A Novel, Efficient, Fast and Inexpensive DNA Extraction Protocol from Whole Blood Applicable for Studying Drug-DNA Interaction

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The DNA molecule has been known to be the cellular target for many cytotoxic anticancer agents for several decades. Understanding how drug molecules interact with DNA has become an active research area in the interface between chemistry, molecular biology and medicine. DNA extraction has been suggested as a main step affecting molecular DNA technology such as PCR and PCR-based methods. Therefore, researchers have used several modified protocols for efficient DNA extraction from whole blood. In this study, we focused on a fast and reliable protocol with inexpensive and non-poisonous reagents for DNA extraction from whole blood. Current method was optimized based on a combination of conventional salting-out and boiling methods. Also the quality and quantity of the extracted DNA were surveyed by gel electrophoresis and Nanodrop spectrophotometry methods, respectively. Results showed that high quantity and quality of isolated DNA by this method is enough to do hundreds of PCR-based reactions and also to be utilized in other DNA manipulation assay such as restriction digestion, drug- DNA interaction and methylation detection survey. In conclusion, we described a fast, low-cost, non-toxic and enzyme free protocol for high yield genomic DNA extraction from whole blood.

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Introduction

DNA as a double helix molecule is composed of four nucleotides including, cytosine, guanine, thymine and adenine. In each strand these nucleotides are connected by phosphodiester bonds. Each strand is joined to another primarily through Watson, Crick hydrogen bonds. The point that the two strands cross each other forms two regions named minor groove [1-3] (Scheme 1).

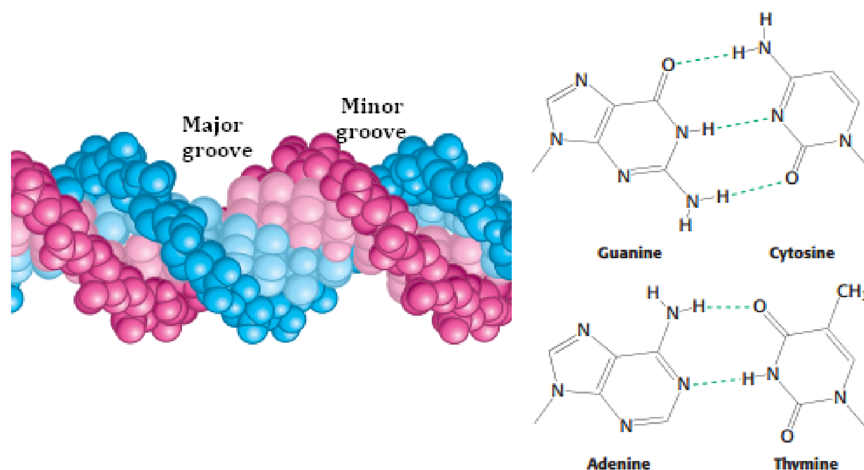
In pharmaceutical studies such as DNA-drug interaction assay, molecular genetics and clinical research, isolation of DNA with high-quality and high-quantity is essential. DNA extraction, the step that affecting molecular DNA technology, is a common and routine work in the clinical and molecular laboratories. Low quality of extracted DNA interferes with follow up reactions, for example, PCR and PCR based methods (Methylation specific PCR, RFLP, and RAPD PCR), Protein-DNA interaction assay and molecular genetics techniques. For the first time, DNA extraction was performed in 1869 by the young Swiss physician Friedrich Miescher[8]. Laboratory experts and researchers need relatively fast, inexpensive, high throughput DNA extraction protocol. Today there are several protocols and commercial kits for genomic DNA isolation from blood or mammalian cells; for example, traditional phenol- chloroform method and its modifications are high yielded methods, but these methods are laborious, time-consuming (at least two days) and poisonous.

Commercial DNA isolation kits are fast and safe, but are usually very expensive and low yielded. Common salting out method is laborious and time-consuming. Therefore, optimized and efficient DNA extraction protocol is an essential step to follow up reaction in genetic engineering and molecular based methods. [9-11]The aim of this research was focused on the optimized protocol for the efficient DNA extraction from common salting out and boiling methods, in addition, receiving to high yielded, inexpensive, simple and also rapid protocol without using hazardous chemical and typical enzyme reagent.

