

## Instructions for Doing a Prinrun of 70-mer Oligos in 3X SSC on our Genetix QArray2 Arrayer

### Quick checklist for starting a run:

- fill and start external humidifier
- turn big red power knob on right side of machine
- press the red reset button on the front
- turn on the computer
- start the software
- check the water level in the nebulizer (see below), and turn on
- wipe down inside with 70% EtOH, and spray with airgun
- fill the water and ethanol bottles, and pull them forwards against the front bar
- you MAY need to refill these during the run, check every few hours!
- make sure the waste bottles are empty
- you MAY need to empty these during the run, check every few hours!
- check for pinched tubing
- sonicate & dry pins (see below)
- load pins
- load one test slide, and fill remainder of column
- vacuum on
- print test (1 slide, 2 fields)
- check test slide, if OK, load blotting slide and print slides
- spin down print plates and load into right-hand stacker (recall A1 goes front-right!)
- load correct protocol in program, confirm parameters are correct, check data tracking, and start

### Quick checklist for ending a run:

- remove plates, re-seal and freeze or start drying in speed-vac
- turn on light, and use head icon to remove the pins, and start them washing
- turn vacuum back on, affix labels to slides, then turn off vacuum, remove slides and place on clean surface to cut label strips
- turn off the software
- turn off the computer
- turn off the machine
- turn off the external humidifier
- pull the arraying head into the middle of the bed, close door.

The Physical Set-UP:

### The pins:

Officially, the 150um tips produce a ~190um spot, use when spotting densities reach 20,000-30,000

The 75um tips produce a 90um spot

Both diameters are “regular volume” \*unless\* otherwise specified – both come in low volume options. **Regular volume is approx 250nl per inking**, low volume is 100-150nl.

In the pin boxes, Cheryl doesn't keep the rubber stoppers on the bottom of the pins, she just pushes them in gently with the top parts flush with the foam so that the pin tips are well clear of the foam. She also numbers the pins in the foam and keeps them in the same order, for tracking purposes

To wash the pins prior to loading in the machine: Place them in the specially-designed tip-washing manifold/stand. Place the stand into a beaker with an approx. 2% solution of aQu clean, or dilute detergent. Place beaker in sonicating water bath. Sonicate 10-15 minutes. She says she RE-USES the 2% aQu solution several times. She also bought just the smallest sonicating water bath that VWR sells and uses that.

Then rinse in Milli-Q H<sub>2</sub>O, sonicating, for 10-15 minutes.

Then either air-dry, or dunk in EtOH.

\*If the pin is persistently clogged, she heats up the 2% soap in the microwave and lets the pins sit sonicating in the warm soapy water.

You NEVER should need to clean the Head in the microarrayer, which actually holds the pins. It's made inside of ball bearings so don't \*ever\* take it apart! Maybe, if the pins aren't sliding in smoothly, use compressed air, gently.

To load pins: Clicking the head icon in the software will bring the head to the front left of the bed for loading. The top of the pins are not radially symmetric, they are rounded and then have one straight edge, this edge should sit flush with the metal bars on the top of the head.

The plates:

Genetix X7022, which has covers and is V-bottom. She recommends no lower than 4ul in them, but has gone as low as 2ul.

Loading plates: Recall they go in REVERSE ORDER (from one perspective), with the first plate being on the BOTTOM of the “in” stack. Also, they go BACKWARDS, with the A1 well going in the bottom front right!! Plates go into the right-hand stacker. Twist the knob to right to lock stacks into place.

The stackers are interchangeable.

Evaporation from stackers. Usually not a problem, but one customer put a plastic bag over the stackers and shoved a humidifier tube up inside, but that was for a 70-plate 30,000-spot run.

The machine:

The tip washing manifold in the arrayer is a flow-through design, first H<sub>2</sub>O, then EtOH. Filled from containers underneath, and each into their own waste container. We filled with Milli-Q water and **80% EtOH**. The smallest container underneath is the equalization bottle – you should *\*never\** see liquid in there, if you do call Tech Support.

Fill the humidifier, from the capped opening on the front right corner of the platter, inside the arrayer hood. Fill with DI-H<sub>2</sub>O or MilliQ. It takes about two liters to fill up, and use a funnel to fill it, and fill it until the level reaches the *bottom* of the plastic aperture through which you're filling. There's a digital display on the front right of the machine that controls the humidity, and holds it at +/- 2.0%. For a run in the winter set to 55-65%. The ambient can get down to 8% in the winter! If the humidity is low, the spots can bleb together, and if it's really low, the spotting solution can dry in the pins and then those pins won't print those spots. Use 1-2 external humidifiers in the room as well, with door closed.

**\*\*Talk to facilities and get the fan turned down in that room during the winter.**

On the START page of the software, clicking the  "Reset Outputs After Run" will stop the humidifier after the run is completed, which is good so that it doesn't run dry – especially a problem in the winter – **if it runs dry the motor on the pump can burn out.**

#### Loading slides:

You have to fill up an entire column of the bed with slides to get a vacuum seal for that column – you don't have to print on them all though, you can just use junk slides to finish filling a column in need be. You can control air flow to each column separately.

