

American Gut Policies for Affiliated Projects

We are always enthusiastic about collaborating with those who have a real interest in putting in the time and effort to run “X Gut” (i.e., Brazilian Gut, Canadian Gut, etc.) projects in other countries or continents around the world.

There are three possibilities for affiliation with the American Gut project—existence as an aggregation site (like Australian Gut), existence as a sister project (like British Gut), or existence as an independent affiliate site.

The aggregation site model: Under this model everything except kit shipping goes through the Knight lab at UCSD. This means that participants sign up on the American Gut Fundrazr page, and samples are processed, sequenced, and analyzed through the Knight lab. The Knight lab sends (in bulk) sample kits to a central location in the local country, and personnel there will mail the kits through the local mail service to participants, collect the samples back from participants, and mail samples in bulk to the Knight lab. This will necessitate local storage of samples before they are shipped back to the Knight lab at UCSD; therefore, the local center must have the capability both to store samples in a scientific grade -20°C freezer and to ship samples back on dry ice to maintain sample integrity. This model greatly reduces postage cost for participants in other countries, a cost which has been prohibitive to interested potential participants in the past.

The sister project model: Sister projects are similar to aggregation sites but are a bit more independent from the Knight lab in that sister projects have their own participant portal maintained by the Knight lab (i.e. microbio.me/britishgut) as well as a project specific Fundrazr campaign, also maintained by the Knight lab. Additionally, personnel from sister projects will have some interaction with local participants and will be expected to successfully run a help account for participant support. The help account must be language specific; for example, if a sister project is located in Russia, the help account should be managed by a Russian speaker. *Note: all appropriate personnel from sister projects must be added to the UCSD IRB document.*

The independent affiliate model: The independent affiliate model is still closely tied to American Gut in that data analysis and delivery of results to participants will occur at UCSD and all identifying data will be stored safely on Knight lab systems at UCSD. However, independent affiliate sites can pack kits, receive samples, process samples, and sequence samples. Independent affiliate labs must undergo the multiple rounds of technical variation that laboratories participating in the [MBQC project](#) underwent. Additional technical validation will be required to account for observed site effects (if any); additionally, it may be necessary to repeat technical validation procedures as protocols change and improve (i.e., changes in sample collection, modifications to PCR primers, etc.). Sequencing data will be accessed by the Knight lab for analysis and production of results. Like sister projects, independent affiliate projects will be expected to manage language specific help accounts for participant support. *Note:*

independent affiliate labs must be added to UCSD's IRB documents and/or be covered by their own site-specific IRB.

Before deciding to pursue affiliation as an American Gut sister or independent affiliation project, please carefully review the following policies:

Policies for sister projects and independent affiliate projects (aggregation sites automatically conform as they are at their root run through American Gut at UCSD):

The American Gut Project is built on several scientifically supported policies that we adhere to and sister and independent affiliate projects either must or are highly encouraged (depending on the policy) to also adhere to these policies. If you do not wish to adhere to these policies, that does not mean that you cannot kick start your own microbiome project similar to American Gut; however, the American Gut Project will not be affiliated with your project and we will place it on a list of “non-affiliated” projects on the americangut.org website. This will enable anyone to easily determine which projects are affiliated with and promoted by American Gut and which are not.

First policy (REQUIRED): IRB compliance

All affiliated projects *will* either be covered by UCSD's IRB or will have their own IRB approval and will remain compliant with the IRB at all times.

Second policy (REQUIRED): use of the Earth Microbiome Project protocols

The American Gut Project uses extensively benchmarked and validated protocols that are indicated for the Earth Microbiome Project (<http://www.earthmicrobiome.org/>), of which American Gut is a part. Any affiliated project that chooses to process and sequence samples on site *must* use these protocols to process and sequence their samples. As described above, such laboratories must undergo the multiple rounds of technical variation that laboratories participating in the MBQC project underwent, and additional technical validation will be required as necessary (i.e., observed site effects, changes in sample collection, modifications to PCR primers, etc.).

