Comparative Analysis of Two DNA Extraction Methods Used in Genomic Research in Cattle

Madalina Alexandra Davidescu[1*], Daniel Simeanu[1], Cristina Simeanu[1], Mihaela Ivancia[1], Ștefofil Creangă[1]

[1]Iasi University of Life Sciences (IULS): Mihail Sadoveanu Alley, no. 3 - 700490, Iasi, Romania

Abstract

DNA (deoxyribonucleic acid) extraction is the first key step in the success of a genetic analysis. This research aims to validate the optimal method of DNA extraction from a number of 30 blood samples collected from Holstein cattle breed. Two methods of DNA extraction were tested, manual method (using Wizard Genomic DNA extraction kit) and automatic method (with Maxwell equipment). The results were interpreted statistically, finding that the average DNA concentration extracted by automatic method was 27.82 µg/µl compared to the average value of 18.01 µg/µl, obtained by manual method, the difference between the two values being quite high, 9.81 µg/µl. Following the application of the T-student test, with unequal variances, a value P = 1.41E-08 < 0.05 resulted, which means that there are statistically significant differences regarding the concentration of the samples of DNA extracted by the two methods, the highest value of the concentration being obtained after the application of the automatic method. The accuracy of the results, the purity of the samples, the short analysis time and the lack of contamination of the samples are just some of the advantages of the automatic method of DNA extraction, which is recommended to be used in molecular genetics studies.

Keywords: automatic extraction of DNA, deoxyribonucleic acid, genetic analysis, manual extraction of DNA

1. Introduction

Many of the possible downstream applications in the field of molecular biology involve the extraction of deoxyribonucleic acid (DNA) [1]. DNA can be extracted from many biological sources, such as hair, nails, bones, but the most widely used source of whole DNA remains blood. DNA extraction protocols are varied, depending largely on the type of method applied, manual or automatic.

Given that DNA isolation is a first step in genomic analysis, the choice of extraction method follows a number of aspects: the purity of the extracted DNA, the efficiency of working time and costs. Blood yields more genomic gDNA and is less fragmented than other less invasive sources, such as saliva or buccal cells [2-3]. RBCs, WBCs-cells, platelets and plasma are all found in whole blood, with gDNA in the nuclei of WBCs. Blood gDNA is of high quality and it is utilized in forensics, cancer diagnosis and a variety of other biological procedures [4]. DNA extraction has evolved from using harsh chemicals such as chloroform to a method called solid phase extraction (SPE) [5] to automatic extraction. Since the middle of 20st century, DNA extraction has grown into a wide range of laboratory procedures. Researchers throughout the world typically choose a method for DNA extraction depending on the availability of equipment, samples, extraction efficiency and technical requirements [6]. The main purpose of this research is to validate the optimal DNA extracting method from blood samples collected from a number of 30 cows, the first key step in genomic analysis in cattle.

*Corresponding author: Madalina Alexandra Davidescu, (+40) 0746511042, mada.davidescu@gmail.com

