DOI 10.31489/2023BMG1/69-75

UDC 57.08

D.I. Kabylbekova[1], Zh. Khamzina[2], K.P. Aubakirova[3], A.A. Ibadullayeva[4*]

[1]*Almaty Technological University, Almaty, Kazakhstan;*
[2]*Al-Farabi Kazakh National University, Almaty, Kazakhstan;*
[3]*M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry, Almaty, Kazakhstan;*
[4]*Kazakh National Agrarian Research University, Almaty, Kazakhstan*
**Corresponding author: akerke.ibadullayeva@kaznaru.edu.kz*

Evaluation and comparison of three methods of DNA extraction from Kazakh horse of the type Zhabe

Horse breeding is one of the main directions in the livestock industry of Kazakhstan. Horse breeding is an economically important part of breeding, as horses have a high productive value. The purpose of this article was to determine the most optimal from the point of view of economic efficiency of the scientific DNA isolation method for genotyping of the Kazakh horse. In this study, we performed the first step (DNA extraction) in genotyping the DNA of the Kazakh horse to further determine the signs of growth, meat and dairy productivity. Nowadays, new generation sequencing technologies have made scientific and technological progress in research. Obtaining highly concentrated and non-contaminated DNA is the main stage of successful analysis. For further genetic studies, a concentration of 50 ng/ml is required, which is considered minimal. In the study, three different DNA extraction methods from tissue were subjected to comparative analysis in order to evaluate and identify the most effective DNA extraction method from horse ear tips. Real-time PCR amplification based on gel electrophoresis and spectrophotometric measurements (Nano Drop) were used to evaluate the isolated DNA's quality and quantity. In addition, energy consumption, time, as well as the cost of analysis were evaluated. According to the obtained results, extraction using the commercial kit protocol was simple to use, effective, but relatively expensive. The phenol-chloroform and CTAB methods are the same in terms of DNA quality, but given the danger of the phenol component used in the phenol-chloroform method, the CTAB method turned out to be the most acceptable for genotyping, because it is safe, not expensive and effective.

Keywords: horse breeding, Kazakh horse, Zhabe, PCR, phenol-chloroform method, CTAB method, genotyping, DNA extraction, electrophoresis.

Buketov University

Introduction

Horse breeding is one of the main branches of livestock production in Kazakhstan. Horses have a great productive value [1].

The country has great opportunities to take a leading position among other countries, as there are extensive pasture lands that allow minimizing the cost of production, and, accordingly, the existing national traditions of horse breeding [2].

Archaeologists consider that the domestication of the horse occurred in the Neolithic and Bronze Age on the territory of Eurasia and for the first time — most likely, between the Syrdariya and Amudaria rivers [3].

According to the research of scientists of horse breeders, more than a thousand years ago a unique breed of horses appeared on the territory of Kazakhstan, which later became known as the Kazakh horse. The breed was formed with the year-round maintenance of the herd. The Kazakh horse of those times was unpretentious to the conditions of maintenance and was considered universal [4]. The Kazakh horse has gained broad popularity mainly due to its exceptional endurance, resistance to harsh climatic conditions, simplicity to feed and year-round maintenance on pasture. These qualities of the Kazakh horse have been developed for centuries. In winter, the horses had to get feed from under the snow, even during freezing or when blizzards blew [5]. All this was genetically fixed in the breed and has practically reached our days. Therefore, today it is important to study indigenous horse breeds at the molecular genetics level, which have rare alleles in their genome.

The genetic capacity of local Kazakh horses is improved mainly using traditional methods and techniques of breeding, creating new factory types and lines with high meat and dairy productivity. Horse breeding in Kazakhstan in the future needs to focus on the genetic resources of the created high-value genotypes of horse breeds [6].

