Sensitive and Robust Workflow for High-Throughput Microbiome Analysis

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Novel, High-Throughput, Automated Procedure for Extraction of Microbial gDNA from Environmental Samples

Microbiome analysis gains significant traction because it is highly relevant for multiple areas, affecting disease status, metabolism, nutrition and many more. Specific areas of interest for microbiome studies include the human gut, nasopharynx or other regions, as well as environmental or animal and plant investigation.

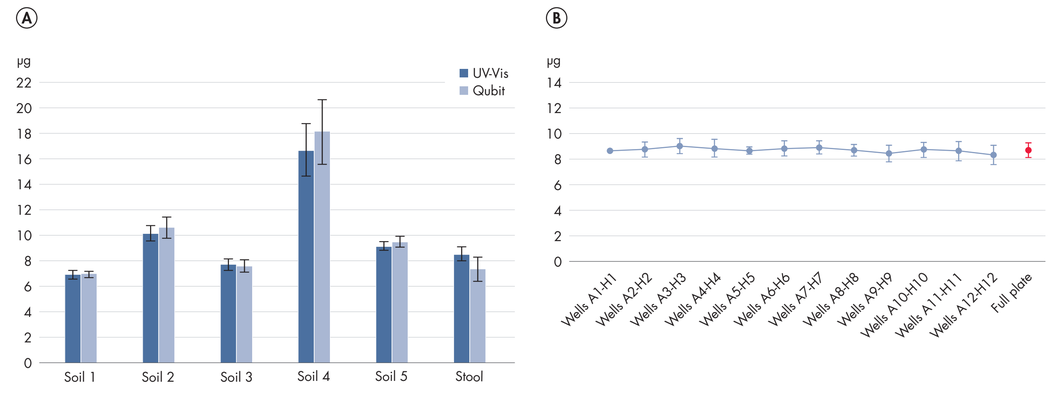
To profile the microbial population of a given sample material, common workflow steps include stabilization and extraction of nucleic acids, amplification of generic target sequences such as 16S or 18S regions, followed by next generation sequencing (NGS) analysis to assess diversity and operational taxonomical unit representation, using appropriate software tools.

Common challenges in the microbiome workflow include complete extraction from all species and sensitive, bias-free amplification and analysis, to reveal the true representation and relationship between microbial population and sample. Specific issues are caused by inhibitors, GC variations or sample processing, particularly in higher-throughput processing.

Here, we demonstrate a comprehensive approach to cover all microbial workflow steps, addressing the challenges to ensure correct results and high workflow efficiency.

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).



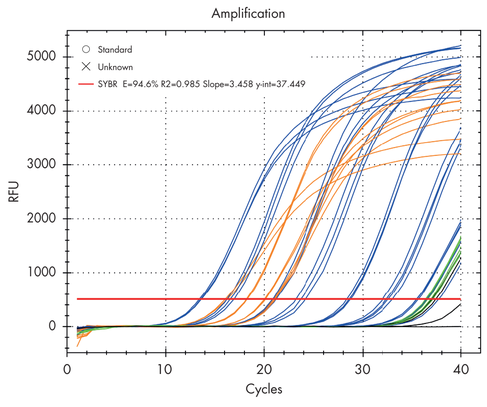
Yield from stool and soil. 250 mg of soil and 100 mg of human stool were prepared using the new DNeasy 96 PowerSoil Pro QIAcube HT Kit. Yields were measured by UV-Vis technology (NanoDrop™) and fluorometric quantification (Qubit®)

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).

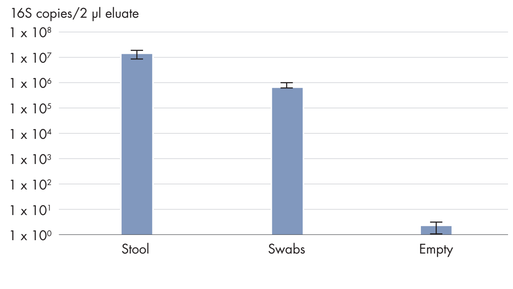
The DNeasy 96 PowerSoil Pro QIAcube HT yields high amounts of DNA from soil and stool samples with high reproducibility and low variance over the 96-well plate.

qPCR Enables Specific Quantification of Bacterial Load and Indicates Absence of Microbial Background

Buccal swabs were processed using the DNeasy 96 PowerSoil Pro QIAcube HT Kit. Using the UCP SYBR® Green 16S Quant Kit which targets the 16S V3/V4 region, the bacterial DNA load was quantified for stabilized stool samples, as well as for buccal swabs. Sterile FLOQSwabs™ were used to monitor background signal.



