OMNIgene®•GUT

OMNIgene®•GUT enables reliable collection of high quality fecal samples for gut microbiome studies

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OMNIgene®•GUT is an all-in-one system for easy self-collection and stabilization of microbial DNA from feces for gut microbiome profiling. In this paper we demonstrate that OMNIgene•GUT allows for reliable and reproducible self-collection of fecal sample. The design of the kit ensures consistent sample input, while the stabilization liquid and mixing properties of the kit ensure that the sample is homogenized and liquefied at the point of collection to simplify handling in the laboratory. DNA extraction from OMNIgene•GUT delivers consistent amounts of high quality DNA sufficient for multiple high-throughput metagenomic analyses. Importantly, the chemical properties of the OMNIgene•GUT stabilization liquid are compatible with commercial processing methods, maintain a neutral profile and do not introduce any bias into downstream applications. These properties simplify workflows in the laboratory, increase efficiency and reduce overhead.

Introduction

For any analysis of the gut microbiota, it is essential to capture an accurate microbiome snapshot of the richness and relative abundance of microbes present at the moment of collection. Current protocols described in the literature suggest a variety of fecal sample transport methods including ambient temperature, 4°C or frozen. Each of these methods has the potential to expose samples to conditions incompatible with microbiome stabilization. Improvements to self-collection, transport and processing are urgently needed in order to address the diversity in form and microbial content of human stool, as well as the range of pre-analytical factors which can introduce variability. OMNIgene•GUT is designed to overcome these challenges and uniquely enable the scalability and reproducibility of metagenomic research and biomarker discovery.

This paper evaluates the suitability of OMNIgene•GUT for use in home self-collection and laboratory processing of fecal samples for microbiome-association studies with a focus on: a) consistency of fecal sample input, b) DNA yield and concentration, c) compatibility with commonly used DNA isolation techniques and d) performance in downstream applications.

Materials and methods

Sample collection

OMNIgene•GUT kits were used by untrained donors to self-collect fecal samples according to the standard instructions provided with the kit. Each of six donors collected three samples from the same bulk fecal sample (n=18 total) using pre-weighed OMNIgene•GUT collection devices. After collection, the kits were weighed, establishing the amount of fecal sample collected. Additionally, an aliquot of fresh feces was collected from the same bulk fecal sample and transported in a styrofoam box with frozen cold packs (as per Human Microbiome Project standard procedure)[1].

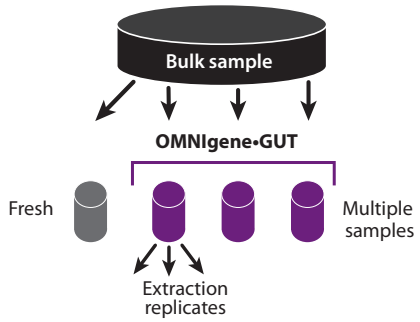

***Figure 1**: Sample collection scheme per donor. Each of six donors collected samples from three different sites of the bulk sample. DNA extraction from all OMNIgene•GUT and fresh samples was performed. Additionally, three DNA extraction replicates were done from one of the OMNIgene•GUT samples.*

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DNA extraction and sample storage

Baseline extractions were performed within three hours of collection. For baseline analysis, a 0.25 mL aliquot was extracted from OMNIgene•GUT samples using the PowerFecal® DNA Isolation Kit (MO BIO Laboratories, Inc.)[2]. Each 0.25 mL sample contained approximately 50 mg of feces and 200 µL stabilizing liquid. Equivalent amounts of fecal sample (approximately 50 mg) were extracted from fresh samples.

DNA analysis

DNA concentration and yield were determined using the Quant-iT™ PicoGreen® reagent (Life Technologies). DNA integrity was evaluated by running approximately 50 ng of purified DNA on a 0.8% agarose gel and staining with ethidium bromide. A Lambda Hind III ladder was used to determine the size of purified DNA.

