Application Note

Automated DNA purification from diverse microbiome samples using dedicated microbiome kits on the QIAcube®

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This application note demonstrates the automation of QIAGEN's new line of DNA sample prep kits for the microbiome. The microbiome of samples as diverse as soil, water and stool was purified using dedicated QIAcube compatible kits. Automation on the QIAcube enabled efficient and reliable use of these samples for sensitive downstream applications such as qPCR and NGS. In addition, the CLC Microbial Genomics Module was successfully employed for metagenome sequencing and identification of microbial composition and diversity.

Introduction

The microbiome shapes our world and the majority of the microbes represent the biological 'dark matter' in this world. They are the often unknown communities within and around us. With the growing interest in the role of the microbiome in human health and disease, researchers are progressing in exploration of these previously unknown communities. A major challenge has been the inability to isolate or culture most microbes in a laboratory, thus making characterization more difficult and providing only a limited view of the vast microbial world. However, the advent of modern sequencing technologies has opened new doors for the analysis of complex microbial ecosystems. With our increasing understanding of the microbial world, it becomes clear that the composition of these communities acts at the interface between humans and their environment. Their interaction highlights differences between healthy and disease states, and there are emerging findings that the microbiome plays a role in a multitude of diseases such as diabetes, obesity and depression as well as infectious diseases and cancer.

The field of microbiome research continues to expand and operate at a much larger scale, creating unique challenges for analysis. Inherent complexities in the composition of microbiome samples, such as stool, soil, water and biofilm, can lead to inefficient lysis and result in an inaccurate representation of the microbial content. Additionally, these samples can contain small molecule inhibitors that may cause unreliable quantification of nucleic acids and negatively impactdownstream applications such as quantitative PCR (qPCR) and next-generation sequencing (NGS).

Optimized bead beating and patented Inhibitor Removal Technology® (IRT) are two innovative features of the new microbiome kits that have enabled lysis of even the toughest samples and successful removal of inhibitors during the purification process. Traditional, labor-intensive and ▷

time-consuming manual purification steps can now be replaced by automating the IRT and purification steps of these new microbiome kits on the QIAcube, saving valuable time and ensuring standardized results.

Material and methods

Experiment 1: Automated isolation of DNA from soil samples using the DNeasy® PowerSoil® Kit

DNA was isolated from 2 soil types, estuary and compost soil, either manually with the DNeasy PowerSoil Kit or by automating the kit on the QIAcube. For both protocols 250 mg of the soil samples were placed in PowerBead Tubes and vortexed for 10 minutes at maximum speed using a 24-sample vortex adapter. To allow for a reliable comparison between manual and automated sample processing, supernatants were pooled after mechanical lysis. This minimizes the effect of sample-to-sample variation when working with heterogeneous sample material such as soil. From this pool of supernatants, 450 µl per sample was then processed following the manual and QIAcube protocols according to the instructions in the *DNeasy PowerSoil Kit Handbook*. Briefly, for the manual protocol, after mechanical lysis, contaminants from the soil samples were removed using the patented IRT in 2 subsequent steps, followed by binding of DNA to a silica-based spin column, washing and elution of DNA in 100 µl of elution buffer. For the QIAcube protocol, after mechanical lysis, 450 µl per sample was transferred into position 2 of the rotor adapter which was then placed into the centrifuge of the QIAcube. All subsequent steps including the removal of inhibitory substances by precipitation were performed on the QIAcube. For each soil type, 12 samples were processed both manually and on the QIAcube. After isolation, DNA yield was determined by fluorometric quantification and DNA quality was assessed by measuring A_{260}/A_{280} ratios. In addition, the DNA was visualized by agarose gel electrophoresis. To quantify the presence of PCR inhibitors, a qPCR assay was performed using the QuantiFast® Pathogen +IC Kit. The DNA eluate containing potential inhibitors was added to a quantitative real-time PCR reaction containing an internal amplification control. The presence of inhibitors was determined by comparing the C_T values of reactions containing DNA eluate and controls.

Experiment 2: Automated isolation of DNA from filtered water samples using the DNeasy PowerWater® Kit

DNA was isolated from 2 water types, ocean and lagoon water, either manually with the DNeasy PowerWater Kit or by automating the kit on the QIAcube. Aliquots of 150 ml of ocean water and 75 ml of lagoon water per sample were filtered through 0.45 µm mixed cellulose ester membranes. The filter membranes were transferred into 5 ml PowerWater DNA Bead Tubes and stored at –20°C. The samples were lysed by placing the bead tubes in a 6-sample vortex adapter at maximum speed for 5 minutes. To minimize the effect of sample-to-sample variation while comparing the manual and automated DNA isolation, supernatants were pooled after mechanical lysis. From this pool, 600 µl of lysate per sample was used as the starting material for the manual and QIAcube protocols. DNA was extracted following the DNeasy PowerWater Kit protocol. Briefly, for the manual protocol, after mechanical lysis, inhibitory substances were removed using the patented IRT, followed by binding of DNA to a silica-based spin column, washing and elution of DNA in 100 µl of elution buffer. For the QIAcube protocol, after mechanical lysis, 600 µl per sample was transferred into position 2 of the rotor adapter which was then placed into the centrifuge of the QIAcube. All subsequent steps including the removal of inhibitors were automated on the QIAcube. For lagoon and ocean water, 6 and 4 samples, respectively, were processed both manually and on the QIAcube. After isolation, DNA yield was determined by fluorometric quantification and DNA quality was assessed by measuring A_{260}/A_{280} ratios. In addition, the DNA extracted from lagoon water was visualized by agarose gel electrophoresis. To quantify the presence of PCR inhibitors, a qPCR assay was performed using the QuantiFast Pathogen +IC Kit. The DNA eluate containing potential

