Total RNA Extraction & cDNA Synthesis

To extract RNA from embryos and make a library of cDNA from cricket embryos, as a more stable alternative to mRNA.

General Notes:
- Be certain to clean bench and pipettes first with water and 70% ethanol.
- Use ~20 embryos per tube.
- Can repeat cDNA reaction with 2x, 3x, 4x etc volume if the reaction fails.

1) Add 200µl Trizol.
   *Note: Steps involving Trizol must be performed under a fume hood and with caution, as Trizol is toxic.
2) Homogenize with pestle, by hand.
3) Add 800µl Trizol; rinse pestle over tube as you pipette, to collect sample stuck to pestle.
4) Spin 10min at 12,000rpm at 4°C in a microfuge.
5) Incubate 5min at room temperature.
6) Add 200µl chloroform.
   * Note: Steps involving chloroform must be performed under a fume hood and with caution, as chloroform is toxic.
7) Vortex for 15sec.
8) Incubate 3min at room temperature.
9) Spin 15min at 12,000rpm at 4°C in a microfuge.
10) Collect supernatant and add 500µl isopropanol.
11) Spin 15min at 12,000rpm at 4°C in a microfuge.
12) Aspirate and discard s/n.
13) Wash with 500µl 70% EtOH in MilliQ H_2O.
14) Spin 15min at 12,000rpm at 4°C in a microfuge.
15) Aspirate and discard s/n.
16) Air dry 2-3min. Do not fully dry, as it can be difficult to dissolve completely dried RNA.
17) Resuspend in 10µl MilliQ H_2O.
    Use water from a new tube, that is certainly clean.
18) Measure concentration with spectrophotometer.
19) Use Invitrogen SuperScript III First-Strand Synthesis System for RT-PCR to generate cDNA.
20) Measure concentration with spectrophotometer. Can also perform a PCR and gel electrophoresis with reliable primers/conditions to test cDNA quality.