

Modified Salting-Out Method: High-Yield, High-Quality Genomic DNA Extraction From Whole Blood Using Laundry Detergent

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Different approaches have been used to extract DNA from whole blood. In most of these methods enzymes (such as proteinase K and RNase A) or toxic organic solvents (such as phenol or guanidine isothiocyanate) are used. Since these enzymes are expensive, and most of the materials that are used routinely are toxic, it is desirable to apply an efficient DNA extraction procedure that does not require the use of such materials. In this study, genomic DNA was extracted by the salting-out method, but instead of using an analytical-grade enzyme and chemical detergents, as normally used for DNA isolation, a common laundry powder was used. Different concentrations of the powder were tested, and proteins were precipitated by NaCl-saturated distilled water. Finally, DNA precipitation was performed with the use of 96% ethanol. From the results, we conclude that the optimum concentration of laundry

powder for the highest yield and purity of isolated DNA is 30 mg/mL. The procedure was optimized, and a final protocol is suggested. Following the same protocol, DNA was extracted from 100 blood samples, and their amounts were found to be >50 µg/mL of whole blood. The integrity of the DNA fragments was confirmed by agarose gel electrophoresis. Furthermore, the extracted DNA was used as a template for PCR reaction. The results obtained from PCR showed that the final solutions of extracted DNA did not contain any inhibitory material for the enzyme used in the PCR reaction, and indicated that the isolated DNA was of good quality. These results show that this method is simple, fast, safe, and cost-effective, and can be used in medical laboratories and research centers. *J. Clin. Lab. Anal.* 19:229–232, 2005.

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Key words: DNA extraction; salting-out; polymerase chain reaction; genomic DNA; laundry detergent

INTRODUCTION

Isolation of genomic DNA is the first and the most important requirement in carrying out a genetic analysis, such as mutation detection or linkage analysis. This step is so important in molecular diagnosis that the success of the subsequent analysis depends on the isolation of DNA fragments that have good purity, integrity, and concentration (1).

The first stage of DNA extraction, which is common to all nucleic acid isolation methods, is cell lysing. DNA isolation is facilitated with the use of proteinase K enzyme, detergents, and chelating agents. Detergents dissolve the cell membrane and denature proteins. Proteinase K digests proteins, while chelating agents bind to bivalent cations of nuclease cofactors and thereby prevent DNA degradation by these enzymes. RNase A is another enzyme that may be used to remove RNA contaminants. In most common procedures, DNA is purified by organic solvents, such as phenol,

chloroform, and isoamyl alcohol. Finally, DNA is precipitated using 96% ethanol (or isopropanol) and dissolved in distilled water or TE buffer for further analysis.

Many different protocols are based on the general procedure described above; however, most of them are time-consuming and cumbersome, and have drawbacks such as the use of toxic materials (e.g., phenol (2) or guanidine isothiocyanate (3)). Furthermore, it is not economical to use enzymes, such as proteinase K (4) or RNase A, especially when a high sample throughput is required.

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Received 26 August 2004; Accepted 5 April 2005

DOI 10.1002/jcla.20083

Published online in Wiley InterScience (www.interscience.wiley.com).

In recent years, many researchers have tried to modify these methods. For example, some investigators have used a salting-out procedure instead of protocols that require the use of toxic materials (5). According to this method, the cell lysate is treated overnight with proteinase K, and then semidigested proteins are dehydrated and precipitated by NaCl-saturated distilled water (6). In addition, attempts have been made to omit incubation with enzymes. In such cases, the proteins are denatured using only detergents and are then precipitated with a 6 M NaCl solution (7).

Although recently introduced innovative methods, such as those using chromatography columns, are efficient and simple to perform, it is not economical to use them for routine diagnostic tests. Therefore, it is not easy to develop a protocol that meets all of the criteria and requirements.

In this study, instead of using analytical-grade enzymes and toxic materials, laundry detergent was used to isolate DNA from whole blood. Some studies have extracted DNA from tissue (8), gels (1), and cadaveric peripheral blood (9) with the use of laundry detergent. The commercial washing powders used in those studies, in addition to detergents and other chemicals such as EDTA, contain enzymes (e.g., proteases) that were cited as the most important agent that contributed to the isolation of DNA. However, in this study we used both enzyme-containing laundry powders (Yekta Co. and Shooma Co. IRI) and multi-purpose washing powders devoid of any enzyme (Darya Co., Rakht Co., and Tage Co. IRI), and found that there was no difference in the results obtained. We simplified and shortened the general protocol described in the above-mentioned studies and optimized it to obtain high-yield, high-quality genomic DNA from small volumes of human whole blood.

