

Evaluation of Biomek® FX SV 96 Method Gripper Moves

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I. Evaluation of Biomek® FX SV 96 Purification Method Gripper Moves

The disassembly and reassembly of the vacuum manifold in preparation for elution of purified nucleic acids is a critical step in performing SV 96 purification methods on the Biomek® FX instrument. We have found that gripper moves for vacuum manifold disassembly and reassembly may differ between Biomek® FX instruments. Failure to evaluate the method gripper moves may result in failure of vacuum manifold disassembly and reassembly, and may cause a gripper crash.

II. Before You Begin

Ensure that both deck and gripper on your Biomek® FX have been accurately framed. An accurately framed deck and gripper are essential for successful implementation of automated liquid handling processes. Information on deck and gripper framing is available in the Help Menu of the Biomek® FX software by selecting “Search for Help on...” and searching for gripper and framing.

If the deck and gripper of your Biomek® FX are accurately framed, then performing the gripper evaluation processes outlined in this document should only involve minor modifications to ensure appropriate and accurate movements. If the gripper moves are grossly inaccurate (>5mm off) during the evaluation process, frame the Biomek® FX deck and gripper again and proceed from a newly framed instrument.

III. Verification of Deck and Labware Offset Values

Prior to beginning evaluation of the gripper moves, verify that deck and labware settings are accurate. If the deck and labware offsets are set appropriately, only minor modifications should be required during the SV 96 Method Gripper Evaluation Procedure (Sections IV and V). Follow the instructions below and refer to Tables 1–3 to verify your deck and labware settings.

1. Check for Accurate Deck Information: Per-Labware Offsets for the SPE and Holder 1 ALP positions

Select “Deck Editor” from the “Tools” menu. Ensure that the current deck in the upper left hand corner is the same as the deck on which the applications will be run. Double-click on the “SPE ALP” position to open the SPE ALP Position Properties window. Click “More” to expand the window. Click on the “Per-labware Offsets” button to open the Per-labware Offsets window. Select the labware indicated in Table 1 and check that the X, Y and Z values match the values listed. If not, change the X, Y and Z values to match those shown in Table 1. Click “OK” to complete. Repeat the procedure for the Holder 1 position. These values will provide the starting point for evaluating gripper moves. Some adjustment to these values may be required during the SV 96 Method Gripper Move Evaluation Procedure due to variability of framing on each Biomek® FX instrument.

Table 1. Per-Labware Offset Settings.

ALP Position	Labware	X	Y	Z
SPE	BCSPE Collar	-0.05	-1.4	0.81
	BCSPE Collar-36mm	-0.05	-1.4	0.81
	BCSPE Collar-23mm	-0.05	-1.4	0.81
Holder 1	BCSPE Collar	0	-1.4	0
	BCSPE Collar-36mm	0	-1.4	0
	BCSPE Collar-23mm	0	-1.4	0

2. Check Application Vacuum Collar and Elution Plate Usage

Each SV 96 purification application uses different vacuum collars and elution plates (Table 2). If you are installing all the SV 96 purification chemistries you will need to verify both vacuum collars and all the elution plates, otherwise you should verify only the vacuum collar and elution plate for the application you are running.

Table 2. SV 96 Application Labware Use.

SV 96 Application	Vacuum Collar Used	Elution Plate Used
Total RNA Isolation	BCSPE Collar-36mm	SBSFlat96Round
Genomic DNA Purification: Mouse Tail Samples	BCSPE Collar	SPECostarDeep96Square
Genomic DNA Purification: Tissue Culture Samples	BCSPE Collar	SPECostarDeep96Square
PCR Clean-Up	BCSPE Collar-36mm	SPEGreiner96UBottom
Plasmid Purification	BCSPE Collar-36mm + BCSPE Collar-23mm or	SBSFlat96Round
	BCSPE Collar	Marsh_22DeepwellSquare

3. Check for Accurate Movement Information and Stacking Offsets for Stackable Labware

Select “Labware Type Editor” from the “Tools” menu. Select the labware type (Table 3) to open the Labware window. Check the Movement Information by highlighting “Movement Information”. Ensure that the X, Y and Z values for Movement Information are the same as those listed in Table 3. Check the Stacking Offsets for stackable labware by highlighting “Stacking” and selecting “Edit” Stack Offsets. Scroll to the selected stackable labware. The selected labware should have a check in the box. To the right, ensure that the X, Y and Z values for the stack offsets are set correctly. If not, change the X, Y and Z values to match those in Table 3.

The values in Table 3 provide the starting point for evaluating gripper moves. Some adjustment may be required during the SV 96 Method Gripper Move Evaluation Procedure due to variability in the framing of each separate Biomek® FX instrument.

