

# Applications

Note 163 | June 2007

## Automated Genomic DNA Purification from Tissue Culture Cells using the Promega Wizard® SV 96 Genomic DNA Purification System and the Eppendorf epMotion® 5075 VAC

*Sarah Shultz, Promega Corporation, 2800 Woods Hollow Road, Madison, Wisconsin, 53711 USA*

### Abstract

Purification of genomic DNA from tissue culture cells in a high-throughput method is essential for many downstream applications in molecular biology. In this report, we have developed an automated method to meet the need for high-throughput genomic DNA purification on the Eppendorf® epMotion® 5075 VAC. Genomic DNA is purified from tissue culture cells with no detectable cross-contamination during the automated isolation procedure. Genomic DNA is then analyzed for yield and purity by measuring sample absorbance at 260 nm and 280 nm, amplified to confirm compatibility for downstream PCR amplification (1.2 kb IL-1B), and compared to genomic DNA extracted from the same tissue culture cells using a manual method.

### Introduction

High-throughput isolation and purification of genomic DNA is an essential step for many researchers in molecular biology. Genomic DNA is traditionally purified from cultured cell samples by mechanically disrupting the cells by proteolysis, followed by phenol extraction. This process can be tedious, labor-intensive and hazardous because of the use of toxic organic compounds. Thus, the development of a simple, hands-free, high-throughput genomic DNA purification procedure capable of isolating high-quality DNA in a relatively short amount of time without contamination between samples is desirable.

The Promega Wizard SV 96 Genomic DNA Purification System provides a high-throughput, membrane-based

technique for consistent preparation of genomic DNA from tissue culture cells. Amplifiable genomic DNA can be isolated from up to  $5 \times 10^6$  cells, without a centrifugation clearing step. The SV 96 genomic DNA isolation procedure involves sample lysate preparation, capture of genomic DNA, washing to remove impurities, and eluting the purified DNA.

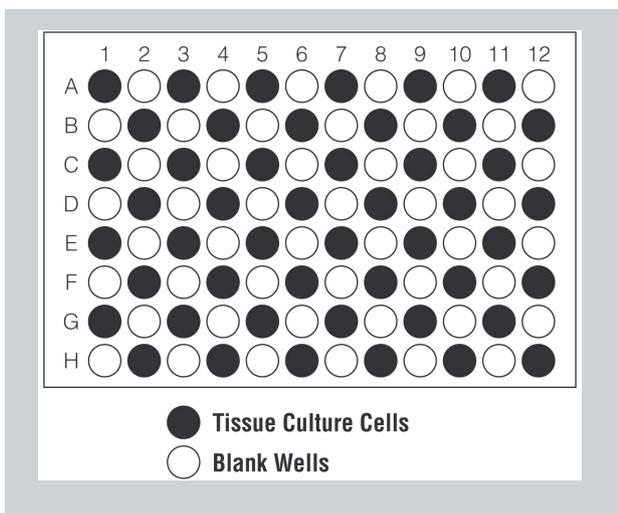
The Eppendorf epMotion 5075 VAC is a flexible automated pipetting system, that can be adapted to various liquid handling tasks. The concept of exchangeable tips and the "Free Jet Dispensing" technology enables this workstation to dispense liquids contact-free and makes it ideal for implementing complex work steps where precision and reliability are required.

**Materials and Methods**

- Eppendorf epMotion 5075 VAC equipped as follows:
- Gripper
- Dispensing tools TM1000-8 and TM300-8
- Vacuum with manifold
- Reservoir Rack
- Height spacers 85 mm and 35 mm
- Vac Frame 2
- Waste Tub
- Eppendorf consumables
- 5 x 100 ml Reagent Reservoirs
- 1 x 1000 µl epTIPS Motion Filtered Tips
- 1 x 300 µl epTIPS Motion Filtered Tips
- Promega Wizard SV 96 Genomic DNA Purification System
- Promega 1.2 ml 96-Well Round-Bottom Deep Well Plate (Elution Plate)
- 96-Well Tissue Culture Plate (Sample Plate)
- Fresh tissue culture cells
- Sterile 1x Phosphate Buffered Saline (PBS)

**Cell and Reagent Preparation**

Two common tissue culture cell lines, HEK-293 and NIH-3T3, were prepared at 1x10<sup>5</sup> cells per well and placed in 96-Well Tissue Culture Plates (Corning, USA) in a checkerboard pattern, as shown in Figure 1.



