American Journal of

Biochemistry and Molecular Biology

ISSN 2150-4210

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS American Journal of Biochemistry and Molecular Biology

ISSN 2150-4210
DOI: 10.3923/ajbmb.2023.28.35

Research Article
Simultaneous Nucleic Acids Separation and Extraction Using Silica Gel-Agarose Matrix Gel

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Abstract

Background and Objective: Amplified product of desired gene and a digested product of DNA cloning vectors using restriction enzymes is proceeding for identification of molecular size, and restriction fragments need to be separated in agarose gel electrophoresis system, both of these steps are time-consuming and expensive procedures in molecular cloning techniques. To perform agarose gel electrophoresis, expensive agarose powder will be required. The extraction of DNA molecules from the gel is a multistep process and expensive kits are required. **Materials and Methods:** Based on the outcomes of the present study, a new matrix was proposed in which DNA molecules can be separated and desired fragments can be extracted and purified without the use of any commercial kit available on the market. For matrix mixture preparation, 0.5% each of agarose and silica gel was taken in 1 x TAE buffer and the agarose and silica mixture was dissolved at boiling temperature. After dissolving substances, 2 µL of Ethidium Bromide (EtBr) of concentration 10 mg mL^{-1} was added and solidified into a gel casting tray. The amplified PCR product and vector digested product was carried out as per the standard procedure. From the gel desired fragment of DNA was extracted. **Results:** The agarose and silica-formed gel are a little clear and bands also seems to be prominent. Recovery of DNA bands was almost 90%. The purity of extracted DNA is within the range. The obtained DNA is suitable for restriction digestion, ligation, PCR, sequencing, transformation, etc. **Conclusion:** The preparation of silica-based gel is very economical and the extraction of DNA from these gels is very simple without the utilization of any commercial gel extraction kits. Its cost is almost one-tenth of the traditional system, and a single step is required for electrophoresis and gel extraction.

Key words: Silica gel, agarose, gel electrophoresis, gel extraction, gel matrix, gene amplification, restriction digestion

Citation: Pasha, S.M., A. Begum, V.D.H.N. Kola, S.A. Pasha, V.K. Chauhan and C. Pasha, 2023. Simultaneous nucleic acids separation and extraction using silica gel-agarose matrix gel. Am. J. Biochem. Mol. Biol., 13: 28-35.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Agarose gel electrophoresis is an effective way of separating DNA fragments ranging from 100 to 25 kb. Agarose is isolated from the seaweed and consists of repeated agarobiose (L and D-galactose) subunits. During gelation, agarose polymers associate non-covalently and form a network of bundles whose pore sizes determine a gel's molecular sieving properties. The use of agarose gel electrophoresis revolutionized the separation of DNA. Agarose being a key component is very expensive. Low-melting agarose used for gel extraction is further expensive.

In molecular biology, agarose gel extraction is a technique to isolate a desired fragment of DNA from an agarose gel following agarose gel electrophoresis. The DNA isolated by gel extraction is used in various applications like genetic engineering[1], blotting techniques, sequencing[2] etc. The success of the experiment depends on the quality and quantity of isolated DNA, as well as the cost of the process[3,4]. Traditional gel extraction consists of four steps: Electrophoresis, identifying the fragment of interest, isolating the desired DNA band and removing the impurities. As the number of steps increases, the loss of DNA also increases and the cost of the process will also increases. The already electrophoresed gel reuse after draining out of DNA gel is the only step to reduce the cost of agarose in repeated experiments[5]. Gel extraction kits contain membrane-bound, filter-plate or columnar silica for DNA binding. Generally, the organic extraction, electroelution, binding of DNA to glass particles, syringe squeeze, centrifugal filtration and ion exchange resins provide pure DNA, but a drawback of such methods is lower yields of DNA, incompatibility of purified DNA for DNA ligase and other enzymes[6]. Even the yields of recovered DNA are lower for larger DNA fragments extracted from agarose gel[7]. Further extracted DNA contains inhibitors for subsequent enzymatic activities[8]. Commercial kits use silica gel polymer to get higher yields of DNA in a rapid and safe manner[9]. In commercial kits, chaotropic salts, sodium iodide and tertiary ammonium salts are used to dissolve the gel but are known to cause denaturation of DNA. The current research papers deal with the development of an agarose-silica gel fused matrix-based gel for DNA electrophoresis and gel extraction of desired DNA. The purified DNA fragments can be used in restriction enzyme modification, hybridization, sequencing, ligation and cloning.

