Differences in Quality of DNA Isolated from Fresh and Frozen Peripheral Blood with Salting-Out Methods

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Abstract: Today, a lot of genomics based research, including genomics, molecular biology, biotechnology and whole genome sequencing, is performed. DNA isolation using commercially available kits from peripheral blood is both costly and inefficient with respect to the amount of DNA isolated. In order to obtain high amounts of fine quality of DNA, the manual precipitation method by salting- out is more efficient. In this study, human fresh peripheral blood tissue and frozen blood tissue were used. DNA isolation from these two different types of blood samples was performed. The purity, quantity and PCR success of the DNA samples were evaluated. As a result, the concentration and purity of DNA isolated from fresh blood samples were determined by Nanodrop spectrophotometer and agarose gel analysis. The fresh blood samples yielded higher quantity, better purity and higher PCR compliance quality than frozen blood samples.

Keywords: DNA Isolation, Salting-Out Methods, Fresh and Frozen Blood.

I. INTRODUCTION

The genomic DNA isolation from human tissue samples is a prelude to all the molecular biology techniques and genetic analyses. However, obtaining the DNA at a desired level of quality from biological samples can be tiresome, time consuming and complicated. A wide range of commercial and lab specific methods are employed widely for the extraction of DNA from mammalian peripheral blood [1].

Currently used techniques for DNA isolation requires a substantial dilution of the sample or precipitation that makes the isolation of DNA from restricted amount of sample cumbersome. Furthermore, removal of proteins is a major goal of clean nucleic acid isolation. Separation of nucleic acids from protein is conducted via a number of chemical procedures as the highly charged phosphate backbone of the nucleic acids is more hydrophilic than protein. Therefore, a vast majority of DNA isolation protocols includes a number of steps to accomplish the goal including cell lysis, enzymatic treatment, differential solubility, and precipitation [2-3].

Some new innovative methods have recently been introduced including chromatography columns or absorption of DNA on silica and coated magnetic bead matrix. Although these methods are easy to execute, they are far from being economical for larger samples [4-6]. Furthermore, an extra step of physical separation of DNA from the matrixes is required prior to PCR or other uses [7-8]. Due to increasing demands for quick, cost effective, and effective DNA extraction procedures in various fields, revisiting DNA protocols for novel, rapid and cost-effective methods gaining importance once again.

Cross comparison of the published methods that evaluate most recent protocols for criteria for cost effectiveness, safety, rapidity, reliability, yield, and purity would be invaluable for selecting the most appropriate method be utilized routinely in small laboratories around the globe. In this study, we evaluated the quality and cost effectiveness of DNA isolation from peripheral blood by the Salting out method [1-2] and compared it with slightly modified version of salt-out method to extract DNA from fresh a blood.

II. MATERIALS AND METHODS

A simple method for DNA extraction from human peripheral blood using salting–out methods is presented that fulfils such criteria. We used two types of peripheral fresh blood and frozen blood. DNA extracted from two types peripheral blood. Blood were taken from volunteers of donors. The DNA was analyzed using optical density, quantity and PCR compliance. Comparisons of results were made on statistical evaluation.

The Standard Procedure for Salting-Out Methods

I. 8 mL blood was taken from volunteers. Whole blood with EDTA was centrifuged at 2500 x g for 15 min and the plasma was separated. **II.** The obtained precipitate was transferred to 50 mL plastic tubes and washed three times with 1X reticulocyte saline solution in low speed centrifugation at 2500 x g at 4 °C, and the

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Table 1: Absorbance Ratio Measured by NanoDrop 1000 Spectrophotometer

Samples	methods	Blood type	DNA (ng/µl)	OD260/OD280 Ratio	OD260/OD230 ratio
1	Salting-Out	Fresh Blood	48,22	1.85	2.1
2	Salting-Out	Frozen Blood	43.77	1.83	2.3
3	Salting-Out	Fresh Blood	50.50	1.86	2.0
4	Salting-Out	Frozen Blood	44.24	1.84	2.3
5	Salting-Out	Fresh Blood	56.21	1.86	2.4
6	Salting-Out	Frozen Blood	45.23	1.84	2.1
7	Salting-Out	Fresh Blood	54.11	1.86	2.2
8	Salting-Out	Frozen Blood	44.76	1.83	2.3
9	Salting-Out	Fresh Blood	58.11	1.85	2.1
10	Salting-Out	Frozen Blood	49.21	1.84	2.3

