EMP Sample Submission Guide

Luke Thompson,Gail Ackermann,Greg Humphrey,Jack Gilbert,Janet Jansson,Rob Knight

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.

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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

BD SWUBE™ collection and transport system 281130 by Bd

Millipore Express PLUS Membrane Filter, polyethersulfone, Hydrophilic, 0.22 µm, 47 mm GPWP04700 by Millipore

JGWP04700 | Omnipore Membrane Filter, PTFE, Hydrophilic JGWP04700 by 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 by Sarstedt

Protocol

Sampling

Step 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

Step 2.

Please choose the one of the following sample types.

Procedures specific to sample types

Step 3 - Bulk unaltered (Examples: soil, sediment, feces).

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).

TEMPERATURE

-80 °C Additional info: Storage

REAGENTS

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

Labeling

Step 4 - Bulk unaltered (Examples: soil, sediment, feces).

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 `doe.99.s003.a05`, 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.

Procedures specific to sample types

Step 3 - Bulk fractionated (Examples: sponges, corals, turbid water).

Fractionate the sample as appropriate for your sample type. Split 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).

TEMPERATURE

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Labeling

Step 4 - Bulk fractionated (Examples: sponges, corals, turbid water).

Label your tubes clearly in permanent marker with a descriptive name. For example, use the PI or contact person’s last name, the study number (provided by the Knight lab), the sample type, and sequential number: “Jones99.soil.1”.

This is the default sample numbering system used in the metadata template (see in Guidelines). Other naming conventions are acceptable, but please make sure the names match those in the metadata CSV file.

Please do not number the aliquots; you should submit 10 identically labeled aliquots for each sample.

Procedures specific to sample types

Step 3 - Swabs (Examples: biofilms, feces).

Take 10 replicate swabs using 5 BD SWUBE™ dual cotton swabs with wooden stick and screw cap. Place cap on swabs and store in -80 °C (or -20 °C).

TEMPERATURE

-80 °C Additional info: Storage

REAGENTS

BD SWUBE™ collection and transport system 281130 by Bd

Labeling

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Procedures specific to sample types

Step 3 - Filters (Example: water).

Filter water through 10 replicate filters: 47 mm diameter, 0.2 um pore size, polyethersulfone (preferred) or hydrophilic PTFE filters. Catalog numbers from Millipore:GPWP04700 (polyethersulfone), JGWP04700 (hydrophilic PTFE "Teflon"). Place filters in 2-mL screw-cap bead beater tubes, flash freeze in liquid nitrogen (if possible), and store at -80 °C (or -20 °C).

REAGENTS

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

using a QR scanner.

Procedures specific to sample types

Step 3.

Place filters in micro centrifuge tubes or cry-vials, flash freeze in liquid nitrogen (if possible), and store at -80 °C (or -20 °C).

TEMPERATURE
-80 °C Additional info: Storage

Warnings

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