

Applications

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Automated purification of PCR products using the Promega Wizard® SV 96 Clean-Up System and Eppendorf epMotion® 5075 VAC automated pipetting system

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Abstract

The Promega Wizard SV 96 PCR Clean-Up System provides a technique for the efficient recovery of purified DNA fragments generated by PCR amplification. A fully automated PCR clean-up method has been developed on the Eppendorf epMotion 5075 VAC automated pipetting system and is presented in this article. Using this hands-free automated method, double-stranded DNA fragments can be purified from 96 samples in approximately 30 minutes.

Introduction

Purification of double-stranded PCR products is an important process in molecular biology research. PCR products are commonly purified to remove excess nucleotides and primers. Purification is traditionally achieved with phenol/chloroform extraction or ethanol precipitation. This process can be labor-intensive and hazardous because of the use of toxic compounds. The development of a simple, hands-free PCR clean-up procedure capable of isolating double-stranded PCR products directly from amplification reactions in a relatively short amount of time is therefore desirable.

The Wizard SV 96 PCR Clean-Up System is designed to purify 100 bp to 10 kb PCR products directly from an amplification reaction with up to 90% recovery. This membrane-based system provides an easy method that can be performed manually or automated on a robotic platform. The DNA can be used for automated fluorescent DNA sequencing, cloning, labeling, restriction enzyme digestion or DNA microarray analysis without further manipulation [1].

The epMotion 5075 VAC is a flexible, modular automated pipetting system workstation that can be adapted to perform various liquid handling and vacuum-based tasks.

The exchangeable tips and jet dispensing technology enables this system to dispense liquids contact-free and makes it ideal for implementing work steps where precision and reliability are required. The epMotion 5075 VAC is well-suited for use with the Wizard SV 96 PCR Clean-Up System as the automated method requires a vacuum manifold, gripper tool and reliable liquid handling capabilities.

Materials and Methods

Materials

1. Eppendorf epMotion 5075 VAC automated pipetting system
 - Gripper
 - Dispensing Tools (TM1000-8 and TM300-8)
 - Vacuum with manifold
 - Reservoir Rack
 - Height Adapter 85 mm
 - Vac Frame 2
 - Vac Holder
 - 400 ml Waste Tub
2. Eppendorf consumables:
 - 3 x 100 ml Reagent Reservoirs
 - 1 x 1000 µl epTIPS Motion Filtertips
 - 1 x 300 µl epTIPS Motion Filtertips

3.Promega consumables:

- Wizard SV 96 PCR Clean-Up System
- 1 x 96-well PCR Clean-Up U-Bottom Plate (Collection Plate)
- Materials supplied by the user:
- 80 % Ethanol (or 95 % Ethanol, for recovery of PCR fragments < 500 bp)
- 1 x 96-well Skirted PCR Plate (Sample Plate) containing amplification products, up to 100 µl per well.

Automated Method

Prior to beginning the automated procedure, ethanol and reagents included in the Wizard SV 96 PCR Clean-Up System should be added to the Reagent Reservoirs as shown in Table 1. *epMotion* tips, plates, and remaining labware are placed onto the *epMotion* 5075 VAC worktable as shown in Figure 1 and Table 2. The automated method can then be started. First, each 100 µl PCR reaction sample is mixed with 100 µl of Membrane Binding Solution.

Table 1: Location, type, and contents of the reservoirs in the Reservoir Rack (Worktable Position B2) when processing 96 samples

Reservoir Rack Position	Reservoir Type	Reagent Reservoir Contents
1	100 ml Reservoir	20 ml Membrane Binding Solution
2	100 ml Reservoir	75 ml 80 % Ethanol*
3	100 ml Reservoir	25 ml Nuclease-Free Water

*Use 95 % Ethanol instead of 80 % Ethanol for recovery of PCR products < 500 bp.

