Research Article

An Effective and Rapid Method of DNA Extraction Protocol from Samples of Human Blood

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ABSTRACT

Introduction: Several different protocols ranging from a variety of manual and automated DNA extraction protocols, are available to extract nucleic acids from whole blood samples, one of the primary sources of DNA. These methods have one or more limitations in terms of low yield, Quality issues, cost, and time efficacy, utilization of toxic organic solvents, and others as well. This study aims to develop an effective protocol for extracting DNA from 500 µl of human blood. **Materials and Methods:** The extraction procedure was standardized using 500 microliters of fresh human blood samples. The disruption and cell lysis done by Lysis Buffers R (RBC) and N (Nucleic acid) contain detergents and salts, followed by the removal of proteins and other contaminants and recovery of DNA. The DNA samples were investigated for quality and quantity by measuring their absorbance at 260 and 280 nm, respectively (A260/A280). **Results:** DNA was checked by Gel docking on 0.8% Agarose gels. According to our protocol, we yielded 19 to 25 µg DNA, respectively, from 500 µL of fresh blood. **Conclusion:** Furthermore, our protocol yields bulk amounts of DNA while avoiding toxic organic solvents like Phenol. Consequently, downstream applications can be performed with the DNA because its quality has not been affected.

Keywords: Buffers, DNA extraction, Human blood.

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INTRODUCTION

In molecular biology, DNA, RNA, and proteins are needed for human health studies. DNA of high quantity and quality is crucial for successful downstream applications.[1-3] Furthermore, the rapid and cost-effective DNA extraction method would make it much more research-friendly.[4]

Blood DNA isolation protocols have been published in several publications. Albarino *et al.*[5] Parzer *et al.*[6] Robbins *et al.*[7] Rudbeck *et al.*[8] Sambrook *et al.*[9] Wang *et al.*[10] and Lahiri *et al.*[11] However, some of these methods require significant amounts of blood samples, making them unsuitable for low volume DNA extraction procedures. Lahiri and Nurnberger *et al.*[12] and Miller *et al.*[13] There were some protocols that used enzymes and organic solvents to get high-quality, PCR-inhibitor-free DNA, while others incorporated salting-out procedures to increase the yield of DNA. Castella *et al.*[14] and Cattaneo *et al.*[15] Therefore, the costs and time involved in some protocols are high by Nasiri *et al.*[16] Besides, in some cases, the quality of the DNA has been compromised. El Bali *et al.*[17] Chacon-Cortes *et al.*[18] and Santos *et al.*[19]

As a result, to meet the requirement for rapid, low volume and cost-effective genomic DNA extraction, our objective was to developed a protocol that would reflect the wide range of scientific interest pertaining to this field for extracting pure DNA from fresh human blood without costly enzymes and toxic organic solvents.

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MATERIALS AND METHODS

Blood Collection

An EDTA-containing vacutainer tube containing blood samples was collected from 30 healthy individuals randomly selected from areas nearby the Swami Rama Himalayan University's campus situated at Jolly Grant, Dehradun, Uttarakhand, India. In order to participate in this study, the volunteers provided consent prior to participation, and no prevalent diseases were present in the volunteers. As a part of the research work, ethics committee approval was obtained prior to the study. The DNA extraction process was performed on fresh blood after 1 hr after collection. While handling the blood samples, appropriate precautions was taken to prevent biohazards. Troutman *et al*.[20]

Chemicals and Reagents

The chemicals used in this method are standard chemicals found at major suppliers, Himedia Chemicals, for this study.

Reagents Preparation

The reagents were prepared using different concentrations as RBC lysis buffer denoted as Lysis buffer R (10X) containing NH_4Cl (1.54 M), $NaHCO_3$ (0.14 M) and 0.5 M EDTA (pH~8) dissolved in 100 mL distilled water and pH adjusted to 8. The solution was converted to the 1X working solution. Another lysis buffer, nucleic acid lysis buffer denoted as Lysis buffer N containing Tris-Cl (50 mM), $MgCl_2$ (50 mM), EDTA (2 mM), NaCl (0.5M), 1% Triton X-100 and 2% of 2- mercaptoethanol dissolved in 25 mL of autoclaved distilled water and pH was adjusted to 8. This protocol also involves 10% SDS followed by high salt concentration preparation of 6M NaCl. Further, reagents preparation involves chemicals like TAE buffer, Chloroform: Isoamyl alcohol (24:1), Isopropanol and 70% Ethanol.

