

Robotic Spotting

Generated on 10/01/2011

Table of Protocols

Robotic spotting of microarrays:	1
<u>FlyChip protocol for adding spotting buffer to 384-well printing plates</u>	2
<u>Library desiccation and rehydration</u>	3
<u>An Introduction to printing spotted microarrays</u>	4
<u>General introduction to optimising the performance of a robotic spotter</u>	8
<u>Printing microarrays with a BioRobotics MicroGrid II 600 or 610 spotter</u>	11
<u>Printing microarrays with a Genetix Qarray2 spotter</u>	13
<u>Full Moon Biosystems (FMB) protocol for processing FMB cDNA slides:</u>	15
<u>Full Moon Biosystems (FMB) protocol for processing FMB PowerMatrix (modified oligo) slides:</u>	16
<u>Full Moon Biosystems QC kit</u>	17
<u>A key to standard microarray spot identity tracking file formats</u>	18

Robotic spotting of microarrays:

Library management

- Adding spotting buffer to the oligonucleotide library ([jump](#))
- Library dessication and rehydration ([jump](#))

Instrument optimisation

- Introduction to printing microarrays ([jump](#))
- Microarray instrument optimisation ([jump](#))

Microarray printing

- Printing microarrays with a BioRobotics MicroGrid II 600 or 610 spotter ([jump](#))
- Printing microarrays with a Genetix Qarray2 spotter ([jump](#))

Microarray processing

- Processing Full Moon Biosystems cDNA slides after spotting ([jump](#))
- Processing Full Moon Biosystems PowerMatrix slides after spotting ([jump](#))

Microarray quality control

- Full Moon Biosystems microarray QC kit ([jump](#))

Microarray spot tracking file formats

- A key to standard microarray spot identity tracking file formats ([jump](#))

FlyChip protocol for adding spotting buffer to 384-well printing plates

Overview

Oligonucleotide libraries are dispatched to us in 384-well microtitre plates. Spotting buffer is added to these plates using a Beckman Coulter Biomek NX^P Liquid Handling Robot (LHR).

Equipment and reagents

- Beckman Coulter Biomek NX^P Liquid Handling Robot (LHR)
- AP96 non-sterile P20 tips (Beckman Coulter; Cat. No. 717254)
- 70% Ethanol
- Spotting buffer
- Hettich Rotina 35 microtitre plate centrifuge
- Adhesive PCR Film (Abgene; Cat. No. AB-0558)
- Horizontal laminar flow work station (Jencons; Cat. No. 566-031)

Procedure

1. Remove the microtitre plates from the -80 °C freezer and leave to defrost
2. Once defrosted, centrifuge all plates at 2000 rpm for 2 minutes
3. Clean the exterior and interior of the LHR using the Dyson vacuum cleaner and wipe with 70% Ethanol
4. Open the program "Hydrate_LIBRARY_YesWash" and home all instrument drives
5. Fill the in-flow wash tank with distilled water and prime the wash station, *e.g.*, for 3-5 min.
6. Load the instrument, as directed by the "Hydrate_LIBRARY_YesWash" program, *i.e.*, fresh box of AP96 P20 tips, reservoir filled with spotting buffer and the plate to be hydrated
 - ◆ Adhesive film should be removed just before the plate is loaded into the LHR
 - ◆ All plates should be loaded in the LHR with well A1 in the top-left corner
7. Start the program and watch to make certain the LHR is working correctly
8. Repeat steps 6 to 7 until all plates have been rehydrated: as each plate is finished, remove from LHR and affix an adhesive PCR film
9. Centrifuge all plates at 2000 rpm for 2 minutes and then incubate the plates at 37 °C for 2 hours to dissolved the probe DNA
10. Clean the LHR to make certain that it has been left ready for others to use
11. Centrifuge all plates at 2000 rpm for 2 minutes and store the plates at -80 °C

R. Auburn (24-02-2009).

Library desiccation and rehydration

Overview

Spotting solution in microtitre plates evaporates during printing. The buffer in the wells at the edge, and especially in the corners of each plate, usually evaporates more quickly than the from the wells in the centre. Evaporation therefore causes variations in probe concentration across the plate. These systematic variations can have a detrimental impact on microarray performance, *e.g.*, variable spot signals, variable spot diameters and variable spot morphologies. These problems are best overcome by desiccating and rehydrating plates between print-runs.

Equipment and reagents

- Beckman Coulter Biomek NX^P Liquid Handling Robot (LHR)
- AP96 non-sterile P20 tips (Beckman Coulter; Cat. No. 717254)
- MilliQ water (unmodified probe DNA) or 0.2 µm filtered distilled water (modified probe DNA)
- Hettich Rotina 35 microtitre plate centrifuge
- Adhesive PCR Film (Abgene; Cat. No. AB-0558)
- 70% ethanol
- Horizontal laminar flow workstation (Jencons; Cat. No. 566-031)

Procedure

Desiccation

1. Remove the microtitre plates from the -80 °C freezer and leave to thaw on a desk
2. Switch the laminar flow workstation on and leave for at least 30 minutes before putting the plates inside
3. Once defrosted, centrifuge all plates at 2000 rpm for 2 minutes
4. Remove the adhesive film from the plates and leave to desiccate in the laminar flow workstation, *e.g.*, four days
5. Once desiccated, the plates can then be resealed and stored at -80 °C

Whilst using the LHR

6. Remove the microtitre plates from the -80 °C freezer and leave to thaw
7. Once thawed, centrifuge all plates at 2000 rpm for 2 minutes
8. Clean the exterior and interior of the LHR using the Dyson vacuum cleaner and wipe with 70% Ethanol
9. Open the program "Rehydrate_LIBRARY_YesWash" and home the instrument drives
10. Fill the in-flow wash tank with distilled water and then prime the wash station by switching the FX Device Controller (HV 1) to 'manual', *e.g.*, for 3-5 min.
11. Load the instrument, as directed by the "Rehydrate_LIBRARY_YesWash" program, *i.e.*, fresh box of AP96 P20 tips, reservoir filled with water and the plate to be rehydrated:
 - ◆ The adhesive PCR film should be removed as the plate is being loaded into the LHR
 - ◆ All plates should be loaded in the LHR with well A1 in the top-left corner
12. Start the program and watch to make certain that the LHR is working correctly
13. Repeat steps 11 to 12 until all plates have been rehydrated: as each plate is finished, remove from LHR and affix an adhesive PCR film
14. Clean the LHR to make certain that it has been left ready for others to use

