Be a "Wizard" at Genomic DNA Purification

Introducing the Wizard® SV and SV 96 Genomic DNA Purification Systems

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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, tissue, 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 workstation. The Wizard® SV Genomic DNA Purification System (Cat.# A2360, A2361) is an easy and versatile spin or vacuum ("SV") format that provides a fast and simple way 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.

The Wizard® SV and SV 96 Genomic DNA Purification Systems simplify genomic DNA purification, providing a fast, practical technique for purifying DNA.

Introduction

The isolation and purification of genomic DNA is a key step for many molecular biology protocols. 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 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".

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 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.

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 tail clippings 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 column as the lysate is pulled through by centrifugation or vacuum. 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

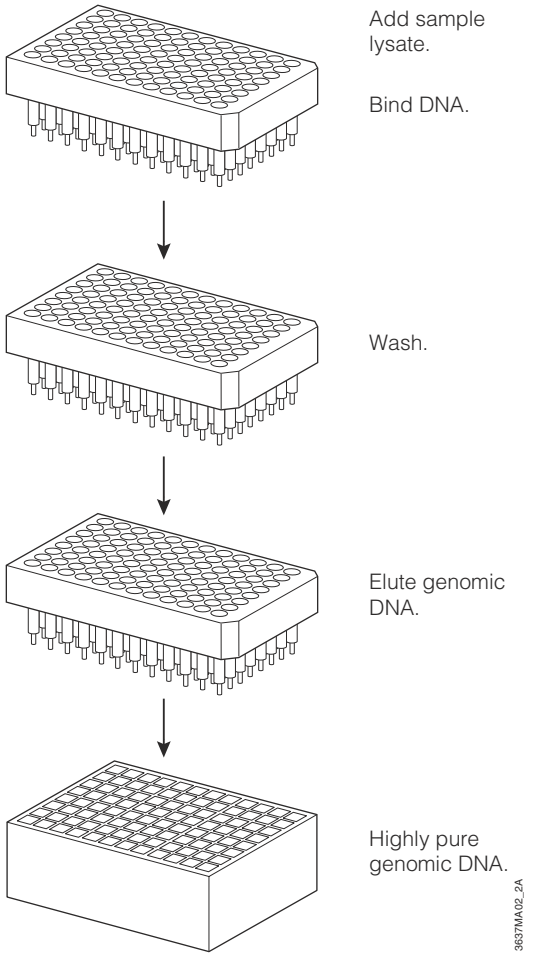

Figure 1. Genomic DNA isolation and purification using the Wizard® SV 96 Genomic DNA Purification System.

