Yeast Microarray: Overview

1. Order the appropriate materials (updated pricing can be obtained from Donna Storton) by emailing the Princeton Microarray facility (genomics-core@genomics.princeton.edu). Order in advance, as it can take up to 2 weeks for some items to arrive. Listed below are common items for performing yeast microarrays (product pricing information from December 2012).

Part Number	Description	Cost
NEL581	Cy5-CTP (10 uL, 100 nmol; 10.0 mM)(ex: 650, em: 668)	$255
NEL580	Cy3-CTP (10 uL, 100 nmol; 10.0 mM)(ex: 550, em: 568)	$255
GASKET4	Backing slides 4 chamber (for 4x44 arrays)	$25.50
GASKET8	Backing slides 8 chamber (for 8x15 arrays)	$25.50
G2509F	Yeast gene expression microarray *8x15k; AMADID: 017566*	$854
G2519F	Yeast gene expression microarray *4x44k; AMADID: 015072*	$750
5190-0447	Quick Amp Labeling Kit, no dye *Contains reagents for ~40 reactions using our protocol*	$696
5190-0404	Hi-RPM Gene Expression Hybridization Kit *Contains enough Fragmentation Buffer, Blocking Agent, and Hybridization Buffer for ~1,000 reactions*	$900
5185-5974	25X Fragmentation Buffer (10 mL)	$214
5188-5281	10X Gene Expression Blocking Agent	$375
5190-0403	2X Hi-RPM Hybridization Buffer (25 mL)	$428
5188-5325	Gene Expression Wash Buffer 1 (4 L)	$150
5188-5326	Gene Expression Wash Buffer 2 (4 L)	$150
5188-5327	Gene Expression Wash Pack *2 L of #1, 1 L of #2*	$280

2. Isolate RNA from yeast cells.
   Use Yeast RNA Extraction protocol and Qiagen RNeasy Clean-up protocol

3. Convert mRNA into dye-labeled cRNA using an Agilent Quick Amp Labeling Kit.
   Use Fluorescent Labeling of yeast RNA for use with Agilent cRNA Arrays protocol and Qiagen RNeasy Clean-up protocol

4. Hybridize labeled cRNA to microarrays, then wash to remove non-bound RNAs and background, and scan the microarray using an Agilent High-Resolution Microarray Scanner.
   Use RNA Hybridization and Washing protocol and Microarray Scanning protocol

5. Use "feature extraction" software to convert spot positions into gene names
   Use Microarray Feature Extraction protocol

6. Analyze microarray data
   There are many options for analyzing the microarray data, the most common in our lab is to use Princeton University MicroArray database (this software can analyze, store, export, and visualize the data). PUMA has a "load data" section for loading individual array information or a batch of arrays. Details for doing this and performing the analysis can be found on the PUMA website.

   One common approach used in our lab (though it depends on the type of data analysis desired) is to "floor" the data (typically using an intensity of 350), so that anything less than the floor is set to the floor value… this prevents artificially large changes in gene expression due to random noise of small numbers.

NOTE: Steps #3 - #5 require advanced sign-up to use microarray facility equipment.

Yeast RNA Extraction
Cell samples are frozen at -80°C in locking-cap 2 mL tubes (either on filters, or just as cell pellets).

1. Before cells thaw, add 400 μL of Lysis Buffer. Vortex to mix cells with buffer.

2. Add 500 μL of phenol (saturated with 0.1 M citrate buffer; from Sigma-Aldrich, P4682), vortex to mix.

3. Incubate for 30 minutes at 65°C, vortexing every 5-10 minutes (filter may or may not dissolve… either way will not affect yield).
   I vortex for 10 second increments with 2 samples at a time to make sure all samples are well mixed

4. Chill samples on ice for 10 minutes.

5. While samples are on ice, spin 2 mL heavy phase lock gel (PLG) tubes for 30 seconds at full speed at room temperature. Set aside.

