Application Note

Improving Microbiome Sequencing using QIAseq® 16S/ITS Panels

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This application note demonstrates the use of QIAseq 16S/ITS Panels to generate bacterial 16S ribosomal RNA (rRNA) and fungal internal transcribed spacer (ITS) libraries for metagenomic analysis in a study that examined links between body mass index (BMI) and the salivary microbiome. QIAseq 16S/ITS panels provide several unique features that greatly improve the quality of targeted sequencing of microbiome samples, including the flexibility to sequence different 16S rRNA variable regions and ITS regions. In this application note, we also establish the need to sequence multiple variable regions to obtain a more complete understanding of microbial diversity.

Introduction

High-throughput sequencing, in particular with the Illumina® MiSeq® platform, has led to a revolution in microbiome research by targeting the 16S rRNA gene for amplicon sequencing. The 16S rRNA gene is an ideal target for bacterial/archaeal classification because it is highly conserved and extensively sequenced.

However, there are a number of issues associated with 16S rRNA gene sequencing and the MiSeq platform, such as: 1) low base diversity and subsequent low read quality; 2) contamination present in mastermixes and in the environment; 3) selecting which 16S rRNA region(s) is/are appropriate for sample type and research study; and 4) data analysis.

The QIAseq 16S/ITS Panels address each of these issues by: 1) incorporating phased primers to increase base diversity throughout the 16S rRNA amplicon; 2) using a low bioburden mastermix with minimal levels of contaminating bacterial/fungal DNA; 3) offering a screening panel that contains seven amplicons covering the entire 16S rRNA gene and fungal ITS region; and 4) integrating the workflow into the CLC Microbial Genomics Module for data analysis.

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The QIAseq 16S/ITS Panels were validated in part by using ATCC® Microbiome Standards 20 Strain Even Mix Genomic Material. The incorporation of phased primers significantly increased base diversity and read quality in sequencing results. Use of the screening panel demonstrated that multiple variable regions may be required for higher taxonomic resolution. Finally, the screening panel was used to investigate the diversity of the human saliva microbiome, and 16S regions V2V3 and V7V9 were chosen for further analyses as they were determined to be the most informative individual regions for saliva samples. In conclusion, we demonstrated that QIAseq 16S/ITS Panels increase base diversity and read quality compared to libraries generated with unphased primers, and that sequencing multiple variable regions increases the accuracy with which microbes are classified.

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.

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.

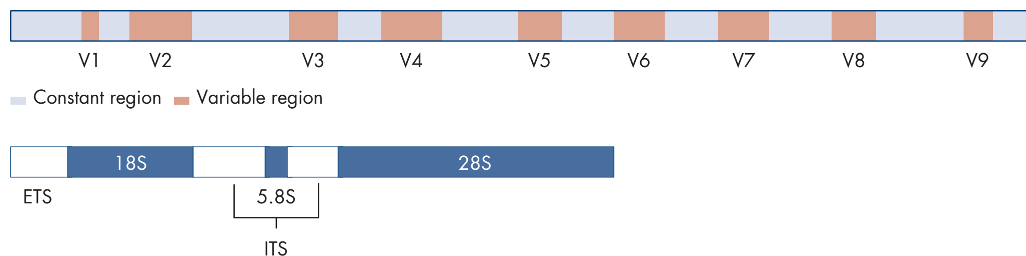

Figure 1. Structure of the bacterial 16S rRNA gene (top) and the fungal ITS region (bottom).

Materials and Methods

Experiment 1: Improving sequence quality using phased primers

To compare sequencing performance between conventional primers and QIAseq 16S/ITS Panels, which utilize phased primer technology, the bacterial 16S rRNA V3V4 region was targeted and libraries were generated using two different library prep methods. Briefly, for unphased primers, the Illumina 16S library prep protocol was followed (Illumina MiSeq 16S Metagenomic Library Preparation Guide).

Sequences for the full-length primers targeting the 16S gene are as follows:

16S PCR Forward Primer
= 5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTACGGGNGGCWGCAG

16S PCR Reverse Primer
= 5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACTACHVGGGTATCTAATCC

The underlined sequences target the V3V4 region of the 16S rRNA gene.

The recommended amount of DNA (12.5 ng) was used for the amplicon PCR step along with 200 nM of each primer in 2X KAPA HiFi HotStart ReadyMix PCR mastermix. The cycling conditions were set to an initial activation step at 95°C for 3 minutes followed by 25 cycles of denaturation (95°C, 30 seconds), annealing (55°C, 30 seconds) and extension (72°C, 30 seconds) with a final extension step at 72°C for 5 minutes.

After the amplicon PCR, AMPure® beads were used to remove free primers. An index PCR was then performed using the Nextera XT DNA Library Prep Kit to add sample indices and Illumina adaptors. The cycling conditions were set to an initial activation step at 95°C for 3 minutes followed by 8 cycles of denaturation (95°C, 30 seconds), annealing (55°C, 30 seconds) and extension (72°C, 30 seconds) with a final extension step at 72°C for 5 minutes. After the index PCR, another round of Ampure bead cleanup was performed to remove free index/adaptor primers.

