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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™ DNA Sequencing Kit
(Illumina Compatible)

Catalog #: 5140-02 (48 reactions)

V11.12

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GENERAL INFORMATION

Product Overview

The NEXTflex™ DNA Sequencing Kit is designed to prepare single, paired-end and multiplexed genomic DNA libraries for sequencing using Illumina® GAIIx, HiSeq 2000/1000 and MiSeq platforms. The enhanced NEXTflex™ DNA Sequencing Kit simplifies workflow by using master mixed reagents and magnetic bead based cleanup, reducing pipetting and eliminating time consuming steps in library preparation. An optional bead-based, gel-free size selection protocol eliminates the need for agarose gel size selection. In addition, the availability of up to 96 unique adapter barcodes makes this a high-throughput kit.

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

Contents, Storage and Shelf Life

The NEXTflex™ DNA Sequencing Kit contains enough material to prepare 48 genomic DNA samples for Illumina® compatible sequencing. The shelf life of all reagents is 12 months when stored properly. DNA Binding Buffer, DNA Wash Buffer and the Clean-Up Spin Columns should be stored at room temperature. All of the other components can be safely stored at -20°C.

Kit Contents	Amount
CLEAR CAP	
NEXTflex™ End Repair Buffer Mix	336 µL
NEXTflex™ End Repair Enzyme Mix	144 µL
RED CAP	
NEXTflex™ Adenylation Mix	168 µL
PURPLE CAP	
NEXTflex™ Ligation Mix	(2) 756 µL
GREEN CAP	
NEXTflex™ PCR Master Mix	576 µL
ORANGE CAP	
6X Loading Dye	500 µL
MW Ladder Ready-to-Load- 100 bp	400 µL
YELLOW CAP	
Column Elution Buffer	(2) 1.8 mL

Kit Contents	Amount
CLEAR CAP-BOTTLE	
Nuclease-free Water	5 mL
Resuspension Buffer	12 mL
DNA Binding Buffer	20 mL
5X DNA Wash Buffer	14 mL
Clean-Up Spin Columns	50

Required Materials not Provided

- 1 µg of fragmented genomic DNA in up to 40 µL nuclease-free water.
- NEXTflex™ DNA Barcodes – 6 / 12 / 24 / 48 (Cat # 514101, 514102, 514103, 514104) or NEXTflex-96™ DNA Barcodes (Cat # 514106)
- 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
- Low melt agarose such as Low Gelling Temperature Agarose with a melt point of 65ºC (Boston Bioproducts, Cat # [illegible]730)
- 1X TAE buffer
- Clean razor or scalpel
- SYBR Gold (Invitrogen, Cat # S11494)
- UV transilluminator or gel documentation instrument
- Gel electrophoresis apparatus
- Electrophoresis power supply
- Vortex

