Norgen's Soil DNA Isolation Kit Outperforms a Competitor with Effective Humic Acid Removal for Metagenomic Studies

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INTRODUCTION

Metagenomics is the study of the variation of species in a complex microbial sample. By using next generation sequencing it is possible to investigate microbial communities by sequencing the marker gene of choice (e.g. 16S) or sample fragments of the whole genome or transcriptome without the need for culturing. Therefore, it is expected that the outcome of these studies may significantly improve soil and crop quality and quantity in agricultural industries. For successful metagenomic studies, the quality of soil DNA is one of the most critical factors. Current soil DNA isolation methods often result in poor DNA quality due to the co-purification of humic acids, which are known to be PCR inhibitors. Labour intensive and time consuming purification steps, including treatment with hazardous chemicals, are often required in order to improve the quality of the isolated DNA.

Norgen's Soil DNA Isolation Kit provides two special features to remove humic acids efficiently from challenging soil samples such as compost and top soil: a specially formulated Organic Substance Removal (OSR) Solution and Humic Acid Removal (HAR) columns. These two components work together synergistically to remove most of the organic substances and humic acids from soil samples. The quality of soil DNA purified using Norgen's kit was compared with a competitor to validate the kit performance. The results indicated that Norgen's soil DNA kit outperformed the competitor, and that the humic acid free DNA is suitable for any type of soil metagenomic studies.

MATERIALS AND METHODS

Soil DNA Extraction

DNA was extracted from 250 mg of clay, top soil and compost using Norgen's Soil DNA Isolation Kit (Cat# 26500), and a competitor's kit (PowerSoil® DNA Isolation Kit), as per the manufacturer's instructions.

Spectrophotometry and Gel Electrophoresis

Soil DNA quantity and quality was measured using the Nanodrop 2000 Spectrophotometer (Thermo Scientific). Ten microliters of each sample was also run on a 1X TAE 1.2% agarose gel for analysis of the DNA integrity.

Real-Time PCR

The purified DNA was then used as the template in a real-time PCR reaction. Briefly, 2 µL or 4 µL of isolated DNA was added to a total of 20 µL of a real-time PCR reaction mixture containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007) spiked with SYBR® Green dye, 5 mM 16S universal primer pair, and nuclease-free water. All PCR samples were amplified under the real-time program; 95°C for 3 minutes for an initial denaturation, 35 cycles of 95°C for 15 seconds for denaturation, 60°C for 30 seconds for annealing and extension. The reaction was run on an CFX96 Real-time system and C1000 touch thermal cycler (Bio-Rad).

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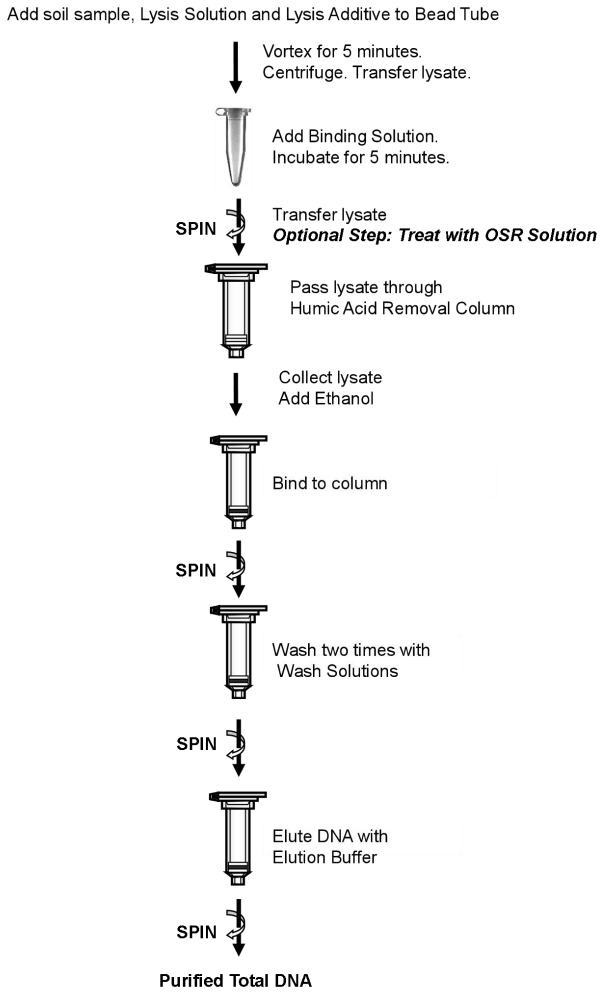

Figure 1. Flowchart for the Purification of Soil DNA using Norgen's Soil DNA Isolation Kit.

