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™ PCR-Free DNA Sequencing Kit

(Illumina Compatible)

Catalog #5142-01 (8 reactions)

 V12.10

This product is for research use only.

NEXTflex™ PCR-Free DNA Sequencing Kit - 5142-01

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Product Overview

The NEXTflex™ PCR-Free DNA Sequencing Kit is designed to prepare single, paired-end and multiplexed genomic DNA libraries for sequencing using Illumina® platforms. The NEXTflex™ PCR-Free DNA Sequencing Kit completely eliminates amplification steps by using specially modified enzymatic master mixes. This significantly improves the number of unique reads, read mapping, and SNP calling while completely eliminating library prep amplification bias. This is especially important in A / T and G / C rich genomic fragments. The NEXTflex™ PCR-Free Kit simplifies workflow by using master mixed reagents and magnetic bead based cleanup, reducing pipetting and eliminating time consuming gel steps in library preparation. The availability of up to 48 unique NEXTflex™ PCR-Free Barcodes enables high-throughput workflows. The NEXTflex™ PCR-Free Sequencing Kit contains the necessary material to take the user's purified and fragmented genomic DNA through preparation for loading onto flow cells for sequencing.

Contents, Storage and Shelf Life

The NEXTflex™ PCR-Free DNA Sequencing Kit contains enough material to prepare 8 genomic DNA samples for Illumina® compatible sequencing. The shelf life of all reagents is 12 months when stored properly at -20°C.

Kit Contents	Amount
CLEAR CAP	
NEXTflex™ PCR-Free End Repair Buffer Mix	56 µL
NEXTflex™ PCR-Free End Repair Enzyme Mix	24 µL
RED CAP	
NEXTflex™ PCR-Free Adenylation Mix	36 µL
PURPLE CAP	
NEXTflex™ PCR-Free Ligation Mix	252 µL
NEXTflex™ PCR-Free DNA Adapter (50 µM)	20 µL
WHITE CAP	
Nuclease-free Water	1.5 mL
Respension Buffer	1.5 mL

Required Materials not Provided

- 500 ng to 3 µg of fragmented genomic DNA in up to 40 µL nuclease-free water.
- If multiplexing: NEXTflex™ PCR-Free Barcodes – 6 / 12 / 24 / 48 (Cat # 514110, 514111, 514112, 514113)
- Ethanol 80% (room temperature)
- AIR™ DNA Fragmentation Kit (Bioo Scientific, Cat # 5135-01) or Covaris System (S2, E210)
- 96-well PCR Plate Non-skirted (Phenix Research, Cat # MPS-499) or similar
- 96-well Library Storage and Pooling Plate (Fisher Scientific, Cat # AB-0765) 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
- Heat block
- Thermocycler
- 2, 10, 20, 200 and 1000 µL pipettes / multichannel pipettes
- Nuclease-free barrier pipette tips
- Microcentrifuge
- 1.5 mL nuclease-free microcentrifuge tubes
- Vortex
- qPCR Library Quantification Kit or the following components:
- qPCR dilution buffer: 10 mM Tris HCl pH 8.0, 0.05% Tween 20
- Control Templates
- qPCR Primer 1 - HPLC Purified - 5'AATGATACGGCGACCACCGA -3'
- qPCR Primer 2 - HPLC Purified - 5'CAAGCAGAAGACGGCATACGA -3'

Warnings and Precautions

NOTICE: This kit utilizes a gel-free method for size selection. If agarose gel size-selection is desired, gel size selection components are available separately, catalog # 514012 (10 samples) or #514013 (50 samples).

