**Trizol isolation from firefly tissue**

*Modified from Lauren Eserman’s protocol by Karolina Heyduk and Margot Popecki (University of Georgia)*

1. To a sample that is in a 1.5mL tube: add 600uL Trizol reagent and grind with electric pestle (~30 seconds)

* *Note: Grind eye samples for 1 minute; must be completely homogenized*
* Incubate at room temperature for 5 minutes

2) Spin samples at 12,000rcf for 5 minutes

3) Incubate at room temperature 5 minutes

4) Add 120ul chloroform and shake the tube by hand for 15 seconds.

* *Note: Vortex eye samples for 30-45 seconds*
* Incubate room temperature for 2-3 minutes

5) Centrifuge 15 minutes at **4**°**C at 12,000 rcf**

6) Remove aqueous phase (clear layer, ~350ml) and place in a new labeled tube

* Add 300 ul 100% isopropanol to the new tube, invert tube 2-3 times.
* Incubate at room temperature for 10 minutes; lay tubes on their side

7) Centrifuge for 10 minutes at **4**°**C at 12,000 rcf**

8) Discard supernatant – should leave behind a pellet

* Add 600ul 75% EtOH
* Vortex until pellet dislodges
* Centrifuge at 7,500 rcf for **5 minutes at 4°C**

9) Discard EtOH. Let samples air dry ~5 minutes. Resuspend in 30 ul H2O

* When resuspending, pipette up and down a few times to dissolve the RNA

**Following RNA Isolation:**

\*\* After extracting RNA, treat sample with DNAase to remove any DNA contaminants

\*\* Clean (remove pigments from eye samples) and concentrate RNA

\*\* Quantitate with Qubit