Automated Isolation of Microbial DNA from Human Samples

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Abstract

The influence of the human microbiome on health has been demonstrated in many studies and is an important area of ongoing investigation for medicine and diagnostics. For these studies, it is crucial to have a reliable, reproducible extraction method that can extract DNA of all microorganisms with comparable efficiency, while simultaneously depleting the inhibitory substances that may affect subsequent downstream analysis. In addition, the increase in number and size of microbiome studies necessitates automation of these extraction methods. Here, we present the results of automating an established extraction method on a magnetic bead handling instrument.

Human microbiome samples (stool, buccal swabs, other) were homogenized and microbial cells (including Gram-negative and -positive bacteria, fungi and archaea) were rapidly and efficiently lysed by bead beating in conjunction with chemical lysis. In a subsequent step, various inhibitory substances were removed from inhibitor-rich sample types, including stool and gut samples. Inhibitor-free DNA was captured on magnetic silica beads, washed and eluted using the QIAsymphony® SP automated system. Extracted DNA quality was assessed for purity and yield, as well as tested in qPCR and 16S rDNA sequencing experiments.

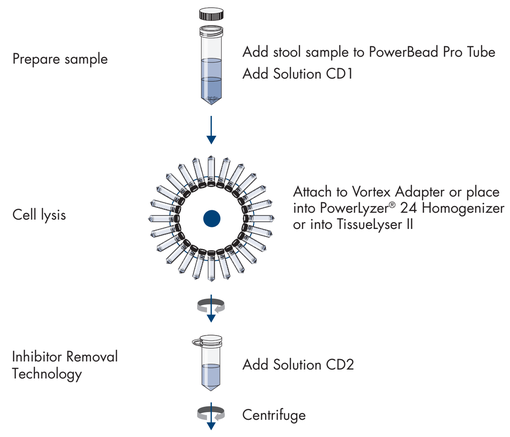
The automated DNA extraction method efficiently extracted microbial DNA from 1 to 96 samples per run, generating consistent results with no detectable cross-contamination. Extracted microbial DNA displayed no inhibition in qPCR with internal control. 16S rDNA sequencing revealed highly complex communities, measured by alpha diversity (observed operational taxonomic units; OTUs), comparable with the manual QIAamp® PowerFecal® Pro reference method and higher than other tested methods. Beta diversity indicated that extraction of multiple replicates of the same sample was extremely consistent.

This workflow enables processing of up to 192 human or environmental samples and delivers high-quality DNA for downstream applications such as PCR amplification and NGS analysis in less than 1 day.

Convenient, Automated, Mid-throughput Workflows

The QIAsymphony PowerFecal Pro DNA Kit comprises a novel method for automated mid-throughput isolation of both microbial and host genomic DNA from stool, soil, buccal swabs and other samples using the second generation of QIAGEN's patented Inhibitor Removal Technology® (IRT).

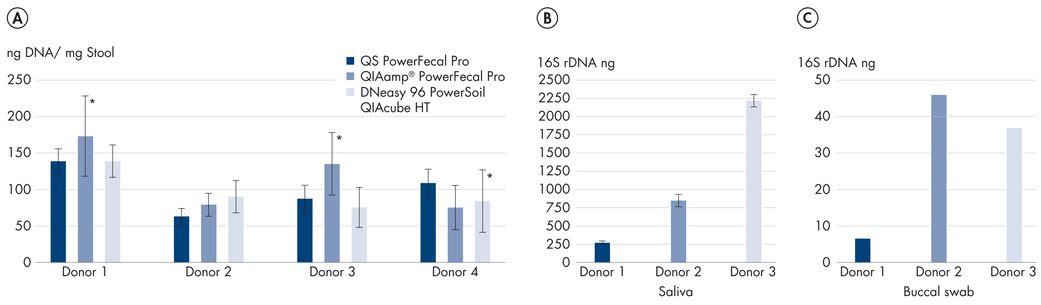
QIAsymphony SP and AS instruments.



QIAsymphony PowerFecal Pro DNA workflow.

