Materials that can be used for sampling

For sampling of human or animal samples we recommend the use of Swubes (Bectonf Dickinson catalogue number 220710) if both metabolomics and amplicon sequencing are to be performed. If amplicon sequencing alone is required (16S, 18S, ITS) a similar swab (that cannot be used for metabolomics) is recommended (Bectonf Dickinson catalogue number BBL 220135). For environmental samples please check with us if the filters used for water samples are compatible with the pipeline.

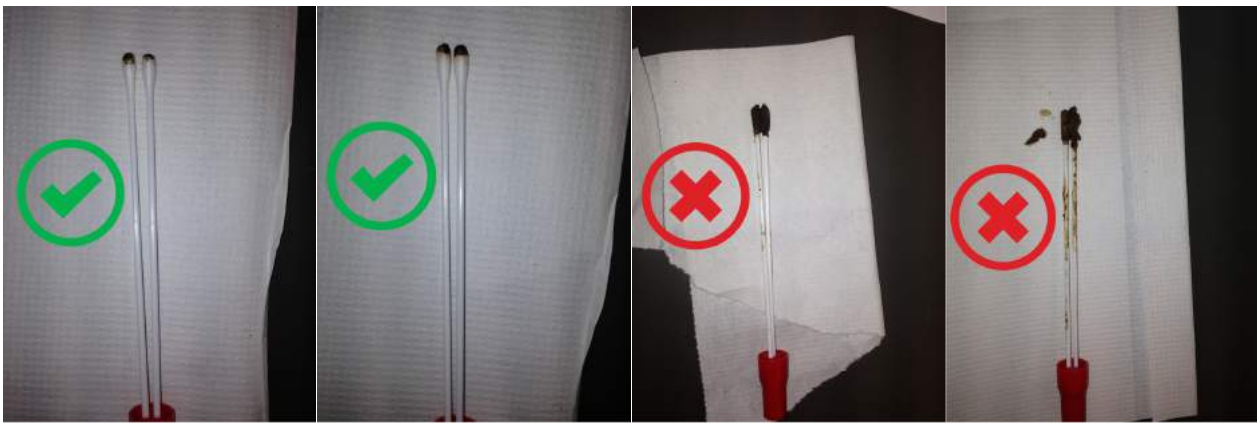
Sample type collection instructions for Seed Grant awardees

1. FECAL: Rub both cotton tips on a fecal specimen (ideally, a used piece of toilet paper). Collect a small amount of biomass, saturating half the swab at maximum. More is not better! The ideal amount of biomass collected is shown in the figure below.

A fecal sample showing an ideal amount of material collected. **Excessive amounts of material will result in the nonrefundable exclusion of your sample**. See below for examples.

It is important that the amount of material collected is small for two reasons. First, the amount of material could bias the result of your sample (e.g. mold growth might contaminate the sample). Second, excess material cannot be handled by our processing pipeline.

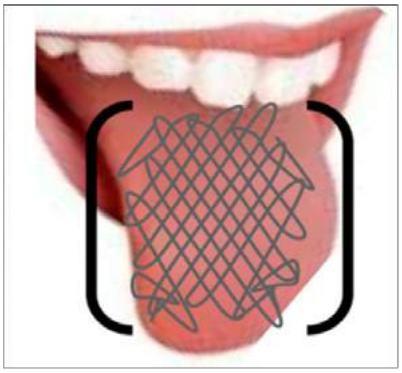
Our molecular methods are incredibly sensitive, and require nanograms (billionths of a gram) of DNA. Previous studies have shown that even a tiny amount of material, like in the picture above, is sufficient to assess the microbial life in your gastrointestinal tract. The figures below show examples of good and bad fecal samples.



Examples of good and bad samples. From left to right, the first two images are good samples and the next two images are bad samples.

