Research article

Evaluation of DNA extracted from hair follicles of four types of Kazakh horses (Zhabe, Naiman, South Altai and Aday)

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Abstract

Background and Aim. This study aimed to assess the quality and concentration of genomic DNA isolated from hair follicles of four Kazakh horse types: Zhabe, Naiman, South Altai, and Aday. A total of 86 hair follicle samples were collected from Kazakh horses originating from four regions of Kazakhstan: Pavlodar, East Kazakhstan, Zhetisu, and Mangystau.

Materials and Methods. Genomic DNA was extracted from these samples using the PureLink Genomic DNA Mini Kit according to the manufacturer's recommended protocol with minor modifications, and the quantity and purity of the extracted DNA were evaluated using a NanoDrop spectrophotometer based on the A260/A280 absorbance ratio.

Results. The analysis revealed that the concentration of extracted DNA ranged from 93.7 to 247.2 ng/µl, while DNA purity values were predominantly within the range of 1.8–1.9, indicating high-quality DNA suitable for subsequent molecular genetic analyses.

Conclusion. The results highlight the potential of hair follicles as a practical and minimally invasive source of genomic DNA in Kazakh horses. The extraction approach provided DNA of sufficient quantity and quality for downstream molecular applications, supporting its use in future genomic studies. Further quality assessment will determine whether the obtained DNA meets the requirements for whole-genome sequencing and subsequent investigation of genetic variation among different types of Kazakh horses.

Keywords: DNA extraction; horse hair follicles; Kazakh horses; PureLink Genomic DNA Kit; Whole genome sequencing.

Introduction

The Kazakh horse is recognized as one of the most ancient horse breeds worldwide. Although several theories have been proposed concerning its origin, the prevailing view is that the contemporary Kazakh horse descended from the ancient Wusun horses, which inhabited Central Asia during the 2nd century BCE [1, 2].

The present-day Kazakh horse has been shaped through centuries of natural selection and selective breeding. Its historical development was closely linked to the lifestyle of nomadic populations, serving as a vital means of transportation along the Great Silk Road and playing an important role in military campaigns and the exchange of horses between Central Asia and China. Today, the breed is mainly found in Kazakhstan, western China, northeastern Kyrgyzstan, Mongolia, and the western Altai region of Russia [3].

According to *Yu. Barmintsev* [4] and *A. Belyaev* [5], the Kazakh horse breed includes several distinct types and local varieties. The emergence of these populations has been largely influenced by regional differences in environmental and climatic conditions, together with variations in livestock management

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and breeding strategies across Kazakhstan. Furthermore, the introduction of genetic material through crossbreeding with other horse breeds, particularly the Mongolian horse, has contributed to the diversification and formation of the breed's intra-population types and local varieties. For instance, the Aday variety of Kazakh horses, distributed in the Caspian desert zone, is characterized by a more pronounced riding-horse body conformation. In the eastern regions of Kazakhstan, local Kazakh horses demonstrate considerable similarity to Kyrgyz, Mongolian, and Altai horses.

The Aday variety was formed in ancient times under the influence of Turkmen horses and the harsh environmental conditions of desert and semi-desert regions. This variety is predominantly classified as a riding-type horse, exhibiting a lean body conformation, agile movements, and favorable speed characteristics. Its development has been strongly influenced by traditional horse husbandry and riding practices, with selective breeding emphasizing individuals capable of sustaining long-distance travel, agility, and high endurance. The desert climate contributed to the development of traits associated with a dry body constitution. Among the seven varieties described by *Yu. Barmintsev*, the Aday variety is the only one that has retained its distinctive specific characteristics. Currently, breeding of purebred Aday horses is carried out in five breeding farms in the Mangystau region [4].

One of the most valuable characteristics of the Aday horse is its exceptional adaptation to the harsh environmental and climatic conditions of the Mangystau desert zone. At the same time, modern representatives of the breed have not yet achieved sufficient uniformity in terms of body conformation and production characteristics. At various stages, attempts were made to increase live body weight through crossbreeding with established and specialized horse breeds. However, these breeding efforts did not produce the expected results [5].