Libraries targeting the V3V4 region were also generated using QIAseq 16S/ITS Panels, which utilize phased primer technology. Phased primers add an additional 0-11 bases to the 5′-end of both the forward and reverse 16S primers. This leads to an increase in base diversity throughout the sequencing run with a subsequent increase in read quality.

Samples that were used for target enrichment with unphased primers were also used for the phased primer protocol. Briefly, 1 ng of sample DNA was combined with QIAseq 16S/ITS Region Panel V3V4 primers, UCP Mastermix and UCP PCR Water. The cycling conditions for the 16S PCR step were: an initial activation step at 95°C for 2 minutes followed by 12 cycles of denaturation (95°C, 30 seconds), annealing (50°C, 30 seconds) and extension (72°C, 2 minutes) with a final extension step at 72°C for 7 minutes.

After the 16S PCR, two rounds of QIAseq bead cleanup was performed to remove all free primers. An index PCR step was run to incorporate sample indices and Illumina adaptors after which a final round of QIAseq bead cleanup was done to remove all free adaptors. The cycling conditions for the index PCR step were: an initial activation step at 95°C for 2 minutes followed by 19 cycles of denaturation (95°C, 30 seconds), annealing (60°C, 30 seconds) and extension (72°C, 2 minutes) with a final extension step at 72°C for 7 minutes.

Library concentration, for both unphased primer libraries and the QIAseq 16S/ITS libraries, was determined using the QIAseq Library Quant System. Both the unphased libraries and libraries constructed with the QIAseq 16S/ITS Panels were sequenced on the Illumina MiSeq using the V3

kit but on separate runs. Paired-end sequencing for both unphased libraries (2 x 300) and QIAseq 16S/ITS libraries (2 x 276 bp) were performed. Sequencing statistics were determined using CLC Genomics Workbench and the Create Sequencing QC Report tool.

Experiment 2: Low bioburden reagents result in low background noise

Background microbial DNA levels were measured in the UCP Multiplex PCR Mastermix and UCP PCR Water by amplifying NTC samples with primer sets that target 16S (all bacteria) or 18S (all fungi) regions. Briefly, 2 to 200 genome copies of *Escherichia coli* were amplified with 16S primers or 3 to 330 genome copies of *Candida albicans* were amplified with 18S primers. UCP PCR Water was also used as the NTC sample. Thirty–eight PCR cycles were performed and the reaction was run on a QIAxcel to detect 16S and 18S amplicons.

Experiment 3: Robust detection of bacterial species

To demonstrate the power and utility of sequencing multiple regions of the 16S rRNA gene, the QIAseq 16S/ITS Screening Panel was used to prepare libraries from a mock community with known composition (ATCC 20 Strain Even Mix Genomic Material). The screening panel includes three pools that contain primers that target different regions of the bacterial 16S rRNA gene and fungal ITS region. Three PCR reactions were set up, each containing one pool from the screening panel in addition to 1 ng of DNA, UCP Mastermix and UCP PCR Water. The cycling conditions for the 16S PCR step were: an initial activation step at 95°C for 2 minutes followed by 12 cycles of denaturation (95°C, 30 seconds), annealing (50°C, 30 seconds) and extension (72°C, 2 minutes) with a final extension step at 72°C for 7 minutes.

After the 16S PCR, the three PCR reactions were combined and two rounds of QIAseq bead cleanup was performed to remove all free primers. An index PCR step was run to incorporate sample indices and Illumina adaptors, after which a final round of QIAseq bead cleanup was performed to remove all free adaptors. The cycling conditions for the index PCR step were: an initial activation step at 95°C for 2 minutes, followed by 14 cycles of denaturation (95°C, 30 seconds), annealing (60°C, 30 seconds) and extension (72°C, 2 minutes) with a final extension step at 72°C for 7 minutes. The library was quantified using the QIAseq Library Quant Kit and paired-end (2 x 276 bp) sequencing performed on an Illumina MiSeq using the V3 kit.

For bioinformatics analysis, the CLC Microbial Genomics Module and QIAseq 16S/ITS Demultiplexer tool was used within CLC Genomics Workbench. Using the QIAseq 16S/ITS Demultiplexer tool, the single FASTQ file generated from the ATCC-derived library was demultiplexed into separate FASTQ files that contained reads from each 16S region amplicon. After demultiplexing, QC/OTU clustering was performed on each region independently using the SILVA database with clustering set at 97% identity. Finally, the different regions were compared at the species level in order to determine the resolving power for each region.

Experiment 4: Determining the diversity of the saliva microbiome

Saliva samples were collected in a blinded manner from volunteers. Prior to donating, each volunteer rinsed their mouth with water and waited 10 minutes before collection. Collected saliva was centrifuged at 10,000 rpm at 2–8°C. The supernatant was removed and the remaining pellet was frozen at –65 to –90°C until DNA extraction.

To extract total DNA, the QIAGEN DNeasy® Powersoil® Kit was used according to the manufacturer's protocol. For microbiome analysis of each saliva sample, the QIAseq 16S/ITS Screening Panel was used to construct 16S/ITS libraries. Three PCR reactions were set up, each containing one pool from the screening panel in addition to 1 ng of DNA, UCP Mastermix and UCP PCR Water. The cycling conditions for the 16S PCR step were: an initial activation step at 95°C for 2 minutes followed by 12 cycles of denaturation (95°C, 30 seconds), annealing (50°C, 30 seconds) and extension (72°C, 2 minutes) with a final extension step at 72°C for 7 minutes.

