

APPLICATION INFORMATION

BioRobotics

Troubleshooting Guide

HIGH-THROUGHPUT AUTOMATED PCR* PURIFICATION ON THE BIOMEK® FX USING PROMEGA'S WIZARD* SV 96 PCR CLEAN-UP SYSTEM

Problems	Possible Cause	Suggestions
Deck mapping and setup difficulties.	Using the wrong deck. Not enough ALP positions.	Refer to the “Deck Mapping on the Biomek FX” (T-1953A).
Low A₂₆₀/A₂₃₀ ratios.	Typically due to guanidine thiocyanate contamination.	Low ratios do not necessarily indicate that the DNA will function poorly in downstream applications. Ethanol precipitate the DNA if the low A ₂₆₀ /A ₂₃₀ ratio is a concern.
Slow filtration rate.	Insufficient vacuum pressure.	A vacuum pressure of >15 inches of Hg is required.
Low DNA yield.	Incorrect ratio of Membrane Binding Solution added to the PCR reaction. 80% ethanol not used. Low vacuum pressure.	<ol style="list-style-type: none"> 1. Verify that an equal volume of Membrane Binding Solution was added to the PCR reaction. 2. Verify that the ethanol solution used to wash the Binding Plate is 80% ethanol. Prepare this fresh. 3. Check to see that vacuum pump is set to the required pressure. A vacuum pressure of >15 inches of Hg is recommended. 4. Make sure that vacuum hoses are in good condition (i.e., no holes or deterioration) and correctly connected (see <i>Biomek FX User's Manual</i>, P/N 719452). 5. Check the collar gasket (P/N 609846) for leakage.

Problems	Possible Cause	Suggestions
No restriction digestion or incomplete restriction digestion.	Concentration of restriction enzyme or length of digestion insufficient.	<ol style="list-style-type: none"> 1. Increase the amount of restriction enzyme and/or length of incubation time. Digest at the appropriate temperature and in the optimal buffer for the restriction enzyme used. 2. Keep volume of PCR to 10% or less of reaction. 3. Use BSA at 0.2 mg/mL as a protein carrier.
Inaccurate elution volume.	<p>Vacuum pump is not working properly.</p> <p>Vacuum leak from worn gaskets.</p> <p>There is not enough nuclease-free water.</p> <p>The aspirating height is not adjusted to the volume of the nuclease-free water.</p> <p>Multichannel pipetting head or Span8 is not working and/or calibrated properly.</p>	<ol style="list-style-type: none"> 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 condition (i.e., no holes or deterioration) and correctly connected (see <i>Biomek FX User's Manual</i>, P/N 719452). 3. Check to make sure the vacuum manifold and collar gaskets are in good condition. 4. Make sure to have enough elution water. Allow 4 mL overage when adding to the reservoir. 5. Adjust the aspirating height according to the amount of nuclease-free water. 6. Verify that the tool is working properly by doing a wet run with water to see if equal volume is achieved.
Uneven reagent distribution.	<p>Multichannel pipetting head not level.</p> <p>Clogged mandrel or tips.</p> <p>Loading partially filled tip boxes.</p>	<ol style="list-style-type: none"> 1. Check that the multichannel pipetting head is level 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 service engineer to re-level the pipetting head before operating the Biomek FX. 2. Need to refurbish head and inspect tips for blocks. Contact Beckman Coulter Service. 3. Only full tip boxes can be used. Partially filled tip boxes create uneven insertion force on the multichannel pipetting head, which may cause damage and pipette inaccuracy to the multichannel pipetting head.

Problems	Possible Cause	Suggestions
Poor results with automated fluorescent sequencing.	<p>Too little DNA added to the sequencing reaction.</p> <p>Too much DNA added to the sequencing reaction.</p> <p>TE buffer used for DNA elution.</p>	<ol style="list-style-type: none"> 1. Increase the amount of DNA used in the sequencing reaction or concentrate the DNA by doing an ethanol precipitation. Up to 7 μL of the eluted DNA can be used per fluorescent sequencing reaction. 2. Too much DNA can interfere with fluorescent sequencing. Use less eluted DNA or dilute DNA. 3. Re-purify the DNA fragments and elute with Nuclease-Free Water.
DNA yields on gel look low compared to spectrophotometric readings.	Trace contaminants in the eluted DNA artificially inflating the spectrophotometric readings.	Use agarose gel/ethidium bromide quantitation to determine yields.
Reagents are not delivered correctly.	Incorrect positioning of reagents.	Consult the initial configuration screen to ensure reagents are placed in the appropriate locations.
Purified DNA floats out of the well when loaded on a gel.	Ethanol carryover.	Be certain that the ethanol wash solution is not carried over from the wash steps into the elution step. Dry the Binding Plate for at least 4 minutes after final wash step goes through the plate.

* *Wizard is a registered trademark of Promega Corporation. The PCR process is covered by patents owned by Roche Molecular Systems and F. Hoffmann-La Roche, Ltd. All other trademarks are the property of their respective owners.*



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