Application Note

Extraction of microbiome DNA using the DNeasy® PowerSoil® Pro Kit manually and automated on the QIAcube® Connect and QIAcube

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Introduction

Microbes are considered as "biological dark matter" in our world, as the majority of microbes have not been isolated or identified. Many microbes cannot be cultured, making characterization difficult.

Metagenomics has, however, enabled researchers to assess this microbial dark matter without relying on culture methods. Furthermore, modern sequencing techniques have opened the door to analyze even highly complex microbial communities, known as the microbiome. With our increasing understanding of the microbial world, it becomes clear that these microbial communities play an important role in health and the environment. Analyzing microbiomes is challenging. Many sample types, including stool and soil, contain substances that inhibit downstream analyses such as PCR or next-generation sequencing (NGS). This inhibition can bias results as some analytes might be more affected than others. Moreover, efficient and uniform lysis of microbes is required to make sure the community representation is as accurate as possible. Here, we show that microbiome analysis on QIAcube Connect and QIAcube helps to ensure unbiased and efficient analysis of the microbiome. Both instruments use proven silica-membrane technology for sample processing, ensuring standardization for research labs and high-quality results.

For the comparisons described in this application note, samples from each soil type were purified by manual and automated procedures. A complete DNA extraction procedure was conducted on on both QIAcube Connect and QIAcube instruments.

Materials and methods

Experiment 1: Comparison of DNA yield and presence of inhibitory substances after isolation from soil samples with the DNeasy PowerSoil Pro Kit manually, and using the Inhibitor Removal Technology® (IRT) protocol on QIAcube Connect and QIAcube

DNA was isolated from soil samples using either the manual protocol or the QIAcube IRT protocols for the DNeasy PowerSoil Pro Kit. Equivalent amounts of two different soil samples (250 mg) from each sample type) were filled into PowerBead Pro tubes. Samples were lysed according to the standard procedure by adding 800 µl lysis buffer (Buffer CD1) followed by vortexing for 10 minutes at maximum speed using a 24-sample vortex adapter. To allow reliable comparison between manual and automated sample processing, supernatants were pooled after mechanical lysis. This minimizes the impact of sample-to-sample variation when working with heterogeneous sample material. From this pool of supernatants, 600 µl per sample was then processed with the manual and QIAcube protocols according to the standard procedure of the *DNeasy PowerSoil Pro Kit Handbook*. Briefly, for the manual protocol, patented Inhibitor Removal Technology (Buffer CD2) was used to remove contaminants from the soil sample. After addition of binding buffer (Buffer CD3), DNA was bound to a silica-based spin column. DNA was washed (Wash solution EA and C5) and eluted in 100 µl of elution buffer (Buffer C6). For processing on QIAcube Connect or QIAcube, 600 µl per mechanically lysed sample was transferred into position 2 of the Rotor Adapter. The Rotor Adapter was placed into the centrifuge of the QIAcube Connect or the QIAcube. All the steps described above, including the removal of inhibitory substances, were performed automatically. For each protocol, 12 samples were processed. After isolation, DNA yield was determined by fluorometric quantification and DNA purity was assessed by photometric measurements of 260/280 nm and 260/230 nm ratios. DNA eluates were spiked with an internal amplification control and assayed using qPCR. Detection of this control would be impaired if the eluate contained inhibitors.

Experiment 2: Comparison of total diversity and community representation in soil DNA samples using the DNeasy PowerSoil Pro Kit manually and automated on QIAcube Connect and QIAcube

DNA was isolated from soil samples using the DNeasy PowerSoil Pro Kit either manually, or automated on QIAcube Connect or on QIAcube as described in “Experiment 1”. For 16S library preparation, barcoded 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 UCP

Multiplex PCR Master Mix, 0.25 µM of each primer and 5 ng of template DNA. The cycling conditions were set to an initial 2-minute activation step at 95°C, 30 cycles of denaturation (95°C, 10 seconds), annealing (50°C, 20 seconds) and extension (72°C, 20 seconds) steps, followed by a final extension step at 72°C for 10 minutes. The 16S amplicons were purified using a bead-based method and quantified with the QIAseq Library Quant Assay Kit. A paired-end sequencing (2 x 250 bp) was performed on a benchtop sequencer. For bioinformatics analysis, the CLC Microbial Genomics Module of the CLC Genomics Workbench was used. For 16S data analysis, FASTQ files were imported into the CLC Microbial Genomics Module and then the OTU (Operational Taxonomic Unit) 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 Silva database and clustering at 97% identity. Next, the OTU table was used to perform diversity analysis.

Results and discussion

Experiment 1: Equal DNA yield and purity and successful removal of inhibitors after DNA isolation from soil samples compared between the manual and automated IRT protocols of the DNeasy PowerSoil Pro Kit.

