Automated MagneSil® ONE, Fixed Yield Plant Genomic DNA Purification Protocol

Automated Protocol #EP022

DESCRIPTION OF THE BIOMEK® FX METHOD FOR PRODUCT MD1370
FOR PURIFICATION OF GENOMIC DNA FROM PLANT TISSUE
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 an automated protocol for DNA purification from plant leaf punches. This protocol is an adaptation of the MagneSil® ONE, Fixed Yield Blood Genomic System.

I. Description

This document describes a modified protocol for automated 96-well isolation of plant genomic DNA using the MagneSil® ONE, Fixed Yield Blood Genomic System. The DNA isolated using this protocol is suitable for molecular biology applications, including SNP genotyping assays (e.g., Third Wave Technology Invader® Platform) and PCR amplification.

Specific instructions are provided for the Beckman Coulter Biomek® FX Automated Workstation. Information about downloading the method for this liquid handling workstation is available at: **www.promega.com/automethods/**

General automation guidelines are provided for adaptation to other liquid handling platforms. For information on the system chemistry please refer to the *MagneSil® ONE, Fixed Yield Blood Genomic System Technical Bulletin* #TB313.

II. Product Components

Product	Size	Cat. #
MagneSil® ONE, Fixed Yield Blood Genomic System	1 × 96 preps	MD1370

For Laboratory Use. Individual reagents are available separately for bulk purchase by custom order.

Includes:

- 160ml Lysis Buffer, Blood
- 25ml MagneSil® PMPs—Fixed Yield[a]
- 120ml Alcohol Wash, Blood
- 45ml Elution Buffer, Blood
- 300µl Anti-Foam Reagent*
  (*not used in this plant genomic DNA isolation protocol)

Storage Conditions: Store all components at room temperature (20–25°C).
Do not freeze the MagneSil® PMPs—Fixed Yield.

Do not use ...the Anti-Foam Reagent for Plant gDNA isolation. Use of this reagent may adversely affect DNA yield from plant material.

Do not freeze the MagneSil® Paramagnetic Particles.

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)
- 96-well Collection Plates (Cat.# A9161 or equivalent)
- 96-well polypropylene round-bottom plates, 1.2ml, 96 round wells (Marsh Bio Products Cat.# AB 0787 or equivalent)
- 3 tip box lids to use as reagent reservoirs
- 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)
- 96-well plant tissue grinder e.g., Geno/Grinder® 2000 (SPEX CertiPrep, Inc.)
- Grinding beads, e.g., 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 MagneSil® ONE, Fixed Yield plant genomic DNA purification protocol. The Anti-Foam Reagent supplied with the product **is not used** with this method as it drastically reduces yield.

Note: This protocol is not compatible with the use of PVPP (polyvinylpolypyrrolidone) to remove phenolic inhibitors from plant material. Thus it may not be suitable for certain plant species (e.g., cotton, strawberry leaf).

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1. Place 1 fresh leaf disk in each well of a 1.2ml, 96-well deep well plate. Add 1–2 grinding beads and 350µl of Lysis Buffer, Blood, to each well. (For thin leaf tissue one bead may be sufficient, for tough or fibrous leaves, two beads will provide greater grinding action.)

   Note: Generally, one leaf punch will yield a minimum of ~100ng DNA, depending on completeness of homogenization.

2. Seal the plate wells firmly with foil tape and process on the Grinder following the manufacturer's instructions. There will be considerable foaming from the detergent in the Lysis Buffer. This will dissipate with centrifugation. After grinding, check the wells to determine that the plant material is sufficiently pulverized. There should be no large chunks of plant tissue remaining. Depending on the sample type, it may be necessary to increase grinding time and/or speed to obtain a homogeneous lysate.

3. Centrifuge the plate at 1,700 × *g* for 15 minutes. Plant debris should be pelleted to the bottom of the wells. If there is suspended material remaining, it may be helpful to refrigerate the plate at 4°C for 1–2 hours then centrifuge again 1,700 × *g* for 15 minutes. It is important to obtain a debris-free lysate to avoid clogging robotic tips during subsequent liquid transfer steps.

B. Preparation of Equipment and Reagents

1. Prepare the wash buffer by adding 1 part 95–100% ethanol and 1 part isopropanol to 2 parts Alcohol Wash, Blood (e.g., 60ml of 95–100% ethanol, 60ml of isopropanol, and 120ml of Alcohol Wash, Blood). 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 two inverted tip box lids with 50ml Lysis Buffer, Blood (do not add Anti-Foam Reagent) and 40ml Elution Buffer or nuclease-free water, respectively, and place on defined positions on the Biomek® FX deck (Figure 1).

3. Thoroughly resuspend the MagneSil® PMPs–Fixed Yield and manually add 15µl to each well of a 96-well Collection Plate. Place this "MagneSil® PMPs" plate on the deck as shown in Figure 1.

4. Turn on and set the recirculating waterbath connected to the Beckman Coulter Heating/Cooling 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.

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

Note: The MagneSil® Particles are best added from a reservoir using a multichannel pipette. Resuspend the particles between each transfer, as they will settle out of solution rapidly.

