Automated Wizard® Magnetic 96 DNA Plant System: Scaled Up Protocol for Increased DNA Yield

Automated Protocol #EP020

DESCRIPTION OF THE BIOMEK® FX METHOD FOR PRODUCTS Z919, A919, A381 and FF377
USING SCALED-UP SAMPLE SIZES FOR HIGH DNA YIELD
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.

Note: This document describes a scaled up, automated protocol for the Wizard® Magnetic 96 DNA Plant System that is designed to obtain increased DNA yields from plant leaf tissue.

I. Description

This document describes an automated protocol for the Wizard® Magnetic 96 DNA Plant System[(a)] that is designed to obtain increased DNA yields (compared to the standard *Automated Wizard® Magnetic 96 DNA Plant System Protocol* #EP006) by scaling up sample size and reagent volume. Yields of 1µg or greater can be obtained from leaf tissue using this protocol. Specific instructions are provided for the Beckman Coulter Biomek® FX Automated Workstation. Information about downloading the validated method for this liquid handling workstation is available at: **www.promega.com/automethods/**

General automation guidelines for adaptation to other liquid handling platforms are also provided. 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.

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Note: To obtain reagents to evaluate the Wizard® Magnetic 96 DNA Plant System Scaled Up Protocol for Increased Yield, please contact Promega Technical Services.

Tel: (608)-274-4330
or (800) 356-9526

Fax: 608-277-2516

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techserv@promega.com

Do not freeze the MagneSil® Paramagnetic Particles.

II. Product Components

Product	Size	Cat. #
Lysis Buffer A, Plant	custom	Z919
Lysis Buffer B, Plant	custom	A919
Wash Buffer, Plant	custom	A381
MagneSil® Paramagnetic Particles	custom	FF377

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

To obtain reagents to evaluate the Wizard® Magnetic 96 DNA Plant System Scaled Up Protocol for Increased Yield, please contact Promega Technical Services.

III. Before You Begin

Materials to Be Supplied by the User

- Deep Well MagnaBot® 96 Magnetic Separation Device (Cat.# V3031)
- 1/4 inch Foam Spacer (Cat.# Z3301)
- Collection Plate (Cat.# A9161)
- Deep Well Plates, 1.2ml, 96 round wells (Marsh Bio Products Cat.# AB 0787)
- Deep Well Plate, 2.2ml, 96 square wells, V-bottom (Corning Cat.# 3960 or equivalent)
- Optional for use on deck without tipwash station: 2 additional Deep Well Plates, 2.2ml, 96 square wells, V-bottom (Corning Cat.# 3960 or equivalent)
- Heat Transfer Block (Cat.# Z3271)
- TE (pH 8.0) or Nuclease-Free Water (Cat.# P1193)
- 96-well plant tissue grinder e.g., Geno/Grinder® 2000 (SPEX CertiPrep, Inc.)
- Geno/Grinder® beads
- Foil sealing tape (3M Scotch brand aluminum foil tape 425: 3 inches × 60 yards)

A. Grinding Plant Material

Plant material should be ground prior to beginning the automated DNA purification procedure.

1. Place 1–5 fresh leaf disks (6mm) in a 1.2ml deep well 96-well plate. Add 350µl of Lysis Buffer A and 1 or 2 grinding beads. (For thin leaf tissue, one bead may be sufficient, for tough or fibrous leaves, 2 beads will provide better grinding action.)

   Note: The optimal number of leaf punches should be determined experimentally for each plant species. The lowest number of punches to consistently obtain the desired yield should be used. For thin leaves such as Canola and Tomato, 5 punches is the maximum sample size and should yield 1µg or more DNA. For thicker leaves, e.g., Corn or Soybean, the maximum number of punches may need to be reduced to avoid chlorophyll carryover into the DNA eluate.

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2. Seal the plate wells firmly with foil tape and process on the grinder following the manufacturer's instructions. After grinding, check the wells to determine that the plant material is sufficiently pulverized. There should be no large chunks remaining. You may need to increase grinding time and/or speed to obtain a homogeneous lysate.

3. Centrifuge the plate briefly at 1700 × *g* to bring down any liquid from the sealer. Remove the seal and add another 300–350µl of Lysis Buffer A to the lysate. Centrifuge the plate again at 1700 × *g* for 10 minutes. Plant debris should be pelleted to the bottom of the wells. If suspended debris remains after this step, it may be helpful to refrigerate the plate at 4°C for 1–2 hours and then centrifuge again at 1700 × *g*. It is important to obtain a debris-free lysate to avoid clogging pipet tips during subsequent liquid transfer steps.

B. Preparation of Equipment and Reagents

1. Prepare the Wash Buffer, Plant by adding 1 part 95–100% ethanol and 1 part isopropanol to 2 parts Wash Buffer and mix well. This is called "Alcohol Wash" on the Biomek® FX Deck. Fill an inverted tip box lid with 140ml of this Wash Buffer and place on the deck at the "Alcohol Wash Dispense" position (Figure 1).

