

SOP - cDNA Microarray

Key References:

- [1]Hasselman, J. (2001). TIGR protocols
- [2]Schut et al., 2001 "DNA microarray analysis of the hyperthermophilic archaeon *Pyrococcus furiosus*: evidence for a new type of sulfur-reducing enzyme complex. J. Bact.
- [3]Hegde, et al., 2000. A concise guide to cDNA microarray analysis. Biotechniques. 29:548-562.

Date Completed	Experiment	Researcher

RNA Sample Info

RNA Tube	Condition	RNA Conc. (ug/uL)	Dye to be Labeled	Date Grown	Media Used	Growth Container	Gel Run on RNA

Slides Info

Slide Barcode	RT OD Cy3/Cy5	Which Tube for Cy3	Which Tube for Cy5

Reverse Transcriptase Reaction:

You need five tubes per slide, per sample. So for a two-condition experiment, with each condition labeled with Cy3 and Cy5, using two slides, you will run ten tubes per sample for a total of twenty tubes. The tubes used are domed PCR tubes for use in the thermocycler

RT reaction:

- 1) Add the following, making the total for all the tubes in each condition in one tube and then dispensing it into individual labeled tubes : Refer to excel worksheet .
- 2) Mix Well and Dispense 15 ul into each tube
- 3) Incubate at 70°C for 10 min.
- ***** **Start making dNTP mix and RT master mix** *****
- 4) Place in a dry ice/EtOH bath for 30 sec.
- 5) microfuge briefly at 13,000 rpm
- 6) Add 5 ul of RT Mix to each tube and mix well
- 7) Incubate at 42°C for 2 hours
- 8) To hydrolyze RNA add 10ul 1N NaOH to each tube
- 9) To stop reaction add 10ul 0.5M EDTA to each tube
- 10) Mix and incubate at 65°C for 15 min.
- 11) Add 10ul 1 N HCl to each tube to neutralize reaction mix

RT cDNA Cleanup

- 1) Combine all five tubes for each sample into a 1.5ml tube
- 2) Add 1250 ul PB Buffer (Qiagen) per RT group (5tubes).
- 3) Add Sln into a Qiagen PCR cleanup Column
Spin at 11.000 rpm for 15sec., empty collection tube, and repeat until all of the solution has passed through the column
- 4) Add 750ul phosphate wash buffer to column, spin for 1min
Phosphate wash buffer (100mL) [5mM KPO4, pH 8.0, 80% EtOH]
1M KPO4, pH 8.5 0.5 mL
MSDW 15.25 mL
95% EtOH 84.25 mL
- 5) Repeat step 4
- 6) Discard the flowthrough, and spin for additional min.
- 7) Transfer column to a new 1.5ml. tube, add 50 of phosphate elution buffer and spin. Do it twice (100ul)
Phosphate elution buffer : 1M KPO4, pH 8.5 to 4mM with MSDW

QC of Reaction

Blank GRL spec with 100 ul TE. Check for cDNA by putting entire reaction into a 100uL cuvette and reading A₂₆₀ and A₂₈₀ or use Nanodrop A260 for a given rxn has to be at least .6 –enough for one dye set (cye3 or cye5 –need 1.2 for dye flip)

Labeling Reaction

- 1) Dry all RT samples completely in speed vac (about 90 min. total, on low heat check every 20 min.)
- 2) Make new 1.0 M Sodium Carbonate Buffer
Buffer Recipe (must be made fresh every time) –
Add 80 ml ultra pure water to small autoclaved glass bottle along with a clean stir bar (measure water using a sterile 50 ml pipette). Add 10.6 g Sodium Carbonate and allow it to dissolve. Adjust pH to 9.0 using 12 N HCl using a 10ml pipette and a bulb to keep track of amount added (should be about 9 ml). bring total volume to 100 ml.
- 3) Make 10 ml of 0.1 M Sodium Carbonate Buffer
Add 9 ml of ultra pure water to a sterile 15 ml Add 9 ml of ultra pure water to a sterile 15 ml screw cap tube and add 1 ml of the fresh 1.0 M
- 4) Resuspend each dried sample cDNA in 4.5 uL of 0.1M sodium carbonate buffer, pH 9.0

**** Move to a Darkened Area the dyes are VERY light sensitive ****

Get aliquots of dyes for each tube, and make sure to label the tubes for which dye will be added to them. Only get the dyes and thaw them from the -70C when ready since they degrade quickly once thawed.

