Applications

Note 178 | May 2008

High-throughput automated genomic DNA purification from mouse tail clippings using the Promega Wizard® SV 96 Genomic DNA Purification System on the epMotion® 5075 VAC automated pipetting system

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

Abstract

In this article, a fast and easy-to-use automated high-throughput genomic DNA isolation method on the Eppendorf ep*Motion* 5075 VAC automated pipetting system is presented. Specifically, the Promega Wizard® SV 96 Genomic DNA Purification System is used to successfully isolate high-quality genomic DNA from mouse tails clippings with comparable yields to manual and other automated isolation techniques. Genomic DNA purified in this manner exhibits no detectable cross-contamination between wells of the same 96-well plate and is compatible directly with downstream PCR amplification.

Introduction

Purification of genomic DNA from mouse tail clippings is important for many downstream applications in molecular biology. Automating this procedure is particularly helpful for researchers. Automation must be timely, precise, and consistent, free from cross-contamination, and produce a pure product of expected yield that can be used directly in downstream applications.

The Wizard SV 96 Genomic DNA Purification System provides a simple membrane-based technique for consistent preparation of genomic DNA from mouse tails. Amplifiable genomic DNA can be isolated from up to 1.2 cm long clippings (20 mg), without a centrifugation clearing step. The SV 96 technology is based on the principle that nucleic acids bind to silica in the presence of chaotropic salts. Once cells or tissues have been disrupted, released nucleic acids bind to the silica membrane of a SV 96 Filter Plate. Subsequently, the bound nucleic acids are washed to remove salt and eluted in nuclease-free water.

The Wizard SV 96 Genomic DNA Purification System is designed for high-throughput genomic DNA isolation in a 96-well format. Therefore, the system can be nicely used with automation, including the Eppendorf ep*Motion* VAC automated pipetting system, in addition to being performed manually. Automating this system enables users to simply setup the worktable labware, start the automated method, and walk-away. This frees up lab personnel from the bench and provides the precision and consistency required for larger sample batches.

The Eppendorf ep*Motion* 5075 liquid handling system is very easy to use and provides the necessary reagent dispensing and vacuum manifold steps for the SV 96 system. The ep*Motion* has an integrated vacuum manifold for vacuum filtration, a gripper for plate transport and reconfiguration of the vacuum chamber, and multiple dispensing tools for precise transfer of liquids in the volume range from 1 µl to 1 ml.

eppendorf

Materials and Methods

- **Eppendorf ep*Motion* 5075 VAC equipped with:**
  - Gripper
  - Dispensing tools TM1000-8
  - Vacuum with manifold
  - Reservoir Rack
  - Height adapters 85 mm and 40 mm
  - Vac Frame 2
  - Waste Tub
- **Eppendorf consumables**
  - Reagent Reservoirs: 5 x 100 ml
  - epT.I.P.S. Motion Filtertips: 2 x 1000 µl
- **Promega Wizard SV 96 Genomic DNA Purification System**
- Promega Wizard SV 96 Genomic DNA Purification System
- Promega 1.2 ml 96-Well Round-Bottom Deep Well Plate (Elution Plate)
- Promega 2.2 ml 96-Well Square-Bottom Deep Well Plate (Sample Plate)
- Frozen Mouse Tails
- Proteinase K

Mouse Tail Preprocessing

Mouse tail tip clippings are sectioned 0.5 to 1.2 cm in length (approximately 15-20 mg), and each section cut into two smaller equally sized pieces. The pieces are placed into a 96-well, deep well plate in a checkerboard pattern, as shown in Figure 1.

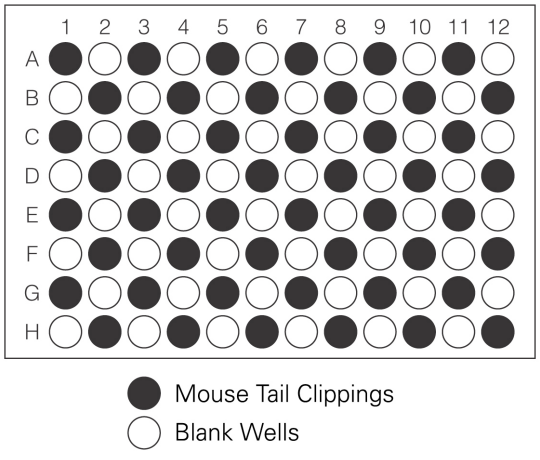

Figure 1: Sample plate layout, Checkerboard pattern.

