

ULTIMATE YEAST PULSE-CHASE

This assay is performed to directly measure the stability of a specific protein in yeast. Following pulse-labeling of yeast cells, recover your target protein by denaturing immunoprecipitation, as described in the 'Ultimate Yeast Denaturing IP' protocol. If you have difficulty detecting your labeled protein, try increasing the amount of labeled methionine used. If you are using temperature-sensitive yeast, shift to the restrictive temperature during the last 30 minutes of the starvation period (B.2).

A. SETTING-UP YEAST CULTURES.

1. Each pulse-chase will require a 20 ml culture of yeast at an OD_{600} of between 0.4 and 0.6. To do this, I usually set up a number of 20 ml cultures the evening before, innoculated at different densities, so that first thing next morning at least one of the cultures will be at an OD_{600} of ~0.5.

B. PULSE-LABELING.

Prepare: 20 ml warm media minus methionine (*I use CSM-Met from Bio101*)

1. When OD_{600} of culture is between 0.4 and 0.6, transfer culture to 50 ml Falcon tube. Spin 5 mins, 2000 rpm, at room temperature.

2. Pour off supernatant. Resuspend yeast pellet in 20 ml warm media minus methionine. Return cells to flask and grow for 1 hour.

Prepare:
(i) 100 µl Tran^{35}S-label (NEN)
(ii) 5 ml warm media minus methionine
(iii) 6 ml warm CHASE media (Met added fresh) in a Falcon 2059 tube
(iv) 4 labeled snap-lock 1.5 ml microfuge tubes (labeled with time points)
(v) a 50 ml Falcon tube for radioactive waste
(vi) ^{35}S waste and radioactive stuff
(vii) liquid nitrogen
(viii) stopwatch or timer
(ix) one labeled snap-lock microfuge tube for radio-labeling.

3. Read OD_{600} of culture. Transfer culture to 50 ml Falcon tube. Spin 5 mins, 2000 rpm, at room temperature.

4. Pour off supernatant. Resuspend pellet in warm media minus methionine to give final OD_{600} of 6.0.

5. Transfer 1 ml of yeast suspension to microfuge tube.

6. Add 100 μl of Tran^{35}S-label. Pipet up and down carefully to mix. Place cells in incubator. Start stopwatch. Incuabte for 10 minutes with shaking. This is the pulse.

7. Pellet cells for 10 seconds at top speed in a microfuge. Remove supernatant with blue-tip/pipetman.

8. Resuspend cells in 1 ml warm media minus methionine to wash. Pellet cells for 10 seconds at top speed in a microfuge. Remove supernatant with blue-tip/pipetman. Repeat this washing step one more time.

9. Resuspend pellet in 1 ml of warm CHASE media. Transfer to the remaining 5 ml of CHASE media in the 2059 tube. Mix thoroughly but carefully.

10. Immediately remove 1.4 ml of cells and transfer to labeled microfuge tube. This is the 'zero' time point. Start stopwatch. Place remaining cell culture in incubator and shake.

11. Pellet the 1.4 ml aliquot of cells for 15 seconds in microfuge tube. Carefully remove supernatant. Freeze pellet in liquid nitrogen.

12. Collect 1.4 ml aliquots at appropriate times (usually 10; 20; and 40 minutes).

Use the ULTIMATE YEAST DENATURING IP PROTOCOL to recover your labeled protein.

CHASE MEDIA is regular drop out media plus 1 mg/ml methionine, added fresh.