After the 16S PCR, the three PCR reactions were combined and two rounds of QIAseq bead cleanup were performed to remove all free primers. An index PCR step was run to incorporate sample indices and Illumina adaptors, after which a final round of QIAseq bead cleanup was performed to remove all free adaptors. The cycling conditions for the index PCR step were: an initial activation step at 95°C for 2 minutes followed by 14 cycles of denaturation (95°C, 30 seconds), annealing (60°C, 30 seconds) and extension (72°C, 2 minutes) with a final extension step at 72°C for 7 minutes. The library was quantified using the QIAseq Library Quant Kit and paired-end (2 x 276 bp) sequencing performed on an Illumina MiSeq using the V3 kit.

For bioinformatics analysis, the CLC Microbial Genomics Module and QIAseq 16S/ITS Demultiplexer tool were used within CLC Genomics Workbench. Using the QIAseq 16S/ITS Demultiplexer tool, the single FASTQ file generated from each saliva library was demultiplexed into separate FASTQ files that contained reads from each 16S region amplicon. After demultiplexing, QC/OTU clustering was performed on each region for all samples independently using the SILVA database with clustering at 97% identity. For the ITS region, QC/OTU clustering was performed using the UNITE database at 97% identity. In order to select regions that may be the most informative in classifying the saliva microbiome, the total number of predicted OTUs and named species were determined for each region. Also, the diversity of each 16S variable region was determined in aggregate for all samples. Finally, the V2V3 and V7V9 regions were selected to show the greatest differences in classification between the two 16S regions.

Experiment 5: Using QIAseq 16S/ITS Region Panels for in-depth analysis of the saliva microbiome

Initial analysis of saliva samples using the QIAseq 16S/ITS Screening Panel showed that regions V2V3 and V7V9 exhibited the highest number of bacterial species calls and that there were differences in taxonomic diversity between these two regions.

To study these regions, and the ITS region, in greater depth, QIAseq 16S/ITS Region Panels V2V3, V7V9 and ITS were used with the same saliva samples. 16S/ITS target enrichment and library construction was performed as described earlier except that the V2V3, V7V9 and ITS primers were used in a single PCR reaction per sample. Bioinformatics analysis was performed as described earlier with the addition of alpha-diversity and differential abundance analysis based on communities (BMI or diet). Differential abundance analysis was performed at the level of both the genus and the species. The analyses are shown in Table 1A (genus) on page 12 and Table 1B (species) on page 13 for both V2V3 and V7V9 regions. Using both regions allows for a more complete picture of the saliva microbiome because some taxa could be identified based on only one region or the other.

Results and Discussion

Experiment 1: Improving sequence quality using phased primers

We compared libraries generated using phased primers, utilized by QIAseq 16S/ITS Panels, with libraries generated using unphased primers that target the V3V4 region. Both sets of libraries were sequenced on Illumina MiSeq platform with the V3 kit. Sequencing single-amplicon libraries often yields low quality results due to the reduced diversity of base composition in the primer regions as shown in fluorescent images of each base at read 1 (Figure 2A) and quality score distributions (Figures 2C). The use of phased primers shifts nucleotide balance and increases base diversity, which leads to increases in quality scores. This can be seen by equal distribution of fluorescent signals in the images for each base (Figures 2B) and higher quality distribution throughout the run (Figures 2D).

Experiment 2: Low bioburden reagents result in 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. This background contamination can decrease the robustness of microbial profiling. The QIAseq 16S/ITS Panels use reagents with low bioburden. To demonstrate the low levels of contaminating DNA associated with these reagents, PCR reactions targeting bacterial DNA (16S) or fungal DNA (18S) were performed using the reagents and no additional template. The results show very low levels of exogenous bacterial/fungal contamination in no template control (NTC) samples: less than the equivalent of two *Candida* or *E. coli* genomes (Figure 3).

