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Introducing a New and Straightforward Approach for DNA Purification from a Gel

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ABSTRACT

In most procedures that involve gene cloning, after the amplification of a target gene by PCR or by Real-time PCR, the purification of the trapped gene on agarose gel is a crucial stage. There are various methods for extracting genes from agarose gel by removing other contaminants. We isolated the amplified *PqHMGR* gene (derived from Ginseng (*Panax quinquefolius*)) from agarose gel by a quasi-electrophoresis device (similar to electro-elution technique). Moreover, the efficiency of this new approach was compared with that of the commercial kit 'Silica Bead DNA Gel Extraction' (Thermo Scientific American Company). Ligation to the PTG-19 plasmid and cloning in *E. coli* bacteria were also done. The results showed successful isolations of targeted DNA, along with a high efficiency in producing recombinant DNA and in concluding a successful cloning procedure through this new device. The invented method provided a better purification ability than the commercial kit, but because of using the TAE 1X buffer as the purified gene storage solution, the plasmid and bacterial transformation rates were slower than the commercial kit method. It was found that using the new method for the purification of nucleotide sequences by electrophoresis and electrophoresis buffer is feasible, and that these purified fragments can be applied in cloning and sequencing. Using the TAE 1X buffer instead of distilled water did not cause problems in gene binding to PTG-19 plasmid. It also allowed a successful transformation of *E. coli* bacteria by the modified plasmid. Nonetheless, using TAE 1X buffer reduced the modification rate of the PTG-19 plasmid and decreased the rate of *E. coli* transformation by the modified plasmid.

Abbreviations:
5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), Complementary DNA (cDNA), Diethyl Pyrocarbonate (DEPC), *Escherichia coli* (*E. coli*), Ethylenediaminetetraacetic acid (EDTA), Isopropyl ß-D-1-thiogalactopyranoside (IPTG), LB (Luria Broth), Optical density 260 (OD260), Optical density 280 (OD280), *Panax quinquefolius* HMGR (PqHMGR), Polymerase chain reaction (PCR), Reverse transcription polymerase chain reaction (RT-PCR), Tris/Borate/EDTA (TBE), Tris-acetate-EDTA. 1X (TAE).

Introduction

To ligate a targeted gene to the plasmid in the cloning process, first, a specific amplified target gene band (i.e. a fragment of the DNA amplified by PCR or RT-PCR) must be efficiently separated from other non-target bands, primer dimers, and other contaminants; otherwise, cloning the gene

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fragment cannot be optimally possible in the next steps. Using agarose gel to separate DNA bands of various sizes has had a long history which started from the discovery of restriction enzymes (Sharp et al. 1973). Although several methods have been devised to purify DNA fragments from agarose gel, the purification of DNA fragments from agarose gel almost always causes problems for further studies. Some of the problems with DNA fragments that are purified from agarose gel (i.e. a decrease in performance, DNA modifications, etc.) have not been satisfactorily resolved (Roberts and Murray 1976). Meanwhile, the purification of amplified bands using agarose gel has always been time-consuming and is usually associated with a decrease in the amount of the targeted DNA band (Boom et al. 1990). Treatments such as NaCl, NaClO4, silica and fine glass beads are often used for purifying DNA bands, with high DNA-binding potential (Marko et al. 1982; Vogelstein and Gillespie 1979; Yang et al. 1979).

There are several different methods for gene purification from agarose gel. Some of these are, namely, the purification of DNA using the phenol/chloroform method (Sambrook 1989), using ion-exchange chromatography with positively charged DEAE-cellulose resin (Dretzen et al. 1981; Zassenhaus 1982), using the DEAE-sephacel method (Sambrook 1989), glass beads (Chen and Thomas Jr 1980), the freeze-squeeze method, the optimized freeze-squeeze method (Tautz and Renz 1983; Thuring et al. 1975), solubilizing the gel with potassium iodide (Blin et al. 1975), using the electro-elution method (Wienand et al. 1979), the ß-Agarase method (Chong and Garcia 1994), the DNA-precipitation method whereby ethanol is used (Sambrook 1989), etc. Each of these methods of gene purification from the gel has its advantages and disadvantages. The most exciting way to isolate and purify DNA fragments is to bind the DNA to powdered glass beads.

The main problem in using glass beads has been DNA disruption at sizes larger than 5 Kbp, which eventually was solved by membrane columns and silica powder, instead of glass beads (Vogelstein and Gillespie 1979). A decrease in DNA concentration during purification was also observed in various commercial kits (Feng et al. 2007; Sulaiman et al. 2005).

