

Dunham Lab Slide Stripping Protocol

(Revised from Young Lab Protocol, March 2013)

Slides should be stripped shortly after being hybridized, within 3-4 days. Leaving the arrays for longer makes them more difficult to strip, and therefore easier to damage.

Make the following stock solutions using glass-distilled water:

1M K_2HPO_4 (87.09g/500mL, filter sterilize)
1M KH_2PO_4 (68.045g/500mL, filter sterilize)

From these, make your fresh slide stripping buffer:

76.2 mL of 1M K_2HPO_4
123.8 mL of 1M KH_2PO_4
top to 2L with glass distilled water
Filter Sterilize

Gather

3 slide washing glass trays with stirbars, and metal rack (optional step 1 only)
1 L glass beaker
500 mL glass beaker
1 thermometer with hanger (to read above 100C)
1 upright metal slide rack
Glass etching pen
Stirbar
1 heating stir plate-with a clean surface!
Tweezers with good grips

1. If you've used the Agilent Stabilization and Drying Solution on the slides, you should do an Acetonitrile wash first. If not, skip to #5. We haven't done this much in this lab, so if you develop a better protocol, please share it.
 - a. Set up 3 glass dishes filled $\frac{3}{4}$ with
 - i. Acetonitrile (stirring GENTLY)
 - ii. 100 mM potassium phosphate buffer, pH 6.6
 - iii. 100 mM potassium phosphate buffer, pH 6.6
 - b. Remove both the Agilent, and the barcode sticker from the slide, and etch the last 4 digits of the barcode on the Agilent/Array/Active side of the slide). Save the barcode sticker for later.
 - c. Load the arrays into the metal rack, and put it into the Acetonitrile bath, stirring GENTLY. Treat slides for 5 minutes. Absolutely no longer than 15 minutes.
 - d. Transfer slides to dish 2. This is just to dilute the acetonitrile away.
 - e. Immediately transfer slides to dish 3. Leave the slides in this buffer until ready to strip as described below.

2. Remove the foil from the 1 L beaker, and set aside. Pour 700 mL of the Slide Stripping Buffer into the beaker, and heat in the microwave on high for 4-5 minutes.
3. While the microwave is going, gather the array slides that you will be stripping (up to 4). If the slide has never been stripped, it will have two stickers. The 'Agilent' sticker is on the array side of the slide, and the barcode # is on the back. Use a razor blade to carefully lift the Agilent sticker, and discard it. Using the glass-etching pen, etch the last few digits of the slide's barcode where the Agilent sticker used to be (on the array side). Then use the razor blade again to carefully remove the barcode sticker, and set it aside for later.
4. Place the slides in the upright metal rack, using the end with the etching as the 'handle'. Be sure the slides are well spaced, and that the array side of the slide is not crowded.
5. After microwaving, the buffer should be around 70C. Use hot mitts to bring the beaker to the hot plate, and turn the heat on high. Add a stir bar and the array rack to the beaker, and adjust the placement and stirring speed so that the stirbar will not hit the arrays.
6. Add the thermometer to the side of the beaker, so that it is submerged in the buffer, but not touching the bottom. Cover the beaker with foil, and place a piece of Styrofoam on top for extra insulation as it heats.
7. Monitor the beaker's temperature, and start timing when the temp reaches 100C. When the buffer reaches a rolling boil (after @10 minutes), remove the Styrofoam and foil, and be prepared to lower the heat in order to prevent the temperature from exceeding 105C. Boil 10 minutes. Fill the 500mL beaker with 425 mL room temp buffer while it is boiling.
8. After 10 minutes, Use grippy tweezers to carefully remove the slide rack to the 500mL beaker with room temp buffer. Leave the slides to cool for 2 minutes, submerged in buffer. Then use the ArrayIt slide spinner to spin them dry, and put them in a slide box labeled appropriately.
9. Use the Axon scanner and Genepix to do a quick scan of the slides, to be sure the stripping worked. The probes on the slide should mostly be green, with dark background. Once stripped, they can be hyb'd right away, or stored for future use.
10. Be sure to put the slides back in the vacuum chamber (and vacuum it).