With the rapid introduction of DNA technology and the success of equine genome research, the total number of marker genes detected in horses already exceeds several hundred, allowing reliable control of a significant part of the genome [7]. Regardless of the purpose of genetic research, the quality of data fundamentally depends on the method of primary extraction of nucleic acids [8]. Despite the importance of the DNA extraction stage, protocols are often chosen without a clear justification and are not formally confirmed [9].

DNA isolated from various biological samples can be used for a wide range of subsequent applications, in particular DNA sequencing, polymerase chain reaction (PCR), random polymorphic DNA amplification (RAPID), genomic library construction, restriction fragment length polymorphism (RFLP), short tandem repeat polymorphism (STRP), single nucleotide polymorphism (SNP), and variable number tandem repeat application (VNTR) [10].

Several different extraction methods have been published. They all have the common property of overcoming specific extraction problems such as high DNA shearing, high contamination, low purity and low yield [11]. A minimum concentration of 50 ng/ml is also necessary in both microarray and NGS analysis [12].

In addition to high quality, purity and quantity, a successful extraction method must meet the additional requirements associated with the representative quality of the sample. Other indicators for scientists choosing an extraction method are high reproducibility, low cost, simplicity and brevity of processing, as well as the possibility of easy transfer to other laboratories [13].

Nowadays, there are many various available procedures for DNA extraction. Most DNA extraction protocols consist of two parts: 1. cell lysis and DNA dissolution; 2. enzymatic or chemical methods for removing polluting proteins, RNA or macromolecules. However, these procedures differ in the cost of consumables, laboratory equipment, the quality and quantity of the obtained DNA, toxicity, time and labor expenditures [14].

Commercially available DNA extraction kits for purifying DNA from whole blood practical and high-performance kits, but their use for processing large volumes of samples is often due to cost-related problems. On the opposite, while traditional methods that utilize organic solvents are a cost-effective option to handle large amounts of samples, the risk to the performer's health often supersedes this advantage [15].

The phenol-chloroform method is a well-proven extraction procedure, although it is known to be time-consuming compared to the alternative methods and contains toxic substance as phenol, requiring special safety precautions in the laboratory. Although this method labor intensive, the output of DNA concentration is high [16].

In 2009 K. Kerkhoff et al. considered that the CTAB procedure was reliable for DNA isolation from hair follicles because it achieved the best results with regard to quantity and PCR suitability of DNA [17].

The purpose of this study was to evaluate three different methods for extraction of genomic DNA from horse hair follicles in terms of DNA quantity, concentration, purity, integrity and real-time PCR suitability, as well as utility and applicability for subsequent DNA genotyping, long-term storage, labor intensity and cost.

Experimental

The material used for DNA extraction was the hair follicles of the Kazakh horse.12 samples were used in the study. Samples were collected from agro firm "Akzhar ondiris" in Pavlodar region. Hair follicles from the tail zone were used as biological material. All the samples were numbered according to the number of the animal and were put in bags.

DNA extraction was carried out by 3 methods: according to the protocol of the Purelink Commercial Kit, CTAB and phenol-chloroform.

DNA extraction. Commercial kit for DNA extraction "Purelink Genomic Kit".

DNA from the samples was isolated by the Purelink Genomic Kit, under the protocol [18]. The PureLink Genomic DNA Mini Kit allows you to extract genomic DNA (gDNA) with high yield and high purity from a wide variety of sample types. It consisted of four steps: sample preparation, DNA binding, washing and elution.

First of all, all the samples should undergo mechanical cleaning. To isolate DNA with a commercial kit, 5–6 good hair follicles will be required.