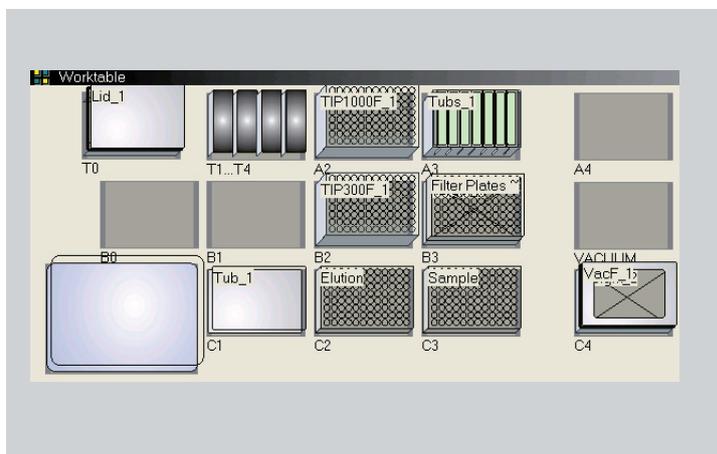
**Figure 1:** Sample Plate checkerboard layout.

Every other well was left empty to serve as a control for method cross-contamination. Reagents were prepared and dispensed into five 100 ml Reagent Reservoirs, as described in Table 1.

**Table 1:** Contents of the Reagent Reservoirs in the Reservoir Rack.

Reservoir Contents	Comments
20ml Wizard® SV Lysis Buffer	
90ml Wizard® SV Wash Solution with EtOH	Add 95% EtOH to the Wizard® SV 96 Wash Solution bottle as directed on the bottle label before dispensing.
90ml Wizard® SV Wash Solution with EtOH	
90ml Wizard® SV Wash Solution with EtOH	
30ml Nuclease-Free Water	

Labware was placed onto the epMotion 5075 VAC worktable, as shown in Figure 2 and Table 2. The automated method was then started.



**Figure 2:** Screenshot from the epMotion Editor showing the epMotion 5075 VAC setup for the Automated Wizard SV 96 Genomic DNA Tissue Culture Method.

**Table 2:** epMotion 5075 Worktable setup details by position.

Position	Labware
A2	1000µl epTIPS Motion Filtered Tips
A3	Reservoir rack with 5 Reagent Reservoirs
A4	Empty
B0	Empty
B1	Empty
B2	300µl epTIPS Motion Filtered Tips
B3	Filter Plate sitting atop 85mm Height Spacer
Vacuum	Empty
C1	Waste Tub with quarter wall separators
C2	Elution Plate: 96-Well 2.2ml Square-Bottom Deep Well Plate
C3	Sample Plate: 96-Well Tissue Culture Plate
C4	Vac Frame 2 atop 35mm Height Spacer

### Automated Method Overview

The automated method begins by dispensing 150  $\mu$ l Lysis Buffer to each well of the Sample Plate. The dispensing tool mixes and transfers the cell lysates to a Filter Plate atop the vacuum manifold apparatus. A vacuum is then applied and lysates are pulled through the Filter Plate. During this step, genomic DNA binds to Filter Plate.

The dispensing tool next dispenses 810  $\mu$ l Wash Solution to each well of the Filter Plate. A vacuum is applied and the Wash Solution is pulled through the Filter Plate. This step is repeated for a total volume of 2.4 ml of Wash Solution per well. After washing, the vacuum remains on for an additional 10 minutes to remove any residual Wash Solution from the Filter Plate. Once the Filter Plate is dry, the Gripper tool disassembles the vacuum manifold stack by moving the Vac Frame 2 and Filter Plate from the manifold to a holding position. The Gripper tool next moves the Elution Plate into the vacuum manifold, to replace the Waste Tub. Then, the Gripper tool reassembles the vacuum manifold stack by moving the Vac Frame 2 and Filter Plate back top the vacuum.

After reassembly, the dispensing tool transfers 200  $\mu$ l Elution Buffer to each well of the Filter Plate. A final vacuum is applied and the Elution Buffer is pulled through the Filter Plate while eluting genomic DNA. At the end of the automated method, purified genomic DNA from the original tissue culture cells is eluted into the Elution Plate. The total processing time for 96 samples using the automated method is just under 1 hour.

