RNA Extraction From Yeast Using Glass Beads

- In advance, prepare 2.0 ml tubes containing 0.25 g acid washed glass beads (0.5 mm diameter) and 250 µl ØOH:CHCl3:isoamyl alcohol (25:24:1). Label and place on ice.

1. For standard preparation of total RNA from yeast, collect 5 OD600 units of yeast (e.g., 10 mls of OD600 = 0.5). Spin in IEC centrifuge for 5 minutes at 2000 rpm at 4°C. Resuspend pellet in 2 mls of ice cold DEPC-treated HE (10 mM HEPES, 1 mM EDTA, pH 8) and transfer to two microfuge tubes. Spin for 30 seconds at top speed at 4°C. Store pellets on ice for immediate use, or at -80°C.

   For pulse-chase labeled cells, start with 0.5 OD600 unit of yeast per time point. Collect cells by centrifugation. Store cell pellets on ice for immediate use, or at -80°C until needed for extraction.

2. Resuspend cell pellet in 250 µl HENS buffer. Quickly transfer to 2.0 ml tube with glass beads and ØOH:CHCl3:IAA. Vortex for 10 seconds. Do one tube at a time. Place tube(s) on ice.

   HENS buffer: 10 mM HEPES-NaOH, pH 7.5   Treat with DEPC.
   1 mM EDTA
   300 mM NaCl
   0.2% SDS

3. Place tubes in multi-tube vortex mixer at 4°C. Vortex for 10-30 minutes (25-75% breakage).

4. Microfuge for 10-30 minutes at 4°C at top speed. If the interface is broad, spin longer.

5. Transfer 200 µl of the supernatant to a 1.5 ml microfuge tube. Do not collect any of the interface.
   - For pulse-chase experiments, collect exactly the same volume of supernatant from each tube.
   - For small numbers of cells, back extract with 100 µl of HENS buffer and pool with 200 µl.


7. Add 3 volumes of 100% EtOH to supernatant. Precipitate 1 hour to overnight at 4°C or -20°C. No need to add additional salt (e.g., don't add NaOAc, pH 5).

8. Spin for 30 minutes at top speed at 4°C. Carefully remove supernatant.

9. Wash pellet with 150 µl of 75% EtOH (DEPC treated).

10. Spin for 5 minutes at top speed. Carefully remove supernatant. Spin briefly and remove remaining droplets.

11. Dry samples in Speed-Vac. Resuspend pellet in 10 µl DEPC-treated ddH2O per 1 OD600 unit. Keep RNA solution on ice when thawed. Store RNA solution at -20°C or -80°C.

   - Alternatives for resuspending RNA: 1 mM DEPC-treated NaCitrate, pH 6.5; DEPC-treated ddH2O + 0.1 % SDS + 0.1 mg/ml proteinase K.