The South Altai horse is regarded as the easternmost regional variety of the Kazakh breed and shares many morphological and conformational characteristics with the Altai horse, including similar body dimensions. In contrast, the Naiman variety, which is native to the Dzungarian Alatau mountain range, displays phenotypic features closely related to those of Mongolian horses. Among the regional varieties of the Kazakh horse, the Naiman type is the smallest in size, yet it is exceptionally well adapted to mountainous terrain and is recognized for its remarkable endurance and efficiency as a pack horse [6].

According to *M.Z. Dyussegaliyev* the Naiman represents the third recognized type and has long been maintained by local communities in the Dzungar Alatau. It is valued as a versatile horse capable of performing effectively under mountainous conditions. In terms of overall conformation, the Naiman type shows similarities to Mongolian horses and is regarded as one of the lighter and more refined types within the Kazakh horse population. The Naiman type of horse possesses a number of valuable economically important traits, including a strong body conformation, robust health, and high reproductive capacity. Under year-round herd management conditions, Naiman horses demonstrate a high foal production rate, which contributes to the economic efficiency of their breeding [6]. In addition to preserving the gene pool, the primary objective of breeding Naiman horses at the Tokzhailau production cooperative is the production of breeding stock. The developed breeding young stock is used to supply horse-breeding farms located in the mountainous regions of southern Kazakhstan [7].

Whole-genome sequencing (WGS) in horses enables the identification of genetic variations at the whole-genome level, including single nucleotide polymorphisms (SNPs) that cannot be detected using marker-based approaches (*Jagannathan* et al., 2019). WGS has also contributed to improving the quality of the horse reference genome, thereby increasing the accuracy of mutation detection and gene annotation (*Kalbfleisch* et al., 2018). These data are used for the identification of hereditary diseases, assessment of breed-specific genetic load, and optimization of breeding programs [8, 9].

Regardless of the objectives of genetic research, the quality of obtained data largely depends on the method used for the initial extraction of nucleic acids [10]. The extracted DNA must possess high quality to ensure successful application in genetic, population, genomic, and forensic studies, while the biological samples used should be easy to collect and process. Hair follicles are considered a non-invasive source of DNA suitable for subsequent genetic investigations [11].

According to *Tozaki* et.al, hair follicles collected from the mane or tail represent a practical source of genomic DNA in horses because sampling is minimally invasive compared with blood collection and the material can be readily preserved for extended periods. Consequently, archived hair-root samples

may provide a useful resource for subsequent genetic and genomic investigations. However, storage conditions have a substantial effect on DNA integrity. Long-term preservation of hair roots at room temperature was associated with considerable DNA degradation, whereas samples maintained under refrigerated conditions of 4–8 °C showed better preservation of DNA quality [12].

Previous studies on equine DNA sampling have emphasized several pre-analytical factors that can influence the suitability of hair-derived material for downstream genomic analyses. These include maintaining samples in a dry condition during prolonged storage, assessing the integrity of collected hairs, applying an appropriate washing procedure before DNA extraction, and preferentially selecting hairs with intact follicles and associated tissue sheaths.

The extraction and quantification of DNA concentration represent fundamental procedures that determine the reliability and efficiency of sample storage and subsequent utilization [13, 14].

Although hair follicles have previously been used as a source of genomic DNA in horses, comparative information regarding DNA quality among different indigenous Kazakh horse types remains limited. Therefore, this study aimed to evaluate the concentration and purity of genomic DNA extracted from hair follicles of four Kazakh horse types using a standardized extraction protocol as a basis for future population genomic investigations.

Materials and Methods

Ethical approval

Institutional Review Board Statement: The study was approved by the Bioethics Commission of Kazakh National Agrarian Research University, Kazakhstan (approval date: 02 November 2025, protocol number 15/09).

The sample collection was conducted between August and November 2025. Hair follicle samples were collected from clinically healthy Kazakh horses of both sexes aged 1.5–9 years that were maintained under traditional herd management systems in four breeding regions of Kazakhstan. The sampling sites represented four distinct horse types: Zhabe, South Altai, Naiman, and Aday. In August, samples from Zhabe-type horses were collected in the Pavlodar region, Maiskiy and Yereimentau districts, at the “Aqshiman-Agro” and “Ata-Zhurt” farms. In November, South Altai-type horses were sampled in Zaisan, East Kazakhstan, at the “Tuyusqan” farm; Naiman-type horses were sampled in Tekeli, Zhetisu, at the “Tekeli”farm; and Aday-type horses were sampled in Ushtagan, Mangystau, at the “Shomaq” and “Miras” farms.

