Product Profile

QIAseq® 16S/ITS Panels

Profiling bacterial and fungal communities using next-generation sequencing

Next-generation sequencing (NGS) of bacterial 16S ribosomal RNA (rRNA) genes or fungal internal transcribed spacer (ITS) regions to profile microbial communities can present several challenges. The new QIAseq 16S/ITS Panels incorporate library prep and bioinformatics solutions to provide a Sample-to-Insight pathway for the robust profiling of bacterial and fungal communities by sequencing 16S rRNA genes and ITS regions on Illumina® platforms. Compared to other approaches, using the QIAseq 16S/ITS Panels provides several advantages, including:

- The power to interrogate all 16S rRNA gene variable regions and fungal ITS regions
- Phased primers that increase the quality of reads and base calling and eliminate the need for PhiX spike-in
- Low bioburden reagents that decrease background contamination
- The ability to monitor library construction steps and user-introduced contamination
- DNA inputs as low as 1 pg to enable analyses of low biomass samples

A brief introduction to 16S rRNA genes and ITS regions

The universal distribution and conserved nature of bacterial 16S rRNA genes and fungal ITS regions have established both as genetic markers that researchers routinely use to identify and classify bacteria and fungi and successfully profile microbial communities.

The 16S rRNA gene consists of both highly conserved and hypervariable regions (Figure 1). The conserved regions serve as primer binding sites for the PCR amplification of the variable regions, which contain sequences that can be used for bacterial identification and classification. ▷

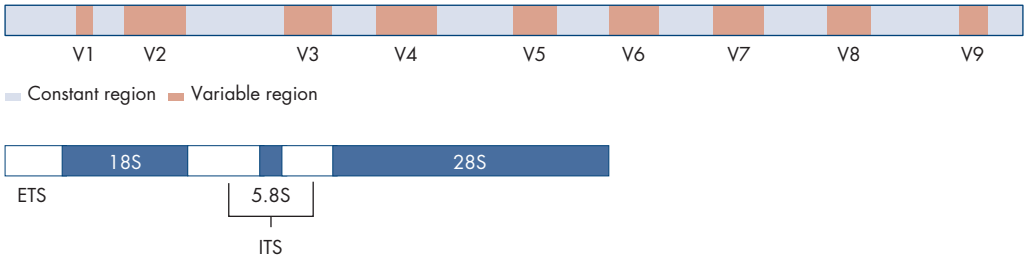

Figure 1. Structure of the bacterial 16S rRNA gene (top) and the fungal ITS region (bottom).

The ITS region is situated between the small and large rRNA subunits. In eukaryotes, there are two ITS regions. ITS1 is located between the 18S rRNA and 5.8S rRNA genes, and ITS2 is located between the 5.8S rRNA and 28S rRNA genes (Figure 1). Identification using ITS regions has the highest probability of success for the broadest range of fungi.

Benefits of using NGS to analyze 16S rRNA genes and ITS regions

NGS provides a culture-free method to analyze microbial communities within biological samples. Libraries from several biological samples can be multiplexed on the same sequencing run, which enables researchers to analyze hundreds of microbial communities simultaneously and cost-effectively.

Using NGS to analyze universal marker genes such as the 16S rRNA genes and the ITS regions is a powerful method for surveying microbial communities, including complex microbiomes. This method allows researchers to profile the majority of microbial taxa in a sample – even those that cannot be cultured – through a single analysis, classify microbes more quickly and accurately than with traditional methods and execute comparative studies of different microbial communities.

Challenges with NGS of 16S rRNA genes and ITS regions

NGS of 16S rRNA genes and ITS regions can present several challenges:

- Poor-quality reads and base calling caused by reduced library complexity
- Inefficient use of flow cell throughput due to the need for considerable amounts of phiX DNA
- Reduced classification specificity for many bacterial taxa due to incomplete coverage of 16S rRNA variable regions
- High background noise because of contaminated reagents

The QIAseq 16S/ITS Panels can help researchers overcome challenges

The QIAGEN QIAseq 16S/ITS Panels can help overcome challenges presented by the sequencing of 16S rRNA genes and ITS regions and deliver a complete Sample-to-Insight solution for profiling microbial communities.

