ISSN: 1220-3173; E-ISSN: 2457-7618

DNA ISOLATION FROM WHOLE BLOOD AND HAIR FOLLICLE SAMPLES IN CARPATHIAN GOATS

IZOLAREA DE ADN DIN SÂNGE INTEGRAL ȘI FOLICULI PILOȘI LA CAPRE DIN RASA CARPATINĂ

Maria Rodica GURĂU[1),*)], Dana Mihaela CREȚU[1)], D. COBZARIU[1)], F. OȚELEA[2)], Elena NEGRU[1)], T. IONESCU[1)], Anca Amalia UDRISTE[1)], Petruța CORNEA[1)], S. BĂRĂITĂREANU[1)]

ABSTRACT | REZUMAT

The use of hair follicle DNA is a simple, effective, and non-invasive method for obtaining the needed DNA for molecular biology diagnostic methods. In a previous study, we proved the value of this sampling method for goat DNA extraction and the success of using hair follicles as an alternative to whole blood samples. DNA quality and quantity must be optimal for the molecular biology tests and there are very important parameters because it intervenes in the quality of sequencing. Therefore, it is very important to choose the biological sample and the DNA isolation method in accordance with the type of the sample. In the present study, DNA was isolated from whole blood samples and hair follicles from Carpathian goats in order to determine the amount of DNA extracted from the two types of samples. The DNA quantity from whole blood samples was higher than the DNA quantity from hair follicles at the DNA values over 20 ng/µL in all 50 samples. Whole blood samples with DNA amounts counted between 10 ng/µL and 20 ng/µL were 34% and in hair follicles samples were 26%. In the case of DNA amounts under 10 ng/µL the hair follicles had a higher percentage of 34% sample while the whole blood samples had only 20%. Although there was less DNA in the hair follicles, the amount isolated from these samples was sufficient to trigger the specific DNA fragment by PCR amplification.

Keywords: Carpathian goats**,** total DNA isolation, hair follicles, whole blood samples

Utilizarea ADN-ului din foliculului piloși este o metodă simplă, eficientă și neinvazivă de obținere a cantității necesare de ADN pentru diagnosticul prin metode de biologie moleculară. Într-un studiu anterior, am demonstrat valoarea acestei metode de prelevare a probelor biologice necesare extracției ADN-ului de capră și succesul utilizării foliculilor piloși ca o alternativă la probele de sânge integral. Calitatea și cantitatea ADN-ului trebuie să fie optime pentru testele de biologie moleculară și sunt parametri foarte importanți deoarece intervin în calitatea secvențierii. Prin urmare, este foarte important să alegi proba biologică și metoda de izolare a ADN ului în funcție de tipul probei. În studiul de față a fost izolat ADN din probe de sânge și foliculi piloși de la capre din rasa Carpatină, pentru a determina cantitatea de ADN extrasă din cele două tipuri de probe. Cantitatea de ADN din probele de sânge a fost mai mare decât cantitatea de ADN din foliculii piloși la valorile ADN de peste 20 ng/µL în toate cele 50 de probe. Probele de sânge cu cantitatea de ADN între 10 ng/µL și 20 ng/µL au fost de 34% din total, iar probele din foliculii piloși au fost de 26%. În cazul unei cantități de ADN sub 10 ng/µL, foliculii piloși au avut un procent mai mare, de 34% probe din total, în timp ce probele de sânge au avut doar 20%. Chiar dacă în cazul foliculilor piloși au fost cuantificate cantități de ADN mai mici, cantitatea izolată din aceste probe a fost suficientă pentru amplificarea fragmentului de ADN specific prin PCR.

Cuvinte cheie: capre din rasa Carpatină, izolare totală de ADN, foliculi piloși, probe de sânge

The sample that is widely used as a source of DNA is the whole blood. The first isolation of DNA from leukocytes was made by Friedrich Miescher in 1869 and its protocols of DNA isolation were published two years later (3, 5, 12). However, to obtain the genomic DNA of an animal, a sufficient amount of tissue is taken.