Real-time PCR

To assess compatibility with third party DNA isolation kits, changes in PCR efficiency (as measured by Cq values) was used to detect potential carry over of inhibitors. Fecal microbial DNA from three donors' samples stabilized in OMNIgene•GUT and fresh samples was amplified using 16S rRNA real-time PCR. PCR primers were chosen from a region of the 16S rRNA gene that is known to be conserved across a wide variety of microorganisms, and is not found in eukaryotes. The primers were BacrRNA173-F 5' ATTACCGCGGCTGCTGG 3' and BacrRNA173-R 5' CCTACGGGAGGCAGCAG 3'. 20 ng of total DNA was taken from each sample to be tested; standard curves were generated using 2-fold serial dilutions of E. coli DNA, from 2.5 ng to 80 ng. All real-time PCR runs were performed on the Corbett Life Sciences Rotor-Gene™. Cq values from the OMNIgene•GUT samples and unstabilized samples were calculated using the default quantitation analysis with the built-in software, Rotor-Gene™ 6000 ver. 1.7. The difference in amplification was quantified using ΔΔCq method[3].

16S rRNA sequencing

16S rRNA library preparation, sequencing and bioinformatics were conducted by Metanome, Microbiome Discovery Service, with additional analytics provided by Resphera Biosciences. V4 hypervariable region paired-end amplicon sequencing was performed using the Illumina® MiSeq®. Using an amplicon length of up-to 500 nt, an average coverage of 25,000 reads per sample was achieved. Using QIIME and custom scripts, sequences were quality filtered. Paired-end reads were assembled and searched against the Greengenes reference database, clustered at 96% by UCLUST. After data normalization, sample-to-sample distance was measured using Bray-Curtis distance on OTU abundance data (performs a pair-wise normalization by dividing the sum of differences by the sum of all abundances). Sample collection methods were compared using the Mann-Whitney test.

Results and discussion

OMNIgene•GUT allows collection of a consistent amount of fecal sample

The volumetric properties of OMNIgene•GUT were tested in a population of 18 samples as per the collection methods described in the materials and methods section. The standard OMNIgene•GUT user collection protocol resulted in samples containing 2 mL stabilizing solution and an average (± SD) of 613 ± 144 mg of feces, with a minimum of 400 mg of feces (Figure 2). Therefore, based on a 0.25 mL input volume for extraction (i.e., PowerFecal DNA Isolation Kit), OMNIgene•GUT samples consisted of at least 50 mg feces per extraction aliquot.

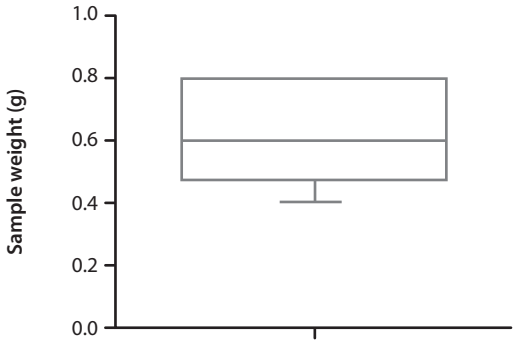

***Figure 2**: Fecal sample amount (g) obtained in 18 OMNIgene•GUT samples (6 donors × 3 samples each). The box plot represents, from bottom to top, minimum, 25th percentile, median, 75th percentile.*

Fecal samples collected with OMNIgene•GUT render reliable DNA yield and concentration

The total DNA yield from OMNIgene•GUT collected samples was 19.18 ± 8.89 µg (mean ± SD), with 90% of samples having ≥ 7.6 µg. DNA yield from multiple

extractions from the same OMNIgene•GUT sample was highly reproducible (see "intra-variability" in Figure 3), demonstrating the value of OMNIgene•GUT's ability to fully homogenize fecal samples and elicit consistent sample aliquots.

