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Fast D NA Purification Methods: Comparative Study

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Abstract: The use of molecular biology methods has increased in the study of many subjects, which has made the urgent need for easy, fast and inexpensive methods to isolate DNA from different tissues. Both diagnosis and characterization purposes need obtaining high yields of integer and pure DNA within sensible cost and time. The eight kits: Monarch® Genomic DNA Purification Kit; Quick-DNA™ Miniprep Kit; EchoLUTION- DNA Extraction Kits; Fast DNA Extraction kit; QIAamp Fast DNA Stool Modified; QIAamp Fast DNA Tissue Kit; QuickExtract™ DNA Extraction Solution; and Rapid Fungal Genomic DNA Isolation Kit were compared with the Chelex-100 methodology. We compared the items of cost, content, storage of the kits and the equipment needed in the methodology. In addition, all items regarding the start material, yield and methodology were compared. Out of eight tested kits, only the EchoLUTION-DNA Extraction Kit is comparable to Chelex-100 methodology. The kit is more expensive than Chelex-100. Except for the suspended impurities, Chelex-100 is regarded the best methodology. In this study, only the EchoLUTION-DNA Extraction Kit is comparable to Chelex-100 methodology.

Keywords: DNA extraction; Fast DNA kits; advantages; disadvantages; DNA methodology.

1. Introduction

DNA extraction and purification is one of the most crucial techniques in molecular biology studies. Many problematic issues have been faced by researchers: (i) highly expensive preparation; (ii) use of hazardous materials; (iii) need for massive sample size; (iv) need for preparation steps before purification; (v) time consuming and laborious protocols; (vi) small amount of yield; (vii) contaminated yield; (viii) sheered DNA yield; and (ix) suitability of the yield for limited applications. For effective purification of DNA, four steps have to be achieved: tissue disturbance and cell lysis; nucleoprotein dissociation; inhibition of nucleases; and elimination of impurities [1].

Several methodologies and kits have been developed to purify DNA from various biological samples [2-3]. Quantity and quality of the DNA product of such methodologies is crucial for successful research studies [4]. Thus choosing the proper and relevant DNA extraction protocol can save money, time and speed up executing the experimental work. Many factors are to be considered by a researcher when selecting DNA extraction protocol. Sample source, preparation, content and quantity [2-3], quality (purity, integrity) and quantity of the DNA should be considered for the intended application, away from the simplicity of method [5].

Herein, we compare between the fast DNA extraction methods and kits. Several criteria were considered in this article to help overcoming research's challenges including: challenges relevant to sample; yield; application; materials; and methodology as well as the expenses.

2. Materials and Methods

A comparison between Chelex-100 method and eight kits for fast extraction of DNA from multiple sources (Monarch® Genomic DNA Purification Kit, Quick-DNA™ Miniprep Kit, EchoLUTION- DNA Extraction Kits, Fast DNA Extraction kit, QIAamp Fast DNA Stool Modified, QIAamp Fast DNA Tissue Kit, QuickExtract™ DNA Extraction Solution, and Rapid Fungal Genomic DNA Isolation Kit) have been studied. All data were retrieved from the manual of the kit and/ or from the website of the manufacturer or provider. Detailed protocol for DNA extraction by Chelex-100 has been retrieved from the article by Walsh et al. [6].

3. Results

Table (1) summarizes comparison of key catalogue and storage data of Chelex-100 resin and eight commercial kits for fast DNA purification from different biological samples. Needed equipment are also summarized (Table 1). Regarding the estimated cost per 100 reactions, Chelex-100 was the cheapest methodology. Meanwhile, the QIAamp Fast DNA Stool Modified kit was the expensivest methodology per 100 reactions. Chelex-100 and 3 of the 8 kits could be stored at room temperature (Table 1). Extra material are needed for all methodologies except in the case of EchoLUTION-DNA Extraction Kits, Fast DNA Extraction kit and QuickExtract™ DNA Extraction Solution (Table 1). The QIAamp Fast DNA Tissue Kit contains toxic material (sodium azide and chaotropic salt). Meanwhile, the other methodology did contain any toxic material (Table 1). All methodologies comprise vortex and centrifuge except QuickExtract™ DNA Extraction Solution kit which comprise vortex only and Rapid Fungal Genomic DNA Isolation Kit which comprise centrifugation without vortex. Thermomixer or water bath are needed in all methodologies except Quick-DNA™ Miniprep Kit. Freezers or refrigerators are needed in the case of Monarch® Genomic DNA Purification Kit, Fast DNA Extraction kit, QIAamp Fast DNA Stool Modified and Rapid Fungal Genomic DNA Isolation Kit. Homogenizer is used in Rapid Fungal Genomic DNA Isolation Kit methodology (Table 1).

