December 2025

QIAseq® 16S/ITS Pro Screening Panel Handbook

For high-throughput targeted next- generation sequencing of metagenomic samples

Sample to Insight

Table of Contents

Kit Contents

QIAseq 16S/ITS Pro Screening Panel Catalog no.	(24) 331782	(96) 331785
Number of reactions	24	96
Pro Screening Panel Pool	3 pools	3 pools
UCP Master Mix	2 x 500 µL tubes	5 x 500 µL tubes
UCP PCR Water	6 x 1.9 mL tubes	18 x 1.9 mL tubes
QIAseq Beads	1 bottle	1 bottle

The QIAseq MUDI index kits are sold separately and are necessary for library construction with UDIs. These index kits contain a UDI sample index plate. To multiplex more than 96 libraries in a single sequencing run, combine different UDI index plates. For example, combining libraries prepared with the QIAseq UDI-A, B, C, and D (96) Kit will allow the generation of up to 384 libraries with different sample indexes for 384-plex sequencing.

QIAseq 16S/ITS 24-index I (for Illumina®) Catalog no. Number of samples	24 UDIs 331802 24
96 well plate with 24 UDI sample indexes	1 plate MUDI-24A

QIAseq 16S/ITS 96 Index Kit Catalog no. Number of reactions	UDI-A (96) 331815 96	UDI-B (96) 331825 96	UDI-C (96) 331835 96	UDI-D (96) 331845 96
96 well plate with 96 UDI sample indexes	1 plate MUDI-96AA	1 plate MUDI-96BA	1 plate MUDI-96CA	1 plate MUDI-96DA

QIAseq 16S/ITS 96 Index Kit Catalog no. Number of reactions	UDI-E (96) 331855 96	UDI-F (96) 331865 96	UDI-G (96) 331875 96	UDI-H (96) 331885 96
96 well plate with 96 UDI sample indexes	1 plate MUDI-96EA	1 plate MUDI-96FA	1 plate MUDI-96GA	1 plate MUDI-96HA

QIAseq 16S/ITS Smart Control Catalog no. Number of reactions	(10) 333832 10
Smart Control	1 tube
UCP PCR Water	1.9 mL tube

Shipping and Storage

The QIAseq 16S/ITS Pro Screening Panel shipped in 2 boxes. Box 1 is shipped on dry ice or blue ice, and Box 2 is shipped on blue ice. Upon receipt, all of the components in Box 1 should be stored immediately at (–30°C to –15°C) in a constant-temperature freezer. All of the components in Box 2 should be stored immediately at (2–8°C).

Intended Use

The QIAseq 16S/ITS Pro Screening Panel is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAseq 16S/ITS Pro Screening Panels, QIAseq UX Index Kits, and QIAseq 16S/ITS Smart Control is tested against predetermined specifications to ensure consistent product quality.

Introduction

Microbial life is present in almost every environment on earth. This microbial life is partly composed of archaea/bacterial and fungal cells, and the composition of each environment or community (i.e., sample type) can be highly unique.

All species of archaea/bacteria contain the ribosomal 16S gene in their genome; likewise, all fungal species contain the ITS gene in their genome. The 16S gene has regions of conserved sequences interspersed with regions of high variability. The highly variable regions of 16S are commonly used to taxonomically classify bacterial species that reside in a sample. The surrounding conserved regions can serve as targets for 16S primers that will amplify all archaea/bacteria species in a sample. Classification of bacterial species in any sample type can be performed by first amplifying regions of the 16S gene and then performing next generation sequencing (NGS) on the variable regions. Furthermore, this is applicable to ITS classification of fungal species. Shifts in archaea/bacterial/fungal communities can be observed by comparing different samples.

When performing 16S NGS experiments, the targeted region of interest depends on the desired classification power. To aid in the selection of the region(s) with the highest discrimination power, the QIAseq 16S/ITS Pro Screening Panel can be used. The QIAseq 16S/ITS Screening Panel separates 6 amplicons into 3 pools that, together, cover the entire ribosomal 16S gene. In addition, the QIAseq 16S/ITS Screening Panel contains primers for ITS to amplify fungal DNA. The contents of each pool are as follows:

- Pool 1: V1V2, V4V5, and ITS
- Pool 2: V2V3 and V5V7
- Pool 3: V3V4 and V7V9

Therefore, in one library construction, all regions of the bacterial 16S gene and the fungal ITS gene are targeted and sequenced. If desired, specific regions can then be chosen for further studies, or the screening panel can also be used.

