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Research Article

COMPARATIVE STUDY ON VARIOUS METHODS FOR RECOVERY OF DEOXYRIBONUCLEIC ACID FROM AGAROSE GELS TO EXPLORE MOLECULAR DIVERSITY

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ABSTRACT

Objective: Although a few techniques exist for recovery of deoxyribonucleic acid (DNA) from agarose gels, they generally require expensive equipment, extended elution times, or considerable handling of the sample after elution. The objective of this study is to determine the effective conventional method for maximum recovery of DNA from agarose gels.

Methods: Different extraction methods, such as dialysis tubing, paper strip, spin column, and phenol-chloroform, were used for a quick and effective recovery with good yield and quality.

Results: Using paper strip method, DNA fragments of 800 bp from an agarose gel slice were easily recovered. The recovery efficiency was about 70%, and the eluted DNA can further be used directly for downstream applications such as polymerase chain reaction and restriction digestion.

Conclusion: This method could also be used to recover large DNA fragments from the agarose gel without degradation since it helps to remove all impurities from agarose gel and it also reduces the clogs during the process.

Keywords: Deoxyribonucleic acid, Agarose gels, Paper strip, Dialysis membrane, Spin column, Electroelution.

INTRODUCTION

Given the increasing emphasis on the role of deoxyribonucleic acid (DNA) in genetics, molecular biology, and recombination technology, its prevention, simple, and cost-effective methods are needed to recover DNA for large-scale studies. Agarose gel electrophoresis was used as a preliminary assay to determine the quality and relative quantity of recovered DNA using an extraction method [1]. Currently, there are several methods available to recover DNA based on different principles. However, the amount and the quality of the DNA obtained by each one of those methods are highly variable [2]. Recovery of DNA from agarose gels by electrophoresis onto DEAE-cellulose membrane is one of the rapid and effective methods. Electroelution is also a good method for DNA recovery, especially for larger DNA fragment [3]. Sephadex G-200 spin column purification was found to be the best method for recovering DNA and removing inhibiting substances and minimizing DNA loss during purification [4]. Larger fragments of DNA can be recovered from agarose gels after electrophoresis by freezing the gel slices and manually squeezing out liquid containing the DNA [5]. Defined RNA fractions can also be recovered from agarose gels by a combination of gel melting and phenol extraction [6]. Procedure for quick and simple elution of DNA from agarose gels is gel slices are equilibrated in a neutral salt buffer are then frozen and centrifuged through a filtration assembly whereby the DNA-containing buffer is squeezed out [7]. DNA can also be efficiently recovered by solubilization of agarose gels with $NaClO_4$, followed by retention of DNA on glass fiber filters. After removal of the $NaClO_4$ by ethanol, the DNA can be extracted with a low salt buffer [8]. The silica-based method is sensitive, inexpensive, and compliant with automation, and the effectiveness of this method has been tested on more than 5000 animal specimens with highly positive results [9]. Electroelution of nucleic acids from agarose or acrylamide gels is also a suitable method to recover DNA, which employs a combination of low- and high-ionic-strength buffers so that nucleic acids electroelute under a high-voltage gradient but accumulate over a high-salt barrier [10]. Typically, methods involving extraction with organic solvents, electroelution, or binding of the DNA to silica particles or ion-exchange resins give quite pure DNA, but yields are relatively low [11]. On the other hand, high-yield techniques tend to be problematic in enzyme reactions. Clearly, there is a distinct trade-off between recovery and the purity of the DNA sample.

METHODS

Blood genomic DNA extraction

Whole blood was collected from volunteer's at the Department of Biotechnology, K L University in EDTA-containing vacutainer tubes; total genomic DNA was extracted using spin column method (Bioserve, Hyderabad). Moreover, the sample was stored at − 20°C for further analysis.

DNA quantification

DNA sample concentrations [12] were determined by a spectrophotometer at the wavelength of 260 nm for the DNA and 280 nm for proteins, and the purity was observed using OD260/OD280, in (Genesys, Thermo, USA) as indicated in Table 1. Concentration results are given in ug/mL, and the DNA purity results are reported as the OD260/OD280. The concentration was calculated with use of nucleic acids calculator from Kenkyuu–Ryuugaku NET, copyright 2002.

Agarose gel electrophoresis

The quality of the DNA yielded by each method was determined by gel electrophoresis with 5 µl sample in a 0.8% TAE-agarose gel, stained with ethidium bromide. 50 ml of 1% agarose (MERCK, LEO Agarose), 1X TAE, and 6 µL EtBr were used. For analytical setups, the sample was mixed with 6X DNA Loading Dye (Fermentas) to a total loading volume of 10 µL. Electrophoresis was performed in running buffer at 100 V, and the gel was illuminated for visualizing DNA bands (UVI-Tech Gel Doc™).

Recovery of DNA from agarose gels

Several techniques for the recovery of DNA have been described Tabak and Flavell [13] which have encountered troubles in achieving good quantity of DNA, probably due to traces of agarose left out in the

solution. We have, therefore, made DNA fragments cut and purified with gel extraction kit (GeneJet Extraction kit, Fermentas) as per the manufacturer's instructions. DNA was eluted in 40 μL 1 mM Tris/HCl pH 8 or sterile ddH_2O to use as control. Samples were again loaded to recover from the gel with various methods such as dialysis tubing, paper strip, silica column, and phenol-chloroform methods.

