# Request for Proposals
# Census of Deep Life Sequencing Opportunities
# Deadline: December 31, 2017

Since 2011, the Deep Carbon Observatory's (DCO; http://deepcarbon.net/) Deep Life Community has sponsored the Census of Deep Life (CoDL) that has supported surveys of the diversity of microbes present in several deep continental and subseafloor environments. The first surveys (2011-2012) were conducted using 454 pyrosequencing and subsequently (2013) Illumina sequencing strategies were adopted. Through this initiative, the Deep Life Community has allowed the characterization of diversity of subsurface microbial communities at numerous sites worldwide including the subseafloor and deep continental locations from a range of geologic settings (e.g., large igneous provinces, subglacial lakes, methane hydrate-rich sediments, cratons). The Illumina platform provides increased numbers of reads for more samples at reduced cost. For DNA samples submitted to the CoDL for sequencing, proponents have the option of obtaining 400-450 nt sequences that span the V4V5 region of Bacterial and Archaeal rRNA coding regions or a greater number of reads for V6 regions that through complete overlap of forward and reverse reads allows detection of lower abundance taxa with reduced stochastic error rates. Shotgun metagenomic DNA sequencing for key samples can also be performed.

This call for proposals aims to support sequencing that represents expanded analyses from ongoing Deep Life Community projects or projects that represent sites and investigators new to the DCO's Deep Life Community. Two independent options, each of which use the Illumina sequencing platform, are available:

*Option 1*: This option offers the opportunity to submit up to 24 samples for 16S rDNA amplicon sequencing (as described above for Bacterial and Archaeal sequences). Note: sequencing of Bacteria AND Archaea in a single sample counts as two samples; therefore a maximum of 12 samples for paired analysis of both Archaea and Bacteria would be acceptable. On a case-by-case basis, sequencing of a reasonable number of additional samples (more than the specified 24) may be requested and justified in the proposal and will be considered by reviewers. Control experiments are important and strongly encouraged in subsurface systems where biomass is low. For this reason investigators may submit control blanks (at the expense of the allocated number of samples for analysis) that combine the reagents used to prepare their libraries. Successful applicants must be able to provide high-quality DNA samples to the Marine Biological Laboratory at Woods Hole for Illumina sequencing **before April 1, 2018.**

*Option 2*: This option offers the opportunity to submit up to **three** select samples from subsurface environments for shotgun metagenomic sequencing. Ideal samples for the metagenomic surveys will have evidence of limited microbial diversity as a result of the prior microbial rDNA community analyses. Successful applicants must be able to provide high-quality DNA samples **before March 1, 2018**. Note: Within the context of the shotgun metagenomic option, we will also consider requests for draft genome sequencing of individual genomes either from cultivars or from single-cell genomic preparations.

For this RFP, in addition to the "classic" CoDL projects that seek sequencing of DNA from samples recovered from unique subsurface settings anywhere in the world, we encourage proposals with the following attributes:

1) **Resequencing:** One of the original CoDL projects sequenced using the 454 pyrosequencing approach can propose to resubmit DNA for re-sequencing using the Illumina platform. Alternatively, one of the original CoDL projects that targeted the V6V4 region of the 16S rDNA

can propose to resubmit DNA for re-sequencing targeting the V4V5 region of the 16S rDNA. Well-preserved DNA samples from the original site are required for this project approach.

2) **New continent:** Projects featuring samples from the subsurface in South America, Australia, Africa, and Asia. These continents are still largely unrepresented in the CoDL database.
3) **High C Flux:** Projects with samples from environments where high carbon flux from the subsurface is occurring. Ideally, such projects will strive to integrate subsurface microbial community data with non-biological data acquired by DCO or other scientists working at the same location (see https://deepcarbon.net// for other DCO communities).

Proposals that target any of the criteria noted above should identify this on the proposal cover sheet.

For proposals please provide the following as a single Word document:

1) A completed cover page (attached below and not part of the two-page proposal limit) with: A) a succinct title that includes the geographic location or site description, B) an indication of whether the proposed sequencing is for Option 1 or 2; C) name, address, e-mail address, phone number of investigators; and D) a brief abstract that includes the project objective.
2) The proposal within a two-page limit that includes:
   - Statement of the question(s) to be addressed by the sequencing opportunity.
   - A statement about which samples would be selected, the status of those samples (How were they/will they be collected? DNA extracted already?), and the availability, concentration, and quantity of DNA, and access to reference genomes for data analysis, if appropriate.
   - DNA extraction protocols including details about sample acquisition and evidence that the samples are representative of a subsurface setting. As noted above, the inclusion of a procedural and/or extraction blank is encouraged as a way to identify the inadvertent inclusion of contaminant sequences in datasets generated from exceedingly low biomass samples common to the subsurface.
   - Information about pre-existing biological diversity and metadata that describes abiological properties of the samples including site coordinates, sampling depth(s), date and time samples were collected or details on how these will be collected, and all other relevant sample properties such as temperature, pH, alkalinity, etc.
   - Proposed experimental and data analysis plan including whether you will require assistance for metagenome assembly (for Option 2).
   - A brief timeline for the study with assurance for sending the DNA samples before **March 1, 2018** for metagenomic sequencing (Option 2) or before **April 1, 2018** for amplicon sequencing (Option 1).
3) A 2-page NSF-style biosketch for each of the primary investigators for the proposed project.

Additional considerations:

- Successful proposals are posted on the CoDL website and are available to the public.
- We do not encourage submission of cDNA for sequencing. However, if clean cDNA preparations that are readily amplifiable are provided to MBL they will be run with no guarantee of success.
- Metadata must be submitted at the same time as DNA samples are submitted for sequencing. Completed DNA sequence data will not be available through the MBL's VAMPS database until metadata submission is complete.
- Successful applicants must agree to submit their sequence data and all relevant metadata to the public domain immediately upon acceptance of a manuscript but no later than 6 months after completion of the DNA sequencing phase.

- Proponents should briefly address how they will include splits from their subsurface samples to be used for analysis of dipicolinic acid and dissolved and/or total organic carbon (performed by Univ. of Bremen; nominally 10 g of each frozen sediment sample) and total cell counts (performed by JAMSTEC; nominally 1 $cm^3$ of each sediment sample but only if samples have been properly fixed and refrigerated at 4°C and not frozen). Details related to shipping these samples will be provided when proposal acceptance is announced.
- PIs may submit separate proposals to each option but they must indicate which proposal (Option 1 or 2) is their priority.
- **Note: This is likely the final call for CoDL proposals.**

Proposals should follow the format employed for prior submissions to the CoDL (see "Project Pages" at http://codl.coas.oregonstate.edu/main/documents.shtml). ***Please send proposals as a single file in Word format*** to: deeplife@coas.oregonstate.edu
**Reviews should be complete by mid-January 2018**.

# Census of Deep Life Cover Sheet

**Project Information**

***Project title (with geographic location or nature of deep subsurface site):***

**Check one:**

**___Option 1 (16S rDNA amplicons) or ___Option 2 (metagenome)**

**Type of proposal**

**__Classic CoDL; __Resequencing; __New continent; __High C flux**

**Latitude/longitude _____**

***Principal Investigator***

**Name:**
**Address:**
**phone #:**
**e-mail:**

***Co-Investigator***

**Name:**
**Address:**
**phone #:**
**e-mail:**

***Co-Investigator***

**Name:**
**Address:**
**phone #:**
**e-mail:**

**Brief project abstract with statement of objective**