

# High throughput tag-sequencing accessions (18S rRNA gene region) and environmental metadata from Axial Seamount, 2013-2015

**Website:** <https://www.bco-dmo.org/dataset/828345>

**Data Type:** Cruise Results

**Version:** 1

**Version Date:** 2020-11-05

## Project

» [Probing seafloor microbial interactions via hydrothermal vent fluids: A focus on protists](#) (Microbial eukaryotes at hydrothermal vents)

## Program

» [Center for Dark Energy Biosphere Investigations](#) (C-DEBI)

Contributors	Affiliation	Role
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## Abstract

High throughput tag-sequencing accessions (18S rRNA gene region) and environmental metadata from Axial Seamount, 2013-2015.

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## Table of Contents

- [Coverage](#)
  - [Dataset Description](#)
    - [Acquisition Description](#)
    - [Processing Description](#)
  - [Related Publications](#)
  - [Parameters](#)
  - [Instruments](#)
  - [Deployments](#)
  - [Project Information](#)
  - [Program Information](#)
  - [Funding](#)
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## Coverage

**Spatial Extent:** N:46.2739 E:-129.7955 S:45.8799 W:-130.0137

**Temporal Extent:** 2013-09 - 2015-08

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## Dataset Description

See SRA BioProject accession: PRJNA641911

(<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA641911>) and BioSample accession IDs: SAMN15376334-SAMN15376347. Sequences are available via SRA (NCBI).

## Acquisition Description

### Sample collection from Axial Seamount:

From Fortunato et al (2018)

Sample collection from Axial Seamount took place in September 2013, August 2014, and August 2015. ROVs ROPOS and JASON were used to collect diffuse hydrothermal venting fluid. At each site, 3-L of diffuse venting fluid was pumped (100-150-ml per minute) onto a 0.22-um 47mm GWSP filter (Millipore). This fluid was collected using the Hydrothermal Fluid and Particle Sampler (HFPS; Butterfield et al. 2004), which was mounted on an ROV. The fluid intake for the HFPS has a temperature sensor to ensure a constant temperature during fluid collection. Filters were preserved in situ with RNALater. Background seawater was collected from a depth of 1500-m using a CTD mounted with 10-L Niskin bottles.

### Extraction and sequencing for 18S tag-sequencing:

For all samples, RNA was extracted and amplified similarly to the protocol described in Hu et al. 2018 (<https://dx.doi.org/10.17504/protocols.io.hk3b4yn>). Frozen filters were thawed and placed into sterile 15-ml falcon tubes with sterile forceps, 1-2 mL of RLT+ buffer (with  $\beta$ -Mercaptoethanol, Qiagen, Valencia, CA, USA) and RNase-free silica beads was added to each tube. Falcon tubes were bead-beaten by vortexing vigorously for 5 minutes. The original sample collection tubes with RNALater were centrifuged to pellet any cellular material left in the RNALater; the RNALater was removed and replaced with 500- $\mu$ l of RLT+ buffer (with  $\beta$ -Mercaptoethanol). This was vortexed and added to the 15-ml falcon tube. RNA was extracted with the RNeasy kit (Qiagen #74104) with the in-line genomic DNA removal step (RNase-free DNase reagents, Qiagen #79254). RNA concentrations were determined using the Ribogreen protocol. Extracted RNA was reverse transcribed into cDNA using a cDNA synthesis kit (iScript Select cDNA Synthesis, BioRad, #1708896, Hercules, CA); the concentration of RNA was normalized for the cDNA synthesis reaction (input -ng of RNA). Primers targeting the V4 hypervariable region of the 18S rRNA gene (Stoeck et al. 2010; Hu et al. 2015) were used in PCR reactions, which consisted of a final concentration of 1X Q5 High Fidelity Master Mix (NEB #M0492S, Ipswich, MA), 0.5  $\mu$ M each of forward and reverse primers, and 1 ng of genetic material. The PCR thermal protocol started with an initial activation step (Q5 specific) of 98°C for 2 min, followed with 10 cycles of 98°C for 10 s, 53°C for 30 s, 72°C for 30 s, and 15 cycles of 98°C for 10 s, 48°C for 30 s, and 72°C for 30 s, and a final extension of 72°C for 2 min (modified from Rodriguez Martinez et al. 2012). The original extract total RNA was also PCR amplified to ensure no genomic DNA was present in the sample. PCR products were checked by confirming the presence of an ~400 bp product on an agarose gel. In cases with no amplification, the PCR reaction was repeated with a higher concentration of cDNA (1.5-2 ng). If this did not yield the expected PCR product, the reaction was repeated with an additional 5 cycles. Three shipboard blanks (MilliQ water) and one extraction blank were also extracted and PCR amplified; while no PCR product was observed in these control samples they were processed similarly to all true samples and sequenced. All PCR products were cleaned using the AMPure bead clean up (Beckman Coulter #A63881, Brea, CA). Samples were multiplexed, pooled at equimolar concentrations and sequenced using the MiSeq 300 x 300 bp PE sequencing at Marine Biological Laboratory Bay Paul Center sequencing facility.

