

<b>STANDARD OPERATING PROCEDURE</b>		
J. David Gladstone Institutes Genomic Core Laboratory		
Title: Using ArrayMaker 2.1 Software with the Linear Servo Arrayer		Page Number: 1
SOP #P002	Version: #1	Date: December 11, 2001
Author: Yanxia Hao		Reviewer: Chris Barker

## **MATERIALS**

1. Resuspended 384-well plates for printing
2. Poly-lysine coated slides, aged 2 weeks
3. 384-well plate, empty, for calibration of tips
4. 384-well plate with 5 $\mu$ l test DNA at 100 ng/ $\mu$ l in 3X SSC for test print
5. DNA in either the first 16 wells (4 rows by 4 columns) or first 32 wells (4 rows by 8 columns) depending on how many tips you are going to print with
6. 95% EtOH
7. Kimwipes or cleanroom wipes
8. Roll of narrow and wide tape
9. Blot pad slides
10. Powder-free gloves
11. Full bottle (500ml) of filtered 0.5X SSC
12. Calculator
13. Scissors
14. Razor blade
15. Label maker and label tape

## **PROTOCOL**

### **PREPARING THE PLATTER:**

1. Scrupulously clean off the platter with Kimwipes moistened with 95% EtOH.
2. With gloved hands, lay down the slides in each column.
  - a. Examine each slide as you put it down. Discard those with big scratches or large pieces of dust.
  - b. Lay down the slides using the pin guides other starting with the slide at the back end of each column. This arrayer can hold up to 265 slides plus the blot pad.
3. Turn on the vacuum (metal lever on the wall behind the arrayer) to hold the slides in place. The vacuum should be quiet. Listen for leaks, and if you find any, adjust the slide positions appropriately so that the leaks are eliminated.
4. Make sure the inside of the dust cover is clean! Put the dust cover back over the slides.

### **BOOTING ARRAYMAKER:**

1. Turn on the computer.
2. Plug in the small and big plugs to the left of the computer. You should hear 2 clicks. (The large circular plug is for the X and Y stages, and the other 3 prong

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plug is for the Z stage.) If you do not hear two clicks, check the LED status lights on top of the amplifiers. All three lights should be glowing green. If they are yellow or red, something is wrong – STOP HERE.

3. Boot the ArrayMaker program.
4. Under the Connect tab:
  - a. Hit Connect to controller. If everything is ok, the Home all Axes button will become available
  - b. *Make sure everyone is out of the way of the path of movement of the arrayer and that the print path is clear of all objects.*
  - c. Hit Home all axes. A warning message will appear on the screen. Click OK. Wait until the arrayer has finished all three axes (“Motor at home position” lights up for all three axes, and you get a dialog box that says all stages are home).

#### **ALIGNING THE ARRAYER:**

Go to the Align tab.

Rinse station:

1. Make sure the sonicator is rinsed out and then filled with 0.5X SSC.
2. If you are unsure of the positions set for the Z axis, click the Reset Z button in the lower left hand panel. This will reset the Z height to the “ready” position, thus preventing any potential collisions. Even if you have only a slight doubt, it’s a good idea to hit this button. It could save you thousands of dollars and a big headache.
3. Go to the rinse position by clicking the Rinse button in the lower left hand panel. The first time the arrayer moves to a position, a warning message will be displayed. (Tips will move to the rinse station.)
4. Using the buttons in the Motion Control section of the page, hit the down arrows at the appropriate step sizes to move the tips until they are at the appropriate level in the liquid (see below).
5. *WHEN MOVING THE TIPS IN THE Z-AXIS AT ANY STEP OF THIS ENTIRE PROTOCOL, NEVER MOVE AT INCREMENTS GREATER THAN 500 MICRONS. IT IS EASIER THAN YOU THINK TO CRUNCH THE TIPS.*
6. At the rinse station control, turn on the sonicator so you can check the level of the tips in the liquid. When the sonicator is on, the level of the liquid should cover the V-point of each tip. Place two pieces of Parafilm, each flanking the path of the tips, over the sonicator. Do not interfere with the path of the tips!!!

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7. *Set and save the alignment.* (Hit the Set button to the right of the station locations and then hit the Save Alignment to Disk button.)
  
