Agilent yeast cRNA arrays
8x15k platform

Use RNase free solutions and plastics throughout.
Remember that the arrays come 8/slide.

RNA

- If starting with crude total RNA, clean up an aliquot with a Qiagen RNeasy column. (15uL)
- Bioanalyze for quality
- Measure concentration with nanodrop.
- Make a 100 ng/µl stock of total RNA.

RNA labeling

The amplification/labeling is done per Agilent instructions with half volume reactions and a half amount of recommended dye.

- Aliquot 4.15 µl (415 ng) total RNA into an eppendorf tube.
- Add 0.6 µl T7 Promoter Primer.
- 65C 10 min
- Ice 5 min

Warm 5X first strand buffer at 80C, with occasional vortexing, until it completely dissolves (3-4 min).
Prepare cDNA master mix, in this order at RT:

1X	
2 µl	5X first strand buffer
1 µl	0.1 M DTT

0.5 µl	10 mM dNTP mix
0.5 µl	MMLV RT
0.25 µl	RNaseOUT

- Add 4.25 µl to each reaction. Mix well
- 40C 2 hours
- 65C 15 min
- Ice 5 min

Warm 50% PEG at 40C until it's resuspended and easy to pipet. (about 1-2 minutes)
Prepare transcription master mix, in this order at RT: Add the appropriate dye to your master mix as that yields better dye incoporation. Make 2 master mixes if using both dyes at the same time.

1X	
8.25 µl	water
10 µl	4X transcription buffer
3 µl	0.1 M DTT
4 µl	NTP mix
3.2 µl	50% PEG
0.25 µl	RNaseOUT
0.3 µl	inorganic pyrophosphatase
0.4 µl	T7 RNA polymerase
1.2 µl	Cy3-CTP or Cy5-CTP

- Add 30.0 µl to each reaction.
- Mix by pipetting.
- 40C 2 hours in the dark

Purify with an RNeasy column. All spins are full speed:

- Bring reaction to 100 µl with 60 µl water.
- Add 350 µl Buffer RLT and mix.
- Add 250 µl ethanol and mix by pipetting.
- Add mix to column.
- Spin 30 sec. The filter should be tinted.
- Move column to new collection tube.
- Add 500 µl Buffer RPE.
- Spin 30 sec. Discard flowthrough.
- Add 500 µl Buffer RPE. Spin 2 min
- Move column to a new collection tube
- Spin 1 min
- Move filter to an elution tube
- Add 30 µl water directly to the membrane. Let sit RT 1 min.
- Spin I min.
- Nanodrop 1.5 ul to check yield and dye incorporation.
- Store RNA at -20C, downstairs.

Hybridization (see sample worksheet)

- Find the amount of sample that gives 2.5-5 pmol dye
- Determine how many ng are in that amount.
- Mix the red and the green reactions such that there is at least 2.5 pmol dye in each channel and there is the same amount of cRNA in each channel. This means that one channel with have more than 2.5 pmol dye
- Final probe will consist of:

Cy3-labeled DNA	at least 2.5pmol
Cy5-labeled DNA	at least 2.5pmol
Water	to 20.9
10X Agilent Blocking Agent	5.5µl
25X Fragmentation Buffer	1.1µl
2X Hi-RPM hybridization buffer	27.5µl
TOTAL VOLUME	55µl
VOLUME LOADED	50µl

- Prepare 10X Agilent blocking agent per tube directions.
- Add 5.5 µl 10X blocking agent to each tube.
- Mix.
- Add 1.1 µl 25X fragmentation buffer. Mix.
- 60C 30 min in the dark
- Add 27.5 µl 2X Hi-RPM hybridization buffer to stop the reaction.
- Mix by pipetting.
- Do a hard spin for 1 min to reduce bubbles
- Place a backing slide, Agilent side up, in a hybe chamber.
- Pipet 50 µl of probe, avoiding bubbles, onto the center of one gasket area. Don't eject the last µl or two in order to avoid bubbles, but don't skimp on the volume or you'll get a hole in the center of the array. Spread the probe around as you pipet, but not too close to the gasket.
- Do the same for the next 7 samples.
- Remove the array from the box. The Agilent side is the Array side. 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. (but not too 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 on the bench to dislodge them. (have someone show you this before you do it!)
- Put the array in the hybe oven. Make sure to balance the rotisserie.
- Hybe 65C for 17 hours at 10 RPM.

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.

Scanning

- Open the Agilent scan control program. If the lasers refuse to warm up, power cycle the scanner. (you will have to do this if you are the first person scanning in the morning)
- Place the slides in the scanner, noting the slot numbers. (they only go in one way)
- Select the appropriate slot numbers from the pulldown menus on the upper left.
- Go to edit
- Select the directory column and click edit values. Browse to find the directory you want to save in (your file on the D drive—after scanning you have to send the files to the server and delete them from the D drive). 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—UNLESS you have a decent amount of dye in one channel, then do the Extended Dynamic Scan making sure to check the box in default settings AND on the scan screen) and with the split and rotate box not checked.

- Scan.
- Open the scanned tiff with the Agilent feature extraction software.
- Check the image to ensure everything looks alright…no bubbles etc
- Using Tiff Splitter, split the Tiff
- Run the appropriate feature extractor protocol. You can extract your files back to the server.