ARIZONA MICROARRAY WORKSHOP 2003

Instructors

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Program of Events

Sunday, March 16

2.00-4.20 pm	Course registration: Marley Building Lobby, University of Arizona.
4:30 pm	Transportation to Temple of Music and Art.
5:00-5:30 pm	Happy Hour.
5:30 pm	Group Dinner: Temple of Music and Art (sponsored by Telechem, Inc.).
7:00-7:45 pm	Lecture 1: Introduction to the workshop and to microarrays (David Galbraith): Temple of Music and Art.
8.00 pm onwards	Discussion of participants' goals.

Monday March 17

All further lectures and lab sessions in the Marley Building.

9:00 – 9:45 am	Lecture 2: Microarray production (David Galbraith).
9:45- 10:30 am	Lecture 3. Issues in experimental setup, hybridization, data extraction and analysis (Rangasamy Elumalai).
10:30-11:00 am	Coffee Break and Demonstration of Printers (Marley room 814). Sponsored by Ventana.
11:00 – 12:30 pm	Lab session 1. Target preparation (RNA isolation and precipitation).
12:30 – 1:30 pm	Lunch sponsored by Qiagen-Operon.
1:30 – 3:00 pm	Lab session 2. Target preparation (Quality checking by RNA gel electrophoresis, and Quantification of RNA using spectrophotometers). Demonstration of Nanodrop instrumentation.
3:00 – 3:30 pm	Coffee Break.

3:30 ~ *6:30 pm	Lab session 3. Target labeling. Array cross-linking and hybridization demonstration. Demonstration of Ventana Discovery Automated Hybridization Workstation.
Evening	Group Dinner at Gentle Bens (no host).

Tuesday March 18

8:30-9:00 am	Agilent Bioanalyzer Demonstration (Dahlia Riley). Breakfast provided.
9:00 – 9:50 am	Guest Lecture: Manufacturing High Quality Microarrays (Todd Martinsky, Telechem, Inc.).
10:00- 10:45 am	Lecture 4. Issues in experimental setup, and alternative platforms (Michael Deyholos, University of Alberta).
10:45- 11:00 am	Coffee Break sponsored by Agilent.
11:00 – 1:00 pm	Lab session 4. Target preparation (purification steps).
1:00 – 2:00 pm	Lunch sponsored by Agilent. Oligonucleotide microarrays: a presentation by Dahlia Riley.
2:00 – 3:00 pm	Lecture 5. Experimental design and data analysis - statistical issues (Cheryl Vanier).
3:00 – 3:30 pm	Coffee Break.
3:30 ~ 5:30 pm	Lab session 5. Microarray cross linking and hybridization – participants using their materials.
5:30- 8:00 pm	Group dinner at El Charro (no host).
8:30 pm	Lab session 5a. Put arrays to hybridize overnight.

Wednesday March 19

8:30 am – 12:30 pm	Lab session 6. Microarray washing and scanning -- participants. Data extraction and analysis -- participants. Presentation of RLS technology (Rachel Formosa, Genicon). Presentation of printing technologies (Jacques Fayet-Faber, GeneMachines).

10:45 – 11:00	Coffee break sponsored by Genicon. GROUP PHOTOGRAPH.
12:30 – 1:30 pm	Lunch sponsored by BioDiscovery.
1:30 – 3:00 pm	Lab session 7. Demonstration of commercial data analysis software (Pankaj Prakash, BioDiscovery).
3:00 pm onwards	Course wrap-up (David Galbraith) and departures. Note: lab will remain open as long as is required by the participants to complete their tasks.

Participation is limited to 20. For the laboratory sessions, participants will work in groups of four, under the direction of individual instructors.

Commercial Participants and Contact Information:

Company	**Contact**	**Email**
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Telechem	Todd Martinsky	todd@arrayit.com
Ventana	Gabriel Longorio	glongoria@ventanamed.com

Laboratory Session 1

Time: Monday March 17 11:00 – 12:30 pm

Location: Room 217 Marley.

Title: Target Preparation I: RNA Isolation and Precipitation.

