International Journal of Research in Medical Sciences
Ali H et al. Int J Res Med Sci. 2016 Jan;4(1):100-104
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20160012

Research Article

Optimized genomic DNA extraction by a modified organic phenol-chloroform method without using PCR for best results

Hamid Ali[1], Sadaf Jafar[2]*, Qurat-ul-Ain[2]

[1]Department of Physiology, Islamabad Medical and Dental College (IMDC), Baharia University, Islamabad, Pakistan
[2]Department of Biochemistry, Islamabad Medical and Dental College (IMDC), Baharia University, Islamabad, Pakistan

Received: 08 October 2015
Revised: 26 November 2015
Accepted: 15 December 2015

***Correspondence:**
Dr. Sadaf Jafar,
E-mail: sadafjafar75@gmail.com

ABSTRACT

Background: The objective of the study was to design a cost effective, efficient and better protocol for genomic deoxyribonucleic acid (DNA) extraction.
Methods: This was an experimental study, which is carried out ARID Research laboratory. The duration of study was four months from March 2015 to June 2015. White blood cells were used for extraction of DNA. Two procedures RBC lysis and extraction of DNA from the pellet was done. The goal was to obtain high quality and quantity DNA yield. Even the amount of blood less than 1 ml can be used for extraction. The extraction of DNA comprise of disruption of nuclear membrane and removal of protein from the pellet. After extraction DNA was purified. DNA was confirmed with the help of Nanodrop technology. Genomic DNA was also confirmed by using horizontal agrose gel electrophoresis and visualized using Gel Doc System. Whole blood approximately 10ml µg, yields 250 µg of DNA, using latest organic phenol chloroform method.
Results: Successful extraction of DNA from the WBCs without compromising quality and quantity was the end result. Shelf life of DNA was increased. Current modified protocol of DNA isolation, extraction and purification of DNA is not only cost-effective and highly yielding but also reproducible.
Conclusions: The DNA extracted with modified organic phenol- chloroform method was of high quality and quantity. The bands of DNA were confirmed with horizontal electrophoresis. Clear DNA band were visualized indicating the precision of latest protocol. Another important advantage was that very small amount of blood sample is required for DNA extraction and the protocol was cost effective and efficient. Present protocol will be very beneficial for genomic studies, requiring DNA extraction. It will enable researchers to work with fewer budgets and less sample size, obtaining best results.

Keywords: DNA, Extraction, WBCs

INTRODUCTION

DNA studies are widely used now-a-days, for this purpose DNA isolation is done, which is the bases for all the genomic studies. In order to find the best results in shortest time, keeping in view cost effectiveness lot of strategies has been used. Different DNA extraction kits are available in market. A genomic DNA from permafrost was isolated. The fast DNA ® SPIN (FDS) kit results were best as compared to Meta-G- Nome TM (MGN) isolation kit.[1]

DNA was isolated from teeth using a modified phenol-chloroform method. Screening and analysis of Yesinia

pestis gene was done to find out about the pandemic and it was found that Y pestis emerged from rodents. [2]

Even for different common diseases like Malaria, DNA isolation is used as tool to study the plasmodium. PCR assay targeting the 18S RNA gene, which was considered for finding the affected plasmodium. Plasmodium Knowlesi was the causative agent in more than 56% of the cases in Malaysia.[3] DNA analysis is also done to find out the treatment of different diseases. It is a big help to physicians, by studying the genome of micro-organisms causing the disease, treatment can be easily sorted out, with help of latest technology.[4] Oral and gut microbe studies are very beneficial for treatment purpose. Genomic studies using a sample of saliva and stool, was indicating the prevalence of the common pathogen. There was a similarity among 41% of microbial transcript.[5]

Lentiviral genomic bar coding is used by researchers to interpret the diversity in growth and differentiation of mammary epithelial cells.[6] In most of the genomic studies DNA isolation is required. DNA extractions are also beneficial to assess DNA damage. Dry olive leaf extract is very effective to attenuate DNA damage produced as a result of reactive oxygen species which is due to excessive adrenaline.[7]

Bead-beating method for extraction of DNA from Giardia duodenalis was the best because it provided highest DNA yields. Spin column extraction with prior freeze-thaw treatment method was also selected because of its desired results.[8]

A modified method from cetyltrimethylammonium bromide (CTAB) method can be used; it was simple, fast and without use of liquid nitrogen. Only chemicals and simple laboratory equipment was needed. The isolated DNA was stable, can be used for DNA finger printing, quantitative traits loci analysis, screening of transformants and digestion by enzymes.[9]

