Total RNA extraction for tissues (BodyMap)

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 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. Remove 25-30mg of tissue from its tube containing RNAlater and place it in a new 2mL safe-lock tube, containing 700ul Qiazol Lysis Reagent and 1 stainless steel bead 5mm.
2. Under the fume hood, place the tube in Tissue Lyser and disrupt for 1-2 minutes at 20-25 Hz. Parameters may vary depending on type of tissue.
3. Let the tubes stand vertically for 5 minutes.
4. Add 140 ul of chloroform and shake it well by hand.
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 form centrifuge and transfer the upper aqueous phase (approximately 350ul) to a new 1.5mL tube.
8. Add exactly 1.5x volume of 100% ethanol (usually 525ul), mix well by pipetting and continue with the next step without delay.
9. Pipet up to 700ul 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 15-20 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 min at > 13000 x g, to remove any remains of previous buffers.
17. Place the column in a new 1.5 ml tube. Add 52 ul of RNAse free water and centrifuge 1 min 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 Bioanalyze. Freeze the remaining 50 ul at - 80 °C as soon as you can.

Reagents

Reagent	Reference
miRNeasy Mini kit (Qiagen)	217004
RNAse free DNAse set (Qiagen)	79254
5mm stainless steel beads	69989
Bioanalyzer RNA 6000 Nano kit	5067-1511
ethanol 100%	
chloroform	
ice	