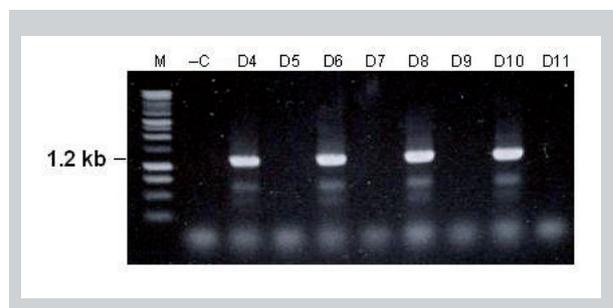
### Analysis of Purified Genomic DNA

Downstream analyses of the purified genomic DNA samples were performed. One microliter samples from the Elution Plate were placed directly onto a ND-1000 Spectrophotometer (Nanodrop, USA). Average yield (A260) and purity (A260/A280) measurements were taken. For eluates purified from NIH-3T3 cells, 1  $\mu$ l purified genomic DNA samples were also PCR amplified using Promega's PCR Master Mix, Cat.# M712B (Promega, USA) and IL-1 $\beta$  primers (Integrated DNA Technologies, USA). A negative control reaction comprised of Elution Buffer only was included in the analysis. The PCR thermal cycling conditions were as follows: 2 minutes at 94  $^{\circ}$ C; followed by 40 cycles of 94  $^{\circ}$ C for 30 seconds, 58  $^{\circ}$ C for 30 seconds, and 72  $^{\circ}$ C for one minute; final extension at 72  $^{\circ}$ C for 5 minutes. After PCR amplification, 10  $\mu$ l of each reaction product was separated on a 1.2 % agarose gel and visualized by ethidium bromide staining.

For additional comparative analysis, the Promega Wizard SV 96 Genomic DNA Purification System was also performed manually using HEK-293 tissue culture cells at  $1 \times 10^5$  cells per well. Cells were prepared in the same checkerboard pattern as described above. Refer to Promega TB#303 for details on the manual processing procedure.

### Results and Discussion

Use of the automated method described in this article incorporating the Promega Wizard SV 96 Genomic DNA Purification System to isolate genomic DNA from tissue culture cells using the epMotion 5075 VAC results in high-quality genomic DNA compatible with downstream PCR amplification. Successful amplification of IL-1 $\beta$  from 1  $\mu$ l purified genomic DNA samples isolated from NIH-3T3 tissue culture cells is shown in Figure 3, lanes D4, D6, D8, and D10.



**Figure 3:** Genomic DNA isolated from NIH-3T3 tissue culture cells using the Wizard SV 96 gDNA Tissue Culture Method on the epMotion 5075 VAC. PCR products were amplified from 1  $\mu$ l of purified sample from each well for IL-1 $\beta$ . Ten microliters of PCR product was run on a 1.2 % agarose gel and visualized by staining with ethidium bromide. Expected PCR product for IL-1 $\beta$  is approximately 1.2 kb, as shown in lanes D4, D6, D8, and D10. No PCR product is expected from blank samples, as shown in lanes D5, D7, D9, and D11, as well as in the negative control lane -C which contained only Elution Buffer. A marker standard was included for size estimation, lane M (Promega BenchTop 1 kb DNA Ladder).

Ten microliters of each PCR product was run on a 1.2 % agarose gel and visualized by staining with ethidium bromide. No cross-contamination between wells of the same Elution Plate was observed, as shown on lanes D5, D7, D9, and D11. A negative control consisting of amplified Elution Buffer shows no amplified product in lane -C, as expected. A marker standard lane M, BenchTop 1 kb DNA Ladder (Promega, USA), was included for size estimation. Expected PCR products size for IL-1 $\beta$  is approximately 1.2 kb.

Genomic DNA purified using this automated method is also of quality yield and purity, as determined by spectrophotometer absorbance measurements at 260 nm and 280 nm. In addition, DNA yields are comparable to those prepared manually. This can be seen in the data presented in Table 3. Spectrophotometer measurements collected from 24 genomic DNA samples processed using the automated and manual methods isolated from HEK-293 tissue culture cells show comparatively equal yields between methods from wells containing  $1 \times 10^5$  cells ( $1.0 \pm 0.2 \mu\text{g}$  and  $0.9 \pm 0.2 \mu\text{g}$ , respectively). Expected average DNA yield is approximately  $0.8 \mu\text{g}$  for the 293 cell line at this density. Spectrophotometer measurements also display excellent genomic DNA purity (A260/A280) for both methods

( $1.8 \pm 0.1$  and  $1.9 \pm 0.2$ , respectively). Expected average purity (A260/A280) is  $\geq 1.7$ . Finally, further support that no cross-contamination exists between wells of the same Elution Plate was evidenced in Table 3, as the average DNA yield for both Blank wells groups analyzed were approximately zero ( $0.1 \pm 0.1 \mu\text{g}$  and  $0.0 \pm 0.1 \mu\text{g}$ , respectively).

