Scientific Papers. Series D. Animal Science. Vol. LXV, No. 1, 2022
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

TESTING THE EFFECTIVENESS OF TWO METHODS OF EXTRACTING DNA FROM BLOOD SAMPLES FROM COWS

Madalina Alexandra DAVIDESCU, Daniel SIMEANU, Cristina SIMEANU, Mihaela IVANCIA, Steofil CREANGA

Iasi University of Life Sciences (IULS), Faculty of Food and Animal Sciences, M. Sadoveanu Alley, no. 3, 700490, Iasi, Romania

Corresponding author email: mada.davidescu@gmail.com

Abstract

This research aims to validate the most effective method of extracting DNA from a number of 20 blood samples collected from cows. Two methods were tested, namely: DNA extraction using a manual extraction kit-Promega and automatic DNA extraction, using the Maxwell 16 LEV Blood DNA kit-Promega. Following the quantification of DNA samples, by spectrophotometry technique, the best results were obtained by applying the automatic extraction method (77.37 ng/µl DNA concentration obtained by automatic extraction compared to 14.95 ng/µl DNA concentration obtained by manual extraction). Therefore, the effectiveness of this technique has been demonstrated, representing a first step in genomic analysis protocols. The accuracy of the subsequent results depends to a large extent on the results obtained by extracting the DNA from the samples. Therefore, the automatic DNA isolation method is recommended because it has a number of advantages: accuracy, reduced analysis time, low costs, reduced labor and ease of application. This technique can be successfully applied in the analysis of genetic diversity of different animal species.

Key words*: blood samples, cows, DNA isolation, genetic analysis.*

INTRODUCTION

The most widely used biological samples in the genomic analysis of cattle are whole blood. DNA (deoxyribonucleic acid) is extracted from these samples by various working protocols. Since its inception in 1869, DNA extraction has progressed significantly. It's the first stage in many of the molecular biology's downstream applications (Tan & Yiap, 2009). These techniques range from extremely simple manual processes to more advanced automated DNA extraction strategies (Chacon-Cortes & Griffiths, 2014). Although the molecule DNA was discovered in 1869, it was not until 1943 that its involvement in genetic heredity was proved. James Watson and Francis Crick discovered that DNA is a double-helix polymer, a spiral made up of two DNA strands twisted around each other, in 1953, with the help of biophysicists Rosalind Franklin and Maurice Wilkins. Scientists gained a better grasp of DNA replication and hereditary control of cellular activity as a result of the breakthrough (Travers & Muskhelishvili, 2002; Watson, 1953). As a result, nucleic acid extraction is an important step in the laboratory processes needed to conduct additional molecular research. While studying the chemical makeup of cells, Friedrich Miescher became the first scientist to isolate DNA. In 1869, he undertook research to extract and identify proteins found in leukocytes obtained from samples on fresh surgical bandages.

During his research, he discovered an unique chemical in the nuclei that he named "nuclein." He then devised two techniques for separating the nucleus of cells from their cytoplasm and isolating this unique substance, now known as DNA (Dahm, 2005). DNA extraction techniques have now been modified to extract DNA from a wide range of biological sources.

The analysis carried out in this research focused on testing the effectiveness of two methods of extracting DNA from blood samples, collected from cows, respectively the manual method and the automatic extraction method. Thus, the main purpose of this research is to validate the optimal DNA extracting method from blood samples collected from a number of 20 cows, the first key step in genomic analysis in cattle.

MATERIALS AND METHODS

In this research, the 20 blood samples were collected by puncturing the jugular vein of cows, using vacutainer with EDTA (ethylene-diamine-tetra-acetic) to prevent clotting. Samples were numbered from C1 to C20 (C-cows). Two methods were used to extract the DNA from blood samples, in order to test their effectiveness, respectively: in the case of the first method, the total genomic DNA was isolated using the Wizard Genomic DNA Purification kit – Promega and in the case of the second method, the isolation of the DNA from the blood samples was done by the automated method, with Maxwell equipment ™ 16 and 16 MDx instruments, using a kit special, 48 Maxwell ™16 MCD LEV-Promega. The purity of the extracted DNA samples was assessed based on the A260/A280 ratio and the concentration of the samples was automatically calculated with the NanoDrop-2000 spectrophotometer software.

