RNA extraction From Tissues (trizol Method)

- **Cleaning Homogenizer**
    - **Wash Homogenizer with DEPC water then 70% EtOH between treatments**
    - **Between samples of same treatment, just wash with DEPC water**
- **When making 75% and 70% ethanol, use DEPC water instead of DI water to dilute**

1. Take a small piece of tissue and place into an autoclaved 5ml tube which is filled with 1ml of Trizol/TRI-Reagent
2. Wash the homogenizer with DEPC water
3. Homogenize the tissue for at least 30 seconds
4. Transfer homogenized lysate to a prelabeled 1.5-2ml tube and incubate at room temperature for 10 minutes
    a. During these 10 minutes you can clean the homogenizer as stated above
5. Turn on the centrifuge and bring the temperature down to 10C
6. Add 200ul of chloroform into the tubes and shake (not invert or vortex) the tubes vigorously for 15-20 seconds and leave the tubes at room temperature for 10 minutes
7. Centrifuge the tubes at 12,000 rpm for 15 minutes at 10C
8. Transfer 500ul of supernatant to a new prelabeled 1.5-2ml tube (discard bottom solution to a **PHENOL WASTE CONTAINER**).
    a. No more than 500ul, can take less than 500ul to prevent taking the bottom layer
9. Add 500ul of isopropanol to the same tubes and invert the tube 5-6 times then leave the tubes for 10 minutes at room temperature.
10. Centrifuge the tubes at 12,000rpm for10 min at 10C
11. Discard the supernatant
12. Wash the pellet with 200ul of 75% ethanol. Vortex the tubes until the pellet dislodge from the bottom of the tubes
13. Centrifuge the tubes at 8000rpm for 8 minutes at 10C
14. Discard the supernatant and dry the tubes for 5 minutes inside of the hood.
    a. This should allow the isopropanol to completely evaporate
15. Add 200ul of TE buffer (pH 7.5) in the tubes and mix it well. IF the pellet does not dissolve properly, you can add 50-75ul of additional TE buffer.
    a. Warm the TE buffer in hot water bath to help dissolve the pellet
16. Measure RNA quantity on nanodrop