

Dial up your cleanup

Increase efficiency with GeneJET, PureLink, and ChargeSwitch kits

Introduction

Efficient purification of PCR products is critical to the success of downstream applications such as DNA sequencing, restriction digestion, cloning, labeling, ligation, *in vitro* transcription, blotting, and *in situ* hybridization. Choosing the right technology, such as silica columns or magnetic beads, is usually a point of confusion, as is the choice of the kits, based on the size of fragments to be recovered as well as the yield of recovery. Here we provide detailed information on several key PCR cleanup kits and highlight their performance in comparison to other commercially available kits, to make your choice easier as well as help you get the most out of your cleanup reactions.

The two main PCR cleanup approaches utilize two technologies—silica spin columns, which are convenient for low-throughput reactions, and magnetic beads, which are designed for higher-throughput applications. Magnetic beads can be used manually or in automated formats with instruments like the Thermo Scientific™ KingFisher™ system. Both technologies allow users to efficiently remove primers, unincorporated dNTPs, enzymes, and salts from PCR mixtures. Selecting the best kit depends on factors such as amplicon size, throughput (number of samples to be processed at a time), and desired DNA yield.

Materials and methods

We tested 3 different types of kits to determine the optimal one based on the size of recovered DNA fragments as well as recovery. Isolations were performed according to the manufacturers' instructions.

For all the reactions, 364 bp and 1,136 bp amplicons were generated in separate PCR reactions, and 50 µL samples were processed using the cleanup kits, in triplicate. DNA yield and purity (A_{260}/A_{280}) were measured using a Thermo Scientific™ NanoDrop™ spectrophotometer, and the fragments were also analyzed using 1% Invitrogen™ E-Gel™ agarose gels.

The following **mini-column** kits were compared side by side for purification of the PCR products:

- Thermo Scientific™ GeneJET™ PCR Purification Kit (Thermo Fisher Scientific, Cat. No. K0701, Lot. No. 00658481)
- QIAquick™ PCR Purification Kit (Qiagen, Cat. No. 28104, Lot. No. 160028882)
- NucleoSpin™ Gel and PCR Clean-up kit (Macherey-Nagel, Cat. No. 740609.10, Lot. No. 1802/009)

The following **micro-column** kits were compared side by side for purification of the PCR products:

- Invitrogen™ PureLink™ PCR Micro Kit (Thermo Fisher Scientific, Cat. No. K310050, Lot. No. 2005524)
- MinElute™ PCR Purification Kit (Qiagen, Cat. No. 28004, Lot. No. 160034624)

The following **magnetic bead-based** kits were compared side by side for purification of the PCR products:

- Invitrogen™ ChargeSwitch™ PCR Clean-Up Kit (Thermo Fisher Scientific, Cat. No. CS12000, Lot. No. 1935125)
- MagAttract™ PowerClean DNA Kit (384) (Qiagen, Cat. No. 27900-4-KF, Lot. No. 160034268)
- Agencourt AMPure XP kit (Beckman Coulter, Cat. No. A63880, Lot. No. 17220600)

All kits were also ranked from 1 (lowest) to 5 (highest) for ease of use based on the number of steps in the protocol, total time required, volume of binding buffer required, confidence regarding cross-contamination, and additional steps outside of the protocol.

Table 1 shows the summary of number of steps, protocol duration for processing 4 samples, relative ease of use, and cost per sample, for each kit.

Results

Mini column-based PCR cleanup

The GeneJET PCR Purification Kit, which contains silica mini spin columns, can be used for purification of DNA fragments from 25 bp to 20 kb with 90–100% recovery. The GeneJET purification columns have a high binding capacity of up to 25 µg of DNA. The entire purification procedure takes only about 5 minutes for a single sample.

The 364 bp and 1,136 bp amplicons were processed separately using the mini-column cleanup kits (50 µL each, in triplicate). Figure 1 shows DNA yield and A_{260}/A_{280} (as measured by the NanoDrop spectrophotometer) as well as analysis by 1% E-Gel agarose gel.

