Fabrication of Daphnia Oligonucleotide Microarrays

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Based on DGRC protocol for DGRC-2 Oligonucleotide production by Kevin Bogart, Lucy Cherbas and Justen Andrews

INTRODUCTION
This protocol describes the procedure of creating Daphnia oligonucleotide microarrays, beginning with lyophilized oligonucleotides and ending with slides ready for use in hybridization experiments. It is important to avoid the use of latex gloves in all matters associated with the fabrication of microarrays. Nitrile gloves should be used at all times.

PART 1. Reconstitution of Oligonucleotide in Sodium Phosphate (NaPO₄) spotting solution

INTRODUCTION
Oligonucleotides were purchased, manufactured, and purified by Invitrogen. They were shipped as lyophilized oligonucleotides. This procedure describes the method by which they were reconstituted in spotting solution for deposition onto glass microarray slides. This section is only completed with new oligonucleotide plates. Please see Part 4 for information on re-suspension after plates are already in use.

EQUIPMENT
Liquid Handling Robot; Beckman-Coulter Biomek FX
Centrifuge; Beckman Coulter Allegra 25R

MATERIALS
Lyophilized Oligonucleotides in 384-well Genetix plates
Ultrapure (DNAse/RNAse free) Distilled Water; Invitrogen: 10977-023
Millipore Stericup 0.45µm vacuum driven disposable filtration system
Thermowell Sealing Tape; Corning-Costar: 6570 / VWR: 29445-080
Biomek FX 20µL sterile tips; ABgene: TN20RS-AFX
Na₂HPO₄ (MW: 141.96 g/mol)
NaH₂PO₄ (MW: 137.99 g/mol)

PROCEDURE
A. Preparation of 150 mM NaPO₄ pH 8.5 spotting buffer
1. To create 100mL of pH 8.5 buffer, mix 97.8mL of 150mM (.15M) Na₂HPO₄ and 2.2 mL 150mM (.15M) NaH₂PO₄. Scale up to create the total amount of buffer needed.
2. To add 10µL of spotting buffer per well for a 384 well plate, you will need approximately 5mL of buffer per plate. Follow the charts below to make 150mM Na₂HPO₄ and 150mM NaH₂PO₄ stock solutions. Use DNase and RNase free water and filter sterilize using 0.45µm Stericup filter.

<table>
<thead>
<tr>
<th>Na₂HPO₄</th>
<th>H₂O</th>
<th>NaH₂PO₄</th>
<th>H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.65 g</td>
<td>500 mL</td>
<td>2.07 g</td>
<td>100 mL</td>
</tr>
</tbody>
</table>

B. Transfer NaPO₄ buffer to plates using the Biomek
1. Even though plates are lyophilized, they should be spun down at 2000 rcf for one minute in order to make sure the dried oligos do not “pop out” when opening the plate seal.
2. Fill a reservoir (a FX tip box lid works well) nearly full with 150mM NaPO$_4$ spotting buffer and place on the deck as instructed by the Biomek FX Instrument setup screen of the method: D:\BiomekFX\Methods\Nicole\Oligo\Fill water 384-well plates
3. Load the Biomek with tips as per instructed by Biomek FX Instrument setup screen. Note: If using only one box of tips, ensure that the Biomek method used does not touch the inside of the wells. If using a program that requires individual boxes of tips (4 per 384-well plate) ensure proper placement based on the Biomek Instrument setup screen.
4. Place 384-well plates in correct configuration. Verify overall deck configuration. Run program specifying how many µL to transfer. If 10µL is desired, it is a good idea to specify 11-12µL to compensate for small variations in dispensing.
5. When one set of plates is finished, continue adding plates and re-filling buffer reservoir until all plates are completed. Seal the finished plates with sealing tape and store at 4°C overnight. Plates are now at a concentration of 30µM.

PART 2. Deposition of Oligonucleotides onto glass slides using an OmniGrid 300 robot

INTRODUCTION
In this procedure, we describe the deposition of oligonucleotides in spotting buffer onto glass-slide microarrays. The Omnigrid 300 should be properly calibrated at all stations before beginning. Before a full print run, one or two test prints should be done to ensure that all pins are printing properly. When 100 slides are printed, they are consistently placed in positions 67-168 to minimize variation between print runs.

EQUIPMENT
OmniGrid 300 Microarrayer, Griddrer software version 4.1.13; GeneMachines
LabLine Orbit Shaker
Holmes Humidifier
DustBuster cordless vacuum cleaner

MATERIALS
384 well plates containing purified oligonucleotides
Corning UltraGAPS Microarray Slides; Fisher: 07-200-256
Thermowell Sealing Tape; Corning-Costar: 6570 / VWR: 29445-080
Approximately 40-60L of 15mM NaPO$_4$ washing solution (10% of the concentration of the spotting solution)

PROCEDURE

A. Gridder Parameters
The following figures show the setup of the Daphnia print run. There are 48 subarrays, each slated to contain a maximum of 225 spots, for a total maximum of 10800 spots. Daphnia slides actually contain 213 spots per subarray, totaling 10224 spots on the entire array (Figure 1). Each subarray is printed by one particular pin as shown in Figure 2.