RESULTS AND DISCUSSIONS

In order to isolate the total genomic DNA, in the case of the first method, 3 stages were completed: cell lysis, nucleus lysis and protein precipitation and DNA precipitation and rehydration. Rehydrated DNA was stored at -20°C temperature. The purity of the extracted DNA samples was assessed based on the A260/A280 ratio and the concentration of the samples was automatically calculated with the NanoDrop 2000 spectrophotometer software. This spectrophotometry method is based on the following principle: most substances of nature shows a characteristic absorption rate in the field of ultraviolet radiation (UV). Thus, the absorption rate of 260 nm corresponds to the DNA/RNA nucleic acids, that of 280 nm for proteins and 230 nm for various contaminants (Cojocaru et al., 2009). According to the literature, DNA is considered pure enough if the ratio of the two readings, respectively A260/A280, has values in the range 1.7-2.0. Values lower than 1.7 indicate protein impurities and higher than 2.0 impurities with other contaminants (Kamangu, 2019). According to Beer Lambert's law, there is a linear relationship between concentration of a compound and its absorbance at a certain wavelength (Piskata et al., 2019). It is based on this fact calculating the concentration of DNA, making assessments on its purity in relation with protein. In the case of the first method of DNA extraction, using manual Wizard Genomic DNA Purification kit- Promega, following spectrophotometry for all DNA samples, DNA concentration values between 7.0 and 28.6 ng/µl were obtained (Table 1).

Table 1. Spectrophotometric quantification of total DNA extracted from blood samples of cows (using manual Wizard Genomic DNA Purification kit- Promega-*first method*)

Samples	Abs260	Abs280	260/280	260/230	DNA conc. (ng/µl)
C1	0.573	0.354	1.62	0.82	28.6
C2	0.442	0.293	1.51	1.00	22.0
C3	0.473	0.310	1.53	1.08	23.6
C4	0.254	0.185	1.37	1.02	12.6
C5	0.183	0.120	1.53	1.00	9.1
C6	0.182	0.106	1.72	1.01	9.0
C7	0.334	0.205	1.63	1.18	16.7
C8	0.272	0.193	1.41	1.13	13.5
C9	0.159	0.113	1.41	0.95	7.90
C10	0.270	0.171	1.58	1.16	13.4
C11	0.272	0.188	1.45	1.30	13.5
C12	0.340	0.218	1.56	1.20	17.0
C13	0.379	0.254	1.49	0.97	18.9
C14	0.266	0.193	1.38	1.09	13.3
Samples	**Abs260**	**Abs280**	**260/280**	**260/230**	**DNA conc. (ng/µl)**
C15	0.243	0.150	1.62	1.11	12.1
C16	0.217	0.152	1.43	1.08	10.8
C17	0.244	0.154	1.58	1.30	12.2
C18	0.146	0.096	1.52	0.98	7.3
C19	0.141	0.108	1.31	1.06	7.0
C20	0.251	0.213	1.18	0.94	12.5

From the table it can be seen that the minimum value of the DNA concentration in the blood samples was 7.0 ng/µl, while the maximum value was 28.6 ng/µl. The ratio of the two absorption rates, respectively A260/A280, presented values between 1.18 and 1.72. Figure 1 shows the values of the DNA concentration in the 20 samples of blood.