CTAB. The hair was mechanically cleaned and washed in alcohol. Added 300 μl of buffer + 6 μl of proteinase K. Left in the thermostat for 12 hours at 37 °C (2.5 hours at 60 °C at 900 rpm). Chloroform was

Buketov University

added 1:1 by volume, then vortexed and centrifuged for 10 minutes at 10000 rpm. The supernatant was transferred to a clean centrifuge tube. To the supernatant, add 0.5 of the volume of 5M NaCl and an equal volume of frosted isopropanol or 90 % alcohol (1:1), vortexed and the sample was stored overnight at –20 °C (The time can be reduced to 2 hours, but the concentration of DNA at the output will drop). Centrifuged for 15 minutes at 14,000 rpm(max), the supernatant was removed without touching the precipitate, 500 µl of 70 % ethanol was added and vortexed for about 5 seconds. The tubes were centrifuged for 5 minutes at 14000 rpm. (max). DNA in the form of a precipitate was dried on a thermoformed at 60–65 °C for 5–10 minutes, and then re-suspended with 50 µl of TE buffer.

Phenol-chloroform. Extraction was performed by adding the same volume of saturated water buffered phenol to an aqueous DNA sample, mixing the mixture, and centrifuging to ensure phase separation. The top layer of water was carefully transferred to a new test tube, avoiding contact with the phenol. Chloroform was then added to extract the remaining phenol from the aqueous phase. Ethanol precipitation was used to concentrate the DNA. The DNA granules were dried and dissolved in a low-salt buffer after being washed with 70 % ethanol [19].

Spectrophotometric analysis of DNA. Quantitation of all samples were extracted in quadruplicate. Extracted DNA was quantized by Thermo Scientific NanoDrop ™ 2000 Spectrophotometer (Thermo Scientific, Germany) using 1 µl of each sample, measuring the absorbance of the DNA extracts at 260 nm, checking for protein impurities at 280 nm. 1 O.D. at 260 nm equaling 50 g/mL DNA [20].

Results and Discussion

Spectrophotometric analysis clearly demonstrates the quantity and quality of the isolated DNA, which is the key point for further analyses. The proportion measured at 260/280 nm, used as an indicator of protein contamination, was within the limits of the range suitable for DNA analyses. Evaluating the wholeness of the nucleic acid extracted by three methods, it had a high molecular weight, which is essential when sequencing the whole genome (Table).

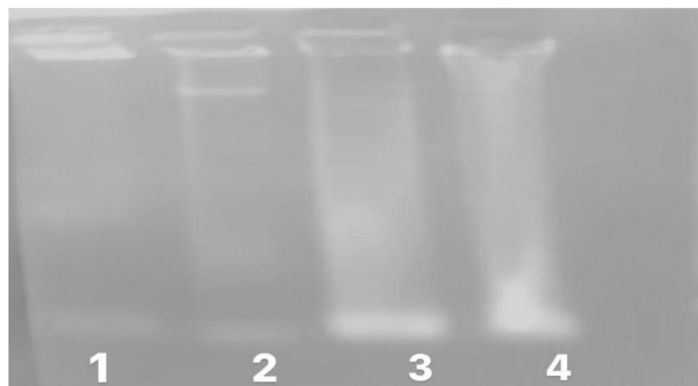
T a b l e

Quantity and purity of horse DNA isolated by 3 methods

Method of extraction		DNA concentration, ng/ml	Unit A260/A280
Commercial kit “Purelink Genomic Kit”	1 Purelink	61.7	1.63
	2 Purelink	66.4	1.32
	3 Purelink	62.6	1.09
	4 Purelink	60.07	1.52
CTAB	1 CTAB	59.2	1.04
	2 CTAB	57.0	1.28
	3 CTAB	54.0	1.42
	4 CTAB	55.4	1.00
Phenol-chloroform	1 Ph/chl	52.5	1.23
	2 Ph/chl	62.2	1.12
	3 Ph/chl	63.3	1.13
	4 Ph/chl	58.4	1.20

After that, the samples were subjected to electrophoresis. Electrophoresis of DNA samples isolated by the classical phenol-chloroform method shows that DNA does not have a clear band, which indicates its small fragmentation (Fig. 1).

