Microbiomics

**APPLICATION NOTE 7** **DANAGENE Microbiome Fecal DNA Kit**

# Novel DANAGENE Microbiome Fecal DNA kit for improved results in stool microbiome and metagenomic analysis

## Introduction

Our **New DANAGENE Microbiome Fecal DNA kit** is even more effective than our original Microbiome Fecal technology and is designed to isolate high yields of pure microbial DNA from stool samples for microbiome and metagenomic analysis.

The kit features a novel **Microbial DNA Column** and optimized chemistry for a more efficient removal of PCR inhibitors (such as polysaccharides or bile) using a novel **Microbiome Lysis Buffer and Microbiome Precipitation Buffer**.

In this application note, we are going to study the DNA yield and Microbial composition (%) using the commercially available gold standard for stool samples, the **QIAamp PowerFecal Pro DNA Kit QIAGEN**, versus the **New DANAGENE Microbiome Fecal DNA Kit.**

We also are going to evaluate the use of our new technology for microbial DNA isolation of preserved stool samples in our 2 systems: DANASTOOL Sample Collection Microbiome Kit and DANASWAB Sample Collection Microbiome kit.

## Material and Methods

### Stool sample collection

We collected a stool sample from a healthy individual and we preserved it in the following way:
a) Fresh sample without preservation solution.
b) 800 mg stool sample in 8.0 ml DANASTOOL Sample Collection MICROBIOME Kit.
c) 200 mg stool sample in 2.0 ml DANASWAB Sample Collection MICROBIOME Kit.

### DNA isolation

DNA was isolated from stool samples using either the QIAamp PowerFecal Pro DNA Kit, the DANAGENE Microbiome Fecal DNA kit or the DANAGENE Microbiome DANASTOOL Kit protocol. For each protocol, three samples were processed.

Briefly, for the New DANAGENE Microbiome Fecal DNA kit, the microorganisms were efficiently lysed by a combination of heat, chemical and mechanical disruption with specialized beads. Proteins and Inhibitors were eliminated by precipitation using a new proprietary inhibitor removal buffer and subsequently pelleted by centrifugation together with the beads and undissolved sample material. The purified lysate was mixed with the Binding Buffer and then applied to a new Microbial DNA column. The DNA that is bound to the column underwent a two-wash step. After a drying step, ready-to-use DNA could be eluted with Elution Buffer (5 mM Tris/HCl, pH 8.5).

### Quality and Quantification of extracted DNA

For DNA quantification, DNA concentration was determined fluorometrically on the Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA) using the QUBIT dsDNA BR Assay Kit.

For DNA quality, DNA purity was determined via 260/280 and 260/230 ratios measured on the NanoDrop (Thermo Fisher Scientific, USA).

### 16S rRNA Gene Sequencing

Genomic DNA amplification was conducted using the 16S Barcoding Kit (SQK-RAB204; Oxford Nanopore Technologies, Oxford, UK) with the following PCR conditions:
Initial denaturation at 95°C for 1 minute, 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.
Amplifies were purified using Agencourt AMPure XP beads (Beckman Coulter) and quantified by fluorometric quantification with Qubit (Thermo Fisher Scientific).

A total of 100 ng of DNA was used for library preparation and sequenced in MiniON flow cells (FLO-FLG001; Oxford Nanopore Technologies) according to the manufacturer's protocol. Sequencing reads were analysed with the metagenomics software EPI2ME.

## Results and discussion

### Experiment 1

We present a comparison of DNA yield and microbial composition (%) from the same fresh stool sample using the commercially available gold standard QIAamp PowerFecal Pro DNA kit versus our New DANAGENE Microbiome Fecal DNA Kit.

We have obtained similar yield and A260/280 and 260/230 ratios (Table 1). The results are the average of three samples.

