

## Agilent yeast DNA arrays (4x44k platform)

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hybrid of Brown and deRisi lab protocols and various Agilent 60-mer oligo microarray processing (SSPE wash) protocols

Remember that the arrays come 4 on a slide.

### DNA

This labeling protocol seems to be rather sensitive to DNA quality. I use the Winston and Hoffman prep with good results. If you're worried about quality, use the Zymo genomic DNA kit, or a Qiagen kit. The Zymo kit can also be used to make DNA from colonies.

Measure starting DNA concentration with a fluorometer. Once it's been purified with the Zymo columns, the spectrophotometer seems to be reasonably accurate.

### DNA Fragmentation via Sonication

Bring 4  $\mu\text{g}$  DNA to 100 $\mu\text{l}$  in a 1.5mL eppendorf tube.

Sonicate 30 pulses power level 1, 1 second on/1 second off.

Run 2  $\mu\text{l}$  on a gel to check fragmentation. Fragments should be a smear around 1 kb.

Purify with zymo DNA clean and concentrator 5 kit.

Elute in 20  $\mu\text{l}$  water.

Nanodrop 1  $\mu\text{l}$ .

DNA can be stored  $-20\text{C}$ .

### DNA labeling

Bring 2  $\mu\text{g}$  to 21 $\mu\text{l}$  with water.

Add 20  $\mu\text{l}$  2.5X random primer/reaction buffer mix:

	STOCKS:	1X:
125mM Tris pH 6.8	0.5M Tris, pH 6.8	5 $\mu\text{l}$
12.5mM MgCl <sub>2</sub>	50mM MgCl <sub>2</sub>	5 $\mu\text{l}$
25mM 2-mercaptoethanol	0.143M 2-mercaptoethanol (dilute fresh)	3.5 $\mu\text{l}$
750 $\mu\text{g}/\text{mL}$ random nonamers	5 $\mu\text{g}/\mu\text{l}$ random nonamers	3 $\mu\text{l}$
	Water to 20 $\mu\text{l}$	3.5 $\mu\text{l}$

95°C 5 minutes.

Ice 5 minutes.

Add 5µl 10X dNTP mix (in TE, pH 8.0):  
 For dCTP Cy dyes: 1.2mM each dATP, dGTP, dTTP, and 0.6mM dCTP  
 For dUTP Cy dyes: 1.2mM each dATP, dGTP, dCTP, and 0.6mM dTTP  
 Add 3µl appropriate Cy-dNTP.  
 Add 1µl Klenow (5U/µl).  
 Incubate 37°C for 2 hours.  
 Add 5µl 0.5M EDTA pH 8.0.  
 Ice.  
 Purify using zymo columns with 0.5 ml binding buffer.  
 Elute in 25 µl water.  
 Nanodrop 1 µl to check yield and dye incorporation.  
 Dye incorporation should be ~20 pmol/µg.

## Hybridization

You may want to randomize the arrays that are neighbors on the arrays. A simple way to do this in Excel is to list the samples in one column and the function =RAND() into each cell in the neighboring column. Copy the random number column, then, with the column still selected, paste special -> values so that the cells won't recalculate. Then, sort both columns by the random number. List the arrays in a third column next to the sorted list. A01 is near the barcode and A04 is farthest from the barcode.

Final hybe reaction will be

Cy3-labeled DNA	100ng
Cy5-labeled DNA	100ng
Water	to 44µl
10X Agilent Blocking Agent	11µl
2X Hi-RPM hybridization buffer	55µl
<b>TOTAL VOLUME</b>	110µl
<b>VOLUME LOADED</b>	100µl

Add 100ng Cy3-labeled DNA and 100ng Cy5-labeled DNA  
 Add water to bring the total volume to 44µl  
 Add 11µl Agilent 10X Blocking Agent (prepare 10X Blocking Agent according to instructions that came with the tube).

