Automated Wizard® Magnetic 96 DNA Plant System

Automated Protocol #EP006

DESCRIPTION OF THE BIOMEK® 2000 AND BIOMEK® FX METHODS FOR PRODUCTS FF3760 AND FF3761
All technical literature is available on the Internet at www.promega.com
Please visit the web site to verify that you are using the most current version of this Automated Protocol.

I. Description

This document describes automation of the Wizard® Magnetic 96 DNA Plant System(a). Specific instructions are provided for the Beckman Biomek® 2000 and Biomek® FX Automated Workstations. Information about downloading the validated methods for these liquid handling workstations is available at:
www.promega.com/automethods/
General automation guidelines are provided for adaptation to other liquid handling platforms. For information on the chemistry and issues related to plant tissue preparation, please refer to the *Wizard® Magnetic 96 DNA Plant System Technical Bulletin* #TB289.

II. Product Components

Product	Size	Cat. #
Wizard® Magnetic 96 DNA Plant System	2 × 96 preps	FF3760

For Laboratory Use.

Includes:

- 68ml Lysis Buffer A, Plant
- 14ml Lysis Buffer B, Plant
- 40ml Wash Buffer, Plant
- 2.25ml MagneSil® Paramagnetic Particles(a)
- 4 Collection Plates
- 1 Protocol

Product	Size	Cat. #
Wizard® Magnetic 96 DNA Plant System	4 × 96 preps	FF3761

For Laboratory Use.

Includes:

- 136ml Lysis Buffer A, Plant
- 28ml Lysis Buffer B, Plant
- 80ml Wash Buffer, Plant
- 4.5ml MagneSil® Paramagnetic Particles(a)
- 8 Collection Plates
- 1 Protocol

Do not freeze the MagneSil® Paramagnetic Particles.

Storage Conditions: All components should be stored at room temperature (20–25°C). **Do not freeze** the MagneSil® Paramagnetic Particles.

III. Before You Begin

Materials to Be Supplied by the User

- MagnaBot® 96 Magnetic Separation Device (Cat.# V8151)
- MagnaBot® Spacer (Cat.# V8381)
- TE (pH 8.0) or Nuclease-Free Water (Cat.# P1193)
- Geno/Grinder™ 2000 (SPEX CertiPrep, Inc.)
  - 96 deep-well plates, 1 or 2ml (polypropylene)
  - sealing tape (3M Scotch® brand aluminum foil tape 425: 3 inches × 60 yards)
  - Geno/Grinder™ beads

OR

- Retsch MM300 Mixer Mill, plate holder and consumables

Grind plant material prior to beginning the automated DNA purification procedure with the Wizard® Magnetic 96 DNA Plant System:

- Place 8mm fresh leaf disks or 1–5 seed samples in a sealed, 96-well deep-well plate (Geno/Grinder®) or capped microtubes (Retsch) in the presence of 300µl of Lysis Buffer A and 1 or 2 grinding beads. Process in the grinder following the manufacturer's instructions. Ground and processed samples are ready for purification on an automated liquid handler.
- Prepare the Wash Buffer by adding 20ml of 95% ethanol and 20ml of 95% isopropanol to the Wash Buffer bottle. Mix well.
- Thoroughly resuspend the MagneSil® PMPs and mix 7ml of Lysis Buffer B with 1.125ml of MagneSil® PMPs. Prepare this solution fresh just prior to use.

IV. Automated Processing Requirements for the Biomek® 2000 Workstation

A. Instrumentation Requirements for the Biomek® 2000

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required to automate the Wizard® Magnetic 96 DNA Plant System on a Beckman Biomek® 2000 instrument.