A total of 86 horses were included in the study, comprising 22 Zhabe, 22 Naiman, 20 South Altai, and 22 Aday horses. One biological sample was collected from each animal. For DNA analysis, hair root samples were obtained from the tail by manually plucking approximately 40–50 hairs with intact follicles from each horse. Only hairs with clearly visible hair bulbs were selected to ensure the presence of sufficient biological material for subsequent DNA extraction. Each sample was placed individually in a sterile paper envelope, labeled with a unique identification number, and stored dry at 4°C until the DNA extraction. To minimize DNA degradation and ensure sample quality, DNA extraction was performed within seven days of sample collection.

Genomic DNA was extracted from hair follicles collected from the roots of horse hairs using the PureLink™ Genomic DNA Mini Kit (Invitrogen, Thermo Fisher Scientific, USA) according to the manufacturer's protocol with minor modifications [14]. Briefly, approximately 1–2 cm of hair containing 5–10 follicles was collected from each sample and transferred to sterile 1.5-ml microcentrifuge tubes after preliminary mechanical cleaning.

The hair follicle samples were subjected to enzymatic digestion using Digestion Buffer and Proteinase K. The samples were incubated at 55 °C until complete digestion was achieved.

Following digestion, the lysates were centrifuged, and the resulting supernatants were transferred to clean microcentrifuge tubes. RNase A treatment was performed according to the manufacturer's protocol. Genomic Lysis/Binding Buffer and ethanol were subsequently added to the lysates, and the resulting mixtures were applied to PureLink spin columns. The columns were centrifuged to allow binding of genomic DNA to the silica membrane.

The columns were washed sequentially with Wash Buffer 1 and Wash Buffer 2, followed by an additional centrifugation step to remove residual wash solution and ethanol. Purified genomic DNA was eluted from the spin columns using Elution Buffer. The extracted DNA was stored at −80 °C until further analysis.

DNA concentration and purity were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

Descriptive statistical analysis was performed using Microsoft Excel. DNA concentration and A260/A280 purity values were expressed as mean ± standard deviation (SD), minimum, and maximum for each horse type.

Results

In the present study, the concentration and purity of genomic DNA extracted from hair follicles of four Kazakh horse types (Zhabe, Naiman, South Altai, and Aday) were evaluated. The quantitative and qualitative parameters of DNA were determined using a NanoDrop spectrophotometer. The obtained results demonstrated variation in DNA concentration among all investigated horse types, which may be associated with individual characteristics of the samples, the number of hair follicles collected, and the efficiency of the extraction process. Nevertheless, all groups yielded DNA concentrations sufficient for further molecular genetic analyses.

In Zhabe-type horses, DNA concentration ranged from 95.4 to 247.2 ng/µl, while the A260/A280 purity ratios varied from 1.76 to 1.94. The majority of samples demonstrated values within the range of 1.8–1.9, indicating good quality of the extracted DNA and minimal contamination with protein impurities (Figure 1).

