Microbiomics

**APPLICATION NOTE 8** **DANAGENE Microbiome Soil DNA Kit**

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

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## 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.**

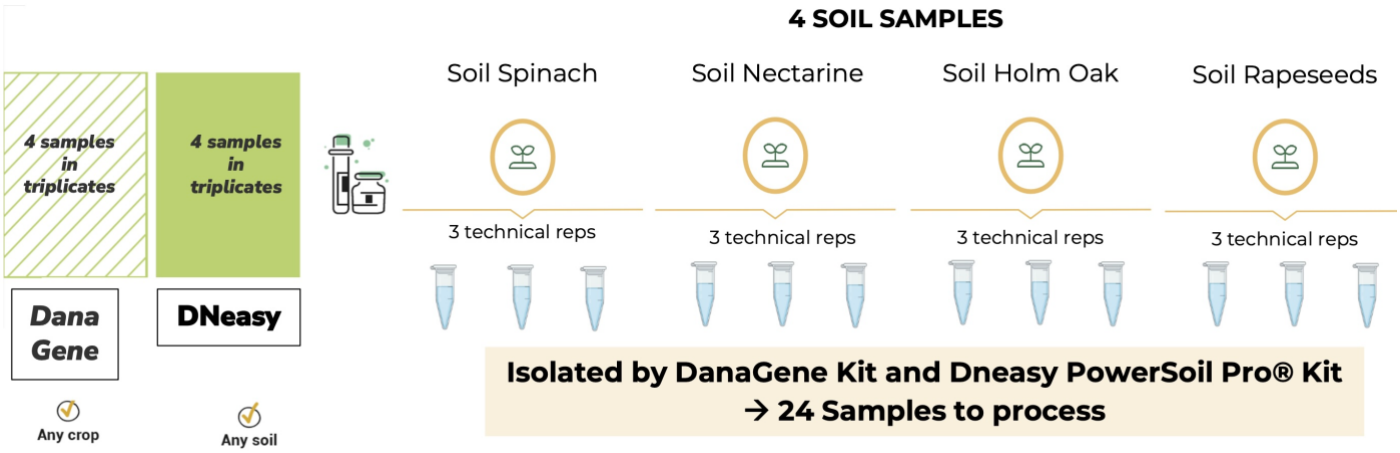
In this application note is compared alfa and beta diversity from four different soil types prepared using the commercially available **DNeasy PowerSoil Pro Kit QIAGEN** versus the **New DANAGENE Microbiome Soil DNA Kit.**

For this, we have used the BeCrop® Test technology (BIOMEMAKERS, Spain), an amplicon sequence analysis of bacteria and fungi applied to soil samples to decipher microbiome identification.

## Material and Methods

### Soil sample collection

We collected four soil samples, each one from a different origin (soil spinach, soil nectarine, soil holm oak and soil rapeseeds), three replicates each were analyzed.



### 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).

### 16S rRNA and ITS Gene Sequencing

Libraries were prepared following the Illumina guidelines using Biome Makers custom primers for amplifying the 16S rRNA V4 region and the ITS1 region described previously (Becares & Fernandez, 2017). Sequencing was conducted in an Illumina MiSeq instrument using pair-end sequencing (2×300 bp).

### BeCrop® indexes

BeCrop® indexes are patented indicators to assess the health status of soils based on metagenomics data as described by (Acedo et al., 2022). Briefly, these indicators assess relevant traits related to soil health ranging from metabolic potential to biocontrol and hormones estimations. BeCrop indexes have been included in previous soil microbiome scientific studies (Milke et al., 2024). Becrop® indexes take scores among 1 and 5.

### Limits of acceptance

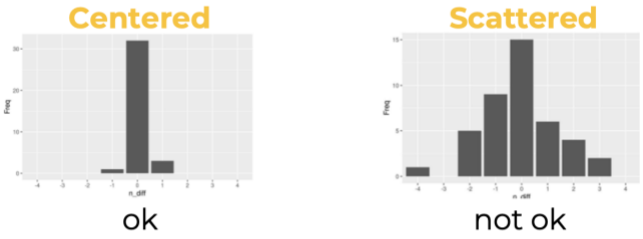
To measure the differences and similarities of the different DNA isolation kits we use:

