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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™ Pre- & Post- Capture Combo Kit- Set A

(Agilent SureSelect^{XT} Compatible)

(Illumina Compatible)

Catalog #5144-61 (16 reactions)

This product is for research use only.

NEXTflex™ Pre- and Post-Capture Combo Kit - 5144-61

Product Overview

The NEXTflex™ Pre- & Post- Capture Combo Kit works seamlessly with the Agilent SureSelect^{XT} system to offer greater multiplexing capabilities along with shortened library prep times. This kit contains library prep reagents, indexed barcodes, blockers, hybridization and wash buffers, and post capture amplification reagents. The NEXTflex Pre- & Post- Capture Kit is designed for 2 hour DNA library construction with as little as 100 ng of fragmented DNA, and optimized for subsequent target enrichment using the Agilent SureSelect^{XT} system. 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. Index-specific barcode blockers used during hybridization lead to higher %-on target metrics than blocking strategies that utilize a common blocker. Post-capture amplification is performed off-bead, providing more robust coverage than on-bead approaches.

There are five main steps involved in preparing DNA libraries for target enrichment: DNA extraction, DNA fragmentation, DNA end repair / adenylation, adapter ligation and PCR amplification. The NEXTflex™ Pre- & Post- Capture Kit contains the necessary material to take the user's purified genomic DNA through library preparation and amplification for target enrichment, hybridization (probes not included), and post-capture sample processing.

Contents, Storage and Shelf Life

The NEXTflex™ Pre- & Post- Capture Combo Kit contains enough material to prepare 16 DNA samples for Illumina® compatible sequencing. The shelf life of all reagents is 12 months when stored properly. NEXTflex™ XT Binding Buffer, Wash Buffer 1, Wash Buffer 2, 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	240 µL
NEXTflex™ End-Repair & Adenylation Enzyme Mix	48 µL
PURPLE CAP	
NEXTflex™ Ligase Enzyme Mix	760 µL
ASSORTED COLOR CAP	
NEXTflex™ DNA Barcoded Adapter (25 µM)	2.5 µL
GREEN CAP	
NEXTflex™ PCR Master Mix	384 µL
NEXTflex™ Primer Mix	64 µL
WHITE CAP	
Nuclease-free Water	2 mL
NEXTflex™ Resuspension Buffer	2 mL

ORANGE CAP	
NEXTflex™ HYB 1	116 µL
RED CAP	
NEXTflex™ HYB 2	6 µL
YELLOW CAP	
NEXTflex™ HYB 3	48 µL
PINK CAP	
NEXTflex™ HYB 4	62 µL
ASSORTED COLOR CAP	
NEXTflex™ Barcode Blockers (250 µM)	2 µL
WHITE CAP	
NEXTflex™ Universal Oligo 1 (500 µM)	20 µL
GREEN CAP	
NEXTflex™ Block 1	43 µL
BLUE CAP	
NEXTflex™ Block 2	43 µL
PURPLE CAP	
NEXTflex™ RNase Block	22 µL
CLEAR CAP BOTTLE	
NEXTflex™ XT Binding Buffer	14 mL
NEXTflex™ Wash Buffer 1	3.5 mL
NEXTflex™ Wash Buffer 2	12 mL

Required Materials Not Provided

- 100 ng - 1 µg of DNA in up to 130 µL nuclease-free water.
- Ethanol 100% (room temperature)
- Ethanol 80% (room temperature)
- Covaris System (S2, E210) and microTUBE Snap-Cap Kit (Cat # 520045)
- 1X Low TE Buffer (10 mM Tris-HCl (pH 8.0), .1 mM EDTA)
- Qubit fluorometer and Quant-iT dsDNA BR Assay Kit (Life Technologies, Cat # Q32850)
- 2100 Bioanalyzer and High Sensitivity DNA Kit (Agilent, Cat # 5067-4626)
- SureSelect^{XT} Capture Library (Agilent)
- 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
- Nuclease free 1.5 mL snap-top centrifuge tubes
- Adhesive PCR Plate Seal (BioRad, Cat # MSB1001)
- Agencourt AMPure XP 60 mL (Beckman Coulter Genomics, Cat # A63880)
- Dynabeads MyOne Streptavidin T1 (Life Technologies, Cat # 65601)
- 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

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.