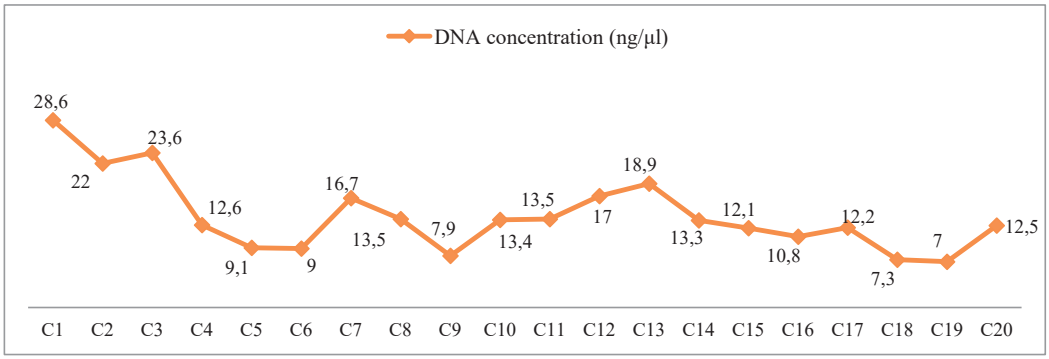

Figure 1. Graphical representation of DNA concentration values, measured with the Nanodrop 2000 (ng/µl) *first method*

Figure 2 shows the difference between the minimum, maximum and average values of the DNA concentration in the blood samples, resulting from the manual extraction method, respectively 7.0/28.6/14.05 ng/µl.

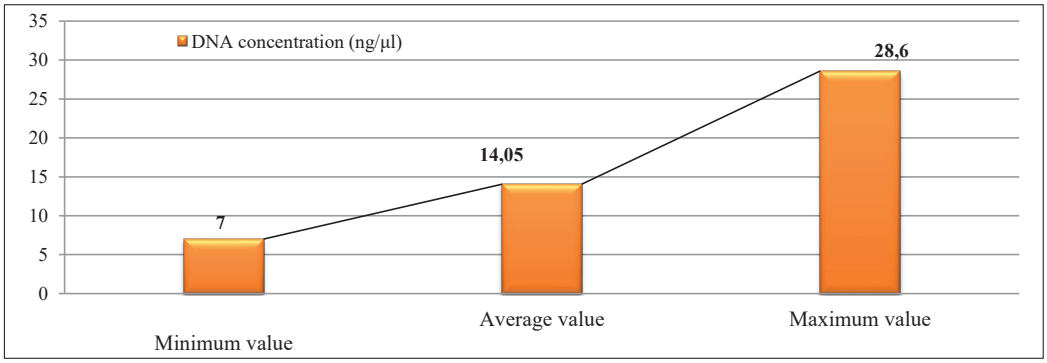

Figure 2. Average value of extracted DNA concentration, relative to minimum / maximum values (ng/µl)- *first method*

Regarding the purity of the extracted DNA, evaluated on the basis of the report of A260/A280 absorbents, the values obtained can be framed in 3 intervals (<1.7; 1.7-2.0). Therefore, it can be stated that insignificant contamination with protein substances. Proteins have the ability to absorb ultraviolet light with a wavelength λ = 280 nm, which leads to an increase in the absorbance value and at the same time a decrease in the ratio absorbents A260/A280, the solution in this situation being the repetition of the process of precipitation of proteins. In the case of the second method of DNA extraction, using Maxwell equipment ™16 and 16 MDx instruments, and a special kit, 48 Maxwell ™16 MCD LEV-Promega, following spectrophotometry for all DNA samples, DNA concentration values between 33.6 and 161.6 ng/µl were obtained (Table 2).
From the Table 2 it can be seen that the minimum value of the DNA concentration in the blood samples was 33.6 ng/µl, while the maximum value was 161.6 ng/µl. The ratio of the two absorption rates, respectively A260/A280, presented values between 1.22 and 2.53. Figure 3 shows the values of the DNA concentration in the 20 samples of blood.