## Processing Description

### BCO-DMO Processing Notes:

- data submitted in Excel files "Axial-Metagenome.environmental.1.0\_JH.xlsx" sheet "tmp" and file "Axial-SRA\_metadata\_acc.xlsx" sheet "SRA\_data" and extracted to csv
- added conventional header with dataset name, PI name, version date
- renamed columns to conform with BCO-DMO naming conventions (removed spaces)
- combined environmental and SRA tables into one table; joined on sample\_name and library\_ID
- removed unpopulated columns (ref\_biomaterial, rel\_to\_oxygen, samp\_collect\_device, samp\_mat\_process, samp\_size, host)
- split lat\_lon into lat and lon columns; changed sign of lon to negative to signify degrees west; reduced precision from variable to 4 decimal places

- replaced commas with semi-colons

[ [table of contents](#) | [back to top](#) ]

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## **Related Publications**

Butterfield, D. A., Roe, K. K., Lilley, M. D., Huber, J. A., Baross, J. A., Embley, R. W., & Massoth, G. J. (2004). Mixing, reaction and microbial activity in the sub-seafloor revealed by temporal and spatial variation in diffuse flow vents at axial volcano. *Geophysical Monograph Series*, 269–289.

doi:10.1029/144gm17 <https://doi.org/10.1029/144GM17>

*Methods*

Hu, S. (2017). RNA (and optional DNA) extraction from environmental samples (filters) v2 (protocols.io.hk3b4yn). *Protocols.io*. doi:[10.17504/protocols.io.hk3b4yn](https://doi.org/10.17504/protocols.io.hk3b4yn)

*Methods*

[ [table of contents](#) | [back to top](#) ]

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## **Parameters**

<b>Parameter</b>	<b>Description</b>	<b>Units</b>
biosample_accession	NCBI BioSample accession number	unitless
library_ID	Short unique identifier for the sequencing library.	unitless
title	Short description that will identify the dataset on public pages.	unitless
library_strategy	Amplicon = Sequencing of overlapping or distinct PCR or RT-PCR products	unitless
library_source	Metagenomic = Mixed material from metagenome	unitless
library_selection	PCR = Source material was selected by designed primers	unitless
library_layout	Paired-end or Single	unitless
platform	Sequencing platforms [Illumina]	unitless
instrument_model	Illumina instrument and model used for sequencing	unitless
design_description	Free-form description of the methods used to create the sequencing library; a brief materials and methods section.	unitless
description	Description of the project	unitless
filetype	file type: fastq	unitless
filename	NCBI R1 filename	unitless
filename2	NCBI R2 filename	unitless
sample_title	NCBI sample title	unitless
bioproject_accession	NCBI BioProject identifier	unitless
organism	Description of sample organism(s)	unitless
isolation_source	Type of source: environmental	unitless
year	year of sample collection	unitless
collection_date	Collection date(s)	unitless
geo_loc_name	Geographic location of sample source	unitless
lat	Latitude; north is positive	decimal degrees
lon	Longitude; east is positive	decimal degrees
source_material_id	Description of methodology	unitless
Vent_site	Description of sample site	unitless
related_Axial_Seamount_projects	List of related BioProjects focused on the Axial Seamount	unitless

[ [table of contents](#) | [back to top](#) ]

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## **Instruments**

<b>Dataset-specific Instrument Name</b>	Niskin bottle
<b>Generic Instrument Name</b>	Niskin bottle
<b>Dataset-specific Description</b>	Used to collect background seawater sample.
<b>Generic Instrument Description</b>	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

