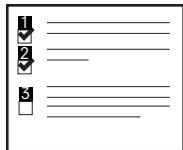




Sampling tip

Sample shaft (**Do Not Handle!**)

Red handling cap



Use these instructions
as a checklist to make
sure you complete each
step in order!



Quick Instructions

Please read through all of these instructions before completing any step. This guide contains a quick, step-by-step instructions sheet, a more detailed set of instructions, and sample type specific collection details. Please do not hesitate to ask questions at info@american gut.org.

- 1** Log in to <http://microbio.me/AmericanGut/>. Your login information can be found on the credentials sheet included in your kit.



- 2** Verify that you received the correct sample tubes and kit. Your verification code should have been sent to you via email. Email info@american gut.org if you have not received your verification code.



- 3** If you will be collecting a human sample, you will need to start a 7 day food diary. Diet summary questions are asked on the survey (see step 4), and will assist us in interpreting your sample. We recommend <http://caloriecount.about.com/>.



- If you are collecting a human sample, please do not proceed to step 4 until after finishing your 7 day food diary.

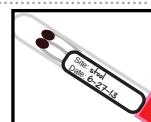
- 4** If you will be collecting a human sample, login (see step 1) and add yourself as a participant. This will take you through the consent process, and the participant questionnaire.



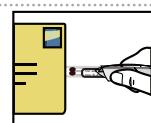
- 5** Collect a sample (see **Appendix A** for specific sample type collection instructions). **Deviations from these instructions are at your expense.**



- 6** Record the date, time (24-hour format) and sample type on **both** the sample tube and on the website (please see **Detailed Instructions**). Note, we intentionally ask for these details to be collected **both** on the sample tube and the website.



- 7** Wrap the sample tube in absorbent tissue, such as facial tissue or paper towels, and mail it back within 48 hours.



If you have multiple samples, it is okay to send them one at a time. If you cannot mail them all at the same time, please store them at room temperature in a dry, cool, location (such as a cabinet or closet).

Please do not freeze or refrigerate samples prior to shipment, unless we have directed you otherwise.

These instructions are extremely important. Deviations will cause issues with sample processing, sequencing, and data analysis. We cannot guarantee that we will be able to process your sample if the instructions are not followed, and **we cannot offer replacements if instructions were not followed**. Please do not hesitate to ask us questions at info@american gut.org.

Detailed instructions

1. Login to <http://microbio.me/AmericanGut/>. Details are found on the Credentials Sheet included in your kit. Please retain this Credentials Sheet for your records! Although you will be able to change your password after logging in, your Kit ID is permanent. The website allows us to obtain the formal consent of each American Gut Project participant. It also serves as a convenient place to collect questionnaire results and sample details. Lastly, we will be presenting data and results (including personalized results for your samples) through the website. For these reasons, it is very important that you are able to log into the website and follow any additional instructions provided for you there.

What you will need to complete step 1:

Credentials Sheet

2. Verify that you received the correct sample tubes and kit. We aim for 100% accuracy when we mail our kits. However, in rare circumstances we might have put the wrong number of sampling tubes in a kit, or associated the wrong Credentials Sheet with the kit, or sent the kit to the wrong address. To reduce the number of these incidences, we employ a verification system that relies on a Kit Verification Code. Your Verification Code will be sent to you via email to the address that you entered when you made your donation; if you made an anonymous donation, please contact us directly. Also note that some email programs' spam filters will incorrectly filter this message, preventing it from reaching your inbox! So, if you can't find the email message and you have already received your kit, please check thoroughly, including in your email program's spam box. The email's sender is "American Gut Project (info@american gut.org)" and the subject line will be "American Gut Kit ID & Verification Code". After you complete step 1 (login), you will see a form asking for your Kit Verification Code and checkboxes next to barcodes for tubes that you should have received with your kit. Please enter your Kit Verification Code in the text box and check the boxes next to the samples you successfully received. If you did not receive *exactly* the samples listed on the form, please contact us immediately.

What you will need to complete step 2:

Successfully complete step 1 (login)

Verification Code from email

3. For human subjects only, record a food diary. If you are collecting an environmental sample or an animal sample and you do not wish to collect a human sample, you should skip step 3 and proceed to step 4. If you *will* be collecting a human sample, you will need to start a 7-day food diary. We strongly recommend using CalorieCount (<http://calorienet.about.com/>) to record your dietary intake. Later, we will be asking for information about your diet such as the percentage of fat in your diet, the number of grams of dietary fiber, etc., and CalorieCount will keep a food diary to determine these summary details with the greatest accuracy. If you are uncomfortable manually calculating what fraction of the calories you consume come from fats, from carbohydrates, or from proteins, you will have difficulty answering the dietary questions without the aid of a food diary such as the one provided on CalorieCount.

