16S V1-V3 Library Preparation Kit for Illumina

Product Insert

Product # 70200, 70210, 70220, 70230, 70240

Contents

Product Overview

The 16S V1-V3 Library Preparation Kit for Illumina consists of the reagents and components required for library preparation of the 16S V1-V3 amplicon libraries to be used for next-generation sequencing on Illumina platforms. All molecular reagents including primers, enzyme mixes, indexes, and buffers are provided. Instructions for PCR clean up with the AMPure XP Magnetic Beads (supplied by customer) are also included for rapid purification of nucleic acid products generated at two steps of the workflow. The library prep workflow could be used for purified DNA inputs from different sources including stool, soil, water, saliva, plant, urine, skin swab, vaginal swab, cheek swab, nasal swab, plasma/serum, tongue swab, gum swab, and others.

The 16S V1-V3 Library Preparation Kit for Illumina has a streamlined procedure that reduces the handling time such that the library prep procedure can be completed in approximately 4 hours (see diagram below). Input DNA is first subjected to targeted PCR to amplify the V1-V3 region of the DNA encoding 16S rRNA. The post-PCR reaction is then cleaned up using AMPure XP beads. Dual index primers are then added using a limited-cycle PCR. The indexed amplicons flanked by 5' and 3' barcoded adaptors are then cleaned using AMPure XP beads. The libraries are then ready for quantification, pooling and sequencing.

Storage Conditions and Product Stability

Norgen's 16S V1-V3 Library Prep Kit for Illumina is shipped as one kit box (for the 24 prep kit) or two sub-component kits (for the 96 prep kit). All kits should be stored at -20°C upon arrival.

<table>
<tr><th></th><th rowspan="2">16S V1-V3 Library Preparation Kit for Illumina (24 preps) #70200</th><th colspan="4">16S V1-V3 Library Preparation Kit for Illumina (96 preps)</th><th rowspan="2">Storage</th></tr>
<tr><th>Set A #70210</th><th>Set B #70220</th><th>Set C #70230</th><th>Set D #70240</th></tr>
<tr><td>16S V1-V3 Library Preparation Reagent Kit</td><td rowspan="2">One kit box including reagents and index primers</td><td colspan="4">16S V1-V3 Library Preparation Reagent Kit (96 Preps)
#70211</td><td>-20°C</td></tr>
<tr><td>Dual Index Kit</td><td>Dual Index Kit – Set A
Product # 70001</td><td>Dual Index Kit – Set B
Product # 70002</td><td>Dual Index Kit – Set C
Product # 70003</td><td>Dual Index Kit – Set D
Product # 70004</td><td>-20°C</td></tr>
</table>

All kit components should remain stable for at least 1 year when stored at the specified storage conditions.

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Library Preparation Kits is tested against predetermined specifications to ensure consistent product quality.

Precautions and Disclaimers

This product is for research use only. Not for use in diagnostic procedures. This manual is proprietary to Norgen Biotek Corp and intended only for customer use in connection with the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose without the prior written consent of Norgen Biotek Corp. Follow the protocol included with the kit for best results.

16S V1-V3 Library Preparation Kit Components

The 16S V1-V3 Library Preparation Reagent Kit includes all reagents required for each step of the library preparation workflow.

Step	Component	Product # 70200 (24 preps)	Products # 70210, 70220, 70230, 70240 (96 preps)	Tube cap
Amplicon PCR (PCR 1)	MGX Master Mix	330 µL	1,320 µL	
	16S V1-V3 Primer Mix	70 µL	280 µL	
Index PCR (PCR 2)	Indexing Master Mix	660 µL	2 x 1,320 µL	
	N7xx Index Primer	50 µL	50 µL	
	S5xx Index Primer	70 µL	70 µL	
PCR Clean-Up	Resuspension Buffer	2 x 1,250 µL	2 x 5,000 µL	
	Nuclease-free water	1,250 µL	1 x 6,000 µL	White

Dual Index Primers

24 prep kit: Index primers are included with the library prep reagents in one kit box.
96 prep kit: Index primers are included in a sub-component kit.
Included indexes in each kit format are as follows:

