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Coral RNA isolation (for large prep's in 15mL Falcon tubes)

Last updated: 29 January 2009 by Mickey DeSalvo

Protocol

1. Grind frozen adult tissue in mortar and pestle:
 - a. Pre-chill mortar and pestle in -80°C freezer overnight (30 min. is probably OK).
 - b. Place pre-chilled mortar and pestle in a bed of dry ice.
 - c. Chisel away the thin layer of adult tissue using a hammer and chisel (also pre-chilled).
 - d. Grind the frozen tissue into a white powder with the pestle.
 - e. Using a pre-chilled spatula, transfer $\sim 0.5\text{mL}$ of powder to a 15mL Falcon tube.
 - f. Keep Falcon tubes on dry ice.
2. Add 8mL Qiazol.
 - a. *Qiazol protocol says to use Qiazol at 10x volume of powder. We have increased this to 16x.*
3. Immediately vortex to re-suspend the frozen powder in Qiazol.
 - a. Add Qiazol and vortex one tube at a time.
 - b. Once powder is homogenized in Qiazol, the tube can sit at RT until all samples have been processed.
4. Vortex each tube for 2 min.
 - a. *I usually use 2 vortexers and vortex 2 tubes/vortexer for 60 sec, then switch to another set of tubes, repeating this twice so that each tube is vortexed for 2 min.*
 - b. *Vortexing each tube for longer may increase yield.*
5. Rotate tubes at RT for 5 min
 - a. *I use a Nutator for this but I am not sure how necessary this step is. My reasoning is that it may break open more cells, and that it takes the place of the 5 min RT incubation in the Qiazol protocol, which is supposed to promote the dissociation of nucleoprotein complexes.*
6. Add 1.6mL of chloroform directly to the Falcon tubes.

a. For every mL of Qiazol, add 0.2mL chloroform.

7. Cap securely and shake vigorously for 30 sec.

8. Let sit at RT for 3 min.

9. Spin at 6,000 rcf for 15 min at 21°C.

a. Tubes can be spun faster but make sure not to exceed the max force of the tubes.

10. Transfer the upper phase (~4mL) to a new Falcon tube.

11. Add an equal volume of chloroform.

12. Cap securely and shake vigorously for 30 sec.

13. Let sit at RT for 3 min.

14. Spin at 6,000 rcf for 15 min at 21°C.

15. Transfer the upper phase (~3mL) to a new tube.

16. Add an equal volume of chloroform.

17. Cap securely and shake vigorously for 30 sec.

18. Let sit at RT for 3 min.

19. Spin at 6,000 rcf for 15 min at 21°C.

20. Transfer the upper phase (~2.5mL) to a new tube.

a. If interface is still dirty, then perform more chloroform steps – this is CRITICAL!

21. Add 4mL isopropanol.

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

22. Vortex to mix.

23. Let sit at RT for 10 min.

24. Centrifuge at 8,000 rcf for 20 min at 4°C to pellet the RNA.

25. Aspirate the supernatant.

26. Add 7mL 70% EtOH to wash the pellet.

27. Centrifuge 8,000 rcf for 15 min at 4°C.

28. Aspirate the EtOH.
29. Add 7mL 70% EtOH.
30. Centrifuge 8,000 rcf for 15 min at 4°C.
31. Aspirate the EtOH.
32. Add 7mL 70% EtOH.
33. Centrifuge 8,000 rcf for 15 min at 4°C.
34. Aspirate the EtOH.
35. Centrifuge 8,000 rcf for 1 min at 4°C and remove the residual EtOH with a pipet.
36. Air-dry the pellet for 5-10 min.
 - a. *DO NOT OVERDRY or it will not re-dissolve.*
37. 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.*
38. Place tubes at 50°C for 5 min to ensure dissolution of RNA.
 - a. If solution is milky-white or cloudy, then spin at 7,500 rcf for 5 min at 4°C.
 - i. *Pelleted materials are contaminants that co-precipitated with the RNA.*
 - ii. *If this happens, this is a bad sign.*
 - iii. *You may have to repeat the extraction with less starting material (or more Qiazol).*
 - b. *>Transfer supernatant to new tube.*
39. NanoDrop to assess yield and purity of RNA.
40. Proceed to RNeasy clean-up (ALWAYS).
 - 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.

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
13. Determine RNA concentration using the Nanodrop and/or Bioanalyzer
14. If concentration is not high enough for subsequent microarray analysis, then precipitate RNA:
 - a. Add 250uL of RNase-free water.
 - b. Add 35uL of 3M NaOAc pH 5.2

- c. Add 875uL 100% EtOH
- d. Vortex and store overnight at -80°C.
- e. The following day, warm samples to 4°C, and vortex.
- f. Spin max speed, 30 min, 4°C.
- g. Aspirate supernatant.
- h. Add 1.5mL 70% EtOH.
- i. Spin max speed, 15 min, 4°C.
- j. Aspirate supernatant.
- k. Add 1mL 70% EtOH.
- l. Spin max speed, 15 min, 4°C.
- m. Aspirate supernatant.
- n. Add 0.5mL 70% EtOH.
- o. Spin max speed, 15 min, 4°C.
- p. Air dry pellet.
- q. Re-suspend in appropriate volume of water – this will vary depending on the yield after RNeasy clean-up – shoot for 1ug/uL.
- r. NanoDrop to assess quantity.