A prominent concern with existing 16S PCR (polymerase chain reaction) protocols is that PCR master mixes and lab water are contaminated, and this will negatively impact the community profile. This is especially important when looking at taxon that may occur at low abundances. To address this problem, the QIAseq 16S/ITS Panels contain a UCP (ultra-clean production) Master Mix and UCP PCR Water that have been produced in an ultra-clean environment and quality controlled to prevent contamination. This produces 16S sequencing reads with a minimal number of contaminating reads and higher confidence in sequencing results.

16S NGS is also challenging because the single amplicon design results in low-base diversity. This results in reduced clusters passing filter and reduced sequence quality (Q30 scores). Ultimately, the consequence is fewer reads that can be used for operational taxonomic units (OTU) analysis. To overcome this issue, the QIAseq 16S/ITS Pro Screening Panels incorporate phased primers that add 0–11 additional bases before the 16S primer. This technology increases the base diversity, which is especially important within the first 11 cycles of sequencing and ultimately leads to higher Q30 scores and usable reads. At the same time, it eliminates the need to add PhiX, increasing the available reads.

Principle and procedure

The QIAseq 16S/ITS Panels utilize a 2-stage PCR workflow for targeted enrichment of 16S and ITS genes (Figure 1). The first PCR step incorporates a phased primer pool to enrich for conserved regions of the 16S gene and ITS gene. Following a reaction cleanup with QIAseq

Beads, library amplification then introduces sample indices and ensures that sufficient target is present for NGS. Following a final cleanup, the libraries are quality-controlled (Bioanalyzer® or TapeStation®) and quantified using the QIAseq Library Quant system. The number of multiplexed samples depends on the number of interrogated variable regions. This is outlined in Table 1.

The QIAseq 16S/ITS Panels offer a 16S/ITS processing control (QIAseq 16S/ITS Smart Control) that monitors both proper library construction and contamination introduced by the environment or user. The process control is used as a sample during library preparation.

After sequencing, any environmental contamination introduced can be identified after bacterial/fungal classification.

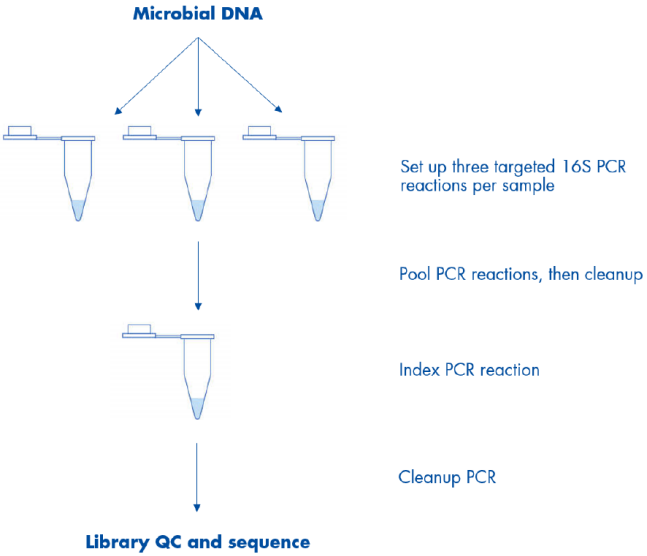

Figure 1. Schematic of QIAseq 16S/ITS Panel workflow. The number of targeted 16S PCR reactions depends on the number of required primer pools. The QIAseq 16S/ITS Screening Panel requires 3 targeted 16S PCR reactions, because 3 primer pools are required.

Data analysis

Sequencing is performed on an Illumina MiSeq i100 or NextSeq 1000/2000 using a 600-cycle kit with 300 × 2 paired-end run; Alternatively, on the MiSeq NGS system using a v3 kit with 276 × 2 paired-end run. It is highly recommended to perform sequencing data analysis with the CLC Genomics Workbench with Microbial Genomics Module and QIAseq 16S Panel Analysis (requires a separate paid license and is not included with the QIAseq 16S kit). Please refer to "Protocol: Data Analysis Using CLC Microbial Genomics Module". Alternatively, the generated FASTQ files can be analyzed with open-source 16S data analysis software. Importantly, when the QIAseq 16S/ITS Screening Panel is used, the individual amplicons must be demultiplexed before bacterial/fungal classification. This can be performed within the CLC Microbial Genomics Module or using the QIAseq 16S Demultiplexer tool on GeneGlobe Data Analysis Center (**geneglobe.qiagen.com/analyze**).

