AQUA-FAANG - Standard Operating Protocol - RNA extraction protocol in carp embryos

Overview

This protocol describes the RNA extraction method used in carp embryo samples frozen in trizol prepared as per the carp embryo collection protocol (1). To perform total RNA extraction (including small RNA species) the miRNeasy Micro Kit was used (Qiagen).

Note: It is recommended to treat the samples with DNase I. This can be achieved using the reagents in the RNase-Free DNase Set (Qiagen).

1. Thaw samples on ice.
2. When trizol is liquid, dissociate the samples by passing them up and down through a 1 ml syringe and a needle until achieving full dissociation (no visible material in suspension). Note: for carp embryos at most stages a 25G needle (Orange, Terumo AGANI®) was suitable to achieve full dissociation. Larger samples (e.g. older larval samples) might require starting with larger needle sizes and then move to the 25G size.
3. Let the tubes stand vertically for 5 minutes.
4. Add 140 µl of chloroform and shake vigorously. Let the tubes stand vertically for 2-3 minutes.
5. Centrifuge 15 minutes 12,000 x g at 4°C.
6. Transfer the upper aqueous phase (approximately 350 µl) to a new 1.5 mL tube.
7. Add 1.5x volume of 100% ethanol (usually 525 µl), mix well by pipetting.
8. Pipet up to 700 µl of the sample into a RNeasy MinElute 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.
9. Repeat the previous step with the remaining volume of sample and with the same column. Discard the flow-through.
10. Add 350 µl of buffer RWT in the column and centrifuge for 15 s at > 8000 x g. Discard the flow-through.

11. Prepare the DNAse I mix per sample of RNA to be extracted (do not vortex):

Reagent	Volume per sample (µl)
DNAse I	**10**
Buffer RDD	**70**
Total	**80**

12. Add 80 µl of DNAse I mix directly to the column membrane and incubate it at room temperature for 15 min.
13. Pipet 350 µl of buffer RWT in the column and centrifuge for 15 s at > 8000 x g. Discard the flow-through.
14. Pipet 500 µl of buffer RPE in the column and centrifuge for 15 s at > 8000 x g. Discard the flow-through.
15. Pipet 500 µl of fresh 80% ethanol on 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 any remains of previous buffers.
17. Place the column in a new 1.5 ml tube. Add 14 µl of RNAse free water, let the tube stand for 2 minutes
18. Centrifuge 1 minute at > 8000 g. Discard the column and place the tube with the flow-through on ice.
19. Quantify your RNA on Nanodrop and check its RIN value and profile on Bioanalyzer. Freeze the remaining 50 µl at - 80 °C as soon as you can.

Bibliography:

1. Jimenez-gonzalez A. Standard Operating Protocol - Dechorionation of carp embryos and embryo preservation for RNA extraction https://data.faang.org/api/fire_api/samples/UOB_SOP_Carp_embryo_collection_RNAseq_20210917.pdf