APARATURA
BADAWCZA I DYDAKTYCZNA

Automation of DNA isolation from biological materials

JUSTYNA STANINSKA[1], ZUZANNA SZCZEPANIAK[2], AGNIESZKA PIOTROWSKA-CYPLIK[2], PAWEŁ CYPLIK[1], JAKUB CZARNY[3]

[1]POZNAN UNIVERSITY OF LIFE SCIENCES, DEPARTMENT OF BIOTECHNOLOGY AND FOOD MICROBIOLOGY
[2]POZNAN UNIVERSITY OF LIFE SCIENCES, FACULTY OF FOOD SCIENCE AND NUTRITION, FOOD OF PLANT ORIGIN INSTITUTE OF TECHNOLOGY
[3]BYDGOSZCZ INSTITUTE OF FORENSIC GENETICS

Keywords: DNA isolation, automation

ABSTRACT

The intensive development of biological sciences, both in the field of medicine and broadly defined biotechnology, created the need to improve the analytical techniques. Molecular biology methods based on the analysis of nucleic acids with high sensitivity and reproducibility deserve special attention. They facilitate precise identification of biological traces, assessing changes in gene expression profiles and complete evaluation of environmental niches biodiversity. The main factor determining effectiveness of analysis is a stage of genetic material isolation. There is a clear trend to increase the capacity and accuracy of DNA isolation. Currently it is possible to overcome the limitations associated with manual isolation techniques. Using of kits and automated robots facilitates working with hundreds of samples simultaneously. The aim of the study was to validate the selected genomic DNA isolation methods. Three commercial kits were used. Two of them, which are dedicated to the automatic platform BioRobot M48 Robotic Workstation (Qiagen), are based on the DNA adsorption on silica beads convenient handling of magnetic particles: QIAsymphony Investigator DNA Kit (Qiagen) and MagAttract® DNA Mini M48 (Qiagen). A third kit Sherlock AX (A&A Biotechnology) for manual isolation of genomic DNA are based on the mechanism of nucleic acin adsorption on ion exchange beads combined with the isopropanol precipitation of DNA. The determination limit, reproducibility and repeatability were evaluated. The results indicate that all of the analysed methods allow the isolation of high-quality genetic material, which can be used in subsequent molecular testing procedures.

Automatyzacja izolacji DNA z materiałów biologicznych

Słowa kluczowe: izolacja DNA, automatyzacja

STRESZCZENIE

Intensywny rozwój nauk biologicznych, zarówno w dziedzinie medycyny, kryminalistyki, jak i szeroko rozumianych badań związanych z biotechnologią, stworzył potrzebę doskonalenia technik analitycznych. Na szczególną uwagę zasługują metody biologii molekularnej bazujące na analizie kwasów nukleinowych, charakteryzujące się wysoką czułością i powtarzalnością. Pozwalają one na precyzyjną identyfikację śladów biologicznych, ocenę zmian w profilach ekspresji genów, a także pełną ewaluację bioróżnorodności nisz środowiskowych. Kluczowym elementem determinującym efektywność przeprowadzanych analiz jest etap pozyskiwania (izolacji) materiału genetycznego. Wyraźnie dostrzega się tendencje rozwoju technologii w kierunku zwiększenia przepustowości oraz dokładności izolacji DNA. Aktualnie możliwe jest pokonanie ograniczeń związanych z metodą manualnej izolacji poprzez zastosowanie gotowych zestawów oraz robotów automatycznych umożliwiających pracę z wieloma setkami prób jednocześnie. Celem badań było przeprowadzenie walidacji wybranych metod izolacji genomowego DNA. Wykorzystano trzy komercyjne zestawy. Dwa z nich, dedykowane platformie automatycznej BioRobot M48 Robotic Workstation (Qiagen), bazowały na zjawisku immobilizacji DNA na złożach z cząstkami paramagnetycznymi: QIAsymphony DNA Investigator Kit (Qiagen) oraz MagAttract® DNA Mini M48 (Qiagen). Trzeci zestaw, Sherlock AX (A&A Biotechnology), przeznaczony był do manualnej izolacji genomowego DNA i wykorzystywał mechanizm adsorbcji kwasów nukleinowych na membranach jonowymiennych połączonej ze strącaniem DNA izopropanolem. Ocenie poddano wykrywalność, powtarzalność, i odtwarzalność. Wyniki wskazują, że wszystkie analizowane metody pozwalają na izolację materiału genetycznego o wysokich standardach, który może być wykorzystany w dalszych procedurach badań molekularnych.