Reproducible, High-quality DNA Yields from Stool, Buccal Swabs and Saliva

High DNA yields were seen when stool, buccal swabs or saliva were processed using the PowerFecal Pro chemistry on the QIAsymphony



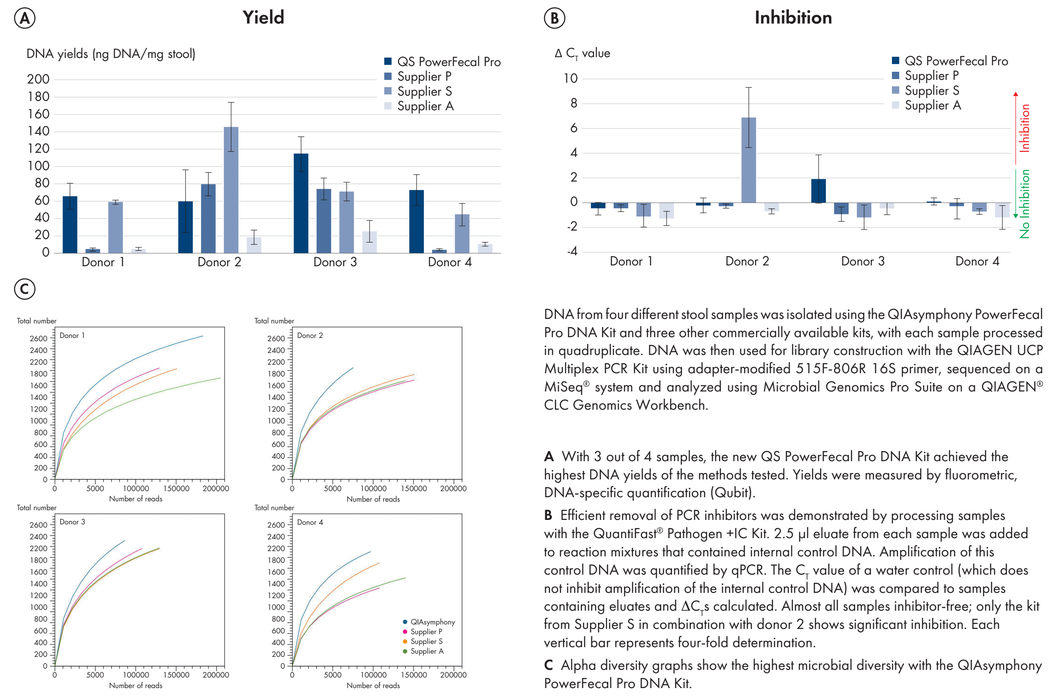
*stool is a very inhomogeneous sample material in which the DNA yields fluctuate.

A 100 mg of human stool (n=5) was prepared using the new QIAsymphony PowerFecal Pro DNA Kit, the manual PowerFecal Pro and the DNeasy 96 PowerSoil® QIAcube® HT Kit. Measurement by fluorometric quantification (Qubit®) showed robust yields for all three DNA extraction kits.

B 250 µl of saliva (n=3) was used for DNA isolation with the QIAsymphony PowerFecal Pro DNA Kit. Up to 2 µg highly pure microbial DNA was extracted depending on the donor. For DNA quantification, qPCR detection of 16S rDNA (QIAGEN UCP SYBR® Green 16S Quant Kit) was used, with a standard curve.

C The QIAsymphony PowerFecal Pro DNA Kit can also be used for isolation of microbial DNA from buccal swabs, though yields depend heavily on the donors. DNA quantification was performed with a 16S rDNA qPCR (QIAGEN UCP SYBR® Green 16S Quant Kit) and a standard curve.

