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EMP Sample Submission Guide

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Earth Microbiome Project

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ABSTRACT

This protocol was designed for collaborators with the Earth Microbiome Project to contribute samples in a standardized fashion. Raw, frozen samples are submitted in individually labeled tubes, 10 aliquots (identical replicates) per sample. Please note that unsolicited samples cannot be accepted.

EXTERNAL LINK

http://www.earthmicrobiome.org/protocols-and-standards/emp500-sample-submission-guide/

GUIDELINES

Samples for this study will ideally meet the following criteria:

- Fresh or frozen sample (no preservatives) in ~10 aliquots
- Metadata well characterized
- Large proportion of unmapped diversity in at least some samples
- Non-human-associated and non-human-perturbed samples will be given priority

Shipping Guidelines

For **non-soil samples** only (e.g., feces, sediments, biofilms, organic material, water filters), no special permits should be necessary. However, material transfer agreements (MTAs) may be required, and these should be negotiated between the sender and recipiment as early as possible.

For **soil samples** only (sediments are not considered soils):

- Regulated domestic soils must be shipped with the Permit to Receive Soil issued through the Animal and Plant Health Inspection Service of the United States Department of Agriculture. Collaborators should know whether or not their soils are regulated. If domestic soils are not regulated, the permit is not needed.
- The PPQ Form 550 Black/White Label (sticker) must be affixed to the outside of the shipment for any soils shipping from outside the USA, Guam, Hawaii, Puerto Rico, or the US Virgin Islands.
- Samples should be shipped with FedEx overnight (or DHL for international shipments). Soil samples must be shipped to the permit holder.
- MTAs may also be required by the sending and receiving institutions, and these should be agreed upon as early as possible.

MATERIALS

NAME	CATALOG #	VENDOR
BD SWUBE™ collection and transport system	281130	Bd
Millipore Express PLUS Membrane Filter, polyethersulfone, Hydrophilic, 0.22 µm, 47 mm	GPWP04700	Millipore
JGWP04700 | Omnipore Membrane Filter, PTFE, Hydrophilic	JGWP04700	Millipore
Screw Cap Micro Tube, 2 ml, PP, with skirted base, with knurls, with assembled cap, no print, sterile, 100 pcs./bag	72.694.005	Sarstedt

STEPS MATERIALS

NAME	CATALOG #	VENDOR

NAME	CATALOG #	VENDOR
Screw Cap Micro Tube, 2 ml, PP, with skirted base, with knurls, with assembled cap, no print, sterile, 100 pcs./bag	72.694.005	Sarstedt
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Screw Cap Micro Tube, 2 ml, PP, with skirted base, with knurls, with assembled cap, no print, sterile, 100 pcs./bag	72.694.005	Sarstedt

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for safety and hazard information.

Sampling

1 Samples should be collected fresh and split into 10 aliquots and then frozen, or collected and frozen and subsequently split into 10 aliquots with minimal perturbation.

Do not use any buffers or solutions to preserve your samples. Do not use RNAlater. Ethanol (50-95%) is acceptable. Aliquot size should be sufficient to yield 10-100 ng genomic DNA, which is approximately 10^7 to 10^8 cells. For low-biomass samples, such as certain water samples and biofilms, please contact us.

Procedures specific to sample types

2 Please choose the one of the following sample types.

step case

Bulk unaltered

Examples: soil, sediment, feces

3 Split fresh (or frozen) sample into 10 2-mL screw-cap bead beater tubes, ideally with at least 200 mg biomass, flash freeze in liquid nitrogen (if possible), and store at -80 °C (or -20 °C).

-80 °C Storage

Screw Cap Micro Tube, 2 ml, PP, with skirted base, with knurls, with assembled cap, no print, sterile, 100 pcs./bag
by Sarstedt
Catalog #: 72.694.005

Labeling

4 To track the provenance of these aliquots, we employ the following QR barcoding scheme:

- Tubes should be 2-mL screw-cap bead beater tubes to enable direct use in DNA extraction.
- Labels should be affixed to aliquot tubes before shipping.
- QR codes have the format

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doe.99.s003.a05
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, where "doe" is the PI name, "99" is the study ID, "s003" is the sample number, and "a05" is the aliquot number.

- QR codes (version 2, 25x25) are printed on Cryogenic Direct Thermal Labels, 1.125" x 0.75" rectangluar labels and 0.437" circular cap labels (GA International, part no. DFP-70) using a Zebra model GK420d printer and ZebraDesigner Pro software for Windows.

- Before aliquots are put away, QR codes are scanned into a sample inventory spreadsheet using a QR scanner.

Metadata

5 High-quality environmental metadata is essential for meaningful analysis and is required for all samples before they are processed.

Please go to the EMP Metadata Guide and read the general instructions for fields required by Qiita (database for EMP data), MIMS (Minimal Information about a Metagenomic Sequence), and your specific environmental package (sample type).

For your environmental package (or more if you have multiple sample types), download the metadata template CSV file and README file, fill in the metadata template, and email it to your contact with the EMP.

Shipping

6 Samples in tubes of appropriate size should be shipped on crushed dry ice in a styrofoam container. Please ensure there is enough dry ice to survive for the duration of the trip.

7 Place samples in dry ice. If there is space between the top of the ice and the lid, fill with paper, or other packing materials, to secure the samples during transit.

8 Tape styrofoam container to secure the lid.

9 Tape a list of samples to the outside of the styrofoam container for sample identification. Sample names should match the sample names in your metadata file.

10 If you are sending additional samples for possible later processing (so-called “Tier 2” samples as discussed with the EMP coordinators), please group these separately and mark as “Tier 2”, with a separate list of samples.

11 Place styrofoam container into larger cardboard shipping box. Pack the box as needed with paper or other packing material as needed to fill up the empty space in the box and secure the styrofoam box during shipping.

12 Seal cardboard box with tape.

13 Ship samples using FedEx overnight (or DHL for international shipments). Ship Monday through Wednesday to avoid samples that are delayed arriving on a weekend.

14 For specifics on **non-soil samples** only (e.g., feces, sediments, biofilms, organic material, water filters) and **soil samples** only (sediments are not considered soils), please see the Guidelines.

Data Release

15 DNA sequence data will be submitted to the European Nucleotide Archive as raw FASTQ files shortly after generation, as required by the funding agencies. Metabolomic data will be submitted to appropriate repositories on a similar timeline. Collaborators will be notified when their data are ready, and are free to access and publish data from their samples.

COMMENTS

Stephanie Rosales Apr 23, 2018 05:56 PM

Hey Luke,

I was wondering why it is recommended to use ethanol and not RNAlater?

Thanks!
Stephanie

Luke Thompson Apr 23, 2018 06:02 PM
National Oceanic and Atmosphe..

Hi Stephanie,

This is because ethanol is compatible with the metabolomics protocols (extraction and chromatography) whereas RNAlater (concentrated ammonium sulfate) is not. If you have no expectation of ever doing metabolomics, and are just interested in DNA and RNA preservation, then RNAlater should be OK.