inhibitors was added to a quantitative real-time PCR reaction containing an internal amplification control. The presence of inhibitors was determined by comparing the C_T values of reactions containing DNA eluate and controls.

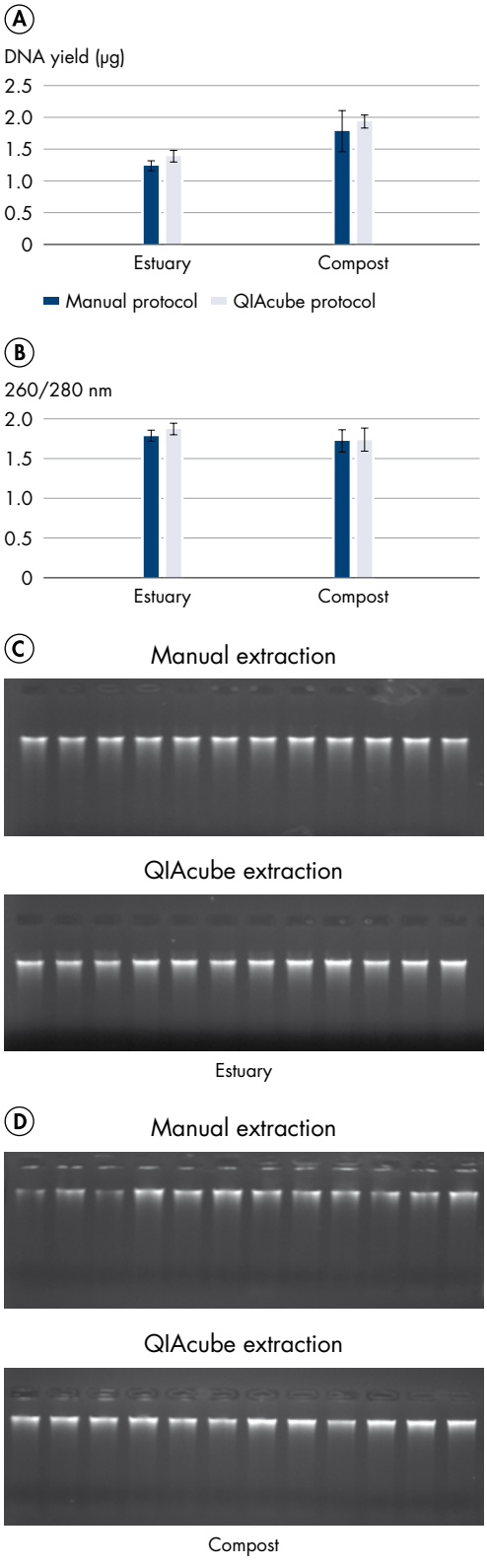
Experiment 3: Automated, low-throughput workflow for microbial analyses of human stool samples using the QIAamp® PowerFecal® DNA Kit and the CLC Genomics Workbench

DNA was isolated from 10 individual human stool samples by automating the QIAamp PowerFecal DNA Kit on the QIAcube. A 200 mg aliquot of stool was placed in Dry Bead Tubes, followed by addition of 750 µl of PowerBead Solution and vortexing of samples for 10 minutes at maximum speed using a 24-sample vortex adapter according to the *QIAamp PowerFecal DNA Kit Handbook*. After lysis, 450 µl of supernatant was transferred into position 2 of the rotor adapter which was then placed into the centrifuge of the QIAcube. All subsequent steps were carried out by the instrument. After isolation, DNA yield was quantified by a fluorometric assay and DNA quality was assessed by measuring A_{260}/A_{280} ratios. In addition, the DNA was visualized by agarose gel electrophoresis. To quantify the presence of PCR inhibitors, a qPCR assay was performed using the QuantiFast Pathogen +IC Kit. The DNA eluate containing potential inhibitors was added to a quantitative real-time PCR reaction containing an internal amplification control. The presence of inhibitors was determined by comparing the C_T values of reactions containing DNA eluate and controls. The resulting DNA was subsequently used for library construction for 16S rRNA gene sequencing as well as whole metagenome sequencing.