High-quality read and base calling

Sequencing single-amplicon libraries often yields low quality results due to the reduced diversity of base composition in the primer regions (Figures 3A and 3C; page 3). To overcome this issue, the QIAseq 16S/ITS Panels use phased primers (Figure 2), which include up to 11 additional bases at their 5'-ends. The use of phased primers shifts nucleotide balance and increases base diversity, which leads to increases in quality scores (Figures 3B and 3D; page 3).

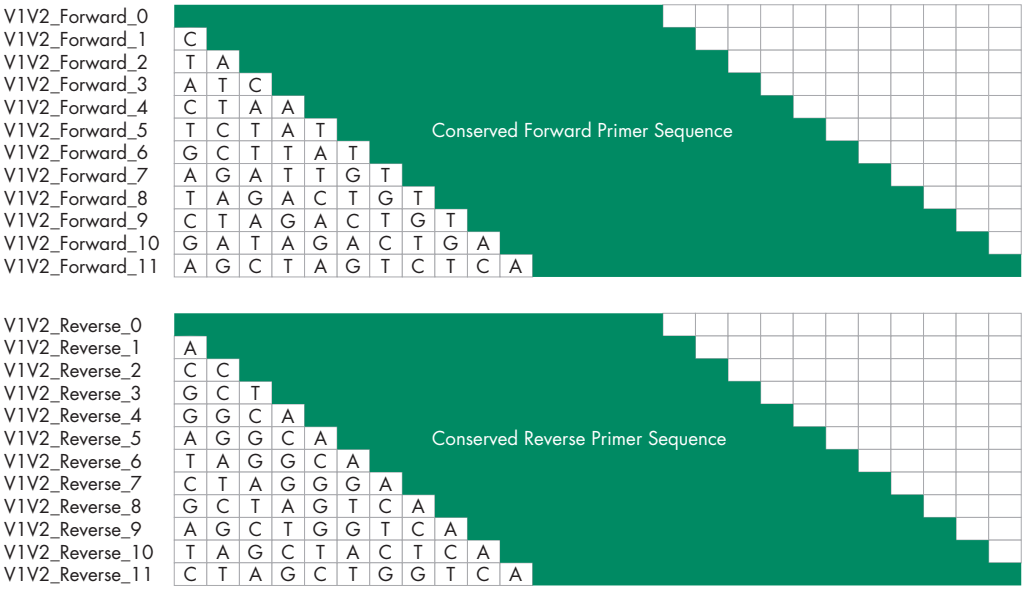

Figure 2. Structure of phased primers used in QIAseq 16S/ITS Panels. Sequences shown may not represent actual primer sequences.

