

SPOTTING ONTO NYLON MEMBRANES WITH THE AFFYMETRIX 417 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:

- Affymetrix 417 Arrayer (cat# Model427)
- Nytran + Supercharge Nylon Membrane (S&S, Inc, Cat# 10416296)
- Six custom cut stainless steel forms
- Krylon repositional adhesive (Krylon, cat# 7020)
- Straight edge razor
- NaOH
- 96-well PCR plates (from previous PCR protocol)
- small square Pyrex dish
- Stratagene UV Crosslinker (cat# 400672)
- Dissecting scope
- Koh-I-Noor rapidograph India Ink Pen
- India Ink (Koh-I-Noor, cat# 3085-F)

Preparation:

A: Set Up of Machine

1. Calibrate the platen.
2. Make sure the platen is firmly seated at all 4 corners, and click “platen calibration”.
3. Click on lower left and verify that #1 pin ring lines up with hole at the lower left position, home.
4. Click on lower right and verify that #1 pin ring lines up with hole at the lower right.
5. Click on upper left and verify that #1 pin ring lines up with hole at the upper left position.
6. Click on dryer position and confirm that all rings enter holes for dryer without touching the walls. **Movement of pin assembly must only be done by pressing the spot directly under the rightmost step motor, NEVER THE PINHEAD ITSELF.**
7. Calibrate the rings (Necessary only if changing type of plates or volume of sample being printed).
8. With sample plate in position 1, click “start ring” and then “new calibration”, depress down arrow until rings **just** enter the liquid of the sample plate. Do not

allow rings to contact the plate bottom. NOTE: When printing plates more than once the volume in each well can vary due to uneven evaporation, in this case it may be necessary to set the rings lower in the wells.

9. Depress wash icon (showerhead) making sure the hose clamps are set three clicks closed. This prevents the wash water from splashing up on the print head.
10. To prevent carry-over, two washes of 2 to 3 seconds each are needed. When printing viscous liquids or high concentration dyes as test prints, more washes may be needed.
11. Dry time should be set for, at least 2.5 seconds.
12. A dry cycle between the 2 washes adds substantially to the total array time.
13. Calibrate the pins (Necessary only if changing type or lot# of print medium).
14. With glass slide or membrane covered form in slide position #22 click “start pin”, then choose either new calibration or adjust calibration. Always be sure to choose new calibration when going from a thinner medium to a thicker one, i.e. glass slides back to membranes. Click the down arrow once and then the space bar can be used to lower the pins 0.1 mm per step until the pins contact the slide. Two clicks **past** this point is usually the proper setting. The tops of the pins should rise about 0.5 mm when spotting.
15. When using a glass slide the pins will make an audible click when they contact the slide.
16. When using a membrane covered slide, an oblique angle high intensity light will allow you to watch the pins and their shadows approach and touch.
17. NOTE: The pin calibration test can be run to determine if the setting is optimum for the slide being used. This test will array a series of 10 spots with settings from -5 to +5 of the indicated, with the chosen setting having a reference line of 14 spots. All settings greater than the indicated setting should spot and at least settings of -1 and -2 should spot. You will need dye/samples in wells A1 and 2 and B1 and 2 of the plate in position 1 and a slide or membrane in slide position #1. When using the 300 micron pins dye can make the spots harder to see due to the spacing. If no spots are observed to the left of the reference line for any pin, lower the setting at least two more clicks. If more than four rows are seen to the left for any pin, raise the setting by one click.
18. Array calibration will use the same dye/ sample in plate 1 and spot a 20x20 array with all four pins. This is used to confirm all pins are spotting normally. The same slide/membrane can be used for this test and the pin test but it will need to be turned end for end so spots do not overlap. Dye may interfere with this test if using 300 micron pins, as the default spacing for this test, at this time is 375 microns.
19. Prime the pump.
20. Move the head out of the way by pressing the maintenance icon, which is between the home and ? icons on the upper tool bar. This moves the head to a position above the third sample plate.
21. Confirm that wash water is in large blue reservoir and waste container is empty. Also make sure that the smaller and longer set of white hoses is in, and at the bottom of the wash container, and the larger and shorter hoses are in the waste reservoir. **There should be no slides or membranes loaded at this time.**

22. Cover the wash station with a folded paper towel to prevent splashing and press the **prime pump** button. Observe as air bubbles are pushed through the fill tubes and out into the wash chamber. Tapping the hoses and fittings under the platen will aid in removal of all air bubbles. When no more air bubbles are seen, stop pump priming by hitting the button again. Wipe up any spilled water and return the pin head to its home position.