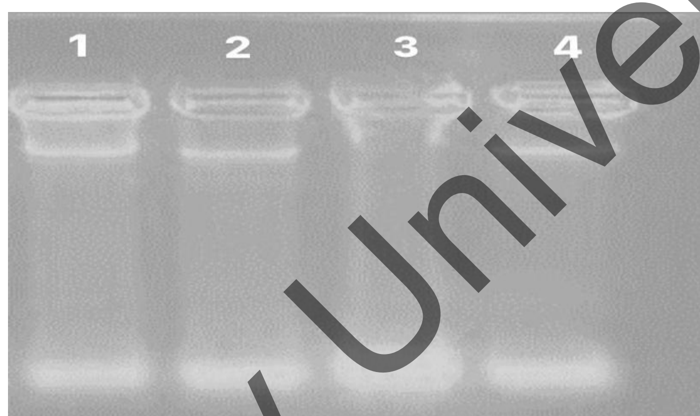
Buketov University



1 — sample Ph/chl1; *2* — sample Ph/chl2; *3* —sample Ph/chl3; *4* — sample Ph/chl4

Figure 1. Electrophoregram of DNA samples isolated by phenol-chloroform method

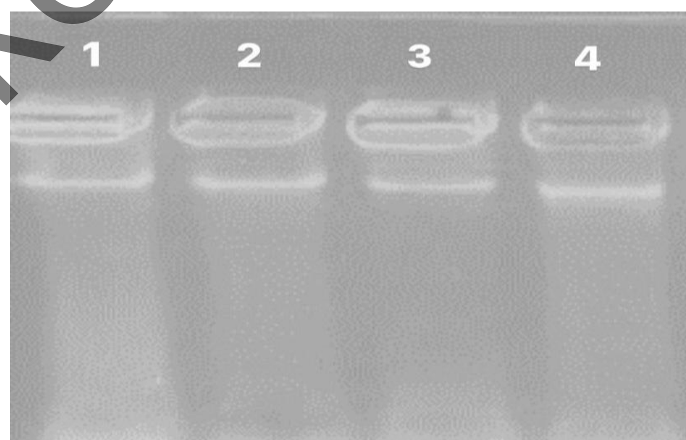
The electrophoregram of DNA isolated according to the protocol of the commercial Purelink Genomic Kit shows clear fragmentation (Fig. 2).



1 — sample 1 Purelink1; *2* — sample Purelink2; *3* — sample Purelink3; *4* — sample Purelink4

Figure 2. Electrophoregram of DNA samples isolated using the Purelink Genomic Kit protocol

The electrophoregram clearly shows that mainly high-molecular DNA fragments were obtained using the CTAB method (Fig. 3).



1 — sample CTAB 1; *2* — sample CTAB 2; *3* — sample CTAB 3; *4* — sample CTAB 4

Figure 3. Electrophoregram of DNA samples isolated by CTAB method

Buketov University

Conclusion

As it is known, there are a lot of methods for extracting DNA from different types of biological samples. But, for analyses like genotyping, the quality of the isolated DNA plays an important role. It is also necessary to take into account time and expenses.

In conclusion, the CTAB method is the most effective for extracting DNA from hair follicles. The purity is high, which makes it possible to widely use DNA for further genetic research, such as genotyping. The use of this method allows receiving of high-molecular DNA with the coveted density and does not require the use of phenol harmful to the organism. Furthermore, phenol/chloroform extraction is time-consuming, and the multiple steps required make this DNA extraction method very susceptible to cross-contamination [21]. Commercially available extraction kits such as Purelink Genomic Kit are much easier to use and eliminate the need for volatile organic solvents. Thus, they do not involve the same serious risks for furthermore, such as phenol/chloroform extraction, and disposal of the reagents used in these kits are also much more convenient since no special precautions need to be taken to remove waste.

DNA extraction with the commercial Purelink Genomic Kit is faster than the other two methods, but also more expensive. Accordingly, the CTAB method of DNA isolation turned out to be the most optimal in terms of quality, effectiveness, safety and cost.

