Bulk RNA extraction from zebrafish embryos

Estimated time: ~2-3 hrs
Notes:

- Gives high quality RNA from 10-50 zebrafish embryos.
- This protocol calls for vortexing embryos for mechanical disruption, which is generally effective for embryos under 32 hpf. Embryos older than 32 hpf or in high batches (tubes of ~30 or more at a time) may require mechanical disruption with a clean pestle, in which case the embryos should be ground in a smaller volume of trizol (~250 µL) then brought to a total volume of 750 µL; this protocol then picks up after step 5.
- Perform all steps involving Trizol and/or chloroform in the fume hood.
- All instructions are per sample/per tube.

1. Harvest dechorionated embryos in a 1.5 mL tube.
2. Remove all embryo media from 1.5 mL tube.
3. Add 600 µL Trizol reagent (deli fridge).
4. Vortex (on max) 2-4 minutes until embryos fully disintegrate.
5. Add 150 µL Trizol reagent to reach a total volume of 750 µL.
6. Incubate 750 µL of embryonic lysate/Trizol for 5 minutes room temperature.
7. Add 200 µL Chloroform per 750 µL Trizol reagent used.
8. Vortex on max for 15 seconds, and hand tumble for 15 seconds (invert several times).
9. Incubate 2 minutes at room temperature.
10. Centrifuge at 12,000 x g for 15 minutes at 4 C (cold room centrifuge).

After centrifugation, the mixture should separate into a top (aqueous) layer containing extracted RNA. The bottom pink-red layer contains Trizol/Chloroform. ***Only transfer the top (aqueous) layer on to the next rounds of purification. For the first round, remove ~400 (or fewer) microliters of aqueous layer. For the second round, remove ~200 (or fewer) microliters of aqueous layer.***

11. Remove ~400 µL of the top (aqueous) layer into a fresh tube containing 200 µL of Chloroform, taking care not to pipette up or disturb the other phases.
12. Repeat steps 7-9 once for a total of 2 chloroform washes.
13. Transfer ~200 µL from aqueous layer of second wash into a fresh tube containing 500 µL Isopropanol.
14. Add 10% of total volume 3M Sodium Acetate pH 5.2 (NaAc or NaOAc) [e.g. for 630 µL isopropanol + aqueous phase, add 70 µL of NaAc for a total vol of 700.]
15. Hand tumble for 30 seconds (invert 10-20 times).
16. Incubate 10 minutes at room temperature.
17. Centrifuge at 12,000 x g for 10 minutes at 4 C (cold room).

At this step, the precipitated RNA will form a white pellet. The pellet size and level of visibility will depend on the RNA concentration.

18. Decant the supernatant alcohol into Trizol:chloroform waste, taking care not to disturb the pellet.
19. Add 1 mL of room temp 75% Ethanol to the pellet.
20. Invert tube 4-6 times.
21. Incubate 5 mins.
22. Centrifuge at 7500 x g for 5 minutes at 4 C (cold room centrifuge).

The remaining steps can be done at the bench.

23. Decant ethanol (down the sink or into waste beaker)
24. Repeat steps 17-21 twice more, for a grand total of 3 75% ethanol washes.
25. After the last decantation, pulse-spin samples and remove residual ethanol with a 20 µL micropipette without disturbing the pellet. If the pellet gets disturbed at this step, add 1 mL 75% EtOH and re-spin 7500xg for 5 mins at 4 C.
26. Dry samples (lid open, sideways tube) for 2-5 min at room temp.
27. Dry samples (lid open) at ~65 C. (recommend using the always-70 C incubator)
28. Transfer to ice once no liquid remains on pellet.
29. Reconstitute with 20-50 µL RNAse-free H2O. May be necessary to scrape pipette tip gently along bottom of tube to resuspend.
30. Quantify RNA quantity and purity on nano-drop and record this info.
31. Aliquot and store at –80 C.