6. Spin lysate at full speed at room temperature for 5 minutes.

7. Transfer top aqueous layer to the PLG tube.

8. Add 500 μL of chloroform. Invert to mix (Do not vortex!). Spin at maximum speed for 3 minutes.

9. Remove aqueous layer to a new 1.5 mL microcentrifuge tube containing 40 μL of 3 M sodium acetate (pH = 5.2). Invert a few times to mix.

10. Add 900 μL of ice-cold 100% ethanol. Invert a few times to mix.

11. Incubate at -20°C for at least 30 minutes (overnight to achieve highest yield).

12. Centrifuge samples at full speed for 5 minutes (at room temperature or 4°C).

13. Wash pellet once with ice-cold 70% ethanol, pulse-spin, aspirate remaining ethanol.

14. Resuspend RNA pellet in 100 μL of water.
    Can leave at room temperature or on ice while working with it, but store at -20°C or -80°C.

15. Nanodrop to determine RNA concentration (typical yield ~700 ng/μL for 5 mL of cells with a Klett value of ~150).

16. If using RNA for microarray analysis, clean it up with a Qiagen RNeasy Kit (Amy Caudy found that this increases labeling efficiency from ~70% of reactions working to 100% of reactions working). After clean-up, Nanodrop to determine concentration again, and dilute to 100 ng/μL for use in the labeling reaction. Note that the RNeasy clean-up will reduce yield by about 2-4 fold.

Lysis Buffer for RNA (make fresh)

	For 100 mL
10 mM EDTA	2 mL of 0.5 M
0.5% (17.3 mM) SDS	5 mL of 10% (346 mM)
10 mM Tris (pH = 7.5)	1 mL of 1 M

Qiagen RNeasy Clean-up
RNeasy Mini Kit (50 reactions); Cat. No. 74104; $240.00
RNeasy Mini Kit (250 reactions); Cat. No. 74106; $1,017.90
We use this protocol to clean up RNA both before labeling for microarray analysis (Amy Caudy found that doing this increased her labeling efficiency from ~70% of reactions working to 100% of reactions working) and also after labeling to separate the labeled probe from unincorporated dye.

NOTE: Buffer RLT contains salt that can precipitate when added to a cold sample. Warm this buffer at 37°C – 42°C for a few minutes before use. Buffer RPE is supposed to get rid of the salt, but doesn't always work well (which is why this protocol uses two RPE washes).

1. Bring sample volume to 100 μL with RNase-free water.

2. Add 350 μL of Buffer RLT and mix by pipetting up and down.

3. Add 250 μL of 100% ethanol to the sample and mix by pipetting up and down.

4. Transfer sample (700 μL) to RNeasy mini spin column placed into a 2 mL collection tube. Close the lid and spin for 30 seconds at maximum speed. Discard flow-through.
   NOTE: If you are purifying labeled probe, at this point the filter in the column should have a colored tint (that is the labeled RNA) which is a preliminary indicatory that the labeling reaction worked.

5. Add 500 μL of Buffer RPE to the spin column. Close the lid and spin for 30 seconds at maximum speed. Discard flow-through.

6. Add 500 μL of Buffer RPE to the spin column. Close the lid and spin for 2 minutes at maximum speed. Discard flow-through.

7. Centrifuge again at maximum speed for 1 minute, then discard the collection tube.

8. Place the spin column into a fresh microcentrifuge tube. Add 50 μL of RNase-free water, close the lid and let sit for 1-3 minutes. Then centrifuge for 1 minute at maximum speed.

9. Use the Nanodrop spectrophotometer to quantify RNA yield (and dye incorporation, if you were purifying labeled RNA).
   - For clean-up of extracted RNA (which has a typical yield of 700 ng/μL), the concentrated cleaned RNA has a typical yield of 350 ng/μL. This RNA should be diluted to 100 ng/μL for use in the labeling reaction, if microarray analysis is the intended application.
   - For clean up of dye-labeled RNA, typical dye incorporation is 4 pmoles/μL; typical RNA yield is 300 350 ng/μL.