Table 2. Spectrophotometric quantification of total DNA extracted from blood samples of cows (using Maxwell equipment ™16 and 16 MDx, with 48 Maxwell ™16 MCD LEV-Promega kit-*second method*)

Samples	A_{260}	A_{280}	A_{260}/A_{280}	A_{260}/A_{230}	DNA conc. (ng/µl)
C1	3.232	1.653	1.96	1.8	161.6
C2	2.231	1.139	1.96	2.17	111.5
C3	1.504	0.754	1.99	1.96	75.2
C4	1.394	0.724	1.93	1.7	69.7
C5	1.244	0.616	2.02	1.95	62.2
C6	1.164	0.566	2.06	2.08	58.2
C7	1.589	0.805	1.97	1.78	79.5
C8	0.671	0.335	2.0	1.22	33.6
C9	1.589	0.847	1.88	2.53	79.4
C10	1.544	0.848	1.82	1.42	77.2
C11	1.579	0.793	1.99	2.16	78.9
C12	1.025	0.497	2.06	2.31	51.3
C13	1.114	0.55	2.03	2.44	55.7
C14	1.927	1.04	1.85	1.76	96.3
C15	1.523	0.754	2.02	2.12	76.1
C16	1.743	0.88	1.98	2.31	87.1
C17	2.073	1.049	1.98	2.37	103.7
C18	1.273	0.637	2.0	2.05	63.6
C19	1.842	0.938	1.96	2.41	92.1
C20	0.691	0.316	2.19	1.81	34.6

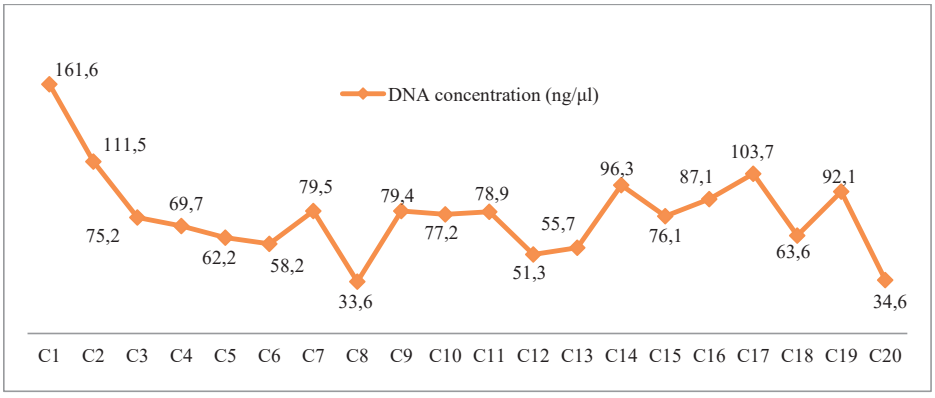

Figure 3. Graphical representation of DNA concentration values, measured with the Nanodrop 2000 (ng/µl)-*second method*

Figure 4 shows the difference between the minimum, maximum and average values of the DNA concentration in the blood samples, resulting from the manual extraction method, respectively 33.6/77.375/161.6 ng/µl.

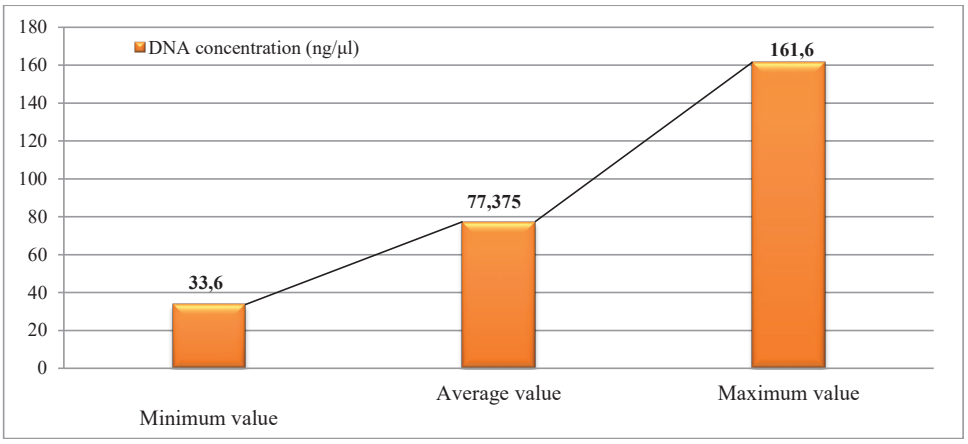

Figure 4. Average value of extracted DNA concentration, relative to minimum/maximum values (ng/µl)- *second method*

Regarding the purity of the extracted DNA, evaluated on the basis of the report of A260/A280 absorbents, the values obtained can be framed in 3 intervals (1.8-2.0; ≥2).

