Perform Automated Nucleic Acid Purification Using the SV Systems and the ep*Motion*® 5075 VAC Workstation

ABSTRACT Automating nucleic acid purification from a variety of sample types is helpful for many high-throughput applications. Automated sample processing must be timely, precise and consistent, producing product that is free from cross-contamination, pure from other contaminants and of an expected yield for direct use in downstream applications. Here we present fast and easy-to-use nucleic acid isolation methods on the Eppendorf ep*Motion*® 5075 VAC liquid handling workstation. The Wizard® SV 96 Genomic DNA Purification System and the SV 96 Total RNA Isolation System are used on the ep*Motion*® 5075 VAC workstation to successfully isolate genomic DNA and total RNA from mouse tails and tissue culture cells with results comparable to manual and other automated isolation techniques.

Sarah Shultz and Cristopher Cowan, Promega Corporation

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INTRODUCTION

The Wizard® SV 96 Genomic DNA Purification System[a] and SV 96 Total RNA Isolation System provide simple techniques for preparing nucleic acids from cultured cells and tissues, including mouse tails. 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 an SV 96 Filter Plate. Subsequently, the bound nucleic acids are washed to remove protein and salt before being eluted in nuclease-free water.

The SV 96 systems are designed for high-throughput nucleic acid isolation in a 96-well format. Nucleic acid purification using these systems has been automated on the Beckman Coulter Biomek® 2000, Biomek® 3000, Biomek® FX, Perkin Elmer Multiprobe® II, and the Caliper Life Sciences Sciclone® 3000 workstations (1–4). Here we describe automated nucleic acid purification on the Eppendorf ep*Motion*® liquid handling workstation. Automating these systems enables users simply to set up the workstation labware, start the automated method, and walk away. This frees lab personnel from the bench and provides the precision and consistency required for larger sample batches.

The Eppendorf ep*Motion*® 5075 VAC liquid handling workstation (Figure 1) is an easy-to-use robotic instrument that provides the necessary reagent dispensing and vacuum manifold functions for the SV 96 nucleic acid purification systems. The workstation 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 volumes ranging from 1 µl to 1 ml (5).

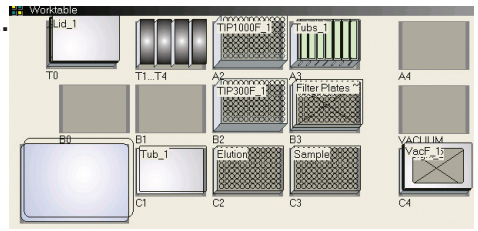
Other advantages of the ep*Motion*® 5075 VA workstation include a simple user interface and a unique optical sensor to check liquid volumes and confirm proper placement of all labware on the worktable. The workstation is nicely suited for mid-throughput systems. No instrument calibration is required, and the user can set up the worktable and then truly walk away during the purification procedure.

Figure 1. The Eppendorf ep*Motion*® 5075 liquid handling workstation.

WORKSTATION SETUP

To perform the automated SV 96 nucleic acid isolation methods, the ep*Motion*® 5075 VAC workstation must be equipped with a gripper, dispensing tools (TM1000-8 and TM300-8), vacuum with manifold and frame, reservoir rack, height spacers, and a waste tub. Cultured cells, tissues and reagents are prepared as recommended in our Technical Literature and Electronic Protocols. Mouse tail clippings are digested overnight at 55 °C in a proteinase K digestion solution. Labware is placed on the worktable for each of the automated methods as shown in Figure 2.

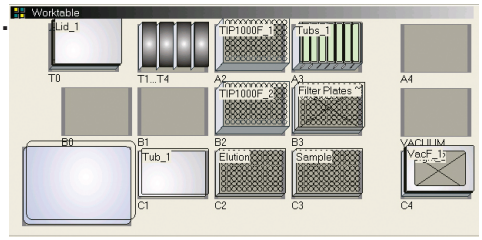
A.



Worktable A. Reservoir Contents.

Position	Contents
1	40 ml Wizard® SV Lysis Buffer
2	90 ml Wizard® SV Wash Solution with ethanol
3	90 ml Wizard® SV Wash Solution with ethanol
4	90 ml Wizard® SV Wash Solution with ethanol
5	30 ml Nuclease-Free Water

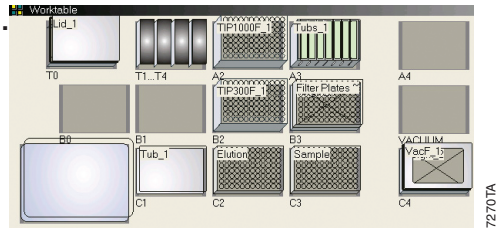
B.



Worktable B. Reservoir Contents.

Position	Contents
1	40 ml Wizard® SV Lysis Buffer
2	90 ml Wizard® SV Wash Solution with ethanol
3	90 ml Wizard® SV Wash Solution with ethanol
4	90 ml Wizard® SV Wash Solution with ethanol
5	30 ml Nuclease-Free Water

C.