8. Dry station:
  1. Go to the dry station:
  2. If you will only be printing with 16 tips, cover the excess dry station holes with a piece of wide tape.
  3. Turn on the vacuum.
  4. Lower the tips down until you hear the vacuum get much quieter.
  5. *Set and save the alignment.*
  
9. Blot pad:
  - a. Go to the blot pad station:
  - b. Using the buttons in the Motion Control section of the page, set the X and Y axis first for the blot pad by moving either left, right, backwards, or forwards. You wish the tips to be left of the center position on the slide. If you are printing with 16 tips, you can do a tandem blot. In this case, you will want to justify the blot position to one side of the slide so that you can fit in a second tandem blot.
  - c. Note that “Left”, “Right”, “Back”, and “Forward” always move the platter. “Up” and “Down” move the tips. Every time you move the platter in the X or Y axis, the tips will also move up to the starting Z position. You may need to move the tips down so that they are close enough to the slide so that you can set the X and Y position relative to the slide.
  - d. Next set the Z-axis. Move the tips down to the surface of the blot pad. Then, moving in 20  $\mu\text{m}$  increments, you will need to lower the tips until the E-clips at the bottom of the springs just move up.
  - e. *Set and save the alignment.*
  
10. Print position:
  - a. You are now setting a temporary print position that you will use later to calibrate the slides. This is not the final print position.
  - b. Go to the print station:
  - c. Using the buttons in the Motion Control section of the page, align the front edge of the tips with the front edge of the slide (i.e. the border between the first slide and the second slide) by using the “Down” button to move the tips. Next set the Z-axis. Move the tips down to the surface of the blot pad. Then, moving in 20  $\mu\text{m}$  increments, you will need to lower the tips until the E-clips at the bottom of the springs just move up.

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d. *Set and save the alignment*

Align the final print position:

1. Under the align tab, go to the Print Station.
2. Using the buttons in the Motion Control section of the page, set the X and Y axes for the tips by moving the platter left, right, forward, and back. You are setting the final print position for the tips. Set the X and Y axis first for the blot pad by moving either left, right, backwards, or forwards. You wish the tips to be left of the center position on the slide. If you are printing with 16 tips, you can do a tandem print. In this case, you will want to justify the print position to one side of the slide so that you can fit in a second tandem print.
3. Next set the Z-axis. Move the tips down to the surface of the slide. Then, moving in 20  $\mu\text{m}$  increments, you will need to lower the tips until the E-clips at the bottom of the springs just move up. Not all the tips may hit the slide at the same Z position. Keep moving the tips down until all the visible E-clips have moved up.
4. *Set and save the alignment.*
  - a. Remember that if you justify the tips so that your array is not perfectly centered, it allows you to have ample space on one side of the slide to place your label. Also, if something goes wrong with the printing of your first plate, you will have space to start the print again on the same set of slides.

Align the plate position:

1. Make sure you have a test plate in the 384-well plate position. This plate should be empty.
2. Under the align tab, go to the Plate position.
3. Generally, the X and Y positions will not need to be changed.
4. Using the buttons in the Motion Control section of the page, lower the tips down toward the wells. If the tips are off-center with the wells, you can adjust the X and Y axes.
5. Continue to move the tips down until the E-clips just push up. This is especially important if you have a small volume in each well.
6. *Set and save the alignment.*
7. In the Calibrate Plate section of the Align tab page, hit the move to last load position. Adjust the tips in the X, Y, and Z axes if necessary. It should usually not be necessary to adjust the axes.

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8. Hit the Calculate Correction button. The arrayer will use the information about the relative position of the first and last wells of the plate to determine the position of the tips in the rest of the plate.
9. *Set and save the alignment.*