For 16S library preparation, modified 515fB and 806rB primers were used to amplify the V4 region of the 16S gene (1, 2, 3, 4). For PCR amplification of the V4 region of the 16S gene, we used the QIAGEN Multiplex PCR master mix, 0.2 µM of each primer and 50 ng of template DNA. The cycling conditions were set to an initial 15 minutes activation step at 95°C, 22 cycles of denaturation (94°C, 30 seconds), annealing (55°C, 90 seconds) and extension (72°C, 60 seconds) steps, followed by a final extension step at 72°C for 30 minutes. The 16S amplicons were purified by a bead-based approach and quantified using a fluorometric assay. Sequencing adapters were then added using the QIASeq™ 1-Step Amplicon Kit. For adapter ligation, 500 ng of amplicon per sample was used as the starting amount. Adapter ligation and library purification were performed according to the kit instructions. Individual samples were labeled with a 6 bp barcode (GeneRead™ 12-plex adapter). All 16S libraries were quantified using the QIASeq Library Quant Kit and paired-end sequencing (2 x 250 bp) was performed on a benchtop sequencer.

For whole metagenome sequencing (WGS), libraries were prepared using the QIASeq FX DNA Library Kit according to the instructions. Briefly, 500 ng of DNA was used as input for the FX fragmentation reaction and incubated for 10 minutes at 32°C, to produce a target fragment peak size of 350 bp. Sequencing adapters were directly added to the fragmentation reaction product. The ligation reaction and library purification steps were performed according to the QIASeq FX DNA Library Kit instructions. The WGS libraries were then quantified using the QIASeq Library Quant Kit and paired-end sequencing (2 x 250 bp) was performed on a benchtop sequencer.

For bioinformatics analysis, the CLC Microbial Genomics Module as part of the CLC Genomics Workbench was used. For 16S data analysis, raw sequencing reads were imported into the CLC Microbial Genomics Module and then the OTU 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 Greengenes database and clustering at 97% identity. Next, OTUs were aligned using muscle and used to construct a Maximum Likelihood phylogenetic tree, followed by alpha and beta diversity analyses. For WGS data analysis, the raw sequencing reads were imported and *de novo* assembled into contigs using the whole metagenome analysis module. Genes and coding DNA sequences were then identified ▷



using MetaGeneMark and functionally annotated with Gene Ontology (GO) terms and Pfam (protein families) domains. Finally, functional profiles were built based on the identified GO terms, which were then statistically analyzed and visualized. For both 16S and WGS analyses, all analysis steps were performed according to the standard specifications in the CLC Microbial Genomics Module.

Results and discussion

Experiment 1: Comparable DNA yield and quality from soil samples with manual and automated protocols of the DNeasy PowerSoil Kit

The performance of the DNeasy PowerSoil Kit protocols on the QIAcube was measured. Yield and purity of DNA isolated using the manual and automated protocols were compared for the 2 different soil types. DNA isolated either manually or on the QIAcube instrument was of comparable yield and quality for both estuary and compost soil samples (Figure 1).

A test for the efficient removal of small molecule PCR inhibitors which can interfere with downstream applications was also performed. Quantitative qPCR analysis confirmed that DNA isolated from both soil types using either the manual or the automated protocol had similar or fewer inhibitors present in the eluates (Figure 2). In particular, the QIAcube protocol completely removed inhibitors from typically inhibitor-rich compost.

Figure 1. Comparable DNA yield and purity between QIAcube and manual protocols of the DNeasy PowerSoil Kit. A DNA yield was measured using a fluorometric-based assay. **B** DNA purity was determined by spectrophotometric measurements at 260 and 280 nm. Displayed is the average yield and purity of 12 samples per soil type per protocol. The standard deviation is shown for each condition. **C** DNA extracted from 250 mg estuary and **D** compost soil was visualized on a 1% TAE agarose gel. Manual and automated DNA extractions are displayed in the upper and lower half of the gel, respectively. Aliquots of 4 and 5 µl from each sample of estuary and compost soil types, respectively were applied to the gel.