Worktable C. Reservoir Contents.

Position	Contents
1	12 ml RNA Lysis Buffer
2	3.125 ml DNase Solution
3	22 ml DNase Stop Solution
4	100 ml RNA Wash Solution
5	12 ml Nuclease-Free Water

Figure 2. Worktable layouts for the SV nucleic acid purification automated methods on the ep*Motion*® 5075 VAC liquid handling station. Panel A. Layout for purification of genomic DNA from tissue culture cells. **Panel B.** Layout for purification of genomic DNA from mouse tail clippings. **Panel C.** Layout for purification of total RNA from tissue culture cells.

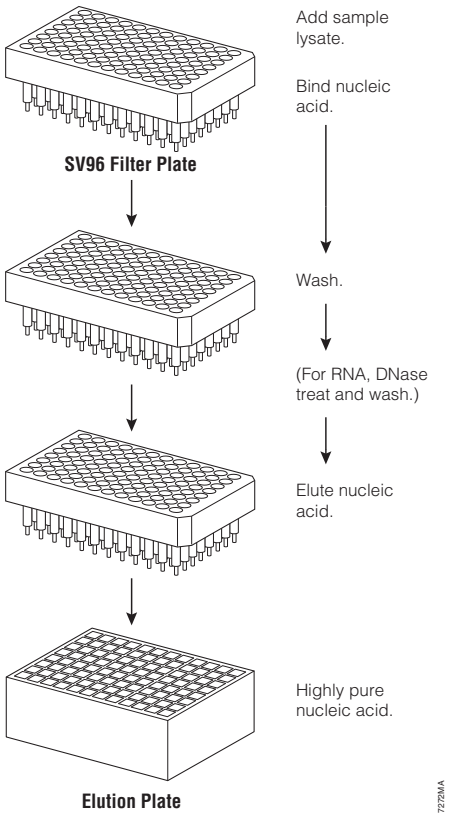


Figure 3. Schematic diagram of procedure for the isolation of genomic DNA or total RNA using the SV 96 Systems.

AUTOMATED SV 96 PROCEDURES

Three new automated methods are available from Promega for use on the ep*Motion*® 5075 VAC workstation: 1) automated Wizard® SV 96 Genomic DNA Purification method for tissue culture cells, 2) automated Wizard® SV 96 Genomic DNA for mouse tails, 3) automated SV 96 Total RNA Purification method for tissue culture cells.

Figure 3 illustrates the procedures using the Wizard® SV 96 Genomic DNA Purification System and SV 96 Total RNA Isolation System. The total processing time for each automated method is less than 1 hour for up to 96 samples. Note that each DNA and RNA isolation method must be performed separately.

The automated SV 96 nucleic acid isolation methods begin by dispensing lysis buffer to each well of a plate containing samples of interest (i.e., fresh tissue culture cells, digested mouse tails). 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.

Nucleic acids are next washed with a low-salt, ethanol-based wash solution to remove any contaminants such as proteins, salts or other cellular impurities. For RNA

Advantages of this system included isolation of nucleic acids from 96 samples in less than one hour, precision and consistency in sample treatment, and comparable yields to other isolation methods.

Table 1. Nucleic Acid Purity and Yield Recovered Using Manual and Automated SV 96 Purification Systems on the ep*Motion*® 5075 VAC Workstation.

Purification System	Automated or Manual	Sample	Input Amount	Average Yield (calculated from A_{260})	Average Purity (A_{260}/A_{280})
Wizard® SV 96 Genomic DNA Purification from Tissue Culture Cells	Automated	293 cells	1 × 10^5	1.0 ± 0.2 µg	1.8 ± 0.1
	Manual	293 cells	1 × 10^5	0.9 ± 0.2 µg	1.9 ± 0.2
Wizard® SV 96 Genomic DNA Purification from Mouse Tails	Automated	mouse tail clipping	20.3 ± 1.7 mg	23.9 ± 5.5 µg	1.9 ± 0.1
	Manual	mouse tail clipping	19.4 ± 2.4 mg	22.2 ± 3.9 µg	1.7 ± 0.1
SV 96 Total RNA Isolation from Tissue Culture Cells	Automated	Jurkat cells	1 × 10^5	0.4 ± 0.1 µg	2.3 ± 0.7
	Manual	Jurkat cells	1 × 10^5	0.3 ± 0.1 µg	1.9 ± 0.2

All purified nucleic acids were high-quality templates for PCR and RT-PCR.

