Promega's SV Membrane Technology: The Evolution of an Indispensable Laboratory Tool

By Kyle Hooper, Ph.D., Promega Corporation

Abstract

The family of Wizard® SV and SV 96 Systems offers optimized reagents for isolation of numerous nucleic acid types, including plasmids, DNA fragments, genomic DNA, PCR products and total RNA. The SV technology uses a silica-impregnated porous membrane to bind DNA or RNA, separating it from contaminants. These membrane-based systems offer several advantages over silica resin columns, including better washing and drying characteristics and greater processing speed.

By exploiting the ability of silica to bind both DNA and RNA, the SV membrane has been adapted to many uses including plasmid, total RNA, genomic DNA and fragment isolation.

Introduction

Promega's SV membrane is a silica-impregnated porous membrane suitable for binding nucleic acids in the presence of a chaotrope. The SV membrane is available in a small basket form for single-sample preparations using either a spin format or, with the addition of our adapters, a vacuum format. Throughput with these single-prep systems is only limited by microcentrifuge rotor capacity or the capacity of the vacuum manifold. For higher throughput, the SV membrane has been scaled up to a 96-well format. Each well of the 96-well plate has the same characteristics and capacity as the single-prep baskets. The 96-well SV 96 systems brought manual or automated capabilities to the SV membrane. Promega has combined this membrane with a variety of reagents to create a large line of systems for isolation of specific nucleic acids from a variety of sources. Systems are available for isolating plasmid DNA, genomic DNA, total RNA, DNA from PCR and DNA from agarose gels (Table 1).

Table 1. Applications available with the SV and SV 96 nucleic acid purification products.

	SV Preps		SV 96 Preps	
Application	**Spin**	**Vacuum**	**Manual**	**Automated**
Plasmid DNA Isolation	√	√	√	√
Genomic DNA Isolation	√	√	√	√
PCR Product Clean-Up	√	√	√	√
DNA Isolation from Gels	√	√	√	√
Total RNA Isolation	√	√	√	√

Plasmid DNA Purification

Single Preps: The SV Systems for plasmid DNA purification offer consistent isolationof high-purity DNA from both high-copy plasmids and low-copy plasmids (1,2; Figure 1). Promega has incorporated a unique alkaline protease digestion into the plasmid preparation, resulting in higher purity DNA (3,4). Due to the large biomass that must be processed to get sufficient quantitites of a low-copy-number plasmid, protein contamination is a real concern. Alkaline protease treatment has been shown to decrease protein carryover in bacterial lysates (5,6). The Wizard® *Plus* SV Minipreps DNA Purification System[a,b] utilizes a simple three-step procedure: lyse, bind, elute.

96-Well Preps: The Wizard® SV 96 Plasmid DNA Purification System[b] is a vacuum-based system for either manual or automated plasmid purification (2). On a suitable robotic platform, the system is truly "walk away" with no manual intervention once the *E. coli* pellets are placed on the deck. The Vac-Man® 96 Manifold