4. After ensuring that the Per-Labware Settings, Movement Information, and Stacking Offset values agree with the values indicated in Tables 1 and 3, save and close all windows.

Table 3. Labware Movement and Stacking Offset Value Settings.

Labware	Movement Information			Stack Offsets for Stackable Labware Selected Stackable			
	X	Y	Z	Labware	X	Y	Z
BCSPE Collar	0	1.4	5.1	PlasmidFilter	0.05	1.4	3.35
BCSPE Collar36mm	0	1.4	2.35	PlasmidFilter	0.05	1.4	0.6
SBSFlat96Round	0	0	-0.2	BCSPECollar36mm	0	-1.4	0.81
SPECostarDeep96Square	0	0	-0.2	BCSPECollar	0	-1.36	0.81
SPEGreinerUBottom	0	0	-0.2	BCSPECollar36mm	0	-1.4	0.81
BCSPE Collar23mm	0	1.4	1.8	Plasmid Filter	0.05	1.41	0.01
BCSPE Collar23mm	0	1.4	1.8	BCSPE Collar36mm	0	0	2.31
Plasmid Filter	0	0	2.6	BCSPE Collar36mm	0	-1.4	2.35
Plasmid Filter	0	0	2.6	BCSPE Collar23mm	0	-1.4	3.35

IV. Running the SV 96 Method Gripper Move Evaluation Procedure

1. Make sure that the “BFXSV96griptest” method you will use for the evaluation procedure is mapped to the same deck as the SV 96 purification method. Refer to the “Biomek® FX Deck Mapping” document for instructions on how to map a method to a new deck.
2. Ensure that the instrument setup of the “BFXSV96griptest” method is using the appropriate labware for the final application (SV 96 Total RNA Isolation, SV 96 Genomic DNA Purification, SV 96 PCR Clean-Up or SV 96 Plasmid Purification). Refer to Table 2 for the list of labware requirements for each application.
3. In the “Method” window, click on “Finish” to validate the method. Any errors in the method will be highlighted in red text. If the method validates without errors, proceed to Section V.

Any method errors need to be resolved before proceeding with evaluation of gripper moves. Two common errors are: 1) a **D-axis Error**, indicating that the “Unsqueeze” setting for the vacuum collar is either too wide or too narrow or 2) a **Stacking Error**, indicating that the vacuum collar is not allowed to stack on the elution plate.

Correcting a D-axis Error

- a. Select “Labware Type Editor” from the “Tools” menu. Double-click on the collar being used in the method (see Table 1).
- b. In the vacuum collar Labware Editing window, click on “Movement Information” (in the menu on the left-hand side of the window). Adjust the unsqueeze value (e.g., if the value is -1.2 , and you are getting an error indicating that the unsqueeze value is too wide try increasing the value to -1.15).
- c. Once the unsqueeze value has been modified, click “Save”, and then “Exit” to leave the Labware Type Editor window. To ensure that the changes you have made take effect, click on the “Instrument Setup” line in the Method window. Drag the PlasmidFilter labware off the existing collar to another position on the deck and then delete the collar by right clicking on it and choosing “Delete”. Replace the collar with the newly saved labware by dragging the new collar onto the SPE position. [Remember to place the PlasmidFilter labware back on top of the collar at the SPE position. Double-Click on the PlasmidFilter figure on the deck and give it the name “Binding”.]
- d. Click “Finish” in the Method window to validate the method and determine whether the unsqueeze value is appropriately set. If not, repeat steps a–c until the unsqueeze value for the collar is set at the largest negative value that does not produce a D-axis error.

Correcting a Stacking Error

- a. Select “Labware Type Editor” from the “Tools” menu. Double-click on the elution plate being used in the method (see Table 2).
- b. In the plate Labware Editing window, click on “Stacking” (in the menu on the left-hand side of the window). In the Stacking Information window, click on the “Edit” button next to “Stack Offsets”.
- c. Once in the Stack Offsets window, click on the box next to the name of the collar used in the method (Table 2) to place a checkmark in that box. Check that the Stack Offset values match those shown in Table 3. Click “OK” in the Stack Offsets window, “Save” in the plate Labware Editing window, and “Exit” in the Labware Type Editor window.
- d. Click “Finish” in the Method window to autovalidate the method and confirm that there are no errors.

Once the method validates, proceed to the SV 96 Method Gripper Move Evaluation Procedure (Section V). If the method does not validate or indicates different errors than those discussed above, please contact a Technical Services Scientist at Beckman Coulter (1-800-551-1150).