In this study, we carried out *PqHMGR* gene purification from agarose gel by a quasi-electrophoresis device, based on the use of electric fields within the TAE 1X buffer, and then evaluated the possibility of cloning the gene by *E. coli* (i.e. the *PqHMGR* gene that was purified and dissolved within the TAE 1X buffer. Finally, the purification and cloning efficiency of the *PqHMGR* gene was determined by the invented electrophoresis-based method. Its efficiency was compared to that of the Silica Bead DNA Gel Extraction Kit which operates normally with silica powder (Thermo Fisher Scientific Company).

Materials and Methods

Selecting plant tissue and materials

Three-year-old American ginseng plants were cultured in peat and perlite (grade-three) in the Aburaihan College, University of Tehran. They were grown at 23 ± 2 °C, in 16 h light and 8 h darkness, with fluorescent light (60-100 µmol/m^2s^2). Given that the highest amount of mRNA were in the leaves of American Ginseng, the leaves were used for sampling (Wu et al. 2010). Then leaves were immediately frozen in liquid nitrogen after sampling and were finally stored at -80 ° C to extract the RNA.

RNA extraction using the Trizol kit

To perform RNA extraction, first, 0.05-0.1 g of leaf tissue was powdered in liquid nitrogen, and then RNA was extracted from the leaves of American Ginseng using 1 mL Trizol kit. All glassware and reagents in this process were treated with 0.1% DEPC. The RNA was separated after running a 1.5% agarose gel electrophoresis using EDTA/ (TBE) buffer at 75 V for 2 hours. To detect the quantity and quality of the extracted RNA (from the band breakage), the gene was compared with the standard 1Kb DNA ladder RTU ((Ready-to-Use) GeneDireX company) on agarose gel. Moreover, the absorption at 230, 260 and 280 nm was measured using the spectrophotometer (PerkinElmer Lamda 25 Spectrophotometer).

Primer design and cDNA synthesis

To synthesize the first strand cDNA, Oligo dt 15 T (A/ C/ G) primers were designed. To confirm cDNA construction, forward and reverse primers of actin housekeeping genes were designed (database ID *V00450*). To synthesize the second-strand cDNA, an online version of Primer3 software (version 0.4.0) was used for designing primers from the *PqHMGR* gene sequence (Accession number FJ755158.1). Sequence characteristics and the melting temperature (Tm) of each designed primer was determined (Table 1). To synthesize the First-strand cDNA, 1 µl of Oligo (dT)15 T(A/C/G) was transferred into 5 µl (2.5 µg) of the extracted RNA, according to guidelines of the RT-PCR Kit (M-Mul V Reverse Transcriptase (10000 units) (Table 2). Then, the cDNA was amplified in a Thermo cycler (BIO-RAD My Cycler company) at 45 °C for 10 min and at 70 °C for 70 min. Finally, the synthesis of the First-strand cDNA was confirmed using the actin housekeeping

gene by its forward and reverse primers (Table 2). The PCR generated the second-strand cDNA in a total volume of 25 µl according to the CinnaGen Master Mix PCR Kit protocol (Table 3) under specific temperature conditions (Table 4). Given that there was a non-specific 1000-bp band along with the *PqHMGR* gene band, to determine the amplified band concentration, the intended band concentration was compared to ladder bands after loading approximately 5 µL of the DNA ladder. The amplified sample ran on a 1.5% agarose gel for 90 minutes. To compare the gene purification efficiency from agarose gel by the invented electrophoresis-based method, with conventional commercial kits, the aforementioned invented device and Silica Bead DNA Gel Extraction Kit (USA Thermo Scientific Company) were used to purify the amplified *PqHMGR* gene. Given that the TAE buffer was used as a solvent for the purified gene in the innovated method, this may have affected the cloning efficiency of the TA cloning method. Therefore, the cloning efficiency of the gene purified by this method was compared to the mentioned commercial kit in terms of the number and percentage of colonies that showed successful cloning.

Table 1. Sequence characteristics and melting temperature of each primer from top to bottom, respectively, including the forward and reverse primers of the *PqHMGR* gene sequence, the actin forward and reverse primers, and the Oligo dt 15 A primer (T/ C/ G).

