RNA labeling (12/5/07)

The protocol for harvesting, preparing, and labeling RNA was taken from Maitreya Dunham's website

The amplification/labeling is done per Agilent instructions with half volume reactions and a quarter amount of recommended dye.

Make a 100 ng/µl stock of total RNA.

Aliquot 3.25 µl (325 ng) total RNA into a PCR tube.
Add 2.5 µl T7 Promoter Primer.
65C 10 min
ice 5 min

Warm 5X first strand buffer at 100C, with occasional vortexing, until it completely dissolves (~1 min).
Prepare cDNA master mix, in this order at RT:

1X	
2 µl	5X first strand buffer
1 µl	0.1 M DTT
0.5 µl	10 mM dNTP mix
0.5 µl	MMLV RT
0.25 µl	RNaseOUT

Add 4.25 µl to each reaction.
40C 2 hours
65C 15 min
ice 5 min

Add 0.8 µl Cy3 CTP to each reference reaction.
Add 0.6 µl Cy5 CTP to each experimental reaction.

Warm 50% PEG at 40C until it's resuspended and easy to pipet.
Prepare transcription master mix, in this order at RT:

1X	
8.25 µl	water
10 µl	4X transcription buffer
3 µl	0.1 M DTT
4 µl	NTP mix
3.2 µl	50% PEG
0.25 µl	RNaseOUT
0.3 µl	inorganic pyrophosphatase
0.4 µl	T7 RNA polymerase

Mix by pipetting.

Add 29.4 µl to each reaction. Mix by pipetting.
40C 2 hours in the dark

Purify with an RNeasy column.
Nanodrop 1 ul to check yield and dye incorporation.
Store RNA at -20C.