Warnings and Precautions

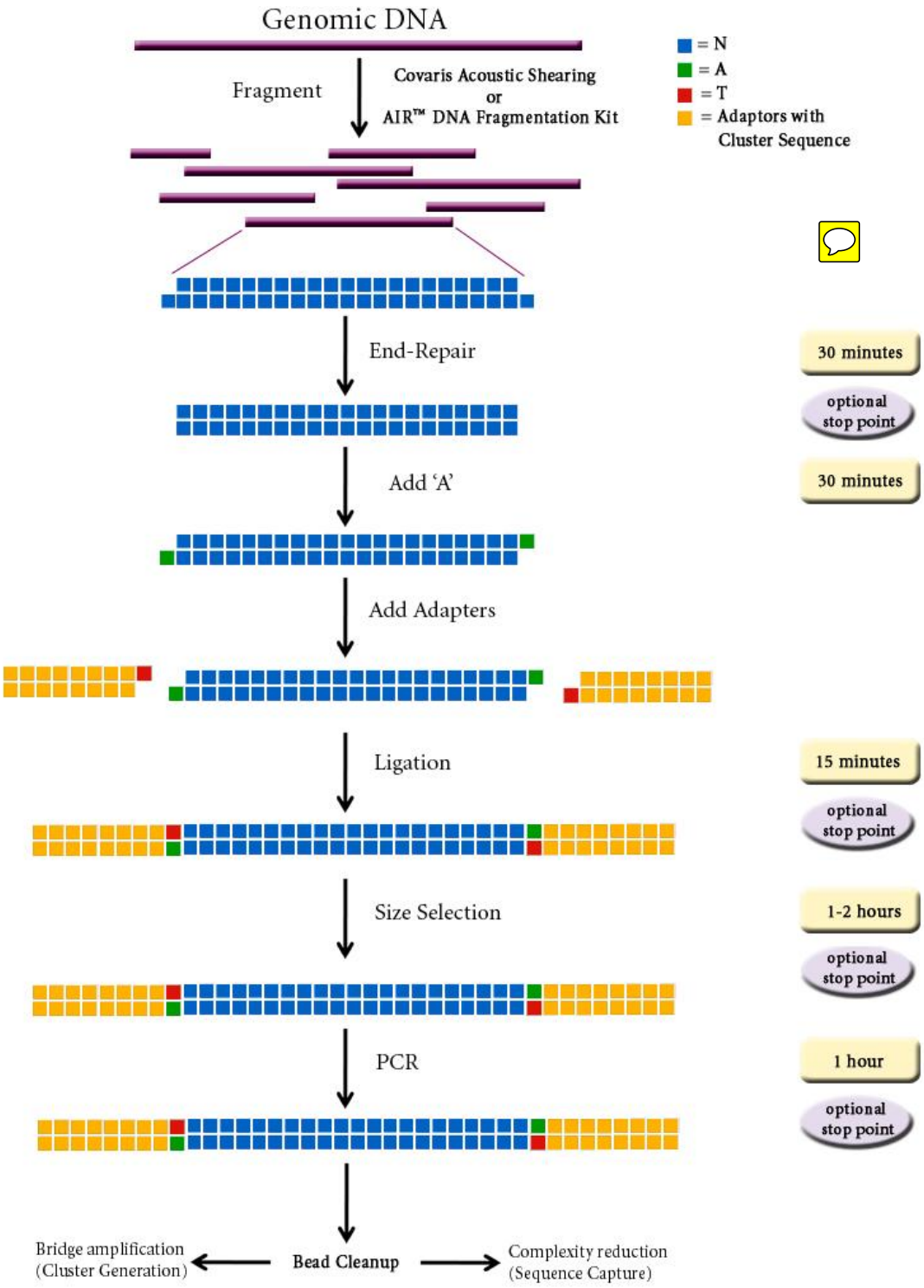
Bioo Scientific strongly recommends that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor or 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 DNA Adapters above room temperature.
- Try to maintain a laboratory temperature of 20º–25ºC (68º–77ºF).
- 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.
- If starting with a DNA input amount greater than or less than 1 µg, adjust the DNA Adapter or DNA Barcoded Adapter volume to preserve the insert to 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.

NEXTflex™ DNA SAMPLE PREPARATION PROTOCOL

NEXTflex™ DNA Sample Preparation Flow Chart

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



Starting Material

The NEXTflex™ DNA Sequencing Kit has been optimized and validated using genomic DNA. Starting with 1 µg of high quality fragmented genomic DNA will allow you to perform at least 8 reactions per adapter or barcoded adapter (see page 3, 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 ™ 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.

4. Add 56 mL of 100% ethanol to the bottle of 5X DNA Wash Buffer. Check box on bottle to show ethanol has been added.

STEP A: End Repair

Materials

Bioo Scientific Supplied

CLEAR CAP
NEXTflex™ End Repair Buffer Mix
NEXTflex™ End Repair Enzyme Mix

WHITE CAP
Nuclease-free H_2O

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 H_2O
_ µL	Fragmented DNA (1 µg)
7 µL	NEXTflex™ End Repair Buffer Mix
3 µL	NEXTflex™ End Repair Enzyme Mix
50 µL	TOTAL

2. Set pipette to 50 µL, gently pipette the entire volume up and down 10 times.

3. Apply adhesive PCR plate seal and incubate on a thermocycler for 30 minutes at 22°C.

STEP B1: Gel-Free Size Selection Clean-Up

NOTICE: If performing agarose gel size selection, please skip Step B1 and proceed with Step B2.

