

Automated Wizard® SV 96 Genomic DNA Purification System for Cell Culture Samples



Automated Protocol #EP018

DESCRIPTION OF THE AUTOMATED METHODS WITH PRODUCTS A2370 AND A2371

Please visit the web site to verify that you are using the most current version of this Automated Protocol.

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1. Description

This protocol describes automation of the Wizard® SV 96 Genomic DNA Purification System to purify genomic DNA from tissue culture cells. Specific instructions are provided for the following automated liquid-handling workstations: Beckman Coulter Biomek® 2000, Biomek® 3000, Biomek® FX and Eppendorf epMotion® 5075 VAC. Information about obtaining validated methods for these automated liquid-handling workstations is available at: www.promega.com/automethods/

General automation guidelines are provided for adaptation to other liquid-handling platforms. To troubleshoot chemistry issues please refer to the *Wizard® SV 96 Genomic DNA Purification System Technical Bulletin #TB303*.

2. Product Components and Storage Conditions

| Product | Size | Cat.# |
|---|--------------|-------|
| Wizard® SV 96 Genomic DNA Purification System | 1 × 96 preps | A2370 |

Each system contains sufficient reagents for 96 isolations. Includes:

- 50ml Nuclei Lysis Solution
- 30ml 0.5M EDTA (pH 8.0)
- 50ml Wizard® SV Lysis Buffer
- 185ml Column Wash Solution (CWA; concentrated)
- 1ml RNase A Solution (4mg/ml)
- 150ml Nuclease-Free Water
- 1 Binding Plate
- 1 96-Well Deep Well Plate

| Product | Size | Cat.# |
|---|--------------|-------|
| Wizard® SV 96 Genomic DNA Purification System | 4 × 96 preps | A2371 |

Each system contains sufficient reagents for 4 × 96 isolations. Includes:

- 2 × 50ml Nuclei Lysis Solution
- 30ml 0.5M EDTA (pH 8.0)
- 3 × 50ml Wizard® SV Lysis Buffer
- 2 × 370ml Column Wash Solution (CWA; concentrated)
- 3 × 1ml RNase A Solution (4mg/ml)
- 2 × 150ml Nuclease-Free Water
- 4 Binding Plates
- 4 96-Well Deep Well Plates

Storage Conditions: Store all components at 22–25°C.

3. Materials to Be Supplied by the User

- 1X phosphate-buffered saline (PBS), sterile
- 55°C water bath
- adhesive plate sealers
- **for Biomek® FX only:** Pyramid-Bottom Reservoir Plates (2)
(Promega Cat.# V6801)

4. Before You Begin

4.A. Preparation of Column Wash Solution (CWA)

Prepare the Column Wash Solution (CWA) prior to beginning the Wizard® SV 96 Genomic DNA Purification System protocol.

Add 95% ethanol to the Column Wash Solution (CWA) bottle as directed on the bottle label. Label the bottle to indicate that ethanol has been added. Seal well, and store at room temperature.

4.B. Preparation of Cell Culture Samples

Before placing cells on the deck of the robot, wash the cells once with 1X PBS. Make sure to remove PBS before placing cells on the deck of the robot for processing. Wizard® SV Lysis Buffer should be added to cells alone.

Use at least 1×10^4 cells and up to 5×10^6 cells per purification. The number of cells may need to be adjusted depending on cell type and function.

5. Biomek® 2000 Workstation Requirements

5.A. Method

1. Check instrument requirements for the Beckman Coulter Biomek® 2000 Wizard® SV 96 Genomic DNA cell culture sample method.

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required to automate the Wizard® SV 96 Genomic DNA Purification System for cell culture samples on a Biomek® 2000 workstation.

| Part Description | Beckman Coulter Part Number |
|--|------------------------------------|
| Biomek® 2000 workstation, 50/60 Hz, 100–120V | 609000 |
| Controller, Biomek® 2000, with BioWorks™ 3.2 for new systems | 267653 |
| Gripper kit | 609001 |
| Eight-channel wash tool | 609027 |
| Wash unit with automatic 6-port valve | 609056 |
| MP200 eight-channel pipetting tool | 609025 |
| Pipette tip rack holder, black (2 for single plate run) | 609121 |
| Labware holder, gray (3) | 609120 |
| Biomek® 2000 96-Filtration System for Promega Wizard® SV 96 Systems (with Vacuum Unit) | 267693 |
| Elution plate spacer | 390792 |
| Tubing kit, wash unit | 609687 |