Part Description		Beckman Part Number
Biomek® 2000 Workstation, 50/60 Hz, 100–120V	1	609000
Biomek® 2000 Controller NT	1	609875
BioWorks™ 3.2 for Beckman Coulter Computer	1	609983
Biomek® 2000 Left Side Module	1	609048
MP200 Pipetting Tool	1	609025
Gripper Tool System for Biomek® 2000	1	609001
Tip Rack Holder	2	609121
Gray Labware Holder	6	609120
Reservoir Holder	1	372795
Quarter Single Reservoirs	2	372790
Quarter Vertical Reservoirs	2	372788

B. Labware Requirements for the Biomek® 2000

Part Description	Quantity	Ordering Information
MagnaBot® 96 Magnetic Separation Device	1	Promega Cat.# V8151
MagnaBot® Spacer	1	Promega Cat.# V8381
2ml deep-well plate (or comparable)	1	Beckman Cat.# 14054
Polystyrene U-bottom multiwell plate (or comparable)	3	Greiner America Cat. # 650101 or Promega Cat.# A9161
Biomek® 2000 Standard 250 tips (rack)	2	Beckman Cat. # 372655

C. Biomek® 2000 Initial Deck Configuration

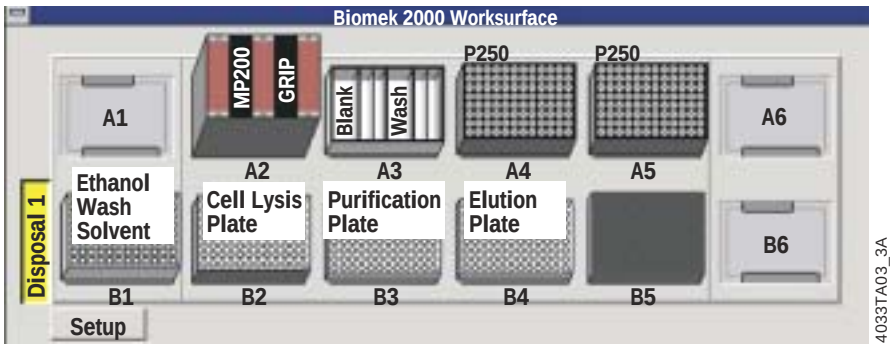

Figure 1. Beckman Biomek® 2000 initial deck configuration.

Position A1: Empty
Position A2: Tool rack containing MP200, and Gripper tools
Position A3: Labware holder, reservoir holder containing a Quarter Vertical and Quarter Single reservoirs (from left to right) (See Figure 3)
Position A4: Tip rack holder, P250 tips
Position A5: Tip rack holder, P250 tips
Position A6: Empty (not used in method)

Position B1: Labware holder, empty 2ml Beckman Deep-Well Square plate
Position B2: Labware holder, deep-well plate containing ground plant samples in Lysis Buffer A (typically a 1.2ml round-bottom plate or a equivalent)
Position B3: Labware holder, empty Greiner 96-well U-bottom plate
Position B4: Labware holder, empty Greiner 96-well U-bottom plate
Position B5: Labware holder, MagnaBot® 96 with a MagnaBot® Spacer on top
Position B6: Empty (not used in method)

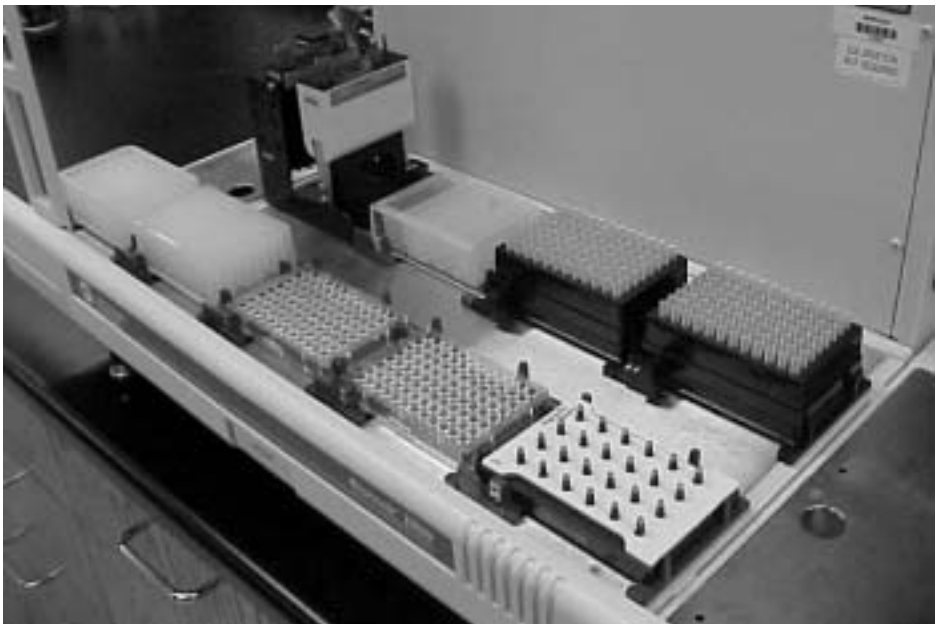

Figure 2. Image of initial deck configuration.

