2	TCCCCAGCGATCTTGGAAAC	20
5	BMP F3	ACCGAATGCTGATGGTCGTT	20
6	BMP R3	CTCGTCTTCCCACAGCTTCC	20

Gel electrophoresis

Agarose powder (1.5 gram) was added in 100 ml of TBE-buffer. Gently mixed and heated the products for standardized solution. The loading of DNA samples into wells of gel. After solidifying the gel placed the gel into gel electrophoresis unit (gel box). However, in the 1st well 1kb DNA ladder as (2ul) of biomolecules was loaded. Remaining of the other shafts were filled up with 2uL of loading dye and 5uL of DNA template (PCR products) so total volume was about 7uL in each well. Afterwards, 70 Volts of an electric current were applied for 50-60 minutes. Subsequently, PCR products were visualized by using Gel Doc documentation machine (Bio-Rad) having ultra-violet light in it. The bands of intensified DNA samples were proceeded for further applications [8, 12, 13].

Data analysis, purification, sequencing of PCR products

The amplified product of PCR samples was sent to Macrogen, company, Korea for purification, sequencing and data analysis. DNA sequence's data were examined by using of available genome browser Ensemble.org and blast the interrogated alignment of BMP-4 gene of nominated goat varieties by using of alignment sequence tools. By using software that plays important role in the comparison of DNA sequencing data with already store data and provide the new sequence of BMP-4 gene [15].

Quantification of DNA performed by using Nano drop Spectrophotometer that measurement the revealed of DNA samples to identify the quantity ranged from 7.22-29.31ng/ul as shown in Table 2 and quantity of DNA sufficient for performance of amplification of PCR. It also verified isolated DNA had absolute purity range subsequently A260/A280 proportion in assortment of 1.09-1.81.

Table **2:** Shown the obtained results of DNA samples through nano drop spectrophotometer measurement**.**

Sample ID	Goat Breeds	DNA Quality (ng/ul)	DNA Purity (260/280nm)
S1	Kamori	7.221 ug/ml	1.77
S2	Kamori	11.357ug/ml	1.14
S3	Kamori	7.866ug/ml	1.43
S4	Bari	9.080ug/ml	1.81
S5	Bari	20.878ug/ml	1.78
S6	Bari	12.309ug/ml	1.70
S7	Baddi	29.314ug/ml	1.61
S8	Baddi	11.063ug/ml	1.09
S9	Baddi	7.403ug/ml	1.63

Visualization of PCR product by gel electrophoresis

Gel electrophoresis procedure played a vital role to visualized amplified PCR product of BMP-4 gene from 9 samples of selected goat breeds and confirmed the size of amplified products as about 1000bp.DNA ladder's bands produced into 1st well of gel and samples started from 2nd well with different ID samples of such as K1, K2, K3, B1, B2, B3, Bd1, Bd2, Bd3 with respect to K= Kamori goat breed, B= Bari goat breed, Bd= Baddi goat breed) to onwards as showed into Figure 1.

Sequencing and identification of mutations

BMP-4 gene's nucleotide sequence from each of goat breeds samples had been analyzed by using Macrogen Company of Korea. Sequence of each nucleotide have been evaluated for the existence of mutations by using software that indicates the alignment sequence tool or blast tool. Consequently, graphs of alignment also display the variation (mutations) that were