2. Check labware requirements for the Beckman Coulter Biomek® 2000 Wizard® SV 96 Genomic DNA cell culture sample method.

| Part Description | Ordering Information |
|--|-----------------------------|
| Reservoir holder | 372795 |
| Quarter reservoir (2) | 372790 |
| 96-Well Deep Well Plate | Provided |
| Binding Plate | Provided |
| 96-well flat-bottom cell culture plate | Not Provided |
| P250 Tips, Sterile, 250µl (2) | Beckman Cat.# 372655 |
| or P250 Tips, Racked, Sterile, 250µl (2) | Axygen Cat.# BT-250-1-R-S |

5.A. Method (continued)

3. Check initial deck configuration for the Beckman Coulter Biomek® 2000 Wizard® SV 96 Genomic DNA Cell Culture Sample Method. The volumes of reagents dispensed in the reservoir at position B4 are shown in Figure 2.

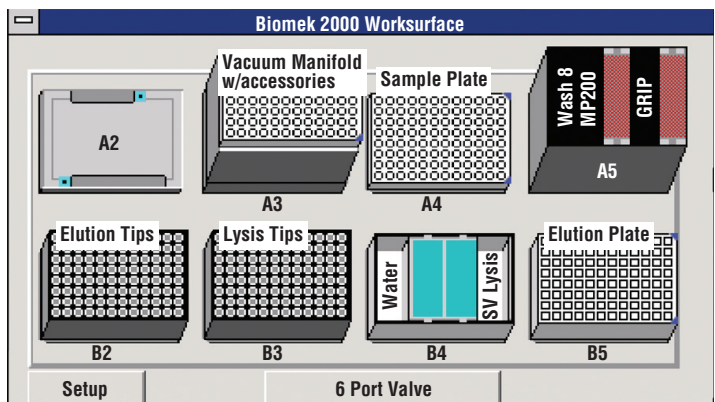
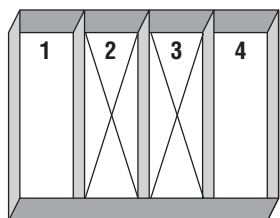


Figure 1. Biomek® 2000 initial deck configuration. Genomic DNA purification from cell culture samples.

Note: Side modules are not part of the initial deck configuration.

| | |
|-------------|--|
| Position A2 | Labware collar holder |
| Position A3 | Vacuum filtration manifold base, elution plate spacer, 65mm collar, Binding Plate |
| Position A4 | Labware holder, 96-well flat-bottom cell culture plate |
| Position A5 | Tool rack containing eight-channel wash tool, MP200 eight-channel pipetting tool and Gripper kit |
| Position B2 | Pipette tip rack holder, P250 tips |
| Position B3 | Pipette tip rack holder, P250 tips |
| Position B4 | Labware holder, reservoir holder, two quarter reservoirs |
| Position B5 | Labware holder, 96-Well Deep Well Plate |



1. 12ml of Nuclease-Free Water
 2. Empty
 3. Empty
 4. 17.5ml of Wizard® SV Lysis Buffer
- Valve 1 of the Biomek® wash unit should be connected to a bottle with at least 250ml of Column Wash Solution (CWA) (ethanol added).

Figure 2. Reagent dispense volumes for the Biomek® 2000. Prior to beginning run, the reagents listed above need to be dispensed appropriately on the deck of the Biomek® 2000.

5.B. Pre-Run Recommendations

Before running the method, import the method into the BioWorks™ software. Please follow the instructions for “Importing Biomek® 2000 Methods” available online at: www.promega.com/automethods/beckman/biomek2000/

6. Biomek® 3000 Workstation Requirements

6.A. Method

1. Check instrument requirements for the Beckman Coulter Biomek® 3000 Wizard® SV 96 Genomic DNA cell culture sample method.

