New Optimized Adapter Concentration

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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 Barcodes - 48
(Illumina Compatible)

Catalog #: 514104 (384 reactions)

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GENERAL INFORMATION

Product Overview

The NEXTflex™ DNA Barcodes are designed to prepare multiplexed single and paired-end genomic DNA libraries for sequencing using Illumina®/Solexa® GAII and HiSeq 2000/1000 platforms. The index and flow cell binding sequence are designed within the NEXTflex™ DNA Barcodes and are attached onto the sample insert during adapter ligation. Pooling with NEXTflex DNA Barcodes allows the user to multiplex several samples in a single flow cell.

Contents, Storage and Shelf Life

The NEXTflex™ DNA Barcodes contain 48 barcoded DNA Adapters with enough material for 8 reactions each, for a total of 384 reactions. The shelf life of each reagent is 12 months when stored at -20°C.

Kit Contents	Amount
NEXTflex™ DNA Barcode Adapter 1 - 48*	20 µL
NEXTflex™ Primer Mix	775 µL

*The Barcode Adapters are supplied in duplex form. Do not heat the adapter above room temperature.

Required Materials not Provided

- 1 µg of fragmented genomic DNA in up to 10 µL nuclease-free water.
- NEXTflex™ DNA Sequencing Kit– 8 / 48 (Cat # 5140-01, 5140-02)
- 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 # P-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

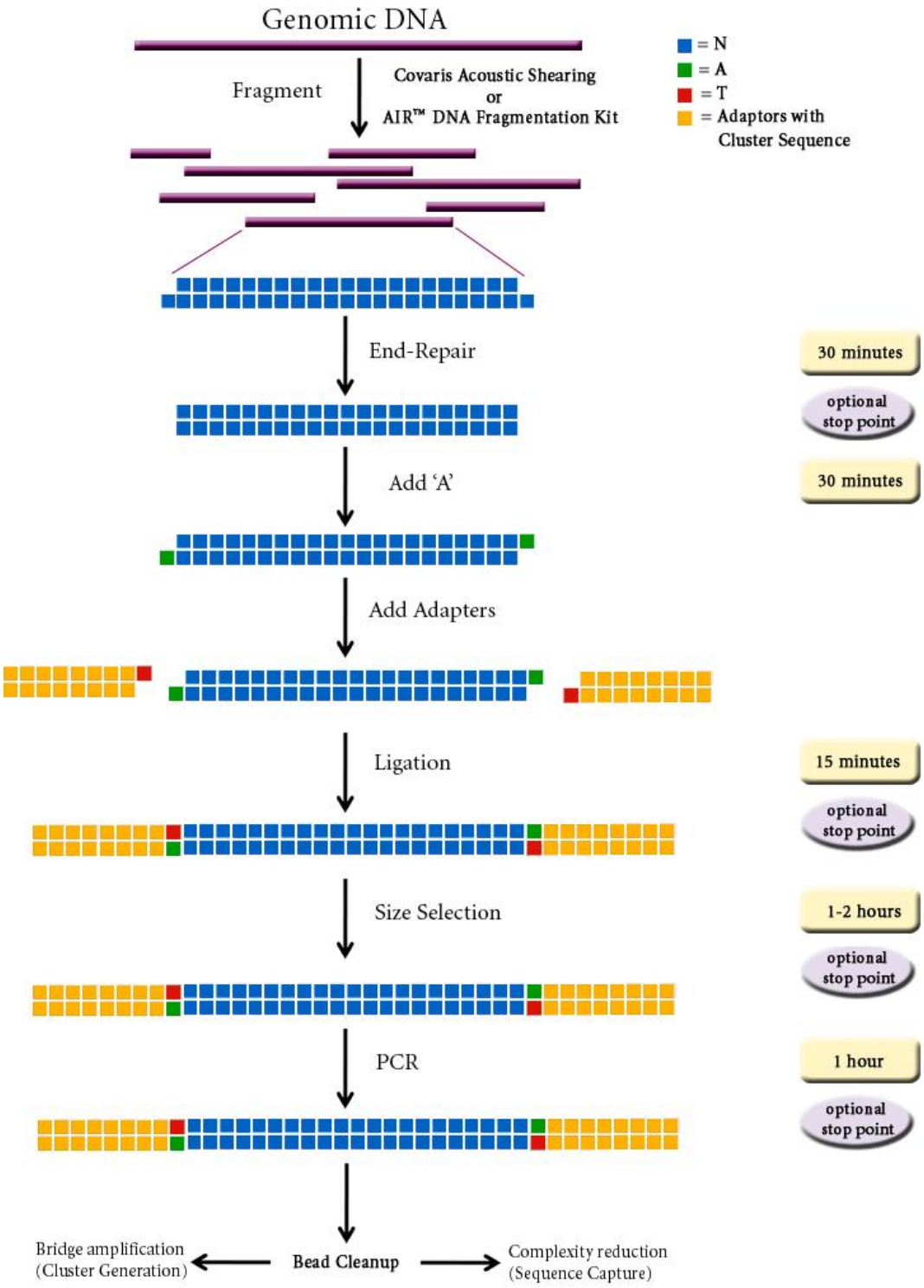
Bioo Scientific strongly recommends that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local 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 Barcode Adapter volume to preserve the insert to adapter ratio.

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 Barcodes and NEXTflex™ DNA Sequencing Kit have 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 2, Warnings and Precautions).

Reagent Preparation

NEXTflex™ DNA Barcodes and DNA Sequencing Kit

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 ethanol to the bottle of DNA Wash Buffer. Check box on bottle to show ethanol has been added.

STEP A: End Repair

Materials

The following components are supplied in Bioo Scientific's NEXTflex™ DNA Sequencing Kit.

CLEAR CAP
NEXTflex™ End Repair Mix

WHITE CAP
Nuclease-free H_2O

User Supplied
Fragmented DNA in 10 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)
10 µL	NEXTflex™ End Repair Mix
20 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 22°C.

STEP B: Clean-Up

Materials

The following components are supplied in Bioo Scientific's NEXTflex™ DNA Sequencing Kit.

WHITE CAP
Resuspension Buffer

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

1. Add 36 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 15 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 54 µL and gently remove 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 sample 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.

STEP C: 3' Adenylation

Materials

The following components are supplied in Bioo Scientific's NEXTflex™ DNA Sequencing Kit.

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 96 well 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

The following components are supplied in Bioo Scientific's NEXTflex™ DNA Barcodes

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

The following components are supplied in Bioo Scientific's NEXTflex™ DNA Sequencing Kit.

PURPLE CAP

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

User Supplied

20.5 µL 3' Adenylated DNA (from STEP C)

1. For each sample, combine the following reagents (in this order) in the 96 well PCR Plate:

20.5 µL	3' Adenylated DNA (from step C)
31.5 µL	NEXTflex™ Ligation Mix
2.5 µL	NEXTflex™ DNA Barcode Adapter 1-48
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

The following components are supplied in Bioo Scientific's NEXTflex™ DNA Sequencing Kit.

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 15 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 100 µL and gently remove 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 sample 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 53 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 50 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.

STEP F: Agarose Gel Size Selection

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 6 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 reference 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.
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 50 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

The following components are supplied in Bioo Scientific's NEXTflex™ DNA Barcodes

NEXTflex™ Primer Mix

The following components are supplied in Bioo Scientific's NEXTflex™ DNA Sequencing Kit.

GREEN CAP

NEXTflex™ PCR Master Mix

User Supplied

Thermocycler
96 Well PCR Plate
Resuspension Buffer
***5 µL Gel Purified Ligation Product (from STEP F)**

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

5 µL	Gel Purified Ligation Product (from Step F)
43 µL	NEXTflex™ PCR Master Mix
2µL	NEXTflex™ Primer Mix
50 µL	TOTAL

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

3. 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.

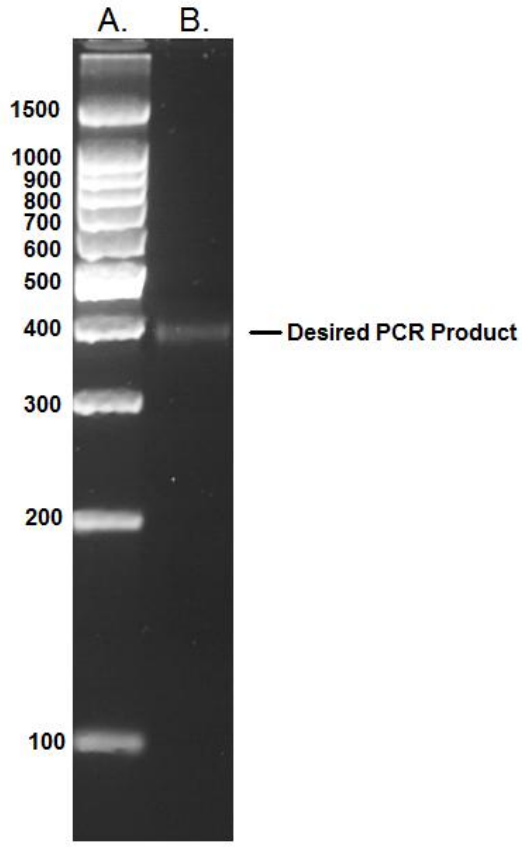
12. For multiplexed libraries transfer 10 µl of each normalized library to be pooled in the well of a new 96 Well PCR Plate. Gently pipette the entire volume up and down 10 times.

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

Figure 1. 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 400 bp PCR product (~300 bp insert).



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 1 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
Primer 1	5'AATGATACGGCGACCACCGAGATCTACAC
Primer 2	5'CAAGCAGAAGACGGCATACGAGAT
DNA Adapter 1	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 2	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACTGACCAATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 3	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACACAGTGATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 4	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGCCAATATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 5	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCAGATCATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 6	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCTTGTAATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 7	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 8	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACTTAGGCATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 9	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACACTTGAATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 10	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGATCAGATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 11	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACTAGCTTATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 12	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGGCTACATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 13	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACAGTCAAATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 14	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACAGTTCCATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 15	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACATGTCAATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 16	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCCGTCCATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 17	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGTAGAGATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 18	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGTCCGCATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 19	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGTGAAAATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 20	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGTGGCCATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 21	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGTTTCGATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 22	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGTACGATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 23	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGAGTGGATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 24	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGGTAGCATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 25	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACACTGATATCTCGTATGCCGTCTTCTGCTTG

NEXTflex™	Sequence
DNA Adapter 26	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACATGAGCATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 27	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACATTCCTATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 28	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCAAAAGATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 29	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCAACTAATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 30	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCACCGGATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 31	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCACGATATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 32	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCACTCAATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 33	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCAGGCGATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 34	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCATGGCATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 35	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCATTTTATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 36	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCCAACAATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 37	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGGAATATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 38	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCTAGCTATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 39	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCTATACATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 40	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCTCAGAATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 41	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGACGACATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 42	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACTAATCGATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 43	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACTACAGCATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 44	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACTATAATATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 45	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACTCATTCATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 46	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACTCCCGAATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 47	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACTCGAAGATCTCGTATGCCGTCTTCTGCTTG
DNA Adapter 48	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACTCGGCAATCTCGTATGCCGTCTTCTGCTTG

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 Sequencing Kit (48 reactions)	5140-02
NEXTflex™ PCR-Free DNA Sequencing Kit (8 reactions)	5142-01
NEXTflex™ PCR-Free DNA Sequencing Kit (48 reactions)	5142-02
NEXTflex™ DNA Barcodes – 6	514101
NEXTflex™ DNA Barcodes – 12	514102
NEXTflex™ DNA Barcodes – 24	514103

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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