**RNA Extraction and Purification**

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Materials:

1. DNase and RNase free water (Sigma )
2. RNase Away
3. TRI Reagent (Sigma: T9424-25ML)
4. 100 % absolute ethanol (NOT the denatured ethanol)
5. 75 % ethanol in RNase free DI water
6. 75 % ethanol in normal DI water for cleaning surfaces.
7. Heavy gel epi tubes (5Prime Phase lock Gel Heavy)
8. Chloroform (Sigma: C2432-1L)
9. Isopropanol (Sigma: )

Notes:

1. Keep the frozen tissue in dry ice all the time. Thawing the tissue might reduce RNA extraction efficiency
2. Use RNase free pipet tips and tubes
3. To avoid dipping of chloroform during pipetting, pump in chloroform in the pipette and then pump out once. Then pump in the chloroform using the same pipet tip, this time chloroform will not dip.

Procedure:

1. Clean the benchtop with 80 % ethanol and then with RNase away
2. Take tissue samples out from -80 C and put into dry ice.
3. Put a weighing boat on dry ice and put a tissue sample on the boat. Cut ~100 mg sample (larger sample will make them harder to homogenize) using a sharp razor blade. Weigh the tissue using another weighing boat, put the sample into a labeled 15 ml round bottom tubes. Keep the tube in dry ice until use.
4. Add 10 ml TRI reagent per 1 g tissue (so for 100 mg you need to add 1 ml TRI reagent) and put the tube in ice (NOT dry ice)
5. Homogenization:
   1. Clear the tip of a mechanical homogenizer (chopper) with Ethanol and Millipore DI water
   2. Homogenize the tissue for 1 min at 75 % power and keep the tube in ice
   3. Look for large chunks of tissue in the solution, if you find any, then homogenization is not done. Repeat homogenization until no visible big tissue remains.
   4. Keep the tubes with the solution into ice.
   5. Between two sample homogenization, clean the homogenizer tip with (1) splaying with DI water and wipe with Kim wipe (2) Physically check if any big tissue is attached to the tip, remove using a forceps (2) spinning the tip into DI water (bicker -1) , wipe with Kim wipe (3) spinning the tip into 100 % ethanol (in ~25ml in a 50 ml tube), wipe with Kim wipe (4) spinning the tip into DI water (bicker-2) and wipe with Kim wipe.
6. Take 1 ml tissue lysate into a heavy gel epi tube and then add 100 ul chloroform in it (see note how to avoid dipping of chloroform during transfer). Mix the solution flipping the tube for a couple of time (~10 times)
7. Incubate the mix for ~5 min at RT
8. Centrifuge at 12,000 g for 15 min at RT.
9. Using 200 ul pipette tips, transfer the top phase into a new epi tube without touching the gel in the interface.
10. Add 500 ul isopropanol in to the tube and mix by flipping the tube.
11. Incubate the solution at RT for 2 min. Over incubation might precipitate unwanted materials.
12. Centrifuge at 12,000 g for 2 min at RT.
13. A visible pellet of RNA should form at the bottom of the tube. Carefully pour down the supernatant.
14. Centrifuge at 12,000 g for 1 min at RT.
15. Using a 200 ul pipette tip, remove the supernatant without disturbing the pellet.
16. Add 1 ml 75% ethanol in RNase free DI water. Vortex for 1 or 2 sec just to detach the pellet into solution.
17. Centrifuge at 12,000 g for 1 min at RT.
18. A visible pellet of RNA should form at the bottom of the tube. Carefully pour down the supernatant.
19. Centrifuge at 12,000 g for 1 min at RT.
20. Using a 20 ul pipette tip, remove the supernatant without disturbing the pellet.
21. Repeat the washing of pellet with ethanol (last 5 steps) for one more time.
22. Air dry the pellet by keeping the tube open on benchtop for 10 min. Dried pellet will convert from white to grayish. Over dry will make it harder to resuspend the pellet in the following steps. If an RNA purification step will be done after extraction, the pellet does not need to be dried much, so a 5 min air dry will be enough.
23. Add 100 ul RNase free DI water. By pipetting-in-and-out resuspend the pellet.
24. Determine the concentration of RNA using a nano-drop

RNA Purification:

1. Use Qiagen kit (RNeasy MinElute Cleanup Kit, Cat: 74204)
2. Capacity of each column in this kit is 45 ug of RNA and putting more than 45 ug of RNA will be waste of RNA. To find the volume of RNA should be purified, use the concentration measured after extraction.