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

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 gently pipette the entire volume up and down 10 times.
2. Incubate sample at room temperature for 5 minutes.
3. Place the 96 well PCR Plate on the magnetic stand at room temperature until the sample appears clear.
4. Set pipette to 90 µL, gently remove and discard clear sample taking care not to disturb beads. Some liquid may remain in wells. This selectively removes DNA below 300 bp.
5. With plate on stand, gently 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 Resuspension Buffer. Gently, pipette entire volume up and down 10 times mixing thoroughly. Ensure beads are no longer attached to the side of the well.
9. Incubate resuspended beads at room temperature for 2 minutes.
10. Place plate on magnetic stand for 5 minutes until the sample appears clear.
11. Gently transfer 50 µL of clear sample to a new well.
12. Add 40 µL of AMPure XP Beads to each clear sample and gently pipette the entire volume up and down 10 times.
13. Incubate at room temperature for 5 minutes.

14. Place the 96 well PCR Plate on the magnetic stand at room temperature until the sample appears clear.

15. **Do not discard clear sample in this step.** Transfer 88 µL of clear sample 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 clear sample and gently pipette the entire volume up and down 10 times.

17. Incubate at room temperature for 5 minutes.

18. Place the 96 well PCR Plate on the magnetic stand at room temperature until the sample appears clear.

19. Once solution clears, gently remove and discard 172 µL of clear sample taking care not to disturb beads. Some liquid may remain in wells.

20. With plate on stand, gently 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 Resuspension Buffer. Gently, pipette entire volume up and down 10 times mixing thoroughly. 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 for 5 minutes until the sample appears clear.

26. Gently transfer 17 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, thaw frozen samples on ice before proceeding.

28. Proceed to Step C.

STEP B2: Clean-Up

NOTICE: Proceed with Step B2 only if performing agarose gel size selection.

Materials

Bioo Scientific Supplied

WHITE CAP
Resuspension Buffer

User Supplied

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

1. Add 55 µL of AMPure XP Beads to each sample and gently pipette the entire volume up and down 10 times.
2. Incubate at room temperature for 5 minutes.
3. Place the 96 well PCR Plate on the magnetic stand at room temperature until the sample appears clear.
4. Set pipette to 100 µL, gently remove and discard clear sample taking care not to disturb beads. Some liquid may remain in wells.
5. With plate on stand, gently add 200 µL of freshly prepared 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully, remove ethanol by pipette.
6. Repeat step 5, for a total of 2 ethanol washes. Ensure all ethanol has been removed.
7. Remove the plate from the magnetic stand and let dry at room temperature for 3 minutes.
8. Resuspend dried beads with 17.5 µL Resuspension Buffer. Gently, pipette entire volume up and down 10 times mixing thoroughly. Ensure beads are no longer attached to the side of the well.
9. Incubate resuspended beads at room temperature for 2 minutes.
10. Place plate on magnetic stand for 5 minutes until the sample appears clear.
11. Gently transfer 17 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, thaw frozen samples on ice before proceeding.
13. Proceed to Step C.

