


Maintenance of quill pins and performance tests			
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Aim

This protocol describes the procedures used in our lab for testing the performance of quill pins used for the generation of DNA microarrays and their cleaning.

Material and equipment

Telechem stealth pins: Chipmaker 2 (CMP4) and SMP3

Biorobotics Microspot pins 2500

Hering sperm DNA (200 ng/μl in 3XSSC/ 1.5 M Betain) in 384 well plates (5-10 μl)


High quality microarray substrates

Pin documentation system (build in house)

Procedure

A Testing the pin performance – Telechem stealth pins

1. Select the suitable print head for the arrayer to be used and the anticipated project and equip it with the required number of pins optimised for their respective position.
2. Spot Hering sperm DNA (200 ng/μl) dissolved in spotting buffer (3X SSC/ 1,5 M Betain) in a 20x20 pattern made of "1". Set the number of spots/ source visit to 400.
3. Verify the transfer for all 400 spots per pin to the slide surface by scanning the slide on the Tecan LS200 scanner in the scatter mode use scatter.lsp stored on the MGTECANLS control computer. Adjust the scan area to the geometry of the addressed on the slide.
4. Save the corresponding image in the Tif format in the folder associated to the project currently run.
5. Run settings:
 - a. Washing time: dH2O 2*3000 msec, technical Ethanol 3000 msec, drying time 10000 msec; wash every first source visit
 - b. Inking-depth: has to be adjusted for every type of plate. It should be as high as possible – however the pins are not to touch the bottom of the well.
 - c. Inking time: 2000 msec
 - d. Number of pre-spots (on separate 8 x 12 cm pre-spotting slide): 25
 - e. Stamp depth: adjusted to each type of slide (for most 1 mm slides 90 μm)
 - f. Stamp time: 400 msec
 - g. Soft touch: yes; distance 1000 μm

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h. Relative humidity: 55%

B Improving the pin performance – Telechem stealth pins

Insufficient performance of the pins has to be addressed by repetitive washing of the pins in the washing station of the Microarrayer. If ten rounds of successive cleaning and testing fail to meet above demands, the pins have to be cleaned in the ultrasonic bath (containing dH₂O, **no deionised water – see below**) for two minutes. After drying the pins with oil-free pressured air, the pins are to be returned to their original position and orientation in the print head and tested again. In case the performance of the Telechem Pins is still insufficient the pins are to be cleaned with arrayit micro cleaning solution according the protocol provided by arrayit :

1. Prepare 1 liter of 1X Micro Cleaning Solution
2. Place 1X Micro Cleaning Solution in sonicator
3. Place microarray pins into sonicator
4. Sonicate for 10 minutes
5. Rinse exhaustively with tap water
6. Sonicate for 5 minutes in dH₂O (**only 1x distilled water**) to remove residual Micro Cleaning Solution. The use of de-ionised water (e.g. from a Millipore Elix water purification system) has to be clearly avoided or the pins will be corroded)
7. Dry pins with oil-free pressured air
8. control pins (quill or split/ slit) in pin documentation system and re-clean the pins if necessary

Re-test the performance of the pins

C testing the pin performance – Biorobotics Microspot 2500 Pins -Only – Don not use these spotting setting for any other types of pins

1. Select the print head for Biorobotics Microspot 2500 Pins and mount it to the Microgrid according to the instruction in the Microgrid manual.
2. Spot Hering sperm DNA (200 ng/μl) dissolved in spotting buffer (3X SSC/ 1,5 M Betain) in a 20x20 pattern made of “1”. Set the number of spots/ source visit to 200.
3. Probe volume: not below 5 μl, opt. 7 - 10 μl, max. 15 μl; volume loss due to evaporation has to be taken into consideration
4. Source visit: Pins go into wells, it is recommended that pins hit the bottom

5. pre-spotting: no soft touch (for pre-spot slides), as pins have to be initialised with 5 to 20 "crashes" before printing
6. Plates setting:
 - a. pin depth - 0.400
 - b. source action - 0.400
 - c. soft touch 4 mm
 - d. soft touch distance 8 mm
 - e. dwell time: lowered to 0.5
7. spots on slides:
 - a. target height to 0.2
 - b. soft touch distance to 0.3
 - c. if any problems during spotting on more than one slide might occur: add a delay before spotting of 0.2 seconds
8. it is recommended to perform a "short" spotting run prior to a long spotting run ("warm up")
9. sonication of pins for 30 sec in H₂O bidest and 20 - 30 wash cycles in the main wash station prior to spotting recommended especially after detergent treatment - apply pin preparation protocol
10. add extra wash cycles at start and end of each spotting run
11. Verify the transfer of at least 100 spots per pin to the slide surface by scanning the slide on the Tecan LS200 scanner in the scatter mode use the scatter.lsp stored on the MGTecan1s control computer. Adjust the scan area to the geometry of the addressed on the slide.
12. Save the corresponding image in the Tif format in the folder associated to the project currently ran.
13. In case the number of spots/ source visit is less than 100 proceed with the washing procedure outlined below.

D Improving the pin performance – Biorobotics Microspot 2500 Pins Only – Do not use the cleaning procedure for any other types of pins

1. Insufficient performance of the pins has to be addressed by repetitive washing of the pins in the washing station of the Microarrayer. If ten rounds of successive cleaning and testing fail to meet above demands, the tips of the pins (in the print head) have to be cleaned in the

