APPLICATION NOTE #107

Norgen's Improved Soil DNA Isolation Plus Kit Produces Yields and Quality Comparable With a Top Competitor

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INTRODUCTION

The soil microbiome is a heterogeneous community of prokaryotes and eukaryotes that is a vital contributor to global ecological processes, provides important ecosystem services, and influences climate change[1].

The health of soil microbial communities can also have a more direct economic impact through industries such as farming[2] , forestry[3] and viticulture[4]. Moreover, new soil sequencing techniques such as eDNA metabarcoding can provide indicators of soil health, and are being used in environmental assessments and monitoring of remediation[5].

To investigate these scientific issues, researchers often use DNA extracted from soil samples in downstream applications such as qPCR or sequencing[6]. These downstream applications require a high quality eluate, free from contaminants and inhibitors.

To this end, we have improved our Soil DNA Isolation Plus Kit (Cat. 64000). The new improved bead beating tube design and new formulation of the lysis buffer results in improved yields, less DNA fragmentation, and less impurities in the eluate. These results are on par with the leading competitor kit for soil DNA extraction.

Methods

DNA EXTRACTION

250 mg of garden soil was used as the input for all extractions. The Norgen Soil DNA Isolation Plus Kit (Cat. 64000) was used following the protocol. The competitor Kit (Qiagen DNeasy PowerSoil Pro Kit) was performed following the protocol.

Norgen Soil DNA Isolation Plus Kit (Cat. 64000)

High Ranger Plus ladder (Cat. 12000)

NUCLEIC ACID QUANTIFICATION

Nucleic acid concentrations and quality were determined using 2 µL of eluate on the Nanodrop ND-1000. Furthermore, 10 µL of the DNA eluate was combined with 2 uL of loading dye and run on a 1.2% agarose gel at 150 V for 25 minutes. 10 uL of the High Ranger Plus ladder (Cat. 12000) was also run.

QUANTITATIVE PCR

Taqman qPCR for 16S and 18S genes was performed in 20 µL reactions with Norgen TaqMan 2X PCR Master Mix (Cat. 28340), 250 pM of primer and probe in the reaction and 1-4 uL of eluate. qPCR was performed on a QuantStudio 7 Pro machine.

Norgen TaqMan 2X PCR Master Mix (Cat. 28340)

Results

THE NORGEN KIT HAS YIELDS SIMILAR TO A LEADING COMPETITOR.

Using 250 mg of soil as input, the eluate from the Norgen kit had an average concentration of 124 ng/uL, which is a yield of 12.4 ug or 49.6 ug per gram of soil (Figure 1). Similarly, the average DNA concentration for the competitor kit was 134 ng/uL, which is a yield of 13.4 ug or 53.6 ug per gram of soil. Moreover, the quality ratios were similar with both kits giving eluate with a 260.280 ratio of 1.9, and a 260/230 ratio of 1.6 and 1.3 for Norgen and the competitor respectively.

In addition, the agarose gel image shows that the extracted DNA is intact and larger than 10,000 bp, which makes it applicable for many downstream processes including sequencing with long-read sequencing technologies (Figure 2).

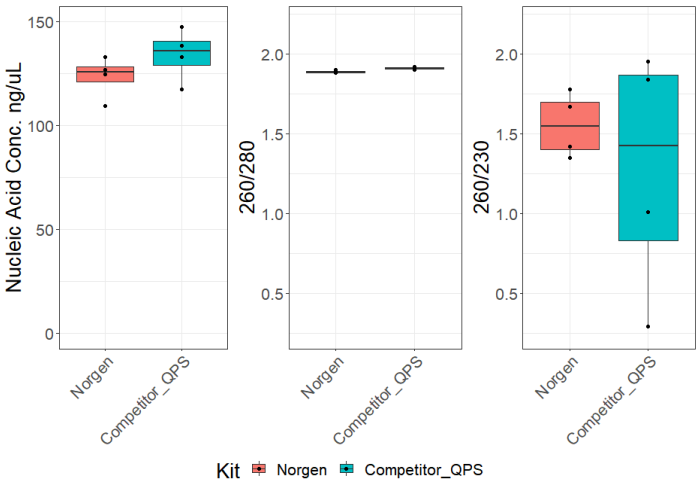

Figure 1 - Nanodrop analysis of DNA extracted from 250 mg of garden soil. Four samples were extracted using the Norgen Soil DNA Isolation Plus Kit (Cat. 64000), while the other four were extracted using a Kit from a leading competitor. The Analysis shows similar results in terms of yield and quality ratios. Norgen kit and the Competitor's kit. They also show similar yields between the automated and manual samples (Figure 2).