Component	Product # 70200 (24 preps)	Products # 70210, 70220, 70230, 70240 (96 preps)
Dual Index Primers	12 of N7xx – 50 µL, each 2 of S5xx – 70 µL, each	12 of N7xx – 50 µL, each 8 of S5xx – 70 µL, each

Included index primers in each kit:

Product # 70200		Product # 70210		Product # 70220		Product # 70230		Product # 70240	
N7xx	**S5xx**	**N7xx**	**S5xx**	**N7xx**	**S5xx**	**N7xx**	**S5xx**	**N7xx**	**S5xx**
N701	S505	N701	S502	N716	S502	N701	S513	N716	S513
N702	S506	N702	S503	N718	S503	N702	S515	N718	S515
N703		N703	S505	N719	S505	N703	S516	N719	S516
N704		N704	S506	N720	S506	N704	S517	N720	S517
N705		N705	S507	N721	S507	N705	S518	N721	S518
N706		N706	S508	N722	S508	N706	S520	N722	S520
N707		N707	S510	N723	S510	N707	S521	N723	S521
N710		N710	S511	N724	S511	N710	S522	N724	S522
N711		N711		N726		N711		N726	
N712		N712		N727		N712		N727	
N714		N714		N728		N714		N728	
N715		N715		N729		N715		N729	

Customer-Supplied Materials and Equipment

- Nuclease-Free PCR Tubes/Plates and their compatible caps/seals
- Nuclease-Free Microcentrifuge Tubes
- Ampure XP Magnetic Beads (Beckman Coulter; Cat. No. A63880)
- Freshly diluted 80% Ethanol
- Thermocycler
- Ice or Cold Block
- Magnetic Rack
- Microcentrifuge

Library Preparation Procedure

Input Consideration

1. Ensure that DNA is isolated using a product that can recover genomic DNA.
2. It is recommended that DNA isolation is performed using Norgen's DNA purification products. All of Norgen's purification products will reliably recover total DNA including microbial DNA.
3. Initial quantification of DNA could be performed by standard procedures including spectrophotometry (such as NanoDrop™), capillary electrophoresis (such as Agilent Bioanalyzer) or fluorescent-based detection (such as Qubit®).
4. The minimum amount of DNA to be used in this kit's protocol is 5 ng/µL.

Step A: Amplicon PCR (PCR 1)

1. Set up the following reaction of DNA, MGX Master Mix, and Primers:

Component	Amount
Microbial DNA (5 ng/µL)	2.5 µL
16S V1-V3 Primer Mix – Blue Cap	2.5 µL
PCR Grade Water – White Cap	7.5 µL
MGX Master Mix – Blue Cap	12.5 µL
Total	**25 µL**

2. Gently pipette up and down 10 times to mix.
3. Cover the plate with cap/seal.
4. Spin the plate for 1 minute (~1000 x g).
5. Perform the PCR in a thermal cycler using the following program:

Temperature (ºC)	Time	Cycles
95	3 min	1
95	30 sec	25
55	30 sec	
72	30 sec	
72	5 min	1
4	∞	

6. Continue with Amplicon PCR Clean-Up, or store sealed at -20ºC.

Step B: PCR Clean-Up

The protocol requires AMPure XP Magnetic Beads to purify the amplicon product from free primers and primer-dimer species.