Funding

This work was carried out within the framework of grant funding for scientific and (or) scientific and technical projects for 2022–2024 of the Ministry Education and Science of the Republic of Kazakhstan AP14869181 “The study of microbiome ecogenomics of Kazakh horse breed by NGS sequencing”.

References

1 Данные по статистике в сельском хозяйстве. — Электронный ресурс. — Режим доступа: https://stat.gov.kz/

2 Султангалиева Л.С. Развитие конкурентоспособности отрасли мясного животноводства Республики Казахстан / Л.С. Султангалиева // Вестн. Казах. нац. ун-та. Сер. Экономика. — 2013. — № 4(98). — С. 93–101.

3 Витт В.О. Конские породы Средней Азии / В.О. Витт. — М., 1937. — 255 с.

4 Нечаев И. Казахская лошадь. Прошлое, настоящее и будущее / И. Нечаев. — Алматы: Эдельвейс, 2005. — 208 с.

5 Керимбаев Д. Коневодство в Казахстане / Д. Керимбаев. — Алма-Ата, 1951. — 276 с.

6 Исхан К.Ж. Зоотехнические особенности табунных лошадей / К.Ж. Исхан, В.А. Демин, Ю.А. Юлдашбаев, А.Д. Баймуканов // Зоотехния и ветеринария. — 2019. — Т. 33, № 9. — С. 57–60.

7 Schiebelhut L.M. A comparison of DNA extraction methods for high-throughput DNA analyses / L.M. Schiebelhut, S.S. Abboud, L.E. Gómez Daglio, H.F. Swift, M.N. Dawson // Molecular Ecology Resources. — 2016. — Vol. 17(4). — P. 721–729. https://doi.orh/10.1111/1755–0998.12620

8 Greathouse L.K. DNA extraction for human microbiome studies: The issue of standardization / L.K. Greathouse, S.R. Vogtmann // Genome Biol. — 2019. — Vol. 20 (1). — P. 212.

9 Shvartsman E. Comparative analysis of DNA extraction and PCR product purification methods for cervicovaginal microbiome analysis using cpn60 microbial profiling / E. Shvartsman, E.I.M. Richmond, J.J. Schellenberg, A. Lamont, C. Perciani et al. // Plos One. — 2022. — Vol. 13, 17(1). — e0262355. https://doi.org/10.1371/journal.pone.0262355.2022

10 Dhaliwal A. DNA extraction and purification / A. Dhaliwal // Mater Methods. — 2013. — Vol. 3. — P. 191.

11 Tabatabaei M. Comparative study of methods for extraction and purification of environmental DNA from high-strength wastewater sludge / M. Tabatabaei, Z.M. Rafein, R. Raha, A. Norhani, W. André-Denis, Sh. Yoshihito, Sh. Mehdi, K. Sakai, H. Mohd // African Journal of Biotechnology. — 2010. — Vol. 9. — P. 4926–4937.

12 Genomics.ed.ac.uk [Электронный ресурс]. Режим доступа: http://genepool.bio.ed.ac.uk/illumina/samples.html

13 Kuhn R. Comparison of ten different DNA extraction procedures with respect to their suitability for environmental samples / R. Kuhn, J. Böllmann, K. Krahl, I.M. Bryant, M. Martienssen // Journal of Microbiological Methods. — 2017. — Vol. 143. https://doi.org/10.1016/j.mimet.2017.10.007

14 Chacon-Cortes D. Methods for extracting genomic DNA from whole blood samples: current perspectives / D. Chacon-Cortes, L.R. Griffiths // Journal of Biorepository Science for Applied Medicine. — 2014. — Vol. 2. — P. 1–9.

15 Koshy L. Evaluating genomic DNA extraction methods from human whole blood using endpoint and real-time PCR assays / L. Koshy, A.L. Anju, S. Harikrishnan, V.R. Kutty, V.T. Jissa, I. Kurikesu, P.R. Sudhakaran // Molecular Biology Reports. — 2016. — Vol. 44(1). — P. 97–108. https://doi.org/10.1007/s11033-016-4085-9

16 Sambrook J. Molecular Cloning: A Laboratory Manual, 2nd ed. / J. Sambrook, E.F. Fritsch, T. Maniatis. — New-York: Cold Spring Harbor Laboratory Press, 1989. — 220 p.