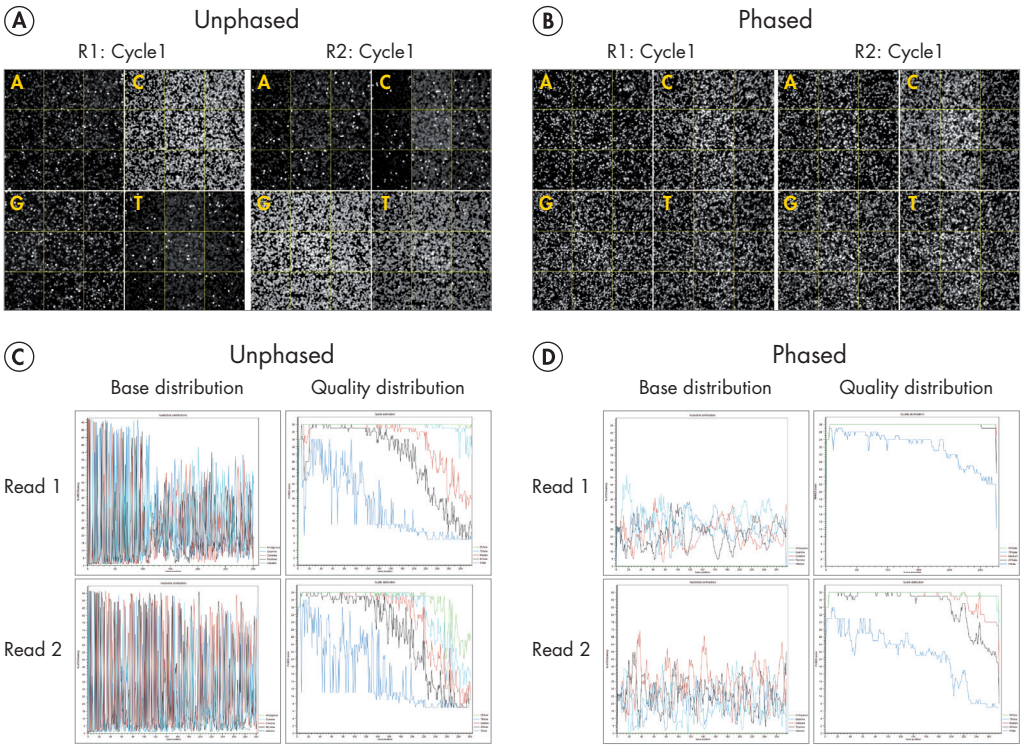

Figure 3. QIAseq 16S/ITS Panels employ phased primers to increases base diversity and quality scores. Fluorescent images (**top row**) taken from the first cycle of read 1 (**R1**) showing uneven base distribution in an unphased primer run **A** and more even base distribution in a phased primer run **B**. Low base diversity and read quality scores (**bottom row**) are apparent in a run using unphased V3V4 primers **C** and significantly improved using phased V3V4 primers **D**.

Efficient use of flow cell throughput

Using phased primers makes libraries produced with the QIAseq 16S/ITS Panels sufficiently complex to eliminate the need for PhiX spike-in, which enables efficient use of flow cell throughput.

Robust classification specificity and detection of microbial species

Identifying members of a bacterial community, based on analysis of the 16S rRNA gene, can depend on which variable regions are sequenced. For example, *Streptococcus mutans* cannot be identified by sequencing variable regions V1V2, V2V3, V3V4 and V4V5. However, the QIAseq 16S/ITS Panels comprehensively profile all *S. mutans* 16S rRNA variable regions, which enable researchers to classify this bacterium (Figure 4; red arrows).

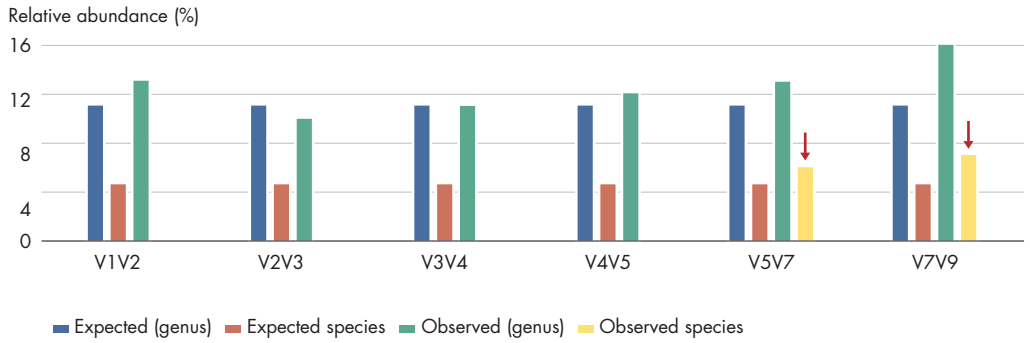

Figure 4. Screening a panel of variable regions provides more robust bacterial profiling compared to screening only individual variable regions. QIAseq 16S/ITS Panels were used to generate libraries from the ATCC® 20 Strain Even Mock Community. Demultiplexing of the variable regions was performed using the CLC Microbial Genomics Workbench and the QIAseq 16S/ITS plugin. Classification was performed for each of the variable regions at the species level using the SILVA database. Results are shown only for *Streptococcus mutans*. Only a subset of the variable regions (**red arrows**) can be used to classify *S. mutans*.