The use of hair follicles as the DNA source is a simple, effective, and non-invasive method for obtaining the needed DNA for molecular biology diagnostic methods. In a previous study, we proved the value of this sampling method for goat DNA extraction and the success of using hair follicles as an alternative to whole blood samples (11). To have an optimal quantity and quality of DNA for the molecular biology assays, the sampling must be done correctly, simply, and following the regulations regarding animal welfare. DNA quality and quantity are important parameters because it in-

1) University of Agronomical Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Bucharest, Romania
2) Brăila County branch of the College of Romanian Veterinarians,
*) *Corresponding author: otelea_maria@yahoo.com*

tervenes in the quality of sequencing (1). Therefore, it is very important to choose the biological sample and the DNA isolation method in accordance with the type of the sample. Since it was developed many DNA isolation protocols based on different principles. There are two main categories: solution-based DNA extraction methods and solid-phase DNA extraction methods. Into the first DNA, isolation categories were included salting out methods and organic solvent/chaotropes methods. Into the second DNA isolation category, Glass milk/silica resin methods, anion exchange methods, and magnetic beads methods were included (2, 3, 17, 19). The solid-phase DNA extraction method from whole blood samples was first described in 1989 by McCormick. Since then numerous procedures have been optimized and used in several commercial extraction kits (4, 6, 10). In order to choose the most appropriate extraction method, it must take into consideration some criteria: the method should be sensitive, easy to do, to be adapted to the equipment from the laboratory, to have minimum risk for the users, and the most important to provide as much as pure and sufficient DNA isolate (3). The commercial kits that are used today can isolate DNA from various biological samples like whole blood, different tissues, fluids, and swabs. To choose a commercial kit that provides a sufficient amount of DNA must take into account that this depends also on the biological sample that it is used (4, 10). A non-invasive sampling method can use as a source of DNA, the animal hair that contains an important amount of DNA necessary for molecular biology tests. It was described in the literature different protocols for DNA isolation from hair, modified protocols because of the protein from the hair samples which requires additional steps in order to break down the cell and expose the DNA. One of these additional steps into the DNA isolation method for hair is the fragmentation of the sample that can be made either by a sterile knife or by a microscopic glass grinder always followed by an organic solvent treatment (7, 8, 9). An alternative to the invasive method of sampling whole blood from animals especially when are tested several samples is the sampling of hair with follicles and using the modified DNA isolation protocol (11). In the present study, DNA was isolated from whole blood samples and hair follicles from Carpathian goats to determine the amount of DNA extracted from the two types of samples.

MATERIALS AND METHODS

Animals and biological samples

Whole blood samples and hair follicles were collected from 50 Carpathian breed goats from Dudescu village (Braila county, Romani). There were 14 goat bucks and 36 does, with ages between 3 years and 17 years. Whole blood samples were collected in tubes with K3-EDTA. Rooting hair samples was done by plucking 30-40 strands of hair (required for one DNA extraction), given that they must contain hair follicles. Disposable gloves were used for each animal, changing gloves after each animal or sampling. The hairs with roots were stored in sterile, flat-bottomed 50 ml centrifuge tubes. On each tube was noted the number of the animal, the type of sample, the date of collection, the location. The samples were stored at room temperature until primary processing.

DNA isolation from whole blood

Isolation of genomic DNA is a non-specific step, in the sense that, in the end, all categories of DNA in the biological sample are isolated. The most commonly used technologies for DNA extraction are based on the ability to selectively bind silica membranes / adsorption of nucleic acids on the surface of paramagnetic particles. The advantages of these approaches are mainly the speed of obtaining nucleic acids with a satisfactory degree of purity for most subsequent applications, ease of use and standardization of workflow, and the possibility of automation of extraction.

The whole blood samples were subjected to DNA isolation using the PureLink Genomic DNA Kits (13). The protocol used for blood DNA isolation was previously described by Gurau et al. (2021). The elution of the DNA was made in 50 µL. After the DNA isolation the samples were stored at -20°C until the quantification step.