Always front-right justify the slides (except for the blotting slide(s) see below), e.g. using forceps, and then turn on the vacuum (icon at top bar of program).

The knobs at the front of each column control the vacuum for that column. When the vacuum didn't come on, Cheryl tried bleeding the line at the thingie on the right side of the machine, lower half, where there's a pressure gauge – she bled the line here (by pushing something in?) until the compressor came on. That didn't fix it, but seemed to be her first trouble-shooting step. Dave had to come and fix it later.

#### Cleaning:

before each run she wipes down the inside – walls, everything – with EtOH and kimwipes. She also blows compressed air *\*gently\** down the grooves on the slide-bed, pushing dust etc. towards the back, and then wipes across the back. If wiping the slide ruts themselves, then wipe right-to-left, since the left-hand side of the slides is less important since that (based on our current config) is where the labels will go.

The Software Set-up:  
you can store protocols.

**WELCOME** tab (meaningless)

**DESCRIPTION** tab: lets you give a name and write lab notes for the printrun

**HEAD CONFIGURATION** tab

you can use a total of 48 pins

they have many configs programmed in the pull-down that let you work from either 96-well or 384-well source plates

you can set up novel configs as defined in a config file

we choose e.g. "16 pin Microarraying head" (default is 384-well)

you don't need to tell it what diameter pins, it doesn't care

it shows you the \*correct\* way to load the pins in the head, based on the config you've selected. Follow its instructions!

**SOURCE** tab:

**Plate Holder:** Stacker Source Plate Holder use stacker

**Plate type:** 7022

**Total Plates:** 1, etc. (If >70 it pauses to let you refill)

**Source order by:**

columns (means the head dips into the source plate proceeding from A1 → P1, so A1, E1...)

rows (means the head dips into the source plate proceeding from A1 → A24, so A1, A5, A9)

**SLIDE DESIGN** tab:

**slide:** 3" x 1" (16pins/ 4fields)

you match this to the actual number of pins being used, and the "field" is the # of times that that printhead could physically fit onto that size slide; e.g., using the 48-pin head config., it could only fit on the slide once.

with a 16-pin config you can actually print at a higher density

**field layout:** can organize replicate fields, etc.

\*\* double-click on any of the spots to open a new screen, allowing you to edit more parameters of slide design:

**spot view:**

layout  
90 for 75um tips

actual (click actual)  
there's good spacing)

**estimated spot size:** 200 for 150um tips,

(she nudges this a little higher so

*Pattern dimensions* tab:

calculate with calculator,

384 pins \* 6 replicates / 16 pins = 144 spots, = a 12x12 matrix given the entered matrix, it automatically gives you the max. pitch for the row and columns; you can decrease the pitch to bring the spots closer if desired.

e.g. if the pitch =300, and the spot size = 200, then there's 100um between spots, which is fine

*Fill tools* tab:

Replicate type:

- Adjacent
- Sector
- Cyclic
- Random

replicate count = 6

where to start (diagrams):

(choose upper left prob)

Statistically, random really is the best

If using marker spots from a separate plate, click on markers, then hit remove all, then highlight the desired spots and type M, these then become red, designated as marker spots, and then when you fill in the spots it will assign the pattern around these marker spots, leaving room for them separately.

**SLIDE LAYOUT** tab:

# slides: \_\_\_\_\_

# blots: \_\_\_\_\_ (uses a sample slide location, and can blot multiply on a single "blotter" which in this case is just a discard slide. You can set the blotting pitch farther apart and overprint the same blotting spots

change blotters      blotters to use:  (this lets you change the number of blotters, it will pause the run for you to change blotters)

Blot overprint method:  same sample

no overprint

**slide order:** (diagrammatic)

can drag slide layout around is you want to . The layout can become important if you want to do humidity testing, arrange the spots to be printed around the edges to check the corners of beds.

**PRINT** tab:

**Max stamps per inking:** (approx. 200-300 in SSC)

**# stamps per spot:** = 1.

You can change this if you're doing e.g. protein microarraying. - can overstamp, or stamp in an offset circle centered around primary spot, etc.

**Stamp time:** 0

She does 0 but you can increase it 20-30 (all in milliseconds) if you're seeing non-uniform features, or have a more viscous fluid.

**Inking time:** = 2000 (this is appropriate for regular volume pins, you can decrease the number for lower volume pins.)

**Print depth adjustment:** she adjusts this so the pins *just barely* hit the slide - \*very\* tough to see, but makes a slight tapping noise during a run. The manual says to do this through Datum Points, and then use the print depth adjustment to just vary if there's a known alternate slide type you use, etc.

**TOUCH OFF** tab:

won't need this for SSC. It's used for more viscous buffers, to wick off the excess solution from the \*outside\* of the pins, by touching the surface of the liquid in the plate.

**STERILIZE** tab:

**Water** - 4 each at 1000ms wash time, 0 dry time, 500 wait time

**EtOH** - 1 - at 2500ms wash time, 7000 dry time, 500 wait time

You can bring the EtOH up to 3500ms and also up the water times if you're starting to worry about cross-contam, but these are the params. she likes

(there's two diff washing philosophies, one as above, the other with longer washes, eg 1 water wash at 5000ms, same EtOH.)