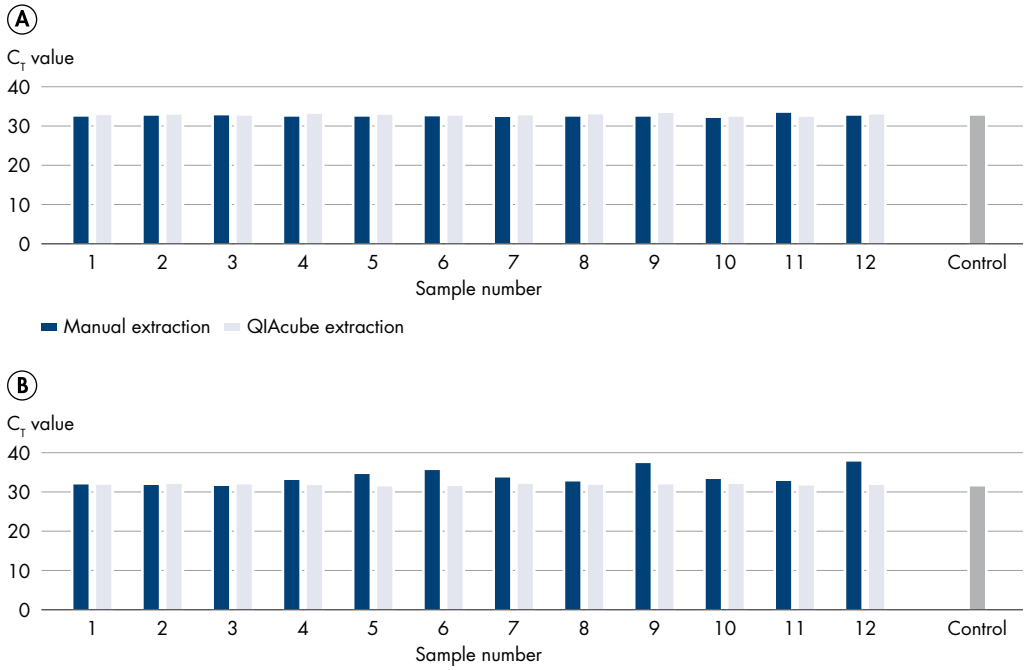


Figure 2. Efficient removal of PCR inhibitors was demonstrated by processing samples isolated with the manual and QIAcube DNeasy PowerSoil Kit protocols using the QuantiFast Pathogen +IC Kit. Aliquots of **A** 7 µl eluate from each estuary sample or **B** 5 µl eluate from each compost sample were added to reaction mixtures containing internal control DNA. Amplification of this control DNA was quantified by qPCR. The C_T values of a water control that does not inhibit amplification of the internal control DNA were compared to samples containing eluates processed using the DNeasy PowerSoil protocol. Eluates from both the manual and the QIAcube protocols did not significantly inhibit amplification of the control DNA as can be seen by a C_T value comparable to the control. Each vertical bar represents an individual sample.

Taken together, these data show that automation of the DNeasy PowerSoil Kit on the QIAcube performs equally well as the manual version of the kit. Automation significantly reduces the hands-on time required 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 NGS and qPCR.

Experiment 2: Comparable DNA yield and quality from water samples with manual and automated protocols of the DNeasy PowerWater Kit

The performance of the DNeasy PowerWater Kit protocols on the QIAcube was measured. Yield of DNA isolated using the manual and automated protocols were compared for the 2 different water types (Figure 3A–B). The two water types were chosen based on their differences in biomass (ocean = low, lagoon = high) and because lagoon water is particularly known to have a high content of difficult-to-remove inhibitors. Purity of DNA isolated using manual and automated protocols were compared for the lagoon water sample alone (Figure 3C–D). DNA isolated either manually or on the QIAcube instrument was of comparable yield and quality for both ocean and lagoon water samples.

A test for the efficient removal of small molecule PCR inhibitors which can interfere with downstream applications was also performed. Quantitative qPCR analysis confirmed that DNA isolated from lagoon water samples using either the manual or the automated protocol were free of inhibitors (Figure 4). In particular, the QIAcube protocol completely removed inhibitors from typically inhibitor-rich lagoon water samples.