MATERIALS AND METHODS

Study area: The work has been carried out in the Departmental Molecular Biology Research Laboratory of Nizam College, Osmania University, Hyderabad, India in the year, 2018 from January to July. The entire study was carried out in 6 months.

Chemicals and reagents: Agarose (Lonza), Silica (Sigma-Aldrich). Tri-base (Sigma-Aldrich), Acetic acid (SRL, India). Ethylenediaminetetraacetic acid ((Sigma-Aldrich). Ethidium bromide (Sigma-Aldrich).

Gel preparation: For the 1% preparation of agarose gel, 2 proportions of silica gel and one agarose (2:1) as well as one proportion of silica gel and one agarose (1:1) (0.5%) were mixed in 1 x TAE buffer prepared using 50 x stock solution (24.2 g-Tris base, 5.7 mL-acetic acid and 10 mL of 0.5 M EDTA made up to 100 mL with distilled water). The mixture was further heated at boiling temperature for solubilization and kept aside to reach a temperature of around 50°C. Before polymerization, 2 µL of ethidium bromide (10 mg mL^{-1}) was added and kept for gel solidification. The matrices of agarose and silica gel (1:2 and 1:1) and normal agarose gel were also prepared for comparing the resolving nature of bands in the three different matrices.

Polymerase chain reaction: The total RNA from fat body tissue[10] (100 mg) was performed using TRIzol (Ambion, Life Technologies, USA) and the experiment was performed by the protocol described by Chauhan *et al.*[11]. The purity of the isolated RNA was passed by using a NanoDrop spectrophotometer (ND-2000, NanoDrop Technologies, USA). The 1 µg of total RNA was used for the synthesis of cDNA using the Revert Aid First Stand cDNA Synthesis Kit (Thermo Scientific, United States of America).

Achaea janata riboflavin-binding gene Polymerase Chain Reaction (PCR) was carried out using a thermocycler (Eppendorf, USA). The 10 µL of each reaction were setup using 5 µL of PCR master mix (2X) (Emerald Amp GT PCR master mix-Clonetech, USA), 3.5 µL nuclease-free water, 0.5 µL forward primer 5'CAGGGTTACGTTGTTAACCACTTAGGC3' (Tm = 59.4) (10 pmol), 0.5 µL reverse primer 5'GCCGTTGTAGTACTGATGTTGATGCG3' (Tm = 59.7°C (10 pmol) and 0.5 µL cDNA template (dilutions of 1:10). The polymerase chain reaction was carried out at 94°C for 2 min (initial denaturation), 94°C for 30 sec (denaturation),

60°C for 30 sec (annealing) and 35 cycles of amplification at 72°C and at 72°C for 10 min (final extension). The amplicon size was 194 bp. Amplified DNA was serially diluted till 1 ng and resolved on a 1% prepared agarose gel.

Gel electrophoresis: The PCR amplicon of ~200 bp size was tested. Amplicon was generated in PCR as described above, and a 25 µL sample was run on a prepared gel. The PCR product sample was allowed to migrate for 1 hr in 1 x TAE buffer until the dye front reached 3/4 of the gel length in a constant 100-volt electric field and was then visualized for excision by UV transillumination (Bangalore GeNei, India).

Gel extraction: After visualizing the desired nucleic acid band (200 bp PCR product), a gel slice is cut and taken out. The sliced gel containing nucleic acid was solubilized in 1 x TAE buffer containing guanidium hydrochloride at room temperature for 5 min with vigorous vortexing. The silica gel-bound DNA was pelleted by centrifugation at 13,000 rpm for 5 min. The pellet was washed twice with 70% alcohol, followed by centrifugation at 13,000 rpm for 5 min. The supernatant was discarded, and the pellet was air-dried. The bound DNA from silica particles was eluted by adding 10 mM Tris-HCl, pH 8.5/Milli-Q water and incubating for 5 min at room temperature with mild agitation. The eluted DNA was taken out of silica gel particles by centrifugation at 13,000 rpm for 5 min. The pure nucleic acids were collected from the supernatant.

