

# Printing microarrays with a BioRobotics MicroGrid II 600 or 610 spotter

## Overview

The following procedure is designed to minimize human error and maximize the performance of the MicroGrid II arrayer and the MicroSpot pins. This procedure assumes the 'Main Pump Unit' has been set to use both re-circulating wash tanks and the main wash station. If you are in any doubt about using the MicroGrid II ask the machines primary operator for assistance. FlyChip accepts no responsibility whatsoever for any damage incurred by following these instructions.

## Equipment and reagents

- MicroGrid II 610 TAS spotter and print-head
- Substrate slides
- 70% Ethanol
- Ultrasonicator (Ultrawave; Cat. No. U100H)
- MilliQ water
- Surface-cleanse/930 (International Products Corporation; Cat. No. S-2001-12)
- Adhesive PCR Film (Abgene; Cat. No. AB-0558)
- Dyson DC05 Vacuum Cleaner

## Procedure

### Weekly and daily maintenance:

1. **Clean the Lab:** Use a Dyson vacuum cleaner to remove dust from the local work area and then wipe with a damp cloth. It is important to keep the working area and the laboratory in a generally clean state. This should be done every week.
2. **Cleaning the MicroGrid II 're-circulating wash tanks':** Turn the MicroGrid II on and start the 'TAS Application'. The MicroGrid II will zero itself. From within the 'Housekeeping Section' select 'Prime The Re-Circulating Baths' to fill the re-circulating wash tanks with Milton Sterilizing Solution (1 to 2 hours). From within the 'Housekeeping Section' select 'Fill the main wash station' (MWS) to fill with MWS 6-litre storage tank with Milton. Then rise each three times for 10 minutes using MilliQ water by following the same steps. Finally, wipe all accessible portions of the re-circulating wash tank and MWS pathway using 70% Ethanol. This should be done once a week.
3. **Cleaning the MicroGrid II interior:** Vacuum the arrayer using the Dyson vacuum cleaner to remove particulates from the arrayer. Wipe the interior of the arrayer using 70% Ethanol and then re-vacuum. This should be done before every print-run.

### Pin care and maintenance:

4. **Cleaning the microspot pins:** These pins are best cleaned using ultrasonication. The pins should be sonicated in either 0.1 x SSC (sodium phosphate spotting buffers) or 2% Surface-cleanse/930 (all other spotting buffers) at room temperature (standard clean) or 65 °C (heavy clean). These sonications should last for between 5 minutes and 2 hours, depending on the level of cleaning required for the pins to function correctly.
5. **Rinsing the MicroSpot pins after cleaning:** After sonication with cleaning solution the pins need to be rinsed by sonicating 3 times for 2 minutes in MilliQ water. The pins can then be used in a print-run without carry-over from the cleaning solutions.

6. **Pin storage after cleaning:** If the pins will be used immediately they can be stored attached to the arrayer whilst the print-run is set-up. Otherwise they should be dried and stored in the BioRobotics pin-tool holder.

### How to perform a print-run:

7. **Fill the arrayer with wash solution:** Turn the MicroGrid II on and start the 'TAS Application'. The MicroGrid II will zero itself. Using the 'Housekeeping Section' 'Prime The Re-Circulating Baths' option fill the left- and right-hand re-circulating wash tanks with MilliQ water. Fill the main wash tank with MilliQ water using the 'Fill 6-litre reservoir' option.
8. **Fill the 'Humidity Control Unit' with MilliQ water:** Fill the 'Humidity Control Unit' with MilliQ water following the manufacturers instructions. Click on the 'Climate' tab from the 'Run Preferences' section. Check the MicroGrid II is using the correct 'Humidity Control Unit' settings. Start the 'Humidity Control Unit' and set the display to update every 1 second. Periodically check the humidity level whilst the clone-set plates defrost.
9. **Thawing the library:** Remove the library from the -80 °C freezer. Leave to defrost at room temperature in a safe place. Centrifuge all plates at 2000 rpm for 2 minutes in the 'Hettich Rotina 35' microtitre plate centrifuge. This will remove surface moisture.
10. **Loading the arrayer:** Load the 'BioBank' with the library and the 'Slide Trays' with the slides. Make sure the plate seals are removed from the plates, the plates are in the correct orientation and the plates are in the correct order. Make sure the slides have been loaded correctly and the vacuum pump is able to keep them in place.
11. **Loading the grid program:** Open the required print run parameters file from the 'My Gridding Runs' directory. Confirm the correct file has been opened. Confirm the settings are correct. If you are in any doubt ask the primary operator for assistance.
12. **Confirm the correct layout will be printed:** Confirm the correct print-run settings file has been opened by comparing the set-up file of this print run with the standard file for the library to be printed. Do not start the run until the target humidity has been reached, otherwise the spots will be the wrong size and the library will rapidly evaporate.
13. **Start the run:** Start the run and periodically observe the MicroGrid II during the print run to be certain everything is OK. If any problems occur get the MicroGrid II primary operator to take a look. The print-run time will depend on the print-run program being used. Please ask the MicroGrid II primary operator to determine how long the print-run will last for.

### After the print-run has finished:

14. **End of the program:** Follow the 'on-screen' instructions to remove the printed slides, library and pin-tool. Store each in its correct location: pin-tool should be cleaned (above) and then stored (above); slides should be stored in a cool, dry and dust-free cupboard; plates should be sealed and put at -80 °C.
15. **Empty solution from the arrayer:** All solutions should be removed from the arrayer and the arrayer itself should then be cleaned using a cloth soaked in 70% ethanol. The re-circulating wash tanks, baths and pipework, the MWS, and the MWS inlet/outlet pipes, should also be cleaned in this way. This will prevent microbial growth within the arrayer, when used in conjunction with the weekly cleaning schedule (above).
16. **Complete all data tracking forms:** All data tracking forms should be completed in full to ensure a complete record of every slide and print run can be maintained. This will be used to track all slides produced by FlyChip that are either given to external groups, or used by FlyChip for external groups experiments.
17. **What's next:** The slides need to be processed and the print-run needs to be quality controlled before any of the slides that have just been printed can be used. Please refer to the appropriate protocols on this web site.

R. Auburn (17-02-2006).