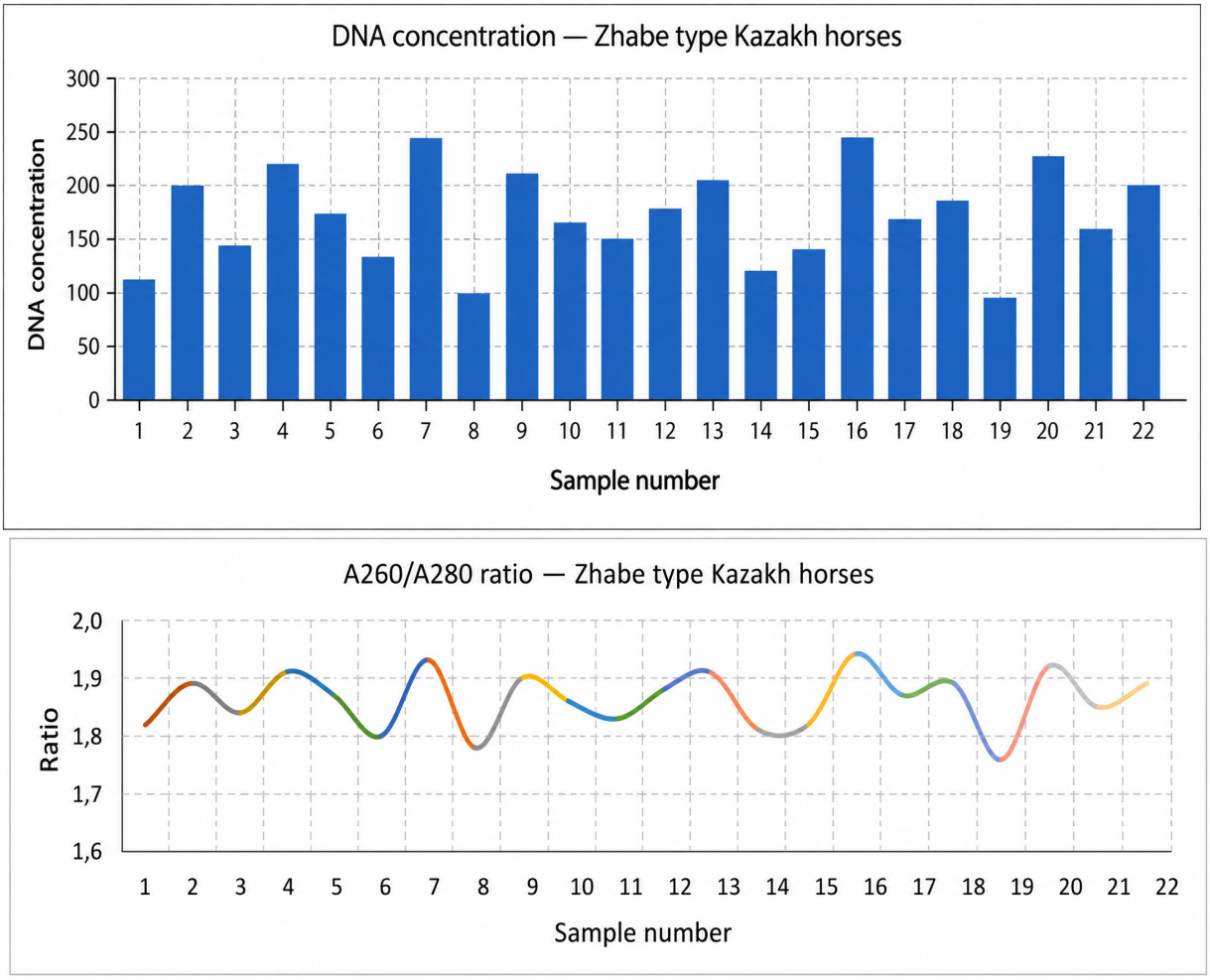

Figure 1. DNA concentration and purity parameters of genomic DNA extracted from hair follicle samples of Zhabe type Kazakh horses, measured using a NanoDrop spectrophotometer

For the Naiman type, DNA concentration ranged from 104.9 to 241.9 ng/µl, while the A260/A280 ratios were within the range of 1.79–1.94. These results also indicate a high degree of DNA purity and confirm the efficiency of the applied extraction method (Figure 2).