Note: If at any point in this exercise you have questions or concerns please contact a Technical Services Scientist at Beckman (1-800-551-1150).

V. SV 96 Method Gripper Move Evaluation Procedure

Open the gripper test method (BFXSV96griptest). Choose “Single Step” from the “Tools” menu. This option allows you to step through all the steps in a method individually. Next, choose “Run” from the “Execution” menu. This will start the method and the Single Step window will prompt you to individually launch each step or to launch all of the steps contained within the Single Step Window.

A. Centering 96-well POD Over Vacuum Collar

1. Start the method in Single Step mode. Choose “Launch All” to perform all of the steps currently listed in the Single Step window. Continue to do this until the head moves over the vacuum manifold at the SPE position.
2. Check that the head is centered over the vacuum collar in the X- and Y-axes. Clear any light curtain errors that arise due to close inspection of the head position. If the Per-labware offsets (Table 1) have not been accurately defined, the head commonly may be shifted about 1.4cm towards the front of the instrument over the collar.
3. If the head is centered over the collar, proceed to Step 5 (Section V.B). If the head is shifted in the X- or Y-axis, choose “Stop” from the “Execution” menu and then “Exit” from the Single Step window. Choose “Labware Type Editor” from the “Tools” menu and double-click on the vacuum collar used in the method (Table 1).
4. A gross misalignment of the head over the vacuum collar may be caused by either: a) inaccurate or missing Per-labware offset values for the ALP positions or b) inaccurate framing.
 - a. Ensure that the Per-Labware offset values match the values in Table 1.
 - b. Re-frame your deck and gripper.

If the problem persists, please contact a Technical Services Scientist at Beckman (1-800-551-1150).

B. Gripping Height for the Vacuum Collar

5. Manually move the vacuum collar from the SPE position to the SPE Holder. Begin the method again in Single Step Mode. “Launch All” until the head has lowered over the SPE position and the grippers have been extended. Hold the vacuum collar next to the grippers, flush with the vacuum manifold base. Check that the extended grippers have cleared the sides of the manifold collar and that the fingers of the grippers are approximately aligned with the grooves near the top of the vacuum collar.
6. If the grippers are at the appropriate height and clear the collar on both sides then proceed to Step 8 (Section V.C). If the grippers are too high or too low, choose “Stop” from the “Execution” menu and then “Exit” from the Single Step window. Choose “Labware Type Editor” from the “Tools” menu and double click on the vacuum collar used in the method.
7. In the Labware Editing window for the vacuum collar, click on “Movement Information” (in the menu on the left-hand side of the window). Modify the Gripper Offset in the Z-field by adding or subtracting the needed offset. Additionally, ensure that the Gripper Offset settings in the X- and Y-fields are set so that the gripper fingers are centered over and clearing the vacuum manifold.

Repeat steps 5–6 until you are sure that the head is at the appropriate height over the 36mm collar.



Remember that, for the Z-axis, negative numbers indicate a move downward.

C. Move Vacuum Collar from SPE ALP to Holder ALP

8. Now that you have assured that the head is appropriately positioned over the vacuum collar, select “Stop” from the “Execution” menu and “Exit” from the Single Step window. Choose “Single Step” from the “Tools” menu and “Run” from the “Execution” menu. Place the vacuum collar back on the SPE position and select “Launch All” from the Single Step window. Continue to choose “Launch All”, making sure that the grippers are clearing the sides of the collar (if not, repeat steps 1–3) and that they are gripping the collar in the grooves near the top (if not, repeat steps 6–7).
9. Next, the head should move the vacuum collar over the SPE Holder position and then attempt to place the collar at that position. Continue to click “Launch All” in the Single Step window until the grippers release the vacuum collar on the SPE Holder position. If the vacuum collar has been placed correctly at the SPE Holder position, proceed to Step 11. If the vacuum collar has been released above the SPE Holder position, or if the head has attempted to place the vacuum collar below the level of the SPE Holder, then a modification needs to be made at this SPE Holder position. Choose “Stop” in the “Execution” menu, then “Exit” from the Single Step window.
10. Select “Deck Editor” from the “Tools” menu. Ensure that the deck you will be modifying is the same deck that is used to run the SV 96 method. If not, click on the “Open” button in the Deck Editor window and select the correct deck. Double-click on the SPE Holder position of the deck. In the Position Properties window, click on the button that says “More>>”, then click the “Per-Labware Offsets” button. From the list of available labware, choose the vacuum collar that is used in the method. Modify the Z offset information to correct the movement of the collar onto the SPE Holder position up or down by adding or subtracting the needed offset.