Methodology for DNA extraction

The standardization of the DNA extraction method was done for the fresh blood samples.

Step 1. A whole Blood sample of only 500 µL from the vacutainer was centrifuged at 2500 rpm for three minutes at 4°C and the serum was carefully aspirated.

Step 2. Approximately 1mL of Lysis buffer R (1X) was added to the pellet, mixed gently with periodic inversions, and was kept for 5 min at room temperature.

Step 3. Centrifugation of the cell's mixture was done for 5 min at 5000 rpm and carefully supernatant was discarded. Repeated the steps 2 and 3 for the pellet becomes white.

Step 4. Approximately, 500µL of Lysis Buffer N was added to the white pellet obtained from the previous steps, was mixed gently with a wide bore pipette, followed by adding 50µL of 10% SDS. The mixture was then incubated at 55-60°C for 30 min.

Step 5. At the end of incubation, 200µL of NaCl was added, vortexed vigorously, and centrifugation was done at 8000 rpm for 5 min.

Step 6. The Supernatant was then taken in a clean Eppendorf tube and added an equal volume of Chloroform: Isoamyl alcohol (24:1). The mixture was mixed well by gentle inversions and centrifuged at 12,000 rpm for 1 min.

Step 7. The Aqueous phase (DNA present) was removed carefully without disturbing the base layer and transferred into a clean Eppendorf tube containing an equal volume of chilled Isopropanol. Then the tube was vigorously shaken for few seconds resulted in fine white threads that appeared in the solution.

Step 8. The floating precipitate was transferred into a clean Eppendorf tube, washed with chilled 70% ethanol to remove any salts trapped with DNA, and was centrifuged at 13,000 rpm for 3 min.

Step 9. The tube was drained and evaporated to remove the ethanol completely. The pellet was then allowed to dried at 37°C.

Step 10. Finally, added sufficient (1X) TAE solution and dissolved the precipitate by light fingertip vibration. A solution of DNA was then stored at -20°C for further use.

RESULTS

A total of thirty human whole blood samples were used for DNA extraction and samples prepared manually showed no failures. Our optimized method was accelerated and economized by using red cell and nucleic acid lysis instead of separation of buffy coats and replacement of proteinase K. Our method observed human whole blood samples with A260/A280 absorbance ratios, a consistent range of 1.8. This effectively indicates that the samples were pure and successfully deproteinized. (Figure 1) It was also assumed that RNA was not found in the extracted DNA

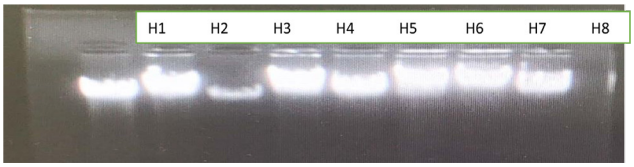

Figure 1: DNA extraction was demonstrated by electrophoresis on agarose gel containing 0.8 percent agarose.

Table 1: Ratios of Optical Density (OD) and the amount of DNA that can be extracted from each 500 µL Human blood DNA.

Coding sample	DNA quantity in ug/500ul blood	OD260/OD280 ratio
H1	23.00	1.78-0.02
H2	24.12	1.82-0.04
H3	19.11	1.72-0.05
H4	25.19	1.81-0.02
H5	22.12	1.85-0.03
H6	25.16	1.89-0.04
H7	22.14	1.87-0.07
H8	23.18	1.86-0.02

samples. In accordance with our protocol, we yielded an average of 25 µg of genomic DNA per 500 µL of fresh blood. The mean concentrations were calculated and tabulated using the experiment results (Table 1).

There were varying intensities of bands in DNA samples run on 0.8% agarose gels. Despite this, all DNA bands were prominent and homogeneous, with minimal lane smearing. It appears that none of the DNA has degraded, despite being exposed to several chemical washes.

