DNA genotek

OMNIgene®•GUT provides easy self-collection and stabilization of liquid fecal samples for microbiome profiling.

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OMNIgene®•GUT is an all-in-one system for easy self-collection and stabilization of microbial DNA from feces/stool for gut microbiome profiling. In this paper, we demonstrate the performance of OMNIgene•GUT in reproducibly collecting and stabilizing gut microbiome profiles from donors undergoing Crohn's and chronic colitis symptoms (Bristol type 6 and 7).

Introduction

Accurate recovery of the microbial profile has been essential for studies linking gut microbiome dynamics and host health. In the past, methods such as 4°C transport or dry ice packing were utilized as means of preservation of stool samples prior to nucleic acid extractions. Over time, these methods were found to be cost prohibitive or incapable of accurately capturing the *in vivo* microbiome state without biased taxonomic changes. In addition, as researchers are rapidly moving towards gut microbiome collections from larger cohorts and/or donors undergoing dysbiosis - an easy to use, accurate and validated method of sample collection is essential to maximize value of the generated data and donor compliance.

In partnership with Crohn's and Colitis Canada, this study evaluates the compatibility and performance of the OMNIgene•GUT collection device for in home self-collection by donors undergoing Crohn's and chronic colitis symptoms. Facets evaluated were: donor compliance, DNA yield and quality, and sample preservation over time. This data works to extend the performance of OMNIgene•GUT into novel use cases, with the intention of expanding gut microbiome researchers' toolboxes and allowing successful study executions with unique cohorts.

Materials and methods

Sample collection

OMNIgene•GUT kits were used by untrained donors recruited through Crohn's and Colitis Canada to self-collect fecal samples. Each donor collected two samples from the same bulk fecal sample, using a different collection tool for each sample. The tools and instructions were: standard OMNIgene•GUT spatula and the DNA Genotek spoon accessory (OM-AC2) designed for donors with dysbiosis. The samples collected with OMNIgene•GUT, along with fresh fecal (bulk) sample from the same donor, were returned to the collection coordinator the same day as collection. The bulk sample was transported using cold chain as per the Human Microbiome Project standard procedure[1]. The collected kits were weighed and compared to an average weight of uncollected kits to determine the amount of fecal sample collected. The bulk samples were stored at 4°C overnight and then distributed among three OMNIgene•GUT kits, and two 5 mL tubes without stabilizer solution.

DNA extraction and sample storage

Baseline extractions were performed within an hour of bulk sample distribution. For all extractions, a 0.35 mL aliquot of stabilized sample or 60 mg of unstabilized/fresh sample was extracted using an adapted Repeat Bead Beating protocol (Yu and Morrison, 2004)[2]. Briefly, the extraction protocol modifications included reduction of lysis buffer to 950 µL and replacement of 0.5 mm beads with two 3 mm beads, four 2 mm beads per extraction tube. After baseline extractions, OMNIgene•GUT samples were stored at room temperature (23±3°C) for 60 days, exposed to simulated transport conditions

(50°C for 3 days) or subjected to 3 cycles of freezing and thawing (each cycle consisted of a minimum of 3 hours at -20°C and a minimum of 3 hours at 35°C) followed by storage at -20°C for 60 days, at which time a second set of 3 freezing and thawing cycles occurred. Unstabilized stool aliquots were stored at room temperature for 14 days and a separate aliquot at -80°C for 60 days. Extractions identical to baseline protocol were performed at each time point.

DNA analysis

DNA concentration and yield were determined using the Quant-iT™ PicoGreen® dsDNA Reagent (Invitrogen). DNA integrity was evaluated by running approximately 70 ng of purified DNA on a 0.8% agarose gel and staining with ethidium bromide. The UltraRanger 1 kb DNA Ladder™ (Norgen) was used to determine the size of purified DNA.

16S sequencing

16S rRNA library preparation, sequencing and bioinformatics were conducted in-house through DNA Genotek's GenoFIND™ service. V3-V4 hypervariable region paired-end amplicon sequencing was performed with a PE-300 V3 kit on an Illumina® MiSeq® platform. Paired-end sequencing reads were merged, screened for length, and filtered for quality using proprietary DNA Genotek scripts. Filtered sequences were aligned to the GreenGenes reference database at 97% identity using the NINJA-OPS algorithm, v1.5.1. Samples were rarefied to 20,000 or 25,000 reads per sample depending on the study. Operational Taxonomic Units (OTUs) that were not present with more than 10 counts per sample in any sample were removed, and all remaining OTUs were collapsed at the species-level (L7) when possible, otherwise were assigned highest available taxonomic resolution. Shannon alpha diversity and Bray-Curtis dissimilarity, were calculated using QIIME v1.9.1 on the collapsed OTU tables. LEfSe (Linear discriminant analysis Effect Size) was used to determine if any organisms were differentially abundant between time points (Segata et al., 2011).[3]

Results

Surveys were filled out by donors to assess ease of use of the kit and characterize their Bristol stool type. Crohn's and Colitis Canada facilitated donor recruitment in order to target active sufferers of inflammatory bowel disease (IBD). As expected, not all donors had Bristol type 6 or 7 stool on the day of collection, with 65% of donors self-reporting Bristol 6 or 7 (Figure 1). Results and discussion will be primarily focusing on samples from those donors.