DNA isolation from hair follicles

A primary processing step was performed before the DNA extraction. At this stage, the hairs with follicles are removed from the tube in which they were stored, placed on a sterile Petri dish, and with the disposable scalpel, only the part that are containing the hair follicles was finely cut. This step and the DNA extraction were performed in a vertical laminar flow cabinet with the same kit used in the DNA blood isolation, PureLink Genomic DNA Kits (13) but with a modified protocol. After crushing the hair follicles, the sample thus processed was placed in a 2 ml Eppendorf tube. Pure Link Genomic Digestion Buffer digestion buffer and proteinase K were added to the hair follicle sample and the tube was incubated at 55 °C with vortexing for 1 hour. After incubation and centrifuge for 3 minutes at maximum speed, the supernatant was transferred to a new Eppendorf tube. Then RNase A was added which was required in the DNA extraction to destroy any amount of RNA and thus obtain a purified DNA extract. The next step was to bind the DNA. PureLink® Genomic DNA kits bind DNA using a silica-based membrane in the presence of salts. The lysate ~ 640 µl was transferred to the colony and centrifuged at 10,000 x g 1

Table 1

Quantification of the total DNA protocol with Qubit dsDNA HS kit

Working solution		Standards	10 µL	Samples	3 µL
Qubit dsDNA HS buffer	199 µL	Working solution	190 µL	Working solution	197 µL
Qubit dsDNA HS reagent	1 µL				
Total	200 µL	Final volume for reading 200 µL			

min after the addition of PureLink Genomic Lysis / Binding Buffer and ethanol 96 ° -100 ° of molecular biology purity. The washing step was performed with 2 wash buffer solutions. DNA elution was performed in 50 µl PureLink Genomic Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA). In the case of hair root samples, a digestion buffer was used before the lysis / binding buffer was added and the incubation time for cell lysis is one hour. (11).

DNA genomic quantification from whole blood and hair follicles

After DNA extraction, DNA quantification was performed in order to observe the results on the DNA concentrations in the biological samples. Total DNA was quantified using the Qubit dsDNA HS test kit (Invitrogen, Thermo Fisher Scientific, Applied Biosystems, US) in conjunction with the Qubit 4 fluorometer (14, 15). A sample concentration DNA is calculated by comparing the relative fluorescence units of the sample to the RFUs of the standards used in calibration. The detection limits of the measurements are specific to each assay. It was used 197 µL of the Qubit working solution and 3 µL DNA for each sample with a total volume of 200 µL (Table 1). For the standards was used 10 µL and 190 µL Qubit working solution. The standards were first read at the fluorometer and then the samples. The result can be measured in ng/µL or in ng/ml.

RESULTS AND DISCUSSIONS

From the data obtained of quantifying the DNA amount in the whole blood samples, the following values were obtained: at 4 samples (8%) values between 30.1 ng/µL (451 ng/ml) and 39.3 ng/µL (590 ng/ml), at 19 samples (38%) values between 21.5 ng/µL (322 ng/ml) and 29.8 ng/µL (447 ng/ml), at 17 samples (34%) values between 11.5 ng/µL (172 ng/ml) and 19.7 ng/µL (295 ng/ml) and at 10 samples (20%) values between 0.284 ng/µL (4.26 ng/ml) and 8.6 ng/µL (129 ng/ml) (Fig. 1; Table 2). The highest DNA amount recorded in whole blood samples was 39.3 ng/µL (590 ng/ml) and the lowest was 0.284 ng/µL (4.26 ng/ml). It can observe that 72% of the whole blood samples had amounts of DNA between 11.5 ng/µL (172 ng/ml) and 29.8 ng/µL (447 ng/ml) and only 8% had a high amount of DNA between 30.1 ng/µL (451 ng/ml) and 39.3 ng/µL (590 ng/ml). The lowest amount of DNA had been registered in only 20% of the whole blood samples.

From the whole blood samples that were collected from the goat bucks, 2 samples (14.28%) had DNA quantities of 30.1 ng/µL (451 ng/ml) and respectively 32.4 ng/µL (486 ng/ml) and in the rest of 12 goat bucks (85.71%) had been DNA amount between 3.21 ng/µL (48.2 ng/ml) and 28.4 ng/µL (426 ng/ml) (Fig. 2; Table 2).

From the whole blood samples that were collected from does, two of them (5.5%) had DNA amount of 39.3 ng/µL (590 ng/ml) and 31.9 ng/µL (478 ng/ml) and the 34 whole blood samples (94.4%) had quantities between 1.33 ng/µL (19.9 ng/ml) and 29.8 ng/µL (447 ng/ml). It can observe that either goat bucks or does had a similar percentage in the DNA amount covered up 29.8 ng/µL (447 ng/ml): (85.71%) goat bucks and (94.4%) does (Fig. 3; Table 2).

