

PCR Clean-Up

Overview

Downstream applications such as T-Vector cloning, restriction digestion and direct sequencing benefit from clean-up of PCR amplimers. T-vector cloning has a tremendous dependence upon PCR product purity. Although unpurified amplification reactions can be used for T-vector cloning, more screening of the resulting colonies is generally necessary to find the specific clone of interest. This is because unpurified PCR amplification reactions can contain primer-dimers and nonspecific amplimers in much higher molar quantities than the PCR product of interest. These nonspecific products compete for ligation with the amplimer of interest. Typically, an experiment with unpurified products will produce a large number of colonies, many of which contain small, nonspecific PCR products as inserts. Thus, the efficiency of the cloning experiment is reduced. In one case the percentage of colonies containing the correct insert was 67% using unpurified PCR products. In contrast, when purified PCR products were used >90% of colonies contained the correct insert (1).

Gel Isolation

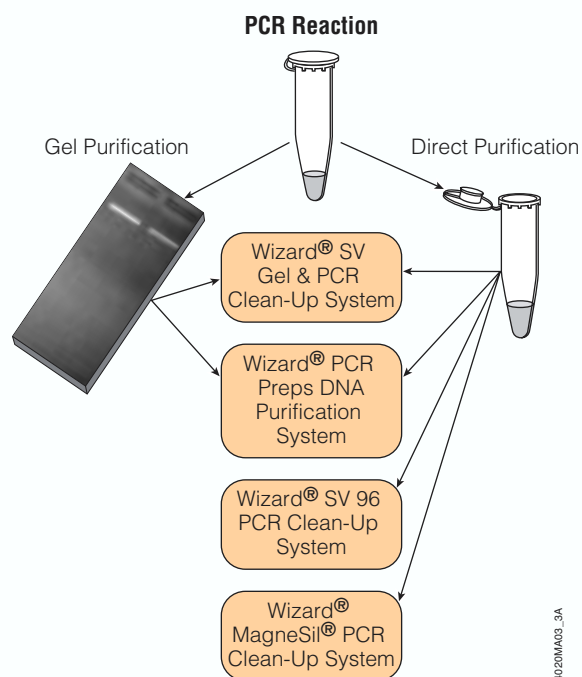
Gel isolation is the most effective way to isolate the PCR product you need for your downstream applications. Agarose gel electrophoresis allows you to separate the desired amplimer from any nonspecific bands, primers and primer-dimers. You visualize your gel quickly on a UV lightbox, using 10mg wavelength UV light, cut out the band of interest and then purify the product. Gel isolation is typically used if the downstream application is cloning and additional bands, representing nonspecific amplimers or primer-dimer, are present on the gel. The agarose is melted and combined with a chaotrope like guanidine, and the DNA is then bound to silica. Agarose and guanidine are quickly and efficiently removed by an alcohol wash, and the purified DNA is eluted in water.

Direct Isolation

Many downstream applications such as DNA sequencing and single nucleotide polymorphism (SNP) analysis require that salts, dNTPs, proteins and primers be removed from the amplification product as they can interfere with further enzymatic reactions. Many researchers use a simple ethanol precipitation prior to sequencing. This removes most dNTPs and salts but leaves behind protein and may also leave primers. Phenol:chloroform extraction can remove protein contaminants but recovery rates can be low, and the use of organics is undesirable. Use of silica technology simplifies the whole process. The amplification reaction products are bound to silica in the presence of a chaotrope like guanidine. Salts, dNTPs and primers pass by the silica. Primer-dimers and nonspecific amplimers smaller than 70bp are not bound efficiently. A simple alcohol wash removes the guanidine and protein while material >70bp is retained. The purified DNA is eluted in water or another low-ionic strength solution. The success of the downstream application is dependent upon the specificity of the amplification reaction, as any nonspecific amplimers will be copurified with the specific product of interest.

