E.coli Total RNA Labeling Protocol for High Density Oligonucleotide Array

(updated/corrected 5-30-2007)

Note:
Start with 10 ug of total RNA for each labeling reaction.
All solutions that can be filtered should be filtered.

RNA Preparation

- If RNA is in ethanol, spin down 10 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 8-10 min at Room Temp (RT). (Caution: if pellet is over dried it is hard to resuspend!)
- Resuspend pellet in 11 ul DEPC H_2O and add 1 ul 0.5 ug/ul Random Hexamer; this is the RNA/Primer Mix.
- Heat to 70°C for 5 min and chill on ice for 2 min, quick spin.
- Mix following reagents:

RNA/Primer Mix		12 ul
dNTP Mix	10mM	3 ul
First-Strand Buffer	5x	12 ul
DTT	0.1M	3 ul
DEPC H_2O		24 ul
SuperScript II	200 U/ul	6 ul
Total		60 ul

- Mix everything except Superscript II. Heat to 42°C for 1 min, then add enzyme. (Can make the mix and add 42 ul mix to RNA/Primer mix)
- Incubate 90 min at 42°C.
- Inactivate the reaction by heating at 70°C for 15 min then chill on ice

Digest RNA and Purification of cDNA

RT reaction		60 ul
RNase H	2 U/ul	1 ul
RNase A	1 ug/ul	1 ul
H_2O		38 ul
Total		100 ul

- Incubate 10 min at 37°C.

Purify cDNA with Qiaquick PCR purification kit

- Add 500 ul of Buffer PB to 100 ul sample.
- Place a QIAquick spin column in a provided 2 ml collection tube.
- Apply the sample to the QIAquick column and centrifuge for 1 min
- Discard flow-through. Place the column back into the same tube.
- Add 750 ul Buffer PE to the column and spin for 1 min.
- Discard flow-through and place the column back in the same tube. Spin for additional 1 min.
- Place the column in a clean 1.5 ml microcentrifuge tube.
- Add 34 ul Buffer EB (10mM Tris.Cl, pH 8.5) or H_2O to the center of the QIAquick membrane, let the column stand for 1 min, and centrifuge the column for 1 min. (Finally volume is about 32 ul)
- Quantitate cDNA in spectrophotometer with 1 ul sample (1 A_{260} = 0.033 ug/ul), save another 1 ul for gel.

cDNA Fragmentation and end labeling

- Dilute 1 U/ul Dnase I to 0.1 U/ul with 1x Dnase I Buffer.
- Mix following reagents:

cDNA		30 ul
DNase I Buffer	10x	4 ul
H_2O		4 ul
DNase I	0.1 U/ul	2 ul
Total		40 ul

- Incubate 10 min at 37°C*
- Heat to 99°C for 10 min to stop the reaction
- Check fragmentation on 2% agarose gel, (normally use 2 ul) avg. length 50-100 bp

** incubation time may need to be adjusted for different batches of DNase I due to varying activity.*

Labeling with Terminal Transferase

- Mix following together

Fragmented cDNA		38 ul
TdT Buffer (NEB Buffer 4)	10x	10 ul
$CoCl_2$	2.5mM	10 ul
Biotin-11-ddATP**	1mM	2.5 ul
H_2O		37 ul
TdT	20 U/ul	2.5 ul
Total		100 ul

- Incubate 2 hours at 37°C
- Optional: Check fragmentation on 2% agarose gel with gel shift assay
- Freeze at -20°C, ready to mix with hybridization cocktail

Reagents and Suppliers

Biotin-11-ddATP**		PerkinElmer	NEL508
SuperScript II	200 U/ul	Invitrogen	18064-014
dNTP set, 100mM solutions		Amersham	27-2035-01

$pd(N)_6$ Random Hexamer***	50 A_{260} U	Amersham	27-2166-01
QIAquick PCR Purification Kit		Qiagen	

*** this protocol originally specified Biotin-N^6-ddATP (PerkinElmer EL508), which is no longer available; according to PerkinElmer, Biotin-11-ddATP can be directly substituted*

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

Note: This protocol was adapted from the original Affymetrix protocol.
