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NEXTFLEX® Rapid DNA-Seq Kit 2.0 (1 ng – 1 µg)

(Illumina Compatible)

Catalog #NOVA-5188-01 (8 reactions)

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NEXTflex® Rapid DNA-Seq Kit 2.0 (1 ng – 1 µg)
NOVA-5188-01

Product Overview

The NEXTflex® Rapid DNA-Seq Kit 2.0 is designed for an approximately 3 hour DNA library construction with as little as 1 ng – 1 µg of fragmented DNA. The kit can be used to prepare multiplexed libraries for single or paired-end sequencing using Illumina® platforms. 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 2.0 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 2.0 DNA Seq Kit contains enough material to prepare 8 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 the Nuclease-free Water and Resuspension Buffer, which can be safely stored at room temperature, and NEXTflex® Cleanup Beads 2.0, which should be stored at 4°C.

Kit Contents	Amount
CLEAR CAP	
NEXTflex® End-Repair & Adenylation Buffer Mix 2.0	120 µL
NEXTflex® End-Repair & Adenylation Enzyme Mix 2.0	24 µL
PURPLE CAP	
NEXTflex® Ligase Buffer Mix 2.0	356 µL
NEXTflex® Ligase Enzyme 2.0	24 µL
GREEN CAP	
NEXTflex® PCR Master Mix 2.0	200 µL
NEXTflex® Primer Mix 2.0	16 µL
WHITE CAP	
Nuclease-free Water	(2) 1.5 mL
Resuspension Buffer	1.5 mL
NEXTflex® Cleanup Beads 2.0	1.5 mL

Required Materials Not Provided

- 1 ng - 1 µg of fragmented DNA in up to 32 µL nuclease-free water.
- Bundled kits (NOVA-5188-11, -12, 13) include the Nextflex-HT barcodes. If multiplexing using other barcodes, they will need to be ordered separately in addition to the non-bundled kits (NOVA-5188-01, -02, -03).
- Ethanol 80% (room temperature)
- Covaris System (S2, E210) or other method for DNA fragmentation
- 96 well PCR Plate Non-skirted (Phenix Research, Cat # MPS-499) or similar
- 96 well Library Storage and Pooling Plate (Thermo Fisher Scientific, Cat # AB-0765) or similar
- Adhesive PCR Plate Seal (Bio-Rad, Cat # MSB1001)
- Magnetic Stand -96 (Thermo Fisher Scientific, Cat # AM10027) or similar
- Thermal cycler
- 2, 10, 20, 200 and 1000 µL pipettes / multichannel pipettes
- Nuclease-free barrier pipette tips
- Vortex

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 bioo.ngs@perkinelmer.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® DNA Barcodes above room temperature.
- To enable multiplexing, please use the appropriate combination of NEXTflex® Barcodes during the Adapter Ligation step if not ordering the bundled products that come with the NEXTflex-HT barcodes.
- 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.
- It is highly recommended that NEXTflex® Primer Mix 2.0 be used during PCR amplification. Inadvertent use of an incorrect primer sequence can potentially result in elimination of the index.

