Original Article

Development of a chamber system for rapid, high yield and cost-effective purification of deoxyribonucleic acid fragments from agarose gel

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Abstract

Background: There are several methods commonly practicing for deoxyribonucleic acid (DNA) purification from agarose gel. In most laboratories, especially in developing countries, present methods for recovering of DNA fragments from the gel are mostly involved organic solvents. However, manual purification using organic solvents are toxic, labor intensive, time consuming and prone to contamination owing to several handling steps. The above mentioned burdens as well as cost and long time to import them, especially in developing countries, prompted us to design and develop a chamber system for rapid, non-toxic, cost-effective and user friendly device for polymerase chain reaction (PCR) products purification from agarose gel.

Materials and Methods: The device was made from plexiglass plates. After amplification of two fragments of 250 and 850 bp, PCR products were electrophoresed. Subsequently, the desired bands were excised and purified with three method: HiPer Mini chamber, phenol extraction method and spin column procedure. To assess the suitability of the purified DNAs, restriction digestion was applied.

Results: Results showed that the yield of recovered DNA in our method was above 95%, whereas the yields obtained with conventional phenol extraction and spin column methods were around 60%.

Conclusion: In conclusion, the current method for DNA elution is quick, inexpensive and robust and it does not require the use of toxic organic solvents. In addition, the purified DNA was well has suited for further manipulations such as restriction digestion, ligation, cloning, sequencing and hybridization.

Key Words: Gel electrophoresis, polymerase chain reaction product purification, phenol extraction

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INTRODUCTION

Recovery of polymerase chain reaction (PCR) products from agarose gel is often required for further molecular experimental procedures on deoxyribonucleic acid (DNA) fragments. Several methods have been introduced to purify PCR products from agarose gel.[1,2] In general, the organic extraction method, electro elution, binding of DNA to silica beads and ion exchange resins provide

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pure DNA, but a drawback of such methods is often lower yields of recovered DNA.[2] In addition, there are other methods such as syringe squeeze[3] and centrifugal filtration[4] that provide higher recovery of DNA; however, the problem with these methods is that the purified DNA is less suitable for enzymatic manipulations.[2] Furthermore, the yield of recovered DNA is lower when larger DNA fragments are going to be extracted from agarose gel.[3] Current methods for recovering of DNA fragments from agarose gel in most laboratories involve utilization of commercially available kits or in some instances in house manually developed procedures in which utilization of mutagenic organic solvents are inevitable. The former method is too expensive for routine use in most laboratories especially in developing countries. In addition, non-availability and long time required to attain these kits in a country like Iran would be troublesome. To overcome all aforesaid difficulties, a fast, economical and high yield without the need of toxic organic solvents is highly demanded.

In the present paper, we are introducing a micro chamber system, for purifying PCR products from agarose gel, which is fast economical and simple to use with high yield and purity compared to the available methods.

MATERIALS AND METHODS

Development of device

The device was made from plexiglass plates, which are inexpensive and easily available. Its dimension is 1 × 1 × 1 cm^3 (the total volume is 1 cm^3). Two short thin platinum electrodes with effective length of 1 cm were fixed in parallel on the base of the chamber opposite to each other [Figure 1]. Owing to its small size and high performance we named the device as high performance mini device or for short "HiPer Mini."

Amplification

To assess and compare the device performance, we amplified just two fragments of 250 and 850 bp being representative of small and large sized DNA fragments.