<b>Dataset-specific Instrument Name</b>	MiSeq 300 x 300 bp PE
<b>Generic Instrument Name</b>	Automated DNA Sequencer
<b>Generic Instrument Description</b>	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

<b>Dataset-specific Instrument Name</b>	
<b>Generic Instrument Name</b>	PCR Thermal Cycler
<b>Generic Instrument Description</b>	General term for a laboratory apparatus commonly used for performing polymerase chain reaction (PCR). The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. (adapted from <a href="http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html">http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html</a> )

<b>Dataset-specific Instrument Name</b>	Hydrothermal Fluid and Particle Sampler (HFPS)
<b>Generic Instrument Name</b>	Alvin Slurp Sampler
<b>Dataset-specific Description</b>	The HFPS pumps vent fluid through a titanium intake nozzle and measures the temperature of the fluid just inside the nozzle and at a second point inside the titanium/Teflon fluid line near the sample containers. Sample containers are either collapsible plastic bags (with valves) within rigid housings, or PVC piston samplers with Teflon spring seals. Teflon check valves prevent the samples from leaking out or being drawn out of the containers. The HFPS was configured to collect 14 discrete fluid samples of up to 800 ml volume each, with the option of filtering eight of those samples. In addition, nine separate filters can concentrate particles from fluids of known temperature by in situ filtration, allowing us to collect and analyze minerals or microbes by a number of techniques. Two additional titanium gas-tight samplers are connected to the HFPS fluid line to collect samples of known temperature.
<b>Generic Instrument Description</b>	Small and large capacity vacuum pump samplers. May have single or multiple chambers. See <a href="http://www.whoi.edu/main/alvin/subsystems/optional-scientific-samplers">http://www.whoi.edu/main/alvin/subsystems/optional-scientific-samplers</a>

<b>Dataset-specific Instrument Name</b>	Beckman Coulter #A63881, Brea, CA
<b>Generic Instrument Name</b>	Coulter Counter
<b>Dataset-specific Description</b>	Used to clean PCR products.
<b>Generic Instrument Description</b>	An apparatus for counting and sizing particles suspended in electrolytes. It is used for cells, bacteria, prokaryotic cells and virus particles. A typical Coulter counter has one or more microchannels that separate two chambers containing electrolyte solutions. from <a href="https://en.wikipedia.org/wiki/Coulter_counter">https://en.wikipedia.org/wiki/Coulter_counter</a>

[ [table of contents](#) | [back to top](#) ]

## Deployments

### FK010

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/666111">https://www.bco-dmo.org/deployment/666111</a>
<b>Platform</b>	R/V Falkor
<b>Report</b>	<a href="https://datadocs.bco-dmo.org/d3/data_docs/Subseafloor_Life/FK010_CruiseReport_Huber_Final.pdf">https://datadocs.bco-dmo.org/d3/data_docs/Subseafloor_Life/FK010_CruiseReport_Huber_Final.pdf</a>
<b>Start Date</b>	2013-09-22
<b>End Date</b>	2013-10-05

### TN300

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/665996">https://www.bco-dmo.org/deployment/665996</a>
<b>Platform</b>	R/V Thomas G. Thompson
<b>Start Date</b>	2013-09-03
<b>End Date</b>	2013-09-19

### RB1403

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/665908">https://www.bco-dmo.org/deployment/665908</a>
<b>Platform</b>	NOAA Ship Ronald H. Brown
<b>Start Date</b>	2014-08-07
<b>End Date</b>	2014-08-19

### TN327

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/664100">https://www.bco-dmo.org/deployment/664100</a>
<b>Platform</b>	R/V Thomas G. Thompson
<b>Start Date</b>	2015-08-14
<b>End Date</b>	2015-08-29
<b>Description</b>	NOAA New Millennium Observatory (NeMO) 2015/Rapid Response to an Eruption

[ [table of contents](#) | [back to top](#) ]

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## Project Information

### Probing seafloor microbial interactions via hydrothermal vent fluids: A focus on protists (Microbial eukaryotes at hydrothermal vents)

**Website:** <https://www.darkenergybiosphere.org/award/probing-subseafloor-microbial-interactions-via-hydrothermal-vent-fluids-a-focus-on-protists/>

**Coverage:** Axial Seamount Juan de Fuca Ridge NE Pacific 46 N 130 W, Gorda Ridge NE Pacific 41 N 127 W, Mid-Cayman Rise Caribbean Sea 18 N 82 W

