OMNIgene®•GUT Sample Collection Kits are Compatible with the Shoreline Complete™ Microbiome Prep Kit for NGS

Introduction

Shoreline Complete™ kits provide a solution for fast and easy microbiome amplicon sample preparation for next generation sequencing (NGS) analysis. All Shoreline Complete kits contain everything a researcher needs to lyse, purify, and amplify samples for microbiome sequencing. The kits improve microbial representation and accelerate high-throughput sample prep. The DNA preparation method includes a novel lysis protocol designed to crack open both Gram-negative as well as Gram-positive microbes missed by other DNA preparation methods, while enabling a 20-fold reduction in hands-on time. The purified DNA is transferred into a plate complete with reagents enabling one step sample barcoding and ribosomal RNA gene amplification. Shoreline Biome offers a range of kit choices include Illumina low resolution V4 and medium resolution V1V3 regions, as well as PacBio species resolution V1V9 region and strain level resolution with our unique 16S-23S long amplicon StrainID kit.

https://www.shorelinebiome.com/our-kits/

The DNA Genotek OMNIgene®•GUT is an all-in-one system for easy self-collection and stabilization of microbial DNA from feces for gut microbiome profiling. OMNIgene•GUT is chosen by many researchers for its ease of use and its ability to preserve DNA integrity and microbial profile during transport and extended storage, addressing key problems researchers face in microbiome studies. The standard volumetric sample input is ideal for high throughput and/or automated processing. This report evaluates the compatibility of OMNIgene•GUT fecal samples with Shoreline Biome's Complete DNA Preparation and PCR Amplification kits.

https://www.dnagenotek.com/US/products/collection-microbiome/omnigene-gut/OMR-200.html

The Shoreline Complete method uses dried reagents to enable direct sample addition from collection tubes for rapid processing of many samples in parallel. The Shoreline Complete dried lysis reagents are designed to be resuspended in water before use, and outperform other commercially available methods for recovery of difficult to lyse bacteria. OMNIgene•GUT kits use a proprietary buffer to store and maintain integrity of fecal samples.

Experiments were designed to assess the compatibility of the OMNIgene•GUT buffer with the Shoreline Biome kit. Performance was assessed by comparing microbial profiles of a variety of fecal samples prepared in parallel using water or OMNIgene•GUT as a substitute for water as direct input into the standard Shoreline Complete kit protocol.

Materials and Methods

DNA purified from frozen fecal samples prepared in OMNIgene•GUT buffer vs. standard Shoreline Biome protocol

Fecal samples (frozen without storage buffer) were thawed on ice. 50ul of sterile water or 50ul of OMNIgene•GUT buffer was added to the 96-well lysis plate to reconstitute the lysis buffer, and ~1-3 mg sample was added to each well. To test performance of input amounts much higher than recommended, a fecal sample was collected directly to the OMNIgene•GUT tube, and after mixing as per manufacturer's instructions, 50 ul (~10mg fecal material) was added to the lysis plate containing dried lysis reagent. For all samples, DNA was subsequently purified as per Shoreline Biome manufacturer's instructions using the Shoreline Complete V4 Kit (Cat# SCV4-96).

DNA Analysis

Nineteen different fecal samples were processed in duplicate, either resuspended in water (Shoreline Complete protocol), or OMNIgene•GUT buffer. Frozen fecal samples were thawed, ~1-3 mg of sample was collected by stabbing the sample with a 1ul sterile loop and suspended by twisting the loop into either water or OMNIgene•GUT buffer. Similar DNA yields were obtained for both methods. Variability is as expected because the amounts transferred can vary two-fold or more depending on sample consistency.