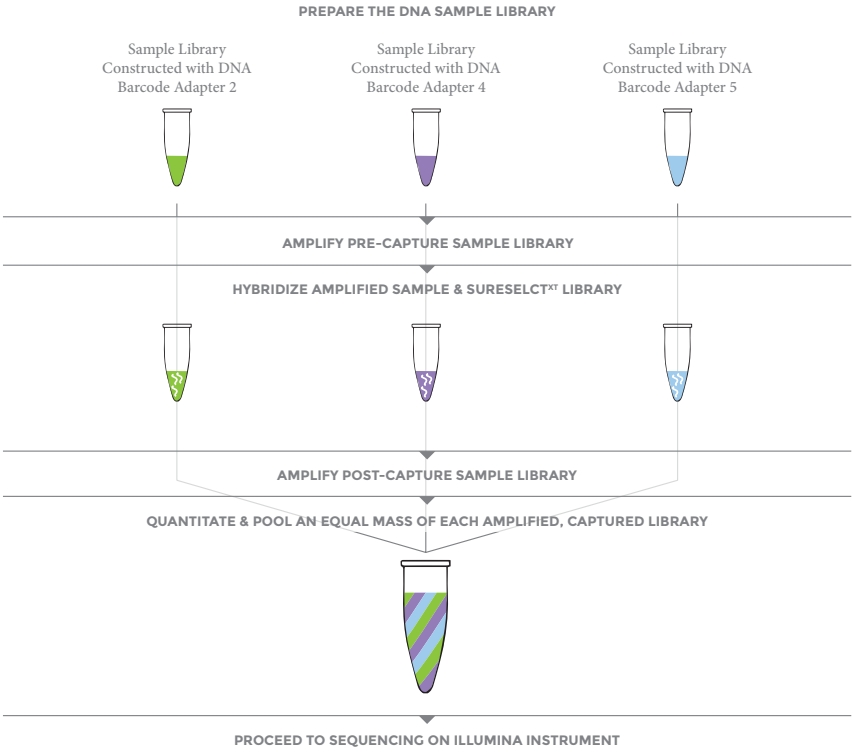
- Do not use the kit past the expiration date.
- DTT in buffers may precipitate after freezing. If precipitate is seen, vortex buffer for 1-2 minutes or until the precipitate is in solution. The performance of the buffer is not affected once the precipitate is in solution.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Do not heat the DNA Barcoded Adapters above room temperature.
- 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.
- 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.

Revision History

Version	Date	Description of Change
V15.09	September 2015	Initial product launch.

NEXTflex™ Pre- & Post-Capture Sample Preparation Flow Chart

Figure 1: Sample flow chart.



Starting Material

The NEXTflex™ Pre- & Post- Capture Combo Kit has been optimized and validated using sheared genomic DNA. Starting with 100 ng - 1 µg of high quality fragmented DNA will allow you to prepare 16 indexed libraries for use with Agilent SureSelect^{XT}.

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.
2. NEXTflex™ XT Binding Buffer, Wash Buffer 1, Wash Buffer 2, Resuspension Buffer, and Nuclease-free Water can be stored at room temperature.
3. 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.
4. Allow Agencourt AMPure XP Beads to come to room temperature and vortex the beads until homogenous.

STEP A: Shear DNA & Assess Quality

Materials

Bioo Scientific Supplied

WHITE CAP - Nuclease-free Water

User Supplied

100 ng - 1 µg Genomic DNA
Qubit dsDNA BR Assay
1X Low TE Buffer
Covaris S2/E210
Covaris microTUBE
Agencourt AMPure XP Magnetic Beads (gDNA Samples <500 ng)

1. For each sample, assess the concentration of the gDNA sample with the Qubit dsDNA BR Assay. Bring sample volume to 130 µL with 1X Low TE Buffer.
2. Shear samples with Covaris microTUBE for target peak of 200 bp according to Covaris recommendations for your particular model.
3. Clean up sheared gDNA samples as follows:
    A. Transfer samples to separate wells of a 96 well PCR plate or snap-top microcentrifuge tube and add 130 µL AMPure XP Magnetic Beads to each sample. Mix well by pipette. Total volume will be ~260 µL.
    B. Incubate samples at room temperature for 5 minutes.
    C. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.
    D. Remove and discard clear supernatant.
    E. 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.
    F. Repeat step E for a total of 2 ethanol washes. Ensure all ethanol has been removed.
    G. Remove the plate from the magnetic stand and let dry at room temperature for 5 minutes or until bead pellet is visibly dry.
    H. Resuspend dried beads with 34 µL Nuclease-free Water. Mix thoroughly until homogenized.
    I. Incubate sample at room temperature for 5 minutes.
    J. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.
    K. Do not discard the sample in this step. Transfer 33 µL of clear sample to a new
4. Assess shear quality with Bioanalyzer DNA 1000 chip or HS DNA chip. Check that electropherogram shows an average DNA fragment size of 150 to 200 bp, and calculate sample quantity. Accurate sample quantification is necessary for appropriate adapter titration and for estimating the required number of PCR cycles.

