Original Article

A Simple, Inexpensive and Safe Method for DNA Extraction of Frigid and Clotted Blood Samples

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Abstract

Background: Extraction of blood genomic DNA is one of the main approaches for clinical and molecular biology studies. Although several methods have been developed for extraction of blood genomic DNA, most of these methods consume long time and use expensive chemicals such as proteinase K and toxic organic solvent such as phenol and chloroform. The objective of this study was to developed easy and safe method for DNA extraction from clotted and frozen whole blood. This method has many advantages: time reducing, using inexpensive materials, without phenol and chloroform, achieving of high molecular weight and good quality genomic DNA.

Materials and Methods: DNA extraction was performed by two methods (new and phenol-chloroform method). Then quantity and quality parameters were evaluated by 1% agarose gel electrophoresis, Nano drop analysis and efficiency of Polymerase Chain Reaction (PCR).

Results: Extracted DNA from 500µL of blood samples were 457.7ng/µl and 212ng/µL and their purity (OD260/OD280) were 1.8 and 1.81 for new recommended and phenol–chloroform methods respectively. The PCR results indicated that D16S539 and CSF1PO loci were amplified.

Conclusion: These results shown that this method is simple, fast, safe and most economical.

Keywords: Genomic DNA extraction, Quality and quantity of DNA, Blood sample

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Introduction

Isolation of DNA with high quality is the first and most important requirement for molecular biology methods and it depends on high purity and high concentration of extracted DNA and removes of PCR inhibitors[1]. On the other hand, the use of a DNA extraction method with high quantity and quality along with simple, safe, rapid and low-cost test is often considered by researchers in molecular genetics. Boiling, salting–out, phenol–chloroform, isopropanol precipitation and commercial kits[2,3] are commonly used for DNA extraction and most common method is phenol-chloroform. This method not only time summing but also the use of toxic organic solvents such as phenol and chloroform are dangerous for the environment and the health of laboratory personnel. Proteinase K increases the cost of extraction and

inhibits Taq DNA polymerase[4]. The use of commercial kits offers a low risk of manipulation and they are faster than conventional protocols, but the amount of recovered DNA is highly variable and kits are expensive[5]. According to the increasing requirements for a fast and efficient method for DNA extraction in fields of biological laboratories, the aim of this study was developed a rapid, affordable and safe method for DNA extraction of human blood samples.

Methods

Blood samples were collected from healthy individuals and all subjects gave written informed consent. Then the blood samples are collected as clotted and in EDTA-tube and were stored at -20°C until the time of DNA extraction.

DNA extraction

DNA extraction by new method

In order to DNA extraction, 500µL of each blood sample were transferred into 2 ml sterile microfuge tubes, 1000µL of RBC lyses buffer (10mM Tris-HCl, 320mM Sucrose, 5mM MgCl2, 1%Triton X-100, pH 8) was added to each sample and mixed well by gently inverting many times and placed on ice for 15 minutes. Then centrifugation was done at 13000rpm for 10 minutes. Upper layer was removed and 500µL of RBC lyses buffer and 500µL of KCl buffer were added. Materials were mixed well and centrifuge at 13000rpm for 5 minutes. Upper layer was removed and repeat this stage until the cell pellets were white or pink–white. 700µL of lysis buffer (400mM Tris-HCl, 150mM NaCl, 60mM EDTA, 10%SDS, pH=8) and 140 µL of sodium perchlorate were added to cell pellet and mixed well by gently pipetting. The reaction tubes were incubated at 65˚C for 90 minute. The tubes were leaved for 1-2 minutes at room temperature afterward, 100µL of sodium acetate and 500µL of butanol were added to each reaction and then mixed well by slowly inverting and centrifuge at 13000rpm for 10 minutes. The bottom layer was transferred into sterile microfuge tube and 120µL of NaCl (5N) and 2 volume of ice-cold absolute ethanol were added. The tubes were mixed by inverting several times and placed at -20°C for 30-45 minutes. After centrifugation at 13000rpm for 15 minutes, supernatant was removed and 500µL of 70% ethanol was added, gently inverted, centrifuged at 13000 rpm for 5min. Supernatant was carefully decanted and allowed the white pellet to air-dried in room temperature until the ethanol was evaporated. Eventually 50µL of ddH2O was added and DNA was stored at 4°C.

DNA extraction with phenol–chloroform method.

DNA extraction from 500µL of blood samples were done by phenol–chloroform method as described previously[6].