Overview:

Isolation of total and polyA^+ RNA

Total RNA can be isolated using variety of procedures depending on plant material and the tissue type. We have provided information about the most basic and simple method of RNA isolation using Trizol, followed by isolation of polyA^+ RNA using magnetic beads. We have found this combination of methods works very well for many different tissue types. It is also very flexible, in that the procedures can be modified to accommodate the differing requirements of specific tissues. For example, if you are working with tissues which contain large quantities of starch or other polysaccharides, such as maize endosperm or root tips, the protocol requires modification to eliminate solubilization of polysaccharide which would interfere with target production. This can be simply achieved by dissolving the pellet containing the RNA in a larger volume than described in the protocol provided here (increasing the volume from 0.1 to 0.5 ml at step 14), and incubating the solution on ice for a few hours. This precipitates most of the dissolved starch from the RNA solution, and it can be removed by centrifugation. If necessary this step can be repeated once or twice more. It should be noted that total RNA can be employed for target production, although for reasons of specificity we prefer use of polyA+ RNA. If total RNA is to be used, we recommend non-phenolic methods using commercial kits, since target labeling is very sensitive to traces of phenol contamination.

The Trizol reagent was developed from the single-step RNA isolation method described by Chomczynski and Sacchi (1987). Trizol is a monophasic mixture of phenol and guanidine isothiocyanate, which during sample homogenization, maintains RNA integrity whilst solubilizing and precipitating other cellular components. For this lab session, Trizol is added to plant materials pulverized by grinding in liquid nitrogen. For smaller samples, homogenization can be done using a glass-in-glass homogenizer. Phase separation is induced by addition of chloroform, and the aqueous phase, containing the RNA, DNA and polysaccharides, is recovered. The nucleic acids (and the polysaccharides) are then precipitated using isopropanol.

What you will learn during this lab session:

- You will be walked through the methods of homogenization and RNA extraction.
- You will then apply to the plant materials either provided by us or brought by you.

- The laboratory ends at the point of RNA precipitation, which will continue over lunch.

Methods:

1. Put gloves on! RNA is exceptionally labile and contamination by ubiquitous RNAses is easy to achieve. All aqueous solutions should be made using DEPC-treated dd-water. Glassware should be autoclaved.
2. Homogenize 200mg of tissue in liquid Nitrogen.
    a. Chill mortar, containing pestle, by addition of ~100 ml of liquid Nitrogen.
    b. Add tissue after Nitrogen has evaporated to one-half its original volume.
    c. Grind tissue with the pestle quickly, but carefully to avoid loss of sample.
    d. When liquid nitrogen has completely evaporated, increase the grinding speed to get a fine talc-like powder.
3. Add 1 ml Trizol per 100 mg of tissue and continue to mix using the pestle.
    a. If the liquid mixture has frozen stiff, wait to allow it to thaw slightly and continue.
4. When mixed thoroughly, wait until melted into a liquid and transfer this to RNAse free tubes using an Eppendorff pipettor.
    a. Cover the mortar with foil while waiting.
    b. Small 1.5 ml RNAse-free tubes are better (1ml liquid per tube).
5. Incubate for 5 minutes @ RT.
6. Add 0.2 ml chloroform per 1 ml volume of Trizol, and vortex for 15 seconds.
7. Incubate for 1 minute @ RT.
8. Centrifuge @ 15,300 rcf for 10 minutes @ 4°C.
9. Transfer the aqueous phase to fresh RNase-free tubes and transfer to ice.
    a. You should see two layers - take from very top and side of tube, leaving a good buffer zone above the second layer.
    b. Put tubes onto ice as soon as liquid is transferred.
10. Precipitate by adding an equal volume of isopropanol.
    a. Mix by inverting tubes twice.
    b. Incubate for 15-30 minutes on ice.

Notes:

Laboratory Session 2

Time: Monday March 17 1:30 – 3:00 pm

Location: Room 217 Marley.

Title: Target Preparation II: RNA Precipitation and QC.

