



Automated Wizard® MagneSil® Sequencing Reaction Clean-Up System Protocol for 96-Well Plates

Automated Protocol No. EP021

DESCRIPTION OF THE BECKMAN COULTER BIOMEK® FX, BIOMEK® 2000 AND TECAN GENESIS® RSP150 METHODS WITH PRODUCTS A1831, A1832 AND A1835.
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.

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I. Description

This document describes automation of the Wizard® MagneSil® Sequencing Reaction Clean-Up System^(a). Specific instructions are provided for the following automated liquid handling workstations: Beckman Biomek® 2000, Beckman Biomek® FX, Tecan Genesis® RSP150. Validated methods for these automated liquid handling workstations are available at: www.promega.com/automethods. General automation guidelines are provided for adaptation to other liquid handling platforms. For troubleshooting chemistry issues please refer to Technical Bulletin #TB287.

II. Product Components

Product	Size	Cat.#
Wizard® MagneSil® Sequencing Reaction Clean-Up System ^(a)	4 x 96	A1831
For Laboratory Use. Each system includes sufficient reagents for processing 4 x 96-well plates. Includes:		

- 1 x 100ml MagneSil® GREEN
- 1 Protocol

Product	Size	Cat.#
Wizard® MagneSil® Sequencing Reaction Clean-Up System ^(a)	8 x 96	A1832
For Laboratory Use. Each system includes sufficient reagents for processing 8 x 96-well plates. Includes:		

- 2 x 100ml MagneSil® GREEN
- 1 Protocol

Product	Size	Cat.#
Wizard® MagneSil® Sequencing Reaction Clean-Up System ^(a)	100 x 96	A1835
For Laboratory Use. Each system includes sufficient reagents for processing 100 x 96-well plates. Includes:		

- 25 x 100ml MagneSil® GREEN
- 1 Protocol

Items Available Separately

Product	Size	Cat.#
MagneSil® GREEN ^(a)	100ml	A8231
For Laboratory Use.		

Storage Conditions: Store all components at room temperature. **Do not freeze.** See product label for product shelf life.

Accessories

Product	Cat.#
Plate Clamp 96 ^(b)	V8251
Plate Stand	V8261
MagnaBot® II Magnetic Separation Device	V8351
MagnaBot® T1 Adaptor	V8481

 **DO NOT**
freeze MagneSil®
Paramagnetic Particles.

III. Before You Begin

Materials to Be Supplied by the User

- MagnaBot® II Magnetic Separation Device (Promega Cat.# V8351)
- 90% ethanol
- elution buffer as appropriate:
 - a. nuclease-free water
 - b. deionized formamide (for example, Hi-Di™ Formamide [Applied Biosystems Cat.# 4311320]), or
 - c. EDTA/Blue Dextran

Preparation of Solutions

Thoroughly resuspend the MagneSil® GREEN by shaking. Ensure that there are no particles of resin remaining at the bottom of the bottle.

IV. Automated Processing Requirements for the Beckman Coulter Biomek® FX Laboratory Workstation

A. Instrumentation Requirements for the Biomek® FX

Part Description	Quantity	Beckman Coulter Part Number
Minimum: Biomek® FX Software version 2.1	1	Contact Beckman
96-channel POD	1	Contact Beckman
Minimum number of Labware Positions by 1 POD	8	Contact Beckman
Tip Loader ALP	1	719356
96 Tipwash ALP (optional)	1	Contact Beckman
Trash ALP (optional)	1	Contact Beckman
MagBead ALP (optional)	1	Contact Beckman
Orbital Shaker ALP (optional)	1	Contact Beckman

B. Labware Requirements for the Biomek® FX

Part Description	Quantity	Ordering Information
MagnaBot® II Magnetic Separation Device	1	Promega Cat.# V8351
2.2ml 96-well, deep square well polypropylene plate (or comparable)	2	Marsh Cat.# AB-0932
1.2ml 96-well deep well round-bottom polypropylene plate (or comparable)	1	Marsh Cat.# AB-0564/BP
Polypropylene 96-well PCR Plate, unskirted (or comparable)	1	Robbins Scientific Cat.# 1055-00-0
Plate Clamp 96 (for use with unskirted PCR plates)	1	Promega Cat.# V8251
Plate Stand (for use with Plate Clamp 96)	1	Promega Cat.# V8261
Collection plates	2	Promega Cat.# A9161
Biomek® AP96 P250 Tips, Nonsterile (rack)	1	BCI Cat.# 717251
Biomek® AP96 P20 Tips, Nonsterile (rack)	1	BCI Cat.# 717254