**1.** Taxonomy correlation
Indicated by **Pearson correlation**

| Size of Correlation | Interpretation |
|---|---|
| .90 to 1.00 (−.90 to −1.00) | Very high positive (negative) correlation |
| .70 to .90 (−.70 to −.90) | High positive (negative) correlation |
| .50 to .70 (−.50 to −.70) | Moderate positive (negative) correlation |
| .30 to .50 (−.30 to −.50) | Low positive (negative) correlation |
| .00 to .30 (.00 to −.30) | negligible correlation |

Limits of acceptance:
- Bacteria Marker (16S) ≥ 0.75
- Fungi Marker (ITS) ≥ 0.68

**2.** Ranks of Reports concordance (expressed in %)




Limits of acceptance:
- Difference 0 AND +1/-1≥ 80%

## Results

### Taxonomy correlation

To measure the differences and similarities between both isolation kits, taxonomy correlation indicated by Pearson correlation was calculated. Figure 1 shows a high correlation of taxonomic abundances for 16S (0,98) and ITS (0,87) was observed between samples isolated with DanaGene kit and DNeasy kit, far above the established limits of acceptability (0,75 for 16S and 0,68 for ITS) established internally in BMK.

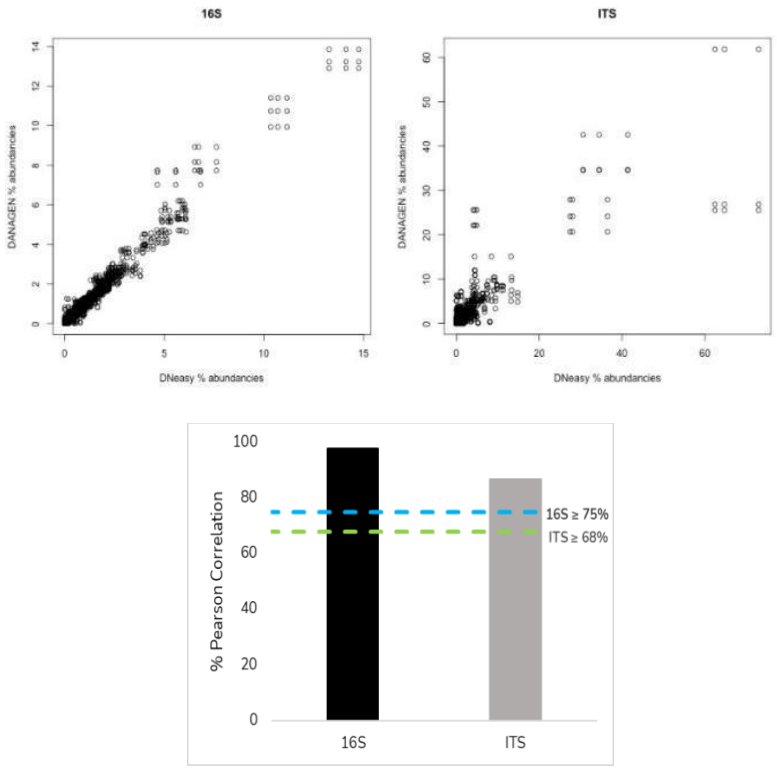



Figure 1. Comparison of taxonomic abundances for 16S (a) and ITS (b) between the DanaGene kit and DNeasy kit. c) Pearson's % correlation between DanaGene kit and DNeasy (c).

The analyses were extended to check similarity among replicates from DanaGene kit, where a high correlation of taxonomic abundances was also observed (0,99 for 16S and 0,81 for ITS) (Figure 2).

