RNA Isolation (Qiagen RNeasy Mini)

Introduction

This protocol is for isolating RNA from mammalian cells and can be used for RT-qPCR.

Materials

- 1 X 10^7 cells maximum in 24 well plate
  - *if they're high RNA yield, you can use less. If RNA yield is unknown, then Qiagen suggests using 3-4 X 10^6 cells
  - *remember, in a 24 well plate, we typically seed 2 X 10^5 per well
- PBS
- Trypsin
- Cell Media with serum
- Pico pure water
- From Qiagen Kit:
  - RNeasy spin columns
  - Buffer RLT
  - Buffer RW1
  - Buffer RPE (*Note: Make sure that ethanol is added)

Procedure

Tissue Culture

1. Put a 50 mL conical of aliquoted media and a 15 mL conical of frozen trypsin in the heat bath to warm up. Takes about 30 minutes.
2. Sign in and set up a tissue culture hood.
3. Calculate the amount of wells needed, usually anywhere between 5-20 wells (1 X 10^6 - 4 X 10^6 cells)
4. Look at cells either using the light microscope by the sink or the EVOS to ensure the cells are healthy and there's no contamination.
5. Aspirate media from each well, making sure not to disturb the cells. This is best done by tipping the plate towards you and aspirating from the side of the well wall. (*Note: if you're doing large amounts of wells, make sure to do this procedure a row at a time so cells don't dry out)
6. Add 200 uL of PBS to each aspirated well. Do this by pipetting slowly down the side of the well so that the cells are not disturbed and make sure to switch out tips each time.

7. Aspirate the PBS

8. Add 200 uL of trypsin to each well (*Note: You don't have to be as gentle here as you were with the PBS. You WANT to disturb cells at this point)

9. You'll want to wait a few minutes for the trypsin to detach the cells from the plate. For HEK293, this should only be a couple of minutes. However, depending on cell type, you might want to also put the plate back into the 37 degree C incubator. DO NOT OVER TRYPSINIZE. You will kill everything.

10. Add 500 uL of cell media to each well to quench the trypsin. (*Note: If you wait to long after this step, the cells may reanneal to the bottom of the plate and everything you've done up to this point will be for nothing.)

11. Transfer cells to a RNase-free 15 mL conical and centrifudge at 300 x g for 5 minutes. Aspirate the supernatant and be careful not to suck up the pellet. The pellet is going to be VERY SMALL!!!! (*Note: make sure to REMOVE ALL OF THE SUPERNANTANT. Leaving any left over may decrease the isolated RNA yield.)

12. **POSSIBLE PAUSE**: If you're short on time or can't use the RNA right away, you can freeze the pellet in the -80 degree C freezer.

Qiagen Kit

13. If using a frozen pellet, make sure to thaw it a little bit using your fingertips. Flick the tube to dislodge the pellet.

14. Add Buffer RLT. Add 350 uL of buffer if you're using less than 5 X 10^6 cells or add 600 uL of buffer is you're using 5 X10^6 cell to 1 X 10^7 cells.

15. Vortex to pipette to mix the pellet with the buffer

16. If using less than 1 X 10^5 cells, homogenize by vortexing for 1 minute. Otherwise, follow one of the options below. (*Note: Incomplete homogenizing will reduce RNA yield and could clog the RNAeasy spin column.)

17. Option 1: Place a QIAshredder spin column into an 2 mL (typical eppendorf tube) collection tube and pipette lysate into spin column

18. Centrifuge spin columns in the collection tubes for full speed in the table top centrifuge for 2 minutes.

19. Option 2: Homogenize lysate for 30 seconds using rotor-stator homogenizer.

20. Option 3: Pass the lysate at least 5 times through a blunt 20-gauge needle (0.9 mm diameter) fitted to a RNase-free syringe

21. **POSSIBLE PAUSE:** You can freeze the homogenized lysate in the -80 degree C freezer for serveral months. When continuing, incubate the lysate in a 37 degree C water bath until it completely thaws and the salts are dissolved. (*Note: prolonged incubation can destabilize the RNA, so if you still have insoluble materal, centrifuge the tube for 5 minutes at 3000-5000 X g. Transfer supernantant to a RNase free tube and continue.)

22. Estimate volumn of lysate using a pipette (probably a little less than 350 ul of 600 ul depending on amount of Buffer RLT used.)

23. Using this estimated volumn, add 70% ethanol to the lysate and mix well by pipetting. (*Note: this may create some precipitate. Don't worry, this is fine.)

24. Transfer up to 700 uL of the sample to an RNeasy spin column placed in a 2 mL collection tube. (*Note: if the sample volumn if bigger than 700 uL, you'll just have to repeat the this and the next step until you've gone through all of your sample.)

25. Gently close the lid and centrifuge for 15 seconds at greater than 8000 x g. Discard the flow through in the collection tube. **DO NOT PUT INTO BLEACH BUCKET!!!!!** Create your own waste tube, like a 50 mL conical and keep it by the other Qiagen buffer waste.

26. Possible DNase digestion?

27. (*Note: Skip if doing DNase digestion) Add 700 uL of Buffer RW1 to the spin column. Close the lid gently and centrifuge for 15 seconds at greater than 8000 x g. Discard flow through into waste tube.

28. Add 500 ul of Buffer RPE to spin column. Close lid gently, and centrifuge for 15 seconds at greater than 8000 x g. Discard flow through into waste tube.

29. Add 500 ul of Buffer RPE to spin column. Close lid gently, and centrifuge for 2 minutes at greater than 8000 x g.

30. Discard old collection tube, place the spin column into a new collection tube, close the lid gently, and centrifuge at full speed for 1 minute.

31. Place the spin column into a new 1.5 mL collection tube and add 30-50 uL RNase-free water (pico pure).

32. Close lid gently and centrifuge for 1 minute at greater than 8000 x g.

33. You have your RNA! Make sure to label appropriately. Either store in the -20 degree C freezer for a day or two or store in the -80 degree C freezer. To have the best RNA stability, it's best to use it as soon as possible. (*Note: Depending on your application, it may be advantageous to aliquot the RNA into single use aliquots before feeezing.)