BLOOD DNA EXTRACTION FOR GENETIC ANALYSIS IN ENDANGERED ROMANIAN GREY CATTLE

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Abstract

The quality and quantity of DNA extracts used for genetic analysis are of utmost importance. The isolation of DNA from different types of samples can be done by classical methods or using specific kits, whose working methodology must strictly follow the protocol established by the manufacturer.

The aim of this research was to evaluate the effectiveness of DNA extraction from 50 blood samples of Romanian Grey cows found in various genetic conservation programs due to their small number in cattle herds. The obtained results are considered satisfactory in terms of the purity of the obtained DNA extracts, with only one sample (2% of the total) having the DNA isolate contaminated with proteins based on the absorbance ratio A260/A280 lower than 1.8.

Key words: DNA quality, DNA quantity, genetic preservation, spectrophotometry

INTRODUCTION

The deoxyribonucleic acid (DNA) molecule, formed by the polymerization of monomeric units named deoxyribonucleotides, provides informational support for storing, inheriting, and expressing of genetic information in protein molecules. Its double-stranded, helical, and antiparallel structure, with the complementarity between nitrogenous bases A=T, G≡C, was described in 1953 by James Watson (1928-) and Francis Crick (1916-2004) in the paper entitled "Molecular structure of Nucleic Acids – A structure for Deoxyribose Nucleic Acids" published in Nature journal, vol. 171, no. 4356, pp. 737-738 [6]. Their contribution and that of Maurice Wilkins (1916-2004) to the development of Medicine was rewarded in 1962 with Nobel Prize. It is worth mentioning that the discoveries of Watson and Crick were based on studies that demonstrated the complementarity of nitrogenous bases A=T, G≡C, published in 1950 by Erwin Chargaff (1905-2002) (1) and X-ray research of Rosalind Franklin (1920-1958) on DNA molecule, which was the mainstay in deducing the spatial configuration of this molecule [2], [5].

Denaturation of DNA under the influence of various thermal (t^0 = 63-100^{0}C) or chemical factors affects its double-stranded structure, with breaking the hydrogen bonds between complementary nitrogenous bases resulting, in this way, a DNA single-stranded structure. Consequently, the nitrogenous bases located inside of DNA molecule now become exposed in the single strand conformation, increasing their ability of ultraviolet radiation absorbance at λ = 260 nm, if we compare to the situation when they were arranged inside the double helix. This hyper chromic effect influences the value of the absorbance ratio of the working solutions at wavelengths of 260 and 280 nm,

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respectively (A260/A280), whose normal limits must be between 1.8 and 2 [3], [2] although some authors admit values between 1.7 and 2 [1], [4]. Values lower than this range indicate higher absorbance and, implicitly, higher protein concentrations in the working samples, which absorb ultraviolet light at λ = 280 nm; on the other hand, ratio values greater than 2 show a RNA contamination.

The aim of this paper is to present a protocol of DNA extraction from cow blood samples of Romanian Grey cattle, as a preliminary work to the genotyping of genetic markers of interest in the conservation of this endangered breed.

MATERIALS AND METHODS

Investigations of molecular biology related to the aim of this study were performed at the Research and Development Station for Bovine Arad. The biological material used in the research consisted of 50 blood samples collected from Romanian Grey cows, a nucleus in genetic preservation at the Research and Development Station for Bovine, Dancu - Iași. Genomic DNA was isolated from 300 µl blood collected in vacutainers containing K3EDTA as anticoagulant; the total genomic DNA was isolated using the manual kit Wizard Genomic DNA Purification Kit (Promega, USA). For the isolation of total genomic DNA, three stages were performed: (i) cell lysis, (ii) nucleus lysis and protein precipitation, and (iii) DNA precipitation and rehydration. The total amount of DNA resulting from precipitation was resuspended in sterile distilled water and spectrophotometrically evaluated with NanoDrop-2000 (Thermo Fisher Scientific, MA, USA). The assessment of DNA concentration was performed using spectrophotometry technique, and its integrity, by agarose gel migration technique. The purity of DNA samples was evaluated based on the absorbance ratio (A260/A280), and its concentration was automatically calculated by NanoDrop 2000 spectrophotometer software.

