

Hybridization and Washing

Aimée M. Dudley

Note: We use these hybridization and washing protocols for both 3D-link and polylysine slides.

Hybridization:

- Concentrate labeled cDNA samples to 26-36 microliters
- Add 2 microliters 10 mg/ml salmon sperm DNA
- Add 2 microliters 10 mg/ml polyadenylic acid
- Heat at 95° C 2-3 min. (Avoid long exposure to heat, since this results in some crossover between the Cy3 and Cy5 channels)
- Add an equal volume of 2X Hyb Buffer (50% formamide, 10X SSC, 0.2% SDS) pre-warmed to 42° C
- Pipette onto slide under Lifter cover slip ([Erie Scientific](#))
- Assemble CMT hybridization chamber ([Corning](#))
- Hybridize at 42-45° C 12-16 hours

Washing:

Note: It is very important not to let the slides dry at all prior to the centrifugation step; this will lead to high background.

- Disassemble the hybridization chamber
- Set up container with a large volume of the first wash solution and a slide washing rack
- Lift slide with cover slip into first wash buffer
- While keeping the slide submerged, tilt slide and allow the cover slip to slide off
- Again while keeping the slide submerged, place the slide into the slide washing rack
- Wash 5 min. at room temperature with shaking in 0.2X SSC/ 0.1% SDS
- Wash 5 min. at room temperature with shaking in 0.2X SSC
- Wash 5 min. at room temperature with shaking in 0.1X SSC
- All washes are large volumes (500 ml)
- Centrifuge dry
- Scan slides