A quick and effective in-house method of DNA purification from agarose gel, suitable for sequencing

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Abstract Sequencing of DNA fragments (e.g., ITS, 16S, 18S, particular genes, and molecular markers) is increasingly required in studies on microbial diversity, microbial genetic population and phylogeny, sequencing of alleles, and searching for SNPs, among others. The cost of obtaining these DNAs, in quantity and quality for sequencing, is high as it involves special kits to recover DNA from gel after PCR, or the cloning and purification of plasmids with commercial kits. Genetic population and other studies require the analyses of many samples, and therefore, the high cost represents an obstacle for carrying out such projects in countries where there is great biodiversity, such as the tropical and subtropical developing countries, where funds are limited. Modifying an already known method for DNA recovery from gel, the first in-house protocol of DNA recovery suitable for direct use in sequencing is presented herein. This protocol is broadly applicable on DNAs from all different living beings, e.g., bacteria, fungi, and plants. Its simplicity, speed, and low cost make this procedure amenable for high-throughput DNA sequencings as required in microbial population studies, development of molecular markers, molecular identification of strains in microbial collections, and others. Recovery of DNA fragments from agarose gel is one of the most common tasks in molecular biology laboratories.

Therefore, its potential of applicability of the protocol presented here is enormous.

Keywords Home-spin columns · DNA purification from gel · Low cost method · DNA for sequencing

Introduction

Recovery of DNA fragments from agarose gel is one of the most common tasks in molecular biology laboratories for downstream applications, such as restriction digestions, ligation, cloning, and sequencing. All these applications require high quantity and purity of the recovered DNA. To date, many methods have been published to extract the desired DNA band from the agarose gel. A number of commercial kits include binding resins such as silica, diatomaceous, or glass fiber matrixes. In these cases, a chaotropic salt denatures the DNA, allowing it to bind to the silica—in-batch or in-spin columns; impurities are eliminated easily and quickly by washing, after which the DNA is eluted in a small volume of sterile water or TE buffer. Silica-based methods are fast, but the inconveniences include low yield and high cost, which are incompatible with sequencing of many samples. Other spin techniques use a tube containing a small-pore-size membrane to recover DNA by spin filtration, e.g., the fast flush extraction. After elution, the DNA is precipitated by standard procedures (Grey and Brendel 1992) which decrease yield importantly. Other methods comprise freeze and thaw-squeeze of the DNA-containing agarose or pressuring the gel plug with the plunger in a plastic syringe, and the use of low melting agarose and DNA release into solution by heating the agarose. Another popular method is electroelution; alternatives include placing the gel fragment

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inside a dialysis bag and electroelutioning onto a paper strip or affinity membrane. All these methods have their advantages and disadvantages. Usually, fast and simple methods result in low yield and poor DNA quality; while methods that result in pure DNA are time-consuming, laborious, and/or expensive. A number of reviews are available dealing with methods for DNA purification from gels (Kurien and Scofield 2002).

Common factors taken into consideration in the selection of the method include speed and simplicity of the protocol, size of the DNA fragment, yield, purity, and very often, low cost. The final selection depends on the planned downstream use of the DNA, and the expense, especially when high-throughput analysis such as sequencing of many samples, is necessary. Cost is a serious limitation, especially in developing countries. In our lab, we frequently need to sequence DNAs, digest DNA for cloning, or use it for downstream PCRs. Usually, we need to fractionate the PCR products in an agarose gel, excise the desired band, and recover it by removing the agarose. Here, we describe a simple, economic in-house procedure for DNA recovery from gel which is directly suitable for PCR, enzymatic restriction, cloning, and, for the first time with an in-house method, sequencing. No in-house protocol has been reported for direct use of the DNA in sequencing after recovery from the gel; further concentration or cloning is usually necessary, thereby increasing time and cost, which, in our case, was not necessary. The protocol describes a procedure starting after electrophoresis of DNA fragments, i.e., PCR products; therefore, it is applicable to DNAs from any living being. Speed, effectiveness, and low cost make this protocol amenable for high-throughput applications as required in ecology, microbial genetics, microbial population analysis, diversity studies, microbial inventory, and development of molecular markers, among others.

