Novel, Automated Procedure for Extraction of Microbial gDNA for High-Throughput Microbiome Analysis

Stefanie Schröer, Dominic O'Neil, Claudia Dienemann, Thomas Deutschmann, Markus Sprenger-Haussels
QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

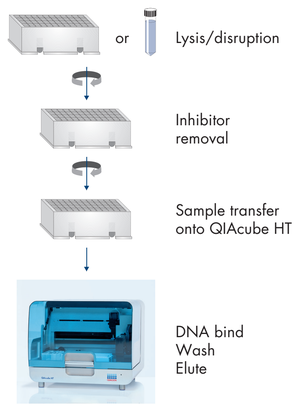
Novel, Procedure for Extraction of Microbial gDNA from Environmental Samples

Studies of the environmental and human microbiome face the problem that sample materials, such as soil and stool, are extremely difficult sample types. This is due to their high abundance of inhibitory substances such as humic and fulvic acids in soil, and bile acids and proteoglycans in stool. These inhibitory substances are often co-purified and have the potential to inhibit enzymatic reactions. Extraction of microbial DNA is therefore challenging and often requires tedious and time-consuming workflows, especially with large sample sizes in high-throughput studies.

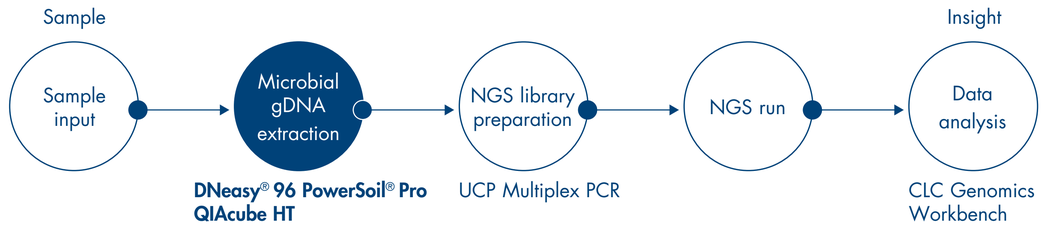
To address these challenges, we have developed a second generation of the QIAGEN®/MO BIO Inhibitor Removal Technology®, which efficiently removes PCR inhibitors from even the most difficult soil types and stool samples. High-throughput bead-beating homogenization allows rapid, thorough, and unbiased lysis by a combination of mechanical and chemical methods. Inhibitory substances are removed by a dedicated precipitation step, all in a fast and streamlined workflow. Subsequent DNA purification is automated on a 96-well silica membrane plate format on the QIAcube® HT instrument using vacuum technology. This technology enables high-throughput processing of environmental samples for downstream applications, such as PCR amplification and NGS analysis, in under 3 hours.

The automated protocol provides equivalent yields and purity to manual workflows, while enabling much higher throughput. 16S sequencing shows equivalent community composition to the manual methods, as shown by alpha and beta diversity measures. The new automated protocol allows for a complete picture of the diversity in the samples, thereby demonstrating that its suitability for high-throughput sample processing of both environmental and stool samples.