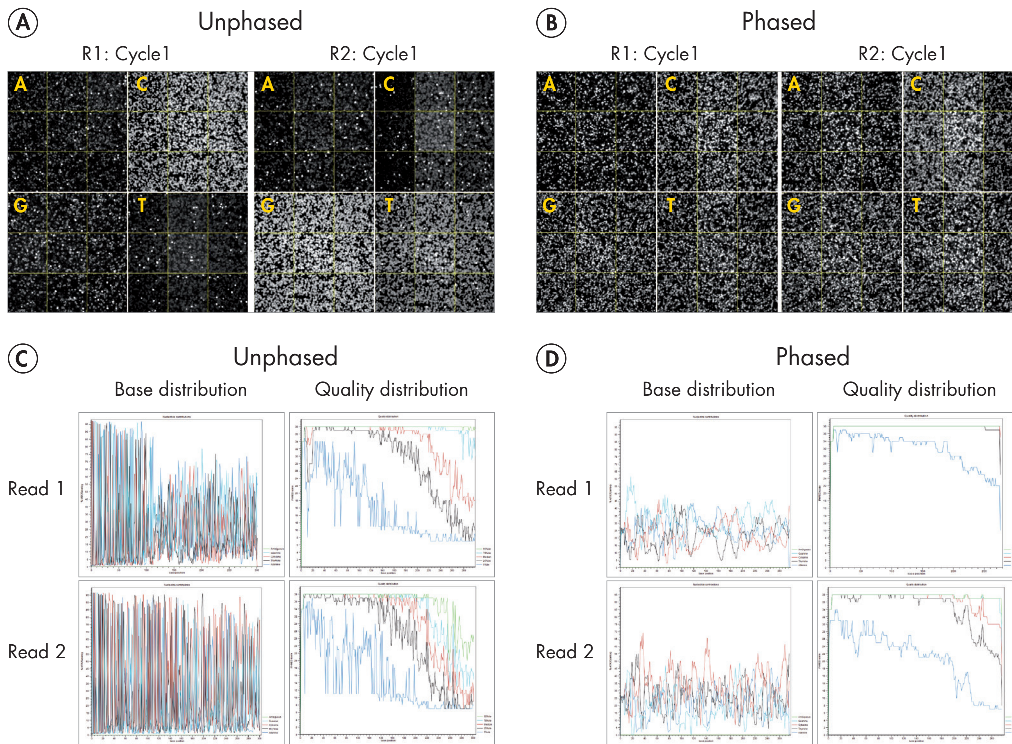

Figure 2. QIAseq 16S/ITS Panels employ phased primers to increases base diversity and quality scores. Fluorescent images (top row) taken from the first cycle of read 1 (R1) or the first cycle of read 2 (R2) showing uneven base distribution in an unphased primer run (A) and more even base distribution 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).

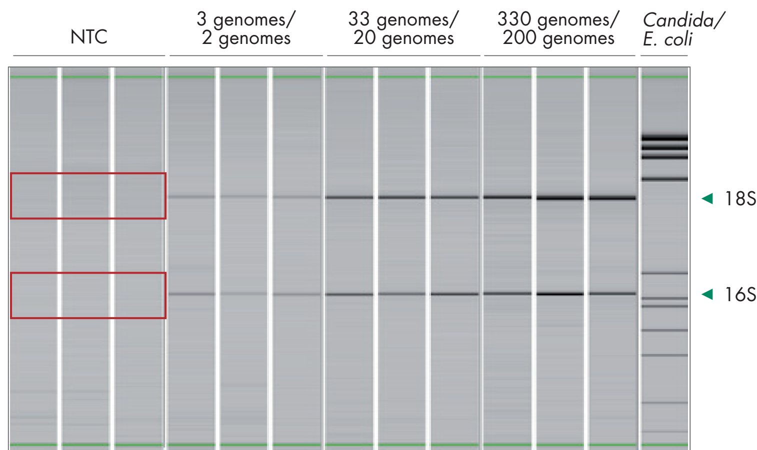

Figure 3. The QIAseq 16S/ITS Panels have very low levels of background contamination due to the use of reagents with low bioburden. Contamination levels were monitored by amplifying with PCR primers targeting 16S (bacteria) and 18S (fungal) rRNA genes with UCP Multiplex Mastermix and UCP PCR Water. 38 cycles of PCR were performed and the reaction was run on a QIAxcel to detect the 16S and 18S amplicons.

Experiment 3: Robust detection of bacterial species

Identifying members of a bacterial community, based on analysis of the 16S rRNA gene, can depend on which variable regions are sequenced. In most studies that sequence the 16S rRNA gene, only one region is interrogated. However, it is known that taxonomic resolution can differ depending on which variable region is being sequenced. For example, *Streptococcus mutans* cannot be identified by sequencing variable regions V1V2, V2V3, V3V4 and V4V5. The QIAseq 16S/ITS Screening Panels (cat. no. 333812 and 333815) comprehensively profile the entire 16S rRNA gene by including primers that target V1V2, V2V3, V3V4, V4V5, V5V7 and V7V9 regions (and also fungal ITS). With the QIAseq 16S/ITS Screening Panels, the V5V7 and V7V9 regions were used to classify *S. mutans* at the species level (Figure 4; red arrows).

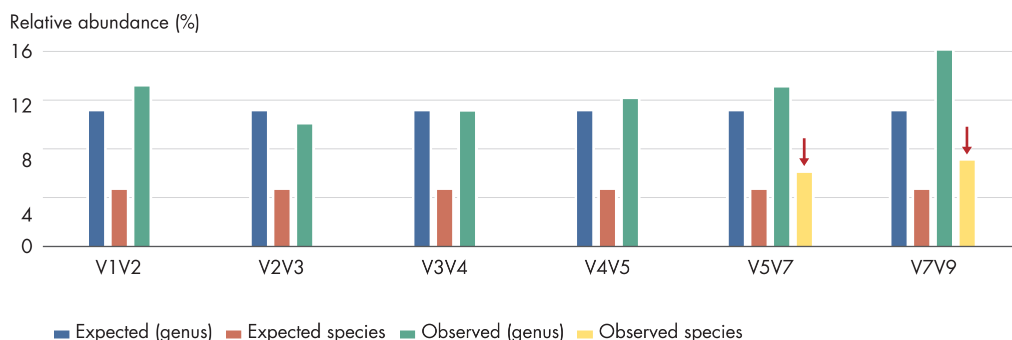

Figure 4. Screening a panel of variable regions provides more robust bacterial profiling compared to screening only individual variable regions. QIAseq 16S/ITS Panels were used to generate libraries from the ATCC 20 Strain Even Mock Community. Demultiplexing of the variable regions was performed using the CLC Microbial Genomics Module 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*.