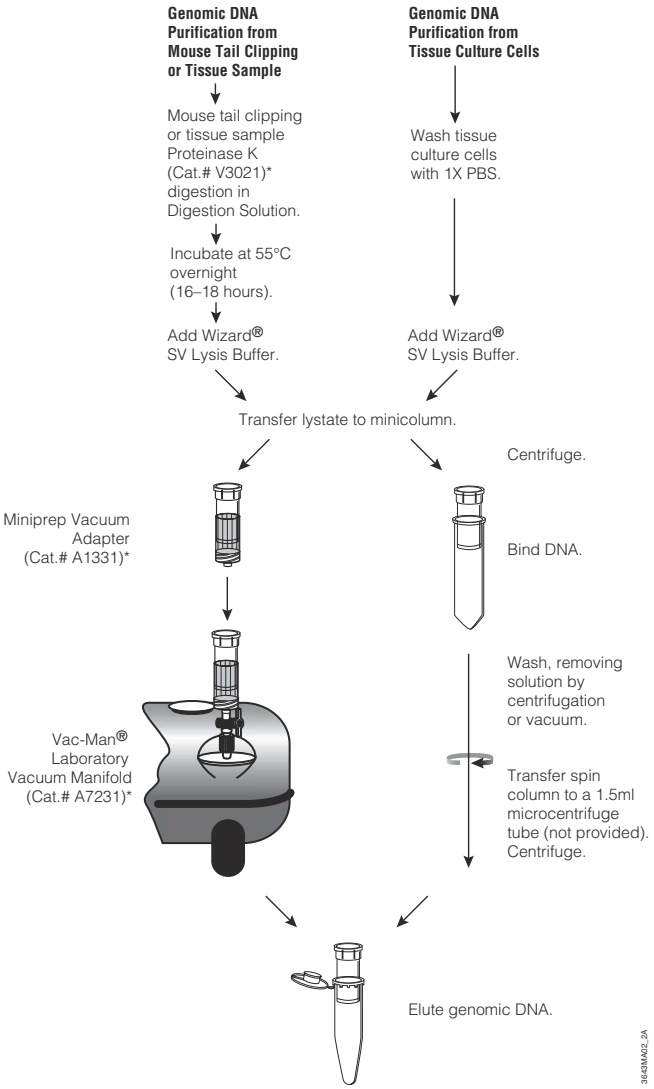

Figure 2. Overview of the Wizard® SV Genomic DNA Purification Spin and Vacuum Protocols. *May be purchased separately.

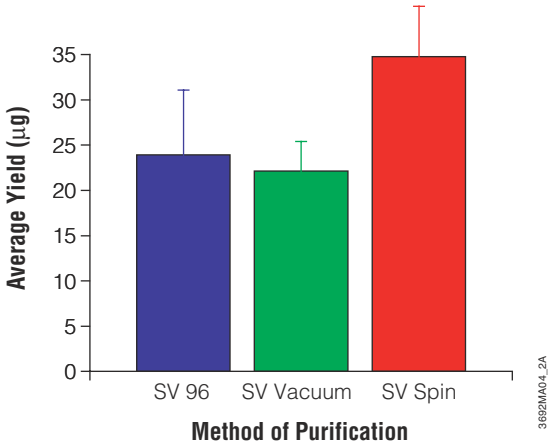

Figure 3. Comparison of DNA yields using the Wizard® SV and SV 96 genomic DNA Purification Systems. Average yield of genomic DNA in micrograms purified from 20mg mouse tail clippings. The average A_{260}/A_{280} ratios are: SV 96, 1.7 ± 0.08; SV vacuum method, 1.7 ± 0.14; SV spin method, 1.7 ± 0.14.

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

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

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 Isolated 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 A_{260}, and the purity was estimated from the A_{260}/A_{280} ratio. High-quality genomic DNA exhibits an A_{260}/A_{280} 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 or mouse tissue samples using the Wizard® SV Genomic DNA Purification System was assessed by PCR amplification. One microliter of genomic DNA purified from 1×10^5 HeLa 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-1β 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 IL-1β. HeLa cell samples were

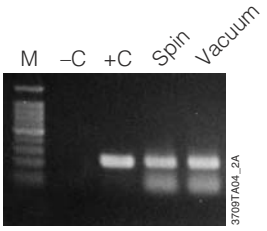

Figure 4. PCR amplification of DNA purified using the Wizard® SV Genomic DNA Purification System. DNA was purified from 1×10^5 HeLa cells, and 1µl of each genomic DNA isolation was amplified using Factor V-specific primers (250bp product). Lanes: –C, negative control, no DNA; +C, positive control, Human Genomic DNA (Cat.# G3041); Spin, genomic DNA purified using the spin protocol; Vacuum, genomic DNA purified using the vacuum protocol. Thermal cycling conditions were: One cycle of 3 minutes at 95°C; 35 cycles of: 30 seconds at 95°C, 1 minute at 60°C, 1 minute at 70°C; and a final extension of 7 minutes at 70°C. Ten microliters of each PCR product was resolved on a 2% agarose gel and visualized by ethidium bromide staining.