C. Biomek® FX Initial Deck Configuration

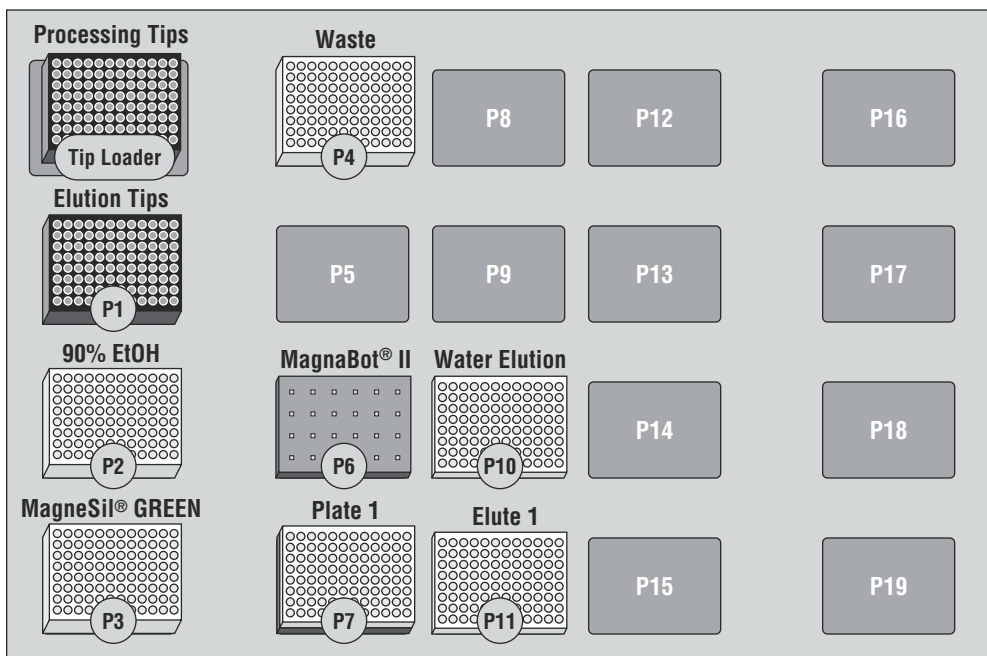


Figure 1. Biomek® FX initial deck configuration.

ALP Name	Equipment
Tip Loader	Biomek® AP96 P250 Tips, Nonsterile
P1	Biomek® AP96 P20 Tips, Nonsterile
P2	2.2ml Deep 96-Well Plate containing 1.0ml of 90% Ethanol per well
P3	1.2ml Deep 96-Well Plate containing 350µl of MagneSil® GREEN per well
P4	Empty 2.2ml Deep 96-Well Plate to be used for waste
P5	Empty
P6	MagnaBot® II Magnetic Separation Device
P7	Plate Clamp 96 with a 96-well PCR Plate containing Sequencing reactions on a Plate Stand
P8	Empty
P9	Empty
P10	Collection plate containing 50µl of nuclease-free water or relevant elution buffer per well
P11	Empty collection plate

D. Biomek® FX Specific Pre-Run Recommendations

The Biomek FX® automated platform allows users to configure the robot's deck according to need. Because of this flexibility, 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. Follow the instructions provided: *Biomek® FX Deck Mapping*. (<http://www.promega.com/automethods/beckman/biomekfx/default.asp>)

**V. Automated Processing Requirements for the Beckman Coulter
Biomek® 2000 Liquid Handling Workstation**
A. Instrumentation Requirements for the Biomek® 2000

