DNA Isolation: A Method for Improving the Efficiency of DNA Extraction from Clotted Blood Samples

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ARTICLE INFO

Keywords:
- Clotted Blood
- DNA Extraction
- Salting-Out Method

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The author has reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/NASETJournal.v1i2.8

1. Introduction

The increasing demand of genome-based analyses in modern evolutionary and disease researches have also increased the need for bulk amount of pure DNA genomic.1,2 DNA can be extracted from many biological samples such as hair, blood, semen, saliva, skin cells, and many more. The one that has gained astounding importance in biological researches is blood. Blood has become an integral part of biochemistry, hematology and clinical studies and forensic investigations [3]. DNA extracted from blood samples is crucial for confirmation of genetic abnormalities, as well as application in epigenetic studies and preventive medicine.4 It serves as an important source of genomic DNA because of the presence of nucleated white blood cells.3

There is a many method to DNA extraction, which are the fenol-chlorom extraction, salting-out, silica-guanadinium thiocyanate, and commercial kits are commonly used5, but it usually comes with high cost.6 Salting-out method is a simple, non-toxic, and unexpensive method for extracting high-molecular-weight DNA from peripheral lymphocytes.6,7 The optimal concentrations of various salts such as Tris-HCl, KCl, MgCl2, and NaCl are used as the buffer to obtain a favorable DNA results.8,7 Xu, et al. in 2010 and Wong, et al. in 2007, showed a physical breakage of the blood clot into small pieces, before extracting DNA, improves the quality of DNA purification from clotted blood.9,10

In this study, researchers developed a simple, safe, and efficient technique for the fragmentation of the clot
before DNA extraction processing. This study used the ball bearing metal shots as a mixture to maximize the fragmentation of the clot and attempted to modify a salting-out method for DNA extraction with the highest possible yield from blood clots with ethylenediaminetetraacetic acid (EDTA) and tri-sodium citrate dehydrate as a blood anticoagulant for both blood homogenization and chelated cations of Mg2+ and Ca2+ which are necessary when using DNAases. Finally, in this study, the rate of the PCR inhibitor and quality of extracted gDNA were compared between a modified salting-out method, a modified QIAamp® DNA Blood Midi Kit, and QIAamp® DNA Blood Midi Kit as the control group.

2. Materials and Methods

Sample collection

Randomly selected 31 clotted blood samples collected as part of the Mashhad Stroke and Heart Atherosclerotic Disorders (MASHAD) study. Ten milliliters of peripheral blood were collected in plain tubes. Samples were spun, and the serum was separated. All tubes containing clotted blood were stored at −80°C. Another 32 samples of clotted blood from this cohort were chosen and considered as a comparator group.

Sample preparation

Before proceeding to DNA extraction using a modified salting-out method and modified QIAamp® DNA Blood Midi Kit, 500 µL phosphate-buffered saline (PBS) (1X) and two ball bearing metal shots, autoclaved in 121°C for 15 minutes, were added to each tube of clotted blood sample and were gently rotated in an electric laboratory rotator for 1 hour at room temperature (18-25°C). In QIAamp® DNA Blood Midi Kit, blood samples were thawed for 1 hour at room temperature (18-25°C).

Modified salting-out method

Two milliliters of blood clot were transferred to a tube containing 7 mL of cell lysis buffer solution (CLB) (0.320 mmol/L sucrose, 10 mmol/L Tris-HCl, 2 mmol/L MgCl₂, 1% Triton X-100, 4 mmol/L trisodium citrate dehydrate) (pH 6.50) (Merck, Germany), and the tubes were mixed well by pulse-vortexing for 2 minutes. After centrifuging at 3800 rpm (1533g) for 10 minutes, the supernatant liquid was carefully discarded to waste and 5 mL CLB was added to pellet again, and tubes were well shaken for 2 minutes and centrifuged at 3800 rpm (1533g) for 10 minutes. After the supernatant was discarded, 5 mL of low salt buffer containing 10 mmol/L Tris-HCl, 4 mmol/L MgCl₂, and 10 mmol/L KCl (known as TKM1) and 0.1 mmol/L Na₂EDTA, pH 4.46 (Merck, Germany), was added to the pellet. The samples were mixed for 1 minute and centrifuged at 3800 rpm (1533g) for 10 minutes at room temperature. After decanting the supernatant, 1.5 mL of high salt buffer TKM2 containing 10 mmol/L Tris-HCl, 4 mmol/L MgCl₂, 8 mmol/L KCl, and 1 mmol/L Na₂EDTA, 390 mmol/L NaCl, pH 4.82 (Merck, Germany), along with 100 µL solution of 10% sodium dode-cyl sulfate (SDS) (w/v) was added to each tube. The samples were then mixed for 1 minute. This mixture was incubated for 1 hour at 65°C. Then, 500 µL of 6 M NaCl (Merck, Germany) was added. After vigorous shaking for 15 seconds, proteins were removed by centrifugation at 3800 rpm (1533g) for 10 minutes. The supernatant liquid was carefully transferred to a new clean tube containing 4 mL cold absolute ethanol (Merck, Germany). The tubes were inverted gently several times, causing long strands of high-molecular-weight DNA to appear. The DNA was transferred to a 1.5-ml sterile microtube along with the addition of cold absolute ethanol. If the DNA cloud were not seen, the solution was transferred to a 1.5-ml sterile microtube centrifuged at 1400 rpm (20817g) for 2 minutes, and the supernatant was then discarded. These steps were repeated several times until the no supernatant solution remained in the tube. The pellet was washed once with 300 µL of 70% ethanol (Merck, Germany) and then centrifuged at 14000 (20817g) rpm for 2 minutes. The DNA pellet allowed to be dried for at least 10
minutes at room temperature (18-25°C) until there was no trace of ethanol. Finally, the DNA was dissolved in 100-200 μL of the sterile distilled water before storage at −20°C.