Adjusted C-DEBI Award Description:

Highly reduced and thermally charged venting fluids from the seafloor mix with surrounding seawater, creating a sharp geochemical gradient which promotes a hub of biological diversity at hydrothermal vent ecosystems. While studies of prokaryotic diversity at hydrothermal vent sites have highlighted the important roles microorganisms play in deep sea carbon cycling and offered a unique window into seafloor microbial communities, depictions of deep-sea marine ecology and food webs are incomplete without characterization of single-celled microbial eukaryotes (protists). I propose to use culture-independent techniques (tag-sequencing and metatranscriptomics) to provide a thorough understanding of protistan biogeography in and near venting fluids, focusing on the vent fluid-seawater interface.

Additionally, these qualitative analyses will be paired with quantitative experiments that measure protistan grazing pressure. Understanding trophic interactions within the protistan community is incredibly important, as these processes form the foundation of deep-sea marine food webs and mediate a significant amount of carbon transferred to higher trophic levels.

C-DEBI project link: <https://www.darkenergybiosphere.org/award/probing-subseafloor-microbial-interactions-via-hydrothermal-vent-fluids-a-focus-on-protists/>

[ [table of contents](#) | [back to top](#) ]

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## Program Information

### Center for Dark Energy Biosphere Investigations (C-DEBI)

**Website:** <http://www.darkenergybiosphere.org>

**Coverage:** Global

The mission of the Center for Dark Energy Biosphere Investigations (C-DEBI) is to explore life beneath the seafloor and make transformative discoveries that advance science, benefit society, and inspire people of all ages and origins.

C-DEBI provides a framework for a large, multi-disciplinary group of scientists to pursue fundamental questions about life deep in the sub-surface environment of Earth. The fundamental science questions of C-DEBI involve exploration and discovery, uncovering the processes that constrain the sub-surface biosphere below the oceans, and implications to the Earth system. What type of life exists in this deep biosphere, how much, and how is it distributed and dispersed? What are the physical-chemical conditions that promote or limit life? What are the important oxidation-reduction processes and are they unique or important to humankind? How does this biosphere influence global energy and material cycles, particularly the carbon cycle? Finally, can we discern how such life evolved in geological settings beneath the ocean floor, and how this might relate to ideas about the origin of life on our planet?

C-DEBI's scientific goals are pursued with a combination of approaches:

- (1) coordinate, integrate, support, and extend the research associated with four major programs—Juan de Fuca Ridge flank (JdF), South Pacific Gyre (SPG), North Pond (NP), and Dorado Outcrop (DO)—and other field sites;
- (2) make substantial investments of resources to support field, laboratory, analytical, and modeling studies of the deep subseafloor ecosystems;
- (3) facilitate and encourage synthesis and thematic understanding of submarine microbiological processes, through funding of scientific and technical activities, coordination and hosting of meetings and workshops, and support of (mostly junior) researchers and graduate students; and
- (4) entrain, educate, inspire, and mentor an interdisciplinary community of researchers and educators, with an emphasis on undergraduate and graduate students and early-career scientists.

Note: Katrina Edwards was a former PI of C-DEBI; James Cowen is a former co-PI.

### Data Management:

C-DEBI is committed to ensuring all the data generated are publically available and deposited in a data repository for long-term storage as stated in their [Data Management Plan \(PDF\)](#) and in compliance with the [NSF Ocean Sciences Sample and Data Policy](#). The data types and products resulting from C-DEBI-supported research include a wide variety of geophysical, geological, geochemical, and biological information, in addition to education and outreach materials, technical documents, and samples. All data and information generated by C-DEBI-supported research projects are required to be made publically available either following publication of research results or within two (2) years of data generation.

To ensure preservation and dissemination of the diverse data-types generated, C-DEBI researchers are working with BCO-DMO Data Managers make data publicly available online. The partnership with BCO-DMO helps ensure that the C-DEBI data are discoverable and available for reuse. Some C-DEBI data is better served by specialized repositories (NCBI's GenBank for sequence data, for example) and, in those cases, BCO-DMO provides dataset documentation (metadata) that includes links to those external repositories.

[ [table of contents](#) | [back to top](#) ]

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## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-0939564</a>

[ [table of contents](#) | [back to top](#) ]