The results of the laboratory determinations were statistically interpreted using a series of basic indicators, such as the arithmetic mean, standard deviation, standard error and confidence limit of the mean, coefficient of variation.

RESULTS AND DISCUSSIONS

There are several researches on methods of DNA isolation from blood, but many of them are time consuming and expensive. In this study a rapid, sensitive, and efficient method of DNA extraction from blood of cattle is presented.

Following the spectrophotometric analysis using the NanoDrop 2000 spectrophotometer, for all DNA samples were obtained values of DNA concentration between a minimum of 25 ng/µl, and a maximum of 161.6 ng/µl, with an average of 65.77 ng/µl (table 1, fig.1). All results obtained for DNA purity and concentration, using the spectrophotometry technique, are found in table 2 and figure 1.

Table 1 Statistical parameters for 50 samples DNA considering its concentrations and A260/A280 absorbance ratio

Statistical parameter	DNA Concentration (ng/µl)	A260/A280
Minimum	25	1.73
Maximum	161.6	2.53
Average	65.77	2.05
Standard deviation	±26.89	0.16
Standard error	±3.80	0.02
Limit of confidence	±7.64	0.04
Coefficient of variation (%)	40.88	7.8

Data presented in Table 1 show an average value of DNA concentration in the investigated samples of 65.77 ng/µl, and an average absorbance ratio of 2.05, slightly above the desired limit range of 2. Analysis of the coefficient of variability for the data

set representing DNA concentration shows that the calculated average value can be considered representative only in a broad sense (the data set is less homogeneous), while the average value of the absorbance ratio is even very representative for the available data set (homogeneous data, grouped around the average) (as observed in figures 1 and 2).

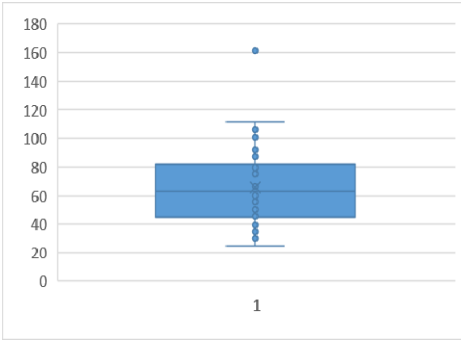

Fig. 1 BoxPlot of the DNA concentration

Fig. 2 BoxPlot of the absorbance 260/A280 ratio

Table 2 The DNA concentration (ng/µl) and A260/A280 ratio calculated using NanoDrop 2000 spectrophotometer

No. of sample	DNA concentration (ng/µl)	A260	A280	A260/A280 ratio
1.	**46.4**	0.927	0.439	2.11
2.	**100.6**	2.012	1.159	1.74
3.	**67.5**	1.35	0.649	2.08
4.	**161.6**	3.232	1.653	1.96
5.	**111.5**	2.231	1.139	1.96
6.	**75.2**	1.504	0.754	1.99
7.	**51.9**	1.037	0.528	1.96
8.	**106.1**	2.123	1.124	1.89
9.	**69.7**	1.394	0.724	1.93
10.	**62.2**	1.244	0.616	2.02
11.	**58.2**	1.164	0.566	2.06
12.	**79.5**	1.589	0.805	1.97
13.	**33.6**	0.671	0.335	2
14.	**79.4**	1.589	0.847	1.88
15.	**56.1**	1.122	0.533	2.11
16.	**80.7**	1.614	0.816	1.98
17.	**30.3**	0.606	0.289	2.09
18.	**83.5**	1.671	0.834	2
19.	**54**	1.081	0.538	2.01
20.	**77.2**	1.544	0.848	1.82
21.	**78.9**	1.579	0.793	1.99
22.	**51.3**	1.025	0.497	2.06
23.	**55.7**	1.114	0.55	2.03
24.	**90.2**	1.803	0.905	1.99
25.	**62.8**	1.257	0.61	2.06
26.	**43**	0.861	0.404	2.13
27.	**96.3**	1.927	1.04	1.85
28.	**87.1**	1.743	0.88	1.98
29.	**81.1**	1.622	0.872	1.86
30.	**88.5**	1.769	0.922	1.92
31.	**103.7**	2.073	1.049	1.98

