Printing *Daphnia* cDNA Microarrays on the Omnigrid 300

by Zach Smith and Elizabeth Bohuski

INTRODUCTION

In this operating procedure we describe the deposition of DNA to glass-slide microarrays. The smooth operation of a print-run depends upon the microarrayer being carefully set-up and calibrated before starting – and then disturbing it as little as possible once it is under way. It is essential that the pins be absolutely clean. All water reservoirs and lines must be clean. The rinse station must be carefully calibrated so that only the quills of the pins (below the bulb) are submerged in the water. It is good practice to run some tests *without* spotting solution to ensure pins are completely dried after the rinse cycle. Likewise it is advisable to run a test *with* spotting buffer to ensure that all pins are printing properly.

Definitions

1. **Printhead**: The printhead is the entire pin assembly, consisting of a metal frame, the pins, the collimeter, foam pad, magnetic plate, metal lid, and screws.
2. **Collimeter**: The collimeter is a flat metal piece with a grid of pin holes that acts as the pin guide. There is a collimeter on the top and bottom of the printhead, holding the pins in place. Should either collimeter move in the horizontal plane, it could decapitate the pins—all 48 at once.
3. **Microarray Printing Blocks**: The microarray printhead holds 48 pins. The printhead must dip into the 384-well print plate 8 times to dip into all the wells. The group of wells in a single dip is a block—there are 8 print blocks on a plate. (See Appendix.)

MATERIALS

**Consumables**

- Plate lids; Genetix, Cat. # X7021
- Thermowell Sealing Tape; Corning-Costar, Cat. # 6570/VWR # 29445-080.
- GAPS II Microarray Coated Slides; Corning, Cat. # 40003/VWR # 77776-867.
- 95% Ethanol.
- Betaine (5M) for spotting buffer.
- Combi-tips (0.5 ml) for repeater-pipetor
- 384-well plates; Genetix, Cat.#

**Re-usables**

- Plates containing purified PCR products reconstituted in spotting solution (see 4.3.27).
- Stainless Steel Slide Rack; ThermoShandon, Cat. #113.
- Old, junk microarray slides.

**Equipment**

- Silicon Pins.
- OmniGrid 300 Microarrayer; GeneMachines, installed with Gridder version 4.1.6 (or better).
- DustBuster cordless vacuum cleaner 9.6 volts; Black & Decker, Cat. # V9610.
- Barcode scanner
PROCEDURE

Print Run Preparations

Stage I (The evening before Day 1...)
- Dry down twelve 384-well sample plates, **overnight**.
- Make printing buffer if low: 3 X SSC & 1.5 M Betaine.

Stage II (Day 1)
- Check dry station foil.
- Check for broken pins.
- Check printhead for "sticky pins."
- Clean printhead, if necessary.
- Replace pins, if necessary.
- Calibrate.
- Replace aluminum foil on dry station, if necessary.

Stage III (Day 1 - Day 2)
- Clean 12 old slides for two pre-print runs.
- Make pre-print run plate: fill wells with printing buffer.
- Run pre-print run with 5 slides. (1st Run, Optional)
- Make a negative control, "Buffer Plate," for the real print run.
- Resuspend printing plates **overnight** at 4°C.
- Fill Rinse Station container.
- Fill humidifier.
- Clean blotting pads.

Stage IV (Day of Print Run: Day 2 or Day 3)
- Lay down 5 old slides (cleaned) for pre-print run.
- Run pre-print run with 5 slides. (2nd Run)
- Lay down new slides for the real print run + 1 new slide for the final pre-print run.
- Scan slides with barcode reader.
- Run pre-print run on a single new slide. (3rd Run)
- Scan through Print Run Checklist.
- Start the real Print Run!
Stage I

Dry down Printing Plates
1. Remove plates from refrigerator.
2. Spin Genetix 384-well plates in stacks of three. Spin at 1000 rcf for 2 min in the normal, Allegra centrifuge.
3. Remove the seals and place the plates in the vacuum centrifuge.
4. Close the lid and turn on the concentrator and then switch the drying rate to high or medium
   - High: 4-6 hours drying time
   - Medium: Overnight drying time
5. Make sure the bleed valve to the suction pump is closed and turn on the pump (the switch is in front, near the table leg).
Note: Make sure that the refrigeration unit is on and cool before running this procedure, or the concentrator will fail to remove the moisture from the samples.
6. Dry down plates until the DNA is concentrated as a white film in the bottom of the wells (about 4 hours or so at high).