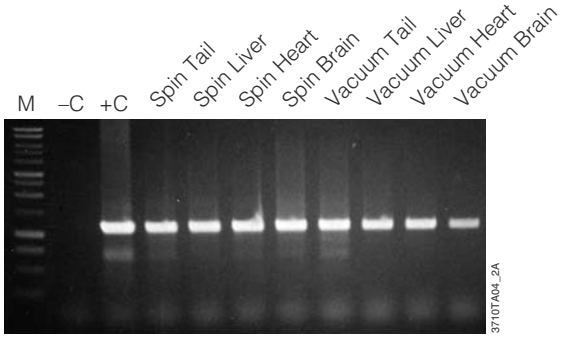

Figure 5. Amplification of genomic DNA isolated from various tissue sources. One microliter of purified genomic DNA was amplified using PCR Master Mix (Cat.# M7502) and mouse-specific IL-1β primers (1.2kb product). Reactions with Mouse Genomic DNA (Cat.# G3091; +C) and without DNA (–C) were performed 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 10µl 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.

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. Once the deck of the Biomek® 2000 has been set up (Figure 7), Start the Biomek® program (Table 2). 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 SV 96 Binding Plate and may even clog the plate. In this case, an optional centrifugation step to collect undigested material at the

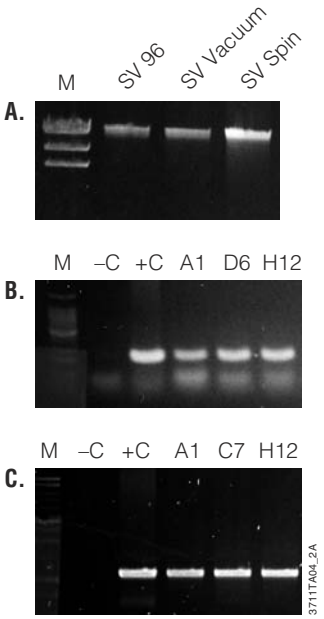

Figure 6. Purified genomic DNA sample analysis. Panel A: Genomic DNA purified from tissue culture cells. Ten microliters of a genomic DNA preparation from 5×10^6 HeLa cells was analyzed by agarose gel electrophoresis on a 1% gel and visualized by ethidium bromide staining. **Panels B and C:** PCR amplification of Factor V DNA from HeLa cell samples and mouse tail clippings. **Panel B:** Expected PCR product size for Factor V amplified from HeLa cells is approximately 250bp. Lanes: M, 100bp DNA Marker; –C, negative control; +C, positive control; A1, D6, H12 sample well 250bp Factor V amplification products. **Panel C:** PCR product run on a 1.5% agarose gel and visualized by staining with ethidium bromide. Expected PCR product size of IL-1β amplified DNA from mouse tail is approximately 1.2kb. M, 1kb DNA marker; –C, negative control; +C, positive control; A1, C7, H12 sample well 1.2kb IL-1β amplification products.

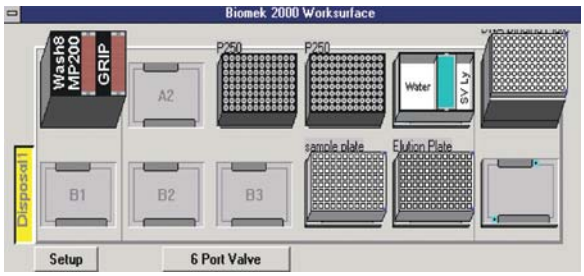

Figure 7. Initial deck configuration of the Biomek® 2000 for purification of genomic DNA. The tools required for the protocol are the Wash 8, MP200, and Gripper all placed at position A1. Positions A2, B1, B2, and B3 remain empty. At positions A3 and A4 are boxes of P250 tips. Position A5 contains a reservoir holding Nuclease-Free Water and Wizard® SV Lysis Buffer. The sample plate containing sample lysate is placed in position B4. (The sample plate shown here is for genomic DNA isolation from mouse tail clipping where a deep well plate contains tissue lysate from an overnight proteinase K digestion of the tissue.) A 96-Well Deep Well Plate for elution is placed at position B5. Position A6 contains a Beckman vacuum manifold, 65mm collar, and SV 96 Binding Plate stacked on top of each other. Position B6 has a collar holder that is used for holding the 65mm collar with the SV 96 Binding Plate during vacuum manifold disassembly and assembly for elution of purified genomic DNA. Additionally, port two of the Biomek® 2000 wash unit must be attached to a bottle containing the SV 96 Wash Solution.