After using the LHR

15. Centrifuge all plates at 2000 rpm for 2 minutes and then incubate at 37 °C for 2 hours to redissolved the probe DNA
16. Clean the LHR to make certain that it has been left ready for others to use
17. Centrifuge all plates at 2000 rpm for 2 minutes and store the plates at -80 °C

An Introduction to printing spotted microarrays

Overview

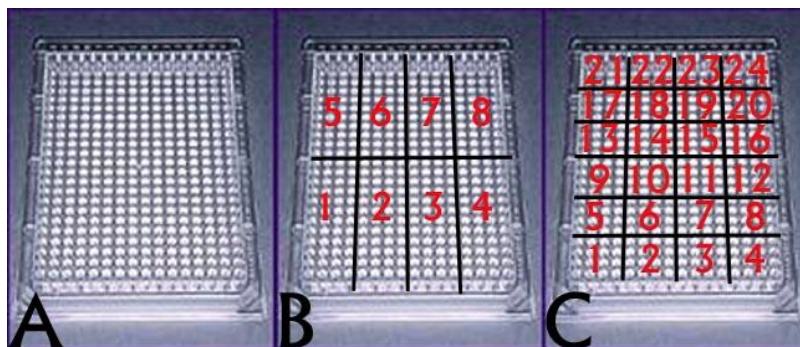
Microarrays are typically produced by transferring gene or transcript specific PCR amplified cDNA clones or long oligonucleotides in either high salt or other denaturing solutions from 384-well microtitre plates to chemically modified 25 x 75 mm glass microscope slides using robotic contact printing instruments. This process is sometimes termed robotic spotting or just spotting. Although robotic spotters were first described by Schena et al. (1995) they are also available commercially.

Printing microarrays

The spotting pins are a vital component because they are the only part of the instrument to contact the probe DNA and the slides. Spotting pins draw fluid into the pins by capillary action when a pin loading is performed. The pins enter adjacent wells of the 384-well microtitre plate because they have a fixed pitch of 450 μm in the print-head. The probe DNA within the microtitre plates therefore needs to be arranged accordingly for any given microarray layout to be printed correctly.

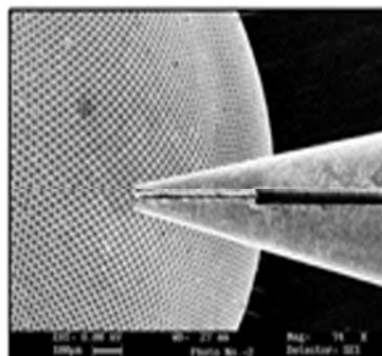
Example source visit layouts

A) A 384-well Genetix plate. B) 12x4 pin-tool source visit layout. C) 4x4 pin-tool source visit layout



When depositing a spot on the slide, surface tension interactions between the substrate surface and spotting buffer lead to spot formation when the pins pull away. Spot diameter is determined by a variety of parameters including the spotting buffer, substrate slide, temperature, relative humidity and the pin itself. Most spotting pins are blunt ended (50 to 100 μm) with a capillary or split and contain a storage reservoir. An example of one such pin is shown in the figure below.

Electron micrograph of a *BioRobotics MicroSpot 2500* pin



Electron micrograph of MicroSpot 2500 pin and the eye of a house fly

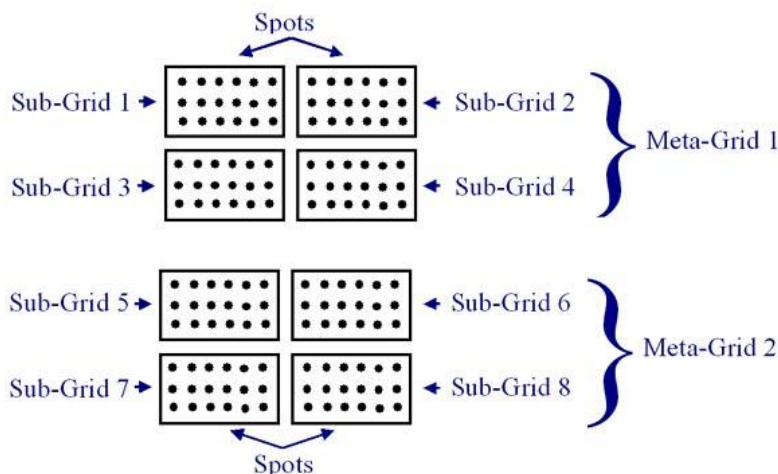
Spot density is determined by the diameter of the printed spots because this dictates the centre-to-centre spot distance that can be used during printing. Additionally, the accuracy and reliability of the robotic spotter will determine the reproducibility of the spot positioning within each microarray. This is sometimes referred to as pin or spot wobble. An imprecise instrument with lots of pin or spot wobble will require a greater

centre-to-centre spot distance, which will reduce the maximum spot density that can be achieved.

Sub-grids and meta-grids

Each pin within the print-head will produce a single pin-patch or a sub-grid of spots. The sum total of sub-grids printed by one pin-tool is called a meta-grid. A single microarray can consist of one or more meta-grids. The number of meta-grids that be printed per microarray is limited by the space available. FlyChip typically spots microarrays using 48 pins and therefore has 48 sub-grids per microarray but just one meta-grid. Other formats are also possible with our set-up and have indeed been produced.

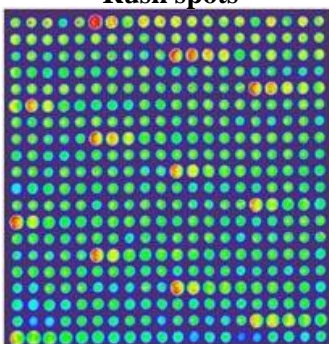
Microarray meta-grid and sub-grid organisation



Rush spots

The first few spots to be printed after a pin loading, the rush spots, are larger than any subsequently printed spots, as when a pin loading is performed some spotting solution will coat the outer surfaces of each pin. Until printing has exhausted this additional spotting solution the printed spots will not have a regular diameter. This problem can be overcome by discarding the first few slides after each pin loading or by using blotting slides that can then be discarded. The later option is preferable when printing arrays with an inter-spot distance that is less than the maximum spot size.