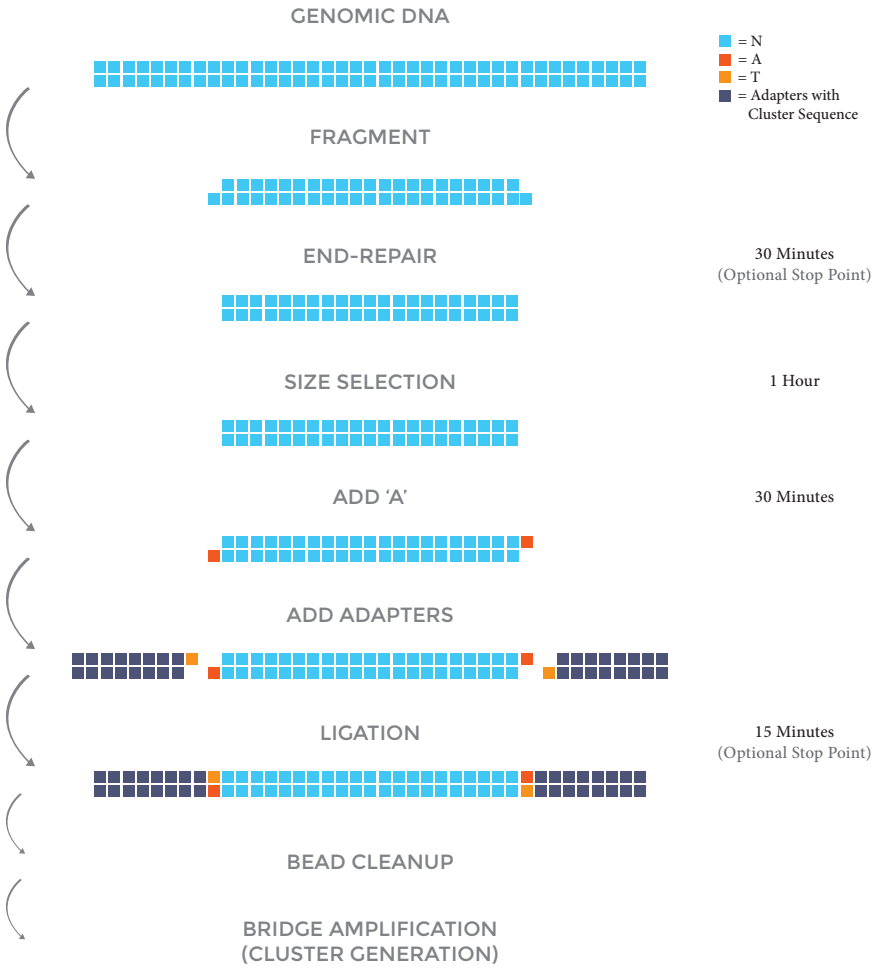
Bioo Scientific strongly recommends that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local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

- Do not use the kit past the expiration date.
- DTT in buffers may precipitate after freezing. If precipitate is seen, vortex buffer for 1-2 minutes or until the precipitate is in solution. The performance of the buffer is not affected once precipitate is in solution.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Do not heat the NEXTflex™ PCR-free DNA Adapters above room temperature.
- This kit contains a single barcoded PCR-free DNA Adapter. To enable multiplexing, please use the appropriate combination of NEXTflex™ PCR-Free DNA Barcodes during the Adapter Ligation step.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- DNA sample quality may vary between preparations. It is the user's responsibility to utilize high quality DNA. 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.
- DNA fragmentation methods that physically break up DNA into pieces of less than 800 bp are compatible with this kit. These methods include the AIR™ DNA Fragmentation Kit (5135-01), based on the nebulization of DNA or acoustic technologies that fragment DNA in a controlled and accurate manner. We do not recommend any enzymatic methods of fragmentation as this may introduce sequence bias into the preparation.
- The following adapter titrations for the given DNA starting input amounts are recommended for the Adapter Ligation step (STEP D):

DNA starting input amount:	Adapter amount:
3 µg	2.5 µL
1 µg	1.25 µL
500 ng	0.625 µL

NEXTflex™ PCR-Free DNA Sample Preparation Flow Chart

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



Starting Material

The NEXTflex™ PCR-Free DNA Sequencing Kit has been optimized and validated using genomic DNA. Best results are obtained when 3 µg of high quality fragmented genomic DNA is used (see page 4, Warnings and Precautions).

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™ PCR-Free Mix just prior to use.
2. DTT in buffers may precipitate after freezing. If precipitate is seen in any mix, vortex for 1 minute or until the precipitate is in solution. The performance of the mix is not affected once precipitate is in solution.
3. Allow Agencourt AMPure XP Beads to come to room temperature and vortex the beads until liquid appears homogenous before every use.