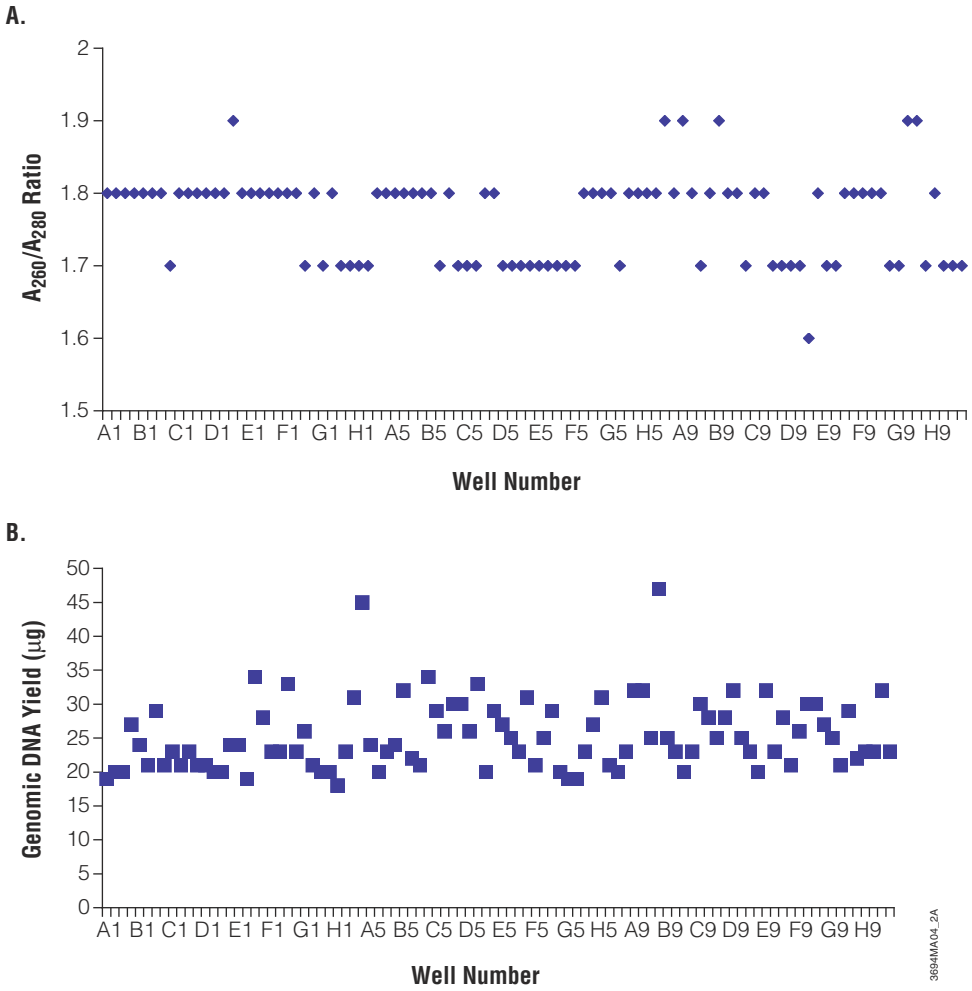

Figure 8. Yield (Panel A) and purity (Panel B) measurements from 96 separate 20mg mouse tail clippings using the Wizard® SV 96 Genomic DNA Purification System. Purity measured by A_{260}/A_{280} ratio and yield measured by absorbance at 260nm.