Repeat steps 8 and 9 to confirm that the vacuum collar is being appropriately placed on the SPE holder position.

D. Centering Elution Plate into Vacuum Manifold Base

11. The next step in the method is to move the elution plate into the manifold base. Confirm that the Elution Spacer has been placed in the manifold base. Start the method in the Single Step window and click “Launch All” until the vacuum collar holder is moved to the SPE Holder. Continue to click “Launch All” for elution plate gripping and movement over the vacuum manifold base. Check that the elution plate is centered over the manifold base. If the elution plate is positioned correctly, proceed to Step 14.
12. If the elution plate is not centered over the manifold bed, estimate the change necessary in the X and Y directions to center the plate over the manifold base. In the “Execution” menu, select “Stop”. Then choose “Manual Control” from the “Tools” menu. In the Manual Control window, click on “Advanced Controls” and choose “Pod1” from the list. In the Pod1-specific window, click on the arrow buttons and the “Go” button to move the plate down close to the deck (this may take several moves). Once the plate is close to the deck surface use the “Unsqueeze/Dispense” button and the “Go” button to release the plate (this may take several Unsqueeze/Dispense commands). Click “Retract Gripper” to retract the grippers. Close the Pod1-specific and main Manual Control windows.



Remember that, for the Z-axis, negative numbers indicate a move downward.

13. Choose “Deck Editor” from the “Tools” menu. Ensure that the method deck is indicated in the Deck Editor window. If not, click “Open” in the Deck Editor window and select the Method Deck. Double-click on the SPE position of the deck. In the Position Properties window, click “More>>”, then select “Per-Labware Offsets”. Choose the appropriate elution plate (Table 2) from the list of available labware. Change the X, Y and Z offset information into the appropriate fields for the ALP.

Repeat steps 11–13 to confirm that the elution plate is now being appropriately positioned over the manifold base.

E. Placement and Un-Grip of Elution Plate into Vacuum Manifold Base

14. Start the method in the Single Step window and click “Launch All” until the elution plate is placed within the manifold base and the gripper has released the elution plate.
15. At this point there are two possible problems: a) **Improper Placement of the Elution Plate** or b) **Gripper Error**. If neither problem occurs, proceed to Step 16 (Section V.F).

a. Improper Placement of Elution Plate

The plate may not have been placed far enough into the manifold base or the robot may have attempted to put the plate too far into the base. If either of these problems occurs:

- (i) Ensure that appropriate offsets for the ALP and stacked labware are entered into the fields (Tables 1 and 3).
- (ii) Start the method in the Single Step window and click “Launch All” until the elution plate has been placed in the manifold base and released.
- (iii) Return to Steps 12 and 13 and make the appropriate changes in the Z offset value for the elution plate.

b. Gripping Error

This error indicates that the gripper is jammed and will not respond. The error occurs when the gripper tries to unsqueeze too far while releasing the elution plate and becomes jammed on the side of the manifold base.

- (i) Choose “Stop” from the “Execution” menu and then “Exit” from the Single Step window. Then select “Manual Control” from the “Tools” menu. In the Manual Control window, click on “Advanced Controls” and choose “Pod1” from the list. In the Pod1-specific window, click on the “Squeeze/Aspirate” button and “Go” to close the grippers (this may take several moves). Close the grippers so that they are off the sides of the manifold base but not gripping the elution plate. Once this is done, click “Move Z-Max” to move the head all the way to the maximum Z-position and out of the way of the manifold. Close the Pod1-specific and main Manual Control windows.
- (ii) In the “Tools” menu, choose “Labware Type Editor”. Scroll down and double-click on the method elution plate. From the list on the left of the window choose “Movement Information” and change the gripper unsqueeze variable to -0.75 . Click “Save” in the window for the method elution plate and close the Labware Type Editor window. Once again, choose “Single Step” from the “Tools” menu and then “Run” from the “Execution” menu. Go back to Step 14.



Remember that, for the X-axis, negative numbers indicate a move to the left. For the Y-axis, negative numbers indicate a move to the back of the instrument.