Dialysis tubing

DNA band is excised from the gel and placed in a bag of the dialysis membrane. This bag is then filled with electrophoresis buffer and placed in an electric field. Electrophoresis was carried out for 30 minutes at 100 V to release any DNA adhered to the inside of the tubing [14]. DNA was recovered from the bag by pooling the TAE fractions, and DNA was precipitated with 0.1 volume of 3 M sodium acetate and 3 volumes of ethanol and or the recovered DNA was analyzed by agarose gel electrophoresis (Fig. 1).

Paper strip method

A slit immediately in front of the band to be extracted is made using a scalpel, and a strip of sterile 3 mm Whatman paper is inserted. The strip was selected to be about the same width as the band but may protrude from the gel by a few mm [15]. The gel is then returned to the electrophoresis tank and a short period of electrophoresis is carried out to move the DNA into the paper. The paper is then transferred to a vial with a small hole in the bottom and centrifuged for about 15 seconds to recover the DNA in TE buffer. The DNA recovered was analyzed or and precipitated in an appropriate volume of autoclaved distilled water or TE buffer for further analysis (Fig. 1).

Silica column method

Gel slices containing the DNA fragment was added with sodium iodide solution in 2.5 volumes and incubated at 50-60°C for 10 minutes or until the gel slice is completely dissolved. To this 15 ul of silica powder is added and left at room temperature with occasional mixing to allow the absorption of DNA molecules of the silica. The sample was then loaded into a silica column (Fermentas) and centrifuged at 12000 rpm for 30 seconds [16]. Ethanol was then loaded for several times to remove any additional residues present in the column and TE buffer was loaded to collect the DNA from the column as eluent. The recovered DNA was analyzed by gel electrophoresis or and precipitated in an appropriate volume of TE buffer for further analysis (Fig. 1).

Table 1: DNA concentration and purity (OD260/OD280) obtained by the four DNA extraction techniques

Method	Average concentration DNA (μg/ml)	OD260/OD280
Dialysis tubing	44.5	1.78/1.13
Paper strip	48	1.92/1.05
Spin column	42	1.68/1.23
Phenol-chloroform	38.75	1.55/0.98

DNA: Deoxyribonucleic acid

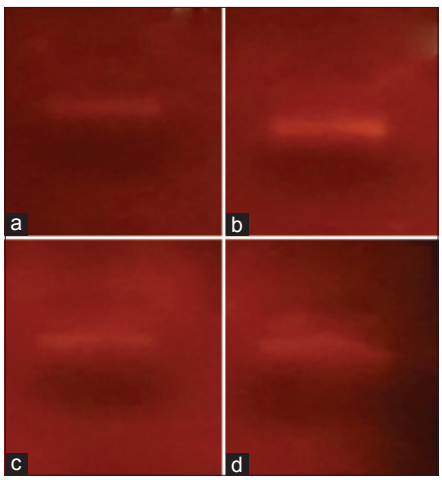

Fig. 1: Molecular characterization of deoxyribonucleic acid recovered from various methods, (a) Dialysis tubing method, (b) Paper strip method, (c) Silica column method, (d) Phenol-chloroform method. Samples were analyzed on 0.8% agarose gel and stained with ethidium bromide

Phenol-chloroform method

Without exposing the DNA to UV light for longer time, the gel slices were cut and transferred into 1.5 ml vials, and the slice was completely melted by exposing to higher temperatures. Equal volumes of phenol-chloroform were then added to the vials and were centrifuged at 12000 rpm for 10 minutes [17]. The aqueous phase was collected and placed in a clean microcentrifuge tube and mixed with alcohol to remove any excess impurities present with the DNA. The alcohol mix to the sample was repeated to enrich the quality of DNA. The recovered DNA was analyzed by gel electrophoresis or and precipitated in an appropriate volume of TE buffer for further analysis (Fig. 1).

RESULTS AND DISCUSSION

Whole blood genomic DNA extraction

Genomic DNA was extracted from 200 ul of whole blood collected before the experiment and spin column method (Bioserve, Hyderabad) was used as per the manufacturer's instructions. The quantity and quality of isolated DNA were evaluated spectrophotometrically and by agarose gel electrophoresis, respectively. The isolated genomic DNA showed an A_{260}/A_{280} ratio of 1.8±0.2, indicating relative purity. 10 ul of the genomic DNA is used for the detection on ethidium bromide-stained agarose gels.

Recovery of DNA from agarose gels

After electrophoresis, the DNA was recovered from agarose gel either by dialysis tubing, paper strip, spin column, or phenol-chloroform method (Fig. 1). Gel slice was cut and the DNA was recovered by either method as per the manufacturer's instructions. DNA was eluted in 40 μL 1 mM Tris/HCl pH 8 or sterile ddH_2O. We obtained relatively pure DNA that gave a considerable yield which is free of RNA, protein, and degrading enzymes (Fig. 1).

CONCLUSION

Gentle, rapid, and efficient recovery of even minute amounts of size-separated genomic DNA fragments from agarose gels can be accomplished using various recovery methods. Preparation provides for quantitative recovery of DNA fragments without the loss or shearing that can occur with electroelution or column purification methods. The DNA recovered from the agarose is often concentrated enough to be used directly for routine molecular biology experiments. Typical yields for silica membrane spin column purification are 60-80% for large DNA fragments. The procedure seems to work best with DNA fragments > 500 bp; smaller-length fragments apparently bind tightly and irreversibly to the silica membrane.

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