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ANIMAL GENETICS

IDENTIFICATION OF *BOS TAURUS* AND *BOS GRUNNIENS* BASED ON SNP

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Abstract. The article examined samples of domestic yak and three breeds of cattle to assess the differentiating potential of the polymorphic variants Chr4:68609356G>T (*JAZF1* gene), Chr14:35695388G>T (*SLCO5A1* gene), and Chr19:63181970C>G (*CEP112* gene). The high accuracy (99.67%), specificity (100%), and sensitivity (100%) of the proposed test model consisting of these three polymorphisms for the identification of domestic yaks and cattle were confirmed. A fast and simple identification method has been developed based on this model using competitive allele-specific PCR (KASP) technology, which can significantly reduce the time and financial costs of molecular genetic analysis, as well as reduce the risk of cross-contamination of samples.

Keywords: *Bos taurus, Bos grunniens, single nucleotide polymorphism, identification, kompetitive allele specific PCR (KASP), in silico genotyping*

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INTRODUCTION

Domestic yak (*Bos grunniens*) is a domesticated form of the wild yak (*Bos mutus*). These animals are known for their ability to survive in extreme conditions, such as low temperatures, hypoxia, and food scarcity, making them an important subject for studying molecular genetic mechanisms of survival and adaptation to high-altitude regions. The long and complex history of yak domestication, as well as the physical and geographical features of its habitat, significantly influence the genetic diversity of modern populations of this animal. Currently, there is active research on *Bos grunniens* worldwide, including studies on population differences, genetic diversity, genogeography, and phylogenetic relationships [1].

In the mountains of Kyrgyzstan, yak breeding is actively developing, while cattle breeding predominates at lower and medium altitudes. Unlike the latter, yaks prefer to use low-growing pasture forage, which indicates their unique adaptive capabilities. The domestic yak has a limited geographical distribution in the highland regions of Central Asia, which is due to its high adaptability to life in mountainous and plateau conditions, similar to its wild relative, the Tibetan yak.

Assessment of the genetic diversity of yaks and cattle breeds raised in Kyrgyzstan, in the context of possible hybridization between species, was conducted in our previous study [2]. Additionally, in study [3], yak hybrids with cattle were investigated, and as a result, a yak-specific pattern of eight ISSR fragments was identified in populations of yaks and first-generation hybrids. Also, based on microsatellite analysis, the allele pool of yaks and their hybrids with *Bos taurus* was previously studied, which showed high genetic diversity for first-generation hybrids compared to the original species [4].

Identification of domestic yak and cattle is important for assessing possible hybridization between these

species. This task is relevant for both breeders and forensic experts, especially in conditions of limited availability of reference data and high costs of STR loci analysis. Assessing the frequency of species-specific SNPs (Single Nucleotide Polymorphism) is also of interest from the perspective of evolutionary change dynamics in domestic animal species compared to their wild relatives, which may be related to artificial selection conducted by humans.

The purpose of this study is to use bioinformatics methods to identify SNPs with high differentiation potential, necessary for identifying sample belonging to both the biological species *Bos taurus* and *Bos grunniens*. Based on the analysis conducted, we propose to develop a test system that includes several SNPs for identification of domestic yak and cattle representatives.

MATERIALS AND METHODS

Biological samples. For molecular genetic research, blood samples were collected from 56 domestic yaks (*Bos grunniens*) from the highland region of Kalmak-Ashuu (Kochkor district, Naryn region, Kyrgyz Republic). This group is designated as YAK. Blood samples were also taken from 146 cows (*Bos taurus*) of three breeds: Aberdeen Angus (n = 45, ABR group), Holstein(n = 51, HOL group), and Alatau (n = 50, ALA group). The collection of these samples formed the COW sample. The biological material was collected by staff of experimental farms and delivered to the laboratory for molecular genetic analysis in Vacutainer tubes (BD Vacutainer® Sodium Citrate Tubes), each of which was labeled with the animal's description (species and breed, age, sex).

