RNA Extraction from Yeast:

References:

- Sambrook, J., E. F. Fritch, and T. Maniatis, 1989. Molecular Cloning, a Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.
- Leeds, P., S. W. Peltz, A. Jacobson, and M. R. Culbertson, 1991. The product of the yeast *UPF1* gene is required for the rapid turnover of mRNAs containing a premature translational termination codon. Genes and Development 5:2303-2314.
- Kohrer, K., and H. Domdey, 1991. Preparation of High Molecular Weight RNA. In Guide to Yeast Genetics and Molecular Biology. Eds. Guthrie, C., and G. R. Fink. Methods in Enzymology 194:398-405.

Materials:

- Selective medium or YEPD
- Sterile dH_2O
- Buffer A

	for 1 L
50 mM NaOAc	16.7 mls 3 M NaOAc
10 mM EDTA	20 mls 0.5 M EDTA
	963.3 mls dH_2O

- Complete Buffer A (0.5 mls per sample)
  for 1 L
  900 mls Buffer A
  100 mls 10 % SDS
  Add 1 % DEPC just before use
- Buffer A saturated phenol (1.2 mls per sample)
- TE saturated phenol: Chloroform (0.6 mls per sample)
- 3 M NaOAc (pH 5.2) (90 µl per sample)
- DEPC-treated dH_2O (1.5 mls per sample)
- Absolute ethanol (2 mls per sample)
- 70 % ethanol (1 ml per sample)

Protocol:

A. Preparation:
1. RNase free tips, microcentrifuge tubes, pipettes.
2. Label the microcentrifuge tubes.
3. Dry ice/ethanol bath.
4. 65°C water bath.
5. Aliquot the solutions required.
6. Add 1% DEPC to Complete Buffer A.

7. Equilibrate the Buffer A saturated phenol to 65°C.
8. Ice.

B. Harvest cells:

1. Grow cells to an OD_{600} of 0.4 - 0.6 in 10 mls of selective medium or 5 mls of rich medium. This can be done in two steps. First, grow an overnight culture to saturation in 3 mls of medium. Second, inoculate 10 mls of selective with 5 µl, 10 µl, 50 µl and 100 µl of the saturated overnight culture the afternoon of the second day. The YEPD can be inoculated with smaller amount of overnight culture. This procedure will generally ensure that at least one of the cultures will be at the correct OD_{600} the following day.
2. Transfer the cultures to sterile 45 ml centrifuge tubes. Pellet the cells by centrifugation at 4,000 g for 5 minutes.
3. Resuspend the pelleted cells in 1.0 mls of sterile dH_2O.
4. Pour the cell slurry into an RNase free 1.5 ml microcentrifuge tube.
   NOTE: all subsequent steps should be using RNase free labware and solutions. Follow the general guidelines in Sambrook et al., 1989 for the preparation of RNase free solutions and labware.
5. Pellet the cells in a microcentrifuge at full speed for 20 seconds.
6. Remove the liquid and freeze in a dry ice/ethanol bath. Store the frozen pellets at -70°C.

C. RNA extractions:

1. Remove the tubes from the -70°C and immediately add 500 µl of **Complete** Buffer A (remember to add 1% DEPC before use). Vortex to resuspend the cells.
2. Add 600 µl of Buffer A saturated phenol equilibrated to 65°C. Mix each tube for ~ 10 seconds. Place tubes in a 65°C water bath. Repeat mixing of each tube every 30 seconds for a total of 5-6 minutes.
3. Centrifuge the tubes in a microcentrifuge for 30 sec at full speed.
4. Remove the phenol layer (yellow, bottom layer) using an RNase free blue tip. **NOTE:** Leave the interface, and the pellet of unbroken cells and cell debris in the tube or you will loose some of the RNA.
5. Add 600 µl of Buffer A saturated phenol equilibrated to 65°C. Repeat mixing and incubation at 65°C for 5-6 min.
6. Centrifuge the tubes in a microcentrifuge for 2-3 minutes at full speed.
7. Remove aqueous layer (top layer) to a new tube.
8. Add 600 µl of 1:1 phenol buffered with TE:chloroform at room temperature. Mix the samples by vortexing for 20 seconds. Separate the layers by centrifuging the tubes in a microcentrifuge for 2-3 minutes at full speed.
9. Remove the aqueous layer (top layer) to a new tube. Add 50 µl of 3 M NaOAc (pH 5.2) and 1 ml of absolute ethanol. Mix. Incubate the microcentrifuge tube on ice for 15 minutes to precipitate the RNA. Centrifuge at full speed in a microcentrifuge for 10 minutes to pellet the RNA. Aspirate. Remove as much of the supernatant as possible. Incubate the open tube at 37°C for 5 minutes to dry the pellet. Be careful not to over dry the pellet. Dehydrated RNA is very difficult to resuspend.
10. Resuspend the pellets in 400 µl of dH_2O. Add 40 µl of 3 M NaOAc and 1 ml of ethanol. Mix and precipitate again as described in Step 9.

11. Wash the pellets by adding 1 ml of 70% ethanol and vortexing for 20 seconds. Centrifuge the microcentrifuge tubes at full speed for 5 minutes.
12. Remove the supernatant by aspiration. Incubate the open tube at 37°C for 5 minutes to dry the pellet.
13. Dissolve the RNA in 50 µl dH_2O. Heat to 65°C for 10 minutes to assist resuspension. Vortex. Centrifuge briefly.
14. Dilute 5 µl RNA into 495 µl of dH_2O. Determine the absorbence at A_{260} and A_{280}.
15. Dilute the RNA to 1 µg/µl.