Primer	Sequence (5′→3′)	Tm (°C)
Primer F	TCCCATAGTTGCCAACCTCC	59.38
Primer R	ACCACACCACCATCTATCCTC	58.88
Primer act F	GGTCGCACAACTGGTATTGTATTG	57.1
Primer act R	CTCAGCAGAGGTGGTGAACA	53.1
Oligo dt15 T(A/ C/ G)	TTTTTTTTTTTTTTT T(A/C/G)	45

Table 2. The mixture used for synthesizing the first-strand cDNA sequence according to CinnaGen Company M-Mul V Reverse Transcriptase Kit protocol (10,000 units).

Material	Concentration (µL)
Template RNA	5
Primer Oligo (dT)15 T(A/C/G)	1
DEPC- treated water	6.5
10X Reaction Buffer	2
Ribolock RNase Inhibitor	2.5
dNTP Mix, 10mM each	2
Revert Aid Reverse Transcriptase	1
Total volume	20 µL

Table 3. The components concentration according to CinnaGen PCR Master Kit protocol for amplifying the *PqHMGR* gene by its primers.

Component of sample	Final concentration	volume (µL)
Master Mix	1X	12.5
Forward Primer	0.1- 1 µM	1
Reverse Primer	0.1- 1 µM	1
Template cDNA	10 pg- 1µg	1
Sterile Deionized Water	-	9.5
Total volume	-	25

Table 4. Thermal cycler program for the amplification of the *PqHMGR* gene by its primers.

Steps	Temperature (°C)	Time (min)	No. of Cycle
Initial Denature	94	5	1
Denature	94	1	35
Annealing	55	1	
Extension	72	2	
Final Extension	72	5	1

Method one: gene purification from agarose gel by electrophoresis

The invented device was used for purifying the gene from agarose gel and consisted of an electrophoretic-like tank with platinum wire in an anode that extended into the syringe needle cover (Fig. 1). A 100 µL space was created by blocking the interior compartment of the Needle Cover through Styrofoam (Fig. 2A). The anode platinum wire was horizontally placed inside the invented device tank, similar to the electrophoresis device. In this device, two syringes are used. One of the syringes was cut off from the opening of its cylinder (which has the cutting cylinder diameter equal to the syringe's cylinder diameter) and cut the agarose gel containing the desired gene band (Fig. 3A and 3B). The other components were a syringe cylinder (where the cut gel was placed), a needle for the transference duct between the syringe cylinder and its needle cover, a needle cover (with a reservoir to store purified gene) and the piston (for transferring the cut gel into the syringe cylinder). A hole was created in front of the syringe cylinder opening to relieve the suction pressure. The pressure was caused by pulling the syringe piston after transferring the cut agarose gel into the syringe.

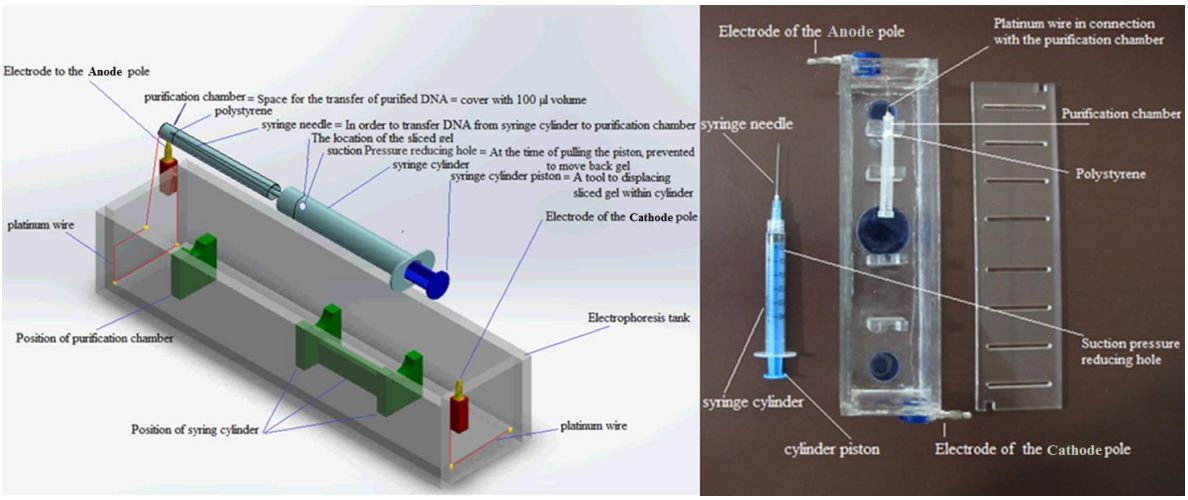

Fig. 1. Different parts of the electro-elution device.