Part Description	Quantity	Beckman Coulter Part Number
Biomek® 2000 Workstation, 50/60Hz, 100–120V	1	609000
Biomek® 2000 Controller NT	1	609875
IBM® Monitor	1	974571
BioWorks™ 3.2 for Beckman Coulter Computer	1	609983
Gripper Tool System for Biomek® 2000	1	609001
MP200 Pipetting Tool	1	609025
MP20 Pipetting Tool	1	609024
Tip Rack Holder	2	609121
Gray Labware Holder	5	609120
2x PCR 8-Strip Tube Holder	1	Promega Cat.# Z3341

B. Labware Requirements for the Biomek® 2000

Part Description	Quantity	Ordering Information
MagnaBot® II Magnetic Separation Device	1	Promega Cat.# V8351
Biomek® P250 Tips, Non-sterile (rack)	1	Beckman Coulter Cat.# 372655
Biomek® P20 Tips, Non-sterile (rack)	1	Beckman Coulter Cat.# 609044
Collection Plate	1	Promega Cat.# A9161
Plate Clamp 96 (for use with unskirted PCR plates)	1	Promega Cat.# V8251
Plate Stand (for use with Plate Clamp 96)	1	Promega Cat.# V8261
Polypropylene 96-well PCR Plate, unskirted (or comparable)	1	Robbins Scientific Cat.# 1055-00-0
MicroAmp® Reaction Tubes (8 Tubes/Strip)	2	ABI Cat.# N801-0580

C. Biomek® 2000 Initial Deck Configuration

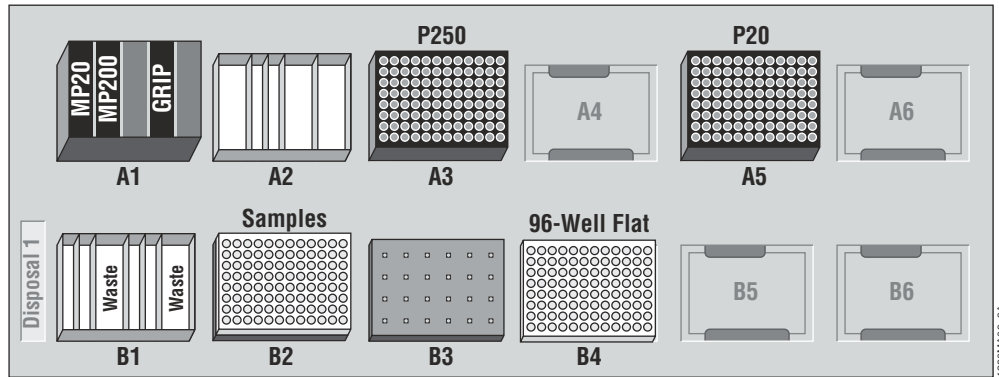


Figure 2. Biomek® 2000 initial deck configuration.

- | | |
|-------------|---|
| Position A1 | Tool rack containing MP20, MP200 and Gripper tools |
| Position A2 | Reservoir holder: one quarter vertical reservoir (see Figure 3 for details) |
| Position A3 | Tip rack holder, Biomek® P250 Tips, non-sterile |
| Position A4 | Empty |
| Position A5 | Tip rack holder, Biomek® P20 Tips, non-sterile |
| Position A6 | Empty |
| Position B1 | Reservoir holder: one quarter vertical reservoirs, two Quarter Reservoirs, and 1 strip tube holder (see Figure 4 for details) |
| Position B2 | Plate Clamp 96 with a 96-well PCR Plate containing Sequencing Reactions on Plate Stand |
| Position B3 | MagnaBot® II Magnetic Separation Device |
| Position B4 | Empty collection plate |
| Position B5 | Empty |
| Position B6 | Empty |

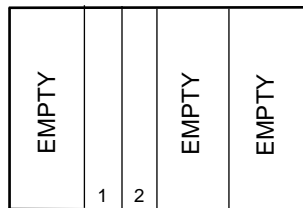


Figure 3. Reagents at Position A2.

1. 19ml 90% ethanol
2. 19ml 90% ethanol

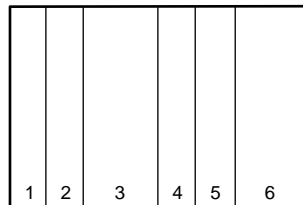


Figure 4. Reagents at Position B1.