10. Store cleaned RNA at -20°C until use.

NOTE: As with any nucleic acid preparation, the 260/280 ratio is indicative of nucleic acid:protein content. Ideally will be greater than 1.8 (indicating a clean preparation). The 260/230 ratio is also a measure of nucleic acid purity. Common contaminants that absorb near 230 nm include EDTA, carbohydrates, and phenol. Again, this ratio will ideally be greater than 1.8 in a pure sample.

Fluorescent Labeling of Yeast RNA for use with Agilent cRNA Arrays

RNA solutions should be prepared with a concentration of 100 ng/μL. If this is the case, this protocol should take approximately 8 hours (resulting in purified, fluorescent RNA).

All temperature incubations can be performed in thermocycler blocks. Use of the hot lid also negates the need to spin samples after each incubation.

Sign-up in advance to use the array facility thermocyclers.

1. Aliquot 4.15 μL (415 ng) total RNA into a PCR tube.

2. Add 0.6 μL of T7 promoter primer. Mix well by pipetting up and down.

3. Place at 65°C for 10 minutes (*to denature RNA secondary structure and allow the primer to bind*).

4. Chill on ice for 5 minutes.

5. Prepare cDNA master mix (in the order listed below) at room temperature:
   *Warm buffer before use at ≥80°C for 3-4 minutes with occasional vortexing.
   **Moloney Murine Leukemia Virus Reverse Transcriptase
   NOTE: Make sure to use the RT enzyme and *NOT* the RNA polymerase for this reaction!
   NOTE: Make sure to use the dNTP mix and *NOT* the NTP mix for this reaction!

	1X	3X	10X	50X	58X
5X First Strand Buffer*	2.0 μL	6 μL	20.0 μL	100 μL	116 μL
0.1 M DTT	1.0 μL	3 μL	10.0 μL	50 μL	58 μL
10 mM dNTP mix	0.5 μL	1.5 μL	5.0 μL	25 μL	29 μL
MMLV RT**	0.5 μL	1.5 μL	5.0 μL	25 μL	29 μL
RNaseOUT	0.25 μL	0.75 μL	2.5 μL	12.5 μL	14.5 μL

6. Add 4.25 μL of cDNA master mix to each reaction. Mix well by pipetting up and down.

7. Incubate at 40°C for 2 hours.

8. Incubate at 65°C for 15 minutes.

9. Chill on ice for 5 minutes.

(TURN)

10. While performing some of the above incubations, prepare the transcription master mix below at room temperature in the order listed (except for the dye – because the dye is extremely ozone sensitive and also light sensitive, it will be added last in the microarray facility in the basement).
    *Warm the 50% PEG at 40°C for a couple minutes before use (for ease of pipetting)
    **By degrading the pyrophoshpate by-products of nucleotide chain synthesis, this reagent strongly promotes the forward reaction of nucleotide chain synthesis.
    ***Add in the ozone-free environment of the microarray facility (with the lights dimmed).
    NOTE: Cy3 (red) is generally used for reference RNA, Cy5 (blue) for experimental RNA
    NOTE: Make sure to use the RNA polymerase and *NOT* the RT enzyme for this reaction!
    NOTE: Make sure to use the NTP mix and *NOT* the dNTP mix for this reaction!

	1X	3X	10X	25X	30X	50X
Water (nuclease-free)	8.25 μL	24.75	82.5	206.25	247.5	412.5
4X Transcription Buffer	10.0 μL	30	100	250	300	500
0.1 M DTT	3.0 μL	9	30.0	75	90	150
NTP mix	4.0 μL	12	40.0	100	120	200
PEG (50%)*	3.2 μL	9.6	32.0	80	96	160
RNaseOUT	0.25 μL	0.75	2.5	6.25	7.5	12.5
Inorganic pyrophosphatase**	0.3 μL	0.9	3	7.5	9	15
T7 RNA polymerase	0.4 μL	1.2	4	10	12	20
Cy3-CTP or Cy5-CTP***	0.6 μL	1.8	6	15	18	30

11. Add 30 μL of Transcription master mix to each reaction. Mix by pipetting.

12. Incubate at 40°C for 2 hours (in the dark).

13. Purify labeled RNA using the Qiagen RNeasy Clean-up protocol/kit.
    Unincorporated dye-labeled nucleotides in the hybridization solution significantly increases background fluorescence on the microarray.