1. Spin the amplicon PCR plate to collect condensation.
2. Vortex the AMPure XP beads for 30 seconds to ensure even distribution of beads in solution.
3. Add 20 µL of AMPure XP beads (vortex thoroughly immediately prior to use). Gently pipette entire volume in each well up and down 10 times. Incubate at room temperature for 5 minutes without shaking.
4. After 5 minutes, place the plate on a magnetic rack for 2 minutes. Ensuring the supernatant is clear of beads, aspirate and discard the supernatant without touching the magnetic beads (while the plate remains on the magnetic plate).
5. Leave the sample plate on the magnetic plate and add 200 µL of freshly prepared 80% ethanol to wash the sample. Incubate at room temperature for 30 seconds and carefully aspirate/discard the supernatant.
6. Repeat the previous step for an additional wash. Remove as much of the ethanol as possible by pipetting before proceeding.
7. With the sample plate remaining on the magnetic rack, allow the beads to air-dry (caps open) for 5-10 minutes.
8. Remove the plate from the magnetic rack and add 52.5 µL of the Resuspension Buffer (provided in the reagent kit). Carefully pipette up and down 10 times to mix, ensuring beads are fully suspended before proceeding. Incubate at room temperature for 2 minutes.
9. Place on magnetic plate and allow to stand for 2 minutes. Carefully transfer 50 µL of the supernatant to a new 96-well elution plate.
10. Continue with Index PCR, or store sealed at -20ºC.

Step C: Indexing PCR (PCR 2)

1. Transfer 5 µL from each well to a new 96-well plate. The remaining 45 µL is not used in the protocol and can be stored sealed at -20°C for other uses.
2. Arrange the indexes using the following arrangements as needed:
    a. Arrange N7xx index primer tubes (orange caps) horizontally, aligned with columns 1 through 12.
    b. Arrange S5xx index primer tubes (white caps) vertically, aligned with rows A through H.
3. Transfer 5 µL of cleaned amplicon PCR product (obtained from step B) into a new 96-well PCR plate.
4. Set up the following reaction of DNA, Index N7xx and Index S5xx, Indexing Master Mix and PCR Grade Water, ensuring that each sample has a unique pair of index primers. Make a record of the primer pair used for each sample:

Component	Amount
Cleaned amplicon PCR product from Step B	5 µL
Index N7xx – Orange Cap	5 µL
Index S5xx – White Cap	5 µL
PCR Grade Water – White Cap	10 µL
Indexing Master Mix – Red Cap	25 µL
Total	**50 µL**

5. Gently pipette up and down 10 times to mix.
6. Cover the plate with cap/seal.
7. Spin the plate for 1 minute (~1000 x g).
8. Perform PCR on a thermal cycler using the following program:

Temperature (ºC)	Time	Total Cycles
95	3 min	1
95	30 sec	8
55	30 sec	
72	30 sec	
72	5 min	1
4	∞	

9. Continue with PCR Clean-Up 2, or store sealed at -20ºC.

Step D: PCR Clean-Up 2

The protocol requires AMPure XP Magnetic Beads to purify the amplicon product from free primers and primer-dimer species.

1. Spin the amplicon PCR plate to collect condensation.
2. Vortex the AMPure XP beads for 30 seconds to ensure even distribution of beads in solution.
3. Add 56 µL of AMPure XP beads (vortex thoroughly immediately prior to use). Gently pipette entire volume in each well up and down 10 times. Incubate at room temperature for 5 minutes without shaking.
4. After 5 minutes, place the plate on a magnetic rack for 2 minutes. Ensuring the supernatant is clear of beads, aspirate and discard the supernatant without touching the magnetic beads (while the plate remains on the magnetic plate).
5. Leave the sample plate on the magnetic plate and add 200 µL of freshly prepared 80% ethanol to wash the sample. Incubate at room temperature for 30 seconds and carefully aspirate/discard the supernatant.
6. Repeat the previous step for an additional wash. Remove as much of the ethanol as possible by pipetting before proceeding.
7. With the sample plate remaining on the magnetic rack, allow the beads to air-dry (caps open) for 5-10 minutes.
8. Remove the plate from the magnetic rack and add 27.5 µL of the Resuspension Buffer (provided in the reagent kit). Carefully pipette up and down 10 times to mix, ensuring beads are fully suspended before proceeding. Incubate at room temperature for 2 minutes.
9. Place on magnetic plate and allow to stand for 2 minutes. Carefully transfer 25 µL of the supernatant to a new 96-well elution plate.
10. Continue with Library quantification, normalization, and pooling, or store sealed at -20ºC.

