RNA purification using RNeasy Lipid Tissue Mini Kit with DNase treatment (RNA-seq)

(adapted from Qiagen RNeasy Lipid Tissue extraction kit Cat. No. 74804 and RNase-Free DNase Set Cat. No. 79254)

Reagents and Equipment

- Qiagen RNeasy Lipid Tissue extraction kit Cat. No. 74804
- RNase-Free DNase Set Cat. No. 79254
- 100% ethanol (AQA Ethyl Alcohol (absolute) ACS Grade 5L no. AL-1551-5000)
- Chloroform (Sigma Cat. No. C2432)
- Pestle (Kimble Chase motor cordless no. 749540)
- Microcentrifuge for centrifugation at 4°C

Protocol for macroareas/whole-brain (small brains) – RNA-seq

1. Disrupt and homogenize tissue in **500** µl Qiazol Lysis Reagent (**1,5** ml eppendorf) using pestle (Samples always on ice - 1 cycle of 45 s, repeat if any tissue is still visible. For samples stored in RNA*later* do 1 cycle of **60** s).

2. Incubate sample at RT for 7 min.

3. Add **100** µl chloroform (kept at 4ºC), and shake vigorously for 15 s (use fume hood).

4. Incubate sample at RT for 5 min.

5. Centrifuge at 14,000 x *g* for 20 min at 4ºC.

Warning: don't jostle the tubes after spinning and don't take every last bit of supernatant off the interphase to avoid gDNA contamination. After step 5 samples should be handled on ice.

6. Transfer upper, aqueous phase to new tube (**1,5** ml eppendorf), add 1 volume of 70% ethanol (kept at 4ºC), and do a short vortex. Do not centrifuge. Proceed at once to step 7.

7. Transfer sample to RNEasy column in 2 ml tube. Wait 5 min. Close lid, centrifuge for 1 min at 14,000 x *g*, and discard flow-through.

8. Add 350 µl Buffer RW1 to Rneasy column. Close lid, centrifuge for 1 min at 14,000 x *g* and discard flow-through.

9. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix gently inverting the tube (**do not vortex** since it will denature DNase I), and centrifuge briefly to collect residual liquid from the sides of the tube.

10. Add 80 µl DNase I incubation mix directly to the RNeasy spin column membrane, and incubate for 15 min at RT.

11. Add 350 µl Buffer RW1 to Rneasy column. Close lid, centrifuge for 1 min at 14,000 x *g* and discard flow-through.

12. Add 500 µl Buffer RPE to Rneasy column. Close lid, centrifuge for 1 min at 14,000 x *g*, and discard flow-through.

13. Add 500 µl Buffer RPE to Rneasy column. Close lid, centrifuge for 2 min at 14,000 x *g*, and discard flow-through.

14. Place RNeasy column in new 2 ml tube, close lid, centrifuge at full speed for 3 min.

15. Place RNeasy column in new 1,5 ml tube. Add **50** µl RNAse-free water, close lid, wait 1 min, and centrifuge for 2 min at 14,000 x *g*.

16. Repeat elution using the same water from previous step to concentrate samples. Close lid, wait 1 min, and centrifuge for 5 min at 14,000 x *g*.

17. Measure RNA concentration in Nanodrop and store at –80 ºC.

Notes:

- Buffer RPE should be always sealed after use with parafilm to avoid ethanol evaporation.
- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex.
- For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

RNA purification using RNeasy Lipid Tissue Mini Kit without DNase Treatment

(adapted from Qiagen RNeasy Lipid Tissue extraction kit Cat. No. 74804)

Reagents and Equipment

- Qiagen RNeasy Lipid Tissue extraction kit Cat. No. 74804
- 100% ethanol (AQA Ethyl Alcohol (absolute) ACS Grade 5L no. AL-1551-5000)
- Chloroform (Sigma Cat. No. C2432)
- Pestle (Kimble Chase motor cordless no. 749540)
- Microcentrifuge for centrifugation at 4°C

Protocol for macroareas/whole-brain (small brains)

1. Disrupt and homogenize tissue in **500** µl Qiazol Lysis Reagent (**1,5** ml eppendorf) using pestle (Samples always on ice - 1 cycle of **45** s, repeat if any tissue is still visible).

2. Incubate sample at RT for 7 min.

3. Add **100** µl chloroform (kept at 4ºC), and shake vigorously for 15 s (use fume hood).

4. Incubate sample at RT for 5 min.

5. Centrifuge at 14,000 x *g* for 20 min at 4ºC.

Warning: don't jostle the tubes after spinning and don't take every last bit of supernatant off the interphase to avoid gDNA contamination. After step 5 samples should be handled on ice.

6. Transfer upper, aqueous phase to new tube (**1,5** ml eppendorf), add 1 volume of 70% ethanol (kept at 4ºC), and do a short vortex. Do not centrifuge. Proceed at once to step 7.

7. Transfer sample to RNEasy column in 2 ml tube. Wait 5 min. Close lid, centrifuge for 1 min at 14,000 x *g*, and discard flow-through.

8. Vortex Buffer RW1 for a few seconds. Add 700 µl Buffer RW1 to Rneasy column. Close lid, centrifuge for 1 min at 14,000 x *g* and discard flow-through.

9. Add 500 µl Buffer RPE to Rneasy column. Close lid, centrifuge for 1 min at 14,000 x *g*, and discard flow-through.

10. Add 500 µl Buffer RPE to Rneasy column. Close lid, centrifuge for 2 min at 14,000 x *g*, and discard flow-through.

11. Place RNeasy column in new 2 ml tube, close lid, centrifuge at full speed for 5 min.

12. Place RNeasy column in new 1,5 ml tube. Add **30-50** µl RNAse-free water, close lid, wait 1 min, and centrifuge for 2 min at 14,000 x *g*.

13. Repeat elution with the same volume of RNAse-free water (to concentrate samples). Close lid, wait 1 min, and centrifuge for 5 min at 14,000 x *g*.

14. Measure RNA concentration in Nanodrop. Store at - 80 ºC.

Notes:

- Buffer RPE should be always sealed after use with parafilm to avoid ethanol evaporation.
- Step 7, if the volume to transfer is higher than 700 µl, this step should be repeated twice using the same column.
- In step 12 the volume of RNAse-free water to use in the elution depends on the required final RNA concentration for follow up analyses. If a high concentration of RNA is needed, in the second elution “re-use” the volume in the eppendorf from the first elution.

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