D. Biomek® 2000 Reagent Dispense Volumes

Prior to beginning a run, the following Wizard® Magnetic 96 DNA Plant System reagents need to be dispensed appropriately on the deck of the Biomek® 2000 set according to the initial deck configuration.

1 2 3

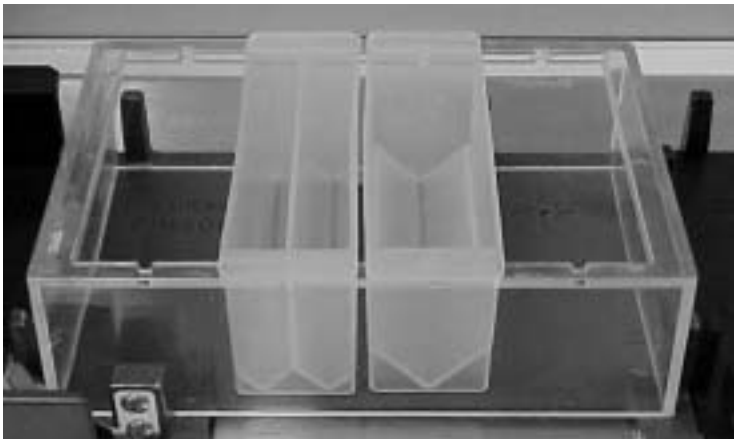

Figure 3. Deck position A3.

1. 7ml MagneSil® PMPs/1.125ml Lysis B mixture
2. 7ml Nuclease-Free Water
3. 34ml Wash Buffer (w/ethanol and isopropanol added)

V. Automated Processing Requirements for the Biomek® FX Workstation

A. Instrumentation Requirements for the Biomek® FX

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required to automate the Wizard® Magnetic 96 DNA Plant System on a Beckman Biomek® FX instrument.

Part Description	Quantity	Beckman Part Number
Biomek® FX Software version 2.1 (minimum)		Contact Beckman
96-channel POD	1	Contact Beckman
Minimum number of Labware Positions by 1 POD	10	Contact Beckman
Tip Loader ALP	1	719356

B. Labware Requirements for the Biomek® FX

Part Description	Quantity	Ordering Information
MagnaBot® 96 Magnetic Separation Device	1	Cat.# V8151
MagnaBot® Spacer	1	Cat.# V8381
2ml deep-well plate (or comparable)	2	Marsh Cat.# AB-0932
1.2ml deep-well plate (or comparable)	1	Marsh Cat.# AB-0564
Polystyrene U-bottom multiwell plate (or comparable)	2	Greiner America Cat.# 650101 or Promega Cat.# A9161
Biomek® FX Standard 200 tips (rack)	3	Beckman Cat.# 717251
Biomek® FX Standard 20 tips (rack)	3	Beckman Cat.# 717254

C. Biomek® FX Initial Deck Configuration

This is an example of the Wizard® Magnetic 96 DNA Plant System deck layout on a Biomek® FX. **Your specific deck layout may be different depending on your Biomek® FX configuration.**

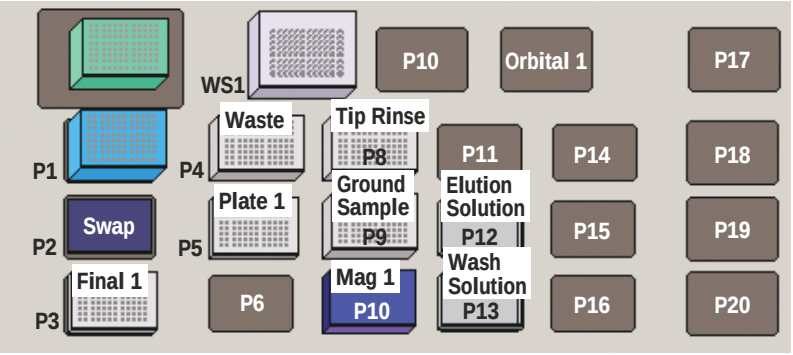

Figure 4. Biomek® FX initial deck configuration.

