Clean-Up with Wizard® SV for Gel and PCR

Wizard® SV Gel and PCR Clean-Up System

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Abstract

The Wizard® SV Gel and PCR Clean-Up System is designed to quickly purify DNA fragments from either agarose gel slices or directly from amplification reactions using the SV Minicolumns. The purified DNA is suitable for a number of downstream applications, including fluorescent sequencing, cloning, labeling, amplification, restriction enzyme digestion, and in vitro transcription/translation.

The Wizard® SV Gel and PCR Clean-Up System is designed to purify DNA fragments from standard- or low-melting-point agarose gels and PCR products directly from amplification reactions.

Introduction

Purification of DNA fragments is a common technique in molecular biology, with downstream applications including PCR, cloning, sequencing, restriction enzyme digestion, and other enzymatic manipulations. DNA is often purified from agarose gel slices using electroelution (1), but this technique is time-consuming and often results in very dilute DNA, which must be further concentrated by ethanol precipitation.

The Wizard® SV Gel and PCR Clean-Up System[a] extracts and purifies DNA fragments of 100bp to 10kb from standard- or low-melting-point agarose gels in either Tris-acetate (TAE) or Tris-borate (TBE) buffer with 70–95% recovery. Agarose concentrations up to 3% have been used successfully with this system, and agarose from different vendors shows no difference in performance. In addition, either low- or high-melting-point agarose may be used with no difference in DNA functionality in downstream applications.

In addition to gel extraction, the Wizard® SV Gel and PCR Clean-Up System purifies PCR products from 100bp to 10kb in size directly from amplification reactions with 80–95% recovery. For many downstream applications, the removal of unincorporated nucleotides, primers, buffer, and enzyme is required or at least advantageous.

This membrane-based system, which can bind up to 40µg of DNA, allows recovery of the isolated DNA in as little as 15 minutes, depending on the number of samples processed. The purified DNA can be used without further manipulations for a number of downstream applications, including automated fluorescent DNA sequencing, cloning, labeling, restriction enzyme digestion, or in vitro transcription/translation.

The Wizard® SV Gel and PCR Clean-Up System is based on the ability of DNA to bind to silica membranes in the presence of chaotropic salts. For purification from gels, the DNA band of interest is excised from the gel following electrophoresis and dissolved in the presence of guanidine isothiocyanate (Membrane Binding Solution). For PCR clean-up, the Membrane Binding Solution is added directly to the amplification reaction. The DNA can then be isolated using either microcentrifugation or vacuum to pull the dissolved gel slice through the membrane while simultaneously binding the DNA on the surface of the silica membrane.