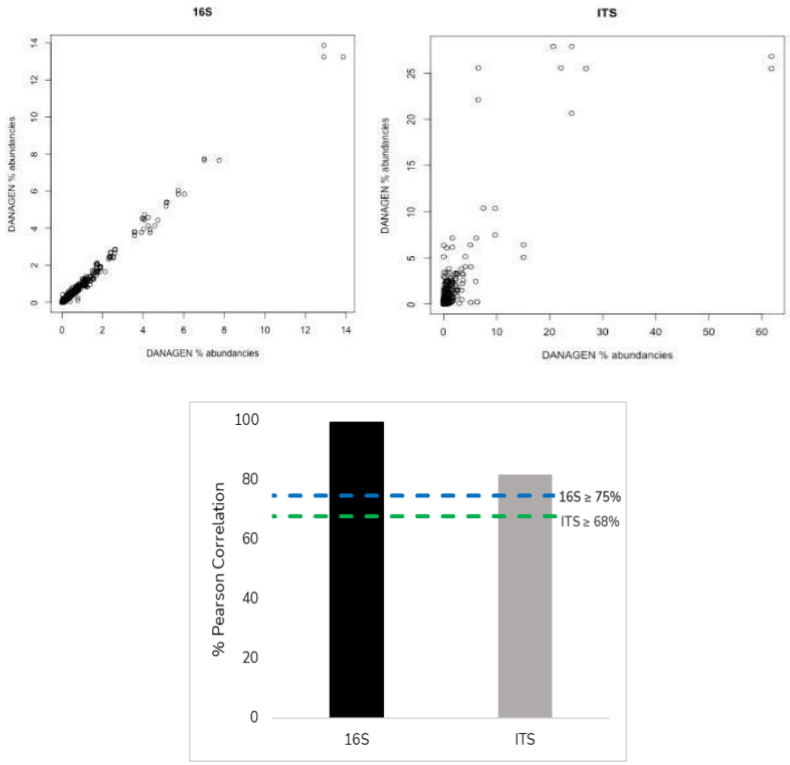



Figure 2. Comparison of taxonomic abundances for 16S (a) and ITS (b) between replicates of DanaGene kit. Pearson's % correlation between replicates of DanaGene kit (c).

## Concordance in BeCrop® indexes

After the taxonomic level comparison, this section presents the results at a higher abstract level, in terms of Becrop indices®. Figure 3 shows the difference between Becrop® score of the two isolation kits. In 86%, 95% and 99% of the cases, there were no differences or slightly low differences (+1 or -1) between the two isolation kits for the results obtained for the BeCrop® indices, classified among impacts, diseases and nutrients (panels a, b and c), respectively. A concordance greater than 80% reflects very high similarity (Figure 3.d).

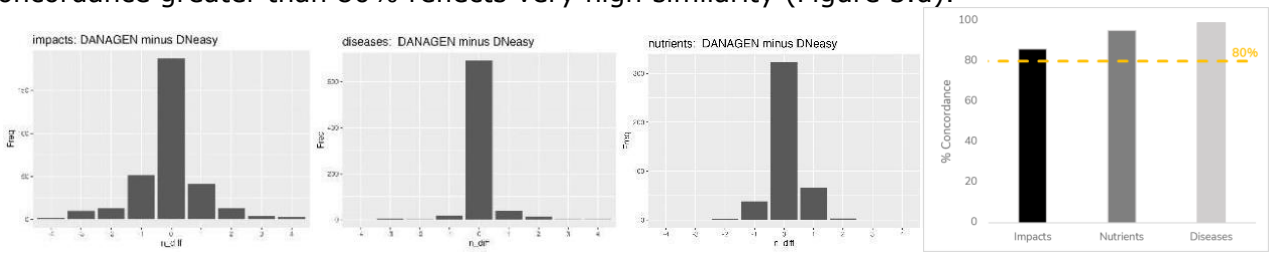



Figure 3. Difference in Becrop ranks for impact (a), disease (b) and nutrient (c) indexes between two isolation methods. Percentage of results that are equal (difference 0) or almost equal (difference -1 or +1) between the two isolation methods (d).

## 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).
The results of this study demonstrate that the new proposed DANAGENE Microbiome Soil DNA kit provides similar results in comparison with the gold standard in microbiome soil sample prep, so it can be used for an efficient and fast DNA isolation for microbiome and metagenomic soil analysis.

## References

Becares A.A. and Fernández A.F. (2017) Microbiome Based Identification, Monitoring and Enhancement of fermentation. Processes and Products.W02017096385A1

Acedo A., Ortega-Arranz H., Almonacid D., Ferrero A. Methods and systems for generating and applying agronomic indices from microbiome-derived parameters. US202/0268756A1, 2022. p. 9.

Milke F., Rodas-Gaitan H., Meissner G., Masson V., Oltmanns M., Möller M., Wohlfahrt Y., Kulig B., Acedo A., Athmann M. and Fritz J. (2024) Enrichment of putative plant growth promoting microorganisms in biodynamic compared to organic agriculture soils. *ISME Communications*, ycae021. https://doi.org/10.1093/ismeco/ycae021.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