Covaris Shear Validation

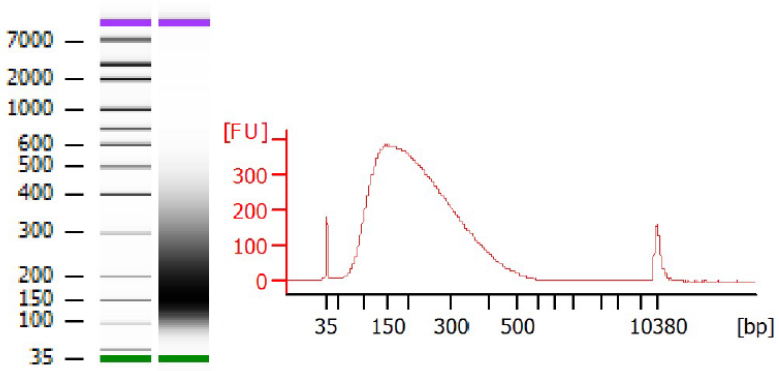


Figure 2. High Sensitivity DNA Chip Ladder / Electropherogram, Jurkat genomic DNA, 1 µg input Covaris shear.

STEP B: End Repair & Adenylation

Materials

Bioo Scientific Supplied

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

User Supplied

32 µL Sheared DNA in 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 in a 96 well PCR plate and mix thoroughly by pipette:

32 µL	Fragmented DNA (100 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 C: Adapter Ligation.

STEP C: Adapter Ligation

Materials

Bioo Scientific Supplied

PURPLE CAP - NEXTflex™ Ligase Enzyme Mix
ASSORTED COLOR CAP - NEXTflex™ DNA Barcoded Adapters
WHITE CAP - Nuclease-free Water

User Supplied

Thermocycler
50 µL of End Repaired & Adenylated DNA (from STEP B)

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 (ng)	Desired Adapter Concentration	Adapter Dilution Required
100 ng	3 µM	1 : 8.3
250 ng	25 µM	None
500 ng	25 µM	None
1 µg	25 µM	None

Each sample will require 2.5 µL of adapter to be added. Perform adapter dilutions with Nuclease-free Water if necessary, depending on sample quantity post-shear.

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 DNA (from Step B)
47.5 µL	NEXTflex™ Ligase Enzyme Mix
2.5 µL	NEXTflex™ DNA Barcoded Adapter
100 µL	TOTAL

3. Apply adhesive PCR plate seal and incubate on a thermocycler for 15 minutes at 22°C.
4. Proceed to Step D: Bead Cleanup.

STEP D: Bead Cleanup

Materials

Bioo Scientific Supplied

WHITE CAP - NEXTflex™ Resuspension Buffer

User Supplied

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

1. Add 60 µL of AMPure XP Beads to each sample. 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.
4. Remove and discard clear supernatant.
5. 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.
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 5 minutes or until bead pellet is visibly dry.
8. Resuspend dried beads with 52 µL Resuspension Buffer. Mix thoroughly until homogenized.
9. Incubate sample at room temperature for 5 minutes.
10. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.
11. Do not discard the sample in this step. Transfer 50 µL of clear sample to a new well.
12. Add 40 µL of AMPure XP Beads to each sample. 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. Remove and discard clear supernatant.
16. 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.
17. Repeat previous step for a total of 2 ethanol washes. Ensure all ethanol has been removed.
18. Remove the plate from the magnetic stand and let dry at room temperature for 5 minutes or until bead pellet is visibly dry.
19. Resuspend dried beads with 22 µL Resuspension Buffer. Mix thoroughly until homogenized.

