High-Throughput Procedure for Rapid Extraction of Microbial DNA

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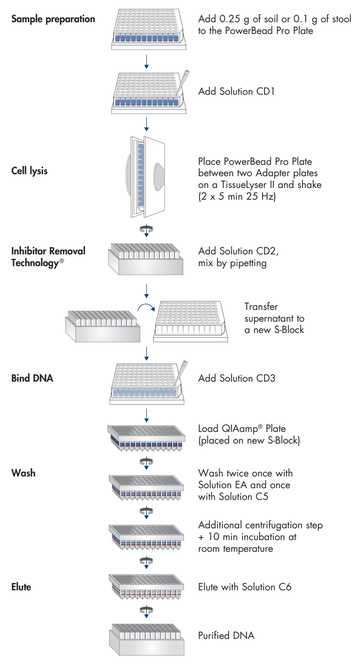
Introduction and Experimental Workflow

Environmental and human microbiome studies often require processing samples that contain inhibitory substances, such as humic and fulvic acids in soil and bile acids and proteoglycans in stool. When co-purified with the extracted DNA, these substances inhibit downstream applications. Usually, the methods to remove these inhibitors are tedious and time-consuming, especially in high-throughput studies.

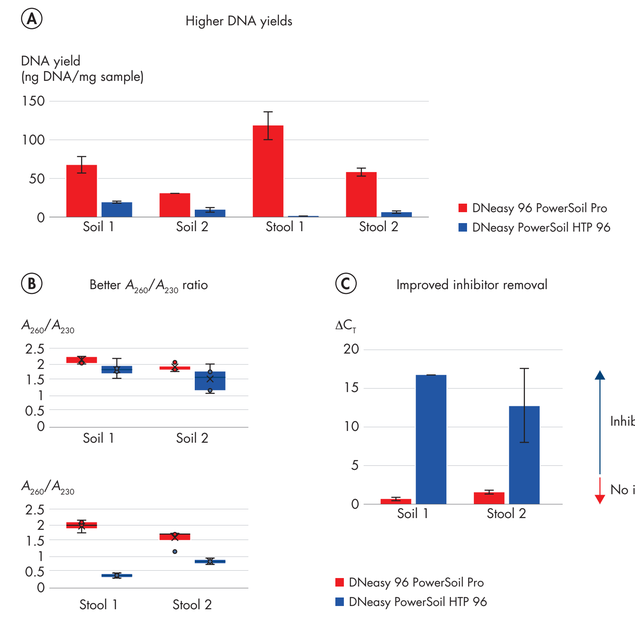
We have developed a streamlined protocol for manual extraction of microbial DNA from all kinds of soil and stool samples – the DNeasy® 96 PowerSoil® Pro Kit. This 96-well format workflow combines rapid and efficient lysis of even difficult-to-lyse microbial cells (e.g., bacteria, fungi and archaea) with efficient removal of inhibitory substances, while requiring fewer centrifugation steps. The inhibitor-free DNA is captured on a 96-well silica membrane spin column, washed and eluted. This workflow enables processing of up to 384 samples for downstream applications, such as PCR amplification and NGS analysis, in less than one day.



DNeasy 96 PowerSoil Pro workflow.

Improved DNA Yield and Quality from Soil and Stool

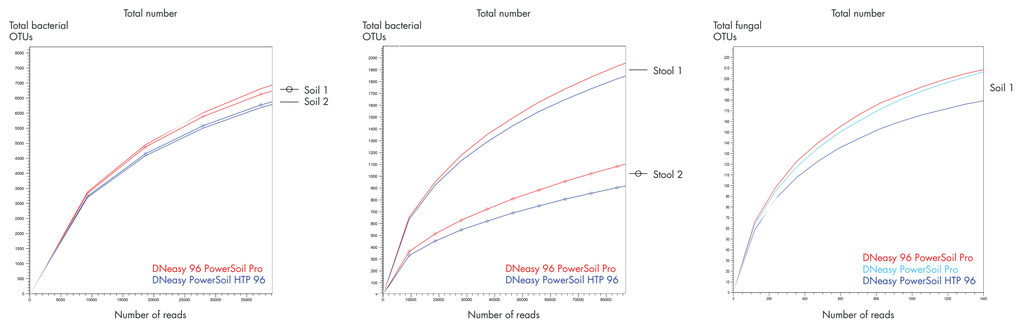
DNA yield and purity were higher and inhibitor removal was improved using the DNeasy 96 PowerSoil Pro Kit compared with the legacy DNeasy PowerSoil HTP 96 Kit.