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required to automate the Wizard® SV 96 Genomic DNA Purification System for cell culture samples on a Biomek® 3000 workstation.

| Part Description | Beckman Coulter Part Number |
|---|------------------------------------|
| Biomek® 3000 workstation, 50/60 Hz, 100–120V | 986120 |
| Biomek® automation controller XP and monitor with Biomek® system software | A16170 |
| Gripper kit | A09053 |
| Eight-channel wash tool | 987370 |
| Wash unit with automatic 6-port valve | 609056 |
| Left-side module | 987264 |
| MP200 pipetting tool | 986146 |
| Pipette tip rack holder, black (2) | 391910 |
| Labware holder, gray (3) | 609120 |
| Biomek® 3000 filtration system with vacuum valve unit | A15925 |
| Collar (65mm) with spacer plate | 609803 |
| Elution plate spacer | 390792 |

2. Check labware requirements for the Beckman Coulter Biomek® 3000 Wizard® SV 96 Genomic DNA cell culture sample method.

| Part Description | Ordering Information |
|---|---|
| Reservoir holder | 372795 |
| Quarter reservoir (2) | 372790 |
| 96-Well Deep Well Plate | Provided |
| Binding Plate | Provided |
| 96-well flat-bottom cell culture plate | Not Provided |
| Biomek® AP96 P250 Tips, Presterile (2) or Biomek® FX Tips, 250µl, Racked, Presterilized (2) | Beckman Cat.# 717252 Axygen Cat.# FX-250-1-R-S |

6.A. Method (continued)

3. Check initial deck configuration for the Beckman Coulter Biomek® 3000 Wizard® SV 96 Genomic DNA cell culture sample method. The volumes of reagents dispensed in the reservoir at position B3 are shown in Figure 4.

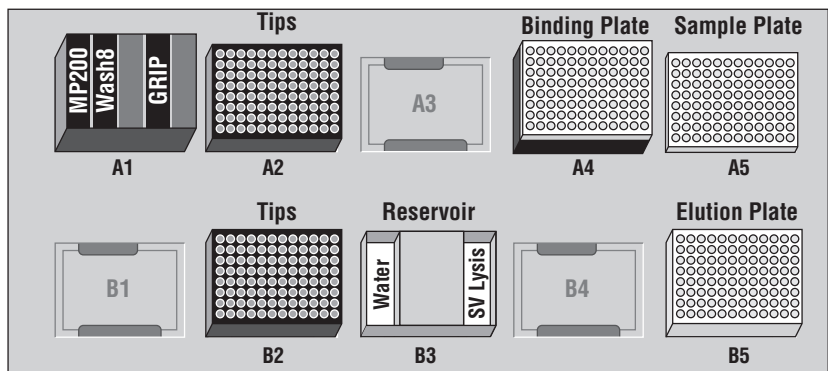
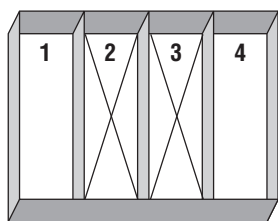


Figure 3. Biomek® 3000 initial deck configuration. Genomic DNA purification from cell culture samples.

| | |
|-------------|---|
| Position A1 | Tool rack containing MP200 pipetting tool, eight-channel wash tool and Gripper kit |
| Position A2 | Pipette tip rack holder, P250 tips |
| Position A3 | Empty |
| Position A4 | Biomek® 3000 filtration system with vacuum valve unit, elution plate spacer, collar (65mm), Binding Plate |
| Position A5 | Labware holder, 96-well flat-bottom cell culture plate |
| Position B1 | Empty |
| Position B2 | Pipette tip rack holder, P250 tips |
| Position B3 | Labware holder, reservoir holder, 2 quarter reservoirs |
| Position B4 | Empty |
| Position B5 | Labware holder, 96-Well Deep Well Plate |



1. 27ml of Nuclease-Free Water
 2. Empty
 3. Empty
 4. 20ml of Wizard® SV Lysis Buffer
- Valve 1 of the Biomek® wash unit should be connected to a bottle of at least 250ml of Column Wash Solution (CWA) (ethanol added).

Figure 4. Reagent dispense volumes for the Biomek® 3000. Prior to beginning run, the reagents listed above need to be dispensed appropriately on the deck of the Biomek® 3000.

7. Biomek® FX Workstation Requirements

7.A. Method

The cell culture sample method will process one or two plates of cultured cells per method run. The instrument requirements for the one- and two-plate runs are identical. However, labware requirements and the starting deck layout for a one-plate or two-plate run are different (see labware requirements list and starting deck layout figures below).

