

WIZARD® SV AND SV 96 GENOMIC DNA PURIFICATION SYSTEMS: HIGH-QUALITY GENOMIC DNA FROM NEURAL SAMPLES

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Abstract

This article introduces the new Wizard® SV and SV 96 Genomic DNA Purification Systems. These systems can be used to purify genomic DNA from mouse tail clippings, brain and other tissues, and tissue culture cells. We have developed the Wizard® SV 96 Genomic DNA Purification System (Cat.# A2370, A2371) to meet the need for high-throughput genomic DNA purification. This system can be used either in a manual format or automated on a liquid handling system such as the Beckman Biomek® 2000 Laboratory Automation Workstation. The Wizard® SV Genomic DNA Purification System (Cat.# A2360, A2361) is a simple and versatile spin or vacuum ("SV") format that provides a fast and simple method to isolate purified and intact genomic DNA in as little as 20 minutes. Genomic DNA can be purified from a variety of sample types with no detectable cross-contamination during the isolation procedure. Both systems provide similar yields of high-quality genomic DNA.

Introduction

The isolation and purification of genomic DNA is a key step for many molecular protocols in neuroscience research. Typically genomic DNA is purified from cell or tissue samples by mechanically disrupting the cell by homogenization or proteolysis, followed by phenol extraction. This process is often tedious, labor-intensive and potentially dangerous because of the use of toxic organic compounds. The Wizard® SV and SV 96 Genomic DNA Purification Systems simplify genomic DNA purification, providing a fast, practical technique for purifying intact genomic DNA that can be used directly in downstream applications without the need for further manipulation or "clean up".

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A high-throughput genomic DNA purification procedure should isolate high-quality DNA in a short amount of time without contamination between sample wells. To address these needs, we have developed the Wizard® SV 96 System, a high-throughput system that isolates genomic

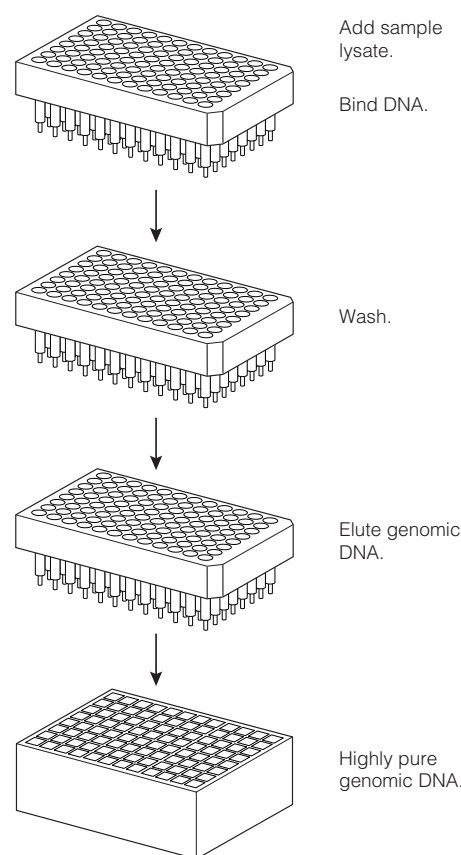


Figure 1. Genomic DNA isolation and purification using the Wizard® SV 96 Genomic DNA Purification System.

DNA from a variety of sample types. Genomic DNA is isolated in a 96-well format in approximately an hour either manually, using a multichannel pipettor, or by using an automated liquid handling system such as a Beckman Biomek® 2000 Laboratory Automation Workstation.

The "SV" Genomic DNA Isolation Procedure

The basic "SV" genomic DNA isolation procedure involves sample lysate preparation, capture of genomic DNA, washing to remove impurities, and eluting the purified DNA. Figure 1 illustrates the procedure using the Wizard® SV 96 Genomic DNA Purification System, and Figure 2 illustrates the procedure using the Wizard® SV Genomic DNA Purification System. Tissue samples such as mouse brain are prepared by performing an overnight digestion with Proteinase K (Cat.# V3021; not provided with system) at 55°C followed by lysis with Wizard® SV Lysis Buffer. Alternatively, tissue culture cells are washed with 1X PBS (not provided with system) and then lysed with Wizard® SV Lysis Buffer. The sample lysate is added to the column, and genomic DNA is captured on the