14. Quantify labeled RNA levels using the “Microarray” program on the nanodrop spectrophotometer.

Labeled cRNA is good for at least one week. Store at -20°C.

RNA Hybridization and Washing (Agilent cRNA microarrays)

Before starting, add 1.25 mL water to lyophilized 10X Blocking Agent. Gently vortex to resuspend. If it doesn't go into solution heat at 37°C for 5 minutes, then centrifuge. This can be stored at -20°C for at least 2 months.

Perform calculations to determine the amount of labeled RNA for each reaction: there needs to be at least 2.5 pmoles of dye in each reaction; there needs to be exactly the same amount of cRNA in each reaction (this means that the amounts of Cy3 and Cy5 in each reaction will not necessarily be equal).

Sign-up in advance to use the microarray facility hybridization oven.

Hybridization

1. Each microarray labeling reaction will be performed in a separate PCR tube. Aliquot appropriate amount of reference (Cy3 labeled) and experimental (Cy5 labeled) cRNA to each tube. Bring this reaction volume to 20.9 μL using RNase-free water.

2. Add 5.5 μL of 10X Agilent Blocking agent to each tube, mix by pipetting.

3. Add 1.1 μL of 25X Fragmentation Buffer, mix by pipetting.

4. Incubate at 60°C for 30 minutes in the dark.

5. Add 27.5 μL of 2X Hi-RPM hybridization buffer to stop the reaction, mix by pipetting (do not vortex – bubbles are undesirable at this point). Total reaction volume at this point: 55 μL.

6. Spin at maximum speed for 1 minute to reduce bubbles.

Reactions can remain at room temperature in the dark at this point until all reactions are at the same point (in case user is performing so many hybridizations that they need to be done in smaller sets).

7. Place backing slide in a hybridization chamber (Agilent side up – sticker on slide has "Agilent" printed only on one side).

8. Pipette 50 μL of probe reaction onto the center of one gasket area (avoid bubbles).
   To avoid bubbles, don't eject the last few microliters. Also spread the probe around while pipetting, but not too close to the rubber gasket.

9. Repeat #8 for all arrays (make sure to note the order of probe placement on the gasket).

10. Carefully lower the microarray slide over the gasket slide, keeping it level.
    The side with "Agilent" printed on the sticker is the side with the arrays. The gasket and array "Agilent" stickers should be facing each other in the final confirmation.

11. Assemble the chamber. Tighten the screw all the way, finger tight.

12. Look at each chamber while rotating the slide. There should be one large bubble that moves freely. A few small bubbles are fine, too, as long as all bubbles are moving freely when you rotate the array. If not, bang the chamber against the table to loosen the stuck bubbles. Before doing this, though, tighten a little extra… otherwise the arrays may leak.

13. Place in the hybridization oven (make sure to have a balance), and hybridize at 65°C for 17 hours at 20 RPM. (NOTE – put Gene Expression Wash Buffer 2 at 37°C to pre-warm overnight)

Washing

Gene Expression Wash Buffers should contain 0.005% Triton X-102 (Add 2 mL of 10% to 4L of Wash Buffer).
Gene Expression Wash Buffer 2 should be pre-warmed to 37°C.
For washing steps, the liquid should be visibly turbulent (due to stir-bar speed) and entire slide submerged.

1. Clean the wash chambers, racks, and stir-bars with RNA-Zap and deionized water.

2. Prepare the following wash chambers:
   A – Containing Gene Expression Wash Buffer 1
   B – Containing Gene Expression Wash Buffer 1 + one rack + small stir-bar (on stir-plate)
   C – Containing Gene Expression Wash Buffer 2 + small stir-bar (on stir-plate)
   D – Containing acetonitrile + small stir-bar (on stir-plate)

3. Disassemble each hybridization chamber one at a time while completely submerged in wash chamber A using plastic tweezer.

