Prehybridization

Prepare each microarray for hybridization as follows:

- Wash slides in SDS 0.2 % (3 ml of SDS 20X in 300 ml H_2O): submerge and take them out vigorously to remove molecules that have not been fixed to the slide.
- Incubate the slides at 55º C during 45' in prehybridization solution (To prepare 300 ml):
    - BSA 1% (3 g of BSA)
    - SSC * 5X (75 ml of SSC 20X)
    - SDS 0.1% (1.5 ml of SDS 20X)
    - H_2O Complete volume to 300 ml
- Wash slides in distilled water in slide baskets: Submerge slide basket and shake vigorously 20 times. Repeat 5 times using a new water each time.
- Wash slides once in isopropanol as before.
- Dry slides with a spin at 850 RPM 5 minutes.
- Hybridize immediately. Hybridization signal diminishes if slides are left for more than an hour.

Hybridization

Prepare hybridazation buffer 2X: (prepare it fresh for each hybridization)

- Formamide 50% (100 μL of formamide)
- SSC * 10 X (100 μL of SSC 20X)
- SDS 0.2% (2μL of SDS 20X)

- Adjust cDNA labeled to 25 μL.
- Add 25 μL of hybridazation buffer 2X.
- Incubate 3' at 95 º C
- Keep on ice
- Spin down tube contents
- Homogenize solution mixing up & down softly without introducing bubbles.
- Place microarray on a Corning® Hybridization Chamber (Product #2551). Cover microarray with a lifter slip (ErieScientific No. 25X601-2-4789).
- Add solution slowly pipeting at the lifter slip edge. Solution will go under lifter slip by capillary.
- Incubate 14-16 hours at 42ºC in hybridization chamber humidified with hybridazation buffer 1X.

Wash

Wash slides during 5 minutes in the following solutions with constant agitation (protect slides from light at all times with paper foil)

Solution 1: (Preheat to 55ºC)

- SDS 1% (2 ml of SDS 20X)
- SSC 2X (40 ml of SSC 20X)
- H_2O (358 ml)

Solution 2: (prepare two containers of wash solution 2, wash briefly slides on first container and then incubate 5 minutes on the second container to avoid SDS carry over from wash sln. 1)

- SSC 1% (20 ml of SSC 20X)
- H2O (380 ml)

Solution 3

- SSC 0.1 % (2 ml de SSC 20X)
- H_2O (398 ml)

Spin at 850 RPM 5 minutes to dry slides. Scan as soon as possible (microarrays can be scanned up to 2 weeks after, without loosing too much signal)