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Gelatin-coated magnetic nanoparticles-based DNA isolation method: A comparison with commercial DNA isolation kits from whole blood

Kelly S Levano[1,2], Luis Jaramillo-Valverde[1,2], Elizabeth Delgado[1,2], Heinner Guio[1,2*]

[1]INBIOMEDIC Research and Technological Center, Lima, Peru;
[2]ALBIOTEC, Lima, Peru;
*Correspondence : Heinner Guio, PhD , E-mail: heinnerguio@gmail.com

ABSTRACT

DNA isolation is the first step for most molecular tests, thus indicating the importance of using a reliable and suitable method for this procedure. The use of magnetic nanoparticles for blood DNA isolation offers several advantages including: subjecting little mechanical stress to your sample, obtaining higher quality and quantity of DNA and non-laborious procedures. In this study, we isolate genomic DNA using gelatin-coated magnetic nanoparticles from blood samples and test its use compare with three commercial blood DNA isolation kits. DNA purity and yield were assessed by measuring absorbance at A260/A280 and by agarose gel electrophoresis. The suitability of the isolated DNA for downstream applications was analyzed by end-point PCR and Sanger sequencing. In this study, gelatin-coated magnetite particles for genomic DNA isolation provide an efficient, simple, and inexpensive method that does not require the use of commercial blood DNA Isolation Kit.

Keywords: *Blood, DNA isolation, Magnetic nanoparticles, PCR*

1 INTRODUCTION

Appropriate DNA isolation method for a specific biological sample (whole blood, saliva, stool, urine, fresh tissue or paraffin-embedded tissue) is a prerequisite for any molecular testing. This selection is dependent on many factors including: yield, purity, time, safety, specialized equipment requirement, trained personnel, intended downstream applications, cost, and sample source.

Blood is an ideal source of human genomic DNA (Cho et al., 2007). However, isolating genomic DNA by traditional methods is a time-consuming process, and phenol and chloroform are toxic reagents that endanger health (Hansen et al., 2013).

Further, traditional methods, such as phenol isolation, isopropanol precipitation, formamide lysate method, nonorganic solvent isolation, and glass particle adsorption, have been found to be ineffective for isolating genomic DNA from trace, dried, and frozen blood. Therefore, it is necessary to find a more convenient and efficient method for obtaining human genomic DNA (He et al., 2013). Saliva samples are a good alternative source of genomic DNA owing to the painless and noninvasive collection (Bux et al., 1995).

Compared to blood, saliva samples are much more

convenient, efficient, and accessible if DNA can be isolated and purified.

A need for a reliable, easy to use, low cost and not requiring special equipment DNA isolation method let us to develop a DNA isolation kit for blood biological sample. The use of magnetic nanoparticles provides several advantages in biotechnological applications such as enzyme purifications, immunoassays, immunotherapy, and nucleic acid isolation (Borlido et al., 2013; Intorasoot et al., 2009)

Even though uncoated magnetic nanoparticles can bind to DNA and can be used for its isolation, polymer-coated magnetite nanoparticles provide a higher recover of DNA. Magnetic nanoparticles are coated with different polymers such as agarose and silica (Taylor et al., 2000; Yoza et al., 2002; Chiang et al., 2005) previously described and used for bacterial cells. In this study, we compare three commercial DNA isolation kit with gelatin-coated magnetic nanoparticles-based method to isolate blood biological samples.

2 MATERIALS AND METHODS

Materials

The following materials were used: gelatin-coated magnetic nanoparticle (GMNPs) genomic DNA isolation method called INBIOMag Genomic DNA Kit (INBIOMEDIC, Peru), QIAamp DNA Blood Mini Kit (Qiagen Cat. No. 51104); GeneJET Genomic DNA Purification Mini Kit (Thermo Scientific, Cat No. K0721) and MagJET Genomic DNA Kit (Thermo Scientific, Cat No. K2721). Oligonucleotide primers were synthesized by Macrogen Inc., Korea. All other chemicals and enzymes used were of high-grade purity.

Sample Collection

Peripheral venous blood samples were collected from 30 participants, who visited INBIOMEDIC Research and Technological Center. After obtaining written informed consent, 3 ml of blood samples were collected in a tube containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Each individual sample was divided into 1 ml aliquots and stored at −20 °C until DNA isolation. DNA isolation from all samples was completed within 3 days after collected blood. The study protocol was approved by INMENSA Ethics Committee, Peru.