1. Check instrument requirements for the Beckman Coulter Biomek® FX Wizard® SV 96 Genomic DNA cell culture sample method.

| Part Description | Beckman Coulter Part Number |
|--|------------------------------------|
| Minimum: Biomek® FX software v2.1 | Contact Beckman Coulter |
| 96-channel POD | Contact Beckman Coulter |
| Minimum number of labware positions by 1 POD (10) | Contact Beckman Coulter |
| Tip loader | 719356 |
| Biomek® FX filtration system (single plate) with vacuum valve unit | 719400 |
| Elution plate spacer | 390792 |

2. Check labware requirements for the Beckman Coulter Biomek® FX Wizard® SV 96 Genomic DNA cell culture sample method.

| Part Description | Quantity | Ordering Information |
|---|--|---|
| Pyramid-bottom reservoir plates | 3 for one-plate method 4 for two-plate method | Promega Cat.# V6801 |
| 96-well flat-bottom cell culture plate | 1 for one-plate method 2 for two-plate method | Not Provided |
| 96-Well Deep Well Plate | 1 for one-plate method 2 for two-plate method | Provided |
| Binding Plate | 1 for one-plate method 2 for two-plate method | Provided |
| Biomek® AP96 P250 Tips, Presterile (2) or Biomek® FX Tips, 250µl, Racked, Presterilized (2) | | Beckman Cat.# 717252 Axygen Cat.# FX-250-1-R-S |

7.A. Method (continued)

3. Check initial deck configuration for Beckman Coulter Biomek® FX Wizard® SV 96 Genomic DNA cell culture sample method.

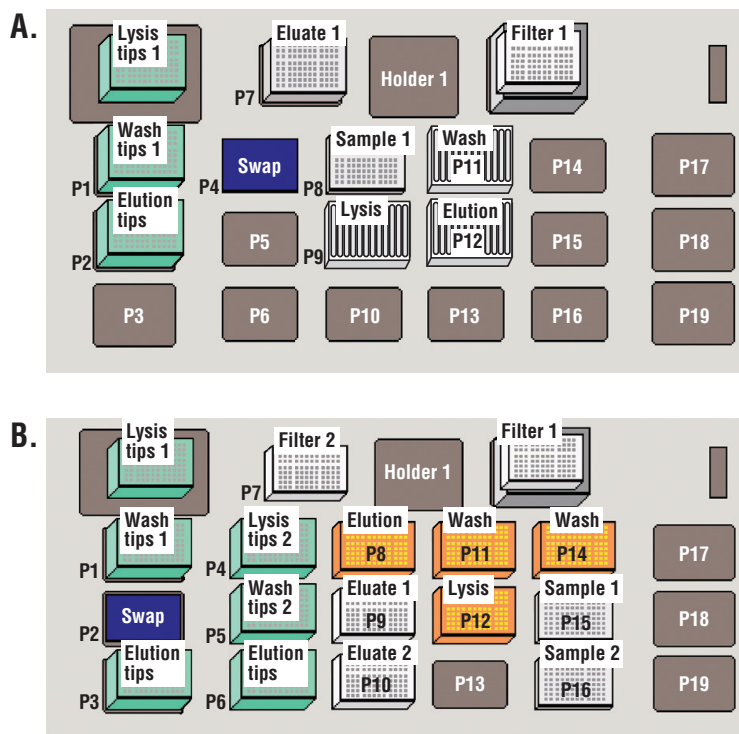


Figure 5. Biomek® FX deck layout. Examples of starting deck layouts for Wizard® SV 96 Genomic DNA Purification on a Biomek® FX. Your specific deck layout may be different depending on your Biomek® FX configuration.

A. Starting Deck Layout for One-Plate Cell Culture Sample Method.

| ALP Name | Part Sitting on ALP |
|------------|---|
| Tip Loader | P250 Biomek® FX tips |
| SPE ALP | Biomek® FX filtration system (single plate) with vacuum valve unit, elution plate spacer, Binding Plate |
| P1 | P250 Biomek® FX tips |
| P2 | P250 Biomek® FX tips |
| P4 | Swap spot |
| P7 | 96-Well Deep Well Plate |
| P8 | 96-well flat-bottom cell culture plate |
| P9 | Pyramid-bottom reservoir plate containing Wizard® SV Lysis Buffer |
| P11 | Pyramid-bottom reservoir plate containing Column Wash Solution (CWA) (ethanol added) |
| P12 | Pyramid-bottom reservoir plate containing Nuclease-Free Water |