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- High-quality nuclease-free water
- Nuclease-free pipette tips and tubes
- LoBind® microfuge tubes (1.5 mL)
- PCR tubes (0.2 mL individual tubes or tube strips) (VWR, cat. no. 20170-012 or 93001 118)
- Ice
- Microcentrifuge
- Thermal cycler
- MagneSphere® Technology Magnetic Separation Stand (Promega, cat. no. Z5342) or DynaMagTM-2 Magnet (Thermo Fisher Scientific, cat. no. 12321D)

Library QC:

Agilent® 2100 Bioanalyzer: High Sensitivity DNA Kit (Agilent, cat. no. 5067-4626) or 4200 TapeStation: High Sensitivity D1000 ScreenTape® (Agilent, cat. No. 5067 5584)

Preferred library quantification method:

qPCR instrument and QIAseq Library Quant System: QIAseq Library Quant Array Kit (cat. no. 333304) or QIAseq Library Quant Assay Kit (cat. no. 333314)

Important Notes

DNA preparation and quality control

High-quality DNA is essential for obtaining optimal sequencing results. The most important prerequisite for DNA sequence analysis is consistent, high-quality DNA from every experimental sample. Therefore, sample handling and DNA isolation procedures are critical to the success of the experiment. Residual traces of proteins, salts, or other contaminants may either degrade the DNA or decrease the efficiency of – if not block completely – the enzyme activities necessary for optimal targeted DNA amplification.

DNA quantification and quality control

For best results, all DNA samples should also demonstrate consistent quality according to the following criteria:

- Concentration and purity determined by UV spectrophotometry
- Concentration and purity of DNA, determined by measuring absorbance using a spectrophotometer such as a Nanodrop®. Prepare dilutions and measure absorbance in 10 mM Tris·Cl, pH 8.0. The spectral properties of nucleic acids are highly dependent on pH.
- A_{260}:A_{280} ratio should be greater than 1.8.

Protocol: QIAseq 16S/ITS Pro Screening Panel

This protocol is designed for the amplification of all 16S/ITS rRNA variable regions.

Important points before starting

- Use high quality DNA.
- If DNA concentration is >1 ng/µL, then dilute to 1 ng/µL. If DNA concentration is ≤1 ng/µL, then use 1 µL of sample per PCR reaction.
- Ensure reactions are thoroughly mixed, as well as prepared and incubated at the recommended temperatures.
- 80% ethanol should be freshly prepared using nuclease-free water and mixed thoroughly by vortexing.
- QIAseq Beads need to be homogenous. Thoroughly resuspend the beads immediately before use and process the beads quickly. If a delay in the protocol occurs, simply vortex the beads again.

Procedure

1. Thaw the following on ice: QIAseq 16S/ITS Screening Panel Pool 1, QIAseq 16S/ITS Screening Panel Pool 2, QIAseq Screening 16S/ITS Panel Pool 3, UCP Multiplex Master Mix, UCP PCR Water, and samples.
2. On ice, prepare 3 PCR reactions per gDNA sample by following Table 1. Briefly centrifuge, mix by pipetting up and down 10 times, and briefly centrifuge again.

Table 1. Preparation of QIAseq 16S/ITS Pro Screening Panel PCR

Component	Panel pool 1 (µL)	Panel pool 2 (µL)	Panel pool 3 (µL)
Microbial DNA sample*	1	1	1
UCP Multiplex Master Mix	2.5	2.5	2.5
Panel pool 1	1	–	–
Panel pool 2	–	1	–
Panel pool 3	–	–	1
UCP PCR Water	5.5	5.5	5.5
Total volume	**10**	**10**	**10**

* The QIAseq 16S/ITS Smart Control can be added instead of a microbial DNA sample. Refer to Appendix A: Preparation and Use of QIAseq 16S/ITS Smart Control.

3. Incubate the reactions in a thermal cycler as described in Table 2.

Table 2. Setup of QIAseq 16S/ITS PCR reaction

Step	Time	Temperature (°C)
Hold	2 min	95
3-step cycling Denaturation Annealing Extension 12 cycles*	 30 s 30 s 2 min	 95 50 72
Final extension (1 cycle)	7 min	72
Hold	∞	4

* If samples contain low bacterial/fungal content, use 20 cycles of 16S PCR.

