Application Note

Novel procedure for improved results in soil microbiome analysis using the DNeasy® PowerSoil® Pro Kit

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This application note demonstrates that the DNeasy PowerSoil Pro Kit improves the process of soil DNA extraction over existing QIAGEN technology and other commercially available kits. The kit enables extraction of higher yields of pure, high-quality DNA from challenging soil samples, and results in higher alpha diversity in 16S rRNA sequencing.

Introduction

Microbiome studies often focus on environmental samples such as soil. Humic acids, fulvic acids and other inhibitors found in soil can prevent accurate DNA analysis through necessary downstream applications such as PCR and sequencing reactions. Technologies used in commercial kits to remove these inhibitory substances often require tedious, time-consuming procedures and still yield low amounts of useful DNA. Efficient and uniform lysis of soil microbes is important to ensure that microbial community representation is as accurate as possible.

By improving on the technology used in the DNeasy PowerSoil Kit, already the gold standard in the field, a new tool for microbiome analysis was designed: the DNeasy PowerSoil Pro Kit. Specifically, newly developed bead tubes incorporate an optimized mix of beads, which in combination with an improved lysis chemistry, provides extremely efficient lysis of microbial cells, including bacteria and fungi. QIAGEN's Inhibitor Removal Technology® (IRT) process was shortened to one instead of two precipitation steps while increasing the efficiency of the removal of downstream-inhibiting substances. Additionally, due to improved binding chemistry, lysate binding can now be achieved in two instead of three loading and centrifugation steps. These advances ensure reliable sample lysis, improve inhibitor removal and simplify sample preparation through streamlined handling.

Bias in sequencing results is also an important issue facing microbiome researchers. Poor lysis methods can lead to an overrepresentation of easy-to-lyse microbes and an underrepresentation of tough fungi and bacterial species, providing a skewed picture of the microbiota. 16S rRNA sequencing results reveal higher alpha diversity, as measured by observed operational taxonomic units (OTUs), with the new DNeasy PowerSoil Pro method compared to the original DNeasy PowerSoil Kit and other tested methods. ▷

Materials and methods

Experiment 1:

Comparison of DNA yield from three different soil types prepared using commercially available sample preparation solutions versus the new DNeasy PowerSoil Pro Kit.

DNA was isolated from soil samples using either the DNeasy PowerSoil Pro Kit, the ZymoBIOMICS® DNA Miniprep Kit, the ZR Soil Microbe DNA MiniPrep® Kit (Zymo Research, Irvine, CA), the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA), the FastDNA® SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA), the Soil DNA Isolation Kit (Norgen Biotek Corp., Thorold, ON, Canada), or the PureLink® Microbiome DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA). Equivalent amounts of compost, clay or top soil (250 mg from each sample type) were used for DNA isolation per the manufacturers' protocols. For each protocol, three samples were processed. Yields were measured by fluorometric quantification (Qubit®). Briefly, for the DNeasy PowerSoil Pro Kit protocol, mechanical lysis of soil samples was performed using new PowerBead Pro tubes containing a ceramic bead mix and 800 µl of Solution CD1 with a Vortex Adapter on a Vortex Genie® 2 for 10 minutes. A one-step IRT procedure was used to remove contaminants from the soil sample, followed by binding of DNA to a silica-based spin column, and washing and elution in 100 µl of Solution EB.

Experiment 2:

Comparison of DNA yield and quality of the original DNeasy PowerSoil Kit versus the new DNeasy PowerSoil Pro Kit via gel electrophoresis.

DNA samples from clay, compost and top soil were prepared using the DNeasy PowerSoil Kit and the new DNeasy PowerSoil Pro Kit as described in Experiment 1. Additionally, 1.8 ml of *Candida albicans* culture was prepared in duplicate using both the DNeasy PowerSoil Kit and the new DNeasy PowerSoil Pro Kit. Briefly, the culture was added to a 2 ml Collection Tube and centrifuged at 10,000 x *g* for 30 seconds at room temperature. The supernatant was decanted andthe pellet centrifuged again at 10,000 x *g* for 30 seconds at room temperature and the media supernatant was completely removed. The cell pellet was then resuspended in 800 µl of Solution CD1 and transferred to a PowerBead Pro Tube. The remaining steps of the protocol were performed as described in Experiment 1. DNA was examined on a 0.8% TAE agarose gel (15 µl per lane).

Experiment 3:

Comparison of inhibitor levels from three different soil types prepared using commercially available sample preparation solutions versus the new DNeasy PowerSoil Pro Kit.

DNA samples were prepared using the DNeasy PowerSoil Pro Kit, the ZymoBIOMICS DNAMiniprep Kit, the ZR Soil Microbe DNA MiniPrep Kit, the E.Z.N.A. Soil DNA Kit, the FastDNA SPIN Kit for Soil, Norgen Biotek's Soil DNA Isolation Kit or the PureLink Microbiome DNAPurification Kit as described in Experiment 1. DNA purity was assessed by measurements of 260/280 nm and 260/230 nm ratios. To quantify the presence of PCR inhibitors, a qPCR assay was used in which the DNA eluate containing potential inhibitors was added into the qPCR reaction containing an internal amplification control. The presence of inhibitors in the DNA eluates was determined by comparing the C_T values of reactions containing DNA eluate with controlsthat did not contain eluate. This assay was performed using the QuantiFast® Pathogen +IC Kit.

Experiment 4:

Comparison of total diversity and community representation in soil DNA samples prepared using the DNeasy PowerSoil Pro Kit versus commercially available sample preparation solutions.

DNA was isolated from soil samples using either the DNeasy PowerSoil Pro Kit, the PureLink Microbiome DNA Purification Kit, the ZR Soil Microbe DNA MiniPrep Kit or the original DNeasy PowerSoil Kit as described in Experiment 1. For 16S library preparation, modified 515fB

and 806rB primers were used to amplify the V4 region of the 16S gene (1–4). For PCR amplification of the V4 region of the 16S gene, we used the QIAGEN Multiplex PCR master mix, 0.2 µM of each primer and 50 ng of template DNA. The cycling conditions were set to an initial 15-minute activation step at 95°C, 22 cycles of denaturation (94°C, 30 seconds), annealing (55°C, 90 seconds) and extension (72°C, 60 seconds) steps, followed by a final extension step at 72°C for 30 minutes. The 16S amplicons were purified by a bead-based approach and quantified using a fluorometric assay. Sequencing adapters were then added using the QIASeq® 1-Step Amplicon Kit. For adapter ligation, 500 ng of amplicon per sample was used as the starting amount. Adapter ligation and library purification were performed per the kit instructions. Individual samples were labeled with a 6 bp barcode (GeneRead™ 12-plex Adapter). All 16S libraries were quantified using the QIASeq Library Quant Kit and paired-end sequencing (2 x 250 bp) was performed on a benchtop sequencer. For bioinformatics analysis, the CLC Microbial Genomics Module as part of the CLC Genomics Workbench was used. For 16S data analysis, raw sequencing reads were imported into the CLC Microbial Genomics Module and then the OTU clustering module and NGS Core Tools were used to merge paired-end reads and perform quality control. OTUs were then picked by mapping sequences against the Greengenes database and clustering at 97% identity. Next, OTUs were aligned using muscle and used to construct a Maximum Likelihood phylogenetic tree, followed by alpha and beta diversity analyses.

Results and discussion

Experiment 1:

Significantly increased DNA yields from challenging soil samples.

The new DNeasy PowerSoil Pro Kit was compared with several commercially available sample prep kits. The DNeasy PowerSoil Pro method outperformed all tested kits in compost, clay and top soil samples. Overall DNA yields were up to 8x higher for the DNeasy PowerSoil Pro Kit than for alternative methods (Figure 1).

Experiment 2:

High-quality DNA from the DNeasy PowerSoil and DNeasy PowerSoil Pro Kits, with increased yield observed in the new method.

The DNeasy PowerSoil Pro method was then compared to the gold standard in microbial sample prep, the DNeasy PowerSoil Kit, for DNA extraction from compost, clay and top soil samples, as well as fungal culture. Samples were analyzed via gel electrophoresis to examine quality and yield. DNA quality was equivalent for samples prepared using the two kits, while DNA yield was increased when the DNeasy PowerSoil Pro Kit was used. This effect was especially important for the challenging fungal samples (Figure 2).

Experiment 3:

Improved Inhibitor Removal Technology increases purity of DNA isolated with the DNeasy PowerSoil Pro Kit compared with alternative methods.

The DNeasy PowerSoil Pro features a streamlined IRT to decrease sample processing time. Here, the new DNeasy PowerSoil Pro Kit was compared with several commercially available sample prep kits. When isolated DNA was examined via UV spectroscopy, DNeasy PowerSoil Pro was the only method showing 260/280 ratios near 1.8 for all soil types (Figure 3A), while also showing the highest 260/230 ratios (Figure 3B), indicating the absence of inhibitors. Less variability was also observed between the samples processed with the DNeasy PowerSoil Pro Kit (Figures 3A and 3B). Inhibitor removal was then visualized with an inhibitor-sensitive PCR with an internal control spiked with eluates from the DNeasy PowerSoil Pro Kit and alternative methods. Samples isolated using the DNeasy PowerSoil Pro Kit showed no inhibition in PCR, in contrast to the significant inhibition observed with the PureLink Kit (Figure 3C) and other commercially available methods tested (data not shown). ▷

Experiment 4:

Unbiased identification of total diversity and community representation in soil samples with DNA isolated using the DNeasy PowerSoil Pro Kit.

To determine the microbial composition of soil samples, we extracted DNA from vegetable gardensoil with low sand content (Figure 4A) and from forest soil (Figure 4B), which is acidic and sandy,and performed 16S rRNA gene sequencing. 16S rRNA gene sequencing can be used to identifythe relative abundance of bacteria present in each sample and to perform alpha and beta diversity analyses, which allow comparison of bacterial diversity both within and between groups of samples. The 16S analysis revealed differences in bacterial composition between samples isolated using the original DNeasy PowerSoil Kit, the DNeasy PowerSoil Pro Kit, the PureLink Microbiome DNA Purification Kit and the ZR Soil Microbe DNA MiniPrep Kit.

We found that the total number of bacteria identified with the DNeasy PowerSoil Pro Kit was higher than all other methods and unique bacteria were identified in these samples that were not present in the other methods. Alpha diversity analyses revealed that the microbiota fromsoil samples isolated with the DNeasy PowerSoil Pro Kit was more diverse than that of samplesprocessed using the other methods tested. This was measured by the number of OTUs.

Analysis of the composition of the samples showed that although the overall composition was very similar between DNeasy PowerSoil Kit and DNeasy PowerSoil Pro Kit, the DNeasy PowerSoil Pro Kit included more of the difficult-to-lyse gram+ *Actinobacteria* and *Firmicutes* (Table 1).

In summary, 16S microbial analyses identified that the soil microbial communities are different between samples prepared using different methods, and that DNA isolated using the DNeasy PowerSoil Pro Kit had increased alpha diversity.

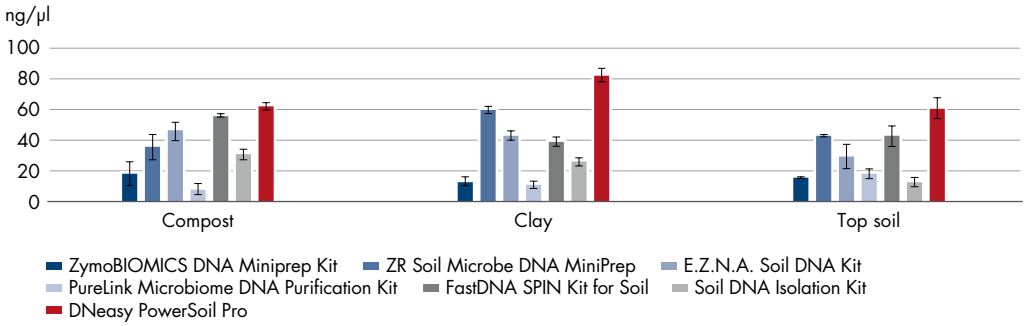

Figure 1. Higher yields of DNA with the new DNeasy PowerSoil Pro Kit. Various soil sample types (250 mg) were prepared using commercially available sample preparation solutions and compared to the new DNeasy PowerSoil Pro Kit. Yields were measured by fluorometric quantification (Qubit).

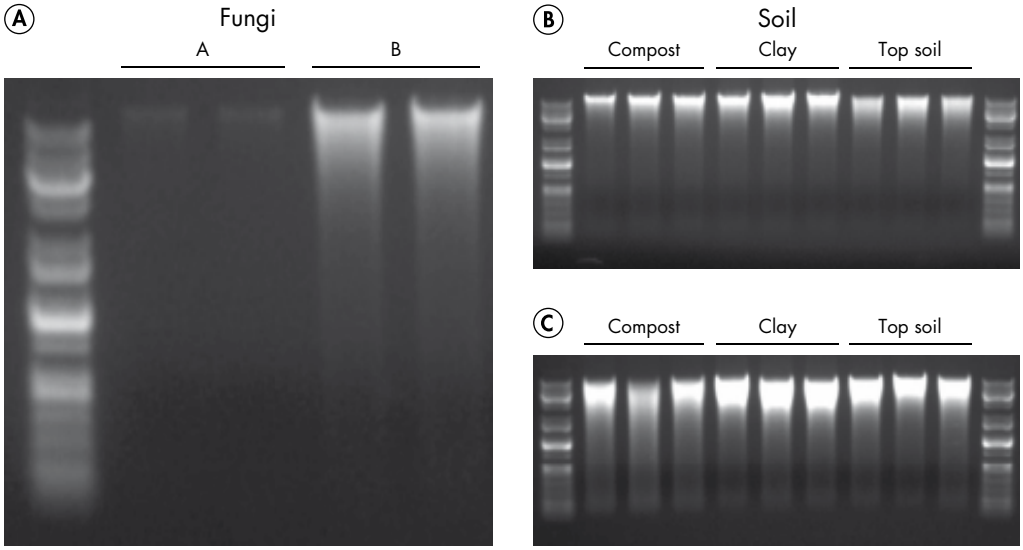

Figure 2. Improved, high-quality DNA yields with the new DNeasy PowerSoil Pro Kit. Gel **A** shows 1.8 ml of *C. albicans* fungal culture processed with the DNeasy PowerSoil Kit (a) compared with the DNeasy PowerSoil Pro Kit (b). Gels **B** and **C** contain various soil samples (250 mg). Gel **B** was processed with the original DNeasy PowerSoil Kit, while Gel **C** was processed using the DNeasy PowerSoil Pro Kit.

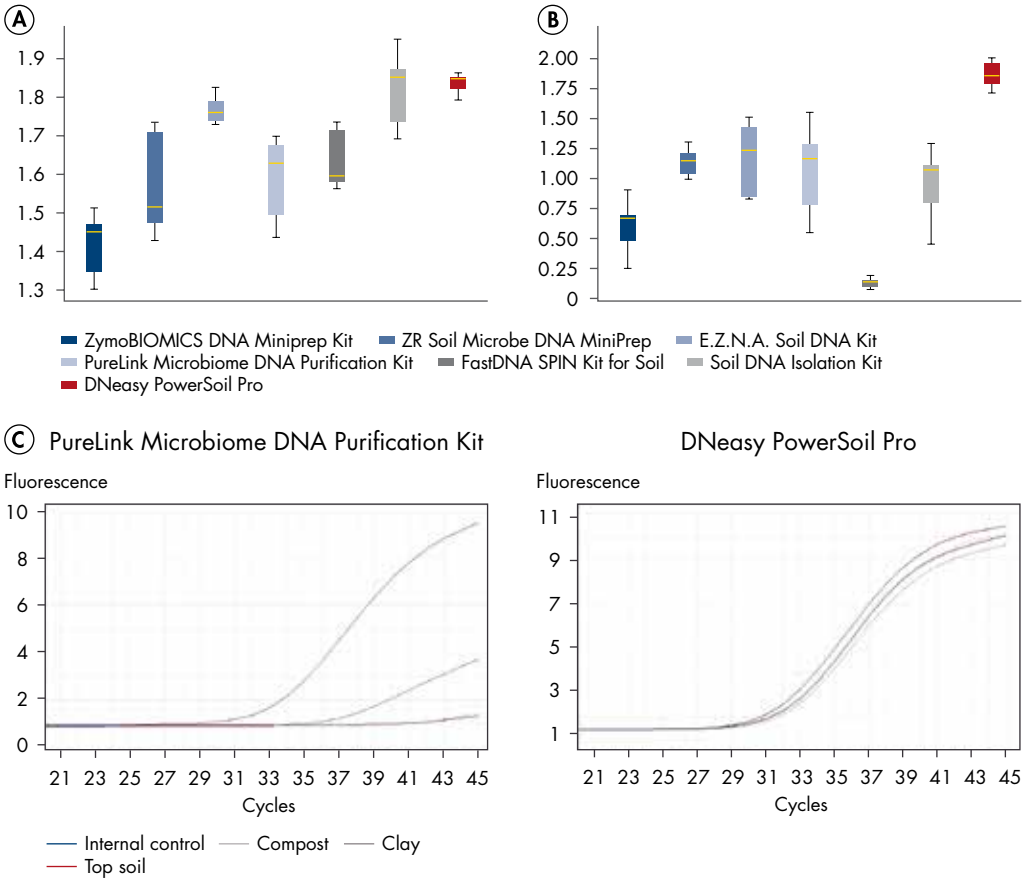

Figure 3. Increased purity and less variability in DNA extracted with the DNeasy PowerSoil Pro Kit. Soil samples (250 mg each) were prepared using commercially available sample preparation solutions and compared to the new DNeasy PowerSoil Pro. DNA purity was depicted via UV measurements and the analysis of ratios 260/280 **A** and 260/230 **B**. Inhibitor removal was visualized with an inhibitor-sensitive PCR with an internal control spiked with eluates from the PureLink Microbiome DNA Purification Kit and the DNeasy PowerSoil Pro Kit. Eluate free PCR-reactions served as internal control **C**.

