

APPLICATION INFORMATION

Genetic Analysis

PCR PURIFICATION USING THE BIOMEK® FX WITH A MAGNABOT® 96 SEPARATION DEVICE AND PROMEGA'S WIZARD® MAGNESIL® PCR® PURIFICATION SYSTEM

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Introduction

Purifying PCR products with MagneSil can be achieved on an automated platform, the Biomek® FX, with or without active Automated Labware Positioners, i.e., ALPs (the Orbital Shaker and the MagBead ALPs). Results obtained when purifying samples with a method utilizing a 96-well MagnaBot Separation Device and the Orbital Shaker are described in this document.

Instrument Setup

The starting instrument setup on the Biomek FX is shown in Figure 1. All reagents and labware needed to process two plates of PCR products are placed on the worksurface of the Biomek FX. No user intervention is required after the initial deck setup. Specific directions for running the method are given in “Configuring the Biomek FX for PCR Purification with This Method,” below.

Agarose Gel Electrophoresis

Purified samples from both plates were separated by electrophoresis on 1.2% agarose gels with unpurified PCR products as controls. The gel analysis demonstrates that primers were efficiently removed from samples when purified on the Biomek FX using the MagneSil chemistry (Figure 2).

Recovery

The percentage recovery was determined using PicoGreen® quantitation, which only detects dsDNA. The use of PicoGreen allows for a direct comparison of the input fragment concentration to the purified

fragment concentration. Overall, approximately 85% of the dsDNA is bound, purified, and eluted using this method (see Table 1).

	<i>MagnaBot Method</i>
Average % Recovery	82.6%
StDev	0.07
CV	5.6%

Configuring the Biomek FX for PCR Purification with this Method

Please reference Beckman Coulter publication A-1933A-QS, “Quick-Start Guide: High-Throughput PCR Reaction Cleanup Using the MagneSil PCR Cleanup System—Two-Plate Protocol,” for detailed instructions.

- Place all ALPs (Orbital Shaker, 4×3, Tip Wash Station, 1×1s, Trash, and Tip Loader) in proper positions (Figure 1). Make sure all active ALP hardware addresses are set to “0.”
- Make sure all worksurface positions, grippers, and ALPs are framed prior to use. General framing instructions can be found in your *Biomek FX User's Manual*. Refer to the proper manuals for framing integrated ALPs.
- Place labware, tips, and reagents on the worksurface of the Biomek FX as indicated (Figure 1).



