Quick-Start Protocol June 2018

QIAseq® 16S/ITS Screening Panel and 16S/ITS Region Panel Library QC and Quantification

Further information

- *QIAseq 16S/ITS Panels Handbook*: **www.qiagen.com/qiaseq-16s-its**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: toll-free 00800-22-44-6000, or **www.qiagen.com/contact**

Important points before starting

- A portion of either the QIAseq 16S/ITS Screening Panel or QIAseq 16S/ITS Region Panel, 25 µl sequencing library is the starting material for the library QC and quantification. When not in use, the QIAseq 16S/ITS Screening Panel or QIAseq 16S/ITS 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 (cat. no. 333304) or QIAseq Library Quant Assay Kit (cat. no. 333314).

Library QC (Agilent 2100 Bioanalyzer)

1. Analyze 1 µl of the QIAseq 16S/ITS Screening Panel or QIAseq 16S/ITS Region Panel sequencing library on an Agilent Bioanalyzer using a High Sensitivity DNA chip according to the manufacturer's instructions.

Library quantification

1. 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 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.

2. Proceed to Protocol: *Sequencing Setup on Illumina MiSeq*.

Sequencing Setup on Illumina MiSeq

Important points before starting

- Recommendations for library dilution concentrations and library loading concentrations are based on QIAGEN's QIAseq Library Quant System.
- QIAseq Read 1 Primer (Custom Read 1 Sequencing Primer) and QIAseq 16S/ITS Read 2 Primer (Custom Read 2 Sequencing Primer) MUST be used when performing sequencing on an Illumina platform.
- Custom Read Primers go into the following specific MiSeq reagent cartridge positions:

  QIAseq Read 1 Primer: MiSeq Position #18

  QIAseq 16S/ITS Read2 Primer: MiSeq Position #20
- Paired-end sequencing should be used for the QIAseq 16S/ITS Screening Panel or QIAseq 16S/ITS Region Panel on an Illumina platform.

- For complete instructions on how to denature sequencing libraries, prepare custom index primers and set up a sequencing run, please refer to the system-specific Illumina documents.

Sequencing Preparations for MiSeq

1. **Sample sheet setup**: Set up a sample sheet with Custom Sequencing Read 1 Primer and Custom Sequencing Read 2 Primer using Illumina Experiment Manager v1.2, or later. The QIAseq 16S/ITS Screening Panel or QIAseq 16S/ITS Region Panel Sample Indexes are compatible with Illumina's TruSeq HT adapter sample index system. Select and check the parameters as follows:
   Category: Other
   Select Application: FASTQ Only
   Sample Prep Kit: TruSeq HT
   Index Reads: 2
   Read Type: Select Paired End Read
   Cycles for Read 1: 276 (251 if using MiSeq V2 500 cycle kit)
   Cycles for Read 2: 276 (251 if using MiSeq V2 500 cycle kit)
   Important: Check Custom Primer for Read 1
   Important: Check Custom Primer for Read 2
   Important: Check Use Adapter Trimming
   Important: Check Use Adapter Trimming Read 2
2. **Sample dilution and pooling**: Dilute the final libraries to 2 nM for the MiSeq. Then, combine libraries with different sample indexes in equimolar amounts if similar sequencing depth is needed for each library.
3. **Library preparation and loading**: Prepare and load the library on a MiSeq according to the MiSeq System Denature and Dilute Libraries Guide. The final denatured library concentration is 10 pM on a MiSeq (V3 kit) or 8 pM (V2 kit).

4. **Custom Sequencing Primer for Read 1 and Read 2 preparation and loading**: Use 597 µl HT1 (Hybridization Buffer) to dilute 3 µl of QIAseq Read 1 Primer to obtain a final concentration of 0.5 µM. Use 597 µl HT1 (Hybridization Buffer) to dilute 3 µl of QIAseq 16S/ITS Read 2 Primer to obtain a final concentration of 0.5 µM. Load 600 µl of the diluted QIAseq Read 1 Primer to Position #18 and load 600 µl of the diluted QIAseq 16S/ITS Read 2 Primer to Position #20 of the MiSeq reagent cartridge. For more details, please refer to Illumina's Protocol: miseq_using_custom_primers_15041638_b.pdf for the MiSeq.
5. Upon completion of the sequencing run, proceed to *Data Analysis using CLC Microbial Genomics Module*.

Scan QR code for handbook.

1114266 06/2018 HB-2563-001