Purification of DNA fragments from agarose gel using HiPer mini

PCR products were electrophoresed in parallel lines on a 1% agarose gel with 0.5 × Tris-borate- ethylenediaminetetraacetic (TBE) buffer (89 mM Tris pH 7.6, 89 mM boric acid, 2 mM ethylenediaminetetraacetic acid (EDTA) pH 8) as conductive media. Subsequently the desired bands were excised from the gel under UV illumination, placed into the HiPer Mini chamber of the device and filed with 0.5 × TBE buffer in such a way to completely cover the excised gel containing DNA fragments. After assembling the device, power supply adjusted on 10 volts and elution continued for 10 min. Upon completion of the run, the gel slice was removed and examined under UV light to check for complete elution of DNA fragments. Then, the TBE buffer containing DNA fragments was transferred into a sterile 1.5 ml micro tube. Equal volume of cold isopropanol was added, mixed by gentle inversion, incubated on ice briefly and then centrifuged at 13000 rpm for 10 min at room temperature. Supernatant was discarded and the pellet was washed twice with 70% cold ethanol, dried and then resuspended in 20 µl of tris-EDTA (TE) buffer (10 mM Tris pH 8, 1 mM EDTA pH 8). Ten µl aliquot of DNA solution was subjected to electrophoresis using 1% agarose gel in parallel to the same amounts of the original PCR products in order to quantify the yield.

Purification of DNA fragments from low melting agarose gel using phenol extraction method

A total of 10 µl of PCR products were electrophoresed using 1% low melting agarose gel and 0.5 × TBE buffer

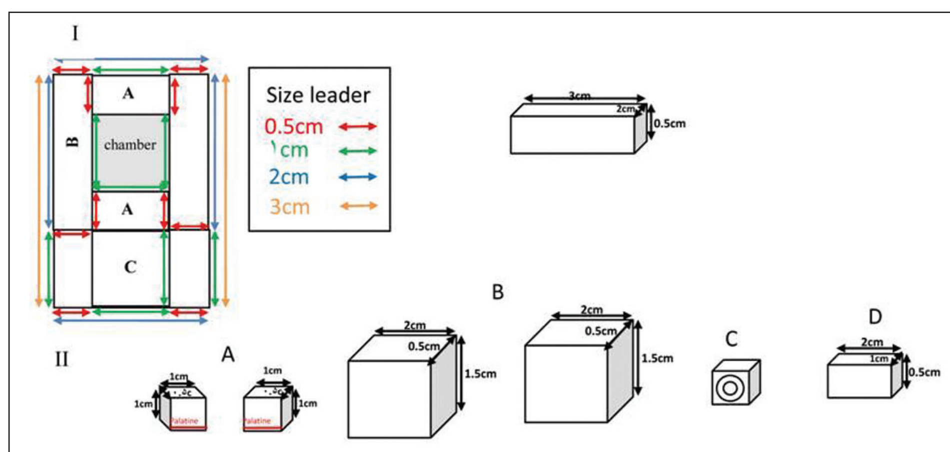

Figure 1: Schematic representation of the device in various orientations. I: The device which was made from plexiglass; II: Various parts of the device

(89 mM Tris pH 7.6, 89 mM Boric acid, 2 mM EDTA pH 8) as conductive media. After running, the band of interest was excised and transferred into a sterile 1.5 ml microtube, dissolved by incubation at 65 degree centigrade for 10 min and the same volume of phenol, chloroform, isoamyl alcohol (25:24:1) was added, mixed by repeated inversion and then centrifuged at 13000 rpm for 5 min. The upper aqueous phase was transferred into a sterile 1.5 ml microtube and equal volume of phenol was added. After mixing and centrifugation at 13000 rpm for 5 min, the upper phase was transferred into a sterile 1.5 ml microtube and equal volume of cold Isopropanol was added. The solution was incubated on ice for at least 20 min and then centrifuged at 13000 rpm for 10 min. The supernatant was discarded and the pellet was washed twice with 70% cold ethanol, dried and resuspended in TE buffer. To assess the yield and quality of the purified PCR products, electrophoresis on 1% agarose gel was performed.

Purification of DNA fragments from agarose gel using spin column procedure

DNA samples (250 bp and 850 bp) were electrophoresis in separate lines on a 1% agarose gel with 0.5 × TBE buffer as conductive media. After running, separated DNA was visualized on a UV transilluminator and the desired bands were excised from the gel and DNA fragments was extracted from gel using the procedure at www.protocol-online.org. The yield of DNA was quantified and analyzed with comparing their concentration with DNA ladder using electrophoresis in 1% agarose gel containing 0.5 μg/ml ethidium bromide.

