RNA ISOLATION PROTOCOL

1. Lable the snap cap tube containing your tissue sample with your initials and the date using a lab marker. Keep the sample stored on ice until you are ready for homogenization.
2. Add 500uL of TriReagent to the 1.5mL snap cap tube containing your tissue. Store on ice.
3. Carefully homogenize the tissue using a disposable pestle. If the tissue is difficult to homogenize, carefully close the tube tightly and briefly vortex the sample.
4. After the sample is completely homogenized, add an additional 500uL of TriReagent to the tube and close the tube tightly.
5. Vortex vigorously for 15s.
6. Stop here for Lab 1 and give your labeled homogenized tissue sample to the TA for storage at -80ºC. You will be finishing your RNA extraction in lab next week.

**RNA EXTRACTION PROTOCOL**   
Continued from Lab 1   
  
1. Turn on heating block to 55°C.   
2. Incubate your homogenized tissue sample (from Lab 1) tube at room temperature (RT) for 5 mins.   
3. In the fume hood, add 200uL of chloroform to your sample and close the tube. **NOTE**: Due to the high volatility of chloroform, pipetting needs to be done carefully and quickly. Have your tube open and close to the container of chloroform before drawing and chloroform into your pipette tip.   
4. Vortex vigorously for 30s. You are vortexing correctly if the solution becomes a milky emulsion.   
5. Incubate tube at RT for 5 mins.   
6. Spin tube in refrigerated microfuge for 15 mins. @ max speed.   
7. Gently remove tube from microfuge. Be sure not to disturb the tube.   
8. Slowly and carefully transfer most of the aqueous phase (the top, clear portion) to a fresh microfuge tube. Do NOT transfer ANY of the interphase (the white, cell debris between the aqueous and organic phase).   
9. Close the tube containing the organic and interphase and properly dispose of the liquid inside the tube as well as the tube itself at the end of the lab.   
10. Add 500uL isopropanol to the new tube containing your RNA and close the tube.   
11. Mix by inverting the tube numerous times until the solution appears uniform. Pay particular attention to the appearance of the solution along the edge of the tube. If mixed properly, it should no longer appear viscous/"lumpy".   
12. Incubate at RT for 10 mins.   
13. Spin in refrigerated microfuge at max speed for 8 mins. When placing your tube in the microfuge position the tube hinge pointing up, away from the center of the microfuge.   
14. A small, white pellet (RNA and salts) should be present. If not, do not fret an continue with the procedure.   
15. Remove supernatant.   
16. Add 1mL of 75% EtOH to pellet. Close tube and vortex briefly to dislodge pellet from the side of the tube. If the pellet does not become dislodged, that is OK.   
17. Spin in refrigerated microfuge at 7500g for 5mins.   
18. Carefully remove supernatant. Pellet may be very loose. Make sure not to remove pellet!   
19. Briefly spin tube (~15s) to pool residual EtOH.   
20. Using a small pipette tip (P10 or P20 tips), remove remaining EtOH.   
21. Leave tube open and allow pellet to dry at RT for no more than 5mins.   
22. Resuspend pellet in 100uL of 0.1%DEPC-H2O by pipetting up and down until pellet is dissolved.   
23. Incubated tube at 55C for 5mins. to help solubilize RNA.   
24. Remove tube from heat, flick a few times to mix and place sample on ice. This will be your stock RNA sample.   
25. Quantitate RNA yield using Nanodrop spectrophotometer.   
  
**RNA QUANTIFICATION**   
NOTE: Always keep your RNA samples on ice!   
1. Pipette 2µL of 0.1%DEPC-H20 onto the Nanodrop pedestal and lower the arm.   
2. Click "Blank", to zero the instrument. NOTE: steps 1 and 2 only need to be done once for the whole class.   
3. Pipette 2µL of your RNA sample onto the Nanodrop pedestal and lower the arm   
4. Click "Measure". Record your RNA concentration (ng/µL), A260/280 ratio and A260/230 ratio. NOTE: The Nanodrop uses the Beer-Lambert Law to calculate RNA concentration for you. See Lab 1 notes on RNA extraction for more information on the calculation and how to evaluate RNA purity using A260/280 and A260/A230 ratios.  
6. Raise the arm and wipe off you sample with a KimWipe   
7. Clearly label your stock RNA sample with the word "RNA", source organism/tissue, your initials, today's date and the concentration in ug/uL.   
8. Give your samples to the TA for storage at -80C.

REVERSE TRANSCRIPTION PROTOCOL

1. Mix your stock RNA sample by inverting tube several times.
2. In a 0.5 ml PCR tube labeled with your initials and “cDNA” combine the following:
   1. 5 μl of YOUR total RNA (extracted and quantified in lab last week)
   2. 1 μl of oligo dT
   3. 4 μl of nuclease free H2O
3. Incubate the mixture for 5 min at 70C on the thermocycler then immediately transfer to ice. Briefly centrifuge you tube and the add the following:
   1. 5 μl of M-MLV 5X Reaction Buffer
   2. 5 ul of dNTPs
   3. 1 μl of M-MLV RT
   4. 4 μl of nuclease free H2O
4. Incubate the mixture for 60 min at 42C and then heat inactivate at 70C for 3 min on the thermocycler.
5. Spin down the sample in a desk top centrifuge.
6. Store on ice or at -20C