STEP A: End Repair

Materials

Bioo Scientific Supplied

CLEAR CAP - NEXTflex™ PCR-Free End Repair Buffer Mix, NEXTflex™ PCR-Free End Repair Enzyme Mix

WHITE CAP - Nuclease-free Water

User Supplied

Fragmented DNA in 40 µL (or less) Nuclease-free Water
96-well PCR Plate
Adhesive PCR Plate Seal
Agencourt AMPure XP Magnetic Beads
Microcentrifuge
Ice

1. For each sample, combine the following reagents on ice in a nuclease-free 96-well PCR Plate:

_ µL	Nuclease-free Water
_ µL	Fragmented DNA (500 ng to 3 µg)
7 µL	NEXTflex™ PCR-Free End Repair Buffer Mix
3 µL	NEXTflex™ PCR-Free End Repair Enzyme Mix
50 µL	TOTAL

2. Mix thoroughly by pipetting.
3. Apply adhesive PCR plate seal and incubate on a thermocycler for 30 minutes at 22°C.
4. Proceed to Step B: Clean-Up.

STEP B: Clean-Up

NOTICE: This kit utilizes a gel-free method for size selection. If agarose gel size-selection is desired, gel size selection components are available separately, catalog # 514012 (10 samples) or #514013 (50 samples).

Size selection using Agencourt AMPure XP Magnetic Beads in this protocol will result in a DNA insert size between 300 – 400 bp with a total length of 400 – 500 bp post adapter ligation.

Materials

Bioo Scientific Supplied
WHITE CAP - Resuspension Buffer

User Supplied
End Repaired DNA (from Step A)
Agencourt AMPure XP Magnetic Beads (room temperature)
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand

1. Add 42.5 µL of AMPure XP Beads to each sample and mix thoroughly by pipetting.
2. Incubate the plate at room temperature for 5 minutes.
3. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes or until the supernatant appears completely clear.
4. Set pipette to 90 µL, remove and discard the supernantant taking care not to disturb beads. Some liquid may remain in wells. This selectively removes DNA below 300 bp.
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 53 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 at room temperature for 5 minutes or until the sample appears completely clear.
11. **Do not discard the sample in this step.** Transfer 50 µL of clear sample to a new well.
12. Add 40 µL of AMPure XP Beads to each well containing sample and mix thoroughly by pipetting.

13. Incubate at room temperature for 5 minutes.
14. Place the 96 well PCR Plate on the magnetic stand for 5 minutes at room temperature or until the sample appears completely clear.
15. **Do not discard the sample in this step.** Transfer 88 µL of the supernatant to a new well. **Be careful not to disrupt the magnetic bead pellet or transfer any magnetic beads with the sample. The bead pellet binds and removes DNA above 400 bp.**
16. Add 88 µL of AMPure XP Beads to each well containing sample and mix thoroughly by pipetting.
17. Incubate at room temperature for 5 minutes.
18. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes or until the supernatant appears clear.
19. Remove and discard 172 µL of the supernatant taking care not to disturb beads. Some liquid may remain in wells.
20. 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.
21. Repeat step 20 for a total of 2 ethanol washes. Ensure all ethanol has been removed.
22. Remove the plate from the magnetic stand and let dry at room temperature for 3 minutes.
23. Resuspend dried beads with 17.5 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure beads are no longer attached to the side of the well.
24. Incubate resuspended beads at room temperature for 2 minutes.
25. Place plate on magnetic stand at room temperature for 5 minutes or until the sample appears clear.
26. Transfer 16 µL of clear supernatant to new well.
27. If you wish to pause your experiment, the procedure may be safely stopped at this step and samples stored at -20°C. To restart, thaw the frozen samples on ice before proceeding.

STEP C: 3' Adenylation

Materials

Bioo Scientific Supplied
RED CAP - NEXTflex™ PCR-Free Adenylation Mix

User Supplied
Purified End Repaired DNA (from Step B)
Thermocycler (set to 37°C)

1. Combine the following in the 96-well PCR Plate:

16 µL	End-Repaired DNA (from Step B)
4.5 µL	NEXTflex™ PCR-Free Adenylation Mix
20.5 µL	TOTAL

2. Mix thoroughly by pipetting.
3. Apply adhesive PCR plate seal and incubate on a thermocycler for 30 minutes at 37°C.
4. Proceed to Step D: Adapter Ligation.