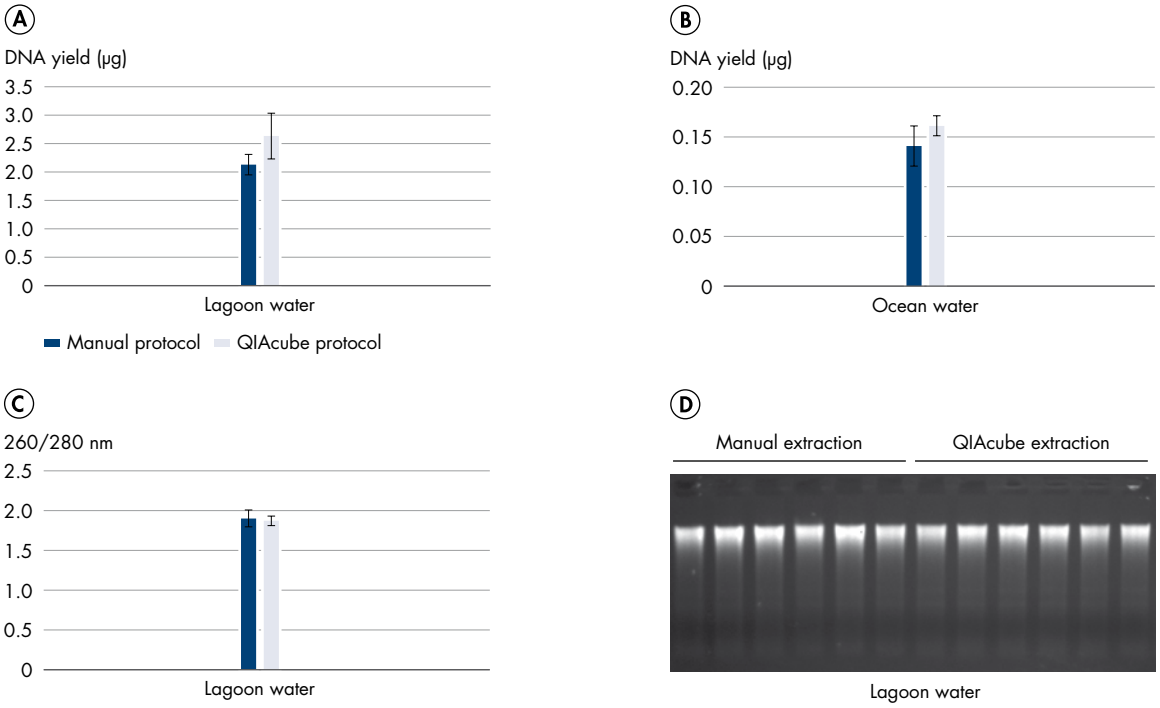


Figure 3. Comparable DNA yield and purity between QIAcube and manual protocols of the DNeasy PowerWater Kit. DNA was isolated from 75 ml lagoon water and 150 ml ocean water using the manual and the QIAcube protocols of the DNeasy PowerWater Kit. **A-B** DNA yield from the two water types compared between the protocols and **C** DNA quality of lagoon water sample compared between the two protocols. DNA yield was determined by a fluorometric assay. Displayed is the average yield of 6 lagoon water samples and 4 ocean water samples per protocol. DNA purity was determined by spectrophotometric measurement at 260 and 280 nm. The ratio of 260/280 nm indicates comparable DNA purity between the manual and the QIAcube protocol. Displayed is the average including standard deviation. **D** DNA extracted from 75 ml lagoon water was visualized on a 1% TAE agarose gel. DNA extracted with the manual protocol is displayed in the first six lanes and the QIAcube-extracted DNA is displayed in the next six. An aliquot of 5 µl per sample of lagoon water was applied to the gel.

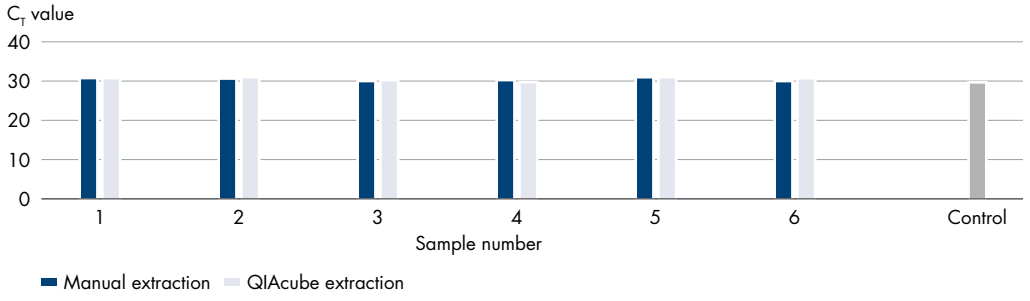


Figure 4. Efficient removal of PCR inhibitors was demonstrated by processing samples isolated with the manual and QIAcube DNeasy PowerWater Kit protocols using the QuantiFast Pathogen +IC Kit. Aliquots of 5 µl eluate from lagoon water samples were added to reaction mixtures containing internal control DNA. Amplification of this control DNA was quantified by qPCR. The C_T value of a water control that does not inhibit amplification of the internal control DNA was compared to samples containing eluates processed using the DNeasy PowerWater protocol. Eluates from both the manual and the QIAcube protocols did not significantly inhibit amplification of the control DNA as can be seen by a C_T value comparable to the control.

Taken together, these data show that automation of the DNeasy PowerWater Kit on the QIAcube performs equally well as the manual version of the kit. Automation significantly reduces the hands-on time required for removal of inhibitory substances and spin-column-based DNA isolation. Thehigh-quality and high-purity DNA can be used directly for downstream applications, including NGS and qPCR.

Experiment 3: Automated, low-throughput workflow for microbial analyses of human stool samples identifies differences between young and old individuals

A healthy gut microbiota is typically characterized by large bacterial taxonomic diversity and functional capacity, whereas frailty and aging are associated with loss of diversity and expansion of more pathogenic bacterial species (5). However, in order to accurately profile changes in microbial communities, the reproducible isolation of high-quality DNA is a critical step. Automation of the QIAamp PowerFecal DNA Kit on the QIAcube allows for fast and reproducible isolation of high-quality DNA from stool sampleswith minimal hands-on time. Isolated DNA can be used directly in NGS applications. In this application note, we present an automated workflow to profile the gut microbiota of young and old individuals. As input, 10 human stool samples were used, 5 samples from individuals in the age group of 28–48 years with an average age of 34 (young) and 5 samples from individuals between 65 and 78 years with an average age of 74 (old). DNA isolated with the QIAcube compatible QIAamp PowerFecal DNA Kit protocol was of sufficient yield and quality as well as free of inhibitors (Figure 5), thus making it directly suitable for NGS library preparation.