B. Starting Deck Layout for Two-Plate Cell Culture Sample Method.

| ALP Name | Part Sitting on ALP |
|------------|---|
| Tip Loader | P250 Biomek® FX tips |
| SPE ALP | Biomek® FX filtration system (single plate) with vacuum valve unit, elution plate spacer, Binding Plate |
| P1 | P250 Biomek® FX tips |
| P2 | Swap spot |
| P3 | P250 Biomek® FX tips |
| P4 | P250 Biomek® FX tips |

Panel B description is continued on next page.

B. Starting Deck Layout for Two-Plate Cell Culture Sample Method. (continued)

| ALP Name | Part Sitting on ALP |
|----------|--|
| P5 | P250 Biomek® FX tips |
| P6 | P250 Biomek® FX tips |
| P7 | Binding Plate for second sample-plate purification |
| P8 | Pyramid-bottom reservoir plate containing Nuclease-Free Water |
| P9 | 96-Well Deep Well Plate #1 |
| P10 | 96-Well Deep Well Plate #2 |
| P11 | Pyramid bottom reservoir plate containing Column Wash Solution (CWA) (ethanol added) |
| P12 | Pyramid bottom reservoir plate containing Wizard® SV Lysis Buffer |
| P14 | Pyramid bottom reservoir plate containing Column Wash Solution (CWA) (ethanol added) |
| P15 | 96-well flat-bottom cell culture plate #1 |
| P16 | 96-well flat-bottom cell culture plate #2 |

Reagent Dispense Volumes for the Biomek® FX.

| ALP Name | Part Sitting on ALP |
|---|--|
| One-Plate Cell Culture Sample Method | |
| P9 Reservoir | 25ml Wizard® SV Lysis Buffer |
| P11 Reservoir | 250ml Column Wash Solution (CWA) (ethanol added) |
| P12 Reservoir | 30ml Nuclease-Free Water |
| Two-Plate Cell Culture Sample Method | |
| P12 Reservoir | 40ml Wizard® SV Lysis Buffer |
| P11 Reservoir | 250ml Column Wash Solution (CWA) (ethanol added) |
| P14 Reservoir | 250ml Column Wash Solution (CWA) (ethanol added) |
| P8 Reservoir | 50ml Nuclease-Free Water |

7.B. 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. Follow the instructions for "Biomek® FX Deck Mapping" available online at:

www.promega.com/automethods/beckman/biomekfx/

Prior to the first run of the Wizard® SV 96 Genomic DNA Purification method on the Biomek® FX, check all gripper moves to ensure that the vacuum manifold disassembly and reassembly for elution is correct. Our experience indicates that proper configuration of the gripper moves is essential to ensure success of Wizard® SV 96 methods on the Biomek® FX. Not performing the gripper evaluation may result in failure of vacuum manifold disassembly and reassembly and may damage your Biomek® FX instrument.

Follow the instructions for "Evaluation of Biomek® FX SV 96 Method Gripper Moves" available online at: **www.promega.com/automethods/beckman/biomekfx/**

"Evaluation of Biomek® FX SV 96 Method Gripper Moves" requires the Biomek® FX grip test method. Please inquire about this method at: **www.promega.com/automethods/beckman/biomekfx/**

8. epMotion® 5075 VAC Workstation Requirements

8.A. Method

1. Check instrument requirements for the Eppendorf epMotion® 5075 VAC Wizard® SV 96 Genomic DNA cell culture sample method.

The following is a list of Eppendorf parts and their corresponding part number that are required to automate the Wizard® SV 96 Genomic DNA Purification System for cell culture samples on an epMotion® 5075 VAC workstation.

| Part Description | Eppendorf Part Number |
|---|------------------------------|
| epMotion® 5075 VAC workstation, gripper and waste tub | 5075 000.016 |
| TM 300-8, 8-channel dispensing tool | 5280 000.231 |
| TM 1000-8, 8-channel dispensing tool | 5280 000.258 |
| Reservoir rack (100ml reagent reservoirs) | 5075 754.002 |
| 85mm height adapter | 5075 751.003 |
| Vac holder | 5075 778.009 |
| Vac frame 2 | Contact Eppendorf |