Making the Spotting Buffer
Note: The 5 M Betaine is packaged in 1.5 ml tubes.

<table>
<thead>
<tr>
<th>Stock Solutions</th>
<th>For 5ml</th>
<th>Stock Solutions</th>
<th>2ml tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betaine (5M)</td>
<td>1.5ml</td>
<td>Betaine (5M)</td>
<td>500ul</td>
</tr>
<tr>
<td>20xSSC</td>
<td>750ul</td>
<td>20xSSC</td>
<td>250ul</td>
</tr>
<tr>
<td>UltraPure H2O</td>
<td>2.75ml</td>
<td>UltraPure H2O</td>
<td>916ul</td>
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</table>
Stage II
Watch your fingers at all times around the printhead. Don't break the pins! Here's a tip. Tape down your ring finger and pinkie to prevent them from inadvertently breaking pins.

Check Dry Station Foil
1. Check the size of the pin-holes in the dry station foil.
   - The holes should only be slightly bigger than the pins.
   - If the holes are big, the vacuum around the pins is not strong enough to dry them.
   - The foil usually needs to be replaced after 2 - 3 print runs.
2. Replace foil when calibrating dry station.

Check for Broken Pins
1. Use a lamp.
2. Look at the pins from the X (front to back) and Y axes (left to right).
3. Look for broken pins and broken pin tips.
4. Replace broken pins after cleaning the printhead.

Check Printhead for Sticky Pins
1. Remove the printhead lid by unscrewing the four screws on top with the special allen-wrench—not the white- or purple-handled allen-wrenches.
2. Remove foam, but leave plastic sleeves to prevent collimeter from moving.
3. With a gloved hand, lightly tap the tips from underneath to see if they slide up with minimal resistance.
   - The pins may not slide back down on their own--this is okay.
   - Taps the pins from the top to slide them back into place.
4. If the pins are sticking, clean the printhead. Otherwise, continue with replacing pins.
5. If no pins need to be replaced, continue with calibrations.

Printhead Cleaning
1. Reassemble the printhead before removing it.
   - Replace foam.
   - Screw on lid securely.

Removing the printhead: Do not under any circumstances touch the magnetic plate on the underside of the printhead, as this secures the collimeter in place. Should the collimeter shift sideways, all 48 pins could be broken.

2. Get a firm grip on the sides of the printhead, while pushing the printhead against the printer arm to hold the printhead in place.
3. Unscrew the two screws in back with the purple-handled allen wrench.
Soaking the printhead
4. Place the printhead on its side.
5. Unscrew the lid and remove the foam.
Note: The pins are now free-floating, so be mindful to angle the printhead downward to prevent the pins from sliding out of the top of the printhead (and collimeter).
6. Fill a clean crystallization dish 2/3-full with Milli-Q water.
7. Immerse the printhead for 5 minutes.

Drying the printhead
8. Remove the printhead from the dish and lay it gently on a layer of kimwipes.
9. Blot the metal frame with kimwipes while avoiding the pins at all cost.
10. Next, use the air brush to dry the pins as much as possible. Set the volume control at 5 (6 is the absolute maximum for this job) and after the initial pressure surge gently remove any remaining water from the pins.
11. Once the printhead is dry, replace the foam pad and the top plate (lid).
12. Place the printhead on its lid, so that the pins are pointing straight up.
13. Remove the magnetic metal plate on the bottom and dry underneath. Be very careful to not move the collimeter that is being held in place by the magnetic plate.
14. Examine the pins under dissecting scope for signs of damage or incorrect installation.
15. Reattach the printhead to the print arm before replacing any pins.
   • The back-screws occupy the lower, more closely spaced set of screw holes.
16. Use the bubble level to make sure the printhead is level.