DNA extraction methods

The four methods are described:

A: Gelatin-coated magnetic nanoparticle genomic DNA isolation method (INBIOMag Genomic DNA Kit): 400 ul of whole blood was used and mix with 1.2 ml of Red Blood Cell lysis (RBC) buffer by vortex. The solution was incubated at room temperature for 2 min and centrifuge 13000 g for 1 min. Pellet was mixed with RBC buffer and centrifuge 13000 g for 1 min. Pellet was mixed with 100 ul of NaCl Solution and 40 ul of Proteinase K, then it was added 600ul of cell lysis buffer and incubate to 56 °C for 10 min. After incubation, 50ul of GMNPs (50 mg/ml) with 400 isopropanol and 400 ul of binding buffer (1.25 M sodium chloride and 10% polyethylene glycol 6000) were added to the lysate. The solution was mixed and incubated at room temperature for 10 min. The magnetic pellet was immobilized in a magnetic rack and the supernatant was removed. The magnetic pellet was washed three times with wash buffer and dried for 15min. The magnetic pellet was then resuspended in 100 ul of Tris-EDTA (TE) buffer and incubated at 65°C for 10 min. The supernatant containing the DNA and was transferred to a fresh tube.

B. QIAamp DNA Blood Mini Kit: 200 ul of sample were incubated with 20 uL of Proteinase K in 200 ul Buffer AL for 10 min at 56°C. 200ul of ethanol was then added. The remainder of the isolation procedure was carried out according to the manufacturer's protocol.

C. GeneJET Genomic DNA Purification Mini Kit: 200 ul of sample were incubated with 20 uL of Proteinase K in 400 ul Lysis Solution for 10 min at 56°C. 200ul of ethanol was then added. The remainder of the isolation procedure was carried out according to the manufacturer's protocol.

D. MagJET Genomic DNA Kit: 3 volumes of sample were mixed with 1X RBC Buffer and incubated on ice for 4-7 minutes. The solution was centrifuge in cold at 7000 g for 5 min. The supernatant were removed. The remainder of the extraction procedure was carried out according to the manufacturer's protocol.

The yield and quality of the DNA isolation methods was analyzed by QIAexpert.

Gel electrophoresis score

To observe possible degradation due to the action of nucleases during the isolation procedure, 1 µg of stock DNA from samples representative of each isolation method was electrophoresed on 1% agarose gel and visualized with blue light. A smeared band was indicative of sheared or degraded DNA samples.

PCR amplification

Genomic DNA isolation quality from whole blood, were analyzed by PCR amplification of *BRCA-1* gene. All PCRs were performed in a 50 ul reaction volume using Platinum Taq Polymerase (Invitrogen) according to the manufacturer´s procedure and using the GTQ cycler 24 (Hain). BRCA-1 specific sequence (242bp) was amplified by primer pairs: BR1c68_69delF (5´-GAAGTTGTCATTTTATAAACCTTT-3´) and BR1c68_69delR (5´ GTATGTAAGGTCAATTCTGTTC-3´) (Lee at al., 2016) using 100 ng of DNA template. Thermal cycling was performed at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 sec, 45 °C for 30 sec, 72 °C for 1 min. PCR products were analyzed on 1.5% agarose gel electrophoresis and visualized with blue light.

DNA sequencing

PCR products obtained from genomic DNA extracted from whole blood, were purified and sent for sequencing (Macrogen) using the primers BR1c68_69delF and BR1c68_69delR**.**

3 RESULTS

DNA concentration and yield

Total concentration and yield of isolated DNA samples were estimated from spectrometric measurements using QIAexpert platform. Summary statistics of 30 samples isolated using four methods are shown in Table 1. Method D extracted the most concentrated DNA with an average of 53.90 ng/µl, followed by method A with an average of 36.78 ng/µl, both are methodologies based on magnetic nanoparticles.

Method B and C showed median value of 16.03 and 10 ng/µl respectively, both are methodologies based in silica columns. Total yield of isolated DNA from each sample was calculated by multiplying DNA concentration with the final elution volume of 100 µl. All samples isolated using method A and D had total DNA yield above 2ug required for sequencing.

Table 1: Summary of total DNA yield and DNA concentration based on QIAexpert measurements obtained from the four extraction methods

Isolate method*	Total DNA yield (µg) per 100 µl	DNA concentration (ng/ul)
	Mean	Mean
A	3.68	36.78
B	1.60	16.03
C	1.00	10.00
D	5.39	53.90

* 30 samples per isolation method

DNA purity

To assess DNA isolated purity by four different methods, absorbance was measured at 230, 260 and 280 nm wavelengths, and the ratio of these absorbances were computed to estimate the relative purity of test samples. A260/A280 and A260/A230 ratios are summarized in Table 2. Methods A, B and D isolated the purest DNA from 30 samples with a mean A260/A280 ratio of 1.62, 1.70 and 1.72, respectively. Method C had the lowest mean A260/A280 ratio of 0.94.

The results of A260/A230 ratio showed that the method A, C and D had mean values >1.0 which was indicative of pure DNA free of organic contaminants (Table 2). Method B however, gave values lower than 1, which suggested the presence of residual phenol or chaotropic salts that strongly absorb at 230 nm.