supernatant was discarded. **III.** The cold lysate (Lysate) solution was added into the precipitate and left to stand on ice for 15 min. It was then centrifuged once or twice at 2500 x g for 15 min. **IV.** The supernatant of the material whose erythrocyte has exploded is discarded. After that, 20 ml of cold STE solution, 10% sodium dodecyl sulfate (SDS, about 1 mL) and 100 mg / ml (approximately 200 mL) was added to the precipitate (10 mL for whole blood). It was stirred gently and left in the water bath at 37 °C overnight. On the next day, if the material in the tube was not homogeneous, it was mixed and kept in the water bath until homogenous. **V.** Saturated phenol was added to the homogeneous tube material. Then, it was centrifuged at 2500 x g for 15 min at 4 °C. **VI.** The upper phase was transferred to another tube and this process was repeated once more. After that, the supernatant was removed. **VII.** At this stage, a mixture of phenol, chloroform, isoamyl alcohol, 25: 24: 1 was added as the same volume as itself. Centrifugation was performed one more time for 15 min at 2500 g. **VIII.** The supernatant was carefully transferred to cold pure ethanol in a flask (it is important not to remove phenol from the bottom) and mixed slowly until the DNA precipitated. Within a few minutes, the DNA is collapsed. **IX.** The filamentous DNA was transferred to a sterile eppendorpf tube using a Pasteur pipette. In the eppendorf tube, the DNA was washed with 70% ethanol. The DNA was centrifuged and the alcohol in the tube was evaporated. **X.** The sterile DNA was dissolved in 1 ml sterile TE buffer. **XI**. The concentration of DNA obtained at this stage was also calculated. 20 ml DNA was diluted 1 ml with distilled water, including 980 mL and the optical density was read on the Nanodrop spectrophotometer at 260 nm [9-11].

III. RESULTS

In this study, we used 50 samples from both groups. We obtained 8 mL of frozen and fresh peripheral blood. DNA isolation was performed by salting-out precipitation method. Quality and quantity of DNA were measured. Randomly at least 5 samples were chosen and NanoDrop results of these samples are given in Table **1**. In addition, agarose gel electrophoresis for PCR compatibility of DNA samples was examined. It was found that the amount of DNA isolated from fresh blood higher and the purity was better. In addition, DNA was amplified by PCR from frozen blood was found to be weaker in the gel electrophoresis. Gel images were given in Figures **1** and **2**.

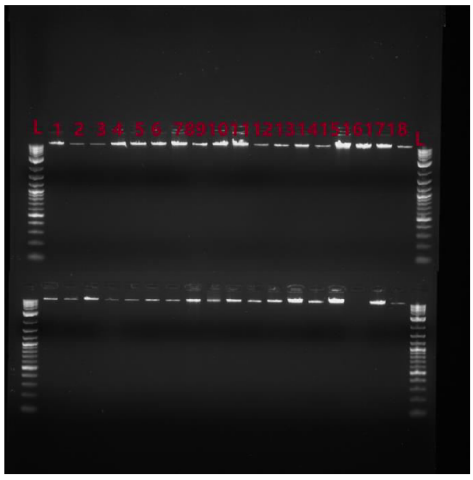

Figure 1: Lane L Marker (1 kb), Lanes 1-18 PCR product of DNA of gel imagine. DNA was isolated from fresh blood.

IV. DISCUSSION

In order to accelerate and economize the standard salt-out method red blood cells were lyzed instead of

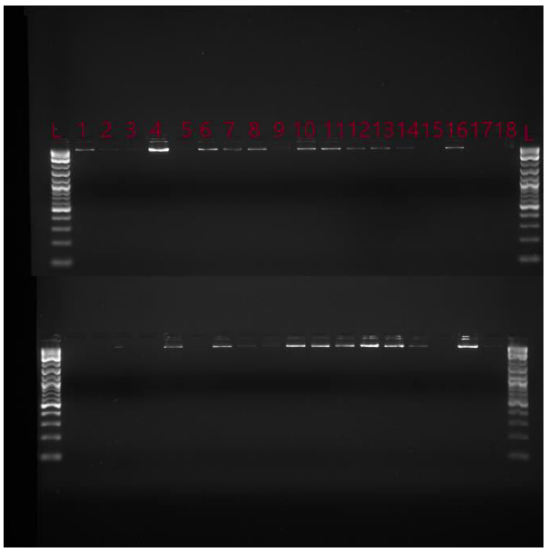

Figure 2: Lane L Marker (1 kb), Lanes 1-18 PCR product of DNA of gel imagine. DNA was isolated from frozen blood.

white blood cell (WBC) separation [1, 4-6]. WBC were incubated with Proteinase K and deproteinization were performed with a saturated NaCl. DNA was precipitated with absolute ethanol. In this study, we used 8 per mL two type of blood (Salting out method; fresh blood 8 mL and frozen blood 8 mL). We compared amount of DNA obtained from fresh blood and the frozen blood DNA by electrophoresis, and OD ratio of 260/280. There was no significant difference between the quality (1.90±0.07; 1.78±0.04; 1.82 ± 0.08). DNA was not degraded and it did not inhibit PCR with sequence specific primers L [9-10]. During one years of use for urgent clinical samples, frozen blood samples had less than 5±0.6 ng for fresh blood sample. Therefore, this fresh blood is useful than frozen blood samples and equal yield of DNA was obtained both in term of quality and quantity. Furthermore, it is a useful technique to obtain efficient DNA extraction for long term stored samples.

V. CONCLUSION

The DNA extraction method described here has had less than 1% failure rate for samples stored for two years for diagnosis of diseases. Qualitative parameters were maintained and comparable to those of other extraction methods. Furthermore, using fresh blood is more efficient to obtain better quality DNA..

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest included in this study.

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