STEP C: 3' Adenylation

Materials

Bioo Scientific Supplied

RED CAP

NEXTflex™ Adenylation Mix

User Supplied

Thermocycler (set to 37°C)

17 µL of End Repaired DNA (from STEP B)

1. Combine the following in the PCR plate:

17 µL	End-Repaired DNA (from Step B)
3.5 µL	NEXTflex™ Adenylation Mix
20.5 µL	TOTAL

2. Set pipette to 20 µL, gently pipette the entire volume up and down 10 times.

3. Apply adhesive PCR plate seal and incubate on a thermocycler for 30 minutes at 37°C.

STEP D: Adapter Ligation

Materials

Bioo Scientific Supplied

PURPLE CAP

NEXTflex™ Ligation Mix (remove right before use and store immediately after use at -20°C)

NEXTflex™ DNA Adapter / or / NEXTflex™ DNA Barcodes – 6 / 12 / 24 / 48 (Cat # 514101, 514103, 514104)

User Supplied

20.5 µL 3' Adenylated DNA (from STEP C)

1. For each sample, combine the following reagents (in this order) in the PCR plate:

20.5 µL	3' Adenylated DNA (from step C)
31.5 µL	NEXTflex™ Ligation Mix
2.5 µL	NEXTflex™ DNA Adapter or Barcode
54.5 µL	TOTAL

2. Set pipette to 50 µL, gently pipette the entire volume up and down 10 times.

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

STEP E: Clean-Up

Materials

Bioo Scientific Supplied

WHITE CAP
Resuspension Buffer

User Supplied

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

1. Add 44 µL of AMPure XP Beads to each sample and gently pipette the entire volume up and down 10 times.
2. Incubate at room temperature for 5 minutes.
3. Place the 96 well PCR Plate on the magnetic stand at room temperature for 15 minutes until the sample appears clear.
4. Set pipette to 96 µL, gently remove and discard clear sample taking care not to disturb beads. Some liquid may remain in wells.
5. With plate on stand, gently 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 and 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 Resuspension Buffer. Gently, pipette entire volume up and down 10 times mixing thoroughly 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 until the sample appears clear.
11. Gently transfer 54.5 µL of clear sample to new well.
12. Repeat steps 1-7.
13. Resuspend dried beads with 22.5 µL Resuspension Buffer. Gently, pipette entire volume up and down 10 times mixing thoroughly. Ensure beads are no longer attached to the side of the well.
14. Incubate resuspended beads at room temperature for 2 minutes.
15. Place plate on magnetic stand for 5 minutes until the sample appears clear.
16. Gently transfer 20 µL of clear sample to new well.
17. 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.

STEP F: Agarose Gel Size Selection

NOTICE: If Step B1 was performed, skip Step F and proceed with Step G.

Materials

Bioo Scientific Supplied

Clean Up Spin Columns

CLEAR CAP-BOTTLE
DNA Binding Buffer
DNA Wash Buffer (ethanol added, see reagent preparation)

YELLOW CAP
Column Elution Buffer

ORANGE CAP
6X Gel Loading Dye
MW Ladder Ready-to-Load 100 bp

User Supplied

2% TAE agarose Gel (Certified Low Gelling Temperature Agarose)
1X TAE Buffer
SYBR Gold
1.5 mL nuclease-free microcentrifuge tubes
Clean razor or scalpel
UV transilluminator or gel documentation instrument
Gel electrophoresis apparatus
Electrophoresis power supply
100% Ethanol (stored at room temperature)
Microcentrifuge

1. Add 4 µL of 6X Gel Loading Dye to each sample.
2. Prepare pre-stained SYBR Gold 2% low melt agarose gel by adding 15 µL of SYBR Gold to every 150 mL of cooled 1X TAE and agarose gel buffer. Mix and then pour into gel tray. Load the entire sample into one lane of the gel. If processing more than one sample, it is recommended to run separate gels or leave several empty wells between samples to avoid cross contamination.
3. Load 4 µL of MW Ladder Ready-to-Load 100 bp into one lane, skipping at least two lanes between it and your sample.
4. Run the gel with 1X TAE buffer at 100-120V for 60 -120 minutes.
5. Visualize the gel on a UV transilluminator or gel documentation instrument.
6. Use a clean razor or scalpel to cut out a slice of gel from each sample lane corresponding to the 400-500 bp marker. This results in an insert size of 300-400 bp (NEXTflex™ Barcode Adapters add ~120 bp to each fragment). The user may choose other insert sizes when appropriate. Keep in mind that sequence reads that overlap into the adapter will result in reads that do not map to the

erence sequence.