Material and Methods

The reagents including, NaCl, Tris base, Triton X-100, Na_2EDTA, $MgCl_2$, Sucrose and Chloroform were purchased from the Merk Company (Germany).

- Buffer A solution (Red blood cell lysis buffer): 10 mMTrisHCl, 0.32 M sucrose, 0.75% Triton-X-100, 5 mM $MgCl_2$ (pH 7.6)
- Buffer B solution (White blood cell lysis buffer): 4 mM Na_2EDTA, 20 mMTris-HCl, 100 mMNaCl (pH 7.4)
- SDS10%



Scheme 1. (**Left**) Structure of DNA molecule, with minor and major grooves. (**Right**) bases (adenine, guanine, thymine and cytosine) of DNA and Watson–Crick pairing between purine and pyrimidine bases in complementary DNA strands.

Optimized DNA extraction protocol

i. The blood sample was collected using venipuncture from healthy volunteer and added into tubes containing Ethylene diamine tetra acetic acid (EDTA).

ii. One milliliter of fresh or frozen (thawed in room temperature) whole blood was transferred into 2 ml micro-tube and mixed in 1 ml of distilled water.

iii. Micro-tube was mixed for 30 Sec and then was centrifuged at 7000 rpm for 5 min. The supernatant was discarded and repeated step ii and iii for 2 times.
Note: In each time the pellet was breakup and mixed by shaking and vortexing.

iv. One ml of buffer A was added to the pellet and again mixed with vortex for breakup and rinse.

v. It was centrifuged at 7000 rpm for 5 min and then supernatant was removed.
Note: If pellet has red blood cells, step v and vi was repeated

vi. In this step, one ml of buffer B and 100 µl of SDS 10% were added and pellet was suspended with vortexing or shaking, then tube incubated in 65°C for 15 min.

vii. Microtube chilled with ice for 3 min and 400 µl of chloroform and 400 µl of saturated Nacl (5M) were added and gently was shacken for 30 Sec and centrifuged at 5000 rpm for 10 min at 4°C.

viii. About one ml of the upper phases transferred into a new 2 ml tube, and equal volume of cold absolute ethanol was added.

ix. Gently shacked the tube; in this step DNA was appeared as a white skein.

x. Again tube was centrifuged at 13000 rpm for 10 min at 4°C.

xi. The supernatant was discarded and the pellet was washed with 1 ml of chilled 70% ethanol, and centrifuged again at 10000 rpm for 10 min.

xii. The supernatant was discarded and the pellet was left at room temperature to be dried.

xiii. 50-100 microlitter of ddH_2O was added to dissolve the pellet and DNA solution stored at -20°C until use.

Note that, for more yielded DNA, we can use more volume of blood, up to 5 ml, in a bigger tube such as 15 ml falcon; all the remaining procedure is the same.

Quantification of extracted DNA

Extracted DNA was monitored and confirmed using gel electrophoresis on 0.8% agarose containing DNA Safe Stain (Invitrogen). The concentration and purity of DNA were assessed with a Nanodrop (Thermo) with 260/280 measurement ratio and at the wavelength of 260 and 280 nm [12].

Results and Discussion

Our results showed that the quantity and the quality of the extracted DNA by this method are high enough to do hundreds of PCR-based reactions and also to be used in other DNA manipulation techniques.

The yield of this method is about 300-500 ng/µl for fresh whole blood. There was no contamination in all extracted samples. The purity of the DNA was assayed with spectrophotometric analysis that showed the A260/A280 ratio of 1.80 to 2. (Fig. 1)