Increased DNA yields and quality compared with the legacy kit. DNA was extracted from 250 mg soil and 50 mg stool (4 replicates) using the DNeasy 96 PowerSoil Pro and DNeasy PowerSoil HTP 96 kits. **A** DNA yields were measured with a Qubit™ Fluorometer. **B** A_{260}/A_{230} ratios were obtained using a QIAxpert®. The overall DNA yields and 260/230 ratios were consistently higher using the DNeasy 96 PowerSoil Pro Kit. **C** Extracted DNA was used to determine the co-extraction of inhibitors. The internal control from the QuantiFast® Pathogen +IC Kit was spiked with 4 µl eluates of the resulting DNA (4 replicates). Distilled water added to the IC was used as a control. The eluate from the DNeasy 96 PowerSoil Pro Kit showed no inhibition.

Increased Microbial Representation

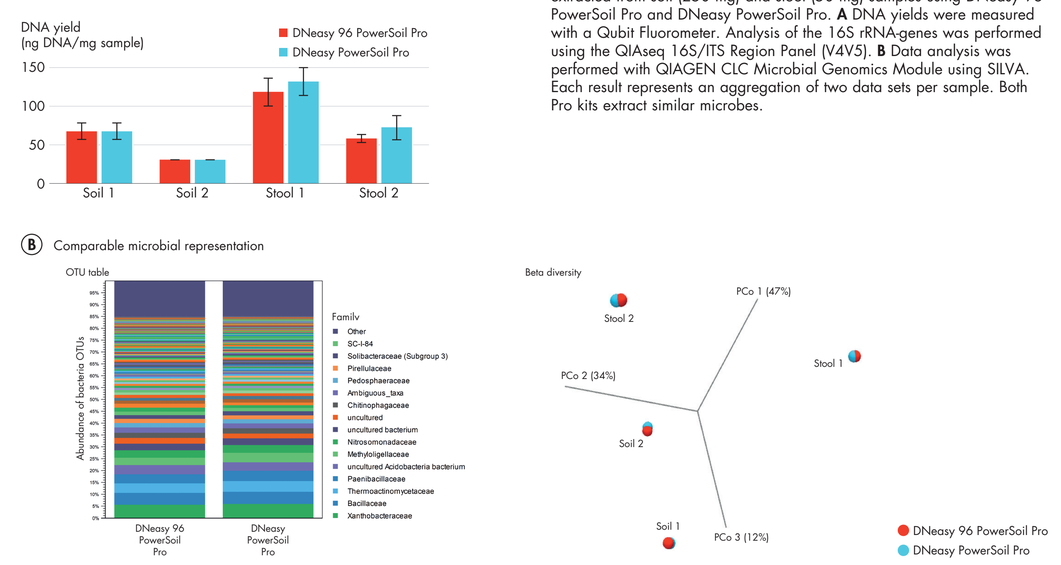
Increased bacterial and fungal operational taxonomic units (OTUs) identified in DNA extracted using the DNeasy 96 PowerSoil Pro Kit compared with the legacy DNeasy PowerSoil HTP 96 Kit.



Increased microbial representation. DNA was extracted from soil and stool samples using DNeasy 96 PowerSoil Pro and DNeasy PowerSoil HTP 96 kits. Analysis of the 16S rRNA genes was performed using the QIAseq® 16S/ITS Region Panel (V4V5) and QIAseq 16S/ITS Index Kit. Data analysis was performed with the QIAGEN® CLC Genomics ProSuite using SILVA. Each result represents an aggregation of two data sets per sample. **A** Alpha diversity was determined by total number of OTUs. The new DNeasy 96 PowerSoil Pro Kit shows increased numbers of identified OTUs compared with the legacy DNeasy PowerSoil HTP 96 Kit. **B** To determine fungal representation, DNA was extracted from soil using DNeasy 96 PowerSoil Pro Kit, the DNeasy PowerSoil Pro Kit and the legacy DNeasy PowerSoil HTP 96 Kit. Analysis of the ITS region was performed using the QIAseq 16S/ITS Region Panel and QIAseq 16S/ITS Index Kit. Data analysis was performed using the QIAGEN CLC Microbial Genomics Module with the database UNITE. Increased fungal diversity is observed using the Pro technology compared with DNeasy PowerSoil HTP 96.