Fig. 2. (A): syringe needle cover. (B): Inserting the syringe cylinder containing the desired cut gene, connected to the 100 µL needle cover space through the syringe needle. (C): filling up the tank with TAE 1X buffer and connecting the invented device electrode to the electric power supply to perform gel electrophoresis at 90 V and 400 mA/gel for 7 minutes.

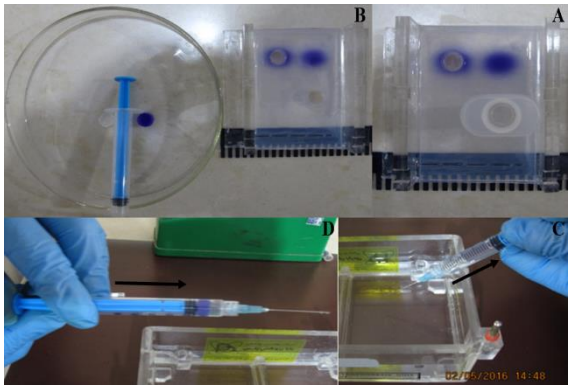

Fig. 3. the invented device has a working protocol as follows: (A): to cut the 1% agarose gel at two points containing the desired gene and at some parts of dye color (to clearly show the cut part on agarose gel) by a syringe cylinder with a slip opening. (B): laying the cut gel parts within the TAE 1X buffer. (C): Filling the syringe cylinder with TAE 1X buffer. (D): Transferring the gel parts containing dye color into the main cylinder of the syringe by the syringe piston.

The protocol comprised 60 µL of PCR product (with the amplified fragment between forward and reverse primers which was about 1857 bp) that ran on a 1% agarose gel electrophoresis (at 75 V and 400 mA for 2 hours). A syringe cylinder was used with an opening to cut the desired gene location on the 1% agarose gel. In the next step, the cut gel was embedded in a syringe cylinder containing TAE 1X buffer until the gel fragment was saturated with TAE 1X buffer. By pulling out the syringe piston, the main cylinder fills with the TAE 1X buffer. At this stage, to fill the syringe cylinder with TAE 1X buffer, the hole at the tip of the syringe needs to be obstructed using a plastic tape. The cut gel was placed inside the main cylinder of the syringe and was then transferred to the primary part of the syringe by pushing its piston. In doing so, the TAE 1X buffer was depleted from the needle tip until an amount of 200 µL TAE 1X buffer remained inside the main cylinder, precisely above the hole at the tip of the syringe cylinder. After inserting the cut agarose gel into the primary part of the main cylinder, to establish a connection between the syringe cylinder and the blocked 100 µL space, a conduit was created by the syringe needle inside the Styrofoam. (The needle cover had an interior space blocker.) Subsequently, by pressing the gel using a syringe piston, about 100 µL of TAE 1X buffer from the main cylinder was depleted into the blocked 100 µL space through the syringe needle (where the cathode platinum wire is located inside the blocked 100 µL space). By fixing the gel in its place and removing the plastic tape, which was done to remove the suction pressure of the piston and prevented the agarose gel from going back into the cylinder, the compressor piston exited the syringe cylinder. After horizontally placing the syringe cylinder with the cut agarose gel into the device tank, the TAE 1X buffer was added into the device tank until it filled up inside the main cylinder. In this step, the negative and positive electrodes were connected to the voltage source, and then 1% agarose gel electrophoresis was performed at 90 V and 400 mA for 7 minutes until the gene was transferred into 100 µl TAE 1X buffer through the syringe needle. Finally, the purified gene within TAE 1X buffer was transferred into a 0.5 µl tube by a sampler. The process of gene purification from agarose gel was done by the electrophoresis-based device (Figures 2 and 3).

Second method: gene purification from agarose gel using the Silica Bead DNA Gel Extraction Kit (Thermo Scientific, USA)

After running a 1% agarose gel electrophoresis (50-60 µL amplified PCR product) and separating the specific band from non-specific bands, they were separated from the gel according to the Silica Bead DNA Gel Extraction Kit protocol (Thermo Scientific, USA). To confirm gene purification from 1% agarose gel using both methods, the purified samples were once again electrophoresed on a 1.5% agarose gel (Fig. 2C). Their quantity and quality were evaluated by running the gel on the spectrophotometer.