4. Remove the tubes/plate from the thermal cycler and briefly centrifuge.

5. Add 20 µL of UCP PCR Water to each of the PCR reactions.

6. Pool the PCR reactions from the same microbial DNA sample into a single LoBind tube or the well of a 96-well PCR plate. The total volume for each microbial DNA sample should be 90 µL.
7. Add 1.1x volume (99 µL) of QIAseq Beads to each combined sample from step 5. Mix well by pipetting up and down 12 times and then briefly centrifuge.
8. Incubate for 5 min at room temperature (15–25°C).
9. Place the tubes/plate on a magnetic rack for 5 min. After the solution has cleared, carefully remove and discard the supernatant.
10. Centrifuge briefly, place the tubes/plate back on a magnetic rack, and carefully remove any residual liquid. Add 55 µL of nuclease-free water. Mix by pipetting up and down 12 times until the beads are fully resuspended. Incubate for 2–5 min at room temperature.

    Note: UCP PCR Water is not needed at this step.
11. Return the tubes/plate to the magnetic rack until the solution has cleared, and then carefully transfer 50 µL of the supernatant that contains the 16S/ITS PCR product to new tubes or plate.
12. Add 1.1x volume (55 µL) of QIAseq Beads to each sample. Mix well by pipetting up and down 12 times and briefly centrifuge.
13. Incubate for 5 min at room temperature.
14. Place the tubes/plate on a magnetic rack for 5 min. After the solution has cleared, carefully remove and discard the supernatant.

    Note: Keep the beads on the magnetic stand while handling the supernatant and during the washing steps.

    Important: Do not discard the beads, because they contain the DNA of interest.

15. Add 200 µL of 80% ethanol while it is on the magnetic rack/plate. Rotate the tube (3 times) or move the plate side to side in the 2 positions of the magnet to wash the beads. Carefully remove and discard the wash.

16. Repeat the ethanol wash as in step 15.

    Important: Completely remove all traces of the ethanol wash after the second wash. To do this, briefly centrifuge and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200 µL pipette, and then use a 10 µL pipette to remove any residual ethanol.

17. With the tubes/plate still on the magnet, air-dry at room temperature for 5 min (up to 15 min when using a plate).

    Note: Visually inspect that the pellet is completely dry. When completely dry, the beads should have a "cracked" appearance.

18. Remove the tubes/plate from the magnetic stand and elute the DNA from the beads by adding 38 µL UCP PCR Water. Mix well by pipetting. Incubate for 2–5 min at room temperature.

19. Return the tubes/plate to the magnetic rack until the solution has cleared.

20. Transfer 35.5 µL of the supernatant to clean tubes/plate.

21. Proceed to "Protocol: Preparation of QIAseq 16S/ITS Pro Screening Panel Sample Index PCR Reaction".

    Alternatively, the cleaned-up products can be stored at –30°C to –15°C in a constant-temperature freezer.

Protocol: Preparation of QIAseq 16S/ITS Pro Screening Panel Sample Index PCR Reaction

This protocol uses the product from "Protocol: QIAseq 16S/ITS Pro Screening Panel".

In this protocol, sample indices and sequencing adaptors are added.

Important points before starting

- Set up the QIAseq 16S/ITS Pro Screening Panel Sample Index PCR Reactions on ice.
- Ensure reactions are thoroughly mixed, as well as prepared and incubated at the recommended temperatures.
- 80% ethanol should be freshly prepared using nuclease-free water and mixed thoroughly by vortexing.
- QIAseq Beads need to be homogenous. Thoroughly resuspend the beads immediately before use and process the beads quickly. If a delay in the protocol occurs, simply vortex the beads again.

Procedure

22. Thaw the following on ice: the required QIAseq MUDI plate, UCP Multiplex Master Mix, and UCP PCR Water.
23. Place on ice the tubes/plate containing the 16S PCR product.
24. Add the components to the tubes/plate containing the 16S PCR product according to Table 3.

    Important: To prevent contamination of UCP Multiplex Master Mix, aliquot a sufficient volume for all samples to a sterile tube and then transfer the required amount to each sample.

Table 3. Preparation of QIAseq 16S/ITS Sample Index PCR Reaction

Component	Per sample (µL)
16S PCR product	35.5
UCP Multiplex Master Mix	12.5
Sample index from a single well of QIAseq MUDI index plate*	2
Total volume	**50**

* Use a unique well from the MUDI index plate for each microbial DNA sample.

25. Incubate the reactions in a thermal cycler according to Table 4.

Table 4. Setup of QIAseq 16S/ITS Sample Index PCR Reaction

	Time	Temperature (°C)
Hold	2 min	95
3-step cycling		
Denaturation	30 s	95
Annealing	30 s	60
Extension	2 min	72
14 cycles		
Final extension (1 cycle)	7 min	72
Hold	∞	4

26. Remove the tubes/plate from the thermal cycler and briefly centrifuge.

27. Add 0.9X volume (45 µL) of QIAseq Beads to each sample from step 5. Mix well by pipetting up and down 12 times and briefly centrifuge.

