

## Direct cDNA Probe Labeling and Purification (V1.0.0)

1. Prepare direct labeling reaction and master mix:

5xRT buffer	120 $\mu$ l
DTT(0.1M)	60 $\mu$ l
dATP(100mM)	3 $\mu$ l
dCTP(100mM)	3 $\mu$ l
dGTP(100mM)	3 $\mu$ l
dTTP(100mM)	0.6 $\mu$ l
nuclease-free water	110.4 $\mu$ l
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Total	300 $\mu$ l

2. This is sufficient for 20 labeling reactions (store at -20°C).  
Note: 5xRT buffer and DTT are supplied with SuperScript II enzyme
3. To 40-50 $\mu$ g total RNA, add 2 $\mu$ g (2 $\mu$ l) oligo(dT) (18-20mer, Invitrogen, cat. #Y01212). Bring total volume to 10 $\mu$ l w/nuclease-free H<sub>2</sub>O.
4. Incubate at 70°C for 10 min, chill on ice for 1 min.
5. To denatured RNA add:

RT master mix	15 $\mu$ l
Cy3 or Cy5 - dUTP (cy3 cat #53022,cy5 cat #55022, Amersham Pharmacia)	2.6 $\mu$ l
SuperscriptII RT (Invitrogen cat #18064-014)	2 $\mu$ l

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7. Mix well and incubate at 42°C for 2 hrs.
8. Add 1.5 $\mu$ l 20mM EDTA to stop rxn.
9. Degrade RNA by adding 1.5 $\mu$ l 500mM NaOH and heat for 10 min at 70°.
10. Neutralize rxn with 1.5 $\mu$ l 500mM HCl.
11. Remove unincorporated nucleotides by GFX columns (Pharmacia, cat # 27-9602-01) purification as per manufacturer instructions.
12. Dry eluted probes in speedvac and resuspend in appropriate amount of TE (pH 8.0).

13. Fluorescent probe incorporation efficiency may be monitored by running 1µl of labeled probe on a 1.7% agarose gel and visualized using an MD Typhoon. The Typhoon will allow you to visualize both Cy3 and Cy5 incorporation on a gel.  
\*Note that EtBr should not be used when running a gel for the Typhoon.

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### Human Cot-1 precipitation

1. Add 1/10 volume of 3M NaAcetate (pH5.5) plus 2 volumes of 100% ethanol. Vortex well.
2. Precipitate at -70°C for 30 min or -20°C O/N.
3. Centrifuge at full speed in an eppendorf microcentrifuge for 10 min.
4. Remove supernatant.
5. Wash pellet with 70% ethanol and spin at 8000xg at 4°C for 5min.
6. Remove all of ethanol and dry pellet.
7. Resuspend pellet in TE (pH 8.0) to give 20µg/µl. If you have precipitated all 500µg of human Cot-1 (Invitrogen, cat#15279011), then resuspend pellet in 25µl TE.
8. Confirm concentration on spectrophotometer via A<sub>260</sub> reading.