Plasmid extraction

A plasmid cloning vector PTG-19T (pTG19-T PCR cloning vector kit) of Cinna Gene Company was used, and the plasmid was recombined with the desired gene according to the protocol of the kit.

Media and bacterial strains

The *E. coli* strain DH5α was prepared using $CaCl_2$. The culture medium that was used for bacterial growth included liquid LB as well as LB agar. This was meant to distinguish between white and blue colonies. Figure 5A illustrates the liquid solution containing *E. coli* bacteria, and Figure 4B shows the linear culture of *DH5α* bacteria that formed a single colony of bacteria on the LB Agar medium.

Cloning procedure of purified samples using the TA cloning method

In this step, a successful modification rate of PTG-19 plasmid involved using the purified genes that were obtained from the two methods (i.e. the invented method and the commercial Silica Bead DNA Gel Extraction Kit). After comparing them with each other, the number of bacterial clones that grew on LB (Luria Broth) Agar media and that contained 1 µg/mL ampicillin was successfully modified through plasmids by the control gene. This

served as the reference for determining the percentage of successful modification. In the following, this comparison was also performed for the successful transformation rate of *E. coli* DH5α by these two kinds of modified plasmids. It also involved another modified plasmid from the control gene. At this stage, the whole number of bacterial clones grown on LB Agar media were supplemented with ampicillin and X-gal. IPTG served as the reference for determining the percentage of bacterial colonies that were successfully transferred. The PTG19 plasmid modification was performed according to the pTG19-T PCR cloning vector kit protocol. The bacterial transformation by modified plasmids was performed according to the TA cloning method which was then subjected to a white-blue assay. In this step, the gene of the pTG19-T PCR cloning vector kit was used as the control.

Comparison of the transfer ratio of the purified gene obtained from the invented method and the commercial kit to PTG-19 and E. coli DH5α.

The percentages of successfully grown bacterial clones (bacterial clones containing PTG-19 plasmids) were determined on LB Agar media containing ampicillin. They were compared with the effect of TAE buffer (i.e. the solvent that was used to solubilize the purified gene in the invented method) in distilled water and the universal solvent to solubilize the purified gene in the Kit method. Comparisons were made in terms of the possibility of PTG-19 plasmid transfer to *E. coli* during the plasmid transfer process to *E. coli* by the heat-shock method.

The successful modification rate of the PTG-19 plasmid = number of bacterial clones that carry the purified gene grown on LB Agar media with ampicillin / number of bacterial clones that carry the control gene grown on LB Agar media containing ampicillin

The percentages of white bacterial clones (clones of bacteria containing the PTG-19 plasmid that carried the control gene and the purified genes obtained by the invented or commercial kit) were determined on LB Agar media containing ampicillin, X-gal, and IPTG. These were used to compare the effect of TAE on distilled water in terms of the modification potential of the PTG-19 plasmid. Since PTG-19 plasmids may be modified by bacterial genes during the process of plasmid transfer into bacteria, the correct modification of the PTG-19 plasmid can be ensured by both purified gene fragments obtained from the invented and commercial kit. These plasmids were extracted from white clones (i.e. the clones with successful transformation by both genes derived from the invented and the commercial kit methods). The extraction involved using the alkaline lysis method (Bimboim and Doly 1979). Subsequently, they were subjected to enzymatic digestion reaction according to the *BamH I* enzyme protocol. The enzyme contained a restriction site within the target gene.

The successful transformation rate of *E. coli* DH5α = number of white clones of bacteria grown on LB Agar media containing ampicillin, X-gal and IPTG / the whole number of bacterial clones grown on LB Agar media containing ampicillin, X-gal and IPTG

Results

The RNA that was extracted from American ginseng leaves had a concentration of 500 ng/ µL. OD280/ OD260 and OD260/ OD230 values were 1.9 and 2, respectively. The shape of the extracted RNA bands on agarose gel was made apparent (Figure 5A). By comparing the concentration of the *PqHMGR* gene and ladder bands, it was found that the specific amplified band concentration was similar to the 500 bp band, which was approximately 18 ng/µl (Fig. 5B). The concentration value of the purified *PqHMGR* gene was actually purified from 1% agarose gel by the invented electrophoresis-based method and Silica Bead DNA Gel Extraction Kit, which yielded values of 17.1 ng/µL (90%) and 16.53 ng/µL (87%), respectively (Fig. 4C). To realize the impact of the purified gene solvent (TAE 1X) on bacterial transformation by PTG-19 plasmid, the successful transformation percentage of *E. coli* DH5α bacteria by the PTG-19 plasmid on LB Agar media (with ampicillin) had a percentage of transferred bacterial clones formed by purified genes. These were derived and assessed by both methods compared to the transferred bacterial clones containing the control gene. In the case of the purified gene that was dissolved in TAE buffer (in the invented method), the success rate was 53.4% (Fig. 4D).