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 for the automated MagneSil® ONE, Fixed Yield plant genomic DNA purification protocol 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	Beckman Coulter Cat.# 719368
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 (96 channel) (Optional)	1	Beckman Coulter #719363
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	Promega Cat.# V3031
1/4 inch Foam Spacer	1	Promega Cat.# Z3301
Deep Well Plate, 2.2ml (or comparable)	1	Corning Cat.# 3960
Deep Well plate, 1.2ml (or comparable)	3	Marsh Cat.# AB-0787
Collection Plate (or comparable)	2	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 Biomek® FX deck layout for the automated MagneSil® ONE, Fixed Yield plant genomic DNA purification protocol. **Your specific deck layout may be different depending on your Biomek® FX configuration.**

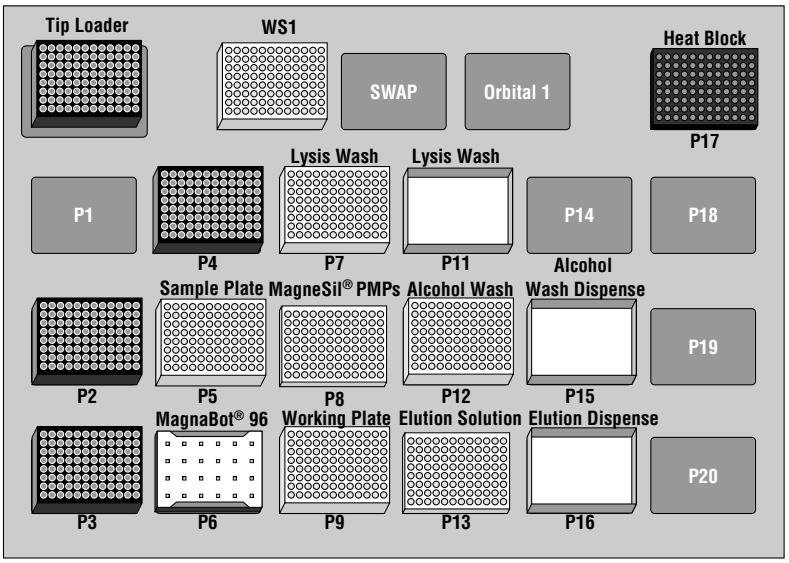

Figure 1. Biomek® FX initial deck configuration.

ALP Name	Equipment
Tip Loader	Biomek® AP96 P250 Tips
P1	Empty
P2	Biomek® AP96 P250 Tips
P3	Biomek® AP96 P250 Tips
P4	Biomek® AP96 P250 Tips
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	Empty 1.2ml deep well 96-well plate (Lysis Wash)
P8	96-well Collection Plate containing 15µl MagneSil® PMPs/well
P9	Empty 1.2ml deep well 96-well plate (Working Plate)
P10	Swap position used by robot to exchange tip boxes
P11	Upside-down tip box lid containing Lysis Buffer, Blood
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 (Alcohol Wash Dispense)
P16	Upside-down tip box lid containing Elution Buffer, Blood or nuclease-free water (Elution Dispense)
P17	Heat Transfer Block
WS1	Tip Wash Station (96-channel) (optional)
Orbital 1	Not used

D. Biomek® FX Specific Pre-Run Recommendations

The Biomek® FX allows users the flexibility to configure the robot's deck according to need. This flexibility makes it likely that the deck used for writing a Biomek® FX method will differ from an end-user's deck. Therefore, it is 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/biomekfx/default.asp).

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

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V. Description of the Automated MagneSil® ONE, Fixed Yield Plant Genomic DNA Purification Protocol

The automated MagneSil® ONE, Fixed Yield plant genomic DNA purification protocol takes approximately 42 minutes to complete. After manual addition of 15µl MagneSil® PMPs–Fixed Yield/well to the "MagneSil® PMPs" plate and filling of the tip box lid dispense positions, the protocol proceeds as follows:

1. **Plant Tissue Lysis and DNA Binding**. Plant tissue lysate (300µl/well) is transferred from the sample plate into the working plate in several pipetting steps. A 100µl aliquot of Lysis Buffer is mixed with the MagneSil® Particles and these are also transferred to the working plate. Sample and particles are then mixed thoroughly by pipetting in all four corners of each well of the working plate to completely resuspend the MagneSil® Particles. The working plate is then transferred to the MagnaBot® Device and the particles are captured. 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. **If a different extraction plate or method is used, re-optimize the tip height before performing the transfer step.**

2. **Washes**. The sample plate is moved off the Deep Well MagnaBot® Device. Lysis Buffer (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.

   Three more washes are performed as above using Alcohol Wash (380µl/well).

3. **Dry**. The MagneSil® particles are allowed to air-dry on the Deep Well MagnaBot® Device for 5 minutes. This is an important step, as it allows residual Alcohol Wash to evaporate. The drying time may be extended if humidity is high in your laboratory. The sample plate is then moved off the MagnaBot® Device and on to the Heat Transfer Block on the heating/cooling ALP to begin the elution step.

4. **Elution.** 150µl of Elution Buffer 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. If necessary, elution volume can be reduced to a minimum of 50µl. Six mixes (by pipetting in all four corners of each well to completely resuspend the MagneSil® Particles) 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.

5. **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.

VI. General Guidelines for Adaptation to Alternative Robotic Platforms

The MagneSil® Particles settle quickly over time. We recommend thoroughly mixing the MagneSil® Particles before dispensing into the working plate and immediately starting the automated protocol.

VII. Troubleshooting

Symptoms	Possible Causes	Comments
Low yield	Alcohol present in eluted material	Make sure all the Alcohol Wash is aspirated before elution.
		Increase drying time before elution.
MagneSil® Particles visible in eluate	MagneSil® Particle carryover into eluted DNA	Allow more time and mixing on the magnet before removing eluted DNA.
		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.
Amplification failure	DNA degrades upon storage	Use TE buffer for elution. Store DNA at –20°C in polypropylene plates or tubes.

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