

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™ Bisulfite-Seq Kit

(Illumina Compatible)
Catalog #5119-01 (8 reactions)



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NEXTflex™ Bisulfite-Seq Kit - 5119-01

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GENERAL INFORMATION

Product Overview

The NEXTflex™ Bisulfite-Seq Kit is designed to enrich and prepare single, paired-end and multiplexed bisulfite converted DNA libraries for sequencing using Illumina® platforms. The NEXTflex™ Bisulfite-Seq Kit contains specially designed enzymes and buffers needed for reduced representation or genome wide bisulfite sequencing. Bisulfite-Seq is a single nucleotide resolution technique that allows the user to study DNA methylation sites and their role in gene regulation.

This kit features "Enhanced Adapter Ligation Technology", resulting in library preps with a larger number of unique sequencing reads. This specially designed NEXTflex™ enzymatic ligation mix allows users to perform ligations with longer adapters and better ligation efficiencies. The kit also contains the NEXTflex™ Bisulfite-Seq U+ PCR Master Mix, a robust polymerase designed to handle bisulfite converted DNA. The NEXTflex™ Bisulfite-Seq Kit simplifies workflow by using master mixed reagents and magnetic bead based cleanups, reducing pipetting and eliminating time consuming steps in library preparation.

Contents, Storage and Shelf Life

The NEXTflex™ Bisulfite-Seq Kit contains enough material to prepare 8 genomic DNA samples for Illumina® compatible sequencing. The shelf life of all reagents is 6 months when stored properly. All components should be stored at -20°C, except for the Nuclease-free Water and Resuspension Buffer, which can be stored at room temperature.

Kit Contents	Amount
CLEAR CAP	
NEXTflex™ Bisulfite-Seq End Repair Buffer Mix	56 μL
NEXTflex™ Bisulfite-Seq End Repair Enzyme Mix	24 μL
RED CAP	
NEXTflex™ Bisulfite-Seq Adenylation Mix	36 μL
PURPLE CAP	
NEXTflex $^{\text{\tiny TM}}$ Bisulfite-Seq Ligation Mix 252 μL	
NEXTflex $^{\text{\tiny TM}}$ Bisulfite-Seq DNA Adapter 24 (25 μM)	20 μL
NEXTflex™ Adapter Dilution Buffer	500 μL
GREEN CAP	
NEXTflex™ Primer Mix (12.5 μM)	16 μL
NEXTflex™ Bisulfite-Seq U+ PCR Master Mix	208 μL
WHITE CAP	

1.5 mL

(2) 1 mL

Nuclease-free Water

Resuspension Buffer

Required Materials not Provided

- 10 ng 1 μ g of fragmented or Msp1 digested genomic DNA in up to 40 μ L nuclease-free water.
- NEXTflex[™] Bisulfite-Seq Barcodes 6 / 12 / 24 (Cat # 511911, 511912, 511913)
- Optional: NEXTflex™ Msp1 8 reactions / 48 reactions (Bioo Scientific, Cat # 511921, 511922)
- EZ DNA Methylation-Gold™ Kit (Zymo Research, Cat # D5005)
- Ethanol 100% (room temperature)
- 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

Revision History

Version	Date	Description of Change
V14.04	April 2014	Initial Product Launch.
V16.04	April 2016	Protocol option for gel size-selection has been removed. Gel-free Size Selection has been optimized. Users who wish to size select libraries by gel size selection should contact BiooNGS@BiooScientific.com.

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.
- 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™ Bisulfite-Seq Adapters above room temperature.
- This kit contains a single Barcoded Adapter. To enable multiplexing, please use the appropriate combination of NEXTflex™ Bisulfite-Seq 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 or Msp1 digestions are compatible with this kit.
- If starting with less than 1 μg of input DNA, follow the 'Adapter Dilution' table in Step D: Adapter Ligation to dilute the methylated adapters. If using Msp1 digested DNA, a greater dilution may be required for samples that are not completely digested. If starting with a DNA input amount greater than 1 μg, inrease the Bisulfite-Seq Adapter volume proportionally. This is important for retaining the optimal input:adapter ratio.
- It is highly recommended that NEXTflex™ Primer Mix be used during PCR amplification.
 Inadvertent use of an incorrect primer sequence can potentially result in elimination of the index.

Starting Material

The NEXTflex™ Bisulfite-Seq Kit has been optimized and validated using fragmented or Msp1 digested genomic DNA.