95°C for 5 minutes (process only 4 samples at a time)  
 Cool @ room temperature for 5 minutes  
 Add 55µl 2X Hi-RPM hybridization buffer, mix by pipetting  
 Spin down  
 Load 100µl onto the gasket slide:  
 Place a backing slide, Agilent side up, in a hybe chamber.

Pipet the whole volume of probe, avoiding bubbles, onto the center of one gasket area. Don't eject the last  $\mu$ l or two in order to avoid bubbles. Spread it around as you pipet, but not too close to the gasket.

Do the same for the other gasket area with the next probe.

Remove the array from the box. The Agilent side is the Array side, and so should face down, onto the probe. Carefully lower the array over the gasket slide, keeping it flat.

Once the array is resting on the gasket slide, place the top of the hybe chamber, and slide the screw over the assembly. Tighten the screw all the way down, finger tight.

Look through the back of the chamber and rotate the slide. There should be one big bubble that moves freely. There may be one big bubble and a couple of little ones stuck to the sides. If they are small and isolated, don't worry too much about them. You will probably do more harm than good trying to remove them. If they seem like they'll interfere with the array, you can try knocking the array with the heel of your hand to dislodge them.

Put the array in the hybe oven. Make sure to balance the rotisserie.

Hybe 17 hrs. @ 65°C, 20RPM

## Washing

Prepare your wash solutions. Be aware of array materials that may be for RNA only use.

Wash A (1 L)

add in this order:

700 ml	Water
300 ml	20X SSPE
0.25 ml	20% N-lauroylsarcosine

Filter. Shake to mix.

Wash B (1 L)

add in this order:

997 ml	Water
3 ml	20X SSPE
0.25 ml	20% N-lauroylsarcosine

Filter. Shake to mix.

Rinse the wash chambers, racks, and stirbars with water.

Set up:

two Wash A chambers, one with a rack and a stirbar on a stirplate.

one Wash B chamber with a stirbar on a stirplate.  
one acetonitrile chamber with a stirbar on a stirplate.

For all stirring steps, the wash liquid should be visibly turbulent. Make sure the entire slide is submerged at all times.

Disassemble each hybe chamber one at a time. Use the plastic tweezers to gently wedge open the sandwich while submerged in Wash A. Transfer slide to the rack in the other Wash A chamber. Leave a gap between each slide and between the slides and the wall.

Once all the slides are in the rack, stir for 1 min.

Start stirring Wash B.

Transfer the rack into Wash B and stir for exactly 1 min. Don't worry about transferring some Wash A into Wash B.

Start stirring the acetonitrile.

Quickly transfer the rack into the acetonitrile, draining off some of the Wash B as you go.

Let stir 30 sec. Slowly and evenly pull the rack out of the acetonitrile. If you see droplets remaining on the slides, submerge them and try again.

Set the rack on a kimwipe.

Load the slides into scanning holders, Agilent side up and barcode sticking out, blotting excess acetonitrile if necessary. The scanner scans through the back of the slide. Don't touch anywhere but the edges and the barcode.

Scan no more than 5 slides at a time to avoid ozone in the scanner.

You can reuse the wash buffers for more slides, replacing the first Wash A for every batch.

## **Scanning**

Open the Agilent scan control program. If the lasers refuse to warm up, power cycle the scanner.

Place the slides in the scanner, noting the slot numbers.

Select the appropriate slot numbers from the pulldown menus on the upper left.

Select the directory column and click edit values. Browse to find the directory you want to save in. Hit set. The column values should change.

Check the default preferences for the correct scanning area (61 x 21.6 mm), resolution (**5  $\mu\text{m}$** ), laser power (100% each) and with the split and rotate box not checked.

Scan.

Open the scanned tif with the Agilent feature extraction software.

Run the appropriate protocol.

Check the visual results to make sure it looks ok. I usually check that it's aligned properly, and that the flagged spots make some sense. If you get a larger than usual number of outlier spots, make a note of it.