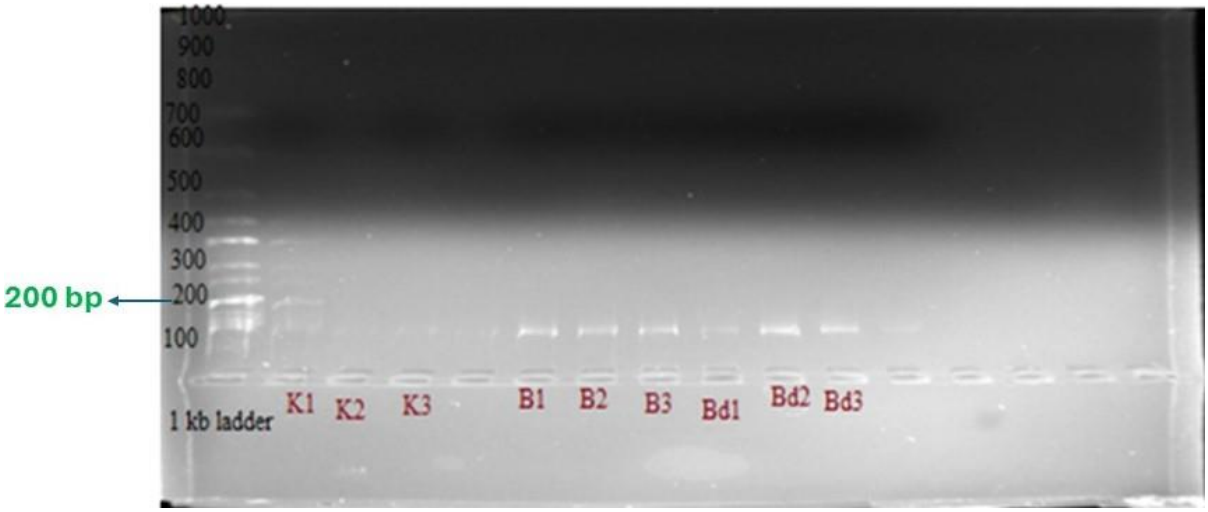

Figure 1. Shown the amplified PCR product of BMP-4 gene in indigenous goats with different ID samples.

found. In Bari goat breed, several point mutations were identified in which alteration occurred on the basis of the genetic codon that leading to changes in base pair in Figure 2. While, in Figure 3 the Kamori goat breed also exhibited mutations that resulted in alterations within the genetic codons. In the Baddi goat breed, only a few mutations were detected which contributed to genetic variations that may also be associated with observable morphological changes in breed as shown in Figure 4.

About 33 mutations were recognized in different Exon 1 region of BMP-4 gene in three indigenous goat breeds. While the mutations based on the variant into genetic codon. In Bari goats breed about 19 different types of mutations while in Baddi goat breed 4 types of mutations and in Kamori breed 8 mutations were found.

BLAST/BLAT Alignment

BLAST/BLAT type	BLASTN
Query location	H220809-003_E17_S5B2_BMPF2.ab1 115 to 317 (+)
Database location	10 40113212 to 40113415 (-)
Genomic location	10 40113212 to 40113415 (-)
Alignment score	219
E-value	1.19e-54
Alignment length	204
Percentage identity	88.725

Markup loaded

```
H220809-003_E17_S5B2_BMPF2.ab1:     115 TTCAGATCGGAGTATGAG-CCCCTATTGGATATACCTGACAAACACACAGCTGTAATATT
                                      1 ||||||||||| ||  || ||||||||| ||| ||||||||||||||||||||||||||||
                                10:40113415 TTCAGATCGGATTACAAGGCCCCTATTGAATAAACCTGACAAACACACAGCTGTAATATT

H220809-003_E17_S5B2_BMPF2.ab1:     175 AAATTCAGTAGGTGCTTTGGAAAAAAAAAAAAAAGGCGAAAAACCTGGCATAAACGGCTTT
                                     61 |||||||||||||||||||||||||||||||||| ||||  |||||||||||||||| ||||||
                                10:40113355 AAATTCAGTAGGTGCTTTGGAAAAAAAAAAAATAGGCAGAAAACCTGGCATAAAAGGCTTT

H220809-003_E17_S5B2_BMPF2.ab1:     235 CATATAGCAAAACCCCGCCGGGGGGGCTAACCCTCTCCCGAGTGGGGTCATTCCCTTCTC
                                    121 |||||||||||| | | |||  |||||||||||| |  | |||||| ||||||||||||||
                                10:40113295 GATATAGCAAAAGCACACCGCAGGGGCTAACCCACGTCTGAGTGGTGTCATTCCCTTCTC

H220809-003_E17_S5B2_BMPF2.ab1:     295 ACTGCCCCCTCCCTTCCCTTCTGT
                                    181  ||| ||||||||||||||||||||
                                10:40113235 GCTGACCCCTCCCTTCCCTTCTGT
```

Figure 2: Presented the Point mutations in Bari goat breed.

