

Illumina MiSeq amplicons of the V4 hyper variable region of 16S rRNA gene from IODP Expedition 336 on R/V JOIDES Resolution in the Mid-Atlantic Ridge in 2011 (North Pond basalts project)

Website: <https://www.bco-dmo.org/dataset/628208>

Data Type: experimental

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Project

» [IODP Expedition 336 Objective Research: The deep biosphere of young and oxic oceanic crust](#) (North Pond basalts)

Programs

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

» [International Ocean Discovery Program](#) (IODP)

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Dataset Description

This dataset contains Illumina MiSeq amplicons of the V4 hyper variable region of 16S rRNA gene. Raw data have been deposited in the NCBI Sequence Read Archive under accession number [SRP068428](#).

Additional data files:

(1) FASTA-formatted quality-controlled 16s rRNA gene sequences, organized by name of the operational taxonomic unit (OTU): [X336_DataReport_Supplemental_filteredOTUs.fasta](#)

(2) Excel file containing a record of the abundance of each OTU in each sample, organized by the name of the IODP Expedition 336 sample, along with the taxonomy of the OTU (based on classification as described in methods) and the closest environmental relative as identified using NCBI's BLAST algorithm (as described in methods): [X336_DataReport_Supplemental_SequenceAbundance.xlsx](#)

Methods & Sampling

Samples were collected from Sites U1382, U1383, and U1384 during IODP Expedition 336.

In the home laboratory, rock samples were crushed in an ethanol- and UV-sterilized steel impact mortar and pestle (Chemplex, Palm City, FL, USA). The mass of rock used for DNA extraction ranged from 7-86 grams. Rock powders were split into 2 ml Lysing Matrix E tubes containing ceramic, silica, and glass beads (MP Biomedicals, Santa Ana, CA, USA). Each tube was filled with 978 ul sodium phosphate buffer and 122 ul MT buffer according to manufacturer protocols for the FastDNA Spin Kit (MP Biomedicals). The tubes were shaken in a FastPrep 24 instrument (MP Biomedicals) twice at a speed of 5.5 for 30 seconds to mechanically extract and homogenize DNA, and the DNA was removed according to manufacturer instructions. Replicate extracts of the same sample were combined and concentrated using an Eppendorf 5301 Vacufuge. To account for

possible sample handling contamination of the low biomass samples, "blank" negative controls were also run through all steps as described above for the rock samples. Resultant DNA was quantified using the Qubit dsDNA HS Assay Kit on a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). The V4 hypervariable region of the 16S rRNA gene was amplified from DNA extracts by a commercial sequencing facility (Mr. DNA, Shallowater, TX) using the Illumina MiSeq platform. The 300bp × 2 kit was used with the Earth Microbiome Project primers (515f (5'-GTG CCA GCM GCC GCG GTA A) and 806r (5'-GGA CTA CHV GGG TWT CTA AT); (Caparaso et al. 2012)) to generate paired end reads. Illumina tag data were processed using mothur v.1.34.4 (Schloss et al. 2009) following the *mothur* Illumina MiSeq Standard Operating Procedure (Kozich et al. 2013). Briefly, paired end reads were joined into contigs, and any sequences with ambiguous base calls were removed. These were then aligned to the *mothur*-recreated SILVA SEED database from release v119 (Yarza et al. 2008). Sequences were then pre-clustered at the 1% dissimilarity level to mitigate the generation of spurious sequences, as recommended elsewhere (Kozich et al. 2013). Chimeras were screened with UCHIME using de novo mode (Edgar et al. 2011) and removed from further processing and analysis. Sequences were clustered into Operational Taxonomic Units (OTUs) at 3% sequence dissimilarity using the average neighbor method. A conservative OTU abundance cutoff threshold of 0.005% of total reads was used for filtering the full dataset before any downstream analysis, as previously suggested (Bokulich et al. 2013). The remaining filtered OTUs were classified using the SILVA Ribosomal 16S gene database (Quast et al. 2013). Closest environmental sequences to the OTUs were identified in the NCBI database using the BLAST algorithm (Altschul et al. 1997). OTUs recovered from the two protocol blanks, which may reflect contaminant DNA from the sample handling or sequencing steps, were removed from the dataset to provide the most conservative estimate of sequences from the deep biosphere, as has been done elsewhere (Inagaki et al. 2015).

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Parameters

Parameters for this dataset have not yet been identified

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Deployments

JRES-336

Website	https://www.bco-dmo.org/deployment/628214
Platform	R/V JOIDES Resolution
Report	http://dmoserv3.whoi.edu/data_docs/C-DEBI/cruise_reports/336PR.pdf
Start Date	2011-09-16
End Date	2011-11-16
Description	More information is available from the IODP website: http://iodp.tamu.edu/scienceops/expeditions/midatlantic_ridge_microbio.html

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Project Information

IODP Expedition 336 Objective Research: The deep biosphere of young and oxic oceanic crust (North Pond basalts)

Website: http://iodp.tamu.edu/scienceops/expeditions/midatlantic_ridge_microbio.html

Coverage: 23N/47W, North Pond, western flank of Mid-Atlantic Ridge

Description from NSF award abstract:

The proposal addresses a fundamental aim of the ocean drilling program, namely to help characterize one of the largest and least studied ecosystems on Earth, the deep biosphere of the igneous crust buried below the ocean floor. The principal scientific objective of IODP expedition 336 is, in particular, to investigate the microbial population in basaltic crust from the North Pond area near the Mid-Atlantic Ridge. The study samples are weathered and porous basalts taken from beneath ~100 m of sediment in the North Pond area. The study proposes to determine both the diversity of the microbe population (using DNA) and its metabolic activity (using RNA). The PIs will investigate the relationship between microbes in the basement and those in the water column and determine which metabolic pathways are used by the deep basement microbes. The study will also provide baseline data for the long-term biological observatories installed in the sub-seafloor basement during expedition 336. Understanding deep biosphere life is a major thrust of the new IODP science plan and has implications for understanding the limits of life.

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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.

International Ocean Discovery Program (IODP)

Website: <http://www.iodp.org/index.php>

Coverage: Global

The International Ocean Discovery Program (IODP) is an international marine research collaboration that explores Earth's history and dynamics using ocean-going research platforms to recover data recorded in seafloor sediments and rocks and to monitor subseafloor environments. IODP depends on facilities funded by three platform providers with financial contributions from five additional partner agencies. Together, these entities represent 26 nations whose scientists are selected to staff IODP research expeditions conducted throughout the world's oceans.

IODP expeditions are developed from hypothesis-driven science proposals aligned with the program's [science plan](#) *Illuminating Earth's Past, Present, and Future*. The science plan identifies 14 challenge questions in the four areas of climate change, deep life, planetary dynamics, and geohazards.

IODP's three platform providers include:

- The U.S. National Science Foundation ([NSF](#))
- Japan's Ministry of Education, Culture, Sports, Science and Technology ([MEXT](#))
- The European Consortium for Ocean Research Drilling ([ECORD](#))

More information on IODP, including the Science Plan and Policies/Procedures, can be found on their website at <http://www.iodp.org/program-documents>.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1233226

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