OMNIgene®•GUT

Impact of fecal collection methods on sample homogenization and extraction reproducibility in gut microbiome profiling

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Introduction

For an accurate analysis of the gut microbiota via fecal sampling, it is critical to capture a snapshot of all of the microbes present in the sample at the time of collection. The complexity and diversity of the microbial community combined with the heterogeneity of the fecal matrix[1] pose a significant challenge when handling and processing samples. Ideally, multiple aliquots extracted from the same sample should have little or no variability and produce very similar results. However, fecal samples can be very heterogeneous and significant microbiome profile differences can arise when multiple aliquots of the same sample are processed. For this reason, proper sample homogenization at the time of collection is critical, as it ensures that all microbes in the sample are exposed to the stabilization chemistry and results in reproducible and consistent microbiome extractions.

DNA Genotek's OMNIgene®•GUT is an all-in-one system that enables high compliance, volumetric self-collection of fecal samples, with built-in homogenization and stabilization of microbial DNA from feces making it ideal for gut microbiome profiling. In this paper we compare the effectiveness and reproducibility of OMNIgene•GUT extracted using QIAamp® PowerFecal® Pro DNA Kit (QIAGEN®), and the DNA/RNA Shield™ Fecal Collection Tube (Zymo Research) extracted using Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research).

Materials and methods

Sample collection

Subjects were asked to collect a bulk fecal sample into a specimen plastic container and transport to the lab in a Styrofoam™ box with a frozen cold pack within three hours of collection. Upon receipt in the lab, each bulk sample was sub-sampled into one OMNIgene•GUT and one Zymo-Shield (DNA/RNA Shield Fecal Collection Tube) according to the manufacturer's instructions.

Post-collection storage and DNA extraction

Samples collected in OMNIgene•GUT and Zymo-Shield were stored at room temperature for 24 hours after which triplicate extractions were performed for each sample using PowerFecal Pro (QIAamp PowerFecal Pro DNA Kit, 250 µL aliquot)[1] and Quick-DNA (Quick-DNA Fecal/Soil Microbe Miniprep Kit, 1 mL aliquot)[2], respectively.

DNA analysis and metagenomic shotgun sequencing

DNA concentration and yield were determined using the Quant-iT™ PicoGreen™ reagent (Thermofisher Scientific). DNA integrity and stability were evaluated using genomic DNA screentapes on the TapeStation (Agilent Technologies).

Metagenomic shotgun sequencing libraries were prepared using the Nextera® XT DNA Library Preparation Kits (Illumina®). Paired-end 150 bp sequencing was conducted on the NextSeq™ 550 (Illumina®) on a high output flow cell. Samples were multiplexed to target a median of 2 million reads per sample.

Metagenomic sequence processing and analysis

Demultiplexed, paired-read FASTQ files were trimmed and quality filtered using in-house scripts. Briefly, reads were filtered and trimmed using kneadData v0.6.1 with Bowtie2[4] v.2.3.4.1 to remove contaminating human genomic and ribosomal RNA genes by mapping against hg37 and SILVA v128 references respectively. Timmomatic v0.38 was used to trim low quality bases (<Q20) and to remove any leftover sequence adapters. The resultant trimmed and filtered reads were mapped using Kaiju[5] v1.6.3 to the bacterial proGenomes database for taxonomic assignment.

