

High-Throughput Wizardry for PCR Purification



Introducing the Wizard® SV 96 PCR Clean-Up System

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Abstract

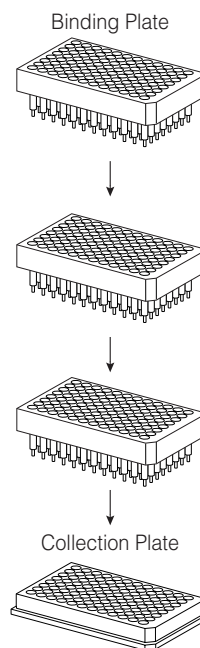
The Wizard® SV 96 PCR Clean-Up System is a high-throughput DNA fragment purification system that purifies double-stranded PCR products directly from amplification reactions. The system provides a flexible method that can be performed manually or automated on a liquid handling workstation such as the Beckman Biomek® 2000 or Biomek® FX.

The Wizard® SV 96 PCR Clean-Up System is designed to allow the rapid purification of PCR fragments in a high-throughput, easily automated format. The system provides consistent and reliable recovery of DNA fragments that are suitable for use in a variety of downstream applications.

Introduction

The Wizard® SV Gel and PCR Clean-Up System^(a) (Cat.# A9281, A9282) introduced a manual, spin column-based system for low-throughput purification of double-stranded DNA fragments. Extending this robust and convenient chemistry into a high-throughput format is now possible using the Wizard® SV 96 PCR Clean-Up System, which integrates the utility of the single, spin-column format into a 96-well, walk-away, automated purification system.

The SV 96 PCR Clean-Up System provides a fast, simple technique for the efficient recovery of purified DNA fragments generated by PCR amplification. The system also provides the ability to automate the purification process on any 96-well liquid handler with a gripper. Using this method, double-stranded DNA fragments are purified from 96 samples in approximately 20 minutes. Purification is achieved without phenol/chloroform extraction or ethanol precipitation. Fully verified and validated automated PCR clean-up methods have been developed for both the Beckman Biomek® 2000 and FX instruments.



Mix PCR sample and Membrane Binding Solution in equal volumes. Apply to Binding Plate.

Apply vacuum to bind PCR product to membrane.

Wash.

Elute bound PCR product into Nuclease-Free Water.

Figure 1. Wizard® SV 96 PCR Clean-Up System protocol overview.

Procedure

The Wizard® SV 96 PCR Clean-Up System efficiently purifies PCR fragments from reactions using 96-well vacuum filtration steps, eliminating the need for laborious centrifugation (Figure 1). An equal volume of Membrane Binding Solution is added to PCR samples, which are then applied to the Binding Plate. Double-stranded PCR products bind to the SV 96 Binding Plate. Washing of the bound PCR fragments does not require disassembly of the manifold, and filtrate waste products are delivered directly to a vacuum trap, eliminating the need to empty waste collection vessels during purification. Purified PCR products are eluted from the Binding Plate with Nuclease-Free Water and collected into a 96-well Collection Plate for easy storage and direct processing for downstream applications.

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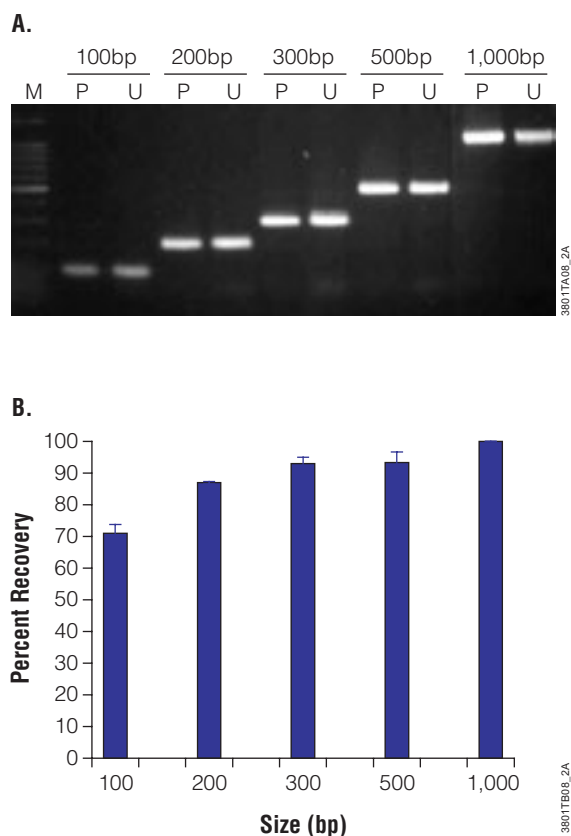