Table 1. Comparative key-table of Chelex-100 and eight commercial kits for fast DNA purification.

Kit	Cat No.	Estimated Cost/ 100 prep	Storage	Extra Material	Hazards	Equipment
Chelex-100	C7901(Sigma-Aldrich)	$ 2.80	Room Temp.	TAE or dH2O	Non-toxic	Pipettes Vortex Centrifuge
Monarch® Genomic DNA Purification Kit	T3010 (BioLabs)	$ 395	Store RNase A and Proteinase K at -20°C.	95% Ethanol	Non-toxic	Pipettes Vortex Centrifuge ThermoMixer Freezer
***Quick*-DNA™ Miniprep Kit**	D3024 & D3025 (Zymo Research)	$ 343	Room Temp.	β-mercaptoethanol	Non-toxic	Pipettes Centrifuge Vortex
EchoLUTION-DNA Extraction Kits	010-001-050 010-011-050 (BioEcho)	$ 298.40-320.88	Room Temp.	NO	Non-toxic	Pipettes Vortex Centrifuge ThermoMixer
Fast DNA Extraction kit	MBK0061 (Diatheva)	$ 246.83-415.13	Room Temp.	NO	Non-toxic	Pipettes Vortex Centrifuge

						ThermoMixer Refrigerator
QIAamp Fast DNA Stool Modified	51604 (Qiagen)	$ 711.32-865.70	Store Proteinase K at -20 °C.	96- 100% Ethanol	Non-toxic	Pipettes Vortex Centrifuge ThermoMixer Freezer
QIAamp Fast DNA Tissue Kit	51404 (Qiagen)	$ 510.74	Store RNase A and Proteinase K at -20 °C.	96- 100% Ethanol Isopropanol	Sodium azide Chaotropic salt	Pipettes Vortex Centrifuge ThermoMixer
QuickExtract™ DNA Extraction Solution	QE09050 (Lucigen)	$ 362.65	Store at –20 °C	NO	Non-toxic	Pipettes Vortex ThermoMixer
Rapid Fungal Genomic DNA Isolation Kit	FT71415 (Biobasic)	$ 86.7	Store at –4 °C	Liquid nitrogen Chloroform Isopropanol 75% Ethanol RNase A solution	Non-toxic	Homogenizer Pipettes Centrifuge ThermoMixer Freezer

Table (2) shows comparative methodology of the nine studied protocols for DNA purification. The least preparation and estimated time were recorded in the case of chelex-100, Quick-DNA™ Miniprep Kit, EchoLUTION-DNA Extraction Kits and QuickExtract™ DNA Extraction Solution. However, the QIAamp Fast DNA Stool Modified Kit records the largest preparation steps and the longest estimated time (Table 2). Not including preparation, chelex-100 methodology exhibits the least total number of steps (5), number of centrifugation (2) and lysis time (10-15 sec). Whilst QIAamp Fast DNA Stool Modified methodology exhibits the largest total number of steps (30) and number of centrifugation (10-12). Meanwhile, Monarch® Genomic DNA Purification Kit exhibits the longest lysis time (35-180 min). In addition, chelex-100 is the fastest protocol for DNA preparation. On the other hand, Monarch® Genomic DNA Purification Kit represents the longest method taking up to 3 h, 15 min for DNA preparation (Table 2).

Table 2. Comparative methodology of nine protocols of DNA purification.

Kit	Preparation steps	Estimated time for preparation	Total actual steps*	No. of centrifugation or spin	Lysis time	Total time of processing**
Chelex-100	One step	1-2 min	5	2	10-15 sec	21 min
Monarch® Genomic DNA Purification Kit	4 steps	4-8 min	18	5-6	35-180 min	18-20 min for cells Up to 3 h, 15 min for tissue
***Quick*-DNA™ Miniprep Kit**	One step	1-2 min	6-7	4-5	5-10 min	15- 24 min
EchoLUTION- DNA	One step	1-2 min	8	3-4	9-22	30 min

Extraction Kits					min	
Fast DNA Extraction kit	2 steps	2-4 min	13	3	22 min	30- 40 min
QIAamp Fast DNA Stool Modified	7 steps	7-14 min	30	10-12	10 min	40- 50 min
QIAamp Fast DNA Tissue Kit	3 steps	3-6 min	23	5-6	10-60 min	1h+ 26-30 min
QuickExtract™ DNA Extraction Solution	One step	1-2 min	8-10	0	7-15 min	18- 20 min
Rapid Fungal Genomic DNA Isolation Kit	3 steps	3-6 min	16	4-6	10-30 min	42- 65 min