Table 2. Biomek® 2000 Genomic DNA Isolation Program.

Step 1.	The Biomek® 2000 adds Wizard® SV Lysis Buffer to the sample. For tissue culture cell genomic DNA purification, the Wizard® SV Lysis Buffer is added directly to adherent cells in a tissue culture plate placed in the sample plate position on the deck. For tissue samples, such as mouse tail clippings, the Wizard® SV Lysis Buffer is added to an overnight proteinase K digestion in a deep well plate (not provided) placed in the sample plate position on the deck.
Step 2.	The Biomek® 2000 then transfers the sample lysate from the 96-well sample plate to the SV 96 Binding Plate. The sample lysate is passed through the plate by vacuum, and the genomic DNA binds to the SV 96 Binding Plate.
Step 3.	The samples are washed with 3 washes of SV 96 Wash Solution using the Wash 8 tool.
Step 4.	The plate is dried briefly by vacuum to remove residual ethanol.
Step 5.	The vacuum manifold is then disassembled using the Gripper tool and reassembled for purified genomic DNA sample elution with a 96-Well Deep Well Plate for elution placed below the SV 96 Binding Plate.
Step 6.	Purified genomic DNA is eluted from the SV 96 Binding Plate into the elution plate with 250µl of Nuclease-Free Water for tissue culture sample and 500µl of Nuclease-Free Water for tissue samples, such as mouse tail clippings.

bottom of the plate containing the lysate is necessary to prevent clogging of the SV 96 Binding Plate.

Figure 8 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. 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 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-1β DNA (Figure 9).

As the figure shows, there is no detectable cross-contamination of DNA into the wells containing only water.

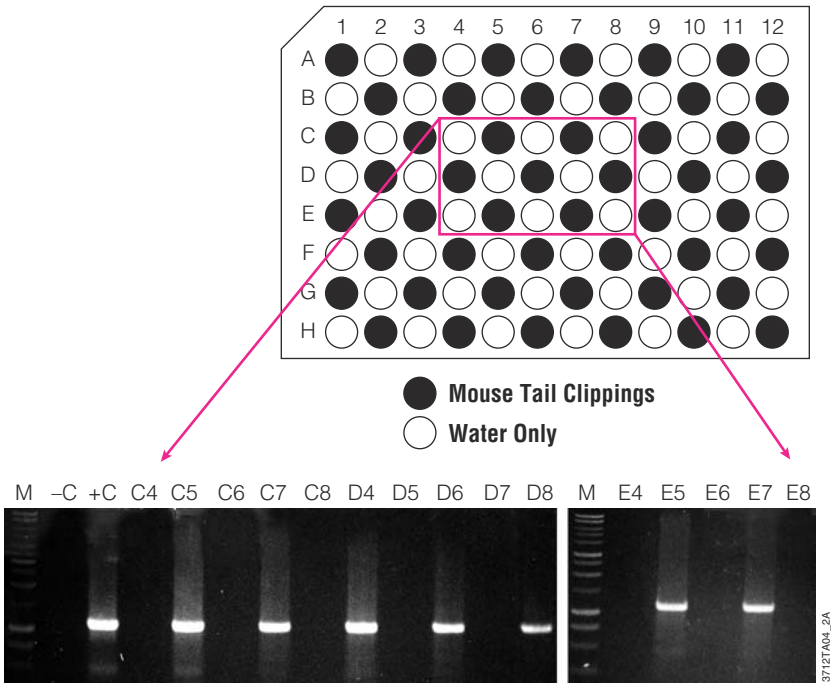

Figure 9. Cross-contamination assay. Genomic DNA purified from mouse tail clipping samples or water samples arrayed in adjacent wells of a 96-well plate. PCR products were amplified from 1µl of purified sample from each well for mouse IL-1β. Ten microliters of PCR product were run on a 1.5% agarose gel and visualized by staining with ethidium bromide. Expected PCR product from mouse tail clippings for IL-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.

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 1µl 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

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