7. Add 400 µL of DNA Binding Buffer to each gel slice containing sample and mix well. Incubate your sample at room temperature and vortex the sample occasionally until the agarose is completely melted.

8. Add 20 µL of 100% ethanol to each sample and mix well.

9. Transfer the sample to a Clean-Up Spin Column.

10. Centrifuge the Clean-Up Spin Column in a microcentrifuge at 14,000 rpm for 1 minute.

11. Decant the flow through and replace the Clean-Up Spin Column into the same collection tube.

12. Add 700 µL of DNA Wash Buffer to each column. *Note: Prior to using the 5X DNA Wash Buffer, 56 mL of 100% ethanol must be added before first use as described in the Reagent Preparation section.*

13. Centrifuge the Clean-Up Spin column in a microcentrifuge at 14,000 rpm for 1 minute.

14. Decant the flow through and replace the Clean Up Spin Column into the same collection tube

15. Repeat steps 12 -14 one time.

16. Centrifuge the Clean-Up Spin column in a microcentrifuge at 14,000 rpm for 1 minute to remove any residual ethanol.

17. Place the Clean-Up Spin Column into a clean 1.5 mL nuclease-free microcentrifuge tube. Add 25 µL of Column Elution Buffer to the center of the column. Incubate the column at room temperature for 1 minute.

18. Centrifuge the Clean-Up Spin Column in a microcentrifuge at 14,000 rpm for 1 minute to elute the clean DNA. 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.

STEP G: PCR Amplification

Materials

Bioo Scientific Supplied

GREEN CAP
NEXTflex™ Primer Mix
NEXTflex™ PCR Master Mix

User Supplied

Thermocycler
96 Well PCR Plate
Resuspension Buffer
***Ligation Product (from STEP E or STEP F)**

1. For each sample, combine the following reagents on ice in the PCR plate. If agarose gel size selection has been performed, use 15 µL of ligation product in PCR. If gel-free size selection has been performed use 10 µL of ligation product.

_ µL	Ligation Product (10 – 15 µL)
_ µL	Nuclease-free H_2O
12 µL	NEXTflex™ PCR Master Mix
2 µL	NEXTflex™ Primer Mix
50 µL	TOTAL

2. Set pipette to 50 µL, gently pipette the entire volume up and down 10 times.

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

2 min	98°C	
30 sec	98°C	
30 sec	65°C	Repeat 10 -15 cycles*
60 sec	72°C	
4 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. Repeat STEP E: Clean-Up, through Step 7 to remove adapter dimers that appear around 120 bp.

5. Resuspend dried beads with 33 µL Resuspension Buffer. Gently, pipette entire volume up and down 10 times mixing thoroughly. Ensure beads are no longer attached to the side of the well.

6. Incubate resuspended beads at room temperature for 2 minutes.

7. Place plate on magnetic stand for 5 minutes until the sample appears clear.

8. Gently transfer 30 µL of clear sample to a well of a new 96 well PCR Plate.

9. To ensure cluster generation it is recommended that you quantify your library by gel or Agilent Bioanalyzer. To quantify by gel, load 2 µL of 6X Gel Loading Dye and 10 µL of PCR Product in a SYBR stained 2% low melt agarose gel.

10. 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.

