E.coli Total RNA Labeling Protocol for Spotted Microarray

(updated/corrected 5-30-2007)

Note:
Start with 20 ug of total RNA for each labeling reaction.
All solutions that can be filtered should be filtered.
Cy dyes are light sensitive and should ALWAYS be handled in dim light.

RNA Preparation

- If RNA is in ethanol, spin down 20 ug of RNA per reaction @ 14000 rpm for 20 min at 4°C.
- Pipette off supernatant and wash pellet with 100 ul of 70% ETOH. (prepared with DEPC H_2O)
- Spin 5 min and remove supernatant without disturbing pellet
- Air dry pellet 15-20 min at Room Temp (RT). (Caution: if pellet is over dried it is hard to resuspend!)
- Resuspend RNA pellet in 12.5 ul DEPC H_2O

RNA		12.5 ul
Random Hexamer*	5 ug/ul	1 ul
Labeling Control (Yeast RNA mix)		1 ul
Total		17 ul

Heat to RNA to 70°C 5 min, ice 2 min, pulse spin

Labeling

Prepare labeling mix (prepare 1 labeling mix for all samples labeled at the same time).

1X labeling mix

First-Strand Buffer	5x	8 ul
DTT	0.1M	4 ul
dNTPs (low dTTP)**	10x	4 ul
RNasin		1 ul
Total		17 ul

- To the RNA/Hexamer mix add 17.0 ul of labeling mix and incubate 10 min at RT
- Add 1.5 ul of appropriate CyDye dUTP (1mM stock) followed by 1.5 ul SSII reverse transcriptase, mix well by tapping and pulse spin
- Incubate 1 hr at 42°C in the dark

- Add an additional 1.5 ul SSII reverse transcriptase, tap, pulse spin and continue incubation 1 hr
- Degrade RNA by addition of 2 ul 1N NaOH, vortex, pulse spin and incubate 15 min at 65°C
- Neutralize by addition of 2 ul 1N HCl, vortex and pulse spin

Clean up Labeled Probes

- Prewash Microcon-30 microfilter by adding 450 ul miliQ H_2O and spinning for 10 min @ 12,000 RPM.
- Add 450 ul miliQ H_2O to each of the probe samples (or total 500 ul). Mix thoroughly by pipetting up and down. Transfer samples to separate Microcon-30 microfilters (Amicon).
- Spin at 12,000 RPM in microfuge for 10 minutes or until 20-40 ul remains in the filter.
- Add 450 ul miliQ H_2O to the probe and gently mix by pipetting up and down. Be careful not to touch the filter at the bottom of the filtration unit.
- Spin 10 min at 12,000 RPM.
- Add 450 ul miliQ H_2O to the probe and gently mix by pipetting up and down.
- Spin 12 min at 12,000 RPM to get smaller volume.
- Invert column into a fresh tube and spin 1 minute at maximum speed to recover probe. Carefully measure recovered probe volume if necessary.
- Transfer recovered probe to the appropriate partner probe.

Note: Probes can be combined after the first wash step.

Probe can be stored at 4°C or -20°C in dark for further purpose.

Reagents and Suppliers

Cy3-dUTP	1mM	PerkinElmer	NEL578
Cy5-dUTP	1mM	PerkinElmer	NEL579
SuperScript II	200U/ul	Invitrogen	18064-014
RNasin	20-40U/ul	Promega	N2515
dNTP set, 100mM solutions**		Amersham	27-2035-01
pd$(N)_6$ Random Hexamer*	50 A_{260} U	Amersham	27-2166-01
Microcon YM-30 column		Amicon	42410

Other reagents: 20X SSC, TE pH 7.4, 10% SDS, 500 mM EDTA, 1M NaOH, 1M Tris-HCl pH 7.5, sterile dH_2O and DEPC H_2O

** supplied as a lyophilized sodium salt with 5' phosphorylated ends; resuspend at desired concentration*

*** for 10X stocks: 5mM each dA, dG, dC and 2mM dT in DEPC H_2O*