***Preparation steps are not included.**

****Preparation time is not included.**

Data in Table (3) summarizes comparison between nine protocols regarding starting sample and yield product of DNA purification. Regarding sample source, Fast DNA Extraction kit is specific for bacteria, QIAamp Fast DNA Stool Modified is specific for feces and sewage samples, and Rapid Fungal Genomic DNA Isolation Kit is specific for fungi. Meanwhile, the other five methodologies are applied to DNA extraction from multiple sample sources (Table 3). Concerning sample quantity, chelex-100 and EchoLUTION-DNA Extraction Kit use the least amount of samples to extract DNA. Whilst, Rapid Fungal Genomic DNA Isolation Kit uses the largest amount of sample to extract DNA (Table 3). Touching quantity, quality and suitability of the yield, eight kits yielded fair quantity of pure and integer DNA which is suitable for many downstream and upstream applications. Whereas chelex-100 methodology yields fair integer DNA which contains suspended impurities. Thus, the resulting DNA by chelex-100 method is suitable for very limited downstream applications like PCR (Table 3).

Table 3. Comparative starting sample and yield product of nine protocols of DNA purification.

Kit	Sample source	Sample amount	Yield	Purity	Integrity	Suitability
Chelex-100	Multiple	Very small	Comparable	Contains suspended impurities	Yes	PCR
Monarch® Genomic DNA Purification Kit	Biological fluids, cell cultures, and solid tissues	-1 x 10^4– 5 x 10^6 cells, -10- 100µl of whole blood, -10- 25 mg animal tissue	-6-10µg -1-8µg/ 100µl, 30- 45µg/ 10µl -4-70µg/ 10 mg	High pure	Yes	Downstream and Upstream
***Quick*-DNA™ Miniprep Kit**	Biological fluids, cell cultures, and solid tissues	-100- 200µl whole blood (4:1)	3-7 µg/ 100 µl blood	High pure	Yes	PCR, Endonuclease digestion, Bisulfite conversion/ Methylation detection, Sequencing,

						Genotyping, etc
EchoLUTION-DNA Extraction Kits	Multiple	Very small	20 µg/ 200 µl blood	Highly pure	Yes	All downstream applications
Fast DNA Extraction kit	Bacteria	1 ml of bacterial culture (1:10 dil)	6-10µg	Highly pure	Yes	Real-Time PCR assay Food testing for pathogens
QIAamp Fast DNA Stool Modified	Feces or sewage	0.2 g of feces or 0.5 g sewage	12 µg 30 µg	Highly pure	Yes	PCR, qRT-PCR
QIAamp Fast DNA Tissue Kit	Fresh, frozen or stabilized tissue	-5–25 mg of fresh, frozen or stabilized tissue	-5-30 µg	Highly pure	Yes	PCR, qRT-PCR
QuickExtract™ DNA Extraction Solution	Multiple	-10^4 cells -0.5-1 cm tissue	2-10 µg/ml cells	Highly pure	Yes	PCR-based analyses, qRT-PCR
Rapid Fungal Genomic DNA Isolation Kit	Fungi	100-500 mg mycelia	2-10 µg/ml of overnight fungi culture.	Highly pure	Yes	qRT-PCR, SNP analysis, REN, hybridizaon, other applicaons.

Table (4) wraps up the advantages and disadvantages of the nine compared protocols of DNA purification. Except for the impurities present in the product DNA, chelex-100 methodology is regarded the cheapest, easy, simple and fast protocol. It is used in extracting DNA from the forensic, hard and multiple sample types. EchoLUTION- DNA Extraction Kit is comparable to chelex-100 in simplicity, time-saving and easiness, but it is apparently costive (Table 4). Other kit-based methodologies yielded fair amount of highly pure and integer DNAs which are suitable for many upstream and downstream applications. But many disadvantages have been recorded including expensiveness, time-consumption, many-steps protocols, use of toxic substances, need for special preparation steps, need for special storage conditions, limited sample sources ….. etc. (Table 4).

Table 4. Advantages and disadvantages of nine protocols of DNA purification.

Kit	Advantages	Disadvantages
Chelex-100	Cheap, easy, simple and fast protocol. Enough yield and 1 µl template for PCR. Suitable for forensic material like blood spot, hair, …etc. Yield could be stored in the freezer until use. Involve no organic solvents. Do not require multiple tube transfers.	Fickle. Sometimes shearing of DNA. Sometimes it needs overnight to work. Sometimes it needs dilution. Degradation of DNA on long term storage. Inhibition of the polymerase chain reaction by impurities.
Monarch® Genomic DNA Purification Kit	Broad range of sample types. Suitable for clinically-relevant samples. Excellent yields of highly-pure DNA. Residual RNA contamination (typically <1%).	Very long protocol. Time consuming and laborious protocol.

