Automated Wizard® SV 96 Genomic DNA Purification System for Tissue Samples

Automated Protocol #EP007

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.

1. Description

This protocol describes automation of the Wizard® SV 96 Genomic DNA Purification System to purify genomic DNA from tissue sample types, such as mouse tail clippings and animal tissue. Specific instructions are provided for the following automated liquid-handling workstations: Beckman Coulter Biomek® 2000, Biomek® 3000, Biomek® FX and Eppendorf ep*Motion*® 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

- proteinase K (Promega Cat.# V3021 or Sigma Cat.# P2308)
- 55°C water bath
- adhesive plate sealers
- 2.2ml, Square-Well Deep Well Plate for proteinase K digestion (Promega Cat.# V6781)
- **for Biomek® FX only:** Pyramid-Bottom Reservoir Plates (2) (Promega Cat.# V6801)

4. Before You Begin

4.A. Preparation of Solutions

Prepare the following solutions prior to beginning the Wizard® SV 96 Genomic DNA Purification System protocol:

1. **Column Wash Solution (CWA):** Add 95% ethanol to the Column Wash Solution bottle as directed on the bottle label. Label the bottle to indicate that ethanol has been added. Seal well, and store at room temperature.
2. **Proteinase K solution:** Resuspend proteinase K (not provided) with nuclease-free water to a concentration of 20mg/ml. Dispense the proteinase K into working volumes determined by the average number of preps done at a time. Store the proteinase K solution at –20°C, and thaw on ice. Avoid multiple freeze-thaw cycles of the proteinase K solution, because this will result in decreased activity.
3. **Digestion solution master mix:** For each sample, combine the following reagents in a tube; store on ice until use.

Digestion Solution Master Mix	Volume per Sample	Total Volume for 96 Samples*
Nuclei Lysis Solution	200µl	22.0ml
0.5M EDTA (pH 8.0)	50µl	5.5ml
proteinase K (20mg/ml)	20µl	2.2ml
RNase A Solution (4mg/ml)	5µl	550µl
Total volume	275µl	30.25ml

*Includes excess volume to cover losses in pipetting.

4.B. Sample Preparation of Mouse Tail and Tissue Lysates Before Automated Processing

1. Use a 0.5–1.2cm mouse tail clipping from the tip of the tail or up to 20mg of other tissues. A 1.2cm mouse tail clipping usually weighs approximately 20mg. Cut the 20mg mouse tail clipping or tissue sample into two equally sized pieces, and place the pieces into a 96-well, deep-well plate (not provided).
2. Add 275µl of the prepared digestion solution master mix to each sample. If the mouse tail clipping or tissue sample is not covered by the Digestion Solution Master Mix, cut the tissue into smaller pieces. Be sure that the sample is completely covered with the digestion solution master mix. Cover the plate with an adhesive plate seal (not provided).
3. Place the plate in a 55°C water bath, and incubate overnight (16–18 hours). Be sure that the water in the incubator does not cover the sample plate. It is not necessary to shake the plate during the incubation.

Optional: If there is undigested hair and cartilage after the overnight proteinase K digestion, centrifuge the plate at 2,000 × *g* to pellet undigested sample. Transfer the supernatant to a new 96-well, deep-well plate (not provided).

4. Following overnight incubation at 55°C, remove the seal and place the warm lysate plate on the sample position of the deck of the liquid-handling robot.

   Note: Sample lysates must be warm for processing to prevent column clogging. Place the warm sample lysates on the deck of the robot just prior to starting the method. If sample lysates cannot be processed immediately, the sample lysate may be frozen at –70°C. However, the lysates need to be warmed to 55°C for one hour before they are processed.

5. Biomek® 2000 Workstation Requirements

5.A. Method

1. Check instrument requirements for the Beckman Coulter Biomek® 2000 Wizard® SV 96 Genomic DNA tissue 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 mouse tail clipping and tissue 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 tissue sample method.

Part Description	Ordering Information
Reservoir holder	372795
Quarter reservoir	372790
Half reservoir	372786
96-Well Deep Well Plate	Provided
Binding Plate	Provided
2.2ml, Square-Well Deep Well Plate	Promega Cat.# V6781
P250 Tips, Sterile, 250µl (2) or P250 Tips, Racked, Sterile, 250µl (2)	Beckman Cat.# 372655 Axygen Cat.# BT-250-1-R-S