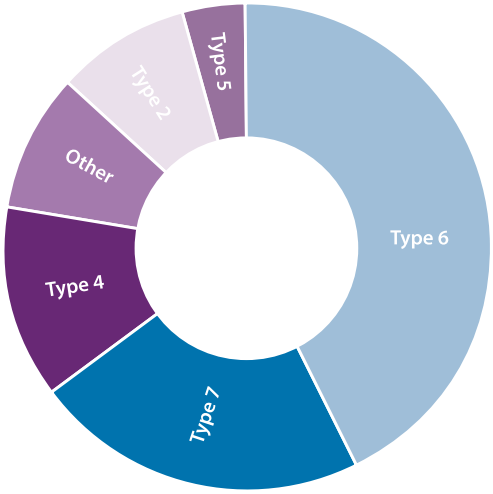

Figure 1. *Distribution of donor-reported Bristol stool type. 65% of donors identified as Bristol 6 or 7. 'Other' encompasses donors that identified more than a single type on the survey.*

Donors were asked to fill the volumetric cavity in the OMNIgene•GUT kit with the spatula or the sampling spoon. The sampling spoon was preferred by all Bristol 7 donors, and collected 580 ± 120 mg (mean ± SD) with a minimum of 430 mg. The spatula was generally preferred for Bristol ≤6 and collected 560 ± 160 mg (mean ± SD) with a minimum of 330 mg. Therefore, based on a 0.35 mL extraction input, OMNIgene•GUT samples contained at least 50 mg of feces per extraction (Figure 2). Both sampling tools were successfully used to collect the required amount of stool into the OMNIgene•GUT device.

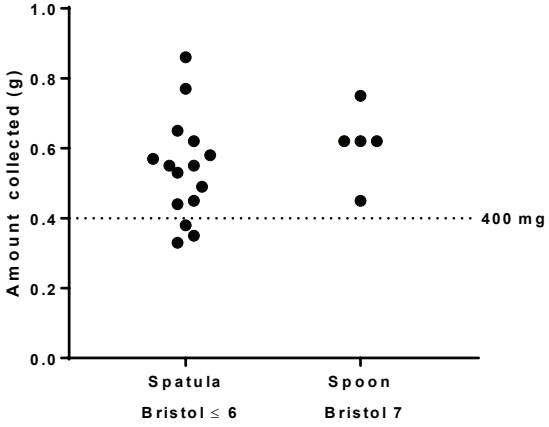

Figure 2. *Amount of fecal sample collected into OMNIgene•GUT by each of the donor preferred tools, spatula for Bristol ≤6 and spoon for Bristol 7. For both sets of donor groups, the preferred tools collected sufficient sample to facilitate at least 50 mg of stool per 350 µL extraction.*

Samples collected from dysbiosis donors with OMNIgene•GUT provide consistent DNA yield and quality, sufficient for downstream sequencing applications

The total DNA yield from Bristol 6 and 7 samples collected into OMNIgene•GUT kits was 31.43 ± 25.67 µg (mean ± SD), with 90% of samples having > 4.05 µg. DNA yield from multiple extractions from the same OMNIgene•GUT sample over time was reproducible (see "intra-variability" in Figure 3), demonstrating the ability of OMNIgene•GUT to fully homogenize samples and provide consistent sample aliquots.

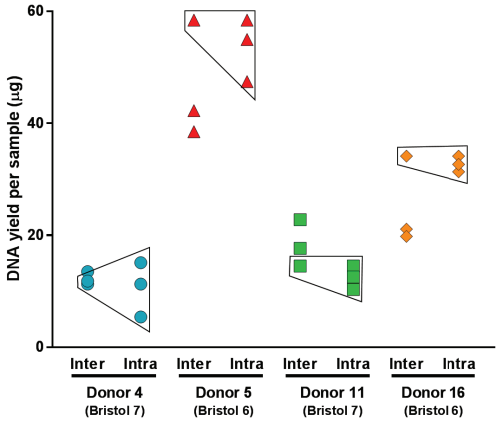

Figure 3. *Total DNA yield from representative Bristol 6 and 7 OMNIgene•GUT collected samples. The figure represents the inter-variability, OMNIgene•GUT samples from the same bulk stool, and intra-variability, extraction aliquots from the same OMNIgene•GUT sample (as outlined by the box).*

The average DNA yield per 0.35 mL OMNIgene•GUT extraction aliquot was 4.49 ± 3.67 µg (mean ± SD). Considering current minimum DNA input requirements for sequencing assays, a single OMNIgene•GUT sample collected by donors undergoing dysbiosis has more than sufficient capacity to provide sufficient DNA for these assays (Table 1).

