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NEXTflex™ Small RNA Sequencing for Small RNA Starting Material

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Abstract

This protocol is for the NEXTflex™ Small RNA Sequencing Kit v2, designed to prepare small RNA libraries for sequencing using Illumina® sequencers. This kit utilizes adapters with randomized ends to greatly reduce sequence bias in small RNA sequencing library construction.

Please see the **full manual** for additional details.

(The protocol below describes preparation of small RNA sequencing libraries from **Small RNA** starting material; for total RNA samples, please see **this** protocol.)

Guidelines

Contents, Storage and Shelf Life

The NEXTflexÃÂ¢ÃÂÃÂ¢ Small RNA Sequencing Kit v2 contains enough material to prepare 24 RNA samples for IlluminaÃÂÃÂ® compatible next-generation sequencing. The shelf life of all reagents is 6 months when stored properly. All components can safely be stored at -20ÃÂÃÂ°C, except: Adapter Depletion Solution, Resuspension Buffer, Nuclease-free Water, and Elution Buffer, which can be stored at room temperature.

Kit Contents	Amount
RED CAP	
3' NEXTflex™ 4N Adenylated Adapter	24 µL
AIR™ Ligase	24 µL
AIR™ Ligase Buffer	60 µL
50% PEG	144 µL
Adapter Depletion Solution	750 µL
RNase Inhibitor	26.5 µL
PURPLE CAP	
5' NEXTflex™ 4N Adapter	36 µL
T4 RNA Ligase 1	36 µL
ATP	36 µL

BLUE CAP	
NEXTflex™ RT Primer	24 µL
M-MuLV Reverse Transcriptase	48 µL
10X M-MuLV Buffer	96 µL
dNTPs	96 µL

GREEN CAP	
NEXTflex™ Universal Primer	24 µL
NEXTflex™ Barcode Primer 1	24 µL
5X DuroTaq Master Mix	120 µL