MATERIALS AND METHODS

Using Different Concentrations of Laundry Detergent

We used 10 EDTA-anticoagulated blood samples, and 5 mL of each sample were placed into five 10-mL glass tubes. Then 8 mL of lysis buffer (0.3 M sucrose, 0.01 M TrisHCl, pH 7.5, 5 mM MgCl₂, 1% Triton X100) were added to each tube. The tubes were centrifuged for 5 min at 2,500 g. The supernatant was discarded and 300 μ L of 10 mM TrisHCl, pH 8, was added to the pellets. The pellets were released from the bottom of the tubes and transferred quickly to fresh microfuge tubes. The sediment was resuspended by vigorous vortexing, and the product was centrifuged for 1 min at 700 g. After the supernatant was discarded, 330 μ L of 10 mM TrisHCl, pH 8; 330 μ L of laundry

powder solution (concentrations: 20, 25, 30, 35, and 40 mg/mL); and a glass bead were added to each tube. The samples were vortexed for 1 min, 250 μ L of 6M NaCl was added, and they were vortexed again for 20 sec. Then the tubes were spun down for 5 min at 15,000 g. The supernatant was transferred to fresh tubes (about 750 μ L) and DNA was precipitated by the addition of 750 μ L of 96% ethanol. DNA precipitate was retrieved using a heat-sealed, thin-end glass pipette, washed twice in 0.5 mL of 70% ethanol, and finally dissolved in 100 μ L of 10 mM TrisHCl, pH 8. The precipitate was completely dissolved by incubation at 70°C for 5 min.

Determining the Purity, Concentration, and Integrity of Isolated DNA

The A₂₆₀ and A₂₆₀/A₂₈₀ ratios were used to determine DNA concentration and purity, respectively (Fig. 1).

To evaluate the probability of DNA degradation, gel electrophoresis was carried out by loading of extracted DNA in parallel with DNA isolated by the standard salting-out procedure on 1% agarose gel.

PCR

A PCR reaction was performed to determine whether any inhibitory material was interfering with the reaction. For this purpose, a fragment of β -globin gene was amplified in 25 μ L reaction containing 1 \times PCR buffer (15 mmol/L (NH₄)₂SO₄, 2 mmol/L MgCl₂, 60 mmol/L TrisHCl, pH 8.5), 200 μ mol/L each dNTP, 0.125 μ g each primer (ACACAAGTGTGTTCACTAGC as the forward primer, and TCATTCGTCTGTTTCCCATT as the reverse primer), and 0.5 U of Taq polymerase. The thermocycling conditions included an initial denatura-

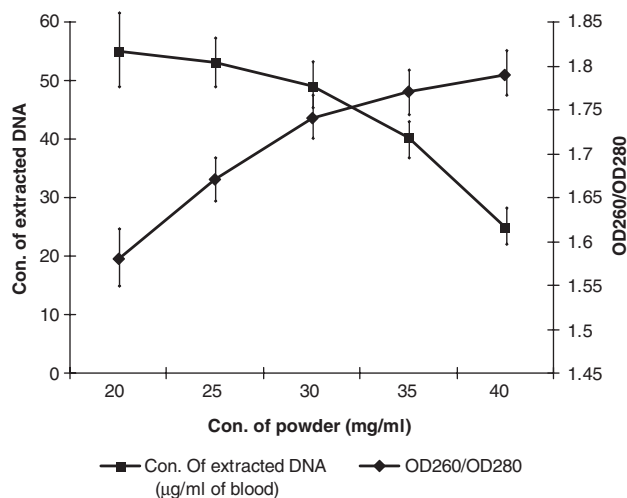


Fig. 1. As the concentration of laundry detergent increases, the yield of DNA decreases but purity increases.