The table below can serve as a guideline in deciding the number of PCR cycles to use in Step G.

Input DNA	10 ng	100 ng	250 ng	500 ng	1 μg
Fragmented Genomic DNA	18 cycles	15-18 cycles	15-18 cycles	12-15 cycles	12-15 cycles
Msp1 Digested DNA	18 cycles	18 cycles	18 cycles	15-18 cycles	12-18 cycles

For Reduced Representation Bisulfite Sequencing (RRBS), Bioo Scientific recommends using NEXTflex™ Msp1 – 8 reactions / 48 reactions (Bioo Scientific, Cat # 511921, 511922)

Reagent Preparation

- 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. 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.

Cluster Sequence

NEXTflex™ Bisulfite-Seq Sample Preparation Flow Chart

Figure 1: Flow chart for sample preparation steps:



NEW PROTOCOL

Please see Revision History on page 3 before starting procedure.

STEP A: End Repair

Materials

Bioo Scientific Supplied

CLEAR CAP - NEXTflex™ Bisulfite-Seq End Repair Buffer Mix, NEXTflex™ Bisulfite-Seq End Repair Enzyme Mix

WHITE CAP - Nuclease-free Water

User Supplied

Fragmented or Msp1 digested genomic DNA in 40 μL (or less) Nuclease-free Water 96 well PCR Plate Adhesive PCR Plate Seal Thermocycler Ice

- For each sample, combine the following reagents on ice in a nuclease-free 96 well PCR Plate:
 - _ μL Nuclease-free Water
 - _ μL Fragmented genomic or Msp1 Digested DNA (10 ng 1 μg)
 - 7 μL NEXTflex™ Bisulfite-Seq End Repair Buffer Mix
 - 3 μL NEXTflex™ Bisulfite-Seq 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

Materials

Bioo Scientific Supplied

WHITE CAP - Resuspension Buffer

User Supplied

 $50~\mu L$ of End Repaired DNA (from Step A) Agencourt AMPure XP Magnetic Beads (room temperature) 80% Ethanol, freshly prepared (room temperature) Magnetic Stand

- 1. Add 90 μL of AMPure XP Beads to each sample and mix thoroughly by pipetting.
- 2. Incubate sample 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 clear.
- Remove and discard clear supernatant. Take care not to disturb beads. Some liquid may remain in wells.
- 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. Note: Do not over dry the beads.
- 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 the plate on magnetic stand for 5 minutes or until the sample appears clear.
- 11. Transfer 16 μL of clear sample to new well.
- 12. If you wish to pause your experiment, the procedure may be safely stopped at this step and samples stored at -20°C. To restart, always thaw your frozen samples on ice before proceeding to Step C: 3' Adenylation.



STEP C: 3' Adenylation

Materials

Bioo Scientific Supplied

RED CAP - NEXTflex™ Bisulfite-Seq Adenylation Mix

User Supplied

 $16~\mu L$ of Purified End Repaired DNA (from Step B) 96 well PCR Plate Adhesive PCR Plate Seal Thermocycler Ice

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

16 μL	Purified End-Repaired DNA (from Step B)
$4.5~\mu L$	NEXTflex™ Bisulfite-Seq 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

PURPLE CAP - NEXTflex™ Bisulfite-Seq Ligation Mix (remove right before use and store immediately after use at -20°C), NEXTflex™ Bisulfite-Seq Adapter 24 or (Optional) NEXTflex™ Bisulfite-Seq Barcodes – 6 / 12 / 24 (Cat # 511911, 511912, 511913), NEXTflex™ Adapter Dilution Buffer

User Supplied

20.5 µL of 3' Adenylated DNA (from Step C) 96 well PCR Plate Adhesive PCR Plate Seal Thermocycler Ice

If starting with less than 1 μ g of input DNA, follow the 'Adapter Dilution' table below to dilute the methylated adapters. This is important for retaining the optimal input:adapter ratio. If using Msp1 digested DNA, a greater dilution may be required for samples that are not completely digested.

Input DNA	10 ng	100 ng	250 ng	500 ng
Bisulfite-Seq Adapter	1 μL	1 μL	1 μL	2 μL
Adapter Dilution Buffer	49 μL	9 μL	3 μL	2 μL
Amount per reaction	2.5 μL	2.5 μL	2.5 μL	2.5 μL

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

$20.5~\mu\mathrm{L}$	3' Adenylated DNA (from Step C)
2.5 μL	NEXTflex™ Bisulfite-Seq Barcode
31.5 μL	NEXTflex™ Bisulfite-Seq Ligation Mix
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: Size Selection Clean-Up.