In this case, the value of the absorbance ratio A260/A280 was in the desired range (1.7-2.0), therefore the presence of other contaminants in the samples is total excluded and thus demonstrates the effectiveness of the automatic method of extracting total genomic DNA from blood samples.

The concentrations of the DNA samples were much lower in the case of the first extraction method compared to the results obtained after the application of the second extraction method (Figure 5).

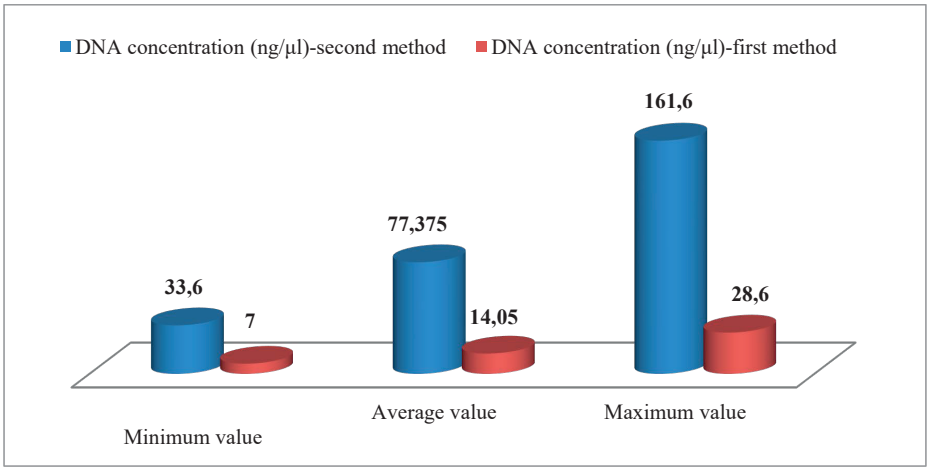

Figure 5. Comparison between the results obtained after performing the two methods of DNA extraction from the 20 blood samples

The major differences between the results are clear. The average values of DNA concentrations resulting from manual kit extraction were only 14.05 ng/µl, while the average values of DNA concentrations resulting from automatic extraction were 77.37 ng/µl. Therefore, a difference of 63.32 ng/µl is observed between the two average values.

CONCLUSIONS

The results of this research demonstrated the effectiveness of the method of automatic extraction of total genomic DNA (using Maxwell equipment ™ 16 and 16 MDx instruments, using a special kit, 48 Maxwell ™ 16 MCD LEV-Promega) from blood samples

collected from cows, the concentration values of the DNA samples obtained by this method, being much higher compared to the results obtained by aliquoting the extraction method using the manual extraction kit (14.05 ng/µl-manual extraction of DNA compared to 77.37 ng/µl-automatic extraction of DNA). Therefore, in performing molecular genetics analysis, it is recommended to use the method of automatic DNA extraction, as a first step to obtain conclusive and highly accurate results.

REFERENCES

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

Cojocaru, D., Toma, O., Cojocaru, S.I., & Ciornea, E. (2009). *Practicum of the biochemistry of proteins and nucleic acids*. Iași, RO: Tehnopress Publishing House (in Romanian).

Dahm, R. (2005). Friedrich Miescher and the discovery of DNA. *Dev Biol*., *278*(2), 274–288.

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, 4*(1), 27-28.

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*, *24*(6), 1188-1194.

Tan, S.C., & Yiap, B.C. (2009). DNA, RNA, and protein extraction: the past and the present. *J. Biomed. Biotechnol*., 574-585.

Travers, A., & Muskhelishvili, G. (2002). DNA structure and function. *FEBS J*., *282*(12), 2279-2295.

Watson, J.D., & Crick, F.H.C. (1953). Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid. *Nature*, 171, 737–738.