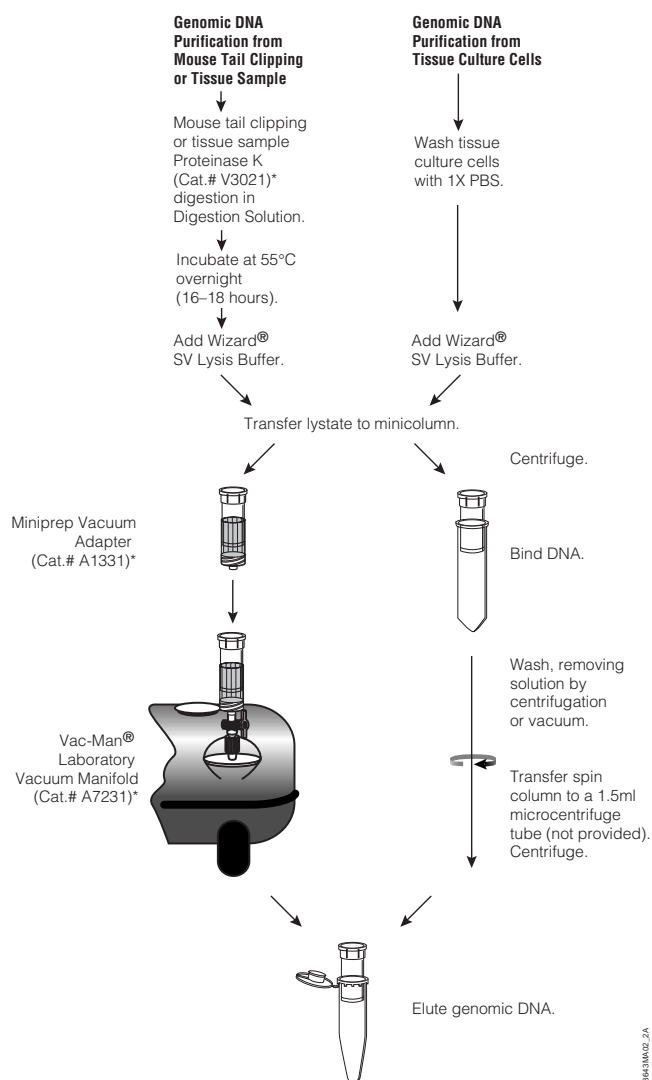


Figure 2. Overview of the Wizard® SV Genomic DNA Purification Spin and Vacuum Protocols. *May be purchased separately.

column as the lysate is pulled through by centrifugation (SV System) or vacuum (SV and SV 96 Systems). Following a series of washes, genomic DNA is eluted with Nuclease-Free Water. The purified genomic DNA is eluted into a 1.5ml microcentrifuge tube in the SV System or into a 96-Well Deep Well Plate in the SV 96 System. High-throughput genomic DNA purification with the SV 96 System uses a convenient vacuum manifold apparatus that does not require disassembly during the genomic DNA binding and wash steps. Filtrate waste products are delivered directly to a vacuum trap, eliminating the need to empty waste collection vessels.

Sample Analysis: Yield and Purity of Genomic DNA

Genomic DNA isolated from mouse tail clippings and tissue culture cells using the Wizard® SV and SV 96

Genomic DNA Purification Systems was analyzed for yield and purity by measuring sample absorbance at 260 and 280nm. Genomic DNA yield was calculated using A₂₆₀, and the purity was estimated from the A₂₆₀/A₂₈₀ ratio. High-quality genomic DNA exhibits an A₂₆₀/A₂₈₀ ratio of 1.7–2.0. Figure 3 shows the average yield and purity of genomic DNA obtained from mouse tail clippings using the spin, vacuum, or SV 96 protocols. Table 1 provides the average yield of genomic DNA isolated from 20mg mouse tail clippings, mouse liver, mouse heart, mouse brain tissue samples or tissue culture cells using each system.