2. Fill 3 inverted tip box lids with 100ml Lysis Buffer A, 50 ml Lysis Buffer B, and 40ml TE (pH 8.0) or Nuclease Free Water, respectively and place on defined positions on the Biomek® FX deck (Figure 1).

Note: For elution, TE (pH 8.0) may be substituted for Nuclease-Free Water.

3. Thoroughly resuspend the MagneSil® Paramagnetic Particles (PMPs) and manually add 20–30µl to each well of a 1.2ml deep well 96-well plate ("working plate") and place on the deck as shown in Figure 1.

4. Turn on and set the recirculating waterbath connected to the heater ALP to 80°C. The temperature is set so that the internal temperature of the 1.2ml deep well plate will be approximately 65°C during elution. You may perform elution without heating, however yields will be lower.

Note: The optimal amount of MagneSil® Paramagnetic Particles to use for your chosen plant species and desired yield must be determined empirically.

5. Place the plates and tips on the Biomek® FX deck as shown in Figure 1. It's important that a 1/4 inch Foam Spacer be placed onto the Deep Well MagnaBot® 96 Separation Device.

IV. Automated Processing Requirements for the Biomek® FX Workstation

A. Instrumentation Requirements for the Biomek® FX

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

Part Description	Quantity	Ordering Information
Biomek® FX Bioworks™ Software version 2.1 (minimum)		Contact Beckman Coulter
96-channel POD	1	Contact Beckman Coulter
Minimum number of Labware Positions by 1 POD	15	Contact Beckman Coulter
Tip Loader ALP	1	Beckman Coulter #719356
Heating/Cooling ALP, Single Position	1	Beckman Coulter #719361
Tip Wash Station	1	Beckman Coulter #710363
Recirculating Water Bath	1	VWR Cat.# 13272-200

B. Labware Requirements for the Biomek® FX

Part Description	Quantity	Ordering Information
Deep Well MagnaBot® 96 Magnetic Separation Device	1	Cat.# V3031
1/4 inch Foam Spacer	1	Cat.# Z3301
Deep Well Plate, 2.2ml (or comparable)	1–3	Corning Cat.# 3960
Deep Well plate, 1.2ml (or comparable)	3	Marsh Cat.# AB-0787
Polystyrene U-bottom multiwell Collection Plate (or comparable)	1	Promega Cat.# A9161 or Greiner America Cat.# 650101
Axygen Wide-Bore Tips (preferred) or Biomek® AP96 P250 tips (rack)	4	Axygen Scientific Cat.# 47743-944 Beckman Coulter Cat.# 717251

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.**

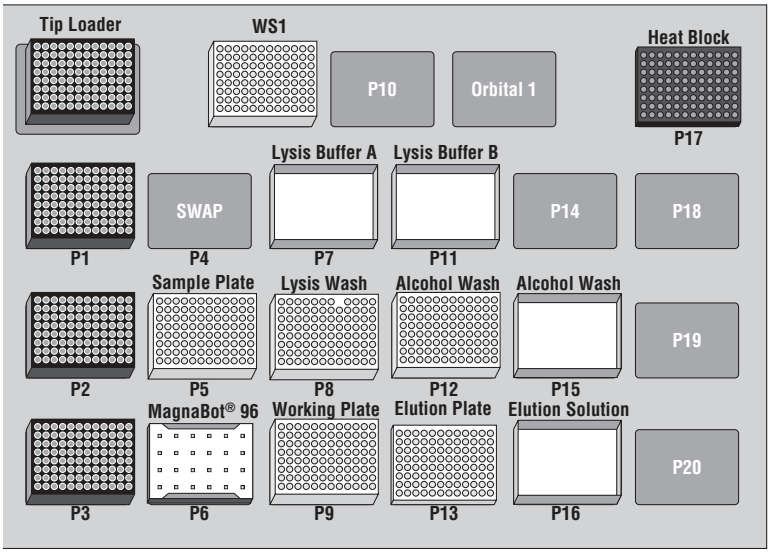

Figure 1. Biomek® FX initial deck configuration.