Rush spots



The above image is a close-up of 1 sub-grid printed with Cy3-labelled sonicated salmon sperm DNA using a BioRobotics MicroGrid II 600 spotter MicroSpot 2500 pins. The spotter started to print spots in the bottom left-hand corner and moved to the right and then up the image. 35 spots were printed per pin loading. This image clearly shows how the spot size initially decreases in diameter before reaching a uniform size. The number of slides to be discarded at the start of each print-run or the number of blotting spots to be printed should be defined in advance.

Spot morphology

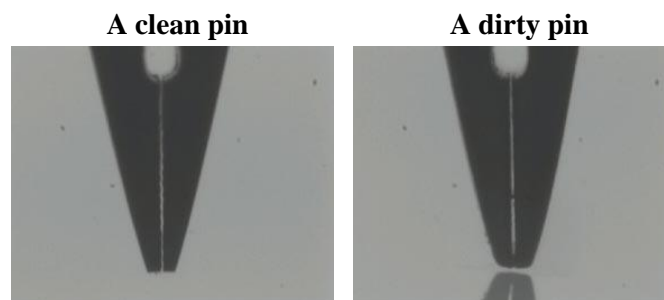
Key determinants of spot morphology have previously been described in the literature. These include substrate chemistry and hydrophobicity, spotter calibration and print settings, spotting buffer viscosity, pH and evaporation, probe DNA concentration, room temperature and relative humidity. Because of these complex interactions it is not possible to predict in advance which combination of conditions should be used for any given clone-set or spotter. For this reason, we routinely check and then optimise the spotting conditions for each of our clone-sets.

Wash cycles

Wash cycles are performed between each pin loading to ensure that the spotting solution from one loading does not contaminate any subsequent pin loadings. The number and length of wash cycles is a key determinant of how long any given print run will last for because the time taken to perform the pin loadings and spot depositions is minimal. Care must therefore be taken to ensure that that wash cycle is optimised for a fast print-run time and low probe DNA carry-over.

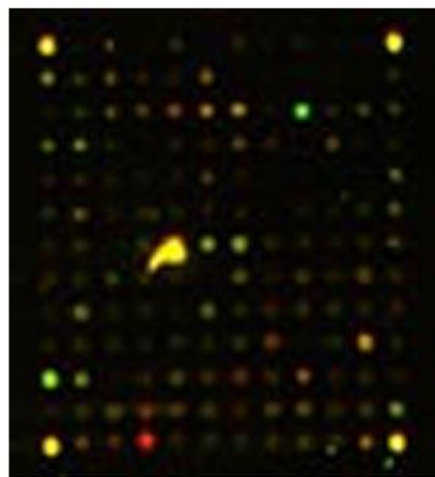
Cleanliness

Microarrays should be printed, stored, hybridised and scanned in a clean dust-free environment to ensure that arrays of the highest possible quality are produced. Spotting pins contain small capillaries and reservoirs that can be easily blocked by dust. Such blockages can lead to pins performing badly or being predisposed to further blockages. Poorly performing pins will either print inconsistently or not at all and a significant amount of potentially valuable data will be lost.



The biological samples being co-hybridised to the microarray are labeled with the Cy3 and Cy5 dyes respectively. Unfortunately, dust and other airborne contaminants are fluorescent under the Cy3 and/or Cy5 wavelengths. The complexities of image analysis mean that any fluorescent contaminant that lies within the spot will adversely affect the measured spot signal and hence, interpretation of the experimental result.

Dust particles



Spot tracking

Each microarray spot has a unique position and each spot corresponds to a specific probe DNA from a specific well of a microtitre plate. There are typically thousands to tens of thousands of probe DNAs distributed between tens of source plates and thousands to tens of thousands of spots on each microarray. A single microarray experiment can consist of a few or a hundred microarray hybridisations. Tracking where and what each probe DNA is on each microarray is therefore an important issue.

Most robotic spotters are supplied with a data tracking program that uses an input file to describe the positions of each probe DNA within the microtitre plates and another file that defines how the microarray was printed to produce a description of where each probe DNA is within each microarray. These spot identities can then be imported into a spot finding and quantification tool that will 'append' the fluorescence spot signal. These data are then analysed to determine what affect any given experimental condition or treatment has had on the gene expression of the samples being compared.

Summary

Many disparate factors need to be considered when printing microarrays. These affect the quality of the microarrays produced, the rate of production and the ease with which any downstream data analysis may be performed. Failure to fully appreciate these limitations will result in sub-standard microarrays being produced and experimental data that may be unreliable. Extreme care and consideration must therefore be taken to ensure that the best quality microarrays are produced.

R. Auburn (17-02-2006).

General introduction to optimising the performance of a robotic spotter

Overview

Many disparate factors need to be considered when printing microarrays. These affect the quality of the microarrays produced, the rate of production and the ease with which any downstream data analysis may be performed. Failure to fully appreciate these limitations will result in sub-standard microarrays being produced and hence experimental data that may be unreliable. Extreme care and consideration must therefore be taken to ensure that microarrays of the best quality are produced.

Loading the spotting pins with probe DNA

Two different approaches can be used to fill spotting pins with spotting solution. Firstly, one can repeatedly dip the pins into the spotting buffer with probe DNA. Secondly, one can use a slow speed single entry into the spotting solution followed by a brief pause. We have found that the second approach is more consistent and reduces the number of rush spots produced after each pin loading.

All of the pin tips need to enter far enough into the microtitre plate wells to ensure that they are all below the meniscus of the spotting solution. We will call this the source target depth and this needs to be determined experimentally. This can be achieved by systematically altering the source target depth and observing when the pins pass through the spotting solution meniscus. One should also then check to ensure that all pins are able print at least 60 spots per pin loading.

If the observed rush is proving to be problematic one could either switch to a different spotting buffer or reduce the source target depth height. If one or a few pins are not printing when all others are, one should check that the pins are all of equivalent length and that none are blocked. If in doubt contact the instrument supplier and pin manufacturer for further assistance.

We have not fully optimised the pin speed. However, a quick appraisal demonstrated that at 4 mm/s the pins would reliably fill with spotting solution without adversely effecting the print-run time. This pin speed therefore provides the best balance between the number of spots that can be printed per pin loading, rush duration and microarray throughput. Other pin types may vary and require different pin speeds.

Depositing the probe DNA on the substrate slide

We shall call the height at which all pins make contact with all slides in all slide positions the target height. Some robotic spotters enable the spotting pins to decelerate to a reduced pin speed at a specified height from the slide surface and we will these the the soft touch speed and soft touch distance. Each of these three parameters will need to be optimised if high quality microarrays are to be produced.

