Yeast homemade array CGH

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hybrid of Brown and deRisi lab protocols and Agilent 60-mer oligo microarray processing 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, 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 100µl in a 1.5mL eppendorf tube.
Sonicate 30 pulses power level 1, 1 second on/1 second off.
Run 2 µl 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 µl water.
Nanodrop 1 µl.
DNA can be stored –20C.

DNA labeling

Bring 2 µg to 21µl with water.
Add 20 µl 2.5X random primer/reaction buffer mix:

	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)	3.5 µl
750µg/mL random nonamers	5µg/µl random nonamers	3 µl
	Water to 20 µl	3.5 µl

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.

Hybe

Mix 1 µg each Cy3 and Cy5 reactions. Bring to 160 µl total with water.
Add 40 µl 10X Agilent block.

Stagger the following steps if you are doing a lot of arrays.
Incubate 95°C 5 minutes.
Incubate RT 5 minutes. Add 200 µl Agilent 2X hybe buffer. You can start adding the hybe solution while the samples are cooling.
Place a single array backing slide, Agilent side up, in a hybe chamber.
Pipet the whole volume of probe, avoiding bubbles, onto the center of the 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.
Remove a crosslinked and blocked array from the box. The barcode 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 quickly rotate the slide to wet all the gaskets. 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, 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 65°C overnight at rotation setting 4.
Prepare for the washes by placing a bottle with 500 ml Wash 1 in the 65°C waterbath.

Washes

Prepare your wash solutions. All solutions should be filtered.

Wash 1 (1 L)

940 ml	MilliQ water
50 ml	20X SSC
10 ml	10% SDS

Wash 2 (1 L)

950 ml	MilliQ water
50 ml	20X SSC

Wash 3 (1 L)

995 ml	MilliQ water
5 ml	20X SSC

Set up a glass chamber with enough Wash 1 to submerge a slide and a 500 ml beaker with the vertical rack with the bent hook and enough Wash 1 to cover the racked slides.
Pour the warmed Wash 1 into a 500 ml beaker in the 65°C bath. Place it on the submergible stirplate and add a stir bar. Keep it covered with aluminum foil.
In the plastic tubs with lids, pour ~500 ml Wash 2 plus a horizontal rack and another with ~500 ml Wash 3.

Disassemble each hybe chamber one at a time. Use the plastic tweezers to gently wedge open the sandwich while submerged in the chamber of Wash 1. Transfer slide to the rack in RT Wash 1 beaker, with a space between each slide (maximum 6 if you place them back to back).
Once all the slides are in the rack, hook the rack on the side of the 65°C Wash 1 beaker.
Stir 10 minutes with enough agitation that the solution is visibly turbulent.
Transfer slides to the rack in Wash 2.
Shake 10 minutes on the orbital shaker.
Transfer rack to Wash 3.
Shake 5 minutes on the orbital shaker.
Spin the slides dry (~15 seconds) one at a time in the little benchtop slide spinner.

Load the slides into scanning holders, barcode side up and barcode sticking out. The scanner scans through the back of the slide. Don't touch anywhere but the edges and the barcode.
Scan at 5 μm resolution.
Scan no more than 6 slides at a time to avoid ozone in the scanner.
Stagger the washes if you're doing multiple batches.
Grid with Genepix.

Slide crosslinking and blocking

Crosslink Corning slides at 700 energy on Donna's Stratalinker.
Make 600 ml 5X SSC, 0.1% SDS, and 6 g Roche Blocking Reagent (1096176).
Warm 65C with occasional agitation until dissolved.
Pour into a 500 ml beaker and warm until 65C.
Add the crosslinked slides in a vertical hanging rack and stir 35 minutes at 65C on submergible stirplate.
Move slides one by one into a new rack in RT water in a tupperware.
Wash on orbital shaker for 5 minutes.
Spin dry.
Use the same day, maybe the next only if absolutely necessary.