2. Check labware requirements for the Eppendorf epMotion® 5075 VAC Wizard® SV 96 Genomic DNA cell culture sample method.

| Part Description | Eppendorf Part Number |
|--|------------------------------|
| 100ml epMotion® reservoirs (5) | 0030 126.513 |
| 300µl epTIPS motion filter tips (1) | 0030 003.977 |
| 1,000µl epTIPS motion filter tips (1) | 0030 003.993 |
| 96-Well Deep Well Plate | Provided |
| Binding Plate | Provided |
| 96-well flat-bottom cell culture plate | Not Provided |

3. Check initial deck configuration for the Eppendorf *epMotion*[®] 5075 VAC Wizard[®] SV 96 Genomic DNA cell culture sample method. The volumes of reagents dispensed in the reservoirs at position A3 are shown in Figure 7.

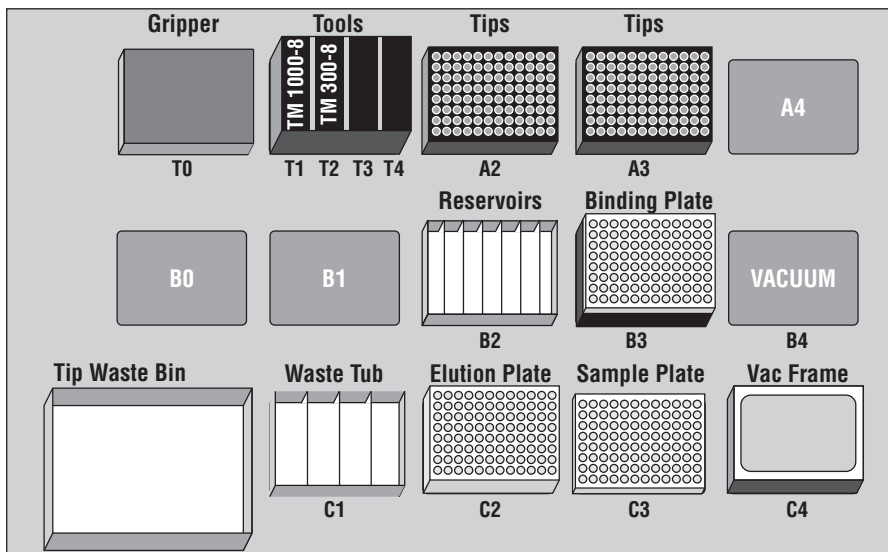
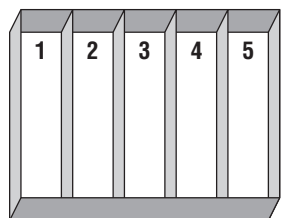


Figure 6. *epMotion*[®] 5075 VAC initial deck configuration. Genomic DNA purification from cell culture samples.

| | |
|-------------|--|
| Position T0 | Gripper |
| Position T1 | TM1000-8, 8-channel dispensing tool |
| Position T2 | TM 300-8, 8-channel dispensing tool |
| Position T3 | Empty |
| Position T4 | Empty |
| Position A2 | 1000µl epTIPS motion filter tips |
| Position A3 | Reservoir rack with 5 reagent reservoirs |
| Position A4 | Empty |
| Position B0 | Empty |
| Position B1 | Empty |
| Position B2 | 300µl epTIPS motion filter tips |
| Position B3 | Binding Plate atop 85mm height adapter |
| Vacuum | Empty |
| Position C1 | Waste tub with quarter wall separators |
| Position C2 | 96-Well Deep Well Plate |
| Position C3 | 96-well cell culture plate |
| Position C4 | Vac frame 2 atop Vac holder |