### TEST PRINT

1. Now it's time to do a test print on the blot pad. Make sure there is a slide in the blot pad position.
  - a. Goals of the test print: To examine the print pattern from each tip and make sure it looks good and to make sure everything is running smoothly.
2. Calculation:
  - a. Figure out how many rows and columns you are going to have in your actual print. Part of this calculation is relevant for the test print. The rest of it is relevant to your real print run.
    - i. There are 4500 microns between tips.
    - ii. To calculate how many spots across you can fit in this space, reason that the spots in a given row will take up 100  $\mu\text{m}$  of space. This gives you 4400  $\mu\text{m}$  between each tip to play with. You have to make sure that both your number of rows down and your number of columns across will fit in this 4400  $\mu\text{m}$  space—otherwise spots from one tip will run into spots from the next tip.
    - iii. Here is a sample calculation for 32 tips:
    - iv. If you are printing from 27 plates, you have 384 spots/plate x 27 plates = 10368 spots/32 tips = 324 spots printed per tip.
    - v. If you were to print at 21 spots across for each tip (21 columns), then you need  $324/21 = 16$  rows down to print all the spots.
    - vi. How much distance do you want between spots?
      1.  $4400/21 = 209.5 =$  maximum distance between spots.
      2. You don't need to be at the maximum distance to fit only 21 columns and 16 rows. (The minimum distance is determined by variables such as how blunt the tips are—blunt tips print bigger spots that take up more room.) For a standard set of tips, 200  $\mu\text{m}$  spacing would be very generous. You can probably go as low as 175  $\mu\text{m}$  if the tips are not too blunt. Remember, closely spaced spots are currently difficult to grid once your arrays are hybridized.  
*Note: row and column refer to the orientation of the spots when you are looking at the slide along its long axis.*

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3. Go to the Test Print tab.
4. Place a plate with test DNA in the plate position.
5. 384-well plate with 5µl test DNA at 100 ng/µl in 3X SSC for test print
6. DNA in either the first 16 wells (4 rows by 4 columns) or first 32 wells (4 rows by 8 columns) depending on how many tips you are going to print with.
  - a. Note that these first 16 or 32 wells are the first “load.” The next 16 or 32 wells (to the right of the first load) are the second load, and so on.
7. Enter the appropriate numbers in each box:
8. Slide and Plate Setup
  - a. Load Number: 1 (This tells the arrayer which load position to use in the 384 well plate.)
  - b. Slide Number: 1 (Tells arrayer which slide to start with. Irrelevant if you are printing on the blot pad.)
  - c. Number of Slides: 1 (Tells arrayer how many slides you are printing.)
  - d. Check the “Print on blot pad” box so you can do your test print on the blot pad.
9. Print Setup
  - a. Total number of taps:
  - b. I want the test print to simulate the number of rows and columns I will see in my real print. In the test print, the tips will load from the plate once and then tap on the blot pad a number of times. If I am printing 21 columns and 16 rows (from the calculation above), I multiply 21 x 16 to give 336. I enter this number in the total number of taps box.
  - c. Number of loads: 1 (If you enter “1”, test print will be from the first load only.)
  - d. Sector width: 21 (= number of columns)
  - e. Spacing: 200 (Depends on spacing you have calculated from above. Ignore the suggested spacing because the software does not calculate whether the number of rows will fit in the available 4400 µm.)
10. Wash and Load Settings
  - a. Wash cycles: 2
  - b. Wash times: 3500 msec (determines how long tips are in sonicator)
  - c. Dry time: 8000 msec (determines how long tips are in vacuum)

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- d. Load time: 1000 msec (determines how long tips are in 384 well plate)
- e. Check the Swish Wash box (provides more vigorous washing in the sonicator). Bounce dry is unnecessary.

#### 11. Tip Setup

- a. Choose the correct number of tips (e.g. usually 16 or 32).

12. Hit the big green START button. If anything goes wrong, you can hit the red STOP button.

13. After the test print is complete, you can examine the blot pad by eye and under the microscope. Remember that this test print will be blotchier than your actual print because the tips were not blotted. You may see that the spots at the left side of each block run together (due to excess liquid) whereas the spots printed afterwards are distinct. The real print will look fine.