The slide trays or beds within most robotic spotters are not perfectly level and the pins themselves are not all the same length. In order to calculate the soft touch distance one firstly needs to determine the absolute difference between the lowest and highest slide position. Secondly, you then need to determine the difference in length between the pins. These values can then be added together and used as the soft touch distance. In order to confirm if this value has been calculated correctly, you just need to check that the longest pin is able to slow to the correct speed before contacting the highest slide position. Failure to do so will result in a poor and highly variable spot structure.

The target height is much easier to determine. Print one slide using a 10 x 15 sub-grid and systematically change the target height after each row of 10 spots has been printed. Use the height at which all pins print reliably plus the difference in height between the slide tray or bed position at which this test was performed and the lowest slide position to define the correct target height. This should enable the shortest pin to make contact with the lowest slide position. We have found that a pin speed of 4 mm/s provides the best balance between spot quality and microarray throughput.

The calculated settings are likely to vary between each instrument and print-head. Additionally, microarray slides from different commercial suppliers also vary in thickness. This means that the above settings need to be determine for each combination of print-head, instrument and substrate chemistry. It is therefore a good

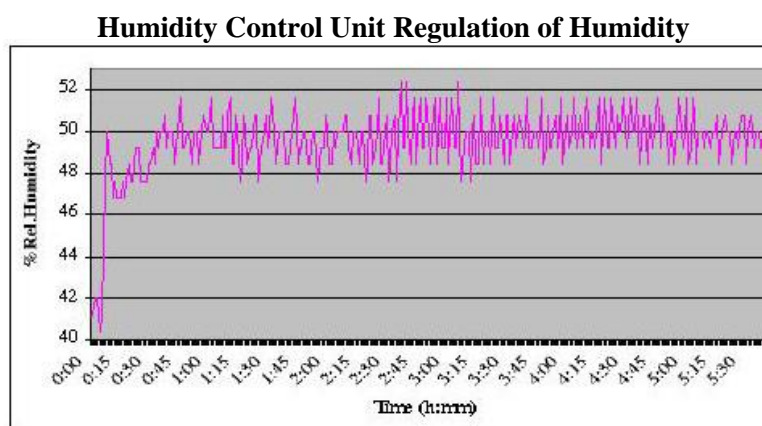
idea to document these settings, otherwise one risks printing microarrays with inappropriate spotter settings.

Selecting an appropriate substrate chemistry and spotting buffer

We have submitted a paper on this topic for publication. Once it has been accepted further details and a link to the abstract will be posted on this web page.

Printing temperature and relative humidity

High temperature and high humidity produces large ill-defined spots, whereas low temperature and low humidity produces small less distinct spots. FlyChip prints microarrays in an air-conditioned room using robotic spotters fitted with humidity control. This ensures that our microarrays are printed under controlled environmental conditions. We typically use a temperature range of between 19 to 21 °C and a relative humidity of 50 to 60%. We vary the humidity to match the slide manufacturers recommendations and are able to maintain the printing environment to within 2% of the target humidity with each of our spotters. We routinely record the temperature and humidity because these can have a profound influence on spot morphology and spot size.



Pin cleaning whilst printing and between print runs

Pins are cleaned between print-runs and between pin loadings to ensure that the spotting solution from one group of wells does not contaminate any subsequent pin loadings or spot depositions. If this were to happen it would effectively reduce the hybridisation stringency because each spot on the microarray would be comprised of the required probe DNA and a variable amount of all other probe DNAs. Each robotic spotter cleans pins in a different way and the amount of probe DNA carry-over between pin loading varies between spotting buffers. The number and length of wash cycles is a key determinant of how long a print run will last for, as the time taken to perform the pin loadings and spot depositions is often minimal.

Fortunately, it is quite straight forward to identify a good wash condition because all one needs to do to is to assess how much probe DNA is carried-over between one pin loading and the next. This can be achieved by using a microtitre plate with three pin loadings. The first and last should only contain spotting buffer, whereas the second should contain spotting buffer with labelled DNA. One can then print test slides using different wash conditions and measure the amount of carry-over between the second and third pin loading. The first can be used to correct for background fluorescence from the spotting buffer itself, e.g. 150 mM NaPO₄. These experiments should be performed using a full set of pins because the technical constraints of most spotters mean that pin number can effect how well pins are cleaned.

Microtitre plate storage and handling

Microtitre plate storage and handling is important because all spotting buffers evaporate and this evaporation is not uniform across the surface of each plate. Specifically, the spotting buffer within the wells at the corners and edge of the microtitre plate will evaporate more rapidly than within the wells towards the centre of each plate. This means that all microtitre plates should be treated with care and not stored uncovered. In fact, it is a good idea to use plate sealers to prevent evaporation leading to a reduction in the spotting buffer volume. One should also cover the plates with lids to prevent damage to the plastic

Robotic Spotting

seals. The plates themselves can be stored in a freezer to further inhibit evaporation.

R. Auburn (17-02-2006).

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

Robotic Spotting

- 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).

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 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 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 maintenance:

4. **Cleaning the aQu 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 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.

Robotic Spotting

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 humidity 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 program 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 conjunction with the weekly cleaning schedule (above).
17. **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 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.

R. Auburn (17-02-2006).

Full Moon Biosystems (FMB) protocol for processing FMB cDNA slides:

Overview

After printing non-modified PCR amplified gene-specific cDNA clones the Full Moon Biosystems (FMB) cDNA slides are then processed to bind the denatured probe DNA to the slide and prevent non-specific hybridisation to the substrate. The outlined protocol is based on the method recommended by Full Moon Biosystems (<http://www.fullmoonbio.com/>).

Equipment and material

- Full Moon Biosystems cDNA slides (Full Moon Biosystems; Cat. No. AS 50)
- Slide staining rack (Philip Harris; Cat. No. B52651)
- Slide staining trough (Philip Harris; Cat. No. B52649)
- UV crosslinker (Ultraviolet products; CL-1000)
- Orbital shaker (Stuart Scientific; mini orbital shaker SO5)
- Hettich Rotina 35 microtitre plate centrifuge
- Microscope slide box (Merck EuroLab; Cat. No. 406/0286/00)
- Horizontal laminar flow work station (Jencons; Cat. No. 566-031)
- Bovine serum albumin (BSA) fraction V (Sigma; Cat. No. A-7906).
- Sodium dodecyl sulfate (SDS), molecular biology grade (Sigma; Cat. No. L-4390)
- Blocking Solution: 4xSSC, 0.1% SDS, 1% BSA
- MilliQ water
- Standard photographic air duster

Protocol

For best results, perform steps 4 onwards just before hybridisation.