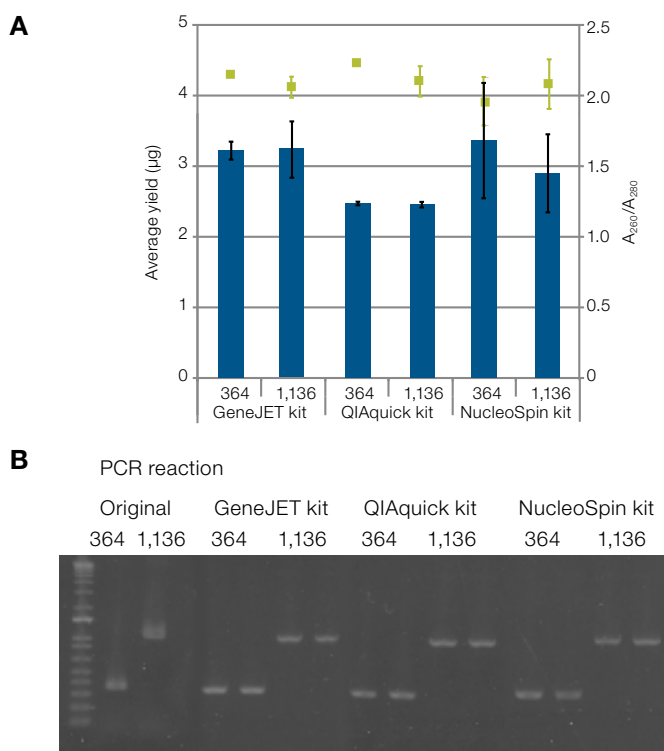


Figure 1. Mini column-based PCR cleanup. (A) DNA yield (blue) and purity (green), and (B) gel analysis of PCR products using 3 different PCR cleanup kits. For gel analysis, 200 ng of input amount was used for 364 bp and 1,136 bp DNA. The Invitrogen™ E-Gel™ 1 Kb Plus DNA Ladder was used as the size standard.

Table 1. Comparative analysis of the PCR cleanup kits.

Method	Mini column			Micro column		Magnetic beads		
	Qiagen	Macherey-Nagel	Thermo Fisher Scientific	Qiagen	Thermo Fisher Scientific	Qiagen	Beckman Coulter	Thermo Fisher Scientific
Brand	Qiagen	Macherey-Nagel	Thermo Fisher Scientific	Qiagen	Thermo Fisher Scientific	Qiagen	Beckman Coulter	Thermo Fisher Scientific
Kit and reaction size	QIAquick PCR purification (50)	NucleoSpin Gel and PCR Clean-up (50)	GeneJET PCR Purification Kit (50)	MinElute PCR Purification Kit (50)	PureLink PCR Micro Kit (50)	MagAttract PowerClean DNA Kit (384)	Agencourt AMPure XP kit (50+)	ChargeSwitch PCR Clean-Up Kit (100)
Number of steps*	7	7	5	8	10	24, 13**	7	20
Total time for 4 samples	15 min	14 min	13 min	15 min	15 min	1 hr 22 min	36 min	25 min
Potential steps outside of protocol	2	1	2	1	None	1	None	None
Ratio of binding buffer to sample volume	5:1	2:1	1:1	5:1	4:1	1:1.2	1.8:1	1:1
Ease of use on a scale of 1 (least favorable) to 5 (most favorable)	3	3	4	3	4	1	4	3
Cost (per rxn)†	\$2.34	\$2.07	\$1.63	\$2.68	\$2.38	\$3.42	\$6.40	\$2.12

* Does not include "Before you begin" steps.

** Manual and automated steps, respectively.

† Price per prep based on list price.

The yield of recovered DNA was higher with GeneJET and NucleoSpin kits than with the QIAquick kit. DNA purity as determined by the NanoDrop spectrophotometer (A_{260}/A_{280}) and agarose gel electrophoresis was high, and comparable for all three kits. The GeneJET kit has the fastest protocol, with fewest steps, as well as the lowest price per prep.

Micro column–based PCR cleanup

The PureLink PCR Micro Kit, which contains silica micro spin columns, is ideal for purifying and concentrating DNA from low-yielding PCR or restriction digestion reactions. The kit provides a simple and rapid method for purifying and concentrating DNA fragments ranging in size from 100 bp to 12 kb. The unique micro-nozzle design allows up to 95% recovery of DNA fragments with as little as 5 μ L of elution volume. Using this kit, the efficiency of removing primers, dNTPs, enzymes, and salts exceeds 98.5%. The PureLink purification micro columns have a binding capacity of up to 5 μ g of double-stranded DNA. The entire purification procedure takes about 6 minutes for a single sample.

The 364 bp and 1,136 bp amplicons were processed using the micro column–based cleanup kits (50 μ L each, in triplicate). Figure 2 shows DNA yield and A_{260}/A_{280} (as measured by the NanoDrop spectrophotometer) as well as analysis by 1% E-Gel agarose gel.

The PureLink kit generated moderately lower DNA yield compared to the MinElute kit; however, purity analyzed by the NanoDrop spectrophotometer (A_{260}/A_{280}) was higher for PureLink samples. DNA purity and integrity as analyzed by agarose gel electrophoresis were high and comparable for the two kits. Duration of the protocol was comparable for the PureLink and MinElute kits. The PureLink PCR Micro Kit has a slightly lower price per prep.

Magnetic bead–based PCR cleanup

The ChargeSwitch PCR Clean-Up Kit, based on magnetic beads, is the method of choice for high-throughput sample processing and is scalable for use on liquid-handling robots. The kit is designed for purification of DNA fragments ranging in size from 90 bp to 40 kb. The ChargeSwitch magnetic beads have a binding capacity of 25 μ g DNA per 1 mg of beads. The entire purification procedure takes about 10 minutes for a single sample.