Materials and methods

PCR amplification

The biological material to establish this protocol can proceed from any living being. Genomic DNA and primers designed on a gene from *Mycosphaerella fijiensis*, a banana foliar pathogen, were used as our lab is currently carrying out research on this microorganism. PCR amplifications were performed in 25 μ L of volume containing 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 mM of each primer (forward: 5'-AGAGTTTGTATCCTGGCTCAG-3'; reverse: 5'-CCG TCAATTCCTTTGAGTTT-3', 1.4 U Taq polymerase (Invitrogen, Carlsbad, CA, USA), and 20 ng of *Mycosphaerella fijiensis* DNA as template (Kantun-Moreno et al. 2013). The expected PCR product is 312 bp.

Conditions of PCR consisted of initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 62 °C for 60 s and 72 °C for 60 s, and a final extension at 72 °C for 10 min. After PCR, products were run on 0.65% agarose and 0.5 $\times$ TAE gel (40 mM Tris-HCl, 20 mM acetic acid, 1 mM EDTA, pH 8.0), containing ethidium bromide (1 μ g/mL) for DNA staining and visualization. The gel was run in 0.5X TAE buffer and photographed using a gel documentation system (Gel Doc EQ, Biorad, Hercules, CA, USA). GeneRuler™ 1 Kb (ThermoFisher scientific™, Lithuania, EU) and 1 Kb Plus DNA Ladder (Invitrogen® Carlsbad, CA, USA) molecular-weight DNA size markers were included for calculation of amount and size of DNA fragment. DNA bands were quantified by densitometry using GelQuant.NET software provided by BiochemLabSolutions (<http://biochemlabsolutions.com>). Selected DNA bands were cut out of agarose gels with surgical blades. The excised gel was as small as possible to avoid diluting the recovered DNA.

DNA purification from agarose gels

To purify the selected DNA bands from agarose gels, a 0.5 mL Eppendorf tube punctured in the center bottom with a syringe needle and packed with a small tassel of cotton (one quarter volume of tube) was used. The cotton was embedded with 0.5 $\times$ TAE and squeezed until no liquid came out. This device was placed into another 1.5 mL Eppendorf tube. The piece of DNA-containing gel was laid on the cotton filter in the tube and then centrifuged at 5200 $\times g$ for 5 min at room temperature. The DNA in the collected liquid was quantified spectrophotometrically by a standard procedure and used for subsequent experiments.

For comparison of effectiveness, commercial commonly used kits QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany), Wizard SV Gel and PCR Clean Up System (Promega, Madison, WI, USA), and GENECLEAN® II kit (MP BIOMEDICALS, Solon, OH, USA) were employed, according to manufacturer's indications. All procedures were carried out in triplicate. The extracted DNAs were quantified using a spectrophotometer at 260 nm and analyzed on 1% agarose gel.

Sequencing of purified fragments

All DNAs were sent to LANBAMA facilities (IPICYT, San Luis Potosí, MX) for sequencing. Quality and size of sequences were examined, respectively, with the programs UGENE (Okonechnikov et al. 2012) and Geneious version 8.1.5 software (Kearse et al. 2012). Phred quality scores were automatically recorded by the sequencer (Applied Biosystems® Sanger Sequencing 3500 Series Genetic Analyzers); Unipro UGENE software was used to export

Phred scores. Statistical analyses were performed using STATGRAPHICS® Centurion XVI software (Statgraphics-Centurion 2009) using one-way ANOVA with 95% level of confidence; a p value <0.05 was considered to be statistically significant. To define the sequence size, each result was aligned with sequences of GenBank accession XM_007929769. The nucleotides in the edited sequences were counted and recorded.

Nested PCR

To test suitability of in-home-purified DNA for PCR reaction, a nested PCR using the primers 5'-CTCCAAA CAAGCAGCTCTGC-3' (forward) and 5'-AGTTCTGGC TGAGGTAGTAC-3' (reverse) resulting in a PCR product of 234 pb was used (Kantun-Moreno et al. 2013). DNAs obtained with commercial kits were also used (20 ng each). All evaluations were conducted in triplicate.

Results

Using the in-house protocol described herein, the collected volume is about 30–45 μL , depending on the amount of agarose gel excised. Recovery of DNA from gel is very fast, requiring only 5 min. The yield was similar to that obtained from commercial kits (Fig. 1; Table 1). The

concentration was 20–26% lower than DNA concentration in samples purified with commercial kits; likewise, yield was 5–20% lower in comparison with commercial kits. However, these yields were satisfactory for downstream applications such as sequencing (Figs. 2, 3) and PCR reamplification (Fig. 4).