1. INTRODUCTION

The discovery of nucleic acids in the nineteenth century has initiated intensive development of research in the field of molecular biology which is nowadays widely used in medical and judicial diagnostic as well as biotechnology. Over the past 100 years there have been many crucial research resulted in the obtaining full knowledge of the chemical and molecular structure of DNA. Modern genetic engineering which is characterized by high sensitivity and reproducibility facilitates efficient cloning, modifying and sequencing of DNA [1]. The step of nucleic acids isolation is a key factor in determining the quality of molecular analyzes. It is essential to obtain the material characterized by high quality, as well as purity and free from PCR inhibitors. Moreover, the selection of reagents with relatively low toxicity in order to ensure the safety of research workers and minimization the impact on the environment are also very important [2]. Each isolation procedure requires cell lysis (mechanical, chemical or enzymatic), inactivation of exonucleases and the final purification of DNA.

Currently, there are many commercially available kits for the isolation and purification of genomic DNA that significantly increase the efficiency of the process, reduce the number of transfers and consequently the risk of contamination. One of the approach, is using technology based on the affinity of nucleic acid molecules to the paramagnetic beads with specific ligands [3]. The diagram of this method is shown on the Figure 1.

The isolation process begins with the enzymatic lysis of the cells using a proteinase k. Then paramagnetic beads with ligands capable of binding to DNA are added to the solution. The system is washed in order to remove contaminants during the magnetic immobilization. The final step consists in the elution of pure genetic material.

Another popular solution is based on ion exchange membranes capable of binding nucleic acids (Fig. 2).

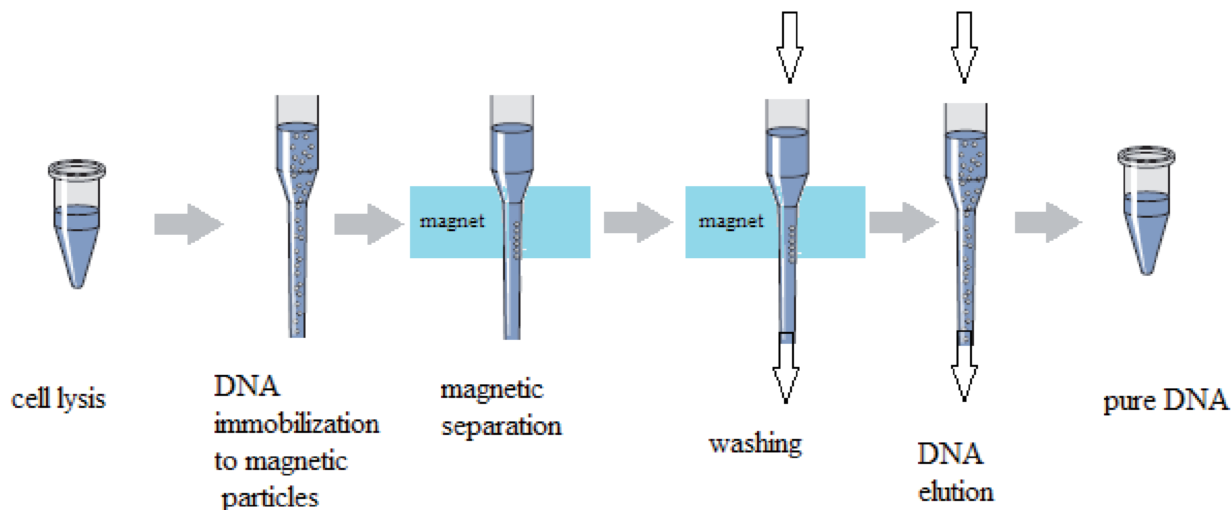

Figure 1 Diagram of separation method based on the affinity of nucleic acid molecules to the paramagnetic beads with specific ligands

