Study of the Comparative Microbiome Profile from Different Fecal Preservation Methods

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INTRODUCTION

The role of the gut microbiome in human health and disease has become a significant research focus in recent years, with researchers moving to explore the relationship between the human microbiome and diseases such as diabetes and cancer[1]. Faecal sampling, along with advances in Next Generation Sequencing (NGS) technology, provide a powerful and non-invasive tool for in-depth analysis of host-microbiome interactions. Using amplicon sequencing targeted to the 16S ribosomal RNA gene, rapid characterization of microbial communities is now possible.

To ensure high-quality results, care must be taken to reduce the introduction of bias at all steps of collection and analysis. Since faecal microbiota sequencing profiles have been shown to change significantly during ambient temperate storage, inappropriate sample handling or storage can critically impact microbiome profiling results[2]. Currently, the gold standard for stool preservation is rapid freezing of the sample to -80°C. However, this rapid freezing may not be feasible for all collection conditions, such as for samples obtained at home.

Through a comparative microbiome profile, this study assessed the quality of the standard -80°C preservation protocol against two popular commercial preservation options: Stool Nucleic Acid Collection and Preservation Tubes (Norgen Biotek), and OMNIgene GUT DNA Stabilization Kit (DNA Genotek).

MATERIALS AND METHODS

Sample collection

A single stool sample each was collected from six healthy, unrelated donors. For each donor, 200mg of sample was preserved using each of the three preservation protocols: the standard protocol of immediate and rapid freezing to -80°C, Norgen Stool Nucleic Acid Collection and Preservation Tubes, and DNA Genotek's OMNIgene GUT DNA Stabilization Kit. Norgen and DNA Genotek samples were stored at room temperature, as detailed in their respective protocols.

DNA Extraction

Stool total DNA isolation was performed using Norgen's Stool DNA Isolation Kit protocol (Cat# 27600). For each of the three treatments, DNA was extracted from 0.2 mL preserved stool or an equivalent amount from the frozen stool at five time points: Day 0, week 1, week 2, week 3, and week 4. Isolated DNA quality and quantity was assessed with gel electrophoresis and Nanodrop quantification.

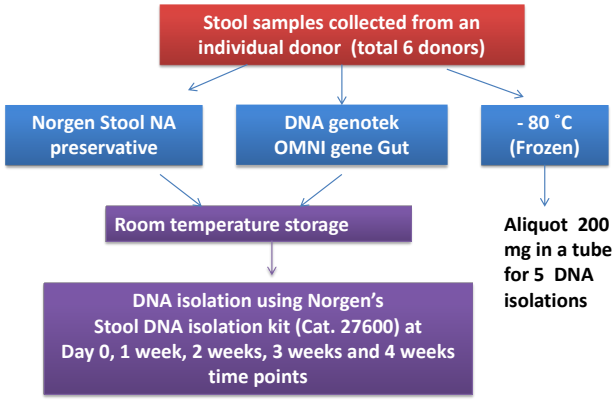

Figure 1. Outline of Sample Collection and DNA Isolation

Next-generation sequencing

Isolated DNA samples were prepared for sequencing using the 16s Metagenomic Sequencing Library Preparation protocol from Illumina. Samples were run on the Illumina MiSeq platform.

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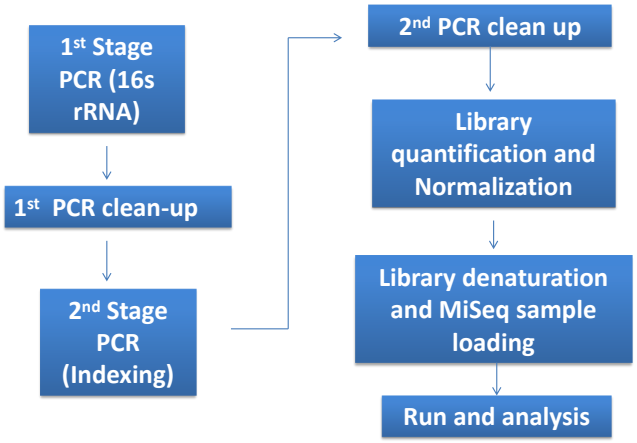

Figure 2. Outline of NGS Strategy

RESULTS AND DISCUSSION

Bioinformatic analysis of the data obtained from next-generation sequencing was contracted by Second Genome Solutions and Norgen Biotek.

Volcano plot

Results indicate that the difference between the stool preservation methods is not significant.