1. 10ml MagneSil® GREEN
2. 10ml MagneSil® GREEN
3. Empty quarter reservoir for waste
4. Strip tubes containing 200µl Nuclease-Free Water per well
5. Strip tubes containing 200µl Nuclease-Free Water per well
6. Empty quarter reservoir for waste

VI. Automated Processing Requirements for the Tecan Genesis® RSP150
A. Instrumentation Requirements for the Genesis® RSP150

Part Description	Quantity	Tecan Part Number
Gemini 3.5 software	1	Contact Tecan
1ml Syringes	8	Contact Tecan
Standard Fixed Tips	8	612501
100ml Reservoir Carriers	1	613020
100ml Reservoirs	3	613021
Robotic Manipulator Arm (RoMa)	1	Contact Tecan
3-Position Microplate Carrier	1	Contact Tecan

B. Labware Requirements for the Genesis® RSP150

Part Description	Quantity	Ordering Information
MagnaBot® II Magnetic Separation Device	1	Promega Cat.# V8351
MagnaBot® Adaptor T1	1	Promega Cat.# V8481
Collection Plate	1	Promega Cat.# A9161
Plate Clamp 96	1	Promega Cat.# V8251
Plate Stand	1	Promega Cat.# V8261
Polystyrene 96-well PCR Plate, unskirted (or comparable)	1	Robbins Scientific Cat.# 1055-00-0

C. Genesis® RSP150 Initial Workspace Configuration

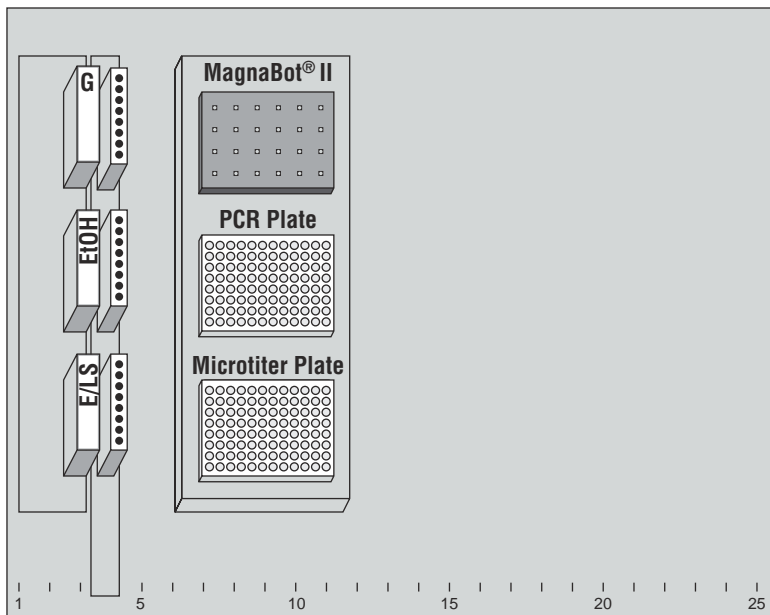


Figure 5. Genesis® RSP150 initial workspace configuration.

Reservoir Carrier Position (Grid 2)	Contents	Microplate Carrier Position (Grid 6)	Rack on Carrier
Back	“G” – 24ml MagneSil® Green	Position 1	MagnaBot® II on MagnaBot® T1 Adaptor
Middle	“EtOH” – 24ml 90% ethanol	Position 2	PCR Plate in Plate Clamp 96 on Plate Stand
Front	“E/LS” – Elution/Loading Solution—Appropriate Volume plus 4ml	Position 3	Empty collection plate

D. Genesis® RSP150 Specific Pre-Run Recommendations

Due to differences in instrument reference positions, carriers and plate types we recommended checking and adjusting the following settings prior to running the method:

- a. X,Y,Z coordinates for each plate and carrier type used in the method
- b. RoMa vectors

VII. Description of Automated Wizard® MagneSil® Sequencing Clean-Up System Protocol

This overview describes general liquid handling steps required for the automated Wizard® MagneSil® Sequencing Reaction Clean-Up System and can be adapted to a variety of automated liquid handling robots. Additional information for adaptation to liquid handling robots other than those referenced above, please see Section VIII, General Guidelines for Adaptation to Alternative Robotic Platforms.