Replacing Pins
1. Remove the top plate and the foam pad.
2. Remove any damaged pins from the printhead, slowly and carefully.
Note: Forceps can be of some use here, but a steady hand is a better tool.

Loading the pins: These pins, as shown below, are similar to the heads of a fountain pen in construction. Notice that one side is smooth to the tip while the opposite side is indented.
3. Remove the plastic sleeve.
4. Place the pin-guide on top of the collimeter, with the “Back” label in the back.
   • It is best to use a gentle, left to right motion to do this.
   • A front to back motion can damage the pins.
5. Remove a pin from its packing case. Be very, very gentle, as these pins snap easily.
6. Position the pin above the correct hole so that the smooth side of the pin is facing you.
7. Lower the pin as far as you can before letting it go to ensure that it does not twist inside the hole before it hits the collimeter.
8. Tap the pin into place using the white-handled allen wrench.
   • Place the allen wrench in the hole above the pin.
   • Tap the pin down the guide and into position in the collimeter. The pin should move easily. If it does not, don’t force it. Call for help if needed. :)
9. Reassemble the printhead: Once all the pins are in place, replace the plastic sleeves, the foam pad, and the printhead lid.
Calibration

1. Remove the top plate and foam pad so that the pins are able to move freely.
2. Do NOT remove the plastic sleeves--they keep the collimeter in place.
3. Open the Gridder software on the Dell and click <Administrate>.
   • Under the heading, "Calibrate," click <Stations>.
   • Midway down the menu is a pulldown menu of stations that need to be calibrated.
4. After Selecting each station, calibrate the X, Y and Z-axises as follows.
   • Click <Buffer <--- Prev> and enter 0 for the Z value.
   • Click <Buffer>. This will send the printhead to the previous position on the X (front to back) and Y (left to right) axis while keeping the Z (up and down) axis raised.
   • Note: Most of the time it should not be necessary to change the X and Y values.
   • Use the arrows on the computer to move the printhead:
     ➢ The double arrows (>>) move the printhead a large distance
     ➢ The single arrow (>) moves the printhead in short increments.
   • Lower the printhead until it is at most a centimeter above the slide.
   • Verify the X and Y axises, then proceed with the Z-axis.
   • Save axises if you make changes and record those changes in the print log.
   Note: Clicking <Save> saves the "Current Position" in all three axises.

Slide Origin

5. Choose Slide Origin in pulldown menu.
6. Place a slide in the top left position.
7. Click <Buffer <--- Prev>. Enter 0 for the Z value. Click <Buffer>.
8. X/Y Alignment: The back row of pins should align with the back edge of the slide to get a correct X axis. The column of pins on the right side of the printhead should align with the right edge of the slide for a correct Y axis. Make any necessary adjustments and note them in the print log.
9. Z-axis Alignment: To calibrate the Z axis, lower the printhead until the pins barely move in the collimeter. If the pins move up too much they will be damaged later in the printrun, as they will be striking the slide too hard. Since the slide deck is not perfectly level, if the pins do not move at all (or too little) they will not make contact with all of the slides. (You should be able to see a slight visible gap between the crosspiece of the pin and collimeter.)
10. Save "Current Position" if you made changes, and record changes in the print log.

Blotting Pad

11. Choose Blotting Pad on the pulldown menu.
12. Place Blotting Pad in Station 1. Click <Prev > Buffer>.
13. Click <Buffer <--- Prev>. Enter 0 for the Z value. Click <Buffer>.
14. X/Y Alignment: In this case, the printhead should be well within the blot pad (not aligned with the edge as with the Slide Origin). There should be enough room for two printhead lengths along the length of the blotting pad.
15. Z-axis Alignment: The pins should make contact with the blotting pad, but in this case, there should be no visible gaps between the pin crosspiece and collimeter.
16. Save "Current Position" if you made changes, and record changes in the print log.
Rinse Station
17. Select Rinse Station on the pulldown menu.
18. Turn the pump control <On>.
19. Click <Buffer <--- Prev>. Enter 0 for the Z value. Click <Buffer>.
20. X/Y Alignment: The printhead should be centered in the wash station.
21. Z-axis Alignment: On the Z axis, the pins should be submerged to the point where the water level is just below the bulbs. See the picture below.
22. Save "Current Position" if you made changes, and record changes in the print log.
Note: A handy trick with this station is to place the light behind the printhead so that the light shines through the bulbs.