1. After arraying, UV cross-link the slides with the cross linker set at 4000 (x100 μ J) = 400mJ
2. Allow slides to dry at room temperature for 30 minutes
3. Slides that are not needed for one month or two months can be stored at this stage:
 - ◆ Place the slides in a clean microscope slide box
 - ◆ Then place the microscope slide box in a pastic bag and seal this bag
 - ◆ Store the sealed bag at 2 to 8 °C for 3 to 6 months
4. Meanwhile prepare and preheat the blocking solution to 55 °C in a water bath
5. Pour the blocking solution into a slide staining trough
6. Transfer the slides to a slide staining rack and place the rack into the slide staining trough
7. Place box on shaker at 50 rpm for 20 minutes at room temperature
8. Remove rack, blot off excess solution by placing on a piece of tissue.
9. Place the slides in the staining rack in the plastic box filled with 2.5 L ultra pure water and put the lid on
10. Place the plastic box with the slides on the orbital shaker at 50 rpm for 15 minutes
11. Remove rack, blot off excess solution by placing on a piece of tissue.
12. Repeat steps 8 to 10 twice (three water washes in total)
13. Transfer slides from the rack to a microscope slide box with fresh tissue in the base
14. Centrifuge at 650 rpm for 15 minutes in a microtitre centrifuge to dry the slides
15. Remove any water droplets from the slide using an air duster
16. Store in a clean sealed slide box at room temperature and in the dark (one to two months) until ready to hybridise

R. Auburn (05-06-2006).

Full Moon Biosystems (FMB) protocol for processing FMB PowerMatrix (modified oligo) slides:

Overview

After printing amino-modified long oligonucleotides the Full Moon Biosystems (FMB) PowerMatrix slides are then processed to bind the single stranded probe DNA to the slide and prevent non-specific hybridisation to the substrate. The outlined protocol is based on the method recommended by Full Moon Biosystems (<http://www.fullmoonbio.com/>).

Equipment and material

- Full Moon Biosystems PowerMatrix slides for modified oligos (Full Moon Biosystems; Cat. No. PXP 50 M)
- Slide staining rack (Philip Harris; Cat. No. B52651)
- Slide staining trough (Philip Harris; Cat. No. B52649)
- Orbital shaker (Stuart Scientific; mini orbital shaker SO5)
- Hettich Rotina 35 microtitre plate centrifuge
- Microscope slide box (Merck EuroLab; Cat. No. 406/0286/00)
- Horizontal laminar flow work station (Jencons; Cat. No. 566-031)
- Bovine serum albumin (BSA) fraction V (Sigma; Cat. No. A-7906).
- Sodium dodecyl sulfate (SDS), molecular biology grade (Sigma; Cat. No. L-4390)
- Blocking Solution: 2xSSC, 0.2%SDS, 0.1%BSA
- Ultra pure water (do not use MilliQ water)
- Standard photographic air duster
- Air tight plastic box (30 x 30 x 18 cm) with lid

Protocol

For best results, perform steps 4 onwards just before hybridisation.

1. Incubate slides in a chamber with 65 to 75 % relative humidity overnight:
 - ◆ Within an air tight plastic box add 100 g solid sodium chloride to 50 ml water
2. Allow slides to dry at room temperature for 30 minutes
3. Slides that are not needed for one or two months can be stored at this stage:
 - ◆ Place the slides in a clean microscope slide box
 - ◆ Then place the microscope slide box in a pastic bag and seal this bag
 - ◆ Store the sealed bag at 2 to 8 °C for 3 to 6 months
4. Meanwhile prepare and preheat the blocking solution to 55 °C in a waterbath
5. Pour the blocking solution into a slide staining trough and then transfer the slides to a slide staining rack and place this rack in the staining trough
6. Place the trough containing the slides on the orbital shaker at 50 rpm for between 20 to 30 minutes at room temperature
7. Remove rack, blot off excess solution by placing on a piece of tissue.
8. Place the slides in the staining rack in the plastic box filled with 2.5 L ultra pure water and put the lid on
9. Place the plastic box with the slides on the orbital shaker at 50 rpm for 15 minutes
10. Remove rack, blot off excess solution by placing on a piece of tissue.
11. Repeat steps 8 to 10 twice (three water washes in total)
12. Transfer slides from the rack to a microscope slide box with fresh tissue in the base
13. Centrifuge at 650 rpm for 15 minutes in a microtitre centrifuge to dry the slides
14. Remove any water droplets from the slide using an air duster
15. Store in a clean sealed slide box at room temperature and in the dark (one to two months) until ready to hybridise

R. Auburn (17-02-2006).

Full Moon Biosystems QC kit

Overview

After printing, a random sample of microarrays is stained and then scanned so that we can assess the print quality and constancy. These checks include substrate defects, sub-grid and meta-grid positioning on the substrate, checking that all spots have been printed and the spot morphology. The outlined protocol is based on the method recommended by Full Moon Biosystems (<http://www.fullmoonbio.com/>).

Equipment and Reagents

- Full Moon Biosystems QC kit
- MilliQ water
- Slide staining rack (Philip Harris; Cat. No. B52651)
- Slide staining trough (Philip Harris; Cat. No. B52649)
- Hettich Rotina 35 microtitre plate centrifuge
- Microscope slide box (Merck EuroLab; Cat. No. 406/0286/00)
- Horizontal laminar flow work station (Jencons; Cat. No. 566-031)
- Lint-free tissue
- Standard photographic air duster

Procedure (4 slides, per batch)

1. Add 25 mL QC solution to a reaction tube
2. Place printed and blocked slides in the reaction tube
3. Incubate on an orbital shaker for 1 minute at room temperature
4. Dip slides in 250 mL 0.05% SDS/0.1 x SSC (5 times)
5. Dip slides in 250 mL 0.1 x SSC (5 times)
6. Dip slides in 250 mL MilliQ water (5 times)
7. Transfer to a microscope slide box with lint-free tissue in the base
8. Centrifuge at 2000 rpm for 2 minutes
9. Remove any water droplets from the slide using an air duster
10. Scan using the cy3-channel of a CCD or dual laser scanner

R. Auburn (18-08-2008).

A key to standard microarray spot identity tracking file formats

Overview

Each microarray spot has a unique position and each spot corresponds to a specific probe DNA from a specific well of a microtitre plate. There are typically thousands to tens of thousands of probe DNAs distributed between tens of source plates and thousands to tens of thousands of spots on each microarray. A single microarray experiment can consist of a few or a hundred microarray hybridisations. Tracking where and what each probe DNA is on each microarray is therefore an important issue.