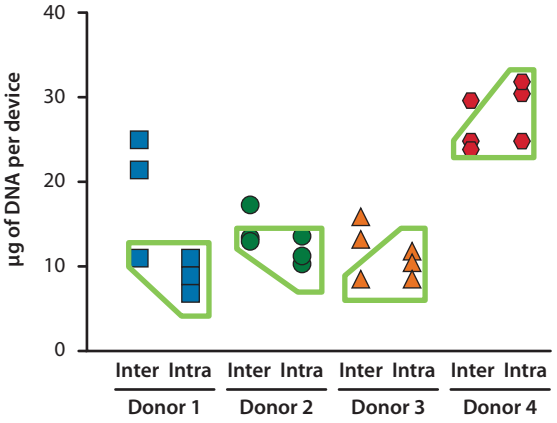

***Figure 3**: Total DNA yield (µg) for OMNIgene•GUT samples. The figure represents the inter-variability (three sites from the same bulk sample) and intra-variability (three aliquots from the same OMNIgene•GUT sample, as outlined by the green box).*

DNA yield from OMNIgene•GUT is sufficient for downstream sequencing applications

The average DNA yield per 0.25 mL extraction aliquot (approximately 50 mg feces) was 2.40 ± 1.11 µg (mean ± SD). Given that 16S rRNA library prep typically requires a minimum of 5 ng purified DNA per assay and metagenomic sequencing requires a minimum of 100 ng per library prep, a single OMNIgene•GUT sample has the capacity to provide sufficient DNA for >3500 16S sequencing assays and 190 metagenomic sequencing assays (Table 1).

	16S rRNA sequencing	Metagenomic sequencing	PCR-free sequencing
Input requirement (per sample)	~5 ng	~100 ng	1–2 µg
# assays possible in one OMNIgene•GUT extraction aliquot†	>475	>20	2
# total assays possible with one OMNIgene•GUT sample†	>3,500	>190	9

***Table 1**: Number of sequencing assays per OMNIgene•GUT sample.*
† Based on mean total DNA yield.

High molecular weight DNA is consistently extracted from samples collected with OMNIgene•GUT

Due to the competitive nature of PCR, DNA degradation in microbiome samples can lead to the introduction of bias since smaller DNA fragments may be preferentially amplified. Therefore, minimizing DNA degradation in fecal samples is critical to ensuring accurate and valid microbiome profiling results. As assessed by agarose gel, the DNA extracted from OMNIgene•GUT samples had a molecular weight greater than 10 kb (Figure 4), thereby reducing a potential source of bias in PCR-based downstream applications.

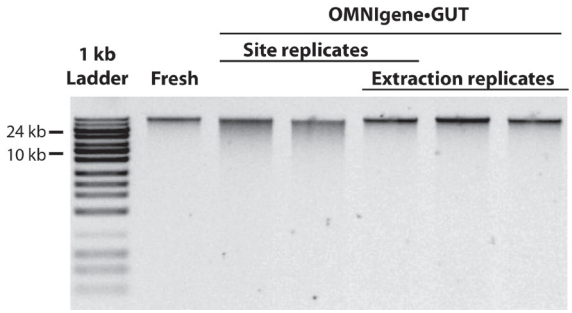

***Figure 4**: Agarose gel of fresh and OMNIgene•GUT fecal samples collected from a representative donor. Site replicates refer to multiple collections from a single fecal sample of one donor. Extraction replicates were performed on a single collection replicate.*

DNA extracted from OMNIgene•GUT stabilized samples demonstrates compatibility and optimal performance on downstream applications

Fecal samples contain several PCR inhibitors including humic acids, polyphenols, polysaccharides and heme, among others. Commercial extraction kits include effective inhibitor-removal systems, ensuring proper performance in downstream applications.

