Stacking up gel extraction kits

Introduction

Efficient extraction of PCR products and DNA fragments from agarose gels is an integral part of a number of downstream applications including DNA sequencing, restriction digestion, cloning, labeling, ligation, *in vitro* transcription, and *in situ* hybridization. Common extraction workflows are based on the ability of DNA to bind to silica membranes in the presence of chaotropic salts. This process efficiently removes primers, unincorporated dNTPs, enzymes, and salts from both PCR and restriction digests. But, do amplicon or DNA fragment sizes affect DNA yields in these protocols? Are there any real differences between kits when they are all based on the same principle? Here we compare the performance of five commercially available gel extraction kits.

Materials and methods

Recovery rates and DNA purity were determined for five commercially available DNA extraction kits. Extractions were performed strictly according to the manufacturers' protocols.

For gel extraction of PCR products, two amplicon sizes were tested: 364 and 1,113 bp. Amplicons were generated in separate PCR runs, and 25 µL samples were run on 1% agarose TAE gels. The PCR product samples were allowed to migrate for 45 minutes in a constant 95-volt electric field, and then visualized for excision by UV transillumination after staining with ethidium bromide. DNA was then extracted from the agarose gel slices using the clean-up kits, in triplicate. DNA yield and A_{260}/A_{280} absorbance ratios were measured using a Thermo Scientific™ NanoDrop™ 8000 spectrophotometer.

Plasmid DNA (pDNA) fragments were generated by restriction digest of a 6,600 bp luciferase plasmid using both HindIII and BamHI restriction enzymes (NEB), for 1 hour at 37°C, resulting in the generation of 4,646 bp and 1,954 bp pDNA fragments. One microgram of pDNA was digested and loaded per well of a 1% agarose TAE gel, and fragments were separated by applying a 100-volt electric field for 45 minutes. The pDNA fragments were separated by gel electrophoresis and excised after visualization as stated above. DNA was then extracted from the agarose gel slices using the indicated clean-up kits, in triplicate. Final pDNA samples were analyzed as stated above. The following gel extraction kits were compared for purification of PCR products and restriction digest fragments:

- Thermo Scientific™ GeneJET™ Gel Extraction Kit (Thermo Fisher Scientific, Cat. No. K0691, Lot. No. 00733508)
- Invitrogen™ PureLink™ Quick Gel Extraction Kit (Thermo Fisher Scientific, Cat. No. K210012, Lot. No. 00736582)
- QIAquick™ Gel Extraction Kit (Qiagen, Cat. No. 28704, Lot. No. 163011203)
- NucleoSpin™ Gel and PCR Clean-up Kit (Macherey-Nagel, Cat. No. 740609.50, Lot. No. 1903/001)
- Wizard™ SV Gel and PCR Clean-Up System (Promega, Cat. No. A9281, Lot. No. 0000363258)

All kits were ranked from 1 (least favorable) to 4 (most favorable) for ease of use based on the number of steps in the protocol, total time required, volume of binding buffer required, and additional steps needed outside of the protocol. Table 1 summarizes of the number of steps, protocol duration for processing 6 samples, relative ease of use, and cost per sample for each kit. Samples were generated following the "purify DNA using a centrifuge" protocol for each.

Table 1. Comparative analysis of the gel extraction kits.

Brand	Thermo Scientific	Qiagen	Macherey-Nagel	Promega	Invitrogen
Kit and no. of preps	GeneJET Gel Extraction Kit (50)	QIAquick Gel Extraction Kit (50)	NucleoSpin Gel and PCR Clean-up Kit (50)	Wizard SV Gel and PCR Clean-up Kit (50)	PureLink Quick Gel Extraction Kit (50)
Column binding capacity	25 µg	10 µg	15 µg	40 µg	15 µg
Column type	Silica	Silica	Silica	Silica	Silica
dsDNA size	25 bp–20 kb	70 bp–10 kb	50 bp–20 kb	100 bp–10 kb	40 bp–10 kb
Number of steps	10	8	8	9	11
Total time for 6 samples	26 min	26 min	32 min	28 min	28 min
Potential steps outside of protocol	2	1	1	None	1
Ratio of binding buffer to gel weight	1:1	3:1	2:1	1:1	3:1
Ease of use on a scale of 1 (least favorable) to 4 (most favorable)	4	4	3	3	3
Cost per rxn (USD)	1.68	2.44	1.74	2.06	2.14