Most robotic spotters are supplied with a data tracking program that uses an input file to describe the positions of each probe DNA within the microtitre plates and another file that defines how the microarray was printed to produce a description of where each probe DNA is within each microarray. These spot identities can then be imported into a spot finding and quantification tool that will 'append' the fluorescence spot signal. These data are then analysed to determine what affect any given experimental condition or treatment has had on the gene expression of the samples being compared.

For a definition of all terminology used below please refer to "Introduction to printing microarrays".

- The microarray layout used in the following examples
- The microarray input file used in the following examples
- Example microarray spot identity file formats
 - ◆ BioRobotics 'TAM' format
 - ◆ Axon 'GAL' format
 - ◆ Molecularware 'MWBR' format
 - ◆ Applied Precision Instruments arrayWoRx 'REF' format
 - ◆ Quantarray file format
 - ◆ Imagene file format
 - ◆ Layout Map Xyxy
 - ◆ Layout Map XYyx
 - ◆ Layout Map YXxy
 - ◆ Layout Map YXyx

The microarray layout used in the following examples

1. Meta-grid Layout:

A single 2x2 (X and Y axis) meta-grid printed on each slide
Numbers below refer to the pins that printed the sub-grids

01 02
03 04

Array position from bottom left of slide: 9.24 mm (X-axis), 34.19 mm (Y-axis)
Meta-grid has an array area of 6.10 mm (X-axis) by 6.10 mm (Y-axis)

2. Sub-grid Layout:

Each sub-grid has 4x4 (16) spots
Centre-to-centre spot distance is 400 microns
Numbers below refer to 'Source Visits'

13 14 15 16
09 10 11 12
05 06 07 08
01 02 03 04

Key to Source Visits
Source '1' to '16' = Imaginary clones

3. Comments:

This is a hypothetical microarray
The arrays contains 64 spots per slide

The microarray input file used in the following examples

The following is a small section of a file that describes a fictitious source plate. Well position is defined by source plate barcode (e.g. TST101), row (A to P) and column (1 to 24). CloneID is a cDNA clone accession code and UniqueID is a well-specific identifier. The columns do not need to be in the order shown. The example shown is a 'tab separated value' (TSV) text file.

CloneID	UniqueID	Row	Column
FC1234	TST101	A	1
FC1235	TST101	A	2
FC1236	TST101	A	3
FC1237	TST101	A	4
Empty	Empty	A	5
Empty	Empty	A	6
Empty	Empty	A	7
Empty	Empty	A	8
Empty	Empty	A	9
Empty	Empty	A	10
Empty	Empty	A	11
Empty	Empty	A	12
Empty	Empty	A	13
Empty	Empty	A	14
Empty	Empty	A	15
Empty	Empty	A	16
Empty	Empty	A	17
Empty	Empty	A	18
Empty	Empty	A	19
Empty	Empty	A	20
Empty	Empty	A	21
Empty	Empty	A	22
Empty	Empty	A	23
Empty	Empty	A	24
FC1238	TST101	B	1
FC1239	TST101	B	2
FC1240	TST101	B	3
FC1241	TST101	B	4
Empty	Empty	B	5
Empty	Empty	B	6
Empty	Empty	B	7

This input file was used to create a series of clone tracking files using the MicroGrid II so that the format structures could be explained using worked examples. These are shown in the following sections, an asterisk denotes an explanation rather than a component of the named file format.

Example microarray spot identity file formats

BioRobotics 'TAM' format

```
[FileInformation]
FileFormat=,1.0 *Version number of the file format
FormatName=,TAM *File format extension
GeneratedBy=,TAS2.1.5.16 *Arrayer software and version
BlockCount=,4 *Number of meta-grids
SpotSize=,180 *Estimated mean spot diameter

[Block1] *Sub-grid being examined
MetaGridX=,1 *Meta-grid X-axis co-ordinate
MetaGridY=,1 *Meta-grid Y-axis co-ordinate
OriginX=,9000 *Distance from top left edge of slide to centre of top left spot
OriginY=,35300 *Distance from top left edge of slide to centre of top left spot
BlockSizeX=,4 *Number of spots in each row
BlockSizeY=,4 *Number of spots in each column
SpacingX=,400 *X-axis centre-to-centre spot spacing
SpacingY=,400 *X-axis centre-to-centre spot spacing
*Information is repeated for each sub-grid on the slide

[mapping] *'comma separated value' spreadsheet that maps clones to wells
1,1,1,1,,1,9,4,"FC1269", "1036",1, {},FC1269,1036,TST101,I,4
```

Robotic Spotting

```
1,1,1,2,,1,1,4,"FC1237","1004",1, {},FC1237,1004,TST101,A,4
1,1,1,3,,1,9,2,"FC1267","1034",1, {},FC1267,1034,TST101,I,2
1,1,1,4,,1,1,2,"FC1235","1002",1, {},FC1235,1002,TST101,A,2
1,1,2,1,,1,11,4,"FC1277","1044",1, {},FC1277,1044,TST101,K,4
```

*Spreadsheet abbreviated for brevity.

*Each row of data relates to each spot on the microarray.

*Origin is top left of slide for all measurements.

