

SPOTTING ONTO NYLON MEMBRANES WITH THE MICROGRID II ARRAYER

Purpose: Protocol to print PCR products onto nylon membrane for use in microarray hybridization experiments. This is our modification of the standard spotting protocol. It is not recommended for spotting onto glass slides.

Materials Needed:

- Microgrid II Arraying System (Apogent Discoveries, cat# BR-14-0)
- Microgrid II 384 Pin Head
- Nytran Supercharge Nylon Membrane (S&S, Inc, cat# 10416296)
- Krylon repositional adhesive (Michael's Crafts, cat# 7020)
- Cyclofoil roller (Apogent Discoveries, cat# 1044-39-2)
- NaOH
- Cresol Red sodium salt (Sigma-Aldrich, cat# 114480)
- 384 well Matrix Screenmates plates (Apogent Discoveries, cat# 4311)
- Low-profile plate lids (Apogent Discoveries, cat# 4320)
- PCR plate sealers (Edge Biosystems, cat# 48461)
- Stratagene UV Crosslinker (cat# 400672)
- Straight edge razor
- Koh-I-Noor rapidograph India Ink Pen
- India Ink (Koh-I-Noor, cat# 3085-F)
- 70% ethanol
- 95% ethanol
- bleach

Procedure:

A: Set Up of Machine

1. Turn on the main power unit, the cooling system, the humidity control, and the computer.
2. Prior to opening the TAS Application Suite, check to make sure there are no plates or equipment obstructing the movement of the machine. When the software is opened, the machine will zero itself and all moving parts will default to a starting position. All operations that require the machine to move **MUST** be done with the lid closed.
3. Choose the module that is required from the main toolbar. By pointing the cursor at any icon and waiting a few seconds, words will appear to identify what the icon does. The DAU uses the "macroarray option," for making low density arrays on porous surfaces.
4. Make sure all the water bottles are filled and all hoses firmly connected.

5. Click the icon for “prime recirculating baths.”

B: Preparation of Plates and Membrane

1. One hour prior to printing, reroll Nytran Supercharge Nylon Membrane to remove the curve. (NOTE: when using nylon rolled onto large diameter cardboard tubes, this step is unnecessary.)
2. Using a paper cutter, cut the membrane so that it is 27cm by 24cm.
3. Lightly spray Krylon repositionable adhesive onto the flat adapter plates.
4. Position the cut membranes on the plates, making sure to cover the screws at the corners of the plates.
5. Roll a cyclofoil roller over the membrane once it is positioned to adhere it to the plate.
6. Using the icons labeled T1 through T4, load the adapter plates into the machine, making sure each slides into place correctly.
7. Add dye and NaOH to a final concentration of 0.2N (see separate protocol for detailed explanation).
8. Load the first Biobank with the plates. Each Biobank holds up to 24 plates (NOTE: some newer models of the MGII only hold up to 20 plates).
9. Click the +BB icon to raise the rails to fit the Biobank in, and load the plates.

C: Settings

1. Under the window titled “macroarray parameters” click on the Source tab.
2. From the drop down menu, choose the type of microplate (# wells and brand name, DAU uses Matrix Screenmates 384).
3. Enter the number of plates being printed, how often to prompt for a Biobank change (DAU sets this parameter for every 24 plates when using a full Biobank), the source action (the movements the tool makes when picking up sample. DAU uses Dwell), and how to remove lids (DAU uses lids and removes them one at a time)
4. In the bottom left-hand corner will be a sentence telling how many source visits have been defined and how many tool spots are available. If the wording is green, the setup has been done correctly. If it is red, the machine will not run. If it is black, settings are less than optimal, but the machine will run.
5. Click on the Target tab. On the right hand side of the window, choose the settings for “Target Action.”
6. Spotting height in mm (DAU -0.752mm)
7. Dwell time (allows pins to pause in the wells, DAU sets this option to 0.00 seconds)
8. Multiple strikes (pins hit the same spots more than once, DAU does not use this option regularly)
9. Delay after spotting (pauses tool for amount of time entered, DAU does not use this function)
10. Tool Array Definition/Edit pattern. Click the “Edit Pattern” button.

