Bioo Scientific®

FOR REFERENCE PURPOSES

This manual is for Reference Purposes Only. DO NOT use this protocol to run your assays. Periodically, optimizations and revisions are made to the kit and protocol, so it is important to always use the protocol included with the kit.

NEXTflex™ 16S V1 - V3 Amplicon-Seq Kit - 4

(Illumina Compatible)

Catalog #4202-01 (8 reactions)

 V16.08

This product is for research use only.

NEXTflex™ 16S V1 - V3 Amplicon-Seq Kit - 4 - 4202-01

Product Overview

The NEXTflex™ 16S V1-V3 Amplicon-Seq Kit is designed to prepare multiplexed amplicon libraries that span the hypervariable domains one through three (V1-V3) of microbial 16S ribosomal RNA (rRNA) genes. These libraries are compatible with paired-end sequencing on the Illumina® MiSeq platform.

There are two main steps involved in 16S V1-V3 amplicon processing: an initial PCR amplification using customized PCR primers that target the V1-V3 domains, and a subsequent PCR amplification that integrates relevant flow cell binding domains and unique 12 base pair sample indices. A limited number of cleanup steps ensures maximum recovery of amplicons for downstream sequencing. It is highly recommended that sequencing of 16S V1-V3 libraries be performed using the MiSeq V3 reagent kit (2x300), which requires the addition of PhiX. The pooled 16S V1-V3 Libraries should contain a final composition of ~5% PhiX control.

Contents, Storage and Shelf Life

The NEXTflex™ 16S V1-V3 Amplicon-Seq Kit contains enough material to prepare eight 16S V1-V3 samples from genomic DNA for Illumina® compatible sequencing. The shelf life of all reagents is 12 months when stored properly. All components can be safely stored at -20°C.

Kit Contents	Amount
GREEN CAP	
NEXTflex™ PCR Master Mix	192 µL
ORANGE CAP	
NEXTflex™ 16S V1-V3 PCR I Primer Mix	16 µL
YELLOW CAP	
NEXTflex™ PCR II Barcoded Primer Mix 1 – 4	4 µL
WHITE CAP	
Resuspension Buffer	1 mL
Nuclease-free Water	1.5 mL

Required Materials not Provided

- 1 ng - 50 ng high-quality genomic DNA in up to 36 µL nuclease-free water for each library
- 96 well PCR Plate Non-skirted (Phenix Research, Cat # MPS-499) or similar
- Adhesive PCR Plate Seal (BioRad, Cat # MSB1001)
- Agencourt AMPure XP 5 mL (Beckman Coulter Genomics, Cat # A63880)
- Magnetic Stand -96 (Ambion, Cat # AM10027) or similar
- Thermocycler
- 2, 10, 20, 200 and 1000 µL pipettes / multichannel pipettes
- Nuclease-free barrier pipette tips
- Vortex
- 80% Ethanol, freshly prepared (room temperature)

Revision History

Version	Date	Description
V14.05	May 2014	Initial Product Launch
V15.03	March 2015	PCR cycling conditions have been optimized.
V16.08	August 2016	NEXTflex Amplicon PCR Master Mix has been replaced with NEXTflex PCR Master Mix. The reagent setup volumes have changed in PCR I and PCR II steps. The number of recommended PCR cycles have changed in PCR I and PCR II steps. The resuspension and elution volumes have changed in PCR I Cleanup.

