Isolation of RNA from RNAlater preserved tissue

Equipment and reagents

RNAlater Stabilisation solution AM7020 Ambion
Dry ice
Wet ice
RNase Zap
Trizol 15596026 100mls Life Technologies
1-bromo-3-chloropropane Sigma # B9673-200ml
Isopropanol P750017EB Fisher Scientific
Ethanol E0650DF17EB Fisher Scientific
RNase free water
Durx 670 clean room wipes SLS CLE4012
FastPrep tissue homogeniser and Lysing Matrix D tubes Fisher # MBR-247-110Y
Or Precellys tissue homogeniser and lysing matrix tubes for RNA for Precellys e.g. CK14
Pipettes and filter tips
RNeasy Mini Kit C-74104EB (Roslin Stores)
Agilent Tapesatation or Bioanalyser and reagents required for RIN QC

Procedure

- Care must be taken during all steps to prevent degradation or contamination of RNA.
- Reagent bottles should be RNase free and kept specifically for RNA prep
- Bench surfaces, racks, ice boxes, pipettes and equipment should be scrupulously clean and treated with RNase Zap
- Sample tubes and reagent bottles should be open only for the minimum time required for each step
- Gut tissues should be washed in several changes of sterile PBS before excising aliquots for freezing

1. Collect tissue into RNA later and leave overnight at 4°C
2. The following day, remove the RNAlater and place 50-100mg of tissue in a 1.5ml screw-topped tube. Ensure that all traces of liquid or crystallised RNAlater are removed from the tissue sample. Residual RNAlater can be a problem with gut tissues for example. If required, quickly blot the sample on a clean room wipe before adding to storage tube. Store tubes at -80°C until required for RNA prep.
3. For each RNA prep, IN FUME HOOD, add 1ml Trizol to a lysing tube and chill on ice.
4. Collect samples from -80°C freezer on dry ice.

5. Set lysing speed and time on homogeniser e.g. for Fastprep
   speed 4m/s,
   time 20 seconds
   Speed and time may change for tissues that are difficult to lyse, but the above settings are suitable for most tissues
6. Add a maximum of 100mg of frozen tissue to 1ml of chilled Trizol in lysing matrix tube.
7. Homogenise samples. A small amount of non-lysed tissue may remain after lysis. Although this will reduce yield, increasing the lysis time and speed can lead to shearing and poor quality RNA.
8. If samples feel warm after homogenising, cool briefly on ice before equilibrating to room temperature.
9. Incubate at room temperature for 5 minutes.
10. IN FUME HOOD add 200ul BCP, shake tube vigorously by hand for 15 seconds.
11. Incubate at room temperature for 3 minutes.
12. Centrifuge samples at 12000 x g for 15 minutes at 4°C.
13. Taking great care not to disturb interface, transfer ~450ul of the upper aqueous phase containing the RNA to a new 1.5ml tube.

<u>RNeasy clean-up (refer to manufacturer's protocol)</u>

14. Add 1 volume of 70% ethanol, and vortex. Do not centrifuge. Proceed at once to step 15.
15. Transfer up to 700 µl of the sample to an RNeasy Mini spin column in 2 ml collection tube (supplied with kit). Centrifuge at room temperature for15 s at 10,000 x g, and discard flow-through.
16. Using the same collection tube, repeat step 15 using the remainder of the sample. Discard the flow-through
17. Add 700 µl Buffer RW1 to RNeasy column. Centrifuge for 15 s at 16,000 x g, and discard flow-through.
18. Add 500 µl Buffer RPE to RNeasy column. Centrifuge for 2min at 16,000 x g, and discard flow-through.
19. Add 500 µl Buffer RPE to RNeasy column. Centrifuge for 15 s at 16,000 x g, and discard flow-through
20. To further dry membrane, place RNeasy column in new 2 ml tube and centrifuge at full speed for 1 min.
21. Place RNeasy column in a new 1.5 ml tube. Add 30–50 µl RNase-free water, leave for I min then centrifuge for 1 min at 10,000 x g. Elution can be repeated with an additional 30 – 50ul RNase-free water to increase yield, or the first elution can be passed though the column for a second time.
22. Place RNA on ice and check RNA concentration and 260/280 and 260/230 ratios on the Nanodrop. Remove a 2ul aliquot for RIN QC. Store remaining stock RNA at -80°C
23. Dilute the 2ul QC RNA if required using RNase free water. Run samples on the Tapestation using the R6K manufacturer's protocol. RIN values of $\geq$ 7 are recommended for RNA seq.