OMNIgene·GUT

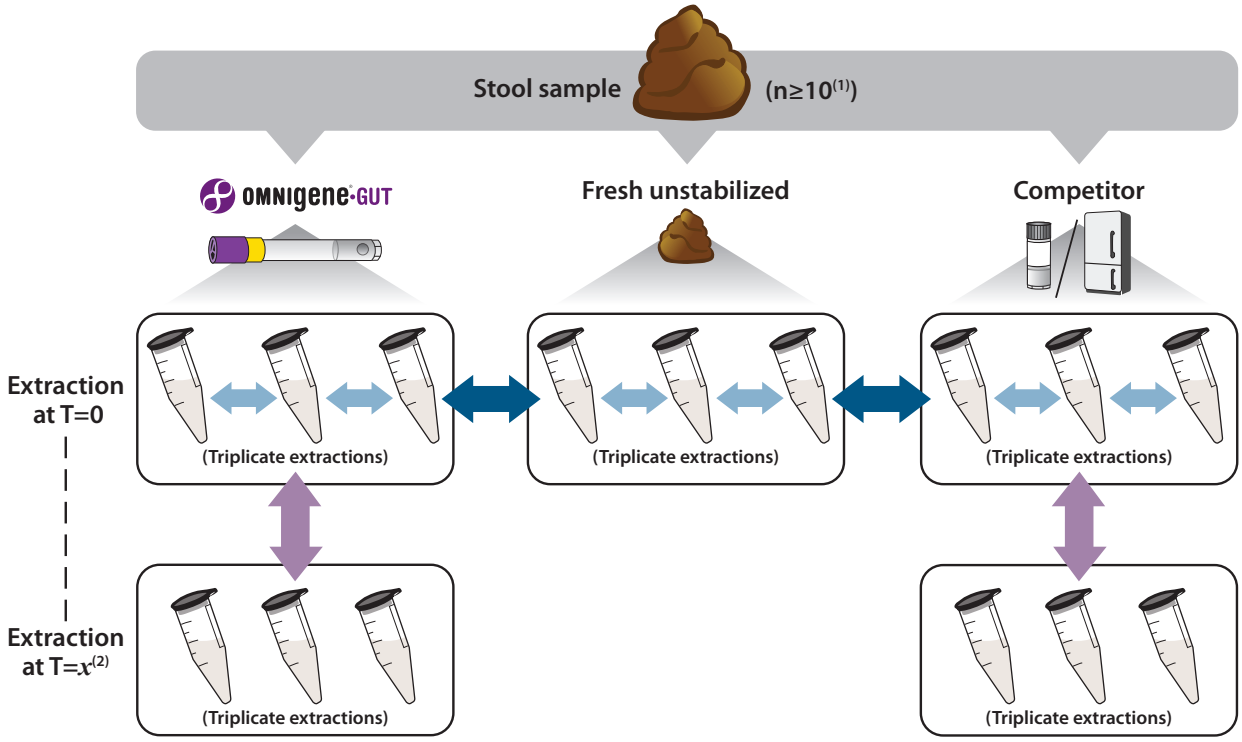
Powering a pilot study: How to design an experimental plan to compare the performance of collection/preservation methods

The goal of this document is to help researchers generate robust, scalable and reliable data to guide the choice of an appropriate sample collection method for gut microbiome studies. Although gut-focused, the suggested study design in this document can be applied to all sample types. A properly designed pilot study will generate the necessary information to choose an appropriate methodology and will allow you to scale from a pilot to a full-scale research project.

In gut microbiome studies, a fresh stool sample extracted immediately is the ideal scenario. However, this method is impractical, especially for large cohorts or field collections; freezing the sample or using a stabilizing solution are considered the best alternatives. We strongly suggest performing a pilot study to accurately determine which sample preservation method will perform best in its ability to:

- Capture an unbiased microbial profile at the time of collection (**neutrality** ⟷)
- Preserve the microbial profile during shipping and storage (**stability** ⟷)
- Rapidly homogenize the collected sample (**reproducibility** ⟷)

To benchmark any preservation method, we recommend the following study design:



(1) We recommend a minimum of 10 different samples (donors)

(2) x is defined by the elapsed time between the collection (T=0) and sample extraction (ex. It takes into account post-collection storage + transportation + pre-processing storage)

Assessement of reproducibility (⟷), neutrality (⟷) and stability (⟷)

***Figure 1**: Recommended pilot study design for comparing different collection and stabilization methods. To make valid comparisons between preservation methods and avoid introducing extraction bias, we recommend using the same extraction method for all samples. Many suitable extraction kits are available; our recommendation for optimal lysis, better DNA quality and higher yields is the QIAGEN® QIAamp® PowerFecal® Pro DNA kit (Evaluation of DNA extraction methods). If you are conducting a study involving mailing of preserved stool, the samples should be exposed to high temperatures, and/or freeze/thaw cycles to simulate real life transport conditions. Samples collected in the field will perform differently than in a controlled laboratory setting. Ideally, the samples tested in your pilot should be collected from subjects that are representative of your cohort of interest (matching Bristol scale stool types, adults vs pediatric donors, etc.).*

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Sample neutrality and stability

How does the collection method affect the sample's taxonomic profile?

Neutrality: A frozen or stabilized sample should be indistinguishable from a fresh stool sample that was extracted immediately. In the suggested experimental design, neutrality is measured by comparing the stabilized samples (at baseline: T=0) to a fresh stool sample that was immediately extracted.

Stability: The microbial profile of a frozen or preserved sample should not change in composition over time and should be identical to a sample extracted immediately after collection, regardless of shipping and storage conditions and/or elapsed time between collection and processing. Stability is measured by comparing the preserved sample extracted at the time of collection (T=0) to the preserved sample extracted post-storage or post simulated shipping conditions (T=***x***).

Lack of neutrality and/or stability will result in a sample with a taxonomic profile different from the fresh sample. This is due to bacterial growth or decay and/or DNA degradation, and results in over/underestimation of select taxa as well as the possible detection of false positives and/or negatives.

Reproducibility

How likely are replicates from the same sample to agree with one another?

Reproducibility: Multiple aliquots extracted from the same specimen should be similar to one another. Stool can be very heterogeneous, so it is critical to fully homogenize the sample in order to ensure data reproducibility across multiple extractions. Reproducibility can be tested by performing triplicate extractions from the same sample (see Figure 1). A wide variety of donors should be included in order to capture the diversity of sample types and their propensity for being homogenized (for example: liquid versus solid stool).

Lack of sample homogeneity can result in a significant taxonomic profile shift due to the lack of reproducibility from duplicate extractions. This is particularly problematic if your study requires a second extraction to perform additional testing/experiments.

In Summary

Neutrality, stability and reproducibility are the three most important criteria to take into consideration when testing or comparing preservation methods. Other important questions you need to ask yourself are: are the quality and yield of the extracted nucleic acids high enough to perform any downstream applications (i.e., NGS, long-read sequencing, etc)? Can the sample be collected in remote locations and be preserved during the shipping process? Have I tested the specific cohort of interest (infants and IBD patients are different from the general adult population)? Does the usability (intuitive and user-friendliness) of the collection method maximize donor compliance?

It is important to arm yourself with an optimal experimental setup to power the decision making process. Our suggested experimental design will allow you to compare the right parameters to ensure the optimal accuracy of each step of your microbiome study.

Technical support is available Monday to Friday (9h00 to 17h00 ET):

- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: +1.613.723.5757, option 6
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