STEP E: Size Selection Clean-Up

Materials

Bioo Scientific Supplied

WHITE CAP - Resuspension Buffer

User Supplied

54.5 µL of 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 clear.
- 4. Remove and discard clear supernatant. Take care not to disturb beads.
- 5. With plate on stand, add 200 μ L of freshly prepared 80% ethanol to each sample and incubate plate at room temperature for 30 seconds. Carefully, remove ethanol by pipette.
- Repeat previous step, for a total of 2 ethanol washes and ensure all ethanol has been removed.
- 7. Remove the plate from the magnetic stand and let dry at room temperature for 3 minutes. Note: Do not over dry the beads.
- 8. Resuspend dried beads with $52 \mu L$ 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 the plate on magnetic stand at room temperature for 5 minutes or until the sample appears clear.
- 11. Transfer 50 μL of clear sample to new well.
- 12. Add 28 μ L of AMPure XP Beads to each sample and mix thoroughly by pipetting.
- 13. Incubate at room temperature for 5 minutes.
- 14. Place the plate on the magnetic stand at room temperature for 5 minutes or until the supernatant appears clear.
- 15. Do not discard clear sample in this step. Transfer 76 μL of clear sample to a new well.
- 16. Add 12 μL of AMPure XP Beads to each sample and mix thoroughly by pipetting.
- 17. Incubate at room temperature for 5 minutes.
- 18. Place the plate on the magnetic stand at room temperature for 5 minutes or until the supernatant appears clear.

- 19. Remove and discard clear supernatant. Take care not to disturb beads.
- 20. With plate on stand, add 200 μ L of freshly prepared 80% ethanol to each sample and incubate plate at room temperature for 30 seconds. Carefully, remove ethanol by pipette.
- 21. Repeat previous step, for a total of 2 ethanol washes and ensure all ethanol has been removed.
- 22. Remove the plate from the magnetic stand and let dry at room temperature for 3 minutes. Note: Do not over dry the beads.
- 23. Resuspend dried beads with 22 μ L 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 the plate on magnetic stand at room temperature for 5 minutes, or until the sample appears clear.
- 26. Transfer 20 µL of clear sample 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, always thaw your frozen samples on ice before proceeding to Step F: Bisulfite Conversion of DNA.

STEP F: Bisulfite Conversion of DNA

Materials

User Supplied

 $20~\mu L$ of Purified Adapter Ligated DNA (from Step E) Thermocycler 100% Ethanol 96~well PCR plate Adhesive PCR Plate Seal

Bisulfite Conversion Kit (This protocol has been thoroughly tested with the EZ DNA Kit. Other conversion kits may also be compatible) - EZ DNA Methylation-Gold™ Kit (Zymo Research Corp., Cat. # D5005)

Reagent Preparation

Preparation of CT Conversion Reagent

The CT Conversion Reagent supplied within EZ DNA Methylation-Gold™ kit is a solid mixture and must be prepared prior to first use. Prepare as follows:

- 1. Add 900 μL of water, 300 μL of M-Dilution Buffer, and 50 μL of M-Dissolving Buffer to a tube of CT Conversion Reagent.
- 2. Mix at room temperature with frequent vortexing or shaking for 10 minutes.

Note: It is normal to see trace amounts of undissolved reagent in the CT Conversion Reagent. Each tube of CT Conversion Reagent is designed for 10 separate DNA treatments.

Storage: The CT Conversion Reagent is light sensitive, so minimize its exposure to light. For best results, the CT Conversion Reagent should be used immediately following preparation. If not used immediately, the CT Conversion Reagent solution can be stored overnight at room temperature, one week at 4°C, or up to one month at -20°C. Stored CT Conversion Reagent solution must be warmed to 37°C, then vortexed prior to use.

Preparation of M-Wash Buffer

Add 24 mL of 100% Ethanol to the 6 mL M-Wash Buffer concentrate (D5005) before use.