DNA extraction. Blood samples were stored at −20°C for 1−2 months or at −80°C for long-term storage. DNA was extracted using the Blood-Animal-Plant DNA Preparation Kit (Jena Bioscience, Germany) according to the manufacturer's instructions. Quantitative assessment of the isolated DNA was performed using NanoDrop 1000 (Thermo Fisher Scientific, USA). The average DNA concentration was 72.4±23.3 ng/μl, with a 260/280 ratio of 1.89±0.09.

KASP. Genotyping for SNPs (Bos_taurus_UMD_3.1.1 genome version, GCF_000003055.6) Chr4:68609356G>T (*JAZF1*), Chr14:35695388G>T (*SLCO5A1*), Chr19:63181970C>G (*CEP112)* was performed using technology based on competitive allele-specific PCR (KASP, Kompetitive allele specific PCR). Genotyping was carried out using KASP Assay mix (KASP by Design, KBD) and KASP Master mix (LGC Biosearch Technologies, UK; Maxim Medical, LLC, Russia) in a volume of 10 μl in a QuantStudio™ 5 Real-Time PCR System (Thermo FS, USA) according to the manufacturer's recommendations. Fig. 1 shows 2D plots of allelic discrimination for SNPs Chr4:68609356G>T (*JAZF1*), Chr14:35695388G>T (*SLCO5A1*), Chr19:63181970C>G (*CEP112),* used in tests for the identification of domestic yaks and cattle.

In silico genotyping. For *in silico* genotyping, animal genomes available in the NCBI database were used, converted to *.fasta format using the SRA-Toolkit package version 2.11. Original GENIS software developed in Python version 3.10 was used for genotype determination. Methodology details are described in paper [5].

Within the framework of the study, genotyping was conducted for 316 domestic yak individuals and 385 cattle individuals. Thus, 701 sequenced genomes were involved in the bioinformatic analysis, the files for which are located in the Sequence Read Archive (SRA) [6]: PRJNA74739 (2012, People's Republic of China [PRC]), PRJNA217895 (2013, PRC), PRJNA285834 (2015, PRC), PRJEB18113 (2016, Switzerland), PRJNA508864 (2018, PRC), PRJNA431934 (2018, Australia), PRJNA531398 (2019, PRC), PRJNA762180 (2021, United Kingdom), PRJNA766811 (2021, PRC), PRJNA842787 (2022, PRC), PRJNA899924 (2022, PRC), PRJNA950586 (2023, PRC).

Statistical data analysis. To evaluate the potential of SNPs as identification markers, ROC analysis (Receiver Operating Characteristic analysis) was used in SPSS version 20.0. The SNP was considered a highly effective marker provided that the lower bound of the asymptotic 95% confidence interval (CI) for the area under the curve (AUC) parameter exceeded 0.8.

A comprehensive assessment of the differentiating potential for a set of SNPs was conducted using the MDR v. 3.0.2 program [7]. The contribution of a specific genotype was determined by the magnitude of entropy H (expressed as a percentage). At H = 100%, the genotype is capable of unambiguously differentiating which group the sample belongs to. In the MDR program, the following highly conservative settings were used to determine the optimal differentiation model: attribute count range – from 1 to n (where n is the number of variables in the model); cross-validation count – 100; track top models – 1,000; search method configuration – exhaustive; ambiguous cell analysis – Fisher's exact test; ambiguous cell assignment – unclassified. The correctness of the model was assessed by the adjusted Balanced Accuracy value. The probability of assigning a sample to one of the