DNAs were sequenced with a Sanger Sequencing Analyzer. The length of the sequence for DNA recovered with the in-house column was 308 bp, 98% of the full template (312 bp) and similar to those lengths obtained for DNAs recovered by commercial methods (Fig. 3, Table 1). Phred index is a parameter measuring DNA sequence quality, where the maximum quality has a value of 62. Phred index for the sequence of the DNA obtained with in-house protocol was 57, close to Phred scores (56–59) of DNA samples recovered from gel with commercial kits (Fig. 2; Table 1). Statistical analysis indicated no significant difference among methods compared here. Therefore, length of the sequence and Phred index confirm that this in-house procedure results in purity and quantity of DNA compatible with DNA sequencing.

To test if DNA purified from gel, using home-column, is suitable for PCR, nested reaction was performed with primers designed internally on the first DNA template, with a nested product of 234 bp. Reamplification was positive and abundance was sufficient, albeit a little less than reamplifications on templates recovered from gel with commercial kits (Fig. 4).

Discussion

The in-house protocol presented here is suitable for DNA sequencing. Many other fast protocols to recover DNA from gel have been reported. Almost all include a last step of precipitation with isopropanol, ethanol, or ethanol/3 M sodium or ammonium acetate precipitation of DNA (Xia et al. 2011; Sun et al. 2012; Kim and Morrison 2009; Li and Ownby 1993; Grey and Brendel 1992); a Savant SpeedVac concentrator (freeze-dried) (Kurien et al. 2001) can also be used. Most of them use TBE buffer to prepare and run the gel (Li and Ownby 1993; Sun et al. 2012; Xia et al. 2011). Borate is known to bind DNA and form complexes (Stellwagen et al. 2000), and it is recognized as an enzyme inhibitor in DNA manipulations (Sambrook and Russell 2001), and thus, the elimination of TBE buffer before the final use of DNA is mandatory. One clear advantage of the current protocol is that no extra steps for DNA purification are required, and DNA can be used directly in $0.5\times$ TAE. This saves time and prevents additional losses of DNA in further purification steps. In our experience, ethanol/acetate salt precipitation usually recovers 54% of the DNA in solution (not shown).

Fig. 1 Comparison of DNA recovery from gel between different methods. **a** Initial DNA, before purification. **b** DNA after purification. In both gels, 4 μL were loaded per lane. DNA markers 1 kb plus (M1) and GeneRuler 1 Kb (M2) were included to estimate size and amount. Lanes 1–3, DNA purified with Geneclean kit; 4–6 purified with Wizard kit; 7–9 purified with Qiagen kit; 10–12 purified with in-home columns and procedure described here

Table 1 Comparison of yield and quality of DNAs recovered with different methods

	In-house columns	Qiagen	Wizard	GenClean
Purification cost for column (US Dollar)	0.5	2.9	2.3	1.3
Time for purification (minutes)	5	30	25	30
Initial DNA concentration (ng/μL)	90	90	90	90
Final DNA concentration obtained (ng/μL)	40.5	49.6	52.3	53.8
Initial total DNA (ng)	2250	2250	2250	2250
Volume obtained (μL)	35	30	30	30
Total DNA after purification (ng)	1417.5	1488	1569	1614
Yield (%)	63	66	69.7	71.7
Sequencing results				
Read length in sequencing (DNA template 312 bp)	308	287	302	293
Quality (Phred grades)	57.08	56.59	59.92	59.63

Fig. 2 PCR amplification test for purified DNAs. Test was performed by Nested PCR on 312 bp template, using internal primers which amplify product with 234 pb. *Lanes 1–3* DNA template purified with Geneclean kit; *lanes 4–6* DNA template purified with Wizard kit; *lanes 7–9* DNA template purified with Qiagen kit; *lanes 10–12* DNA template purified with in-house columns **Fig. 3** Quality comparison of DNA sequences purified with different methods. Phred quality score outputs from sequencer were used. For statistical analysis, ANOVA was performed, with a level of 95% confidence and p value <0.05

Concentration of the collected DNA is only needed when the desired DNA is low in the sample loaded on the gel for electrophoresis. Ohyama (1993) published a method for the

recovery of DNA which can be directly used for enzymatic digestion and ligation; however, it is more laborious given that his protocol requires the construction of an electroelution device using a platinum wire electrode.