Experiment 4: Determining the diversity of the saliva microbiome

Saliva is an easily accessible sample that can be collected non-invasively and used to study the oral microbiome. To assay for the diversity of the oral microbiome, QIAseq 16S/ITS Screening Panels were used to generate libraries from a collection of saliva samples. Microbial diversity, determined by analyzing each variable region, was compared by aggregating data from all samples for each different variable region and performing alpha-diversity analysis and determining the total number of OTUs. Comparing the alpha-diversity for each region, V4V5 exhibited the highest number of total OTUs, suggesting that this region may provide the broadest coverage of taxa to be identified. Next, relative abundance, determined by analyzing different variable regions, was assessed at the species level. While there were differences in relative abundance between all the different regions,

V2V3 and V7V9 had the most calls of named species, indicating that these two regions allow for the highest taxonomic resolution. Also, the relative abundance between these two regions for the same sample showed differences, which indicates that more than one region is required for comprehensive analysis. Therefore, for this collection of saliva samples, V2V3, V4V5 and V7V9 are the most informative regions.

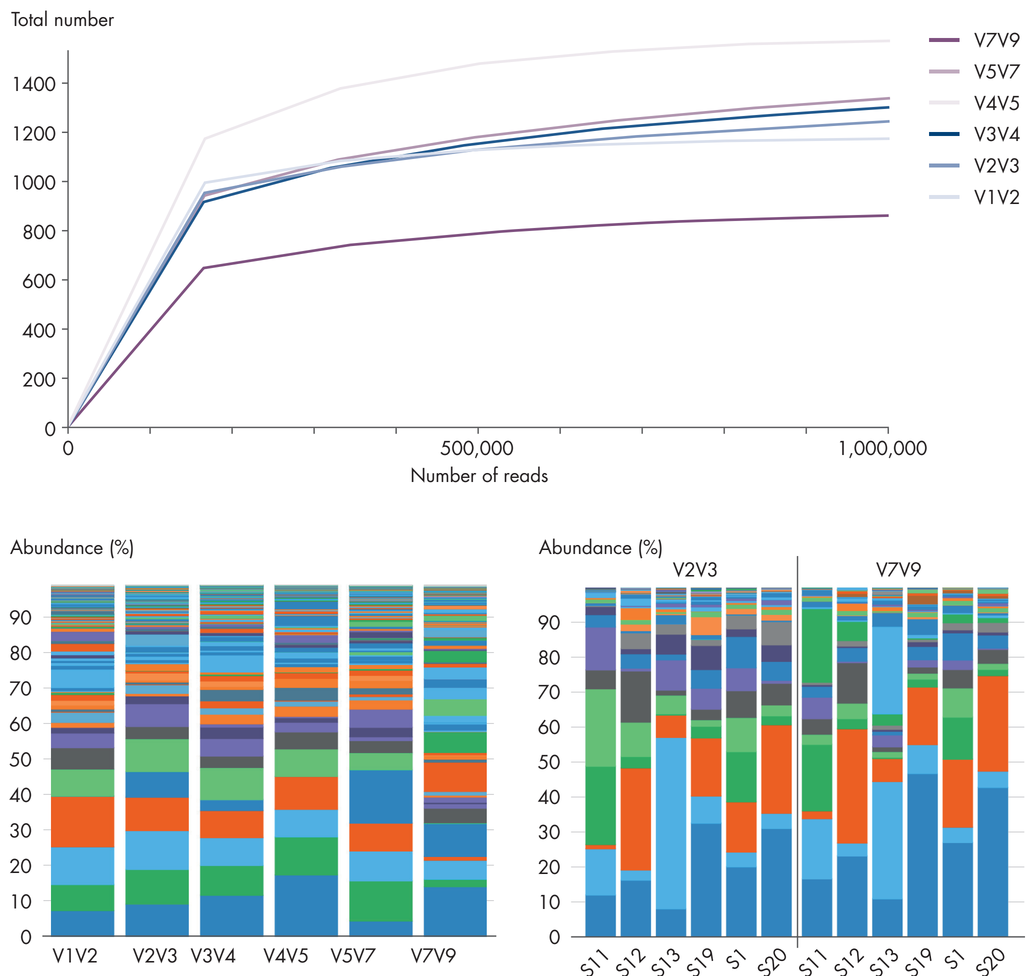

Figure 5. Determining the diversity of the saliva microbiome. Libraries of 16S regions were generated from saliva samples (DNA was extracted with the DNeasy PowerSoil Kit) using the QIAseq 16S/ITS Panels. Data analysis was performed using CLC Microbial Genomics Module. (Top) Demultiplexed FASTQ files were aggregated by 16S variable region; alpha diversity as measured by total number of OTUs was determined. (Bottom left) Diversity at species level of different 16S rRNA regions; V2V3 and V7V9 regions were selected for further analysis due to the number of named species each region was able to call. (Bottom right) Diversity of saliva microbiome as shown by V2V3 and V7V9 regions at the family level. Both regions show high inter-individual diversity. In addition, the two regions show differences in classification for the same sample.