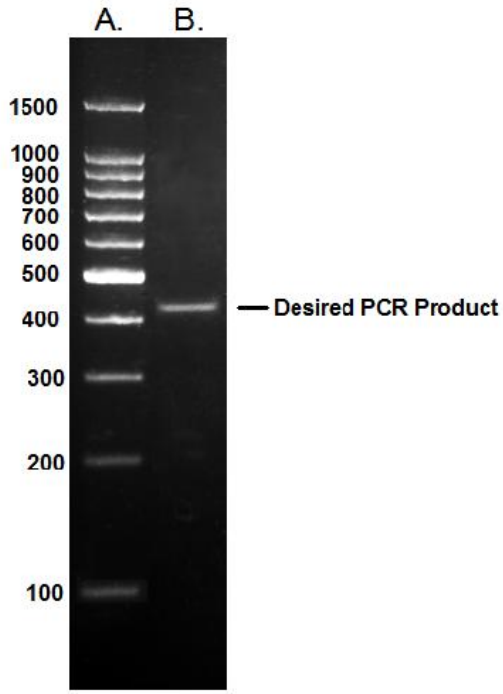
11. Non-multiplexed DNA libraries can be normalized to 10 nM using Tris-HCl (10 mM), pH 8.5 with 0.1% Tween 20. The library is now ready for cluster generation per the standard Illumina protocol.

12. For multiplexed libraries, transfer 10 µL of each normalized library to be pooled in the well of a new PCR plate. Gently pipette the entire volume up and down 10 times.

13. Proceed to cluster generation or seal with Adhesive PCR Plate Seal and store at -20°C.

LIBRARY VALIDATION

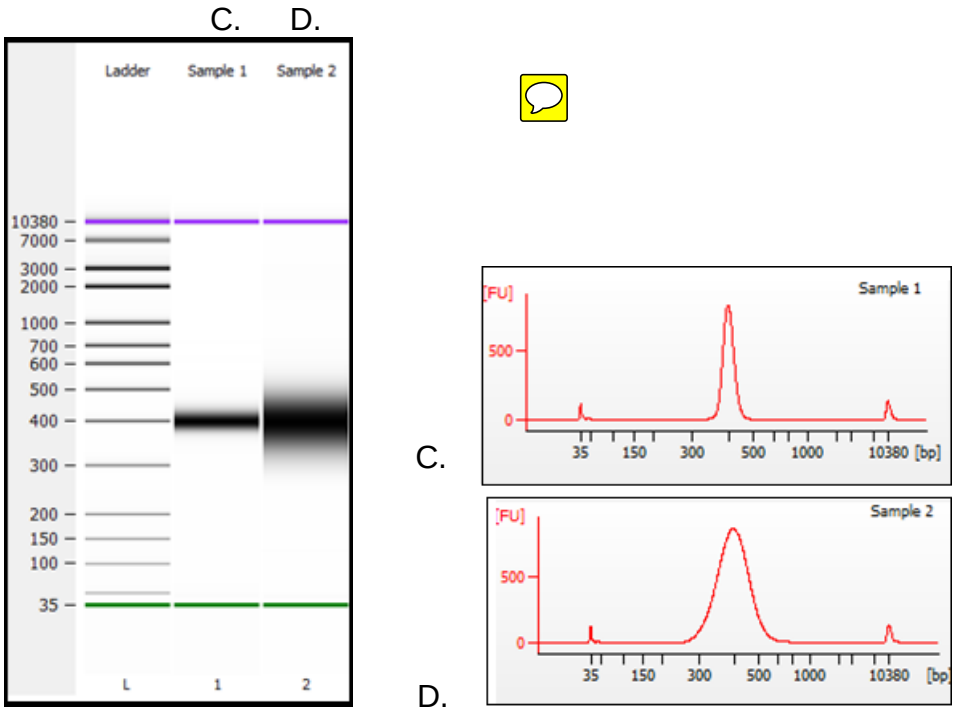
Figure 2. Gel validation of the NEXTflex™ DNA PCR product (10 cycles)



2% Agarose gel

A) MW Ladder Ready-to-Load- 100 bp
B) NEXTflex™ 10 cycle PCR product (agarose gel size selection)

Figure 3. Bioanalyzer validation of NEXTflex PCR product (10 cycles) with gel and gel-free size selection.



High Sensitivity DNA Chip Ladder / Electropherogram

C) NEXTflex™10 cycle PCR product (agarose gel size selection).
D) NEXTflex™10 cycle PCR product (gel-free size selection).

