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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™ Rapid DNA-Seq Kit
(1 ng – 1 µg)

(Illumina Compatible)

Catalog #5144-01 (8 reactions)

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NEXTflex™ Rapid DNA-Seq Kit (1 ng – 1 μg) - 5144-01

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Product Overview

The NEXTflex™ Rapid DNA-Seq Kit is designed for 2 hour DNA library construction with as little as 1 ng – 1 µg of fragmented DNA. The kit can be used to prepare single, paired-end and multiplexed DNA libraries for sequencing using Illumina® platforms. The NEXTflex™ 1-step End Repair and Adenylation simplifies workflow and shortens hands-on library construction time. A bead-based, gel-free size selection protocol eliminates the need for agarose gel size selection. In addition, the availability of up to 384 unique adapter barcodes facilitates high-throughput applications.

There are five main steps involved in preparing DNA for sequencing: DNA extraction, DNA fragmentation, DNA end repair / adenylation, adapter ligation and PCR amplification. The NEXTflex™ Rapid DNA-Seq Kit contains the necessary material to take the user's purified and fragmented DNA through preparation and amplification for loading onto flow cells for sequencing.

Contents, Storage and Shelf Life

The NEXTflex™ Rapid DNA-Seq Kit contains enough material to prepare 8 DNA samples for Illumina® compatible sequencing. The shelf life of all reagents is 12 months when stored properly. The Resuspension Buffer and Nuclease-free Water can be stored at room temperature. All other components should be stored at -20°C.

Kit Contents	Amount
CLEAR CAP	
NEXTflex™ End-Repair & Adenylation Buffer Mix	120 µL
NEXTflex™ End-Repair & Adenylation Enzyme Mix	24 µL
PURPLE CAP	
NEXTflex™ Ligase Enzyme Mix	380 µL
NEXTflex™ DNA-Seq Adapter 1	20 µL
GREEN CAP	
NEXTflex™ PCR Master Mix	96 µL
NEXTflex™ Primer Mix	16 µL
WHITE CAP	
Nuclease-free Water	1.5 mL
Resuspension Buffer	(2) 1 mL

Required Materials Not Provided

- 1 ng - 1 µg of fragmented DNA in up to 32 µL nuclease-free water.
- If multiplexing: NEXTflex™ DNA Barcodes – 6 / 12 / 24 / 48 (Cat # 514101, 514102, 514103, 514104) or NEXTflex-96™ DNA Barcodes (Cat # 514106) or NEXTflex™ ChIP-Seq Barcodes – 6 / 12 / 24 / 48 (Cat # 514120, 514121, 514122, 514123) or NEXTflex-96™ ChIP-Seq Barcodes (Cat # 514124) or NEXTflex-HT™ Barcodes (Cat # 514170, 514174, 514175, 514176, 514177)
- 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 60 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

Revision History

Version	Date	Description of Change
V14.02	February 2014	Instructions for preparing libraries with size selection have been separated from the instructions for preparing libraries without size selection. Bead clean up volumes have been optimized for both protocols. PCR cycle recommendations have been optimized for preparing libraries without size selection.
V15.08	August 2015	The volume of Adapter Ligated DNA that is used and cycling recommendations in Step D: PCR Amplification, have been optimized.
V15.10	October 2015	In Option 2, Step C2 Bead Size Selection has been optimized and no longer requires the use of Sizing Solution.

