## Aim

This protocol describes the generation of Microarrays presenting PCR products or oligonucleotides as DNA probes for different applications such as expression profiling.

## Prerequisites/ boundary conditions

This protocol takes advantage of isopropanol purified PCR products (re-suspended in 3X SSC/ 1.5 M Betain) prepared according to the MPI/AG protocol 001. Alternatively the settings given here are used for the generation of oligonucleotide based DNA microarrays. This protocol is successfully used for the fabrication of cDNA microarray on a modified Genetix QArray and a Biorobotics Microgrid II using Telechem stealth pins and Biorobotics 2500 tungsten pins. This protocols works in our laboratory on different microarray substrates such as Telechem Superamine slides (Cat. #SMM-25), Schott Epoxy Microarray Slides (Cat. #39-1066643), Advalytix Epoxy microarray (Cat. #AD100-25) and in-house poly lysine coated microarray substrates (http://cmgm.stanford.edu/pbrown/protocols/1_slides.html) superior to other protocols. It should be noted that the suitability of this protocol for codelink microarray substrates (Amersham Biosciences #30011-4PK) is rather limited.

## Material and equipment

1. High precision microarraying robots (a in-house modified Genetix QArray – details in preparation for publication, non-modified Biorobotics Microgrid II).
2. Telechem split Pins Chipmaker 2 (or CMP3 Pins) or SMP3 Pins
3. Milli Q Water (for humidifying unit of each Microarrayer)
4. Microarray substrates (slides)
5. DNA-probes dissolved in 3XSSC (pH 7) supplemented with 1.5 M Betain:
   a. cDNA [PCR Product] > 100 ng/ µl
   b. Oligonucleotides: 100 µM (100 pmol/µl) for spotting on Epoxy and Telechem Superamine slides
   c. Oligonucleotides: 50 µM (50 pmol/µl) for spotting on Polylysin slides

## Procedure

### A. General outline

1. Maintain a clean environment inside the arrayer room – i.e. prevent any sort of dust from entering the arrayer – any dust/ powder will impair the generation of DNA microarrays.
2. Prior to opening the arrayers (before and after an arraying run) the outside of the arrayers has to be cleaned with a moistened Kim Wipes cloth.
3. Any part interior part of the arrayers may be only touched while wearing powder free gloves.
4. Printing heads are to be assembled outside of the arrayer.

5. No alterations on the cooling unit for the MTPs are to be made by anyone without prior consent of the engineer in charge of the machines and of the head of the lab. In case of an approval the alterations have to be clearly marked on the outside of the machine.
6. No spotting run is to be started without a complete MTP descriptor file.
7. The performance of all pins has to verified and improved according to the MPI/AG protocol No 2.
8. Broken/ cracked microtitre plates, plates with contaminated lids, plates with remainders of sealing tape must not be loaded in any arrayer.

**B1. Array fabrication – modified Genetix QArray**

1. Prior to opening the arrayers (before and after an arraying run) the outside of the arrayers has to be cleaned with a moistened Kim Wipes cloth.
2. Refill the humidifying unit with de-ionised water.
3. Fix the print head in the holder only with two screws marked in green. Any alteration to the set-up of the printing head has to be approved by the engineer in charge of the machines and the head of the lab.
4. Verify the performance of the pins as outlined in protocol MPI/AG 002.
5. Define all run settings have or import them form the planning folder created for this project.
6. Suitable microarray substrates (named above) are to be placed at positions inside the Microarrayer according to the outline in the planning file.
7. Thaw MTPs containing the DNA solutions to be spotted.
8. Spin down the plates, and remove their sealing tape after bringing them to the arrayer room. Only after verification that no tape remained on the plates, load the plates in the stacker unit (QA1) or place them in the plate holders (QA2 and QA3) according to the plate order defined in the run settings file.
9. Verify that the A1 position of every plate has to direct to the front right corner of the machine.
10. For QA1 load the plates closed with lids into the stacker unit. Note that the barcodes (and plate position P24) have to face the backside of the stacker unit. Encapsulate the stacker unit after loading the humidity units.
11. For QA2 and QA3 load the plate to the plate holders and remove the lids from the MTPs.
12. Load pre-spotting Slides
13. Activate the humidity
14. All parameters for the arraying run have to be re-checked
15. Start the calculation of the arraying script – for QA1 define the barcode list
16. Start the arraying run
17. Every plate has to be sealed after removal from the Microarrayer and stored at –20°C.
18. Upon completion of the arraying run the Gal file of this run has to be created and named according to the guidelines agreed upon in the lab.