Comparable Results Across DNeasy PowerSoil Pro Formats

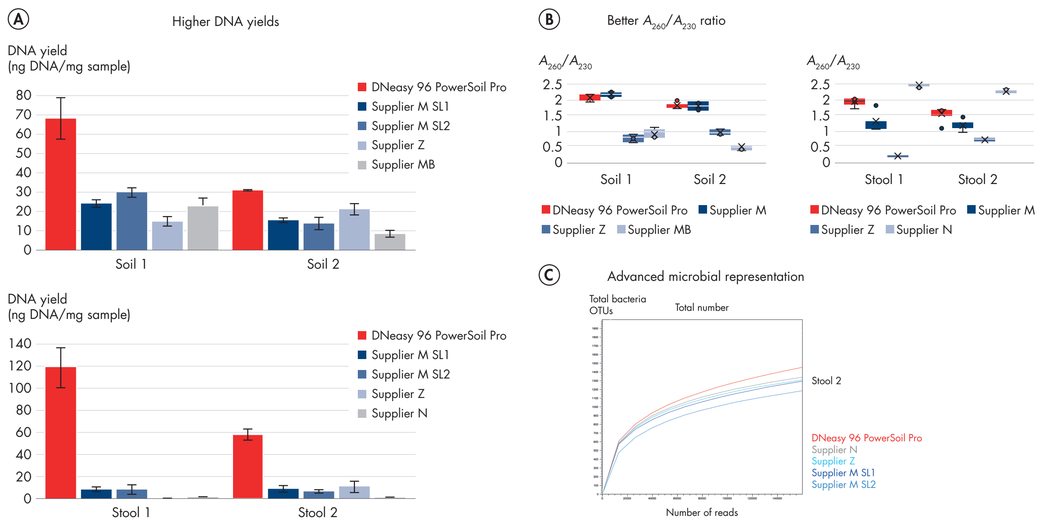
DNA extraction from soil and stool using DNeasy 96 PowerSoil Pro is comparable to the manual low-throughput DNeasy PowerSoil Pro kit (single column).



Comparable results using different formats of Pro technology. DNA was extracted from soil (250 mg) and stool (50 mg) samples using DNeasy 96 PowerSoil Pro and DNeasy PowerSoil Pro. **A** DNA yields were measured with a Qubit Fluorometer. Analysis of the 16S rRNA-genes was performed using the QIAseq 16S/ITS Region Panel (V4V5). **B** Data analysis was performed with QIAGEN CLC Microbial Genomics Module using SILVA. Each result represents an aggregation of two data sets per sample. Both Pro kits extract similar microbes.

Technology Comparison: DNA Yield and Quality

Compared with kits from other suppliers, the DNeasy 96 PowerSoil Pro Kit gave better DNA yield and quality, resulting in improved microbial representation.



Significantly increased DNA yield and quality compared with other suppliers. DNA was extracted using the DNeasy 96 PowerSoil Pro Kit and kits from Supplier M, Supplier Z, Supplier N and Supplier MB (4 replicates). **A** DNA yields were measured with a Qubit Fluorometer. **B** DNA purity (A_{260}/A_{230} ratio) was determined using QIAxpert. **C** 16S libraries were prepared using the QIAGEN UCP Multiplex PCR Kit, with data analysis on the QIAGEN CLC Microbial Genomics Module using SILVA. Each result represents an aggregation of two data sets per sample. Alpha diversity was determined by total number of OTUs.

Conclusions

The new DNeasy 96 PowerSoil Pro Kit:

- Has a streamlined, user-friendly protocol
- Gives higher DNA yields and better inhibitor removal than the legacy DNeasy PowerSoil HTP 96 Kit
- Shows better bacterial and fungal representation than the legacy DNeasy PowerSoil HTP 96 Kit and is comparable to the DNeasy PowerSoil Pro Kit
- Extracts significantly more DNA, with higher quality and species richness, than competitor technologies
- Provides DNA of optimal quality for downstream applications, including PCR, qPCR and NGS
- Requires less plastic than the legacy DNeasy PowerSoil HTP 96 Kit

The DNeasy 96 PowerSoil Pro Kit is intended for molecular biology applications. This product 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.

Sample to Insight