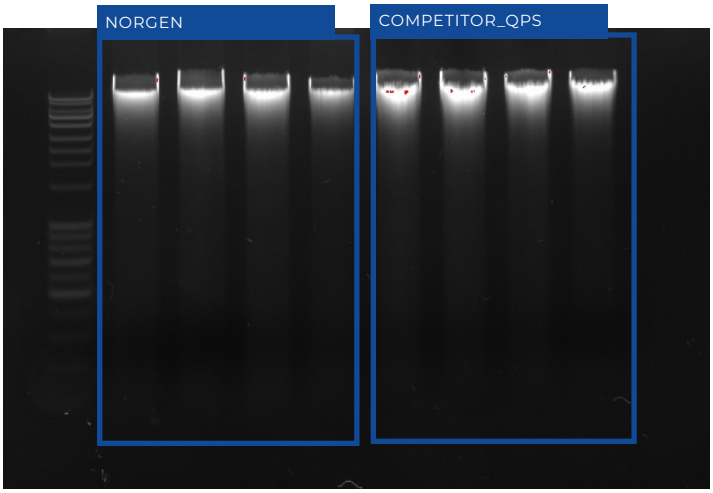

Figure 2 - An image of DNA on a 1.2 % agarose gel. 10 uL of each eluate was combined with 2 uL of loading dye and run on a 1.2 % agarose gel at 150 V for 20 minutes. 10 uL of the High Ranger Plus ladder (Cat 12000)

THE NORGEN ELUATE HAS MINIMAL PCR INHIBITORS

To evaluate the presence of PCR inhibitors in the eluate, 16S qPCR was performed with multiple volumes of eluate. The fact that the Cq decreases with every doubling of elulate input, even up to 4 uL, demonstrates that the eluate is of high quality with minimal PCR inhibitors.

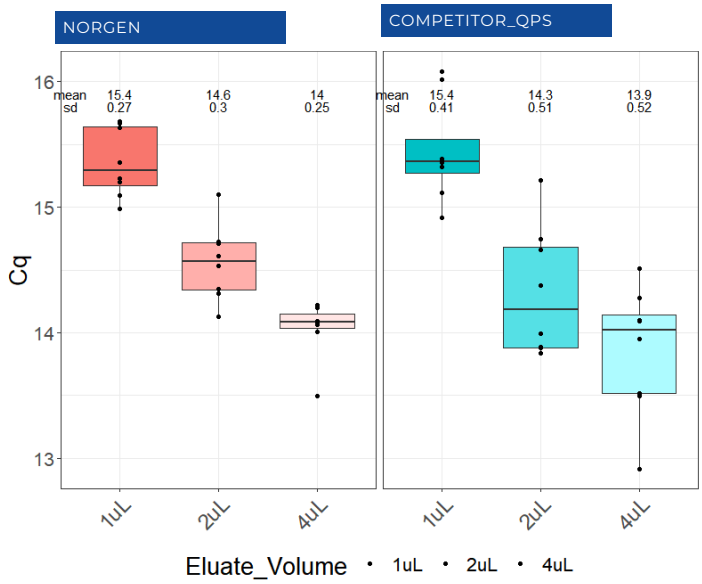

Figure 3 - 16S qPCR with increasing eluate concentrations.

BACTERIAL AND EUKARYOTIC SPECIES CAN BE QUANTIFIED IN THE ELUATE

Quantitative PCR of the eluate from both the Norgen and the competitor kits shows that both microbial (16S - Figure 4) and eukaryotic (18S - Figure 5) genes can be identified.