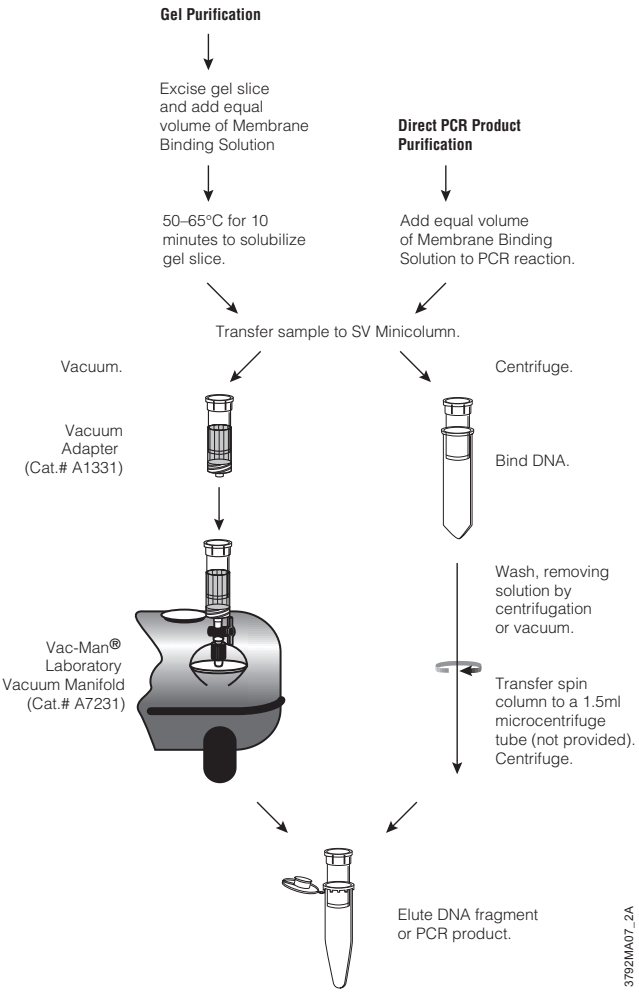

Figure 1. Flow chart of DNA fragment gel purification or direct PCR product purification using the Wizard® SV Gel and PCR Clean-Up System.

After washing the bound DNA fragments with the Membrane Wash Solution, the DNA is eluted in water. This system may be used with linear DNA fragments and PCR products, supercoiled plasmid DNA, and linear or circular single-stranded DNA. Yields with single-stranded DNA may be lower than with double-stranded DNA.

Product Characteristics

The Wizard® SV Gel and PCR Clean-Up System uses a simple protocol, which is outlined in Figure 1. The procedure takes approximately 15 minutes, depending on the number and type of samples to be processed, and requires only a microcentrifuge.

The Wizard® SV Gel and PCR Clean-Up System effectively purifies 0.1–10kb DNA fragments (Table 1 and Figure 2). Percent recovery was determined by gel analysis of DNA purified from 1% agarose/TAE gel slices weighing 350mg each; one slice was purified per column. Higher efficiencies for larger DNA fragments may be achieved by using heated water (65–80°C) for elution. Each column can accommodate up to 40µg of DNA; however, the system has been used successfully to purify as little as 10ng of DNA from a single gel slice.

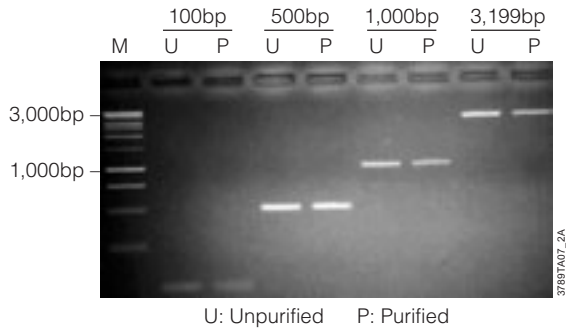

Figure 2. Gel analysis of PCR products before and after gel extraction using the Wizard® SV Gel and PCR Clean-Up System. Both unpurified (U) and purified (P) DNA fragments of the sizes indicated were analyzed. Lane 1 contains the 1kb DNA Ladder (Cat.# G5711).

Table 1. Recovery versus DNA fragment size using the Wizard® SV Gel and PCR Clean-Up System tp purify DNA from an agarose gel.

DNA Fragment Size	Percent Recovery
55bp	26%
70bp	39%
85bp	55%
100bp	84%
500bp	89%
1,000bp	92%
3,199bp	95%
9,416bp	95%
23,130bp	47%

Figure 3 and Table 2 demonstrate the average recovery of various-sized PCR products purified with the direct purification method. The 100bp, 200bp, 500bp, 1,000bp, and 1,500bp PCR products were generated using PCR Master Mix[c] (Cat.# M7501, M7502, M7505), while the 3,199bp DNA product was linearized pGEM®-3Zf(+) Vector[b] DNA purified from a mock reaction. Recovery was determined by gel analysis. The system can effectively remove unincorporated primers from the amplification reaction, and mineral oil does not interfere with purification.

Table 2. Recovery versus DNA fragment size using the Wizard® SV Gel and PCR Clean-Up System with various-sized PCR products.