Experiment 5: Using QIAseq 16S/ITS Region Panels for in-depth analysis of the saliva microbiome

To examine links between body mass index (BMI) and the salivary microbiome, the V2V3 and V7V9 regions of the bacterial 16S rRNA gene and the fungal ITS region were sequenced with the QIAseq 16S/ITS Panels.

At the phylum level, the regions have relatively similar distributions in taxonomic abundance between normal-weight and obese individuals, particularly the proteobacteria to firmicutes ratio.

Alpha diversity and Shannon diversity show that normal-weight individuals have a higher diversity of bacteria in their saliva microbiome. While the V2V3 region showed a difference in alpha diversity between normal-weight and obese individuals, the effect was greater for the V7V9 region.

There were differences in abundance between normal-weight and obese individuals at both the genus and the species level (Table 1 and Table 2). Importantly, this shows that multiple regions need to be sequenced to obtain a more comprehensive picture of taxonomic diversity as some taxa can be identified by sequencing one region but not another.

Fungal taxa could be identified in some samples by sequencing the ITS amplicon, but there was no correlation with BMI (Figure 7).

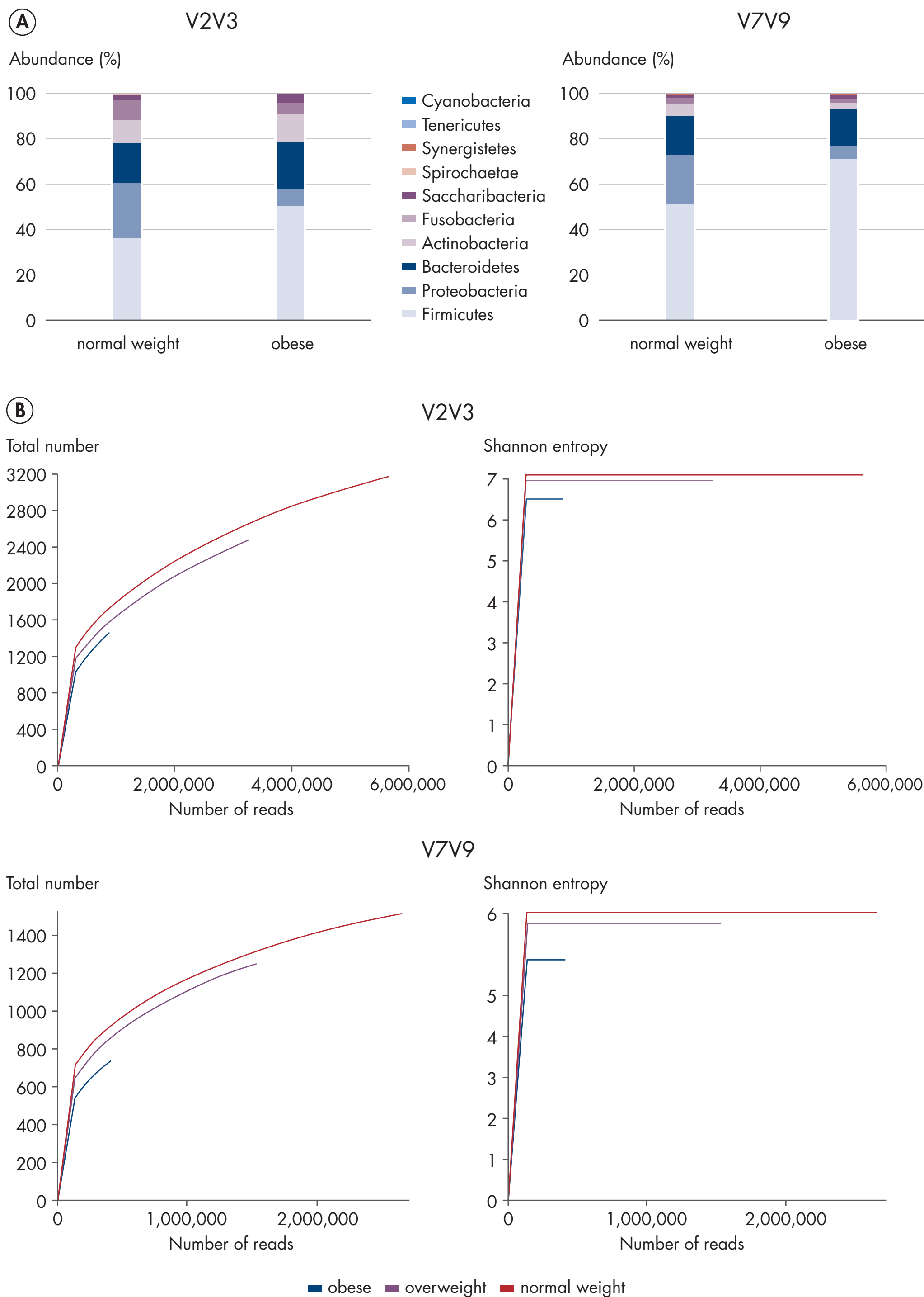

Figure 6. Differences in saliva microbiome due to BMI were determined using QIAseq 16S/ITS Region Panels and CLC Microbial Genomics Module. (A) Samples from normal-weight and obese individuals were aggregated and abundance distribution was determined for the V2V3 and V7V9 region at the phylum level. (B) Alpha diversity, as measured by total number of OTUs, and Shannon diversity was determined for each BMI category (normal-weight, overweight and obese).