Table 2 Summary of A260/A280 and A260/A230 ratios obtained from the four extraction methods

Isolate method*	A260/A280	A260/A230
	Mean	Mean
A	1.62	1.00
B	1.70	0.94
C	0.94	1.13
D	1.72	1.33

* 30 samples per extraction method

DNA integrity

DNA integrity was checked by electrophoresis of stock DNA samples representative of four isolation methods, as shown in Figure 1. All four isolation methods were capable of isolating non-degraded, slow-migrating genomic DNA with high-molecular weight >10 kb. A method sample showed least amount of shearing compared to B, C and D methods samples.

End-point assay: PCR

BRCA-1 amplification by PCR was followed by separation PCR products on 1.5 % agarose gel. As shown in Figure 2, amplification pattern was consistent for all samples using method A and were further confirmed by direct sequencing.

DNA sequencing

Sanger sequencing, was performed on 5 DNAs samples isolated by A method. The alignment in Figure 3 showed successful detection of candidate mutations for each sample. The sequencing performance in the DNA isolated from Gelatin-coated magnetic nanoparticle genomic DNA method is optimal.

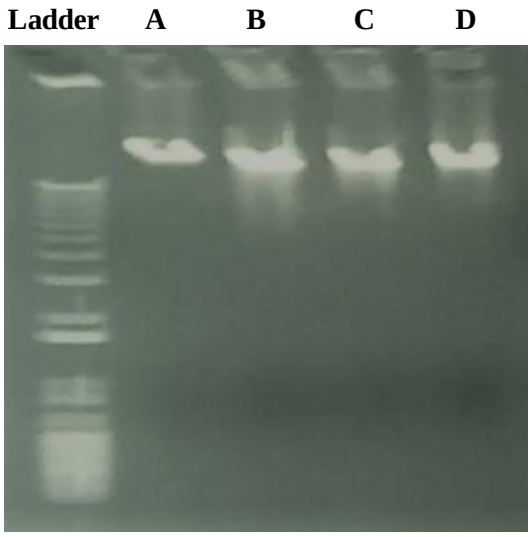

Figure 1 Gel electrophoresis analysis of genomic DNA samples isolated from human whole blood using four different methods. Method A (lane 1), method B (lane 2), method C (lane 3) and method D (lane 4). Molecular weight marker: 1 kb Plus DNA Ladder

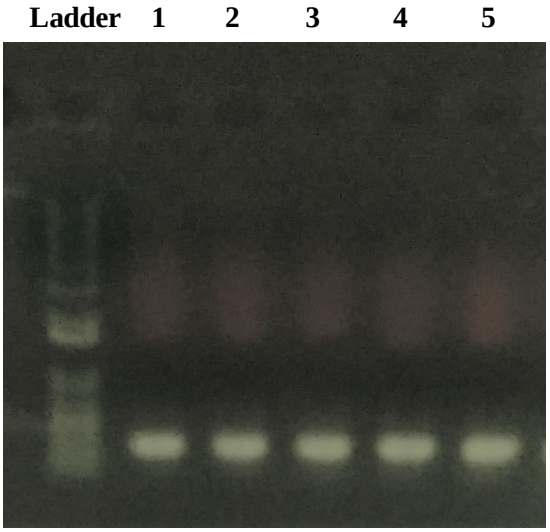

Figure 2 Gel electrophoresis analysis of PCR products isolated from human whole blood using method A in five samples. Molecular weight marker: 1 kb Plus DNA Ladder

Assessment of time, cost and labor intensity

Other desirable factors considered for routine DNA isolation are the time involved, cost incurred and the intensity of labor that the operator must put in. The fastest isolation protocol was the column-based method B followed by the method C. Method A and D was the most time-consuming procedures. Relatively, the method A was the cheapest since it did not require the use of complex equipment.

4. CONCLUSION

In summary, the gelatin-coated magnetite particles for genomic DNA isolation method (INBIOMag Genomic DNA Kit) developed in this study provide an efficient, simple, and inexpensive method that does not require the use of commercial DNA Isolation Kit.

Acknowledgements

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Conflicts of interest

The authors declare no conflicts of interest.

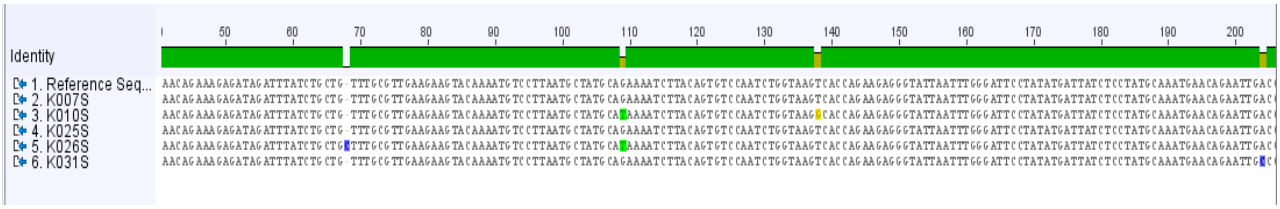

Figure 3 Sanger sequencing for BRCA-1 gene (242bp) alignment analysis using *Geneious* 4.8.4.

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