NEXTflex® Rapid 2.0 DNA Sample Preparation Flow Chart

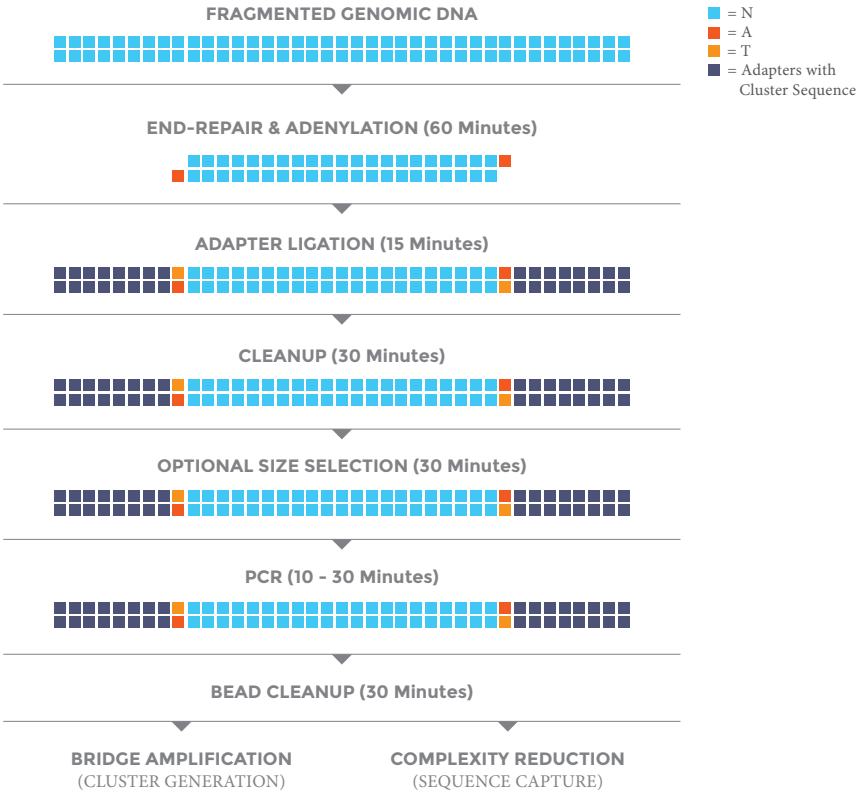

Figure 1: Sample flow chart with approximate times necessary for each step.

Starting Material

The NEXTflex® Rapid DNA Seq Kit 2.0 has been optimized and validated using genomic DNA. Starting with 1 ng - 1 µg of high quality fragmented DNA will allow you to perform at least 8 reactions (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® 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 1-2 minutes or until the precipitate is in solution. The performance of the mix is not affected once the precipitate is in solution.
3. Allow NEXTflex® Cleanup Beads 2.0 to come to room temperature and vortex the beads until homogenous.

STEP A: End-Repair & Adenylation

Materials

Bioo Scientific Supplied

CLEAR CAP -NEXTflex® End-Repair & Adenylation Buffer Mix 2.0, NEXTflex® End-Repair & Adenylation Enzyme Mix 2.0
WHITE CAP - Nuclease-free Water

User Supplied

Fragmented DNA in 32 µL (or less) nuclease-free water
Thermal cycler
96 well PCR Plate
Adhesive PCR Plate Seal
Microcentrifuge
Ice

1. Thaw NEXTflex® End-Repair & Adenylation Buffer Mix on ice, and vortex for 5-10 seconds.
2. 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 2.0*
3 µL	NEXTflex® End-Repair & Adenylation Enzyme Mix 2.0*
50 µL	TOTAL

* *These components can be premixed and added in a single step.*

3. Apply adhesive PCR plate seal and incubate in a thermal cycler using the following program:

30 min	20 °C
30 min	65 °C
end	4 °C

4. Proceed to Step B: Adapter Ligation.

STEP B: Adapter Ligation

Materials

Bioo Scientific Supplied

PURPLE CAP - NEXTflex® Ligase Buffer Mix 2.0, NEXTflex® Ligase Enzyme 2.0
WHITE CAP - Nuclease-free Water, Resuspension Buffer, NEXTflex® Cleanup Beads 2.0

User Supplied

50 µL of End-Repaired and Adenylated DNA (from Step A)
Thermal cycler
Adhesive PCR Plate Seal
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand

NEXTflex® Unique Dual Index Barcodes – 96 (Cat # 514150, 514151) or
NEXTflex® Dual Index Barcodes – 96 (Cat # 514160, 514161) or
NEXTflex-HT™ Barcodes – 6 / 96 (Cat # 514170, 514174, 514175, 514176, 514177) or
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)

1. Thaw NEXTflex® Ligase Buffer Mix 2.0 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 for all listed barcodes except ChIP-Seq barcodes:

Input DNA	Desired Adapter Concentration	Adapter Dilution Required
1 ng	0.3 µM	1 / 80
10 ng	0.6 µM	1 / 40
100 ng	6.25 µM	1 / 4
250 ng	25 µM	None
500 ng	25 µM	None
1 µg	25 µM	None

* ChIP-Seq barcodes may be used in some cases, but require a different dilution. Please inquire for details.