Every other well is left empty to serve as a control for method cross-contamination. A volume of 275 µl Digestion Solution Master Mix containing Proteinase K [22 ml Nuclei Lysis Solution, 5.5 ml 0.5 M EDTA (pH 8.0), 2.2 ml proteinase K (20 mg/ml), 550 µl RNase A Solution (4 mg/ml)] is added to each well of the 96-well deep well plate using a multichannel pipettor. The plate is covered with an aluminum foil adhesive seal and placed in a 55 °C water bath to incubate overnight (16 to 18 hours).

Reagent Preparation and Worktable Setup

Following overnight incubation of the mouse tail sections, reagents are prepared and dispensed into five 100 ml reagent reservoirs, as described in Table 1.

Reservoir Rack Position	Reservoir Size	Reservoir Contents	Reagent Preparation Instructions*
1	100 ml	40 ml Wizard SV Lysis Buffer	Add 1 ml BME to 50 ml RNA Lysis Buffer
2	100 ml	90 ml Wizard SV Wash Solution with EtOH	Add 312.5 µl Nuclease-Free Water to DNase I and swirl gently to mix
3	100 ml	90 ml Wizard SV Wash Solution with EtOH	Add 20 ml 95 % EtOH to DNase Stop Solution
4	100 ml	90 ml Wizard SV Wash Solution with EtOH	Add 95 % EtOH to the RNA Wash Solution bottle as directed on the label
5	100 ml	30 ml Nuclease-Free Water	

Table 1: Contents of the five Reagent Reservoirs in the Reservoir Rack

All remaining labware is then placed onto the ep*Motion* 5075 worktable, as shown in Figure 2 and Table 2.

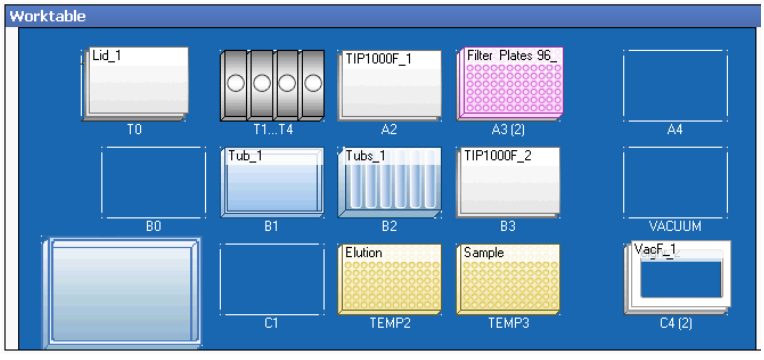

Figure 2: Screenshot from the epMotion Editor showing the ep*Motion* 5075 VAC worktable setup for the Automated Wizard SV 96 Genomic DNA Mouse Tail Method.

Table 2: ep*Motion* 5075 Worktable setup, details by position.

Worktable Position	Labware
A2	1000 µl epT.I.P.S. Motion Filtertips
A3	300 µl epT.I.P.S. Motion Filtertips
A4	Empty
B0	Empty
B1	Empty
B2	Reservoir Rack with 5 Reagent Reservoirs
B3	1000 µl epT.I.P.S. Motion Filtertips
Vacuum	Empty
C1	Waste Tub with quarter wall separators
C2	Elution Plate: 96-Well 1.2 ml U-Bottom Deep Well Plate
C3	Sample Plate: 96-Well 2.2 ml Square-Bottom Deep Well Plate
C4	Vac Frame 2 atop 35 mm Height Spacer

The aluminum foil seal on the deep well plate containing digested mouse tails can be carefully removed and the plate placed onto the worktable at position C3. The automated method can then be started.

Automated Method Overview

The automated method begins by dispensing 275 µl Lysis Buffer to each well of the plate containing digested mouse tails. Cell lysates are mixed thoroughly and transferred to the SV 96 Filter Plate atop the vacuum manifold. A vacuum is applied and lysates are pulled through the filter plate in each well. During this step, nucleic acids bind to the silica membrane in the filter plate. The vacuum process eliminates the need for centrifugation steps.

The dispensing tool next dispenses 810 µl Wash Solution to each well of the Filter Plate. The Wash Solution is a low salt ethanol-based wash to remove any contaminants such as proteins, salts or other cellular impurities. The wash steps occur a total of three times while the filter plate remains on the vacuum, thus no disassembly of the manifold to remove filtrate waste is required during binding and wash steps. The vacuum remains on for an additional 6 minutes to remove any residual ethanol from the filter plate.

The gripper disassembles the vacuum manifold by moving the binding plate from the vacuum to a holding position. The gripper next places an elution plate inside the vacuum manifold and replaces the SV 96 Filter Plate on top. This allows the eluate flow through to be collected in an elution plate. Nuclease-free water (500 µl) is added to each well of the filter plate and incubates at room temperature to release the nucleic acids.