Assessment of the quality and quantity of genomic DNA

The quantity and quality of extracted DNA were measured by nano drop spectrophotometer and electrophoresed on 1% agarose gel respectively.

Polymerase Chain Reaction (PCR)

To determine inhibitory materials for biological reactions, we used two primer sets for STR loci (D16S539 and CSF1PO) and PCR reactions were performed. Primer sequences and PCR reaction conditions are shown in table 1.

PCR reactions were consisting of: 100ng genomic DNA, 10 pmol of each primer, 0.2mM dNTP, 1.5mM MgCl2, 1 unit of Taq DNA polymerase in 25µL final volume. PCR Products were electrophoresed on 2% agarose gel, stained by ethidium bromide and DNA bands were observed by 260nm wavelength under UV Tran illuminator device.

Results

Quantity of extracted DNA from 500µL of blood samples were 457.7ng/µl and 212ng/µL and their purity (OD260/OD280) were 1.8 and 1.81 for new recommended and phenol–chloroform methods respectively as shown in table 2.

The results of PCR reactions indicated that D16S539 and CSF1PO loci were amplified. Content of extracted DNA with recommended new method is sufficient for many PCR reactions in order to genotypic analysis (such as analysis of microsatellites and single nucleotide polymorphisms) (Figure 1, 2).

Table 1: Sequence of primers and conditions of PCR cycles.

Conditions OF PCR cycles			Primer Sequence	Locous
1 cycle	94°C	3 min		CSF1PO
	94°C	30 sec	F: 5`-	
40 cycle	64°C	30 sec	ACAGTAACTGCCTTCATAGATAG-3`	
	72°C	1 min	R: 5`- GTGTCAGACCCTGTTCTAAGTA-	
1 sycle	72°C	5 min	3`	
1 cycle	94°C	3 min	F: 5`-ATACAGACAGACAGACAGGTG-	D16S539
	94°C	30 sec	3`	
40 cycle	56°C	30 sec	R: 5`-	
	72°C	1 min	GCATGTATCTATCATCCATCTCT-3`	
1 cycle	72°C	5 min		

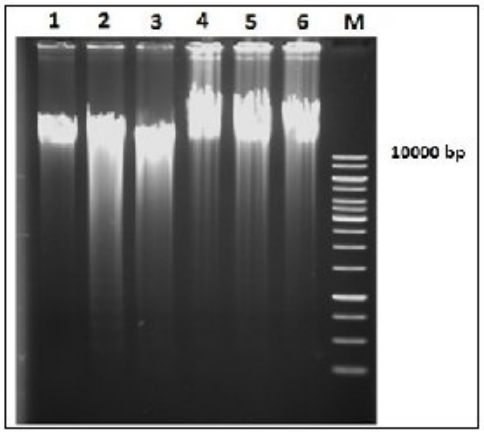

Figure 1. Electrophoretic analysis of total genomic DNA of blood samples on 1% agarose gel; Lines 1-6: extracted DNA by new method, M: 1 kb DNA ladder marker

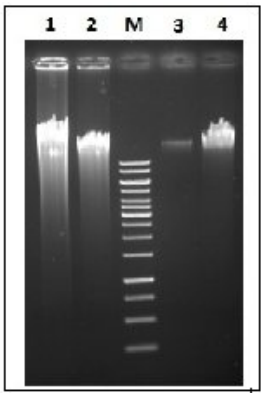

Figure 2. Electrophoretic analysis of total genomic DNA of blood samples on 1% agarose gel; Lines 1, 2: extracted DNA by new method, Lines 3, 4: extracted DNA by phenol – chloroform method,
M: 1 kb DNA ladder marker

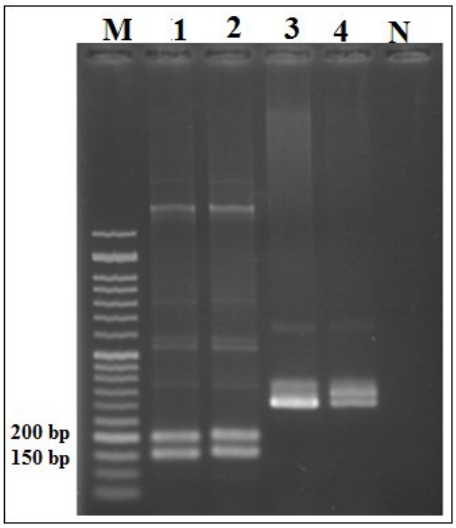

Figure 3. PCR amplification of STR primers; Lines 1, 3: PCR products of D16S539 locus, Lines 4. 5: PCR products of CSF1PO locus, N: negative control, M: 50bp DNA ladder marker