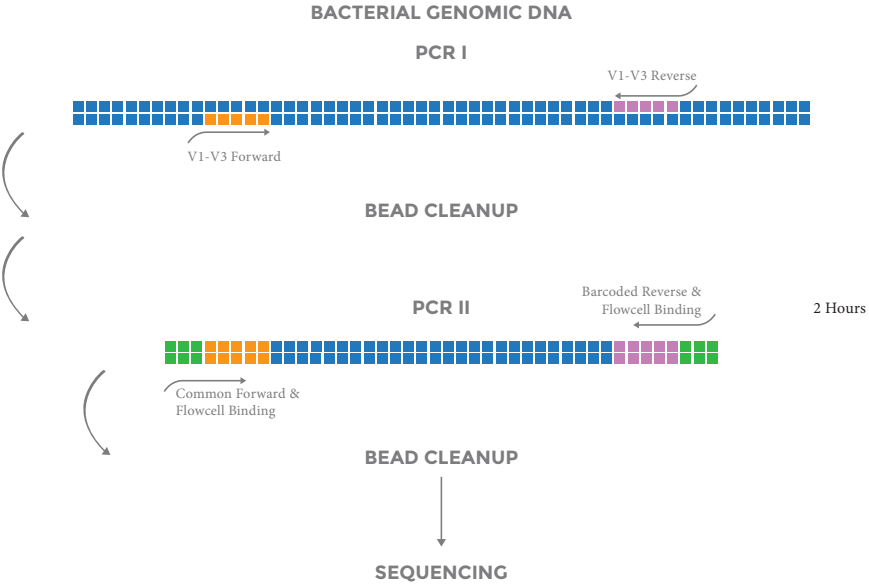
Warnings and Precautions

Bioo Scientific strongly recommends that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor or Bioo Scientific at nextgen@biooscientific.com.

- Do not use the kit past the expiration date.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- Genomic DNA sample quality may vary between preparations. It is the user's responsibility to utilize high quality Genomic DNA. Genomic DNA that is heavily nicked or damaged may cause library preparation failure. Absorbance measurements at 260 nm are commonly used to quantify DNA and 260 nm / 280 nm ratios of 1.8 - 2.0 usually indicate relatively pure DNA. Other quantification methods using fluorescent dyes may also be used. The user should be aware that contaminating RNA, nucleotides and single-stranded DNA may affect the amount of usable DNA in a sample preparation.
- It is required that NEXTflex™ 16S V1-V3 PCR I & PCR II Primer Mixes are used during PCR amplification.

NEXTflex™ 16S V1-V3 Amplicon Sample Preparation Flow Chart

Figure 1: Sample flow chart with approximate times necessary for each step.



Starting Material

The NEXTflex™ 16S V1-V3 Amplicon-Seq Kit has been optimized and validated using 1 ng - 50 ng of high-quality bacterial genomic DNA.

Reagent Preparation

1. Briefly spin down each component to ensure material has not lodged in the cap or side of tube. Keep on ice and vortex each NEXTflex™ Mix just prior to use.
2. Allow Agencourt AMPure XP Beads to come to room temperature and vortex the beads until liquid appears homogenous before every use.

STEP A: PCR I Amplification

Materials

Bioo Scientific Supplied

GREEN CAP - NEXTflex™ PCR Master Mix
ORANGE CAP - NEXTflex™ 16S V1-V3 PCR I Primer Mix
WHITE CAP - Nuclease-Free Water

User Supplied

Thermocycler
96 Well PCR Plate
1 ng - 50 ng High-Quality Genomic DNA (in up to 36 µL Nuclease-Free Water)

1. For each sample, combine the following reagents on ice in the PCR plate.

_ µL	High-Quality Genomic DNA (in Nuclease-free Water)
_ µL	Nuclease-free Water
12 µL	NEXTflex™ PCR Master Mix
2 µL	16S V1-V3 PCR I Primer Mix
50 µL	TOTAL

2. Mix reaction well by pipetting.
3. Apply adhesive PCR plate seal and place in thermocycler for the following PCR cycles:

4 min	98°	
30 sec	98°	
30 sec	60°	Repeat 8 cycles
30 sec	72°	
4 min	72°	

STEP B: PCR I Cleanup

Materials

Bioo Scientific Supplied
WHITE CAP - Resuspension Buffer

User Supplied
Agencourt AMPure XP Magnetic Beads (room temperature)
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand

1. Add 50 µL of AMPure XP Beads to each clear sample mix thoroughly by pipetting.
2. Incubate at room temperature for 5 minutes.
3. Place the 96 well PCR Plate on the magnetic stand at room temperature until the supernatant appears completely clear.
4. Remove and discard the supernatant. Do not disturb beads. Some liquid may remain in wells.
5. With plate on stand, add 200 µL of freshly prepared 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully, remove ethanol by pipette.
6. Repeat previous step, for a total of 2 ethanol washes. Ensure all ethanol has been removed.
7. Remove the plate from the magnetic stand and let dry at room temperature for 3 minutes.
8. Resuspend dried beads with 38 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure beads are no longer attached to the side of the well.
9. Incubate resuspended beads at room temperature for 2 minutes.
10. Place plate on magnetic stand for 5 minutes until the sample appears clear.
11. Transfer 36 µL of clear supernatant (purified PCR I product) to new well.

