RNA Extraction From *Fundulus* Embryos

1. Embryos collected at day 10 of development weighed approximately 4 mg.
2. Homogenize embryo by adding 200ul of Trizol.
3. After homogenizing, add 100ul chloroform, mix a few times by inversion, and let sit for 5min at room temperature.
4. Spin samples at 15,000xg for 15min at 4˚C.
5. While samples are spinning, wipe down bench and pipettes with 10% ROCALL to destroy any RNases. Put on fresh gloves before handling samples.
6. Transfer supernatant to fresh tube (should be 100-150ul) and add equal volume of 70% ethanol. Invert a few times to mix (DNA extraction can be performed on remaining volume).
7. Immediately transfer supernatant to RNeasy column (RNeasy Qiagen Kit Cat#74104).
8. Spin at 12,000xg (Eppendorf Mini-Spin 13,400rpm) for 15sec at room temperature. Pour off liquid in catch tube.
9. Add 700uL RW1 Buffer and spin at 12,000xg (Eppendorf Mini-Spin 13,400rpm) for 15sec at room temperature.
10. Transfer column to a new collection tube.
11. Add 500uL RPE buffer and spin at 12,000xg (Eppendorf Mini-Spin 13,400rpm) for 15sec at room temperature. Pour off liquid in catch tube.
12. Add 500uL RPE buffer and spin at 12,000xg (Eppendorf Mini-Spin 13,400rpm) for 3min at room temperature.
13. Place column into a new tube and elute with 20uL RNase free H_2O. Incubate for 5min at room temperature.
14. Spin at 12,000xg (Eppendorf Mini-Spin 13,400rpm) for 1min.
15. Alternatively, you can also elute once with 10ul RNase-free H_2O and incubate for 5min at room temperature. Spin at 12,000xg for 1 min. Place column into a new tube and elute with another 10ul RNase-free H_2O and incubate for 5min at room temperature. Spin at 12,000xg for 1 min.
16. Spec on Nanodrop
17. Store at -80˚C or at -4˚C with 2.5 volumes 100% etoh and 1/10 volume 3M sodium acetate.