F. Restack Vacuum Collar Over Elution Plate on SPE ALP

16. Continue method run in the Single Step Window and “Launch All” until the vacuum collar has been gripped and moved over the manifold base. At this point, the software thinks that the vacuum collar is being stacked on top of the Elution Plate rather than on the manifold base. Therefore, it may not be appropriately centered over the manifold base. Check that the vacuum collar is centered over the manifold base. If the collar is centered proceed to Step 20 (Section V.G). If not, estimate the distance in the X and Y-axes that the collar needs to move to be centered.
17. Select “Stop” from the “Execution” menu and “Manual Control” from the “Tools” menu. Choose “Advanced Control” in the Manual Control window and select “Pod1” from the list. In the Pod1-specific window, click on the arrow buttons and the “Go” button to move the collar close to the deck at the SPE (this may take several moves). Once the collar is close to the deck surface use the “Unsqueeze/Dispense” button and the “Go” button to release the collar (this may take several “Unsqueeze/Dispense” commands). Click the “Retract Gripper” button to retract the grippers. Close the Pod1-specific window and main Manual Control window.
18. From the “Tools” menu, choose “Labware Type Editor”, scroll down to the appropriate elution plate for your method and double-click. Click on “Stacking” in the list on the left of the window. Click on the “Edit...” button next to “Stack Offsets” and then click on the name of the vacuum collar used in the method. Place a check in the box to the left of the collar name by clicking on that box. Change the values in the “Stack Offset X” and “Stack Offset Y” spaces on the right side of the window to reflect the values needed to center the collar over the manifold base by adding or subtracting the needed offset. Once this is done click “OK” in the Stack Offsets window, “Save” in the Elution Plate window and “Exit” from the Labware Type Editor window.
19. To ensure that the changes you have made take effect, in the method itself click on the “Instrument Setup” line. Right-click on the elution plate and click “Delete”. Next, select the required elution plate (Table 2) from the available labware shown and drag onto the image of the deck at the same position. Double-click on the new elution plate and type “Elution Plate” (exactly as it is shown) next to “Name” in the window that comes up. From the “Tools” menu, choose “Single Step” and then select “Run” from the “Execution” menu. Go back to Step 18 and confirm that the collar is now centered over the manifold base.



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G. Pipetting to SV 96 Binding Plate in Vacuum Collar on SPE ALP

20. Continue method run in “Single Step” mode and choose “Launch All” until the collar has been placed on the manifold base and released. The next step in the method is to check pipetting to the DNA binding plate on the vacuum collar. Choose “Launch All” until the pipet tips are moved over the DNA binding plate. Evaluate that the pipet tips are centered over the individual wells of the DNA binding plate. If not, estimate the distance in the X- and Y-axes that the tips need to move to be centered. If the tips are centered proceed to Section V.H.

21. Select “Stop” from the “Execution” menu and “Manual Control” from the “Tools” menu. Once in the Manual Control window, select “Advanced Controls” and choose “Pod1” from the list. In the Pod1-specific window, click on the arrow buttons and the “Go” button to move the tips back into the holes in the tip box on the Tip Loader station (this may take several moves). Once the tips are in the holes of the tip box use the “Unsqueeze/Dispense” button and the “Go” button to drop the tips into the tip box (this may take several Unsqueeze/Dispense commands). Close the Pod1-specific and main Manual Control windows.

From the “Tools” menu, choose “Labware Type Editor”, scroll down to the collar used in the method and double-click. Click on “Stacking” in the list on the left of the window for the collar. Then click on the “Edit...” button next to “Stack Offsets” and select “PlasmidFilter”. Place a check in the box to the left of the PlasmidFilter by clicking on that box. Change the values in the “Stack Offset X” and “Stack Offset Y” spaces on the right side of the window to reflect the values needed to center pipet tips over the SV 96 binding plate by adding or subtracting the needed offset. Once this is done, click “OK” in the Stack Offsets window, “Save” in the collar window and “Exit” from the Labware Type Editor window.

22. To ensure that the changes you have made take effect, in the method itself click on the “Instrument Setup” line. Click on “PlasmidFilter” and drag it to an open spot on the deck. Right click on “PlasmidFilter” and select “Delete”. Next, replace the PlasmidFilter by selecting it from the available labware shown and dragging it onto the image of the deck, stacked on top of the vacuum collar at the SPE position. Double-click on this new plate and type “Binding” (exactly as it is shown) next to “Name” in the window. Choose “Single Step” from the “Tools” menu and then “Run” from the “Execution” menu. Go back to Step 20 and confirm that the collar is now centered over the manifold base.
23. To save all the changes you have made during the procedure, click “File”, “Save Workspace”. This will ensure that the changes are permanent.

H. Completion of Gripper Move Evaluation Procedure

You should now be ready to run your SV 96 method. To check that everything has gone well, click “Exit” in the Single Step window (this will cause the method that is running to finish the remaining steps). Next, choose “Run” from the “Execution” menu and allow the “grip only” test method to run all the way through. This makes a final check that all of the gripper movements and pipetting steps are working well.

If at any point in this exercise you have questions or concerns please contact a Technical Services Scientist at Beckman (1-800-551-1150).



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