**Sample collection and DNA Extraction Procedure**
**Project code:** AOT
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**Sample collection** – Water samples were sequentially filtered: first through a 3 μm, 142 mm diameter membrane (to collect the "eukaryotic fraction" of the community), and then onto 0.2 μm, 142 mm diameter isopore polycarbonate filters (to collect the "prokaryotic fraction"). Millipore catalog numbers for these filters are: TSTP14250 (3 μm) and GTTP14250 (0.2 μm). Total volume of water that was filtered was between 40 and 60 L. Filters were frozen in approximately 10 ml of TENS buffer in a small whirlpack, and stored at -20 C.

*TENS Solution:* 50mM Tris – HCL (pH 8.0), 20mM EDTA, 400mM NaCl, 0.75 M Sucrose

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**DNA Extraction Protocol –** Revised from MoBIO Labs Water DNA Kit (Cat # 14880-25)

*Only the 0.2 ul filters were used for the ICoMM samples.*

1. Using sterile forceps and scissors, cut filter into pieces and divide filter and TENS buffer from bag evenly between 3 Water Bead Tubes (MoBio Cat # 14800-25-BT).

2. Add Bead Solution to 10 ml line (MoBio Cat # 14880-25-BS).

3. Vortex 30 sec

4. Add 750 μl of Solution WD1 (MoBio Cat # 14800-10-1) to each tube and vortex 30 sec.

5. Tape tubes horizontally to vortex pad and vortex at max speed for 15min.

6. Combine filter pieces, solution, and beads from same sample into one (1) 50ml tube. Centrifuge new tubes at 2500g for 1min.

8. Transfer supernatant to clean 50ml tube (expect about 20-25ml of supernatant).

9. Add 4.0ml of Solution WD2 (MoBio Cat # 14800-25-2) to supernatant and vortex 5 sec.

10. Incubate at 4ºC for 15 min (refrigerator).

11. Centrifuge tubes for 5 min at 2500g.

12. Transfer supernatant to clean 50 ml tube (expect about 20-25ml of supernatant).

13. Add 40ml of Solution WD3 (MoBio Cat # 14880-10-3) to the supernatant and vortex 5 sec (may require 2 tubes - split sample and liquid evenly between tubes)

14. Load supernatant onto 50 ml spin filter tube and centrifuge for 2 min at 2500g.

15. Discard flow through.

16. Repeat load, spin, and discard until entire solution has passed through spin column.

17. Add 3 ml of Solution WD4 (MoBio Cat # 14800-25-4) to spin filter.

18. Centrifuge for 3 min at 2500g. Discard flow through.

19. Centrifuge for 5 min at 2500g. Then move spin filter in a new 50 ml tube. Be very careful not to splash any liquid on the filter basket

20. Add 3 ml of Solution WD5 (MoBio Cat # 14800-25-5) to center of white filter membrane. Let stand 3-4 minutes.

21. Centrifuge tubes for 2 min at 2500g. Discard spin filter.

22. Add 3 ml of WD3, vortex well.

23. Load 700 µl of liquid onto 2 small spin filters (From PowerSoil Kit, MoBio Cat # 12888-100-SF)

24. Switch to microcentrifuge. Centrifuge at 10,000 g for 1 min, discard flow through.

25. Repeat steps 24 and 25 until all liquid has been filtered and discarded.

26. Add 300 µl of WD4 to the top of each spin filter.

27. Centrifuge 30 seconds at 10,000 g.

28. Discard flow through.

29. Centrifuge again for 1 min, carefully transfers the spin basket to a clean tube.

30. Add 50 µl WD5 to the center of the white filter membrane, let sit for 3 to 4 minutes.

31. Centrifuge 30 seconds at 10,000 g.

34. Discard the spin filter. The purified DNA is in the tube.

35. To dry the DNA, add 10 µl of 5 M NaCl, vortex. Then add 500 µl of ice cold ethanol, vortex.

36. Centrifuge at 2500 g for 20 minutes at 4 C.

37. Decant all liquid.

38. Dry residual ethanol in speed vac or dessicator or ambient air.