**DATA TRACKING** tab:

First, on the desktop, change the comma-delimited .csv file from Excel to .txt, then open the separate Data Tracking Program. The **username: dtuser,**  
**password: dtuserpw**

admin username: sa, pword: genetixsapw

Tools → Import Process File, Files of type Qsoft... etc.

Close Data Tracking Program, and back in Qsoft under Data Tracking tab:

File name: (for gal file) File format: gal4.1 works

Open Groups → OligoPrototypeArray (whatever group you want, you've loaded)  
highlight the plates wanted, and click Add.

**the TOP plate in the software = the first plate out of stacker = the BOTTOM plate in stacker**

**Always** backup the Database before reading new (e.g. rubbish) data in!

**BARCODING** tab: N/A

**START** tab:

Normal  
 Test plate (inks ONLY from the first 16-pin quadrant, just to print a few slides)

Print Test (inks and then prints onto just the front left slide, without re-inking. Let's you see if all the pins are clean, see the numbers of times you can print from a given inking, and the # of times you might need to blot, etc.)

○ Data tracking export only (just generates the gal file for a given data tracking import)

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### Other Important thing on the Software:

If you ever want to just get the gal file made for a given run, or made alone, you find it through: My Computer → C → Program Files → Genetix → QArray → logs → gal

**Configuration Icon** on Toolbar → Defined Objects → 3x1 (16pins/4 fields) (double click)

scroll down:

offset field 1x	this controls how far from the skinny “top” edge the field starts. She changed it from 1000 to 1500, for the purposes of the print test.
offset field 1y	this controls how far from the long “side” edge the field starts. She changed it from 3900 to 4100, eventually to 4300, to get the field more centered on the slide.

(offset field x, y just control the distance between fields)

**Inking depth:** This is how deep into a plate you go. She has it set for their plates, and set for 4-10ul volume. If we go above 10, to 15 or so, it’s worth changing the defined object inking depth so we waste less oligo on the outside of the pin. Currently it’s set for about 200um above the bottom of the plate. You can change the inking depth for a new defined object, and save, or you can change it within a given run only.

**Print depth:** This needs to be done for particular slides. Go to **Configure**

**Datum Points** in the toolbar, and set the heights for the slides in **each column** empirically. To adjust the print depth, negative numbers = up higher.

She saved a protocol called “Print Test” which prints two fields, and prints one slide only. She changed the field layout for it.

**Hammer and Screwdriver** icon at top = Diagnostic button, lets you move things around, etc. You can just drag the head where you want it, make a wash happen, etc.

Slide layout for first run was:

sample slides: 14

# blots: 5

blot pitch: 300

# overprints: 1

BUT check off No overprint, below.

Water = 2000 wash time, 4 replicates, 0 dry time, wait 500  
EtOH = 4000 wash time, once, 10000 dry time, wait 500

save routine icon at top, looks like save as (double disk)

**To back-up datum points and protocols:**

Robot config → Database → Back-up (on C drive, → Qsoft back-up)

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Maintenance and Troubleshooting:

If the arrayer is not going to be used for  $\geq$  week or so, she says to empty the bottles and leave everything dry, so that nothing grows. She also says you may want/need to wash the tubing once in a while: says some customers have run a mild fungicide through and then flushed with water – this is under I/O diagnostic section, Microarraying Wash, click on Port 1 (=wash), Switch, and Vacuum, and then the wash runs continuously through both lines until you stop it.

To test if the pins are drying post-wash, and if they're washing enough or if there's carryover, cut a small piece of e.g. nylon and tape it on a slide. Then print (print test) on it and see if it's wicking off moisture, and if it's colored (use a dilute blue food coloring in your spotting buffer to see if there's carryover). Use an empty source plate if you're just trying to see if the dry time is sufficient – it needs a plate in there to go through the printing motions.

Blotting:

**5-10 blots OK for 3xSSC**

she set for 5, all on 1 slide (program figures how many slides automatically) the blot slide doesn't stick to field size but starts at far left hand side – for this reason, this slide should NOT be bottom-right justified but be a little more centered!

Troubleshooting: back pins not printing, tho freshly cleaned. Tried washing tray again, still no. Switched pins front-to-back, and back half still not printing – so pins are OK. Checked balance of head and bed with a mini-balance. Turns out head itself is not level.

Cheryl's free advice on plate-sealers (any gunk left on top may prevent top from coming off plate cleanly, and screw up the machine):

- small hand-held heat sealers units (for sealing foil) from Marsh (now bought by Fisher)
- or HyperTask, small local company, have foil plate-sealers she's liked and not had probs with.

U.K. Tech Support (US toll free #):

1-877-436-3275

favorite dude: Tim Roberts, ext. 4796, **expert** on QArray2

[tim.roberts@genetix.com](mailto:tim.roberts@genetix.com)

U.S. Tech Support:

1-877-436-3849

spoke previously to Joe Jordan there.

Another local tech/rep person is Ken Adams, 617-549-6050