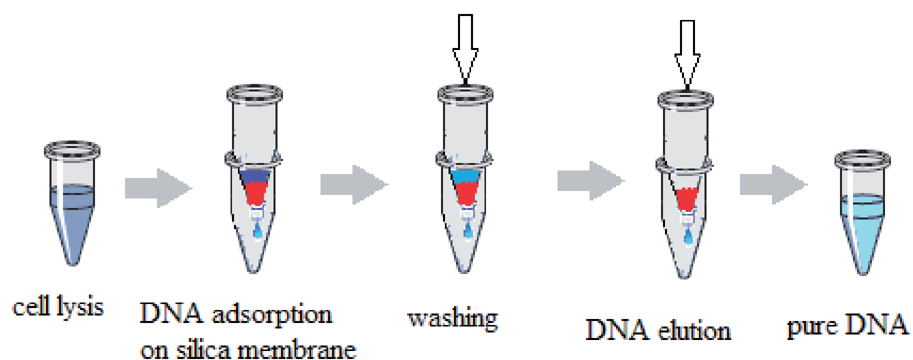

Figure 2 Diagram of DNA separation method using ion exchange membranes

Enzymatic cell lysis is the first step of the process. Next, DNA adsorption on the silica membranes and the filtration in order to remove contaminants are conducted. Then, the solution is subjected to further filtration in the presence of chaotropic salts in high concentrations. In the next step, samples are washed several times in order to remove proteins, PCR inhibitors and other contaminants. Finally, the DNA is eluted with a solution characterized by high ionic strength. The obtained eluate with nucleic acids is salted out and concentrated by alcohol precipitation.

Currently, automatic apparatus for nucleic acids isolation are becoming more and more popular. Modern workstations facilitate simultaneous work with several samples with minimum of involvement in the direct operation of the process. Full automation reduces the risk of human error, external contamination and ensures high throughput of isolation. The possibility of adding internal standards allows full control of the quality and yield of genetic material. Moreover, biorobots with optimized procedures isolation from different matrices with optional possibility of modification in order to increase efficiency in problematic experimental systems are also available. In addition, during one cycle of work, biorobots automatically dispense reagent volumes based on the amount of the analyzed samples, which significantly reduces the burden of the environment compared to conventional methods and allows full control of costs [2, 4]. It is worth noticing that in the case of some platforms there is a risk of considerable variability in the quality of DNA isolation due to the fact that they do not require the use of dedicated disposable materials which quality have a significant impact.

The aim of this study was to validate the selected genomic DNA isolation methods: immobilization on paramagnetic beads using automatic platform with dedicated kits and DNA adsorption on ion exchange membranes using kit for manual isolation.

2. EXPERIMENTAL

2.1 The isolation of genetic material

The human blood and saliva samples were examined during experiments. Commercially available kits for DNA isolation: working on the BioRobot M48 Robotic Workstation (Qiagen): QIAsymphony DNA Investigator Kit (Qiagen) and MagAttract® DNA Mini M48 (Qiagen) and also dedicated for standard, manual isolation: Sherlock AX (A&A Biotechnology) were used. The genomic DNA concentrations in extracts were evaluated with the use of Quantifiler Human DNA Quantification Kit. Water for dilution was characterized by the highest purity (Sigma).

2.2 Detection of method

Three series of samples containing blood and saliva in dilutions: 1:2, 1:10, 1:20, 1:100 and a negative control sample containing water were prepared. Samples were prepared in a volume of 10 ml using dilution water and applied to a clean cotton cloth. Then isolation of genomic DNA and evaluation of concentration according to the procedures recommended by the manufacturer was performed in triplicate. The samples of cloth with applied blood were excised by sterile scalpel and placed in a DNA extraction basket (NAO Baskets, Copan). This technology allowed all genetic material to recover from the absorbing material, and therefore the absorption capacity don't affect the evaluation of DNA concentration.

The limit of genomic DNA detection was characterized by the smallest amount of biological material for which at least in one attempt the concentration of 0.007 ng/μl was obtained. This is the experimentally determined limit of quantification of PCR amplification polymorphism of multiplex STR loci typing systems, which is currently the most effective way to identify samples of human biological material [5].

