

# The Inside Scoop on Poop: The Top 5 Tips for Microbial DNA Isolation from Fecal Specimens

## Introduction

We often receive questions from researchers starting fecal microbiome studies about recommended procedures and expected yields for microbial DNA isolation, as well as proper storage, processing, and shipping conditions.

So, to help you successfully plan your project, we've tried to do the dirty work of gathering tips and tricks from leading experts in fecal DNA isolation and microbiome analysis so that you can get to the...um...dirtier work of starting your own microbiome study.

To gather the best information out there, we spoke with Joseph Petrosino, Assistant Professor of Molecular Virology & Microbiology at Baylor College of Medicine, who helped lead procedure development for specimen collection and DNA isolation for the Human Microbiome Project (HMP), Suzanne Kennedy, Director of Research and Development at MO BIO Laboratories where they have pioneered tools for microbial DNA isolation, and Kate Patterson, research associate at TGA Sciences where they support the pharmaceutical, biotechnology, and academic communities by providing laboratory services for DNA isolation.

The following 5 hints summarize solutions that have worked in their laboratories and will hopefully extend to your project.

## 1. Catching the specimen

For rodents, collecting fecal pellets is relatively straightforward. While there are cages that you can purchase that collect the feces away from the cage floor, they can be expensive and may be overkill for this type of study. Researchers can pick up fecal pellets with sterile gloved hands and place them in a sterile container like a BD Falcon™ or Eppendorf tube.

With human stool, it gets a little messier, so to speak, but talking to our experts, there is a "lot of forgiveness" in the different methods. Many labs provide patients/subjects with a bucket-like tray, sometimes referred to as a "hat" that fits under the toilet seat for catching the specimen. Another alternative is for the subject to use newspaper to catch the specimen, which allows more moisture to be absorbed. It's key that the subject is provided with instructions for avoiding contamination from toilet water or the side of the toilet. These types of instructions should also include a recommendation that the subject urinate first to avoid specimen contamination.

If you are collecting swabs from the anal area, be sure to use a sterile swab, allow it to air dry, and enclose the swab in a capped sterile transport container. Swabs should be allowed to dry for an hour, to avoid microbial DNA degradation upon freezing and storage.

## 2. Transporting and storing of the specimen

Once the specimen is caught it can be kept in the same container and stored prior to processing. Alternatively, the specimen may be transferred into a specific sterile collection container.

If the specimen is collected in the subject's home, the specimen collection container should be frozen in the subject's freezer and shipped on ice packs to the laboratory for processing. For specimens collected in a laboratory environment, specimens should also be frozen immediately. For the HMP project, specimens were frozen in their collection buckets at -80°C and processed for DNA isolation within 24 to 48 hours. One of our experts observed that the microbial community profiles of specimens that were frozen for a year did not exhibit large variation from specimens that were processed immediately.

For microbial DNA profiling, our experts agreed that using a stabilization solution like Qiagen RNeasy for storage is probably not necessary and may interfere with downstream DNA isolation. One of our experts observed that stool samples stored in RNeasy separated into different solution phases when processed and DNA recovery was decreased.

Technical replication allows for processing and measurement variability to be estimated. In general, technical replicate analysis is unnecessary when microbial analysis is conducted in a laboratory compliant with Good Laboratory Practices and Quality Management Systems running quality control and quality assurance programs. Additionally, each PhyloChip assay has numerous quality controls built into the assay and the process.

## 3. Handling human stool from healthy and diseased individuals

Human fecal samples from healthy or diseased individuals should be processed under BSL-2 conditions, including the use of a face mask, gloves, and lab coat. Basically, treat all samples as if you don't know whether they contain an infectious agent like HIV.

Stool from individuals experiencing constipation may require additional subsequent bead-beating as part of the DNA isolation process and loose stool may require a pre-processing step to spin out excess liquid.

## 4. Isolating DNA

While there are many published methods for microbial DNA isolation from fecal samples, all of our experts recommended the use of bead-beating methods and MO BIO PowerSoil® isolation kits. Non-bead beating methods don't recover gram-positive bacterial DNA as well. With the MO BIO PowerSoil® isolation kits, the final eluted DNA is enriched for microbial DNA, making PCR more sensitive. Human or animal epithelial cells will lyse quickly under the heating and beating conditions used for microbial lysis, releasing the free DNA into the supernatant. The free DNA is subjected to



Figure 1. Human feces collection kit available through Second Genome

prolonged mechanical breakage resulting in small fragment sizes that are washed out of the column during the binding step. The microbial DNA remains as high molecular weight fragments and is retained on the column.

Our experts observed that bead-beading methods like those used in the PowerSoil® kit generally yield approximately 100 µl of 2 to 20 ng/µl of total genomic DNA from 0.1 g of stool. Starting with a range of 0.1 g to 0.25 g of human feces is ideal. For dry mouse feces, our experts recommended working with less starting material, closer to 0.1 g, or diluting the samples 1:4 or 1:5 so that sufficient lysis solution can be absorbed. They suggest using the beads provided in the kit and found other separately purchased glass or silica bead products to be unnecessary.

One expert has observed that yields from human stool retain approximately 2% human DNA. In contrast, other types of samples, like vaginal swabs, may contain up to 80% human DNA post-isolation.

## 5. Removing PCR inhibitors

Stool is similar to soil in that it contains a high concentration of PCR inhibitors. Further, these inhibitors will vary between individuals based on their diet. These inhibitors often bind to the silica membranes of spin columns and co-elute with DNA. Samples with this problem will often show a brown or yellow color after purification and low purities when measured with spectrophotometry. Again, because of these properties, our experts recommended using the MO BIO PowerSoil® DNA Isolation kit because it incorporates Inhibitor Removal Technology® (IRT) to remove humic acids, polyphenols, polysaccharides, heme, and dyes prior to binding of lysates to the spin column, resulting in high purity DNA and more accurate microbial profiles after amplification.

## Final Thoughts

If you have additional questions about these recommendations, don't hesitate to contact us at [info@secondgenome.com](mailto:info@secondgenome.com). Or, if you have your own tips or tricks that you'd like to add, please post a comment to this article.

Finally, Second Genome offers DNA isolation services for human stool, animal feces, solid tissue, swabs, blood, and frozen water filters. We also offer specimen collection and transport kits. If you'd like to learn more about these solutions or our microbial profiling services in general, contact us at [info@secondgenome.com](mailto:info@secondgenome.com).

## References

1. [Impact of DNA extraction method on bacterial community composition measured by denaturing gradient gel electrophoresis](#). Julia R. deLipthay et al. (2004) Soil Biology and Biochemistry. 36;10:1607-1614.
2. [Manual of Procedures for Human Microbiome Project](#).