Trizol RNA Extraction – Detailed version
UPDATED 150803, Irene Liao

It is important to keep everything RNase free (wipe everything with RNase-away), use filter tips and RNase free materials, and do the protocol under the hood (hazardous chemicals). Wear a lab coat!

1. Freeze and grind tissue.
   There are many ways to do this.
   Preferred method:
   a. Collect tissue in 1.7 mL tubes, and immediately drop it into a container with liquid nitrogen. These can be stored in the -80 ° C freezer if they are not going to be extracted on the day of.
   b. Fill a white Styrofoam box with a layer (1 cm?) of liquid nitrogen. Put a Styrofoam tube rack into the box with liquid nitrogen.
   c. Place the samples in the rack in liquid nitrogen.
   d. Wipe an autoclaved plastic pestle* with RNase-away.
   e. Open the tube with the sample (in liquid nitrogen). Gently push the RNase-free plastic pestle to the bottom of the tube. Place the open tube with the pestle into the rack in the liquid nitrogen to cool the pestle (a few seconds to minutes).
   f. Once the pestle is cold, grind the tissue into a powder. If the tissue begins to thaw, place the tube back into the liquid nitrogen.
   g. Once all of the samples have been processed, proceed to the next step.

2. Add 1 mL Trizol/Tri-Reagent. Mix well.
   a. Keep the pestle in the tube as you do this. The Trizol will freeze and reach the top of the tube. Once the Trizol has thawed (keep and eye out), using the pestle, grind/mix the tissue and the Trizol.
   b. Trizol contains phenol and guanidine salts – be careful! Wear double gloves. Discard gloves if it has some phenol on it.

3. Incubate samples at RT for 5 min.
   a. Start the timer after the last sample has been mixed well with the Trizol.

4. Add 200 µl chloroform.
5. Shake vigorously for 15 sec.
6. Incubate at RT for 2-3 min.
7. Centrifuge at 12,000xg, 15 min, 4 ° C.
   a. Remember to put the tabletop centrifuge in the cold room the night before!
   b. Set the timer for 14 min after you start the centrifuge in the cold room. For all other spins, subtract 1 min from the amount of time in the centrifuge so that you know when the spin is done.

8. During spin: in new (labeled) 1.7 mL tubes, add 500 µl isopropanol, 1 µl glycogen (5 µg/ µl).

a. Glycogen is optional – it is mainly important for small amounts of RNA.
b. If more glycogen is needed, the stock solution is 20 µg/µl.
c. It is also possible to use a salt to help with the precipitation of the RNA, but I would have to look it up.

9. Removed 450 µl of the aqueous layer. Don't take the interphase. Add to tubes with isopropanol.
    a. Use a P200 to draw up 150 µl at a time. This yields plenty of RNA and prevents drawing up/touching the interphase.

10. Incubate at RT, 10 min.
11. Centrifuge at 12,000xg, 10 min, 4 ° C.
12. Discard supernatant.
13. Wash pellet with 1 mL 75% EtOH.
14. Flick tube to dislodge pellet.
15. Centrifuge at 12,000xg, 5 min, 4 ° C.
16. Discard wash.
17. Repeat 13-16 (EtOH wash) 2X. (Total washes = 3)
    a. Theoretically, you can perform as many washes as needed. This removes any salts (phenol, guanidine salts) that might contaminate the sample. Could probably do a total of 5 washes.

18. Air dry pellet (5-15 min). Check tubes every 5 min.
    a. After pouring out the EtOH, centrifuge the tubes briefly to collect any remaining EtOH.
    b. Pipet out the remaining EtOH without disturbing the pellet.
    c. Invert the tube over kimwipes to let the pellet dry.
    d. Do not over dry the pellets! When they become clear/transparent, close the tubes or add water (next step).

19. Resuspend pellet in 30 µl RNase-free water. Pipet 20-50x to resuspend.
    a. With leaf tissue, you can also resuspend it in 50 µl. I prefer the RNA to be more concentrated with the option of diluting it after quantification with the Nanodrop.
    b. If you are anticipating less RNA (i.e. using small amounts of tissue), resuspend in less water (i.e. 15 µl).

20. Nanodrop.

*Cleaning and preparing plastic pestles
    a. After use in extractions, wash the pestles with Alconox to remove phenol and other plant tissues.
    b. Incubate the pestles in a NaOH solution at 37 ° C for at least 2 hours.
    c. Rinse the pestles with DEPC water 2X.
    d. Rinse the pestles with 100% EtOH. Let it semi-dry.
    e. Wrap the pestles with aluminum foil for autoclaving (20 min).

Cleaning and preparing ceramic mortars and pestles

a. Wrap the mortars, pestles, and metal spatulas in foil.
b. Bake the materials at 200 ° C for 2 hours.