2.3 Repeatability and reproducibility

Five FTA paper discs carrying the saliva of 3 mm and 1.2 mm diameters were prepared. Isolation of genomic DNA and evaluation of concentration were performed in five replicates. Reproducibility assay was carried out in different series of experiments conducted at different times, as positive controls. Precision (repeatability and determination) of methods as a measure of agreement between individual results of the analyses was assessed on the basis of the coefficient of variation (CV) [6], defined as:

$$\boldsymbol{CV} = \frac{\boldsymbol{SD}}{\overline{\boldsymbol{x}}} \times \mathbf{100}\% \qquad (1)$$

where: SD – standard deviation, $\overline{x}$ – the average amount of DNA ($\overline{x} \neq 0$)

3. RESULTS

Results of detection range for the isolation of DNA from blood and saliva by various methods are presented in Table 1.

The results indicate that the genomic DNA isolation method based on the mechanism of nucleic acids adsorption on ion exchange beads (Sherlock AX) provides the greatest efficiency in both variants of the matrix (blood and saliva). In the case of MagAttract® DNA Mini M48 isolation kit, detection levels are established on 1 μl (blood) and 0.5 μl (saliva). QIAsymphony DNA Investigator Kit and Sherlock AX are characterized by a level of detection equals 0.1 μl for both matrix. Literature reports confirm that the extraction of DNA based on the affinity to the magnetic beads has a slightly lower efficiency compared to the extraction by adsorption to a solid phase. This may be a consequence of intense flushing and washing out weakly adsorbed nucleic acids [7]. It is worth noting that the use of BioRobot M48 Robotic Workstation (Qiagen) based on affinity to paramagnetic beads increases the amount of obtained genetic material. High throughput and effective DNA isolation confirm the usefulness in research requiring the rapid analysis of a large number of samples in the short time.

The results of genomic DNA isolation precision are shown in Table 2 (reproducibility) and Table 3 (determination).

Obtained results indicate that the method based on the paramagnetic beads is characterized by higher precision than the method based on ion exchange membranes. Studies conducted by Shyiang et al. (2013) concerning comparison of bacterial DNA isolation methods from human plasma samples, also show the highest reproducibility of the magnetic method [9]. Berensmeier (2006) emphasizes that elimination of the sample centrifugation in a magnetic separation method reduces the nucleic acid degradation due to shear forces. This may determine the high repeatability

Table 1 DNA detection with standard deviations (SD) of dilution series of human blood and saliva with selected isolation kits. Gray color highlights the results below prescribed level of detection

	MagAttract® DNA Mini M48		QIAsymphony DNA Investigator Kit		Sherlock AX	
	The average amount of DNA	SD	The average amount of DNA	SD	The average amount of DNA	SD
	[ng]					
Blood						
5 µl	0.1231	0.0827	1.9000	0.7118	2.9233	0.6984
1 µl	0.0090	0.0036	0.1400	0.0432	0.5247	0.1774
0,5 µl	0.0038	0.0033	0.0867	0.0262	0.2460	0.0193
0,1 µl	0.0008	0.0012	0.0153	0.0105	0.0413	0.0077
Saliva						
5 µl	1.0295	0.1014	0.7000	0.0000	4.3573	0.2318
1 µl	0.0525	0.0189	0.3333	0.1247	0.8457	0.1245
0,5 µl	0.0163	0.0057	0.1267	0.0249	0.4047	0.0859
0,1 µl	0.0000	0.0000	0.0087	0.0049	0.0713	0.0048

Table 2 Reproducibility of DNA isolation from human saliva using selected kits for isolation with a standard deviation (SD) and coefficients of variation (CV)

The diameter of filter [mm]	MagAttract® DNA Mini M48			QIAsymphony DNA Investigator Kit			Sherlock AX		
	The average amount of DNA	SD	CV	The average amount of DNA	SD	CV	The average amount of DNA	SD	CV
	[ng/µl]		[%]	[ng/µl]		[%]	[ng/µl]		[%]
3	3.600	0.157	4.347	0.640	0.102	15.938	8.7858	1.3289	15.126
1	0.516	0.034	6.552	0.268	0.0264	9.851	2.077	0.314	15.115

Table 3 Determination of DNA isolation from human saliva using selected kits, with standard deviations (SD) and coefficients of variation (CV)

