Hyaseq Bacterial 16S (V3-V4) Amplicon Library Prep Kit for Illumina

Cat. No.: HYD621

Product Description

Hyaseq Bacterial 16S (V3-V4) Amplicon Library Prep Kit for Illumina is designed for high-throughput sequencing of the V3–V4 regions of bacterial 16S rDNA. Combined with bioinformatics analysis, it enables the identification and characterization of microbial communities, primarily bacteria, in various environmental samples, providing valuable insights into taxonomic composition, relative abundance, community structure, and phylogenetic relationships.The kit includes all reagents required for bacterial 16S rDNA amplification. The 16S F/R primers amplify an approximately 500 bp fragment spanning the V3–V4 regions of bacterial 16S rDNA. When used in conjunction with the NGS Tagment Index Kit for Illumina (Cat.#HYD342), it enables amplification of full-length library constructs for sequencing. All reagents undergo rigorous quality control and functional validation to ensure consistent and reproducible library preparation.

Components

Components	24T	96T
2×Multiplex PCR Master Mix	300 µL	1200 µL
16S Primer Mix	48 µL	192 µL
2× HiFi Amplification Mix	300 µL	1200 µL

This kit is compatible with Illumina sequencing platforms and must be used in combination with the NGS Tagment Index Kit for Illumina (Cat. # HYD342), specifically designed for Illumina platforms.

Storage

Store at -25 ~ -15°C, valid for 1 year.

Instructions

I. Required Materials and Equipment (User-Supplied)

1) DNA Purification Beads: NGS DNA Selection Beads (Cat. # HYD511), AMPure XP Beads (Cat. #A63880), or equivalent products.
2) DNA Quality Control: Agilent Technologies 2100 Bioanalyzer or equivalent instruments.
3) Second-Round PCR Primer Mix: NGS Tagment Index Kit for Illumina (Cat. # HYD342).
4) Other Materials and Equipment: Absolute ethanol, nuclease-free ultrapure water, low-retention microcentrifuge tubes, PCR tubes, magnetic racks, PCR thermal cycler, and other standard laboratory supplies.

II. Workflow

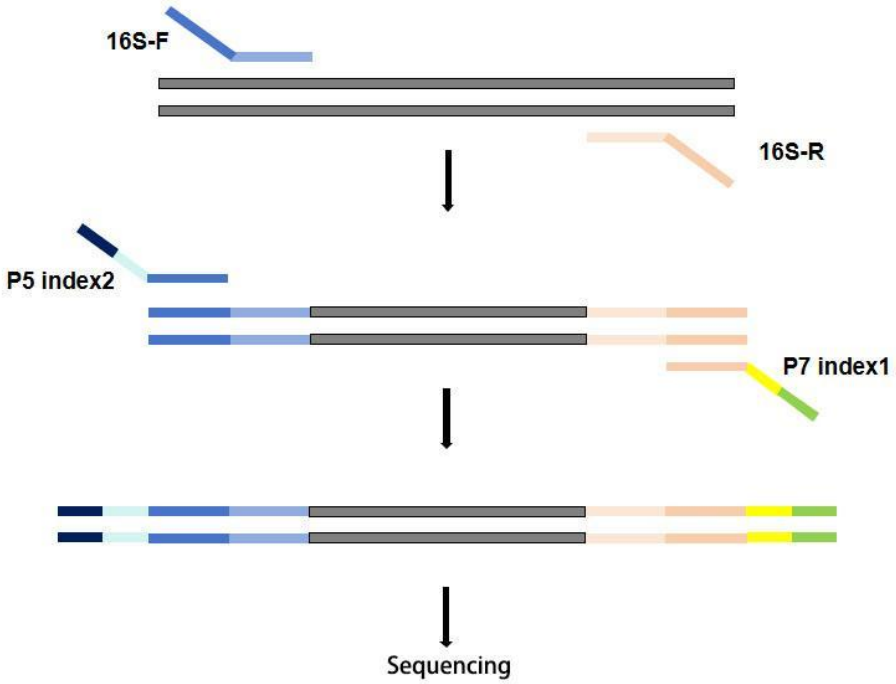

Figure 1. Library Preparation Workflow

III. Procedure

1. First-Round PCR (1st PCR)

Amplify the V3–V4 region of bacterial 16S rDNA from metagenomic samples.

1) Thaw the reagents, mix thoroughly by inversion, and place on ice until use.
2) Prepare the PCR reaction mixture in a nuclease-free PCR tube according to Table 1.

Table 1. PCR Reaction System

Components	Volume
Metagenomic DNA	x μL
2×Multiplex PCR Master Mix	12.5 μL
16S Primer Mix	2 μL
ddH_2O	Up to 25 μL

3) Gently mix by pipetting up and down or vortexing, then briefly centrifuge to collect the reaction mixture at the bottom of the tube.