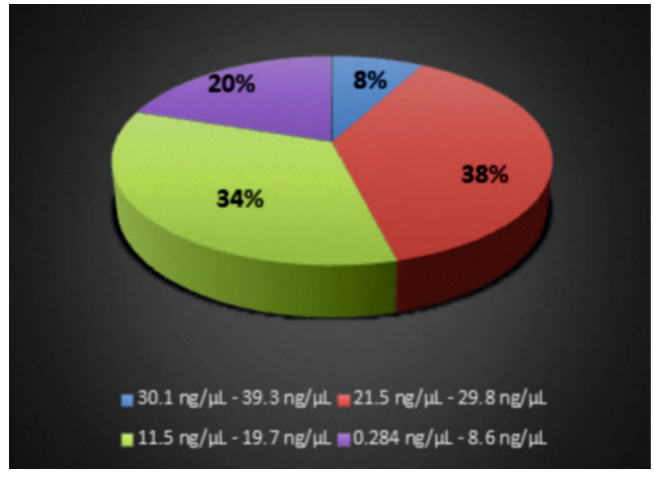

Fig. 1. Blood DNA quantities in 50 Carpathian breed goat samples

From the data obtained of quantifying the DNA amount in the hair follicle samples, the following values were obtained: at 8 samples (16%) values between 30 ng/µL (450 ng/ml) and > 600 ng/ml, at 12 samples (24%) values between 20.7 ng/µL (311 ng/ml) and

Table 2

The results of total DNA quantification of whole blood and hair follicle samples in 36 does

No.	Sample name	Sex	Whole blood ng/µL	Whole blood ng/ml	Hair follicle ng/µl	Hair follicle ng/ml
1	1C	M	28.4	426	3.77	56.5
2	2C	M	13.2	198	9.27	139
3	3C	M	7.67	115	12.1	182
4	4C	M	15.3	229	21.7	326
5	5C	M	16.3	245	18.3	274
6	6C	M	17.3	260	6.37	95.5
7	7C	M	6.8	102	24.8	372
8	8C	M	15.4	231	25.9	388
9	9C	M	18.5	278	29.9	449
10	26C	M	30.1	451	15.6	234
11	27C	M	3.21	48.2	18.6	279
12	28C	M	4.53	67.9	18.4	276
13	29C	M	22.9	343	20.7	311
14	30C	M	32.4	486	1.09	15.9
15	10C	F	24.4	366	3.73	55.9
16	11C	F	17.1	256	34.7	520
17	12C	F	25.1	376	28.3	424
18	13C	F	8.6	129	3.04	45.6
19	14C	F	14	210	8.8	132
20	15C	F	6.8	102	6.65	99.7
21	16C	F	13.1	197	9.13	137
22	17C	F	0.284	4.26	4.13	62
23	18C	F	29.1	436	4.45	66.7
24	19C	F	5.77	86.6	1.45	21.7
25	20C	F	39.3	590	30.5	458
26	21C	F	16.9	253	11.7	175
27	22C	F	15.3	230	2.73	41
28	23C	F	21.5	322	10.3	155
29	24C	F	28.6	429	12.9	193
30	25C	F	7.67	115	3.73	34.4
31	31C	F	15.7	235	6.47	97
32	32C	F	22.1	332	24.6	369
33	33C	F	1.33	19.9	24.7	370
34	34C	F	19.7	296	10.9	164
35	35C	F	23.9	358	11.1	167
36	36C	F	21.6	324	12.4	186
37	37C	F	11.5	172	16.7	251
38	38C	F	19.7	295	27.3	410
39	39C	F	24.1	362	16.3	244
40	40C	F	25.9	388	14.9	224
41	41C	F	25.3	379	34.7	520
42	42C	F	26.4	396	2.11	31.6
43	43C	F	29.6	444	31.9	478
44	44C	F	29.8	447	25.1	376
45	46C	F	17.5	263	22.9	344
46	47C	F	16.4	246	38.7	580
47	48C	F	29.3	440	31.3	469
48	49C	F	28.5	428	30	450
49	50C	F	28.1	421	21.1	317
50	51C	F	31.9	478	Too high	>600.0