Sample Analysis: Quality of Genomic DNA

The quality of genomic DNA isolated from either tissue culture cells or mouse tissue samples using the Wizard®

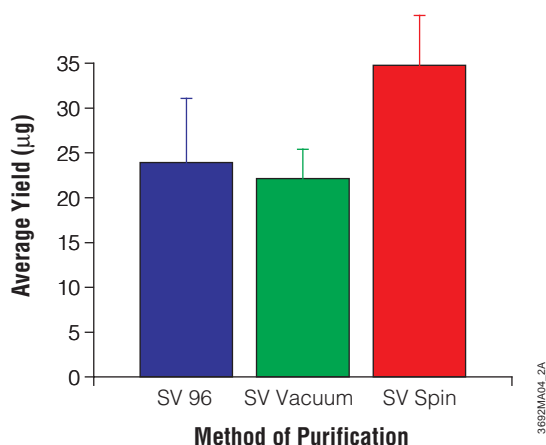


Figure 3. Comparison of DNA yields using the Wizard® SV and SV 96 Genomic DNA Purification Systems. Average yield of genomic DNA in micrograms purified from 20mg mouse tail clippings. The average A_{260}/A_{280} ratios are: SV 96, 1.7 ± 0.08 ; SV vacuum method, 1.7 ± 0.14 ; SV spin method, 1.7 ± 0.14 .

SV Genomic DNA Purification System was assessed by PCR amplification. One microliter of genomic DNA purified from 3×10^6 SH-SY5Y neuroblastoma cells was amplifiable by PCR using Factor V-specific primers (Figure 4). One microliter of purified genomic DNA from 20mg of mouse liver, mouse heart or mouse brain tissue was amplifiable by PCR using mouse-specific IL-1 β primers (Figure 5).

Quality of genomic DNA isolated using the Wizard® SV 96 Genomic DNA Purification System was similarly evaluated. One microliter of the eluted genomic DNA prepared from either mouse tail or tissue culture samples was amplified by PCR. Mouse tail samples were evaluated by amplifying the IL-1 β gene. HeLa cell samples were evaluated by amplifying Factor V DNA (Figure 6). All "SV"-purified genomic DNA samples were good templates for PCR amplification.

Automated Isolation of Genomic DNA

Using the Beckman Biomek® 2000, we have purified genomic DNA from both tissue culture cell samples and mouse tail clipping samples with the Wizard® SV 96 Genomic DNA Purification System. Once the deck of the Biomek® 2000 has been set up, simply start the Biomek® program. The Biomek® 2000 isolates genomic DNA from up to one plate of 96 samples in approximately 1 hour.

A potential limitation of this automated genomic DNA isolation system is the processing of viscous sample lysates. Sample lysates that contain too much cellular debris will not move easily into the Binding Plate and may even clog the plate. In this case, an optional centrifugation step to collect undigested material at the bottom of the digestion plate is necessary to prevent clogging of the Binding Plate.

Table 1. Average Yield of Genomic DNA Using the Wizard® SV and SV 96 Genomic DNA Purification Systems.

Sample Type	Starting Amount	Average Yield
Mouse Brain	20mg	6µg
Mouse Tail Clipping	20mg	20µg
Mouse Liver	20mg	15µg
Mouse Heart	20mg	10µg
SH-SY5Y Cells	3×10^6 cells	25µg
CHO Cells	1×10^6 cells	5µg
NIH3T3 Cells	1×10^6 cells	9µg
293 Cells	1×10^6 cells	8µg

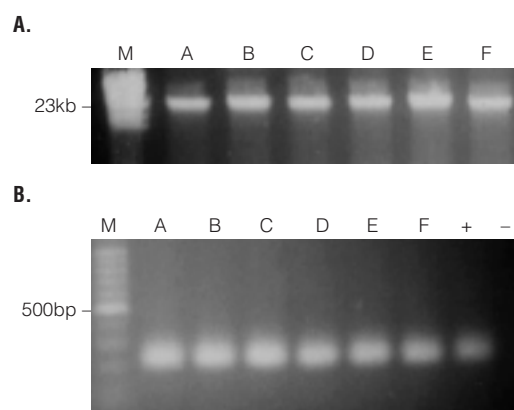


Figure 4. PCR amplification of DNA purified using the Wizard® SV Genomic DNA Purification System. Panel A. DNA was purified from 3×10^5 SH-SY5Y neuroblastoma cells, and 15µl of each genomic DNA isolation was visualized on a 1% agarose gel and stained with ethidium bromide. Six separate (lanes A–F) genomic DNA purifications; M = Lambda DNA *Hind* III Markers (Cat.# G1711). **Panel B.** Factor V amplification from 1µl of purified genomic DNA of each of the samples (Lanes A–F). The 250bp amplicon is visualized on a 1% agarose gel stained with ethidium bromide. M = 100bp DNA Ladder (Cat.# G2101).