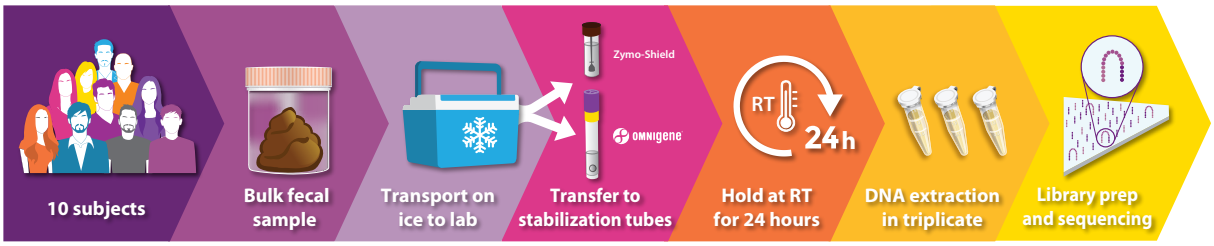

***Figure 1**: Sample collection, transport and processing workflow.*

The mapped read counts table was filtered to keep reads assigned to taxonomic bins occurring in at least 2 samples and having a total of at least 10 mapped reads. Samples had a median of 2,509,820 (IQR: 1,033,238) mapped reads, post-filtering. Using the ALDEx2 R package[6], read counts were transformed to centre-log-ratios (CLR), and the Euclidean distance between the CLR (the Aitchison Distance) was used as a measure of similarity between samples. A lower Aitchison distance between samples represents more similarity in the relative composition of the microbiome. Original figures were generated in R using the ggplot2 package and modified for publication[7]. Statistical analyses were performed in R.

Results

Fecal samples collected with OMNIgene•GUT produce higher DNA yields and higher molecular weight DNA

The mean DNA yield per 250 µL aliquot of OMNIgene•GUT-stabilized feces (approximately 50 mg feces) was significantly higher than from the same volume aliquot of feces stabilized in Zymo-Shield (11.85 +/- 3.52 µg vs 1.14 +/- 0.7 µg; paired t-test $p < 0.01$) as shown in Figure 2.

The average molecular weight of DNA extracted from OMNIgene•GUT was significantly higher than the Zymo-Shield (16.7 kb vs 3.2 kb; paired t-test $P < 0.01$). DNA extraction using the Quick-DNA resulted in significant shearing of the DNA compared to DNA extracted using PowerFecal Pro as seen in Figure 3.

DNA quality and quantity

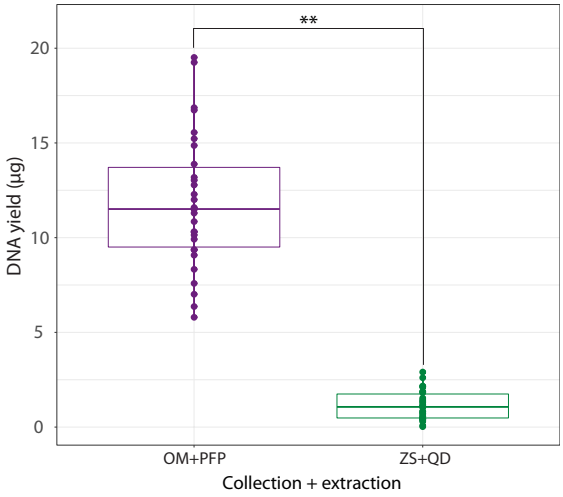

***Figure 2**: Total DNA yield (µg) from a 250 µL aliquot OMNIgene•GUT (purple) and Zymo-Shield (green) fecal sample extracted with PowerFecal Pro and Quick-DNA respectively.*

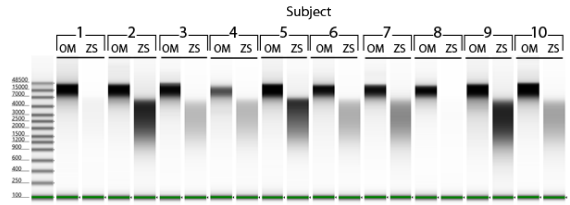

***Figure 3**: Pseudo-gel image generated by Agilent TapeStation gDNA Assay showing DNA extracted from OMNIgene•GUT (OM) and matching Zymo-Shield (ZS) samples extracted with PowerFecal Pro and Quick-DNA respectively.*

Metagenomic sequencing analysis

Samples collected in OMNIgene•GUT have higher subsampling reproducibility and introduce less variability in the microbiome profile

The magnitude of change in the total microbiome (Aitchison distance) is lower and the amount of variance between replicate samples is smaller for samples collected using OMNIgene•GUT and

extracted with PowerFecal Pro (mean Aitchison distance 89.74 and coefficient of variance 3.22 as shown in Figure 4 and Figure 5). In contrast, samples collected using Zymo-Shield and extracted with Quick-DNA protocol showed the most variability between replicates and replicates were less similar in their microbiome profiles (mean Aitchison distance 104.63 and coefficient of variance 7.09).