Protocol:

- 1. Add 130 μ L of the CT Conversion Reagent to the 20 μ L of Purified Adapter Ligated DNA (from Step E) in the PCR plate.
- Mix the each sample thoroughly by pipetting, then centrifuge the liquid to the bottom of the tube.
- 3. Place the sample in a thermocycler and incubate as follows:

10 min 98°C 2.5 hrs 64°C hold 4°C

- 4. Add 600 μL of M-Binding Buffer to a Zymo-Spin™ IC Column and place the column into the provided Collection Tube.
- Load the sample (from Step 3) into the Zymo-Spin™ IC Column containing the M-Binding Buffer. Close the cap and mix by inverting the column several times.
- 6. Centrifuge at full speed (14000 rpm) for 30 seconds. Discard the flow-through.
- Add 100 µL of M-Wash Buffer to the column. Centrifuge at full speed for 30 seconds. Discard the flow through.
- 8. Add 200 μ L of M-Desulphonation Buffer to the column and incubate at room temperature (20-30°C) for 17 minutes. After the incubation, centrifuge at full speed for 30 seconds.
- 9. Add 200 μ L of M-Wash Buffer to the column. Centrifuge at full speed for 30 seconds. Add another 200 μ L of M-Wash Buffer and centrifuge at full speed for an additional 30 seconds.
- 10. Discard the flow-through and centrifuge at full speed for 10 seconds to remove residual ethanol
- 11. Place the column into a clean 1.5 mL microcentrifuge tube and add 17 μ L of M-Elution Buffer directly to the column matrix.
- 12. Incubate at room temperature for 1 minute.
- 13. Centrifuge for 30 seconds at full speed to elute the DNA.
- 14. 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 frozen samples on ice before proceeding to Step G: PCR Amplification.



STEP G: PCR Amplification

Materials

Bioo Scientific Supplied

GREEN CAP - NEXTflex™ Bisulfite-Seq U+ PCR Master Mix, NEXTflex™ Primer Mix

WHITE CAP - Nuclease-free Water, Resuspension Buffer

User Supplied

Bisulfite Converted DNA Product (from Step F)
Thermocycler
96 Well PCR Plate
Adhesive PCR Plate Seal
Agencourt AMPure XP Magnetic Beads (room temperature)
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand
Ice

The table below can serve as a guideline in deciding the number of PCR cycles.

Input DNA	10 ng	100 ng	250 ng	500 ng	1 μg
Fragmented Genomic DNA	18 cycles	15-18 cycles	15-18 cycles	12-15 cycles	12-15 cycles
Msp1 Digested DNA	18 cycles	18 cycles	18 cycles	15-18 cycles	12-18 cycles

1. For each sample, combine the following reagents on ice in the 96 well PCR plate:

$_{-}\mu L$	Bisulfite Converted DNA Product (from Step F)
$_{-}\mu L$	Nuclease free Water
$2~\mu\mathrm{L}$	NEXTflex™ Primer Mix
$26~\mu L$	NEXTflex™ Bisulfite-Seq U+ PCR Master Mix
50 μL	TOTAL

Mix thoroughly by pipetting.

3. PCR Cycles:

10 min	95°C	
30 sec	95°C	
30 sec	65°C	Repeat as suggested in above table*
45 sec	72°C	
7 min	72°C	

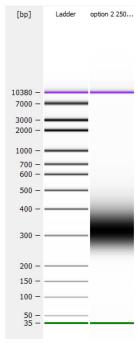
^{*}PCR cycles will vary depending on the amount of starting material and quality of your sample. Further optimization may be necessary. Always use the least number of cycles possible.