Figure 2. Purification and recovery of PCR products. PCR fragments of 100, 200, 300, 500 and 1,000 base pairs were purified using the Wizard® SV 96 PCR Clean-Up System on the Biomek® 2000 robotic workstation. **Panel A.** Agarose gel analysis. Purified (P) and unpurified (U) fragments were separated on an ethidium bromide-stained, 2% agarose gel. **Panel B.** Percent recovery of purified PCR products. Percent recovery was quantitated using a Hitachi FMBIO® Fluorescent Scanner. Results show the mean and standard deviation for 6 purified fragments of each size. For small PCR fragments (<500bp), optimal recovery requires a 95% ethanol wash. For larger fragments (>500bp), optimal results are achieved using an 80% ethanol wash.

System Performance

As previously described, Promega's Wizard® SV technology purifies double-stranded PCR fragments efficiently from a variety of PCR conditions (see article on page 2). The Wizard® SV 96 PCR Clean-Up System allows automated purification and recovery of PCR products, and provides the flexibility to efficiently purify both small and large double-stranded DNA fragments (Figure 2). Purification of smaller fragments (<500bp) is achieved using a 95% ethanol wash. This simple modification of the protocol, coupled with the selectivity of silica chemistry, provides the advantage of efficient recovery of small PCR products, something that cannot be accomplished using size exclusion-based DNA purification systems.

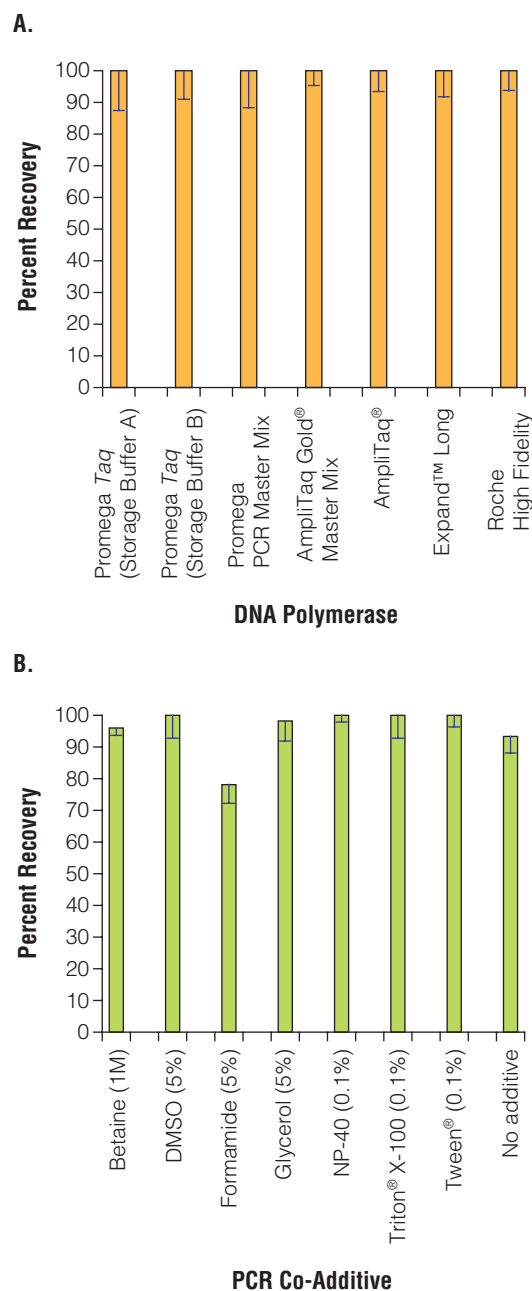


Figure 3. Recovery of PCR products is independent of variable amplification conditions. Percent recovery of purified PCR products generated using multiple polymerases (**Panel A**) and PCR co-additives (**Panel B**) was evaluated. Results show the mean and standard deviation from 4 purifications.