ALP Name	Equipment
Tip Loader	Biomek® AP96 P250 Tips or Axygen Wide Bore Tips
P1	Biomek® AP96 P250 Tips or Axygen Wide Bore Tips
P2	Biomek® AP96 P250 Tips or Axygen Wide Bore Tips
P3	Biomek® AP96 P250 Tips or Axygen Wide Bore Tips
P4	Swap position used by robot to exchange tip boxes
P5	1.2ml deep-well sample plate containing lysed and centrifuged plant tissue
P6	Deep Well MagnaBot® 96 Magnetic Separation Device with 1/4 inch Foam Spacer
P7	Upside-down tip box containing Lysis Buffer A (lysis wash)
P8	Empty 1.2ml deep well 96-well plate (lysis wash)
P9	1.2ml deep well 96-well plate containing MagneSil® PMPs (working plate)
P10	Empty
P11	Upside-down tip box lid containing Lysis Buffer B
P12	Empty 2.2ml deep well 96-well plate (alcohol wash)
P13	Empty 96-well Collection Plate (elution plate)
P14	Empty
P15	Upside-down tip box lid containing Alcohol Wash (Wash Buffer, Plant)
P16	Upside-down tip box lid containing elution solution (TE [pH 8.0] or nuclease-free water)
P17	Heat Transfer Block
WS1	Tip Wash Station (96-channel) (optional)

D. Biomek® FX Specific Pre-Run Recommendations

The Biomek® FX automated platform allows users the flexibility to configure the robot's deck 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 generally be 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

V. Description of the Automated Wizard® Magnetic 96 DNA Plant System Scaled Up Protocol

The scaled up, automated Wizard® Magnetic 96 DNA Plant System protocol takes approximately 50 minutes from start to finish. After manual addition of MagneSil® Particles to the working plate and filling of tip box lid dispense positions, the robotic protocol proceeds as follows:

1. **Reagent Dispensing.** The robot fills the empty 96-well plates with Lysis Buffers, Wash Buffer and elution solution. At this step 250µl Lysis Buffer B is also added to the 1.2ml deep well plate containing MagneSil® Particles.

2. **Plant Tissue Lysis and DNA Binding**. Plant tissue lysate is transferred in several pipetting steps from the sample plate into the 1.2ml deep well 96-well plate containing Lysis Buffer B and MagneSil® Particles. The MagneSil® Particles are mixed well by pipetting, then captured on the MagnaBot® Device. The supernatant is removed to waste.

   Note: During the transfer of plant tissue lysate it is critical to have the tips high enough from the bottom of the wells to avoid the pelleted cell debris and grinding beads, but low enough 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.**

3. **Washes**. The sample plate is moved off the Deep Well MagnaBot® Device. Lysis Buffer A (380µl) is added to the samples in two steps and mixed thoroughly by pipetting in all four corners of each well to completely resuspend the MagneSil® Particles. The particles are then captured on the MagnaBot® Device and the supernatant is removed. This Lysis Buffer wash procedure is repeated once.

   Three more washes are performed as above using Wash Buffer, Plant (Alcohol Wash).

4. **Dry**. The sample plate is moved off the Deep Well MagnaBot® Device and placed on the Heat Transfer Block on the heating/cooling ALP. The MagneSil® particles are allowed to air-dry for 5 minutes. This important step allows any residual Wash Buffer to evaporate.

5. **Elution.** 50–200µl of TE (pH 8.0) or nuclease-free water is added to the samples and mixed by pipetting. The volume used depends on the plant species and the desired DNA concentration. Several mixes and pauses ensure that all the MagneSil® Particles are resuspended and the DNA is released into solution. The particles are then captured on the Deep Well MagnaBot® Device. The supernatant containing the DNA is removed to a clean 96-well Collection Plate.

6. **Method Ends.** Purified genomic DNA has been eluted into the Collection Plate.

Note: During supernatant removal steps, it is important to make minor adjustments to tip heights to ensure that all liquid is removed at each wash step.

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

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

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 just prior to dispensing into the 1.2ml working plate. Resuspension of the MagneSil® Particles can be accomplished by thorough tip mixing or shaking.

VII. Troubleshooting

Symptoms	Possible Causes	Comments
Low yield	Alcohol present in eluted material	Make sure all the Wash Buffer is aspirated before elution.
		Increase drying time on the Heat Transfer Block.
	Insufficient amount of starting material	The optimal amount of starting material should be determined for each plant species. See note in Section III.A, Step 1, for guidelines for leaf tissue samples.
Green or cloudy DNA	Too much starting material	Decrease the amount of starting material by 30% and re-isolate DNA.
		Centrifuge the plate and transfer the supernatant.
	Cold laboratory temperatures may cause reagent precipitation	Centrifuge the plate and transfer the supernatant.
MagneSil® Particles visible in eluate	MagneSil® Particle carryover into eluted DNA	Allow more time and mixing on the magnet before removing eluted DNA.
		Use the minimal amount of MagneSil® Particles necessary to obtain the desired yield.
		Remove particles by centrifuging the plate and transferring the supernatant.
DNA too dilute	Too much elution solution used	Decrease elution volume to a minimum of 50µl.
	Insufficient amount of starting material	The optimal amount of starting material should be determined for each plant species. See note in Section III.A, Step 1, for guidelines.
Amplification failure	DNA degrades upon storage	Use TE buffer for elution. Store DNA at –20°C in polypropylene plates or tubes.
	Plant may contain phenolics	Add 10mg insoluble PVPP (polyvinylpolypyrrolidone) to plant material before lysis and grinding step.

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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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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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