Comparison of Extraction Methods for Stool Samples



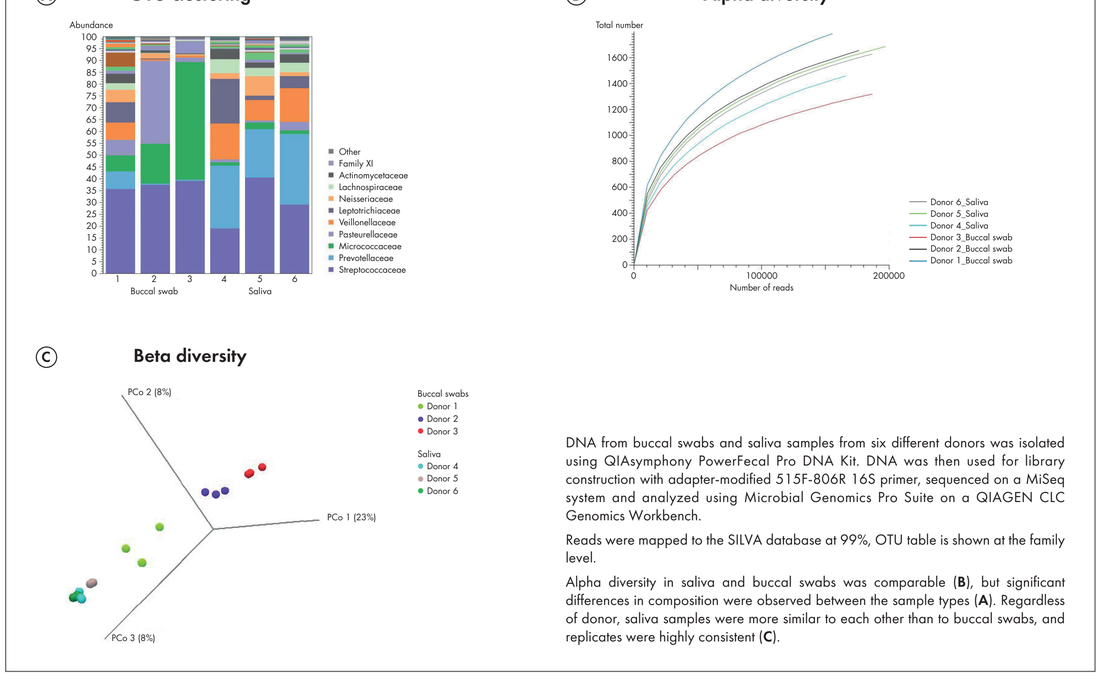
DNA from four different stool samples was isolated using the QIAsymphony PowerFecal Pro DNA Kit and three other commercially available kits, with each sample processed in quadruplicate. DNA was then used for library construction with the QIAGEN UCP Multiplex PCR Kit using adapter-modified 515F-806R 16S primer, sequenced on a MiSeq® system and analyzed using Microbial Genomics Pro Suite on a QIAGEN® CLC Genomics Workbench.

A With 3 out of 4 samples, the new QS PowerFecal Pro DNA Kit achieved the highest DNA yields of the methods tested. Yields were measured by fluorometric, DNA-specific quantification (Qubit).

B Efficient removal of PCR inhibitors was demonstrated by processing samples with the QuantiFast® Pathogen +IC Kit. 2.5 µl eluate from each sample was added to reaction mixtures that contained internal control DNA. Amplification of this control DNA was quantified by qPCR. The C_T value of a water control (which does not inhibit amplification of the internal control DNA) was compared to samples containing eluates and ΔC_Ts calculated. Almost all samples inhibitor-free; only the kit from Supplier S in combination with donor 2 shows significant inhibition. Each vertical bar represents four-fold determination.

C Alpha diversity graphs show the highest microbial diversity with the QIAsymphony PowerFecal Pro DNA Kit.

Diversity and Community in Buccal Swab and Saliva Samples



DNA from buccal swabs and saliva samples from six different donors was isolated using QIAsymphony PowerFecal Pro DNA 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 on a QIAGEN CLC Genomics Workbench.

Reads were mapped to the SILVA database at 99%, OTU table is shown at the family level.

Alpha diversity in saliva and buccal swabs was comparable (**B**), but significant differences in composition were observed between the sample types (**A**). Regardless of donor, saliva samples were more similar to each other than to buccal swabs, and replicates were highly consistent (**C**).

Conclusions

Highly multiplexed 16S sequencing enables studies of large sample size. Such studies require improved sample extraction techniques in order to enable true high-throughput studies. We have demonstrated that the QIAsymphony PowerFecal Pro DNA Kit provides a robust, high-performance DNA extraction that facilitates automation:

- Processing samples on the QIAsymphony SP Instrument enables mid-throughput extraction of DNA.
- DNA quality and yield is robust and reproducible.
- Accurate representation of microbial communities is maintained when extracting DNA in 96-well and automated procedures.
- Different sample materials (stool, swab, saliva, soil, etc.) can be effectively processed with the QIAsymphony PowerFecal Pro DNA Kit.

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

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Sample to Insight