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C·MORE linking genomes to biomes

C-MORE/HOT: Microbial DNA Collection and Processing

Cruise: HOT 194-215 (Aug 2007 – Sep 2009)
Publication: Bryant et. al, 2015, Wind and sunlight shape microbial diversity in surface waters of the North Pacific Subtropical Gyre. ISME J. *in press*

Goal: Collect microbial cell fraction (1.6 µm GFA pre-filter, >0.22 µm sterivex GV filter) from HOT hydrocasts at 25 m and 500 m.

Summary: A total of 4x12 L bottles were collected from each depth. For DNA, two sterivex (0.22 µm) filters are used to collect 20 liters per filter. Two depths are processed simultaneously with the below protocol and a 4-head peristaltic pump manifold.

Cells for DNA extraction (use one 4-head pump manifold for processing two depths).

1. Set up a four-head Cole Parmer peristaltic pump placing 47 mm Whatman GF/A filters into each of the pre-filter housings.
2. Mount one 0.22 µm 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 20 L of water from each depth sampled.
4. Place the pump tubes into the respective carboys and "prime" the tubing. Loosen the vent valve on the pre-filter housings and pump until the water drips out of the vents and all air is removed from the pre-filter housings. Continue pumping while tightening the vents on the pre-filter housings.
5. Filter each 20 L sample onto one Sterivex filter at a moderate pump rate.
6. When 20 L has been collected, connect a syringe to the luer fitting and purge remaining seawater in the filter housing.
7. Cap off the small end of the Sterivex with a plastic syringe cap or by wrapping tightly with parafilm. Using a 3 cc syringe, add 2mL of sterile DNA Storage Buffer (50 mM Tris-HCl, 40 mM EDTA and 0.75 M sucrose) 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 -80°C.
8. 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.

DNA Extraction and Sequencing Protocol

1. Lyse cells directly in Sterivex filter units and purify crude DNA lysates on a Quick-Gene 610L system (Fujifilm, Tokyo, Japan) using DNA Tissue Kit L (Autogen, Holliston, MA, USA). Modifications made to the manufacturer's cell lysis protocol are described in Sharma et al., 2013 and below.

2. Modifications: 50 mg of lysozyme was added to 1 mL of lysis buffer (50 mM Tris-HCl, 40 mM EDTA and 0.75 M sucrose) and mixed by vortexing before 40 µL was added to thawed Sterivex filters. Filters were set in a rotating incubator at 37°C for 45 min. Following this, 100 µL each of the kit buffers EDT and MDT were added to the filter, which was incubated at 55°C for 2 h with rotation. The lysate was decanted from the filter using a syringe; 2 mL LDT solution was added to the lysate, mixed by inversion, and incubated at 55°C for a further 15 min without rotation. 2.7 mL EtOH was added and vigorously mixed by vortexing, at which point the sample was immediately loaded onto the QuickGene column and placed in the Quick-Gene 610 L instrument (Fujifilm, Tokyo, Japan) for purification according to the manufacturer's DNA Tissue protocol, with an elution volume of 400 µL.
3. **Metagenomic shotgun sequencing libraries** were prepped with FLX or Titanium Rapid Library Preparation protocols, quantified using the Titanium Slingshot kit (Fluidigm, San Francisco, CA, USA), and added to emulsion PCR reactions at 0.1 molecules per bead.
4. Metagenomics sequencing done with shotgun pyrosequencing on a Roche Genome Sequencer FLX instrument according to manufacturer's recommendations (Indianapolis, IN, USA).
5. **Bacterial amplicon libraries** (V1-V3 SSU rRNA) were generated using the protocol established for the human Microbiome Project (Jumpstart Consortium Human Microbiome Project Data Generation Working Group, 2012).
6. To increase yield and keep the number of amplification cycles low, perform triplicate PCR reactions using 20 amplification cycles and then pool.
7. Purified amplicons using the QiaQuick PCR Clean-Up kit (Qiagen, Valencia, CA, USA)
8. Verify their size with agarose gel electrophoresis.
9. Pool equal quantities of all PCR reactions from the same ocean depth.
10. Sequence using a 454 Genome Sequencer, with the Titanium Rapid Library Preparation protocol.
11. Notes: Amplicon libraries were not diluted with AMPure XP beads before size selection. Also 1/4 of the recommended volume of amplification primers was used for emulsion PCR to accommodate for amplicon DNA fragments being shorter than the fragment length targeted by the kit.