32.	**63.6**	1.273	0.637	2
33.	**92.1**	1.842	0.938	1.96
34.	**83.5**	1.67	0.854	1.96
35.	**30.9**	0.619	0.252	2.46
36.	**34.6**	0.691	0.316	2.19
37.	**45.7**	0.914	0.499	1.83
38.	**39.9**	0.798	0.378	2.11
39.	**76.3**	1.526	0.773	1.97
40.	**50.2**	1.005	0.469	2.14
41.	**39.5**	0.79	0.363	2.17
42.	**31**	0.62	0.278	2.23
43.	**25**	0.5	0.198	2.53
44.	**31.5**	0.631	0.256	2.46
45.	**32.5**	0.649	0.272	2.38
46.	**52.3**	1.047	0.49	2.14
47.	**33.3**	0.666	0.285	2.34
48.	**66.5**	1.33	0.631	2.11
49.	**60.1**	1.201	0.55	2.18

The analysis of the data presented in table 2 shows that out of the total of the 50 samples, half had values of the absorbance ratio A260/A280 higher than the ideal value 2. In the interval that is considered a sample with pure isolated DNA (1.8-2) are found 24 samples (48%), one sample (2%) having an absorbance ratio value less than 1.8. As aforementioned, a value of the absorbance ratio less than 1.8 characterizes a contamination of the DNA isolate with proteins, which absorb ultraviolet light with a wavelength of 280 nm, increasing the value of their absorbance and causing a decrease in the total value of absorbance ratio A260/A280. From a technical point of view, in such situations the step of protein precipitation of the DNA extraction protocol is repeated. On the other hand, a value of the absorbance ratio exceeding the range 1.8-2 shows a contamination of the DNA extract with RNA which usually does not affect the DNA analysis (at much higher values, the degradation of the contaminating RNA is done by use of RNA enzymes).

CONCLUSIONS

Laboratory analysis of 50 blood samples of Romanian Grey cows for DNA extraction demonstrated the effectiveness of the manual kits for isolating DNA samples. With the exception of a single sample (2% of the total) for which the value of the A260/A280 absorbance ratio indicates a protein contamination, the remaining samples either met the normal purity parameters or had minor RNA contaminations that did not interfere with the next stages of work.

REFERENCES

[1] Chargaff E., 1950: Chemical specificity of nucleic acids and mechanism of their enzymatic degradation. Experientia nr. 6, p. 201–209.
[2] Dnagenotek, 2020 – Animal Genetics and Microbiome, Available online at: https://dnagenotek.com/ROW/products/collection-animals/index.html, (Accessed on: 20.09.2020).
[3] Kamangu N. E., 2019: Comparison of the quality of the DNA extracted from different media at the Laboratory of molecular biology of the faculty of medicine of UNIKIN. International Journal of Molecular Biology nr. 4(1): p. 27-28.
[4] Piskata Z., Servusova E., Babak V., Nesvadbova M., Borilova G., 2019: The Quality of DNA Isolated from Processed Food and Feed via Different Extraction Procedures. Molecules nr. 24(6): p. 1188.
[5] Schmitzer H., Tierney D., Gregory Braun G., 2010: How Rosalind Franklin Discovered the Helical Structure of DNA: Experiments in Diffraction. The Physics Teacher nr. 49(3): p. 1021.
[6] Watson J.D., Crick F. H. C., 1953: Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid. Nature, nr. 171: p. 737–738.