B: Set Up of Plates and Membrane

1. Prepare the membrane (NOTE: Gloves must be worn any time the forms and membrane are being handled.)
2. The 417 arrayer has six sections on the printing platen that accommodate 7 glass slides each for a total capacity of 42 glass slides. Six stainless steel forms were fabricated to fit onto the printing surface in the space that would normally hold the individual glass slides. These forms are approximately 160 mm x 80 mm x 3 mm each.
3. Krylon repositionable adhesive (cat # 7020) is then sprayed **lightly** onto each form. The glue is allowed to dry a few minutes. Too much glue can cause the reverse side of the nylon membrane to pull off when the membranes are removed from the forms after printing. Too little glue and the membrane can come free and get twisted in the print head causing damage to the arrayer.
4. Nytran⁺ Supercharge (S&S, Inc., cat # 10416296) nylon membrane is cut to fit each form exactly keeping the protective red paper covering on top of the filter membranes.
5. The cut nylon membranes are then placed on a clean surface and the form is placed on-top with the glue side down. Even pressure is applied to the entire form. Any overlapping edges of the membrane are trimmed with a razor.
6. The form/membrane/paper units are seated into the arrayer slots all the way to the top stop and pushed to the left to engage the springs. The protective paper can now be removed from the membrane. This process is repeated to fill the entire platen with form/membrane units. This allows 42 microscope slide size areas to be printed.
7. Prepare the plates.
8. Add NaOH to a final concentration of 0.2N (see separate protocol for detailed explanation).
9. Load first 3 plates into machine.

C: Set Up of Arrayer

1. Click "microplates" and then "auto generate plates".
2. If the "normal" spacing of 665um is used then 24 plates will fit on one slide or 12 plates with a replicate spot on the same membrane.
3. The setting for 24 plates is zero replicates, one replicate for 12 plates, and two replicates for 6 plates.
4. The default setting for the location of replicate spots is directly on top of the original spot, so Y values must be chosen that move the replicates further along

- but not off the end of the membrane.
5. Center to center, the spacing is 665um.
 6. Once the above settings are made click "OK" and the program returns to the array screen. At this time the number of slides to be printed must be chosen.
 7. For prints of 7, 14, 21, 28, 35 or 42 slides, the print order is top to bottom on the first, third, and fifth column of slides and bottom to top on the second, fourth and sixth column. For any other number of slides, print order is top to bottom for all columns.

Procedure:

1. Once the first three plates are ready and the filters are in the arrayer the print job is ready to start.
2. Turn on the vacuum pump, check for water in the wash bottle and make sure the waste bottle is empty. Clicking the "start" button will begin the print.
3. The rings and pins will first go to the wash station and go through a wash cycle. Make sure the rings are actually submerged in the wash water, and that the rings are not making contact when entering the dryer holes.
4. The pins will next move to the first plate position and pick up samples A1, B1, A2 and B2 and then move to slide #1 to start arraying. Make sure the pins are hitting on the membrane on all slides for these first spots.
5. The computer will estimate the time remaining in the entire print as well as the time remaining for each group of three plates. Use this time to estimate when the next set of three plates need to be taken out of the freezer and prepared for printing.
6. When the last plate has been printed a "**job completed**" box appears. Click **OK** and then click on the **file** menu, and **export file** to save the arrayer file. Chose a unique name with date of print and store this file in **my documents** in the correct folder, i.e. LG or LI.
7. Remove the filters one at a time and number in the upper right hand corner their print position 1-6.
8. Filters are then UV crosslinked once using the auto crosslink program, 120,000 microjoules.
9. At this time the membranes should be QC'ed by sight under the dissecting scope to note any missing spots.
10. Any position without a dent in the membrane means the sample was probably not picked up by the ring, so no DNA was deposited.
11. These positions should be marked on the array map to store with the filters.
12. Membranes are then carefully pulled off the supports and placed between pieces of printer paper.
13. Each individual filter from 1-42 is then labeled with the name, print #, date and a sequential number, usually 1-42, cut apart and stored until use.

Comments:

The main reason that this works well, is because this arrayer spots using blunt pins, not split pins. We have no experience spotting with split, Telechem pins, or pins by other manufacturers.

Caution:

With the pins moving quickly over the entire platen during the entire printing run it is very important that the membrane stay completely glued to the forms. If a corner comes loose, and the loaded rings pass over and touch it before printing, the sample can be absorbed out of the ring and no spotting will occur. In the worst case, the rings can catch the filter pull it completely up, potentially damaging the rings or pins.

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For frequently asked questions go to the following address:

<http://www.grc.nia.nih.gov/branches/rrb/dna/protocolFAQs.htm>