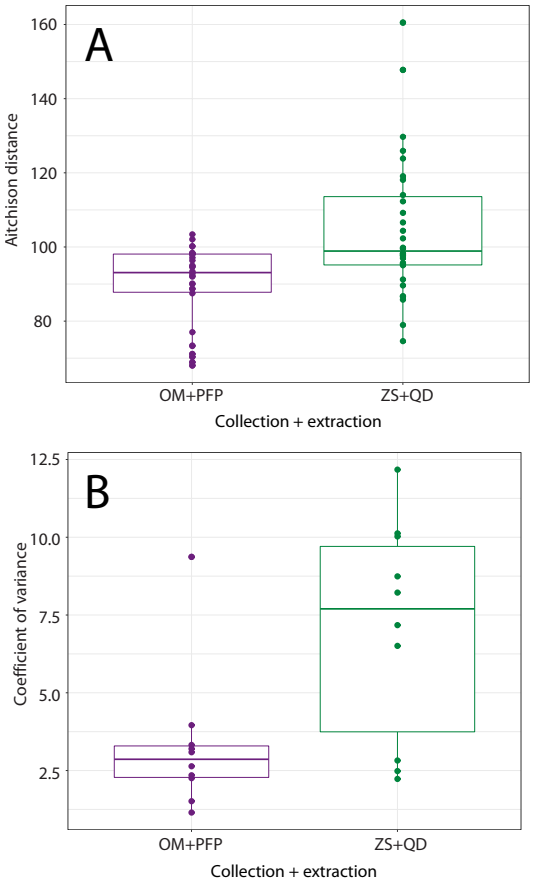

***Figure 4**: Groupwise A) Difference in Aitchison distance and B) Coefficient of variance of the Aitchison distance between replicate samples collected with OMNIgene•GUT extracted with PowerFecal Pro (purple) and replicate samples collected with Zymo-Shield extracted with Quick-DNA (green).*

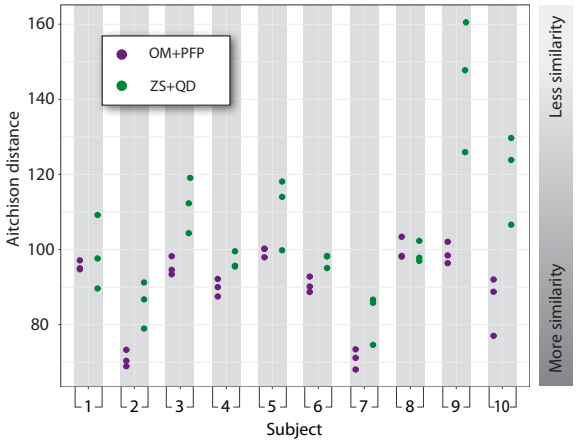

***Figure 5**: The between-replicate Aitchison distances are plotted per-donor (x-axis) and colored according to collection+extraction methodology: OMNIgene•GUT samples extracted with PowerFecal Pro (left side-purple dots) and Zymo-Shield samples extracted with Quick-DNA (right side-green dots). A lower Aitchison distance represents microbiota profiles that are more similar to each other. In addition, the spread between triplicate points represents the variability in the measured microbiota in replicated extractions.*

Conclusions

- DNA yield and molecular weight from OMNIgene•GUT samples was significantly higher than samples collected using Zymo-Shield. In addition, OMNIgene•GUT samples extracted with PowerFecal Pro showed less DNA fragmentation.
- Samples collected using OMNIgene•GUT showed reduced intra-sample replicate variability resulting in higher sampling reproducibility compared to samples collected using Zymo-Shield. This reduced technical variability gives confidence that a single sample aliquot is representative of the entire sample, and that multiple extractions from the same sample will produce the same results.
- The unique design of the OMNIgene•GUT collection kit ensures that the sample is collected volumetrically and is properly homogenized, resulting in more consistent and reproducible microbiome profile.
- Collection device form factor is a critical consideration when it comes to choosing a reliable and reproducible collection and stabilization device.

References

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