RNA extraction for BLiMMP project

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Solutions/supplies needed

- Lysis matrix A in MP Bio bead-beating tubes
- TRIzol
- Internal standard, at ~0.75-1.25 ng/µl
- Nuclease-free 1.5 ml Eppendorf tubes (4 per extraction)
- Chloroform
- 100% isopropanol
- 100% ethanol, cold
- 70% ethanol, cold
- 3M sodium acetate, pH = 5.5
- Turbo DNase kit

Prep work before starting extractions

1. Cool centrifuge to 4˚C.
2. Set up and label four 1.5 ml Eppendorf tubes for each extraction
3. Clean everything with RNase Zap.

Physical lysis and Trizol extraction

1. Sterilize and RNase Zap the PVC cutter, a razor blade, and two tweezers.
2. Cut open Sterivex filter cartridge with the PVC cutter and remove the filter using the razor and the tweezers.
3. Add filter to beadbeating tube with lysis matrix A in it. Immediately add 1 ml of Trizol and bead-beat for 1 minute.
    a. While the sample is bead-beating, wipe down and RNase Zap the tweezers, razor, and PVC cutter
4. Store sample in fridge.
5. Repeat for all the samples that need processing on this day.
6. Now process all the samples together. Add 12 ng of internal standard to each sample. For the internal standard prepped on 2022-03-29, that's 14 µL of the 500X diluted internal standard. Invert repeatedly for 1 min.
7. Centrifuge for 5 minutes at 16,100 x g at 4˚C .
8. Transfer 800 µl of supernatant to new 1.5 ml Eppendorf tube.
9. Add 300 µL of the chloroform to Trizol/sample mix. Invert repeatedly for 90 seconds, then incubate at room temperature for 3 minutes.
10. Centrifuge for 15 minutes at 13,200 x g at 4˚C.
11. Transfer 500 µL of the aqueous phase to a new 1.5 ml Eppendorf minicentrifuge tube.

Purification by isopropanol and ethanol precipitation

12. Add 500 µL of 100% isopropanol to sample. Invert several times to mix.
13. Incubate at room temperature for 20 minutes.
14. Spin down at 16,100 x g for 25 minutes at 4˚C.
15. Carefully remove the supernatant by pipetting it off.
16. Resuspend pellet in 100 µl of DEPC-treated H2O.
17. Add 10 µl of 3 M NaOAc at pH 5.5. Mix well.
18. Add 250 µl of cold 100% EtOH. Mix well, store at -20˚C for 20 minutes.
19. Centrifuge for 20 min at 16100xg at 4˚C.
20. Add 200 µl of 70% EtOH, and vortex to mix.
21. Spin down at 16,100 x g for 20 minutes at 4˚C.
22. Carefully pipette off the supernatant and allow RNA to dry on the countertop.
23. Resuspend in 50 µl of nuclease-free water.

DNA removal with Turbo DNase

24 Add 5 µl DNase Buffer from Turbo DNA kit. Mix gently
25 Add 1.5 µl of TURBO DNase. Mix gently. Incubate at 37 ˚C for 25 minutes.
26 Add 5µl of DNase Inactivation Reagent (mix regent well first). Incubate 5 min at room temp, mixing periodically.
27 Centrifuge for 90 seconds. Transfer supernatant to new tube, take ~52 µl. Be sure not to get any of the inactivation reagent.

Purification by ethanol precipitation

28 Add 148 µl of nuclease free H_2O to sample, mix well
29 Add 20 µl of 3 M sodium acetate (pH 5.5) to the sample and mix well.
30 Add 500 µl of cold 100% ethanol to sample, mix well.
31 Store at -20˚C for 20 minutes.
32 Centrifuge sample for 20 minutes at 16,100 RCF and at 4˚C.
33 Pipet off supernatant.
34 Add 350 µl of cold 70% ethanol. Vortex briefly.
35 Centrifuge sample for 20 minutes at 16,100 RCF and at 4˚C.
36 Pipet off supernatant, allow RNA to dry on bench top.
37 Resuspend in 54 µl of nuclease-free water.
38 Remove 4 µl aliquot for Qubit (both DNA and RNA) and Nanodrop, immediately store the remainder at -80˚C.