Low background noise

Microbial DNA is present in every corner of our lives, which increases the risk of contamination during the handling and processing of biological samples. Also, manufacturing and processing of enzymes and reagents can introduce exogenous bacterial DNA into samples. Background contamination can decrease the robustness of microbial profiling. The QIAseq 16S/ITS Panels use reagents with low bioburden, which show very low levels of exogenous bacterial/fungal contamination in no template control (NTC) analyses (Figure 5).

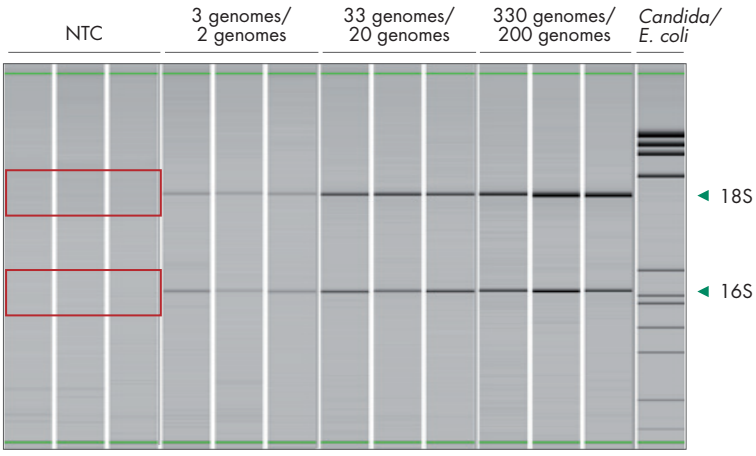

Figure 5. The QIAseq 16S/ITS Panels have very low levels of background contamination due to the use of reagents with low bioburden. Contamination levels were monitored by amplifying with PCR primers targeting 16S (bacteria) and 18S (fungal) rRNA genes with UCP Multiplex Mastermix and UCP PCR Water. 38 cycles of PCR were performed and the reaction was run on a QIAxcel to detect the 16S and 18S amplicons.

Small amounts of input DNA

The QIAseq 16S/ITS Panels can be used with inputs ranging between 1 pg and 1 ng bacterial DNA, allowing users to profile bacterial communities in samples with low biomass (Figure 6).