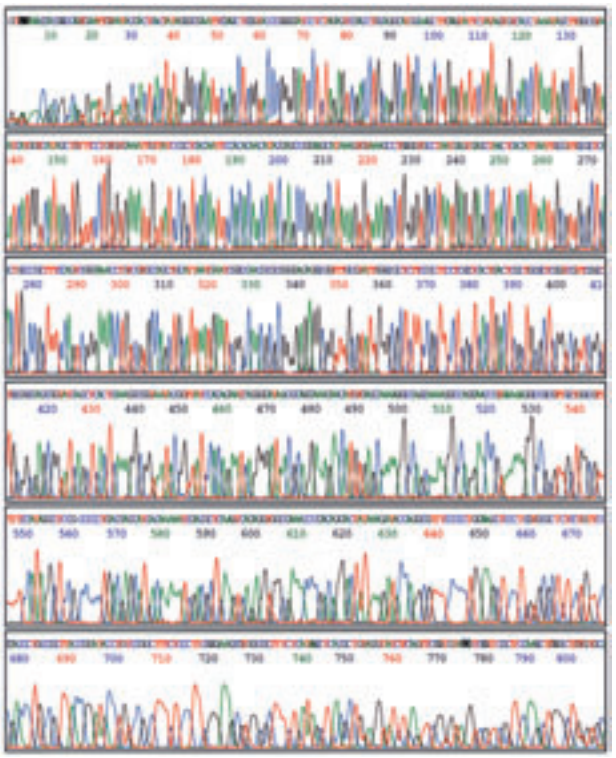

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Figure 1. Fluorescent BigDye® Terminator sequencing of pGEM® plasmid purified using the Wizard® *Plus* SV Minipreps DNA Purification System. The system is tested to ensure that purified plasmid DNA can be sequenced using dye-terminator chemistry and an ABI PRISM® 377 DNA Sequencer at ≥98% accuracy over 600 bases.

allows washing of the bound DNA and requires no disassembly of the manifold. Filtrate waste products are delivered directly to a vacuum trap, eliminating the need to empty waste collection trays. The SV 96 System incorporates lysate clearing with concurrent plasmid binding through the combined use of the Lysate Clearing Plate and SV 96 Binding Plate (6). To meet the needs of customers processing large quantities of plates per day, the SV 96 System is available in a large-quantity format called the Wizard® SV 9600 Plasmid DNA Purification System[b], which contains sufficient material to process 100 × 96-well plates. (7)

DNA Fragment Purification from Agarose Gels

Silica binds DNA in both supercoiled and linear forms, so the easy-to-use spin and vacuum methods led to the development of the Wizard® SV Gel and PCR Clean-Up System[c] (8) for the isolation of supercoiled and linear DNA from agarose gels and PCR samples. Researchers have used the Wizard® *Plus* SV Minipreps System to clean-up sodium bisulfite reactions of genomic DNA for methylation-specific PCR (9,10) and even used the system to gel-purify DNA fragments (11). The optimized recovery reagents and SV membranes allow purification of a wide range of fragments from standard or low-melting-temperature agarose as well as TBE or TAE gels. The gel slice is melted in the membrane binding solution, vacuumed through the SV Membrane, washed and eluted (12). Recovery of 84–95% is possible when the starting material is double-stranded DNA fragments from 100bp to 10kb. For more information on the system, please see the article on page 2 of this issue.

PCR Product Purification

The Wizard® SV Gel and PCR Clean-Up System is intended for amplimer purification directly from PCR and presumably from any enzymatic reaction. DNA amounts from 10ng to 40µg have been purified on a single column (8). Up to 1ml of PCR product can be purified in a single preparation. Elution volumes can be decreased to as little as 15µl with a minimal loss in yield. The purified DNA can be used directly in cloning applications with the pGEM®-T or pGEM®-T Easy Vector Systems[d,e], fluorescent sequencing, restriction digestion, or in vitro transcription/translation with the TNT® Systems.[f,g,h,i]

The SV 96 Binding Plate has been adapted for direct purification of PCR products through the Wizard® SV 96 PCR Clean-Up System (13). The system allows purification of 96 PCR products using either a manual or robotic platform (e.g., the Biomek® FX or Biomek® 2000 instruments; 14). The purification process is not hindered by common PCR additives such as betaine or DMSO, and mineral oil need not be removed from the reactions. PCR products can be purified from reactions prepared with *Taq* DNA Polymerase[j] in Storage Buffer A, Storage Buffer B or in the PCR Master Mix[j,k] (13).