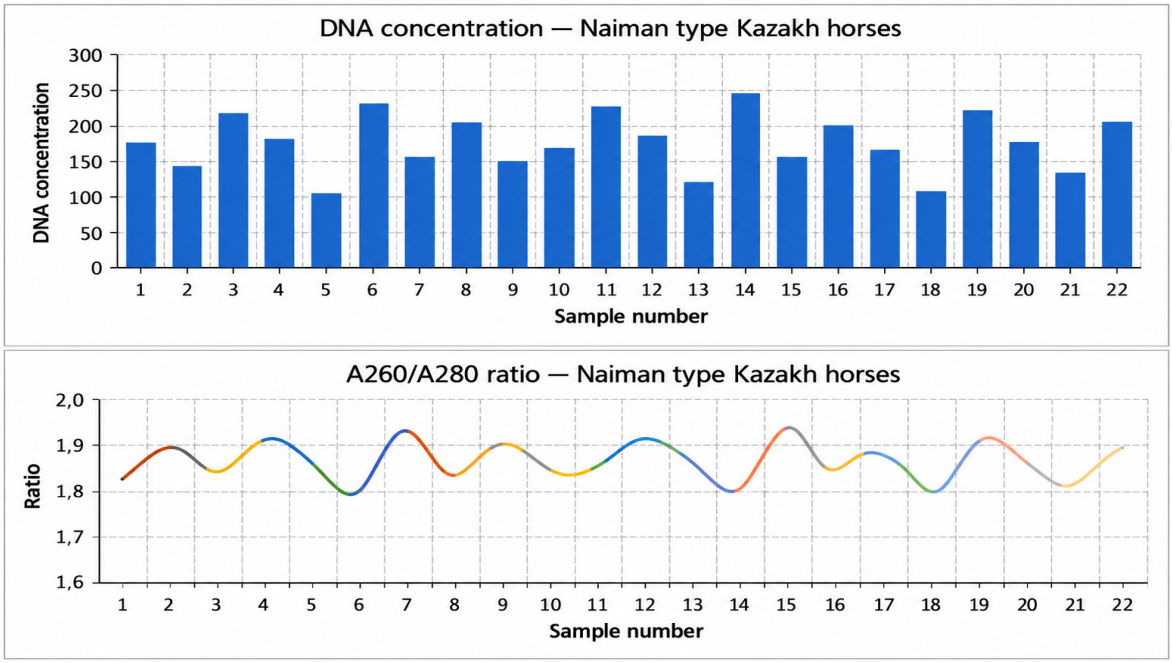

Figure 2. DNA concentration and purity parameters of genomic DNA extracted from hair follicle samples of Naiman type Kazakh horses, measured using a NanoDrop spectrophotometer

For the South Altai type samples, DNA concentration ranged from 93.7 to 243.5 ng/µl. The A260/A280 values ranged from 1.72 to 1.94. Slightly reduced purity values were observed in some individual samples (samples No. 9 and No. 19), which may indicate the presence of minor protein contaminants or residual reagents. However, the majority of samples corresponded to the recommended quality parameters for molecular genetic studies (Figure 3).

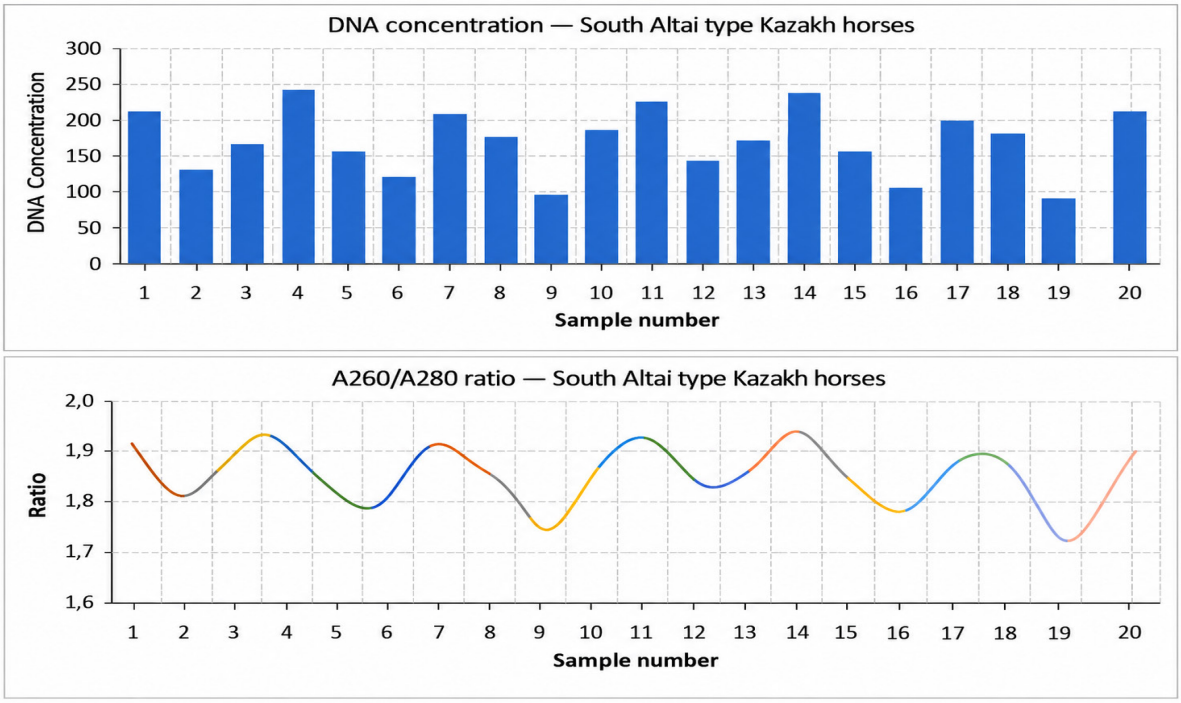

Figure 3. DNA concentration and purity parameters of genomic DNA extracted from hair follicle samples of South Altai type Kazakh horses, measured using a NanoDrop spectrophotometer