The performance (yield, quality, inhibitor removal) of the DNeasy PowerSoil Pro protocol was compared between QIAcube Connect, QIAcube and the manual procedure. No significant deviation was detected between all three methods (Figures 1 and 2).

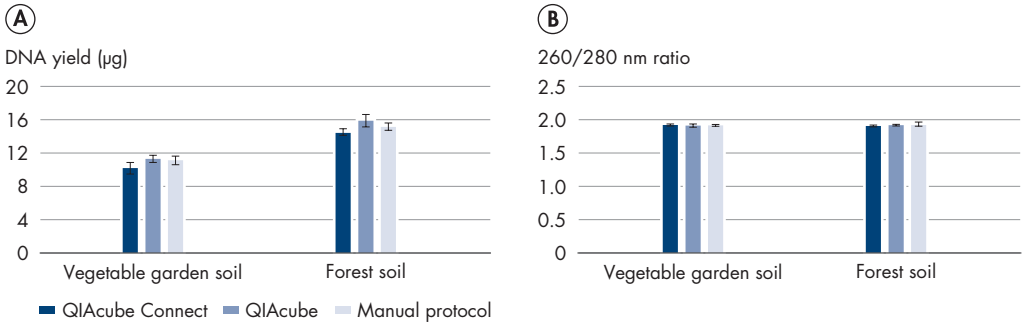

Figure 1. Comparable DNA yields. Comparison of QIAcube Connect, QIAcube and manual DNeasy PowerSoil protocols. **A:** DNA yield was measured using a fluorometric, DNA-specific assay. **B:** DNA purity was determined by spectrophotometric measurements at 260 nm and 280 nm. Each column represents the mean of six extractions from a soil sample; each bar represents the standard deviation.

Efficient removal of PCR inhibitors, which can interfere with downstream applications, was also tested. This analysis showed that none of the eluates impaired the control qPCR reaction demonstrating successful removal of inhibitors with all methods (Figure 2).

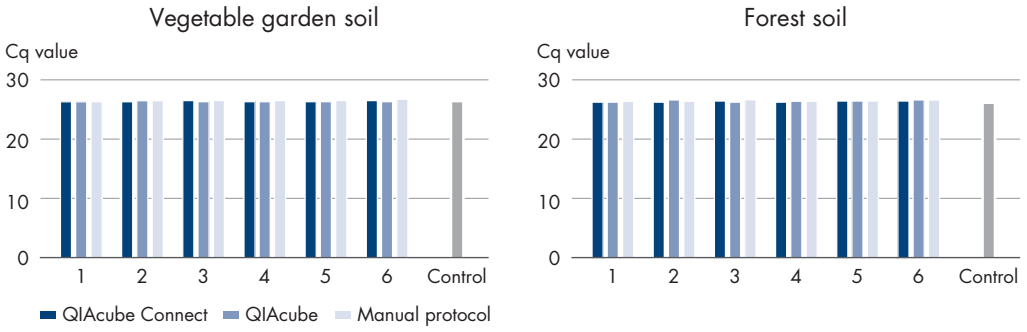

Figure 2. Efficient removal of PCR inhibitors. Analysis of samples isolated manually, on QIAcube Connect, or QIAcube using DNeasy PowerSoil Pro Kit protocols. Eluate from each sample (4 µl) was added to reaction mixtures that contained internal control DNA. Amplification of this control DNA was quantified by qPCR.
The Cq value of a water control, which does not inhibit amplification of the internal control DNA, was compared to samples containing eluates from the DNeasy PowerSoil Pro protocol. Equal Cq values confirmed that all methods resulted in complete removal of inhibitory substances.

Experiment 2: 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 garden soil with low sand content and from forest soil, which is acidic and sandy. 16S rRNA gene sequencing was used to identify the 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.

We found that the total number of bacteria identified with the DNeasy PowerSoil Pro Kit was comparable for all protocols, whether automated or manual. Alpha diversity analyses measured by the number of operational taxonomic units (OTU), revealed that the microbiota from soil samples isolated with the DNeasy PowerSoil Pro Kit was almost the same for all three protocols tested (Figure 3).