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 2.0 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 & Adenylated DNA (from Step A)
44.5 µL	NEXTflex® Ligase Buffer Mix 2.0*
2.5 µL	NEXTflex® Barcoded Adapter
3.0 µL	NEXTflex® Ligase Enzyme 2.0*
100 µL	TOTAL

** These components can be premixed and added in a single step. Adapter should not be premixed in order to prevent excess adapter dimer formation.*

3. Apply adhesive PCR plate seal and incubate in a thermal cycler with heated lid turned off or open for 15 minutes at 20°C, followed by a 4°C hold.
4. Add 65 µL of Nuclease-free water and 35 µL NEXTflex® Cleanup Beads 2.0 to each sample. Mix thoroughly until homogenized. The NEXTflex® Cleanup Beads 2.0 and Nuclease-free water can be premixed and added in a single step.
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 or until supernatant appears completely clear.
7. Remove and discard clear supernatant, taking care not to disturb beads. Some liquid may remain in wells.
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 96 well PCR plate from the magnetic stand and let dry at room temperature for 3 minutes.
11. Resuspend dried beads with 25 µL of Resuspension Buffer. Mix thoroughly until homogenized.
12. Incubate sample at room temperature for 2 minutes.
13. Place the 96 well PCR Plate on the magnetic stand at room temperature for 2 minutes or until the supernatant appears completely clear.
14. Do not discard the supernatant in this step. Transfer 23 µL of clear sample to a new well. Remove the 96 well PCR plate from the magnetic stand.
15. The procedure may be safely stopped at this step with samples stored at -20°C if needed. To restart, thaw the frozen samples on ice before proceeding with Step C. If input DNA amount was 500 ng or greater, PCR amplification may not be necessary depending on the 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 quantitation kit compatible with Illumina® platforms.

Note: Included library cleanup reagents are compatible with size selection methods. Contact Bioo Scientific at bioo.ngs@perkinelmer.com.

STEP C: PCR Amplification

Materials

Bioo Scientific Supplied

GREEN CAP - NEXTflex® PCR Master Mix 2.0, NEXTflex® Primer Mix 2.0
WHITE CAP - Nuclease-free Water, Resuspension Buffer, NEXTflex® Cleanup Beads 2.0

User Supplied

23 µL of Adapter Ligated DNA (from Step B)
Thermal cycler
Adhesive PCR Plate Seal
96 Well PCR Plate
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand

*The following table lists recommended PCR cycles for varying DNA input amounts.

	Number of PCR cycles to produce	
Input DNA (ng)	**100 ng libraries**	**1 µg libraries**
1	10 - 12	13 - 15
10	6 - 8	9 - 11
100	2 - 3	6 - 7
250	1 - 2	4 - 5
500	0	3 - 4
1000	0	2 - 3

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

23 µL	Adapter Ligated DNA (from Step B)
25 µL	NEXTflex® PCR Master Mix 2.0*
2 µL	NEXTflex® Primer Mix 2.0*
50 µL	TOTAL

* These components can be premixed and added in a single step.

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

30 sec	98°C	
15 sec	98°C	Repeat as suggested in above table
30 sec	65°C	
30 sec	72°C	
2 min	72°C	

3. Add 45 µL of NEXTflex® Cleanup Beads 2.0 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 or until the supernatant appears completely clear.
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 the 96 well PCR plate from the magnetic stand and let dry at room temperature for 3 minutes.
10. Resuspend dried beads with 33 µL of Resuspension Buffer. Mix thoroughly until homogenized.
11. Incubate resuspended beads at room temperature for 2 minutes.
12. Place the 96 well PCR Plate on the magnetic stand at room temperature for 2 minutes or until the supernatant appears completely clear.
13. Do not discard the supernatant in this step. Transfer 30 µL of clear sample to a new well. Remove the 96 well PCR plate from the magnetic stand.
14. Examine library by electrophoresis to ensure proper library sizing and to verify exclusion of contaminating small and large fragments (recommended: LabChip® GXII Touch™ HT instrument (PerkinElmer®).
15. qPCR is recommended to quantify DNA library templates for optimal cluster density. This can be performed using any qPCR quantification kit for Illumina® platforms.
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.

Oligonucleotide Sequences

NEXTflex™	Sequence (5' → 3')
PCR Primer 1	AATGATACGGCGACCACCGAGATCTACAC
PCR Primer 2	CAAGCAGAAGACGGCATACGAGAT

For low level multiplexing recommendations, see Appendix A in the NEXTflex® barcode manual.

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!

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THE NGS EXPERTS™

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