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Sample collection/stabilization and DNA/RNA extraction from swab samples for microbiome or metagenome analyses

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INTRODUCTION

Good preservation and storage are essential to preserving microorganisms' genetic material in microbial communities from wide array of sample inputs and accurately represent the bacterial composition for further analysis and applications.

The objective is to develop a proper preservation and storage medium to preserve DNA and RNA from those microorganisms.

DANAGEN-BIOTED has developed a new product to deal with this problem:

a) DANASWAB Sample Collection MICROBIOME Kit: is designed for the collection, ambient storage and transport from samples collected using a swab wherever a swab may be deemed appropriate per application.

MATERIAL AND METHODS

Sample Collection

200 mg of human stool samples were collected using DANASWAB Sample Collection MICROBIOME Kit and stored at room temperature for 1 month.

Microbial DNA/RNA isolation

Preserved stool samples were obtained at the indicated time points (Figure 1) and processed following the DANAGENE MICROBIOME SWAB DNA kit protocol for DNA isolation and the DANAGENE Microbial RNA kit for RNA isolation (Figure 2).

PCR amplification of 16S rRNA genes and targeted library preparations and sequencing

Genomic DNA amplification was conducted out in duplicate, using the 16S 1-24 Barcode Kit (SQK-16S024; Oxford Nanopore Technologies, Oxford, UK) with the following PCR conditions:
Initial denaturation at 95°C for 5 minutes, 25 cycles of 95°C for 20s, 55°C for 30s, and 65°C for 2 minutes, followed by a final extension at 65°C for 5 minutes (Kai *et al.*, 2019).
Amplifies were purified using CleanNGS (CleanNA, PH Waddinxveen, The Netherlands) and quantified by fluorometric quantification with Qubit (Thermo Fisher Scientific).
A total of 333ng of DNA was used for library preparation and sequenced in MiniON flow cells (FLO-FLG001; Oxford Nanopore Technologies) according to the manufacturer's protocol. After 24 hours of sequencing, the total number of reads for each sample ranged from 35,000 to 43,000.

RESULTS

The microbiome composition and abundance of stool samples conserved 0 and 30 days in DANASWAB Sample Collection MICROBIOME Kit designed to preserve DNA and RNA do not present significant differences. The composition of both samples is constant (Figure 1 and Table 1).
Microbial RNA isolated with the DANAGENE Microbial RNA kit presents an optimal quality. Quality was assessed using the Agilent 4150 TapeStation System (Figure 2), showing clearly RNA fragments corresponding to 23S, 16S, microRNAs, and an absence of degradation.

	Day 0	Day 30
Species	238	238
Individuals	22978	26714
Simpson-D	0.9753	0.9725
Shannon-H	4,315	4,269
Sorensen	100%	

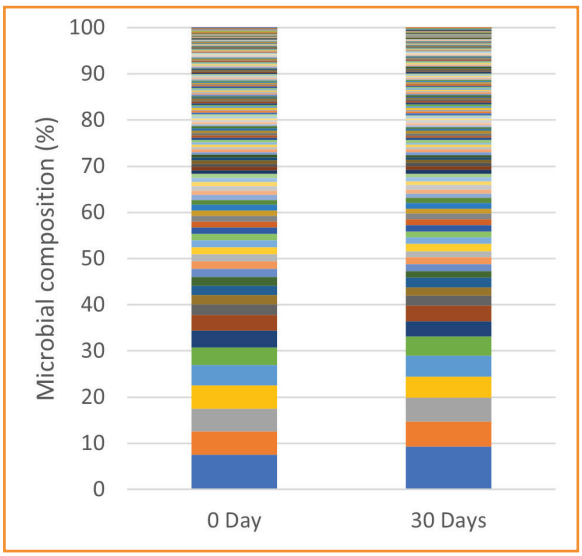

Figure 1.

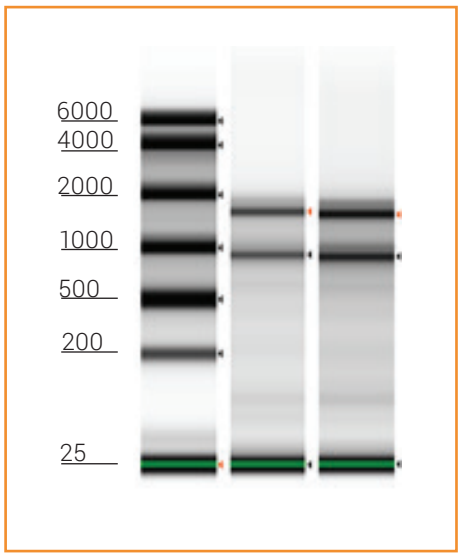

Figure 2.

CONCLUSIONS

According to the Shannon and Simpson index, it can be determined that both samples show high biodiversity, and a qualitative similarity of 100% between them.

The MICROBIOME Stabilization Solution takes a microbial snapshot and preserves RNA of a sample while inactivating pathogens making samples safe and ready for transport. Samples stored in the these microtubes are stable at ambient temperature for 1 month, and can be frozen for longer-term storage.

REFERENCE

Kai S, Matsuo Y, Nakagawa S, Kryukov K, Matsukawa S, Tanaka H, Iwai T, Imanishi T & Hirota K. Rapid bacterial identification by direct PCR amplification of 16S rRNA genes using the MinIONTM nanopore sequencer. *FEBS Open Bio* 9(3), 548–557 (2019). https://doi.org/10.1002/2211-5463.12590

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