Microarray Protocols

Protocols listed below were adapted from protocols developed by the DeRisi Lab (UCSF) and the Kapur Lab (U of MN). For original protocols please see the following links:

http://www.agac.umn.edu/microarray/protocols/protocols.htm
and
http://www.microarrays.org/protocols.html

Post Processing

RNA Isolation

Reverse Transcription and Amino-allyl Coupling

Array Hybridization

Array Washing

POST PROCESSING

Before beginning:
- Start boiling water on heat plate or in microwave, enough to cover slide rack.
- Turn on heat block (highest setting) to be used for snap drying.
- Use powder free gloves throughout protocol.
- DO NOT splash any solutions on array during hydration and heat fixing.

HYDRATION/ HEAT FIXING:

- Pick out about 5-10 slides to be processed.
- On back of slide, etch two lines above and below array to designate array area after processing. Post processing causes the array to disappear.
- Pour enough 0.5X SSC into hydration tray to just below the level of the slides.
- Set slide array side down (be careful not to get the slides wet) and observe spots until proper hydration is achieved. Hydration times will vary depending on printing. You may want to standardize this time for yourself. Under hydration will cause spots to be too small for proper quantitation and over-hydration will cause spots to run together. The purpose of hydration is to "fill in" the spotted DNA/SSC which immediately dries in a ring afterspotting. Good hydration will cause the DNA to spread out evenly over the entirearea of the spot. I have found 20-30 minutes at room temp to work well.
- Upon reaching proper hydration, immediately snap dry slide by setting the slide array side up on the heat block set at approx. 140°C for a few seconds until the array is completely dry.
- UV crosslink at 65 mJoules.
- Continue to surface blocking step.

SURFACE BLOCKING:

(Use fume hood when working with 1-methyl-2-pyrrolidinone)
Store succinic anhydride in vacuum desiccator until ready to use.

- Measure 335 ml 1-methyl-2-pyrrolidinone into designated clean, dry beaker with stir bar. (Don't use 1-methyl-2-pyrrolidinone if it appears yellow - it should be colorless)
- Dissolve 5.5 g succinic anhydride completely.
- IMMEDIATELY after succinic anhydride dissolves, add 15 ml 1M NaBorate (pH 8.0) and pour into designated clean, dry slide dish. Plunge slides rapidly in blocking solution and shake evenly under level of solution for at least 1 min. (NaBorate is made with boric acid and pH is adjusted with NaOH. The succinic anhydride reacts with the poly-l-lysine coating and prevents non-specific hybridization on the slide.)
- Soak slides in solution on shaker for 15 min.
- Before 15 min. incubation is done, reduce heat on boiling water so that temp is approx. 95°C. Drain excess blocking solution off slides briefly and transfer slide rack to the almost boiling water, plunging a couple times, and incubate for 90 seconds. The boiling water denatures the spotted DNA and makes it accessible for hybridization.
- Transfer rack to dish of 95% EtOH and plunge 5X. Spin downslides in tabletop centrifuge.
- Arrays may be used immediately or stored for future use. Store slides desiccated in the dark at room temperature.

RNA ISOLATION

Use your favorite method to isolate RNA. Below is just one of many methods.

RNA preparation from Candida (from Dana Davis)

1. Harvest 20-50 ml of culture at OD600=0.2-0.6 by vacuum filtration and store pellets at –80°C.
2. Set up 2ml screw cap tubes containing:
   set 1 250µl acid washed 0.5µm glass beads and
   700µl phenol:chloroform:IAA 25:24:1
   set 2 500µl phenol:chloroform:IAA 25:24:1.
   Store tubes on ice or at 4°C prior to use.
3. Resuspend cell pellet in 800µl LETS buffer (10mM Tris-Cl pH7.4, 10mM EDTA, 100mM LiCl, 0.2% SDS) and transfer immediately to an ice-cold **set 1** tube (glass beads and phenol:chloroform) and mix by inversion.
4. Vortex tubes 4-6 times for 30sec intervals followed by ≥30sec on ice. Do these by hand as multivortexers give poor yields.
5. Spin 10min at 14k rpm in the cold room.
6. Remove aqueous (top) layer to ice-cold **set 2** tube.
7. Vortex 2 times for 30sec intervals followed by ≥30sec on ice.
8. Spin 10min at 14k rpm at 4°C.
9. Remove aqueous (top) layer (~750µl) to a fresh 2ml screw cap tube and add 1/20th vol 4M LiCl (~38µl).
10. Fill tube with 100% EtOH and ppt ON. (If possible use ice-cold EtOH. Also, RNA can be ppt after 2hrs at –20°C)
11. Pellet RNA 15 minutes at 14k rpm at 4°C.
12. Pour off supernatant and gently rinse pellets with ~250µl ice-cold 70% EtOH.

13. Dry pellet 5-10 minutes in speed-vac. (Follow instructions on speed-vac except, do NOT use heat.)
14. Resuspend pellet in 50-100µl H_20 and store samples at –20°C.

– DNAse sample using your favorite method. (RNase free DNase I from Roche, DNA-free DNase Treatment and Removal Reagents from Ambion or other.)