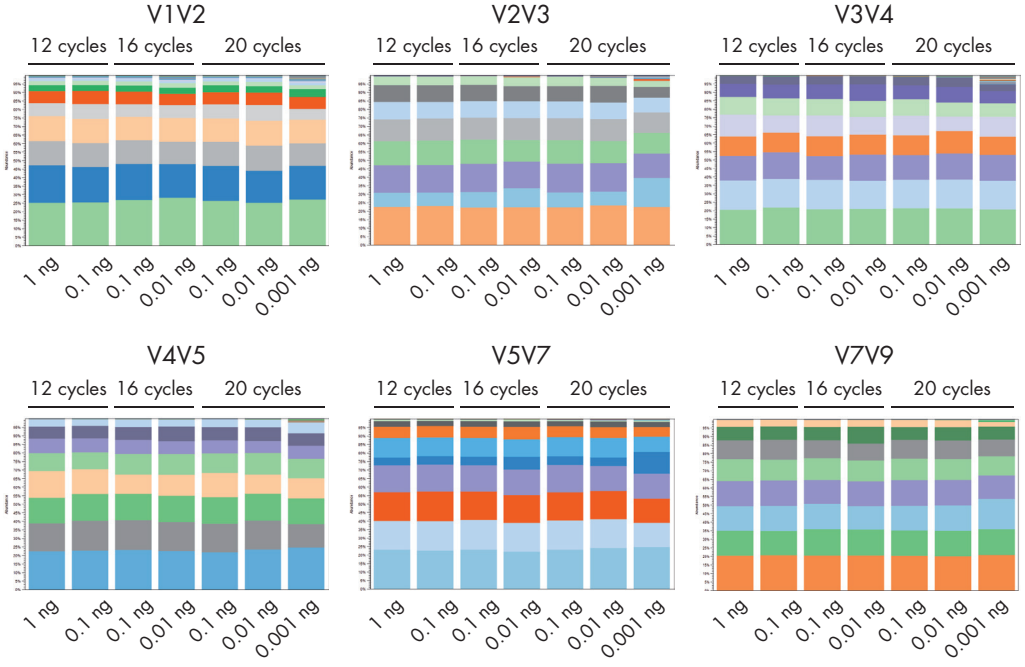

Figure 6. The QIAseq 16S/ITS Panels can be used with as little as 1 pg of input DNA. Serial dilutions of a mixed bacterial DNA sample were used to generate libraries using the QIAseq 16S/ITS Region Panel. PCR for 12, 16 or 20 cycles was carried out followed by index PCR. The CLC Microbial Genome Module and SILVA database were used to perform classification.

QIAseq 16S/ITS products, workflow and data analysis

Products

Panels

QIAseq 16S/ITS Region Panels (cat. no. 333802 or 333805) contain phased primers to analyze the variable regions of the 16S rRNA gene or ITS region. Users can select up to three variable regions using a custom primer builder at **www.qiagen.com/16Scustom**. Primers for the selected regions will be included, separately, in the kit ordered. Each kit also contains low-bioburden mastermix and water.

Available regions: V1V2, V2V3, V3V4, V4V5, V5V7, V7V9 and ITS

QIAseq 16S/ITS Screening Panels (cat. no. 333812 or 333815) can be used to interrogate all nine variable regions of the 16s rRNA gene or ITS region. Each panel contains phased primers, low-bioburden mastermix and water.

Indices

In addition to the QIAseq 16S/ITS Panels, a set of indices is required to generate libraries that can be quantified, qualified and sequenced.

The QIAseq 16S/ITS 24-Index (cat. no. 333822) can be used to multiplex up to 24 samples on a sequencing run. As each index can be used to process four samples, the kit contains enough indices to process up to 96 samples. The QIAseq 16S/ITS 96-Index (cat. no. 333825) can be used to multiplex up to 96 samples on a sequencing run. As each index can be used to process four samples, the kit contains enough indices to process up to 384 samples.

Smart Control

QIAseq 16S/ITS Smart Control (cat. no. 333832) contains synthetic DNA to assess library construction steps and user-introduced contamination while using QIAseq 16S/ITS Panels.

The Smart Control DNA contains primer binding sites from *E. coli*. Artificial sequences originating from *Arabidopsis thaliana* replace the hypervariable 16S rRNA region between the primer binding sites (Figure 7). The artificial sequence cannot be classified as bacterial or fungal. Therefore, any sequences that are classified as bacterial or fungal will be due to environmental contamination introduced during library construction.

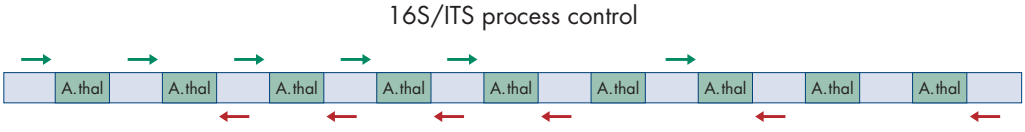

Figure 7. Structure of the QIAseq 16S/ITS Smart Control DNA.

Workflow

The QIAseq 16S/ITS Panels utilize a 2-stage PCR workflow. Starting with DNA extracted from microbial communities, the 16S rRNA variable and ITS regions are first enriched using phased primers. After a round of bead clean-up, library adapters are added onto the ends of amplicons in the second stage. Finally, another round of bead clean-up yields libraries that can be quantified and sequenced (Figure 8). The recommended sequencing chemistry is V3 2x300.