Restriction digestion

To assess the suitability of the purified DNAs for molecular manipulations, DNA fragments obtained by application of three different protocols were digested using AvaII restriction endonuclease. The digestion products were evaluated on 1% agarose gel containing 0.5 μg/ml ethidium bromide.

RESULTS

The mean concentrations of amplicons were 150 ng/μl and 210 ng/μl for small and large fragments respectively. After DNA purification from agarose gel, it was shown that the yield of recovered DNA from agarose gel using current optimized procedure with HiPer Mini device was on average 95-98%, whereas the yields obtained with conventional phenol extraction and spin column methods were in the range of 50-60% [Figure 2]. All PCR products purified by 3 different methods were digested using AvaII restriction endonuclease [Figure 3]. Furthermore, plasmid purification from agarose gel using three methods had the same quality [Figure 4].

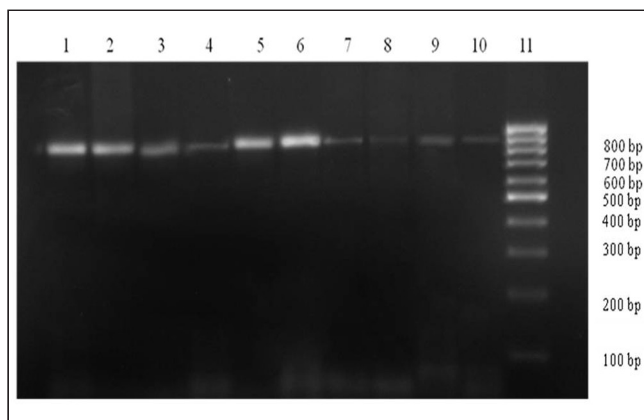

Figure 2: Agarose gel electrophoresis of the purified PCR products of 850 bp with three methods. Lanes 1-2: Spin column method; lanes 3-4 and 7-10: Conventional phenol extraction; lanes 5-6: Current optimized procedure with our device; lane 11: 100 bp DNA ladder

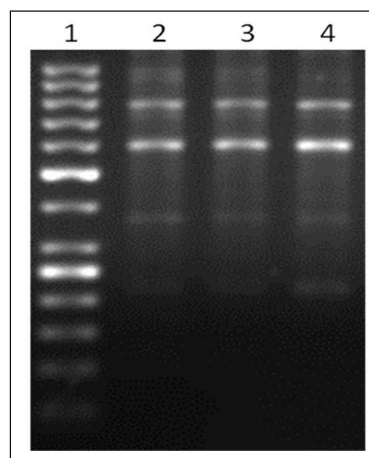

Figure 3: Restriction enzyme (AvaII) digestion of DNA fragments purified using three different methods. Lane1: ladder 50 bp; Lane 2: conventional phenol extraftion; Lane 3: Spin column method; Lane 4: current optimized procedure with device

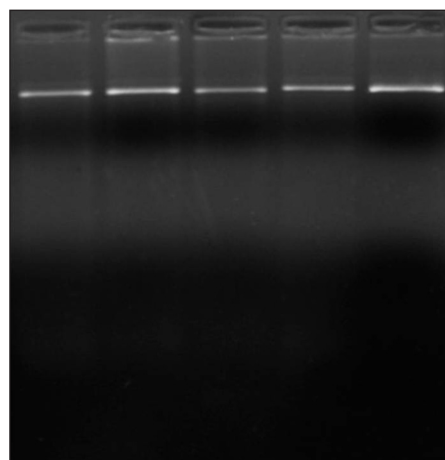

Figure 4: Plasmid purification with three methods. From the left side, Lanes 1-2: Spin column method; Lane 3: Conventional phenol extraction; Lanes 4-5: Current optimized procedure with our device

DISCUSSION

Three major platforms of electrophoresis comprise capillary electrophoresis, micro fabricated devices and slab gel electrophoresis. The latter named electro elution of DNA is the most common form of DNA electrophoresis, briefly molding a polymer like agarose and applying a voltage in a conductive medium so that multiple samples migrate in parallel.[5]

