

Sampling protocol - CMORE RNA and DNA. August 30, 2007

Goal : Collect microbial cell fraction (1.6um prefiltered, >0.22um) from HOT hydrocasts, for RNA and DNA for downstream analyses by CMORE investigators. Collection depths are 25m, 45m, 75m, 125m, 200m, 500m, 770m and 1000m.

Summary: A total of 4x12 liter bottles will be collected from each depth. 8 liters will be collected onto 4-25 mm filters for RNA extraction. For DNA, two sterivex filters will be used to collect 20 liters each. Two depth can be processed simultaneously with the below protocol and two 4-head peristaltic pump manifolds.

When water is on deck, proceed immediately to step 3, below, "Cells for RNA extraction". Wear gloves throughout the entire RNA collection process.

- A. Cells for RNA extraction (use one 4-head pump manifold for each depth).
 1. Set up two, 4 -head, Cole Parmer peristaltic pumps placing a 42.5mm Whatman GFA filter into each prefilter housing.
 2. Mount a 25mm X 0.22um, Millipore Durapore filter in each of four polypropylene filter holders (Swinex-like screw-on filter housings). Wait to attach the 0.22um filter housing to the luer lock fittings of the peristaltic pump tubes until after step 3.
 3. Continue to wear gloves during the sampling process! As soon as the CTD is on deck, quickly collect 8 liters of seawater in a carboy and place all four collection tubes from the peristaltic pump manifold into the carboy. Loosen the vent valve on the prefilter housings in preparation for priming the pump tubing. Pump until the water drips out of the vents and all air is removed from the prefilter housings. Continue pumping while tightening the vents on the prefilter housings. When water reaches the end of the tubing, pump an additional 500mL through to flush the system.
 4. Mount the filter housings on the four luer locks and turn on the pump at setting 3. Time the collection. Water should be collected in 10-15 minutes max.
 5. Continue collection on all four filters until all the water is filtered. Using forceps, immediately place the filter into a pre-numbered, 2mL, cryotube containing 200uL RNALater. Invert several times to coat filter. Record sample information and filtering times on the C-MORE RNA Filtering log sheet corresponding to the cryotube number. Flash-freeze the tubes in liquid nitrogen and place at -80C.

- B. Cells for DNA extraction (perform after step A, "Cells for RNA extraction") - one four-head peristaltic pump can be used to process two depths at a time.
 1. Set up a four-head Cole Parmer peristaltic pump placing 42.5mm Whatman GFA filters into each of the prefilter housings.
 2. Mount one 0.22um Sterivex GV filter onto each luer lock fitting. Using two Sterivex filters for each depth, label each with the HOT cruise number and

- “DNA” with a sample number corresponding to the C-MORE DNA log sheet.
3. Fill two carboys with 20L of water from each depth sampled.
 4. Place the pump tubes into the respective carboys and “prime” as described in RNA step #3, above.
 5. Filter each 20L sample onto one Sterivex filter at a moderate pump rate.
 6. When 20L has been collected, cap off the small end of the Sterivex with a plastic syringe cap or by wrapping tightly with parafilm. Using a 3cc syringe, add two mL of sterile DNA Storage Buffer to the filter through the luer fitting. Cap off the Sterivex with a plastic luer cap. Record sample information and filtering times on the C-MORE DNA Filtering log sheet corresponding to the Sterivex number. Place the Sterivex cartridge containing the sample at -80C.
- C. When all sampling is completed, rinse all pump tubes and filter housings with D.I. Water. This is best accomplished by assembling the housings and pump tubes without filters and pumping the D.I. Water through the system.