Pulse-Chase Labeling of Yeast with $[^{3}\text{H}]$-Methyl Methionine or $[^{3}\text{H}]$-Uracil

- Plan for: 0.5 $A_{600}$ unit of cells per chase time,
  0.5 ml of labeling medium per $A_{600}$ unit,
  25 µCi of radioactive label per $A_{600}$ unit,
  5 µl of volume to resuspend RNA pellet from 1 $A_{600}$ unit of cells (enough for 2 gels).

- For $[^{3}\text{H}]$methyl methionine labeling, use methionine-free medium.

- For uracil labeling of uracil auxotrophs, use 1/3 the normal amount of uracil supplement.

**Medium or Temperature Shift**

1. Prepare a starter culture of log phase yeast ($A_{600} \leq 0.5$). For a standard depletion experiment, grow in SGal plus supplements. For a standard temperature shift experiment, use SD plus supplements. Plan for both experimental and control cell cultures.

2. Medium Shift: If you plan to change the medium, wash once in sterile ddH$_2$O at room temperature. Spin down cells at 2000 rpm for 5 minutes in IEC centrifuge.

   Temperature Shift. If you plan to raise the temperature to 37°C, add 50°C medium to 37°C, and transfer to incubator-shaker pre-warmed to 37°C.

3. Plan to collect a total of 2.5 $A_{600}$ units of cells at $A_{600} < 0.5$ for the pulse-chase labeling. For this, dilute cells into the appropriate medium such that you will have enough cells at the desired time in the desired medium at the desired temperature. Plan to grow excess culture volume (5-10 $A_{600}$ units of cells) and discard the cells that are not needed.

**Labeling**

1. Just prior to collection, check $A_{600}$ to determine exact yield of cells. Spin down cells at 2000 rpm for 5 minutes in IEC centrifuge. Wash pellet with 1 ml SD medium without uracil or methionine.

2. Resuspend 2.5 $A_{600}$ units of cells in 2.5 ml of SD medium without uracil or methionine in a 15 ml tube. For medium shift, use SD at room temperature and do the labeling and chase on the bench. For temperature shift, use SD at 37°C and do the labeling and chase at 37°C.

3. Add 125 µCi of $[^{3}\text{H}]$-methyl methionine or 125 µCi of $[^{3}\text{H}]$-uracil and mix.
   - For $[^{3}\text{H}]$-uracil: label for 4 minutes. Chase with ~1.5 mM uracil: add 250 µl of "100X" uracil stock. Collect 0 minute time point immediately. Other timepoints are 4, 8, 16, and 32 minutes.
   - For $[^{3}\text{H}]$-methyl]-methionine: label for 2 minutes. Chase with ~5 mM cold methionine: add 250 µl of 50 mM stock (250 mM aliquots at -70°C). Collect 0, 2, 4, 8 and 16 minute timepoints.

**Time Points:**

1. Have on ice: labeled 1.5 ml microfuge tubes filled with ice to ~1 ml mark. At timepoint, transfer 0.55 ml of labeled cells to a tube and vortex. Keep on ice until all time points are collected.

2. Microfuge tubes for 30 seconds at top speed. Remove all supernatant (radioactive liquid waste) and immediately freeze pellet on dry ice. Store at -70°C until ready for RNA extraction.
   - Use the phenol/glass beads to extract RNAs. The hot phenol extraction method also works well.