Buketov University

17 Kerkhoff K. Development of a DNA-analytical Method for the Identification of Animal Hair Fibers in Textiles / K. Kerkhoff, G. Cescutti, L. Kruse, J. Müssig // Textile Research Journal. — 2009. — Vol. 79(1). — P. 69–75. https://doi.org/10.1177/0040517508090488

18 Thermofisher.com [Электронный ресурс]. — Режим доступа: https://tools.thermofisher.com/content/sfs/manuals/purelink_genomic_man.pdf

19 Köchl S. DNA Extraction and Quantitation of Forensic Samples Using the Phenol–Chloroform Method and Real-Time PCR / S. Köchl, H. Niederstätter, W. Parson // Forensic DNA Typing Protocols, 013–030. https://doi.org/10.1385/1-59259-867-6:013

20 Sambrook J. Molecular Cloning: A Laboratory Manual (2nd ed.) / J. Sambrook, E.R. Fritsch, T. Maniatis // Cold Spring Harbor, the Use of DNA Extraction for Molecular Biology and Biotechnology Training: A Practical and Alternative Approach. — New-York: Cold Spring Harbor Laboratory Press, 1989. — P. 90–105.

21 Chan P.K.S. Evaluation of extraction methods from paraffin wax embedded tissues for PCR amplification of human and viral DNA / P. K. S. Chan // J. Clin. Pathol. — 2001. — Vol. 54. — P. 401–403.

Д.И. Кабылбекова, Ж. Хамзина, К.П. Аубакирова, А.А. Ибадуллаева

Қазақтың бақа басты жылқысының түрін ДНҚ экстракциялаудың үш түрлі әдісін салыстыру және бағалау

Жылқы шаруашылығы Қазақстанның мал шаруашылығы саласының басты бағыттардың бірі. Жылқы өсіру селекцияның экономикалық маңызды бөлігі болып табылады, өйткені жылқылардың өнімділігі жоғары. Мақаланың мақсаты — қазақ жылқысын генотиптеу үшін ДНҚ-ны экстракциялаудың экономикалық тиімділігі тұрғысынан неғұрлым оңтайлы ғылыми әдісін анықтау. Бұл зерттеуде қазақ жылқысының өсу белгілерін, ет және сүт өнімділігін одан әрі анықтау үшін ДНҚ генотиптеуінің бірінші кезеңіне (ДНҚ экстракциясы) жүргізілген. Бүгінгі таңда жаңа буын секвенирлеу технологиялары зерттеулерде ғылыми-техникалық прогреске қол жеткізді. Жоғары тығыздықты және контаминацияға ұшырамаған ДНҚ сәтті талдаудың негізгі кезеңі болып саналады. Әрі қарай генетикалық зерттеулер үшін минималды деп саналатын 50 нг/мкл концентрациясы қажет. Зерттеуде жылқылардың шаш фолликулаларынан ДНҚ алудың ең тиімді әдісін бағалау және анықтау үшін ДНҚ-ны шаш түктерінен алудың үш түрлі әдісі салыстырмалы түрде талданды. Алынған ДНҚ-ны сапалы және сандық бағалау спектрофотометриялық өлшеулер (NanoDrop), гель электрофорезіне негізделген нақты уақыт режимінде ПТР арқылы жүргізілді. Сонымен қатар, энергия шығыны, уақыт, сондай-ақ талдау құны бағаланды. Зерттеу нәтижелері бойынша коммерциялық жиынтық хаттамасы бойынша экстракция жасау оңай және тиімді, бірақ салыстырмалы түрде қымбат болып шықты. Фенол-хлороформ және ЦТАБ әдістері коммерциялық жинақ әдісімен салыстырғанда ДНҚ сапасы бірдей, бірақ фенол-хлороформ әдісінде қолданылатын компоненттің, фенолдың қауіптілігін ескере отырып, ЦТАБ әдісі генотиптеу үшін ең қолайлы болып шықты, өйткені ол қауіпсіз, қымбат емес және тиімді.

