# Validation of DANAGENE Microbiome DNA Kits for Microbiome Analysis

## Introduction

Bias in nucleic acid extraction procedures is a major contributor to inaccurate microbial profiling due to inferior cell lysis methods failing to extract DNA uniformly from diverse microbes.

There are several reports in the literature citing variations in microbial composition profiling caused by the use of different DNA extraction methods.

With identification and abundance being the most important factors in a microbiome analysis, lysis efficiency and bioburden/background contamination should be major considerations when using a DNA isolation system. Problems with these two factors can completely distort the truth.

DANAGENE Microbiome DNA kits were built specifically for microbiome analysis and were designed with these new requirements in mind. To determine if a microbial DNA extraction process is biased or not, one needs a microbial sample of defined composition.

Unbiased cellular lysis was validated using one Microbial Community Standard.

## Materials and Methods

### Microbial Community Standard

We prepared one microbial community standard with the following composition in Table 1:

### DNA Extraction from Microbial Community Standard

75 µl of Standard were used to compare different DNA extractions protocols:

a) DANAGENE Microbiome Fecal DNA Kit.
b) DANAGENE Microbiome Swab DNA Kit.
c) Supplier Z.
d) Supplier Q.

### Targeted Library Preparations, Sequencing and Bioinformatics Analysis

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, 32 cycles of 95 ° C for 30s, 53 ° C for 45s, and 65°C for 2 minutes and 15s, followed by a final extension at 65 ° C for 5 minutes.
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.

| Species | Theoretical Abun. (%) |
|---|---|
| Veillonella rogosae | 48.50 |
| Faecalibacterium prausnitzii | 22.50 |
| Eschericiha coli | 7.50 |
| Lactobacillus fermentum | 6.50 |
| Roseburia hominis | 6.50 |
| Clostridium difficile | 2.50 |
| Prevotella corporis | 2.00 |
| Bacteroides fragilis | 1.50 |
| Fusobacterium nucleatum | 1.50 |
| Salmonella enterica | 0.50 |
| Akkermansia muciniphilia | 0.50 |

Table 1

## Results

We can observe a little variation in microbial composition compared with the standard composition using our DANAGENE Microbiome Extraction kits for fecal and soil samples. Despite these kits used different chemistry and beads for mechanical lysis. (Fig.1)

In addition, we also have good results with the species in low percentages and better results than Supplier Z and Q.

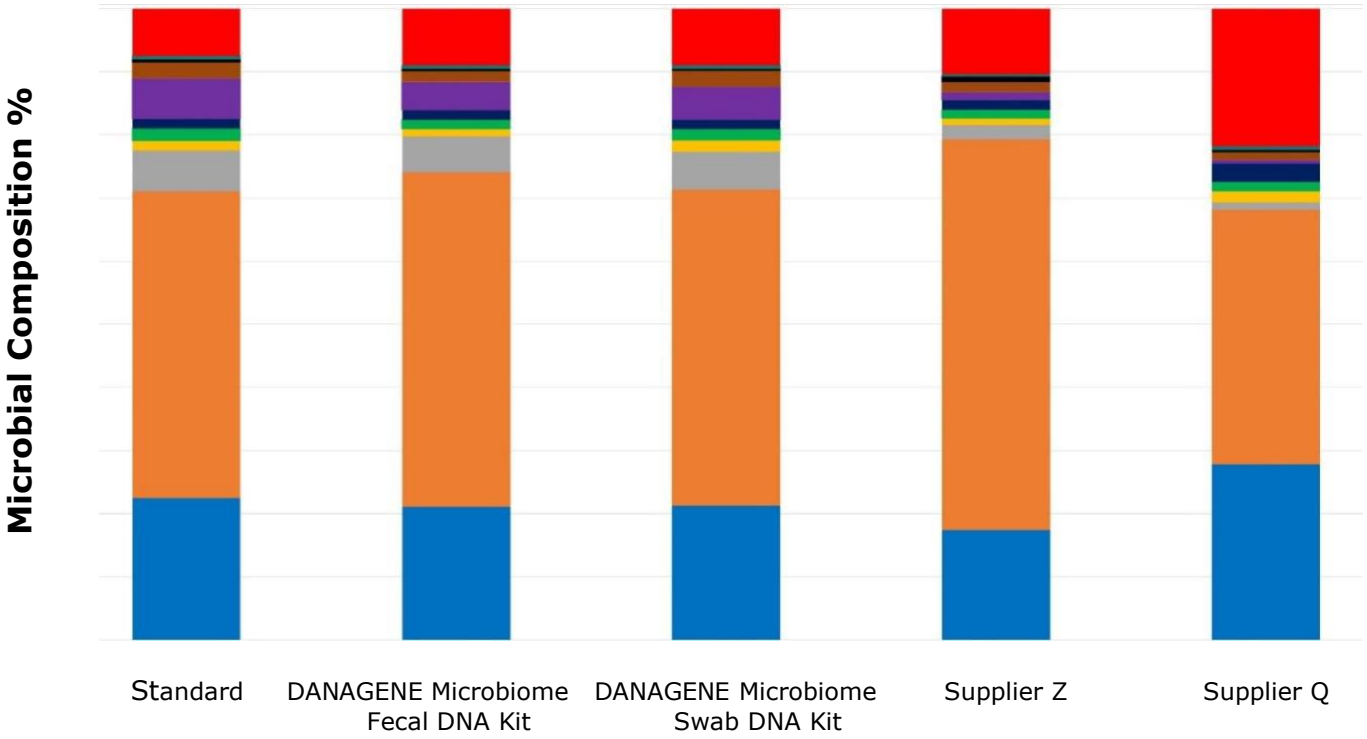


**Fig.1**

## Conclusion

The goal of this study was the validation of our DANAGENE Microbiome DNA extraction kits for microbiome analysis due to the fact that the DNA extraction can be biased because of uneven microbial cell lysis or low bioburden.

For this, we prepared one Microbial community standard for comparing different DNA extraction protocols.

In conclusion, our data demonstrates that our isolation kits for stool and soil samples can be used for an efficient DNA isolation for microbiome analysis.