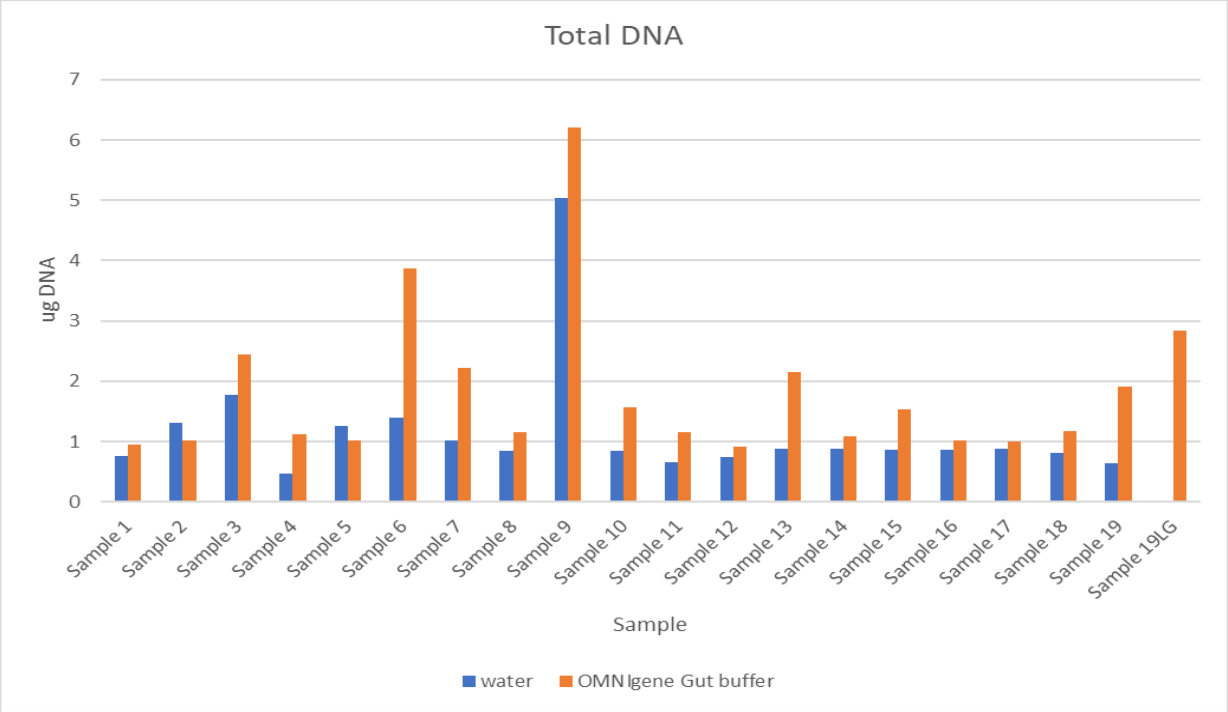

Figure 1: Total DNA yield from samples containing standard buffer or OMNIgene•GUT buffer. *Sample DNA was purified using the Shoreline Biome lysis and DNA purification protocols. For samples 1-19, either 50ul of sterile water (blue) or 50ul of OMNIgene•GUT buffer (orange) was added to the 96-well lysis plate to reconstitute the lysis reagent, and ~1-3 mg sample was added to each well. For sample 19LG, fecal sample was collected directly to the OMNIgene•GUT tube (~500mg in 2ml) as per manufacturer's instructions. Yield of purified DNA was measured using the Promega QuantiFluor ssDNA System (Cat# E3190), as per manufacturer's instructions.*

PCR and Pooling

The DNA quantitated in Figure 1 was amplified using the Shoreline Complete V4 Kit (Cat# SCV4-96). The primers in the kit target conserved regions on either side of the V4 region near positions 513 and 806 in the 16S rRNA gene, and are constructed with unique dual ended barcodes and Illumina® sequence adaptors that are added during one-step PCR. PCR reactions were performed as per the Shoreline Biome kit manufacturer's instructions, briefly, 10 ul of purified DNA was added to well containing dried barcoded PCR primers and 2x PCR polymerase mix, and subjected to 35-cycle PCR. After PCR, a 5 ul aliquot of each reaction was combined to create a pool of all barcoded samples, and the amplicon pool was purified using the QIAGEN MinElute® PCR Purification Kit (cat. # 28004). Amplicon library size was confirmed using Agilent 2200 TapeStation system and the final library concentration measured by Qubit Fluorometer as per manufacturer's instructions. Because all barcoding and sequencing primers were added during PCR, no further processing was required for sequencing.

16S rRNA V4 Region Sequencing

The purified pool of barcoded samples was diluted to the proper concentration for Illumina sequencing. 16S V4 rRNA sequencing was performed using the Illumina MiSeq® (2x300 chemistry) as per manufacturer's instructions (Cat# MS-102-3003). Sequencing coverage averaged 56,000 reads per sample. Demultiplexing and taxonomic classification of 16S rRNA targeted amplicon reads was performed using the Illumina demultiplexer and the Illumina 16S Metagenomics application. The

Metagenomics application uses an Illumina-curated version of the GreenGenes taxonomic database for read classification. The algorithm is a high-performance implementation of the Ribosomal Database Project (RDP) Classifier (described in Wang Q. *et. al*). Representative sample results are reported in Figures 3 and 4 below. The top 11 genera were listed for clarity.