	DNA is suitable for downstream applications, including PCR, qRT-PCR and NGS. Excellent choice upstream of long-read sequencing platforms. Kit components available separately.	
***Quick*-DNA™ Miniprep Kit**	Excludes the use of Proteinase K and organic denaturants. Compatible with commonly used anticoagulants.	Sometimes DNA degradation. Sometimes DNA is not performing well. Sometimes RNA contamination of the yield.
EchoLUTION- DNA Extraction Kit	Single-step spin column-based purification of genomic DNA. Flexible input from 200 µl to 1 ml or 5 to 60 µl liquid blood & dried blood spots. Suitable for forensic material like blood spot, hair, …etc. Inhibitor-free highly pure DNA for reliable results. Improved yields. Fast, half the hands-on time, and fewer steps. 70% less plastic waste, and no toxic chemicals.	Average of 310 USD/ 100 preparations.
Fast DNA Extraction kit	The protocol is based on thermal lysis that permits to obtain, in only 30 minutes, a DNA extracted suitable for Real-Time PCR assay. Stored at room temperature.	Could not be used for the isolation of bacterial DNA from primary production samples.
QIAamp Fast DNA Stool Modified	Higher DNA yield. Both Proteinase K and Buffer AL are supplied with the kit. Additional volumes can be purchased separately.	Very long protocol. Time consuming and laborious protocol. Average of 788.51 USD/ 100 preparations.
QIAamp Fast DNA Tissue Kit	Proteinase K is stable at room temperature for at least 1 year. Store at 2–8 °C for more than 1 year. Suitable for fresh, frozen or stabilized samples of different tissue types. Uses lyse, bind, wash and elute protocol, which can be automated (QIAcube) or done manually. Allows rapid purification of DNA from soft and solid tissue. High pure yield.	Contains sodium azide as a preservative. Contains a chaotropic salt. Average of 510.74 USD/ 100 preparations.
QuickExtract™ DNA Extraction Solution	Fast, simple, and inexpensive method. No use of toxic chemicals or spin columns. DNA is suitable for all PCR-based analyses.	Optimization of the PCR may be necessary. An average of $ 362.65 USD/100 preparations.
Rapid Fungal Genomic DNA Isolation Kit	Suitable for downstream applications including qRT-PCR, SNP, REN, hybridization and other applications.	Suitable only for fungal tissue. Needs liquid nitrogen for grinding sample tissue.

4. Discussion

The present study compared nine of the fast DNA extraction methods. Chelex-100 is a non-toxic resin in distilled water or TAE buffer, stored in room temperature. It was the cheapest methodology costing 2.8 USD/ 100 reaction. Above all, it is still the easiest, the fastest and the simplest protocol for DNA extraction. The main concern that should be taken is the impurities of the yielded DNA. Singh et al. [6] have developed a modified chelex-100 protocol to remove such impurities from the DNA. The main drop point of the protocol is that it became laborious and time consuming [6]. Becker et al. [7] investigated six kits to isolate chromosomal and plasmid DNA from a single isolate of bacterial species. A little difference on suitability of yielded DNA for sequencing and sequence reads [7]. Furthermore, six kits have tested for DNA recovery from dilutions of cytomegalovirus (CMV) added to whole blood, cerebral spinal fluid, bronchoalveolar lavage, and plasma. The produced DNA was PCR-inhibitor free even at 200 PFU/ml of whole CMV. The PG and NS kits produced invariably positive results. The cost of one test is $0.23 in the case of PG kit and $4.00 per test for the NS. Diversified processing time was observed between kits (55 min for GCC to 4 h 39 min for PG), as well [8]. Many published articles clarified that QIAamp columns gave the best results (DNA suitable for PCR) between all methods whatever commercial and noncommercial [9-11]. Ferrand et al. [12] evaluated 7 methodologies for DNA extraction from bacteria in cecal and fecal samples of mice. They reported that the FastDNA® SPIN Kit was the best method for extracting DNA from soil. Moreover, 5 commercial DNA purification kits have been compared for purification of bacteria from human faeces. The automated QS kit provided practical advantages by supplying the best quality and highest yield of DNA [13]. Recent study has demonstrated fast, durable, fair, and cheap methodology to isolate a good yield of pure high molecular weight metagenomic DNA [14]. Ramón-Laca et al. [15] reported a time saving and cost-effective method that provided better quality DNA for PCR applications. In addition, Menu et al. [16] compared EZ1® (Qiagen) and QIAamp® DNA Stool Mini Kit (Qiagen) for diagnosis of pathogens by using PCR. They found that both yielded a comparable realization for detecting *Cryptosporidium* spp. and *D. fragilis.* A better performance of EZ1® on the five remaining pathogens (*Blastocystis* spp., *Cyclospora cayetanensis*, *Giardia intestinalis*, *Cystoisospora belli* and *Enterocytozoon bieneusi*) was observed [16].