14. Put a fresh slide on the blot pad.

### **PRINT RUN**

1. Now you are ready to print!
2. Place your first 384-well plate (spun down at 500-600 rpm for 10 minutes) in the plate position.
3. Go to the Print Run tab.
4. Enter the appropriate values. I have put sample values below for plate number and tips, etc., but obviously you need to enter the appropriate values for your print plates and setup.
5. Print Setup
  - a. Total number of plates: 27 (Equals the total # of 384-well plates.)
  - b. Tips: 32
  - c. Spots per load: 1 (Increase the number to print replicates, if so desired)
  - d. If you are printing the entire platter's worth of slides, check the Entire Platter box.
  - e. If you are printing a tandem array (two arrays per slide), check the Tandem Print box. *Note that you cannot print a tandem array with 32 tips since that takes you off the edge of the slide!*
6. Station Settings
  - a. Wash Cycles: 2
  - b. Wash time: 3000 msec
  - c. Dry time: 8500 msec
  - d. Load time: 500 msec
  - e. Check the swish wash box. Bounce dry is not necessary.

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## 7. Start Settings

- a. Start from Plate: 1 (Designates which 384-well plate you are starting with.)
- b. Start from Load: 1 (Designates which position in the plate you are starting with.)
- c. Blot pad Taps: 21 (in our example with 21 columns per tip)
- d. This number determines the configuration used by the tips to blot the DNA on the blot pad for each load. Blotting is crucial to getting nice-looking spots.
- e. This is a very important number to enter correctly.
- f. If you are printing with 32 tips, you want your blot pad taps  $\leq$  sector width where the sector width is the number of columns. If the blot pad taps are set at  $>$  the sector width, the arrayer will try to do a tandem blot (next to the first blotting area on the blot pad). With 32 tips, you cannot fit a tandem blot on one slide!
- g. If you are printing with 16 tips and you want to do a tandem blot, you can set the blot pad taps at 2 x sector width.
- h. Tandem Print Offset: 19500
- i. This number determines the distance between the two tandem arrays and blots (only possible if printing with 16 tips). The units are in microns. A typical 16-pin array is 18000 microns across.
- j. Check the "Use Blot Pad" box. This will allow you to blot on the blot pad.
- k. Check the "Use Re-Fill" box only if your printing tips are not capable of carrying enough liquid to print across the entire platter. Checking this box will force the arrayer to go back to the printing plate for another dip into the DNA in the middle of each platter.

## 8. Slide Setup

- a. Begin on slide: 1 (Designates on which slide the actual print will begin after blotting.)
- b. End on slide: 225 if using entire platter. You can print as few slides as you want.
- c. Sector width: 21 in our example. Indicates number of columns printed by each tip.
- d. Spacing: 200 in our example. (Depends on spacing you have calculated from above. Ignore the suggested spacing because the software does not calculate whether the number of rows will fit in the available 4400  $\mu\text{m}$ .)

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9. Plate control

- a. Type in a designation for your Plate I.D.: e.g. “Hc8 Plate 1” (*Histoplasma capsulatum* Print 8, 1<sup>st</sup> plate).
- b. Hit the Record Plate List button to have the computer record your plate I.D.’s.

10. Control Panel

- a. Check over the numbers and if they are okay, hit the big green START button. You will get a window reminding you to change the blot pad if you have not already. At any time, if you have a problem you can hit the STOP button. For emergency stops, however, hit the emergency stop button (physically connected to the arrayer, not via the computer).
- b. After each plate has printed, take a look at the spots through a loop (flashlight helps) to make sure each tip is printing and to make sure there are no dust particles or hairs clogging the tips.
- c. For each plate you will spin it down (plates can be spun down two at time—e.g. spin plates 1 and 2 together before printing plate 1. Then spin plates 3 and 4 before printing plate 3, etc.), change the blot pad, enter the new plate I.D., and hit START.
- d. It is helpful to fill the humidifier and turn it on before printing.

**FINISHING UP**

1. Tips: Change the liquid in the sonicator to Millipore water and sonicate for approximately 5 minutes. Change the liquid in the sonicator to 95% EtOH and sonicate the tips for a few minutes. Dry the tips in the dry station. The rationale for this step is to prevent rust formation on the tips.
2. Make labels for the slides and put them on one column at a time. After laying down on the labels, cut the labels with a razor blade, turn the vacuum off, and remove the slides one at a time, placing them in a clean slide box in numerical order.
3. Log out of the arrayer computer.
4. Unplug the arrayer.

Adapted from:

“Using ArrayMaker 2.1 with the Linear Servo Arrayer”  
Anita Sil, June 2001