A

Name	obese vs. normal weight					
	V2V3			V7V9		
	Max group mean	Fold change	P-value	Max group mean	Fold change	P-value
Family XIII UCG-001	23.72	-18.42	0.0010	8.55	-18.13	0.0054
Leuconostoc	21.59	-32.23	0.0086	10.79	-45.65	0.0200
Macrococcus	40.34	-109.49	0.0030	19.55	-81.41	0.0077
Moraxella	87.83	-76.02	0.0007	20.28	-175.11	0.0020
Pseudomonas	40.79	-178.18	0.0010	12.45	-52.61	0.0100
Defluviitaleaceae UCG-011	46.34	-13.55	0.0021	26.66	-8.16	0.0100
Actinobacillus	509.14	-21.52	0.0001	0.34	-2.79	0.4500
Alloscardovia	161.33	12.58	0.0200	10.17	6.07	0.0500
Johnsonella	106.28	-4.93	0.0200	6.52	-11.4	0.0200
Uncultured cyanobacterium	0	0	0.0000	26	217.21	0.0000
Prevotella 1	27.1	-4.6	0.0400	2.86	-15.05	0.1000
Actinomyces	12360	2.93	0.0028	462.67	1.79	0.0400
Alloprevotella	1474.28	-3.43	0.0010	977.86	-3.46	0.0025
Fusobacterium	10035.79	-2.42	0.0055	3195.52	-3.01	0.0008
Neisseria	22682.38	-5.74	0.0005	2504.03	-8.12	0.0003
Parvimonas	176.93	-2.91	0.0400	69.59	-3.11	0.0300
Porphyromonas	1649.52	-4.29	0.0004	755.07	-4.65	0.0002
Prevotella	1244	-2.84	0.0036	2208.31	-2.01	0.0200
Prevotella 2	925	-5.84	0.0002	788.33	2.63	0.0100
Tannerella	210	-4.28	0.0046	39.34	-4.42	0.0300
Treponema 2	160.24	-3.87	0.0300	220.69	-4.12	0.0100
Veillonella	49889.83	2.76	0.0026	29290.5	2.23	0.0100
Corynebacterium 1	94.24	-7.94	0.0046	9.67	2.17	0.1600
Filifactor	104.45	-4.74	0.0400	52.31	-4.92	0.0900
Lactococcus	120.83	8.12	0.0072	5	2.39	0.3200
Megasphaera	2494	3.31	0.0200	2856	2.98	0.0500
Mannheimia	0	0	0.0000	192.97	-1.08	0.9000
Massilia	0	0	0.0000	24.62	-2.21	0.1700
Microbacter	0	0	0.0000	35.34	-4.38	0.0800
Morganella	0	0	0.0000	1291.59	-1.82	0.2500
Providencia	0	0	0.0000	3631.28	-2.39	0.0500

Table 1. Determining differences in the saliva microbiome between normal-weight and obese individuals. Differential abundance analysis at the genus level (A) and species level (B) was performed for both V2V3 and V7V9 regions. A value of zero for both max group mean and fold change indicates that the taxa was not identified using the indicated region.

