Total RNA Isolation

(Reinke/Kim protocol edited by Erik Andersen April 30, 2010)

1. Wash worms off five 10 cm plates (spotted with 12,500 L1s) with H_2O.
2. Centrifuge in table-top centrifuge for one minute at 1000 rpm.
3. Wash four times with H_2O.
4. Resuspend worms in four volumes of Trizol.
5. Vortex vigorously until completely resuspended (longer than 1 minute).
6. Flash freeze slurry in liquid nitrogen.
7. Thaw at 37°C and vortex for one minute.
8. Repeat steps 5 and 6.
9. Store at -80°C until all samples are ready to be processed.
10. Thaw at 37°C.
11. Add two volumes of Trizol and vortex vigorously.
12. Add two volumes of chloroform and shake by hand for 15 seconds.
13. Incubate at room temperature for three minutes.
14. Centrifuge at 12,000 g (about 11,500 rpm in microcentrifuge) at 4°C for 15 minutes.
15. Remove the top aqueous layer to a fresh tube taking care not to disturb the interphase.
16. Add an equal volume of isopropanol and mix the tubes well.
17. Incubate at room temperature for 10 minutes.
18. Pellet RNA by spinning at top speed in a microcentrifuge at 4°C for 15 minutes.
19. Wash RNA pellets in 70% EtOH, spin at 7500g (about 9000rpm in a microcentrifuge) for five minutes at 4°C. Remove ethanol wash.
20. Spin again for five seconds in a microcentrifuge at 4°C and remove remaining ethanol.
21. Air dry the pellets briefly.
22. Dissolve them in RNAse-free water (0.5 ml-1 ml per ml of packed worms). Starting with five 10 cm plates, one should resuspend in 100 μl.
23. Take concentration of the RNA. A_{260}/A_{280} should be between 1.6 and 1.9. Multiply A_{260} by 40 ng/ml to determine RNA concentration.