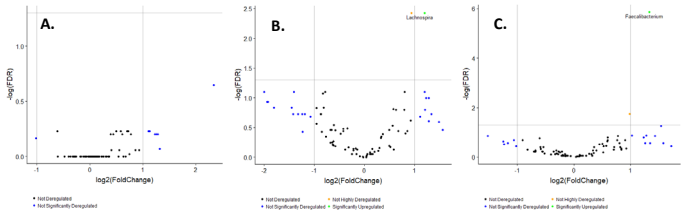

Figure 3. Volcano plot representation of differential results between the three study groups, showing fold change (log2 fold change, x-axis) and significance (-log FDR, y-axis). Features with a significant change should have more than 2-fold change (> 1 or <-1 on x-axis) and an FDR of less than 0.05 (> 1.3 on y-axis). A: Frozen vs Norgen, B: Frozen vs OMNIgene GUT, C: Norgen vs OMNIgene GUT. The dots represent genera.

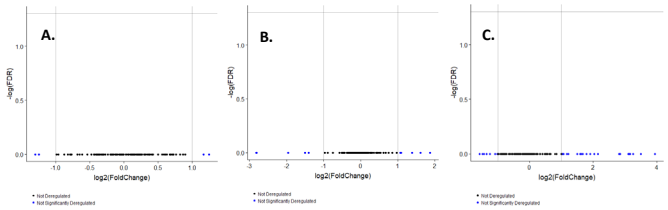

Figure 4. Volcano plot representation of differential results between two time points in each method: time 0 and one month. The plot shows fold change (log2 fold change, x-axis) and significance (-log FDR, y-axis). Features with a significant change should have more than 2-fold change (> 1 or <-1 on x-axis) and an FDR of less than 0.05 (> 1.3 on y-axis). Plot A: Frozen; Plot B: Norgen; Plot C: OMNIgene GUT.

Heatmap

Heatmap analysis was generated using Z-scores of the 50 most variable features in each of the methods used, and illustrates the similarity relationship of genera based on Bray-Curtis distance and Ward's hierarchical clustering method. Under each of the six donors, the three different storage treatments demonstrated similar heat map microorganism profiles without significant differences, indicating that all three storage conditions were able to preserve stool DNA at room temperature for up to four weeks.

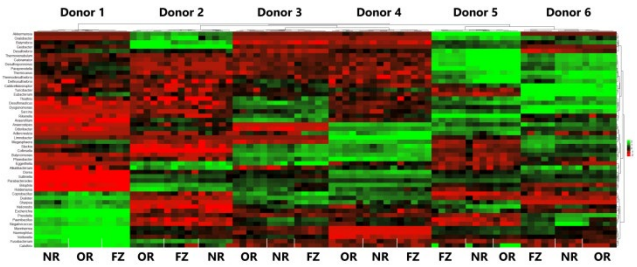

Figure 5. Heatmap illustrating the distribution of the major genera under three different storage conditions for all donors. Storage conditions are indicated by NR (Norgen), OR (OMNIgene GUT) and FZ (Frozen at -80°C). The dendrogram shows distinct clustering according to donor rather than the used method.

Alpha diversity estimates

The effect of storage conditions on faecal microbiota alpha diversity was assessed based on OTU richness (measured based on the absolute number of taxa) and diversity (Shannon H'). OTU richness and Shannon diversity did not differ significantly between storage conditions.

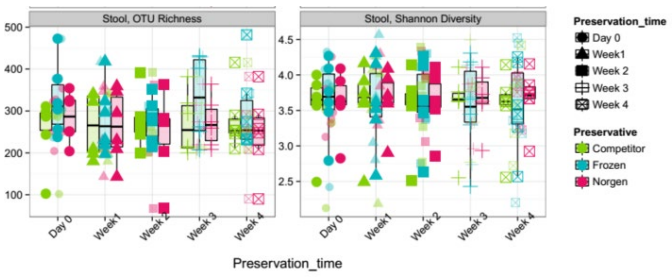

Figure 6. Left panel: OTU richness represents the number of OTUs present in each sample. Right panel: Shannon Diversity Index takes into account the richness and evenness of OTUs within a sample.

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Table 1. Mean and standard deviation (sd) values for alpha-diversity metrics.

Preservative	OTU Richness	Shannon Diversity	
OMNIgene GUT	269 (68.3)	3.55	(0.505)
Frozen	303 (83.1)	3.59	(0.566)
Norgen	263 (77.4)	3.64	(0.39)

Weighted Ordination using Abundance

No significant difference in inter-sample relationship ($p < 0.05$ has a statistical significance) between Norgen and OMNIgene.GUT.

