

# APPLICATION INFORMATION

BioRobotics

## Troubleshooting Guide

### **HIGH-THROUGHPUT GENOMIC DNA ISOLATION FROM CELL CULTURES AND MOUSE TAILS ON THE BIOMEK® FX LIQUID HANDLING SYSTEM USING PROMEGA'S WIZARD\* SV 96 GENOMIC DNA PURIFICATION SYSTEM**

Problems	Possible Cause	Suggestions
<b>Deck mapping and setup difficulties</b>	Calling up the wrong deck. Not enough ALP positions.	Refer to Beckman Coulter Application Information Bulletin T-1953, "Deck Mapping on the Biomek® FX."
<b>Low DNA yield (i.e., low A<sub>260</sub>).</b>	Sample contained too few cells.	<ol style="list-style-type: none"> <li>1. Obtain fresh new cell samples.</li> <li>2. Check to see if cell count is within the recommended range.</li> </ol>
	Tissue lysate is stored at -20 or -70°C.	1. For optimal yield, purify the DNA as soon as the lysate is prepared. Generally, if the lysate has been frozen it may have a decreased amount of genomic DNA.
	Sample subjected to many freeze-thaw cycles.	1. Freezing and thawing samples repeatedly could result in DNA degradation. Use fresh samples whenever possible.
	Tissue culture cells were low in genomic DNA.	1. Genomic DNA yield varies depending on the number of cells used for the isolation. Increase the starting amount processed to a maximum of 20 mg of tissue or 5 X 10 <sup>6</sup> tissue culture cells.
	Sample did not receive any or enough Wizard SV Lysis Buffer.	1. Check to make sure the pipetting head is working properly and the aspirating height is optimized to deliver correctly.

<b>Problems</b>	<b>Possible Cause</b>	<b>Suggestions</b>
<b>Low DNA yield (i.e., low A<sub>260</sub>) (continued).</b>	Frozen mouse tail lysates were not thawed and warmed to 55°C sufficiently.	1. Process lysates at 55°C incubation. If samples have cooled, it may be difficult to purify due to viscosity. Incubate at 55°C for 1 hour and continue with the purification.
	Lysed cells or lysates were not re-suspended completely.	1. Increase number of sample mixes to ensure re-suspension before transferring to the DNA filter plate.
	No ethanol or incorrect volume of ethanol added to the Wizard SV Wash Solution.	1. Prepare the solutions as instructed in Promega's Technical Bulletin No. 303 Sections III.A and IV.A before beginning the procedure.
	Low vacuum pressure.	1. Check vacuum pump for correct pressure (15 inches of Hg). 2. Check vacuum lines and seals for leakage.
<b>Inaccurate elution volume.</b>	There is not enough nuclease-free water.	1. Make sure to have enough elution water.
		2. Allow 10 mL overage when adding to the reservoir.
	The aspirating height is not adjusted to the volume of the nuclease-free water.	1. Adjust the aspirating height according to the amount of nuclease-free water.
	Vacuum pump is not working properly.	1. Check to see that vacuum pump is set to the required pressure. A vacuum pressure of >15 inches of Hg is recommended.
2. Make sure that vacuum hoses are in good conditions (i.e., no holes or deterioration) and correctly connected—see the Biomek® FX User's Manual #719452.		
3. Check the manifold and collar gaskets for leakage.		

<b>Problems</b>	<b>Possible Cause</b>	<b>Suggestions</b>
<b>Clogged column.</b>	Lysate was too viscous to pipette easily.	1. Dilute lysate with Wizard SV Lysis Buffer until it becomes easy to pipette. Then apply the entire lysate to the column.
	Too much tissue sample was used in the lysate preparation.	1. The maximum recommended weight of mouse tail tissue used for lysate preparation is 20 mg.
	Too many cells were being processed.	1. The maximum recommended amount of tissue culture cells being processed on the column membrane is $5 \times 10^6$ cells.
	Tissue lysates were too viscous when cooled.	1. Process lysates at 55°C incubation. If samples have cooled, it may be difficult to purify due to viscosity. Incubate at 55°C for 1 hour and continue with the purification.
	Differential vacuum pressure in filter plate.	1. Manually place Vacuum Assist film from Whatman, Cat. # 7705-0112, on the filter plate during vacuum steps to ensure even vacuum pull in all 96 columns.
<b>Uneven reagent distribution.</b>	Uneven multi-channel pipetting head.	1. Inspect the levelness of the multi-channel pipetting head by lowering the pod via manual control to touch a 1X1 ALP on the deck. Uneven contact of the head to the ALP signifies a tilted head. Contact a service engineer to re-level the pipetting head before operating the Biomek® FX.
	Clogged mandrel or tips.	1. Need to refurbish head and inspect tips for blocks.
	Loading partially filled tip boxes.	1. Only full tip boxes can be used. Partially filled tip boxes creates uneven insertion force on the multi-channel pipetting head, which may cause damage and pipette inaccuracy to the multichannel pipetting head.

Problems	Possible Cause	Suggestions
<b>Reagents are not delivered correctly.</b>	Incorrect positioning of reagents.	1. Consult the instrument setup screen to ensure reagents are placed in the appropriate location. 2. Check to make sure that lysis buffer, wash solution, and nuclease-free water are added to the appropriate labware and placed in the correct deck position.
	Reagents were not delivered to the desired position in wells.	1. Frame deck properly.
<b>RNA contamination.</b>	RNase A was not added to the tissue lysate digestion solution.	1. Add 2 µL of RNase A solution to final eluate and incubate at room temperature for 10 minutes.
	RNA was co-purified with genomic DNA from tissue culture cells.	1. Add 2 µL of RNase A solution to final eluate and incubate at room temperature for 10 minutes.
<b>Contamination of wells.</b>	Outside sources introduced during sample preparation.	1. Make sure that Biomek® FX and reagents are free of contaminants.

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