DNA Product Size	Percent Recovery
100bp	85%
200bp	87%
500bp	91%
1,000bp	92%
1,500bp	92%
3,199bp	87%

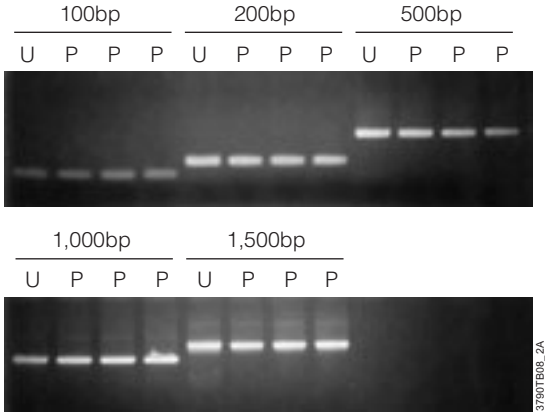

Figure 3. Gel analysis of PCR products before and after direct purification using the Wizard® SV Gel and PCR Clean-Up System. DNA fragments of the sizes indicated were analyzed before (U) and after (P) purification

The Wizard® SV Gel and PCR Clean-Up System is compatible with a variety of amplification enzymes, buffers, and PCR-enhancing additives. We investigated the effect of 13 different PCR additives on the recovery efficiency of a 1,000bp PCR product. The results are shown in Table 3 and demonstrate that all of the additives tested were compatible with direct purification using the Wizard® SV Gel and PCR Clean-Up System.

The standard protocol includes an elution step into 50µl of Nuclease-Free Water. However, elution volumes as small as 15µl may be used to purify DNA in a more concentrated manner. Table 4 shows the yields of a 700bp PCR product, which was purified directly, eluted with various volumes of Nuclease-Free Water, and quantified by ethidium bromide gel analysis in triplicate. Elution

Table 3. Effect of various PCR additives on percent recovery of a 1,000bp PCR product using the direct purification method and the Wizard® SV Gel and PCR Clean-Up System.

PCR Additive	Percent Recovery*
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-Pyrollidone**	95%
1mM Tartrazine	100%
1% Ficoll®-400	100%

*Percent recovery shown is relative to the "no additive" recovery.
**See references 2–4 for more information on these PCR additives.

volumes of 10µl or less are not recommended, since 10µl is not sufficient to completely wet the silica membrane in the spin basket and resulting in reduced yields.

Functionality

A 1,000bp PCR product was gel-purified using this system and tested in T-vector cloning, restriction enzyme digestion, and automated fluorescent sequencing. The purified DNA was efficiently ligated into the pGEM®-T Easy Vector[b,d] and digested completely with all of the restriction enzymes tested (*Bam*H I, *Eco*R I, and *Hind* III; data not shown). The purified fragment was compatible with ABI BigDye® Terminator fluorescent sequencing chemistry with an average read length >700bp with >98% accuracy (Figure 4). No difference in recovery, cloning, restriction digestion, or sequencing was seen between TAE and TBE agarose gel slices (data not shown). Similar results in terms of cloning and sequencing efficiency were observed when the same 1,000bp PCR product was purified directly from the amplification reaction (data not shown).

The gel-purified DNA fragments are also suitable templates for in vitro transcription/translation reactions using Promega's TNT® Systems without further manipulations (data not shown). In particular, the TNT® T7 Quick[e,f,g,h,i] and TNT® T7 Quick for PCR DNA[f] Systems work well with linear DNA templates.

Competitor Analysis

We compared the recovery of different-sized DNA fragments purified with the Wizard® SV Gel and PCR Clean-Up System and competitor Q's Gel Extraction and PCR Purification Kits. The Wizard® SV Gel and PCR Clean-Up System performed as well as, or better than,

Table 4. Elution volume versus recovery of a 700bp PCR product purified directly from an amplification reaction.

Elution Volume	Percent Recovery*
10µl	35%
15µl	98%
25µl	98%
50µl	100%
75µl	100%
100µl	100%

*Percent recovery compared to recovery using a 50µl elution volume.

the competitor Q's Kits (Table 5). In addition, DNA purified using either system exhibited comparable functionality (data not shown).

In general, a single gel slice of up to 350mg may be processed per spin basket. However, the SV Minicolumns can actually accommodate up to 3.5g of total of melted agarose, which corresponds to 10 × 350mg 1% agarose gel slices. No difference in yield or function was seen when an equal amount of DNA was purified from either 1, 2, 5 or 10 gel slices (data not shown). This would be advantageous when a single DNA fragment is spread over multiple gel lanes or is contained within a single, large slice. Competitor Q's Gel Extraction Kit protocol does not recommend greater than 400mg of melted agarose per spin column. Thus DNA samples in a larger mass of agarose must be processed through multiple columns and the eluted DNA pooled and most likely concentrated further by ethanol precipitation. This is time-consuming and may result in loss of the DNA fragment during precipitation.