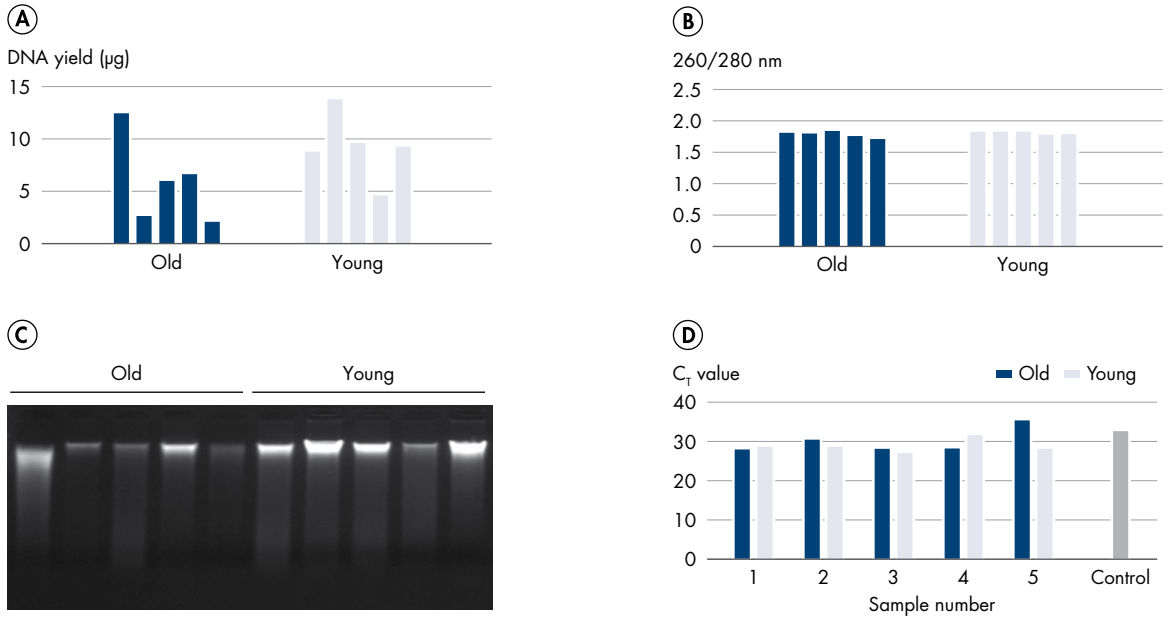


Figure 5. Isolation of high-quality, inhibitor-free DNA with the QIAamp PowerFecal QIAcube protocol. DNA was isolated from 200 mg of human stool samples from old and young individuals. **A** DNA yield was determined by a fluorometric assay. **B** DNA purity was determined by spectrophotometric measurements at 260 and 280 nm. **C** Isolated DNA from old and young individuals were visualized on a 1% TAE agarose gel. Five microliters of samples were applied. **D** PCR inhibition was measured using the QuantiFast Pathogen PCR +IC Kit. Aliquots of 5 µl eluates from human stool samples were added to reaction mixtures containing 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 processed using the QIAamp PowerFecal DNA Kit protocol. Most eluates did not significantly inhibit amplification of the control DNA as indicated by the C_T values comparable to the control.

To determine the microbial composition of human stool samples from young and old individuals, we performed both 16S rRNA gene and whole metagenome sequencing. The 16S rRNA gene sequencing can be 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. The 16S analysis revealed differences in bacterial composition between young and old individuals characterized by an increase in *Fusobacterium* and *Anaerostipes* with age (Figure 6A). Diversity analyses revealed that the microbiota from young individuals is more diverse than that of old individuals as measured by the number of Observed Taxonomic Units (OTUs) and that these communities are significantly different from one another (Figure 6B–C). In sum, 16S microbial analyses identified that the gut microbial communities are remarkably different between young and old individuals and that young individuals have a more diverse gut microbiota.