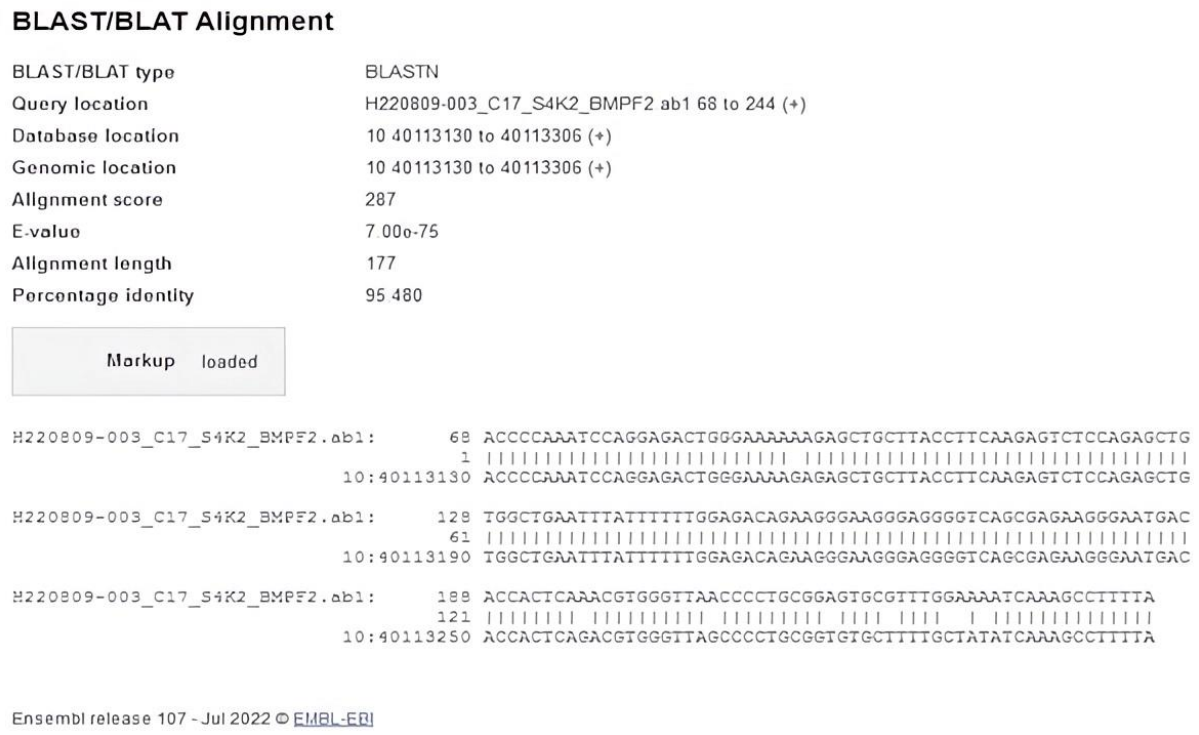

Figure 3: Shown the different types of mutations in Kamori goat breed.

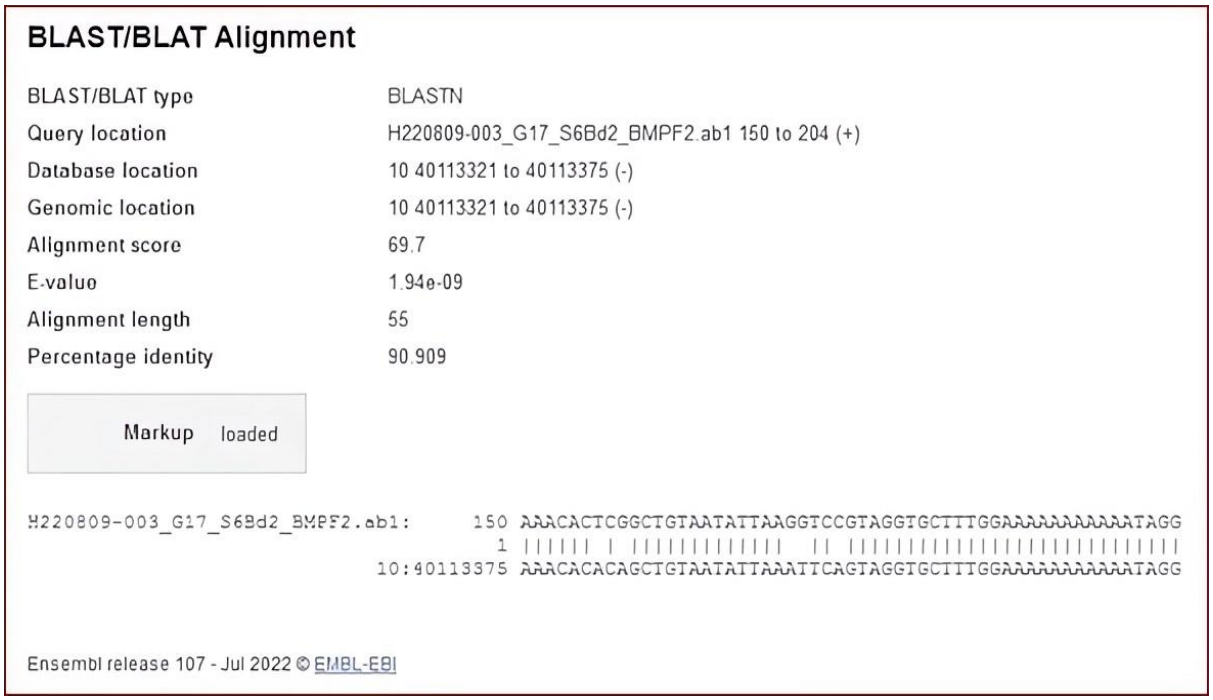

Figure 4: Shows the different types of mutations in the Baddi goat breed.