20. Incubate resuspended beads at room temperature for 5 minutes.
21. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.
22. Transfer 20 µL of clear sample to a new well.
23. Proceed to Step E: PCR Amplification.

STEP E: PCR Amplification

Materials

Bioo Scientific Supplied

GREEN CAP - NEXTflex™ PCR Master Mix, NEXTflex™ Primer Mix
WHITE CAP - Nuclease-free Water, NEXTflex™ Resuspension Buffer

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 D)

*The following table lists recommended PCR cycles:

Input DNA (ng)	PCR cycles
100	8
250	6
500	5
1000	4

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

20 µL	Adapter Ligated DNA
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	
30 sec	65°C	Repeat 4 -8 cycles (*as suggested in above table)
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 17 µL Nuclease-free Water.
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 16 µL of clear sample to a new well.
14. Examine your library on Agilent Bioanalyzer. Ensure that electropherogram shows no adapter dimers or primer dimers. Assess concentration of samples by Qubit.
15. 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 target enrichment with Agilent SureSelect^{XT}.

Library Validation

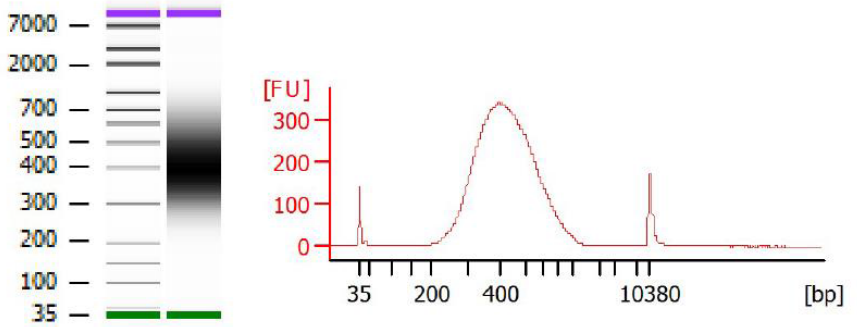


Figure 3. High Sensitivity DNA Chip Ladder / Electropherogram, 100 ng input NEXTflex™8 cycle PCR product.

Target Enrichment Hybridization with Agilent SureSelect^{XT}

STEP F: Hybridize DNA Library Pools to SureSelect^{XT} Capture Library

Materials

Bioo Scientific Supplied

ORANGE CAP - NEXTflex™ HYB 1
RED CAP - NEXTflex™ HYB 2
YELLOW CAP - NEXTflex™ HYB 3
PINK CAP - NEXTflex™ HYB 4
ASSORTED COLOR CAP - NEXTflex™ Barcode Blockers
WHITE CAP - NEXTflex™ Universal Oligo 1, Nuclease-free Water
GREEN CAP - NEXTflex™ Block 1
BLUE CAP - NEXTflex™ Block 2
PURPLE CAP - NEXTflex™ RNase Block

User Supplied

Snap-top centrifuge tubes
Parafilm
SureSelect^{XT} Capture Library
Thermocycler
96 Well PCR Plate or Strip Tubes with Caps
Magnetic Stand
DNA Libraries (from STEP E)

This step requires an incubation at 65°C with a heated lid at 105°C for 24 hours. Before performing the hybridization, it is recommended to test that the plate and capping method are appropriate for the thermocylcer to be used. A test run of 27 µL of Nuclease-free Water can be performed to ensure that no more than 4 µL is lost to evaporation under the conditions used for hybridization.

1. Each hybridization reaction requires 750 ng of prepared DNA in a volume of 3.4 µL, for a concentration of 221 ng/µL.
2. For libraries with DNA concentrations above 221 ng/µL, prepare 3.4 µL of a 221 ng/µL dilution of each library.
3. For prepped libraries with DNA concentrations below 221 ng/µL, use a vacuum concentrator to concentrate the samples at ≤ 45°C. Add the entire 16 µL volume of prepped library to an Eppendorf tube. Pierce the cap or parafilm cover with a narrow gauge needle. Reconstitute with Nuclease-free Water to a final concentration of 221 ng/µL. Pipette up and down along the sides of the tube for optimal recovery. Mix well on a vortex mixer and spin in a centrifuge for 1 minute.
4. Transfer each 3.4 µL gDNA library sample (750 ng) to a separate well of a 96-well plate or strip tube. Seal the wells and keep on ice.