In this print run, plates are loaded in groups of 6 (Figure 3). A Daphnia print run has 26 plates, including a control plate and one plate with virgin 150mM NaPO$_4$ spotting buffer. The control plate has various control spots including Actin5C, Ambion 1, 3, 5 and 7. The first three spots serve as a quality control measure in the following way: on each subarray, spotting buffer is printed first, then the uniformly expressed Actin5C
is printed from the control plate, followed by a print of the same original well of the spotting buffer plate (Figure 3 shows how the gridder begins with plate 1, then plate 2, then back to plate 1). After nonamer hybridization, it is thus possible to see whether the third spot on each subarray is blank, which in-turn indicates whether or not the pins are being cleaned properly between samples (Figures 4a and 4b).

Figure 1: Gridder software array design parameters

Figure 2: Silicon Pin Configuration

Figure 3: Gridder parameters for plate and printing sequence
B. Pre-print run preparations
1. Retrieve oligonucleotide plates from 4°C and shake in the LabLine Orbit shaker at 200-300 rcf for a minimum of 60 minutes, then centrifuge at 2000 rcf for 1 minute. Store at 4°C until printed.
2. Vacuum the interior space of the OmniGrid 300. Ensure the area within the chamber that houses the robot is dust-free. Carefully vacuum the slide deck.
3. Securely place UltraGAPS slides in positions 67 – 168 with barcodes facing up and towards the right of the platen.
4. Re-vacuum the interior space of the OmniGrid 300, working from the back to the front of the slide platen and keeping the mouth of the vacuum cleaner ~10 mm above the surface of the slides.
5. Clean and place glass blotting pads in the two blotting positions of the deck.
6. Ensure the humidifier reservoir is full (~7L) of MilliQ water. Turn the Speed Control and Mist Volume to High. Allow the humidity to equilibrate.

C. Printing
1. Load 6 unsealed Plates into Carousel 1 in positions 1-1 through 1-6. The first set contains spotting buffer plate, control plate, and plates 1-4. The subsequent sets contains 5-10, 11-16 and so on.
   Note: Remaining plates should be stored at 4°C and retrieved as required. When a plate is finished with, it should be resealed and stored at 4°C. Unsealed plates are vulnerable—use caution when loading and unloading plates from the carousel.
2. Run the Daphnia print-run protocol (Daphnia_Oligo_March_2007). Print at slide positions 67 – 168 (be sure to select “Each sample” under the slides heading of the Run parameters). Un-check the De-lid plates option. Use the plate sequence Daphnia_Oligo_March_2007 which prints in batches of 6 plates (Figure 5).
D. Post-printing
1. Turn the Holmes Humidifier off. 

We generally leave the slides setting in the Omnigrid 300 printer overnight after completing a print run in ambient humidity. This allows the spots to evaporate and concentrates the sample before UV immobilization.

PART 3. Immobilization

INTRODUCTION
This procedure describes the process of fixing oligonucleotides to the microarray slide in preparation for long-term-storage, shipping, and prehybridization. The most effective method for immobilizing oligonucleotides to UltraGaps slides is UV Crosslinking.

EQUIPMENT
Ultra LUM UV Crosslinking Oven
Vacuum Sealer PRO 2300

MATERIALS
Ultra-GAPS slides spotted with oligonucleotides
Barcode scanner
6” x 6” Black Mylar Foil Pouches; IMPAK Corp: 06MFS06
6” x 12” Vacuum Sealer Bags (cut in half); Westson Supply

PROCEDURE

A. Barcode Scanning
1. Using excel and the barcode scanner (connected by USB to the computer), scan each slide in the correct order (67-168) into a column in excel. Be aware that the scanner often reads a barcode more than once and you will need to check for repeats in the list. Holding the scanner as close as possible to the slides can
minimize this. Another tactic is to insert a second, temporary column and perform the scanning procedure twice and compare the lists.

**B. UV Crosslinking**

1. Remove the slides from the deck by picking up a single black slide platen containing 11 slides. Place the slides in the UltraLUM UV crosslinker. Multiple platens may be irradiated at once. Set the crosslinker to irradiate with 600mJ energy and press start. The crosslinking should take < 10 seconds.

**C. Packaging**

1. Slides are packed in groups of 5 (simply return them to the Corning packages they come in). Heat seal the package in the first mylar bag first and then vacuum and heat seal the outer, clear vacuum bag. Store at room temperature and humidity.

**PART 4. Post-printing re-suspension**

**INTRODUCTION**

After each print run, the volume in the plates should be checked by spot-checking a few wells. If there is 5µL or less left, plates need to be dried down and re-suspended. Now however, plates should be re-suspended in pure, DNAse, RNAse free water to maintain the proper salt concentration. Plates should be dried down prior to re-suspension simply by removing their seals and placing them for one or two nights in the horizontal flow, particle-free hood. The procedure for re-suspension this is identical to the original reconstitution procedure (Part 1) except that the reservoir should now be filled with pure water.