- 4. Add 40 μL of AMPure XP Beads to each sample and mix thoroughly by pipetting.
- 5. Incubate at room temperature for 5 minutes.
- 6. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes or until the supernatant appears clear.
- 7. Remove and discard clear supernatant. Take care not to disturb beads.
- 8. With plate on stand, add 200 μ L of freshly prepared 80% ethanol to each sample and incubate plate at room temperature for 30 seconds. Carefully, remove ethanol by pipette.
- 9. Repeat previous step, for a total of 2 ethanol washes and ensure all ethanol has been removed.
- 10. Remove the plate from the magnetic stand and let dry at room temperature for 3 minutes. Note: Do not over dry the beads.
- 11. Resuspend dried beads with 52 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure beads are no longer attached to the side of the well.
- 12. Incubate resuspended beads at room temperature for 2 minutes.
- 13. Place the plate on magnetic stand at room temperature for 5 minutes or until the sample appears clear.
- 14. Transfer 50 μL of clear sample to new well.
- 15. Add 40 μL of AMPure XP Beads to each sample and mix thoroughly by pipetting.
- 16. Incubate at room temperature for 5 minutes.
- 17. Place the plate on the magnetic stand at room temperature for 5 minutes or until the supernatant appears clear.
- 18. Remove and discard clear supernatant. Take care not to disturb beads.
- 19. With plate on stand, add 200 μ L of freshly prepared 80% ethanol to each sample and incubate plate at room temperature for 30 seconds. Carefully, remove ethanol by pipette.
- 20. Repeat previous step, for a total of 2 ethanol washes and ensure all ethanol has been removed.
- 21. Remove the plate from the magnetic stand and let dry at room temperature for 3 minutes. Note: Do not over dry the beads.
- 22. Resuspend dried beads with 11.5 μ L of Resuspension Buffer. Mix thoroughly by pipetting. Ensure beads are no longer attached to the side of the well.
- 23. Incubate resuspended beads at room temperature for 2 minutes.
- 24. Place the plate on magnetic stand for 5 minutes or until the sample appears clear.
- 25. Transfer 10 μL of clear sample to a well of a new 96 well PCR Plate.
- Examine your library with a fluorometer and check the size using an Agilent Bioanalyzer or 2% agarose gel.



- 27. qPCR is recommended to quantitate DNA library templates for optimal cluster density. This can be performed using any qPCR quantification kit with the NEXTflex™ Primer Mix.
- 28. The library is now ready for cluster generation per the standard Illumina protocol. Proceed to cluster generation or seal with Adhesive PCR Plate Seal and store at -20°C.

LIBRARY VALIDATION



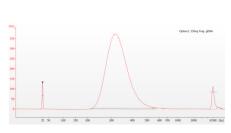


Figure 3: Bioanalyzer Electropherogram Image

Figure 2: Bioanalyzer Gel Image

Figures 2 & 3: 250 ng of fragmented genomic DNA was used. A total of 15 cycles of PCR were performed after bisulfite conversion. 1 μ L of the resulting library was run on an Agilent High Sensitivity DNA chip to verify size.

APPENDIX A

Oligonucleotide Sequences

NEXTflex™	Sequence
Primer 1	5'AATGATACGGCGACCACCGAGATCTACAC
Primer 2	5'CAAGCAGAAGACGGCATACGAGAT
Bisulfite-Seq Adapter 24	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCAC <u>GGTAGC</u> ATCTCGTATGCCGTCTTCTGCTTG

For sequences of the NEXTflex $^{\bowtie}$ Bisulfite-Seq Barcodes, please refer to their respective manuals or contact us at nextgen@biooscientific.com.

RELATED PRODUCTS

Illumina Compatible RNA NGS Kits and Adapters

NEXTflex™ Rapid Directional RNA-Seq Kit

NEXTflex™ RNA-Seq Barcodes

NEXTflex-96™ RNA-Seq Barcodes

NEXTflex™ Rapid Directional qRNA-Seq™ Kit

NEXTflex™ Small RNA Sequencing Kit v2

NEXTlfex™ Small RNA Barcode Primers

NEXTflex™ Poly(A) Beads

Illumina Compatible DNA NGS Kits and Adapters

NEXTflex™ 16S V4 Amplicon-Seq Kit

NEXTflex™ 16S V4 Amplicon-Seq Kit 2.0

NEXTflex™ 16S V1-V3 Amplicon-Seq Kit

NEXTflex™ 18S ITS Amplicon-Seq Kit

NEXTflex™ Rapid DNA-Seq Kit

NEXTflex™ Cell Free DNA-Seq Kit

NEXTflex™ DNA Barcodes

NEXTflex-96™ DNA Barcodes

NEXTflex-HT[™] Barcodes

NEXTflex™ Dual-Indexed DNA Barcodes

NEXTflex™ Bisulfite-Seq Kit

NEXTflex[™] Bisulfite-Seq Barcodes

NEXTflex™ Methyl-Seq 1 Kit

NEXTflex™ Msp 1

NEXTflex[™] ChIP-Seq Kit

NEXTflex™ ChIP-Seq Barcodes

NEXTflex-96™ ChIP-Seq Barcodes

NEXTflex[™] Pre-Capture Combo Kit

NEXTflex™ Rapid Pre-Capture Combo Kit

NEXTflex™ DNA Barcode Blockers

NEXTflex™ PCR-Free DNA Sequencing Kit

NEXTflex[™] PCR-Free Barcodes





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We can't wait to hear from you!





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