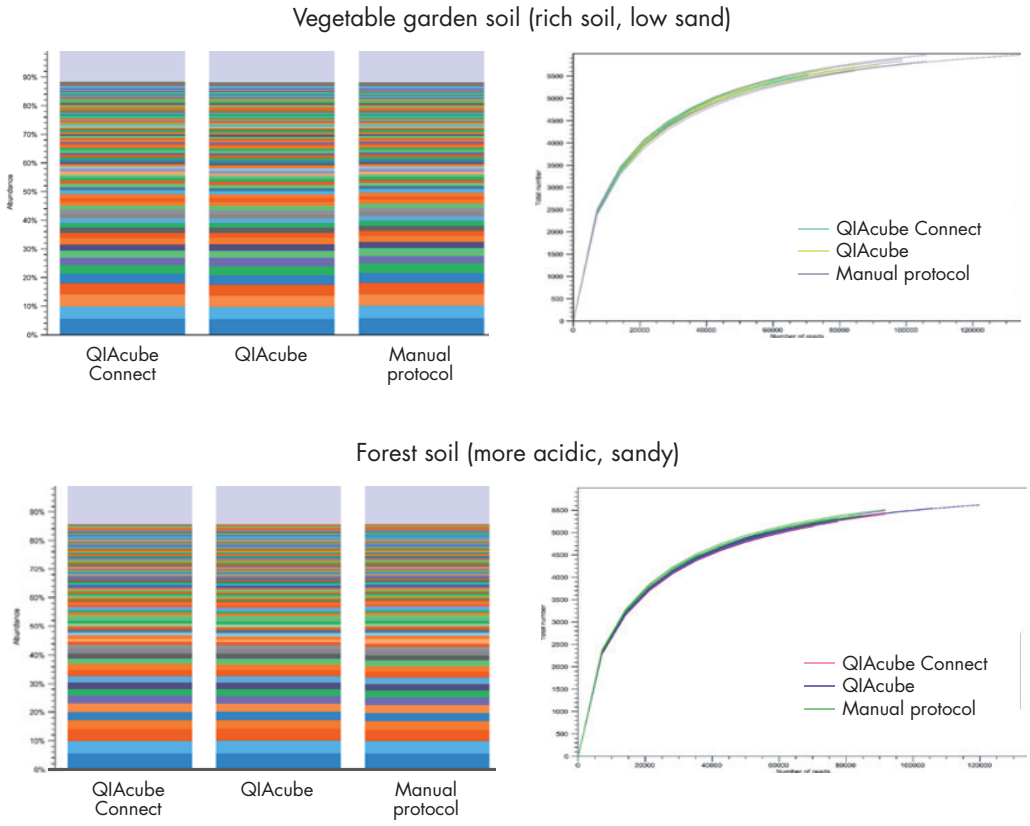

Figure 3. Unbiased identification of total diversity and community representation. For two different soil samples, eluates were obtained with the manual protocol and automated DNeasy PowerSoil Pro protocols on QIAcube Connect and QIAcube. In 16S sequencing, these preparations resulted in practically identical OTU tables (left: tables show identified microbial taxonomic units, with each color signifying a different genus) and alpha diversity data (right: curve shows the number of detected species at a given sequencing depth).

Conclusions

These data show that automation of the DNeasy PowerSoil Pro Kit on QIAcube Connect and QIAcube performs equally to the manual kit protocol while significantly reducing hands-on time for removal of inhibitory substances and spin column-based DNA isolation. The extracted DNA is of high quality and can be used immediately in downstream reactions, including next-generation sequencing and qPCR.

Automation of the DNeasy PowerSoil Pro Kit:

- Enables convenient and efficient isolation of high-quality DNA from difficult sample types
- Helps to ensure quality and quantity of isolated DNA
- Provides unbiased detectable diversity for microbiome analysis

QIAcube Connect's advanced digital capabilities and full connectivity redefines the benefits of automated sample processing. QIAcube Connect enables any research lab to standardize the first step in their workflows using proven QIAGEN technologies to gain valuable insights.

- Standardized workflows at the push of a button
- Automation of over 80 spin column kits
- Connection to QIAsphere® for remote instrument management and monitoring
- Built-in UV light for highest safety standards

QIAsphere ready

For more information on QIAcube Connect, check out the online demo tool **www.qiagen.com/QIAcubeConnect-demo**.

Ordering Information

Product	Description	Cat. no.
QIAcube Connect	For automation of lyse, bind, wash and elution steps of QIAGEN spin columns for DNA, RNA and protein purification	Inquire
DNeasy PowerSoil Pro Kit (50)	For the isolation of microbial genomic DNA from all soil types	47014
DNeasy PowerSoil Pro Kit (250)	For the isolation of microbial genomic DNA from all soil types	47016
Vortex Adapter for 24 (1.5–2.0 ml) tubes	For vortexing 1.5 and 2 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-24
UCP Multiplex PCR Kit (100)	For 500 x 20 µl PCR reactions	206742
QIAseq Library Quant Assay Kit	qPCR assays for quantifying Illumina® libraries	QSTF-ILZ
CLC Genomics Workbench	For analyzing, comparing, and visualizing Next Generation Sequencing data	Inquire

QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

QIAcube Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

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