Results

Protocol observations

Overall, protocols for all five kits are similar but there are slight differences that do affect the experience. The QIAquick and PureLink kits include an optional isopropanol (IPA) step for increased yield. The NucleoSpin kit has 30 sec spins for the first 3 steps and then switches to 1 min spins for the last 3 steps. The microcentrifuge used did not have a 30 sec spin option, so it was necessary to either hold the short spin button or to stop the spin after 30 sec of a 60 sec spin. The QIAquick kit leaked wash solution during the wash step, which removed tube labels because of the high ethanol concentration. The Wizard and NucleoSpin kits had multiple wash steps and the Wizard kit was the only kit with a 5 min spin. For the QIAquick and PureLink kits, if a large piece of agarose was used in the extraction (<250 g), an additional step was needed to load the entire sample onto the column because of the 3:1 binding buffer to gel weight ratio.

The GeneJET Gel Extraction Kit contains silica mini spin columns that can be used for purification of DNA fragments from 25 bp to 20 kb in size and has a recovery rate of up to 95% in the 100 bp–10 kb range. Each GeneJET purification column has a DNA binding capacity of up to 25 µg and can process up to 1 g of agarose. The PureLink Quick Gel Extraction Kit contains silica mini spin columns that can be used for purification of DNA fragments from 40 bp to 10 kb with up to 95% recovery. Each PureLink purification column has a DNA binding capacity of up to 15 µg. The entire purification procedure takes about 25 min for a single sample.

PCR product gel extraction

The 364 and 1,113 bp amplicons were processed separately using the extraction kits (25 µL each, in triplicate). Figure 1 shows DNA yield and A_{260}/A_{280} ratio (as measured by the NanoDrop spectrophotometer) as well as analysis by 1% agarose TAE gel.

The yield of recovered DNA for both amplicons was highest with the PureLink kit. The NucleoSpin Kit had a similar DNA yield for the 1,113 bp amplicon but only half the yield for the 364 bp amplicon compared to the PureLink kit. DNA purity as determined by the NanoDrop spectrophotometer (A_{260}/A_{280}) and agarose gel electrophoresis was high and comparable for all five kits except for the QIAquick and Wizard SV kits with the 364 bp amplicon.

Restriction digest fragment extraction

A 6,600 bp luciferase plasmid (1 µg pDNA) was digested with HindIII and BamHI restriction enzymes (NEB). This digestion generated two DNA fragments, 4,646 bp and 1,954 bp, which were separated on a 1% agarose gel. The two DNA fragments were then excised and extracted separately using the clean-up kits. Figure 2 shows DNA yield and A_{260}/A_{280} ratio (as measured by the NanoDrop spectrophotometer) as well as analysis by 1% agarose TAE gel.

The yield of recovered DNA fragments was highest for the PureLink Kit. DNA purity as analyzed by the NanoDrop spectrophotometer (A_{260}/A_{280}) was more variable for these DNA fragments when compared to the PCR amplicons (shown in Figure 1). This may be due to the different nature of contaminants in the restriction digestion reaction compared to PCR amplification. DNA integrity analysis by gel electrophoresis was comparable for all five kits. However, the PureLink protocol was 2 min slower than the GeneJET and NucleoSpin Kits.