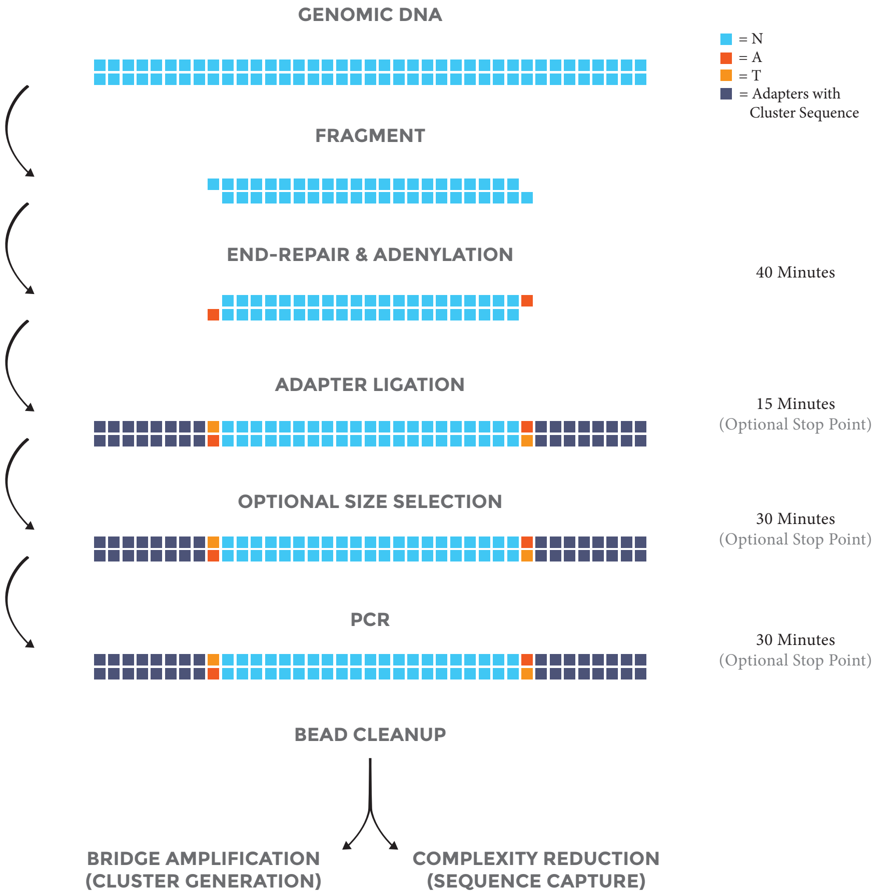
Warnings and Precautions

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 contact 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 the precipitate is in solution.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Do not heat the NEXTflex™ Barcodes above room temperature.
- This kit contains a single Barcoded Adapter. To enable multiplexing, please use the appropriate combination of NEXTflex™ Barcodes during the Adapter Ligation step.
- 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 enzymatic methods of fragmentation as this may introduce sequence bias into the preparation.
- 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.

NEXTflex™ Rapid DNA Sample Preparation Flow Chart

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



Starting Material

The NEXTflex™ Rapid DNA-Seq Kit has been optimized and validated using 1 ng - 1 µg of fragmented genomic DNA.

There are two Rapid DNA-Seq protocol options to choose from:

Option 1 is intended for users who do not wish to size select their libraries. Clean up steps throughout are designed to eliminate only unwanted low molecular weight material.

Option 2 is designed for users who wish to size select their libraries. The user can choose from five size selection ranges, found in Step C2: Bead Size Selection. Size Selection may not be optimal for inputs ≤ 5 ng. Please consider the amount of starting material that will be excluded by size selection when choosing input amount and desired size range.

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 ™ component just prior to use. Nuclease-free Water and Resuspension Buffer can be stored at room temperature.
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 the precipitate is in solution.
3. Allow Agencourt AMPure XP Beads to come to room temperature and vortex the beads until homogenous.

Option 1 is designed for users who do not wish to size select their libraries. Clean-up steps throughout are designed to eliminate only unwanted low-molecular weight material. If you wish to size select your libraries, please follow Option 2.

STEP A1: End-Repair & Adenylation

Materials

Bioo Scientific Supplied

CLEAR CAP -NEXTflex™ End-Repair & Adenylation Buffer Mix, NEXTflex™ End-Repair & Adenylation Enzyme Mix

WHITE CAP - Nuclease-free Water

User Supplied

Fragmented DNA in 32 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 (1 ng - 1 µg)
15 µL	NEXTflex™ End-Repair & Adenylation Buffer Mix
3 µL	NEXTflex™ End-Repair & Adenylation Enzyme Mix
50 µL	TOTAL

2. Apply adhesive PCR plate seal and incubate on a thermocycler using the following program:

20 min	22 °C
20 min	72 °C
end	4 °C

3. Proceed to Step B1: Adapter Ligation.

STEP B1: Adapter Ligation

Materials

Bioo Scientific Supplied

PURPLE CAP - NEXTflex™ Ligase Enzyme Mix, NEXTflex™ DNA Adapter 1 (25 µM)

WHITE CAP - Nuclease-free Water

User Supplied

Thermocycler
50 µL of End Repaired and Adenylated DNA (from STEP A1)

Optional

NEXTflex™ DNA Barcodes, NEXTflex-96™ DNA Barcodes, NEXTflex™ ChIP-Seq Barcodes, NEXTflex-96™ ChIP-Seq Barcodes, or NEXTflex-HT™ Barcodes

1. Thaw NEXTflex™ Ligase Enzyme Mix to room temperature, and vortex for 5-10 seconds. Do not spin down tube, as this may cause components of the mix to separate and affect performance.