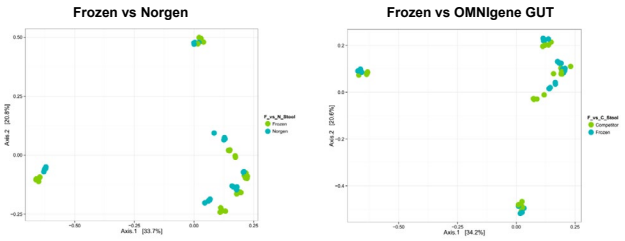

Figure 7. Dimensional reduction of the Bray-Curtis dissimilarity between microbiome samples, using the PCoA ordination method.

Table 2. Covariate significance summary table.

Variable	P value	Classes	Sample count
Frozen vs Norgen	0.073	Frozen, Norgen	30, 30
Frozen vs OMNIgene GUT	0.113	Frozen, OMNIgene GUT	30, 30

$p < 0.05$ has a statistical significance

Clustering of samples by PCoA based on Bray-Curtis similarity distance

Samples from different donors (indicated D 1 to 6) are well clustered regardless of sample storage condition (stool preservatives and frozen). More importantly, clustering was well maintained among weekly storage duration (indicated W 0 to 4). This data suggests that there was no significant changes in the 6 samples tested between stool preservation methods as well as storage time up to 4 weeks at room temperature.

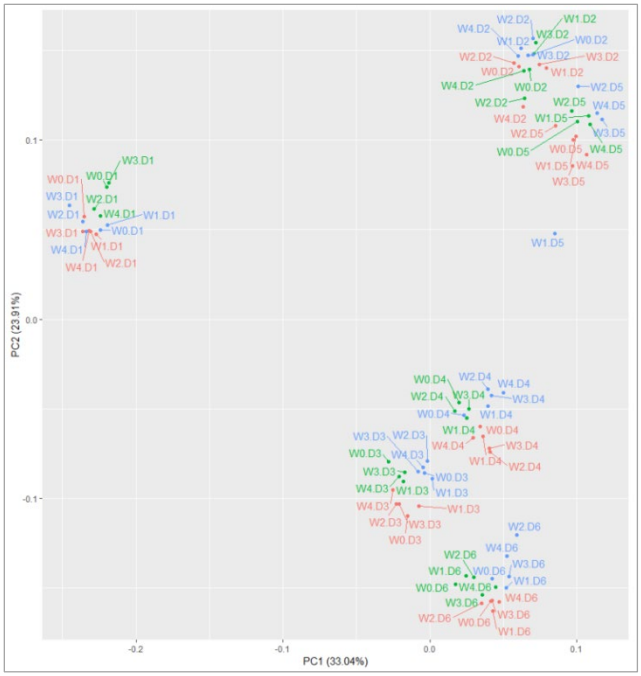

Figure 6. Principal component analysis of the most variable features in each method. Analysis of the most variable 50 features was based on TMM-normalized counts. Close clustering is shown between samples of each donors regardless of the used method or time point. Red: Norgen, Green: Norgen, Blue: OMNIgene GUT.

CONCLUSIONS

Our study reveals that the three stool storage conditions could preserve stool microorganisms profile with no significant changes. In particular we observed that Norgen's Stool Nucleic Acid Collection and Preservation Tubes (Norgen Biotek) and DNA Genotek's OMNIgene.GUT DNA Stabilization Kit performed in a similar manner to the traditional method of preserving stool DNA at room temperature.

From the data presented in this report, the following can be concluded:

1) OTU richness and Shannon diversity did not differ significantly between storage conditions.

2) No significant difference in inter-sample relationship ($p < 0.05$ has a statistical significance) between Norgen's and DNA Genotek's stool preservatives.

3) No significant differentially abundant features detected between storage conditions from volcano plot.

4) Clustering of samples by PCoA based on Bray-Curtis similarity distance showed a tight clustering between the two preservatives and frozen samples.

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REFERENCES

1. Cani, Patrice. (2018). Human gut microbiome: hopes, threats and promises. *Gut.* 67:1716-1725.

Related Products	Product #
Stool Nucleic Acid Collection and Preservation Tubes	45660
Stool Nucleic Acid Collection and Preservation System	63700
Fecal DNA Collection & Preservation Mini Tubes	27650
Fecal Swab Collection and Preservation System	45670
Stool DNA Isolation Kit	27600
Stool DNA Isolation Kit Dx	Dx27600
Stool DNA Isolation Kit (Magnetic Bead System)	55700
Stool Total RNA Purification Kit	49500
Stool Total RNA Purification Kit Dx	Dx49500
Stool Nucleic Acid Isolation Kit	45600

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