The efficiency of nucleic acid extractor system was compared with QIA amp DNA blood mini kit using whole blood volume. The time for DNA extraction was 20 minutes where as other methods took 50 minutes and 40 minutes. The latest method was very useful for diagnostic testing for clinical purpose.[10]

METHODS

This original study was approved by ARID Research Committee at Rawalpindi. The blood samples were taken after verbal consent of the patients by the doctor. WBCs were the target cell for the extraction of DNA from the whole blood. The procedure for DNA Extraction from blood was split into:

1. RBCs lysis
2. Extraction of DNA from pellet

Remaining pellet of things have to kept side by side while performing destruction of RBCs. Extraction of DNA was our goal" HIGH QUALITY DNA AND HIGH QUANTITY" yield. If quantity was low especially below, PCR optimization might become difficult and if quality was substandard, then production of dimers and other PCR inhibitors would increase .Moreover this high yield protocol was also of worth weight age when amount of blood is ≤1 ml. We a group molecular Scientists modified Existing protocols to achieve our desire goal.

The composition of RBC lysis Solution A (RBC Lysis Buffer) is:

(i) 0.32 M sucrose
(ii) 10 mM Tris (pH 7.5)
(iii) 5mM MgCl2
(iv) 1% v/v Triton X¬100 1.

Methodology for RBC lysis

750 µl of blood was taken in a 1.5 ml Eppendorf tube and 750 µl of solution A was added into it. Tube was incubated at room temperature for 10 min. Tube was centrifuged for 3 min at 5000 RPM. Supernatant was discarded and pellet was resuspend with 500 µl of solution A and centrifuge again for 3 min at 5000 RPM. Washing two times with solution is necessary for complete destruction of RBCs. Remaining whitish pellet was saved for DNA extraction.

Methodology for nuclear lysis

Disruption of nuclear membrane and removal of proteins form the pellet.

The composition of solution B:

1. 10 mM Tris (pH 7.5)
2. 400 mM NaCl
3. 2 mM EDTA (pH 8.0)

Next step: >400 µl of solution was added to tube containing pellet and 30 µl of 20% SDS and 5 µl of Proteinase K (stock 10 mg/ml) and incubated at 37 for overnight.

Methodology for DNA extraction and purification

DNA was extracted and purified on the 2nd day.

The composition of solution C:

Buffered Phenol PH 8.4

The composition of solution D:

Chloroform Isoamylalcohol mixture (24:1) 500 µl of fresh mixture of solution C and D and centrifuged at 6000 RPM at 4ºĊ. Supernatant was shifted into a new tube.

Tube was placed in crushed ice pad for 5 min. 500 µl of solution D was added and centrifuged at 6000 RPM at 4ºC it in a refrigerated centrifuge. Upper layer was shifted into a new tube. Sodium acetate 50µl of 3M with pH 6 and 350 µl of ice cold 10% ethanol. Gently mix it to precipitate DNA. DNA threads will be visible as whitish threads. Tubes were again placed in crushed ice pack for 10 min. Tubes were centrifuged for 3 min at 6000 RPM at 4ºC. Discard the supernatant and pellet was washed with 300 µl of 70% ethanol. Supernatant was discarded and tubes were dried by inverting them on sterile filter paper. The pellet was dissolved in 150ul of TE buffer.

All these steps need to be performed in a fume hood wearing gloves.

DNA confirmation

Quantification of DNA was done using Nanodrop technology (NanoDrop instruments measure nucleic acid concentrations using only 1 µL of sample) and confirmation of genomic DNA was made by running the sample on 0.8-1% prestained agarose gel with Ethidium bromide in a technique called horizontal agarose gel electrophoresis and subsequently visualizing images using Gel Doc System. Minimum quantity required for PCR is 50 ng/µl. We extracted and purified DNA from 3-4ml of whole sample i.e. 100 ≥400 ng. Ideally it was estimated that 10 ml of whole blood, yields approximately 250 µg of DNA using organic phenol chloroform method.

RESULTS

Successful extraction of DNA from the WBCs without compromising quality and quantity is a desire goal of every molecular genetics analysis lab. The quantities of chemicals used and time allowed for each process either increases or decreases the overall yield of DNA, so a costless, efficient optimized DNA protocol was the need of time. We, successfully not only isolated the DNA but the quality of DNA was very good. Very less number of dimers and other secondary structures were formed during the process of subsequent PCR reaction for the amplification of ADD1 gene. Moreover, beside the quantity and quality of DNA, overall shelf life of DNA was increased as reveled by the picture of DNA bands run on 0.5% Agarose gel and visualized by gel documentation system and UV Trans- illuminator and quantified by nanodrop technology. Successful results clearly indicate that the current modified protocol of DNA isolation, extraction and purification of DNA is not only cost-effective and highly yielding but also reproducible. (Figure 1-3).