What you will need to complete step 3:

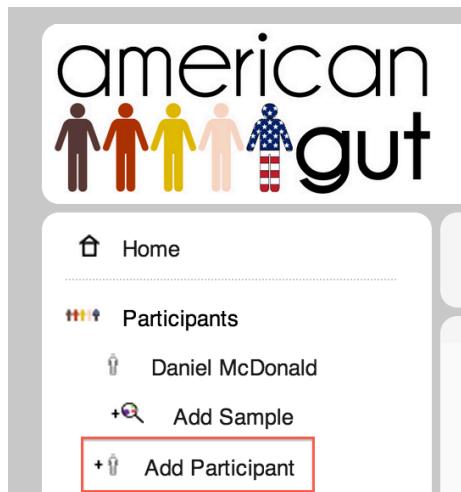
Successfully complete steps 1 (login)

You are taking a sample from a human subject

CalorieCount account and food diary (highly recommended)

IMPORTANT: If you are collecting a human sample, please do not proceed to step 4 until after finishing your 7 day food diary. You will not be able to complete step 4 accurately and completely without this food diary.

4. If you will be collecting a sample from a human or animal subject, add the subject as a participant (see the figure below). This will take you to the consent form (if applicable; we do not collect consent from animal participants) and the participant questionnaire.



To add a participant, click on the “Add Participant” link on the left hand navigation menu. This is highlighted in red in the figure above.

Please note that we require all human subjects sampled to provide consent online via this website. If you wish to participate in the study by providing a human sample, **providing consent is mandatory!** Prior to processing samples taken from human subjects, we are required by the Institutional Review Board at the University of Colorado to obtain the explicit consent of all human subjects using the provided consent document. Moreover, we are required to destroy any human subject’s sample we receive for which we do not have the explicit consent of the subject. **We cannot refund or replace samples in these circumstances.**

What you will need to complete step 4:

Successfully complete steps 1 (login)

You are taking a sample from a human or animal subject

Successfully complete step 3 (7-day food diary, only if human subject)

Some time (30-45 mins for human subject, or 10 mins for animal subject)

5. Collect a sample (see **Appendix A**, below, for specific sample type collection instructions). The sample tube provided is completely sterile on the inside. Our molecular techniques are incredibly sensitive, so it is critical to handle the sample by the red cap only. It is equally as important to touch the cotton tips only to the surface of the sample being collected. Any other handling of the swabs or accidental contact of the cotton tips with other surfaces or substances will likely result in **contamination** of your sample. Holding on to the red cap with one hand and the plastic sheath with the other, slide the cotton swabs out of the sheath. Obtain sample (refer to instructions in **Appendix A**). Replace swabs inside sheath. Press red cap down firmly to seal shut. We understand that accidents happen, and that sometimes samples are accidentally contaminated. Please contact us if your sample is contaminated and we will work with you to figure out a solution. It may not be possible for us to replace the sample free of charge.

What you will need to complete step 5:

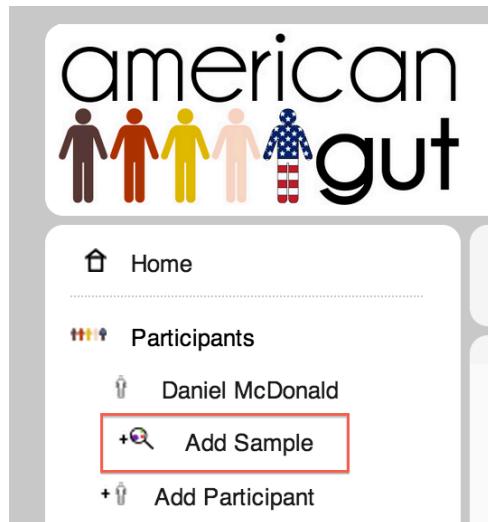
Successfully complete steps 1-4

Sampling tube

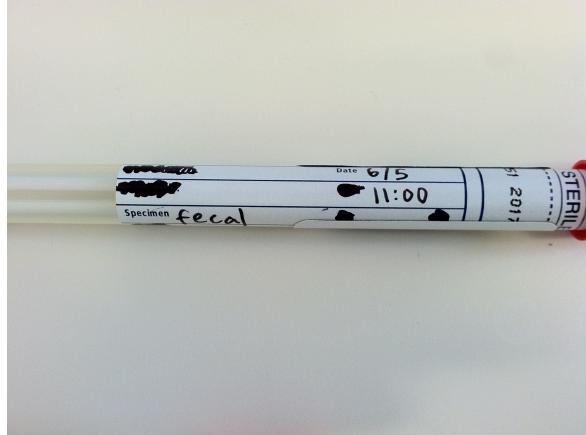
Material being sampled (e.g., used bathroom tissue)

Appendix A instructions (below)

6. Record the date, time (24-hour format), and sample type both on the sample tube and on the website (see the figures below). Note, we intentionally ask for these details to be collected both on the sample tube and on the website. Simple redundancies like this have previously proved invaluable teasing apart the inevitable human error that arises in large research projects.