Overview:

RNA quality is a critical factor which determines the quality of hybridization. RNA that is of poor quality (i.e. degraded) is not efficiently labeled, and will not give good hybridization signals. Polysaccharide and polyphenolic contamination not only adversely affects RNA labeling but also interferes with RNA quantification. As a general rule: Good RNA = Good Hybridization.

What you will learn during this lab session:

- You will take the extracted RNA for quantification by spectrophotometry, and quality analysis using gel electrophoresis.
- You will see a demonstration of the Nanodrop spectrophotometer.
- You will see a demonstration of the Agilent 2100 Bioanalyzer.

Methods:

11. Take the tubes of precipitating RNA from the morning session and centrifuge @ 15,000 rcf for 10 minutes @ 4°C.
    a. A small white pellet should be visible (this depends on the amounts of starting material, but for the 200mg tissue that we are using, the pellet should be obvious). Occasionally, the pellet may appear purple or brown depending on the type of starting tissues used or the conditions under which the plants are grown.
12. Discard supernatant by decanting.
    a. Centrifuge briefly (1 min) in the microfuge, and remove remaining supernatant using an Eppendorff 200μL pipette.
13. Wash pellet by addition of 1.0 mL 75% ethanol made using RNAse-free (DEPC-treated) water; be careful, since the pellet may be loose. Remove most of the ethanol using a pipettor.
14. Air dry the pellet for 5 min at RT by inverting the tube onto a Kimwipe (DO NOT let it dry for longer, since the pellet will become very difficult to resuspend).
15. Add 25μL RNAse-free water.

a. Resuspend by pipetting using a 200μL Eppendorff pipette (disrupting the pellet assists it to dissolve). Incubate in ice for at least 1 hour with occasional resuspension using the pipettor.

16. Prepare agarose gel for RNA analysis.
    a. For quick RNA quality check, use a 1% agarose gel prepared in TBE buffer.
    b. Melt 1.0 g agarose in 100 mL 1X TBE buffer (prepared in twice-autoclaved ddH_2O), and cast the gel within a RNAse free gel box. It is good practice to keep a gel box separately for RNA use only; after each run, it should be cleaned by rinsing with DEPC-treated water). **Note:** Please follow standard denaturing gel electrophoresis if you are planning to do Northern analysis.