The diameter of filter [mm]	MagAttract® DNA Mini M48			QIAsymphony DNA Investigator Kit			Sherlock AX		
	The average amount of DNA	SD	CV	The average amount of DNA	SD	CV	The average amount of DNA	SD	CV
	[ng/µl]		[%]	[ng/µl]		[%]	[ng/µl]		[%]
3	1.7266	0.0948	5.491	1.0234	0.1579	15.429	8.241	1.0759	13.055
1	1.208	0.1195	9.892	2.8392	0.2001	7.048	1.8972	0.2429	12.803

of describing method [8]. Literature data indicate that in the case of precision (repeatability and reproducibility), coefficients of variation should achieve the smallest value. The result can be considered as a precise, when coefficient of variation (CV) does not exceed 15%. The tolerance range up to 20% for samples with low concentrations, such as systems used throughout the currently described experiments, is also acceptable [6]. Since the obtained analysis are characterized by the coefficient of variation smaller than 20%, it can be stated that all kits for the isolation meet the criteria for reproducibility and repeatability and thus have application potential in the molecular sciences fields.

4. CONCLUSIONS

The validation has confirmed that all of the analyzed genomic DNA isolation kits are characterized by the application potential in the molecular sciences fields. The method based on ion exchange membranes represented by Sherlock AX kit indicates the highest efficiency of extraction. In addition, automatic MagAttract® DNA Mini M48 and QIAsymphony DNA Investigator Kit based on paramagnetic beads are characterized by the highest precision. Despite the less extraction efficiency, isolation of DNA using automatic platforms eliminates human errors during manual labor. It is worth noticing that the use of BioRobot M48 Robotic Workstation (Qiagen) is described by high throughput, repeatability as well as efficiency of isolation. It can be assumed that a wide range of commercially available DNA isolation kits contribute to the progress of intensive research using molecular tools.

5. ACKNOWLEDGEMENTS

Research work funded by financial resources for science as a research project Opus no 2013/11/B/NZ9/01908.

REFERENCES

[1] Gabryelska M., Szymański M., Barciszewski J., DNA – cząsteczka, która zmieniła naukę. Krótka historia odkryć, Nauka, 2(2009), 111-134.
[2] Gabryelczyk P., Zastosowanie aparatu NucliSens easyMag do automatycznej izolacji kwasów nukleinowych w diagnostyce medycznej, J Trasf Med, 3(2010), 1-8.
[3] Dębska M., Drabik J., Porównanie efektywności różnych metod izolacji genomowego DNA ze śladów biologicznych, Probl Krym, 264(2009), 11-25.
[4] Małodobra M., Jonkisz A., Kowalczyk E., Lebioda A., Bartnik B., Świątek B., Wydajność trzech komercyjnych zestawów do izolacji DNA i RNA ze zróżnicowanego materiału klinicznego i dowodowego przy użyciu automatycznej stacji Janus, Arch Med Sąd Kryminol, 61(2011), 51-57.
[5] Pepiński W., Niemcunowicz-Janica A., Skawrońska M., Koc-Żórawska E., Janica J., Sołtyszewski I., Berent J., Polimorfizm wybranych loci mikrosatelitarnych wśród ludności Polski północno-wschodniej w aspektach zróżnicowania etnicznego i przydatności w badaniach medyczno-sądowych, Rocz PAM, 53(2007), 71-75.
[6] Spas A., Zbieć-Piekarska R., Wewnętrzna walidacja metody kwantyfikacji DNA z wykorzystaniem zestawu Quantifiler® Human DNA Quantification Kit i aparatu 7500 Real-Time PCR System wraz z oprogramowaniem Hid Real-Time PCR Analysis Software V. 1.1 w Zakładzie Biologii Centralnego Laboratorium Kryminalistycznego Policji, Probl Krym, 284(2014), 1-11.
[7] Grody W., Nakamura R., Kiechle F., Strom Ch., Molecular Diagnostics: Techniques and Applications for the Clinical Laboratory, Boston, Academic Press, 2010, 38-40.
[8] Berensmeier S., Magnetic particles for the separation and purification of nucleic acids, Appl Microbiol Biotechnol, 73(2006), 495-504.
[9] Shiyang P., Bing G., Hong W., Zihe W., Peng W., Hao P., Weiping X., Dan Ch., Genyan L., Comparison of four DNA extraction methods for detecting Mycobacterium tuberculosis by real time PCR and its clinical application in pulmonary tuberculosis, J Thorac Dis, 5(2013), 251-257.