The microbial composition resulting from each extraction method (DANAGEN versus QIAGEN) is summarized graphically in Figure 1. Similar results can be observed in terms of microbial composition between our kit and the gold standard for stool samples.

| | 100 mg Fresh Stool Sample | |
|---|---|---|
| **KIT** | **DANAGENE Microbiome Fecal DNA Kit** | **QIAamp PowerFecal Pro DNA Kit** |
| **Yield (ng/µl) QUBIT** | 164,50 | 152 |
| **A260/280** | 1,85 | 1,84 |
| **A260/230** | 1,92 | 1,94 |

**Table 1. DNA concentrations of total DNA obtained and 260/280 and 260/230 ratios**

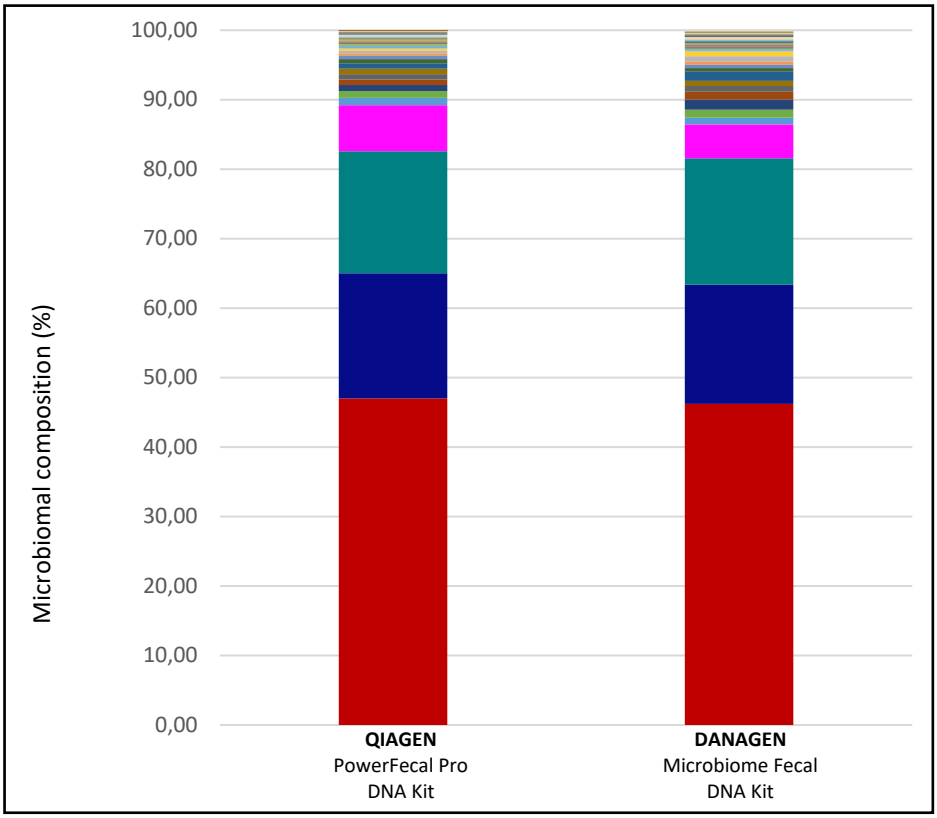


**Figure 1. Microbial composition of stool samples. Results are summarized at the genus level**
DNA was extracted from the same fresh stool sample (100 mg) Kit using the QIAamp PowerFecal Pro DNA kit versus our New DANAGENE Microbiome Fecal DNA Kit. The bacterial composition of each sample type resulting from each extraction protocol is summarized graphically at the genus level

## Experiment 2

The goal of this experiment was to evaluate whether the new kit is compatible with our fecal sample preservation systems.

We present a comparison of DNA yield and microbial composition (%) using our New DANAGENE Microbiome Fecal DNA Kit from the same stool sample preserved in different ways.

We have obtained similar yield and A260/280 and 260/230 ratios (Table 2). The results are the average of three samples.

We have obtained good results but the best ones are from the fresh stool sample because we can only process 250 µl and 400 µl from DANASTOOL and DANASWAB samples, respectively. The chemistry of both preservative solutions does not allow processing larger samples.