Dry Station
23. Select Dry Station from the pulldown menu.
24. Verify that the holes in the foil on the dry station are not too big.
   • Replace the foil if the holes are much bigger than the pins. (See Replacing Foil.)
   • The foil is needed to maintain suction when drying the pins.
25. Start Vacuum Pump: The vacuum pump should be on while calibrating to suck the pins through the foil and reduce resistance against the pin's downward motion.
   • Unplug the Vacuum power cord from the Omnigrid 300.
   • Attach the extension cord.
   • Plug extension cord into the power strip with the surge protector.
26. Click <Buffer <--- Prev>. Enter 0 for the Z value. Click <Buffer>.
27. X/Y Alignment: Center the pins above the holes in the foil.
28. Z axis Alignment: The pins should be high enough to where the foil is just below the bulb. Try not to widen the holes in the foil, if possible, as this leads to loss of suction.
   • Lower pins until they move slightly; lightly tap the pins down with gloved finger.
   • Continue lowering the pins (as above) until they are at the correct height.

Note: Never "Go To" <Previous> or "Go To" <Buffer> without zeroing-out the Z-Value while there is foil on the dry station (even foil with holes) unless you first:
   • Replace the foam and printhead lid to prevent pins from lifting in the printhead.
   • Turn on the vacuum (to suck the pins through foil).

29. When finished, plug the vacuum back into the Omnigrid 300 power source.
30. Save "Current Position" if you made changes, and record changes in the print log.
Replace Foil (if necessary)
Note: Be aware that it is not the same foil for sealing the 96/384 well plates.

31. Center a rectangular piece of ultra-thin foil over the dry station holes.
32. Turn on the vacuum pump.
   - Under Administrate, Click <Test Aux. Devices>. Click Dry Station <On>.
33. Once you can see the holes beneath the foil, verify that the foil is centered.
   - If it is not centered, turn off the vacuum.
   - Re-center the foil.
   - Turn on vacuum again, etc.
34. Use a gloved finger to smooth out all the wrinkles in the foil. (The wrinkles could allow air to leak, decreasing the vacuum pressure around the pins.)
35. Tape along the edges of the foil.
36. Click <Administrable>. Click <Stations>. Choose "Dry Station".
37. Click <Buffer <--- Prev>. Enter 0 for the Z value. Click <Buffer>.
38. Poke holes in the foil by slowly lowering the pins onto the foil.
   - Make sure the printhead lid and foam are removed.
   - Make sure the vacuum is on.
   - Administrate>Calibrate>Dry Station
   - X/Y Alignment: Center the pins above the dry station holes (viewable as circles in the foil) while the pins are about 1 mm above the foil.
   - Z-axis Alignment:
     - Lower the pins onto the foil until pins move up in the printhead slightly.
     - Tap the pins on top with a gloved finger, poking the pins through the foil.
     - Lower the pins, again, until they move slightly, then push the pins down.
     - Continue lowering the pins as above until the bulb is just below the foil.
     - Raise the pins until the foil is just below the bulb.
40. Continue with 384-Well Plate Station.

Note: A handy trick for calibrating this station is to place the light behind the printhead so that the light shines through the bulbs.
384-Well Plate Station
41. From main menu, click <Administrate>. Under the heading "Sample Plate Type," click <Edit Existing>.
42. Place a clean Genetix 384-well plate in the cassette so that position A1 on the plate corresponds to the corner of the cassette labeled A1.
43. Select 384 Genetix plate on the computer. Click
   - Make sure that there is a lid on the plate and that it is position so that the lid does not overhang the lip of the cassette on the back.
   - The software will go through a series of checks to make sure the lid is on, that the server arm is in position, etc. Calibrate once the plate is in loading position.
44. Click <Buffer <--- Prev>. Enter 0 for the Z value. Click <Buffer>.
45. X/Y Alignment: The pins should be centered above the wells of the plate.
46. Z-axis Alignment: The pins should not move at all.
   - You should not need to change this setting.
   - To fix/verify the calibration, lower the pins until they move up slightly.
   - Move the printhead up about 500 units. Save position.
   - The pins should be low enough in the wells that they pick up low volumes of sample (~2 µl), but not so low as to touch the bottom of the plate.
47. Save "Current Position" if you made changes, and record changes in the print log.
Stage III