Discussion

High milk production is one of a dairy farmer's top concerns. In addition to this milk composition features a new breeding goal has been established to meet the demands of a better diet [16]. As a result, choosing farm animals with better dairy implementation is crucial for dual breeders and consumers. DNA markers are important in animal breeding programs. Genetic mapping and genes of all animal and plants generally have been transformed by DNA markers. SNPs also called "snips"

are the extremely predominant variety of genetic variations found in mammals.

SNPs stands for single nucleotide polymorphism. The majority of the time, these modifications are found between genes. SNPs are recognized as biological markers that help researchers find genes associated with disease or genes involved in the expression of characteristics. SNPs that are located inside or close to a gene's regulatory domain may affect how genes function more directly. These mutations (SNPs) may cause sickness or occasionally have good effects such as increasing the output of milk or beef [17].

Researchers have previously looked into the relationship between SNPs in the BMP4 gene and the quantity and quality of milk globally [18]. Therefore, the goat of this study was to find unique SNPs in the BMP4 gene of three different indigenous goat breeds namely as (Kamori, Bari, Baddi) found in the district Khairpur, Sindh Pakistan. The work had been on title BMP4 gene and their relationship between these breed's marker traits.

PCR-Gel electrophoresis followed by DNA sequence techniques revealed that about (31) point mutations were identified in all three different indigenous goat breeds followed by missense mutation (28), (2) nonsense mutations, (1) deletion mutation. Based on genetic codon maximum number of mutations were noted (19) in Bari goat breed followed by (8) Kamori goat breed and (4) mutations were classified into Baddi goat breed. This suggests that SNPs could affect positive or negative depending upon production of milk and quality in Bari goat breed. In future time period it may validate phenotypically.

The high number of missense mutations suggests significant potential impact on meat and milk production traits and could have a substantial impact on meat quantities and yielding of milk of that goat breed, meanwhile changed genomic codon produced alteration of dispensable amino acid into indispensable amino acid at numerous locations [19]. These results agreed with the research of [20] and disagreed with study conducted by [21].

In this study there were not any kind of silent mutations found because it would not be effective on the qualities and quantities of milk, subsequently, the phenotypical gene expression would not be affected because of the amino acid code by that gene endure similar. Non-sense mutations were found only 2 times then it remains good mark, while its negative consequence is the termination codon (stop codon) partly produced protein.

Deletion mutation was found in only Bari goat breed. The type of change would have harmful effect on milk attribute then the mutated codons would not be coded for any amino acid, shortening the structure of protein and reduction of milk characteristics and quantities.

If any kind of mutation occurred in DNA sequences are less than 1% considered mutation and if it is greater than 1% than called SNPs. Results of that research specified that one sample of Bari goat, two samples of Kamori goat and one sample of Baddi goat breeds showed the SNPs. While none of them showed just type of mutations. From the result of Bari goat breed performed to be healthier breed for variety of admixture to progress milk and meat qualities and quantities. Although, minimum types of mutations detected in Kamori as well as Baddi goat breeds recommend that varieties are resistant to severe ecological situations hence, the characteristic can also be actual beneficial for admixture of breeds to bear changeable environmental situations.

Conclusion

Present study revealed that total of (31)

mutations in BMP-4 gene were found in all three different indigenous goat breeds such as Bari, Kamori, and Baddi goat breeds. Mutations were identified by the help of PCR sequencing technique based on Genetic codon, these mutations were classified and explained. In Bari goat breed (16) Missense mutation, (2) Nonsense mutation, (1) Deletion mutation was found. Identification of missense mutation is concerned with coding for various amino acids. In Kamori goat breed a total of (8) Missense mutations were recognized. In Baddi goat breed about (4) Missense mutations were classified. In conclusion, our result is the assortment in genomic makeup that could be useful for breed admixture and possibly better for meat and milk characteristics.

Acknowledgement

The authors would like to greatly appreciate the Department of Zoology, Shah Abdul Latif University Khairpur Mir's Sindh, Pakistan for providing the resources and assistance required to carry out this research work.

Conflicts

The author declares that they have no conflict of interests.

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