4) Place the PCR tube in a thermal cycler and run the PCR program specified in Table 2.

Table 2. PCR Reaction Program

Temperature	Time	Cycles
Heat Lid 105°C	On	-
99 °C	2 min	1
99 °C	15 s	x cycle (determined by the amount of metagenomic sample input) 0.1 ng – 35 cycle 1 ng – 31 cycle 10 ng – 28 cycle
60 °C	2 min	
72 °C	10 min	1
4 °C	Hold	-

2. 1st PCR Product Purification (Magnetic Bead Cleanup)

1) Preparation: Remove NGS DNA Selection Beads from storage and allow them to equilibrate to room temperature for at least 30 minutes. Prepare fresh 80% ethanol.

2) Thoroughly resuspend the beads by vortexing or repeated inversion.

3) Add 22.5 μL of NGS DNA Selection Beads (0.9×; bead-to-DNA ratio = 0.9:1) to the PCR product. Mix thoroughly by vortexing or gently pipetting up and down. Incubate at room temperature for 5 minutes.

4) Briefly centrifuge the PCR tube and place it on a magnetic rack to separate the beads from the solution. Once the solution has cleared (approximately 5 minutes), carefully remove the supernatant.

5) Keep the PCR tube on the magnetic rack. Add 200 µL of freshly prepared 80% ethanol to wash the beads. Incubate at room temperature for 30 seconds, then carefully remove the supernatant.
6) Repeat Step 5 once more, for a total of two washes.
7) Keep the PCR tube on the magnetic rack, open the tube lid, and air-dry the beads until cracks just begin to appear (no more than 5 minutes).
8) Remove the PCR tube from the magnetic rack for elution. Add 21 µL of ddH_2O and thoroughly resuspend the beads by vortexing or gently pipetting up and down. Incubate at room temperature for 5 minutes. Briefly centrifuge the tube and place it on the magnetic rack. Once the solution has cleared (approximately 5 minutes), carefully transfer 20 µL of the supernatant to a new PCR tube without disturbing the beads.
9) Use Hyasen dsDNA Standard 1 and dsDNA Standard 2 as standards to calibrate the Qubit fluorometer with diluted 1× dsDNA Reagent dye. After calibration, mix 199 µL of 1× dsDNA Reagent dye with 1 µL of the purified PCR product. Vortex thoroughly and briefly centrifuge before measuring the DNA concentration using the Qubit fluorometer.

3. 2nd PCR Reaction System

Use 100 ng of the purified first-round PCR product as the template for the second-round PCR amplification and enrichment. This step must be performed in combination with the NGS Tagment Index Kit for Illumina (Cat. # HYD342).

1) Thaw the reagents listed in Table 3, mix thoroughly by inversion, and place on ice until use.
2) Prepare the PCR reaction mixture in a nuclease-free PCR tube according to Table 3.

Table 3. PCR Amplification Reaction System

Components	Volume
Purified DNA (Product from step 2)	100 ng
2× HiFi Amplification Mix	12.5 µL*
N5xx*(Cat. # HYD342)	1 µL**
N7xx*(Cat. # HYD342)	1 µL
Total	25 µL

*Index Selection Strategy: Illumina systems use green fluorescence to label dG/dT and red fluorescence to label dC/dA. To ensure successful sequencing, both green and red fluorescent signals must be present in each cycle. Therefore, the balance between green and red signals must be considered when selecting Indexes. The user manual for product #HYD342 (NGS Tagment Index

Kit for Illumina) provides recommended Index combinations based on the number of samples. Please read the manual before use.

3) Gently mix by pipetting up and down or vortexing, then briefly centrifuge to collect the reaction mixture at the bottom of the tube.
4) Place the PCR tube in a thermal cycler and run the PCR amplification program specified in Table 4

Table 4. PCR Amplification Reaction Program

Temperature	Time	Cycles
98 ℃	45 s	1
98 ℃	15 s	
60 ℃	30 s	6
72 ℃	30 s	
72 ℃	1 min	1
4 ℃	Hold	-

4. 2nd PCR Product Purification (Magnetic Bead Cleanup)

Follow the same magnetic bead purification procedure as described for the first-round PCR cleanup (Section 3.2). Purify the second-round PCR products using NGS DNA Selection Beads at a 0.9× bead-to-sample volume ratio (Beads = 0.9:1).

5. Library Quality Control

In general, the quality of prepared libraries can be assessed by measuring DNA concentration using a Qubit fluorometer and analyzing fragment size distribution by agarose gel electrophoresis.

6. Sequencing

The sequencing mode is set to MiSeq PE300. The sequencing data volume distribution is 0.05 M clean reads per sample.