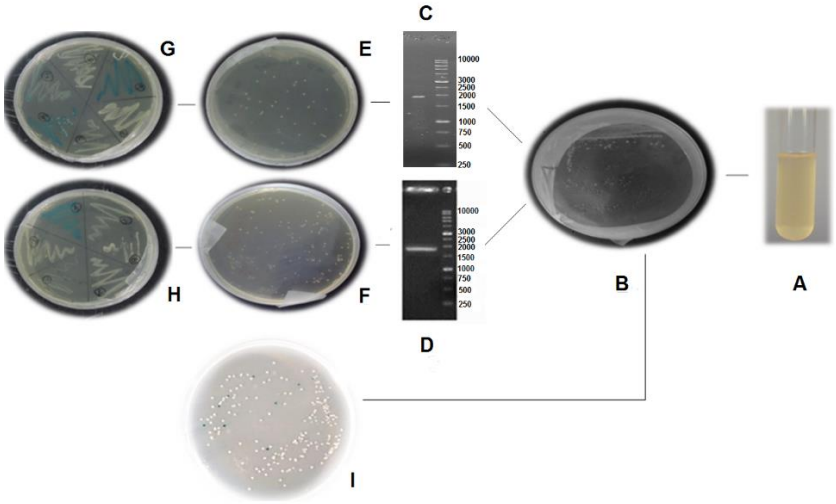

Fig. 4. (A): DH5α bacteria solution. (B): DH5α bacteria linear culture. (C): Loading the purified gene bands obtained by the invented method (16.53 ng / µLit). (D): Loading the purified gene bands obtained by the kit method (16.53 ng/µLit) on a 1.5% agarose gel. (E): Formation of transferred bacterial clone carrying the purified gene derived from the invented method LB Agar media that contained ampicillin (53.4%). (F): Formation of transferred bacterial clone carrying the purified gene obtained by the commercial kit method on LB Agar media containing ampicillin (almost 78.12%). The number of successfully modified plasmids by the control gene was used as a reference for determining the percentage of successful modification which included the number of bacterial clones in petri dish I). (G and H): Success rate of transferred bacteria formation containing the PTG-19 plasmid that carried the purified *PqHMGR* gene derived from [(G): The Invented method (40.3%) and (H): commercial Kit method (47.92%) on LB Agar media containing ampicillin, X-gal, IPTG]. (I): Success rate of transferred bacteria formation containing the PTG-19 plasmid that carried the control gene (95.83%) on LB Agar media containing ampicillin, X-gal, and IPTG. The number of whole bacterial clones grown on LB Agar media containing ampicillin, X-gal, and IPTG in each method was used as a reference for determining the percentage of successful bacterial transformation.

In the case of the purified gene dissolved in sterile distilled water (in the commercial kit method), the success rate was 78.12% (Fig. 4F). The proportion of white clones to whole clones in all three invented, kit, and control methods on the LB Agar media, including ampicillin, X-gal, and IPTG were 40.3% (Fig. 4A), 47.92% (Fig. 4G), and 95.83% (Fig. 4H), respectively. To identify white clones with the purified fragment using the invented method (after selecting recombinant white clones from the blue ones), the plasmids were extracted from the white clones. The extracted plasmid bands had three supercoil, linear, and double-stranded DNA bands (Figure 6A). Figure 6B shows bands derived from plasmid digestion using the *BamH I* enzyme that comprised a linear plasmid (PTG-19 plasmid cleavage with the *BamH I* enzyme). It had two cleaved *PqHMGR* fragments (i.e. the *BamH I* enzyme cleavage within the sequence).