Gel extraction rates and DNA purity were determined for the Thermo scientific GeneJET Gel Extraction Kit, which is commercially available (Waltham, Massachusetts, United States of America). Extractions were performed strictly according to the manufacturer's protocols. For gel extraction of PCR product, 200 bp. Amplicon has generated in the PCR run above and 25 µL samples were run on a 1% agarose TAE gel. The PCR product was allowed to migrate for 1 hr in a constant 100-volt electric field and then visualized for excision by UV transillumination. The DNA was then extracted from the agarose gel slices using the clean-up kits, in triplicate.

Plasmid DNA (pDNA) fragments were generated by the restriction digestion of a 4,400 bp recombinant pPICZ alpha A plasmid using both EcoRI and KpnI restriction enzymes (NEB), for 1 hr at 37°C, resulting in the generation of 3,600 bp and 700 bp pDNA fragments. The 1 µg of pDNA and digested pDNA were loaded per well of a 1% agarose TAE gel and novel matrix-prepared gel and separated by applying a 100-volt electric field for 1 hr. The pDNA fragments were separated by gel electrophoresis and excised after visualization. The DNA was then extracted from the gel slices using the indicated clean-up kits and a novel method reported in triplicate. Final pDNA samples were analyzed as stated above.

Evaluation of extracted DNA quality: A 260 absorbance was taken to quantitate DNA. The A260/A280 absorbance ratios were measured to check the DNA purity. Both absorbencies were taken using a SPECTRONIC™ 200 Spectrophotometer (Thermo Scientific, USA).

Polymerase chain reaction of eluted DNA and sanger sequencing: The eluted amplified PCR DNA is further used as a template (1:50 dilution) for further amplification to check if the eluted product has any impurities that interfere with the PCR reaction. The eluted PCR DNA proceeded for Sanger's sequencing.

Restriction digestion and ligation of eluted plasmid DNA: The plasmid eluted from the prepared gel (Agarose:Silica gel: 1:1) was kept for restriction digestion to determine whether any particles attached to the DNA interfered with enzymatic activity. Ligation of both methods gel extracted 3,600 and 700 bp was carried out with T4 Ligase (Invitrogen) as per the manufacturer's instructions.

Assessment of transformation efficiency: The pPICZαA recombinant plasmid was transformed in *E. coli* (DH5α) by the calcium chloride method, and recombinant colonies were screened on Zeocin LB Agar.

RESULTS

Gel preparation and gel electrophoresis: Agarose and silica gel molecules interacted together to make a jelly matrix, and its assumed structure was given in Fig. 1.

The prepared gel electrophoresis was compared with normal agarose gel and visualized under a UV-trans illuminator. The gel formed is opaque, but the bands formed are prominent. The band running pattern was slower than the normal agarose gel. But DNA is separated based on size. The DNA bands are prominent in prepared gel compared to agarose gel even at lower concentrations as shown in Fig. 2.

In agarose gel, up to 10 ng DNA, only clear bands were seen, whereas in modified gel bands to 1 ng are clear as shown in Fig. 3.

Evaluation of extracted DNA quality and recovery

Purity: Purity index 260/280 is in the range of pure with 2.97 (Kit extracted) and 2.91 (prepared gel extracted) as well

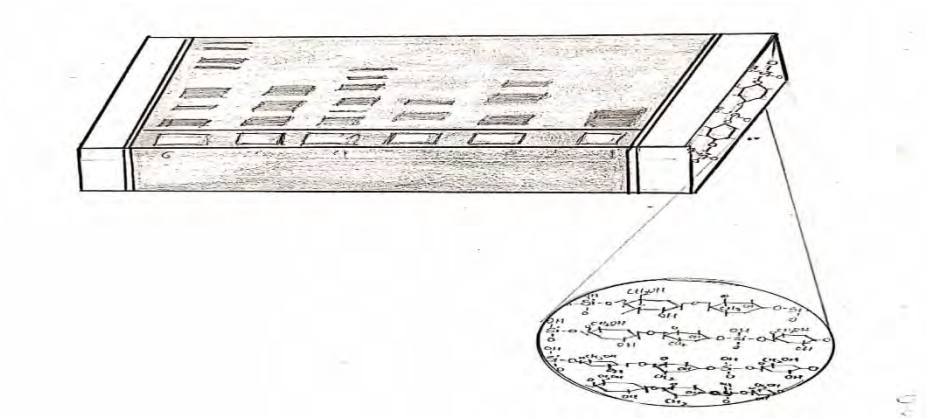

Fig. 1: Schematic representation of the interaction of agarose and silica to form a jelly matrix