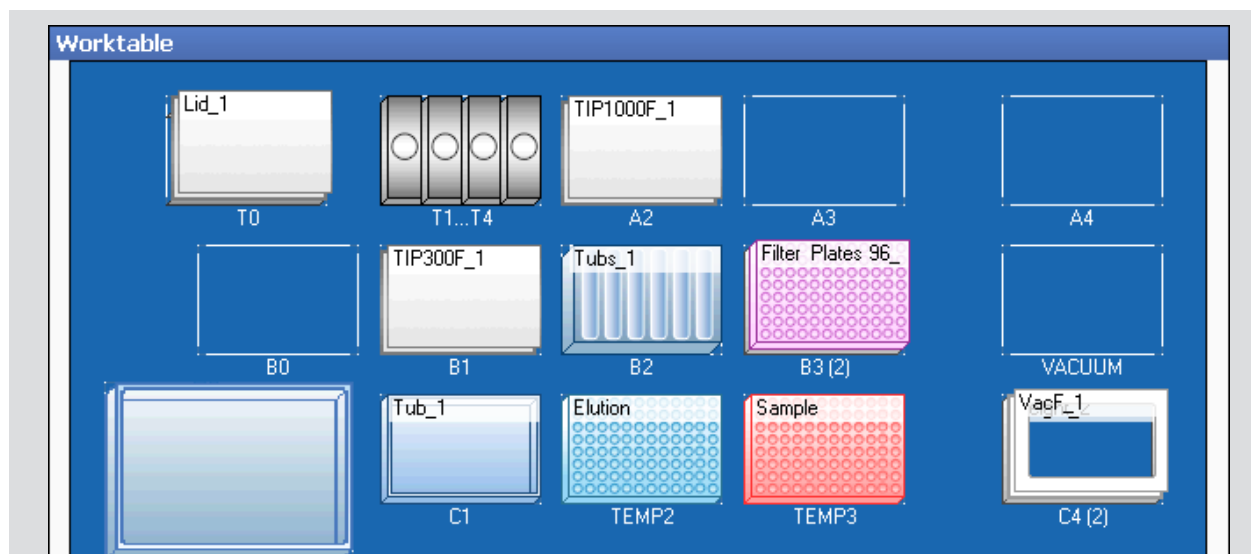


Figure 1: Screenshot of the *epMotion* Editor showing the *epMotion* 5075 VAC worktable setup for the automated Wizard SV 96 PCR Clean-Up method.

Table2: *epMotion* 5075 VAC Worktable labware setup by position

Worktable Position	Labware
A2	1000 µl epTIPS Motion Filtertips
A3	Empty
A4	Empty
B0	Empty
B1	300 µl epTIPS Motion Filtertips
B2	Reservoir Rack with 3 x 100 ml Reagent Reservoirs
B3	SV 96 Binding Plate on 85 mm Height Adapter
Vacuum	Empty
C1	Waste Tub
C2	Collection Plate: 96-well PCR clean-up U-Bottom Plate or Eppendorf Microplate 96/U
C3	Sample Plate: 96-well PCR Plate
C4	Vac Frame 2 on Vac Holder

The PCR products are then transferred to the SV 96 Binding Plate on top of the vacuum manifold and allowed to bind to the silica membrane for 1 minute. The Wizard SV 96 PCR Clean-Up System is based on the ability of DNA to bind to silica membranes in the presence of chaotropic salts. PCR products are purified using 96-well vacuum filtration, eliminating the need for centrifugation. The vacuum is applied and the Membrane Binding Solution is pulled through the plate. PCR products are then washed three times with 80% or 95% Ethanol. Washing the bound DNA requires no disassembly of the manifold and filtrate waste products are delivered directly to the Waste Tub. After washing, the vacuum remains on for an additional 4 minutes to remove any residual Ethanol from the SV 96 Binding Plate. Once the plate is dry, the Gripper reassembles the vacuum manifold stack by placing the Elution Plate inside the vacuum and moving the SV 96 Binding Plate back atop the vacuum manifold. After the stack is reassembled, purified PCR products are eluted into the Elution Plate by the addition of Nuclease-Free Water to the SV 96 Binding Plate. The total processing time for this automated method is just under 30 minutes.

Results

PCR fragments of 500 bp and 1,000 bp were purified using the automated Wizard SV 96 PCR Clean-Up System

method on the *epMotion* 5075 VAC system. Agarose gel analysis of purified (P) and unpurified (U) fragments separated on a 1.3 % agarose gel and visualized by ethidium bromide staining is shown in Figure 2A. Percent recovery of purified PCR products is shown in Figure 2B as quantitated using a Hitachi FMBIO® Fluorescent Scanner. Results show the mean and standard deviation for 5 purified fragments of each size. Average percent recovery for both 500 bp and 1000 bp PCR fragments were greater than 90 %. Recovery data for samples purified using this automated procedure also compare equally to samples purified manually [1]. Refer to Promega TB#311 [2] for details on the manual processing procedure. Use of the automated method results in successful isolation and recovery of double-stranded PCR products directly from amplification reactions.

Conclusion

The Promega Wizard SV 96 PCR Clean-Up System is a high-throughput DNA fragment purification system providing rapid and reliable recovery of purified DNA fragments that are suitable for many downstream applications. The system is well-suited for automation on the Eppendorf *epMotion* 5075 VAC automated pipetting system.