4. Transfer array slide to rack in wash chamber B. Repeat #3 and #4 with all arrays. Leave a gap between slides.

5. Once all slides are in the rack, stir for 1 minute. While stirring, start the stirring mechanism in wash chamber C.

6. Transfer rack to wash chamber C, and stir for exactly one minute. While stirring, start the stirring mechanism in wash chamber D.

7. Transfer the rack to wash chamber D, and stir for 30 seconds. Slowly and evenly pull the rack out of the acetonitrile. The slide should be completely dry. If drops remain on the slides, submerge and try again.

8. Set the rack onto a kimwipe.
   Do not touch anywhere on the slides except the edges and the barcode area.

9. Load the slides into the plastic scanning chambers. The printed "Agilent" side should be facing up and the barcode sticking out. The scanner reads each spot through the back of the slide.

10. Scan the array.
    The array slide can be stored at room temperature, in a desiccated, ozone-free, dark environment for at least a few months and still successfully re-scanned.

Note the layout of samples placed on the array slide for future analysis

Arrays

	Array 1_1	Array 1_2	Array 1_3	Array 1_4
BARCODE	Sample:	Sample:	Sample:	Sample:
	Sample:	Sample:	Sample:	Sample:
	Array 2_1	Array 2_2	Array 2_3	Array 2_4

Barcode Number ____________

Microarray Scanning (Agilent cRNA microarrays)

Scanning is performed on the Agilent High-Resolution Microarray Scanner in the microarray facility. Feature Extraction is performed on a separate computer to allow other users to scan array slides.

After scanning, if microarray slides are kept in the dark in a desiccator, they can be re-scanned (Donna Storton has scanned properly stored arrays after 6 months and they were fine)

Sign-up in advance to use the microarray facility array scanner.

1. Place slides in the scanner, noting the slot numbers (the slides can only be inserted in one orientation).

2. Open the "Agilent Scan Control" program.

3. Confirm/edit settings: In the "Settings" tab, click "modify default settings"
   a. In the "Scan Configuration" section:
      a. Set scan region: 61 x 21.6 mm (this is a full slide)
         i. There is an option to "attempt to retrieve from XML (GEML) files," which is a design file for microarrays. This allows users to do an instant feature extraction with the scanning. We don't use this feature because it ties up the scanner's computer. So keep this box unchecked.
      b. Set the dye channel: Red and Green
      c. Set the scan resolution: 5 µm
      d. Set the PMT Sensitivity Level: check the "extended dynamic range" box (this function allows the scanner to re-scan any spots that were above a threshold intensity with 10% laser power to more accurately distinguish the signal)
      e. Set the 5 µm Scanning Mode: Single Pass
         i. A double pass would scan the entire slide twice, then average all the results (empirical testing indicates that this is completely unnecessary, and doesn't improve the data)

   b. In the "Scan Image File Handling" section:
      a. Set the output path:
         i. D:\Botstein\(user name)
      b. Set the file naming convention:
         i. Prefix 1: custom (lab_initials_date)
         ii. Prefix 2: array barcode
         iii. Final: Prefix1_Prefix2_scannumber.tif
            i. Note that the scan number will be designated as "S01_scan1" unless it finds an "S01" in the folder already, in which case, it will have an "S02" prefix. Files are followed by "_high" or "_low" for high- and low-intensity laser scans, respectively.
         iv. Do not check the "Split and Rotate TIFF" or "Compress TIFF" boxes (this function was important for getting separate files for red and green, which PUMA needed for microarray analysis, but PUMA can now parse a single, combined image)

   c. Click "ok."

4. To update the scan control window with settings changes, click "Slot #" to highlight all, then click the "reset selection" button.

