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 very 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 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.

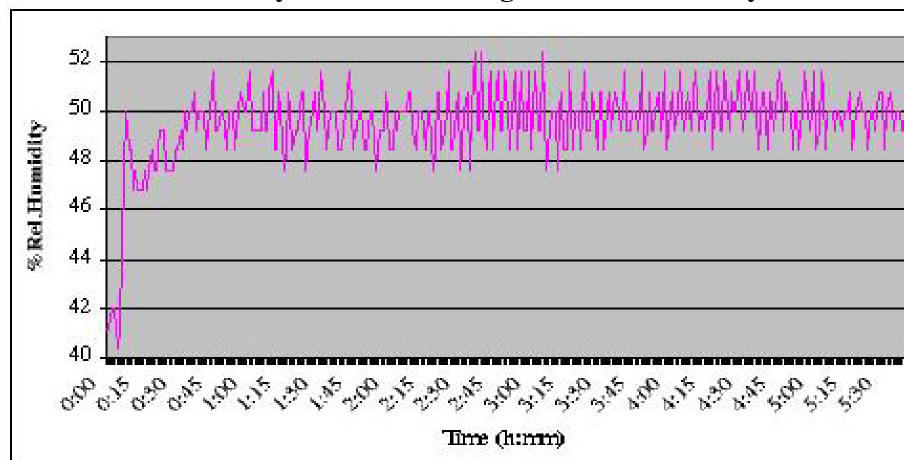
Selecting an appropriate substrate chemistry and spotting buffer

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.

Humidity Control Unit Regulation of Humidity



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 microtire 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 flourscense from the spotting buffer itself, e.g. 150 mM NaPO[4]. These experiments should be perfomed 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 evapourate and this evapouration is not uniform accross the surface of each plate. Specifically, the spotting buffer within the wells at the corners and edge of the microtitre plate will evapourate more rapidly than within the wells towards the centre of each plate. This means that all microtire plates should be treated with care and not stored uncovered. In fact, it is a good idea to use plate sealers to prevent evapouration leading to a reduction in the spotting buffer volume. One should also cover the plates with lids to prevent damage to the plastic seals. The plates themselves can be stored in a freezer to further inhibit evpouration.

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