APPLICATION NOTE

Evaluation of Humic Acid Removal from DNA Purified via Omni International's Soil DNA Purification Kit.

Shari Garrett, Omni International, Inc.

Introduction

Soil is one of the most biodiverse ecosystems on Earth, comprising about 10^9-10^{10} microbial cells per gram of material (1). Each gram of soil contains greater than 100,000 different species of bacteria, archaea, protists and fungi (1). This diverse community plays a huge role in regulating soil productivity by nitrogen fixation, nutrient cycling and disease suppression (2). Methods such as plating or observation by microscopy can only provide information of a small minority of microbes present in soil (3). However, by using metagenomic studies, scientists can better explore the interactions between plants and microbes using PCR directed sequencing to analyze microbial diversity (4). A critical step in genome sequencing is extracting microbial DNA from the soil via methods such as spin-column based technology. However, soils typically contain compounds like humic acids that bind to DNA and inhibit enzymes used in PCR (5). Omni's Soil DNA Purification kit contains a specially formulated reagent (HTR) that is designed to remove inhibitory substances from purified DNA products.

Herein, we evaluate the Omni Soil Purification Mini kit's ability to effectively remove humic acids from purified microbial DNA. PCR inhibition was analyzed via both end-point and qPCR methods.

Materials and Methods

- **Omni Bead Ruptor 24 Elite (Cat#19-040E)**
- **Bead Ruptor 2 ml Tube Carriage Kit (Cat#19-010-310)**
- **Omni Soil DNA Purification Kit (Cat #26-013G)**
- **Humic Acid (TeraVite SP-90 100% Soluble Powder)**

Omni Soil DNA Purification Kit
Cat#26-013G

Bead Ruptor Elite
Cat#19-040E

Sample Preparation and Separation

Soil samples were taken from top soil located in Kennesaw, GA. Two 250 mg 20% humic acid (HA)/ soil mixtures were prepared and added to a prefilled 2 ml garnet bead tube (Cat. # 19-624). In addition, 1 soil sample was prepared without HA and all samples were processed in 750 µl of lysis buffer on the Bead Ruptor 24 Elite at 5 m/s for 45 seconds. After homogenization, the remainder of the Omni Soil DNA Extraction kit protocol was followed but with the following changes; HTR was added to only one duplicate of soil with HA and soil without HA samples. Each sample was subsequently eluted in 100 µl elution buffer. After elution, 1 µl of each sample was analyzed on the NanoDrop Spectrophotometer at 280 nm. 200 ng of each sample was separated by gel electrophoresis by mixing with 6 x TBE/Urea Buffer (Bio-Rad #161-0768). Each sample was separated on a 1% agarose gel at 140V and stained with ethidium bromide. The gel was washed with DD H20 and visualized on a GelDoc EZ System.

PCR Assay and Fragment Analysis

To each elutant, 95 ng of pGLO plasmid DNA was added and subjected to end point PCR. The following primers were used to amplify a 714 bp fragment from the pGLO plasmid (6). Primers were synthesized by Integrated DNA Technologies.

Primer	Sequence
GFP-F	5'-ATGGCTAGCAAAGGAGAAGAA - 3'
GFP-R	5' - GTAGAGCTCATCCATGCCATGTG - 3'

Table 1

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Each reaction was established by adding 1x reaction buffer; 10 mM Tris-HCL, 50 mM KCL, 1.5 mM MgCL2, 0.2 dNTPs, 5% Glycerol, 25 U Taq (New England Biolabs #M0496S)), 0.5 µM of each primer and 1 ng of each pGLO/soil DNA sample. Amplification was carried out using the T100 Thermal Cycler by Bio-Rad per the settings in table 2.

	Temperature	Time
Hot Start/ Denaturation	95°C	2 min
30 Cycles	95°C	30 s
	59°C	30 s
	72°C	60 s
Final Extension	72°C	5 min

Table 2

PCR products were analyzed on a 2% agarose gel and stained using ethidium bromide. Fragments were visualized and analyzed via Bio-Rad's Gel Doc EZ System

After end-point PCR, DNA samples were sent to ARQ Genetics for qPCR analysis using the same primer sequences from the end point PCR.