STEP D: Adapter Ligation

Materials

Bioo Scientific Supplied

(Optional) NEXTflex™ PCR-Free Barcodes – 6 / 12 / 24 / 48 (Cat # 514110, 514111, 514112, 514113)

PURPLE CAP - NEXTflex™ PCR-Free Ligation Mix (remove right before use and store immediately after use at -20°C), NEXTflex™ PCR-free DNA Adapter

WHITE CAP - Nuclease-free Water

User Supplied

3' Adenylated DNA (from Step C)
Thermocycler (set to 22°C)

The following adapter titrations for the given DNA starting input amounts are recommended:

DNA starting input amount:	Adapter amount:
3 µg	2.5 µL
1 µg	1.25 µL
500 ng	0.625 µL

1. For each sample, combine the following reagents (in this order) in the 96-well PCR Plate:

20.5 µL	3' Adenylated DNA (from Step C)
31.5 µL	NEXTflex™ PCR-Free Ligation Mix
_ µL	NEXTflex™ PCR-Free DNA Adapter or NEXTflex™ PCR-Free Barcode
_ µL	Nuclease-free Water
54.5 µL	TOTAL

2. Mix thoroughly by pipetting.
3. Apply adhesive PCR plate seal and incubate on a thermocycler for 15 minutes at 22°C.
4. Proceed to Step E: Clean-Up.

STEP E: Clean-Up

Materials

Bioo Scientific Supplied
WHITE CAP - Resuspension Buffer

User Supplied
Adapter Ligated DNA (from Step D)
Agencourt AMPure XP Magnetic Beads (room temperature)
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand

1. Add 44 µL of AMPure XP Beads to each sample and 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 for 5 minutes or until the supernatant appears completely clear.
4. Remove clear supernatant taking care not to 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 57 µL of Resuspension Buffer. Mix thoroughly by pipetting and ensuring 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 or until the supernatant appears completely clear.
11. Transfer 54.5 µL of clear sample to new well.
12. Add 44 µL of AMPure XP Beads to each sample and mix thoroughly by pipetting.
13. Incubate at room temperature for 5 minutes.
14. Place the 96-well PCR Plate on the magnetic stand at room temperature for 5 minutes or until the supernatant appears completely clear.
15. Remove clear supernatant taking care not to disturb beads. Some liquid may remain in wells.
16. 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.
17. Repeat step 16 for a total of 2 ethanol washes. Ensure all ethanol has been removed.

18. Remove the plate from the magnetic stand and let dry at room temperature for 3 minutes.
19. Resuspend dried beads with 22.5 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure beads are no longer attached to the side of the well.
20. Incubate resuspended beads at room temperature for 2 minutes.
21. Place plate on magnetic stand for 5 minutes or until the supernatant appears completely clear.
22. Transfer 20 µL of clear supernatant to a new well.
23. If you wish to pause your experiment, the procedure may be safely stopped at this step and samples stored at -20°C. To restart, thaw the frozen samples on ice before proceeding.

STEP F: Quantification

Materials

User Supplied

qPCR Thermocycler
96-well PCR Plate
qPCR Library Quantification Kit /or/ the following components:
qPCR Dilution Buffer: 10 mM Tris HCl pH 8.0, 0.05% Tween 20
Control Templates
qPCR Primer 1 - HPLC Purified - 5'AATGATACGGCGACCACCGA -3'
qPCR Primer 2 - HPLC Purified - 5'CAAGCAGAAGACGGCATACGA -3'
2.5 µL Ligation Product (from STEP E)

1. qPCR is recommended to quantitate DNA library templates for optimal cluster density as it selectively measures only those templates with both adapter sequences on either end. qPCR is also a sensitive method for measuring DNA libraries whose concentrations are below the threshold of detection using spectrophotometric methods. NEXTflex™ PCR-Free library templates will require quantification by qPCR. This can be performed using any qPCR quantification kit with at least three dilutions of a previously used library with known cluster numbers. If performing quantification without a kit, a protocol is provided below.

2. Choose a control template as similar as possible to your experimental template size, GC content and library type.

3. Using qPCR dilution buffer, make 6 serial dilutions of the control template in a range from 0.01 pM to 100 pM. Ensure that these dilutions are made fresh for immediate use before qPCR. Make three replicate, independent serial dilutions of each control template. Triplicate results are important for qPCR analysis.

