

MOLECULAR-GENETIC IDENTIFICATION AND ALLELIC PROFILING OF THE CSN2 GENE IN BUSHUYEV CATTLE

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Abstract

This study aims to perform the molecular-genetic characterization of the β-casein (CSN2) gene, a critical quantitative trait locus (QTL) associated with milk protein composition and technological properties, in the indigenous Bushuyev cattle breed. The CSN2 gene plays a crucial role in encoding β-casein, influencing milk yield, nutritional value, and allergenicity profiles. Genomic DNA (gDNA) was extracted from biological samples of Bushuyev cattle using standard extraction protocols. Polymerase Chain Reaction (PCR) conditions were optimized using locus-specific primers to amplify the targeted exon region of the CSN2 gene. The resulting amplicons were identified and validated via agarose gel electrophoresis based on expected base pair size. The molecular data obtained provides a critical scientific baseline for allelic profiling, evaluating genetic diversity within the endangered Bushuyev breed, and implementing future marker-assisted selection (MAS) strategies aimed at improving milk quality traits.

Keywords: Bushuyev cattle, CSN2 gene, β-casein, PCR amplification, agarose gel electrophoresis, molecular identification, marker-assisted selection.

Introduction In modern livestock genetics, the molecular characterization of indigenous cattle breeds represents a strategic imperative for conserving genetic diversity and identifying quantitative trait loci (QTLs) linked to economically

important traits. Integrating molecular markers into classical breeding programs facilitates precise genotype-based selection. Milk protein fractions are predominantly governed by the casein gene cluster (CSN1S1, CSN1S2, CSN2, and CSN3), encoding αS1, αS2, β, and κ-caseins, respectively. Among these, the beta-casein gene (CSN2) plays a pivotal role in dictating milk protein composition, technological cheese-making properties, and nutritional/digestibility profiles. The Bushuyev breed represents a unique local genetic pool in Uzbekistan, highly adapted to arid environmental conditions. Therefore, establishing the molecular profiling of the CSN2 gene in Bushuyev cattle holds significant scientific and practical value.

Objective This study aimed to perform the locus-specific PCR identification of the targeted CSN2 gene fragment in Bushuyev cattle, thereby establishing a robust molecular platform for subsequent allelic profiling and polymorphism analysis.

Materials and Methods Biological samples were harvested from a representative population of Bushuyev cattle. Genomic DNA (gDNA) was extracted following standard molecular protocols, with DNA concentration and purity verified spectrophotometrically/fluorometrically.

Target regions of the CSN2 gene were amplified via Polymerase Chain Reaction (PCR) using locus-specific primers. The PCR reaction mixture contained gDNA matrix, forward and reverse primers, dNTPs, PCR buffer, $MgCl_2$, and thermostable Taq DNA Polymerase. Amplification proceeded under optimized thermocycling parameters encompassing initial denaturation, cycle denaturation, primer annealing, and strand elongation. The resulting amplicons were resolved using 1.5-2.0% agarose gel electrophoresis and visualized under UV transillumination to confirm the expected molecular size relative to a DNA size standard.

Results and Discussion

Electrophoretic analysis confirmed the successful amplification of the target CSN2 gene fragment of the expected base-pair size from the Bushuyev gDNA samples.

The presence of single, sharp electrophoretic bands corroborated the high specificity of the PCR assay and validated the locus presence at the molecular level.

These findings establish a fundamental molecular baseline for characterizing the genetic structure of Bushuyev cattle regarding milk quality traits. The next phase of this research involves Sanger sequencing of the amplified fragments followed by bioinformatic alignment (BLAST, MEGA) against NCBI RefSeq datasets.

Further investigation will focus on detecting Single Nucleotide Polymorphisms (SNPs)-specifically identifying the Pro67His mutation defining A1/A2 beta-casein variants-calculating allelic and genotypic frequencies, and performing association studies with milk yield and composition. Ultimately, this comprehensive approach will advance Marker-Assisted Selection (MAS) protocols and aid in conserving the unique genetic resources of the Bushuyev breed.

Conclusion

Successful Molecular Identification: The target fragment of the β-casein (CSN2) gene-a key quantitative trait locus (QTL) governing the nutritional, technological, and immunological properties of milk protein-was successfully identified and validated in the indigenous Bushuyev cattle breed using locus-specific PCR amplification and agarose gel electrophoresis.

Baseline for Genetic Profiling: The empirical data obtained serve as a foundational molecular baseline for mapping milk protein gene clusters, evaluating genetic diversity, and establishing the overall genetic architecture of the unique Bushuyev breed at the DNA level.

Future Perspectives & Genomic Approaches: Future research will prioritize Sanger sequencing of the amplified CSN2 amplicons, detection of Single Nucleotide Polymorphisms (SNPs, specifically the Pro67His mutation defining A1/A2 beta-casein allelic variants), evaluation of allele and genotype frequencies under Hardy-Weinberg

equilibrium, and performing genotype-phenotype association studies with milk yield and physicochemical parameters.

Selectional & Conservation Value: These findings provide a solid scientific foundation for implementing Marker-Assisted Selection (MAS), optimizing molecular-genetic monitoring, and supporting sustainable conservation strategies for the endangered Bushuyev cattle genetic resources.

References

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