



RESOURCES

Spotted Microarray Hybridization and Scan

Setup Hybridization

Note: for single spotted arrays halve volumes

- Mix two color samples (for genomic DNA, Normally 0.5ug), add 2ml Block Mix (10mg/ml salmon sperm DNA (BRL) + 4mg/ml yeast tRNA)
- Use Speed Vec to dry the sample to a small volume, normally less than 5ml, do NOT overdry.
- Add 50ml Sigma ArrayHyb™ Hybridization Buffer
- Heat mixture to 100°C 5 minutes, mix by pipetting up and down if necessary
- Spin for 5 minutes at 14,000 RPM to pellet any particulate material
- Place the Microarray(s) in a hybridization chamber (TeleChem or GeneMachines)(To prevent arrays from drying, seal array in hybridization chamber with Whatmann paper moistened with 100 ml of H₂O.)
- Carefully transfer the probe solution to a new tube avoiding any pelleted material.
- Carefully pipette the hybridization mix onto the array (between marks etched on back of array)
- Overlay with a clean glass cover slip being careful to avoid bubbles under the cover slip
- Seal chamber and place in 60°C water bath. Incubate 18-20 hours

Wash Slides

- Dry the exterior of hybridization chamber
- Carefully open hybridization chamber to avoid H₂O entering the chamber.
- Remove slide from the chamber and quickly place the slide, array side down, in a container filled with 0.2X SSC, 0.03% SDS and a slide holder. Position the slide upside down and at a slight angle so the coverslip falls away from the slide. Keep slide in this position until the coverslip has fallen off. When cover slip falls off, transfer the slide to the other wash chamber containing wash buffer 1 and incubate for 5min at room temperature with gentle mixing on an orbital shaker (speed 3-4).
- Transfer ONLY the slide to a container with 0.2X SSC (filtered) and a slide holder. Incubate for 5min at room temperature with gentle mixing on an orbital shaker (speed 3-4).
- Transfer the slide holder with the slide to fresh 0.2X SSC (filtered) and repeat wash 5min.
- Transfer the slide holder with the slide to 0.05X SSC (filtered) and wash for 45 sec.
- Spin in slide holder seated in a Plexiglass carrier on microtiter plate carrier for 5 minutes at 500 RPM to dry slide.

Scan slide.

Scan Slides

Currently we use Packard Science SA5000 scanner to scan our microarrays. to obtain good signal and low signal/noise, different PMT setting might be used. However, to compare probe quality and hybridization quality, it is suggested to scan all slide at the same power and PMT setting. Now we use the setting:

- For Cy3, which normally is the RNA channel, Power 85, PMT 75.
- For Cy5, which normally is the genomic DNA channel, Power 85, PMT 70.

Reagents and Suppliers

Hybridization Chambers	Telechem	Cat # AHC-1
ArrayHyb Buffer	Sigma	Cat # A-7718

Please let me know of any problems, suggestions or updates to this protocol.

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