Results

In this study, we investigated Omni's Soil DNA Purification kit's ability to effectively remove humic acids from purified DNA without interfering with DNA yield or quality. Obtaining PCR-quality DNA from soil is quite challenging due to the presence of PCR inhibitory compounds such as humic acids. Humic acids interfere with genomic analysis by binding to template DNA, disturbing the DNA polymerase or chelating with magnesium ions used in PCR reactions (3). In this assay, soil samples were inoculated with humic acids and genomic DNA was extracted via the Omni Soil Purification Mini kit. Each DNA sample was spiked with pGLO plasmid DNA and the GFP gene was detected via end-point PCR and quantitative PCR. The extent of inhibition was compared amongst all samples.

Genomic DNA Quantification and Visualization

The isolated soil DNA was quantified by spectrophotometry. Soil samples without the use of HTR have relatively high DNA yields and high absorbance values at 340 nm. Because humic acids absorb optimally at 320 nm, the elevated 340 reading demonstrates that humic acids have been copurified with DNA during the extraction process(table 3).

Sample	Average DNA Yield (ng/µl)	Average 340 Raw
Soil + HA - HTR	4678.75	135
Soil + HA + HTR	12.4	0.444
Soil - HA - HTR	9.65	0.159

Table 3

Lastly, the isolated DNA was analyzed via agarose gel electrophoresis. The gel image shows that despite the elevated DNA yields the samples not treated with HTR are significantly lower in abundance (Fig 1).

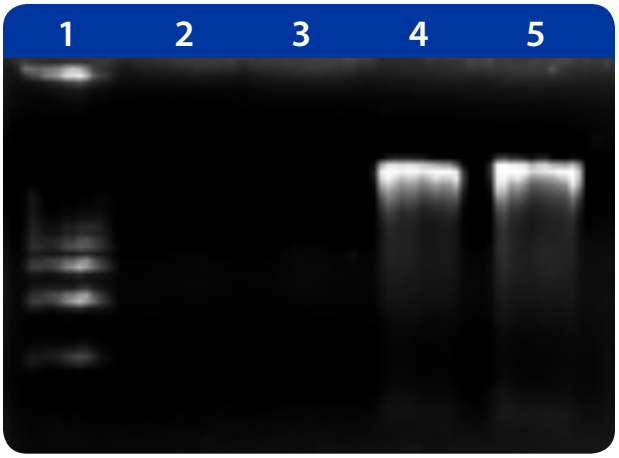

Figure 1. Purified Genomic DNA from Soil
Lane 1: Lambda Ladder
Lane 2: Empty
Lane 3: Soil +HA + HTR
Lane 4: Soil +HA -HTR
Lane 5: Soil -HA -HTR

End-Point PCR Detection

To qualitatively assess PCR inhibition, each genomic DNA sample was inoculated with pGLO plasmid DNA. Each sample was then subjected to end-point PCR to amplify the GFP gene from the pGLO plasmid. 10 µl of each amplicon was analyzed on a 2% agarose gel. The size of the target fragment is 714 bp. Severe PCR inhibition was observed in the humic acid inoculated sample that was not treated with HTR. In addition, slight inhibition was also shown in the soil samples that were not inoculated with humic acid (Fig 2). Because this sample was not treated with HTR, residual humic acids in the original soil sample were carried over, causing PCR inhibition.

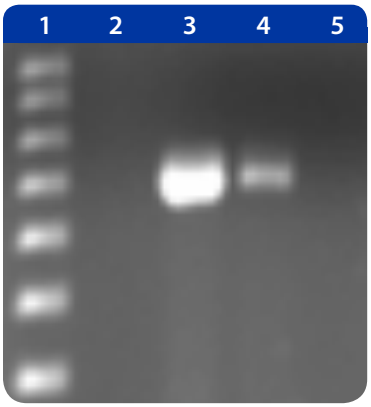

Figure 2: GFP Gene Detection
Lane 1: 100 bp ladder
Lane 2: Empty
Lane 3: Soil +HA + HTR
Lane 4: Soil -HA – HTR
Lane 5: Soil +HA -HTR