A final vacuum is applied and eluates are pulled through the SV 96 Filter Plate, eluting the genomic DNA to collect into the final elution plate.

At the end of the automated method, purified genomic DNA from the original mouse tail clippings is eluted into the Elution Plate. The total processing time for the automated method is less than 1 hour for up to 96 samples.

Analysis of Genomic DNA Purified Using the Automated Method

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 (A_{260}) and purity ($A_{260/A280}$) measurements were taken.

One µl purified genomic DNA samples were also PCR amplified using Promega's PCR Master Mix (Promega, USA) and IL-1ß 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 °C; followed by 40 cycles of 94 °C for 30 seconds, 58 °C for 30 seconds, and 72 °C for one minute; final extension at 72 °C for 5 minutes. After PCR amplification, 10 µl of each reaction product was separated on a 1.2 % agarose gel and visualized by ethidium bromide staining.

For comparative analysis, the Promega Wizard SV 96 Genomic DNA Purification System was also performed manually using mouse tails arrayed in the same checkerboard pattern and preprocessed in the same manner as described above. Refer to Promega TB#303 for details on the manual processing procedure.

Results and summary

Use of the automated Wizard SV 96 Genomic DNA Purification System method to isolate genomic DNA from mouse tail clippings on the epMotion 5075 VAC results in high-quality genomic DNA of expected yield, compatible with downstream PCR amplification.

Average genomic DNA yield and purity absorbance measurements are presented in Table 3. Genomic DNA samples isolated from sectioned mouse tail clippings (20 ± 1.1 mg) processed using the automated and manual methods show equal yields (23.91 ± 5.54 µg and 23.00 ± 4.12 µg, respectively). Expected DNA yield is approximately 20 µg for 20 mg mouse tail clippings. Absorbance measurements (A_{260}/A_{280}) also display genomic DNA purity for both automated (1.85 ± 0.05) and manual (1.78 ± 0.18) methods.

Expected purity (A_{260}/A_{280}) is ≥1.70 for this sample type. Support that no cross-contamination exists between wells of the same plate is evidenced in Table 3, as the average DNA yield for both Blank Wells groups analyzed were approximately zero (0.08 ± 0.17 µg and 0.40 ± 0.31 µg).

This simple, automated high-throughput genomic DNA purification procedure on the epMotion 5075 VAC automated pipetting system successfully isolates high-quality DNA in a relatively short amount of time without contamination between samples. This method nicely fits the needs of researchers requiring considerable sample sets of genomic DNA from mouse tail clippings for downstream applications in molecular biology.

Table 3: Average genomic DNA purity and yield recovered using manual and automated (epMotion 5075 workstation) Wizard SV 96 Genomic DNA Purification System

	Automated			Manual		
	Amount	DNA Yield	DNA Purity A_{260}/A_{280}	Amount	DNA Yield	DNA Purity A_{260}/A_{280}
Mouse Tails	20 ± 1.1 mg	23.91 ± 5.54 µg	1.85 ± 0.05	20 ± 1.4 mg	23.00 ± 4.12 µg	1.78 ± 0.18
Blank Wells	0 ± 0 mg	0.08 ± 0.17 µg	0.40 ± 1.63	0 ± 0 mg	0.40 ± 0.31 µg	2.81 ± 4.50

Eppendorf Ordering Information

Product		Order no. international	Order no. North America
epMotion 5075 VAC	With integrated vacuum station	5075 000.016	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

Promega Ordering Information

Product	Cat.#.
Promega Wizard SV 96 Genomic DNA Purification System	A2370 and A2371
Proteinase K	V3021
Round-Bottom Deep Well Plate, 1.2 ml	V6771
Square-Bottom Deep Well Plate, 2.2 ml	AB0932
PCR Master Mix	M712B
BenchTop 1kb DNA Ladder	G754A

Promega

eppendorf

In touch with life

Your local distributor: 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

Eppendorf North America, Inc. · One Cantiague Road, P.O. Box 1019 · Westbury, N.Y. 11590-0207 · USA

Tel: +1 516 334 7500 · Toll free phone: +1 800-645-3050 · Fax: +1 516 334 7506 · E-mail: info@eppendorf.com

Application Support Europe, International: Tel: +49 1803 666 789 · E-mail: support@eppendorf.com

North America: Tel: +1 800 645 3050 ext. 2258 · E-mail: support_na@eppendorf.com

Asia Pacific: Tel: +60 3 8023 6869 · E-mail: support_asiapacific@eppendorf.com

Order-No. AA1 78PW 020/GB1/0508/1T/PPM/STEF