Institute for Systems Biology
Hybridization of Yeast 70-mer oligo Microarrays

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Slide Pretreatment

Some slides need a pretreatment, others, such as those prepared for GCAT at Washington University, do not. This process is used to redistribute the DNA on the slides, and helps with spot morphology and hybridization.

1. Steam the DNA side of the slide over boiling dH_2O. 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^o 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^oC) 3x SSC, 0.1% SDS, 0.1 mg/ml sonicated salmon sperm (ss) or herring sperm (hs) or calf thymus (ct) DNA. The slide must be completely immersed. Two slides can be placed back to back (ie. arrays facing outward).
2. Agitate gently for 30-60 minutes at room temp.
3. Quickly transfer the slide to a 50 ml tube with dH_2O. Dip briefly several times.
4. Blow the slide dry with air (connect a piece of tubing with a filter tip on the end to house air or to a nitrogen tank). Don't blast the slide too hard or too long. Rather, 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.

   An alternative to blow drying is to place the slide into a 50 ml tube with a crumpled kimwipe in the bottom and spin 5 minutes at 500 rpm for 1 min in a clinical centrifuge.

Labeled Sample Preparation and Hybridization

1. Combine the two labeled cDNAs (30 pmol of each dye, or all of it) into a single tube and speed-vac to 1-2 μl. If sample dries, add 2 μl DEPC-H2O and let stand

2 minutes. If the pellet is still not dissolved, heat at 37°C for 5 minutes. Reminder: keep the Cy-labeled samples in the dark as much as is practical.

2. Add 40 µl of the following Hybridization Buffer
    a. 36.4 µl DIG Easy Hyb
    b. 1.8 µl of 10 mg/ml hs DNA (or ct DNA)
    c. 1.8 µl of 10 mg/ml yeast tRNA
3. Mix the sample and heat at 90°C for 1 minute.
4. Cool at room temp for 1 minute.
5. Spin down, then keep in the dark under foil until ready to pipette onto array.
6. Blow dust off microarray slide if necessary.
7. Apply sample to array by one of these methods:
    a. The preferred method is to pipette sample onto one end of the array (opposite the markings). Place one end of the cover slip onto the end with the sample. Use a fine gauge needle to lower the other end of the cover slip but raise it back up after the solution has made its way about half way, then allow it to settle on the array. This serves to mix the sample while it is applied to the array.
    b. Pipette sample along the length of the cover slip. Invert the microarray slide, make contact with the cover slip, then flip the slide back over.
8. Some chasing of bubbles can be done, but do not make major adjustments to the coverslip once it is on the array. You want to avoid scratching the array.
9. Place the slide in a hybridization chamber and incubate at 37°C for 15-16 hours (notes: different hybridization solutions require different hybe temps; the time of hybridization can be increased to 2-3 days if care is taken to prevent drying of the array in the chamber).

Posthybridization Washing

Caution: In order to protect the Cy dyes, keep the hybridized slides in the dark as much as possible. Use of aluminum foil sleeves on 50 ml tubes is recommended. Additional protection is afforded by the 1 mM DTT added to each wash solution. It is very important that the microarray not be allowed to dry between washes.

1. Heat 50 ml 1X SSC / 0.1% SDS / 1 mM DTT and 0.5X SSC / 1 mM DTT to 55°C.
2. Using forceps, transfer slide to heated 1X SSC / 0.1% SDS / 1 mM DTT (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. Transfer quickly to new tube with fresh 1X SSC / 0.1% SDS / 1 mM DTT at room temperature. Agitate gently for 5 minutes under foil. Do not let the slide dry at all.

4. Transfer quickly to new tube with 55^{o}C 0.5X SSC / 1 mM DTT. Agitate gently for 5 minutes under foil, inverting the tube a couple of times. Do not let the slide dry at all.
5. Transfer quickly to new tube with 0.1X SSC / 1 mM DTT 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. Dip slide quickly in a 50 ml tube with 1 mM DTT.
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. The alternative method, briefly centrifuging in a 50 ml conical is also possible at this step.
8. Keep dry and in the dark until scanning.