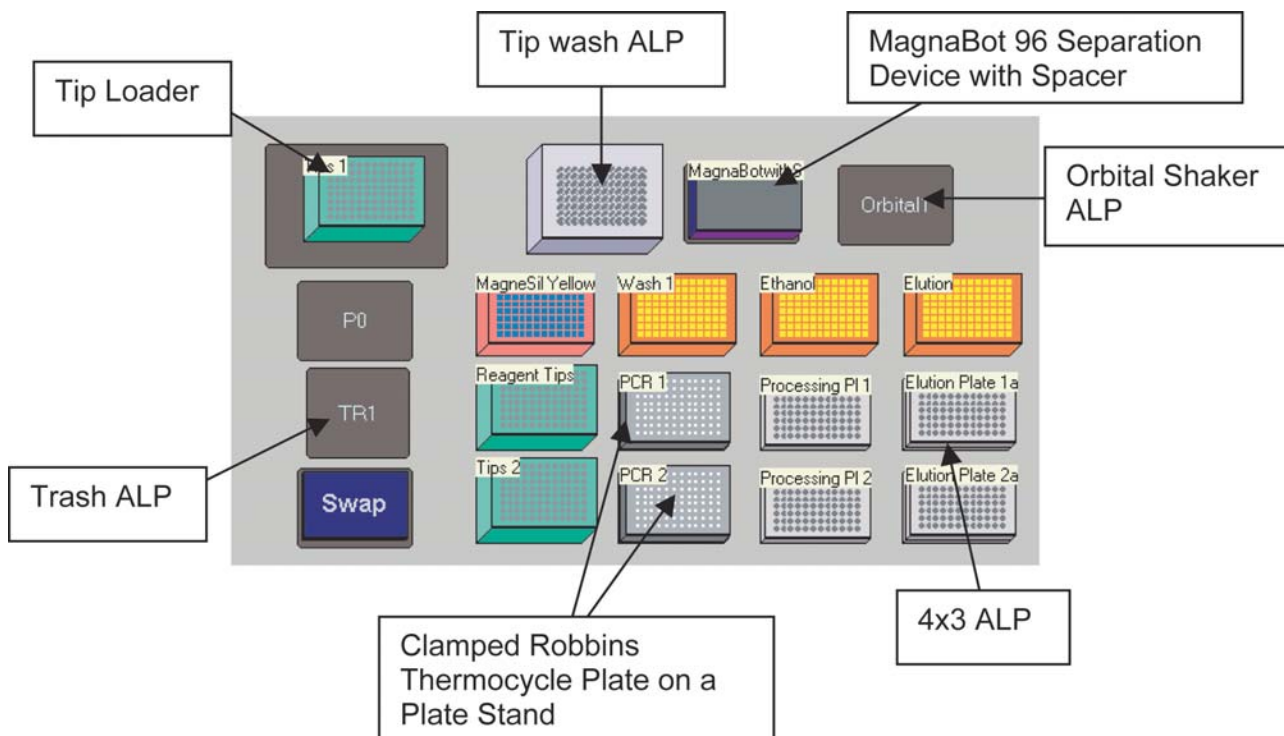


Figure 1. Starting Instrument Setup for PCR Purification using MagneSil chemistry and the MagnaBot 96 Separation Device.

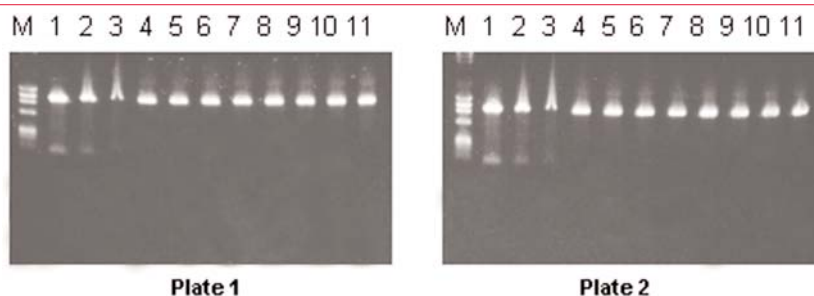


Figure 2. Agarose electrophoresis of PCR products purified by using the Biomek® FX and the MagnaBot 96 Separation Device. Samples purified from plate as indicated. Lanes are: M= λ H3/PhiXHaeIII molecular marker; 1: 100% crude sample; 2: 50% crude sample; 3: 25% crude sample; 4-11: purified samples from wells of indicated plate.

- Place a MagnaBot 96 Separation Device with a 3/16-inch spacer on P14.
- When mapping this method onto an existing deck, you will need 1 Orbital Shaker ALP, 1 Wash Station, 1 Trash ALP, and 1 Tip Loader ALP on the deck. Besides these ALPS, make sure there are at least 15 open ALP positions (labeled P0-P14) for labware placements.
- Please reference the Beckman Coulter document A-1946A, “Deck Mapping on the Biomek FX,” for greater detail on how to map methods onto different decks.

Troubleshooting

Please reference Beckman Coulter publication A-1933A-TS, “Troubleshooting Guide: High-Throughput PCR Reaction Cleanup Using the MagneSil PCR Cleanup System—Biomek FX Two-Plate Protocol.”

Cautions

The following describes cautions for aspirating MagneSil paramagnetic particles.

- It is critical not to aspirate from the bottom of the well, plate, or reservoir when mixing or aspirating MagneSil paramagnetic particles. Doing so may create a vacuum in the pipette tips, causing the paramagnetic particles to be aspirated into the pipetting head, which can cause irreversible damage.
- To optimize the mixing of particles, aspirate first from a comfortable height and then adjust that height downward. Under no circumstances should you mix MagneSil using 0 mm as an aspiration height (see Figure 3).

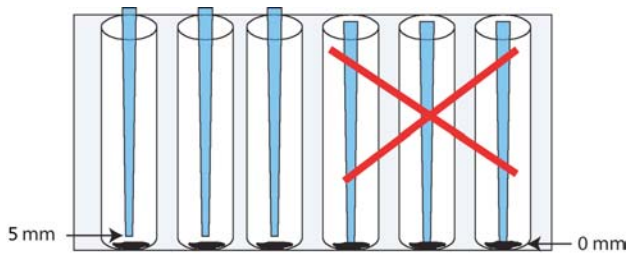


Figure 3. Pipette tip aspiration height warning.

* The PCR process is covered by patents owned by Roche Molecular Systems, Inc., and F. Hoffmann-La Roche, Ltd. MagneSil is a trademark and MagnaBot and Wizard are registered trademarks of Promega Corporation. PicoGreen is a registered trademark of Molecular Probes, Inc. All other trademarks are the property of their respective owners.



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