http://www.flychip.org.uk/printsop2.php

Printing microarrays with a Genetix Qarray2 spotter

Overview

The following procedure is designed to minimize human error and maximize the performance of the Qarray2 arrayer and the aQu pins. This procedure assumes the stacker has a humidity control unit fitted. If you are in any doubt about using the Qarray2 ask the machines primary operator for assistance. FlyChip accepts no responsibility whatsoever for any damage incurred by following these instructions.

Equipment and reagents

- Qarray2 with humidity controlled stacker
- Substrate slides
- 80% Ethanol
- 70% Ethanol
- Ultrasonicator (Ultrawave; Cat. No. U100H)
- Water - distilled and MilliQ
- 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 maintanence:

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 Qarray2 'wash tanks':** Turn the Qarray2 on then press the reset button and start the 'Qarray MicroArray' software. The Qarray2 will zero itself. Fill the water (distilled water) and ethanol (80% ethanol) bottles. From within the 'Robot Diagnostics' select 'Wash Head' and then 'Water' to rinse the wash tanks with water (repeat three times). Then, from 'Robot Diagnostics' select 'Wash Head' and then 'Ethanol' to rinse the wash tanks with ethanol. This should be done once a week.
3. **Cleaning the Qarray2 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 maintanence:

4. **Cleaning the aQu pins:** These pins are best cleaned using ultrasonciation. 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 aQu 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 dry in a pin-tool holder.

How to perform a print-run:

7. **Fill the arrayer with wash solution:** Turn the Qarray2 on then press the reset button and start the 'Qarray MicroArray' software. The Qarray2 will zero itself. Fill the water (distilled water) and ethanol (80% ethanol) bottles. From within the 'Robot Diagnostics' select 'Wash Head' and then 'Water' to rinse the wash tanks with water. Then, from 'Robot Diagnostics' select 'Wash Head' and then 'Ethanol' to rinse the wash tanks with ethanol.
8. **Fill the 'humidifiers' with MilliQ water:** Fill the humidifiers with MilliQ water following the manufacturers instructions. Click on the 'Humidity' button to start the huidity control units. Periodically check the humidity level whilst the library to be printed defrosts.
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. The pins can be left printing water whilst this step is being performed.
10. **Loading the arrayer:** Load the 'Stacker' with the library plates and the 'Slide Bed' with the slides. Make sure the plate seals are removed from the microtitre plates, the microtitre 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 spotting script:** Open the required script file. 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 Qarray2 during the print run to be certain everything is OK. If any problems occur get the Qarray2 primary operator to take a look. The print-run time will depend on the print-run prgram being used. Please ask the Qarray2 primary operator to determine how long the print-run will last for.
14. **Refill the water wash bottles:** The Qarray2 water bottles do not store sufficient water for long print-runs. Ask the Qarray2 primary operator if this will be needed for this print-run and then seek his help when the bottles need to be refilled.

After the print-run has finished:

15. **End of the program:** 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.
16. **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. This will prevent microbial growth within the arrayer, when used in conjunctoion with the weekly cleaning schedule (above).
17. **Compete 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 in experiments for external groups.
18. **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.

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