Figure 7 illustrates typical yields and purity of DNA isolated from 96 separate 20mg mouse tail clippings using the SV 96 System procedure automated on a Beckman Biomek® 2000 workstation. Notice that the A_{260}/A_{280} ratio of the DNA isolated using this procedure usually falls between 1.7–2.0, indicating that the genomic DNA generated from this high-throughput protocol is of high quality.

Cross-contamination of the automated SV 96 purification system was tested by running an array of mouse tail clipping samples in a 96-well plate dispersed between

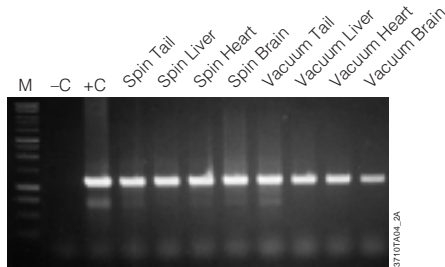


Figure 5. Amplification of genomic DNA isolated from various tissue sources. One microliter of purified genomic DNA was amplified using PCR Master Mix(a,b) (Cat.# M7502) and mouse-specific IL-1 β primers (1.2kb product). Reactions with Mouse Genomic DNA (Cat.# G3091; +C) and without DNA (-C) were performed as positive and negative controls, respectively. Thermal cycling conditions were: 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. All lanes contained 10 μ l of reaction product separated on a 1% agarose gel. PCR products were visualized by ethidium bromide staining. "Spin" and "Vacuum" designations indicate the protocol used for genomic DNA isolation.

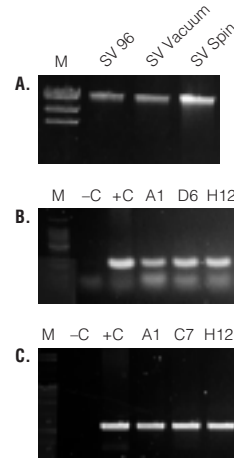


Figure 6. Purified genomic DNA sample analysis. Panel A. Genomic DNA purified from tissue culture cells. Ten microliters of a genomic DNA prepared using the Wizart® SV 96 System from 5×10^6 HeLa cells was analyzed on a 1% agarose gel. **Panel B.** Expected PCR product size for Factor V amplified from HeLa cells is approximately 250bp. Lanes: M, 100bp DNA Ladder; -C, negative control; +C, positive control; A1, D6, H12 sample well, 250bp Factor V amplification products. **Panel C.** PCR product visualized on a 1.5% agarose gel. Expected PCR product size of IL-1 β -amplified DNA from mouse tail is approximately 1.2kb. M, 1kb DNA ladder; -C, negative control; +C, positive control; A1, C7, H12 sample well, 1.2kb IL-1 β amplification products.