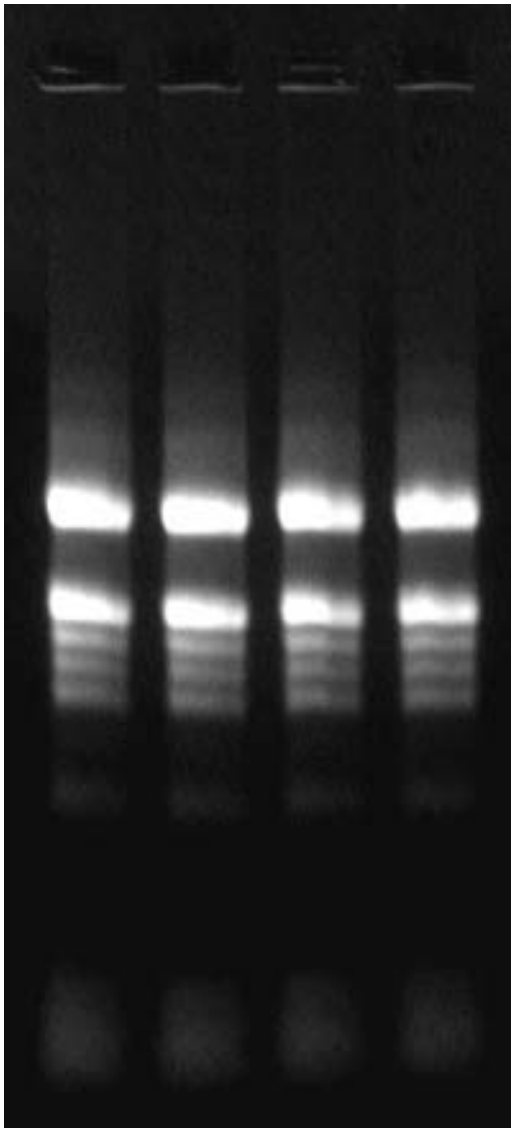
10 X TBE Buffer

ddH2O	800 mL
Tris base	108.0 g
Boric acid	55.0 g
EDTA	9.3 g

Adjust volume to 1L with ddH_2O

17. Centrifuge solubilized RNA (from step #5a) @ 20,000 rcf for 15 minutes @ 4°C.
18. Transfer supernatant to a new RNAse-free tube.
    a. Note that you don't always get a clear distinction between the supernatant and the unwanted debris layer underneath.
    b. To avoid transferring debris, pipette slowly from the very surface of the supernatant.
19. OPTIONAL: Steps #9-12 are only needed for "difficult" tissues (for maize endosperm, precipitation is done three times; for root tissues, it is repeated once). Repeat precipitation by addition of 10% volume 3M sodium acetate and an equal volume of 100% isopropanol.
    a. Precipitate on ice for 1 hour (or overnight @ –80°C).
20. Centrifuge @ 20,000 rcf for 20 minutes @ 4°C.
21. Dissolve in 25μL RNAse-free water for 1 hr on ice, with resuspension.
22. Centrifuge @ 20,000 rcf for 20 minutes @ 4°C.
23. Measure RNA concentration using conventional spectrophotometer or NanoDrop instrument.
    a. Repeat isopropanol precipitation if concentration is less than 100 ng/uL.
24. Run agarose gel to check RNA integrity.
    a. 1% Agarose gel in 1X TBE in an RNAse free gel box.
    b. Load 5μL sample and 2μL dye.
    c. Run at 100V until the dye reaches the end of the gel (~30 minutes).
25. Store remaining RNA at –80°C.

Figure: Image typical of total RNA analyzed using agarose gel electrophoresis. This RNA is suitable for target preparation. Critical is the non-smeared appearance of the major bands, which correspond to ribosomal RNA. The mRNA is not visible, being a collection of molecules of different lengths.



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Laboratory Session 3

Time: Monday March 17 3:30 – 6:30 pm

Location: Room 217 Marley.

Title: Target Preparation III: Target labeling.

- Demonstration of microarray cross-linking, and of hybridization will also be done during this session.

Overview:

Target labeling is conventionally done in a single-step process by direct incorporation of Cy3- and Cy5-substituted deoxyribonucleotides using Reverse Transcriptase (RT). These reagents are expensive, and alternatives include the two-step approach of RT-based incorporation of aminoallyl-dNTPs followed by direct labeling of the terminal amino groups using reactive fluorochromes. The advantages of this second approach include: (a) the fluorochromes are considerably cheaper than the fluor-substituted dNTPs, (b) a wide variety of fluorochromes are available, allowing flexible selection of different combinations of fluor (including >2 fluor experiments), (c) historically, aminoallyl-dNTPs were found to be incorporated more readily than fluor-substituted dNTPs, and (d) dye-specific incorporation artifacts were eliminated.

The primary disadvantage of the two-step approach is that it is less convenient than the single step approach. Further, engineered improvements in RT performance have largely eliminated problems of incorporation and dye-bias.

What you will learn during this lab session:

- How to label RNA for hybridization using the direct incorporation method.
- In this session each group will be provided with 1 μg poly A^+ RNA, and you will be labeling total RNA as well as poly A^+ RNA.

Methods:

Production of Fluorescent Targets from total RNA for Array Hybridization

1. Label two 0.5mL tubes "Cy3" and "Cy5" respectively.
2. Cover tubes with foil to protect from light (dyes are light-sensitive).
    a. Each hybridization is done using a pair of Cy3- and Cy5-labeled RNA targets, which are separately prepared, then mixed prior to hybridization.
    b. Keep RNA on ice at all times, unless otherwise indicated