2. Materials and methods

The biological material consisted of 30 DNA samples, isolated from a lot of 30 females of Holstein breed, age 12 to 26 months. For each cow, the DNA sample were isolated from a quantity of 300 µl blood, collected through the jugular vein puncture, in EDTA anticoagulant tubes. The effectiveness of two methods of DNA extraction was tested, namely the manual method (using the Wizard Genomic DNA extraction kit, Promega USA) and automatic method (with Maxwell equipment ™ 16 and 16 MDx instruments, using a special kit, 48 Maxwell ™ 16 MCD LEV-Promega). The principle of both methods is based on the extraction of DNA from blood samples using either the manual kit - Wizard Genomic DNA extraction kit, in this case, or an automatic extraction equipment, in this case Maxwell® 16 LEV Blood DNA Kit. The steps of the manual extraction method protocol involve three main steps, respectively: Stage I-Cell lysis; Stage II-Lysis of nuclei and protein precipitation and Stage III-DNA precipitation and rehydration. The automatic method involves the lysis stages of cells by treatment with specific buffers, lysis nuclei, protein precipitation and DNA precipitation, processing up to 16 samples in 40 minutes and the extracted DNA can be used in a variety of applications, including PCR. The effective DNA extraction method was validated after spectrophotometric quantification of the DNA samples, using the Nanodrop-2000 spectrophotometer and also the purity of the DNA extracts obtained by both manual and automatic extraction was followed. The Excel database consisted of two sets of data, namely a set of data on the concentration of DNA extracted by the manual method and another set of data on the concentration of DNA extracted by the automatic method, concentrations measured by spectrophotometry and expressed in µg/µl. Statistical interpretation of the results started from formulation of statistical hypotheses: null and alternative, respectively H_0 and H_A; Null hypothesis: H_0 - the data are not related, they are independent/the compared values do not differ from each other (there are no statistically significant differences in the concentration of DNA samples extracted by the two methods - automatic and manual); Alternative hypothesis: H_A - the data are related, are dependent/the compared values differ from each other (there are statistically significant differences in the concentration of DNA samples extracted by the two methods - automatic and manual). The validation of the effectiveness of the DNA extraction methods was performed following the testing of the two statistical hypotheses, applying the T-student statistical test. The Student's t test (also known as the T test) compares the means of two groups without the requirement for multiple comparisons because a single P value is seen [7-8]. It's one of the most common statistical hypothesis tests [9-10]. The statistical analysis of the data was carried out based on the Excel stat program.

3. Results and discussion

DNA samples isolated from the blood by the two methods - manual and automatic, were quantified by spectrophotometry, using the 2000 Nanodrop spectrophotometer.

Spectrophotometry 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 [11]. It is based on this fact calculation of the DNA concentration, making assessments on its purity in relation with proteins [11]. The yield of DNA results by spectrophotometric quantification (µg/300 µl blood) are shown in table 1.

In the case of the automatic extraction method, the values of the DNA concentration, for the 30 samples, measured by spectrophotometry, vary from the minimum value of 18.4 µg to 42.2 µg/ 300 µl blood *(Fig. 1)* and in the case of the manual extraction method the range of values is between 11.5 µg and 27.63 µg/ 300 µl blood *(Fig. 2)*. There is a significant difference between the minimum and maximum values of the DNA concentration extracted by the two methods (18.4 µg/300 µl blood, the minimum value of the DNA concentration, resulting from the automatic method compared to the minimum value of the DNA concentration resulting from the manual method, which was only 11.5 µg, with 6.9 µg/ 300 µl blood less in the case of the second method, a significant difference in the case of genomic analysis)

Table 1. Values of DNA concentration extracted by manual or automatic method (µg/300 µl blood)

Samples No.	Results of DNA concentration (µg/300 µl blood) extracted by:		Difference (1-2) (µg/µl)
	automatic method (1)	manual method (2)	
1.	35.6	27.63	7.97
2.	29.2	21.01	8.19
3.	28.6	23.45	5.15
4.	22.0	12.31	9.69
5.	21.0	12.3	8.7
6.	26.1	11.5	14.6
7.	23.4	15.87	7.53
8.	32.4	13.21	19.19
9.	32.1	21.0	11.1
10.	29.8	13.23	16.57
11.	27.7	13.0	14.7
12.	24.5	16.87	7.63
13.	18.9	18.65	0.25
14.	21.8	13.11	8.69
15.	24.6	11.87	12.73
16.	24.2	17.32	6.88
17.	22.3	22.02	0.28
18.	35.7	20.2	15.5
19.	18.4	18.21	0.19
20.	24.3	24.11	0.19
21.	42.2	15.5	26.7
22.	21.4	18.22	3.18
23.	19.2	19.0	0.2
24.	29.4	25.2	4.2
25.	39.7	21.5	18.2
26.	20.2	20.01	0.19
27.	30.1	22.1	8.0
28.	41.0	19.4	21.6
29.	38.6	16.7	21.9
30.	30.2	16.0	14.2

.