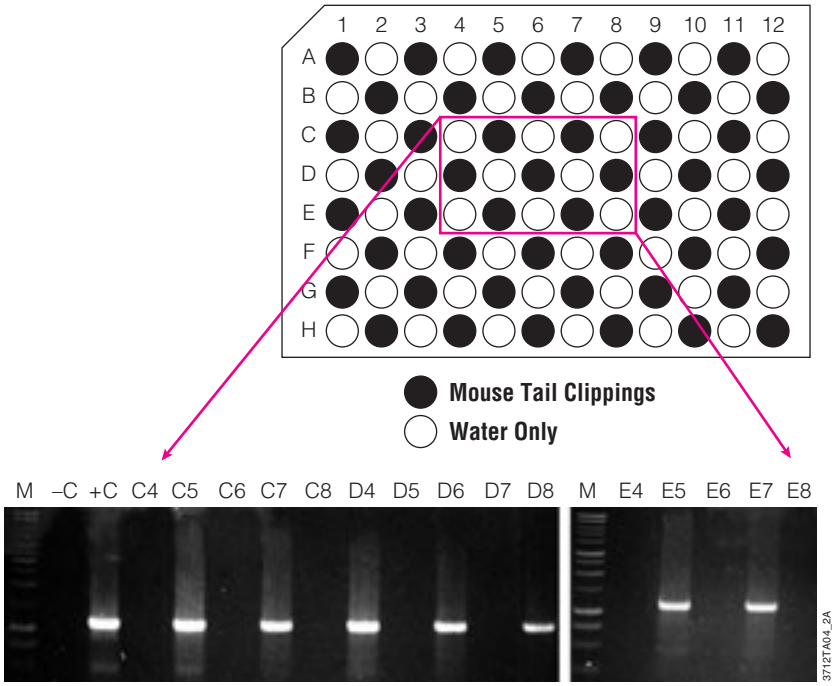

Figure 2. Cross contamination assay. Genomic DNA purified from mouse tail clipping samples or water samples arrayed in adjacent wells of a 96-well plate. PCR products were amplified from 1µl of purified samples from each well for mouse IL-1β. Ten micoroliters pf PCR product were run on a 1.5% agarose gel and visualized by staining with ethidium bromide. Expected PCR product from mouse tail clippings for mouse IL-1β is approximately 1.2kb. No PCR product was expected from water samples. PCR protocol: One cycle of 3 minutes at 95°C; followed by 30 cycles of: 95°C for 30 seconds, 60°C for one minute, 70°C for one minute and thirty seconds; final extension at 70°C for seven minutes; 4°C soak.

Genomic DNA Purification

Genomic DNA purification was an obvious extension for the SV membrane technology because total nucleic acids could be isolated by direct capture from cell or tissue lysates. The SV membrane was adapted for the purification of genomic DNA from blood (15–17), buccal swabs (14,16), mammalian tissues (16,18) and cultured cells (16,19). This membrane can even purify DNA from apoptotic cells (19,20). In addition, the protocol can be modified in such a way that both RNA and DNA can be purified from a single sample (16).

Optimal genomic DNA isolation can now be accomplished with the new Wizard® SV Genomic DNA Purification System (21). The system allows rapid, direct isolation of genomic DNA from up to 5×10^6 cultured cells. The speed of the system becomes obvious when tissues like mouse tail snips, commonly used for transgenic mouse genotyping, are used. After lysis of the tail snip with Proteinase K and the buffers provided, it takes only 20 minutes to purify genomic DNA that is ready for amplification (22).

A 96-well version is available of the Wizard® SV 96 Genomic DNA Purification System. This system has been used on the Biomek® FX, Biomek® 2000 and Packard Multiprobe® II instruments. A plate of 96 mouse tail snips can be completely processed in 45–60 minutes after lysis with no intervention. Processing of 96-wells of cultured cells can be accomplished in a little as 30 minutes (23). The design of the 96-well plate makes the purifications worry-free, with no cross contamination detectable between adjacent wells (Figure 2).

Total RNA Isolation

Because silica will bind any nucleic acid, the SV membranes were adapted for total RNA purification. The SV Total RNA Isolation System[a,l] is a highly versatile total RNA isolation system, allowing purification from cultured mammalian cells, mammalian tissue, plant tissue, yeast and bacteria (24,25). The SV protocol includes a preferential precipitation of the RNA from the genomic DNA prior to membrane binding, as well as an innovative, patented DNase treatment performed directly on the membrane. The SV membrane allows on-membrane digestion and washes to remove the DNase from the membrane prior to elution (26). The eluted material is then ready for downstream applications such as RT-PCR.

As with the Wizard® *Plus* SV Minipreps System, throughput of the SV Total RNA System was limited by microcentrifuge rotor capacity. Thus, the SV 96 Total RNA Isolation System[l] was introduced (27). Lysed target material is loaded into the SV 96 RNA Binding plate, DNase-treated, then washed and eluted. No centrifugation is required once the lysate is loaded onto the plates. The total RNA is eluted directly into a 96-well plate (25). This system is readily automated, and purifications have been performed with the Biomek® FX, Biomek® 2000, Tecan Genesis, and Packard Multiprobe® instruments. The RNA purified with the system is compatible with downstream applications such as RT-PCR, real-time and quantitative RT-PCR (Figure 3).