High-Throughput Wizardry...continued

Using the Wizard® SV 96 PCR Clean-Up System, recovery of PCR products is independent of variable PCR amplification conditions that may include different DNA polymerases or PCR additives (Figure 3). The purified PCR fragments are suitable for direct, automated fluorescent sequencing. Average Phred 20 scores for a 1,000bp, purified PCR fragment are >600. The Phred score is a quality value that indicates the probability that a base call is correct. A score of 20 indicates a 99% probability that the base call is correct (1,2).

PCR fragments purified using the Wizard® SV 96 PCR Clean-Up System are also suitable for microarray analysis (Figure 4). Spotting PCR products generated in the presence of PCR co-additives sometimes causes failure in microarray analysis (data not shown). Figure 4 shows that the presence of the co-additive, betaine, in the PCR mix did not affect microarray analysis performed after PCR product purification using the Wizard® SV 96 PCR Clean-Up System.

Gel Purification Application

We have also purified DNA from gel slices in a 96-well format using the Wizard® SV 96 PCR Clean-Up System. A gel slice containing the DNA fragment is added to an equal weight/volume of Membrane Binding Solution in a 96-well deep well plate. After incubation at 65°C for 10 minutes to melt the agarose, this solution is applied to the SV 96 Binding Plate. A vacuum is then applied to bind the DNA fragment to the plate. Wash and elution steps are unchanged from the standard protocol (Figure 1). Using this modified procedure, high-throughput purification of DNA fragments from gel slices is achieved. Percent recovery of DNA fragments purified from gel slices is reduced compared with recovery directly from PCR. Generally, percent recovery of DNA fragments recovered from gel slices using the Wizard® SV 96 PCR Clean-Up System is >70% (data not shown). For more information on this application, please contact Promega Technical Services.

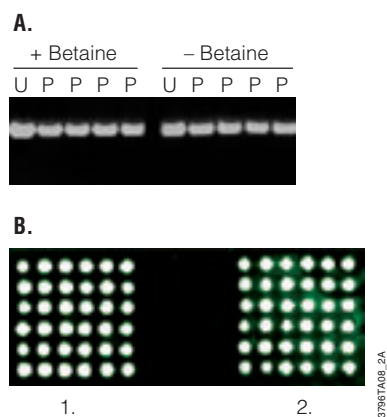
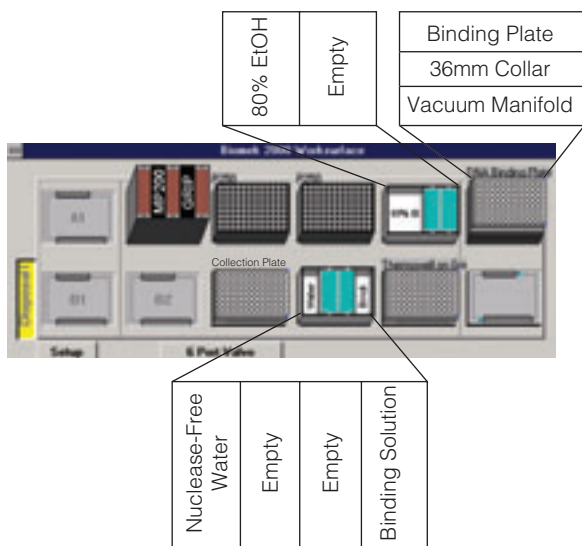


Figure 4. Microarray of purified PCR products. PCR products (300bp) were amplified in the presence or absence of 1M betaine, then purified using the Wizard® SV 96 PCR Clean-Up System. **Panel A.** Agarose gel analysis. Purified (P) and unpurified (U) PCR products amplified with (+) or without (-) betaine were separated on an ethidium bromide-stained, 2% agarose gel. **Panel B.** Representative microarray blocks of purified PCR product hybridized to complementary Cy®3-labeled cDNA. 1) PCR product amplified under standard amplification conditions (-betaine). 2) 1M betaine added to the PCR mix.