*Column 01: Meta-grid X-axis co-ordinate

*Column 02: Meta-grid Y-axis co-ordinate

*Column 03: Sub-grid Y-axis co-ordinate

*Column 04: Sub-grid X-axis co-ordinate

*Column 05: Plate Barcode

*Column 06: Plate Number

*Column 07: Row Number

*Column 08: Column Number

*Column 09: Sample Name

*Column 10: Sample ID

*Column 11: Block number

*Column 12: Termination of additional fields

*Column 13 to End: Source data from the operator (See Example Array Input File)

Axon 'GAL' format

```
ATF      1.0
10      5 *first term is the number of headers, not including this, the previous or
        the column header row. The second term is the number of columns
"Type=GenePix ArrayList V1.0" *Version number of the file format
"BlockCount=4" *Number of sub-grids
"BlockType=0"
"Block1=9000, 35300, 180, 4, 400, 4, 400" *Mapping information for sub-grid 1
"Block2=13500, 35300, 180, 4, 400, 4, 400" *Mapping information for sub-grid 2
"Block3=9000, 39800, 180, 4, 400, 4, 400" *Mapping information for sub-grid 3
"Block4=13500, 39800, 180, 4, 400, 4, 400" *Mapping information for sub-grid 4
"Supplier=BioRobotics" *Arrayer manufacturer
"ArrayerSoftwareName=TAS Application Suite (MicroGrid II)" *Arrayer software name
"ArrayerSoftwareVersion=2.1.5.16" *Arrayer software version
"Block" "Column" "Row" "ID" "Name"
*Header for clone mapping 'tab separated value' (TSV) spreadsheet
1      1      1      1036      FC1269
1      1      2      1004      FC1237
1      1      3      1034      FC1267
1      1      4      1002      FC1235
1      2      1      1044      FC1277
1      2      2      1012      FC1245
1      2      3      1042      FC1275
1      2      4      1010      FC1243
1      3      1      1052      FC1285
1      3      2      1020      FC1253
1      3      3      1050      FC1283
1      3      4      1018      FC1251
1      4      1      1060      FC1293
1      4      2      1028      FC1261
1      4      3      1058      FC1291
1      4      4      1026      FC1259
2      1      1      1040      FC1273
2      1      2      1008      FC1241
2      1      3      1038      FC1271
2      1      4      1006      FC1239
2      2      1      1048      FC1281
2      2      2      1016      FC1249
2      2      3      1046      FC1279
2      2      4      1014      FC1247
2      3      1      1056      FC1289
2      3      2      1024      FC1257
```

*Spreadsheet abbreviated for brevity.

*Each row of data relates to each spot on the microarray.

*Origin is top left of slide for all measurements.

*Block: Sub-grid identification

*Column: Sub-grid Y-axis co-ordinate

Robotic Spotting

*Row: Sub-grid X-axis co-ordinate
*ID: Sample ID (See Example Array Input File)
*Name: Sample Name (See Example Array Input File)

Molecularware 'MWBR' format

[FileInformation]

FileFormat=,1.0.2 *Version number of the file format
FormatName=,MwBr *File format extension
GeneratedBy=,TAS2.1.5.16 *Arrayer software and version
BlockCount=,4 *Number of sub-grids
SpotSize=,180 *Estimated mean spot diameter

[Source]

Comment=,Genetix384 *Source plate type
XWells=,16 *Number of wells in X-axis
YWells=,24 *Number of wells in Y-axis
XPitch=,4.5 *Well spacing in X-axis
YPitch=,4.5 *Well spacing in Y-axis
PlateCount=,1 *Number of source plates

[Tool]

Description=,2x2 (384 well) split pin *MicroSpot II pin number and type
PinsX=,2 *Number of MicroSpot pins in X-axis
PinsY=,2 *Number of MicroSpot pins in Y-axis
PinPitch=,4500 *Pin-to-pin spacing in the pin-tool

[Target]

TargetWidth=,25000 *Width of the glass microscope slide
TargetHeight=,75000 *Height of the glass microscope slide
LeftMargin=,9239 *Meta-grid distance from left of slide
RightMargin=,9239 *Meta-grid distance from right of slide
TopMargin=,34189 *Meta-grid distance from top of slide
BottomMargin=,34189 *Meta-grid distance from bottom of slide
XSpacing=,0 *Spacing between meta-grids in X-axis
YSpacing=,0 *Spacing between meta-grids in Y-axis
NumberOfCopies=,27 *Number of slides being printed

[slides] *Mapping of the slide the microarray was printed on

Slide1=,1,20000,243000, *List abbreviated to save space

[Block1] *Sub-grid being examined

MetaGridX=,1 *Meta-grid X-axis co-ordinate
MetaGridY=,1 *Meta-grid Y-axis co-ordinate
OriginX=,9000 *Distance from top left edge of slide to centre of top left spot
OriginY=,35300 *Distance from top left edge of slide to centre of top left spot
BlockSizeX=,4 *Number of spots in each row
BlockSizeY=,4 *Number of spots in each column
SpacingX=,400 *X-axis centre-to-centre spot spacing
SpacingY=,400 *X-axis centre-to-centre spot spacing
*Information is repeated for each sub-grid on the slide

[mapping]*As for TAM format expect column 3 and 4 are in the reverse order

1,1,1,1,,1,9,4,"FC1269","1036",1, {},FC1269,1036,TST101,I,4
1,1,1,2,,1,1,4,"FC1237","1004",1, {},FC1237,1004,TST101,A,4

*Spreadsheet abbreviated for brevity.

*Each row of data relates to each spot on the microarray.

*Origin is top left of slide for all measurements.

Applied Precision Instruments arrayWoRx 'REF' format

#ArrayWoRx Reference File *File Format

#Tag 0x00FF040C

#Version: 2.10 *File format version

#GridOrigin: 34190 9660

#GridRotation: 0

#GridType: Irregular

#GridColumnsRows: 64 1

#GridWidthHeight: 6100 6100 *Array size: X-axis, Y-axis

#NomSpotSpacingColumnRow: 400 400 *X-axis Y-axis centre-to-centre spot distance

#OddRowOffset: 0

Robotic Spotting

#Number_UniqueID_Types: 2
#UniqueID_Type 1: 0
#UniqueID_Type 2: 0
#Column 1: Spot number *Key to the clone spreadsheet
#Column 2: Spot name 1 [gene name] *Key to the clone spreadsheet
#Column 3: Spot name 2 [chromosome] *Key to the clone spreadsheet
#Column 4: Spot type [1=experiment; 2=ratio control] *Key to the clone spreadsheet
#Column 5: Unique ID 1 *Key to the clone spreadsheet
#Column 6: Unique ID 2 *Key to the clone spreadsheet
#Column 7: X coordinate *Key to the clone spreadsheet
#Column 8: Y coordinate *Key to the clone spreadsheet
#Column 9: Spot size 1 [width in um] *Key to the clone spreadsheet
#Column 10: Spot size 2 [height in um] *Key to the clone spreadsheet
#Column 11: Bounding box width [ROI about the spot] *Key to the clone spreadsheet
#Column 12: Bounding box height [ROI about the spot] *Key to the clone spreadsheet
#Column 13: Reserved column 1 *Key to the clone spreadsheet
#Column 14: Reserved column 2 *Key to the clone spreadsheet
#Column 15: Reserved column 3 *Key to the clone spreadsheet
#Column 16: Reserved column 4 *Key to the clone spreadsheet
#Column 17: Reserved column 5 *Key to the clone spreadsheet
#Column 18: Reserved column 6 *Key to the clone spreadsheet
#Column 19: Reserved column 7 *Key to the clone spreadsheet
#Column 20: Reserved column 8 *Key to the clone spreadsheet
#Column 21: Include flag [0=exclude; 1=include] *Key to the clone spreadsheet
#Column 22: Description [optional] *Key to the clone spreadsheet