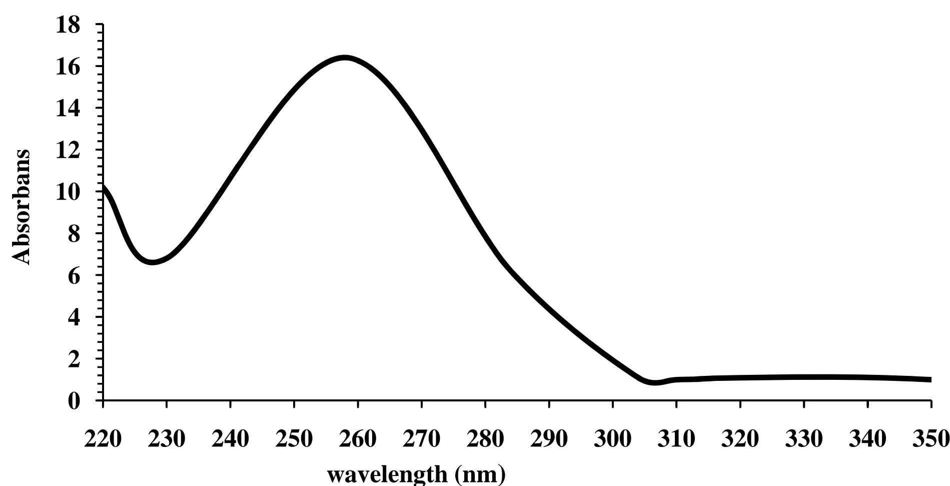


Fig. 1. UV–Visible absorption spectrum of the purified DNA.

Therefore, based on spectrophotometric assay results, purity of extracted DNA and efficacy of current optimized protocol was confirmed; also there is no sign of degraded DNA during preparation and band of extracted DNA was sharp on 0.8% gel electrophoresis (Fig. 2).

1 2 3

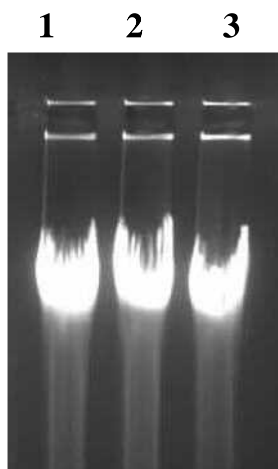


Fig. 2. Agarose gel electrophoresis (0.8 %) for extracted DNA, isolated by optimized protocol (Lane 1-3).

Samples with crude DNA generally contain potent PCR amplification inhibitors[2-4]. To test polymerase enzyme performance and to make sure whether extracted DNA is intact and naked, *β-actin* gene (as a representative of constitutive genes) was amplified. Figure 3 shows the result of agarose gel electrophoresis for 300-bp *β-actin* gene fragment that amplified by PCR. In PCR reaction 200 ng of extracted DNA was used as a template.

1 2 3 4

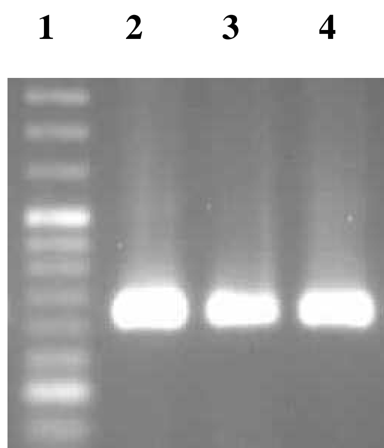


Fig. 3. Agarose gel electrophoresis for human β-actin gene that amplified by PCR method. Lane 1 shows 50 bp molecular DNA size markers, lane 2-4, show 330 bp product bands.

DNA extraction has been suggested as a main step affecting molecular DNA technology such as PCR and PCR based methods, therefore, researchers have used several modified protocols for efficient DNA extraction from blood or animal and plant cell samples [13-17]. In recent decades, attempts in success of rapid, less hazardous, inexpensive and optimized protocol for DNA extraction with high quality were increased. Samadi Shams *et al.* [14] reported highly effective DNA extraction method from fresh, frozen, dried and clotted blood samples, however, in this protocol Cetyltrimethyl ammonium bromide (CTAB) has been used that reduces the final yield of extracted DNA. Kumar Sahu*et al*. [15] have reported the DNA extraction protocol for plant with high levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol. Also, Amani*et al*. [16] have used a simple and rapid method for DNA extraction from leaf.

Conclusion

In the work presented here, we described a fast, inexpensive and efficient protocol for high yield genomic DNA extraction from whole blood. Also, in current optimized protocol, we omitted the need to use of CTAB, hazardous or toxic chemical such as phenol and enzymatic agents like RNase and Proteinase K. Our results, derived at laboratory scale, can be used as a basis for further investigation involving for DNA extraction from mammalian cells and animal or plant tissues.

Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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