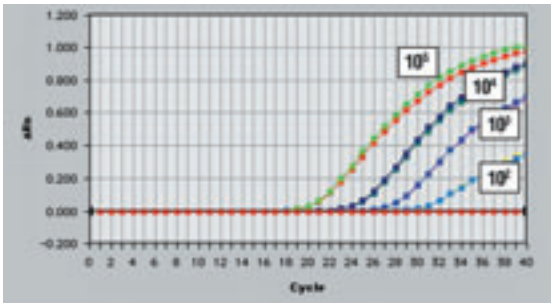

Figure 3. TaqMan® assay for GAPDH of total RNA isolation from different numbers of HeLa cells with the SV 96 Total RNA Isolation System. Duplicate RNA samples were purified from HeLa cells ranging in concentration from 1×10^2 to 1×10^5 total cells. The RNA was eluted in 100µl of Nuclease-Free Water, and 1µl of each sample was analyzed with the GAPDH assay kit (Applied Biosystems) on an ABI PRISM® 7700 sequence detection system.

Conclusion

The SV and SV 96 membranes simple and versatile solutions for nucleic acid purification, allowing researchers to increase throughput with no failure in downstream applications. Systems containing reagents optimized for plasmid, DNA fragment, PCR fragment, genomic DNA or total RNA isolation are available. A researcher can begin with the SV, increase throughput with manual 96-well purification and even automate the 96-well purifications on a wide variety of robotic platforms.

References

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Protocols

- *Wizard®* Plus *Minipreps DNA Purification System Technical Bulletin #*TB117, Promega Corporation. (www.promega.com/tbs/tb117/tb117.html)
- *Wizard® SV 96 Plasmid DNA Purification System Technical Bulletin* #TB272, Promega Corporation. (www.promega.com/tbs/tb272/tb272.html)
- *Wizard® SV 9600 Plasmid DNA Purification System Technical Bulletin* #TB292, Promega Corporation. (www.promega.com/tbs/tb292/tb292.html)
- *Wizard® SV Gel and PCR Clean-Up System Technical Bulletin* #TB308, Promega Corporation. (www.promega.com/tbs/tb308/tb308.html)
- *Wizard® SV Genomic DNA Purification System Technical Bulletin* #TB302, Promega Corporation. (www.promega.com/tbs/tb302/tb302.html)
- *SV 96 Total RNA Isolation System Technical Bulletin #*TB294, Promega Corporation. (www.promega.com/tbs/tb294/tb294.html)

Kyle Hooper, Ph.D.
Product Manager

Ordering Information

Product	Size	Cat.#
Wizard® *Plus* SV Minipreps DNA Purification System*	50 preps	A1330
	250 preps	A1460
Wizard® SV 96 Plasmid DNA Purification System*	1 × 96 preps	A2250
	5 × 96 preps	A2255
Wizard® SV 9600 Plasmid DNA Purification System	100 × 96 preps	A2258
Wizard® SV 96 PCR Clean-Up System*	1 × 96 preps	A9340
	4 × 96 preps	A9341
	8 × 96 preps	A9342
Wizard® SV 96 Genomic DNA Purification System	1 × 96 preps	A2370
	4 × 96 preps	A2371
SV 96 Total RNA Isolation System*	1 × 96 preps	Z3500
	5 × 96 preps	Z3505

*For Laboratory Use.

(a) Australian Pat. No. 730718 and other patents pending.
(b) U.S. Pat. No. 5,981,235, Australian Pat. No. 729932 and other patents pending.
(c) U.S. Pat. Nos. 5,658,548, 5,808,041, Australian Pat. No. 689815 and other patents pending.
(d) U.S. Pat. No. 4,766,072.
(e) Licensed under one or both of U.S. Patent No. 5,487,993 and European pat. no. 0 550 693.
(f) 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.
(g) U.S. Pat. Nos. 5,324,637, 5,492,817, 5,665,563, Australian Pat. No. 660329 and other patents.
(h) 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.
(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.
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