PRINTING MICROARRAYS
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A microarray printing run of more than a hundred microarrays with many thousands of elements each is always a fun and rewarding activity, but without the right attitude, it can become a chore that you dread. A typical print run takes about a day of non-stop printing depending on the number of printing tips and elements to be printed. Someone knowledgeable needs to be near the machine constantly to make sure everything is going smoothly. Before you begin the run, you need the following:

1. DNA to be printed, aliquoted in printing plates: It s best to have this in 384-well plates. The best printing plates are Genetix 384-well plates (see DNA prep and precipitation section for details). For printing, the DNA needs to be in about 4 ul of 3X SSC. Obviously, you should know how the DNA was aliquoted. The best method of storing print plates is to dry them down. If DNA in water was dried down, you need to add 3X SSC and if it was dried down in 3X SSC, you will be adding water. This might seem like an obvious point, but when there s a production pipeline going with many people doing different steps, it s easy to lose track! If dried down DNA needs to be resuspended, add the water or SSC a few hours before beginning the print run. After it is resuspended, keep the plate tightly sealed to minimize evaporative losses.
2. Familiarity with the ArrayMaker software: You should know how to do alignments and what the different parameters in the test print and print window mean.
3. Glass slides: You need plenty (~150) of coated glass slides from batches that have been tested with test prints and hybridizations. See poly-L-lysine coating and test print sections.
4. Printing tips: You should have many spares in addition to the 16 or 32 that you are planning to print with. Obviously, these should all be in good condition.
5. Other materials: 3X SSC, double distilled water, test print DNA (100 ng/ul sheared salmon sperm DNA in 3X SSC), _ wide labelling tape, magnifying glass. Also useful is a dissecting microscope to inspect printing tips, and a camera attached to a monitor that will let you observe the arrays being printed.

Details for some of the following steps are covered in the software section:

Turn on the main power and computer, run ArrayMaker and home the stages: See the Mguide and software section for details.

Align all positions: It is necessary to make sure that all the positions are correctly aligned at the start of every print run. Although none of the positions might have changed, there s always the possibility that someone had last printed with a weird configuration of tips in a 32-tip holder and aligned that to the dry station and print plate, or used a slightly different print plate. If you are not absolutely sure that all of the positions are perfect, align everything. It s a good idea to make sure anyway. Clicking on the Align button on the main window takes you to the align window. In the Align window, clicking on the Reset Z button resets all the vertical positions to the ready position, which is safely above everything. You can then click on the buttons for the various positions and carry out alignments. See the software section for details on alignment. The tips in the rinse station (sonicator) should be aligned with only the slot immersed in the

liquid. Remember to set and save before exiting this window. If you are printing with less than 32-tips (and using a 32-tip holder and dry station), cover the unused holes in the dry station with a piece of tape.

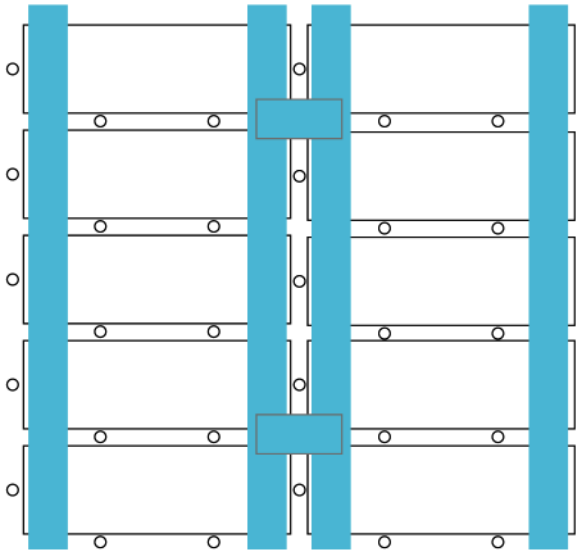
Printing tips: The quality of microarrays you print depends a lot on the printing tips (the DNA in the printing plates is another major factor). The current generation printing tips pick up ~ 0.5 µl of solution and deposit a few nl with every tap on the glass surface. A good printing tip should be able to print 100-200 spots before it needs to be loaded again. A good set of 16 or 32 tips should produce uniformly sized, distinct, well spaced spots across the entire array. Before beginning, inspect the tips carefully for obvious imperfections under a dissecting microscope. You can observe how well a tip picks up liquid by just touching the tip to the surface of a 3X SSC solution. With a good tip, you should see the solution wicking up in the slot in the center of the tip. Make sure the outside is free of any salt or corrosive build-up, especially at the retaining clip or inside the slot. Once the tips are in the holder and the top is on, use forceps or needle nose pliers to make sure all the tips slide up smoothly and that the spring pushes them back down when released. You might need to clean the tip-holder holes of any build up. Gentle sanding of the outside of the tip (where it rubs against the tip-holder) might also be called for occasionally. Use a fine grade sandpaper for this. If the shaft of the printing tip is slightly bent, it affects the smooth up and down movement and also alters the register of the different sectors of the test print array. If this is the case, replace the tip to get a set that is well matched and evenly spaced. Tips should be cleaned after every print run by sonicating in warm water and rinsing with ethanol. Handle tips with great care. Their points should never hit anything hard except the slides while printing. Putting them in 200 ul pipette tips is a convenient way to store them.