3. In one 0.5mL tube, mix the following:
    a. Total RNA [50] 30.0μL
    b. dNTP (10mM dATP, dCTP, dGTP, 2mM dTTP) 2.0μL
    c. Cy3- or Cy5-dUTP 2.0μL
    d. Oligo dT primer 2.0μL
4. Incubate the mix in a 65°C water bath for 5 minutes.
5. To the heated mixture, add:
    a. 5x First Strand Buffer 12.0μL
    b. 0.1M DTT 6.0μL
    c. RNAse inhibitor 1.0μL
    d. Powerscript 1.0μL
    e. H2O 4.0uL
    60.0μL
6. Incubate the tubes at 42°C for 2 hours.
    a. Program the PCR machine to hold at 42°C for 2 hours.
7. Add:
    a. 5μL 0.5M EDTA
    b. 5μL 1N NaOH
8. Incubate in a 65°C water bath for 10 minutes.
9. Add:
    a. 25μL 1M Tris-HCL (pH 8.0)
    b. 100μL TE
    c. Store on ice, or freeze in -20°C freezer overnight, until ready to purify.
    d. Be sure tubes are completely wrapped in foil.

Production of Fluorescent Targets from Poly A^+ RNA

Label two 0.5mL tubes "Cy3" and "Cy5" respectively.

10. Cover tubes with foil to protect from light (dyes are light-sensitive).
    a. Each hybridization is done using a pair of Cy3- and Cy5-labeled RNA targets, which are separately prepared, then mixed prior to hybridization.
    b. Keep RNA on ice at all times, unless otherwise indicated
11. In one 0.5mL tube, mix the following:
    a. Poly A^+ RNA [1-2μg] 20.0μL
    b. dNTP (10mM dATP, dCTP, dGTP, 2mM dTTP) 2.0μL
    c. Cy3- or Cy5-dUTP 2.0μL
    d. Random Primer 2.0μL
12. Incubate the mix in a 65°C water bath for 5 minutes.
13. To the heated mixture, add:
    a. 5x First Strand Buffer 8.0μL
    b. 0.1M DTT 4.0μL
    c. RNAse inhibitor 1.0μL
    d. Powerscript 1.0μL
    40.0μL
14. Incubate the tubes at 42°C for 2 hours.
    a. Program the PCR machine to hold at 42°C for 2 hours.

15. Add:
    a. 5μL 0.5M EDTA
    b. 5μL 1N NaOH
16. Incubate in a 65°C water bath for 10 minutes.
17. Add:
    a. 25μL 1M Tris-HCL (pH 8.0)
    b. 100μL TE
    c. Store on ice, or freeze in –20°C freezer overnight, until ready to purify.
    d. Be sure tubes are completely wrapped in foil.

Microarray Immobilization and Hybridization Demonstration

This lab session will end with a demonstration by one of the instructors to introduce the participants to methods of microarray immobilization and hybridization (these methods will be done by the participants in a following lab session).

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Laboratory Session 4

Time: Tuesday March 18 11:00 – 1:00 pm

Location: Room 217 Marley.

Title: Target Preparation III: Target purification.

Overview:

Target purification is done based on size exclusion, using nitrocellulose filters contained in the Microcon YM30 columns. These filters retain single-stranded DNA molecules that are larger than 55 bp or other molecules of MW > 30,000. Target purification is one of the most critical steps in hybridization. If you do not purify the targets sufficiently well, you will end up with very high background on your slides.

What you will learn during this lab session:

- How to purify the fluorescent targets prior to hybridization.

Methods:

Target Purification using Millipore YM-30 Columns

1. Write labels on both the column and tube for each tube of labeled target that will be purified.
2. Using a 200μL Eppendorf pipette, transfer the labeled targets to the spin column – without touching the membrane.
3. Spin at 11700 rcf for 15 minutes at 15°C (make sure no liquid is left in the column).
4. Add 100μL of TE to each column.
    a. Mix the solution by pipetting few time, be careful not to damage the membrane.
    b. Don't forget to change tips between tubes!
    c. Save the wash out (in case the membrane brakes)
5. Spin columns at 11700 rcf for 15 minutes @ 15°C.
6. Repeat TE wash 4X total (steps #4-#6)
7. After 4th wash and final spin, add 40μL TE onto the column.
    a. Mix carefully with Eppendorf pipette.
8. Leave for 2 minutes @ RT.
9. Turn column upside-down into a NEW spin tube.
    a. Label new tube.
10. Spin @ 11700 rcf for 1 min @ 15°C.