Third policy (REQUIRED): standardized sample collection

The way in which a sample was collected can have an impact on the data that is obtained from that sample. All basic participation level samples-fecal, skin, oral, animal, and environmental-that are collected through the American Gut project are done so using a single type of swab with standardized sample collection instructions sent to each participant. The samples are then mailed back to the Knight lab. Participation levels that require more sample material or preservation of RNA are given a separate sample collection receptacle with a different set of collection and mailing instructions (i.e., on -20°C ice packs or on dry ice). All affiliated projects are required to use the same sample collection materials and instructions to

ensure comparability of data without necessitating additional computational steps to remove, for example, irrelevant sequences due to differences in sample collection and handling.

Fourth policy (REQUIRED): standardized metadata

We have implemented a health and lifestyle questionnaire that all American Gut participants complete. The data obtained from this questionnaire has enabled us to detect interesting associations between the microbiome and various health and lifestyle factors, enabling us to more fully understand the role of the human microbiome in health and disease. Affiliated projects may certainly add their own third party surveys or questions to the official American Gut questionnaire (i.e., those that may be specific to the geographical region or culture in which the study is occurring); however, *because all American Gut project questions are approved by the Institutional Review Board at UCSD, any additional questions from affiliated projects must either a) be added to and approved by the IRB at UCSD or b) approved and covered by a locally approved IRB.*

Fifth policy (REQUIRED): open access

The American Gut is first and foremost an open access project. This means that, to the greatest extent ethically possible, the project is freely and publicly available. Open access projects add more to the research community by enabling anyone with an interesting research question to access the data and analyze it, either alone or in combination with data produced from other studies. Open access policies surrounding processing and analysis additionally lower barriers to novel analysis by providing insight into exactly what is being done to the data. **American Gut affiliated projects *must* adhere to an open access data policy. This includes any code written to perform affiliated project specific analyses in addition to the regular analysis pipeline (which will be run through the Knight lab); such code can be made available on GitHub. Importantly, ALL data *must* be de-identified and free of protected health information (PHI).** Note that American Gut is partner with OpenHumans.org which has significant international sharing operational now.

Sixth Policy (REQUIRED): standardized data analysis

All American Gut and affiliate samples are processed through a single data analysis workflow, which is based on QIIME (Quantitative Insights Into Microbial Ecology), an analysis software originally developed in the Knight lab. Independent affiliate projects may also analyze the data and produce participant results locally if they wish (note, this will require local maintenance of a participant portal). *However, if an affiliate lab chooses this route, they must follow the exact pipeline used by the Knight lab to produce American Gut, British Gut, and Australian Gut results,* as identical protocols minimize technical variability of the data. Affiliate labs must deposit raw sequence data into Qiita (qiita.ucsd.edu) and subsequently upload sequences to EBI through this system. Affiliate labs will either utilize Knight lab compute resources or will work with the Knight lab to acquire specific compute time (i.e., the XSEDE grant) to facilitate data analysis and results production. **If affiliate labs analyze data and produce results they must**

be added to the UCSD IRB document. Affiliate labs will not be allowed to analyze data or access UCSD compute resources without prior IRB approval.

Seventh Policy (HIGHLY RECOMMENDED): a citizen science model

The American Gut project and current sister/sub-projects (British Gut and Australian Gut) operate as citizen science projects. Participants contribute varying amounts of money for different participation levels; in essence, the participant funds his or her own sample, which is combined and analyzed together with the collective cohort. While it is not required that affiliated projects operate on a citizen science model, it is *highly* suggested and is preferable. **Please note that any affiliated projects that do not operate on the citizen science model are required to either provide their own funding or to work with the Knight lab to identify other means for funding the project (i.e. through grants, charitable donations, or sponsorships).**

Eight Policy (ENCOURAGED BUT NOT REQUIRED): contributions to development effort

The American Gut participant portal and analysis pipeline code are freely available on GitHub, and analyses exist as Jupyter notebooks. While affiliate projects are not required to contribute to the code base for the participant portal or data analysis pipeline, we always welcome those willing to contribute development hours to the project, especially if an affiliate site has an idea for an interesting analysis not currently included in our processing notebooks.

We are happy to discuss further with any interested investigators about the possibility of a project collaboration or affiliation. We also highly encourage interested parties to leverage the American Gut for focused, grant, scholarship, or charitable donation-funded science projects and are happy to discuss such opportunities. For any questions or concerns about the above policies, or if you have an “X Gut” project that you’d like to be affiliated with American Gut, please contact American Gut project manager Dr. Embriette Hyde at ehyde@ucsd.edu or at info@americangut.org.

We look forward to working with you!