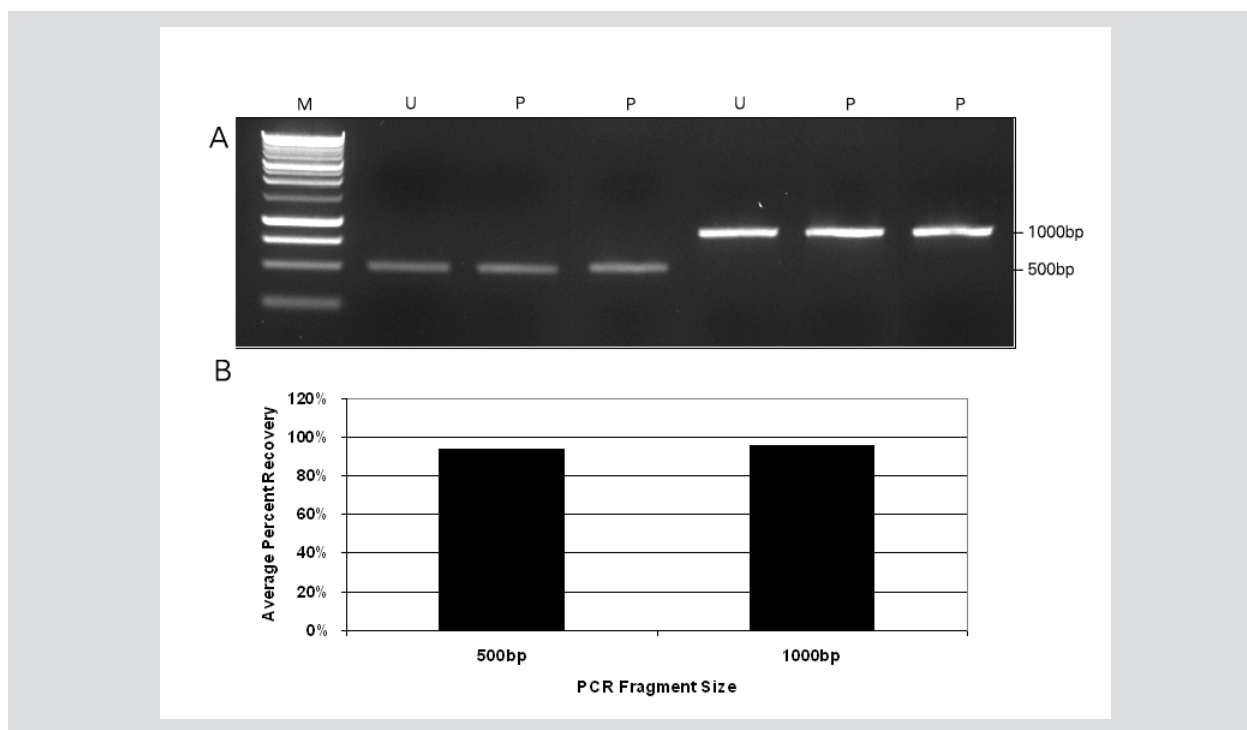


Figure 2: Purification and recovery of PCR products.

Panel A: Agarose gel analysis. Purified (P) and unpurified (U) fragments were separated on a 1.3 % agarose gel and visualized by ethidium bromide staining.

Panel B: Percent recovery of purified PCR products. Percent recovery was quantitated using a Hitachi FMBIO Fluorescent Scanner. Results show the mean and standard deviation for 12 purified fragments of each size.

References

- [1] Buros M et al. (2002) High-Throughput Wizardry for PCR Purification, Promega Notes 82, Pages 6-9.
 [2]. Wizard SV 96 PCR Clean-Up System Technical Bulletin #TB311, Promega Corporation.
 (<http://www.promega.com/tbs/tb311/tb311.html>)

Ordering Information Eppendorf

Product	Order no. international	Order no. North America
epMotion® 5075 VAC (vacuum chamber included)	5075 000.164	960020014
Height Adapter 85 mm	5075 751.003	960002105
Reservoir 400 ml	5075 777.002	960002229
Vac Frame 2	5075 785.005	960002261
Dispensing tool TM 1000-8	5280 000.258	960001061
Dispensing tool TM 300-8	5280 000.231	960001052
Reservoir Rack	5075 754.002	960002148
Reservoirs 100 ml (10 x 5 reservoirs in bags/case, PCR clean)	0030 126.513	960051017
epTIPS Motion 1000 µl Filter	0030 003.993	960050100
epTIPS Motion 300 µl Filter	0030 003.977	960050061
Eppendorf Microplate 96/U (80 plates, Clear, PCR clean)	0030 601.203	951040048

Ordering Information Promega

Product	Cat. #
Wizard® SV 96 PCR Clean-Up System	A9340, A9341, or 9342
1 x 96-well PCR Clean-Up U-Bottom Plate (Elution Plate)	A1260

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 FMBIO is a registered trademark of Hitachi Software Engineering Company, Ltd.



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