28. Incubate for 5 min at room temperature.

29. Place the tubes/plate on a magnetic rack for 5 min. After the solution has cleared, carefully remove and discard the supernatant.

Note: Keep the beads on the magnetic stand while handling the supernatant and during the washing steps.

Important: Do not discard the beads as they contain the DNA of interest.

30. Add 200 µL of 80% ethanol while it is on the magnetic rack/plate. Rotate the tube (3 times) or move the plate side to side in the 2 positions of the magnet to wash the beads. Carefully remove and discard the wash.

31. Repeat the ethanol wash.

    Important: Completely remove all traces of the ethanol wash after the second wash. To do this, briefly centrifuge and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200 µL pipette, and then use a 10 µL pipette to remove any residual ethanol.

32. With the tubes/plate still on the magnet, air-dry at room temperature for 5 min (up to 15 min when using a plate).

    Note: Visually inspect that the pellet is completely dry. When completely dry, the beads should have a "cracked" appearance.

33. Remove the tubes/plate from the magnetic stand, and elute the DNA from the beads by adding 30 µL nuclease-free water. Mix well by pipetting. Incubate for 2–5 min at room temperature.

    Note: UCP PCR Water is not needed at this step.

34. Return the tubes/plate to the magnetic rack until the solution has cleared.

35. Transfer 25 µL of the supernatant to clean tubes/plate. This is the final Microbial 16S/ITS Screening Panel Sequencing Library.

36. Proceed to "Protocol: Library QC and Quantification". Alternatively, the completed QIAseq 16S/ITS Screening Panel Sequencing Library can be stored at –30°C to –15°C in a constant-temperature freezer.

Protocol: Library QC and Quantification

This protocol determines the quality and quantity of each QIAseq 16S/ITS library.

Important points before starting

- A portion of either the QIAseq 16S/ITS Screening Panel or QIAseq 16S/ITS Region Panel, 25 µL total volume of sequencing library, is the starting material for the library QC and quantification. When not in use, the QIAseq 16S/ITS Screening or Region Panel sequencing library should be stored on ice.
- Library QC involves the use of an Agilent 2100 Bioanalyzer or TapeStation.
- Library quantification involves the use of QIAGEN's QIAseq Library Quant System: QIAseq Library Quant Array Kit or QIAseq Library Quant Assay Kit.

Library QC (Agilent 2100 Bioanalyzer or TapeStation)

37. Analyze 1 µL of the QIAseq 16S/ITS Screening Panel or QIAseq 16S/ITS Region Panel sequencing library on an Agilent Bioanalyzer or TapeStation using a high-sensitivity DNA chip according to the manufacturer's instructions. A typical QIAseq 16S/ITS Screening Panel or QIAseq 16S/ITS Region Panel sequencing library is shown in Figure 2. Figure 3 shows a library that has been constructed from the QIAseq 16S/ITS Smart Control. These libraries have an extra ~440 base pair peak present as this represents the ITS amplicon, which is at equal abundance to the other 16S amplicons.

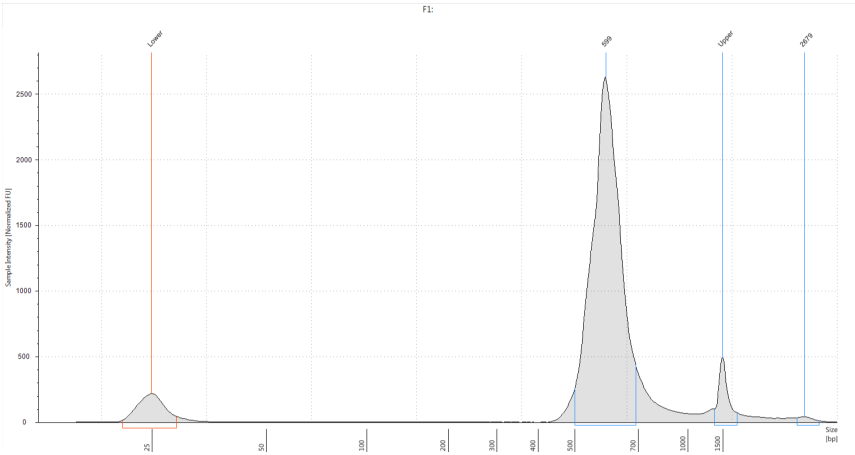

Figure 2. Bioanalyzer trace of library prepared with QIAseq 16S/ITS Screening Panel or QIAseq 16S/ITS Region Panel.