Convenient, Automated, High-Throughput Workflow

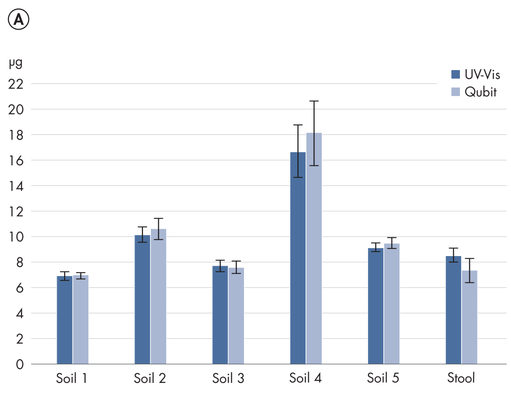


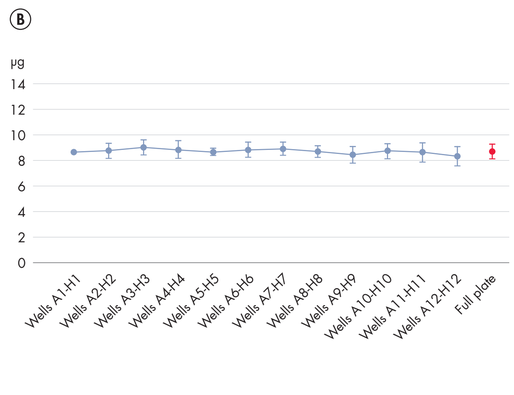
- New bead technology for efficient and unbiased lysis
- Next-generation chemistry for lysis, inhibitor removal and DNA binding
- Automated purification of 24–96 samples simultaneously minimizes experiment-to-experiment deviation
- High degree of automation allows for minimal hands-on time
- User-friendly and intuitive software for easy run setup and data management
- Harmonized interplay of QIAGEN instrument and kit technologies



Reproducible, High-Quality DNA Yields from Soil and Stool

Soil and human stool samples were prepared using the new DNeasy 96 PowerSoil Pro QIAcube HT Kit. Microbial gDNA was either extracted from individually processed samples (A) or from aliquots of a lysate pool (B).





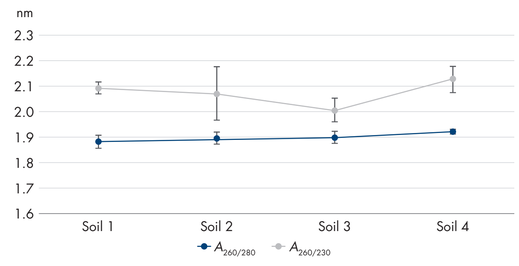
Yield from stool and soil. 250 mg of soil and 100 mg of human stool were prepared using the new DNeasy 96 PowerSoil Pro QIAcube HT Kit. Yields were measured by UV-Vis technology (NanoDrop™) and fluorometric quantification (Qubit®)

Consistency of yield across 96-well plates. DNA from a soil sample lysate pool was isolated in a full 96-well plate run on the QIAcube HT and yield determined by UV-Vis technology (NanoDrop).

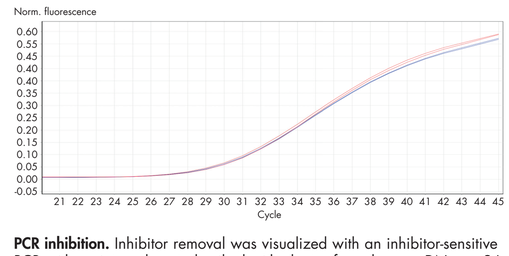
The DNeasy 96 PowerSoil Pro QIAcube HT yields high amounts of DNA from soil and stool samples with high reproducibility and low variance over the 96-well plate.

Inhibitor Removal Technology Increases Purity of Isolated DNA

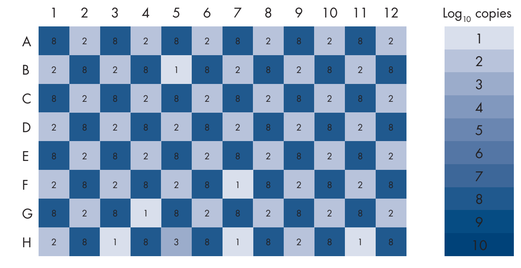
Soil samples were processed with the new DNeasy 96 PowerSoil Pro QIAcube HT Kit.



***A*260/280 and *A*260/230 ratios.** DNA purity shown as UV measurements and the analysis of ratios 260/280 (blue line) and 260/230 (grey line).



PCR inhibition. Inhibitor removal was visualized with an inhibitor-sensitive PCR with an internal control spiked with eluates from the new DNeasy 96 PowerSoil Pro QIAcube HT Kit (blue lines). Eluate-free PCR reactions served as controls (red lines).