29.9 ng/µL (449 ng/ml), at 13 samples (26%) values between 10.3 ng/µL (155 ng/ml) and 18.6 ng/µL (279 ng/ml) and at 17 samples (34%) values between 1.09 ng/µL (15.9 ng/ml) and 9.27 ng/µL (139 ng/ml) (Fig. 4; Table 2). The highest DNA amount recorded in hair follicle samples was > 600 ng/ml in the sample name 51C, from a doe, where the value in ng/µL was read as too high. The lowest DNA amount in hair follicles was 1.09 ng/µL (15.9 ng/ml).Also, it can observe that 50% of the hair follicle samples had amounts of DNA between 10.3 ng/µL (155 ng/ml) and 29.9 ng/µL (449 ng/ml) and only 16% had a high amount of DNA between 30 ng/µL (450 ng/ml) and > 600 ng/ml. The lowest amount of DNA had been registered in 34% of the hair follicle samples with values between 1.09 ng/µL (15.9 ng/ml) and 9.27 ng/µL (139 ng/ml).

From the hair follicles samples that were collected from the goat bucks, none of them had high DNA quantities between 30 ng/µL (450 ng/ml) and > 600 ng/ml. All the 14 hair follicle samples (100%) from goat bucks had DNA amounts between 1.09 ng/µL (15.9 ng/ml) and 29.9 ng/µL (449 ng/ml) (Fig. 2; Table 2).

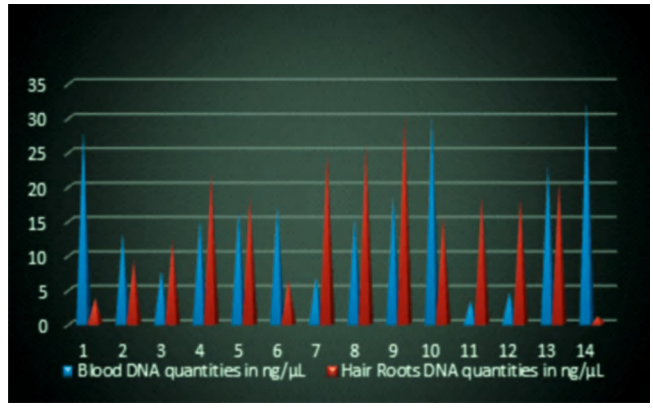

Fig. 2. Total DNA quantities in whole blood and hair follicle samples from bucks

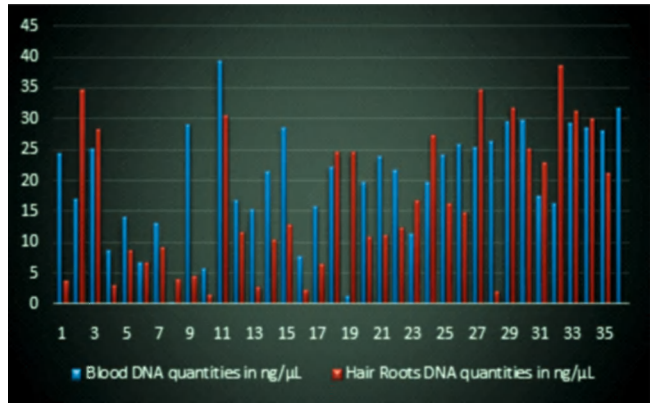

Fig. 3. Total DNA quantities in whole blood and hair follicle samples from does

From the hair follicle samples that were collected from does, 8 of them (16%) had DNA amount values between 30 ng/µL (450 ng/ml) and > 600 ng/ml and 28 hair follicles samples (77.77%) had quantities between 1.45 ng/µL (21.7 ng/ml) and 28.3 ng/µL (424 ng/ml). It can observe that there is a difference between the percentage in the DNA amount from goat bucks and does with 22.23% less for does of the DNA

values that are up to 28.3 ng/μL (424 ng/ml) (Fig. 3; table 2). Whole blood samples with DNA values above 20 ng/μL and more were 46% while hair follicle samples accounted 40% with DNA values above 20 ng/μL.