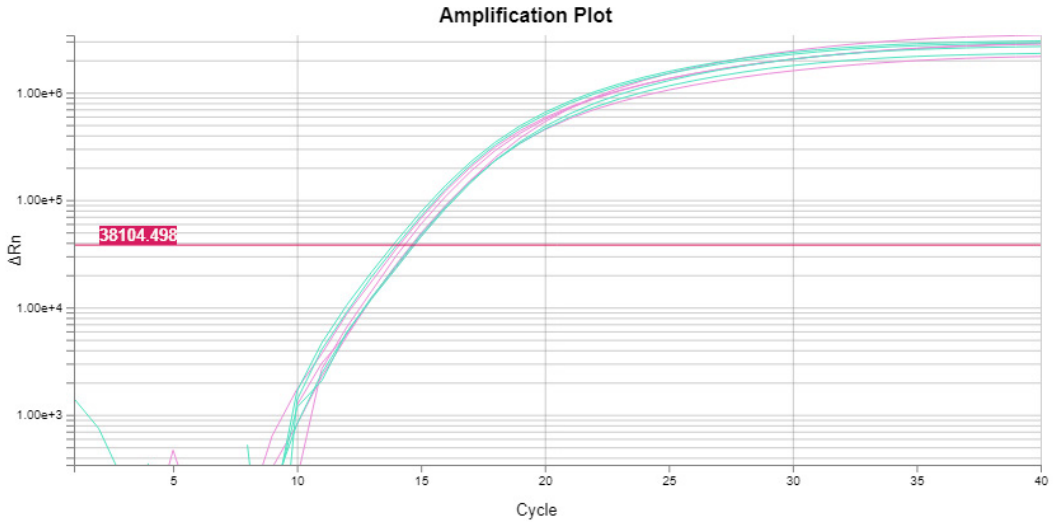

Figure 4 - 16S qPCR using 2uL of eluate from both the Norgen Kit (pink) and the Competitor Kit (turquoise)

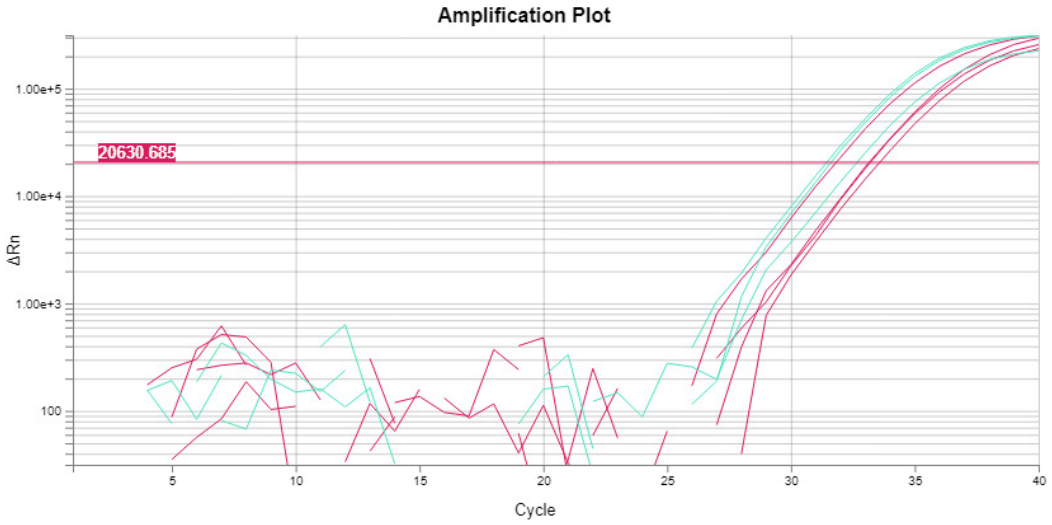

Figure 5 - 18S qPCR using 2uL of eluate from both the Norgen Kit (pink) and the Competitor Kit (turquoise)

Conclusions and Summary

In conclusion, the improved Norgen Soil DNA Isolation Plus Kit (Cat. 64000) contains a new bead beating tube design and a new formulation of lysis buffer that minimizes DNA shearing, and increases DNA yield and eluate quality.

Nanodrop, gel and qPCR analyses confirm that the Norgen kit performs similarly to a leading competitor's kit. qPCR analysis of increasing eluate volumes confirms that there are negligible PCR inhibitors in the eluate, and amplification of 16S and 18S genes indicates that this kit can be used to extract microbial and eukaryotic DNA from soil.

A review of the literature also indicates that Norgen's Soil DNA Isolation Plus Kit can be used for a variety of similar sample types, such as wastewater samples 7 , filters 8, and lake sediment 9 , and can be used to detect a variety of organisms, ranging from fish 9 to nematode 10 . This soil DNA is also applicable for applications such as metagenomic library preparation and sequencing, and has been used in metabarcoding studies 11 . This kit improvement should pave the way for even more exciting research opportunities.

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RELATED PRODUCTS

Description	Prep Size	Cat. #
Soil DNA Isolation 96-Well Kit	2 x 96	26560
Soil DNA Isolation 96-Well Kit (Magnetic Bead System)	2 x 96	62800
Soil DNA Isolation Kit (Magnetic Bead System)	50 preps	58100
Soil Total RNA Purification Kit	50 preps	27750
Soil DNA Isolation Plus Kit	50 preps	64000
Soil DNA Isolation Maxi Kit	10 preps	62000
16S V3-V4 Library Preparation Kit for Illumina	96 preps set	70410
ITS1 Library Preparation Kit for Illumina	96 preps set	71010

Norgen also offers full NGS sequencing services.

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