TROUBLESHOOTING

DNA Not Visible During Size Selection

Possible Causes	Recommended Action
Low DNA recovery after clean up steps	Make sure beads are fully resuspended after magnetic clean up. Ensure that the entire volume of sample is mixed 10 times before and after the addition of magnetic beads.
Nuclease contamination	Ensure that your starting DNA material is nuclease free. Use nuclease-free pipettes, pipette tips (filter tips are preferred), tubes and reagents. Perform protocol under nuclease-free conditions. Wear gloves.
Not enough starting material	Use 750 ng -2 µg of fragmented DNA.

No Visible PCR Product

Possible Causes	Recommended Action
Adapter/Primer Error	Make sure that you vortex and briefly spin down each adapter or primer before removing material. Ensure that the right volumes of adapters or primers were added at each step of the procedure.
Adapter or Enzyme Degradation	Ensure that all adapters and enzymes mixes are kept at -20°C when not in use.
Adapters did not anneal to DNA fragments	Ensure that adapters have not been warmed above room temperature and thus denatured.
Gel slice was not fully dissolved after size selection	Vortex every two minutes to dissolve the gel slice. Add ethanol to DNA Binding Buffer after gel slice has dissolved and before applying to the column. If necessary, add more DNA Binding Buffer to fully dissolve the gel slice. Increase the amount of ethanol to 5% of the volume of binding buffer used.
Gel band was not excised carefully after size selection	Carefully excise desired band avoiding other bands.

Adapter/Primer Contamination in Sequencing Data

Possible Causes	Recommended Action
Dimer bands were not properly removed post PCR.	Perform a second bead clean up to ensure all adapter / primer bands have been removed.

APPENDIX A

Oligonucleotide Sequences

NEXTflex™	Sequence
DNA Adapter	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTATCTCGTATGCCGTCTTCTGCTTG
Primer 1	5'AATGATACGGCGACCACCGAGATCTACAC
Primer 2	5'CAAGCAGAAGACGGCATACGAGAT

RELATED PRODUCTS

DNA Fragmentation

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

DNA Next Generation Sequencing Kits

Product	Catalog Number
NEXTflex™ DNA Sequencing Kit (8 reactions)	5140-01
NEXTflex™ DNA Barcodes – 6	514101
NEXTflex™ DNA Barcodes – 12	514102
NEXTflex™ DNA Barcodes – 24	514103
NEXTflex™ DNA Barcodes – 48	514104
NEXTflex-96™ DNA Barcodes	514106
NEXTflex™ ChIP-Seq DNA Sequencing Kit (8 reactions)	5143-01
NEXTflex™ ChIP-Seq DNA Sequencing Kit (48 reactions)	5143-02
NEXTflex™ ChIP-Seq Barcodes – 6	514120
NEXTflex™ ChIP-Seq Barcodes – 12	514121
NEXTflex™ ChIP-Seq Barcodes – 24	514122
NEXTflex™ ChIP-Seq Barcodes – 48	514123
NEXTflex-96™ ChIP-Seq Barcodes	514124
NEXTflex™ PCR-Free DNA Sequencing Kit (8 reactions)	5142-01
NEXTflex™ PCR-Free DNA Sequencing Kit (48 reactions)	5142-02
NEXTflex™ PCR-Free Barcodes – 6	514110
NEXTflex™ PCR-Free Barcodes – 12	514111
NEXTflex™ PCR-Free Barcodes – 24	514112
NEXTflex™ PCR-Free Barcodes – 48	514113

RNA Next Generation Sequencing Kits and Adapters

Product	Catalog Number
NEXTflex™ Small RNA Sequencing Kit (24 reactions)	5132-01
NEXTflex™ Small RNA Sequencing Kit (48 reactions)	5132-02
NEXTflex™ Small RNA Barcodes – Set A	513301
NEXTflex™ Small RNA Barcodes – Set B	513302
NEXTflex™ Small RNA Barcodes – Set C	513303
NEXTflex™ Small RNA Barcodes – Set D	513304

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