Reference

1. Buros, M. and Betz, N. (2002) Removal of ethidium bromide and calf intestinal alkaline phosphatase using the Wizard® SV Gel and PCR Clean-Up System. *eNotes*: www.promega.com/enotes/applications/ap0045_tabs.htm



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PCR Clean-Up

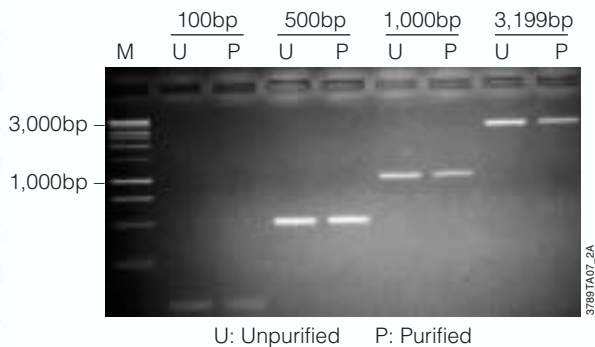
Wizard® SV Gel and PCR Clean-Up System

The Wizard SV Gel and PCR Clean-Up System is designed to extract and purify DNA fragments directly from PCR reactions or from agarose gels. Fragments of 100bp–10kb can be recovered from standard or low-melt agarose gels in either Tris acetate (TAE) or Tris borate (TBE) buffer. Up to 95% recovery is achieved, depending upon the DNA fragment size. This membrane-based system, which can bind up to 40µg of DNA, allows recovery of isolated DNA fragments or PCR products in as little as 15 minutes, depending on the number of samples processed and the protocol used. Samples can be eluted in as little as 15µl of Nuclease-Free Water. The purified DNA can be used for automated fluorescent sequencing, cloning, labeling, restriction enzyme digestion or in vitro transcription/translation without further manipulation.

Wizard® SV Gel and PCR Clean-Up System

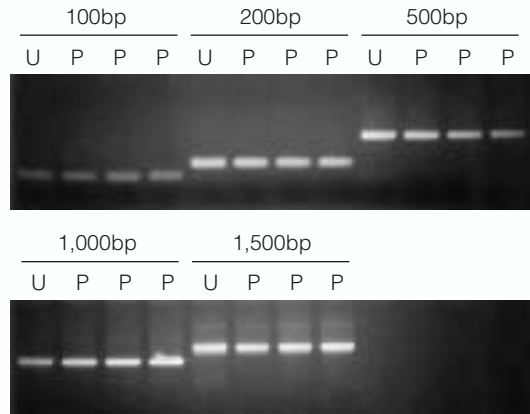
Cat.#: A9281 (50 preps)
A9282 (250 preps)

Protocol:
www.promega.com/tbs/tb308/tb308.html

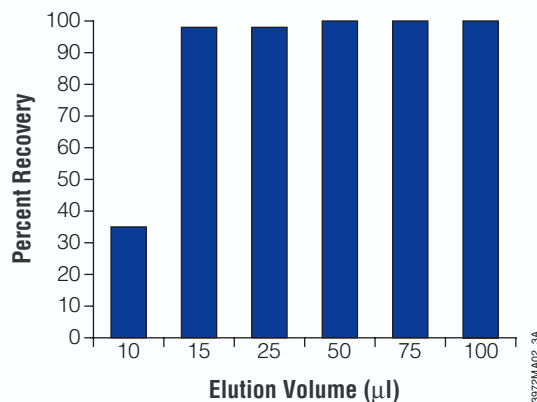


Gel analysis of PCR products before and after gel extraction using the Wizard SV Gel and PCR Clean-Up System. Recovery of various sizes of unpurified (U) and purified (P) PCR products. Purified products were extracted from a 1% agarose gel run with TAE buffer. Lane M, 1kb DNA Ladder (Cat.# G5711).

Linear DNA as big as 10kb can be purified with up to 95% recovery.



Gel analysis of PCR products before and after direct purification using the Wizard SV Gel and PCR Clean-Up System. DNA fragments of the sizes indicated were analyzed before (U) and after (P) direct purification from amplification reactions.



Elution volume versus recovery for a 700bp PCR product purified directly from an amplification reaction using the Wizard SV Gel and PCR Clean-Up System. One hundred percent represents recovery with 50µl elution volume. Adapted from Table 4 in Betz, N. and Strader, T. (2002) Clean up with Wizard SV for Gel and PCR. *Promega Notes* 82, 2–5.

Effect of Various PCR Additives on Recovery of a 1,000bp PCR Product Using the Wizard SV Gel and PCR Clean-Up System Direct Purification Method.