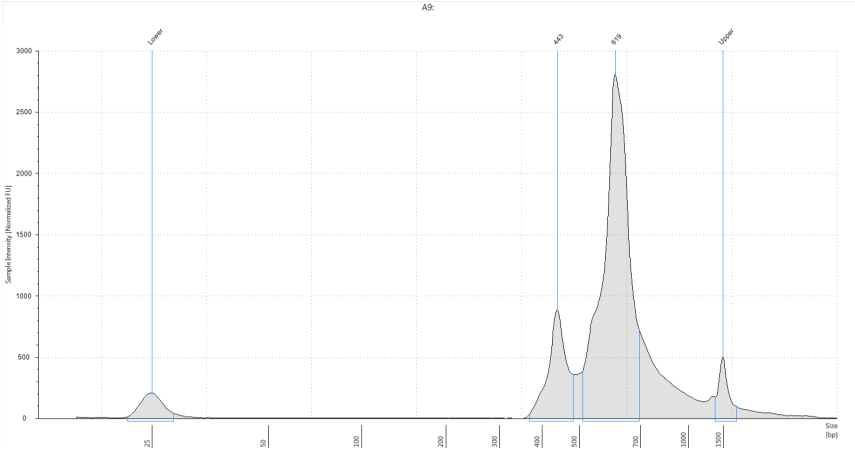

Figure 3. Bioanalyzer trace of library prepared with QIAseq 16S/ITS Smart Control (as DNA sample) and the QIAseq 16S/ITS Screening Panel.

38. Proceed to “Library Quantification”.

Library Quantification

39. The library yield measurements of the Bioanalyzer or TapeStation system use fluorescent dyes that intercalate into DNA or RNA, and cannot discriminate between DNA with or without adapter sequences. Real-time PCR-based methods provide an accurate quantification of complete QIAseq 16S/ITS Screening Panel or QIAseq 16S/ITS Region Panel sequencing libraries with full adapter sequences. Therefore, QIAGEN's QIAseq Library Quant Array Kit or Assay Kit, which contains laboratory-verified forward and reverse primers, together with a high-DNA standard, is highly recommended for accurate quantification of the prepared library.

2 nM of QIAseq 16S/ITS Screening Panel or QIAseq 16S/ITS Region Panel libraries should be used as input for the denaturation procedure to load the MiSeq sequencing instrument.

40. Proceed with "Protocol: Illumina MiSeq/MiSeq i100/NextSeq 1000/NextSeq 2000 Sequencing Setup".

Protocol: Illumina MiSeq/MiSeq i100/NextSeq 1000/NextSeq 2000 Sequencing Setup

Important points before starting

- Recommendations for library dilution concentrations and library loading concentrations are based on QIAGEN's QIAseq Library Quant System (see "Protocol: Library QC and Quantification").
- Add at least 1–2% phiX to final library pool before loading.
- Paired-end sequencing must be used for the QIAseq 16S/ITS Screening Panel or QIAseq 16S/ITS Region Panel on an Illumina platform.
- Please refer to the system-specific Illumina documents for complete instructions on how to denature sequencing libraries, prepare phiX, and set up a sequencing run.

Table 5. MiSeq Sequencing Kit compatibility and sample multiplexing guideline

Panel	MiSeq Reagent Kit*	No. of samples†	Library concentration loaded on MiSeq
Screening Panel	MiSeq v3 (600-cycle kit)	12–24‡	10 pM

*When using MiSeq v3 Reagent Kit, setup with paired end read program = 276 x 2.

† Number of samples multiplexed on each MiSeq sequencing run depends on sample type and complexity. This needs to be experimentally determined.

Table 6. MiSeq i100 and NextSeq 1000/2000 Sequencing Kit compatibility and sample multiplexing guideline

Panel	Sequencing Reagent Kit	No. of samples†	Library concentration loaded on instrument*
Screening Panel	MiSeq i100 5M (600-cycle kit)	3–6	65 pM

Table 6. MiSeq i100 and NextSeq 1000/2000 Sequencing Kit compatibility and sample multiplexing guideline (continued)

Panel	Sequencing Reagent Kit	No. of samples†	Library concentration loaded on instrument*
Screening Panel	MiSeq i100 25 M (600-cycle kit)	16–32	65 pM
Screening Panel	MiSeq i100 50M (600-cycle kit)	30–60	65 pM
Screening Panel	NextSeq P1 (600-cycle kit)	75–150	650 pM
Screening Panel	NextSeq P2 (600-cycle kit)	200–400	650 pM
Screening Panel	NextSeq P3 (600-cycle kit)	500–768	650 pM

*This is recommended starting point for library concentration to load. It may need to be optimized.