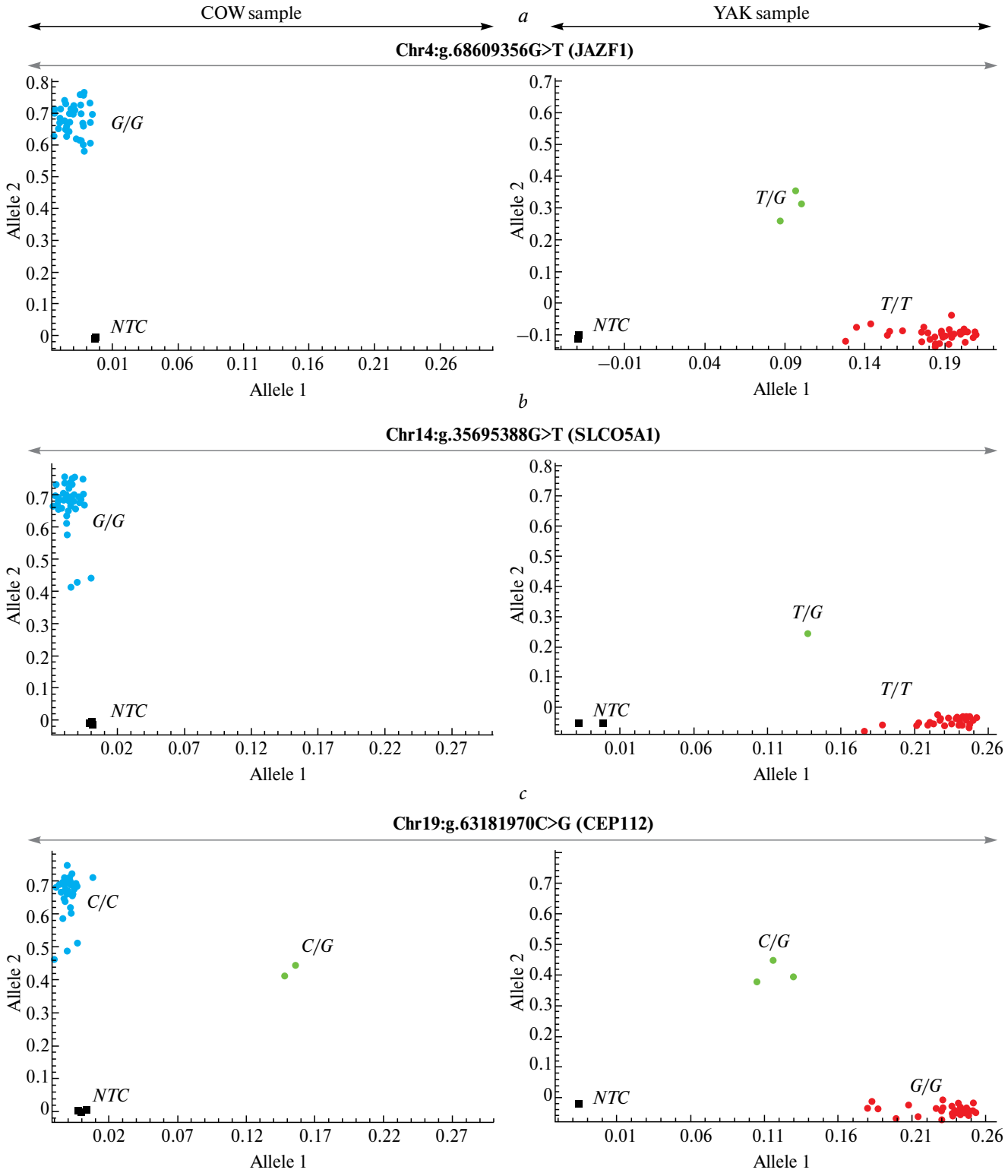

Fig. 1. Allelic discrimination plot of KASP results for polymorphisms: *a* – Chr4:68609356G>T (JAZF1), *b* – Chr14:35695388G>T (SLCO5A1), *c* – Chr19:63181970C>G (CEP112)

two groups — YAK or COW — was calculated in SPSS v.20.0 using logistic regression.

RESULTS AND DISCUSSION

The selection of the most informative SNPs included two stages. At the first stage, two test groups were formed with randomly included samples: BG-1 (domestic yak, n = 56) and BT-1 (cattle, n = 60), for which genotypes were determined for 947 SNPs (the list of SNPs is presented in the file "Supplementary materials", Table D-1). The list of potentially informative SNPs included both previously described SNPs [8] and their flanking SNPs from the BovineHD BeadChip from Illumina© [9].