**Modified QIAamp® DNA blood midi kit**

A 500 μL phosphate-buffered saline (PBS) (1X) and two ball bearing metal shots were added to each tube of clotted blood sample. They were then gently rotated for 1 hour. DNA extraction of the clotted blood samples was performed with the QIAamp® DNA Blood Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations.

**QIAamp® DNA blood midi kit to compare the results**

A 22 frozen clotted blood samples as the control group were chosen from the MASHAD cohort and thawed at room temperature (18-25°C) for 1 hour, followed by DNA extraction which was performed using QIAamp® DNA Blood Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

**Statistical analysis**

This study using g SPSS 17.0 (SPSS Inc, Chicago, Illinois) for statistical analysis and using the Shapiro-Wilk test the data normality. Non-normally distributed data are presented as median and percentiles (IQ1-IQ3). The Kruskal-Wallis test was used for comparing non-normal data among the groups. Statistical significance was set at P < 0.05.

3. Results

The mean result and purity of DNA obtained by these three methods were significantly different (P < 0.001) and a significant difference was also observed in the A260/A280 ratios among those methods (P = 0.01). The quality of the DNA was fairly uniform in both modified salting-out and modified QIAamp® DNA Blood Midi Kit methods. DNA purified from clotted blood samples could be used to amplify and genotype the exon 3 of the crystalline gamma-D (CRYGD) gene and SNP rs1333049 successfully showed by the analysis of PCR products and real-time PCR.

**Salting-out method for DNA isolation**

An alternative method was developed in this study to the isolation of high quality DNA with a large number of blood clots, which in use of the ball bearing metal shots to homogenize and break down the blood clot, and adding phosphate-buffered saline (PBS) to maximize the volume of blood in liquid form that believe will increase the buffy coat volume [11]. The higher quantity of DNA using a mechanical device showed by the increase of DNA extraction in modified salting-out method and modified QIAamp® DNA Blood Midi Kit looks significant when compared with QIAamp® DNA Blood Midi Kit (P<0.001, 75.43 ± 47.12 and 91.48 ± 63.07 vs 42.46 ± 26.21). This observation is consistent with previous results which indicated a mechanical breakage of the clots is useful for enhancing the amount of DNA.12,9

It seems that the use of ball bearing metal shots in blood clot containing tubes is a simple and safe mechanical device to break down the blood clot. A minimized handling the clotted sample, risk of possible external contamination of the samples can be reduced, improving the DNA yield through the efficient disintegration of the clot, speeding up the extraction procedure as blood clots may hinder the conventional extraction procedures, and improving the safety are the superiority in use of ball bearing metal shots. PCR genotyping was successfully extracting the DNA. Real-time PCR is more accurately in gDNA quantification. An inhibitory factors is not identified in the solution. These findings demonstrated the quality and quantity of DNA samples purified from clotted blood, and this method would also help to use frozen blood clots as a source of DNA in many areas of molecular biology.

Pipetting each step to dissolve the pellet is challenging. Modified QIAamp® DNA Blood Midi Kit is a suitable choice for enhancement of the speed of DNA purification and minimizes the possibility of cross-
contamination. The reduction time for DNA extraction, and quality improvement and quantity of DNA is fairly reduced showed by using physical and chemical methods.

4. Conclusion

A modified salting-out method for DNA isolation from frozen blood clots is a simple, safe, and low cost in routine laboratory tasks. The utilization the ball bearing metal shots for the fresh blood as a homogeneous suspension may be skipped although this method is beneficial and practical for either fresh or clotted blood.

5. Acknowledgments

We express our gratitude to the researcher who had been modified this DNA isolation in good purpose, we hope this manuscript may contribute to the DNA isolation related to DNA extraction.

6. References

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