5. It is not necessary to enter any text into the "operator" window.

6. Double-check to confirm that the "Extended Dynamic Range" is selected.

8. Select carousel slots to be scanned ("check carousel" will indicate which slots are occupied).

9. Check that the "Scanner Status" indicates "Scanner Ready."
   a. Every once-in-a-while, the scanner stalls in the "lasers warming" stage (if this takes more than 10 minutes, it is stalled); if this happens, close the "Agilent Scan Control" program, turn of the scanner for a few seconds, turn the scanner back on, wait 2-3 minutes for it to establish a communication with the computer, then re-open the "Agilent Scan Control" program.

10. Modify the "Edit Slot Values" settings:
    a. Starts with default settings; if changes are made, they will trump the defaults
    b. Check the box "read barcode from carousel" (this will read each slide barcode automatically)

11. Click "SCAN SLOT(S)."
    This takes ~25 minutes per 8x15 slide.

12. After scanning is complete, the files will be saved in the D:\Botstein\(user name)

13. Move the files to the network drive (drive Z:) to the following directory (files in this directory are accessible by PUMA):
    \\arrayfiles\arraydata\people\(user name)

14. Delete all your files from the local D: drive.

15. Remove microarrays from scanner (can store in the dark in a desiccator and re-scan for at least up to six months).

16. Log out.

17. Perform feature extraction on a different computer using the Microarray Feature Extraction protocol.

Microarray Feature Extraction (Agilent cRNA microarrays)

The version of Feature Extraction software that contains the standard grid files for mapping spots to genes is only on one of the computers in the array facility (currently sitting on top of a cabinet across from Donna's desk). This version is Feature Extraction 9.5.3.1.

You should open the array scan .tiff files to make sure there are no severe problems (large completely blank portions of the array, for example, suggesting that a bubble prevented hybridization). ImageJ or Adobe Illustrator can open these large images of the entire slide, but Preview cannot. The microarray images can later be examined after feature extraction in PUMA or as individual files that are now small enough for Preview to open.

Sign-up in advance to use the microarray facility feature extraction computer.

1. Open Feature Extraction 9.5.3.1

2. There are two options for running a feature extraction protocol: a) use an existing protocol (there are a number of them for our custom yeast arrays, and they work well), or b) create a new protocol (details for doing this are in a separate section below).

3. The existing protocol that I use (and is already set up) is: BOT_PG_GE_8x15_20100311 (this protocol will work for both 8x15 and 4x44 arrays).

4. To load desired protocol, go to "Project Explorer" (in the left-most tile), right-click on "FE Project," select "Add Extraction," and choose file from the network drive (only the "-high" image needs to be selected; the corresponding "-low" file will be accessed automatically). This can extract from more than one image, so repeast this process to add all images that you want processed.

5. In the Extraction Set Configuration tab, a number of options will appear:
    a. Extraction Set Name – this will automatically be designated as the slide name
    b. Grid Name – this should auto-select based on slide barcode (the grid file defines which spots correspond to which genes – our 8x15 arrays use the following: 017566_D_F_20070822; our 4x44 arrays use the following: 015072_D_20060913)
    c. Protocol Name – pull-down this menu, click on the protocol that you want to use. Now the red "x" under FE Project should disappear because all fields will be automatically filled-in.
    d. Output File Name – automatically generated (output file writes to same folder as input files)
    e. Scan File Name – auto-selected based on files already added to the extraction project
    f. XDR 2nd Scan File Name – automatically lists low pass scan file
    g. Scan File Path – automatically lists output file path

6. Go to "File" and "Save As" to save file in the network location (Z: drive).

7. Click on the "Project" tab then "Start Extracting"
    (this process takes about 10-12 minutes per slide for an individual slide… if there are more slides, the software has a more optimal efficiency of about 20 minutes per 3 slides)
    (if this is the first time that you have extracted on this computer, you may get an error… the software requires you to change the results folder to your personal storage folder in the Project Properties tab the first time you extract. For subsequent extractions from your account, this information remains stored)