Up to 1ml of amplification reaction may be processed through a single SV Minicolumn. This allows for the concentration of dilute PCR products or purification from larger reaction volumes. In contrast, competitor Q's PCR Purification Kit recommends no more than 100µl of reaction per column. Additionally, PCR products from reactions as small as 10µl have been successfully purified with the Wizard® SV Gel and PCR Clean-Up System.

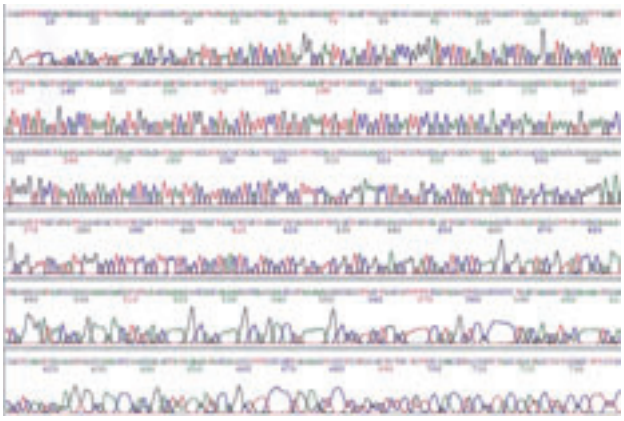

Figure 4. Sequence of a 1,000bp PCR product purified from a 350mg 1% agarose/TAE gel slice using ABI BigDye® Terminator chemistry.

Table 5. Comparison of percent recovery for different-sized DNA fragments or PCR products between the Wizard® SV Gel and PCR Clean-Up System and competitor Q's Gel Extraction PCR Purification Kits.

Product Size	Wizard® SV Gel and PCR Clean-Up System	Competitor Q's Gel Extraction Kit
55bp	26%	32%
70bp	39%	41%
85bp	55%	61%
100bp	84%	65%
500bp	89%	91%
1,000bp	92%	75%

Product Size	Wizard® SV Gel and PCR Clean-Up System	Competitor Q's PCR Purification Kit
100bp	85%	85%
200bp	86%	84%
500bp	91%	94%
1,000bp	92%	90%

Conclusions

The Wizard® SV Gel and PCR Clean-Up System can be used to purify DNA fragments from either standard agarose gel slices or directly from amplification reactions. The system is easy to perform and efficiently purifies DNA fragments between 100bp to 10kb with 70–95% recovery. The purified products are functional in numerous downstream applications.

Acknowledgments

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4. Chakrabarti, R. and Schutt, C.E. (2001) *Nucl. Acids Res.* **29**, 2377–2381.

Protocols

- *Wizard® SV Gel and PCR Clean-Up System Technical Bulletin #TB308, Promega Corporation.*
  (www.promega.com/tbs/tb308/tb308.html)

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Ordering Information

Product	Size	Cat.#
Wizard® SV Gel and PCR Clean-Up System*	50 preps	A9281
Wizard® SV Gel and PCR Clean-Up System*	250 preps	A9282
Vac-Man® Laboratory Vacuum Manifold, 20-sample capacity	1 each	A7231
Vac-Man® Jr. Laboratory Vacuum Manifold, 2-sample capacity	1 each	A7660
Vacuum Adapters	20 each	A1331

*For Laboratory Use.

(a) U.S. Pat. Nos. 5,658,548, 5,808,041, Australian Pat. No. 689815 and other patents pending.

(b) U.S. Pat. No. 4,766,072.

(c) The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

(d) Licensed under one or both of U.S. Pat. No. 5,487,993 and European Pat. No. 0 550 693.

(e) U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289, 5,814,471, Australian Pat. No. 649289 and other patents and patents pending.

(f) U.S. Pat. No. 5,552,302, Australian Pat. No. 646803 and other patents.

(g) The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

(h) U.S. Pat. Nos. 5,324,637, 5,492,817, 5,665,563, Australian Pat. No. 660329 and other patents.

(i) U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.