5. Prepare the Hybridization Buffer by mixing the following components at room temperature:

1 RXN	16 RXN	Component
6.63 µL	116 µL	NEXTflex™ HYB 1
0.27 µL	4.7 µL	NEXTflex™ HYB 2
2.65 µL	46.4 µL	NEXTflex™ HYB 3
3.45 µL	60.4 µL	NEXTflex™ HYB 4
13 µL	227.5 µL	TOTAL

*Prepare Hybridization Buffer for at elast 5 reaction equivalents to allow accurate pipetting volumes.
If a precipitate forms, warm the Hybridization Buffer at 65°C for 5 minutes. Keep the prepared Hybridization Buffer at room temperature until it is used in later steps.

6. For each library, prepare the Block Mix by mixing the following components:

1 RXN	16 RXN	Component
2.5 µL	42.5 µL	NEXTflex™ Block 1
2.5 µL	42.5 µL	NEXTflex™ Block 2
1.2 µL	20.4 µL	NEXTflex™ Universal Oligo 1
6.2 µL	85 µL	TOTAL

7. To each DNA library sample well prepared in step 4, add 6.2 µL of the Block mix prepared in the previous step.
8. Add 0.6 µL of the appropriate NEXTflex™ Barcode Blocker to each sample. It is critical to use the NEXTflex™ Barcode Blocker that corresponds to the NEXTflex™ DNA Barcoded Adapter used during the Adapter Ligation step.
9. Mix each sample well by pipetting.
10. Cap the wells, and transfer the sealed plate or strip tube to the thermocycler with the following program:

5 min	95°C
Hold	65°C

Use a heated lid set at 105°C to hold the temperature at 65°C. Make sure that the DNA sample and Block Mix are held at 65°C for at least 5 minutes before adding the remaining hybridization reaction components below.

11. Prepare the appropriate dilution of NEXTflex™ RNase Block, based on the size of your Capture Library. Keep the mixture on ice until it is used in subesequent steps.

For Capture Libraries < 3.0 Mb, make a 10% dilution of the RNase Block by adding 1 part RNase Block to 9 parts Nuclease-free Water. A total of 5 µL is needed for each target capture reaction.

For target captures ≥ 3.0 Mb, make a 25% dilution of the RNase Block by adding 1 part RNase Block to 3 parts Nuclease-free Water. A total of 2 µL is needed for each target capture reaction.

12. Prepare the Capture Library Hybridization Mix appropriate for your Capture Library Size. Mix well by vortexing at high speed for 5 seconds then spin down briefly. Keep the mixture at room temperature until use in the next step.

For Capture Libraries < 3.0 Mb:

1 RXN	16 RXN	Component
13 µL	221 µL	Hybridization Buffer mixture from Step 5
5 µL	85 µL	10% RNAse Block solution from Step 11
2 µL	34 µL	Capture Library
20 µL	340 µL	TOTAL

For Capture Libraries ≥ 3.0 Mb:

1 RXN	16 RXN	Component
13 µL	221 µL	Hybridization Buffer mixture from Step 5
2 µL	34 µL	25% RNAse Block solution from Step 11
5 µL	85 µL	Capture Library
20 µL	340 µL	TOTAL

13. Maintain the gDNA library & Block Mix plate or strip tube at 65°C while you add 20 µL of the Capture Library Hybridization Mix from Step 12 to each sample well. Mix well by pipetting. The hybridization reaction wells now contain approximately 28 µL - 30 µL, depending on the degree of evaporation during the thermocycler incumation.
14. Seal the wells with strip caps. Make sure that all wells are completely sealed.
15. Incubate the hybrdization mixture for 16 - 24 hours at 65°C with a heated lid at 105°C. Proceed to Step G: Prepare Streptavidin Beads.