Pre-Print Run Preparations
1. Clean 12 old slides for pre-print run.
   - Rinse old barcoded slides in the following order:
     ➢ Very hot tap water
     ➢ MilliQ water
     ➢ 95% ethanol (to dry the slides)
   - Dry with a Kimwipe.
2. Make pre-print run plate.
   - Aliquot 6.0 ul of spotting buffer into each well of print block 1.
   - (See Stage I for spotting buffer.)
   - (See Appendix for print block 1.)

Pre-Print Run (Day before the Print Run, Optional)
This pre-print run is intended to catch major problems (like broken, chipped, or sticky pins) the day before the real print run.
1. Fill torch with gas per manufacturers instructions.
2. Remove the lid and foam pad from the printhead, as well as the plastic sleeves.
3. Flame the pins until they glow red using the bright, interior part of the flame.
   - It is best to flame the pins from all angles to ensure coverage of the interior ones.
   - This step removes any DNA contamination remaining on the pins.
   - Allow the pins to cool for 20 minutes before reassembling the printhead.
4. Place 5 old slides that have been cleaned of any spots onto the printdeck.
   - Use the third or fourth column so you can see the slides as they print.
   - This step is to determine if all the pins are printing properly.
5. Place the Pre-Print Run plate into position 1 of cassette 1 so that position A1 on the plate corresponds to the corner of the cassette labeled A1.
6. Run Method> EB_PinBreakIn
   - Enter the slide numbers (start and end), where the test slides are located.
   - Click "Each Sample" next to the slide position (instead of the default).
   - Under the heading, "Sequences", Click <Choose...>
     ➢ Select plate sequence, "DGRC Test"
   - Check the "De-lid" and "Replace lid" boxes.
   - Click Run.
7. Check the slides after the print run to make sure that all the pins are printing properly:
   - Make sure that the spots are orderly and not bleeding into each other.
8. Re-run Pre-Print Run if pins are misbehaving. Otherwise continue.
Resuspend Printing Plates

Note: The tips used to resuspend the print plates have been used in the past to resuspend print plates or to transfer purified PCR product from Millipore plates to storage Nunc plates. Biomek FX tips are quite expensive, so they are re-used whenever possible. "Dirty tips," tips that may have been contaminated with DNA, can only be re-used in the same print plate wells to prevent cross-contamination. Because the tips are "dirty," a single reservoir of water cannot be used as the source for distributing water into dried-down print plates. In order to re-use dirty tips, Ultra Pure water is first distributed into clean 384-well plates--one for each print plate--and these plates are used as source plates in the biomek protocols. The water is then transferred with the dirty tips from the water-filled 384-well source plates to the "destination" 384-well print plates.

1. Using the Biomek, resuspend the DNA in **6.0 ul of Ultra Pure water**.

2. There are two Biomek methods for resuspending the print plate DNA and buffer.
   C://Program Files/ Biomek FX/ Methods/ Daphnia/ 384Resuspend_PostDryDown/

   - /MulitplePrintRuns_DirtyTips/ 384_Resuspend_DirtyTips_Resuspending
     - This method resuspends the DNA using pre-made 384-well source plates. These source plates are created with 50 ul water, and can be re-used about 7 times until the water level is too low. The plates simply need to be sealed between print runs/ resuspensions with clear plastic seals and stored at 4 °C.
     - Create the 384-well source plates using the method in the same folder:
       /MulitplePrintRuns_DirtyTips/ 384_Resuspend_DirtyTips_CreatingSource