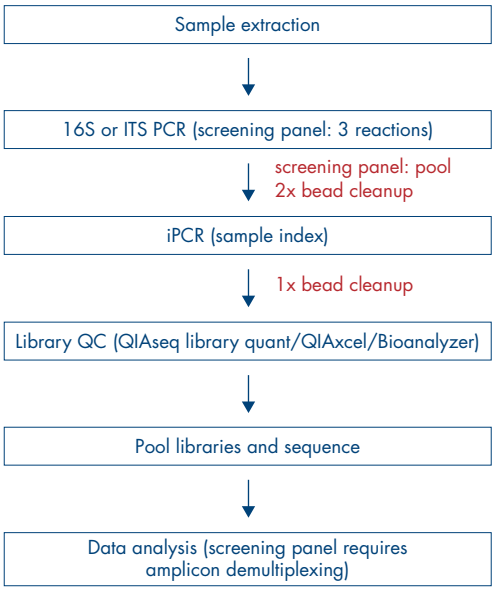

Figure 8. Workflow of the QIAseq 16S/ITS Panels.

Multiplexing

Each QIAseq 16S/ITS Panel can be used to multiplex up to 96 samples on a MiSeq® run using the V3 sequencing chemistry at 2x300. The appropriate QIAseq 16S/ITS Index Kit (cat no. 333822 or 333825) is necessary to multiplex the required number of samples.

Data analysis

Post sequencing, data are analyzed using QIAGEN Bioinformatics CLC Genomic Workbench (CLC GWB) and Microbial Genomics Pro Suite Module. A custom workflow, which can be further edited as needed, is available to automate FASTQ file import, amplicon demultiplexing, quality-controlled filtering and trimming, OTU clustering and secondary bioinformatics analysis.

QIAGEN Bioinformatics CLC Genomics Workbench with Microbial Genomics Pro Suite Module enables researchers to output standardized reports on sample library quality metrics and organism abundance tables and provide numerous options for downstream interactive analyses of microbiome profiles and reporting experimental results. The Microbial Genomics Pro Suite Module also includes tools for calculating diversity metrics and for comparing the microbial profiles of different samples. ▷

The CLC Genomics Workbench also produces publication-quality figures in formats that are readily useable for researchers to present their results.

The QIAseq 16S/ITS Panels are designed to help you overcome the challenges around sequencing 16S rRNA genes or ITS regions to successfully profile microbial communities. Using these panels, you can robustly classify and identify a wide range of bacteria and fungi.

Ordering Information

Product	Contents	Cat. no.
QIAseq 16S/ITS Region Panel (24)	For 24 samples: contains all reagents (except indexes) for sequencing either specific variable regions of the 16S bacterial gene or the fungal ITS gene	333802
QIAseq 16S/ITS Region Panel (96)	For 96 samples: contains all reagents (except indexes) for sequencing either specific variable regions of the 16S bacterial gene or the fungal ITS gene	333805
QIAseq 16S/ITS Screening Panel (24)	For 24 samples: contains all reagents (except indexes) for sequencing all variable regions of the 16S bacterial gene and the fungal ITS gene	333812
QIAseq 16S/ITS Screening Panel (96)	For 96 samples: contains all reagents (except indexes) for sequencing all variable regions of the 16S bacterial gene and the fungal ITS gene	333815
Related Products		
QIAseq 16S/ITS 24-Index I (96)	For indexing up to 24 samples for 16S/ITS sequencing using Illumina platforms: contains library adapters for 96 samples	333822
QIAseq 16S/ITS 96-Index I (384)	For indexing up to 96 samples for 16S/ITS sequencing using Illumina platforms: contains library adapters for 384 samples	333825
QIAseq 16S/ITS Smart Control	For 10 samples: contains synthetic template that can be as positive control with QIAseq16S/ITS Panels	333832

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.

Profile microbial communities your way. Visit **www.qiagen.com/16S** to learn more.