Illumina 16S NGS Library Kit (Two-Step PCR Method)

【Product Name】 Illumina 16S NGS Library Kit (Two-Step PCR Method)

【Packing Size】 YST0123-01，8 reactions
YST0123-02，24 reactions
YST0123-03，96 reactions

【Product Usage 】

Illumina 16S NGS Library Kit (Two-Step PCR Method) is a bacterial DNA library preparation kit for the Illumina high-throughput sequencing platforms. This kit is used for the direct amplification and preparation of the stool sample DNA library, and the DNA NGS library can be directly obtained without the tedious DNA extraction process.

【Product Introduction 】

This kit is capable of generate 16S DNA libraries form stool directly from stool without tedious and messy feces' DNA purification. The kit is easy to use and only requires two steps of amplification. Bacteria V3 and V4 regions are amplified in the first step of PCR and then Illumina sequencing dual-index adapters are added in the second step of PCR. The resulted 16S DNA libraries can be sequenced on MiSeq. All the reagents provided in the kit have been thoroughly tested for quality control to ensure high performance and reproducibility.

【Kit Components】

Item
HiFi Nucleic Acid-Direct Extraction reagent
PK reagent
GTX Buffer B
2X Ultra HiFi PCR Master Mix
5X Illumina 16S NGS Primer Pool
2X HiFi PCR Master Mix
10X Illumina Library Amplification Primer Mix
Nextera XT Index 1 Primers (N7XX)
Nextera XT Index 2 Primers (S5XX)

【16S Library Preparation Method】

Stool sample processing

1. Take 5-10mg of stool sample and put it in a 0.5mL tube.
2. Add 100μL of HiFi Nucleic Acid-Direct Extraction reagent and 1μLof PK reagent to the tube, cover the tube, vortex and mix for 15 seconds, heat and shake at 650 rpm on a dry thermostat at 56°C for 30 minutes.

3. Increase temperature and heat at 95°C for 1 minutes. Then remove the centrifuge tube and place it at room temperature.
4. Cool to room temperature, add 100uL GTX Buffer B to the centrifuge tube after incubation, vortex and mix for 10 seconds, centrifuge at 12000 rpm/min for 1 minute, then transfer the supernatant to a clean centrifuge tube for use. This is the bacterial DNA sample for amplification.

PCR Amplification

1. Sep up the following reaction of DNA, 2X Ultra HiFi PCR Master Mix, and primers:

Component	Volume
2X Ultra HiFi PCR Master Mix	12.5μL
5X Illumina 16S NGS Primer Pool	5μL
H2O	5μL
Bacterial DNA from last step	2.5μL
Total	25μL

2. Seal plate and perform PCR in a thermal cycler using the following program:

95℃ for 10 minutes
25 cycles of:
    95℃ for 30 seconds
    55℃ for 30 seconds
    72℃ for 30 seconds
72℃ for 5 minutes
Hold at 4℃

3. After the PCR amplification is completed, the amplification solution is centrifuged briefly.

Purification

1. Freshly prepared 80% Ethanol.
2. Bring the magnetic beads to room temperature. Vortex the magnetic beads for 30 seconds to make sure that the beads are evenly dispersed.
3. Add 25μL beads to each PCR well and mix by pipetting up and down.
4. Incubate at room temperature for 5 minutes.
5. Place the plate on a magnetic stand for 3 minutes.
6. With the plate on the magnetic stand, use pipette to remove and discard the supernatant.
7. Add 125μL of freshly prepared 80% ethanol to each well, incubate the plate on the magnetic stand for 30 seconds. Carefully remove and discard the supernatant.
8. Perform a second ethanol wash. Add 125μLof freshly prepared 80% ethanol to each sample well, incubate the plate on the magnetic stand for 30 seconds. Carefully remove and discard

the supernatant.

9. Use a P20 multichannel pipette with fine pipette tips to remove the residual ethanol.
10. With the Amplicon PCR plate on the magnetic stand, allow the beads to air-dry for 5 minutes.
11. Add 10 μL ddH2O to each beads' well. Gently mix, incubate at room temperature for 3 minutes.
12. Place the plate on a magnetic stand for 3 minutes or until the supernatant has cleared.
13. Carefully transfer the supernatant to a new tube. This is the amplified 16 DNA.

PCR to Add Adapter Index to 16S DNA

1. Step up the following reaction:

Component	Volume
2X HiFi PCR Master Mix	6.25μL
Nextera XT Index 1 Primers(N7XX)	0.5μL
Nextera XT Index 2 Primers(S5XX)	0.5μL
PCR product DNA	5.25μL
Total	12.5μL

2. Perform PCR on a thermal cycler using the following program:

95℃ for 2 minutes
8 cycles of:
    95℃ for 30 seconds
    55℃ for 30 seconds
    72℃ for 30 seconds
72℃ for 5 minutes
Hold at 4℃

Purification

1. Freshly prepared 80% Ethanol.
2. Bring the magnetic beads to room temperature. Vortex the magnetic beads for 30 seconds to make sure that the beads are evenly dispersed.
3. Add 12.5μL beads to each PCR well and mix by pipetting up and down.
4. Incubate at room temperature for 5 minutes.
5. Place the plate on a magnetic stand for 3 minutes.
6. With the plate on the magnetic stand, use pipette to remove and discard the supernatant.
7. Add 125μL of freshly prepared 80% ethanol to each well, incubate the plate on the magnetic stand for 30 seconds. Carefully remove and discard the supernatant.
8. Perform a second ethanol wash. Add 125μLof freshly prepared 80% ethanol to each sample

well, incubate the plate on the magnetic stand for 30 seconds. Carefully remove and discard the supernatant.

9. Use a P20 multichannel pipette with fine pipette tips to remove the residual ethanol.
10. With the Amplicon PCR plate on the magnetic stand, allow the beads to air-dry for 5 minutes.
11. Add 15µL ddH2O to each beads' well. Gently mix, incubate at room temperature for 3 minutes.
12. Place the plate on a magnetic stand for 3 minutes or until the supernatant has cleared.
13. Carefully transfer the supernatant to a new tube. This is the final 16 DNA library for sequencing.