2. The following table lists recommended adapter concentration dilutions for various input amounts:

Input DNA	Barcodes Used	Desired Adapter Concentration	Adapter Dilution Required
1 ng	ChIP-Seq (0.6 µM)	0.6 µM	None
10 ng	ChIP-Seq (0.6 µM)	0.6 µM	None
100 ng	DNA or HT (25 µM)	3 µM	1 : 8.3
250 ng	DNA or HT (25 µM)	25 µM	None
500 ng	DNA or HT (25 µM)	25 µM	None
1 µg	DNA or HT (25 µM)	25 µM	None

   Each sample will require 2.5 µL of adapter to be added. Perform adapter dilutions if necessary with Nuclease-free Water, depending on input amount and starting adapter concentration. The following reaction must be mixed thoroughly. The NEXTflex™ Ligase Enzyme Mix is very viscous. Thorough mixing of the reaction below is critical to obtaining optimal results. Suggestion: To mix, pipette up and down 15 times; visually inspect tubes to ensure proper homogenization. Combine the following in the PCR plate and mix thoroughly by pipette:

50 µL	End Repaired and Adenylated DNA (from Step A1)
47.5 µL	NEXTflex™ Ligase Enzyme Mix
2.5 µL	NEXTflex™ Barcodes
100 µL	TOTAL

3. Apply adhesive PCR plate seal and incubate on a thermocycler for 15 minutes at 22°C.

4. Add 50 µL of AMPure XP Beads to each sample. Mix thoroughly until homogenized.

5. Incubate sample at room temperature for 5 minutes.
6. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.
7. Remove and discard clear supernatant.
8. With plate on stand, add 200 µL of 80% ethanol to each magnetic bead pellet 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. Ensure all ethanol has been removed.
10. Remove the plate from the magnetic stand and let dry at room temperature for 5 minutes or until bead pellet is visibly dry.
11. Resuspend dried beads with 52µL of Resuspension Buffer. Mix thoroughly until homogenized.
12. Incubate sample at room temperature for 5 minutes.
13. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.
14. Do not discard the sample in this step. Transfer 50 µL of clear sample to a new well. Remove the 96 well PCR plate from the magnetic stand.
15. Add 40 µL of AMPure XP Beads to each sample. Mix thoroughly until homogenized.
16. Incubate sample at room temperature for 5 minutes.
17. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.
18. Remove and discard clear supernatant.
19. With plate on stand, add 200 µL of 80% ethanol to each magnetic bead pellet 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. Ensure all ethanol has been removed.
21. Remove the 96 well PCR plate from the magnetic stand and let dry at room temperature for 5 minutes or until bead pellet is visibly dry.
22. Resuspend dried beads with 22µL of Resuspension Buffer. Mix thoroughly until homogenized.
23. Incubate resuspended beads at room temperature for 5 minutes.
24. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.
25. Transfer 20 µL of clear sample to a new well.
26. If you wish to pause your experiment, the procedure may be safely stopped at this step with samples stored at -20°C. To restart, thaw frozen samples on ice before proceeding to Step C1: PCR Amplification. If Input DNA amount was 500 ng or greater, PCR Amplification may not be necessary, depending on sequencing application. Users starting with greater than 500 ng of Input DNA are advised to quantify their unamplified libraries by qPCR at this point to determine if PCR Amplification is necessary. This can be performed using any qPCR quantification kit with the NEXTflex™ Primer Mix.