Many fast methods for recovering DNA from gel have shown to be suitable for PCR (Sun et al. 2012; Watanabe 1999), Nick translation and random priming labeling of probes (Matitashvili and Zavizion 1997), enzymatic restriction and cloning (Kim and Morrison 2009; Kurien et al. 2001; Ohyama 1993; Watanabe 1999; Xia et al. 2011), transformation (Kurien et al. 2001), or transfection (Kim and Morrison 2009), but none of them allows DNA sequencing. Sequencing facilities usually recommend a small list of options for DNA purification kits. In our experience, the most commonly recommended kit is QIAGEN (QIAquick Gel Extraction Kit) (Davis Sequencing, Inc., USA; LAMBAMA, IPICYT, Mexico; MACROGEN, Korea).

Frequently, financial constraints push researchers to look for methods which achieve quality of samples for their particular goals, at the lowest possible cost. In our case, we need to sequence many DNA samples and the procedure described here meets both criteria, it is cheap and the DNA preparation works in sequencing. This is the first in-house protocol which has shown to be suitable for this application, and its speed and easiness make it amenable for massive sequencing. To date, our lab has sequenced 180 DNA samples in an ongoing project involving molecular phylogeny. The sizes of molecular markers isolated with this method have ranged between 144 and 1880 bp and effectiveness in further sequencing has been similar to that obtained in our past experience with commercial kits. This protocol could be used in population studies, molecular phylogeny, systematics, the development of SCAR (Sequence Characterized Amplified Region) markers, and massive molecular identification of biological collections.

Fig. 4 Graphical representation of multi-alignment by Geneious version 8.1.5 software of DNA sequences obtained by each purification method. Solid grey bars represent the size of sequences obtained in each case. Colours at beginning and ends inside the grey

bars correspond to mistakes at sequencings. The two upper bars correspond to consensus sequence, the green bar to identical regions, and the coloured bars represent codes as follows: red adenine, blue cytosine, green thymine, and yellow guanine

Other papers have published the use of spin in-house columns as used here (Li and Ownby 1993; Sun et al. 2012; Watanabe 1999), but they prepare and run the gel with TBE and need to precipitate DNA before it is used downstream (for digestion and cloning in the three reports). Although our proposed change was simple (electrophoresis on 0.65% agarose gel and the use of $0.5 \times$ TAE instead of $1 \times$ TBE), the result was remarkable. The merit of this protocol is its simplicity, low cost, and suitability for multiple purposes; including, for the first time in an in-house protocol, DNA sequencing. It is important to emphasize that this protocol works successfully in downstream applications when the recovered DNA has high concentration, which avoid the use of a high volume of eluate. Higher concentrations of components of TAE can interfere in some downstream reactions: EDTA (>10 mM) inhibits the activity of alkaline phosphatase, ligases (Sambrook and Russell 2001), and S1 nuclease (Rittié and Perbal 2008); and acetate (>5 mM) can inhibit PCR reactions (van Pelt-Verkuil et al. 2008). Although sufficient for our purpose, nested PCR reactions shown in lanes 11 and 12 in Fig. 2 were less efficient than those using DNA templates recovered by commercial kits that eliminate TAE buffer. More sensitive applications such as NGS library preparation were not tested here, and therefore, caution is advised as TAE can reduce the effectiveness of high-throughput sequencing.

Since the protocol starts after electrophoresis of DNA fragments, i.e., PCR products, it is obviously universally applicable on DNAs from any living being. In addition to the results presented in this work, we have used this method to prepare bacterial, fungal, or plant DNAs. In addition, this method has enabled us to obtain DNA inserts for ligation in an expression vector or ligation in TOPO-TA (Invitrogen). DNA templates were successfully used in those ligations with no need of previous precipitation (data not shown).