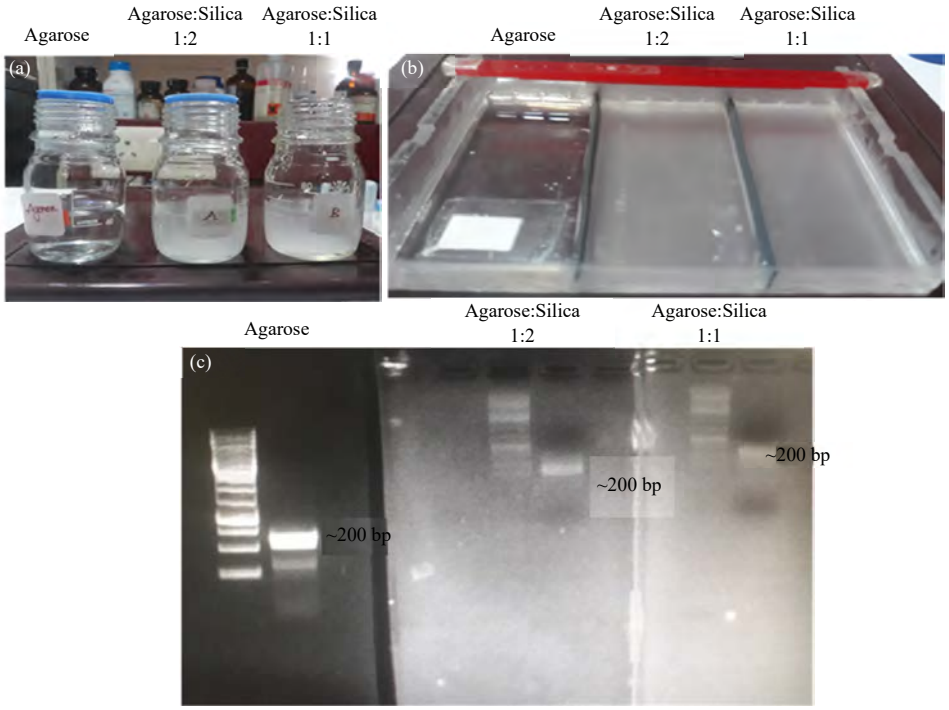

Fig. 2(a-c): Gels preparation and separation of DNA, (a) Agarose, (b) Agarose and (c) Silica gel-based

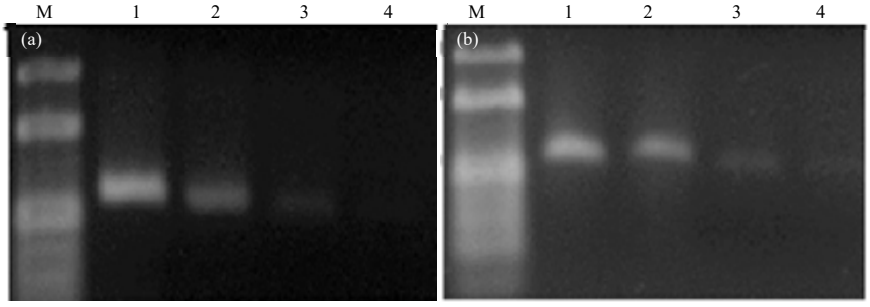

Fig. 3(a-c): Different quantities of 200 bp PCR DNA resolved on (a) Agarose gel and (b) Prepared gel. M: MW marker, 1:1000 ng, 2:100 ng, 3:10 ng and 4:1 ng

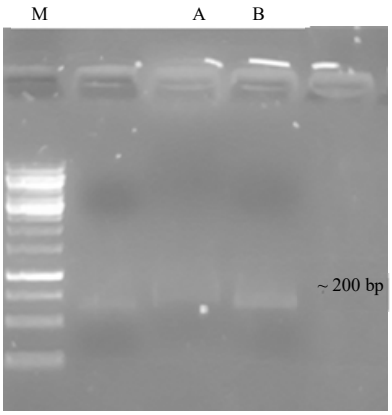

Fig. 4: PCR amplification of gel extracted DNA (A) PCR amplified product from eluted templated from kit based and (B) Proceed from silica agarose gel