RESULTS AND DISCUSSION

Humic Acids Removal

Since humic acids are often covalently bound to the DNA, they will co-elute with the DNA if they are not effectively removed prior to the column binding step. Figure 2 indicates the effective humic acid removal with Norgen's Soil DNA Isolation Kit using the provided Organic Substance Removal (OSR) Solution and Humic Acid Removal (HAR) columns, while the competitor (MO) still showed visible amounts of humic acids before the binding step.

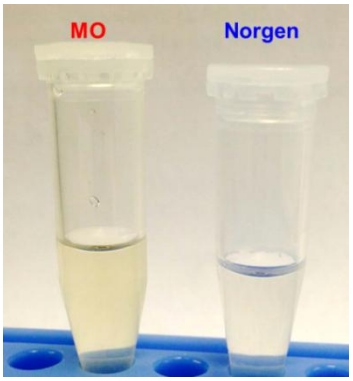

Figure 2. Superior Removal of Humic Acid versus Competitor Kit (MO). Samples of clay (250 mg) were processed using Norgen's Soil DNA Isolation Kit and a competitor's kit (MO). As it can be seen, Norgen's kit successfully removed more humic acid than the competitor, as evidenced by the removal of the brown colour in Norgen's lysed sample. Humic acids in soil are known PCR inhibitors and inhibit a number of downstream applications.

DNA Quantity and Quality

DNA quantity and quality were assessed using both gel electrophoresis **(Figure 3)** and spectrophotometry **(Figure 4)**.

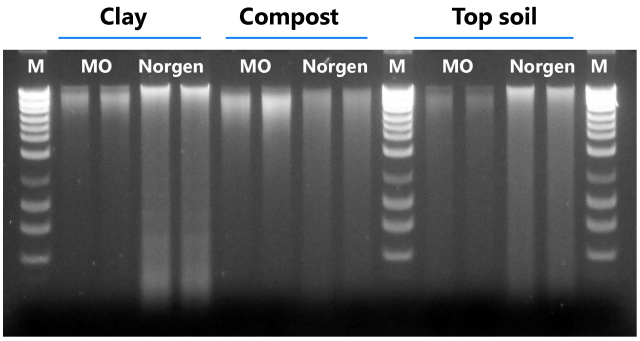

Figure 3. High Yields of Genomic DNA. DNA was isolated from 250 mg of clay, compost and top soil using Norgen's kit and a competitor's kit (MO). Following isolation, 10 µL from each 100 µL elution was loaded on 1% TAE agarose gel. Lane M: Norgen's HighRanger 1kb DNA Ladder.

As can be seen in Figure 3, Norgen's Soil DNA Isolation Kit resulted in higher yields of DNA from two of the soil samples tested (clay and top soil).

Spectrophotometry provides data for evaluating the purity of a DNA sample. The absorbance ratio A260/A280 provides information on the amount of contaminating proteins and organic compounds that are present in a sample. Samples with an A260/A280 outside of the ideal range of 1.8-2.0 have a significant amount of protein contamination, and may have issues with PCR amplification. In Figure 4 the superior quality of Norgen's soil DNA can be seen, as evidenced by the higher A260/280 ratios when

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compared to the competitor.

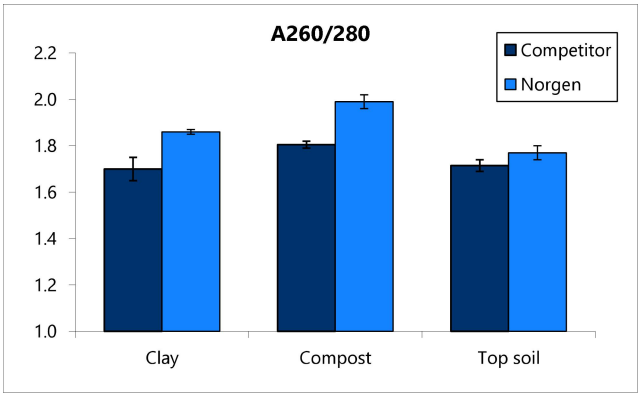

Figure 4. High Purity of DNA Samples Isolated from Clay, Compost and Top Soil. DNA was isolated from 250 mg samples of clay, compost and top soil using Norgen's Soil DNA Isolation Kit and a competitor's kit (MO). DNA purity was determined using NanoDrop for the DNA isolated using Norgen's kit (260/280 = Blue) and the competitor's kit (MO) (260/280 = Red). For all soil types, Norgen's DNA was found to have a higher A260/A280 ratio, indicating the high quality of the purified DNA.

Table 1. Spectrophotometer Reading of DNA from Compost Samples

Kit	260/280	Ave	260/230	Ave	Con (ng/ul)	Ave
Competitor MO	1.79	1.805	0.97	1.205	13.2	16.3
	1.82		1.44		19.4	
Norgen	1.96	1.99	0.26	0.145	12.8	10.45
	2.02		0.03		8.1	

Quality of Soil DNA for a Metagenomic Study

PCR is one of the powerful tools used for metagenomic studies to understand the soil microorganism community. In order to obtain reliable results, the DNA sample has to be of the highest quality in order to deliver detailed information from the soil sample.