	16S rRNA sequencing	Metagenomic sequencing	PCR-free sequencing
Assay input requirement (per sample)	~50 ng	~100 ng	1-2 µg
# assays possible in one OMNIgene•GUT extraction aliquot†	>800	>40	4
# assays possible with one OMNIgene•GUT sample†	>6000	>300	31

Table 1. *Number of sequencing assays per OMNIgene•GUT sample. †Based on mean DNA yield and an assumption of 7 extraction aliquots per sample.*

DNA extracted from OMNIgene•GUT samples had a molecular weight greater than 10 kb, thereby reducing a potential source of bias in PCR-based downstream applications that could preferentially amplify smaller DNA fragments (Figure 4).

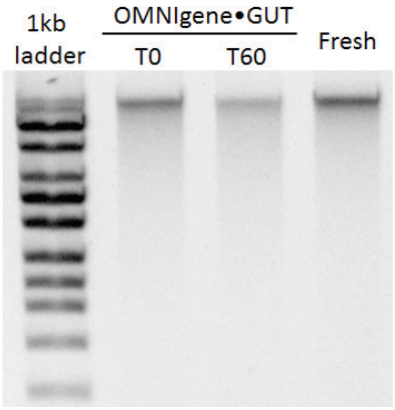

Figure 4. *Agarose gel of OMNIgene•GUT and unstabilized stool samples from a representative Bristol 7 donor. Stabilized samples were extracted at baseline and post storage at room temperature for 60 days, control fresh sample from matched donor was extracted at baseline.*

Microbiome profile neutrality of donors with dysbiosis is maintained in OMNIgene•GUT

A crucial aspect of microbiome studies is that any sample taken represents the *in vivo* microbial community present in the donor. Storage at -80°C remains the standard for preservation of the microbiota, but is not feasible or cost effective in many situations – particularly for in home collections. Chemical stabilization buffers offer the benefits of being free from additional expense and inconvenience of cold chain preservation, but need to demonstrate neutrality where-in the microbial profile is not altered by DNA degradation or growth of specific microbes. Neutrality was established by comparing OMNIgene•GUT baseline to fresh sample extractions, this dissimilarity was then statistically tested against dissimilarity between replicate extractions of baseline fresh samples (Figure 5). Finding no statistical significance confirmed that stabilization within OMNIgene•GUT did not introduce microbiome profile changes beyond what is typically observed through biological sampling.

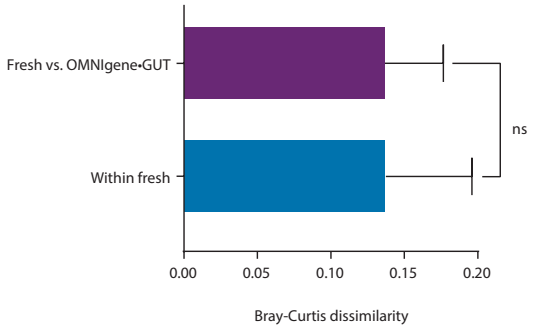

Figure 5. *Bray-Curtis dissimilarity of OMNIgene•GUT baseline compared to fresh (marked in purple), as well as, baseline variability within fresh aliquots (marked in blue); no statistical difference was found between the two sets of dissimilarity values. Bray-Curtis dissimilarity results for fresh aliquots (marked in blue) were used from the OMNIgene•GUT validation study[4], with triplicate aliquots from 28 donors.*

Gut microbiome profiles of donors with dysbiosis are preserved for up to 60 days in OMNIgene•GUT

OMNIgene•GUT samples were kept at room temperature for 60 days and exposed to simulated transport conditions: 50°C for 3 days and freeze-thaw cycling with interim storage at -20°C for 60 days. Additionally, control unstabilized sample aliquots were kept at room temperature for 14 days and -80°C for 60 days. The microbiome profile changes of samples at baseline and after storage was evaluated using Bray-Curtis dissimilarity analysis (Figure 6). As expected, unstabilized samples stored for 14 days at room temperature showed a large increase in Bray-Curtis dissimilarity from baseline. Mann-Whitney analysis showed significant differences between OMNIgene•GUT samples and unstabilized samples, both held at -80°C and room temperature. This confirms that stool samples collected with OMNIgene•GUT from donors undergoing dysbiosis show significantly less profile change over time than unstabilized samples held at -80°C.