This simple, high-throughput, automated genomic DNA purification procedure on the epMotion 5075 VAC successfully isolates high-quality DNA in less than one hour without contamination between samples. This method nicely fits the needs of researchers requiring considerable sample sets of genomic DNA for downstream applications in molecular biology.

**Table 3:** Spectrophotometer measurements collected from 24 samples processed using the Automated Wizard SV 96 Genomic DNA Tissue Culture Method on the epMotion 5075 VAC and manual Wizard SV 96 Genomic DNA Purification System. Expected average DNA yield from wells containing  $1 \times 10^5$  293 cells is approximately  $0.8 \mu\text{g}$ . Expected average purity (A260/A280) is  $\geq 1.7$ .

		Automated			Manual		
		DNA ( $\mu\text{g}$ )	260/280	ng/ $\mu\text{l}$	DNA ( $\mu\text{g}$ )	260/280	ng/ $\mu\text{l}$
<b><math>1 \times 10^5</math> 293 cells (n=12)</b>	<b>AVG</b>	1.0	1.8	4.5	0.9	1.9	4.1
	STDEV	0.2	0.1	1.1	0.2	0.2	0.9
<b>Blank Wells (n=12)</b>	<b>AVG</b>	0.1	1.0	0.3	0.0	0.1	-0.2
	STDEV	0.1	2.2	0.3	0.1	0.6	0.5

## Eppendorf Ordering Information

Product		Order no. international	Order no. North America
epMotion 5075 VAC 230V	With integrated vacuum station	5075 000.016	N/A
epMotion 5075 VAC 120V		5075 000.164	960020014
Dispensing tool TM 1000-8	8-channel dispensing tool for the volume range from 40 to 1000 µl	5280 000.258	960001061
Dispensing tool TM 300-8	8-channel dispensing tool for the volume range from 20 to 300 µl	5280 000.231	960001052
Reservoir rack	for use with 30 mL and 100 mL reagent reservoirs	5075 754.002	960002148
Height adapter 85 mm		5075 751.003	960002105
Height adapter 55mm		5075 752.000	960002113
Height adapter 40 mm		5075 755.009	960002121
Vac Frame 2	Frame to adapt filter plates to the vacuum manifold	5075 785.005	960002261
epMotion Reservoir 100 mL		0030 126.513	960051017
epTIPS Motion 1000 µL, Filter	Volume range 40 - 1000 µL, 15 x 96 Filtertips in racks, PCR clean	0030 003.993	960050100
epTIPS Motion 300 µL, Filter	Volume range 20 - 300 µL, 15 x 96 Filtertips in racks, PCR clean	0030 003.977	960050061

Wizard is a registered trademark of Promega Corp., Madison WI, USA

## Promega Ordering Information

Product		Order no. international	Order no. North America
Promega Wizard SV 96 Genomic DNA Purification System		A2371	
1.2 ml, Round-Bottom Deep Well Plates		V6771	
PCR Master Mix		M712B	
BenchTop 1 kb DNA Ladder		G754A	



**eppendorf**  
In touch with life

Your local distributor: [www.eppendorf.com/worldwide](http://www.eppendorf.com/worldwide)

Eppendorf AG · 22331 Hamburg · Germany · Tel. +49 40 53801-0 · Fax +49 40 538 01-556 · E-Mail: [eppendorf@eppendorf.com](mailto:eppendorf@eppendorf.com)  
Eppendorf North America Inc. · One Cantiague Road, P.O. Box 1019 · Westbury, N.Y. 11590-0207 USA  
Tel. 516 334 7500 · Toll free phone 800-645-3050 · Fax +1 516 334 7506 · E-Mail: [info@eppendorf.com](mailto:info@eppendorf.com)

## Application Support

Europe, International: Tel. +49 1803 666 789 · E-Mail: [support@eppendorf.com](mailto:support@eppendorf.com)  
North America: Tel. 800 645 3050 ext. 2258 · E-Mail: [support\\_NA@eppendorf.com](mailto:support_NA@eppendorf.com)  
Asia, Pacific: Tel. +603 8023 2769 · E-Mail: [support\\_AsiaPacific@eppendorf.com](mailto:support_AsiaPacific@eppendorf.com)