B

Name	obese vs. normal weight					
	V2V3			V7V9		
	Max group mean	Fold change	P-value	Max group mean	Fold change	P-value
Macrococcus caseolyticus	27.9	-125.03	0.0023	19.48	-72.87	0.0088
Streptococcus cristatus	11.17	28.15	0.0000	62.62	-14.05	0.0012
Catonella sp. oral clone FL037	32.34	-127	0.0015	0	0	0.0000
Bacterium NLAE-zl-C503	32.17	-61.17	0.0007	0	0	0.0000
Leuconostoc sp. MEC9	11.69	-53.26	0.0100	0	0	0.0000
Mitsuokella sp. oral taxon G68	137.5	43.96	0.0002	0	0	0.0000
Streptococcus australis	208.34	-1172.49	0.0000	0.03	1.07	0.9600
Treponema calligyrum	0	0	0.0000	22.1	-100.76	0.0035
Prevotella buccae D17	19.72	-3.58	0.1300	5.45	-11.01	0.0400
Haemophilus haemolyticus	13.5	2.13	0.4300	7.66	-20.21	0.0200
Porphyromonas sp. oral clone ASCH03	113.67	3.35	0.1700	2.79	-18.52	0.0600
Leptotrichia goodfellowii DSM 19756	14	-7.85	0.0300	0	0	0.0000
Neisseria meningitidis NM82	0	0	0.0000	81.59	-7.4	0.0200
Capnocytophaga sp. oral clone DZ074	0	0	0.0000	116.9	-4.58	0.0044
Prevotella sp. oral clone AH125	0.5	3.71	0.0400	170.45	-5.83	0.0100
Prevotella sp. oral clone P4PB_83 P2	13.93	-9.73	0.0059	148.59	-2.22	0.1900
Eubacterium sp. oral clone EW049	0	0	0.0000	172.03	-3.16	0.0500
Granulicatella sp. oral clone ASCA05	0	0	0.0000	11.76	-1.71	0.5300
Streptococcus suis	0	0	0.0000	106.31	-1.62	0.3200
Leptotrichia-like sp. oral clone BB135	0	0	0.0000	175.1	-1.46	0.4600
Gemella sp. oral clone ASCF12	0	0	0.0000	441.79	-1.07	0.8800
Mannheimia ruminalis	0	0	0.0000	187.28	-1.01	0.9900
Selenomonas sputigena ATCC 35185	0	0	0.0000	50.97	1.01	0.9900
Solobacterium moorei F0204	0	0	0.0000	91.34	1.09	0.8400
Eubacterium sp. oral clone DZ073	0	0	0.0000	23.5	1.43	0.5500
Streptococcus oricebi	0	0	0.0000	13.67	1.91	0.5000
Streptococcus thermophilus TH1435	0	0	0.0000	56	5.58	0.0600
Actinomyces graevenitzii C83	1805	3.41	0.0100	4.5	2.61	0.2700
Streptococcus sp. oral clone ASCE10	82.28	-4.34	0.1500	0	0	0.0000
Dialister pneumosintes	21.97	-4.11	0.0800	0	0	0.0000
Streptococcus anginosus subsp. *anginosus*	30.1	-3.21	0.1900	0	0	0.0000
Streptococcus sp. oral clone ASCB12	0.9	-2.59	0.2700	6242	3.26	0.0200
Kingella genomosp. P1 oral cone MB2_C20	0.28	-2.48	0.5100	151.33	5.35	0.0030
Streptococcus sp. XJ149-N3-2	216.21	-1.32	0.7100	0	0	0.0000
Abiotrophia sp. oral clone OH2A	78.34	-1.3	0.6700	0	0	0.0000
Streptococcus sp. oral clone ASB02	11.21	-1.13	0.8900	0	0	0.0000
Streptococcus sp. oral clone ASCC01	13.76	-1.11	0.8600	0	0	0.0000
Prevotella salivae	1598.17	1.92	0.2400	0	0	0.0000
Streptococcus sanguinis	495	2.85	0.1000	0	0	0.0000
Streptococcus mutans	0.17	3.11	0.2200	22.5	5.68	0.0300

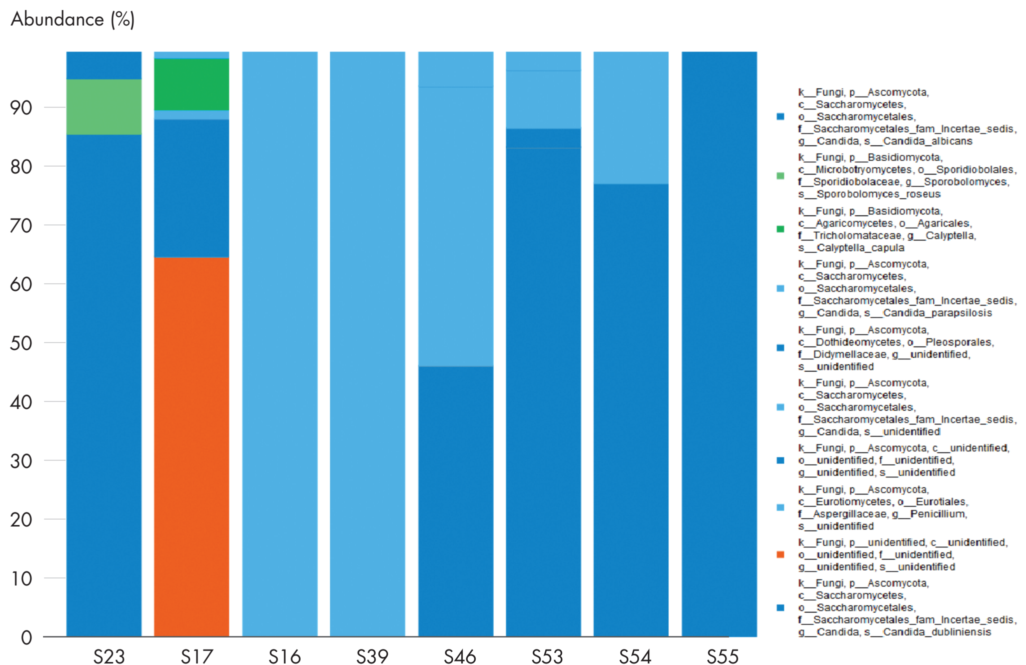

Figure 7. The diversity of fungal species in saliva microbiome was analyzed using reads derived from the ITS amplicon. No correlation was found between BMI and fungal diversity.

Conclusions

The QIAseq 16S/ITS Panels include:

- Phased primers, which shift nucleotide balance and increase base diversity, leading to increases in quality scores.
- Low bioburden reagents, which result in low background noise, reducing interference with microbial profiling.
- The ability to interrogate more 16S variable regions, yielding more robust results. The QIAseq 16S/ITS Panels enable sequencing of all nine 16S rRNA variable regions and the fungal ITS regions, providing a more complete understanding of microbial diversity.

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	333842
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	333845
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

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

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