STEP C1: PCR Amplification

Materials

Bioo Scientific Supplied

GREEN CAP - NEXTflex™ PCR Master Mix, NEXTflex™ Primer Mix

WHITE CAP - Resuspension Buffer, Nuclease-free Water

User Supplied

Thermocycler
96 Well PCR Plate
Agencourt AMPure XP Magnetic Beads (room temperature)
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand
20 µL of Adapter Ligated DNA (from STEP B1)

*The following table lists recommended PCR cycles:

Input DNA (ng)	PCR cycles
1	13 - 15
10	10 - 12
100	5 - 7
250	4 - 6
500	0 - 4
1000	0 - 4

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

20 µL	Adapter Ligated DNA (from Step B1)
16 µL	Nuclease-free Water
12 µL	NEXTflex™ PCR Master Mix
2 µL	NEXTflex™ Primer Mix
50 µL	TOTAL

2. Apply adhesive PCR plate seal and place in thermocycler for the following PCR cycles:

2 min	98°C	
30 sec	98°C	Repeat as suggested in above table.
30 sec	65°C	
60 sec	72°C	
4 min	72°C	

3. Add 40 µL of AMPure XP Beads to each sample. Mix thoroughly until homogenized.

4. Incubate at room temperature for 5 minutes.
5. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.
6. Remove and discard clear supernatant taking care not to disturb beads. Some liquid may remain in wells.
7. With plate on stand, add 200 µL of 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipette.
8. Repeat previous step, for a total of 2 ethanol washes. Ensure all ethanol has been removed.
9. Remove plate from magnetic stand and let dry at room temperature for 5 minutes or until bead pellet is visibly dry.
10. Resuspend dried beads with 21µL of Resuspension Buffer. Mix thoroughly until homogenized.
11. Incubate resuspended beads at room temperature for 5 minutes.
12. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.
13. Transfer 20 µL of clear sample to a new well.
14. Examine your library by electrophoresis gel or Agilent Bioanalyzer.
15. qPCR is recommended to quantify DNA library templates for optimal cluster density. This can be performed using any qPCR quantification kit with the NEXTflex™ Primer Mix.
16. 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.

Option Two is designed for users who wish to size select their libraries. The user can choose from five selection ranges, found in Step C2: Bead Size Selection. If you do not wish to size select your libraries, please follow Option 1.

STEP A2: End-Repair & Adenylation

Materials

Bioo Scientific Supplied
CLEAR CAP - NEXTflex™ End-Repair & Adenylation Buffer Mix, NEXTflex™ End-Repair & Adenylation Enzyme Mix

WHITE CAP - Nuclease-free Water

User Supplied
Fragmented DNA in 32 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 (1 ng - 1 µg)
15 µL	NEXTflex™ End-Repair & Adenylation Buffer Mix
3 µL	NEXTflex™ End-Repair & Adenylation Enzyme Mix
50 µL	TOTAL

2. Apply adhesive PCR plate seal and incubate on a thermocycler using the following program:

20 min	22 °C
20 min	72 °C
end	4 °C

3. Proceed to Step B2: Adapter Ligation.

STEP B2: Adapter Ligation

Materials

Bioo Scientific Supplied

PURPLE CAP - NEXTflex™ Ligase Enzyme Mix, NEXTflex™ DNA Adapter 1 (25 µM)

WHITE CAP - Nuclease-free Water, Resuspension Buffer

User Supplied

Thermocycler
50 µL of End Repaired and Adenylated DNA (from STEP A2)

Optional

NEXTflex™ DNA Barcodes, NEXTflex-96™ DNA Barcodes, NEXTflex™ ChIP-Seq Barcodes, NEXTflex-96™ ChIP-Seq Barcodes, or NEXTflex-HT™ Barcodes

1. Thaw NEXTflex™ Ligase Enzyme Mix to room temperature, and vortex for 5-10 seconds. Do not spin down tube, as this may cause components of the mix to separate and affect performance.
2. The following table lists recommended adapter concentration dilutions for various input amounts:

Input DNA	Barcodes Used	Desired Adapter Concentration	Adapter Dilution Required
1 ng	ChIP-Seq (0.6 µM)	0.6 µM	None
10 ng	ChIP-Seq (0.6 µM)	0.6 µM	None
100 ng	DNA or HT (25 µM)	3 µM	1 : 8.3
250 ng	DNA or HT (25 µM)	25 µM	None
500 ng	DNA or HT (25 µM)	25 µM	None
1 µg	DNA or HT (25 µM)	25 µM	None

