

## RNA Extraction

1. All reagents, tips, tubes etc should be RNase free. If something is dirty, touched by ungloved hands or open to the air then it is probably not RNase free. Get new clean tubes or tips from the TA if this is the case.
2. Obtain a cryogenically frozen soybean embryo from the TA. Add directly to a tube containing 700 ul of RNA extraction buffer.
3. *Immediately* homogenize with a mini-pestle until you have a uniform suspension.
4. Add 500 ul of *acidic* phenol:chloroform (PC), cap well, and vortex for 30 seconds. Be careful!!!!!! Wear gloves and safety glasses!
5. Centrifuge the tubes for 5 minutes at full speed.
6. Remove the upper aqueous phase (~ 600 ul), containing the nucleic acid, and add it to a new 1.5 ml tube. Then add 500 ul of chloroform (CH). Cap the tube securely, and vortex for 1 minute.
7. Centrifuge the tube for 3 minutes and remove the upper aqueous phase, placing it into a new 1.5 ml tube. You should have about 500 ul. Do not contaminate the samples with RNase.
8. Add 1/3 volume of 8M LiCl (about 166 ul); mix thoroughly by inverting the tube. Incubate the LiCl precipitation for 30 minutes at -20°C. The LiCl will precipitate the RNA, while the DNA remains in solution. Be sure to write your name and / or group number on the tube!
9. Centrifuge the tubes five minutes to pellet the RNA.
10. Remove the supernatant, leaving the RNA pellet in the tube (Very pure RNA forms a clear precipitate, it may not be visible by eye).
11. Add 300 ul of sterile DEPC H<sub>2</sub>O to the tube and dissolve the pellet.
12. Add 30 ul of 3M sodium acetate, mix, and then add 700 ul of ethanol, mix. Place the tube on dry ice for 10 minutes to precipitate the RNA.
13. Centrifuge the RNA at high speed for 10 minutes, then remove the supernatant with a pipette being careful not to lose your RNA pellet.
14. Dry the pellet in the speedvac for 5 minutes, then dissolve your RNA pellet in 50 ul of DEPC H<sub>2</sub>O. Leave it on ice with the TA.
14. The TA will read the OD at 260/280nm and provide this data next week.

## RNA EXTRACTION BUFFERS

### Complete RNA Extraction Buffer

<u>Final Concentrations:</u>	<u>Chemicals:</u>	<u>Mol Wt</u>
0.1 M Tris-HCl, pH 8.0	Trizma base	121 (Sigma T1503)
2 M NaCl	NaCl	58.4
25 mM EDTA	EDTA (disodium)	372.2
2% CTAB	Cetyl Trimethyl Ammonium Bromide	
2% PVP	Polyvinyl Pyrrolidone	
0.05% Spermidine	Spermidine	
2% B-ME	Beta-Mercaptoethanol (add just before use)	

### REAGENTS FOR RNA EXTRACTION

8 M LiCl      Lithium chloride      34g / 100 ml H<sub>2</sub>O, autoclave, store 4 C  
MW42.4

Acidic phenol-chloroform:  
Phenol:chloroform:isoamyl alcohol 125:24:1; pH 4.2-4.5

Buy premade from Ambion or Fisher

Chloroform:isoamyl alcohol (24:1) Prepare in a media bottle that does not have a rubber cap. Chloroform will dissolve the rubber. Prepare under the hood.

Chloroform            192 ml  
Isoamyl alcohol      8 ml

DEPC water:

Add 1ml fresh Diethyl pyrocarbonate (DEPC) to 1000ml distilled, deionized water. Leave overnight and then autoclave for 40 minutes on liquid cycle to destroy DEPC.