1. REVERSE TRANSCRIPTION AND AMINO-ALLYL COUPLING

RNA 10 ug total RNA
Oligo dT 2 ul of 0.5 ug/ul
Bring volume to 15.5 ul with ddH_2O

(You may want to try different amounts of total RNA, 5 - 20 ug)

- Incubate at 70°C for 10 min.
- Chill on ice 10 min.
- Add 14.5 ul reverse transcription mix:

(Buffers are supplied with SuperScript II, GibcoBRL)

RT Mix	ul Per rxn
5 X Buffer	6
50 X aa-dUTP/dNTP*	0.6
0.1 M DTT	3
ddH_2O	3.9
SuperScript II	1
	14.5

*Altering the ratio of aa-dUTP's:dTTP's may be required. I've had success with a ratio of 4:1. You may also want to try ratios of 2:3 and 3:2 to see what works best in your hands. Below is the recipe for a 50 X mix at a ratio of 4:1.

50 X recipe for 4:1
10 ul each 100 mM dA, dG, dC
8 ul 100 mM aa-dUTP
2 ul 100 mM dT

- Incubate RT reaction at 42°C for 2 hours.

HYDROLYSIS

- Add 14 ul H_2O

- Add 5 ul 1M NaOH
- Add 1 ul 0.5 M EDTA
- Incubate 10 min. at 65°C
- Neutralize with 50 ul 1 M HEPES pH 7.0, mix well

CLEANUP

- Add 400 ul water to each reaction.
- Filter through Microcon-30 (12K for 6-8 min)
- Repeat process 2X, refilling original filter.
- Elute.
- Check volumes, bring to 10 ul with H_2O.
- Samples may be stored at -20°C indefinitely.

COUPLING

- Add 10 ul 0.1 M NaBicarbonate Buffer pH 9.0 to cDNA
- Resuspend dye* with cDNA
- Incubate 1 hour at room temperature in dark.

  * Resuspend tube of Cy3 or Cy5 in 32 ul DMSO. Aliquot 4 ul into 8 tubes and dry in speed vac. Store dried aliquots at 4°C, in dark, with dessicant.

CLEANUP (QiaQuick PCR Purification columns from Qiagen)

- Add 500 ul Buffer PB.
- Apply to Qia-quick column and spin at 13,000 rpm in microfuge for 30 – 60 sec.
- Aspirate off flo-thru.
- Add 750 ul Buffer PE and spin 30 – 60 sec.
- Aspirate off flo-thru and repeat.
- Aspirate flo-thru and spin for 1 min. at high speed to dry column.
- Transfer to fresh eppi. tube.
- Add 60 ul Buffer EB to center of filter and incubate for 5 min. at RT.
- Spin 13,000 rpm for 1 min.
- Repeat elution step again.

– Dry down eluate in speed vac (or concentrate in Microcon-30 filter).
– Continue to hybridization

ARRAY HYBRIDIZATION

The following protocol was adapted from protocols obtained from the Tronto MicroArray Consortium at the Ontario Cancer Institute and the Berman Lab (U of MN).
http://www.uhnres.utoronto.ca/services/microarray/

Notes:
The protocol below uses DIG EasyHyb (Roch) as the hybridization solution. I have not directly compared this solution to hybridization in SSC/SDS . See http://www.agac.umn.edu/microarray/protocols/protocols.htm
and http://www.microarrays.org/protocols.html for hybridization protocols using SSC/SDS.

Lifterslips are coverslips with thin strips on the sides. They help avoid bubbles and are easier to use however regular coverslips can also be used.

HYBRIDIZATION

- Resuspend and combine dried down labeled cDNAs in 70 ul DIG Easy Hyb solution.
- Filter probe in Milipore 0.45 um membrane
- Set up slide for hybridization: Blow slide briefly with compressed air to remove any dust or debris. Set slide in hybridization chamber. Apply fresh, clean LifterSlip (cleaned with acetone) to slide.
- Heat filtered probe at 65°C for 5 min, transfer to 37°C.
- Slowly inject the probe under one corner of the LifterSlip until the array surface is covered. Continue to apply remaining probe at the other corners. Try to avoid bubbles.
- Add 10 ul of DIG Hyb solution to each end of slide as a drop. This is to ensure a constant humidity in the chamber during the hybridization and will prevent the array from drying out (very bad!).
- Tightly screw down chamber lid and carefully place chamber in a 37°C water bath. Keep chamber flat during transfer and hybridization.
- Hybridize overnight.

ARRAY WASHING

It is recommended that all wash solutions be filtered before using.
Prepare wash solutions in glass slide dishes, with each dish having its own rack.

Wash Soln. I
255 mL millipure water
7.5 mL 20X SSC 1 mL 20X SSC
0.75 mL 10% SDS

Wash Soln. II
262 mL millipure water
0.75 mL 20X SSC

- Carefully remove array from water bath, making sure to keep chamber level. Dry array with paper towels and attempt to "wick" any water away from chamber seams.
- Unscrew chamber and remove array. Some water may enter chamber and pool under slide at this time. If so, it is helpful to have a pair of forceps to pry array away from chamber.
- Keep array level when submerging in Wash I. Once submerged, tilt array and gently dump off coverslip. It may be necessary to lightly swish array under solution to dislodge the slip.
- Plunge rack up and down for 1 minute.
- Individually transfer slide to slide dish containing Wash II, do not transfer entire slide rack as this will cause too much SDS carryover. Once slide is in Wash II, remove additional hybs from water bath.
- Once all slides are in Wash II, plunge rack 10 - 20 times.
- Dry arrays in room temperature table top centrifuge at 800 rpm for 5 min.
- Try to scan array within hours of washing as the Cy dyes are unstable and degrade differentially.