RT Labeling from total RNA source & Qiagen Clean-Up (Cy-dCTPs)

Cy3 Lot#_____
Cy5 Lot#_____
SSRT Lot#_____

I. Labeling Date: Agent: Dose Timept.

Tube #	Sample	PI/Project ID/Exp ID	Location	Conc µg/µl	Label	µg RNA	µl RNA	µl H2O 12µl total	Chip	MAPS L H# QA/QC
1					Cy3				**p s**	
2					Cy5					
3					Cy3				**p s**	
4					Cy5					
5					Cy3				**p s**	
6					Cy5					
7					Cy3				**p s**	
8					Cy5					
9					Cy3				**p s**	
10					Cy5					
11					Cy3				**p s**	
12					Cy5					
13					Cy3				**p s**	
14					Cy5					
15					Cy3				**p s**	
16					Cy5					
17					Cy3				**p s**	
18					Cy5					
19					Cy3				**p s**	
20					Cy5					
21					Cy3				**p s**	
22					Cy5					
23					Cy3				**p s**	
24					Cy5					

Master mix (1 tube/probe)		**2 pairs**	**3pairs**	**4pairs**	**5pairs**	**6pairs**	**7pairs**	**8pairs**	**12prs.**
5X buffer	6µl	12	18	24	30	36	42	48	72
DTT	3µl	6	9	12	15	18	21	24	36
Cy3 or Cy5	**1µl**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**12**
dNTPs(low CTP)(___/___)	1.5µl	3	4.5	6	7.5	9	10.5	12	18
RNAsin (___/___)	1µl	2	3	4	5	6	7	8	12
Superscript RT	2µl (add last)	4	6	8	10	12	14	16	24

I. First add water to each tube (12µl total RNA + water), then add:
2 µl oligo dT (___/___) and 1 µl RNAsin to each tube,
Place tubes **on ice** and add RNA to each tube.
Incubate @ **65°C** for 10 min.
Chill on ice 2 min. (**reduce thermal cycler to 42°C**)
Record (mo/yr) from tubes of dNTPs, RNAsin, oligo dT

II. Add 14.5µl master mix/tube.
Incubate @ **42°C** for 1 hour, 15 min.
Add 2µl Superscript II Reverse Transcriptase (SSRT).
Incubate @ **42 C** for 1.5 hours.
Add 30 µl 0.1M NaOH, incubate @ **70°C** for 30 min.
Add 30µl 0.1M HCl (use fresh 1.5ml aliquot every 2 mos. of 0.1M NaOH & 0.1M HCl)

II. Probe Clean-Up

***Turn on 100°C heating block and 65°C water bath, check water level, turn on speed vac, warm up 35% guanidine HCl to RT.**

Add 500 μl Buffer PB to each100 μl reaction mix. Into QIAquick column and tube, combine 300 μl each probe. Spin* 1 min, discard flow through.
Add remaining probes, spin 1 min, discard flow through.
Wash with 750 μl **RT** 35% guanidine HCl** and spin 1 min, discard flow through.
Add 750 μl Buffer PE*** to column and spin 1 min. Discard flow through and spin again for 1 min.
Place column into clean 1.5 ml tube. Add 30μl Buffer EB to membrane, let stand for 2 min at RT and spin 1 min. There will be ~ 30 μl left, so speed vac10 min (5 min) to concentrate (final volume of < 10 μl) (final volume of < 20 μl)
Add:
7 μl 1ul/10ugRNA Cot human DNA (35Cy3 +35Cy5 = 70ug = 7 μl Cot) [**minimum** of 5μl Cot 1 must be added]
1μl poly A RNA (8μg/μl)
5 μl tRNA yeast (4μg/μl)
Add TE for final volume of 26 μl for 2x4 array or 14.8 μl for 2x2 array or 32-35μl for 2x4 array.

* spin at 12K
** 35 g guanidine HCl, water to 100 mL : exp. date, 30 days at RT; 6 mos. at 4°C
*** be sure 100% ethanol has been added as directed to PE buffer provided in kit.

III. Hybridization

22 x 40 mm coverslip	22 x 22 coverslip	22 x 50 mm coverslip
For 26 μl add:	For 14.8 μl add:	For 32-35 μl add:
5.2 μl 20X SSC (3X final) 1:6.7	3 μl 20X SSC	6.7 μl 20X SSC
2.7 μl 25X Denhardt's (2X final) 1:12.5	1.6μl 25X Denhardt's	3.6 μl 25 X Denhardt's
1.1 μl 10% SDS (0.3X final) 1:33	0.6 μl 10% SDS	1.36 μl 10% SDS
Total 35 μl	Total 20 μl	Total ~44 μl

Heat for 2 min @ 100°C.
Incubate @ RT for 10 min.
Filter through prewashed (with 10 μl H2O) millipore filter, spin @ 12K for 3 minutes.

Add entire volume of probe to slide, carefully apply coverslip, insert into chamber, add 10 μl of 3X SSC along inside bottom edge of chamber, to provide humidity and incubate 16-24 hrs @ 65°C

IV. Wash and Scan

Wash Notes:

Scan date:
Scanner:

1	2	3	4	5	6	7	8	9	10	11	12
p s	p s	p s	p s	p s	p s	p s	p s	p s	p s	p s	p s
Red/Grn	Red/Grn	Red/Grn	Red/Grn	Red/Grn	Red/Grn	Red/Grn	Red/Grn	Red/Grn	Red/Grn	Red/Grn	Red/Grn
/	/	/	/	/	/	/	/	/	/	/	/
min											
max											

Notes: