# Superior Performance of Omega Bio-tek's E.Z.N.A.® Soil DNA Kit Over Company M's Soil DNA Isolation Kit for DNA Extraction from Soil Samples

## Introduction

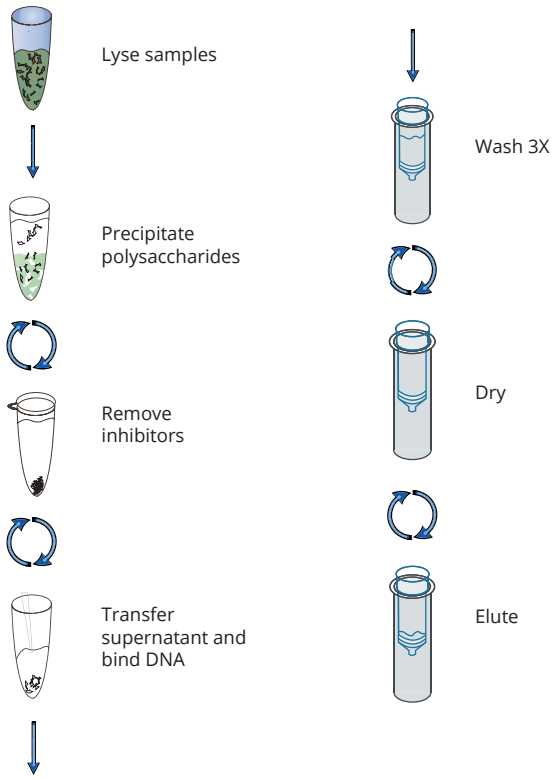
Molecular analysis of soil DNA offers a direct solution for detecting microorganisms residing in soil and studying microbial diversity. Isolation of DNA from soils is often challenging because of the presence of many contaminants like humic acid that can interfere with the extraction process and are 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 Omega Bio-tek's E.Z.N.A.® Soil DNA Kit (D5625) 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 & Methods

Total DNA was isolated from 200 mg of outdoor soil spiked with 10 µL of ZymoBIOMICS™ Microbial Community Standard (Zymo Research) using E.Z.N.A.® Soil DNA Kit (Omega Bio-tek) and DNA Isolation kit (Company M). ZymoBIOMICS™ Microbial Community Standard is comprised of 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 2 kits. The soil samples were homogenized using the Omni Bead Ruptor 24 and the isolations were done in triplicate following manufacturer's recommended protocols for each kit. The protocol times were approximately 60 minutes and 70 minutes for Company M and Omega Bio-tek's extractions respectively, and the ease of use was comparable.

Purified DNA was eluted in 100 µL of each kit's elution buffer and quantitated using Promega's QuantiFluor® dsDNA system. The quality of the purified 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 cerevisiae* (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.

## Illustrated Protocol



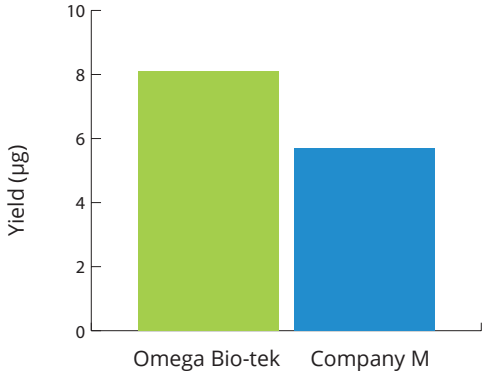




**Figure 1.** Average DNA yield from both the kits using Omni Bead Ruptor 24 as the homogenizer (*p < 0.05).

## Results & Discussion

The DNA yields from the soil samples spiked with ZymoBIOMICS™ using the Omega kit and Company M kit are shown in Figure 1. The performance of the Omega 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 $C_t$ values generated from a qPCR reaction. Table 1 shows the average $C_t$ values obtained on serial dilutions of the purified DNA using 16S bacterial-specific primers. The $C_t$s seem to be slightly lower (~0.5) with the Omega Bio-tek extractions.

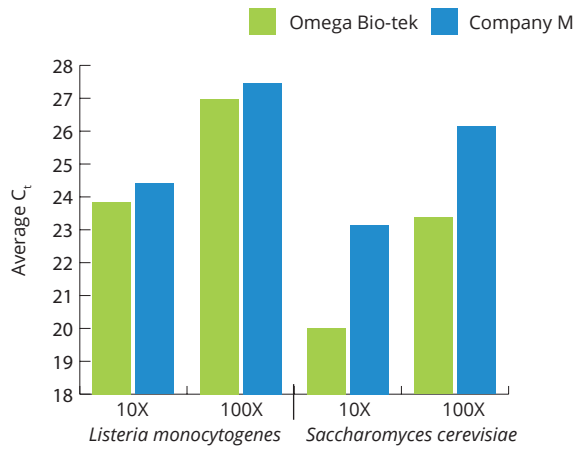



**Figure 2**. Average $C_t$ values obtained amplifying the purified DNA from Omega Bio-tek and Company M kits with *Listeria* and *Saccharomyces*-specific primers.

Figure 2 shows average $C_t$ values obtained using *Listeria* and *Saccharomyces*-specific primers using 10X and 100X diluted purified DNA as the template. The organisms (*Listeria monocytogenes* and *Saccharomyces cerevisiae*) investigated for are both tough-to-lyse and the results demonstrate the $C_t$'s with Omega kit were significantly lower than Company M's ($p<0.05$; *Tukey's post-hoc* analysis). $C_t$'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 Omega kit when compared to the Company M kit. The $\Delta C_t$ between the serial dilutions were comparable for both the kits (~3.1 for *Listeria* and ~3.4 for *Saccharomyces*). This data suggests that Omega Bio-tek's kit performed as well as the Company M kit in eliminating PCR inhibitors from the isolations but with superior yields. The $C_t$ values obtained on qPCR corroborate with the yields obtained, with Omega Bio-tek's E.Z.N.A.® Soil DNA Kit excelling on both fronts.

**Table 1.** Average $C_t$ values from 10X, 100X, 1000X dilutions of purified DNA using Omega Bio-tek and Company M kits and 16S bacterial-specific primers.

| | $C_t$ | | |
|---|---|---|---|
| | 10X | 100X | 1000X |
| Omega Bio-tek | 14.93 | 17.42 | 21.46 |
| Company M | 15.34 | 18.22 | 21.97 |

## Conclusions

The Omega Bio-tek 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 Omega kit effectively removed the PCR inhibitors exemplified by the fact that the Omega-isolated DNA amplified consistently sooner than the Company M-isolated DNA represented by their lower $C_t$ values. The lower $C_t$ values may also indicate higher quality of the eluted DNA obtained using the Omega Bio-tek kit. Overall, the results show that the Omega Bio-tek E.Z.N.A.® Soil DNA Kit isolates high yielding, high quality DNA compatible with various downstream applications such as qPCR, next-generation sequencing, etc.

## Product Information

| Description | Product No. | Preps |
|---|---|---|
| E.Z.N.A.® Soil DNA Kit | D5625-00 | 5 |
| | D5625-01 | 50 |
| | D5625-02 | 200 |

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