8. Data files will be saved on the network drive, and can be loaded from there into PUMA using the PUMA interface.

Create a New Feature Extraction Protocol

1. Go to the "Protocol Browser" and double click to view – this opens the FE Protocol Editor
    a. There are a number of existing generic protocols that can be changed, if desired:
        i. GE1 prefix – Gene Expression 1-color
        ii. GE2 prefix – Gene Expression 2-color
        iii. GE2_V5_95_Feb07 – most recent generic protocol for Agilent yeast microarrays

2. Within the FE Protocol Editor, there are many options that can be changes; the default is appropriate for the majority of these changes. Below is a description of the optional changes:
    a. Protocol Properties: **there is nothing in this menu that needs to be changed**
    b. Protocol Steps:
        i. Place Grid:
        Don't change the defaults. The default setting is "automatically determined" by AMADID Number (this ID number is encoded within the slide barcode and automatically identified by the software).

        ii. Find and Measure Spots:
        Don't change the defaults. The default setting is "automatically determined." This step will reject outlier pixels. The standard setting for 1.42 means that features or background outside of this interquartile range will be rejected. The statistical method that it uses is set to "mean/st. dev."

        iii. Flag Outliers:
        Don't change the defaults. This setting flags data outliers, but we don't use this data. PUMA has its own analysis for identifying and flagging outliers.

        iv. Compute Background, Bias, and Error:
        Don't change the defaults. The "background subtraction method default" is set to "no background subtraction." The arrays we use are really high quality with a background intensity signal around 30 and an average feature intensity signal around 3,000 (therefore the background is insignificant). There is also a "significance" option set to "use error model for significance", and a "use surrogates" default set to "TRUE" (which floors "0" intensities to "1" for ease of calculations). The next option defaults are fine (Choose propagated error – most conservative; MultErrorGreen – 0.1000; MultErrorRed – 0.1000; Auto Estimate Add Error Red – True; AutoEstimate Add Error Green – True; Use Surrogates – True).

        v. Correct Dye Biases:
        There are options here that users may want to change. The default settings are appropriate for "Dye Normalization Probe Selection Method" set at "Use Rank Consistent Probes," "Rank Tolerance" set at "0.050," "Omit Background Population Outliers" set at "False," "Allow Positive and Negative Controls" set at "False," and "Signal Characteristics" set at "OnlyPositiveAndSignificantSignals." The "Normalization Correction Method" is something that users may want to change: Linear for CGH, Linear and Lowess for gene expression, or Lowess (which normalizes based on nearby spots).

        vi. Compute Ratios:
        There is an option here that users may want to change. The default "Peg Log Ratio Value" is set to "4." This means that the highest value for a green:red ratio is 10^4. Because some gene expression signals may be have more than four orders of magnitude change, we typically change this value to "200."

vii. Calculate Metrics:
There is an option here that users may want to change. This window includes an option to designate whether or not you used artificial RNA molecules "spike-ins" that Agilent sells so you can do QC statistics. Our lab generally doesn't use this, so we set the "Spikein Target Used" option to "False." The next option defaults are fine (Min Population for Replicate Stats = 3; Max difference between Grid – 10.00; PValue for Differential Expression – 0.01)

viii. Generate Results:
Don't change the defaults. Generate Single Text File = True. JPEG Down Sample Factor = 4.

3. Save the protocol as a unique name (other than the original name). In our microarray facility, they prefer the following format:
    a. (first three letters of PI last name)_(your initials)_(AMIDAD number)_(yearmonthday)
        for example: BOT_DG_014893_20061107

    b. The AMADID (agilent microarray design identification) of an array is encoded within the array barcode. It is the 5 digits immediately following the "25" at the beginning of the barcode, with a prefix of the digit "0." This number is a unique identifier assigned by Agilent to all microarray designs that are submitted for manufacturing. This number is also used to identify the correct grid file.

Note on Grid Files:

Grid files (and grid file annotation updates) can be downloaded from the Agilent eArray web service. However, access to this service requires a login identification and password. Further, users need administrative permission to install grid files or updates on microarray facility feature extraction computers. Therefore, whenever I have needed a grid file downloaded/installed on a computer, Donna Storton in the microarray facility has assisted.