STEP G: Prepare Streptavidin Beads

Materials

Bioo Scientific Supplied

CLEAR CAP BOTTLE - NEXTflex™ Wash Buffer 2, NEXTflex™ XT Binding Buffer

User Supplied

65°C Circulating Water Bath or Heat Block
96 Well PCR Plate
Dynabeads MyOne Streptavidin T1
Magnetic Stand

1. Prewarm the NEXTflex™ Wash Buffer 2 at 65°C in a circulating water bath or heat block for use in subsequent steps.
2. Vigorously resuspend the Dynabeads MyOne Streptavidin T1 magnetic beads by vortexing.
3. For each target capture reaction, add 50 µL of the resuspended beads to a fresh PCR plate.
4. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes.
5. Remove and discard clear supernatant.
6. Resuspend dried beads with 200 µL NEXTflex™ XT Binding Buffer.
7. Incubate resuspended beads at room temperature for 2 minutes.
8. Place the 96 well PCR Plate on the magnetic stand at room temperature until supernatant has completely cleared.
9. Repeat steps 5 - 8 for a total of three washes.
10. Resuspend the beads by adding 200 µL NEXTflex™ XT Binding Buffer.
11. Proceed to Step H: Capture the Hybridized DNA Using Streptavidin Beads.

STEP H: Capture the Hybridized DNA Using Streptavidin Beads

Materials

Bioo Scientific Supplied

CLEAR CAP BOTTLE - NEXTflex™ Wash Buffer 1, NEXTflex™ Wash Buffer 2*, NEXTflex™ XT Binding Buffer
WHITE CAP - Nuclease-free Water

User Supplied

Dynabeads MyOne Streptavidin T1
Hybridized DNA Library Pools (from STEP F)
Washed Streptavidin Beads (from STEP G)
Nutator mixer or equivalent
Thermocycler
96 Well PCR Plate and strip tube caps or 1.5 mL Snap-top Centrifuge Tubes (must be compatible with water bath/heat block)
Agencourt AMPure XP Magnetic Beads (room temperature)
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand
65°C Water Bath or Heat Block

*This step uses the pre-warmed NEXTflex Wash Buffer 2 at 65°C in a water bath or heat block from STEP G.

The following steps may be performed in a 96 well PCR plate or 1.5 mL snap-top centrifuge tubes.

1. Estimate and record the volume of hybridization solution that remains after the 24 hour incubation.
2. Maintain the hybridization plate at 65°C while transferring the entire volume (approximately 25 - 30 µL) of each hybridization mixture to the plate wells containing 200 µL of washed Streptavidin beads. Mix well by pipetting.
3. Cap the wells and incubate the capture plate on a Nutator mixer or equivalent for 30 minutes at room temperature. Make sure that samples are properly mixing in the wells.
4. Briefly spin the plate in a centrifuge or mini-plate spinner.
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 the supernatant.
7. Resuspend the beads in 200 µL of NEXTflex Wash Buffer 1. Mix by pipetting the volume up and down 10 to 20 times.
8. Incubate for 15 minutes at room temperature. Briefly spin in a centrifuge or mini-plate spinner.
9. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes,

or until the supernatant appears completely clear.

10. Remove and discard the supernatant.

11. It is important to maintain bead suspensions at 65°C during the washing procedure to ensure specificity of the capture. Wash the beads with the pre-warmed 65°C NEXTflex™ Wash Buffer 2 (from STEP G) as follows:
    A. Resuspend the beads in 200 µL of 65°C NEXTflex™ Wash Buffer 2.
    B. Cap the wells and mix on a vortex mixer for 5 seconds to resuspend the beads.
    C. Incubate the sample plate for 10 minutes at 65°C.
    D. Briefly spin the plate in a centrifuge or mini-plate spinner.
    E. Place the plate on the magnetic stand for 5 minutes, or until the supernatant appears completely clear.
    F. Remove and discard the supernatant.
    G. Repeat steps A through F for a total of 3 washes. Briefly spin in a centrifuge or mini-plate spinner.

12. Add 36 µL Nuclease-free Water to each well, and mix well to resuspend the beads.

13. Keep the samples on ice until they are used in STEP I: PCR Amplification.

STEP I: Post-Capture PCR Amplification

Materials

Bioo Scientific Supplied

GREEN CAP - NEXTflex™ PCR Master Mix, NEXTflex™ Primer Mix
WHITE CAP - NEXTflex™ Resuspension Buffer

User Supplied

Thermocycler
96 Well PCR Plate
Agencourt AMPure XP Magnetic Beads (room temperature)
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand
36 µL of Hybridized Library (from STEP H)

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

36 µL	Hybridized Library
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	
30 sec	65°C	Repeat for a total of 12 cycles
60 sec	72°C	
4 min	72°C	

3. Add 50 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 17 µL 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 16 µL of clear sample to a new well.
14. Examine your library by Agilent Bioanalyzer. Ensure that electropherogram shows no adapter dimers or primer dimers. Assess concentration of samples by Qubit, and proceed to Illumina sequencing preparation.