PCR Additive	Percent Recovery Relative to "No Additive"
No Additive	100%
1M Betaine	94%
1M Q-Solution	97%
0.1% Triton® X-100	92%
0.1% Tween® 20	87%
0.1% NP-40	82%
5% Glycerol	87%
5% Formamide	90%
5% DMSO	87%
0.5M Tetramethylene Sulfoxide	94%
0.4M Sulfolane	94%
0.4M 2-Pyrrolidone	95%
1mM Tartrazine	100%
1% Ficoll®-400	100%

PCR Clean-Up

Wizard® SV 96 PCR Clean-Up System

The Wizard SV 96 PCR Clean-Up System provides a fast, simple technique for the efficient isolation of purified DNA fragments generated by PCR amplification. Walkaway automation is easily achieved on any 96-well liquid handling workstation equipped with a gripper and vacuum apparatus. Double-stranded DNA fragments can be purified from 96 samples in less than 20 minutes. Purification is achieved without phenol:chloroform extraction or ethanol precipitation. Optimized methods are available for the Beckman Coulter Biomek® 2000 and FX instruments.

If it works in SV,
it'll work in SV 96.



3445CA06_1A

Recommended automated system for reactions prepared in the presence of PCR enhancers like betaine, DMSO, etc. Just like the SV system, SV 96 works with a wide variety of PCR additives.

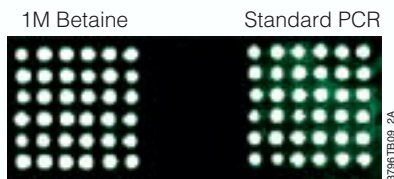
Manual or automated 96-well PCR purification.

Wizard® SV 96 PCR Clean-Up System

Cat.#: A9340 (1 × 96 preps)
A9341 (4 × 96 preps)
A9342 (8 × 96 preps)

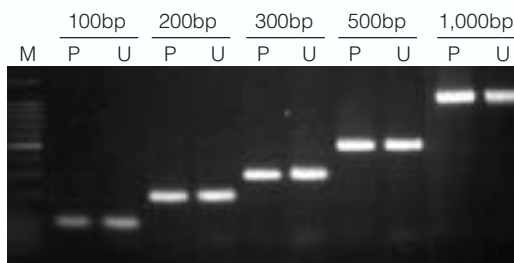
Protocol:
www.promega.com/tbs/tb311/tb311.html

For information on automated methods, visit:
www.promega.com/automethods/



3796TB06_2A

Microarray of purified PCR products. Representative microarray blocks of PCR product purified using the Wizard SV 96 PCR Clean-Up System and hybridized to complementary Cy³-labeled cDNA. PCR DNA was isolated from a standard amplification reaction or from a reaction containing 1M betaine. No effect of betaine is observed.



380TA06_2A

Agarose gel analysis of PCR fragments purified on the Beckman Coulter Biomek® 2000 liquid handler. PCR fragments of 100, 200, 300, 500 and 1,000bp were purified using the Wizard SV 96 PCR Clean-Up System on the Beckman Coulter Biomek® 2000 robotic workstation. Both purified (P) and unpurified (U) fragments were separated on an ethidium bromide-stained 2% agarose gel. Percent recovery ranged from 71 ± 3% for the 100bp fragment to 100 ± 1% for the 1,000bp fragment. Lane M, 100bp DNA Ladder (Cat.# G2101).

Want to do 96-well agarose gel isolations?

Request Application Note #AN096.



PCR Clean-Up

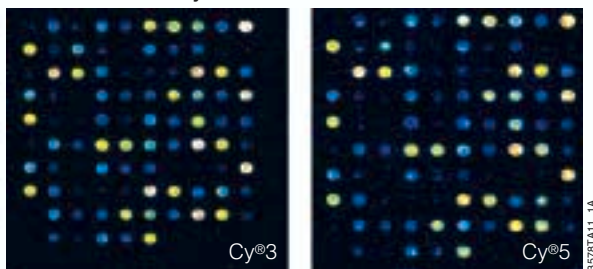
Wizard® MagneSil® PCR Clean-Up System

The Wizard MagneSil PCR Clean-Up System^(a) removes impurities from PCR reactions, giving high quality and yield of double-stranded amplicons. The system removes excess nucleotides, primers and small amplification products such as primer-dimers from PCR reactions. The system is designed for automation on laboratory robotic systems for unattended 96- to 384-well purification.

PCR clean-up is performed using Promega's unique MagneSil Paramagnetic Particles^(a) and the MagnaBot® 96 Magnetic Separation Device (Cat.# V8151) fitted with a 3/16" MagnaBot Spacer (Cat.# V8381). The MagnaBot 96 Magnetic Separation Device is designed to work with most robotic platforms. A MagnaBot 384 Magnetic Separation Device is also available (Cat.# V8241).