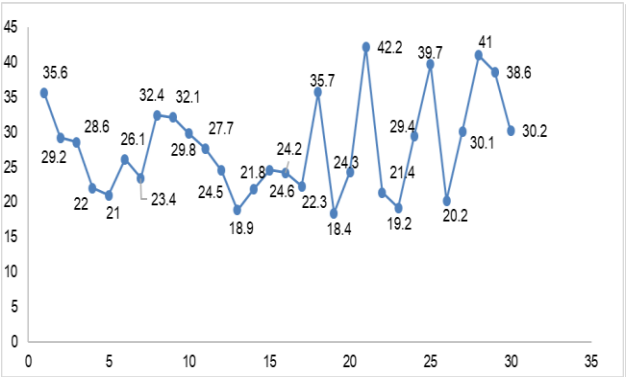

Figure 2. Graphical representation of DNA concentration values–automatic extraction method (µg/300 µl blood)

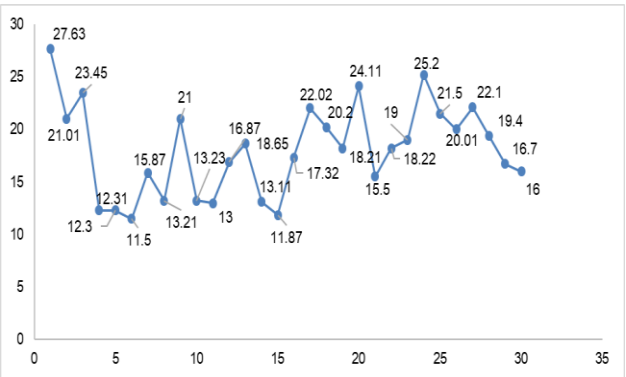

Figure 2. Graphical representation of DNA concentration values–manual extraction method (µg/300 µl blood)

The average DNA concentration for the 30 samples extracted by the automatic method was 27.82 µg / 300 µl blood compared to the average value of 18.01 µg/300 µl blood, obtained by extracting the 30 DNA samples by the manual method, the difference between the two values being quite high, exactly 9.81 µg/ 300 µl blood (Fig. 3 and Fig. 4).

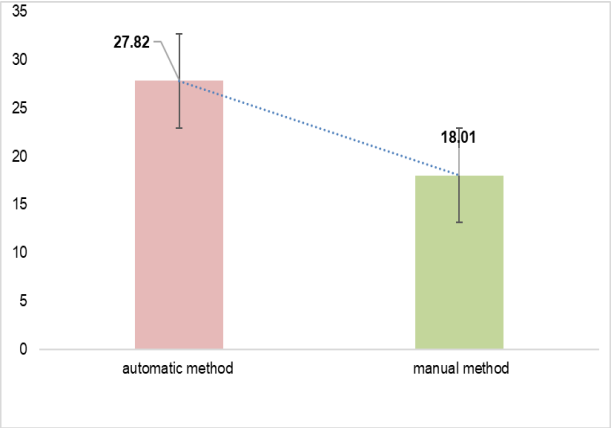

Figure 3. Graphical comparison between the average values of the DNA concentration, (µg/300 µl blood)

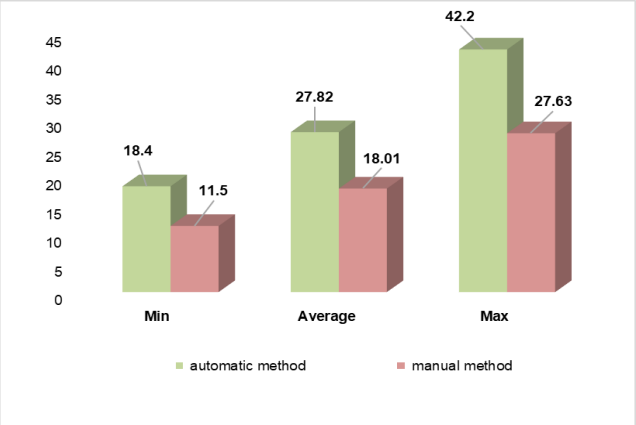

Figure 4. Graphical comparison between min., max. and average values of the DNA concentration, (µg/300 µl blood)