† Number of samples multiplexed on each Illumina sequencing run depends on sample type and complexity. This needs to be experimentally determined.

Sequencing

- **Important**: The following guidelines outline the most important settings for Illumina instruments. More detailed instructions on how to configure a run and how to create a sample sheet can be found in the "Product Resources" section for QIAseq MUDI indexes at **www.qiagen.com/QIAseq16S-ITSregionpanels**
- Always ensure that libraries have been quantified using QIAseq Library Quant Assay or a compatible method to enable equal library representation within the sequencing pool and exact pool concentrations for optimal flow cell loading and best sequencing performance.
- For complete instructions on how to denature sequencing libraries and set up a sequencing run, please refer to the system-specific Illumina documents.

1. **Sample dilution and pooling**:

   Dilute the final libraries to 2 nM.

Then, combine libraries with different sample indices in equimolar amounts if similar sequencing depth is needed for each library.

Note: Recommendations for the library dilution concentrations are based on the QIAseq Library Quant System.

2. Dilute according to the recommended starting point for library concentration depending on instrument and flowcell.

Protocol: Data Analysis Using CLC Microbial Genomics Module

Important points before starting

- If using the CLC Microbial Genomics Module, refer to the *CLC Microbial Genomics Module User Manual*.

Bacterial and Fungal Classification

1. Import FASTQ files.

   Import > **Illumina** > check **Paired reads** > select FASTQ files

2. Demultiplex amplicons (for use with Screening Panel or multiple Region Panels).

   Toolbox > **QIAGEN 16S Panel Analysis** > **Demultiplex Reads by Barcode** > If demultiplexing more than one sample, check **Batch** > Select FASTQ files > In Metadata table, select **barcodes all** file; in Barcode, select **Barcode** > check **Save**

3. Bacterial Classification

   Toolbox > **Microbial Genomics Module** > **Metagenomics** > **Amplicon Based OTU Clustering** > **Workflows** > **Data QC and OTU Clustering** > If Screening Panel or multiple Region Panels were used, select same region for all samples (i.e., Samples 1–24: V1V2) > Quality limit = 0.05 (default) > OTU picking = Reference based OTU > Select OTU database > Check **Save**

   If applicable, repeat for each region.

   After OTU clustering analysis, OTU tables can be merged into one table, if desired.

   Toolbox > **Microbial Genomics Module** > **Metagenomics** > **Abundance Analysis** > **Merge Abundance Tables**

4. Fungal Classification

   Toolbox > **Microbial Genomics Module** > **Metagenomics** > **Amplicon Based OTU Clustering** > **Workflows** > **Data QC and OTU Clustering** > Select demultiplexed FASTQ files. Select ITS region for each sample (i.e., Samples 1–24, ITS region) > Quality limit = 0.05 (default) > OTU picking = Reference based OTU > Select UNITE > Check Save

5. Comparing 16S variable regions – to compare performance of different 16S variable regions in terms of diversity.

   To determine which region contains the most OTU sequences and, therefore, potentially has the highest diversity, check the "Total predicted OTUs" in the OTU report for each region analysis.

   Alternatively, using the merged abundance table from step 3 (in Table view), select Region under "Aggregate sample." Then select the region that contains the highest number of features with greater than 5–10 reads at target taxonomy level (i.e., family, genus or species). Also, alpha diversity can be measured to determine which region exhibits the highest diversity.

6. For more in-depth analysis, please refer to the *CLC Microbial Genomics Module User Manual*.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx** (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Low library yield	
a) DNA contains PCR inhibitors carried over from sample preparation	If samples contain high concentration of PCR inhibitors (such as stool or soil), use appropriate sample DNA extraction kit.
b) Improper reaction setup	Ensure reactions are thoroughly mixed, prepared, and incubated at recommended temperatures.
c) Excess ethanol not removed during bead cleanup steps	After each second ethanol wash, ensure that excess ethanol is removed. Briefly centrifuge and return the tubes or plate to the magnetic stand. Remove with a 10 µL pipette to remove any residual ethanol. In addition, allow beads to dry for the appropriate amount of time.
d) Insufficient starting material	If starting material is significantly less than 1 ng bacterial or fungal DNA, up to 4 µL of microbial DNA sample can be added per QIAseq 16S/ITS reaction. Adjust UCP PCR Water so that total 16S/ITS PCR reaction volume equals 10 µL. Also, use 20 cycles at the 16S/ITS PCR step. If library yields are still low, then additional cycles (3–5) can be added at the sample index PCR step.
Primer-dimers observed	
a) Excess ethanol not removed during bead cleanup steps	After each second ethanol wash, ensure that excess ethanol is removed. Briefly centrifuge and return the tubes or plate to the magnetic stand. Remove with a 10 µL pipette to remove any residual ethanol. In addition, allow beads to dry for the appropriate amount of time.
b) Improper mixing of QIAseq beads and PCR reaction	After adding QIAseq beads, mix by pipetting up and down 12 times.