Test prints: It is important to do test prints with the tips that will be used for the print run, immediately before beginning the run. Tips that have performed well in the past may gradually deteriorate with improper cleaning and other abuse. Load a sample print plate that is identical to the real print plates with 4-5 ul test print DNA in the top left 16 or 32 wells (beginning at A1) and use this for test printing onto slides from the same batch that will be printed on. The test print parameters (spacing and number of spots across) should match what the real array is going to have. This can be calculated depending on how many elements total you need to print (see software section for details). Tape down a glass slide in position 1 on the platter (closest to the plate holder) for test printing. If printing with 16 or 32 tips, it s very convenient to simply print 24 spots across in each quad. This way, one row of spots on the array corresponds to 1 or 2 384-well plates and makes it easier to localize imperfections to specific print plates. However, this will limit the total number of spots you can print. You can set the tips to load multiple times, which allows you to print thousands of spots on the same test-print slide. You can use the same wash and dry parameters as for the actual run or shorten them slightly.

The liquid in the sonicator for cleaning the tips can be either water or SSC (0.5 X or 1 X). Using SSC leads to somewhat better priming of the tips and they can then print more spots before running dry, but depending on the DNA in the print plates, it could also lead to spots that tend to spread. You should do a test print run using a spare sample plate of the actual DNA that will be printed to see how different wash solutions affect the spot quality. Do test prints from different load positions on the 384-well plate to check the plate alignment, and on different slide positions on the platter to check the slide height adjustment on the platter. Use of the plate positioner is strongly recommended.

Printing the real microarray: Once you have a set of printing tips working perfectly on the test print, start the array print run immediately. Set the print parameters in the Print Array window. To prevent carryover on the tips, it is necessary to do at least 3 cycles of cleaning, with 5-6 sec sonication and 8 sec dry times. A few other things to note: You of course know how many printing plates total you have, but set the spacing so that you have room for more. Printing a plate of controls is useful. The control plate can include λ DNA, salmon sperm DNA, poly dA, Cot1 or other low complexity repeat containing DNA, genomic DNA, 3X SSC, dilutions of fluorescently labelled cDNAs, etc as positive and negative controls. You should also try to leave room for re-printing a few plates should there be a problem with some of them.

When all the parameters are properly set, you are ready to begin. Spray off the dust and glass shards on the platter and wipe the surface with a moist kimwipe. Place your slides (wearing powder-free gloves) on the platter, making sure they are aligned straight and lying flat. If printing less than 137 slides (a no-no!) you should fill the platter in the order they will get printed. Use the _ tape to tape down all slides along the edge. Don t put the tape on top of the dowel pins on the platter. Use pieces of tape to tape across columns. Taping securely is important as you don t want your slides moving around during the print run.



Taping diagram

Spin down every print plate just before putting it into the plate holder. You want to ensure there s no liquid on the sides of the wells. Be careful when taking off the seal. Make sure the plate is placed in the proper orientation in the holder. Again, the plate positioner helps. You will need a spreadsheet listing the contents of each well in the printing plates. The order and orientation on the slide platter determines where each DNA is going to end up on the array, and only you can keep track of this. It s very useful to keep a log at the arrayer where the orientation and plate number of every plate can be recorded for each print run. When everything is ready, drop the first plate in and click the start button!

Monitoring the run: Close monitoring is essential throughout the run. A live CCD camera with a macro lens or attached to a microscope, and hooked to a monitor, makes things a lot easier. You basically need to ensure that all the tips are printing well on all the slides. It s sometimes necessary to use the reload feature if some tips are unable to print till the end of the platter. Often, the first few slides have larger spots; this is normal. If they begin running in to each other a lot in the beginning of the platter, you might want to turn on the blot pad feature. Check the level of liquid in the sonicator every few plates. In case of printing problems, you can stop the run after making note of the plate and load number. Then remove the first slide, put in a fresh one, and do a few test prints (with test print DNA) and diagnose the problem. Is a single tip printing large spots? The tip could be blunted or the slot could be misshapen or something could be clogging the tip. Look under the scope and try cleaning it gently. Has a tip stopped printing? Make sure it continues to slide smoothly and that it s touching the slide on the test print. Are all the tips misbehaving? It could be due to salt or corrosive build up on the tips. You can move the stage to the right to immerse the tips briefly in warm water, and then inspect them. After this make sure the tip holder bottom holes are dry and free of crud. Are some isolated slides on the platter missing spots? Maybe the Z-height has not been corrected for those positions.

Remember, many seemingly disastrous runs are salvageable. If a bad tip problem crops up in the middle of a plate, and you lose a lot of spots on the array, you can print that plate again at the end of the run if you ve managed to fix the problem. It s usually not worth filling in holes on the array as you have to keep careful track of which slides are missing which spots and so on. It s far easier to reprint an entire plate at the end, which is why it s good to leave room for a few extra plates. When you stop and start up again, make sure that the printing starts exactly where you stopped by entering in the appropriate values for the current plate, slide and load in the Print window. Stopping a print run to catch some sleep at night is unseemly. Tag team instead and carry on the through the night. You ll get the arrays faster that way.

Ending the run: Once all plates are printed, you can begin another run immediately with the same plates. If you are not up to that (or if you don t have enough slides), the plates are best dried down in a speedvac and sealed for the next run. The first thing though, is to remove the slides and clean the tips. Carefully peel off the tape without touching the surface of the arrays. It helps to have an extra person to hold down one edge of the slides while the other peels tape from the other end. Alternatively you can use the edge of a long strip of metal like a ruler to hold down the slides when the tape is pulled off. Some people like to label the slides at this point, especially if using stick on labels. Put the tips in fresh warm water in the sonicator and clean for a few minutes. Inspect tips for any corrosive or salt crud inside the slot. Cleaning with ethanol may also help. Store tips dry.