* **Note:** TRIzol & chloroform are carcinogens & corrosive = work in the West side hood. Always wear PPE. Dispose of waste properly. This is assay uses both phenol- and column based methodology to extract total RNA from finicky marine gastropod larvae.
* RT=Room Temperature
* **Before Starting:**

1. Keep everything clean. RNases are everywhere, especially on you.
2. Items **\***marked**\*** must be chilled on ice prior to use.
3. Abalone larval samples were rinsed in distilled water, flash frozen in 200uL TRIzol using liquid nitrogen
4. Homogenize sample with in 10 minutes of adding/thawing TRIzol
5. Larval samples were homogenized using a combination of pestle and sonicator. Samples were thawed on ice.
6. Pestle homogenization ~ 1 minute
7. Sonicator; 50% Amplitude, 2 second pulse (3) times
8. RT; 5 minutes; Vortex every 30 seconds
9. Transfer sample to clean, labeled 1.5mL tube
10. Add 200uL \*chloroform\* (1:1 TRIzol) to each sample
11. Invert samples 6 times
12. RT; 1 minute; vortex every 15 seconds
13. Centrifuge 15,000g; 4°C; 10 min
14. Change gloves
15. RNase away bench top and pipettes
16. Pipette off clear, aqueous top later into a fresh-labeled 2mL tube
    1. Take extreme care to not get any of the other fractions, this will contaminate your sample
    2. Start by pipetting off 50uL supernatant at a time and transfer into a fresh labeled 2mL tube
    3. If other layers are compromised, vortex homogenate and re- centrifuge and try again.
    4. Keep aqueous supernatant on ice

* **Qiagen Kit**

1. Add 700uL RLT Buffer (Qiagen Kit) to each sample supernatant
   1. Pipette mix

* Add 500uL 100% EtOH (aliquoted fresh daily) to each sample supernatant
  1. Vortex sample supernatant

1. Transfer 700uL sample to Qiagen RNeasy spin column
2. Centrifuge 8,000g; 15 seconds; RT
3. Discard filtrate
4. Repeat steps 18-20 until all RLT-EtOH-supernatant is washed through spin-column. Do not add more than 700ul at a time. Discard filtrate.
5. Add 350uL RW1 buffer to sample column
6. Centrifuge 8,000g; RT; 15 seconds
7. **Prepare DNase I Cocktail** – I prepare this before I get started if I have a lot of samples to process.
   1. Add 10 uL of DNase I stock (-20°C) to 70uL RDD buffer (+4°C)
   2. Mix gently by inverting tube. Do not vortex.
8. Add 80ul DNase I Cocktail to each sample column
9. Incubate RT on counter; 15 minutes
10. Add 350ul RW1 Buffer to each column
11. Centrifuge 8,000g; 15 seconds; RT
12. Discard filtrate and save collection tube
13. Add 500uL RPE to each column
14. Centrifuge 8,000g; 15 seconds; RT
15. Discard filtrate and save collection tube
16. Add 500uL RPE buffer to each column
17. Centrifuge 8,000g; 2 minutes; RT
18. Discard filtrate and transfer column into new collection tube (Qiagen provided)
19. Centrifuge 16,100g; 1 min; RT
20. Put sample column in labeled for storage 1.5mL tube
21. Add 20uL nuclease-free water; incubate RT; 1 min
22. Centrifuge 8,000g; RT; 1 min
23. **Save** filtrate, as this is your **eluted RNA**.
24. Repeat 38-40
25. Aliquot 5uL in PCR tube for bioanalyzer and Qubit analyses
26. Store remaining total RNA -80°C