To assess the informativeness of SNPs, the ROC analysis was performed, which allowed us to identify 64 SNPs with the highest values of the AUC (area under ROC curve) parameter — the area limited by the ROC curve and the axis of the proportion of false positive classifications. These markers were selected for further research. In the second, expanded phase, genotypes of these 64 SNPs were determined for an additional 260 animals *Bos grunniens* (group BG-2) and 325 animals *Bos taurus* (BT-2 group). List of selected for genotyping *in silico* animals is presented in Table D-2 (Supplementary Materials). Repeated ROC analysis was performed to refine the SNPs with the highest discriminatory potential for distinguishing between domestic yak and cattle. The obtained results were used to construct ROC curves and evaluate the AUC indicator. According to the results of bioinformatics analysis, the AUC values for the selected SNPs were in the range from 0.899 to 0.999, indicating their high differentiating potential (Supplementary Materials, Table D-3).

To study SNP interactions, the multivariate dimensionality reduction (MDR v.3.0.2) method was used. Based on the data obtained during the SRA bioinformatics analysis, three SNPs with the greatest differentiating potential were selected: Chr4:68609356G>T (*JAZF1*), Chr14:35695388G>T (*SLCO5A1*) and Chr19:63181970C>G (*CEP112*). The H entropy values for these SNPs were 78.19, 77.79, and 77.07% respectively. Next, using KASP technology, genotypes were determined for 202 samples of domestic yak and cattle from Kyrgyzstan. Re-analysis using the MDR method allowed calculation of the entropy value H for each SNP: for Chr4:68609356G>T (*JAZF1*) H = 85.16%, for Chr14:35695388G>T(*SLCO5A1*) H = 85.16%, for Chr19:63181970C>G (*CEP112*) H = 82.76%. Genotyping results are presented in Table D-4 (Supplementary materials).

As a result of ROC analysis, it was established that all three SNPs have the highest differentiating potential for distinguishing domestic yak and cattle. The AUC values for SNPs were: Chr4:68609356G>T (*JAZF1*) AUC = 1.0, 95% CI = [1.0–1.0], $p = 4.17*10^{-28}$; Chr14:35695388G>T (*SLCO5A1*) AUC = 1.0, 95% CI = [1.0–1.0], $p = 4.17*10^{-28}$; Chr19:63181970C>G (*CEP112*) AUC = 1.0, 95% CI = [0.999–1.0], $p = 4.56*10^{-28}$. Allele frequencies also confirmed their significance: G allele for Chr4:68609356G>T in the COW sample was 100%, *G* allele for Chr14:35695388G>T – 100%, *C* allele for Chr19:63181970C>G – 99.32%. In the YAK sample, allele frequencies were as follows: *T* allele for Chr4:68609356G>T – 97.32%, *T* allele for Chr14:35695388G>T – 99.11%, *G* allele for Chr19:63181970C>G – 97.32%.

Thus, three SNPs were included in the test system, a graphical interpretation of the model based on the analysis of 202 samples is presented in Fig. 2*a*. According to the results obtained, the adjusted balanced accuracy of identification of domestic yak and cattle when analyzing 202 samples by SNP Chr4:68609356G>T (*JAZF1*), Chr14:35695388G>T (*SLCO5A1*), and Chr19:63181970C>G (*CEP112*) was 98.87% (model specificity – 100%, sensitivity – 100%). When combining two data arrays – samples from Kyrgyzstan and samples from NCBI-SRA, for which data on three SNPs were available — followed by analysis in MDR, it was determined that the adjusted balanced accuracy of identification of domestic yak and cattle was 99.67% (model specificity – 100%, sensitivity – 100%). The graphical interpretation of the model based on the analysis of 611 samples is presented in Fig. 2*b*.