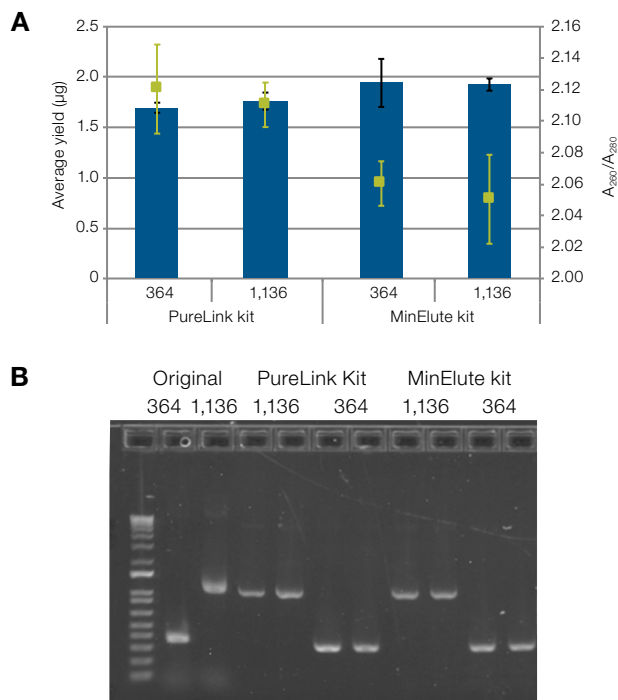


Figure 2. Micro column–based PCR cleanup. (A) DNA yield (blue) and purity (green) were analyzed using the NanoDrop spectrophotometer. (B) Gel analysis of PCR products following purification with 2 different PCR cleanup kits. For gel analysis, 200 ng of input amount was used for 364 bp and 1,136 bp DNA. The E-Gel 1 Kb Plus DNA Ladder was used as the size standard.

The 364 bp and 1,136 bp amplicons were processed using the magnetic bead–based cleanup kits (50 μ L each, in triplicate). Figure 3 shows DNA yield and A_{260}/A_{280} (as measured by the NanoDrop spectrophotometer) as well as analysis by 1% E-Gel agarose gel.

The ChargeSwitch kit recovered DNA at higher yields and purity (A_{260}/A_{280}), than the MagAttract and Agencourt AMPure kits. DNA purity as analyzed by agarose gel electrophoresis was high, and was comparable for all three kits. The ChargeSwitch kit provided the fastest protocol as well as the lowest price per prep.

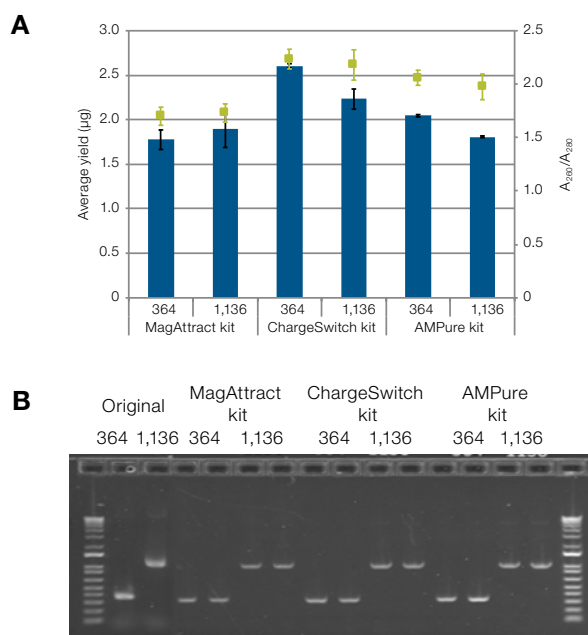


Figure 3. Magnetic bead–based PCR cleanup. (A) DNA yield (blue) and purity (green), and (B) gel analysis of PCR products using 3 different PCR cleanup kits. For gel analysis, 200 ng of input amount was used for 364 bp and 1,136 bp DNA. The E-Gel 1 Kb Plus DNA Ladder was used as the size standard.

Conclusions

There are several options for PCR cleanup.

- The GeneJET PCR Purification Kit is the best-performing spin column–based kit tested in this study (mini-column binding capacity: up to 25 μ g); for low to medium sample throughput (1–24 samples at a time).
- The PureLink PCR Micro Kit is a competitive spin column–based kit for low-yielding or low-volume PCR reaction cleanup (micro-column DNA binding capacity: up to 5 μ g); for low to medium sample throughput (1–24 samples at a time).
- The ChargeSwitch PCR Clean-Up Kit is the best-performing magnetic bead–based kit tested in this study; for medium to high sample throughput (up to 96 samples at a time).

Find more at [thermofisher.com/cleanup](https://www.thermofisher.com/cleanup)

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