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

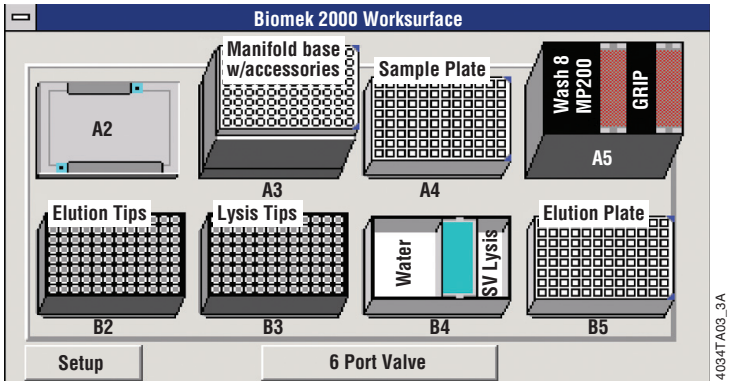
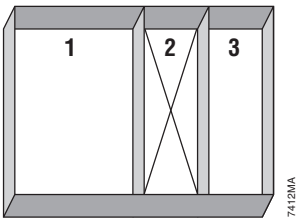


Figure 1. Biomek® 2000 initial deck configuration. Genomic DNA purification from mouse tail clipping or tissue sample.

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; 2.2ml, Square-Well Deep Well 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, half reservoir, quarter reservoir
Position B5	Labware holder, 96-Well Deep Well Plate



1. 50ml of Nuclease-Free Water
2. Empty
3. 25ml 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 tissue 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 mouse tail clipping and tissue 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 tissue sample method.

Part Description	Ordering Information
Reservoir holder	372795
Quarter reservoir	372790
Half reservoir	372786
96-Well Deep Well Plate	Provided
Binding Plate	Provided
2.2ml, Square-Well Deep Well Plate	Promega Cat.# V6781
Biomek® AP96 P250 Tips, Presterile (2)	Beckman Cat.# 717252
or Biomek® FX Tips, 250µl, Racked, Presterilized (2)	Axygen Cat.# FX-250-1-R-S

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

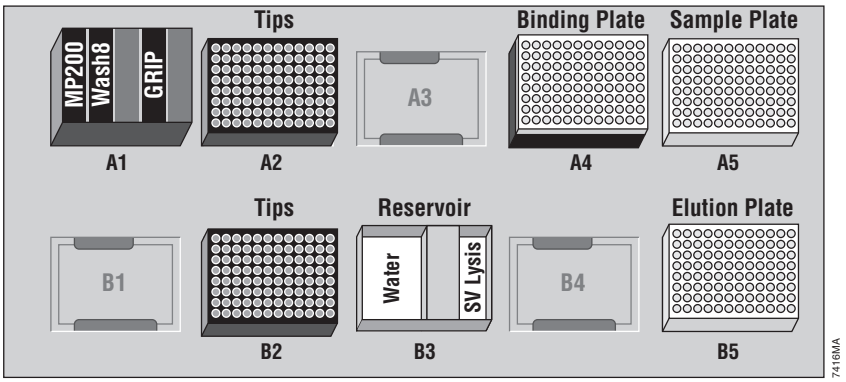
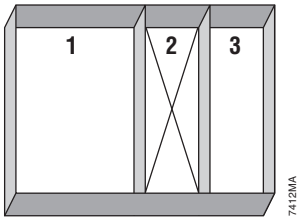


Figure 3. Biomek® 3000 initial deck configuration. Genomic DNA purification from mouse tail clipping or tissue sample.

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; 2.2ml, Square-Well Deep Well Plate
Position B1	Empty
Position B2	Pipette tip rack holder, P250 tips
Position B3	Labware holder, reservoir holder, 1 half reservoir, 1 quarter reservoir
Position B4	Empty
Position B5	Labware holder, 96-Well Deep Well Plate



1. 42ml of Nuclease-Free Water
2. Empty
3. 30ml 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

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

Part Description	Ordering Information
Pyramid-bottom reservoir plate (3)	Promega Cat.# V6801
2.2ml, Square-Well Deep Well Plate	Promega Cat.# V6781
96-Well Deep Well Plate	Provided
Binding Plate	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

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

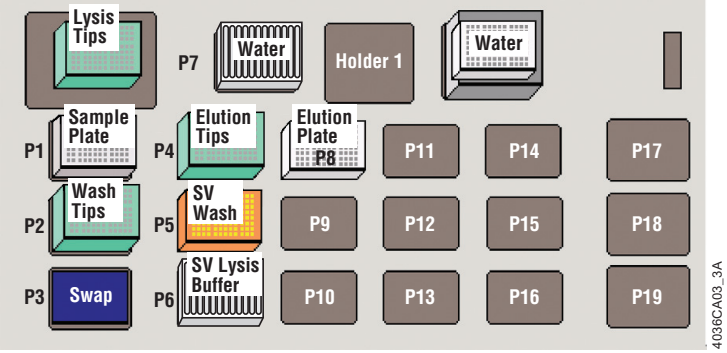


Figure 5. Biomek® FX deck layout. This is an example of a Wizard® SV 96 Genomic DNA Purification deck layout on a Biomek® FX for purification from mouse tail clipping or tissue sample lysates. Your specific deck layout may be different depending on your Biomek® FX configuration.

Starting Deck Layout for Mouse Tail Clipping or Tissue Samples.