STEP C: PCR II Amplification

Materials

Bioo Scientific Supplied

GREEN CAP - NEXTflex™ PCR Master Mix
YELLOW CAP - NEXTflex™ PCR II Primer Mix

User Supplied

Thermocycler
96 Well PCR Plate
Purified PCR I product (from Step B)

1. For each sample, combine the following reagents on ice in the PCR plate.

36 µL	Purified PCR I product (from Step B)
12 µL	NEXTflex™ PCR Master Mix
2 µL	NEXTflex™ PCR II Barcoded Primer Mix
50 µL	TOTAL

2. Mix well by pipette.
3. Apply adhesive PCR plate seal and place in thermocycler for the following PCR cycles:

4 min	98°
30 sec	98°
30 sec	60°
30 sec	72°
4 min	72°

Repeat cycles as recommended in table below

Input to PCR I (ng)	PCR II Cycles
1	20
5	18
10	16
25	12
50	10

STEP D: PCR II Cleanup

Materials

Bioo Scientific Supplied

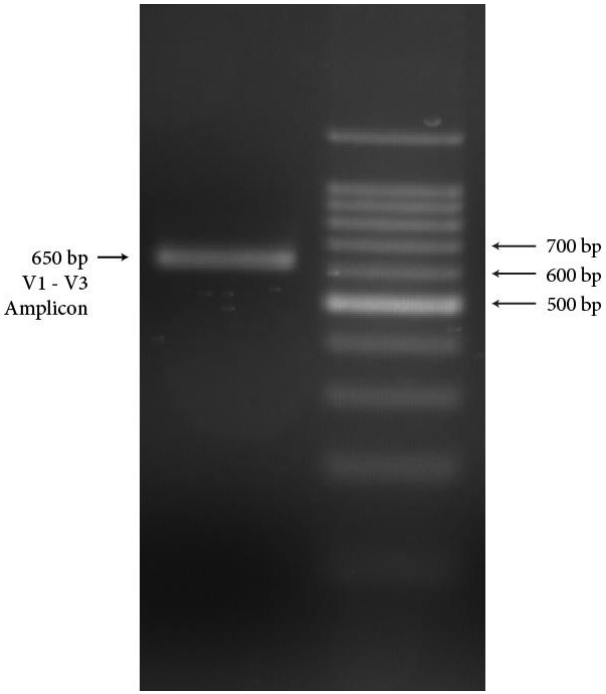
WHITE CAP - Resuspension Buffer

User Supplied

Agencourt AMPure XP Magnetic Beads (room temperature)
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand

1. Add 50 µL of AMPure XP Beads to each clear sample mix thoroughly by pipetting.
2. Incubate at room temperature for 5 minutes.
3. Place the 96 well PCR Plate on the magnetic stand at room temperature until the supernatant appears completely clear.
4. Remove and discard the supernatant. Do not disturb beads. Some liquid may remain in wells.
5. With plate on stand, add 200 µL of freshly prepared 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully, remove ethanol by pipette.
6. Repeat step 5, for a total of 2 ethanol washes. Ensure all ethanol has been removed.
7. Remove the plate from the magnetic stand and let dry at room temperature for 3 minutes.
8. Resuspend dried beads with 17 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure beads are no longer attached to the side of the well.
9. Incubate resuspended beads at room temperature for 2 minutes.
10. Place plate on magnetic stand for 5 minutes until the sample appears clear.
11. Transfer 16 µL of clear supernatant to new well.
12. To ensure cluster generation, it is recommended that you quantify your library by gel or Agilent Bioanalyzer. To quantify by gel, load 2 µL of 6X Gel Loading Dye and 6-10 µL of PCR Product on a 2% low melt agarose gel + SYBR Gold.
13. Quantitate DNA library templates for optimal cluster density.

Figure 2. 2% Agarose + EtBr Gel validation of the NEXTflex™ 16S V1-V3 PCR product (20 ng E. coli gDNA; 15 cycles of PCR).



*Important note – Bacterial hypervariable regions vary in base composition and length. For community studies, expect bands that are ~650 bp.