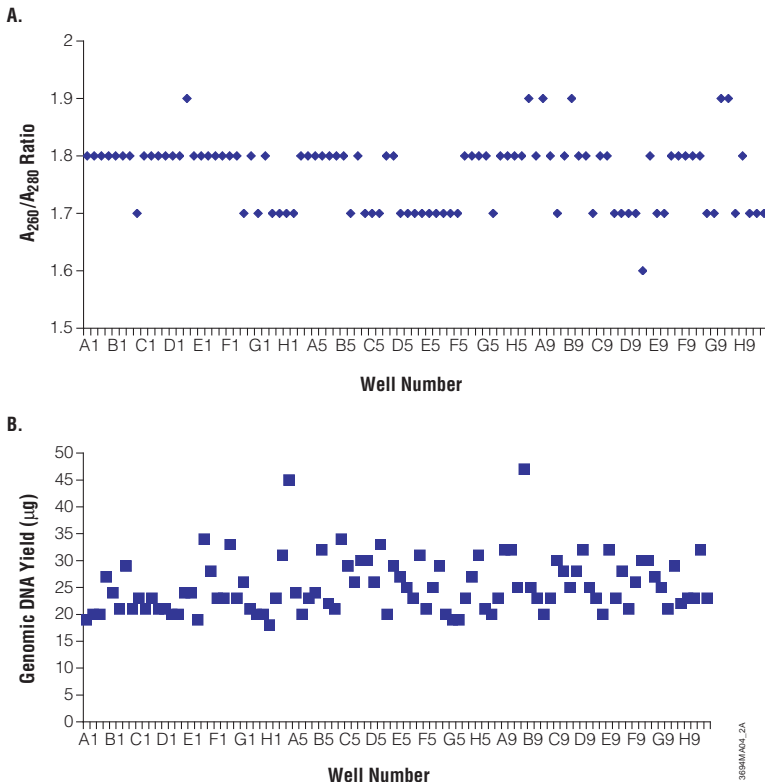


Figure 7. Purity (Panel A) and yield (Panel B) measurements from 96 separate 20mg mouse tail clippings using the Wizart® SV 96 Genomic DNA Purification System. Purity measured by A_{260}/A_{280} ratio and yield measured by absorbance at 260nm.

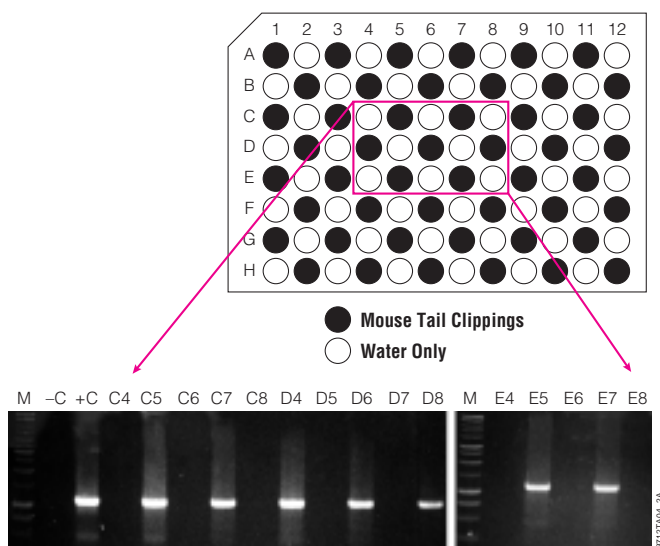


Figure 8. Cross-contamination assay. Genomic DNA purified from mouse tail clipping samples or water samples arrayed in adjacent wells of a 96-well plate. PCR products were amplified using mouse IL-1 β primers from 1 μ l of purified sample from each well. Ten microliters of PCR product were run on a 1.5% agarose gel and visualized by staining with ethidium bromide. Expected PCR product from mouse tail clippings for IL-1 β 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.

samples of water (negative control). Genomic DNA was purified from the arrayed plate both manually (data not shown) and by using the Beckman Biomek® 2000 workstation. Cross-contamination between samples was assayed by amplifying mouse IL-1 β DNA (Figure 8).

As the figure shows, there is no detectable cross-contamination of DNA into the wells containing only water. Automated genomic DNA purification using the Wizard® SV 96 Genomic DNA Purification System and the Beckman Biomek® 2000 workstation generates largely intact genomic DNA greater than 23kb in length. Genomic DNA purified from tissue culture cells may contain some contaminating RNA, but this can be removed by adding 1 μ l of RNase solution to eluted DNA or by adding RNase solution to the Nuclease-Free Water used for elution. The purified genomic DNA is amplifiable by PCR and suitable for many downstream applications. The processing time for isolating genomic DNA is approximately one hour of hands-off instrument time for 96 samples. Multiple sample types can be processed in one run without evidence of cross-contamination.

Summary

The Wizard® SV and SV 96 Genomic DNA Purification Systems provide high-quality genomic DNA in low-, medium-, or high-throughput research settings. These user-friendly systems consistently provide PCR-quality genomic DNA without the

need for further manipulation. These flexible systems will purify genomic DNA from a variety of sample types.

Protocols

Wizard® SV Genomic DNA Purification System Technical Bulletin #TB302, Promega Corporation.

www.promega.com/tbs/tb302/tb302.html

Wizard® SV 96 Genomic DNA Purification System Technical Bulletin #TB303, Promega Corporation.

www.promega.com/tbs/tb303/tb303.html

Ordering Information

Product	Size	Cat.#
Wizard® SV Genomic DNA Purification System	50 preps	A2360
	250 preps	A2361
Wizard® SV 96 Genomic DNA Purification System	1 × 96	A2370
	4 × 96	A2371

(a)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.

(b)U.S. Pat. No. 6,242,235 has been issued to Promega Corporation for polymerase stabilization by polyethoxylated amine surfactants. Other patents are pending.

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