Each sample will require 2.5 µL of adapter to be added. Perform adapter dilutions if necessary with Nuclease-free Water, depending on input amount and starting adapter concentration. The following reaction must be mixed thoroughly. The NEXTflex™ Ligase Enzyme Mix is very viscous. Thorough mixing of the reaction below is critical to obtaining optimal results. Suggestion: To mix, pipette up and down 15 times; visually inspect tubes to ensure proper homogenization. Combine the following in the PCR plate and mix thoroughly by pipette:

50 µL	End Repaired and Adenylated DNA (from Step A2)
47.5 µL	NEXTflex™ Ligase Enzyme Mix
2.5 µL	NEXTflex™ Barcode Adapter
100 µL	TOTAL

3. Apply adhesive PCR plate seal and incubate on a thermocycler for 15 minutes at 22°C.
4. Add 50 µL of AMPure XP Beads to each sample. Mix thoroughly until homogenized.

5. Incubate sample at room temperature for 5 minutes.
6. Place the 96 well PCR plate on the magnetic stand at room temperature for 5 minutes.
7. Remove and discard clear supernatant.
8. With plate on stand, add 200 µL of 80% ethanol to each magnetic bead pellet 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. Ensure all ethanol has been removed.
10. Remove the plate from the magnetic stand and let dry at room temperature for 5 minutes or until bead pellet is visibly dry.
11. Resuspend dried beads with 52µL of Resuspension Buffer. Mix thoroughly until homogenized.
12. Incubate sample at room temperature for 5 minutes.
13. Place the 96 well PCR plate on the magnetic stand at room temperature for 5 minutes.
14. Do not discard the sample in this step. Transfer 50 µL of clear sample to a new well.
15. If you wish to pause your experiment, the procedure may be safely stopped at this step with samples stored at -20°C. To restart, thaw frozen samples on ice before proceeding to Step C2: Bead Size Selection.

STEP C2: Bead Size Selection

Materials

Bioo Scientific Supplied

CLEAR CAP BOTTLE - Resuspension Buffer

User Supplied

Agencourt AMPure XP Magnetic Beads (room temperature)
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand
50 µL of Purified Adapter Ligated DNA (from STEP B2)

Size Selection may not be optimal for all samples. The size ranges listed in tables below reflect the total library size, including the insert and NEXTflex™ Barcode Adapters. NEXTflex™ Barcode Adapters add ~120bp to the insert length. Yield and specificity of size selection is affected by size distribution of starting material. It is important to select for an insert size that is compatible with the size range of the starting material.

Ensure all reagents are at room temperature. Vortex AMPure XP thoroughly prior to use. Use a fresh dilution of 80% ethanol during wash steps.

The following table lists the appropriate AMPure XP bead volumes to be used to result in the following approximate library peak sizes:

Approximate Insert Peak Size	150-250bp	250-350bp	300-500bp	400-600bp	500-700bp
Approximate Library Peak Size	270-370bp	370-470bp	420-620bp	520-720bp	620-820bp
Bead Volume #1	35 µl	32 µl	27 µl	24 µl	22 µl
Bead Volume #2	12 µl	9 µl	8 µl	8 µl	8 µl

1. Add Bead Volume #1 as indicated in above chart that corresponds to desired size range. Mix thoroughly until homogenized.
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 solution is completely clear.
4. Do not discard the supernatant in this step. Transfer the clear supernatant to a new well. Be careful not to disrupt the magnetic bead pellet or transfer any magnetic beads with the sample.
5. Add Bead Volume #2 as indicated in above chart that corresponds to desired size range. Mix thoroughly until homogenized.
6. Incubate sample at room temperature for 5 minutes.
7. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes

8. Remove and discard clear supernatant taking care not to disturb beads. Some liquid may remain in wells.

9. With 96 well PCR plate on stand, add 200 µL of 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipette.

10. Repeat previous step for a total of 2 ethanol washes. Ensure all ethanol has been removed.

11. Remove the 96 well PCR plate from the magnetic stand and let dry at room temperature for 5 minutes or until bead pellet is visibly dry.

12. Resuspend dried beads with 22 µL of Resuspension Buffer. Mix thoroughly until homogenized.

13. Incubate sample at room temperature for 5 minutes.

14. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.