11. Use the labeled target immediately for hybridization, or you can store it in a -20°C freezer covered with aluminum foil.

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Laboratory Session 5

Time: Tuesday, March 18, 3:30 ~ 6:30 pm

Location: Room 217 Marley

Title: Microarray Cross-linking and Hybridization

Overview:

Microarray elements (long oligonucleotides, or PCR amplicons) are deposited onto specially modified glass surfaces during the print process, but the DNA is not irreversibly bound to the surface until the surface carrying the DNA is exposed to UV. **It is essential not to apply any liquid to the microarray surface before cross-linking.** Cross-linking is done by exposing the microarray slides directly to short wave UV light. Prior to this step, the printed microarrays must be rehydrated. The rehydration/snapdrying process described below was devised to maximize the number of DNA molecules that have direct contact with the glass surface. Care must be taken during rehydration. The timing of rehydration must be carefully controlled; excessive exposure to hot water vapor will cause the array elements to expand in volume to the point that merging of neighboring spots can occur. This would result in between-spot contamination.

What you will learn during this lab session:

- How to rehydrate the microarrays
- How to crosslink the microarrays prior to hybridization.

Methods:

Microarray Immobilization

This can be performed at any time before hybridization.

1. Mark the corner boundaries of the array on a separate glass slide (template).
    a. Once spots have been immobilized and the slide is washed, the array elements will not be visible (the SSC dissolves).
    b. You need to know the boundaries of the array in order to correctly place the coverslip over the microarray during hybridization.
2. Re-hydrate slide over a 60°C water bath for 10 seconds.
    a. Hold slide label side down over the water vapor.
    b. Watch spots carefully to avoid over-hydration (the spots will begin to merge together).
3. Snap dry the slide on a 65°C heating block for 2 seconds.

a. Place slide label-side-up on the heating block. The elements should reappear as white dots (SSC).
b. Allow slide to cool for 1 minute.

4. Repeat steps #3-4 a total of five times.
5. UV cross-link the slide by exposing the slides label-side-up to 120mJ in a commercial cross-linker machine. We use a Stratalinker, but other cross-linkers should be fine.
6. Wash the slide in 1% SDS for 5 minutes at RT on a shaker or agitate by hand.
7. Remove SDS by dipping slide into 100% ethanol for 30 seconds with gentle agitation.
8. Spin dry slide in centrifuge at no more than 1000 rpm for 2-4 minutes.
    a. Pack bottom of 50 mL plastic disposable centrifuge tube with Kimwipes.
    b. Using forceps, carefully place the slide into tube with label at the bottom.
    c. Repeat spin if any liquid remains on slide.
9. Repeat ethanol wash if any visible streaks remain after the spin dry step.
10. Store slide in a lint-free lightproof box @ RT under conditions of low humidity.

Hybridization Chamber

The chamber should be prepared at least 30 minutes before hybridizing slides. You may use a variety of commercially available chambers, or you can construct one as follows:

Required Components:

Large round petri dish with lid (150 x 15mm).
Small square petri dish lacking lid.
H_2O.
Incubator set to 60-62°C.
Level the surface inside chamber.

1. Invert the small square dish inside the large round dish.
2. Fill large dish with enough H_2O so that the open surface is covered but square dish is not floating.
3. Cover large dish with lid.
    a. Make sure that the lid and square dish do not touch, and leaving enough space for the slide to sit on top of the square dish without making contact with the lid.
4. Place chamber onto level surface in a 60-62°C incubator 30 minutes prior to hybridization.
    a. Chamber must sit on a level surface otherwise hybridization will be uneven.
5. Two slides can be hybridized per chamber.

