

Total RNA Isolation from *C. elegans* for Affymetrix Microarrays Using TRIzol

Reagents needed

- Trizol (Invitrogen)
 - RNeasy kit (Qiagen)
 - Chloroform
 - 70% EtOH
 - RNAase free water
1. Sort 500-1000 worms directly into 1.5ml tubes using the Biosort
 2. Spin tubes briefly to pellet worms (~5seconds in mini centrifuge)
 3. Remove supernatant, leaving 100µl of solution on top of the sample
 4. Add 400µl of Trizol reagent
 5. Vortex for 2 minutes at room temperature
 6. Freeze at -80° by placing tubes in the blue freezing chamber (PapaCooler) -
(STOPPING POINT)
 7. Freeze/thaw the sample twice at 37° and -80°
 8. Add 200µl of Trizol and let the samples set at room temperature for 5 minutes
 9. Add 140µl of chloroform, shake vigorously for 15 seconds to mix.
 10. Incubate at room temperature for 2 minutes.
 11. Centrifuge at NO MORE THAN 12,000xG for 15 minutes in the cold room (4°)
 12. Remove the top aqueous phase to a new 1.5ml tube
 13. Slowly add an equal volume of 70% EtOH by pipetting.
 14. Transfer the mixture to a Qiagen RNeasy spin column.
 15. Spin at max speed for 15 seconds; discard flow through
 16. Add the remaining sample to the spin column and repeat #15.
 17. Add 700µl of buffer RW1; spin at max speed for 15 seconds; discard supernatant
 18. Add 500µl of buffer RPE; spin at max speed for 15 seconds; discard supernatant
 19. Add 500ul of buffer RPE; spin at max speed for 2 minutes; discard supernatant
 20. Spin at max speed for 1 minute to remove any residual buffer
 21. Transfer column to new labeled 1.5 ml tube
 22. Add 50µl of RNAase-free water to the center of the filter (do NOT use DEPC treated water)
 23. Wait 1 minute
 24. Collect eluted RNA by spinning at max speed for 30 seconds
 25. Transfer elution back onto the filter; wait one minute, collect by spinning at max speed for 30 seconds.
 26. Check the concentration and purity on the spectrophotometer
 - a. To used for microarrays, samples need to be ~300-500 ng/µl and have A260/280 ratios of 1.9-2.0.
 - b. Make sure the spec is set to correct the reading to the A320 reading