**RNA extraction for BLiMMP project**

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**Solutions/supplies needed**

* Lysis matrix A in bead-beating tubes
* TRIzol
* Internal standard, at 0.75-1.25 ng/µl
* Eppendorf tubes (3 per extraction)
* Chloroform
* 100% Isopropanol
* 70% ethanol
* Turbo DNase kit

**Prep work before starting extractions**

1. Cool centrifuge to 4˚C
2. Set up and label 4 eppendorf centrifuge tubes for each extraction
3. Clean everything with RNaseZap

**Extraction protocols**

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.
   1. 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 100X diluted internal standard. Invert repeatedly for 1 min.
7. Centrifuge for 5 minutes at 16,100 x g at 4˚C.
8. Transfer 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.
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. Add 750 µl of 70% EtOH, and invert to mix.
17. Spin down at 16,100 x g for 25 minutes at 4˚C.
18. Carefully pipette off the supernatant.
19. Allow RNA to dry on the countertop.
20. Resuspend in 20 µl of nuclease-free water.
21. Add 3µl DNase Buffer from Turbo DNA kit
22. Add 1µl of TURBO DNase. Mix gently. Incubate at 37C for 25 minutes.
23. Add 3µl of DNase Inactivation Reagent (mix regent well first). Incubate 5 min at room temp, mixing periodically.
24. Centrifuge for 90 seconds. Transfer supernatant to new tube, hopefully ~25 µl
25. Remove 4 µl aliquot for Qubit (both DNA and RNA) and nanodrop, immediately store the remainder at -80˚C.