Hybridization

1. Mix the following in a 1.5mL tube:

20X SSC	25μL
Liquid Block	15μL

2% SDS	10μL
Both Labeled Targets	160μL
H_2O	40μL
	250μL

2. Denature labeled target by incubating tube in boiling water for 2 minutes.
3. Transfer tube to ice immediately.
4. Cut two 1.5 mm strips of coverslip, and remove the coating on both sides.
5. Place microarray slide (label-side-up), and add a drop (15μL) of hybridization mix at the edge of the slide.
6. Carefully place the 1.5 mm strip along the edge, and place the second strip along the other edge.
7. Apply remaining target down the middle of the array, and place the coverslip over the array.
    a. Using forceps gently lay down the cover slip from one end to the other to avoid trapping any bubbles.
    b. Avoid applying pressure to the coverslip which would thereby expel liquid.
    c. Place slide in Hybridization Chamber and incubate @ 62°C for 8-12 hours. (Do not exceed 12 h, since longer incubation results in evaporation which will increase background).

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Laboratory Session 6

Time: Wednesday, March 19, 9:00 am – 12:30 pm

Location: Room 217 Marley

Title:
1. Microarray Washing and Scanning
2. Data Extraction and Analysis

Overview:

After hybridization, it is necessary to wash the slides to remove non-hybridized target. Proper wash conditions are required to eliminate both background and potential cross-hybridization. High stringency washing is required to remove non-specific hybridization. Low SSC concentration and higher temperature will increase stringency. Note that some people employ formamide-containing hybridization buffers, and hybridization is done at lower temperatures.

What you will learn during this lab session:

- How to wash the microarrays following hybridization.
- How to use microarray scanner
- How to extract data from TIFF image
- How to make false color overlay image

Methods:

Microarray Washing

1. Wash slide in the following solutions for 5 minutes each:
   2x SSC, 0.5% SDS @ 55°C
   0.5x SSC @ RT
   0.05x SSC @ RT
2. Washing is done by immersing the slides in a glass slide-staining jar containing the appropriate volume of wash buffer, followed by placing it on a belly shaker at 60 rpm. Pre-heat the first wash solution, and make sure the slides are completely immersed in wash buffer.

3. After completion of the washes, spin dry the slide in centrifuge at no more than 1000 rpm for 2-4 minutes.
   a. Pack bottom of 50 mL plastic disposable centrifuge tube with Kimwipes.
   b. Using forceps, carefully place slide into tube with label at the bottom.

c. Repeat spin if any liquid remains on the slide.

4. Scan slide immediately, or store in a light proof box @ room temp under dry conditions.
    a. Immediate scanning is recommended, however we have observed that properly stored slides (light protected-dry- RT) can retain the signal up to a month. **Note:** some reports indicate environmental pollutants (ozone) can drastically affect fluorescence.
    b. Examine the scanned images immediately to determine the number of elements that are near zero or are saturated (for a 16-bit scanner, that represents a value of 65,400). The proportion of these elements should be acceptably low, since information is lost in either case.
    c. It is much more preferable to rescan with altered gain settings on the scanner than to proceed with analysis of images containing large proportions of zero or saturated elements. As we can tell, even though the absolute value of the spots may be reduced by scanning a second or third time, the relative fluorescence is preserved, so the information is not lost (see figures for an illustration of this problem – C. Vanier will explain).

Data Extraction.