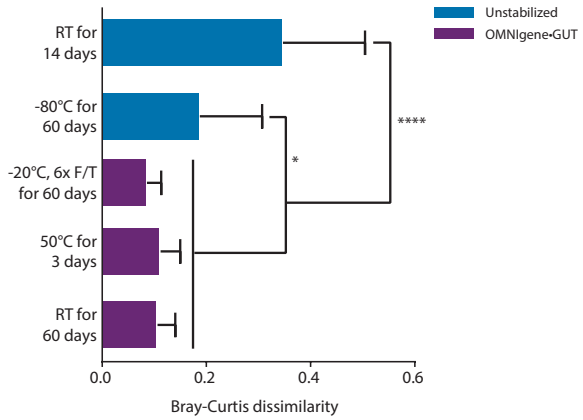

Figure 6. *Bray-Curtis dissimilarity of stool samples collected with OMNIgene•GUT from donors undergoing dysbiosis and then exposed to storage and simulated shipping conditions. Dissimilarity was generated by comparing sample's microbial profile at baseline to its paired time held sample. Significant difference in microbiome profile change was observed in unstabilized (marked in blue) samples held at RT or -80°C when compared to paired OMNIgene•GUT (marked in purple) stabilized samples (*P≤0.05 and ****P≤0.0005, respectively).*

Additionally, LEfSe analysis of unstabilized samples stored for 14 days at room temperature and compared to baseline found 13 significant taxonomic differences, ranging from family to species level. Contrary, no significant differences were found after storage of stool samples in OMNIgene•GUT kits for 60 days at room temperature or exposed to simulated transport conditions. As a control, LEfSe analysis was carried out on stool samples that were immediately stored at -80°C after collection and held for 60 days until extraction, no significant differences were identified. Together this evidence shows that the OMNIgene•GUT device can effectively stabilize microbiome signals in stool collected by donors undergoing dysbiosis, allowing extended storage conditions without a need for cold chain.

Enrichment and depletion of microbes linked to IBD are observable in gut microbiome profiles of donors with dysbiosis

We observe a total of 17 microbiota markers for Colonic Crohn's disease (CCD) in our cohort and 53% of those markers follow published enrichment and depletion trends (Walters, Xu and Knight, 2014)[5] when compared to control donors. For example, *Parabacteroides distasonis* is depleted in donors with dysbiosis by ~14 fold when compared to controls. We also observe a total of 23 microbiota markers for Ileal Crohn's disease (ICD) and 65% of those markers follow published trends (Walters, Xu and Knight, 2014)[5]. As an example, we observed a ~35 fold enrichment of *Veillonella dispar* in our dysbiosis cohort. These CCD and ICD findings are preliminary and the remaining data not following published trends may be a result of the particular cohort or study size. Broad taxonomic shifts that have been published regarding IBD-linked microbes include a depletion of Firmicutes and Bacteroidetes and enrichment of Proteobacteria and Actinobacteria (Sartor and Mazmanian, 2012)[6], similar trends are also observable in our cohort (Figure 7).

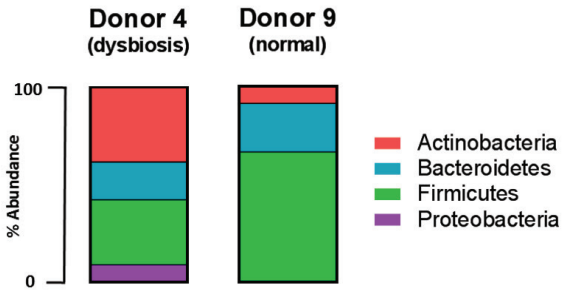

Figure 7. *Phylum-level abundance charts of representative donors illustrating previously published depletion and enrichment trends for IBD-linked microbes.*

Conclusions

The OMNIgene•GUT kit provides the means to effectively collect and stabilize microbiome profiles from donors undergoing dysbiosis. These stool samples are soft or liquid in consistency and common for people suffering from inflammatory bowel disease, Crohn's or chronic colitis. The intuitive design of the spoon accessory combined with OMNIgene•GUT allows for easier collection process and in home use for donors. Samples collected in OMNIgene•GUT recover DNA yields and quality sufficient for multiple downstream applications. In addition, these samples maintain their microbial profile neutrality without any introduced bias; therefore, ensuring the *in vivo* dysbiosis profile of the donor can be accurately interrogated. Profile preservation is achieved for up to 60 days at room temperature and various transport conditions, allowing for banking of samples and repeat analysis.

References

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