Agilent yeast DNA arrays

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

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 (Yeastar), 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 µg DNA to 350µl in a 1.5mL eppendorf tube.
Sonicate using Program 2, which is Amplitude=40, 15 seconds with 1 second on/1 second off. Don't touch the probe to the bottom of the tube!
Run 10 µl on a gel to check fragmentation. Fragments should be a smear around 750 bp.
Purify with Zymo DNA clean and concentrator 5 kit.
Elute in 20 µl water.
Nanodrop 1 µl, and store in –20C.

DNA labeling

Random primer reaction mix (per Rxn):

	STOCKS:	1X:
125mM Tris pH 6.8	0.5M Tris, pH 6.8	5 µl
12.5mM MgCl2	50mM MgCl2	5 µl
25mM 2-mercaptoethanol	0.143M 2-mercaptoethanol (dilute fresh, 1:100 of stock)	3.5 µl
750µg/mL random nonamers	5µg/µl random nonamers	3 µl
	Water to 20 µl	3.5 µl

Bring 2 µg DNA to 21µl with water, and add 20µl of Rxn mix.
95°C 5 minutes.
Ice 5 minutes.

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

Hybridization

Since we borrow the Eichler Lab scanner, HYB chambers and oven, you'll need to coordinate with them, to be sure they will be available for you. Always be courteous.

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.

Thaw 10X Agilent blocking agent (in –20C Labeling Box). If there is not a frozen aliquot, there should be a fresh tube in the RNAse free supplies. Add 1250µl* RNAse free water to the lyophilized pellet. Vortex and incubate at 37C until pellet is resuspended.
*We buy the Large volume Hi-RPM kit, the amount of water will be different if you're using the regular sized kit.

Handle all reagents as for RNA.
Choose a table below to make appropriate sized HYB Cocktail.
process only 4 samples at a time.

1 x 244k

Cy3-labeled DNA	490 ng
Cy5-labeled DNA	490 ng
Add Water	to 255 µl total

Add 10X Agilent Blocking Agent	5.2 µl
95°C for 5 minutes, Cool to RT	
Add 2X Hi-RPM hybridization buffer	260 µl
Mix by pipetting, Spin 1min, 13k rpm	
TOTAL VOLUME	520 µl
VOLUME LOADED	490 µl

2 x 105k

Cy3-labeled DNA	245 ng
Cy5-labeled DNA	245 ng
Add Water	to 104 µl total
Add 10X Agilent Blocking Agent	26 µl
95°C for 5 minutes, Cool to RT	
Add 2X Hi-RPM hybridization buffer	130 µl
Mix by pipetting, Spin 1min, 13k rpm	
TOTAL VOLUME	260 µl
VOLUME LOADED	245 µl

4 x 44k

Cy3-labeled DNA	100 ng
Cy5-labeled DNA	100 ng
Add Water	to 44 µl total
Add 10X Agilent Blocking Agent	11 µl
95°C for 5 minutes, Cool to RT	
Add 2X Hi-RPM hybridization buffer	55 µl
Mix by pipetting, Spin 1min, 13k rpm	
TOTAL VOLUME	110 µl
VOLUME LOADED	100 µl

8 x 15k

Cy3-labeled DNA	100 ng
Cy5-labeled DNA	100 ng
Add Water	to 20 µl total
Add 10X Agilent Blocking Agent	5 µl
95°C for 5 minutes, Cool to RT	
Add 2X Hi-RPM hybridization buffer	25 µl
Mix by pipetting, Spin 1min, 13k rpm	
TOTAL VOLUME	50 µl
VOLUME LOADED	40 µl

Place a gasket slide, Agilent side up, in a hybe chamber.
Pipet the appropriate volume of probe, avoiding bubbles, onto the center of one gasket area. Don't eject the last µl 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. For new arrays, the Agilent side is the Array side, and so should face down, onto the probe. However, some arrays have been stripped, and those have only one sticker which is on the array side of the slide. Carefully lower the array over the gasket slide, keeping it level.
Once the array is resting on the gasket slide, put the top of the hybe chamber on, and slide the screw assembly on. Tighten the screw all the way down, 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, making sure to balance the rotisserie.

Hybridize at 65°C, 20RPM.
40 hours for 1 x 244k and 2 x 105k arrays
24 hours for 4 x 44k, and 8 x 15k arrays

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
0.5 ml	10% N-lauroylsarcosine

Filter. Shake to mix.

Wash B (1 L)
add in this order:

997 ml	Water
3 ml	20X SSPE
0.5 ml	10% N-lauroylsarcosine

Filter. Shake to mix.

Rinse the wash chambers, racks, and stirbars with di water.
Set up:
One Wash A chamber for disassembling the sandwich (a small jar or beaker works- just be sure that the sandwich can be submerged).
One Wash A chamber 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
One optional chamber with Agilent's Stabilization and Drying Solution.
Only do this one if the ozone is above 30ppb.
Check by Going to this website
(http://www.pscleanair.org/airq/ozonetrend/default.aspx?area=Beacon+Hill)

All the washes should be stirring so that they are visibly turbulent (Setting 7). Make sure the entire slide is submerged at all times.

Disassemble each hybe chamber one at a time using 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.
Transfer the rack into Wash B and stir for exactly 1 min. Don't worry about transferring some Wash A into Wash B.
Quickly transfer the rack into the acetonitrile, draining off some of the Wash B as you go.
Let stir 30 sec (only 10 sec if using Stabilization and drying solution, then Quickly transfer to Stabilization and Drying solution, and stir for 30 sec).
Slowly and evenly pull the rack out of the final wash chamber (acetonitrile, or acetonitrile based Stabilization and drying solution). 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, Active side up (If the slide has 2 stickers, the Agilent side is the Active side. If the slide has been stripped, there will only be one sticker and it will be on the active side). 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 in the same day, replacing the first Wash A for every batch.

Scanning

Open the Agilent scan control program. Wait a minute to be sure it can find the scanner, so it can begin warming up. It takes about 20 minutes.

Place the slides in the scanner. Take note of which barcodes are in which slot.
Select the appropriate slot numbers from the pulldown menu 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 µm**), laser power (100% each) and with the split and rotate box not checked.
Scan.
Open the scanned tif with the Agilent feature extraction software.
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.
Run the appropriate protocol.

Type of Array	Protocol
8 x 15k Gene Expression	GE2-v5_95_Feb07
4 x 44k chip-chip	cgh_105_Dec08-CP
2 x 99k S. bayanus (design id 017940)	CGH-v4_10_Apr08
1 x 244k Hybrid array (design id 019037)	

If you get a larger than usual number of outlier spots, make a note of it.