ORANGE CAP	
6X Loading Dye	150 µL
Ready to Load Low MW Ladder	300 µL

YELLOW CAP	
Resuspension Buffer	1000 µL

WHITE CAP	
Nuclease-free Water	1.5 mL

CLEAR CAP	
microRNA Control	10 µL

CLEAR CAP BOTTLE	
Elution Buffer (10X)*	2 mL

*see Reagent Preparation for dilution procedure

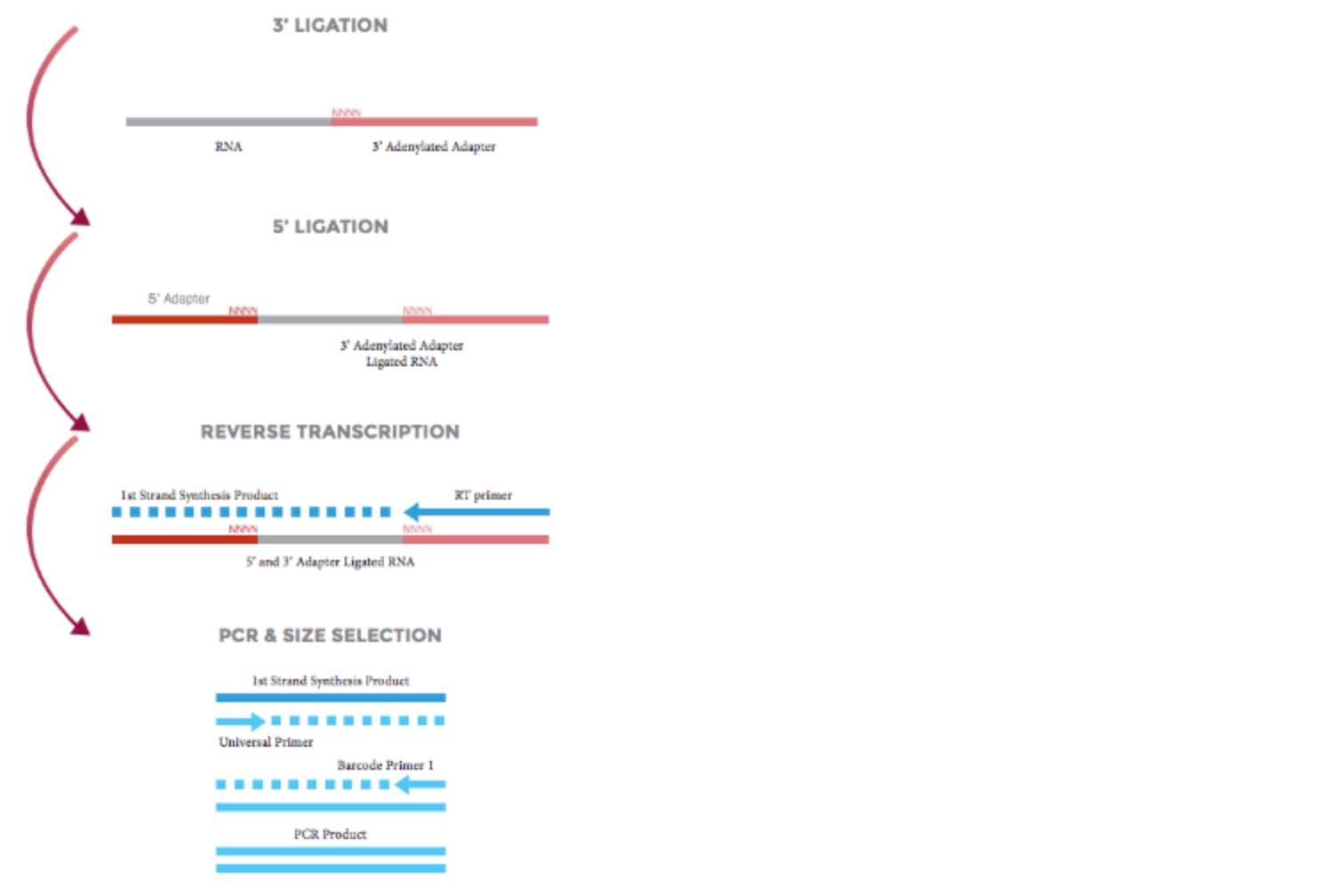
Required Materials Not Provided

ÃÂ¢ÃÂÃÂ¢ 1-10 ÃÂÃÂµg total RNA or purified small RNA from 1-10 ÃÂÃÂµg total RNA in up to 4 ÃÂÃÂµL Nucleasefree Water
ÃÂ¢ÃÂÃÂ¢ Isopropanol
ÃÂ¢ÃÂÃÂ¢ 80% Ethanol
ÃÂ¢ÃÂÃÂ¢ 2, 10, 20, 200 and 1000 ÃÂÃÂµL pipettes
ÃÂ¢ÃÂÃÂ¢ RNase-free pipette tips
ÃÂ¢ÃÂÃÂ¢ Microcentrifuge
ÃÂ¢ÃÂÃÂ¢Nuclease-free 1.7 mL microcentrifuge tubes
ÃÂ¢ÃÂÃÂ¢ 96 well PCR Plate Non-skirted (Phenix Research, Cat # MPS-499) or similar
ÃÂ¢ÃÂÃÂ¢ Sterile disposable pestles (Fisher Cat # K749521-1500 or similar)
ÃÂ¢ÃÂÃÂ¢ Thin wall nuclease-free PCR tubes
ÃÂ¢ÃÂÃÂ¢ Thermocycler
ÃÂ¢ÃÂÃÂ¢ Heat block
ÃÂ¢ÃÂÃÂ¢ 6% TBE PAGE gels (1.0 mm) (Life Technologies Cat # EC6265BOX)

ÃÂ¢ÃÂÃÂ¢ 1X TBE buffer
ÃÂ¢ÃÂÃÂ¢ Clean razor or scalpel
ÃÂ¢ÃÂÃÂ¢ Nucleic acid stain such as SYBR Gold (Invitrogen)
ÃÂ¢ÃÂÃÂ¢ UV transilluminator or gel documentation instrument
ÃÂ¢ÃÂÃÂ¢ Gel Electrophoresis apparatus
ÃÂ¢ÃÂÃÂ¢ Electrophoresis power supply
ÃÂ¢ÃÂÃÂ¢ Vortex
ÃÂ¢ÃÂÃÂ¢ 0.45ÃÂÃÂµm, 2 mL Spin-X Centrifuge tube (Sigma Cat # CLS8162)
ÃÂ¢ÃÂÃÂ¢ Agencourt AMPure XP 60 mL (Beckman Coulter Genomics, Cat # A63881)
ÃÂ¢ÃÂÃÂ¢ Magnetic Stand -96 (Ambion, Cat # AM10027) or similar
ÃÂ¢ÃÂÃÂ¢ Magnetic stand for micrcentrifuge tubes (Life Technologies DynaMagÃÂ¢ÃÂÃÂ¢-2 or similar)

Optional Materials Not Provided
ÃÂ¢ÃÂÃÂ¢ÃÂÃÂ If multiplexing: NEXTflexÃÂ¢ÃÂÃÂ¢ Small RNA Barcodes A ÃÂ¢ÃÂÃÂ D (513305, 513306, 513307, 513308)

