Version 6 Jan 2016. ABD.

RNA extraction from Trizol. Protocol for insects.

Before Starting:

- Use good RNA practices throughout. Handle everything as quickly as possible, minimize the time that all bottles and tubes are open, even the pipet tip boxes. Change gloves often, don't touch your hair/skin, etc. Mostly common sense stuff!
- Use sterile, RNAse and DNAse free plastics at all times, filter tips, and trusted (clean) pipettes.
- Be aware of safety practices when working with Trizol. Wear gloves whenever in contact with Trizol or containers holding it. One layer of latex and one nitrile is probably a good idea. Stay in a well-ventilated **chemical** hood at all times.
- Before beginning, collect all plastics you will need in the hood, and wipe down everything with RNAzap (or similar).
- Rinse cleaned micropestles with 95% + ethanol and leave to dry on large kimwipe.
- Prepare/find: (a) chloroform, (b) isopropanol, (c) 75% ethanol, (d) RNAse free water

Part 1: RNA extraction

1. Homogenize tissue thoroughly and quickly in 1 ml Trizol.

   For insect samples with exoskeletons, a micro-pestle in a 1.5ml microcentrifuge tube works well. It is usually easier to homogenize the tissue in 500ul Trizol, and then add the remaining 500ul to wash the pestle at the end.
   *wipe down pestles and leave to evaporate in a hood for at least 24 hours before removing and washing.

2. To increase yield, leave to sit **overnight** at room temperature in a hood. This step is optional and depends on your application.

3. Phase separation: add 200ml cold chloroform and cap the tube securely. To get the full volume, coat the pipet tip by carefully pipetting the chloroform up and down once first. Shake the tube vigorously by hand for a full 15 seconds. Let sit at room temperature 2-3 minutes. You should see the two mixtures separating almost immediately: pink on the bottom and clear on top.

4. Centrifuge the mixture 15 min centrifugation at 12,000 x g and 4˚C. Any denatured proteins might appear as a white interface. Avoiding this interface, **carefully** move the clear top liquid to a new clean, labeled tube. Leave behind some sample if needed.

   *** To isolate DNA: discard all of the interface and remaining clear liquid. Close the tube and save the bottom liquid for later DNA isolation. Make sure it is well labeled.

5. Precipitate the RNA by adding 500ul cold isopropanol. Incubate **overnight** in a 4˚C fridge (Optional but increases yield. Otherwise let sit for 5 minutes- 1 hour at RT or in fridge. Leaving to precipitate in the -80 is not recommended because it might cause contaminants to co-precipitate).

6. Pellet the RNA precipitate by spinning 10 min centrifugation at 12,000 x g and 4˚C. You may see the pellet as a clear, gel –like ball at the base of the tube, or a small white smear. You could see nothing. Discard supernatant by tipping out and blotting the rim. Keep an eye on the pellet (if you can) to make sure it doesn't get tipped out.

7. Wash the RNA pellet **2 times** using 1ml of 75% ethanol (made fresh weekly). Invert the tube (or vortex) each time to rinse all of its surfaces. Between washes, spin the sample at 7,500 x g for 5 minutes at 4˚C.

8. After the final wash, remove as much of the ethanol as possible. Use a pipette if the pellet is visible or carefully tip out the liquid and blot the tube edge. Let air dry until all liquid is gone. Be careful not to over-dry the samples, as they will not go back into solution. If you are walking away to wait for them to dry, cover the open tubes with a kimwipe. Or leave upside down on a clean kimwipe to dry.

9. Re-suspend the pellet in 30-50 ul of Sigma water, or a 0.5%SDS solution for longer storage.

10. Check that all tubes are well labeled with the sample number, date, and your initials. Store at -80˚ C, or in fridge if needed in the next few days.

AFTER EXTRACTIONS

*Wash any pestles that have sat to evaporate overnight. Use soapy water and rinse well. Soak in bleach, then water for sterilization. Rinse in ethanol if you want to speed up drying time.

*Wipe down the hood and all surfaces

Part 2: DNA extraction from Trizol.

Before Starting:

- Prepare (a) 0.1 M Sodium citrate in 10% ethanol, (b) 8mM NaOH, (c) 100% ethanol, 8d) 75% ethanol, (d) RNAse free water
- Wipe down all surfaces and continue working in a hood for as many steps as possible (certainly

DNA extraction

11. Remove all of the interface and top layer from the chloroform isolation step above (step 4). If necessary, centrifuge sample anew for 10 minutes at 4˚C.

12. Precipitate DNA with 300ul 100% ethanol (fresh), mix well by inversion. Store at RT for several minutes, or overnight in the fridge (to increase yield).

13. Centrifuge at 2,000 x g and 4˚C for 5 minutes.

14. Discard supernatant by tipping out and blotting the rim. Keep an eye on the pellet (if you can) to make sure it doesn't get tipped.

15. Wash **2 time** using 1ml of 0.1 M sodium citrate in 10% ethanol. At each wash, leave the pellet for 30 minutes at room temperature, with periodic mixing. Pellet the DNA after each wash by centrifuging at 2,000 x g and 4˚C for 5 minutes.

16. Let pellet air dry for 5-15 minutes in an open tube. Longer drying may be necessary to ensure no more drops are visible in the tube.

17. Re-elute in 30ul Sigma water. Note, if yield is high and DNA will be stored for longer, this volume should be adjusted accordingly, and 8mM NaOH should be used.