The microbial composition resulting from each extraction protocol is summarized graphically in Figure 2.

| Sample | Fresh Stool Sample | Preserved Stool Sample in DANASTOOL | Preserved Stool Sample in DANASWAB |
|---|---|---|---|
| **Sample Quantity** | 100 mg | 250 µl | 400 µl |
| **Yield (ng/µl) QUBIT** | 178 | 40 | 106 |
| **A260/280** | 1,87 | 1,84 | 1,85 |
| **A260/230** | 2,01 | 1,95 | 1,82 |

**Table 2. DNA concentrations of total DNA obtained and 260/280 and 260/230 ratios**

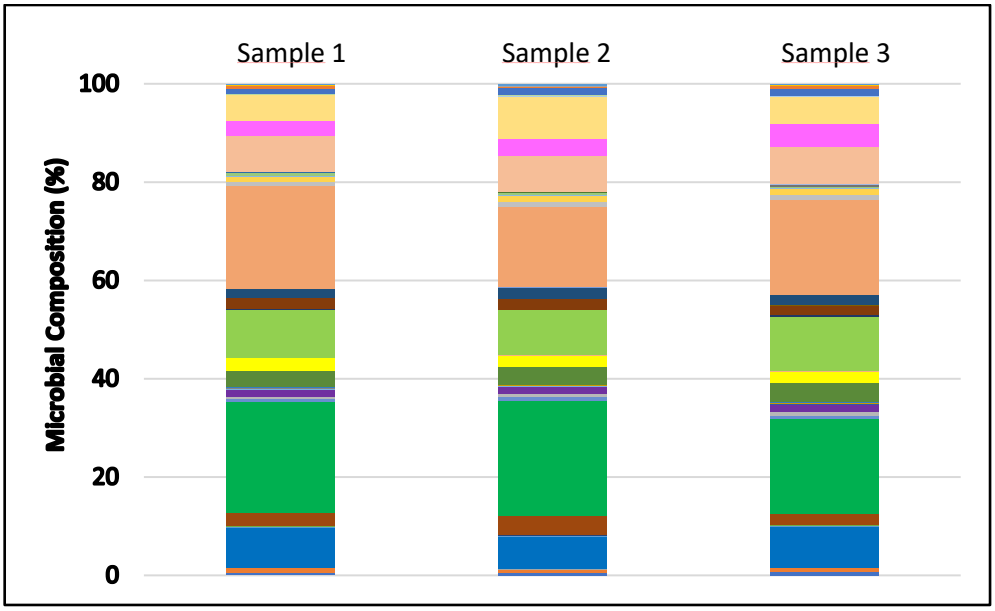


**Figure 2. Microbial composition of stool samples. Results are summarized at the genus level**
DNA was extracted from the same stool sample preserved in different conditions using the New DANAGENE Microbiome Fecal DNA kit. The bacterial composition of each sample type resulting from each extraction protocol is summarized graphically at the genus level. For the preservation of stool samples, we used our 2 solutions.
Sample 1: Fresh Stool sample. Sample 2: Preserved stool sample in DANASTOOL Sample Collection MICROBIOME Kit. Sample 3: Preserved stool sample in DANASWAB Sample Collection MICROBIOME Kit

## Experiment 3

The goal of this experiment was to evaluate the best option for microbial DNA isolation from preserved stool samples in our gold preservation system DANASTOOL Sample MICROBIOME Collection Kit.

We present a comparison of DNA yield and microbial composition (%) using our DANAGENE Microbiome DANASTOOL Kit specifically developed for this kind of samples versus of our New DANAGENE Microbiome Fecal DNA Kit and QIAamp PowerFecal Pro DNA kit.

We have obtained similar A260/280 and 260/230 ratios but total DNA yields were 2.5 times higher for the DANAGENE Microbiome DANASTOOL Kit than for alternative methods (Table 3). The results are the average of three samples.

The microbial composition resulting from each extraction protocol is summarized graphically in Figure 3. Similar results can be observed in terms of microbial composition between the three kits.

| Preserved Stool Sample in DANASTOOL Sample MICROBIOME Collection KIt | | | |
|---|---|---|---|
| **KIT** | **DANAGENE Microbiome DANASTOOL Kit** | **DANAGENE Microbiome Fecal DNA Kit** | **QIAamp PowerFecal Pro DNA Kit** |
| **Sample Quantity** | 1.0 ml | 250 µl | 250 µl |
| **Yield (ng/µl) QUBIT** | 108 | 166 | 160 |
| **Elution Volume (µl)** | 200 | 50 | 50 |
| **Total Yield (µg)** | 21,60 | 8,30 | 8,0 |
| **A260/280** | 1,90 | 1,86 | 1,87 |
| **A260/230** | 2,06 | 2,07 | 2,01 |