Library Quantification, Normalization, and Pooling

Quantify the libraries. We recommend qPCR-based library quantification for optimal normalization. Once quantified, dilute each library to 4 nM. Aliquot 5 µL of 4 nM DNA from each library and mix by vortexing to create the library pool.

Pool Quantification

Quantify the pooled libraries. We recommend qPCR or PicoGreen platforms for quantification at this step. Once the pool is quantified, you are ready to proceed with denaturing and sample loading on an Illumina sequencing platform.

Appendix

Amplicon PCR Primers

16S V1-V3 Forward (5'-3'):
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGAGTTTGATCCTGGCTCAG

16S V1-V3 Reverse (5'-3'):
GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATTACCGCGGCTGCTGG

Index PCR Primers

N7xx (5'-3'):
CAAGCAGAAGACGGCATACGAGATNNNNNNNNGTCTCGTGGGCTCGG

S5xx (5'-3'):
AATGATACGGCGACCACCGAGATCTACACNNNNNNNNTCGTCGGCAGCGTC

List of Indexes

N7xx

Index ID	Bases for Sample Sheet
N701	TAAGGCGA
N702	CGTACTAG
N703	AGGCAGAA
N704	TCCTGAGC
N705	GGACTCCT
N706	TAGGCATG
N707	CTCTCTAC
N710	CGAGGCTG
N711	AAGAGGCA
N712	GTAGAGGA
N714	GCTCATGA
N715	ATCTCAGG
N716	ACTCGCTA
N718	GGAGCTAC
N719	GCGTAGTA
N720	CGGAGCCT
N721	TACGCTGC
N722	ATGCGCAG
N723	TAGCGCTC
N724	ACTGAGCG
N726	CCTAAGAC
N727	CGATCAGT
N728	TGCAGCTA
N729	TCGACGTC

S5xx

Index ID	Bases for Sample Sheet HiSeq 2000/2500 & MiSeq	Bases for Sample Sheet NextSeq & HiSeq 3000/4000
S502	CTCTCTAT	ATAGAGAG
S503	TATCCTCT	AGAGGATA
S505	GTAAGGAG	CTCCTTAC
S506	ACTGCATA	TATGCAGT
S507	AAGGAGTA	TACTCCTT
S508	CTAAGCCT	AGGCTTAG
S510	CGTCTAAT	ATTAGACG
S511	TCTCTCCG	CGGAGAGA
S513	TCGACTAG	CTAGTCGA
S515	TTCTAGCT	AGCTAGAA
S516	CCTAGAGT	ACTCTAGG
S517	GCGTAAGA	TCTTACGC
S518	CTATTAAG	CTTAATAG
S520	AAGGCTAT	ATAGCCTT
S521	GAGCCTTA	TAAGGCTC
S522	TTATGCGA	TCGCATAA

Related products

Related Sample Isolation/Purification Products	Product #
Bacterial Genomic DNA Isolation Kit	17900
Biofilm DNA Isolation Kit	62300
Cells and Tissue DNA Isolation Kit	53100
Fecal DNA Collection & Preservation Mini Tubes	27650
Fecal Swab Collection and Preservation System	45670
Food DNA Isolation Kit	54500
Genomic DNA Isolation Kit	24700
Microbiome DNA Isolation Kit	64100
Milk Bacterial DNA Isolation Kit	21550
Olive Oil DNA Isolation Kit	61700
Plant RNA/DNA Purification Kit	24400
Plant/Fungi DNA Isolation Kit	26200
Saliva DNA Collection, Preservation and Isolation Kit	35700
Saliva DNA Isolation Kit	45400
Soil DNA Isolation Kit	26500
Stool DNA Isolation Kit	27600
Stool DNA Isolation Kit (Magnetic Bead System)	55700
Stool Nucleic Acid Collection and Preservation Tubes	45660
Swab Collection and DNA Preservation Kit	45690
Synovial Fluid Bacterial Genomic DNA Isolation Kit	67900
Urine DNA Isolation Kit for Exfoliated Cells or Bacteria	47050

Technical Support

Contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Norgen's purification technology and NGS-associated technologies are patented and/or patent pending. See www.norgenbiotek.com/patents

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