4. Using qPCR dilution buffer, make a dilution of your library (Ligation Product from STEP E) for quantification. Libraries will need to be diluted so that they fall within the range as the control template. Although this range depends on your sample and amount of starting genomic material, recommended dilutions include 1:500, 1:1,000, 1:2,000, 1:5,000, 1:10,000. Make three replicate, independent dilutions for each unknown library.

5. Prepare 4 µM qPCR Primer 1 and qPCR Primer 2 stock solutions. Mix equal volumes of both primers to achieve a 2 µM qPCR Primer Mix.

6. For each sample, prepare a master mix by combining the following reagents on ice:

4 µL	Nuclease-free Water
10 µL	2X SYBR Master Mix
2 µL	qPCR Primer Mix (2 µM)
16 µL	TOTAL

7. Add 4 µL of the diluted control template or unknown library dilutions to each well, ensure that you have triplicates for each sample. Add 16 µL of the master mix into each corresponding well.

8. Mix thoroughly by pipetting.
9. Centrifuge plate for 1 minute at 200 x g.
10. Quantify the libraries using the following qPCR cycles (note cycle conditions may vary according to SYBR Mix manufacturer):

5 min	95°C	
30 sec	95°C	Repeat 35 cycles
45 sec	60°C	

11. Analyze the libraries ensuring there is good amplification for each control template. Remove outlying or bad replicates.

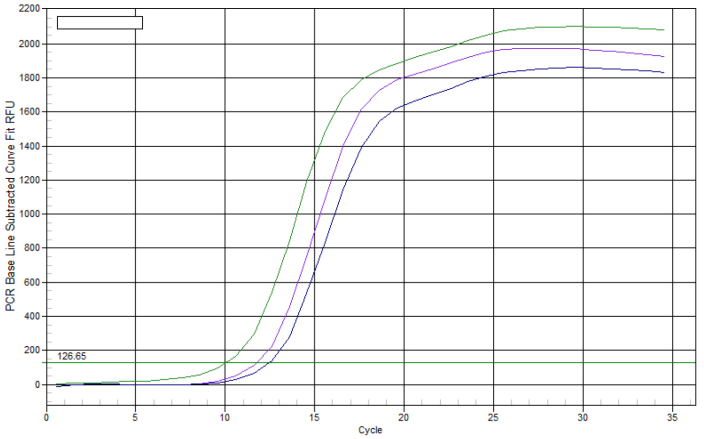


Figure 2. Example of qPCR Template Amplification for 500 ng (blue), 1 µg (purple) and 3 µg (green) input DNA dilutions. The Ct value for 3 µg is 1.58 Ct lower than the 1 µg; the Ct value for 1 µg is 1 Ct less than 500 ng

12. Generate a standard curve from the control template by plotting Ct values against the log initial concentration. Efficiency of the amplification should be 90-110% and the R2 should be greater than 0.95. Calculate the initial concentration of your unknown library templates based on the standard curve and the dilution factor of your unknown sample.

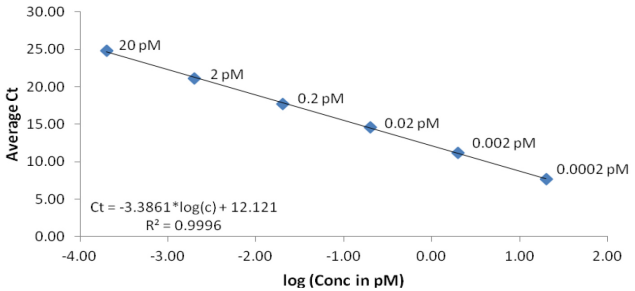


Figure 3. Standard curve generated by plotting the log concentration of library DNA (pM) against average Ct values.

13. Once you have quantified your library, dilute to the appropriate concentration for clustering. If your library concentration is less than 1 nM, follow Bioo Scientific's Denaturation of Sub-nanomolar DNA Libraries Protocol, available by contacting nextgen@biooscientific.com. If multiplexing libraries, transfer equal amounts of each normalized library to be pooled in the well of a new 96-well PCR plate. Mix thoroughly by pipetting.

14. Proceed to cluster generation.

Oligonucleotide Sequences

NEXTflex™	Sequence
DNA Adapter 1	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTATCTCGTATGCCGTCTTCTGCTTG

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™

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