To add a sample, click “Add Sample” on the left hand navigation menu. This is highlighted in red in the above figure.



An example of a sample tube with details filled in. The above sample tube contains a fecal sample collected on June 5th, at 11:00am.

We ask that you provide time and date of collection because we want to know if there is a seasonal effect on samples (date of collection) and if there is an effect driven by circadian rhythms (time of collection). We also request that you record the sample type as it is not always apparent by visual inspection.

What you will need to complete step 6:

Successfully complete steps 1-5 (omit step 3 for non-human subjects; omit step 4 for environmental samples)

Permanent marker

7. Wrap the sample tube in absorbent tissue, such as facial tissue or paper towels, and mail it back as soon as possible. Shipping within the US should be less than \$1.50, but we recommend taking the sample to the post office to get the proper postage. Getting the postage right on the first try is important since samples that spend a long time in transit will likely not produce the highest quality results. We also recommend using a reinforced envelope to reduce the chance of losing your sample due to damaged

packaging. The absorbent tissue will help to keep the relative humidity within the package low. The sooner we receive your sample, the sooner we can get it stored in our -20C freezers and ready for processing! However, if you cannot send your sample immediately, please do not freeze or refrigerate the sample prior to shipment unless we have directed you otherwise.

If you have multiple samples, it is best to send them one at a time. If you cannot return your samples one at a time, please contact us via email at info@americanagut.org and describe your particular situation.

What you will need to complete step 7:

Successfully complete steps 1-6 (omit step 3 for non-human subjects; omit step 4 for environmental samples)

Absorbent tissue

Proper postage

Sturdy mailing envelope

Appendix A: Sample type collection instructions

FECAL: Rub both cotton tips on a fecal specimen (ideally a used piece toilet paper). Collect a small amount of biomass (maximum collection would be to saturate 1/2 a swab). 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 off, 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.

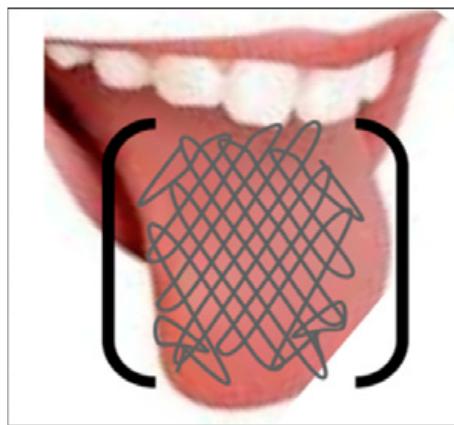
Our molecular methods are incredibly sensitive, and require nanograms (billions of a gram) of DNA. Previous studies have shown that even a tiny amount of material, like in the picture above, is sufficient to assess the microbial life in your gastrointestinal tract. The figures below show examples of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

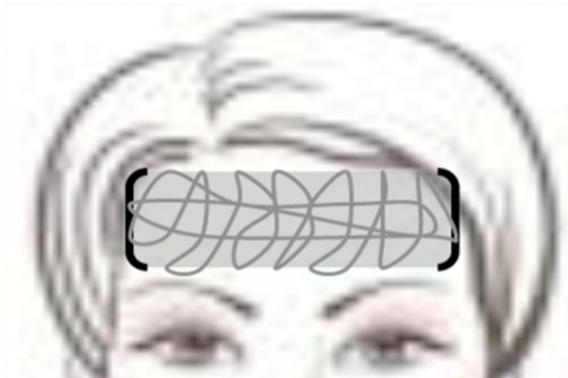
DIFFICULT TO OBTAIN FECAL SAMPLES: We recognize that some participants have chronic constipation. In these instances, what we recommend is the “catch and release” method, using 2-4 paper towels (enough to not get any on you, but to also avoid clogging the toilet). If the specimen is not soft, please 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.

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 cheeks, teeth, or lips. An example of the area of sampling for the tongue is shown below.



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

SKIN: Firmly rub both sides of both cotton tips over the skin surface being sampled for 20 seconds. An example of the area of sampling (for a forehead sample) is shown below.



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