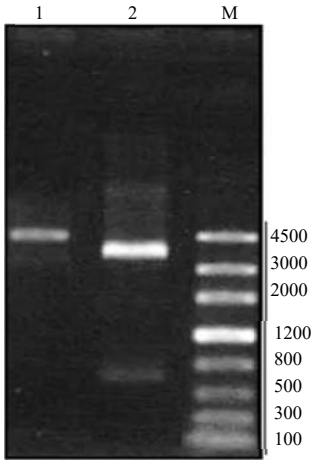

Fig. 5: Restriction analysis of recombinant pPICZαA
Lane 1: Recombinant plasmid (~4300 bps), Lane 2: Recombinant plasmid digested with EcoRI and KpnI, (~3600 and ~700 bps) and Lane M: DNA Marker III (4.5 Kb ladder)

as 54 of 60 µg DNA was extracted from prepared gel to give 90% recovery, whereas 87% DNA was recovered with a commercial kit (Table 1).

Polymerase chain reaction: The eluted DNA was used as a template for further amplification to check the eluted product has any impurities that interfere with the PCR reaction. The eluted product from the polymerized gel (Agarose:Silica gel:1:1) was better enough for PCR amplification (B) than the kit eluted DNA (A) (Fig. 4).

Sequencing: Extracted PCR DNA was used for sequencing, and a 100% identical product was obtained.

Restriction digestion and ligation: The plasmid eluted from the polymerized gel (Agarose:Silica gel:1:1) was kept for restriction digestion to know if any particles attached to the DNA interfered with enzymatic activity. The plasmid was digested well and there was no problem in enzyme activity (A 4,400 bp pPICZαA plasmid (1 µg pDNA) was digested with EcoRI and KpnI restriction enzymes (New England Biolabs Inc.). This digestion generated 2 DNA fragments, 3,600 and 700 bp, which were separated on a 1% agarose gel (Fig. 5). Restriction-digested fragments extracted from the prepared gel was ligated with T4 ligase, and a band of 4.3 kb was observed (Fig. 6).

Transformation efficiency: Transformation efficiency is calculated with TE = Colonies/µg × Final volume/Volume plated colonies = number of colonies observed on the plate in µg = amount of transformed DNA in µg dilution = Total DNA diluted before plating, TE for commercial kit extracted

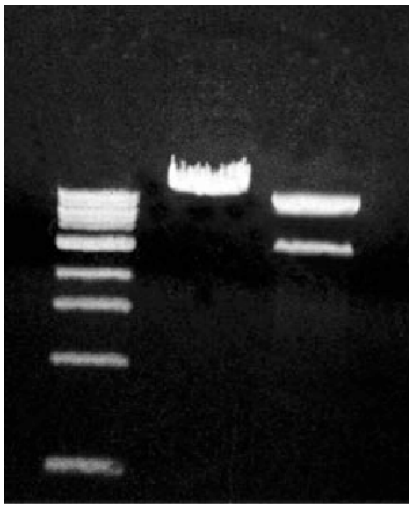

Fig. 6: Digestion of plasmid vector eluted from fusion gel

Table 1: Evaluation of eluted DNA quality and recovery

Feature	Commercial kit agarose extracted	Prepared gel extracted
260/280 purity	2.97	2.91
260 quantization	52 µg	54 µg
Recovery	87%	90%
PCR	Good	Good
Sequencing	Good	Good
Restriction digestion	Good	Good
Ligation	Good	Good
Transformation efficiency	1.6×10^3/µgDNA	1.2×10^4/µgDNA

plasmid: 40/0.05 µg×0.5/0.25 mL = 1600 1.6×10^3/µg DNA and TE prepared gel extracted plasmid: 300/0.05 µg×0.5/0.25 mL = 12000 1.2×10^4/µg DNA.

DISCUSSION

In the present study, agarose silica spheres in a matrix or loosely bound to the DNA were separated in the electrical field and easily extracted in a single step. After the electrophoresis and visualization under UV, the desired fragment is sliced and DNA can be extracted with Milli-Q water or a low concentration of buffer. At 90% of the DNA loaded in the gel matrix is extracted by this method. The opacity of the gel and the band migration was less like the low melting gel in the present gel, but the recovery was higher. The DNA extracted was suitable for PCR, restriction digestion and transformation. Digestibility and transformation efficiency are improved with the developed gel extraction method.