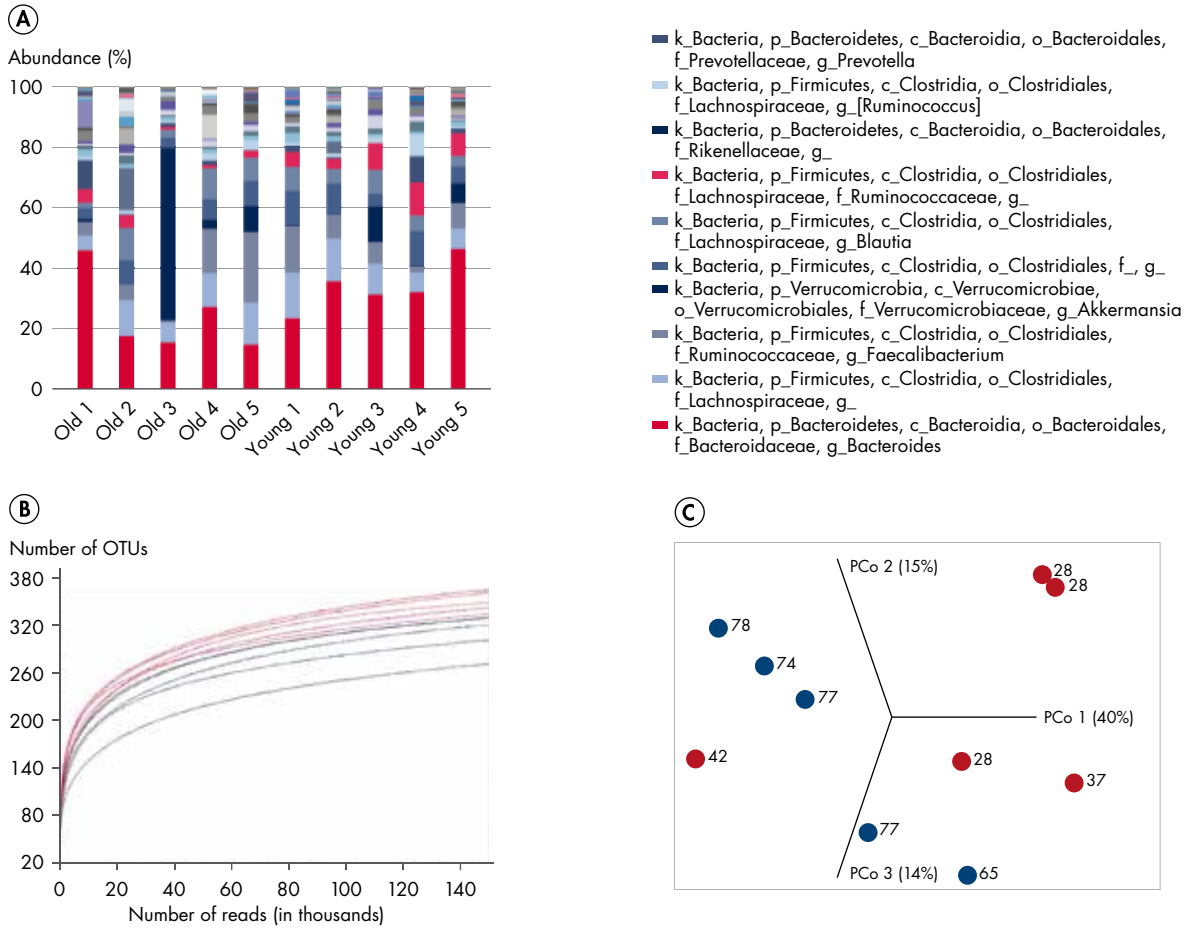


Figure 6. 16S data analysis with the CLC Microbial Genomics Module identified differences in microbial community composition and diversity between young and old individuals. FASTQ files were imported into the CLC Genomics Workbench and processed with the Microbial Genomics Module using the OTU clustering workflows. **A** Taxonomic assignment to determine bacterial composition was performed by mapping sequences against the Greengenes database and clustered at 97% identity. Sequences that did not map were then clustered *de novo*. Results are summarized at the genus level. **B** Alpha diversity analysis revealed that the human stool microbiota from young individuals is more diverse compared to older individuals. Diversity was measured by the number of bacteria identified. Each line represents a single individual. Sampling depth = 150,000. **C** Unweighted UniFrac analysis was used to measure beta diversity and revealed that the gut microbiota from young individuals is significantly different from that of the old. Blue indicates old individuals whereas pink indicates young individuals. Individual ages are also displayed. P = 0.047 as determined by PERMANOVA.

In a following experiment, WGS was used to analyze these microbial communities. WGS can be used to determine the taxonomic composition, similar to 16S data, and to analyze the metagenome of a microbial community in order to determine its functional capacity. We compared the metagenomes from young and old individuals using the CLC Microbial Genomics Module. This analysis revealed marked differences between the total functional capacity of young and old metagenomes which tended to cluster separately with some overlap (Figure 7).

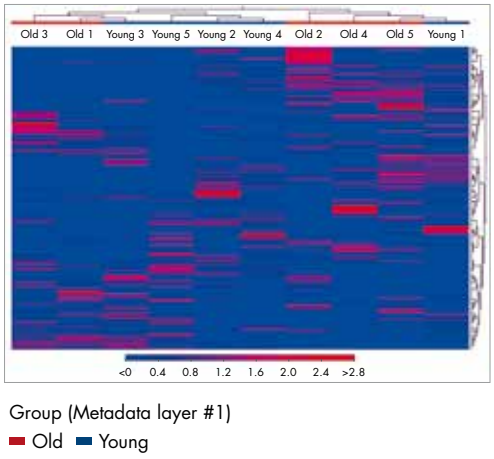


Figure 7. Whole metagenome analysis with the CLC Microbial Genomics Module identified differences in the microbiomes of young and old individuals. Raw NGS sequences were imported into the CLC Genomics Workbench and processed with the Microbial Genomics Module using the whole metagenome analysis workflows. A heatmap and dendrogram based on Euclidean distance were used to assess the similarities between the samples. The heat map shows the functional profiles comparing metagenomes from young and old individuals.

A differential abundance analysis was performed comparing the mean number of mapped reads for young and old metagenomes to identify specific functional categories that differ between the two metagenomes. A number of functional categories, based on Gene Ontology terms, were identified to be overrepresented in either the young or the old individuals. Listed here are top 10 differences in either young or old metagenomes (Table 1). This analysis is particularly useful in determining specific and significant differences between groups of interest.

Table 1. Differential abundance analysis comparing metagenomes from young and old individuals.