Кілт сөздер: жылқы шаруашылығы, қазақ жылқысы, бақа басты жылқы, ПТР, фенол-хлороформ әдісі, ЦТАБ әдісі, генотиптеу, ДНҚ экстракциясы, электрофорез.

Д.И. Кабылбекова, Ж. Хамзина, К.П. Аубакирова, А.А. Ибадуллаева

Оценка и сравнение трех методов экстракции ДНК у казахской лошади типа жабе

Коневодство является одним из главных направлений в животноводческой отрасли Казахстана. Разведение лошадей является экономически важной частью селекции, так как лошади имеют высокую продуктивную ценность. Целью данной статьи было определение наиболее оптимального с точки зрения экономической эффективности научного метода экстракции ДНК для генотипирования казахской лошади. В данном исследовании мы выполнили первый шаг (экстракция ДНК) в генотипировании ДНК казахской лошади для дальнейшего определения признаков роста, мясомолочной продуктивности. На сегодняшний день технологии секвенирования нового поколения произвели научно-технический прогресс в исследованиях. Получение высококонцентрированной и неконтаминированной ДНК является главным этапом успешного анализа. Для дальнейших генетических исследований требуется концентрация 50 нг/мкл, которая считается минимальной. В исследовании были подвергнуты к сравнительному анализу три различных метода извлечения ДНК из ткани, чтобы оценить и идентифицировать наиболее эффективную методику выделения ДНК из волосяных луковиц лошадей. Качественная и количественная оценка извлеченной ДНК проводилась с помощью спектрофотометрических измерений (NanoDrop), амплификация ПЦР в реальном времени на основе гель-электрофореза. Помимо этого, к оценке подверглись энергозатратность, время, а также стоимость анализа. По результатам исследова-

Buketov University

ния, экстракция по протоколу коммерческого набора оказалась простой в применении и эффективной, но относительно дорогостоящей. Фенол-хлороформный и ЦТАБ методы по качеству ДНК одинаковы, но, учитывая опасность компонента фенола, применяемого в фенол-хлороформном методе, ЦТАБ метод оказался наиболее приемлемым для генотипирования, так как является безопасным, недорогим и результативным.

Ключевые слова: коневодство, казахская лошадь, жабе, ПЦР, фенол-хлороформный метод, ЦТАБ метод, генотипирование, экстракция ДНК, элекрофорез.

References

1 *Dannye po statistike v selskom khoziaistve [Data on statistics in agriculture]*. Retrieved from https://stat.gov.kz/ [in Russian].

2 Sultangalieva, L.S. (2013). Razvitie konkurentosposobnosti otrasli miasnogo zhivotnovodstva Respubliki Kazakhstan [Development of competitiveness of the meat livestock industry of the Republic of Kazakhstan]. *Vestnik Kazakhskogo natsionalnogo universiteta. Seriia Ekonomika — Bulletin of Kazakh National University. Series Economics, 4(98);* 93–101 [in Russian].

3 Vitt, V.O. (1937). *Konskie porody Srednei Azii [Horse breeds of Central Asia]*. Moscow [in Russian].

4 Nechaev, I. (2005). *Kazakhskaia loshad. Proshloe, nastoiashchee i budushchee [Kazakh horse. Past, present and future].* Almaty: Edelveis [in Russian].

5 Kerimbaev, D. (1951). *Konevodstvo v Kazakhstane [Horse breeding in Kazakhstan]*. Alma-Ata [in Russian].

6 Iskhan, K.Zh., Demin, V.A., Iuldashbaev, Yu.A. & Baimukanov, A.D. (2019). Zootekhnicheskie osobennosti tabunnykh loshadei [Zootechnical features of herding horses]. *Zootekhniia i veterinariia — Zootechnics and veterinary medicine, 33 (9)*; 57–60 [in Russian].