Table 1. Biomek® Program for Purification of PCR Fragments.

Step	Process
1	Biomek® transfers 100µl of Binding Solution to PCR samples, then mixes.
2	Samples are transferred to the Binding Plate. DNA binding occurs as samples are pulled through the plate by vacuum.
3	Samples are washed 3X with 200µl 80% ethanol*.
4	Binding Plate is dried briefly by vacuum.
5	Binding Plate is transferred to the collar holder and the Collection Plate is positioned in the vacuum manifold. The Binding Plate is then placed over the Collection Plate.
6	Fragment DNA is eluted into the Collection Plate by the addition of Nuclease-Free Water to the Binding Plate.

*For fragments <500bp, 95% ethanol is recommended.

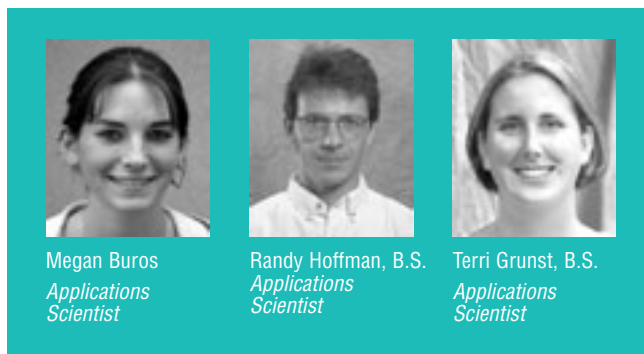


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Figure 5. Initial deck configuration of the Biomek® 2000. Tools required for use of the Wizard® SV 96 PCR Clean-Up System include: MP200 and Gripper, both placed at position A2. Positions A1, B1 and B2 remain empty. Position A3 and A4 contain boxes of P250 tips. Position A5 contains a reservoir holding 80% ethanol wash solution. The vacuum assembly, containing a Beckman vacuum manifold with a Beckman 36mm collar stacked on top, is at position A6. The Binding Plate is stacked on top of this collar. Position B3 contains the 96-well Collection Plate. Position B4 contains a reservoir holding Nuclease-Free Water and Binding Solution. Position B5 holds a 96-well PCR plate placed on top of a 96-well U-bottom plate. At position B6 is a collar holder used for vacuum assembly, disassembly, and reassembly for DNA elution from the Binding Plate.

Automated PCR Clean-Up on the Beckman Biomek® 2000 and FX Robotic Workstations.

The Wizard® SV 96 PCR Clean-Up System protocol is easily automated on liquid handling workstations. We demonstrate the use of this system on both the Beckman Biomek® 2000 and FX robots. The initial deck configuration for the Biomek® 2000 is outlined in Figure 5. Deck configuration on the Biomek® FX may vary. Minimally, FX methods require the SPE and SPE holder ALPS, tip loader and eight positions available to the 96-well head. Use of the Wizard® SV 96 PCR Clean-Up System on automated liquid handling workstations requires vacuum manifolds specific to each workstation. The Biomek® program for purification of PCR fragments is given in Table 1.



Conclusions

The Wizard® SV 96 PCR Clean-Up System is a high-throughput DNA fragment purification system with a flexible format that allows rapid manual or automated PCR product purification. The system provides consistent and reliable recovery of purified DNA fragments that are suitable for downstream applications such as automated fluorescent sequencing and microarray analysis.

References

1. Ewing, B. *et al.* (1998) *Genome Res.* **8**, 175–185.
2. Ewing, B. and Green, P. (1998) *Genome Res.* **8**, 186–194.

Protocols

- ◆ *Wizard® SV 96 PCR Clean-Up System Technical Bulletin #TB311*, Promega Corporation. (www.promega.com/tbs/tb311/tb311.html)

Ordering Information

Product	Size	Cat.#
Wizard® SV 96 PCR Clean-Up System	1 × 96	A9340
	4 × 96	A9341
	8 × 96	A9342

For Laboratory Use.

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

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