1. **Binding DNA Sequencing Extension Products to MagneSil® GREEN Paramagnetic Particles**

- MagneSil® GREEN Transfer: MagneSil® GREEN paramagnetic particles (PMPs) are resuspended by pipetting. 180µl of MagneSil® GREEN PMPs is transferred from the MagneSil® GREEN reservoir to the sample plate.
- Binding Mix: The sequencing reactions and MagneSil® GREEN PMPs are mixed. During this step the amplification products are bound to the MagneSil® GREEN PMPs. Mixing can be performed by pipetting or shaking intermittently for 1–3 minutes. The sample plate is transferred to the MagnaBot® II Magnetic Separation Device and paused briefly to capture PMPs to the sides of each well. The liquid can be mixed by pipetting, and then the plate paused briefly a second time to ensure complete capture of PMPs. The entire supernatant is then transferred to waste. It is critical to ensure complete removal of the supernatant at this step.

2. **Ethanol Wash of MagneSil® GREEN PMPs**

- The sample plate is transferred off the MagnaBot® II Magnetic Separation Device. Ethanol (90%) is transferred [100µl for the Biomek® 2000 and Tecan Genesis®, and 125µl for the Biomek® FX] to the sample plate.
- Wash Solution and MagneSil® GREEN PMPs are mixed. Mixing can be performed by pipetting or shaking intermittently for 1–3 minutes. The sample plate is transferred to the MagnaBot® II Magnetic Separation Device, and paused briefly to capture PMPs to the sides of each well. The liquid can be mixed by pipetting, and then the plate paused briefly a second time to ensure complete capture of PMPs. The entire supernatant is then transferred to waste. It is critical to ensure complete removal of the supernatant at this step.
- Steps 1–2 are repeated for a total of 3 washes when using the Biomek® 2000 and Biomek® FX Automated Laboratory Workstations, or 2 washes if using the Tecan Genesis® RSP150 Workstation.

3. **Drying the MagneSil® GREEN PMPs**

- Optional: A final low-volume supernatant removal is performed to ensure complete removal of ethanol from the sample plate.
- Drying MagneSil® GREEN PMPs: The sample plate is paused on the MagnaBot® II Magnetic Separation Device for 5–15 minutes. This allows any ethanol remaining on the PMPs or in the plate to evaporate. Depending on the humidity level in your lab, this drying time may be increased or decreased.

4. **Eluting DNA Sequencing Extension Products from MagneSil® GREEN PMPs**

- The sample plate is transferred off the MagnaBot® II Magnetic Separation Device.
- Elution Water/Loading Solution transfer: Elution Water/Loading Solution (volume appropriate for downstream application) is transferred to the sample plate and mixed by pipetting to resuspend. The plate is paused and mixed intermittently for 2–3 minutes to elute the purified DNA sequencing extension products off the MagneSil® GREEN PMPs.

For questions not addressed here, please contact your local Promega branch office or distributor. Contact information available at: www.promega.com.

E-mail: techserv@promega.com

- The sample plate is transferred to the MagnaBot® II Magnetic Separation Device. After a brief pause to capture the PMPs to the sides of each well, the liquid is mixed with pipette tips to ensure complete capture of PMPs. The eluted Sequencing Reactions are then transferred to the Elution Plate.
- The samples are now ready for direct loading onto an automated fluorescent sequence analyzer.

VIII. General Guidelines for Adaptation to Alternative Robotic Platforms

The MagneSil® GREEN particles settle rapidly over time. It is recommended to thoroughly mix the MagneSil® GREEN particles on the automated platform prior to dispensing into samples. Resuspension of the MagneSil® GREEN particles can be accomplished by thorough tip mixing.

It is important to completely remove supernatants during binding and washing steps. Failure to do so may result in poor sequence read quality.

^(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.

^(b)Patent 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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