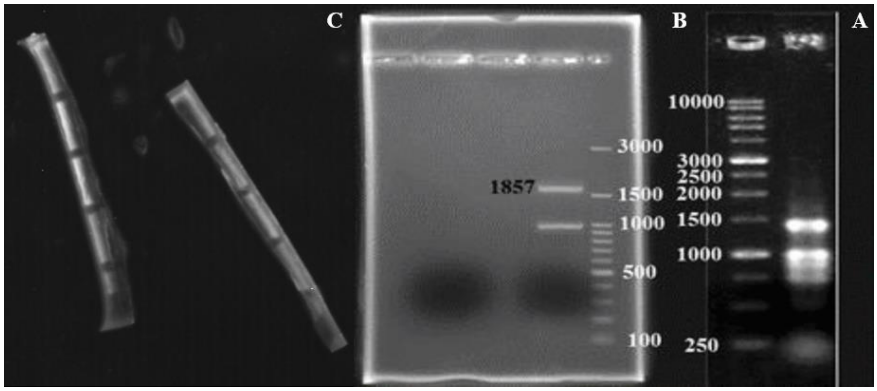

Fig. 5. (A): extracted RNA from Ginseng plant leaves. (B): A specific band corresponding to a part of the 1857 bp *PqHMGR* gene and a non-target of almost 1000 bp band. (C): provision of the cut gel by scalpel for purification through the commercial kit.

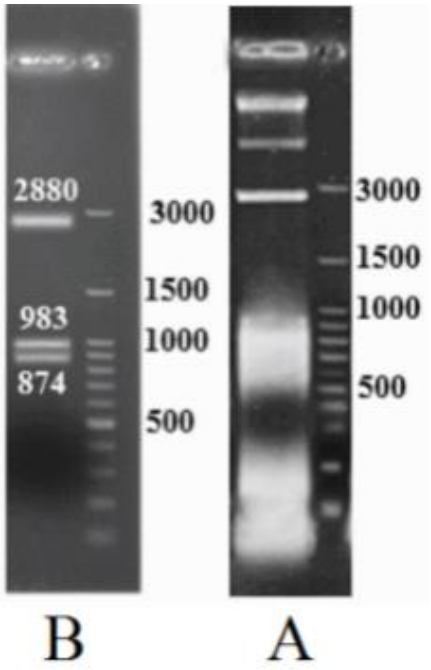

Fig. 6. A: PTG-19 plasmid extraction containing the *PqHMGR* gene, including supercoil, linear and double-stranded DNA bands. B: The above-mentioned plasmid cleavage with *BamH I* enzyme forms three bands that comprise a linear plasmid (i.e. *BamH I* enzyme cleavage on PTG-19 plasmid) and two cleaved *PqHMGR* fragments (i.e. *BamH I* enzyme cleavage within the sequence).

Discussion

In the available literature, various methods have been described for the isolation of nucleic acids from gels, including phenol/chloroform, DEAE-cellulose resin, DEAE-sephacel, glass beads, freeze-squeeze, optimized freeze-squeeze, potassium iodide, electro-elution, ß-Agarase and DNA Precipitation (Sambrook 1989) (Dretzen et al. 1981; Zassenhaus 1982), (Sambrook 1989), (Chen and Thomas Jr 1980), (Tautz and Renz 1983; Thuring et al. 1975), (Blin et al. 1975), (Wienand et al. 1979), (Chong and Garcia 1994), (Sambrook 1989). Most of these methods have limitations. Some are time-consuming or require special equipment. Sometimes, there are impurities in the gel or salts in the isolation protocol, thereby reducing the quality of the purified nucleic acids. In addition, in some methods, the breakage of highly-sequenced nucleic acid fragments may occur during isolation. There are a variety of gene purification methods that use agarose gel or PCR solutions with high purification efficiencies. The Silica/Guanidinium salt method involves a purification process for PCR solution and agarose gel. It makes a yield of 90-80% and 80%, respectively, in 5 minutes (for the PCR solution) and in 60 minutes (for the agarose gel). The advantage of this method includes eliminating the contaminants in the shortest possible time in just one step, but its disadvantage is the possible breakage of nucleic acid fragments (in sizes greater than 5 Kbp) (Ausubel M 1998).

The filtering cartridges in combination with or without freezing is a method that involves a purification yield of over 95% in 2-5 minutes, depending on the purified sample concentration. The advantages of this method are its high-speed in operation, the increase in sample concentration, and elimination of unwanted salts. Its only disadvantage is the inability to eliminate large contaminants such as proteins (Ausubel M, 1998).

The ethanol or isopropanol precipitation method involves a purification yield of over 95% in 2-5 minutes or in 1 day, depending on the purified sample concentration. The advantages of this method are the provision of visibility to the purified plate, high performance, and usability with other extractable salts. Its disadvantages are the time-consuming nature of the process and the difficulty in using multiple samples simultaneously. The possibility of losing the plate and, ultimately, the possibility of not removing the contaminants are other problems of this method (Ausubel M 1998; Sambrook 1989).