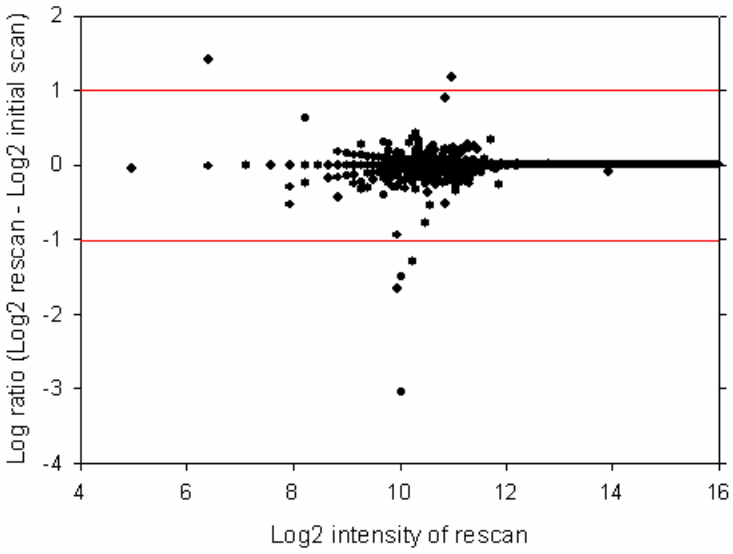
1. Data extraction is done using spot-finding programs such as Imagene (BioDiscovery).
    a. Build a template based on one of the slides in a print run, then to allow the software to fit the template to each slide.
    b. Spend time going through the entire slide twice: once to look for mismatches in the grid, and the second time to manually flag obvious problems. Look for: smears, streaks, or dust where the spot is obviously the same intensity or lighter than the smear it is sitting in, individual spots that are misplaced with respect to the grid (even if nothing is there, chances are the position is being dictated by a small noisy area, visible or not), spots that have bled into adjacent spots, and anything else that looks suspicious. We would like to keep as much data as possible, but dealing with an unbalanced statistical design is preferable to allowing outliers into the data set.

False color overlay image

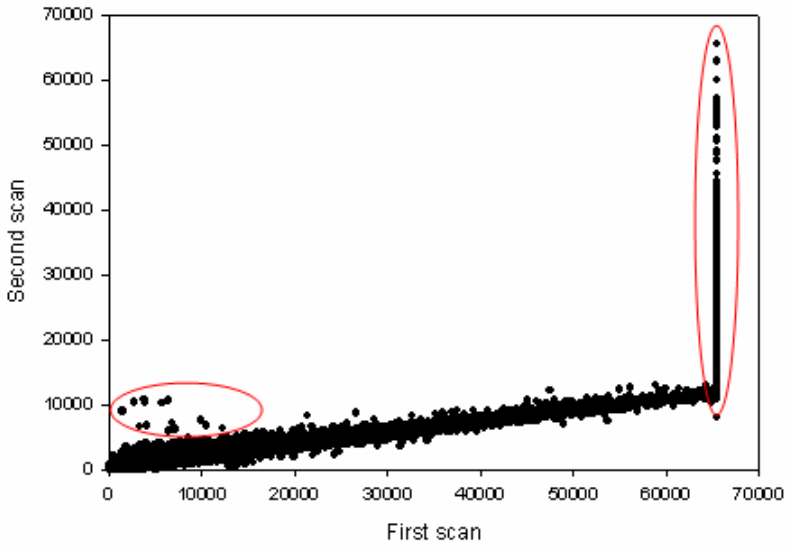
1. Open the Control TIFF image in Adobe Photoshop, convert the image to 8 bit, copy entire image.
2. Open a new image as RGB color, and select the green channel and paste the first image.
3. Open the Experiment TIFF image and convert it to 8 bit, copy entire image and go to the new image (where you have already pasted the control image on green channel), select the red channel and paste the experiment image.
4. Click the RGB channel and you will see the red, green and yellow spots. You can adjust the brightness and contrast to get appropriate levels in the image.

Notes:

Failure to identify problematic spots can cause large differences between replicate spots.



The effects of rescanning and careful spot-finding



Notes:

Laboratory Session 7

Time: Wednesday, March 19, 1:30 am – 3:00 pm

Location: Room 217 Marley

Title: Demonstrations of commercial data analysis software (BioDiscovery).

Part 1.

Introduction to GeneSight
Data upload (flip/swap dye experiment, combining replicates and computing ratios)
Review of all different data transformation features and options
Scatter plot
Histogram
Determination of differentially regulated genes
Confidence analysis
Retrieving annotations from NCBI for selected gene(s)

Part 2.

Data upload (without computing ratios and combining replicates)
Data transformation
Making partitions in data
Significance analysis (ANOVA, t-test)
Sorting and creating subsets of genes with low p-values
Generating reports, viewing report in Excel

Notes:

Notes:

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