Results and Discussion

DNA purification using samples suspended in OMNIgene•GUT buffer was compared to the Shoreline Complete standard protocol for frozen fecal material for 19 samples. Similar microbial profiles were obtained for all donors. In Figure 3, the 11 most abundant genus classifications for three representative donors are shown.

Figure 3: Taxonomic profiles of samples prepared using Shoreline Biome Kit comparing fecal samples suspended in water or OMNIgene•GUT buffer. *Amplicons were prepared using the Shoreline Biome 16S V4 extraction and amplification kit and sequenced on Illumina MiSeq (2x300). The top 11 taxa for each sample are shown.*

Because each fecal sample in Figure 4 is from a different donor, the top 11 taxa for each sample vary by both type and amount, as expected. However, the overall profile of the identity and relative abundance of the top 11 genera is similar in each sample, an indication that the representation of the easily lysed Bacteriodetes and the difficult to lyse Gram-positive Firmicutes were not significantly affected by the OMNIgene•GUT buffer during the lysis step. Investigators using OMNIgene•GUT for convenient sample collection and storage gain added benefits of using the fecal suspension directly as input into the high-throughput Shoreline Complete kits. Direct input of the suspended sample reduces the handling steps required for each sample, limiting the chance for contamination, sample switching errors, and further decreases time to result.

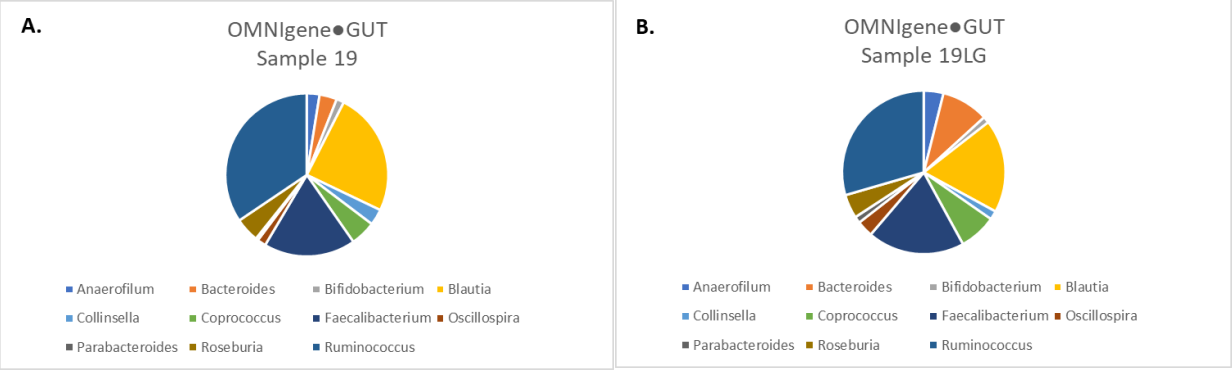

Figure 4: Comparison of taxonomic profiling with a 10-fold difference in fecal sample input. *Sample 19 used (A) ~1mg sample in 50ul of* OMNIgene•GUT *buffer or (B) was added directly to the OMNIgene•GUT tube per manufacturer's instructions and 50 ul (~10mg) was added to the lysis plate.*

Figure 4A and 4B compare taxonomic profiles of the same sample processed using ~1 mg of frozen fecal material vs ~10 mg stabilized stool from an OMNIgene•GUT collection tube. The taxonomic profile and abundance are similar regardless of the differential in input amount. The comparison shows that the lysis protocol is capable of uniform results using a combination of the OMNIgene•GUT buffer and a 3-fold larger than recommended input of fecal material. To account for variations in sample input for the OMNIgene•GUT collection system and to maintain a working range for the Shoreline Biome kits, we recommend using 10ul of OMNIgene•GUT sample/buffer mix.

Conclusions

The DNA Genotek OMNIgene•GUT collection system is compatible with the Shoreline Complete microbiome sample preparation kit allowing for convenient stabilization of fecal microbial samples followed by high-throughput lysis and amplification for microbiome NGS profiling. The direct use of 10ul of sample from the OMNIgene•GUT collection tube with 40ul of water (approximately 2mg of feces) enables direct transfer of stabilized sample into the Shoreline Complete kit without intervening steps. Profiling is consistent over more than a 4-fold range of input fecal material. As a result, it is possible to move directly from the OMNIgene•GUT stored sample to the Shoreline Complete PCR amplicon prep method for microbiome NGS analysis.