	Comments and suggestions
c) Input DNA amount too low	If input DNA is equivalent to less than 1 pg, then primer-dimers may be formed. If starting material is significantly less than 1 ng, up to 4 µL of microbial DNA sample can be added per QIAseq 16S/ITS reaction. Adjust UCP PCR Water so that total 16S/ITS PCR reaction volume equals 10 µL. Also, use 20 cycles of 16S/ITS PCR.

Sequencing Issues

a) Too low or too high cluster density	Accurate library quantification is the key for optimal cluster density on any sequencing instrument. PCR-based quantification method is recommended. Other methods may lead to the incorrect quantification of the library, especially when there is overamplification.
b) Very low clusters passing filter	Make sure the library is accurately quantified and that the correct amount is loaded onto the sequencing instrument. Adding more than 2% phiX may help with clusters passing filter.

References

1. *CLC Microbial Genomics Module User Manual*. June 20, 2025. resources.qiagenbioinformatics.com/manuals/clcmgm/current/User_Manual.pdf

Appendix A: Preparation and Use of QIAseq 16S/ITS Smart Control

QIAseq 16S/ITS Smart Control is a synthetic DNA construct that contains target sequences for all QIAseq 16S/ITS primers interspersed with nonbacterial/nonfungal sequences. When the Smart Control is included during library preparation (as a separate sample), it can monitor both the library construction process and contamination introduced by the environment or user. Any contaminating reads detected by the Smart Control can then be subtracted for all samples that were prepared.

Preparation and use

When working with high bacterial content samples, simply add 1 µL of the QIAseq 16S/ITS Smart Control to each primer pool PCR reaction as described in the protocol.

When working with low bacterial content or low biomass samples, dilute the QIAseq 16S/ITS Smart Control immediately before use. Prepare dilution by adding 2 µL of stock Smart Control + 198 µL of UCP PCR Water. Discard after use. Do not store.

Ordering Information

Product	Contents	Cat. no.
QIAseq 16S/ITS Pro Screening Panel (24)	For targeted enrichment of 16S/ITS from 24 microbial DNA samples	331782
QIAseq 16S/ITS Pro Screening Panel (96)	For targeted enrichment of 16S/ITS from 96 microbial DNA samples	331785
QIAseq 16S/ITS Smart Control (10)	Process control for monitoring library construction and contamination with the QIAseq 16S/ITS Panels	333832
QIAseq UX 24 Index Kit IL UDI (24)	Twenty-four unique dual indices, and custom read primers compatible with Illumina platforms; enough to process a total of 96 samples	331802
QIAseq UX 96 Index Kit IL (96)	Sample Index Kit Sets for 96 libraries using unique dual indices on Illumina NGS instruments; indices are supplied as liquid in a 96 well plate with a pierceable foil seal	331815; 331825; 331835; 331845; 331855; 331865; 331875; 331885
Related products		
QIAseq Library Quant Array Kit	Reagents for quantification of libraries prepared for Illumina or Ion Torrent® platforms; array format	333304
QIAseq Library Quant Assay Kit	Reagents for quantification of libraries prepared for Illumina or Ion Torrent platforms; assay format	333314
DNeasy® PowerSoil Pro Kit (50)	For the isolation of microbial genomic DNA from all soil types	47014
QIAamp PowerFecal Pro DNA Kit (50)	For the isolation of DNA from stool, gut material, and biosolids	51804
QIAamp DNA Host-Free Microbiome (50)	The QIAamp DNA Host-Free Microbiome Kit selectively isolates microbial DNA from samples rich in host DNA, such as tissues, bodily fluids and swabs, promoting accurate microbiome analysis due to efficient host nucleic acid depletion.	51904

Document Revision History

Revision	Description
12/2025	Initial release.

Notes.

Limited License Agreement for QIAseq® 16S/ITS Pro Screening Panel

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this Instructions for Use and for use with components contained in the panel only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this panel with any components not included within this panel except as described in the protocols provided with the product, this Instructions for Use, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this panel and/or its use(s) do not infringe the rights of third-parties.
3. This panel and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the panel agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the panel and/or its components.

For updated license terms, see **www.qiagen.com**