Oct 02, 2020

NOAA-CalCOFI Ocean Genomics (NCOG) Sample Collection

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1 Works for me dx.doi.org/10.17504/protocols.io.bmubk6sn

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ABSTRACT

This protocol describes our sampling strategy and techniques for the NOAA-CalCOFI Ocean Genomics (NCOG) project.

In summary, seawater for DNA and RNA from multiple depths is filtered onto Sterivex filters at each station. Although the protocol is specific to this project, it is easily adaptable for other field-based sampling.

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PROTOCOL CITATION

Ariel Rabines, Rob Lampe, Lisa Zeigler Allen, Andrew E Allen 2020. NOAA-CalCOFI Ocean Genomics (NCOG) Sample Collection. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bmubk6sn

KEYWORDS

Oceanography, genomics, CalCOFI, RNA, DNA, amplicon, metatranscriptomics, marine microbiology, eDNA, environmental DNA

CREATED

Sep 29, 2020

LAST MODIFIED

Oct 02, 2020

PROTOCOL INTEGER ID

42595

ABSTRACT

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Station sampling and depths

1 For station positions and cruise patterns, refer to the CalCOFI website.

Stations:

Prodo stations refers to stations sampled around the same time of day (late morning) when primary productivity is measured. Cardinal stations refers to stations at a certain location to be sampled no matter what time of day. A station can be both a prodo and a cardinal station during a cruise.

- Sample all Prodo stations on lines 93.3, 86.7, 83.3, and 76.7
- Sample all Prodo and Cardinal Stations on lines 80 (55.0, 70.0, 80.0, 100.0) and 90 (120.0, 90.0, 70.0, 53.0, 37.0)
- Sample Station 081.8 046.9 (Santa Barabara Basin)

Depths:

Normally 4 depths are sampled with 6 filters total (2 DNA + 4 RNA). RNA is collected at higher volumes.

Depth	DNA/RNA	RNA Vol (L)	DNA Vol (L)	Description
10 m	DNA + RNA	4	2	Mixed layer / near surface
Chl max	DNA + RNA	4	2	Subsurface chlorophyll max
170 m	RNA	6		DIC typically sampled here
515 m	RNA	6		DIC typically sampled here

Exceptions:
- Less than 515 m: sample 10 m, Chl max, and 170 m
- Less than 170 m : sample 10 m, Chl max depth
- Less than 170 m and Chl max is around 10m: sample 10 m

Water budget:
- Ask for a duplicate bottle at 10m and chl max whenever possible
- Size Fractionation Stations: ask for 2 extra 10 m bottles and 1 extra Chl max bottle
- Please keep in mind whether the station is a DIC or Size Fractionation station. If two DIC samples are taken at one of the four depths it may be best to ask for a duplicate bottle
- Do **NOT** mix water from different depth. Prioritize RNA. If short on water DNA volumes can be reduced to .5L then RNA can be reduced in 0.5L increments

Filtration setup

2 We use Masterflex persistaltic pumps and tubing for everything. As there are 4 RNA filters, we use a 4-channel pump head on one pump with two-stop tubing. The other pump has 2 single-channel pump heads for the 2 DNA filters. 6 L bottles are used for RNA collection and 2 L bottles are used for DNA. Bottles are wrapped in black tape to keep the seawater in the dark, but graduations are still visible on one side. Lines have 10 mL serological pipettes that have been cut to have no filter and go into the bottles and are connected to the tubing with luer connections for sterivex on the outflow.

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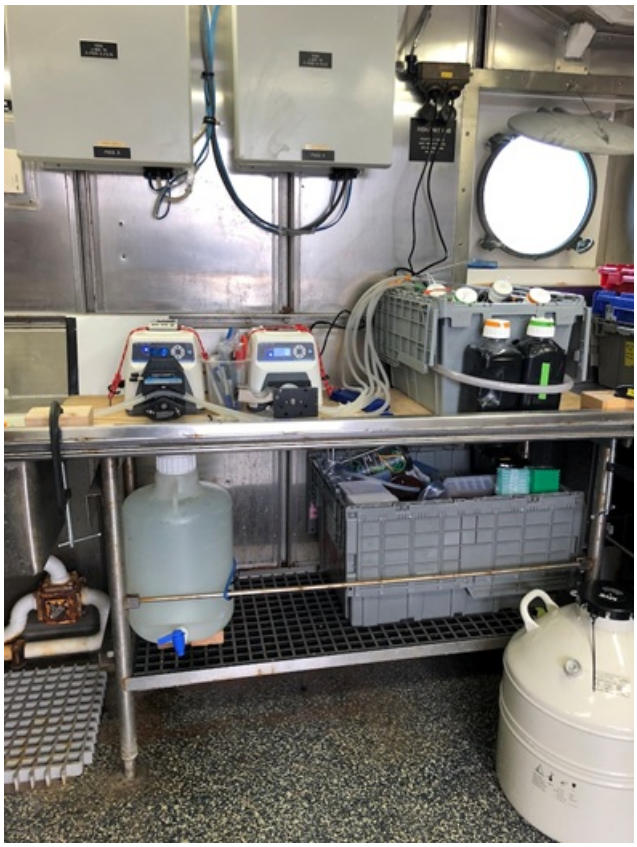

A typical sampling set up. Bottles are stored in the crate on the bench. Two peristaltic pumps are used. MilliQ water, liquid nitrogen, and extra supplies are stored beneath the bench.

Notes:

- Change two-stop tubing approximately halfway through the cruise (8 days)
- Periodically change the 10 mL pipettes

Sample collection

3 1. See the table above for sampling depths.
2. Label sterviex filters and aluminum foil for each filter with Cruise, Station, Cast, Bottle, Depth and DNA/RNA. Keep sterivex in plastic sleeve until ready to use. Labeling example for DNA:

Sterivex: 201511 DNA	Foil: 1511 DNA
093.3 026.7	93.27 C1
C1 B22 10 m	B22 10 m

Example Sterivex label

3. Collect water in 2L bottles for DNA or 6L bottles for RNA. Rinse bottles with seawater 3x before collecting samples. Overfill beyond the 2L and 4L or 6L mark and bring to the lab.
4. Prime the lines by running the extra water you collected through the lines without the filter.
5. Pause the pump, attach filter, start the pump, and filter the sample. Start pump at 150 rpm and reduce if flow rate significantly decreases. For RNA, start a 30 minute timer and if the sample has not finished filtering at the end of 30 minutes, pause the pump and remove the filter. Measure the remaining volume with a graduated cylinder and record

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the volume filtered after the steps below.
6. When filtration is done, release the line and remove the filter. The pump can be paused if all samples are done.
7. Use a 20-50 mL syringe to purge extra water out of the filter 3x.
8. Plug the large end of the sterivex with a luer-lock plug. Plug the small end with putty (Hemato-Seal).
9. Wrap the sterivex in the labeled foil, flash freeze in liquid nitrogen, and store in the dewar.

Clean up

4 Flush all lines with 2L MilliQ water. When finished, open the pump heads to release pressure on the lines and turn off the pump.

Rinse all collection bottles 3x with MilliQ water.

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