RNA and DNA extractions from *Fundulus* embryos

Chaos Buffer
4.5M Guanidinium thiocyanate
2% N-lauroylsarcosine
50mM EDTA, pH 8.0
25mM Tris-HCl, pH 7.5
0.1M b-Mercaptoethanol
0.2% Antifoam A

Homogenize embryo in 400µl Chaos Buffer and remove 100µl for RNA extraction. The remaining 300µl is used for DNA extraction.

Chaos RNA extraction
1. Remove 100µl from 400µl Chaos homogenate.
2. Add 10µl 2M NaOAc and mix by vortexing
3. Add 100µl acidic phenol and mix by vortexing
4. Add 50µl chloroform:isoamyl - mix again
5. Let sit on ice for 10 minutes.
6. Centrifuge on maximum speed for 20 min @4°C
7. Remove supernatant (to avoid contamination by DNA trapped at interface, do not take lowest part of aqueous phase) and add equal volume of 70% isopropanol. Invert a few times to mix.
8. Immediately transfer supernatant to RNeasy column (RNeasy Qiagen Kit Cat#74104).
9. Spin at 12,000xg (Eppendorf Mini-Spin 13,400rpm) for 15sec at room temperature. Pour off liquid in catch tube.
10. Add 700 µl RW1 Buffer and spin at 12,000xg (Eppendorf Mini-Spin 13,400rpm) for 15sec at room temperature.
11. Transfer column to a new collection tube.
12. Add 500µl RPE buffer and spin at 12,000xg (Eppendorf Mini-Spin 13,400rpm) for 15sec at room temperature. Pour off liquid in catch tube.
13. Add 500µl RPE buffer and spin at 12,000xg (Eppendorf Mini-Spin 13,400rpm) for 3min at room temperature.
14. Place column into a new tube and elute with 35-50µl RNase free H_2O.
15. Spin at 12,000xg (Eppendorf Mini-Spin 13,400rpm) for 1min.
16. Store at -80˚C.

DNA extraction (Qiagen DNeasy Blood and Tissue kit #69504)
* Pre-heat Buffer AE (elution buffer) to 70°C
1. Add 200-300µl Buffer AL to sample and mix thoroughly by vortexing. Add 200-300 µl 100% ethanol and mix again thoroughly by vortexing.
2. Add mixture to a DNeasy mini spin-column placed in a 2ml collection tube. Centrifuge at 6,000g (8,000rpm) for 1 min. Discard flow-through and collection tube.
3. Place spin-column into a new collection tube and add 500µl Buffer AW1. Add 5µl RNase A (Ambion) to Buffer AW1 in spin-column and let sit for 10 min at RT. Centrifuge for 1 min at 6,000g (8,000rpm). Discard flow-through and collection tube.

4. Place spin-column into a new collection tube and add 500µl Buffer AW2. Centrifuge for 3 min at 20,000g (14,000rpm). Discard flow-through and collection tube.
5. Place the spin column into a clean 1.5 or 2 ml microcentrifuge tube and add 200µl Buffer AE, preheated to 70°C, directly onto the DNeasy membrane. Incubate the spin column at 70°C for 10 min then centrifuge for 1 min at 2,000g (8,000 rpm) to elute.