Oligonucleotide Sequences

NEXTflex™ 16S V1-V3 PCR I Primer Mix	
NEXTflex™	**Sequence 5' → 3'**
16S V1-V3 Forward	CTCTTTCCCTACACGACGCTCTTCCGATCTAGAGTTTGATCCTGGCTCAG
16S V1-V3 Reverse	CTGGAGTTCAGACGTGTGCTCTTCCGATCTGTATTACCGCGGCTGCTGG

NEXTflex™ PCR II Barcoded Primer Mix	
NEXTflex™	**Sequence 5' → 3'**
PCR II Forward	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
PCR II Reverse	CAAGCAGAAGACGGCATACGAGAT[1]XXXXXXXXXXXX[1]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

[1]XXXXXXXXXXXX denotes the index region of adapter. The index sequences and the respective reverse complement sequences contained in each adapter are listed below.

Reverse Primer Index Sequences and Reverse Complements

Barcoded Primer	Index Sequence (5' → 3')	Reverse Complement
1	GGCCGGCTAGAT	ATCTAGCCGGCC
2	AAGGAAGAGATA	TATCTCTTCCTT
3	GGACGGCATCTA	TAGATGCCGTCC
4	AAGGAAGGAGCG	CGCTCCTTCCTT

For an electronic list of the 16S V1-V3 Primers visit our webpage.

Low Level Multiplexing

Use the following reverse primer combinations for low level multiplexing in this kit:
Pool of 2: (Barcodes 1 & 2) OR (Barcodes 3 & 4)
Pool of 3: (Barcodes 1, 2, & 3), (Barcodes 1, 2, 4), (Barcodes 1, 3, 4), OR (Barcodes 2, 3, 4)

Illumina Compatible RNA NGS Kits and Adapters

Catalog #	Product
5138-01	NEXTflex™ Rapid RNA-Seq Kit (8 reactions)
5138-02	NEXTflex™ Rapid RNA-Seq Kit (48 reactions)
5138-07	NEXTflex™ Rapid Directional RNA-Seq Kit (8 reactions)
5138-08	NEXTflex™ Rapid Directional RNA-Seq Kit (48 reactions)
512911	NEXTflex™ RNA-Seq Barcodes –6
512912	NEXTflex™ RNA-Seq Barcodes – 12
512913	NEXTflex™ RNA-Seq Barcodes – 24
512914	NEXTflex™ RNA-Seq Barcodes – 48
512916	NEXTflex-96™ RNA-Seq Barcodes
5130-01	NEXTflex™ qRNA-Seq™ Kit 4 barcodes (8 reactions)
5130-02	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set A (48 reactions)
5130-03	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set B (48 reactions)
5130-04	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set C (48 reactions)
5130-05	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set D (48 reactions)
5130-01D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 4 barcodes (8 reactions)
5130-02D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set A (48 reactions)
5130-03D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set B (48 reactions)
5130-04D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set C (48 reactions)
5130-05D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set D (48 reactions)
5132-01	NEXTflex™ Small RNA Sequencing Kit (24 reactions)
5132-02	NEXTflex™ Small RNA Sequencing Kit (48 reactions)
5132-03	NEXTflex™ Small RNA Sequencing Kit v2 (24 reactions)
5132-04	NEXTflex™ Small RNA Sequencing Kit v2 (48 reactions)
513305	NEXTlfex™ Small RNA Barcode Primers -12 (Set A)
513306	NEXTlfex™ Small RNA Barcode Primers -12 (Set B)
513307	NEXTlfex™ Small RNA Barcode Primers -12 (Set C)
513308	NEXTlfex™ Small RNA Barcode Primers -12 (Set D)
512979	NEXTflex™ Poly(A) Beads (8 reactions)
512980	NEXTflex™ Poly(A) Beads (48 reactions)
512981	NEXTflex™ Poly(A) Beads (100 reactions)

