Microbiomics

APPLICATION NOTE 6 **DANAGENE Microbiome Soil DNA Kit**

Novel DANAGENE Microbiome Soil DNA kit for improved results in soil microbiome analysis

Introduction

The **New DANAGENE Microbiome Soil DNA kit** has been designed for the isolation of high molecular weight genomic DNA from microorganisms such as Gram positive and Gram negative bacteria, archaea and fungi **from environmental samples of all soil types.**

The kit uses new **Microbial DNA Columns**, **Microbiome Lysis Buffer and Microbiome Precipitation Buffer** specifically designed for use with environmental samples containing high humic substances content, including difficult soil types.

In this application note, we compare DNA yield and Microbial composition (%) from two different soil types prepared using the commercially available **DNeasy PowerSoil Pro Kit QIAGEN** versus the **New DANAGENE Microbiome Soil DNA Kit.**

Material and Methods

Soil sample collection

We collected two soil samples, each one from a different origin.

DNA isolation

DNA was isolated from soil samples using either the DNeasy PowerSoil Pro Kit or the DANAGENE Microbiome Soil DNA kit protocol. For each protocol, three 250 mg samples were processed.

Briefly, for the DANAGENE Microbiome Soil 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

Microbiome Analysis

The microbial composition of each soil sample type (Soil 1 and Soil 2) resulting from each extraction method (DANAGEN versus QIAGEN) are summarized graphically in Figure 1.

Similar results can be observed in terms of microbial composition between our kit and the gold standard for soil samples.

We also obtained similar yield, A260/280 and 260/230 ratios (Table 1). The results are the average of three samples. Remarkably, we acquired close to twice the yield of QIAGEN for soil 1. Regarding the values of the A260/230 ratio, even though they are slightly low, the difference between our kit and that of the gold standard is substantial for soil 2.

	SOIL 1		SOIL 2	
PROTOCOL	**DANAGEN**	**QIAGEN**	**DANAGEN**	**QIAGEN**
YIELD (ng/µl)	23,25	13,50	49,51	50,23
A260/280	1,88	1,89	1,90	1,90
A260/230	1,78	1,82	1,75	1,25

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

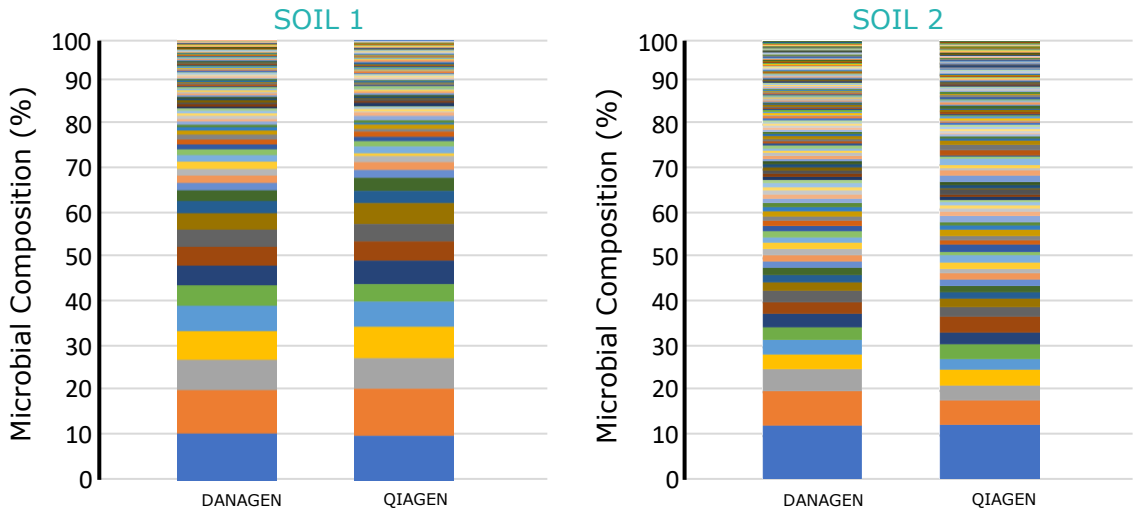

Figure 1. Microbial composition of soil samples. Results are summarized at the genus level

Conclusion

The new DANAGENE Microbiome Soil DNA kit method was compared to the gold standard in microbial soil sample prep, the DNeasy PowerSoil Pro Kit (QIAGEN).

Our data demonstrate that our new method has better results than our previous version of DNA isolation kit for soil samples (data not shown) and we obtained similar results in comparison with the gold standard in microbiome soil sample prep.

In conclusion, the DANAGENE Microbiome Soil DNA kit can be used for an efficient and fast DNA isolation for microbiome soil analysis.

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