qPCR Detection

To quantitatively assess the extent of PCR inhibition each of the inoculated soil samples were amplified via qPCR. The qPCR results show that when HTR is used the Ct values are much lower indicating that there are high amounts of target DNA (Fig 3). However, as shown in the amplification curves, samples not treated with HTR indicate inconsistent Ct values between dilutions as well as high levels of contamination (Fig 4-6). Also, without the use of the HTR reagent, PCR efficiencies are reduced to less than 100%.

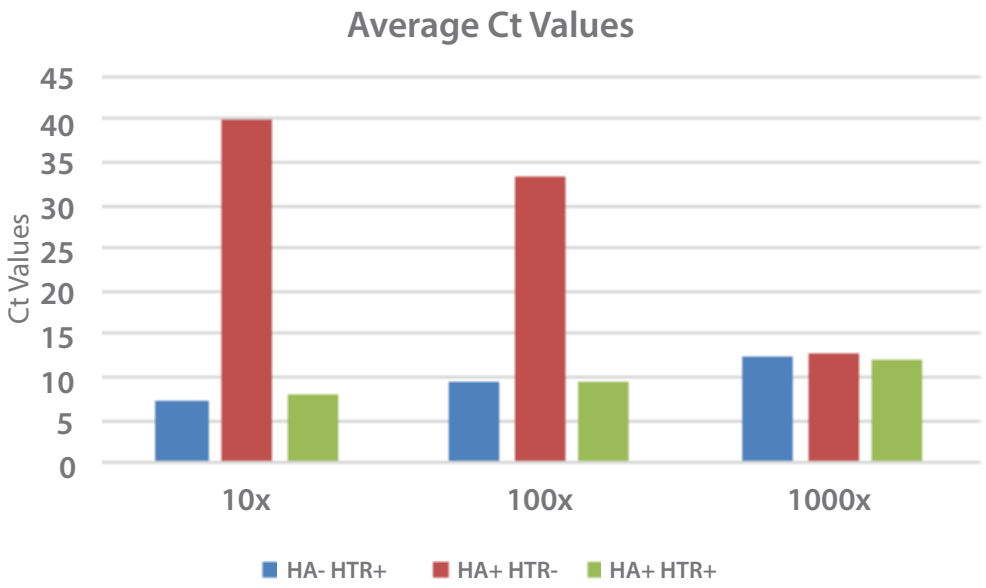

Fig 3. Average Ct Values of the Amplification of GFP Gene from Purified DNA Via Soil DNA Purification Kit.