When assessing the accuracy of assigning a sample to one of two groups: domestic yak or cattle, using logistic regression, the following data were obtained (Supplementary materials, Table D-5):

– in the presence of genotype CC (Chr19: 63181970C>G) /GG (Chr4:68609356G>T) / GG (Chr14:35695388G>T), the probability of assigning the sample to the COW group is 100% (the total prevalence of individuals with these genotypes in the COW group is 94.79%);

– in the presence of genotype CG (Chr19: 63181970C>G) /GG (Chr4:68609356G>T) / GG (Chr14:35695388G>T) or CC (Chr19:63181970C>G) / GG (Chr4:68609356G>T) / TG (Chr14:35695388G>T), the probability of assigning the sample to the COW group is 100% (the total prevalence of individuals with these genotypes in the COW group is 4.22%);

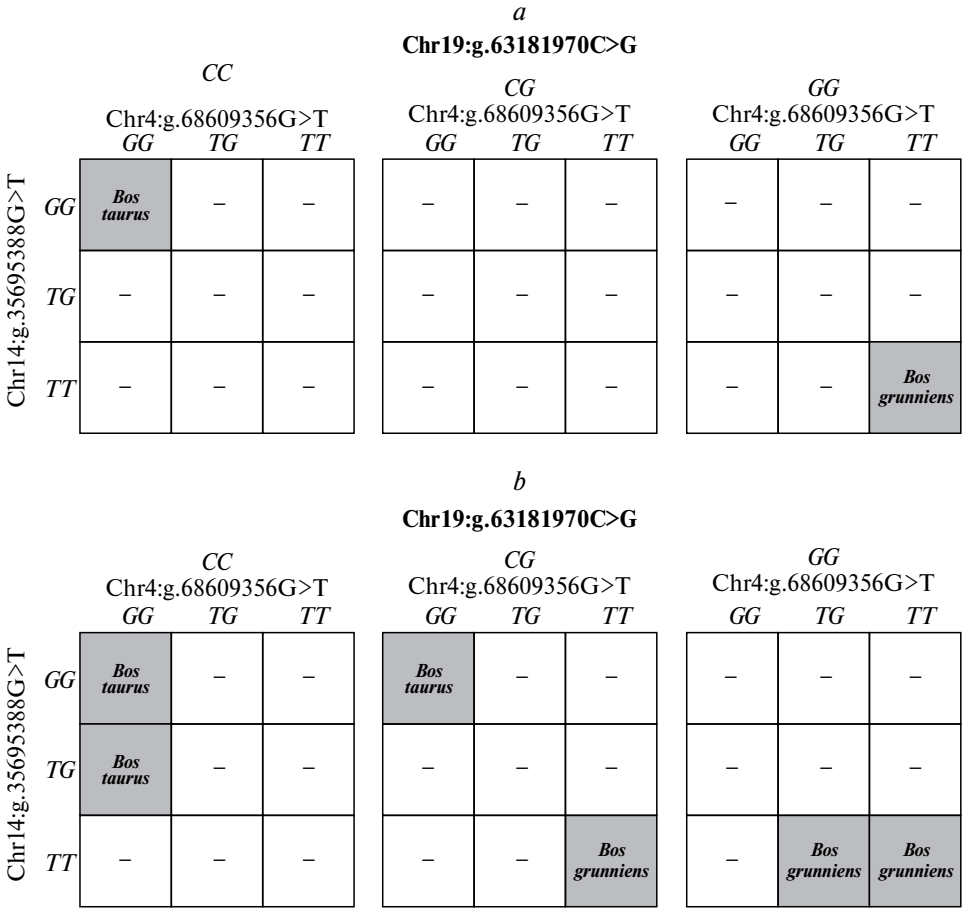

Fig. 2. Graphical representation of the three-polymorphism model for the identification of domestic yak and cattle: *a* – 202 samples (samples from Kyrgyzstan), *b* – 611 samples (samples from Kyrgyzstan and SRA data)

– in the presence of genotype GG (Chr19: 63181970C>G) / TT (Chr4:68609356G>T) / TT (Chr14:35695388G>T), the probability of assigning the sample to the YAK group is 100% (the total prevalence of individuals with these genotypes in the COW group is 90.87%);

– in the presence of genotype CG or GG (Chr19: 63181970C>G) / TT or TG (Chr4:68609356G>T) / TT (Chr14:35695388G>T), the probability of assigning the sample to the YAK group is 100% (the total prevalence of individuals with these genotypes in the COW group is 7.21%);

– in 1.31% of cases, the individual could not be assigned to any cluster with the level of accuracy of 99.0% declared in MDR.