15. Transfer 20 µL of clear sample to a new well.

16. If you wish to pause your experiment, the procedure may be safely stopped at this step with samples stored at -20°C. To restart, thaw frozen samples on ice before proceeding to Step D2: PCR Amplification. If Input DNA amount was 500 ng or greater, PCR Amplification may not be necessary, depending on sequencing application. Users starting with greater than 500 ng of Input DNA are advised to quantify their unamplified libraries by qPCR at this point to determine if PCR Amplification is necessary. This can be performed using any qPCR quantification kit with the NEXTflex™ Primer Mix.

STEP D2: PCR Amplification

Materials

Bioo Scientific Supplied
GREEN CAP - NEXTflex™ PCR Master Mix, NEXTflex™ Primer Mix

WHITE CAP - Resuspension Buffer, Nuclease-free Water

User Supplied
Thermocycler
96 Well PCR Plate
Agencourt AMPure XP Magnetic Beads (room temperature)
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand
20 µL of Size Selected Adapter Ligated DNA (from STEP C2)

*The following table lists recommended PCR cycles:

Input DNA (ng)	PCR cycles
1	15
10	11 - 13
100	6 - 8
250	5 - 7
500	2 - 4
1000	0 - 4

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

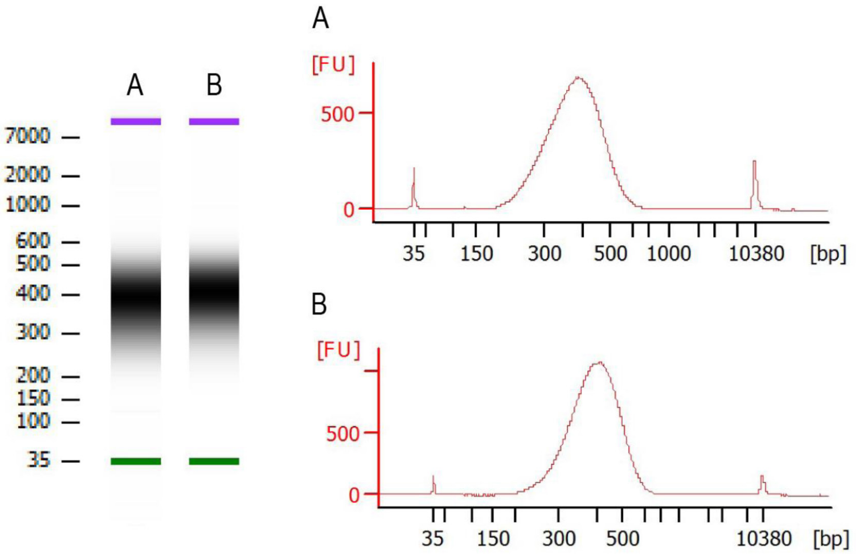
20 µL	Size Selected Adapter Ligated DNA (from Step C2)
16 µL	Nuclease-free Water
12 µL	NEXTflex™ PCR Master Mix
2 µL	NEXTflex™ Primer Mix
50 µL	TOTAL

2. Apply adhesive PCR plate seal and place in thermocycler for the following PCR cycles:

2 min	98°C	
30 sec	98°C	Repeat as suggested in above table.
30 sec	65°C	
60 sec	72°C	
4 min	72°C	

3. Add 40 µL of AMPure XP Beads to each sample. Mix thoroughly until homogenized.

4. Incubate at room temperature for 5 minutes.
5. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.
6. Remove and discard clear supernatant taking care not to disturb beads. Some liquid may remain in wells.
7. With plate on stand, add 200 µL of 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipette.
8. Repeat previous step for a total of 2 ethanol washes. Ensure all ethanol has been removed.
9. Remove plate from magnetic stand and let dry at room temperature for 5 minutes or until bead pellet is visibly dry.
10. Resuspend dried beads with 21µL of Resuspension Buffer. Mix thoroughly until homogenized.
11. Incubate resuspended beads at room temperature for 5 minutes.
12. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.
13. Transfer 20 µL of clear sample to a new well.
14. Examine your library by electrophoresis gel or Agilent Bioanalyzer.
15. qPCR is recommended to quantify DNA library templates for optimal cluster density. This can be performed using any qPCR quantification kit with the NEXTflex™ Primer Mix.
16. 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.



High Sensitivity DNA Chip Ladder / Electropherogram

A) 1 ng input NEXTflex™ 15 cycle PCR product.
B) 1 µg input NEXTflex™ 4 cycle PCR product.

Oligonucleotide Sequences

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

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NOTES

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)

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