- 5) Add 4.5 uL NHS-ester Cy5 dye (aliquots prepared in DMSO)
- 6) Incubate for 1 hour at room temperature in absolute Darkness
- 7) Add 35uL of 100mM NaOAc pH 5.2 To each tube and mix
- 8) Add 250ul Buffer PB to each tube and mix
- 9) Add each mix to a labeled Qiagen DNA Column and spin at 13,000 rpm, empty tube
- 10) Wash each column with 750 uL Buffer PE (Qiagen) and empty tube
- 11) Spin an additional 1 minute at 13,000 rpm and throw away collection tube
- 12) Elute into a new labeled 1.5 ml tube by adding 50ul EB buffer (Qiagen) letting stand 1min at room temperature. Then spin at 13,000rpm. Repeat step final vol 100ul.
- 13) Combine different labeled probes as specified in the experimental design and dry in a speed vac low heat (70min).
- 14) Resuspend combined tubes in 20ul MSDW

Optional: Check cDNA and incorporation:

Measure OD at 260, 550, 650.

cDNA => 1 OD = 37 ng/uL; nucleotide pmols =

$[\text{OD}260 \times \text{vol. (uL)} \times 37 \text{ ng/uL} \times 1000 \text{ pg/ng}] / (324.5 \text{ pg/pmol})$; pmol Cy3 =

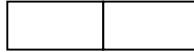
$[\text{OD}550 \times \text{vol. (uL)}] / 0.15$; pmol Cy5 = $[\text{OD}650 \times \text{vol. (uL)}] / 0.25$

nucleotides/dye ratio = pmol cDNA/pmol Cy dye

ratio is best with >200 pmol dye incorporated per sample and a ratio less than 50

Prehybridization (Start when dyes are nearly dry in SpeedVac)

- 1) Prepare Prehyb buffer fresh (225 ml water, 75 ml 20xSSC, 0.3g SDS, 3.0g BSA). Preheat 45 min at 42C.
- 2) Place slides in Copling jar, prehybridize at 42°C for 45 min. Handle slides only by barcode. Turn w/bath 95C
- 3) Wash slides by dipping 5 times in MSDW at room temp
- 4) Dip twice in isopropanol at room temp,
- 5) Air dry (not more than 30min) Prehyb-time



Hybridization:

**** Everything is completed in the Dark **** Use high quality SDS, stock sln. 10% @

- 1) Add 1uL of COT1-DNA (20ug/uL) to each tube that will be hybridized to a slide
- 2) Heat probe mixture to 95°C for 2 min., do a quick spin
- 3) Add 21 ul of 2x hyb. buffer that has been preheated to 42°C to each tube (50% formamide, 10XSSC +0.2SDS)
- 4) Apply hyb. mix to slide and cover with a 20x60 mm polyethylene hydrophobic coverslip
- 5) Add 10ul of MSDW to both ends of chamber and seal it
- 6) Place in 42°C bath for 16-20 hours, cover very well with foil

Slide Wash and Scan

**** Must be completed in the Dark ****

- 1) Place slides in dish containing low-stringency wash buffer (1x SSC and 0.2% SDS at 42°C) Agitate for 4 min.
- 2) Wash slides at high stringency wash buffer (0.1x SSC and 0.2% SDS) at room temp. for 4 min.
- 3) Wash slides in 0.1x SSC for 4 min.
- 4) Warm up scanners Lasers for 15min
- 5) Dry slide with nitrogen, use Kimwipes to blot edges. Be gentle. During summer don't let dry slides for too long >1.5hours exposed
- 6) Scan, quick scan on Cy3 <80% then Line scan, after laser power is balanced scan starting with Cy5.

Stratascript RT Stratagene Cat# 600085-51 cost approx w/shipping \$193.00 (200ul)
Random Primers Life Technologies Cat48190011 cost approx w/shipping \$110 (60ul)
Aminoallyl dUTP Ambion Cat8439 cost approx w/shipping \$130 (50ul)
SDS DNASE, RNASE, Protease free 327315000 Fisher
Sodium Carbonate Sigma S-6139
Water from RICCA, Arlington Texas.