

# APPLICATION INFORMATION

## Genetic Analysis

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

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#### Introduction

Automated plasmid purification from bacterial pellets using MagneSil paramagnetic particles can be achieved on the Biomek® FX using a static magnet (96-well MagnaBot Separation Device, Promega, Inc.) to provide a magnetic field for particle capture and the Biomek FX Orbital Shaker automated labware positioner (ALP) to efficiently resuspend bacterial pellets and mix the paramagnetic particles. Results obtained when purifying plasmids with a method utilizing this hardware configuration 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 one plate of bacterial pellets to purified plasmids 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 Plasmid Purification with This Method,” below.

#### Agarose Gel Electrophoresis

Two plates of bacterial pellets containing DH5α (Cell Density=4 OD<sub>600</sub>) with pGEM-3Zf(+) were purified using the MagnaBot method. Purified plasmids from the two independent runs of the method were separated by electrophoresis on 1.2% agarose gels. Gel analyses indicate that high quality plasmid DNA was generated with the MagneSil chemistry on the Biomek FX.

#### Yield and Purity

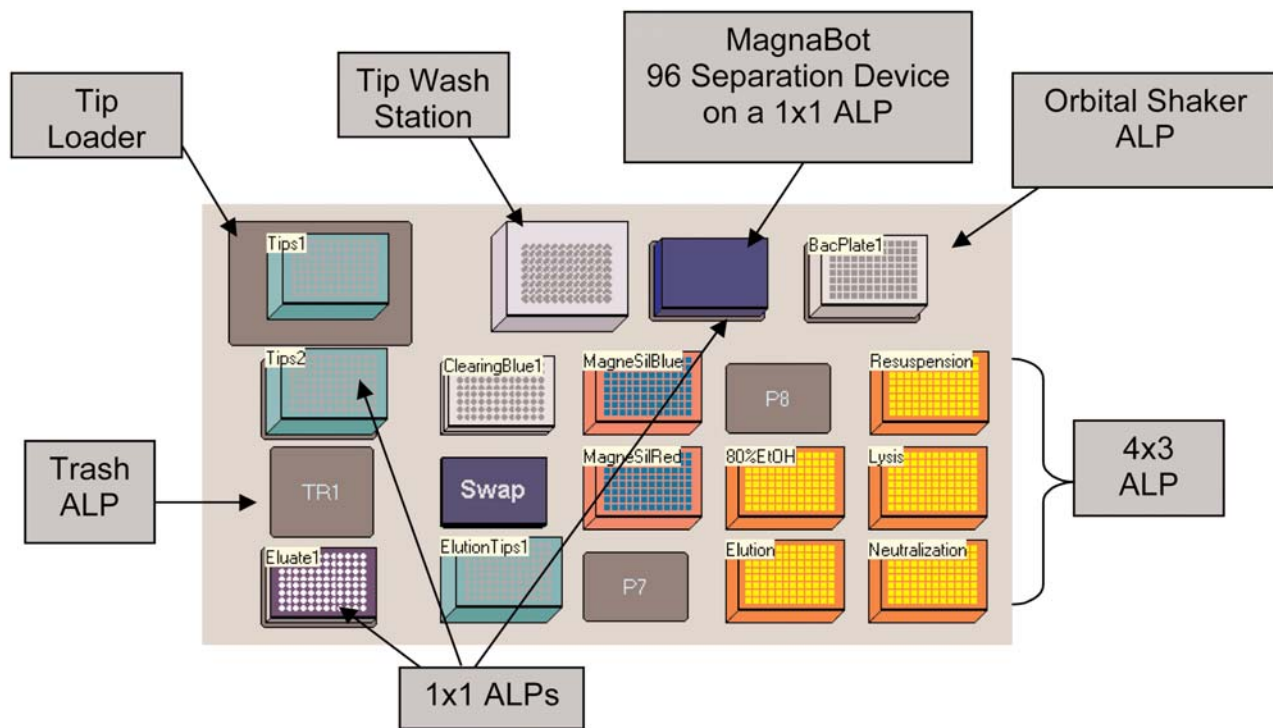
The yield and purity of the purified plasmids was determined by spectrophotometric analysis. The average quality of the plasmids (260/280 ratio) and the average concentration of plasmids (µg/mL) generated with the MagnaBot method are given in Table 1.

Run	Average Quality (260/280)	Average Concentration (µg/mL)
First	1.77 ± 0.08	145.6 ± 35.0
Second	1.77 ± 0.06	171.2 ± 49.8

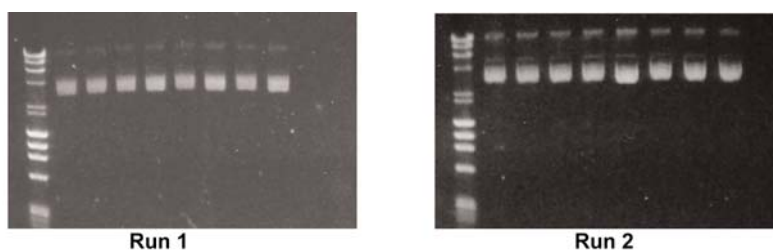
#### Configuring the Biomek FX for Plasmid Purification with this Method

Refer to Beckman Coulter publication A-1934A-QS, “Quick-Start Guide: High-Throughput Plasmid Purification Using the Wizard MagneSil Plasmid Purification Kit—Two-Plate Protocol,” for detailed instructions regarding growth of bacterial cultures, labware and reagent preparation, as well as technique selection.





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



**Figure 2.** Agarose electrophoresis of plasmids purified by using the Biomek<sup>®</sup> FX and the MagnaBot 96 Separation Device. Samples purified from two independent runs of the method as indicated. For both gels, the first lane is  $\lambda$ H3/PhiXHaeIII molecular marker; Remaining lanes are samples from column 2 of both plates.

- Place all ALPs (Orbital Shaker, 4x3, Tip Wash Station, 1x1s, Trash, and Tip Loader) in proper positions (Figure 1). Make sure all active ALP hardware addresses are set to “0.”
- Make sure all deck positions, grippers, and ALPs are framed prior to use. General framing instructions can be found in your *Biomek<sup>®</sup> FX User’s Manual*. Refer to the proper manuals for framing integrated ALPs.
- Place labware, tips and reagents on the work surface of the Biomek FX as indicated (Figure 1).
- Place a MagnaBot 96 Separation Device on P14.
- When mapping this method onto an existing deck, you will need 1 Orbital Shaker, 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 (P0-P14) for labware placements.

- Refer to Beckman Coulter publication *A-1946A*, “Deck Mapping on the Biomek FX,” for greater detail on how to map methods onto different decks.

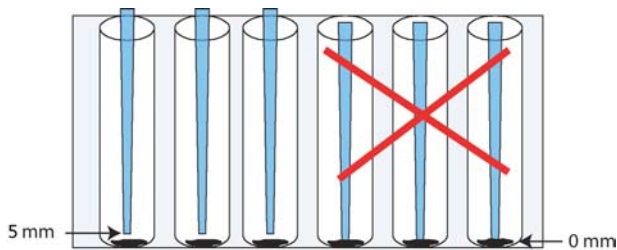
## Troubleshooting

Refer to Beckman Coulter publication *A-1934A-TS*, “Troubleshooting Guide: High-Throughput Plasmid Purification Using the Wizard MagneSil Plasmid Purification Kit—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.

\* MagneSil is a trademark and MagnaBot and Wizard are registered trademarks of Promega Corporation. All other trademarks are the property of their respective owners.



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