The DNA is separated by gel electrophoresis. The separation gel matrices included agar, agarose, polyacrylamide and composite agarose-acrylamide[12] gels. Agarose is mainly used to separate DNA in an electric field by electrophoresis[13]. However, agarose is very expensive and not readily available in developing countries[14]. Agarose extracted from the seaweed Gelidium and Gracilaria[15]. During gelation, agarose polymers associate non-covalently and form pore sizes to determine a gel's molecular sieving properties. High-concentration agarose gels up to 14% were prepared for resolving 2-5 bp differences of DNAs in the 25-200 bp region[16]. Separation and extraction of 20-500 base pairs (bp), DNA was achieved in a glass lab-on-a-chip (LOC) containing a crossed injector, 25 mm long separator, Y-shaped extractor and glass reservoirs for fluid handling in less than 2 min[17]. In the present agarose silica gel matrix gel, smaller fragments are well separated. The use of agarose gel for separating DNA, followed by gel extraction to isolate the desired DNA is a routine process in molecular biology[18,19]. But, the purification of DNA from agarose gel has long been problematic[20]. The DNA is modified and damaged during gel extraction[21]. Agarose is modified to low-melting agarose through hydroxyethylation. Low-melting agarose is used for gel extraction of DNA. Hydroxyethylation reduces the packing density of the agarose bundles, reducing their pore size[22]. The DNA fragments of the same size will take longer to move through a low-melting agarose gel as opposed to a standard agarose gel[23], slow migration in the present agarose silica

matrix gel can be explained by reduced pore side with covalent binding of agarose with silica. DNA fragments are separated according to size by electrophoresis on low-melting-temperature agarose and then recovered by melting the agarose[24]. Low-melting agarose and commercial kits used for gel extraction are expensive. Even though several techniques have been devised for gel extraction[1], none are satisfactory in eliminating the problems of impurities in DNA, degradation of DNA, modification of DNA, low yield, high cost, inconvenience, etc.[7,25]. Good-quality DNA requires the isolation of pure, intact, highly concentrated double-stranded DNA. The DNA quantity provides indices of extraction efficiency, while quality provides information on its purity and integrity, indicating its suitability for downstream experiments[19]. Commercial kits use silica gel polymer to get higher yields of DNA in a rapid and safe manner[9] Crystalline silica gel also causes DNA damage. In gel extraction kits, the gel is dissolved in chaotropic salts like sodium perchlorate or potassium iodide which are known to degrade high molecular DNA[7]. The current research papers deal with the development of an Agarose-silica gel fused matrix-based gel for DNA electrophoresis and gel extraction of desired DNA. Due to the fusion with agarose, silica will become soft, and the degradation of DNA will be reduced. In commercial kits, chaotropic salts, sodium iodide and tertiary ammonium salts are used to dissolve the gel, which is known to cause the denaturation of DNA. The simple gel matrix can be used for the separation and gel extraction of DNA without the use of any kits.

Study results implied that the preparation of gel is very simple and cost-effective. The additional gel extraction kit is not required. The purity is almost similar to a traditional gel extraction kit. One can easily reproduce the experimental procure in a cost-effective manner.

CONCLUSION

The present study shows that gel electrophoresis and the extraction of DNA from the gel are very economical, the cost of the entire procedure is reduced by almost 10 times lower cost than the traditional agarose gel preparations, and gel extraction total cost is almost one-tenth that of the market gel extraction system and only a single step is required for gel extraction. Gel formed is a little opaque, but the bands are prominent. The quality and quantity of DNA purified from the proposed agarose-silica fusion gel are up to the mark when compared with commercial kits available in the market and the obtained DNA is suitable for various steps used in molecular cloning like restriction digestion, ligation, PCR, sequencing, transformation, etc.

SIGNIFICANCE STATEMENT

The idea of the present study is to reduce the cost of agarose as well use of the use of a commercial gel extraction kit that is available in the market. The preparation of silica-based agarose gel is very economical and easy to cast and the extraction of DNA from these gels is very simple without using a gel extraction kit. The intensity of the DNA band is prominent in this hybrid gel, and well as recovery of DNA is almost 90% pure.

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