Thus, in the present work, we have shown that the balanced accuracy of differentiation between *Bos taurus* and *Bos grunniens* was more than 99%, which is quite sufficient for solving most population genetics problems. However, in 1.31% of cases (according to the model, Fig. 2*b*), domestic yak and cattle samples cannot be correctly differentiated with a significance level of $p < 0.01$. This is due to the relatively small number of individuals with rare genotypes, such as *CC* (Chr19:63181970C>G) / *TG* (Chr4:68609356G>T) / *GG* (Chr14:35695388G>T) for cattle or *GG* (Chr19:63181970C>G) / *TT* (Chr4:68609356G>T) / *TG* (Chr14:35695388G>T) for domestic yak. If a significance level of $p < 0.05$ is used in MDR analysis, then all 611 samples are correctly differentiated. A similar situation is shown for the differentiation results using logistic regression.

In the future, to increase the model accuracy, especially for forensic applications, it may be recommended to increase the sample size by including new samples with known species identity. Increasing the number of rare genotypes while maintaining their relative frequency in domestic yak and cattle samples will reduce the percentage of samples that cannot be correctly differentiated.

Characterization of the studied genes

The *JAZF1* gene (JAZF zinc finger 1, NCBI Gene ID 616701) is located on chromosome 4 (NC_037331.1 (67992971..68321145), ARS-UCD2.0 (GCF_002263795.3)). The ortholog of this gene in humans encodes a nuclear protein with three C2H2-type zinc fingers and acts as a transcription repressor. This gene is involved in lipid metabolism, suppressing lipogenesis and enhancing lipolysis, which leads to a decrease in lipid accumulation in adipose tissue. *JAZF1* is also involved in glucose homeostasis, improving glucose metabolism and insulin sensitivity. JAZF1 proteins in cattle and humans have similar sizes. In the study by Eusebi G.P. et al., gene expression profiles in the prefrontal cortex of aggressive cattle breeds were examined, including *JAZF1*, which showed decreased expression in the Spanish Lidia breed, known for its agonistic behavior [10]. Jin M. et al. found that *JAZF1* participates in the adaptation of sheep *Ovis aries* to high-altitude conditions [11]. Zhao F. et al. identified *JAZF1* as one of the key genes affecting productive characteristics of cattle [12]. *JAZF1* polymorphism was associated with growth in human [13].

The gene *SLCO5A1* (solute carrier organic anion transporter family member 5A1, NCBI Gene ID 535202) is located on chromosome 14 (NC_037341.1 (33483001..33770931), ARS-UCD2.0 (GCF_002263795.3)). The ortholog of this gene in humans is responsible for the activity of the organic anion transmembrane transporter, independent of sodium. The SLCO5A1 protein is located in intracellular membrane-bound organelles and the plasma membrane. The size of proteins in cattle and humans differs slightly – 846 and 848 amino acids respectively. In an associative analysis of reproductive traits in *Sus scrofa domesicus* and gene networks based on genome-wide studies, it was shown that the gene *SLCO5A1* is associated with transcription factors involved in the functioning of the mammary gland and skeletal muscles [14]. In the work of Gaddis K.L.P. et al., a connection between the gene *SLCO5A1* and susceptibility to ketosis in Jersey cattle was found [15]. In another study, Zeng X. identified a region on chromosome 14 of the *Bos taurus* (Aberdeen Angus) genome with potentially high association with birth weight, where the gene *SLCO5A1* is also located [16].