**Table 3. DNA concentrations of total DNA obtained and 260/280 and 260/230 ratios**

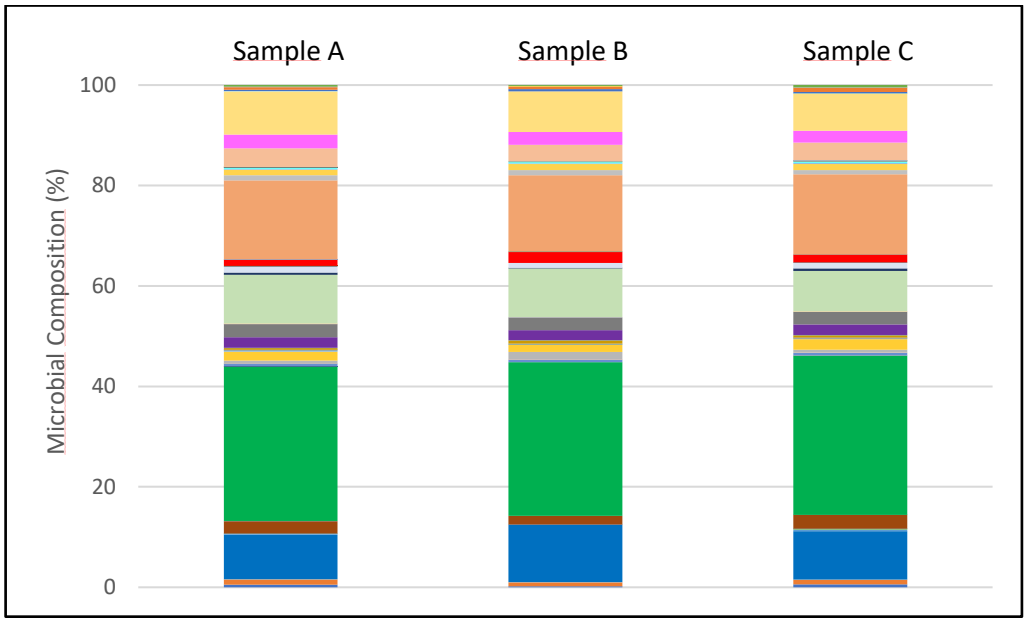


**Figure 3. Microbial composition of stool samples. Results are summarized at the genus level**
DNA was extracted from the same preserved stool sample in DANASTOOL Sample Collection MICROBIOME Kit using different DNA isolation kits. The bacterial composition of each sample type resulting from each extraction protocol is summarized graphically at the genus level.
Sample A: DANAGENE Microbiome DANASTOOL Kit. Sample B: DANAGENE Microbiome Fecal DNA kit. Sample 3: QIAamp PowerFecal Pro DNA kit.

## Conclusion

- Our data demonstrate that our new DANAGENE Microbiome Fecal DNA kit has better results than our previous version of DNA isolation kit for fresh stool samples (data not shown) and we obtained similar results in comparison with the gold standard in microbiome stool sample prep, the QIAamp PowerFecal Pro DNA kit (QIAGEN).

- The DANAGENE Microbiome Fecal DNA kit can be used for a fast and efficient microbial DNA isolation from fresh stool samples and preserved stool samples in DANASTOOL and DANASWAB sample Collection MICROBIOME Kit.

- The best option for microbial DNA isolation from fresh stool samples is the New DANAGENE Microbiome Fecal DNA kit.

- The best option for microbial DNA from preserved stool samples in DANASTOOL Sample Collection Microbiome Kit is the old DANAGENE Microbiome DANASTOOL Kit.

## Ordering information

| Reference | Product | Preps |
|---|---|---|
| 0620 | DANAGENE Microbiome FECAL DNA Kit | 50 |
| 0620.50 DANASTOOL | DANAGENE Microbiome DANASTOOL Kit | 50 |
| 0620.250 DANASTOOL | DANAGENE Microbiome DANASTOOL Kit | 250 |
| 0617 | DANASTOOL Sample Collection MICROBIOME kit | 50 |
| 0618 | DANASTOOL Sample Collection MICROBIOME kit | 250 |
| 0626.100 | DANASWAB Sample Collection MICROBIOME Kit | 100 |