

Hughes Lab
RNA purification using Trizol and RNeasy columns from
muscle tissue

Samples run through the protocol
today:

Date: _____

1. RNaseZap gloves, pipettes, tubes + rack, and spray the bench paper across the work area
2. Place 1 stainless steel bead (5mm) into each 2ml tube
3. Place the bead containing tube for 15min on dry ice
4. Turn on 4 degree centrifuge
5. Transfer ~ 20 mg fresh or frozen tissue to the precooled tube, incubate for another 15 min on dry ice
6. Place tubes into tube rack for < 2min

Note: this is to avoid freezing of Trizol in step 6. Do not incubate for longer than 2 min, otherwise the tissue will thaw, resulting in potential RNA degradation

7. Add 500ul Trizol immediately (in the hood)
8. Homogenize samples for 2-5 min (2 mins. + 2 + 1 (+ 1 or 2 at a time as needed)) @ 50 Hz until it is homogenized well (no tissue debris is visible), prepare 70% EtOH
9. While homogenizing, spin down phase lock gel at 10k rpm, 1 minute
10. Incubate homogenized samples, 5', RT
11. Transfer Trizol samples to Phase-locked gels
12. Add 100 ul Chloroform:Isoamyl Alcohol (24:1), mix vigorously with shaking
13. Spin down at 10k rpm, 10', 4 degrees
14. Transfer aq. phase to new tubes (~250ul)
15. Add equal volume (~250ul) 70% EtOH
16. Mix gently
17. Transfer to Qiagen RNeasy columns
18. Spin down, 30s, 4° C, 12k rpm
19. Dump Flow Thru or save for miR. Add 700ul RW1, spin down, 15s, 4° C, 12k rpm
20. Dump Flow Thru, 500 ul RPE, spin down, 15s, 4° C, 12k rpm
21. Dump Flow Thru, 500 ul RPE, spin down, 30s, 4° C, 12k rpm
22. Dump Flow Thru, move column to fresh tube, spin down, max speed, 2 minutes, 4° C.
Wipe off excess EtOH from the columns
23. Elute with 30ul RNase-free H₂O (let columns sit 1' before spin @ RT, 2 mins, 12k rpm)
24. Ice, Nanodrop/qubit/BioA, Freeze at -80