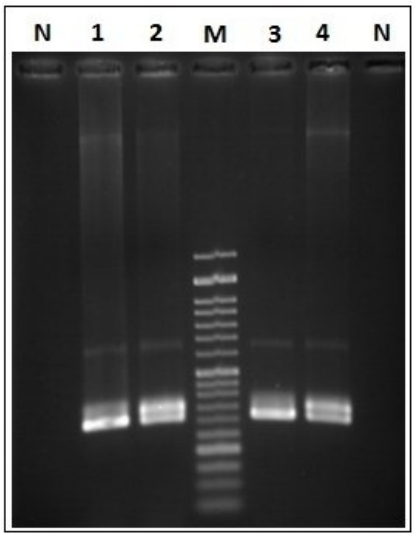

Figure 4. PCR amplification of CSF1PO locus: Lines 1, 2: PCR products of extracted DNA by new method, Lines 3. 4: PCR products of extracted DNA by phenol – chloroform method, N: negative control, M: 50bp DNA ladder marker

Discussion

Sambrook et al. have offered phenol – chloroform as standard method of DNA extraction for first time[7]. Because of its disadvantages, researchers have tried to develop some methods for DNA extraction; for example Barbaro et al., in order to compare efficiency and validation of DNA extraction with commercial kits, different samples such as fresh blood, cigarette stump, hair and semen stains were studied by some kits: DNA IQ™ system (Promega), Invisorb Forensik Kit I (Invitek), Chelex (Bio-Rad), QIAamp DNA blood mini kit (Quiagen, Hilden, Germany) and phenol – chloroform standard method. They were indicated that quantity and quality of extracted DNA

Table 2: Quantity and quality of extracted DNA.

Quality of DNA (OD260/OD 280		Quantity of DNA(ng/ µL)		Blood sample
phenol – chloroform method	New method	phenol – chloroform method	New method	
1.75	1.79	44	707.9	Frigid blood
1.78	1.8	99	278	Frigid blood
1.82	1.88	125	424.7	Frigid blood
1.85	1.78	731	865	Frigid blood
1.86	1.8	186.8	357	Frigid blood
1.83	1.78	87.6	114	Clotted blood
1.74	1.87	8.8	740.5	Clotted blood

by these commercial kits is further than phenol–chloroform method[6]. Dokanehiifard and Bidmeshkipour, compared three DNA extraction methods for PCR of hepatitis B virus infected sera and demonstrated that results of PCR reactions were positive 86%, 62% and 40% with QIAamp MinElute Virus Spin kit, Dnptm Kit and phenol – chloroform method respectively[8]. Samadi-Shams et al. introduced a blood DNA extraction kit, achieved in simple, inexpensive, rapid and less-hazardous protocol for extracting high quality DNA from frozen, fresh, clotted and dried blood samples. They compared their kit results with the others commercial kits (TAKARA and QIAGEN), but they were used chloroform which is toxic substance[1]. Recently Radheshyam et al., in order to evaluation of modified salt-out DNA extraction method, examined blood samples with three methods phenol – chloroform, salting-out and QIAamp DNA Mini kits; DNA concentration were 40.8±4.3µg/mL, 38.5±7.3µg/mL and 35.3±5.4µg/mL for salting-out DNA extraction method, Phenol-chloroform extraction and QIAamp DNA mini kit respectively[9]. Steffen Bank et al. introduced Maxwell 16 Blood purification kit for DNA extraction, although offered a simple and rapid protocol from frozen archival blood clots; the kit is very expensive[10].

Our introduced method not only giving sufficient quantity and purity of DNA from frigid and clotted blood samples, but also it dose possible to access the product in the shortest time with lower costs and safer materials. DNA extraction by phenol-chloroform method provides a good product, but the use of toxic organic solvents and destructive, such as phenol and chloroform not only is dangerous for the environment but also it is a threatening the laboratory personnel health. In addition, the phenol – chloroform method used proteinase K, which it raises the cost of extraction as well as inhibits Taq DNA polymerase[11,12]. Therefore, new method not only hasn`t defects of phenol-chloroform but also it is simple, rapid, safe and inexpensive method and extracted DNA has suitable quantity and quality. Thus in genotyping analysis, at laboratories and research centers where have limited financial resources and time, it seems appropriate method.

Conclusion

These results shown that this method is simple, fast, safe and most economical.

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