The DNA quantity from whole blood samples was higher than the DNA quantity from hair follicles at the DNA values over 20 ng/μL in all 50 samples. Whole blood samples with DNA amount counted between 10 ng/μL and 20 ng/μL were 34% and hair follicles samples were 26%. It can observe that whole blood samples had also higher values of DNA at the range between 10 ng/μL and 20 ng/μL. But in the case of DNA amount under 10 ng/μL the hair follicles had a higher percentage of 34% sample while the whole blood samples had only 20%. Even in the hair follicles were less quantity of DNA, the amount isolated from these samples was sufficient to trigger the specific DNA fragment by PCR amplification. The kit that was used for the detection of the amount of DNA dsDNA High-Sensitivity (HS) Assay Kit, can detect DNA from range 0,1 ng, meaning that is suitable for the samples of hair follicles that have a lower DNA quantity.

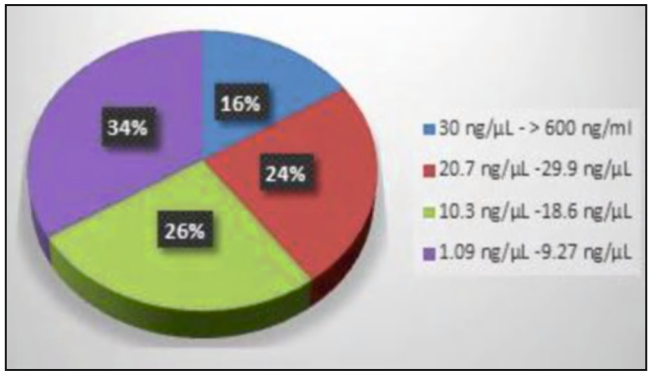

Fig. 4. The quantities of DNA from hair follicle samples

The use of a kit similar to the one used in this study has been cited in the literature and the DNA quantities that they succeed in isolation from hair was less than 120 ng/μL (18). Another scientific article compared methods of DNA isolation from hair and the amount of DNA was from 29.5 ng/μL to 218.5 ng/μL (20). This large range was dependent on the number of hair follicles used in the extraction and by the method. Pure Link Genomic DNA Kit used in this study has a principle based on the silica membrane selective binding of the DNA in the presence of salts and ethanol. The digestion is done by the proteinase K. It is also cited in the scientific articles that, in hair follicles samples, by the method based on ethanol precipitation were obtained DNA amounts from 29.5 ng/μL and by the methods based on proteinase K, from 188 ng/μL (20). Compared with the previous studies the results obtained in the case of DNA amount from hair follicles in this study are similar, 29.5 ng/μL in Zabek et al. (2005) study and 1.09 ng/μL- 38.7 ng/μL and > 600 ng/ml in our study. In the Zabek et al. 2005, the maximum DNA quantity was 218.5 ng/μL (20) witch is lower than in our study with more than 600 ng/ml. Ghatak et al. (2013) obtained values between 49 and 72 ng/μL of DNA amount in hair follicles and Otaño-Rivera et al. (2017) values between 50 and 150 ng/μL (7, 16). According to these studies, the DNA isolated from hair follicles in our study was in almost the same ranges.

CONCLUSIONS

DNA from hair follicles can be extracted by several methods, with some modification in the isolation protocol, adapted to the sample. Even in the hair folliclesA High-Sensitivity (HS) Assay Kit, can detect DNA from range 0,1 ng, meaning that is suitable for the samples of hair follicles that have a lower DNA quantity. The hair is an important sample and source of DNA, especially since the collection of the hair follicles is a non-invasive method that can replace the invasive method of whole blood sampling.

ACKNOWLEDGMENTS

The work was performed as part of CaPriRo project. CaPriRo has received funding from the USAMV Bucharest, Internal Research Projects 2021 under Grant Agreement No 2021-0004/13.07.2021.