   - /384_Resuspend_DirtyTips_SinglePrintRun
     - This method resuspends the DNA after first creating the 384-well source plates from a reservoir of Ultra Pure, all within the same method.
     - The downside is that the 384-well source plates have to be tossed after each Biomek run--they cannot be re-used because there is too little leftover water.
Real Print Run Preparations

1. Make Print Run "Buffer Plate."
   - This is a negative-control plate for the real print run.
   - Use repeater-pipetor with 0.5 ml Combi-Tip.
   - Dispense 5 µl of printing buffer (3 X SSC, 1.5M Betaine) into each well of printing blocks 1 - 5. (See Appendix.)

2. Fill Rinse Station container.
   - Set the Milli-Q pump to 35 liters to completely fill container (about 20 liters).

3. Fill humidifier.
   - Set the Milli-Q pump to 12 liters to completely fill chamber (about 7 liters).

4. Clean both glass blotting pads.
   - Run the blotting pads under HOT tap water for about 5 minutes.
   - Periodically (~3 times) wipe from one end of the blotting pad to the other end with a paper towel (to wipe off the betaine).
   - Rinse with Milli-Q water.
   - Dry with clean paper towels or Kimwipes.

Note: Insufficient cleaning will leave a coating of betaine on the blotting pad, causing the spots to pool on the blotting pad quickly.
Stage IV (Day of Print Run)

Print Run Setup

1. Layout 5 old slides that have been cleaned of any spots onto the printdeck.
2. Flame the pins as follows, if the pins have not been flamed since calibrating.
   - Fill torch with gas per manufacturers instructions.
   - Remove the lid and foam pad from the printhead, as well as the plastic sleeves.
   - Flame the pins until they glow red using the bright, interior part of the flame.
     - Flame the pins from all angles to ensure coverage of the interior pins.
     - This step removes any DNA contamination remaining on the pins.
     - Allow the pins to cool for 20 minutes before reassembling the printhead.
3. Execute the Pre-Print Run on the five old slides.
   - Run Method> EB_NewPinBreakIn
     - Enter the slide numbers (start and end), where the test slides are located.
     - Click "Each Sample" next to the slide position (instead of the default).
     - Under the heading, "Sequences", Click <Choose...>
       - Select plate sequence: "DGRC Test"
     - Check the "De-lid plates" and "Replace lids after dipping" boxes.
     - Click Run.
   - Repeat the Pre-Print Run if all the pins are not printing properly.
   - Check for broken pins and re-flame if a second Pre-Print Run is unsuccessful.
4. Lay out the requisite number of slides on the printdeck.
5. Scan Barcodes
   - Plug the barcode scanner into the USB port on the front of the Dell.
   - Open the file, Genomics > Daphnia_2003-2005 > SlideOrderTemplate.
   - When scanning the barcodes, keep the scanner close to the slides. After scanning each column, check the numbers on the excel spreadsheet to make sure there are no duplicates. To follow the slide order, scan in a zig-zag pattern among columns.
   - Rename and Save the spreadsheet and minimize it for later use.
6. Place one extra new slide ahead of the others for a pre-print run on a fresh slide.
7. Execute the Pre-Print Run on the single new slide.
   - Place a clean blot pad in the 1st blot pad position.
   - Run Method> EB_NewPinBreakIn_Blot
     - Enter the slide numbers (start and end), where the test slides are located.
     - Click "Each Sample" next to the slide position (instead of the default).
     - Under the heading, "Sequences", Click <Choose...>
       - Select plate sequence, "DGRC Test"
     - Check the "De-lid plates" and "Replace lids after dipping" boxes.
     - Click Run.
   - Check the slide and the blot pad to verify that all the pins are printing properly.
8. If all the pins are printing well during the entire pre-print run, continue with the real print run. Otherwise, try refiring the pins, or replacing the bad pins and then refiring.
9. Clean the blot pad.
Starting the Real Print Run
10. Place a clean blotting pad in both the 1st and 2nd blot pad position.
11. Load a fresh Buffer Plate into Position 1 in the cassette and a fresh Positive/Negative Control Plate into Position 2.
12. Run Method> EB_M6_011005
   - Enter the slide numbers (start and end), where the test slides are located.
   - Click "Each Sample" next to the slide position (instead of the default).
   - Under the heading, "Sequences", Click <Choose...> ➢ Select plate sequence, "M6_011005"
   - Check the "De-lid plates" and "Replace lids after dipping" boxes.
   - Click Run.
13. After the Buffer and Positive/Negative Controls have been spotted onto slides, begin loading the sample plates in the proper order in the cassette and allow the program to proceed. (See Appendix: Print Plate Order in Cassette)
14. One will have to swap out blotting pads and printing plates periodically, and not normally at the same time.
15. Clean the glass blotting pads between uses.
   - Run the blotting pads under HOT tap water for about 5 minutes.
   - Periodically (~3 times) wipe from one end of the blotting pad to the other end with a paper towel (to wipe off the betaine).
   - Rinse with Milli-Q water.
   - Dry with clean paper towels or Kimwipes.

