Institute for Systems Biology
Hybridization of Yeast 70-mer oligo Microarrays
Secrets of the Pros that Improve Results
(Prepared by Todd Eckdahl; August 2003)

Slide Pretreatment

In order to redistribute the DNA on the slides – helps spot morphology and hybridization.

1. Steam the DNA side of the slide over boiling dH2O. Do not allow visible droplets to form on the slide.
2. Immediately place the slide (DNA side up) on a heat block or hot plate set to 100 C or slightly less to snap dry. Take off after 5 seconds.
3. Repeat steam step, followed by drying step. Allow the slide to sit on the heat block for 1 minute this time. Allow slide to cool.

Prehybridization and Blocking

1. Place slide into a 50 ml tube filled with warm (55 C) 3x SSC, 0.1% SDS, 0.1 mg/ml Sonicated Salmon DNA. The slide must be completely immersed. This solution can be re-used, and two slides can be placed back to back.
2. Agitate gently for 30-60 minutes at room temp.
3. Quickly transfer the slide to a 50 ml tube with dH2O. Dip several times.
4. Blow the slide dry with air. The idea is to chase all the drops of water off the slide while it is held at an angle on a towel. If drops of water start to dry in place on the array, quickly immerse the slide back into water and start again. You are not trying to blow dry the slide, rather you are trying to push the liquid away from the spots. If you see streaks at this stage, rewet the slide. If you see dried on streaks, you will have streaks in your final scan.

Labeled Sample Preparation and Hybridization

1. Combine 30 pmol of each dye into a single tube and Speed-vac to 1-2 ul. If sample dries, add 2 ul DEPC-H2O and let stand 2 minutes (slight warming is OK)
2. Add 40 ul of the following Hybridization Buffer
    a. 36.4 ul DIG Easy Hyb
    b. 1.8 ul of 10 mg/ml ss DNA (or ct DNA)
    c. 1.8 ul of 10 mg/ml yeast tRNA
    d. 0.14 ul of 1 ug/ul oligo dA (50)
3. Mix the sample and heat at 90 C for 1 minute.
4. Place on ice 1 minute.
5. Heat at 90 C for 5 seconds, then spin down. Keep in the dark under foil until used.
6. Blow dust off microarray slide if necessary.

7. Prepare cover slip by dipping in 100% isopropanol and blow off liquid. You want to blow liquid off slide more than dry it.
8. Pipette sample onto one end of the array. Place one end of the cover slip onto the end with the sample. Use a fine gauge needle to lower the other end (between 70% and 95% of the spots should be wetted) of the cover slip but raise it back up before the solution makes its way over the entire area. This serves to mix the sample while it is applied to the array.
9. Place the slide in a hybridization chamber and incubate at 37 C for 15-16 hours.

Posthybridization Washing

1. Heat 50 ml 1X SSC / 0.1% SDS and 0.5X SSC to 55 C.
2. Transfer slide to heated 1X SSC / 0.1% SDS (50 mL conical tube is good). Place slide with cover slip facing down and agitate gently until coverslip falls away from the slide. Pull up the slide just enough to make sure the coverslip is completely detached from the slide and resubmerge the slide. Remove the cover slip with a forceps. Agitate the slide gently for 5 minutes under foil.
3. Dump wash solution and fill immediately with fresh 1X SSC / 0.1% SDS at room temperature. Agitate gently for 5 minutes under foil. Do not let the slide dry at all.
4. Dump wash solution and fill tube with 55 C 0.5X SSC. Agitate gently for 5 minutes under foil, inverting the tube a couple of times.Do not let the slide dry at all.
5. Dump wash solution and fill tube with 0.1X SSC at room temperature. Agitate gently for 2 minutes under foil, inverting the tube a couple of times. Do not let the slide dry at all.
6. Transfer slide quickly to a 50 ml tube with dH2O.
7. Blow the slide dry with air by pushing the liquid away from the spots and towards the outer edges. The idea is to chase all the drops of water off the slide while it is held at an angle on a towel. If drops of water start to dry in place on the array, quickly immerse the slide back into water and start again.
8. Keep dry and in the dark until scanning.