Illumina Compatible DNA NGS Kits and Adapters

Catalog #	Product
4201-01	NEXTflex™ 16S V4 Amplicon-Seq Kit – 4
4201-02	NEXTflex™ 16S V4 Amplicon-Seq kit – 12
4201-03	NEXTflex™ 16S V4 Amplicon-Seq kit – 24
4201-04	NEXTflex™ 16S V4 Amplicon-Seq kit – 48
4201-05	NEXTflex™ 16S V4 Amplicon-Seq kit – 96
4201-06	NEXTflex™ 16S V4 Amplicon-Seq kit – 192
4201-07	NEXTflex™ 16S V4 Amplicon-Seq kit – 288
4202-01	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 4
4202-02	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 12
4202-03	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 48
4202-04	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 1-96
4202-05	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 97-192
4202-06	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 193-288
4202-07	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 289-384
5140-01	NEXTflex™ DNA Sequencing Kit (8 reactions)
5140-02	NEXTflex™ DNA Sequencing Kit (48 reactions)
5144-01	NEXTflex™ Rapid DNA-Seq Kit (8 reactions)
5144-02	NEXTflex™ Rapid DNA-Seq Kit (48 reactions)
5150-01	NEXTflex™ Cell Free DNA-Seq Kit (8 reactions)
5150-02	NEXTflex™ Cell Free DNA-Seq Kit (48 reactions)
514101	NEXTflex™ DNA Barcodes – 6
514102	NEXTflex™ DNA Barcodes – 12
514103	NEXTflex™ DNA Barcodes – 24
514104	NEXTflex™ DNA Barcodes – 48
514105	NEXTflex-96™ DNA Barcodes (Plate Format)
514106	NEXTflex-96™ DNA Barcodes (Tube Format)
514160	NEXTflex™ Dual-Indexed DNA Barcodes (1-96)
514161	NEXTflex™ Dual-Indexed DNA Barcodes (97-192)
5119-01	NEXTflex™ Bisulfite-Seq kit (8 reactions)
5119-02	NEXTflex™ Bisulfite-Seq kit (48 reactions)
511911	NEXTflex™ Bisulfite-Seq Barcodes – 6
511912	NEXTflex™ Bisulfite-Seq Barcodes – 12
511913	NEXTflex™ Bisulfite-Seq Barcodes - 24
5118-01	NEXTflex™ Methyl-Seq 1 Kit (8 reactions)
5118-02	NEXTflex™ Methyl-Seq 1 Kit (48 reactions)

511921	NEXTflex™ Msp 1 (8 reactions)
511922	NEXTflex™ Msp 1 (48 reactions)

5143-01	NEXTflex™ ChIP-Seq Kit (8 reactions)
5143-02	NEXTflex™ ChIP-Seq Kit (48 reactions)
514120	NEXTflex™ ChIP-Seq Barcodes – 6
514121	NEXTflex™ ChIP-Seq Barcodes – 12
514122	NEXTflex™ ChIP-Seq Barcodes – 24
514123	NEXTflex™ ChIP-Seq Barcodes – 48
514124	NEXTflex-96™ ChIP-Seq Barcodes

5140-51	NEXTflex™ Pre-Capture Combo Kit (6 barcodes)
5140-52	NEXTflex™ Pre-Capture Combo Kit (12 barcodes)
5140-53	NEXTflex™ Pre-Capture Combo Kit (24 barcodes)
5140-56	NEXTflex™ Pre-Capture Combo Kit (48 barcodes)
5140-54	NEXTflex™ Pre-Capture Combo Kit (96 barcodes)
514131	NEXTflex™ DNA Barcode Blockers - 6 for SeqCap
514132	NEXTflex™ DNA Barcode Blockers - 12 for SeqCap
514133	NEXTflex™ DNA Barcode Blockers - 24 for SeqCap
514136	NEXTflex™ DNA Barcode Blockers - 48 for SeqCap
514134	NEXTflex™ DNA Barcode Blockers - 96 for SeqCap

5142-01	NEXTflex™ PCR-Free DNA Sequencing Kit (8 reactions)
5142-02	NEXTflex™ PCR-Free DNA Sequencing Kit (48 reactions)
514110	NEXTflex™ PCR-Free Barcodes – 6
514111	NEXTflex™ PCR-Free Barcodes – 12
514112	NEXTflex™ PCR-Free Barcodes – 24
514113	NEXTflex™ PCR-Free Barcodes – 48

DNA Fragmentation

Catalog #	Product
5135-01	AIR™ DNA Fragmentation Kit (10 reactions)
5135-02	AIR™ DNA Fragmentation Kit (40 reactions)

WE WANT TO HEAR FROM YOU!

Your feedback is important to us. Tell us what you think of our kits by scanning the QR code or visiting our website at www.biooscientific.com/NGSfeedback. We can't wait to hear from you!

Bioo Scientific®

THE NGS EXPERTS™

Bioo Scientific Corporation · 7050 Burleson Road, Austin, Texas 78744 · BiooScientific.com
P: 1.888.208.2246 · F: 512.707.8122 · Bioo Research Products Group · nextgen@biooscientific.com
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