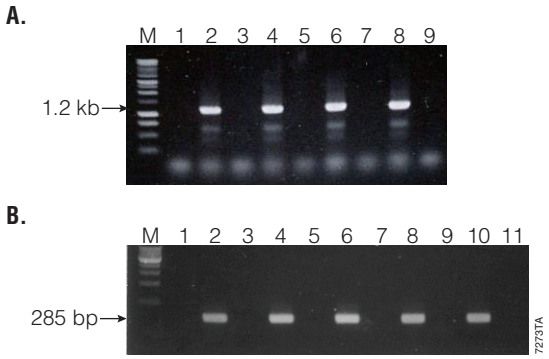


Figure 4. Gel images of amplified genomic DNA (Panel A) and RNA (Panel B) purified on the ep*Motion*® 5075 VAC liquid handling workstation using the SV 96 systems. Ten microliters of amplification product was run on a 1.2% agarose gel and visualized by staining with ethidium bromide. Amplification products were clearly observed in even-numbered lanes. No PCR products were observed from blank well samples. **Panel A.** Expected PCR product for IL-1β is approximately 1.2 kb (lanes 2,4,6, and 8). No PCR product is obtained from the blank samples (lanes 3,5,7 and 9) or amplified elution buffer (lane 1). Lane M, Promega BenchTop 1 kb DNA Ladder (Cat.# G7541). **Panel B.** The expected RT-PCR product for β-actin is approximately 285 bp (lanes 2,4,6,8, and 10). No product was obtained from blank samples (lanes 3,5,7,9 and 11) or amplified Elution Buffer (lane 1). Lane M, Promega BenchTop 1 kb DNA Ladder.

isolation, DNase is added to each well of the binding plate and incubated to digest genomic DNA. Following the incubation, the DNase is inactivated with the addition of a DNase Stop Solution. The wash steps occur 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 5–6 minutes to remove any residual ethanol from the filter plate.

After the drying step, the gripper disassembles the vacuum manifold by moving the binding plate from the vacuum to a holding position. Next the gripper places the 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 the elution plate. Nuclease-free water (100–500 µl) is added to each well of the filter plate and incubated at room temperature to release the nucleic acids. A final vacuum is applied, and eluates are pulled through the SV 96 filter plate. The eluted genomic DNA or total RNA is collected into the final elution plate.

ANALYSIS OF NUCLEIC ACIDS PURIFIED USING THE AUTOMATED PROCEDURES

Tissue culture cells and mouse tail tip clippings are two common sample types that were processed using the automated SV 96 nucleic acid purification methods on the ep*Motion*® 5075 VAC workstation. Yield, purity, quality and cross-contamination were assessed.

Purified genomic DNA and total RNA samples were evaluated spectrophotometrically by relative absorbance measurements for yield and purity (i.e., A_{260} and A_{260}/A_{280}) using a NANOdop Spectrophotometer. All purified samples were of expected purity and yield as shown in Table 1.

One microliter of purified genomic DNA and total RNA samples were also amplified by PCR and RT-PCR, respectively, using IL-1ß and ß-actin primers. All samples were high-quality templates for amplification as shown in Figure 4.

Cross-contamination was accessed by comparing nucleic acid yields in neighboring wells of checkerboard and alternating row plates. Results show no detectable cross-contamination between wells of the same plate as shown in Figure 4.

SUMMARY

The automated methods described in this article incorporating the Wizard® SV 96 Genomic DNA Purification System and SV 96 Total RNA Isolation System on the ep*Motion*® 5075 VAC workstation result in the successful high-throughput isolation of high-quality DNA and RNA. Advantages of this automated system include isolation of nucleic acids from 96 samples in less than one hour, precision and consistency in sample treatment, and comparable yields and purity to nucleic acids isolated using other manual or automated isolation procedures. In

addition, nucleic acids isolated using this system may be used directly for downstream applications including amplification and have no detectable cross-contamination between wells of the same 96-well plate.

ACKNOWLEDGEMENTS

We wish to acknowledge Eppendorf for the use of their ep*Motion*® 5075 VAC workstation.

REFERENCES

1. Grunst, T. (2001) *Promega Notes* **79**, 29–32.
2. Grunst, T. *et al.* (2002) *Promega Notes* **81**, 9–13.
3. Grunst, T. and Tack, L. (2002) Lab Automation 2002 Scientific Poster #M086.
4. Grunst, T. and Tack, L. (2002) Lab Automation 2002 Scientific Poster #M085.
5. Eppendorf Workstation ep*Motion*® 5075 Manual.

PROTOCOLS

- *Wizard*® *SV 96 Genomic DNA Purification System Technical Bulletin* #TB303, Promega Corporation
  www.promega.com/tbs/tb303/tb303.html
- *Wizard*® *SV 96 Genomic DNA Purification System Automated Protocol* #EP007, Promega Corporation
  www.promega.com/tbs/ep007/ep007.html
- *SV 96 Total RNA Isolation System Technical Bulletin* #TB294, Promega Corporation
  www.promega.com/tbs/tb294/tb294.html
- *SV 96 Total RNA Isolation System Automated Protocol* #EP003, Promega Corporation
  www.promega.com/tbs/ep003/ep003.html

ORDERING INFORMATION

Product	Size	Cat.#
Wizard® SV 96 Genomic DNA Purification System	1 × 96	A2370
	4 × 96	A2371
SV 96 Total RNA Isolation System*	1 × 96	Z3500
	5 × 96	Z3505

For ep*Motion*™ 5075 instrument and consumables, contact Eppendorf.

*For Laboratory Use.