The gene *CEP112* (centrosomal protein 112, NCBI Gene ID 617266) is located on chromosome 19 (NC_037346.1 (62280043..62600661), ARS-UCD2.0 (GCF_002263795.3)). The ortholog of this gene in humans encodes a protein with a coiled-coil domain, which belongs to the family of effector proteins that control cell division. CEP112 has been identified as a component of the human centrosome. This gene is characterized by alternative splicing, which leads to the formation of multiple transcript variants. In the study by Kim S. et al., it was established that the polymorphism rs42699274 of the *CEP112* gene can serve as a genetic marker in assessing genetic diversity and divergence among Korean cattle breeds [17]. In another study aimed at improving the accuracy of genomic prediction of body conformation traits in Korean Holstein cattle, it was shown that polymorphism of the *CEP112* gene is an important marker for understanding the genetic basis of these traits and can serve as a reliable foundation for predictions based on genomic data [18].

It is known that direct transfer of results obtained for one biological species to another species is incorrect and requires additional research. Nevertheless, the obtained results can be useful for further studying the evolution of the phylogeographic structure of domestic yak populations and cattle breeds in Kyrgyzstan. They can also contribute to the conservation of genetic resources for future breeding research and expand understanding of how animals can adapt to climate change. All three SNPs included in the test system are located in intronic regions of genes and should not affect the functioning of expressed proteins. However, different domestication events, adaptation to various climatic zones, and divergent selection for productive traits have shaped the genomic differences between the studied species.

Previously, we have shown that in the analysis of five STR loci (BM1818, BM1824, BM2113, CSSM66, and ILSTS006), the classification accuracy for *Bos grunniens* was 98.8±3.4%, and for *Bos taurus* – 99.1±1.2% [2]. When using three SNPs (Chr4:68609356G>T, Chr14:35695388G>T, and Chr19:63181970C>G), the classification accuracy was 99.67% with the maximum possible values of specificity and sensitivity. At the same time, samples YAK_107, YAK_117, and YAK_131, for which the accuracy of assignment to their cluster based on STR analysis was 70.3, 75.5, and 72.3% respectively, were unambiguously (100% accuracy) classified as domestic yaks according to SNP analysis.

Thus, in the present study, we proposed a test system for differentiating domestic yak (*Bos grunniens*) and cattle (*Bos taurus*) based on the analysis of three SNPs in the genes *JAZF1*, *SLCO5A1*, and *CEP112*. Using bioinformatics methods, SNPs with high differentiating potential were identified. These polymorphic variants confirmed their effectiveness in practice. The model based on three SNPs (Chr4:68609356G>T in the *JAZF1* gene, Chr14:35695388G>T in the *SLCO5A1* gene, and

Chr19:63181970C>G in the *CEP112* gene) demonstrated high balanced accuracy (at least 99.67%) when analyzing 611 samples. The application of this approach allowed achieving 100% sensitivity and 100% specificity in differentiating the two species. The KASP method used in molecular genetic analysis is characterized by being a single-stage process, which reduces the risk of cross-contamination and decreases labor costs by eliminating restriction and electrophoresis steps. Further expansion of the sample database through genotyping, especially *in silico*, remains a relevant task for improving and applying this test system.

FUNDING

The GENIS software was developed as part of the research project "Bioinformatic approach to whole-genome sequencing data analysis for identifying single nucleotide substitutions capable of differentiating closely related biological species" (BRFFR, 2023–2025, B23-060, Reg. No. 20231076). The collection of biological samples from domestic yak and cattle was carried out within the framework of the government assignment of the Ministry of Education and Science of the Kyrgyz Republic, Contract No. 30-21 dated 02.15.2021 No. 129/1.

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ETHICS DECLARATIONS

The study was approved by the Ethics Committee of the Research Institute of Molecular Biology and Medicine (Bishkek, Kyrgyz Republic), 12.06.2024, protocol No. 6.

STATEMENT OF COMPLIANCE WITH ETHICS REQUIREMENTS

All applicable international, national and/or institutional principles for the care and use of animals were followed.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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