

PREPARATION OF PLATES FOR PRINTING ONTO NYLON MEMBRANES

Purpose: to prepare PCR product for arraying in the Affymetrix 417 or MicroGrid II arrayer

Materials Needed:

- Plate sealers (Edge Biosystems, cat# 48461)
- 250ul Matrix barrier tips (Apogent Discoveries, cat# 7252)
- 10N NaOH
- cyclofoil roller (Apogent Discoveries, cat# 1044-39-2)
- 1.3 mg/ml Cresol red (in DEPC water, filtered) (Sigma-Aldrich cat#114480)
- 384 well Matrix Screenmates plates (Apogent Discoveries, cat# 4311)
- Sorvall Super T-21centrifuge (Sorvall, cat#)

Procedure:

A: For printing on the Affymetrix-417 arrayer

1. Carefully remove the lids from the evaporated plates and dry the tops with a paper towel.
2. Dilute the 10N NaOH stock to 1N. Each plate uses approximately one ml of solution.
3. Set the multi-channel pipetter to fill with 250ul and dispense 10ul.
4. Deliver 10ul of 1N NaOH to each well. The setting of the pipetter will allow for 24 rows to be filled, expelling the final aliquot to account for pipet variability.
5. Dry the tops of the plates again if necessary and seal tightly, rolling the edges with the cyclofoil roller. It is especially important that there be no bubbles in the plate sealer to prevent contamination.
6. Vortex the plates thoroughly, first right side up, then turning them over and vortexing again, and then right side up one more time.
7. Centrifuge plates at 3000 rpm for one minute.
8. Heat plates to 42 degrees for five minutes. This can be done using a MJ Research PTC-225 Peltier Thermal Cycler.
9. Remove the plates from heat and place on ice. Once the plates have cooled, they are ready for arraying.

B: For printing on the MicroGrid II arrayer

1. Carefully remove the lids from the 96 well PCR plates and dry the tops with a paper towel.
2. Dilute the 10N NaOH to 1.6N. Each plate uses approximately 1.5ml solution.
3. Set the multi-channel pipetter to fill with 250ul and dispense 20ul.

4. Mix a 25/75 solution of cresol red and 1.6N NaOH. The solution will turn bright purple.
5. Deliver 20ul of NaOH/Cresol solution to each well. The setting of the pipetter will allow for 12 rows to be filled, expelling the final aliquot to account for pipet variability.
6. Dry the tops of the plates again if necessary and seal tightly, rolling the edges with the cyclofoil roller. It is especially important that there be no bubbles in the plate sealer to prevent contamination.
7. Vortex the plates thoroughly, first right side up, then turning them over and vortexing again, and then right side up one more time.
8. Centrifuge plates at 3000 rpm for one minute.
9. Heat plates to 42 degrees for five minutes. This can be done using a MJ Research PTC-225 Peltier Thermal Cycler.
10. Remove the plates from heat and place on ice.
11. Once plates are cooled, transfer the PCR product from 96-well plates to 384 well plates, patterning if needed.
12. Seal plates and centrifuge. Plates are now ready for arraying.

Comments:

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

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