Oligonucleotide Sequences

NEXTflex™	Sequence
Primer 1	5'AATGATACGGCGACCACCGAGATCTACAC
Primer 2	5'CAAGCAGAAGACGGCATACGAGAT
Barcoded Adapter	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**XXXXXXXX**[1]ATCTCGTATGCCGTCTTCTGCTTG

[1]XXXXXXXX denotes the index region of the adapter. The index sequence of each adapter is listed below.

Index 1	AACGTGAT	Index 63	TCTTCACA
Index 3	ATGCCTAA	Index 72	AATCCGTC
Index 9	CGCTGATC	Index 73	AATGTTGC
Index 16	AAGACGGA	Index 74	ACACGACC
Index 22	ACGTATCA	Index 75	ACAGATTC
Index 28	ATCCTGTA	Index 76	AGATGTAC
Index 51	GCGAGTAA	Index 77	AGCACCTC
Index 57	GTCGTAGA	Index 92	GAACAGGC

NEXTflex™	Sequence
HE Universal Oligo 1	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Barcode Blocker	5'CAAGCAGAAGACGGCATACGAGAT**XXXXXXXX**[2]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT/3INVdT/

[2]XXXXXXXX denotes the index region of the barcode blocker. The sequence of each barcode blocker is listed below.

Index 1	ATCACGTT	Index 63	TGTGAAGA
Index 3	TTAGGCAT	Index 72	GACGGATT
Index 9	GATCAGCG	Index 73	GCAACATT
Index 16	TCCGTCTT	Index 74	GGTCGTGT
Index 22	TGATACGT	Index 75	GAATCTGT
Index 28	TACAGGAT	Index 76	GTACATCT
Index 51	TTACTCGC	Index 77	GAGGTGCT
Index 57	TCTACGAC	Index 92	GCCTGTTC

Illumina Compatible RNA NGS Kits and Adapters

Catalog #	Product
5138-01	NEXTflex™ Rapid RNA-Seq Kit (8 reactions)
5138-02	NEXTflex™ Rapid RNA-Seq Kit (48 reactions)
5138-07	NEXTflex™ Rapid Directional RNA-Seq Kit (8 reactions)
5138-08	NEXTflex™ Rapid Directional RNA-Seq Kit (48 reactions)
512911	NEXTflex™ RNA-Seq Barcodes –6
512912	NEXTflex™ RNA-Seq Barcodes – 12
512913	NEXTflex™ RNA-Seq Barcodes – 24
512914	NEXTflex™ RNA-Seq Barcodes – 48
512916	NEXTflex-96™ RNA-Seq Barcodes
5130-01	NEXTflex™ qRNA-Seq™ Kit 4 barcodes (8 reactions)
5130-02	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set A (48 reactions)
5130-03	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set B (48 reactions)
5130-04	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set C (48 reactions)
5130-05	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set D (48 reactions)
5130-01D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 4 barcodes (8 reactions)
5130-02D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set A (48 reactions)
5130-03D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set B (48 reactions)
5130-04D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set C (48 reactions)
5130-05D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set D (48 reactions)
5132-01	NEXTflex™ Small RNA Sequencing Kit (24 reactions)
5132-02	NEXTflex™ Small RNA Sequencing Kit (48 reactions)
5132-03	NEXTflex™ Small RNA Sequencing Kit v2 (24 reactions)
5132-04	NEXTflex™ Small RNA Sequencing Kit v2 (48 reactions)
513305	NEXTlfex™ Small RNA Barcode Primers -12 (Set A)
513306	NEXTlfex™ Small RNA Barcode Primers -12 (Set B)
513307	NEXTlfex™ Small RNA Barcode Primers -12 (Set C)
513308	NEXTlfex™ Small RNA Barcode Primers -12 (Set D)
512979	NEXTflex™ Poly(A) Beads (8 reactions)
512980	NEXTflex™ Poly(A) Beads (48 reactions)
512981	NEXTflex™ Poly(A) Beads (100 reactions)