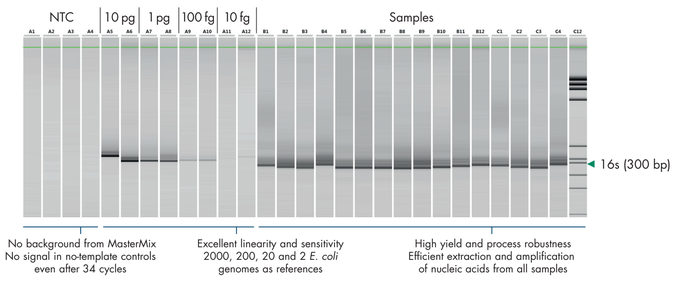
Blue: *E. coli* DNA standard dilution series from 10 ng/reaction to 10 fg/reaction. **Orange:** Buccal swabs; 2 µl of eluate used for quantification. **Green:** Empty FLOQSwabs. Results indicate a background signal of ~5 fg/µl eluate. **Black:** No-template control.



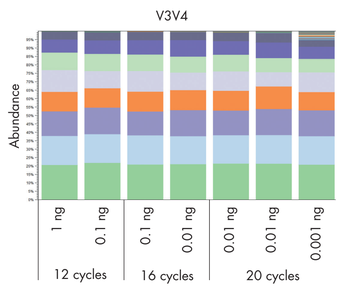
Quantification of buccal swabs and stool samples in one run. The bacterial loads show significant differences.

UCP Multiplex PCR Chemistry Secures Quantitative Distribution of Bacterial Strains in the Sample

Fecal samples were purified using the QIAcube HT and eluates were subjected to an automated 16S library PCR setup using the UCP Multiplex PCR Kit on a QIAgility® instrument. PCR amplicons were resolved on the QIAxcel®. *E. coli* DNA was used to generate a standard curve. In a second experiment, the QIAseq® 16S/ITS Kit was used to amplify and analyze a bacterial mock community. No differences in the abundance of the different strains were observed across the input range (1 ng to 1 pg).

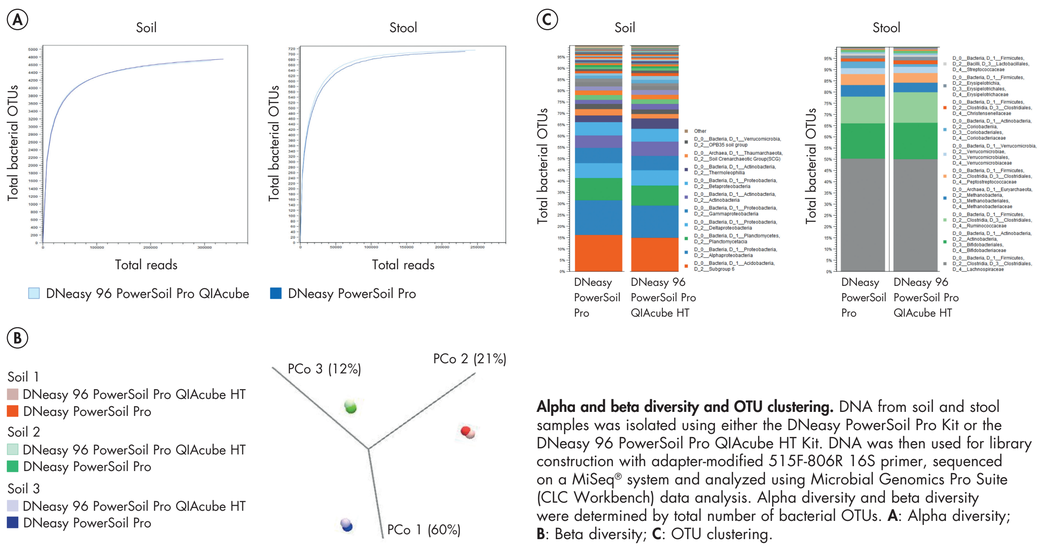


QIAxcel gel image of processed frozen stool samples (200 mg).



Analysis of a mock bacterial community.

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

- The DNeasy 96 PowerSoil Pro QIAcube HT Kit enables the operator to easily streamline and process a high number of different samples for microbiome analysis.
- The kit enables extraction of bacterial DNA from stool, as well as from a number of other interesting sample types, e.g., buccal swabs.
- Automation, together with the wide range of potential starting sample types, provides the possibility of a one-for-all, harmonized workflow that can be further improved by using RNA*later*® as a sample stabilizing agent.
- Utilization of QIAGEN's UCP PCR kits for quantification, as well as the UCP Multiplex PCR Kit or QIAseq 16S/ITS kits for library PCR ensure unbiased amplification of the microbial communities, which can then be seamlessly analyzed with the CLC Genomics Workbench.

In summary, this poster demonstrates a highly automated high-throughput workflow to extract, quantify and analyze a variety of samples in a microbiome workflow.

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

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Sample to Insight