The statistical significance of the data is of great importance in genomic analysis. In this case, in order to see the level of statistical significance of the data obtained on the concentration of DNA extracted by the two types of methods, we performed the T-student statistical test. In the first phase, the two statistical hypotheses were formulated: zero and alternative, respectively H_0 and H_A. The null hypothesis holds that the data are not related, are independent/the compared values do not differ from each other, while the alternative hypothesis: H_A argues that the data have statistically significant links between them, are dependent/the compared values differ from each other. Such a statistical test results in a p-

value which is in fact a number between 0 and 1 and represents the probability of making an error if we reject hypothesis H_0. This value p is compared with the significance threshold, α which has a value of 0.05 and expresses the level accepted by the scientific community to reject or not to reject the null hypothesis [12]. To choose the type of T-student test, we first performed the F test to see the type of variance (equal or unequal). Several parameters, such as species, tissue preservation strategy, and extraction procedure, can all have an impact on DNA isolation. Depending on the origin of the biological sample, many sorts of contaminations might be obtained during the DNA extraction processes. Phenolic and other secondary chemicals induce DNA damage and/or block enzymatic processes, reducing the quality and quantity of samples [13]. Jorge C. Pereira and other co-authors published an article in 2011 regarding to comparative analyze of the quality and yield of genomic DNA from blood using the automated extraction (including the Maxwell 16 System (Promega UK, Southampton, UK) and manual extraction (whole blood: 300 µl of proteinase K mixture was added (0.5 M EDTA, [pH 8.0], 20% SDS, 20 mg/mL Proteinase K). The automated Maxwell System produced the highest amount and quality DNA across the sample range examined. Using the Maxwell System, an average yield of 28.7 µg of genomic DNA was recovered from 450 µl of blood buffy coat (n = 10 animals). Although the manual approach produced a higher yield of blood buffy coat (41.8 µg), the difference was not statistically significant (P>0.05) [14]. The automated process resulted in much greater DNA quality [15].

Table 2. F-Test Two-Sample for Variances

F-Test Two-Sample for Variances		
	Variable 1	Variable 2
Mean	27.82	18.01667
Variance	47.51476	18.78473
No. of samples	30	30
[1]Df	29	29
[2]F-value	2.529436	-
P(F<=f) one-tail	0.007441	P = 0.007441 < 0.05
[3]F Critical one-tail	1.860811	

[1]Df-(degrees of freedom)
[2]F-value (the ratio of two variances)
[3]f-(probability distribution)

Following the application of the F test, the value P=0.007441<0.05 resulted (P-value, the probability of obtaining a f-value with an absolute value at least as large as the one we actually observed in the sample data if the null hypothesis is actually true) *(Table 2)*, which means that the variances show statistically significant differences, so we applied the T-student Statistical Test, with unequal variances *(Table 3).*

Table 3. t-Test: Two-Sample Assuming Unequal Variances

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	Variable 2
Mean	27.82	18.01667
Variance	47.51476	18.78473
No. of samples	30	30
Hypothesized Mean Difference	0	-
[1]Df	49	-
[2]T Stat	6.594461	-
P(T<=t) one-tail	1.41E-08	
[3]t Critical one-tail	1.676551	
P(T<=t) two-tail	2.82E-08	P = 1.41E-08 < 0.05
t Critical two-tail	2.009575	

[1]Df-(degrees of freedom)
[2]T-value indicates the difference between sample data and the null hypothesis
[3]t-(quantify the difference between the sample means)

Following the application of the T-student test, with unequal variances, a value P = 1.41E-08 < 0.05 resulted (P-value - the probability of obtaining a t-value with an absolute value at least as large as the one we actually observed in the sample data if the null hypothesis is actually true) (Table 3), which means that there are statistically significant differences, therefore the alternative Hypothesis H_A is accepted according to which there are statistically significant differences regarding the concentration of the samples of DNA extracted by the two applied methods, manual and automatic, the highest value of the concentration being obtained after the application of the automatic method, using a special kit, 48 Maxwell™16 MCD LEV-Promega. In conclusion, the result of the statistical test allows the validation of the alternative hypothesis.

Conclusions

Obtaining optimal and sufficient DNA concentrations after the extraction stage is a key step for the success of a genetic analysis. In this research, the spectrophotometric quantification of DNA samples extracted from the 30 females of cattle validated quantitatively and qualitatively deoxyribonucleic acid isolation step by automatic extraction method. In addition to the fact that the automatic DNA extraction method leads to higher values of the DNA concentration in the samples, compared to the manual method, in the case of the automatic method, the amount of DNA in the

samples and the accuracy of the results. In conclusion, the results obtained make it possible to move on to the next steps of genomic analysis, the concentration of DNA samples resulting from the automatic method, allowing us to successfully amplify DNA sequences from these samples by PCR (Polymerase Chain Reaction) technique, which will be the next step in continuing this research.