5. Conclusions

Conclusively, choosing the extraction methodology of DNA is a matter of the requested quantity and quality of yield and of the starting sample, as well. Except the suspended impurities of the produced DNA, chelex-100 method is still the cheapest ($ 0.02 per reaction), fastest (≈20 min), simplest (5 steps), and easiest (vortex and spin) methodology ever. It can be used for DNA isolation from multiple samples with very little amount including blood spot and other forensic materials.

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References

1. Doyle, K. (1996). The source of discovery: protocols and applications guide. Madison: PROMEGA.
2. Thatcher, S. A. (2015). DNA/RNA preparation for molecular detection. Clinical chemistry, 61(1), 89-99.
3. Mullegama, S. V., Alberti, M. O., Au, C., Li, Y., Toy, T., Tomasian, V., & Xian, R. R. (2019). Nucleic acid extraction from human biological samples. Biobanking, 359-383.
4. Cseke, L. J., Kirakosyan, A., Kaufman, P. B., & Westfall, M. V. (Eds.). (2011). Handbook of molecular and cellular methods in biology and medicine. CRC press.
5. Dhaliwal, A. (2013). DNA extraction and purification. Mater Methods, 3, 191.

6. Singh, U. A., Kumari, M., & Iyengar, S. (2018). Method for improving the quality of genomic DNA obtained from minute quantities of tissue and blood samples using Chelex 100 resin. Biological procedures online, 20(1), 1-8.
7. Becker, L., Steglich, M., Fuchs, S., Werner, G., & Nübel, U. (2016). Comparison of six commercial kits to extract bacterial chromosome and plasmid DNA for MiSeq sequencing. Scientific reports, 6(1), 1-5.
8. Fahle, G. A., & Fischer, S. H. (2000). Comparison of six commercial DNA extraction kits for recovery of cytomegalovirus DNA from spiked human specimens. Journal of clinical microbiology, 38(10), 3860-3863.
9. Daugharty, H., Skelton, S. K., & Messmer, T. (1998). Chlamydia DNA extraction for use in PCR: stability and sensitivity in detection. Journal of clinical laboratory analysis, 12(1), 47-53.
10. Klein, A., Barsuk, R., Dagan, S., Nusbaum, O., Shouval, D., & Galun, E. (1997). Comparison of methods for extraction of nucleic acid from hemolytic serum for PCR amplification of hepatitis B virus DNA sequences. Journal of clinical microbiology, 35(7), 1897-1899.
11. Vince, A., Poljak, M., & Seme, K. (1998). DNA extraction from archival Giemsa-stained bone-marrow slides: comparison of six rapid methods. British journal of haematology, 101(2), 349-351.
12. Ferrand, J., Patron, K., Legrand-Frossi, C., Frippiat, J. P., Merlin, C., Alauzet, C., & Lozniewski, A. (2014). Comparison of seven methods for extraction of bacterial DNA from fecal and cecal samples of mice. Journal of microbiological methods, 105, 180-185.
13. Claassen, S., du Toit, E., Kaba, M., Moodley, C., Zar, H. J., & Nicol, M. P. (2013). A comparison of the efficiency of five different commercial DNA extraction kits for extraction of DNA from faecal samples. Journal of microbiological methods, 94(2), 103-110.
14. Kumar, J., Kumar, M., Gupta, S., Ahmed, V., Bhambi, M., Pandey, R., & Chauhan, N. S. (2016). An improved methodology to overcome key issues in human fecal metagenomic DNA extraction. Genomics, proteomics & bioinformatics, 14(6), 371-378.
15. Ramón-Laca, A., Soriano, L., Gleeson, D., & Godoy, J. A. (2015). A simple and effective method for obtaining mammal DNA from faeces. Wildlife Biology, 21(4), 195-203.
16. Menu, E., Mary, C., Toga, I., Raoult, D., Ranque, S., & Bittar, F. (2018). Evaluation of two DNA extraction methods for the PCR-based detection of eukaryotic enteric pathogens in fecal samples. BMC research notes, 11(1), 1-6.