REFERENCES

1. *Brajkovic V., Duvnjak I., Ferenčaković M., Špehar M., Raguž N., Lukić B., Curik I., Cubriccurik V.*, (2018), The effect of DNA quality on the sequencing success of cattle. J Cent Eur Agric, 19(4):804-809
2. *Carpi F.M., Di Pietro F., Vincenzetti S., Mignini F., Napolioni V.*, (2011), Human DNA extraction methods: patents and applications. Recent Pat DNA Gene Seq, 5(1):1-7
3. *Chacon-Cortes D., Griffiths L.R.*, (2014), Methods for extracting genomic DNA from whole blood samples:current perspectives. J Biorepos Sci Appl Med, 2:1-9
4. *Clark D.P., Pazdernik N.J.*, (2013), Polymerase Chain Reaction, in Molecular Biology, 2nd ed., (Ed.) Elsevier BV, Amsterdam, Netherlands, 163-193
5. *Dahm R.*, (2005), Friedrich Miescher and the discovery of DNA. Dev Biol, 278(2):274-288
6. *Eslami G., Khalatbari-Limaki S., Ehrampoush M. H., Gholamrezaei M., Hajimohammadi B., Oryan A.*, (2017), Comparison of three different DNA extraction methods for *Linguatula serrata* as a food born pathogen. Iran J Parasitol, 12(2):236-242
7. *Ghatak S., Muthukumaran R.B., Nachimuthu S.K.*,

(2013), A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. J Biomol Tech, 24(4):224-231
8. *Gilbert M.T.P., Djurhuus D., Melchior L., Lynnerup N., Worobey M, Wilson A.S., Andreasen C., Dissing J.*, (2007), mtDNA from hair and nail clarifies the genetic relationship of the 15th century Qilakitsoq inuit mummies. Am J Phys Anthropol, 133(2):847-853
9. *Guan Z., Zhou Y., Liu J., Jiang X., Li S., Yang S., Chen A.*, (2013), A simple method to extract DNA from hair shafts using enzymatic laundry powder. PloS one, 8(7):e69588
10. *Gupta N.*, (2019), DNA extraction and polymerase chain reaction. J Cytol, 36(2):116-117
11. *Gurău M.R., Crețu D.M., Negru E., Ionescu T., Udriste A.A., Cornea P., Bărăităreanu S.,* (2021), Comparative analysis of total DNA isolation procedure from blood and hair follicle samples in goats. Revista Romana de Medicina Veterinara, 31(4):82-86
12. *Holmes F.L.*, (2001), Meselson, Stahl, and the Replication of DNA: A History of the Most Beautiful Experiment in Biology, (Ed.) Yale University Press, New Haven, CT, USA
13. *Invitrogen*, (2012), Manual Kit PureLink Genomic DNA Kits. Thermo Fisher Scientific, Applied Biosystems, USA, Available at: https://tools.thermofisher.com/content/sfs/manuals/purelink_genomic_man.pdf (Accessed: October 14, 2021)
14. *Invitrogen*, (2015), Manual Kit Qubit dsDNA HS Assay Kits. Thermo Fisher Scientific, Applied Biosystems, USA, Available at: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/Qubit_dsDNA_HS_Assay_UG.pdf (Accessed: October 14, 2021)
15. *Invitrogen*, (2015), Qubit dsDNA HS and BR Assay Kits. Thermo Fisher Scientific, Applied Biosystems, USA, Available at: https://www.thermofisher.com/order/catalog/product/Q32851 (Accessed: October 14, 2021)
16. *Otaño-Rivera V., Boakye A., Grobe N., Almutairi M.M., Kursan S., Mattis L.K., Castrop H., Gurley S.B., Elased K.M., Boivin G.P., Di Fulvio M.*, (2017), A highly efficient strategy to determine genotypes of genetically-engineered mice using genomic DNA purified from hair roots. Laboratory animals, 51 (2):138-146
17. *Sambrook J., Russell D.W.,* (2001), Molecular cloning: a laboratory manual, (Ed.) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA
18. *Suenaga E., Nakamura H.*, (2005), Evaluation of three methods for effective extraction of DNA from human hair. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, 820(1):137-141
19. *Tan S.C., Yiap B.C.,* (2009), DNA, RNA, and protein extraction: the past and the present. J Biomed Biotechnol, 2009:574398
20. *Zabek T., Radko A., Slota E.*, (2005), Implications for the use of horse hair follicles as a DNA source for microsatellite typing. Czech J Anim Sci, 50(11): 499-502.