References

1. Chacon-Cortes, D., Griffiths, L., Methods for extracting genomic DNA from whole blood samples: current perspectives. Journal of Biorepository Science for Applied Medicine, 2014, 2, pp. 1-9, DOI: doi.org/10.2147/BSAM.S46573.
2. Koshy, L., Anju, A. L., Harikrishnan, S., Kutty, V. R., Jissa, V. T., Kurikesu, I., Evaluating genomic DNA extraction methods from human whole blood using endpoint and real-time PCR assays. Mol. Biol. Rep., 2017, 44, pp. 97–108, DOI: 10.1007/s11033-016-4085-9.
3. Abd El-Aal, A.A., Abd-Elghany, N.A., Mohamadin, A.M., El-Badry, A.A., Comparative study of five methods for DNA extraction from whole blood samples. International Journal of Health Science, 2010, 3(1), pp. 285–287.
4. Lee, K., Tripathi, A., Parallel DNA Extraction from Whole Blood for Rapid Sample Generation in Genetic Epidemiological Studies. Front. Genet., 2010, 11, pp. 374. DOI: 10.3389/fgene.2020.00374.
5. Ali, N., Rampazzo, R., de, C. P., Costa, A. D. T., and Krieger, M. A., Current nucleic acid extraction methods and their implications to pointof-care diagnostics. BioMed Res. Int., 2017, 9, pp. 306-314, DOI: 10.1155/2017/9306564.
6. Chacon-Cortes, D., Haupt, L., Lea, R., Griffiths, L., Comparison of genomic DNA extraction techniques from whole blood samples: a time, cost and quality evaluation study. Mol Biol Rep., 2012, 39, pp. 5961–5966.
7. Mishra, P., Singh, U., Pandey, C.M., Pandey, G., Application of student's t-test, analysis of variance, and covariance. Ann Card Anaesth, 2019, 22(4), pp. 407-411. DOI: 10.4103/aca.ACA_94_19.
8. Feng, Y., Huang, Y., Ma, X., The application of Student's t-test in internal quality control of clinical laboratory. Frontiers in Laboratory Medicine, 2017, 1(3), pp. 125-128, DOI.org/10.1016/j.flm.2017.09.002.
9. Yim, K.H., Nahm, F.S., Han, K.A., Park, S.Y., Analysis of statistical methods and errors in the articles published in the korean journal of pain. Korean J Pain, 2010, 23, pp. 35–41.
10. Kim, T.K., T test as a parametric statistic. Korean J Anesthesiol, 2015, 68(6), pp. 540-546. DOI:10.4097/kjae.2015.68.6.540.
11. Cojocaru, D.C., Sandu, M., Protein and nucleic acid biochemistry. Publishing house PIM, Iasi, 2004, pp. 311-322.
12. Martins, J.P., Student t-statistic distribution for non-Gaussian populations, Proceedings of the ITI, 32nd International Conference on Information Technology Interfaces, 2010, pp. 563-568.
13. Lee, H.J., Park, Y., Choi, J.R., Lee, E.K., Kim, H.S., Comparisons of three automated systems for genomic DNA extraction in a clinical diagnostic laboratory. Yonsei Med. J., 2010, 51, pp. 104-110.
14. Foley, C., O'Farrelly, Meade, K.G., Technical note: Comparative analyses of the quality and yield of genomic DNA from invasive and noninvasive, automated and manual extraction methods, Journal of Dairy Science, 2011, 94(6), pp. 3159-3165.
15. Khokhar, S.K., Mitui, M., Leos, N.K., Rogers, B.B., Park, J.Y., Evaluation of Maxwell® 16 for automated DNA extraction from whole blood and formalin-fixed paraffin embedded (FFPE) tissue. Clin. Chem. Lab. Med., 2011, 50(2), pp. 267-72.