ALP Name	Equipment
Tip Loader	200µl Biomek® FX tips
P1	20µl Biomek® FX tips
P2	Empty: Tip box Swap Spot
P3	Empty Greiner 96-well round-bottom plate (labeled "Final 1")
P4	Empty Marsh 2ml deep-well square plate (labeled "Waste")
P5	Empty Greiner 96-well round-bottom plate (labeled "Plate 1")
P8	Marsh 2ml deep-well square plate filled with water (labeled "Tip Rinse")
P9	Marsh 1.2ml deep-well round plate containing ground plant sample (labeled "Ground Sample")
P10	MagnaBot® Magnetic Separation Device with MagnaBot® Spacer on top
P12	Upside-down tip box lid containing Elution Solution (Labeled "Elution Solution")
P13	Upside-down tip box lid containing Wash Solution (Labeled "Wash Solution")
Orbital1	Not used in method
WS1	Tip Wash Station (96-channel) (not necessary for method)

D. Biomek® FX Specific Pre-Run Recommendations

The Biomek® FX automated platform allows users the flexibility to configure the robot's deck configuration according to need. Because of this flexibility in deck configuration, it is likely that the deck used for writing a Biomek® FX method will differ from an end-user's deck. Therefore, it will be generally necessary to map an imported method onto an end-user's deck configuration. To map an imported method onto your deck, please follow the instructions provided in the document *Biomek® FX Deck Mapping* (www.promega.com/automethods/beckman/biomek/default.asp).

Note: Instructions to map Biomek® FX methods onto your deck configuration are available at www.promega.com/automethods/beckman/biomek/default.asp

VI. Description of the Automated Wizard® Magnetic 96 DNA Plant System

1. **Plant Tissue Lysis and DNA Binding**. Plant tissue lysate is transferred from the Cell Lysate plate into a U-bottom 96-well plate containing 62.5µl of Lysis B/MagneSil® particles. The MagneSil® particles are mixed well by pipetting. The particles are captured on the MagnaBot® Device, and the supernatant is removed to waste.

   Note: During the transfer of plant tissue lysate from the Cell Lysate plate, it is critical to have the tips high enough from the bottom of the well to avoid the pelleted cell debris, but low enough in the well that the tips are submerged in the lysate or below any oil layer that may be present. To determine this height prior to running the method with real samples, mark a plate containing ground samples at the lowest and highest acceptable pipetting height. Take an empty plate and adjust the Aspirate Height in the method so the tips are positioned appropriately within the plate. **In addition, if a different extraction plate or method is used, it will be necessary to re-optimize the tip height before performing the transfer step.**

   IMPORTANT: ...Re-calibrate the pipetting tip height every time you use a different plate or method.

2. **Washes**. The sample plate is moved off of the MagnaBot® Device. 150µl of Wash Buffer is added and mixed by pipetting. The particles are captured on the MagnaBot® Device. The supernatant is removed, and this wash procedure is repeated using 100µl of Wash Buffer for the second wash.

3. **Dry**. The MagneSil® particles are allowed to air-dry on the MagnaBot® Device for 5 minutes. This is important to allow any residual Wash Buffer to evaporate. The sample plate is then moved off of the MagnaBot® Device to begin the elution step.

4. **Elution.** 50µl of Nuclease-Free Water is added to the samples and mixed by pipetting. TE (pH 8.0) may be substituted for Nuclease-Free Water. The particles are then captured on the MagnaBot® Device. DNA-containing supernatant is removed and saved in a clean 96-well U-bottom collection plate.

   Note: TE (pH8.0) may be substituted for Nuclease-Free Water.

5. **Method Ends.** Purified genomic DNA has been eluted into a clean 96-well U-bottom plate.

VI. General Guidelines for Adaptation to Alternative Robotic Platforms

The MagneSil® particles settle over time. We recommend thoroughly mixing the MagneSil® particles on the automated platform prior to dispensing into samples. Resuspension of the MagneSil® particles can also be accomplished by thorough tip mixing or shaking.

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[a]U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756, and Japanese Pat. No. 3253638 have been issued to Promega Corporation for methods of isolating biological target materials using silica magnetic particles. Other patents are pending.

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All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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