Figure 1: Graphical concentration of DNA obtained from nano drop.

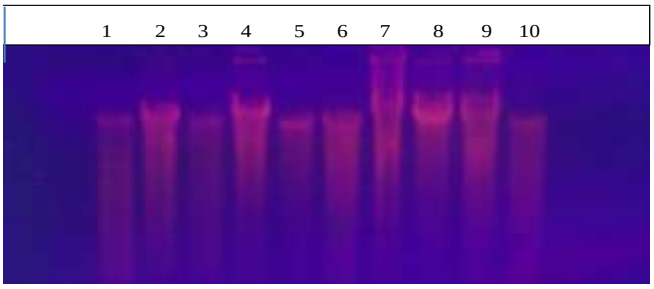

Figure 2: Analyses of stock human genomic DNA from human blood are run in well 1-10 to check out its quantity and quality.

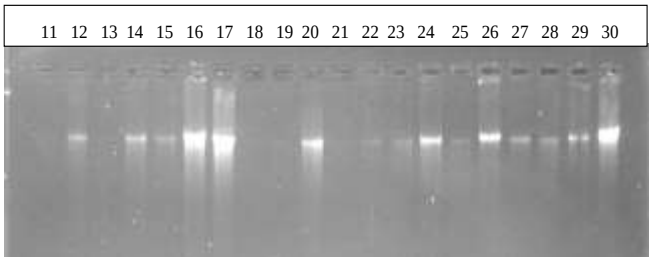

Figure 3: Analyses of 10X human genomic DNA from human blood are run in well 12-30 to check out its quantity and quality.

DISCUSSION

Different drug response can be studied in the field of genetics by using DNA extraction technology. Even the pesticides can cause mutations in genes. In our study we sorted out a new regimen for DNA extraction which may be very efficient and beneficial in upcoming research programmes.[11]

Another isolation of DNA, from human peripheral whole blood was carried out using non enzymatic procedure, based on the principle of salting out. Chloroform, phenol and isoamyl alcohol were avoided. The whole procedure took sixty five minutes, without proteinase K. Even the quantity was 100 -150 µg of DNA, which was extracted from one milliliter of blood. The purity was also suitable for genotyping. As compare to our study different

chemicals are used without proteinase K and the results were successful.[12]

In comparison of three methods, salt precipitation, silica column based extraction and chloroform phenol extraction the first two methods gave good results for DNA extraction as compared to the third one while in our study a new protocol for DNA extraction was created.[13]

Another direct method for DNA extraction is often used by the researchers by amplifying genomic DNA directly from human specimens while extraction and purification steps are bypassed. In our study modified organic phenol-chloroform method for DNA extraction was introduced which is less time consuming.[14]

DNA was extracted from urine, both human and microbial DNA were extracted. It was simple and noninvasive and urine sample was collected at different moments. Different commercial kits for DNA extraction were used. In our study only one method for DNA extraction was used and instead of urine, blood samples were used.[15]

Viral human papilloma DNA was extracted using Silica based method. Chemicals are not used; even sophisticated equipment is not required. It was less expensive and highly reliable. Similarly our DNA extraction protocol was found to be reliable and cost effective.[16]

Modified cetyltrimethyl ammonium bromide protocol was used to isolate genomic DNA. Genetic patterns were used to analyze parentage of offspring. In our study the protocol can also be used to study the genetic parentage.[17]

Quality evaluation of DNA samples by amplification of 16SrRNA gene with PCR and restriction fragment length polymorphism. With the help of this study a good quality metagenomic DNA from the soil was obtained. The present study also deals with the similar DNA extraction protocol in which 250µg of DNA was extracted from 100ml of whole blood sample.[18]

Species of Cryptosporidium was studied in HIV positive patient and their subtypes along with clinical manifestation. Present protocol yields high quality DNA which is beneficial for different type of studies.[19]

The relationship between vascular access failure and the genetic polymorphism of ACE and TNF-gene was found. So it was considered that TNF-α-308G>A may be the genetic marker in hemodialysis patients. In our study genetic patterns can be easily studied with the help of efficient and cost effective protocol.[20]