Note: Insufficient cleaning will leave a coating of betaine on the blotting pad, causing the spots to pool on the blotting pad quickly.

Note: If you exit a run on the gridder software, and then restart the run, the pins may not home to exactly the same X/Y positions. This homing problem will cause the spots to not line-up in a clean grid formation. Analyzing the slide images will be more difficult.
## APPENDIX

### Print Plate Order in Cassette
"EB_M6_011005"

<table>
<thead>
<tr>
<th>Plate Sequence</th>
<th>Cassette Position</th>
<th>Plate Label</th>
<th>Sample Sequence (Blocks)</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Buffer Plate</td>
<td>Block 1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Positive/ Negative Plate</td>
<td>EM_M6_Plate2 (1, 2, 3, 5)</td>
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</tr>
<tr>
<td>Reload</td>
<td>Reload</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>6A</td>
<td>Vertical (1 - 8)</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>7A</td>
<td>Vertical (1 - 8)</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>8A</td>
<td>Vertical (1 - 8)</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>9A</td>
<td>Vertical (1 - 8)</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>10A</td>
<td>Vertical (1 - 8)</td>
</tr>
<tr>
<td>Reload</td>
<td>Reload</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1</td>
<td>Wfg, Sans Fille, Fruit</td>
<td>Block 5</td>
</tr>
<tr>
<td>17</td>
<td>2</td>
<td>Positive/ Negative</td>
<td>Block 5</td>
</tr>
<tr>
<td>18</td>
<td>3</td>
<td>Buffer</td>
<td>Block 4</td>
</tr>
<tr>
<td>19</td>
<td>2</td>
<td>---</td>
<td>Block 6</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>---</td>
<td>Block 5</td>
</tr>
</tbody>
</table>

### Microarray Printing Blocks
Print Run Preparations Checklist

Stage I (The evening before Day 1...)
- Dry down twelve 384-well sample plates, overnight.
- Make printing buffer if low: 3 X SSC & 1.5 M Betaine.

Stage II (Day 1)
- Check dry station foil.
- Check for broken pins.
- Check printhead for "sticky pins."
- Clean printhead, if necessary.
- Replace pins, if necessary.
- Calibrate.
- Replace aluminum foil on dry station, if necessary.

Stage III (Day 1 - Day 2)
- Clean 12 old slides for two pre-print runs.
- Make pre-print run plate: fill wells with printing buffer.
- Run pre-print run with 5 slides. (1st Run, Optional)
- Make a negative control, "Buffer Plate," for the real print run.
- Resuspend printing plates overnight at 4°C.
- Fill Rinse Station container.
- Fill humidifier.
- Clean blotting pads.

Stage IV (Day of Print Run: Day 2 or Day 3)
- Lay down 5 old slides (cleaned) for pre-print run.
- Run pre-print run with 5 slides. (2nd Run)
- Lay down new slides for the real print run + 1 new slide for the final pre-print run.
- Scan slides with barcode reader.
- Run pre-print run on a single new slide. (3rd Run)
- Scan through Print Run Checklist.
- Start the real Print Run!