

PRODUCTION PROTOCOL FOR REARRAYING PRIMERS USING THE SPAN8 ROBOT

Production Author: Jason Morrison

Revision Date: 12/17/04

Version 1.0

PURPOSE: This protocol describes how to rearray Mutational Profiling primers to be dried down from Matrix tubes into MJ 384-well plates using the Biomek FX - SPAN8 robot.

MATERIALS AND EQUIPMENT:

Biomek FX - SPAN8 robot
Primers (pooled) @ 0.6uM in Matrix tubes
Matrix tube rack
MJ 384-well hard-shell plates
de-gassed DI water
Sevant Speedvac with rotor
Jouan GR422 centrifuge
Adhesive plate sealers
Tape
Sharpie marker

PROCEDURE:

1. Prepare Biomek FX - SPAN8 robot for rearray

- 1.1. Fill the water carboy on the Biomek FX - SPAN8 with de-gassed, distilled water being sure that the rubber tubing reaches to the bottom of the carboy.
- 1.2. Empty the waste carboy.
- 1.3. Double click the **Biomek-FX** icon on the desktop to open the Biomek software.
- 1.4. If running the first run of the day or if supply water and waste have been changed, the system will need to be purged. This is done by clicking on **Tools > Manual Control > Advanced Control > Pod 1**. When the Advanced Controls window pops up hit the **Purge System** button. A box will appear asking to verify the tips, Click OK. Another box will appear asking to Click OK when all bubbles are flushed from the pumps. Watch the pumps and when all bubbles are purged **Click OK**.

2. Prepare Reagents and Materials

- 2.1. Determine the appropriate primers in Matrix tubes to be used. Primers should be pooled to a final concentration of 0.6uM. Place the primer tubes in a Matrix tube rack, putting the primers in order from left to right starting with the upper, left (A1) well position.
- 2.2. Determine the correct number of MJ 384-well hard-shell plates to be used and label each.

3. Rearranging primers from the Matrix tube rack into MJ 384-well plates

- 3.1. Select the appropriate program for primer rearray:
 - 3.1.1. Click on the **File** tab and select **Open**.
 - 3.1.2. In the Methods folder, double-click on the **Resequencing** folder.
 - 3.1.3. Within the Resequencing folder, click on the correct program for the number and pattern of primers to be rearrayed (e.g. #_Primer_Addition_quadrant(s).bmt) and then click the **Open** button.
- 3.2. Place the Matrix tube rack with the appropriate primers on the SPAN-8 deck as indicated by the Instrument Set-Up diagram.
- 3.3. Place the MJ 384-well hard-shell plates directly on the deck as indicated by the Instrument Set-Up diagram.
- 3.4. Click the **green arrow** at the top of the program screen. The program will dispense 2uL of primer into each appropriate well of the MJ 384-well plate(s).

PRODUCTION PROTOCOL FOR REARRAYING PRIMERS USING THE SPAN8 ROBOT

Production Author: Jason Morrison

Revision Date: 12/17/04

Version 1.0

- 3.5. After the program as completely finished, remove the MJ plates and quickspin them at 1500rpm for 30 seconds on a centrifuge. After centrifugation, verify that the liquid levels within each MJ plate well are equal. If not, report the problem to a coordinator.
- 3.6. When all primers have been rearrayed, clean the Biomek FX - SPAN8 robot and close out of all programs.

4. Drying down primers in MJ plates

- 4.1. Place each MJ plate with rearrayed primers on plate positions of the rotor in a Sevant Speedvac.
- 4.2. Be sure the seal on the speedvac is in the correct position.
- 4.3. Turn the Vacuum and Concentrator to ON. Set the Drying Rate to Medium.
- 4.4. Check to be sure the speedvac is sealed and allow the plates to dry for 30 minutes under these condition.
- 4.5. After 30 minutes, turn the speedvac off and check to be sure no liquid remains within each well. Stack the trays together, seal the top plate with an adhesive plate sealer, and tape the stack together. Label the stack with initials, date, and primer information. Store the trays in a drawer until they are needed for PCR. (UNDER TESTING!!!!!!!!!!!!!!!)