CONCLUSION

The DNA extracted with modified organic phenol-chloroform method was of high quality and quantity. The bands of DNA were confirmed with horizontal electrophoresis. Clear DNA band were visualized indicating the precision of latest protocol. Another important advantage was that very small amount of blood sample is required for DNA extraction and the protocol was cost effective and efficient. Present protocol will be very beneficial for genomic studies, requiring DNA extraction. It will enable researchers to work with fewer budgets and less sample size, obtaining best results.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Tatiana V, Alice L, Maggie L. Commercial DNA extraction kits impact observed microbial community composition in permafrost samples. FEMS Microbiol Ecol. 2014;17(87):217-30.
2. Wagner DM, Klunk J, Harbeck M. Yersinia pestis and the plague of Justinian 541-543 AD: A genomic analysis. Lancet Infect Dis.2014;14:319-26.
3. Ruhani Y, Yee L, Rohela M. High proportion of Knowlesi malaria in recent malaria cases in Malaysia. Malaria Journal. 2014;13:168.
4. Andrea T, Michelle S, Mark S. Analyzing the human microbiome: A "how to" guide for physicians. Am J Gastroenterol. 2014;109:983-93.
5. Franzosa EA, Morgan XC, Segata N. Relating the metatranscriptome and metagenome of the human gut. Proc Natl Acad Sci USA. 2014;3:111.
6. Long VN, Maisam M, Annaick C. Clonal Analysis via Barcoding Reveals Diverse Growth and Differentiation of Transplant Mouse and Human Mammary Stem cells. 2014;14(2):253-63.
7. Cabarkapa A, Zivkovic L, Zukovec D. Protective effect of dry olive leaf extract in adrenaline induced DNA damage evaluated using in vitro comet assay with human peripheral leukocytes. Toxicol in vitro. 2014;28:451-6.
8. Elwin K, Fairclough V, Hadfield J. Giardia duodenalis typing from stools. A comparison of three approaches to extracting DNA, and validation of a probe-based real-time PCR typing assay. J Med Microbiol. 2014;63:34-44.
9. Ferdous, Jannatual M, Mohamed H. A quick DNA extraction protocol: without liquid nitrogen in ambient temperature. African Journal of Biotechnology. 2012;11:6956-64.
10. Tae-Dong J, Young C, Woochang L. An efficient genomic DNA extraction from whole blood using Nextractor. Clinica Chimica Acta. 2014; 435:14-7.
11. Preety B, Asha C. Genotoxicity evaluation of acephate and profenofos by the PCR-RFLP assay. Toxicol Int. 2014;21:84-8.
12. Senthikumar K. The Rapid and Non-enzymatic isolation of DNA from the human peripheral whole blood suitable for genotyping. European Journal of Biotechnology and Bioscience. 2014;1:1-16.
13. Muhammad A, Bindu B, Jini R. Evaluation of different DNA extraction methods for the detection of adulteration in raw and processed meat through Polymerase chain reaction-restriction fragment

polymorphism (PCR-RFLP). J Food Sci Technol. 2015;52:514-20.
14. Ariefdjohan M. DNA Extraction Methods for Human Studies. Encyclopedia of Metagenomics. 2014;6:1-4.
15. Bali L, Diman A, Bernard A, Roosens NH, De Keersmaecker SC. Comparative Study of Seven Commercial Kits for Human DNA Extraction from Urine Samples Suitable for DNA Biomarker-Based Public Health Studies. Journal of biomolecular techniques: JBT. 2014;25(4):96.
16. Boumba L, Assoumou SZ, Moukassa D, Touil N, Mestoui O, Khattabi A, et al. Optimization of amanual and rapid Silica-based DNA Extraction Method Applied to Human Papillomavirus detection using fresh cervical biopsies samples. International Journal of Scientific Reseach. 2015;5:2250-3153.
17. Mirimin L, Roodt R. Testing and validating a modified CTAB DNA extraction method to enable molecular parentage analysis of fertilized eggs and larvae of an emerging South African. J Fish Biol. 2015;86:1218-23.
18. Embarcadero-Jimenez S, Yang FL, Freye-Hernandez R, Trujillo-Cabrera Y, Orduna FN, Yuan HL, et al. An improved protocol for extraction of metagenomic DNA from high humus alkaline and saline soil of Chinamp a for T-RFLP finger printing analysis. British Microbiology Research Journal. 2014;4(7):821-30.
19. Adamu H, Petros B, Zhang G, Kassa H, Amer S, Ye J, et al. Distribution and clinical manifestation of cryptosporidium species and subtypes in HIV/AIDS patient in Ethiopia. PLoS Neg I Trop Dis. 2014;8(4):e2831.
20. Sener EF, Taheri S, Korkmaz K, Zararsiz G, Serhatlioglu F, Unal A, et al. Association of TNF-α-308 G > A and ACE/D gene polymorphism in hemodialysis patient with arterivenous fistula thrombosis. Int Urol Nephrol. 2014;46(7):1419-25.

Cite this article as: Ali H, Jafar S, Ain QU. Optimized genomic DNA extraction by a modified organic phenol- chloroform method without using PCR for best results. Int J Res Med Sci 2016;4:100-4.