For PCR analysis, 16S rDNA was amplified according to the PCR conditions described in Materials and Methods. A similar Ct was detected between Norgen and competitor (MO) DNA samples isolated from clay and top soil (data not shown). However, a difference was observed with the

PCR result from the compost DNA. Despite the fact that the competitor isolated more DNA than Norgen (Figure 3 and Table 1), the detection of 16S rDNA was somehow inhibited by delaying about 2 Ct when compared with Norgen (Figure 5A). Furthermore, the melting curve analysis indicated that the amplification did not match with the expected Tm (single peak at 85°C), which could be explained by unspecific PCR products generated during the PCR processing (Figure 5B). In other words, the competitor failed to detect 16S rDNA from the compost sample even with the fairly acceptable DNA quality based on spectrophotometer reading (Table 1). Residual humic acid or unremoved salt in the competitor's DNA sample might be a possible explanation for these results.

Another intriguing observation was that the low 260/230 from Norgen's compost DNA (Table 1) seemed to have no effect on the PCR performance. Even higher template volumes (from 2 µL to 4 µL) did not show any sign of PCR interference and the amplicon was successfully matched with the correct Tm in the melting curve analysis (data not shown). While spectrophotometer reading (yield, 260/280 and 260/230) is still widely accepted as a bench mark to assess the DNA quality, this study showed that real-time PCR is the ultimate tool to validate the quality of soil DNA that can be used for metagenomics and other downstream applications. Therefore, Norgen's Soil DNA Isolation Kit successfully outperformed a competitor's kit to demonstrate the highest DNA quality for challenging soil samples.

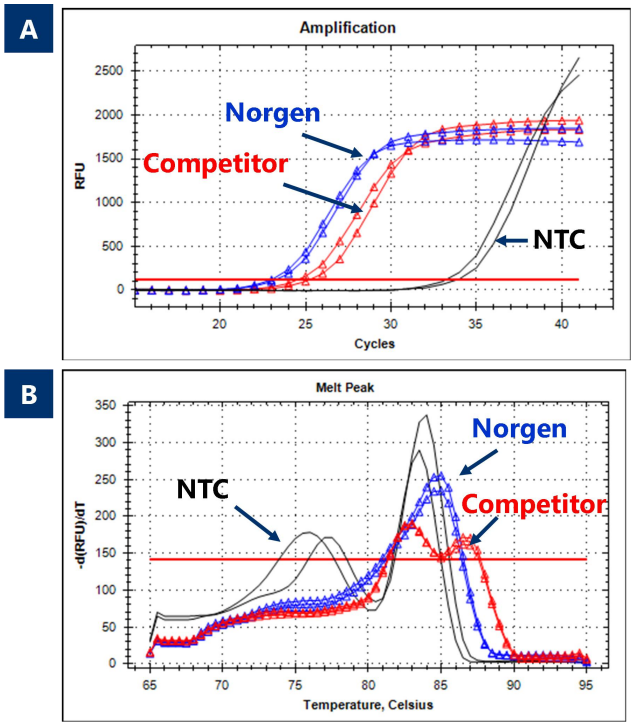

Figure 5. High Quality DNA Free From PCR Inhibitors - Superior Quality Versus a Competitor Kit (MO). Total DNA was isolated from samples of compost using Norgen's kit and a competitors kit (MO). Four µL was then used as the template in 20 µL PCR reactions using universal prokaryotic 16s rDNA primers in real-time PCR (SYBR Green). Norgen's DNA was successfully amplified, indicating the high quality of the inhibitor-free DNA (A), whereas competitor-isolated DNA showed unspecific PCR amplification as indicated in the melting curve analysis (B). Note: The recombinant Taq polymerase used showed detection of *E.coli* DNA as a background from No template control (NTC).

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CONCLUSIONS

From the data presented in this report, the following can be concluded:

1. Norgen's Soil DNA Isolation Kit can remove humic acids effectively from various soil samples containing different levels of humic acid using the OSR (Organic Substance Removal) Solution and HAR (Humic Acid Removal) column system.
2. The high quality DNA isolated using Norgen's Soil DNA Isolation Kit can be used directly for PCR and sequencing as a part of metagenomic studies.
3. Results suggest that Real-time PCR is the most reliable method to validate the quality of soil DNA.
4. Relatively low A260/230 from Norgen Soil DNA Isolation Kit did not effect the PCR performance.
5. Norgen's Soil DNA Isolation Kit outperforms a competitor (MO) to detect 16S rDNA from microorganisms in challenging compost soil samples.

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