ALP Name	Part Sitting on ALP
Tip loader	P250 Biomek® FX tips
P1	2.2ml, Square-Well Deep Well Plate
P2	P250 Biomek® FX tips
P3	Swap spot
P4	P250 Biomek® FX tips
P5	Pyramid-bottom reservoir plate containing Column Wash Solution (CWA) (ethanol added)
SPE ALP	Biomek® FX filtration system (single plate) with vacuum valve unit, elution plate spacer, collar (65mm), Binding Plate
P6	Pyramid-bottom reservoir plate containing Wizard® SV Lysis Buffer
P7	Pyramid-bottom reservoir plate containing Nuclease-Free Water
P8	96-Well Deep Well Plate

Reagent Dispense Volumes for the Biomek® FX.

ALP Name	Part Sitting on ALP
P5 Reservoir	250ml Column Wash Solution (CWA)
P6 Reservoir	35ml Wizard® SV Lysis Buffer
P7 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 reassemble 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. ep*Motion*® 5075 VAC Workstation Requirements

8.A. Method

1. Check instrument requirements for the Eppendorf ep*Motion*® 5075 VAC Wizard® SV 96 Genomic DNA tissue sample method.

   The following is a list of Eppendorf parts and their corresponding part numbers that are required to automate the Wizard® SV 96 Genomic DNA Purification System for mouse tail clipping and tissue samples on an ep*Motion*® 5075 VAC workstation.

Part Description	Eppendorf Part Number
ep*Motion*® 5075 VAC workstation, gripper and waste tub	5075 000.016
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 ep*Motion*® 5075 VAC Wizard® SV 96 Genomic DNA tissue sample method.

Part Description	Eppendorf Part Number
100ml ep*Motion*® reservoirs (5)	0030 126.513
1,000µl epTIPS motion filter tips (2)	0030 003.993
96-Well Deep Well Plate	Provided
Binding Plate	Provided
2.2ml, Square-Well Deep Well Plate	Promega Cat.# V6781

8.A. Method (continued)

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

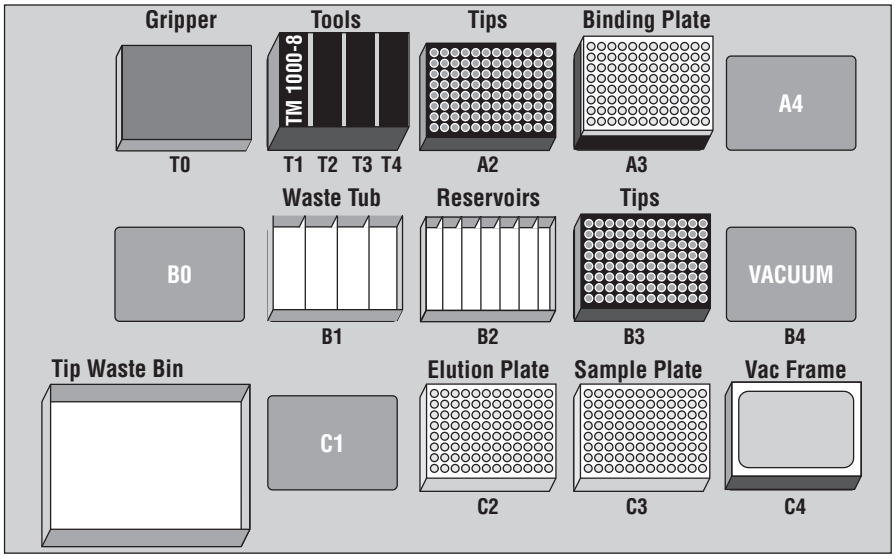
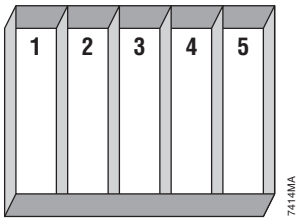


Figure 6. ep*Motion*® 5075 VAC initial deck configuration. Genomic DNA purification from tissue samples.

Position T0	Gripper
Position T1	TM1000-8, 8-channel dispensing tool
Position T2	Empty
Position T3	Empty
Position T4	Empty
Position A2	1,000µl epTIPS motion filter tips
Position A3	Reservoir rack with 5 reagent reservoirs
Position A4	Empty
Position B0	Empty
Position B1	Empty
Position B2	1,000µl 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	2.2ml, Square-Well Deep Well 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 ep*Motion*® 5075 VAC workstation. Prior to beginning run, the reagents listed above need to be dispensed appropriately on the deck of the ep*Motion*® 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 Tissue.** Transfer 250µl 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.

Disassembly of vacuum manifold | **Placement of elution plate** | **Reassembly of vacuum manifold**

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.

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

7. **Elute Purified Genomic DNA.** Transfer 400µl 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 400µl is recommended for optimal DNA yield from tissue samples. However, the elution volume may need to be optimized depending on the 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 and may cause column clogging when processing mouse tail clippings and tissue lysates.

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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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.