The MagneSil PCR Clean-Up procedure is fast and reliable. PCR products bind to MagneSil particles in the presence of guanidine hydrochloride and remain tightly bound during washing. Purified DNA is eluted in water and may be used for automated fluorescent sequencing and microarray spotting. The MagneSil PCR Clean-Up System is ideally suited for reactions prepared using PCR Master Mix and GoTaq® DNA Polymerase. It also works well with reactions prepared using AmpliTaq® and AmpliTaq Gold® DNA Polymerase.

E. coli Control Array



Array images after hybridization with Cy3 and Cy5 fluorescent control *E. coli* targets. *E. coli* genomic DNA was amplified using 96 unique primer pairs. The Wizard MagneSil PCR Clean-Up System-purified PCR products were printed onto a poly-L-lysine-coated slide and hybridized to Cy3- or Cy5-labeled *E. coli* cDNA. For further details, see Splinter BonDurant, S. *et al.* (2002) MagneSil Paramagnetic Particles: A high-throughput PCR purification system for microarrays. *Promega Notes* 80, 14–16.

Wizard® MagneSil® PCR Clean-Up System

Cat.#: A1930 (4 × 96 preps)
A1931 (8 × 96 preps)
A1935 (100 × 96 preps)

Protocols:

Automated 96-well plate protocol

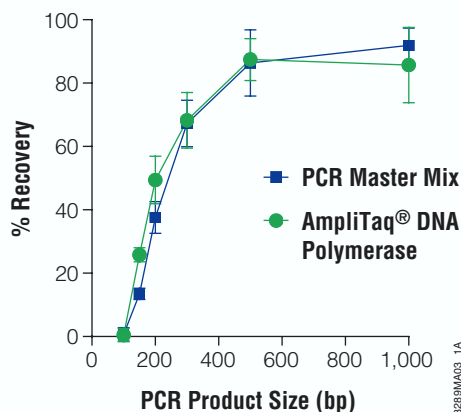
www.promega.com/tbs/tb290/tb290.html

Automated 384-well plate protocol

www.promega.com/tbs/ep009/ep009.html

For information on automated methods visit:

www.promega.com/automethods/



Percent recovery of PCR products purified using the Wizard MagneSil PCR Clean-Up System. PCR amplicons were purified from standard amplification reactions performed using either PCR Master Mix or AmpliTaq® DNA Polymerase.

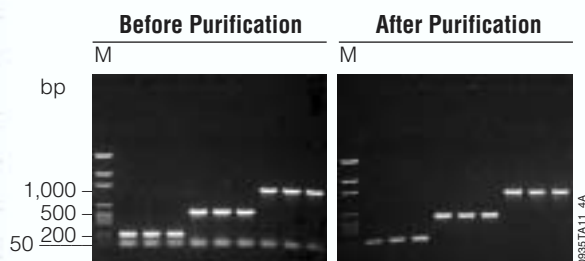
Quality tested for success in fluorescent BigDye® Sequencing with >98% accuracy over 600 bases read.

This system is capable of 384-well purifications.

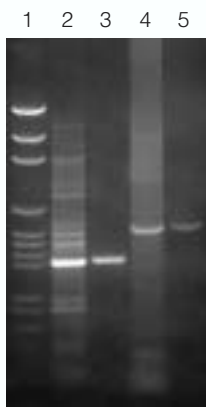
PCR Clean-Up

Wizard® PCR Preps DNA Purification System

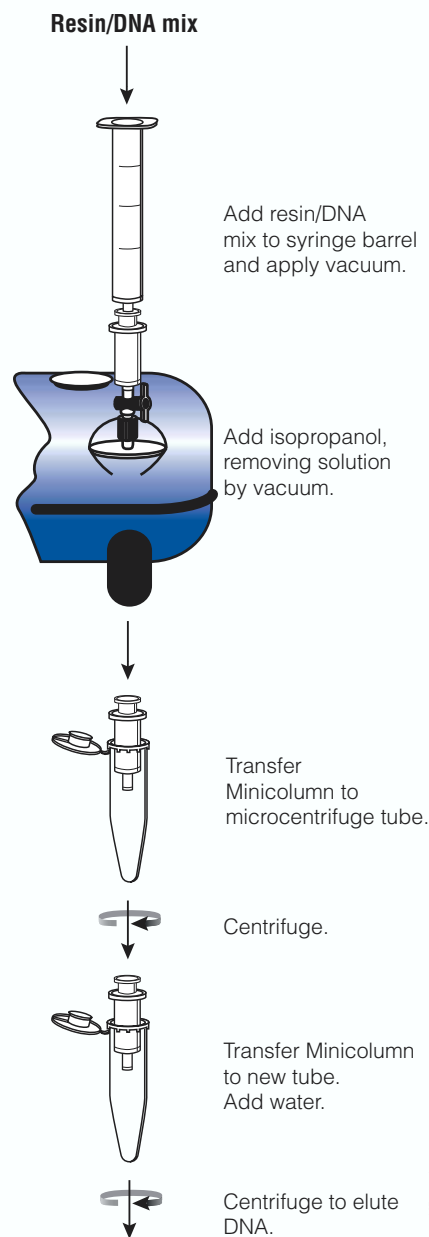
The Wizard PCR Preps DNA Purification System^(k) provides a simple, reliable way to purify double-stranded, PCR-amplified DNA. PCR products are effectively separated from contaminants, including primer-dimers and amplification primers. This system can also be used to purify DNA fragments from agarose gels. The DNA can be eluted from the Wizard PCR Preps DNA Purification Resin in water or TE buffer, with little salt or macromolecular contamination. A unique feature of this resin-based method is its size cutoff capability. The resin does not appreciably bind double-stranded DNA smaller than 200bp, virtually assuring the removal of primer-dimers from the reaction. Multiple PCR preps can be easily processed at one time using the Vac-Man® Laboratory Vacuum Manifold (Cat.# A7231).