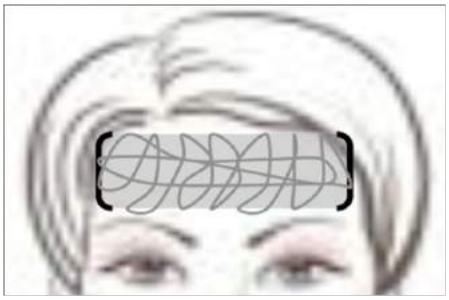
DIFFICULTY WITH OBTAINING FECAL SPECIMENS**:** We recognize that some participants have chronic constipation. In these instances, what we recommend is the "catch and release" method, using 2-4 paper towels (enough to not get any on you, but to also avoid clogging the toilet). If the specimen is not soft, please just rub both sides of the cotton swabs on it to get a light smear. And of course, make sure to wash your hands afterwards.

2. ORAL: Firmly rub both sides of both cotton tips on the surface of the tongue for 20 seconds. Take great caution not to touch the inside of your cheeks, teeth, or lips. An example of the area of sampling for the tongue is shown on the next page.

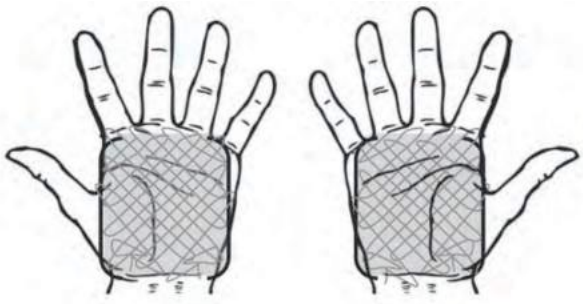


The area of the tongue to cover with the cotton swabs.

3. SKIN: Firmly rub both sides of both cotton tips over the skin surface being sampled for 20 seconds. Some examples of the area of sampling are shown below. Note that for skin samples, it is necessary to press relatively hard with the sampling tips. Also, do not wash the skin surface for at least two hours prior to sampling.



An example of where to rub the cotton swabs if you are collecting a forehead sample.



An example of where to rub the cotton swabs if you are collecting a sample from the palm

4. Environmental samples: Here are the instructions for other sample types (per EMP).

Procedures specific to sample types are as follows:

- **Bulk unaltered** (e.g. soil, sediment, feces) — 200mg biomass in microcentrifuge tubes, or cryovials, flash freeze in liquid nitrogen (if possible), and store at -80°C (or -20°C). For soil and sediment provide additional metadata such as pH, pH method, carbon:nitrogen ratio, moisture content etc.
- **Bulk fractionated** (e.g. sponges, corals, turbid water) — Fractionate the sample as appropriate for your

sample type. Provide at least 200 mg biomass, flash freeze in liquid nitrogen (if possible), and store at -80°C (or -20°C).

- **Swabs** (e.g. biofilms) — Sample using a BD SWUBE dual cotton swabs with wooden stick and screw cap (part number BD 281130). Cap swabs and place in -80°C (or -20°C).
- **Filters** (e.g. water) — Filter water through filter: 47 mm diameter, 0.2 um pore size, polyethersulfone (preferred) or hydrophilic PTFE filters. Part numbers from Millipore: GPWP04700(polyethersulfone), JGWP04700 (hydrophilic PTFE "Teflon"). Place filter in microcentrifuge tubes or cryo-vials, flash freeze in liquid nitrogen (if possible), and store at -80°C (or -20°C).

Step 2. Labeling

Label your tubes clearly in permanent marker with a descriptive name. For example, use the PI or contact person's last name, an an alphanumeric identifier. Please make sure the names match those in the metadata .txt file.

Step 3. Metadata

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).

If you have further questions or issues: contact Gail Ackermann (ackermag@ucsd.edu)

All samples should be mailed to or delivered to:

University of California, San Diego
Knight Lab/ATTN: Greg Humphrey
BRF II Room 1220D
9500 Gilman Drive, MC 0763
La Jolla CA 92093-0763