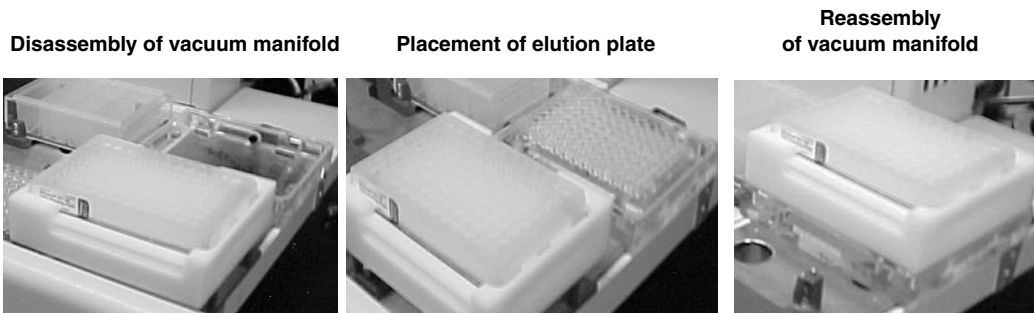
1. 100ml reservoir: 20ml of Wizard[®] SV Lysis Buffer
2. 100ml reservoir: 100ml of Column Wash Solution (CWA) (ethanol added)
3. 100ml reservoir: 100ml of Column Wash Solution (CWA) (ethanol added)
4. 100ml reservoir: 100ml of Column Wash Solution (CWA) (ethanol added)
5. 100ml reservoir: 30ml of Nuclease-Free Water

Figure 7. Reagent dispense volumes for the *epMotion*[®] 5075 VAC workstation. Prior to beginning run, the reagents listed above need to be dispensed appropriately on the deck of the *epMotion*[®] 5075 VAC workstation.

9. Description of Automated Wizard® SV 96 Genomic DNA Purification

This overview describes general liquid-handling steps required for automated Wizard® SV 96 Genomic DNA Purification and can be adapted to a variety of automated liquid-handling robots. Additional information about adaptation to liquid-handling robots other than those referenced above, please see Section 10.

1. **Lyse Cells.** Transfer 150µl of Wizard® SV Lysis Buffer from a reservoir to each well of the 96-well sample plate. Mix by pipetting.
2. **Transfer Cell Lysates.** Transfer the cell lysate contained in the sample plate to the Binding Plate sitting on top of the vacuum manifold apparatus.
3. **Bind Genomic DNA to the Binding Plate.** Once the cell lysates have been transferred to the Binding Plate, apply the vacuum and cell lysate is pulled through the Binding Plate by vacuum. Vacuum time may vary depending on sample type. During this vacuum step, genomic DNA binds to the Binding Plate.
4. **Wash Binding Plate.** Dispense 500µl of Column Wash Solution (CWA) (ethanol added) to each well of the Binding Plate. Apply the vacuum, and the wash solution is pulled through the Binding Plate. This step is repeated for a total volume of 2.5ml of Column Wash Solution (CWA) per well.
5. **Dry to Remove Residual Alcohol.** Apply the vacuum for 6–10 minutes to remove any residual ethanol from the Binding Plate.
6. **Prepare for Elution.** After the final vacuum step there is a one-minute pause to allow complete vacuum ventilation before disassembly and reassembly for the final elution step. A gripper tool disassembles the vacuum manifold stack by removing the Binding Plate from the vacuum manifold to a holding position. The gripper then moves the deep-well elution plate into the vacuum manifold. The gripper then reassembles the vacuum manifold stack by moving the Binding Plate back onto the vacuum manifold on the top of the elution plate.



Example of vacuum manifold stack disassembly, placement of elution plate and reassembly of vacuum manifold stack to elute purified DNA on the Biomek® 2000 workstation.

7. **Elute Purified Genomic DNA.** Transfer 200µl of Nuclease-Free Water from the reservoir to each well of the Binding Plate. Apply the vacuum, and the Nuclease-Free Water is pulled through the Binding Plate, eluting the genomic DNA into the elution plate. An elution volume of 200µl is recommended for optimal DNA yield from tissue culture cells. However, the elution volume may need to be optimized depending on amount of sample being processed and desired final concentration of eluted genomic DNA. Smaller elution volumes will increase concentration but may decrease the total DNA yield.
8. **Method Ends.** Purified genomic DNA has been eluted into the 96-Well Deep Well Plate sitting in the vacuum manifold. Dispose of the Binding Plate after use.

10. General Guidelines for Adaptation to Alternative Robotic Platforms

This method uses vacuum filtration to bind, wash and elute DNA samples. Make sure that your vacuum pump is set to pull a vacuum of 15–20 inches Hg. Vacuum pressure less than 15 inches of Hg will result in reduced genomic DNA yield and purity.

Following the second wash, it is critical to dry the Binding Plate for at least 6–10 additional minutes. Ethanol contamination in the DNA eluate can inhibit downstream applications such as PCR.

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