APPLICATION NOTE

Superior Performance of Omni International's Soil DNA Kit Over Company M's Soil DNA Isolation Kit for DNA Extraction from Soil Samples

Shari Garrett, Omni International, Inc.

Introduction

Molecular analysis of soil DNA offers a direct solution for detecting microorganisms residing in soil and for studying microbial diversity. Isolation of DNA from soils is often challenging because of the presence of many contaminants, like humic acid, that can interfere with the extraction process and are inhibitory to several downstream applications. An ideal DNA extraction method should effectively eliminate inhibitory substances and maximize DNA yields. The main objective of this study was to compare the performance of Omni International's Soil DNA Kit (26-013G/B) to that of Company M's Soil DNA Isolation Kit in terms of DNA yield and quality, as well as amplification potential and sensitivity of detection using real-time PCR.

Materials and Methods

Equipment

- Bead Ruptor Elite (Cat#19-040E)
- Bead Ruptor 2 ml Tube Carriage Kit (Cat#19-010-310)
- Soil DNA Kit (Cat#26-103G)

Bead Ruptor Elite
Cat#19-040E

Total DNA was isolated from 200 mg of outdoor soil spiked with 10 µL of ZymoBIOMICS™ Microbial Community Standard (Zymo Research) using the Omni International Soil DNA Kit and DNA Isolation Kit (Company M). ZymoBIOMICS™ Microbial Community Standard comprises 10 microbial strains -- 3 easy-to-lyse Gram-negative bacteria, 5 tough-to-lyse Gram positive bacteria, and 2 tough-to-lyse yeasts and serves as a benchmark for performance comparison of the two kits. The soil samples were homogenized using the Omni Bead Ruptor Elite and the isolations were done in triplicate following the manufacturer's recommended protocols for each kit. The protocol times were approximately 60 minutes and 70 minutes for Company M and Omni International extractions respectively, and the ease of use was comparable.

Purified DNA was eluted in 100 µL of each kit's elution buffer and quantified using the Promega QuantiFluor® dsDNA system. The quality of the purifed DNA from both the kits was analyzed by performing real-time PCR using 16S bacterial specific primers on 10X, 100X, and 1000X dilutions of the purified DNA. It was further tested for relative abundance of 2 tough-to-lyse strains of Listeria monocytogenes (Gram-positive bacteria) and Saccharomyces cerevisae (yeast) on 10X and 100X dilutions employing either Listeria specific primers or Saccharomyces specific primers. Briefly, a qPCR reaction was set up to a total volume of 20 µL using Agilent's Brilliant III 2X SYBR® as the master mix and 2 µL of template DNA at appropriate dilutions amplified with suitable primers following a standard protocol on the ABI 7900.

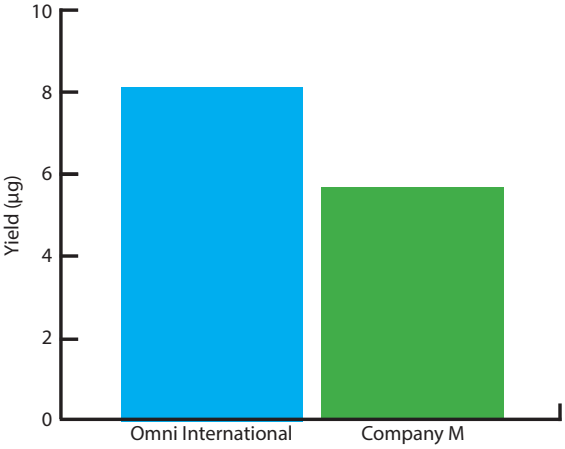

Figure 1. Average DNA yield from both the kits using Omni Bead Ruptor Elite as the homogenizer. (*p < 0.05)

Soil DNA Kit (Cat#26-103G)