Name	Max group mean	Fold change	P-value
RNA ligase activity	151.20	–3.12	0.0002
Cellular potassium ion transport	47.00	32.84	0.0008
Potassium ion transmembrane transport	47.00	32.80	0.0008
Acetyltransferase activity	1229.80	–1.30	0.0010
N-acyltransferase activity	1039.00	–1.35	0.0010
Aminoglycoside 3-N-acetyltransferase activity	22.60	–188.37	0.0011
Aminoglycoside N-acetyltransferase activity	22.60	–188.37	0.0011
N-acetyltransferase activity	1039.00	–1.35	0.0011
Cysteine-type peptidase activity	615.20	1.48	0.0014
Plasmid maintenance	145.20	12.85	0.0014
Galacturonan metabolic process	16.80	–137.19	0.0022
Carbon-oxygen lyase activity, acting on polysaccharides	16.80	–137.19	0.0022
Pectin catabolic process	16.80	–137.19	0.0023
Pectin metabolic process	16.80	–137.19	0.0023
Oligogalacturonide lyase activity	15.80	–129.63	0.0026
Lysozyme activity	178.20	1.69	0.0178
Acireductone dioxygenase [iron(II)-requiring] activity	8.00	72.76	0.0178
Cell wall macromolecule catabolic process	178.20	1.69	0.0180
Deoxyribonucleotide metabolic process	323.00	1.77	0.0191
External encapsulating structure part	7.60	57.94	0.0202
3-beta-hydroxy-delta5-steroid dehydrogenase activity	6.00	49.64	0.0214

Collectively, the results presented here show an easy-to-use and efficient automated workflow for isolation of high-quality DNA from human stool samples which is free of inhibitors and can be used directly in downstream NGS applications. This workflow will help simplify and streamline the DNA extraction process and library construction from samples with high inhibitor content. Furthermore, the CLC Microbial Genomics Module provides a highly accurate and easy-to-use bioinformatics package for subsequent analyses to determine the bacterial composition, diversity and functional capability as demonstrated here with the example of microbial communities in young and old individuals.

Conclusions

- Automation of the DNeasy PowerSoil, DNeasy PowerWater and the QIAamp PowerFecal DNA protocols on the QIAcube allows efficient isolation of high-quality DNA from inhibitor-rich samples with minimal hands-on time.
- DNA extracted from diverse sample types such as soil, water and stool using the QIAcube is of high quality and purity, ready for sensitive downstream applications such as qPCR and NGS.
- Additional protocols for a range of sample materials with Inhibitor Removal Technology are available on the QIAcube, including the DNeasy PowerLyzer® PowerSoil Kit, DNeasy PowerPlant® Pro Kit, DNeasy PowerClean® Pro Cleanup Kit, DNeasy Ultraclean® Microbial Kit and the RNeasy® PowerMicrobiome® Kit.

References

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Ordering Information

Product	Contents	Cat. no.
QIAcube	Robotic workstation for automated purification of DNA, RNA or proteins using QIAGEN spin-column kits: includes 1-year warranty on parts and labor	Inquire
DNeasy PowerSoil Kit (50)*	For the isolation of microbial genomic DNA from all soil types	12888-50
DNeasy PowerWater Kit (50)*	For the isolation of genomic DNA from filtered water samples, including turbid water	14900-50-NF
PowerBead Tubes, Glass 0.1 mm (50)	Ready-to-use bead tubes for rapid and reliable biological sample lysis from a wide variety of starting materials	13118-50
PowerWater DNA Bead Tube (50)	Bead Tubes for the DNeasy PowerWater Kit	14900-50-NF-BT
PowerBead Solution	For dispersing sample particles, homogenization and lysis	12955-4-BS
Dry Bead Tubes (50)	Bead Tubes for the QIAamp PowerFecal DNA Kit	12830-50-BT
QIAamp PowerFecal DNA Kit	For the isolation of DNA from stool, gut material and biosolids	12830-50
QIAseq FX DNA Library Kit (24)*	Buffers and reagents for DNA fragmentation (including end repair and A-addition), ligation and library amplification; for use with Illumina instruments; includes a plate containing 24 adapters with different barcodes (pierceable foil seal, allowing usage of defined parts of plate)	180473
QIAseq 1-Step Amplicon Library Kit (12)*	For 12 reactions: 1-Step Amplicon Enzyme Mix, 4x 1-Step Amplicon Buffer, Primer Mix Illumina Library Amp, HiFi PCR Master Mix, RNase-Free Water. Adapters sold separately	180412
QIAseq Library Quant System*	For qPCR-enabled quantification of NGS libraries	QSTF-ILZ-Q
QuantiFast Pathogen PCR +IC Kit (100)*	For 100 x 25 µl reactions: Master Mix, lyophilized Internal Control Assay, lyophilized Internal Control DNA, ROX Dye Solution, High-ROX Dye Solution, RNase-Free Water, Nucleic Acid Dilution Buffer, Buffer TE	211352
Vortex Adapter for 24 (1.5–2.0 ml) tubes	For vortexing 1.7, 2, 5, 15 and 50 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-24
Vortex Adapter for 6 (5.0 ml) tubes	For vortexing 1.7, 2, 5, 15 and 50 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-5

* Other kit sizes or formats available; see **www.qiagen.com**.

The QIAcube and all the kits mentioned here are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease. The applications described here are not intended for diagnostic use. No claim or representation is intended to provide information for the diagnosis, prevention or treatment of a disease.

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