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Coral RNA isolation protocol (for small prep's in 1.5mL or 2mL tubes)
Last updated: 17 March 2009 by Kevin Portune

Protocol

1. For adult tissue, follow these steps first:

    a. Label appropriate number of 2.0mL tubes (screw caps are preferred).

    b. Fill pre-labeled tubes with 1.5mL Qiazol.

    c. Proceed to step 3b or 3c.

2. For eggs and larval samples, proceed to step 3a.

3. Homogenize tissue sample.

    a. Eggs or larvae in RNA later:

i. Pipet off RNA later.

ii. Add 500uL of Qiazol.

iii. Homogenize with pellet pestle, syringe lysing, or glass beads.

    b. Adult tissue in RNA later:

i. Clip small nubbin from sample.

ii. Place in pre-labeled, pre-filled tube.

iii. Add glass beads.

    c. Flash-frozen adult tissue:

i. Pre-chill mortar and pestle in -80^{o}C freezer overnight.

ii. Place pre-chilled mortar and pestle in a bed of dry ice.

iii. Chisel away the thin layer of adult tissue using a hammer and chisel (also pre-chilled).

iv. Grind the frozen tissue into a white powder with the pestle.

v. Using a pre-chilled spatula, transfer ~100uL of powder to a pre-filled tube.

4. Add Qiazol to final volume that is 10x volume of starting material.

a. Seal samples with parafilm to avoid leakage during subsequent vortexing (or use screw-cap tubes).

5. Vortex for 10 min.

6. Spin at 12,000 rcf for 10 min at 4^{o}C to pellet skeletal waste.

7. Transfer supernatant to a new tube.

8. Leave at room temp for 5 min.

9. For every 1mL of Qiazol that you added to your sample, add 0.2 mL chloroform directly to the new tube (if followed steps above, then add 0.3mL chloroform à 1.5mL Qiazol).

10. Cap securely and shake vigorously for 30 sec.

11. Let sit at RT for 3 min.

12. Spin at 12,000 rcf for 15 min at 4^{o}C.

13. Transfer supernatant to a new tube.

14. Add an equal volume of chloroform.

15. Cap securely and shake vigorously for 30 sec.

16. Let sit at RT for 3 min.

17. Spin at 12,000 rcf for 15 min at 4^{o}C.

18. Transfer the upper (aqueous) phase to a new tube.

19. Add 5uL glycogen (20mg/mL).

20. Add 0.5mL 100% isopropanol for every 1 mL Qiazol used at the beginning.

21. Vortex to mix.

22. Let sit at room temp for 10 min.

23. Centrifuge at 12,000 rcf for 10 min at 4^{o}C to pellet the RNA.

24. Decant the isopropanol.

25. Add 1.5mL 70% EtOH and wash the pellet by flicking the tube sharply.

26. Centrifuge max speed for 5 min at 4 degrees

27. Decant the EtOH.

28. Add 1.0mL 70% EtOH and wash the pellet by flicking the tube sharply.

29. Centrifuge max speed for 5 min at 4 degrees

30. Decant the EtOH.

31. Spin tubes for 30 sec at max speed (4^{o}C) and remove the residual EtOH with a pipet.

32. Air-dry the pellet for 5-10 min.

    a. DO NOT OVERDRY or it will not re-dissolve.

33. Dissolve the RNA in 100uL water.

    a. Flicking the tubes, dragging the tubes across the holes of a tube rack, and vortexing will help to re-dissolve the pellet.

34. Place tubes at 50^{o}C for 5 min to ensure dissolution of RNA.

35. If solution is milky-white or cloudy, then spin at max speed for 5 min at 4^{o}C.

36. Transfer supernatant to new tube.

    a. Pelleted materials are contaminants that co-precipitated with the RNA.

37. NanoDrop to assess yield and purity of RNA.

38. Proceed to RNeasy clean-up (if needed).

    a. In my experience a clean RNA prep has 260/280 ~ 2.1 and 260/230 greater than or equal to 2.1 (these values are routinely obtained after RNeasy clean-up, but rarely obtained before RNeasy clean-up).

    b. DO NOT use RNeasy clean-up protocol if RNA is highly contaminated (260/230 ratio < 0.5), as RNA will not bind column and will be lost.

    c. RNA suitable for RNeasy will have 260/280 ranging from 1.8-2.1 and 260/230 ranging from 1.0-2.0.

    d. High levels of contamination can be avoided by adding appropriate amounts of starting material and Qiazol.

    e. However, some samples seem to have high contamination even when being strict about the amount of starting material.

    f. If the sample is highly contaminated, then perform a basic sodium acetate/ethanol precipitation.

i. After pelleting RNA, perform at least 2 70% EtOH washes.

ii. RNA should be less-contaminated, and thus RNeasy clean-up will be possible.

RNeasy CLEAN-UP using the RNeasy kit

All centrifugations are to be performed at room temp.

1. Label an appropriate number of spin columns.
2. To your 100uL RNA sample, add 350uL Buffer RLT and vortex to mix.
3. Add 250uL EtOH (96-100%) and vortex to mix. DO NOT STOP HERE – proceed quickly to step 4.
4. Transfer mixture to an RNeasy spin column and centrifuge 30 sec at 16,000 rcf.
5. Discard flow through.
6. Pipet 500uL Buffer RPE onto column and centrifuge 30 sec at 16,000 rcf.
7. Discard flow through.
8. Pipet 500uL Buffer RPE onto column and centrifuge 2 min at 16,000 rcf.
9. Transfer spin column to a new 2mL collection tube.
10. Spin again for 1 min (max speed) to completely dry the membrane
11. Transfer the column to a new 1.5mL tube.
12. Elute the RNA with 30-50ul RNase-free water.
    a. IMPORTANT à pipet water directly onto the membrane.
    b. Spin 1 min at 16,000 rcf.
    c. Repeat elution if yield is expected to be > 30ug

DETERMINE RNA concentration using the Nanodrop and/or Bioanalyzer