*Tab separated value (TSV) clone spreadsheet

```
1 FC1262 unknown 1 1029 Unknown 34190 9660 180 180 200 200 * * * * * * * * 1 Plate 1, Well H1
2 FC1294 unknown 1 1061 Unknown 34590 9660 180 180 200 200 * * * * * * * * 1 Plate 1, Well P1
3 FC1264 unknown 1 1031 Unknown 34990 9660 180 180 200 200 * * * * * * * * 1 Plate 1, Well H3
4 FC1296 unknown 1 1063 Unknown 35390 9660 180 180 200 200 * * * * * * * * 1 Plate 1, Well P3
```

*Spreadsheet abbreviated for brevity.

*Each row of data relates to each spot on the microarray.

*Origin is bottom left of slide for all measurements.

Quantarray file format

*'Tab separated value' (TSV) text file

```
1      1      1      1      1036
1      1      2      1      1004
1      1      3      1      1034
1      1      4      1      1002
1      1      1      2      1044
1      1      2      2      1012
1      1      3      2      1042
1      1      4      2      1010
1      1      1      3      1052
1      1      2      3      1020
1      1      3      3      1050
1      1      4      3      1018
```

*Spreadsheet abbreviated for brevity.

*Each row of data relates to each spot on the microarray.

*Origin is top left of slide for all measurements.

*Column 01: Meta-grid X-axis co-ordinate

*Column 02: Meta-grid Y-axis co-ordinate

*Column 03: Sub-grid Y-axis co-ordinate

*Column 04: Sub-grid X-axis co-ordinate

*Column 05: 'UniqueID' (See [Example Array Input File](#))

Imagene file format

*'Tab separated value' (TSV) text file

```
1      1      1      1      1036
1      1      1      2      1004
1      1      1      3      1034
1      1      1      4      1002
```

Robotic Spotting

1	1	2	1	1044
1	1	2	2	1012
1	1	2	3	1042
1	1	2	4	1010
1	1	3	1	1052
1	1	3	2	1020
1	1	3	3	1050
1	1	3	4	1018

*Spreadsheet abbreviated for brevity.

*Each row of data relates to each spot on the microarray.

*Origin is top left of slide for all measurements.

*Column 01: Meta-grid X-axis co-ordinate

*Column 02: Meta-grid Y-axis co-ordinate

*Column 03: Sub-grid X-axis co-ordinate

*Column 04: Sub-grid Y-axis co-ordinate

*Column 05: 'UniqueID' (See [Example Array Input File](#))

Layout Map XYxy

*'Tab separated value' (TSV) text file

1	1	1	1	1036
1	1	1	2	1004
1	1	1	3	1034
1	1	1	4	1002
1	1	2	1	1044
1	1	2	2	1012
1	1	2	3	1042
1	1	2	4	1010
1	1	3	1	1052
1	1	3	2	1020
1	1	3	3	1050
1	1	3	4	1018

*Spreadsheet abbreviated for brevity.

*Each row of data relates to each spot on the microarray.

*Origin is top left of slide for all measurements.

*Column 01: Meta-grid X-axis co-ordinate

*Column 02: Meta-grid Y-axis co-ordinate

*Column 03: Sub-grid X-axis co-ordinate

*Column 04: Sub-grid Y-axis co-ordinate

*Column 05: 'UniqueID' (See [Example Array Input File](#))

Layout Map XYyx

*'Tab separated value' (TSV) text file

1	1	1	1	1036
1	1	2	1	1004
1	1	3	1	1034
1	1	4	1	1002
1	1	1	2	1044
1	1	2	2	1012
1	1	3	2	1042
1	1	4	2	1010
1	1	1	3	1052
1	1	2	3	1020
1	1	3	3	1050
1	1	4	3	1018

*Spreadsheet abbreviated for brevity.

*Each row of data relates to each spot on the microarray.

*Origin is top left of slide for all measurements.

*Column 01: Meta-grid X-axis co-ordinate

*Column 02: Meta-grid Y-axis co-ordinate

*Column 03: Sub-grid Y-axis co-ordinate

*Column 04: Sub-grid X-axis co-ordinate

*Column 05: 'UniqueID' (See [Example Array Input File](#))

Layout Map YXxy

*'Tab separated value' (TSV) text file

1	1	1	1	1036
1	1	1	2	1004
1	1	1	3	1034
1	1	1	4	1002
1	1	2	1	1044
1	1	2	2	1012
1	1	2	3	1042
1	1	2	4	1010
1	1	3	1	1052
1	1	3	2	1020
1	1	3	3	1050
1	1	3	4	1018

*Spreadsheet abbreviated for brevity.

*Each row of data relates to each spot on the microarray.

*Origin is top left of slide for all measurements.

*Column 01: Meta-grid Y-axis co-ordinate

*Column 02: Meta-grid X-axis co-ordinate

*Column 03: Sub-grid X-axis co-ordinate

*Column 04: Sub-grid Y-axis co-ordinate

*Column 05: 'UniqueID' (See [Example Array Input File](#))

Layout Map YXyx

*'Tab separated value' (TSV) text file

1	1	1	1	1036
1	1	2	1	1004
1	1	3	1	1034
1	1	4	1	1002
1	1	1	2	1044
1	1	2	2	1012
1	1	3	2	1042
1	1	4	2	1010
1	1	1	3	1052
1	1	2	3	1020
1	1	3	3	1050
1	1	4	3	1018

*Spreadsheet abbreviated for brevity.

*Each row of data relates to each spot on the microarray.

*Origin is top left of slide for all measurements.

*Column 01: Meta-grid Y-axis co-ordinate

*Column 02: Meta-grid X-axis co-ordinate

*Column 03: Sub-grid Y-axis co-ordinate

*Column 04: Sub-grid X-axis co-ordinate

*Column 05: 'UniqueID' (See [Example Array Input File](#))

R. Auburn (17-02-2006).