In Aday horses, the DNA concentration ranged from 97.3 to 245.3 ng/µl, and the purity values were 1.77–1.94. High values of the A260/A280 ratio confirm the receipt of high-quality genetic material suitable for subsequent sequencing and genomic analysis (Figure 4, Table 1).

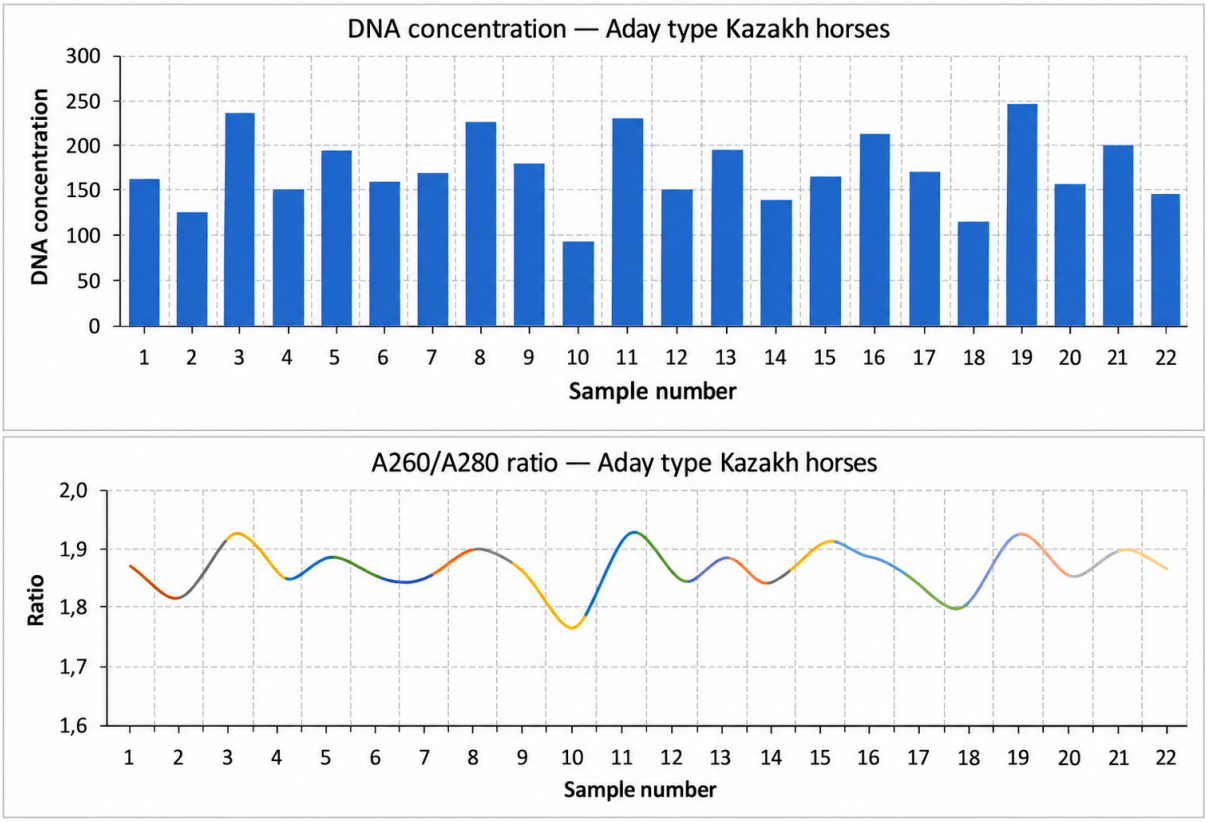

Figure 4. DNA concentration and purity parameters of genomic DNA extracted from hair follicle samples of Aday type Kazakh horses, measured using a NanoDrop spectrophotometer