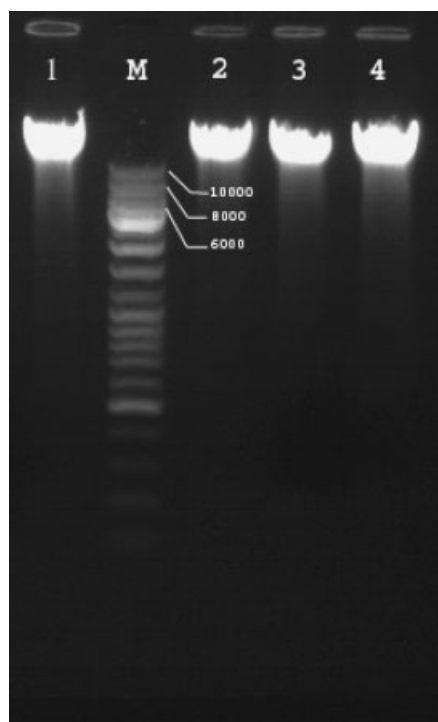


Fig. 2. Agarose gel electrophoresis of genomic DNA isolated by laundry detergent (lanes 2–4) and standard salting-out (lane 1) procedures.

tion step at 94°C for 5 min, followed by 35 cycles of denaturation at 95°C for 15 sec, annealing at 55°C for 30 sec, and polymerization at 72°C for 45 sec. Cycling culminated with a final extension at 72°C for 10 min (10).

RESULTS

In this work different concentrations of laundry powder were used to isolate DNA, and subsequently the concentration and purity of the isolated DNA were tested (Fig. 1). We found that 30 mg/mL is the optimum concentration of laundry powder. We also used different powders with different formulations, and observed no meaningful differences in the quality and quantity of the extracted DNA (data not shown).

In some cases, after cell lysing was performed the pellet was found to be red. Such a situation occurs when red blood cells (RBCs) are trapped between nuclei. We recommend that the cell-lysing step be repeated one more time. For this purpose, 2 mL of lysis buffer should be added to the pellet, and after resuspension and centrifugation the subsequent steps are followed. We also found that lengthening the centrifugation time from 5 min to 10 min after adding NaCl-saturated distilled water also increases the purity of isolated DNA by

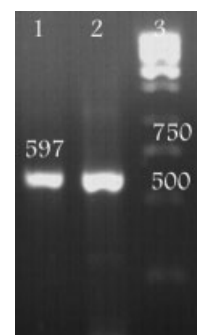


Fig. 3. Agarose gel displaying the result of PCR reaction performed on DNA extracted by laundry detergent (lane 1) and the standard salting-out (lane 2) procedure.

facilitating isolation of the supernatant from the protein precipitate (data not shown).

Following the above optimizations, 100 samples of whole blood were tested for DNA extraction. The average yield of DNA isolated from these samples was 56.3 ± 7.2 µg/mL of blood, and the A_{260}/A_{280} ratio (as the indicator of purity) was 1.77 ± 0.11 .

The results obtained from electrophoresis also showed that there was no smear on the sample lane (Fig. 2).

The results of the PCR reaction on isolated DNA in parallel with the PCR products of DNA extracted by the common salting-out method were visualized by subsequent gel electrophoresis (Fig. 3).

DISCUSSION

Previous studies have isolated DNA from different sources using commercial laundry detergents. Most of those works used biological detergents containing proteases, and it has been reported that enzymes (particularly proteases) are probably the most important component of washing powders for facilitating DNA isolation. However, we observed no significant difference in the results obtained using both biological and nonbiological detergents. Therefore, we conclude that the major agent that enables DNA isolation is one (or several) of the chemical substances that are common to those commercial detergents. This may be supported by the short incubation time (10 min) considered after the addition of detergent, which may not be enough time for the enzymes to digest the substrate. We are continuing our work to find the simplest formulation for isolating DNA. However, this method may not have much practical value as far as cost is concerned, since using pure chemical components of laundry powder (other than commercially available forms) may not be economical. Moreover, in this study one of our main objectives, in addition to developing a fast procedure, was to devise an economical method for DNA isolation

that could be used in most molecular biology procedures (e.g., in molecular ecology and field studies).

Gel electrophoresis of the high-molecular-weight, isolated DNA samples showed that the DNA fragments had acceptable quality and yield, and were not degraded during this procedure. PCR amplification with the isolated DNA was performed successfully, indicating there was no prominent inhibitory factor in the final solution.

Our results show that the DNA produced by our simple and fast protocol can be reliably used for PCR-based procedures. Furthermore, laundry detergents are available everywhere and their components are non-toxic. Therefore, the efficiency, speed, and use of low-cost chemicals make this procedure an appropriate alternative to existing standard methods for extracting genomic DNA, and since it meets most criteria for the selection of an appropriate method, it can be used routinely in many areas of molecular biology.

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