Revised: June 19, 2003

Measurement of mRNA Stability

References:

- Parker et al., 1991. Measurement of mRNA decay rates in *Saccharomyces cerevisiae.* Methods in Enzymology, Vol. 194:415-423.
- Nonet et al., 1987. Eukaryotic RNA polymerase conditional mutant that rapidly ceases mRNA synthesis. Molecular and Cellular Biology, Vol. 7:1602-1611.

Materials:

- Yeast Strain: Use *rpb1-1* background. Grow at 28°C.
- Culture medium
- Dry Ice

Procedure:

1. Prepare:
   - 100-150 ml culture in log phase (i.e. OD_{600} of 0.4 - 0.6)
   - 15 ml of medium equilibrated to the growth temperature
   - 60°C water bath with 15 ml of medium in a test tube for each culture
   - 37°C water bath
   - Microcentrifuge - must be one that decelerates rapidly like the Tomy Capsule microcentrifuge.
   - Ethanol/dry ice bath
   - 10 ml pipettes
   - pre-labeled microcentrifuge tubes
2. Pellet cells in sterile 45 ml screw cap centrifuge tubes at 4,000 rpm for 5 minutes in a high speed centrifuge.
3. Resuspend cells in 15 ml of medium. Equilibrate at growth temperature for a few minutes while preparing to take the first sample.
4. Add 15 ml of pre-warmed (60°C) medium and place in the 39°C water bath. **Note:** *rpb1-1* mutants are usually transferred to the non-permissive temperature of 37°C for half-life measurement. However, we have found that transcription does not arrest until ~3 minutes at 37°C while arrest of transcription is essentially immediate at 39°C.
5. Sample immediately: Remove ~3 ml and distribute to 2 microcentrifuge tubes. Pellet cells for 10 seconds in a microcentrifuge. Pour off supernatant and freeze in dry ice/ethanol bath. **NOTE** - Ideally this step should take less than one minute to do. This is especially important for mRNAs with a short half-life. It is also important that this step take approximately the same time for each time point in order to get an accurate half-life.
6. Sample times: 0, 3, 6, 9, 12, 18, 25, 35 min. **NOTE** - These times may have to be adjusted for individual mRNAs.
7. Store at -70°C until RNA extractions can be done. RNA can be extracted from the cells at each time point using the RNA Extraction from Yeast protocol.