At this point, DNA can be quantified by spectrophotometer and diluted to a working concentration, or you can simply use 1-2 µL per for the PCR reaction by Huberman *et al*.[21]

DISCUSSION

Genomic DNA extracted from whole blood samples is being used for diagnostic and genotype studies, thereby facilitating personalized medicine. DNA extraction protocols are designed according to the chemical activity of the reagents on various organelles of cells by Push *et al*.[22] and Tan *et al*.[23] The RBC Lysis Buffer contains NH_4Cl, $NaHCO_3$, and EDTA. NH_4Cl produces an increase in osmotic pressure within RBCs until they burst due to water influx. Despite this, it has a negligible effect on other cellular contents of the blood, especially on leucocytes. EDTA and $NaHCO_3$ act as buffer components, and $NaHCO_3$ increases the RBCs' swelling rate by Thoms *et al*.[24] A higher concentration of Tris buffer was used for the DNA extraction buffer, which maintains the buffer's pH at a steady level. Following this, EDTA was added to the extraction buffer to bind calcium and divalent magnesium cations that maintain the membrane integrity. Triton X-100 is a non-ionic surfactant to lyse cells and maintains DNA integrity, but higher concentrations lead to cell death by Yee *et al*.[25] NaCl forms ionic bonds with the phosphates in DNA that neutralize the negative charges, otherwise causing DNA molecules to repel one another and keep the DNA in solution. Anionic detergents like SDS can solubilize lipids and proteins by assisting in breaking down the membranes and nuclear envelopes, exposing the DNA-containing chromosomes. 2-mercaptoethanol is a powerful reducing agent used in DNA extraction buffer through disulfide bond breaking. It linearizes proteins, causing the molecules' denaturation and removal during the centrifugation by Koley *et al*.[26] The next step involves the addition of chloroform and isoamyl alcohol, which help to bind and precipitate proteins and lipids of cell membranes. DNA was generated in an aqueous phase, and lipids and proteins were formed in a non-aqueous phase. The hydration shell is formed by water molecules surrounding DNA at this stage. The DNA can therefore be separated from the remaining soluble components by centrifugation by adding Isopropanol, which acts as a dehydrating agent and disrupts the hydration shell.

Thomas *et al*.[27] published an earlier DNA extraction protocol using CTAB, but the buffer compositions and the sequences of steps differed considerably from our experiment. However, our present method is unique because it is simpler, faster, and more robust than many other methods for separating DNA from human blood samples. Additionally, there are no toxic reagents used in this protocol., so extractions are safe. Ness *et al*.[28] The average DNA yield was within the normal range using our protocol, and it takes roughly 2-3 hr for the protocol to be successfully completed.

CONCLUSION

This protocol could prove to be efficient in obtaining considerable quantities of genomic DNA from fresh human blood samples. The versatility of this universal method can be extended from fresh to frozen samples. Additionally, eliminating time-consuming steps like enzymatic incubation of Proteinase K and RNAase treatment and the absence of toxic organic solvents such as Phenol permitted an efficient and time-saving protocol in a way that could be used for advanced molecular biological techniques. Further, laboratories with limited funds may benefit from it by pursuing basic molecular biological research.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

µl: Microlitre; **nm:** Nanogram; **µg:** Microgram; **A260/A280:** Absorbance; **OD:** Optical density; **EDTA:** Ethylenediamine tetraacetic acid; **NaCl:** Sodium Chloride; **$NaHCO_3$:** Sodium bicarbonate; **NH_4Cl:** Ammonium chloride; **SDS:** Sodium Dodecyl Sulfate; **TAE:** Tris-acetate-EDTA.

SUMMARY

A successful application of this method has been made in freshly isolated human whole blood samples. Genomic DNA isolated by this method has an average quantity of 25 µg, and according to the measurements, this DNA had a quality of 1.7 to 1.8. Further agarose gel electrophoresis was performed to ensure that the DNA obtained was of high quality, without RNA and protein contamination (Figure 1). In this method, however, only a few chemicals were used in addition to ethanol (tris-HCl, EDTA, NaCl, and SDS), and they are readily available in every routine laboratory worldwide.

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