Illumina Compatible DNA NGS Kits and Adapters

Catalog #	Product
4201-01	NEXTflex™ 16S V4 Amplicon-Seq Kit – 4
4201-02	NEXTflex™ 16S V4 Amplicon-Seq kit – 12
4201-03	NEXTflex™ 16S V4 Amplicon-Seq kit – 24
4201-04	NEXTflex™ 16S V4 Amplicon-Seq kit – 48
4201-05	NEXTflex™ 16S V4 Amplicon-Seq kit – 96
4201-06	NEXTflex™ 16S V4 Amplicon-Seq kit – 192
4201-07	NEXTflex™ 16S V4 Amplicon-Seq kit – 288
4202-01	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 4
4202-02	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 12
4202-03	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 48
4202-04	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 1-96
4202-05	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 97-192
4202-06	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 193-288
4202-07	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 289-384
5140-01	NEXTflex™ DNA Sequencing Kit (8 reactions)
5140-02	NEXTflex™ DNA Sequencing Kit (48 reactions)
5144-01	NEXTflex™ Rapid DNA-Seq Kit (8 reactions)
5144-02	NEXTflex™ Rapid DNA-Seq Kit (48 reactions)
5150-01	NEXTflex™ Cell Free DNA-Seq Kit (8 reactions)
5150-02	NEXTflex™ Cell Free DNA-Seq Kit (48 reactions)
514101	NEXTflex™ DNA Barcodes – 6
514102	NEXTflex™ DNA Barcodes – 12
514103	NEXTflex™ DNA Barcodes – 24
514104	NEXTflex™ DNA Barcodes – 48
514105	NEXTflex-96™ DNA Barcodes (Plate Format)
514106	NEXTflex-96™ DNA Barcodes (Tube Format)
514160	NEXTflex™ Dual-Indexed DNA Barcodes (1-96)
514161	NEXTflex™ Dual-Indexed DNA Barcodes (97-192)
5119-01	NEXTflex™ Bisulfite-Seq kit (8 reactions)
5119-02	NEXTflex™ Bisulfite-Seq kit (48 reactions)
511911	NEXTflex™ Bisulfite-Seq Barcodes – 6
511912	NEXTflex™ Bisulfite-Seq Barcodes – 12
511913	NEXTflex™ Bisulfite-Seq Barcodes - 24
5118-01	NEXTflex™ Methyl-Seq 1 Kit (8 reactions)
5118-02	NEXTflex™ Methyl-Seq 1 Kit (48 reactions)

511921	NEXTflex™ Msp 1 (8 reactions)
511922	NEXTflex™ Msp 1 (48 reactions)

5143-01	NEXTflex™ ChIP-Seq Kit (8 reactions)
5143-02	NEXTflex™ ChIP-Seq Kit (48 reactions)
514120	NEXTflex™ ChIP-Seq Barcodes – 6
514121	NEXTflex™ ChIP-Seq Barcodes – 12
514122	NEXTflex™ ChIP-Seq Barcodes – 24
514123	NEXTflex™ ChIP-Seq Barcodes – 48
514124	NEXTflex-96™ ChIP-Seq Barcodes

5140-51	NEXTflex™ Pre-Capture Combo Kit (6 barcodes)
5140-52	NEXTflex™ Pre-Capture Combo Kit (12 barcodes)
5140-53	NEXTflex™ Pre-Capture Combo Kit (24 barcodes)
5140-56	NEXTflex™ Pre-Capture Combo Kit (48 barcodes)
5140-54	NEXTflex™ Pre-Capture Combo Kit (96 barcodes)
514131	NEXTflex™ DNA Barcode Blockers - 6 for SeqCap
514132	NEXTflex™ DNA Barcode Blockers - 12 for SeqCap
514133	NEXTflex™ DNA Barcode Blockers - 24 for SeqCap
514136	NEXTflex™ DNA Barcode Blockers - 48 for SeqCap
514134	NEXTflex™ DNA Barcode Blockers - 96 for SeqCap

5142-01	NEXTflex™ PCR-Free DNA Sequencing Kit (8 reactions)
5142-02	NEXTflex™ PCR-Free DNA Sequencing Kit (48 reactions)
514110	NEXTflex™ PCR-Free Barcodes – 6
514111	NEXTflex™ PCR-Free Barcodes – 12
514112	NEXTflex™ PCR-Free Barcodes – 24
514113	NEXTflex™ PCR-Free Barcodes – 48

DNA Fragmentation

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

WE WANT TO HEAR FROM YOU!

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