Recovery of PCR products using the Wizard PCR Preps DNA Purification System. Equivalent amounts of a PCR reaction taken before and after purification were separated on a 1% agarose gel and stained with ethidium bromide.



Purification and analysis of PCR products from low-melting agarose using the Wizard PCR Preps System. Reactions are shown before (lanes 2 and 4) and after (lanes 3 and 5) purification.



Overview of PCR product purification using the Wizard PCR Preps DNA Purification System and the Vac-Man Laboratory Vacuum Manifold.

Wizard® PCR Preps DNA Purification System

Cat.#: A7170 (50 preps)
A2180 (250 preps)

Protocol:
www.promega.com/tbs/tb118/tb118.html

Citations for use available at:
www.promega.com/citations/

Syringe-based protocol also provided (where no vacuum is required).

PCR Clean-Up

PCR Clean-Up Systems and Accessories

Product	Size	Cat.#
Wizard® SV Gel and PCR Clean-Up System ^(k)	50 preps	A9281
	250 preps	A9282

For Laboratory Use. Manual spin-basket system for direct PCR purification and gel isolation from standard agarose gels.

Product	Size	Cat.#
Wizard® SV 96 PCR Clean-Up System*	1 × 96 preps	A9340
	4 × 96 preps	A9341
	8 × 96 preps	A9342
Vac-Man® 96 Vacuum Manifold	1 each	A2291

* For Laboratory Use. The Wizard® SV 96 PCR Clean-Up System provides a manual or automated system for 96-well direct PCR purification by vacuum. Compatible with a wide variety of PCR additives and all Promega amplification reaction buffers.

Product	Size	Cat.#
Wizard® MagneSil® PCR Clean-Up System ^{(a)*}	4 × 96 preps	A1930
	8 × 96 preps	A1931
Wizard® MagneSil® PCR Clean-Up System, HTP1 ^{(a)*}	100 × 96 preps	A1935
MagnaBot® 96 Magnetic Separation Device	1 each	V8151
MagnaBot® 384 Magnetic Separation Device	1 each	V8241
MagnaBot® Spacer (3/16")	1 each	V8381
384-Well Plate, Flat	10 pack	V5291

* For Laboratory Use. The Wizard® MagneSil® PCR Clean-Up System provides an automated 96- or 384-well system for direct purification of PCR products. Compatible with standard reactions using Promega's PCR Master Mix or GoTaq® DNA Polymerase.

Product	Size	Cat.#
Wizard® PCR Preps DNA Purification System ^{(k)*}	50 preps	A7170
	250 preps	A2180
Vac-Man® Laboratory Vacuum Manifold, 20-sample capacity	1 each	A7231

* For Laboratory Use. The Wizard® PCR Preps DNA Purification System is a manual, resin-based vacuum system for direct purification or isolation from agarose gels.

Related Products

Product	Size	Cat.#
Agarose, LE, Analytical Grade	100g	V3121
	500g	V3125
Agarose, Low Melting Point, Analytical Grade	25g	V2111
Blue/Orange Loading Dye, 6X*	3ml (3 × 1ml)	G1881
TAE Buffer, 10X	1,000ml	V4271
TAE Buffer, 40X	1,000ml	V4281
TBE Buffer, 10X	1,000ml	V4251

*For Laboratory Use.