Tropical and subtropical developing countries are the most biodiverse, but the characterization of their ecosystems is limited by the cost of required facilities and kits. The characteristics of this protocol are useful to enable developing countries to study and characterize their own biodiversity.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

References

- Grey M, Brendel M (1992) Rapid and simple isolation of DNA from agarose gels. *Curr Genet* 22(1):83–84
- Kantun-Moreno N, Vazquez-Euan R, Tzecz-Sima M, Peraza-Echeverria L, Grijalva-Arango R, Rodriguez-Garcia C, James AC, Ramirez-Prado J, Islas-Flores I, Canto-Canche B (2013) Genome-wide in silico identification of GPI proteins in *Mycosphaerella fijiensis* and transcriptional analysis of two GPI-anchored beta-1,3-glucanotransferases. *Mycologia* 105(2):285–296. doi:10.3852/12-103
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12):1647–1649. doi:10.1093/bioinformatics/bts199
- Kim Y-C, Morrison SL (2009) A rapid and economic in-house DNA purification method using glass syringe filters. *PLoS ONE* 4(11):e7750. doi:10.1371/journal.pone.0007750

- Kurien BT, Scofield RH (2002) Extraction of nucleic acid fragments from gels. *Anal Biochem* 302(1):1–9. doi:[10.1006/abio.2001.5526](https://doi.org/10.1006/abio.2001.5526)
- Kurien BT, Kaufman KM, Harley JB, Scofield RH (2001) Pellet pestle homogenization of agarose gel slices at 45 degrees C for deoxyribonucleic acid extraction. *Anal Biochem* 296(2):162–166. doi:[10.1006/abio.2001.5299](https://doi.org/10.1006/abio.2001.5299)
- Li Q, Ownby CL (1993) A rapid method for extraction of DNA from agarose gels using a syringe. *Biotechniques* 15(6):976–978
- Matitashvili E, Zavizion B (1997) One-tube extraction of DNA or RNA from agarose gel. *Anal Biochem* 246(2):260–262. doi:[10.1006/abio.1997.2027](https://doi.org/10.1006/abio.1997.2027)
- Ohyama T (1993) An ultrarapid method for the recovery of DNA from gels. *Anal Biochem* 208(1):209–211. doi:[10.1006/abio.1993.1030](https://doi.org/10.1006/abio.1993.1030)
- Okonechnikov K, Golosova O, Fursov M, team tU (2012) Unipro UGENE: a unified bioinformatics toolkit. *Bioinformatics* 28(8):1166–1167. doi:[10.1093/bioinformatics/bts091](https://doi.org/10.1093/bioinformatics/bts091)
- Rittié L, Perbal B (2008) Enzymes used in molecular biology: a useful guide. *J Cell Commun Signal* 2(1–2):25–45. doi:[10.1007/s12079-008-0026-2](https://doi.org/10.1007/s12079-008-0026-2)
- Sambrook J, Russell DW (2001) *Molecular cloning: a laboratory manual*, vol 1. Protocol 1. Agarose Gel Electrophoresis, 3rd edn. Cold Spring Harbor Laboratory Press, New York
- Statgraphics-Centurion (2009) Versión XVI. StatPoint Technologies, Inc., Warrenton
- Stellwagen NC, Bossi A, Gelfi C, Righetti PG (2000) DNA and buffers: are there any noninteracting, neutral pH buffers? *Anal Biochem* 287(1):167–175. doi:[10.1006/abio.2000.4848](https://doi.org/10.1006/abio.2000.4848)
- Sun Y, Sriramajayam K, Luo D, Liao DJ (2012) A quick, cost-free method of purification of DNA fragments from agarose gel. *J Cancer* 3:93–95. doi:[10.7150/jca.4163](https://doi.org/10.7150/jca.4163)
- Van Pelt-Verkuil E, van Belkum A, Hays JP (2008) Deoxynucleotide triphosphates and buffer components (chapter 6). *Principles and technical aspects of PCR amplification*, 1st edn. Springer, Netherlands, pp 91–101
- Watanabe M (1999) Rapid and inexpensive recovery method of DNA fragments from agarose and polyacrylamide gels by a cotton-wool column tube. *Nucleic Acids Symp Ser* 42(1):101–102. doi:[10.1093/nass/42.1.101](https://doi.org/10.1093/nass/42.1.101)
- Xia H, Tan Z, Qiao J, Liang C (2011) Recovery of DNA from agarose gel by trap method. *Afr J Biotech* 10(50):10280–10286