Phenol-chloroform RNA isolation from *Nematostella*

1. Collect embryos or small polyps by swirling them around in the dish, pipetting off garbage, and pipetting embryos into 1.5 ml centrifuge tube, minimizing water. Pipette off excess water.
2. In the hood, add 1 mL Trizol to each tube of embryos. Swirl to dissolve; vortex lightly if needed. Make sure all embryos are dissolved. If extracting from small polyps, start with 0.5 mL Trizol, homogenize, then add another 0.5 mL Trizol.
3. Spin down heavy phase lock tubes, keeping centrifuge at 4°C. Transfer the phenol containing dissolved embryos into the heavy phase lock tubes.
4. Add 200 µL chloroform to each tube and shake well for 15 seconds.
5. Incubate 10 minutes on ice.
6. Spin down at max speed for 15 minutes at 4°C.
7. Spin down new, empty phase lock tubes for 1 minute. Transfer the aqueous phase to the new phase lock tubes.
8. Add 600 µL phenol-chloroform-isoamyl-alcohol to each tube and shake for 15 seconds.
9. Incubate on ice for 5 minutes.
10. Spin down at max speed 15 minutes at 4°C.
11. Transfer aqueous phase to new, clean 1.5 mL tube. Use a barrier tip and be very careful the tube does not touch the bench.
12. Add 1 µl glycogen.
13. Add 500 µl isopropanol, shake, and incubate at room temperature 10-20 minutes.
14. Spin at max speed for 15 minutes at 4°C.
15. Remove liquid being careful to avoid pellet. Spin again for 10 seconds and remove more liquid.
16. Add ~890 µl RNase-free 70% EtOH stored at -20°C. Vortex.
17. Remove liquid, avoiding pellet. Spin down briefly and remove liquid again.
18. Repeat (16) & (17).
19. Let pellet dry thoroughly and add 10µl RNase free water.