

## **cDNA Synthesis and Labeling**

**Aimée M. Dudley**

We use the Atlas Glass Labeling Kit from Clontech ([Catalog # K1037-1](#)) as directed by the manufacturer. This kit, which employs an aminoallyl-dUTP incorporation method, has some advantages over methods employing the direct incorporation of Cy3 and Cy5 labeled nucleotides (see [Brown lab protocol](#)). The first advantage is cost, the Clontech kit costs about \$65 per slide, while the direct labeling method costs about \$95 per slide (mostly due to the expense of the labeled nucleotides). The second advantage is the lack of differential incorporation of Cy3 and Cy5 nucleotides by the polymerase, since the aminoallyl-dUTP is incorporated during the cDNA synthesis step and the dyes are chemically attached in a subsequent step. There are other protocols that also employ reverse transcription in the presence of aminoallyl-dUTP with subsequent chemical attachment of Cy3 or Cy5 (see [Brown lab protocol](#)). At approximately \$22 per slide, these methods are considerably cheaper than the Clontech kit. However, in our hands, they were less robust and reproducible than the Clontech kit. There are several differences between the protocols that could account for these differences, and we have not investigated these.

**Notes about my version of the protocol:**

- **I start with 20 micrograms total yeast RNA.**
- **My source of oligo dT is a 16-mer of all Ts synthesized by my favorite oligo synthesis company with no purification. I resuspended the oligo in water at a concentration of 2 micrograms/microliter and use 3.5 microliters per reaction. This is much cheaper than most commercial sources of oligo dT and works just as well.**
- **I don't do any of the reactions in a thermocycler. I just use a couple of heat blocks and spin down any obvious condensation on the top of the tube as needed.**
- **At the end of the protocol you will have about 90 microliters per sample or 180 microliters per slide. This is too much volume to apply to the slide. I pool the samples that I am going to hybridize together and concentrate them down to 30-40 microliters final volume using a microcon YM-30 filter (Millipore).**
- **The kit gives some advice on measuring cDNA synthesis and dye incorporation by absorbance ratios. I've never done this, so I can't comment on the correlation between these measurements and the success of my experiments. However, my sample is always obviously colored.**
- **Our major reason for using this kit is that, in our hands, it reproducibly results in a robustly labeled product. We have had people with little or no molecular biology experience successfully use this kit in our lab.**