Electro elution of DNA from agarose gels is a standard procedure in molecular biological applications. One method is placing the gel slices inside the dialysis tube containing the buffer. Its disadvantage is taking a relatively long time.[6-8] Some documents developed electro elution into troughs excised ahead of band, filled with polyethylene glycol.[9] Disadvantages of this procedure are the need for constant vigilance and removing buffer from the trough frequently. Stabile and Wurtzel designed electro elution into wells cut out on the left side of the band.[10] This method showed an average recovery of 79% for fragments ranged from 7.6-22 kb in size. The recovery decreased for the DNA with higher masses (67%). Constant vigilance is needed for this method and the procedure as a whole is tedious in terms of time and labor. Kormanec *et al*. developed a procedure, which is similar to electro elution into the trough except for the fact that DEAE paper is used to trap the eluted DNA.[11] The yield of this method was higher than the typical elution procedures involving membranes inserted into troughs ahead of the band of interest (about 80-90% for fragments from 100 bp to 4 kb), but no information provided for fragments greater than 4 kb. Our device performance was better comparing to the aforesaid procedures from the points of view of time, cost, yield and quality.

As shown in the Figure 1, the device is very simple and could be used in every laboratory, especially in developing countries where sometimes the resources are seriously limited. So far, a number of complicated apparatus were made for electroeluting DNA from gel slices;[11-16] however, all these methods proved to be inconvenient to operate in less developed laboratories, time-consuming and expensive.

DNA mobility on agarose gel-electrophoresis depends on the composition and ionic strength of electrode buffer and the agarose concentrations. Tris-acetate-EDTA (TAE) and TBE are the most commonly used running buffers for preparative works.[17] Although the resolution of supercoiled DNAs is better in TAE than TBE,[18] TBE has higher buffering capacity and more stability than TAE. There is a report that TBE is more effective than TAE to obtain high resolution of smaller DNA (300 bp) while TBE and TAE have the same applicability for the middle size of the DNA fragments ranging from 300-bp to 20-kb. As, most applicable purified DNAs are under 2 kb, we decided to apply 0.5 × TBE (89 mM Tris pH 7.6, 89 mM boric acid, 2 mM EDTA pH 8) as the running buffer in this study. As this method could be used for plasmid purification, we proposed TAE buffer for extracting it.

Agarose molecules with 1, 3-linked β-D-galactose and 1, 4-linked 3,6-anhydro-α-L-galactose and infrequently with carboxylate, pyruvate and/or sulfate residues,[7,19,20] make a random coil structure at high temperatures in solution[20-22] and upon cooling, the non-covalent hydrogen bonds in junction zone form the agarose gel.[21,23-27] As the median pore radius size of agarose gel is 1%,[28] we decided to use this concentration.

The mobility of DNA molecules in agarose gels are highly dependent on the electric field applied to the gel,[28,29] most likely because the electric field disrupts the hydrogen bonds in the junction zones, allowing the gel fibers and fiber bundles to orient in the electric field.[30,31] As, our target was DNA molecules elution from agarose gel, we decided to select 10 V/cm in order to eluting the DNA fragments from gel.

This purification method was optimized to directly elute the desired fragments of DNA from agarose gel. The current method was compared with conventional phenol extraction[1] and spin column procedures. The DNA yield obtained with the device was 95-98% in comparison to DNA yield of 50-60% for phenol extraction and spin column procedures. Elution of DNA fragments was repeated in triplicate for checking the robustness and reproducibility and the results were found to be consistent.

The isolated DNA was also checked for its compatibility with restriction endonuclease digestion. The results showed a successful restriction digestion, indicating that enzyme inhibitors were absent and that the purified DNA can be used for further manipulation.

CONCLUSION

The most important factors that should be taken into account to extract DNA fragments from gels are speed, simplicity, the yield and purity of recovered DNA and cost of the system. As mentioned before, our device showed satisfactory competency in quick DNA fragments elution from agarose gel, inexpensive and vigorous and it does not require the use of organic solvents. Furthermore, the purified DNA demonstrated suitable quality for further manipulations like restriction digestion.

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