7 Schiebelhut, L.M., Abboud, S.S., Gómez Daglio, L.E., Swift, H.F. & Dawson, M.N. (2016). A comparison of DNA extraction methods for high-throughput DNA analyses. *Molecular Ecology Resources, 17(4)*; 721–729. https://doi.orh/10.1111/1755–0998.12620

8 Greathouse, L.K. & Vogtmann, S.R. (2019). DNA extraction for human microbiome studies: The issue of standardization. *Genome Biol., 20 (1)*; 212.

9 Shvartsman, E., Richmond, E.I.M., Schellenberg, J.J., Lamont, A., Perciani, C. et al. (2022). Comparative analysis of DNA extraction and PCR product purification methods for cervicovaginal microbiome analysis using cpn60 microbial profiling. *Plos One, 13, 17(1)*; e0262355. https://doi.org/10.1371/journal.pone.0262355.2022

10 Dhaliwal, A. (2013). DNA extraction and purification. *Mater Methods, 3*; 191.

11 Tabatabaei, M., Rafein, Z.M., Raha, R., Norhani, A., André-Denis, W., Yoshihito, Sh., Mehdi, Sh., Sakai, K. & Mohd, H. (2010). Comparative study of methods for extraction and purification of environmental DNA from high-strength wastewater sludge. *African Journal of Biotechnology, 9*; 4926–4937.

12 Genomics.ed.ac.uk. Retrieved from http://genepool.bio.ed.ac.uk/illumina/samples.html

13 Kuhn, R., Böllmann, J., Krahl, K., Bryant, I.M. & Martienssen, M. (2017). Comparison of ten different DNA extraction procedures with respect to their suitability for environmental samples. *Journal of Microbiological Methods, 143*. https://doi.org/10.1016/j.mimet.2017.10.007

14 Chacon-Cortes, D. & Griffiths, LR. (2014). Methods for extracting genomic DNA from whole blood samples: current perspectives. *Journal of Biorepository Science for Applied Medicine, 2*; 1–9.

15 Koshy, L., Anju, A.L., Harikrishnan, S., Kutty, V.R., Jissa, V.T., Kurikesu, I. & Sudhakaran, P.R. (2016). Evaluating genomic DNA extraction methods from human whole blood using endpoint and real-time PCR assays. *Molecular Biology Reports, 44(1)*; 97–108. https://doi.org/10.1007/s11033-016-4085-9

16 Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd ed. New-York: Cold Spring Harbor Laboratory Press.

17 Kerkhoff, K., Cescutti, G., Kruse, L. & Müssig, J. (2009). Development of a DNA-analytical Method for the Identification of Animal Hair Fibers in Textiles. *Textile Research Journal, 79(1)*; 69–75. https://doi.org/10.1177/0040517508090488

18 Thermofisher.com. Retrieved from https://tools.thermofisher.com/content/sfs/manuals/purelink_genomic_man.pdf

19 Köchl, S., Niederstätter, H. & Parson, W. (2013). DNA Extraction and Quantitation of Forensic Samples Using the Phenol–Chloroform Method and Real-Time PCR. *Forensic DNA Typing Protocols,* 013–030. https://doi.org/10.1385/1-59259-867-6:013

20 Sambrook, J., Fritsch, E.R. & Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual (2nd ed.). *Cold Spring Harbor, the Use of DNA Extraction for Molecular Biology and Biotechnology Training: A Practical and Alternative Approach*. New-York: Cold Spring Harbor Laboratory Press, 90–105.

21 Chan, P.K.S. (2001). Evaluation of extraction methods from paraffin wax embedded tissues for PCR amplification of human and viral DNA. *J. Clin. Pathol., 54*; 401–403.

Buketov University