OMNI International

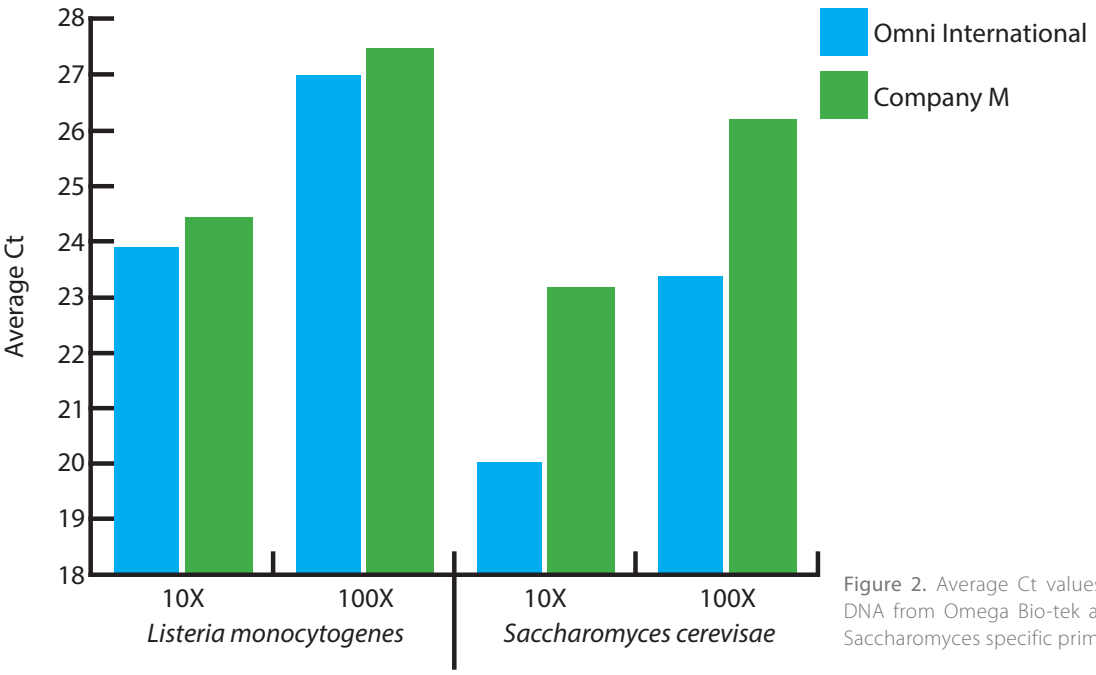

Figure 2. Average Ct values obtained by amplifying the purified DNA from Omega Bio-tek and Company M kits with Listeria and Saccharomyces specific primers.

Results

The DNA yields from the soil samples spiked with ZymoBIOMICS™ using the Omni International kit and Company M kit are as shown in Figure 1. The performance of the Omi International kit was significantly better compared to Company M's with a 40% increase in yield when eluted in 100 µL final volume (8.1 µg vs. 5.7 µg) ($p < 0.05$; Tukey's post-hoc analysis). The quality of the DNA obtained from each extraction was determined based on the Ct values generated from a qPCR reaction. Table 1 shows the average Ct values obtained on serial dilutions of the purified DNA using 16S bacterial specific primers. The Cts seem to be slightly lower (~ 0.5) with the Omni International extractions.

Table 1. Average Ct values from 10X, 100X, 1000X dilutions of purified DNA using Omni International and Company M kits and 16S bacterial specific primers.

	Ct		
	10X	**100X**	**1000X**
Omni International	14.93	17.42	21.46
Company M	15.34	18.22	21.97

Figure 2 shows average Ct values obtained using Listeria and Saccharomyces specific primers using 10X and 100X diluted purified DNA as the template. The organisms (Listeria monocytogenes and Saccharomyces cerevisae) are both tough-to-lyse and the results demonstrate the Ct's with the Omni International kit were significantly lower than Company M's ($p<0.05$; Tukey's post-hoc analysis). Ct's were lower by 1 cycle for the Gram-positive bacterium, Listeria and almost 3 cycles lower for the yeast strain, Saccharomyces; that is, a two-fold and eight-fold higher yield of Listeria and Saccharomyces with the Omni International kit when compared to the Company M kit. The ΔCt between the serial dilutions were comparable for both the kits (~3.1 for Listeria and ~3.4 for Saccharomyces). This data suggests that Omni International's kit performed as well as the Company M kit in eliminating PCR inhibitors from the isolations but with superior yields. The Ct values obtained on qPCR corroborate with the yields obtained, with the Omni International Soil DNA Kit excelling on both fronts.

Conclusion

The Omni International kit isolated DNA with significantly higher yields and was better able to isolate tough-to-lyse organisms than the Company M kit for the soil sample tested. The Omni International kit effectively removed the PCR inhibitors exemplified by the fact that the Omni-isolated DNA amplified consistently sooner than the Company M-isolated DNA represented by their lower Ct values. The lower Ct values may also indicate higher quality of the eluted DNA obtained using the Omni International kit. Overall, the results show that the Omni International Soil DNA Kit isolates high yield, high quality DNA compatible with various downstream applications such as qPCR, next-generation sequencing.