The electro elution method involves a purification yield of over 95% of the fragments that are smaller than 1 Kbp long. For very small fragments, the yield is usually 50-60%, and for large fragments in 120-240 or 60-160 min, the yield is less than 20%. To dissolve the compartments in the DEAE liquid, this method has the advantage of being non-toxic, with high performance in purification. Its disadvantages are the difficulty in seeing the purified band and the limited use in the range of 0.05-20 Kbp fragments (Ausubel M 1998; Bostian et al. 1979; Dretzen et al. 1981; Girvitz et al. 1980; Henrich et al. 1982; Smith 1980; Strongin et al. 1977; Tabak and Flavell 1978).

The process described here can be used to purify nucleic acid fragments, RNA, protein and DNA (i.e. DNA from the extraction process or genes from the PCR process). In the invented method, about 90% of the genes were extracted from the agarose gel, which can be a proof of its higher purification efficiency compared with the Silica/Guanidinium salt method. The amount of time required for gene purification from agarose gel in the invented method was a little longer than the time required by the method of "Filtering cartridges in combination with or without freezing". The invented method has the advantage of eliminating protein contamination based on a quasi-electro-elution method. It resulted in the purification of an 1857 bp nucleotide fragment without breakage. Other large and small fragments must be tested to determine the quantity and quality of the gene

fragments and their purification. Additionally, this method is not only inexpensive and straightforward to purify the gene from agarose gel, but it also provides convenience in working with the purified samples, thereby eliminating pollutions that are sometimes created through the purification process of salts. Its only disadvantage is the purified gene that dissolves in the TAE 1X buffer. This happens because of using a salty liquid instead of sterile distilled water. By experimenting, it was found that although using the TAE 1X buffer, instead of distilled water, does not cause the general problem of gene binding to the PTG-19 plasmid, the TAE 1X buffer reduced the modification rate of the PTG-19 plasmid and decreased the transformation rate of *E. coli* bacteria.

The invented device can be used to purify short DNA, RNA and protein fragments as well as large DNA fragments from other DNA, RNA or protein contaminants. This method allows the purification of DNA fragments with different sizes in the shortest time. The quality of DNA recovery is high enough to reach an acceptable level. It is independent of the amount of loaded nucleic acid, which is because this method allows the nucleic acid content of the purification solution to be concentrated in the same purification solution. It is possible to use isolated nucleic acids in other molecular processes. In general, this device can be used for a variety of DNA, RNA and protein fragments, while having the capacity to incorporate slight changes in the purification solution (1X TAE for DNA and TBE.1X for RNA), voltage and time, without a fundamental change in the protocol. The only problem with using this method is the limited amount of purification and the percentage of successful *E. coli* bacteria using these fragments. To increase the concentration of the purified gene, gel fragments containing the desired gene can be entered into the device several times. In summary, this device offers a cheap, safe and simple method for isolating nucleic acid components from agarose gels, compartments that can also be used for other molecular processes such as cloning and sequencing. This device requires few reagents and can be used easily and cheaply. Using this method can reduce the amount of contaminants in the purification solution within the shortest possible time. It can be used frequently in purifying various sizes, ranging from large to small nucleotide sequences and proteins. The available literature suggests the use of dialysis membranes to trap the purified gene, followed by precipitation by ethanol to solve this problem in later steps (Wienand et al. 1979). It is worth mentioning that in the manufactured device, the purification path has been narrowed to a needle between the gene fragment and the 100 µL space. It does not involve using the dialysis membrane within the 100 µL space and in front of the anode platinum wire. Thus, it shortens the required time from 1-3 hours to about 7 minutes, although this also reduces the recovery rate of the purified gene from agarose gel. It is suggested that the dialysis membrane can be used for separating the purified gene within distilled water. Separating the purified gene from the dialysis membrane surface via ethanol leads to plate deposition and, finally, dilution within a certain amount of distilled water. The disadvantages of this device are the absence of a dialysis membrane that would otherwise separate the platinum wire from the purified gene location within the TAE 1X buffer, the likelihood of platinum wire breakage, even as the wire is the connector between the needle cover and cathode, and a lack of a free-surface within the headspace at the needle cover of the anode in the device.

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Short description of the contributions made by ea ch listed author

Kajvan Saed and Ali Izadi conceived and designed the research. Kajvan Saed, Nima Namjoo and Namjoo Saed Mocheshi conducted the experiments. All authors read and approved the manuscript.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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