19. The humidity/ temperature logger (Testo) hast to be read out.
20. All files describing the arraying run – humidity (Testo-) log, arrayer log file, rsp, the pat, the barcode list file (QA1 only), and the Gal-file file are immediately transferred to the run settings folder of this arraying project folder.

**B2. Array fabrication –Biorobotics Microgrid II**

1. Refill the humidifying unit and all water tanks of the.
2. Define the spotting pattern/ field arrangement and the remaining run settings or loaded from a suitable descriptor file stored in the current project folder.
3. Spin down the plates containing thawed PCR products in 3X SSC/ 1.5 M Betain, bring the plates to the arrayer room, remove their seal, and after verification that no tape remained on the plates, their lids have to be carefully checked for cracks or any sources of contamination that can impair the lid lifter in it's performance. After verifying the correct state of the plate lids, load the lidded plates in the plate hotel (stacker unit). The barcode (and well position A1) of the MTPs has to face the front side of the Microgrid.
4. Suitable number of pre-spotting slides and production slides are to be loaded on the trays according to the display on the control software. Positions not occupied by production/ pre-spotting slides are to be occupied by blank slides for the generation of vacuum required for fixing the slides on the trays.
5. The run is to be started by increasing the upper boundary of humidity setting. After app. 15 Minutes this upper boundary shall be lowered to app 55% rH.
6. Upon completion of the run all plates have to be sealed and stored at –20$^{o}$C.
7. The slides are to be incubated at app 55% rH over nicht inside the arrayer and subsequently snap-dried on a heating plate (200$^{o}$C for 10 sec).
8. A gal file describing the spotting run has to be generated.
9. All log files have to be copied to the run settings subfolder in the project folder.

**C. Quality control – scatter mode scan**

Upon completion of the spotting run, the slides are to be incubated at app 55% rH inside the arrayers and subsequently snap-dried on a heating plate (200$^{o}$C for 10 sec). Thereafter load the slides in slide holders of the Tecan Scanner – DNA Side facing upwards and scan all slides in the scatter mode. Use the scanning settings defined in the scatter.lsp but adjust the scan area according to the needs of the project.

**D. Slide processing**

prior to processing all slides should remain in the arrayers for at least 12 hours at 55% relative humidity.

**D1. Telechem Superamine substrates**

1. snap-dry the slide on a heating plate (200$^{o}$C for ~ 10 sec) DNA side up!
2. 2x times /Cross link (Stratagene, UV-Stratalinker) spotted target DNA to substrate surface at 1200 µjoules

3. In a fume hood: prepare the blocking solution by:
4. Dissolving 1g succinic anhydride (Sigma-Aldrich) in- 200ml anhydrous 1,2-dichlorethane (DCE; Fluka), and then add 2,5 ml N-methylimidazol (Fluka)
5. 1x incubate in blocking solution / at r.t. / for 1h (10 Slides/ Jar
6. 1x times / wash briefly in 200ml fresh DCE
7. 1x times / incubate in boiling water for DNA denaturation / for 2 min
8. 1x times / rinse briefly in 95% ethanol p.a.
9. Dry in centrifuge / Falcon Tubes, 130 rcf for 10min
10. QC 2 slides in a primer/ background hybridization (refer to protocol….)
11. Store the slides in a dry (descicator) environment at 5$^{o}$C.

**D2. Advalytix Epoxy Slides**

1. snap-dry the slide on a heating plate (200$^{o}$C for ~ 10 sec) DNA Side up!
2. Rehydrate the slides over night in a humid chamber (Incubator with an additional beaker of dd$H_2O$) at 42$^{o}$C.
3. Wash for 2 minutes in 0,5 % SDS.
4. Wash thoroughly in $H_2O$ (five times for 2 minutes on a rotary shaker, replace the water).
5. Incubate the slides in $H_2O$ (at 50$^{o}$C)
6. Dry slides by centrifugation / Falcon Tubes, 130 rcf for 10min
7. Quality control of two slides in a primer/ background hybridization (refer to protocol MPI/AG 004)
8. Store the slides in a dry (descicator) environment at 5$^{o}$C.

| Version | Tracking of changes | Name | Date |
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