The compatibility of samples collected with OMNIgene•GUT with the PowerFecal DNA Isolation Kit was assessed. Three donors collected one fresh sample and one OMNIgene•GUT sample from the same bulk fecal sample. DNA was extracted from an aliquot of each sample. DNA performance was determined by 16S rRNA real-time PCR using equal amounts of DNA. No difference in Cq values were observed between DNA extracted from the control or OMNIgene•GUT samples, indicating

that OMNIgene•GUT method of stabilization did not impact inhibitor removal or real-time PCR performance (Figure 5).

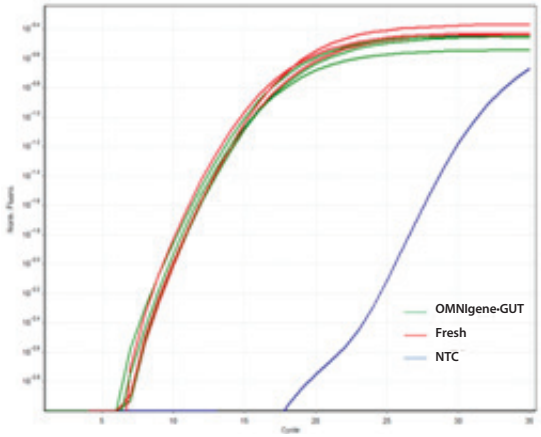

***Figure 5**: Real-time 16S rRNA PCR of fecal DNA extracted from 3 donors using OMNIgene•GUT or fresh samples (ΔΔCq = 0.54). NTC-no template control.*

OMNIgene•GUT stabilization liquid maintains a neutral microbiome profile and does not introduce bias

The use of chemical stabilization buffers can potentially modify the microbial composition of the sample by accelerating growth of some microbes while allowing the decay of others. In ideal conditions, the stabilization liquid should be neutral (i.e., it should not introduce any bias to the microbiome profile). To test neutrality, fresh samples and OMNIgene•GUT collected samples were compared using 16S rRNA sequencing. Bray-Curtis analysis of microbiome profiles obtained using 16S rRNA sequencing showed that OMNIgene•GUT does not introduce any bias to the microbiome profile.

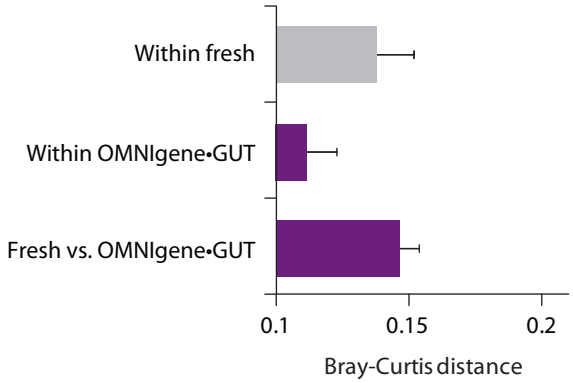

***Figure 6**: Dissimilarity within and between donor matched fresh and OMNIgene•GUT collected fecal samples. Bray-Curtis analysis showed no statistical difference in dissimilarity between fresh and OMNIgene•GUT.*

Conclusions

OMNIgene•GUT provides a means to collect samples containing sufficient material for microbiome-wide association studies to bank and share for repeat analyses. The OMNIgene•GUT stabilization liquid is compatible with a commercially available extraction process, and DNA quality is suitable for downstream applications including qPCR and sequencing. Furthermore, the preservative function of OMNIgene•GUT is capable of maintaining DNA integrity, thereby minimizing bias in commonly used microbial profiling applications. Reproducible and reliable self-collection with OMNIgene•GUT uniquely enables access to study participants, facilitating population-based microbiome-wide association studies.

References

1 Manual of Procedures - Human Microbiome Project (2010).
2 *OMNIgene*•GUT gut microbial DNA purification using MoBio PowerFecal DNA Isolation Kit. DNA Genotek. PD-PR-00434.
3 Pfaffl, M. A new mathematical model for relative quantification in real-time RT–PCR, *Nucleic Acids Res.* 29(9): E45 (2001).

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