



Baylor College of Medicine Microarray Core Facility

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RNA Labeling (Dye Primer)

1. Add the following into a nuclease-free microcentrifuge tube:
 - **For mRNA**
 - 1 ul labeled oligo dT Cy3 or Cy5 (2.0 ug/ul)
 - 1 ug mRNA
 - 1 ul Yeast RNA (Positive Control)
 - RNase free water to 10.5 ul
 - **For total RNA**
 - 1 ul labeled oligo dT **Cy3** or **Cy5** (2.0 ug/ul)
 - 10 ug RNA
 - 1ul Yeast RNA (Positive Control)
 - RNase free water to 13 ul
2. Heat to 70°C for 10 minutes, then quick chill on ice.
3. Centrifuge briefly
4. Prepare Mastermix: the following amounts are for **one** reaction
 - 4 ul 5X First Strand Buffer
 - 2 ul 0.1M DTT
 - 1 ul Nucleotide Mix (10mM each dNTP's)
 - 2 ul RNasin (10 unit/ul)
 - 1 ul SuperScript II
5. Add 10ul of Mastermix to reaction and mix by pipetting up and down
6. Incubate at 42°C for 2 hours and 30 minutes
7. Denature cDNA/mRNA with 1 ul 5M NaOH and incubate at 37°C for 10 minutes.
8. Add 5 ul 1M Tris-HCl pH 7.5 and 1 ul 5M HCl.
9. Purify probe using EtOH Purification; add 1/10 total volume 3M NaOAc pH 5.2 and twice the total volume 100% EtOH to each tube; store at -20°C for 5 minutes (in order to keep solution cold); spin at 14,000 rpm for 10 minutes.
10. Wash the pellet with twice the total volume 70% EtOH; spin again at 14,000 rpm for 10 minutes.
11. Take off EtOH and let pellet air dry; move on to Hybridization.