Table 1. Descriptive statistics of DNA concentration and A260/A280 purity ratio in four Kazakh horse types

Horse type	Zhabe	Naiman	South Altai	Aday
N	22	22	20	22
DNA concentration, mean ± SD (ng/µL)	172.8 ± 43.3	176.9 ± 40.6	171.8 ± 44.9	175.3 ± 40.3
Min–Max	95.4–247.2	104.9–241.9	93.7–243.5	97.3–245.3
A260/A280, mean ± SD	1.86 ± 0.05	1.87 ± 0.04	1.85 ± 0.06	1.87 ± 0.04
Min–Max	1.76–1.94	1.79–1.94	1.72–1.94	1.77–1.94

The mean DNA concentration was 172.8 ± 43.3 ng/µl in Zhabe horses, 176.9 ± 40.6 ng/µl in Naiman horses, 171.8 ± 44.9 ng/µl in South Altai horses, and 175.3 ± 40.3 ng/µl in Aday horses. The corresponding concentration ranges were 95.4–247.2, 104.9–241.9, 93.7–243.5, and 97.3–245.3 ng/µl, respectively.

The mean A260/A280 ratios were 1.86 ± 0.05 in Zhabe horses, 1.87 ± 0.04 in Naiman horses, 1.85 ± 0.06 in South Altai horses, and 1.87 ± 0.04 in Aday horses. The corresponding ranges were 1.76–1.94, 1.79–1.94, 1.72–1.94, and 1.77–1.94, respectively. Overall, the obtained values indicated a generally satisfactory level of DNA purity in all four horse types.

Discussion

In the present study, the PureLink Genomic DNA Mini Kit demonstrated reliable and efficient performance for DNA extraction from hair follicles of different Kazakh horse types. The mean A260/A280 ratios ranged from 1.85 to 1.87 across the four horse types. This indicates that the extracted DNA was of sufficient quality for subsequent molecular analyses. Together with previous reports,

our findings confirm that hair follicles represent a practical and minimally invasive biological material for obtaining genomic DNA, while commercial extraction kits can provide reproducible DNA quality suitable for downstream molecular applications. *Kabylbekova* et al. compared three methods of DNA extraction from the hair follicles of Kazakh horses of the Zhabe type: a commercial kit, CTAB, and the phenol-chloroform method. Although the methods produced DNA of comparable quality, the authors recommended CTAB as the most suitable method for genotyping, considering its safety, cost-effectiveness, and efficiency [15, 16]. These findings highlight the importance of selecting an extraction method according to the intended downstream application and the characteristics of the biological material. *Ibadullayeva* et al. demonstrated that the PureLink Microbiome Purification Kit enables efficient DNA extraction from microbiome samples of Kazakh horses of the Jabe and Aday types, yielding DNA suitable for subsequent 16S rRNA sequencing [17]. *Similarly, Villacrez-Arce* et al. showed that high-quality genomic DNA suitable for genomic studies and genotyping-by-sequencing (GBS) can be obtained non-invasively from horse tail and mane hair. The highest DNA yield was obtained using 80 hair follicles (22.99 ng/µl), with DNA quality parameters within the requirements for sequencing platforms [18]. These studies support the suitability of hair-derived material as a convenient and minimally invasive source of genomic DNA for molecular and genomic analyses in horses.

A limitation of the present study is that DNA quality was evaluated using the A260/A280 absorbance ratio only. Electrophoretic assessments of DNA integrity were not performed and should be included in future investigations.

Conclusion

The obtained DNA was of satisfactory concentration and purity for subsequent molecular genetic analyses. Its suitability for whole-genome sequencing requires further confirmation through additional assessment of DNA integrity and purity. In future studies, sequencing is planned to study genetic diversity, identify genetic variants, and compare four types of Kazakh horses.

Authors' Contributions

AI, and AKh: conceptualized and designed the study, conducted a comprehensive literature search, analyzed the collected data, and prepared the manuscript. TA, TSh, AK: conducted final editing and proofreading of the manuscript. All authors read, reviewed, and approved the final version of the manuscript.

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Conflict of Interest

The authors declare that they have no known financial interests or personal relationships that could have influenced the results presented in this work.

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