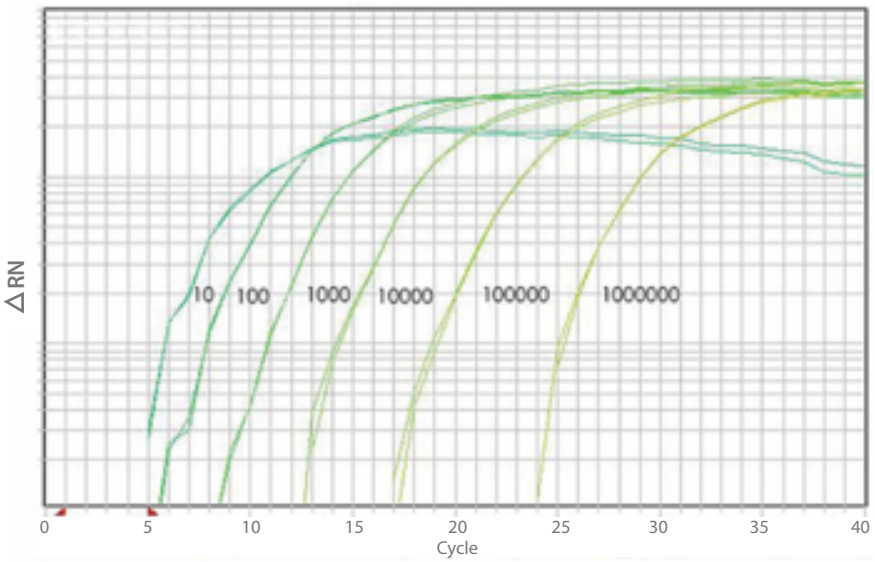

Fig 4: Soil HA+ HTR+ Amplification Plot

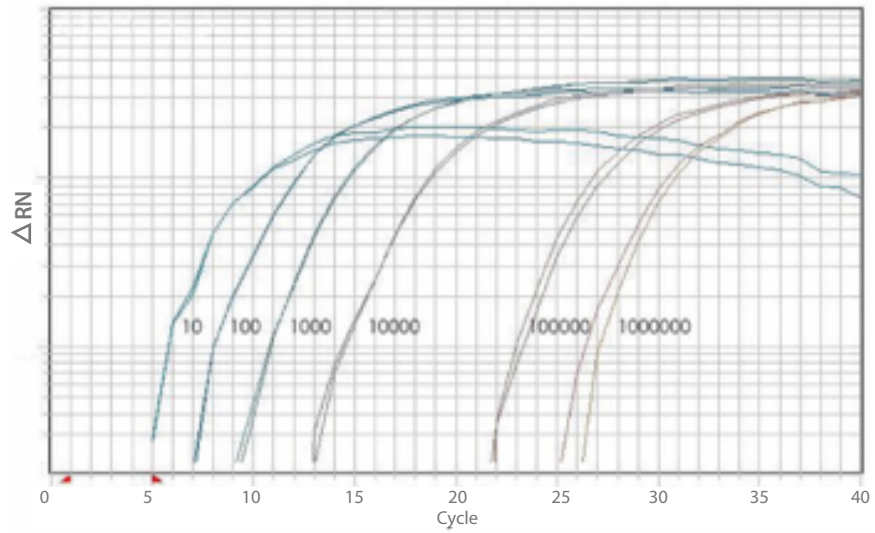

Fig 5. Soil HA – HTR – Amplification Plot

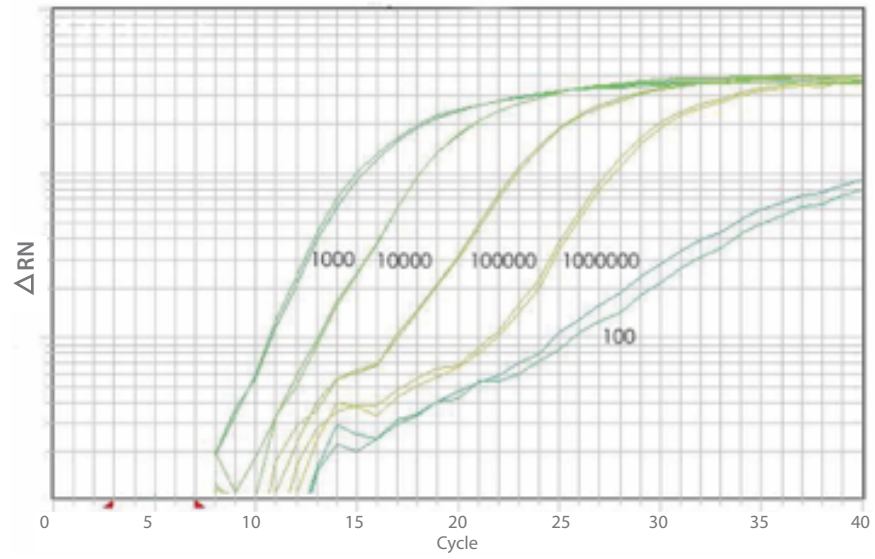

Fig. 6 Soil HA+ HTR –

Conclusion

Omni International's Soil DNA Purification kit can extract high yielding genomic DNA from a variety of soil matrices. The Soil DNA kit is a rapid and cost-effective method for isolating high quality PCR-ready DNA from soil. Omni International's purification kit provides superior reagents designed to remove PCR inhibitors, resulting in purified DNA that is ready for most downstream applications.

References

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