11. In the window that appears, use the number keys to tell the arrayer where each plate should be printed (horizontal vs. vertical).
12. Choose the grid size and the pitch between spots (DAU 5X5, 0.850)
13. The Adapter Plate and Slide Layout/Edit layout section defines the type of membrane to be macroarrayed onto, and the number of array replicates.
14. The machine defaults to one 22cm membrane, and 6 separate tool arrays on that membrane.
15. To change these settings, click the “Edit Layout” button.
16. On the new “Layout editor” window that appears, click on the “Adapter Layout” tab.
17. Choose the size membrane from the drop down menu. DAU uses 24cm adapter.
18. Under “Number of copies” choose the number of membranes to be printed on. One membrane corresponds to one plate.
19. Click on the “Membrane Layout” tab. Use the options to define the position of the tool-array and choose the spacing of the arrays from each other.
20. Top margin—9mm (Click the “mirror vertical margins” button)
21. Left margin—9mm (Click the “mirror horizontal margins” button)
22. X axis—8mm
23. Y axis—11mm
24. To print identical filters on each 22cm membrane, using the mouse and the number buttons, change the number on the layout on the right hand side of the screen to “1”

D: Options

1. If the microplate type has already been chosen, the tool type will be automatically adjusted. If the tool type is changed, the microplate type will be updated as well.
2. DAU uses the “wash after every completed sample” option. Using this option directs the arrayer to wash the pin tool after each plate has been printed on all membranes.
3. Washing is highly recommended when printing more than one plate to prevent contamination.
4. From the main toolbar, choose Window and “Run Preferences.”
5. This section contains fine-tuning parameters. There are several tabs under this section.
6. Target Action—used for rearray and replicate only.
7. MWS—options for draining and filling main wash station.
8. Baths 1&2—wash cycle parameters (DAU time in left bath—10 seconds, time in right bath—10 seconds, time in vacuum dryer—10 seconds)
9. Softtouch—not used by DAU
10. Climate—not used by DAU
11. Barcodes—not used by DAU
12. General—contains choices for alerts prior to beginning printing. If all choices are clicked off, the machine will start to print as soon as “Go” is clicked. If choices are clicked on, a reminder will pop up to perform a function prior to printing.

13. Source Action--#wiggles (DAU 0), #dips (DAU 1), inter-dip delay (DAU 0), dwell time (DAU 0), pin depth (The more negative the number, the further the pins will descend into the wells—DAU -2 to -4)
14. Click the “Go” icon to start the print.

E: Post Arraying Procedures

For the Arrayer:

1. Remove biobank. The rails will raise to a position from which the Biobank can be removed.
2. Remove tool. The tool will move a position from which the cover can be put back on, and the tool removed. It is important to remove the tool when prompted to avoid damage to the machine.
3. Remove adapter plates. Each tray will move to a position from which they can be removed. Avoid touching the membranes without gloves.
4. Empty wash baths.
5. Empty wash bottles. Replace the water in the wash bottles immediately after completing a run.
6. Shut down. Close the software and shut down the computer. Turn off the main power unit, the cooling system, and the humidity control. The lid will lock once the machine is turned off.

For the Membranes:

1. Number. Using a pencil and wearing gloves, number each large membrane as the adapter plate is removed from the arrayer.
2. UV Crosslink. Filters are then UV crosslinked once using the auto crosslink program, 120,000 microjoules.
3. QC for Abnormal spots. Visually inspect the membranes for abnormal spotting, large spots, and erratically aligned subarrays.
4. Label with Name and Cut. Label the filters with number and name of print, cut apart with a razor, and store between clean sheets of printer paper.

F: Periodic Maintenance of Arrayer

1. On a regular basis, wipe down the base plate with 70% ethanol to remove contaminants and dust. Pay careful attention to the microplate sampling area to remove accumulation of plastic dust from plates and lids.
2. The arraying tool pins should be cleaned at regular intervals using distilled water in an ultrasonic bath. Alternatively, pins can be submerged overnight in 95% ethanol.
3. Be careful not to submerge the tool up to the top, as this can cause pins to stick and cause problems. After soaking, rinse with deionized water.

4. After every run, be sure to immediately change the water in the two 2 liter bottles. This should also drain the recirculating wash baths. Wipe out the empty recirculating baths on a regular basis with ethanol.
5. As needed, flush 10% bleach solution through the system.
6. The main wash station (MWS), housed within the main power unit, holds 6 liters of water, and is emptied and filled using the hoses on the front of the machine.
7. Empty the MWS by placing the hose with the open end in a bucket, and clicking the icon on the screen that reads “empty main wash station.” (The icon looks like a container filled with water, and can be used to determine how much fluid remains inside.)
8. Empty the MWS halfway, mix 600ml bleach with 2400ml water, and refill the MWS. Place the hose with the closed end in the bucket and click on the MWS “refill” icon.
9. Fill outside wash bottles with 10% bleach solution.
10. Click the “prime recirculating baths” icon, and let the baths sit for 15 minutes. Drain the bottles and MWS of all water, and refill with fresh water. As desired, repeat procedure.

Comments:

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For frequently asked questions go to the following address:
<http://www.grc.nia.nih.gov/branches/rrb/dna/protocolFAQs.htm>

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