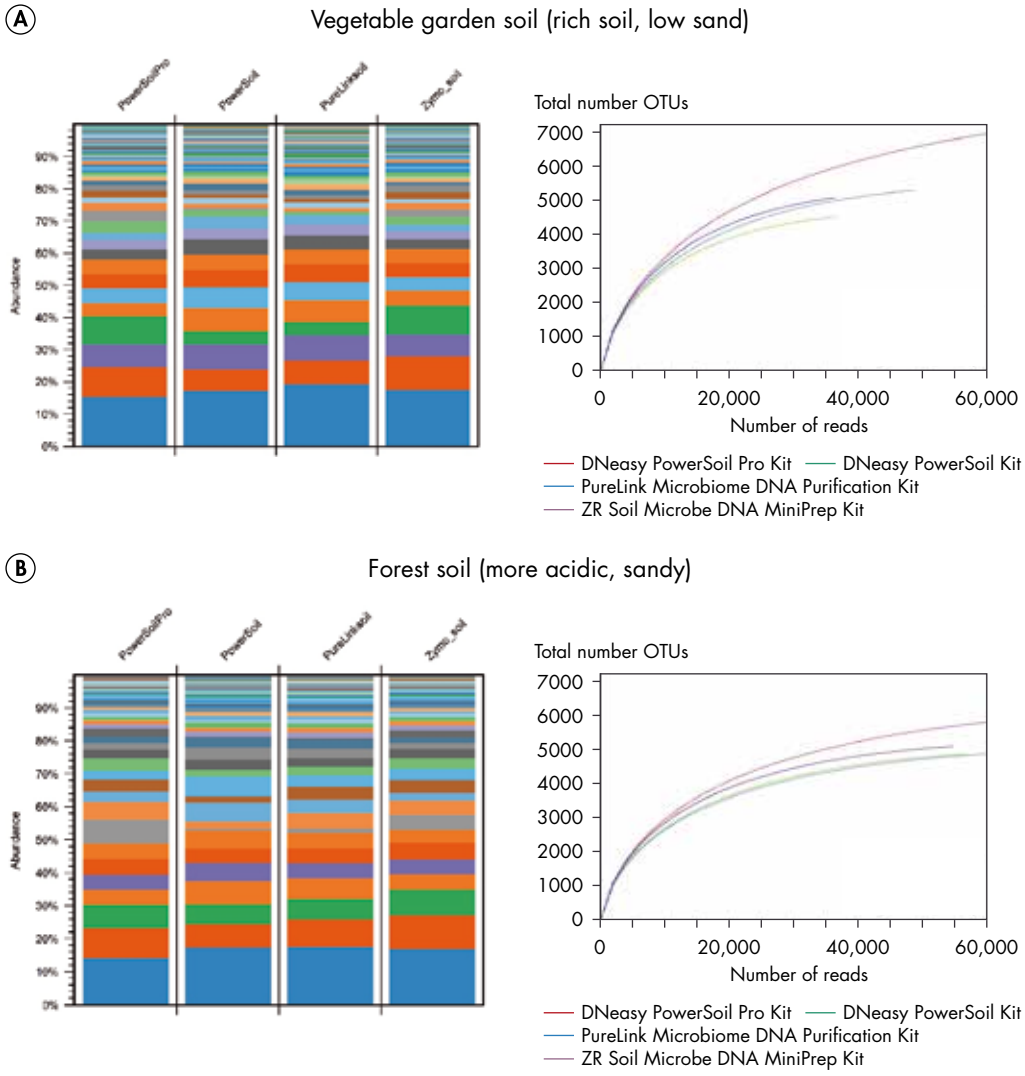

Figure 4. CLC Microbial Genomics Module 16S data analysis identified differences in microbial community composition and diversity between DNA samples isolated using various extraction methods. DNA was extracted from vegetable garden soil with low sand content **A** and forest soil **B**, which is acidic and sandy, using different sample preparation solutions. For each sample type, FASTQ files were imported into the CLC Genomics Workbench and processed with the Microbial Genomics Module using the OTU clustering workflows. The bacterial composition of each sample type resulting from each extraction method are summarized graphically on the left. Taxonomic assignments were performed by mapping sequences against the Greengenes database and clustered at 97% identity. Sequences that did not map were then clustered de novo. Results are summarized at the genus level. Alpha diversity analyses for each sample are depicted on the right. DNA isolated using the DNeasy PowerSoil Pro Kit was more diverse than DNA extracted using other methods. Diversity was measured by the number of operational taxonomic units (OTUs) identified. Sampling depth = 150,000.

Table 1. DNeasy PowerSoil Pro Kit isolates more difficult to lyse microbes.

Phylum (aggregated)	DNeasy PowerSoil Pro Kit	DNeasy PowerSoil Kit
Thaumarchaeota	5%	2%
Acidobacteria	22%	29%
Actinobacteria	12%	6%
Armatimonadetes	0%	0%
Bacteroidetes	6%	11%
Chloroflexi	4%	4%
Cyanobacteria	0%	0%
Firmicutes	10%	1%
Gemmatimonadetes	1%	1%
Latescibacteria	0%	1%
Nitrospirae	2%	2%
Planctomycetes	9%	8%
Proteobacteria	24%	25%
Tectomicrobia	0%	0%
Verrucomicrobia	6%	9%

Conclusions

Taken together, these data show that the DNeasy PowerSoil Pro Kit outperforms the gold standard original DNeasy PowerSoil Kit, as well as all other commercially available methods tested. The DNeasy PowerSoil Pro Kit isolates higher yields of high-quality DNA from soil samples and fungal cultures. DNA isolated using the DNeasy PowerSoil Pro Kit also demonstrates better 260/280 and 260/230 ratios compared with other tested methods, and an inhibitor removal assay shows no detectable PCR inhibitors, in stark contrast to competitor methods. The DNA isolated using the DNeasy PowerSoil Pro Kit was also used for 16S rRNA sequencing, and compared with the original DNeasy PowerSoil Kit and other commercially available methods. Results indicated that the alpha diversity was higher in the samples isolated with the DNeasy PowerSoil Pro Kit, and more bacteria (OTUs) were detected. The total number of bacteria detected with the DNeasy PowerSoil Pro Kit was the highest of the kits tested, and different bacteria were identified that were not found with other methods. Thus, the new DNeasy PowerSoil Pro enables the lysis and sequencing of species of bacteria that are not identified with the other methods tested, without the loss of most other species that are also identified with alternative methods.

In summary, the DNeasy PowerSoil Pro Kit provides a streamlined, improved method of DNA isolation from soil samples, enabling:

- Efficient lysis of bacteria and fungi in all soil types, including compost, clay and top soil
- Isolation of up to 8-fold higher yields of DNA compared to alternative methods
- Recovery of inhibitor-free DNA, ready to use directly in downstream NGS applications
- Unbiased results, with higher alpha diversity in sequencing as compared to other methods

Ordering Information

Product	Contents	Cat. no.
DNeasy PowerSoil Pro Kit (50)	For 50 DNA minipreps: Buffers, PowerBead Tubes, Spin Filters, 2 ml Collection Tubes	47014
DNeasy PowerSoil Pro Kit (250)	For 250 DNA minipreps: Buffers, PowerBead Tubes, Spin Filters, 2 ml Collection Tubes	47016

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Visit **www.qiagen.com** or contact QIAGEN today to discover how DNeasy PowerSoil Pro Kits can enhance your soil DNA extraction!

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