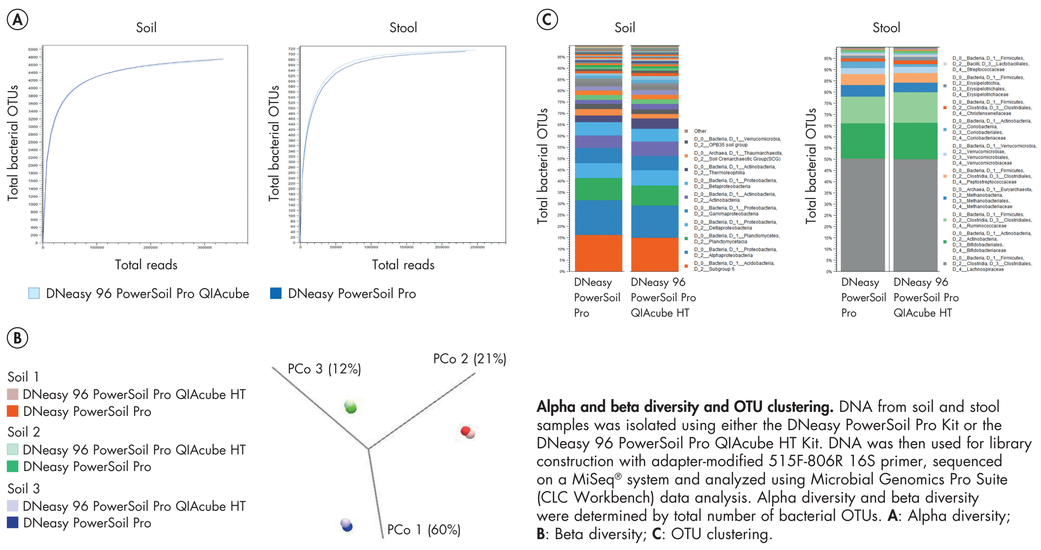


Cross-contamination. Aliquots from a soil sample lysate pool and H_2O as substitute sample were distributed in a 96-well plate in a checkerboard pattern and processed on the QIAcube HT using the DNeasy 96 PowerSoil Pro protocol. Bacterial gDNA was determined by 16S qPCR and DNA content per well pictured by color intensity of blue. Dark blue: Soil samples with up to 3.9 x 10^8 copies/well; Light blue: H_2O samples with <100 copies/well.

DNeasy 96 PowerSoil Pro QIAcube HT shows:

- A_{260}/A_{280} ratios ~1.9
- High A_{260}/A_{230} ratios over 2
- No inhibition in PCR
- Well controlled cross-contamination

Identification of Total Diversity and Community Representation in Soil and Stool Samples



Alpha and beta diversity and OTU clustering. DNA from soil and stool samples was isolated using either the DNeasy PowerSoil Pro Kit or the DNeasy 96 PowerSoil Pro QIAcube HT Kit. DNA was then used for library construction with adapter-modified 515F-806R 16S primer, sequenced on a MiSeq® system and analyzed using Microbial Genomics Pro Suite (CLC Workbench) data analysis. Alpha diversity and beta diversity were determined by total number of bacterial OTUs. **A**: Alpha diversity; **B**: Beta diversity; **C**: OTU clustering.

Total bacterial OTUs identified in DNA isolated with the DNeasy 96 PowerSoil Pro QIAcube HT Kit and the DNeasy PowerSoil Pro Kit are comparable, showing an equally high alpha diversity and identical beta diversity.

Conclusions

Relatively inexpensive, highly multiplexed 16S sequencing enables studies of large sample size, for which improved sample extraction techniques are needed in order to enable true high-throughput studies. A new semi-automated, high-throughput method is presented that:

- Allows simultaneous purification of microbial genomic DNA of up to 96 samples in a convenient semi-automated workflow using the QIAcube HT instrument
- Efficiently lyses bacteria in soil and stool samples
- Provides high yields of useful, pure DNA
- Removes inhibitors commonly present in soil and stool samples, allowing eluates to be used directly in downstream NGS applications.

These data show that the new high-throughput method on the QIAcube HT achieves successful DNA isolation from soil and stool samples in a semi-automated 96-well format.

The method presented here is intended for molecular biology applications. This method is not intended for the diagnosis, prevention, or treatment of a disease.

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.

1117162 04/2019

Sample to Insight