Total RNA extraction for frozen cells (ImmunoMap)

This protocol is a modification of the original NMBU protocol "Total RNA extraction for tissues (BodyMap)"
https://data.faang.org/api/fire_api/experiments/NMBU_SOP_RNAextraction_protocol_20200503.pdf

Preparation

- Pre-chill a fixed rotor centrifuge with capacity for 2 mL tubes to 4˚C, under the fume hood
- All steps should be done at room temperature, except the 1st and 2nd centrifugation (step 6)
- Al steps should be done under the fume hood
- Prior to first use, prepare the stock DNAse I mix, the buffer RWT and RPE according to manufacturer's instructions

Protocol

1. Centrifuge 5 minutes 500 x g at 4ºC. Carefully remove the supernatant without disturbing the pelleted cells. Add 700 ul Qiazol Lysis.
2. Under the fume hood, vortex the tube and disrupt for 1 minute at max speed.
3. Let the tubes stand vertically for 5 minutes.
4. Add 140 ul of chloroform and vortex for 15 s.
5. Let the tubes stand vertically for 2-3 minutes.
6. Centrifuge 15 minutes 12,000 x g at 4°C. After this step, heat the centrifuge up to room temperature.
7. Carefully remove the tube from centrifuge and transfer the upper aqueous phase (approximately 350 ul)to a new 1.5 mL tube.
8. Add exactly 1.5x volume of 100% ethanol (usually 525 ul), mix well by pipetting and continue with thenext step without delay.
9. Pipet up to 700 ul of the sample into a RNeasy Mini spin column placed into a 2 ml collection tube. Centrifuge at >8000 x g for 15 s at room temperature and discard the flow-through.
10. Repeat the previous step with the remaining volume of sample and with the same column. Discard the flow-through.
11. Add 350 ul of buffer RWT in the column and centrifuge for 15 s at > 8000 x g. Discard the flow-through.
12. Add 80 ul of DNAse I mix and incubate it at room temperature for 15 min.

13. Pipet 350 ul of buffer RWT in the column and centrifuge for 15 s at > 8000 x g. Discard the flow-through.
14. Pipet 500 ul of buffer RPE in the column and centrifuge for 15 s at > 8000 x g. Discard the flow-through.
15. Pipet another 500 ul of buffer RPE in the column and centrifuge for **2 minutes** at > 8000 x g. Discard the flow-through.
16. Place the column in a new 2 ml collection tube and centrifuge 1 minute at > 13000 x g, to remove anyremains of previous buffers.
17. Place the column in a new 1.5 ml tube. Add 50 ul of RNAse free water and centrifuge 1 minute at > 8000 g.Discard the column and place the tube with the flow-through on ice.
18. Quantify your RNA on Nanodrop and check its RIN value and profile on Bioanalyzer. Freeze the remaining50 ul at - 80 °C as soon as you can.

Reagents and Equipment

Reagent	Reference
miRNeasy Mini kit (Qiagen)	217004
RNAse free DNAse set (Qiagen)	79254
Bioanalyzer RNA 6000 Nano kit	5067-1511
Microcentrifuge thermoregulated	
Ethanol 100%	
Chloroform	
Ice	