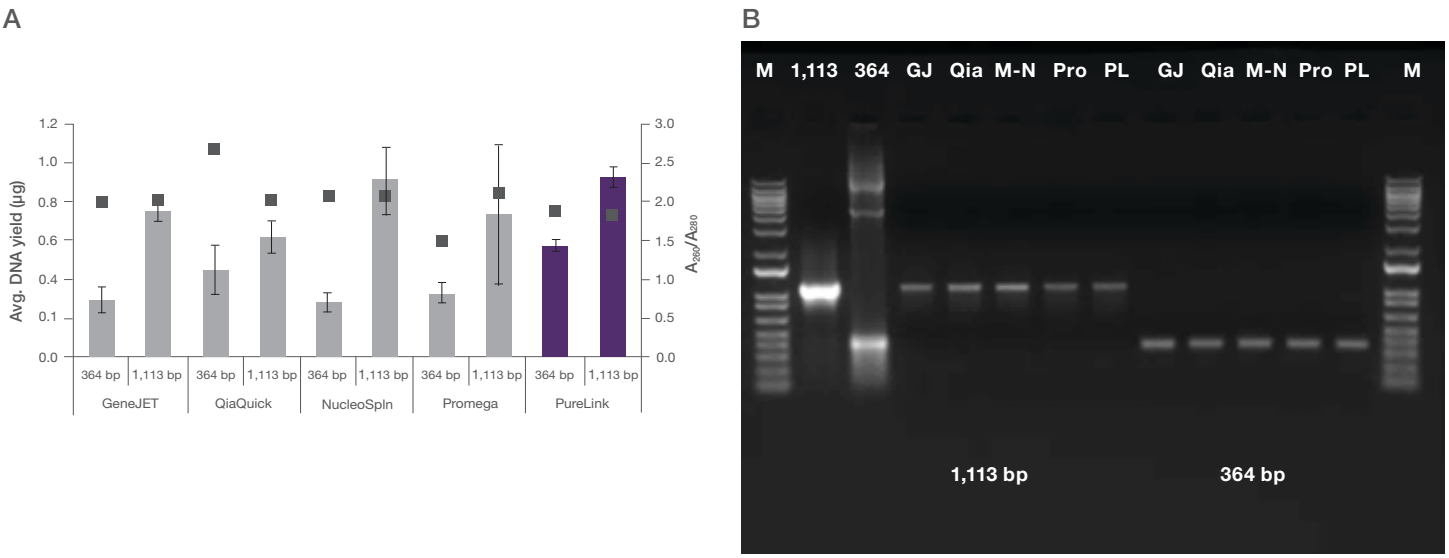

Figure 1. Gel extraction of PCR products. (A) DNA yield (bars) and purity (■), and **(B)** gel analysis of restriction digest fragments using five different gel extraction kits. For gel analysis, 50 ng of input was used for both 364 bp and 1,113 bp DNA. The Invitrogen™ E-Gel™ 1 kb Plus DNA Ladder was used as the size standard.

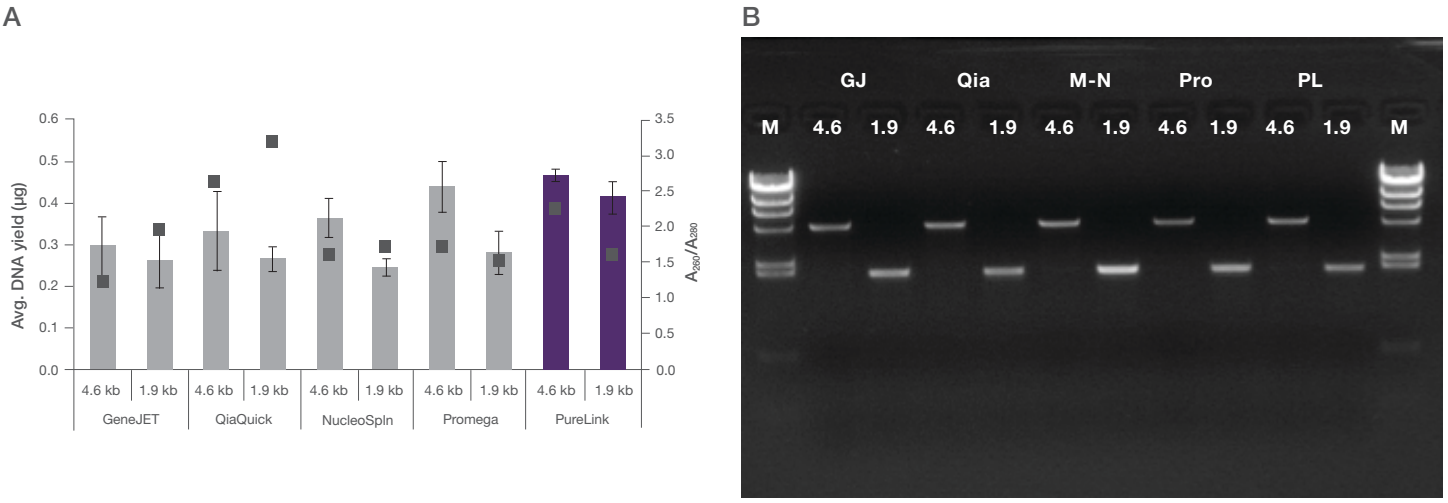

Figure 2. Gel extraction of restriction digest fragments. (A) DNA yield (bars) and purity (■), and **(B)** gel analysis of restriction digest fragments using five different gel extraction kits. For gel analysis, 50 ng of input was used for 4,646 bp and 1,954 bp pDNA fragments. The E-Gel 1 kb Plus DNA Ladder was used as the size standard.

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Conclusions

Our testing demonstrated that the two top options for gel extraction clean-up of DNA samples are:

- The PureLink Quick Gel Extraction Kit is the best-performing kit identified in this study; it delivers the highest yield for both small and large PCR amplicons as well small and large restriction digest fragments.
- The GeneJET Gel Extraction Kit offers competitive performance at a value price with an easy-to-use protocol.

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