NEXTflexÃÂ¢ÃÂÃÂ¢ Small RNA Sample Preparation Flow Chart



Starting Material

The NEXTflexÃÂ¢ÃÂÃÂ¢ Small RNA Sequencing Kit v2 has been optimized and validated using total RNA (1 - 10 ÃÂÃÂµg), purified small RNA (from 1 - 10 ÃÂÃÂµg total RNA), and a synthetic miRNA pool (ÃÂ¢ÃÂÃÂ¥100 pg). Best results are obtained with high quality starting material. The use of degraded RNA may result in poor yields or lack of sequencing output data. Bioo Scientific recommends running total RNA on a 1 - 2% agarose gel or examining its integrity using an Agilent Bioanalyzer. High quality total RNA preparations should have an 28S band that is twice as intense as the 18S band of ribosomal RNA. At low concentrations, small RNA is difficult to detect on a gel; however it can be detected using an Agilent Bioanalyzer Small RNA assay (see Figure 2 in Appendix A). We recommend beginning with total RNA or a preparation of highly enriched small RNA by PAGE selecting small RNAs around 15-45 nt long or using a phenol-based small RNA isolation method such as BiooPureÃÂ¢ÃÂÃÂ¢ (Cat # 5301) for enrichment.

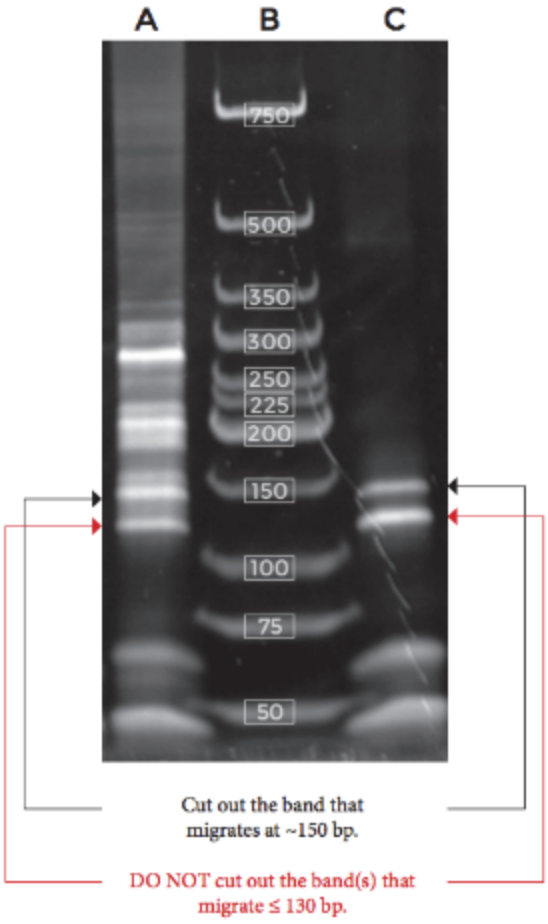
If the user is performing the procedure for the first time, we recommend using the microRNA Control included in the kit: 5ÃÂ¢ÃÂÃÂPhos/CUCAGGAUGGCGGAGCGGUCU/3ÃÂ¢ÃÂÃÂ. This positive control sample consists of 21 RNA nucleotides and does not match any known sequence in miRBase. When running a positive control reaction, the user should add 1 ÃÂÃÂµL of the microRNA Control in STEP A instead of their small RNA sample and expect to observe a 147 nt PCR product. The microRNA control may degrade with multiple freeze thaw cycles or exposure to nucleases. If you plan on using the control multiple times, we recommend aliquoting into several tubes and storing at -20ÃÂÃÂ°C. For a total RNA positive control, human brain total RNA (Ambion catalog number AM7962 or similar) is recommended.

Reagent Preparation

1. Vortex and micro centrifuge each component prior to use, to ensure material has not lodged in the cap or the side of the tube.
2. Add 18 mL of molecular biology-grade water to each bottle of Elution Buffer (10X) to make a 1X Elution Buffer. Check box on bottle to show water has been added.

Figure 2

6% TBE-PAGE gel
A. PCR product from library constructed from 1 ÃÂÃÂµg human brain total RNA
B. Ready to Load Low MW Ladder
C. PCR product from library constructed from 1 ÃÂÃÂµL miRNA control
NOTE: 25 bp band not shown. 225 bp & 250 bp bands may run as a single band.



References

Jayaprakash et al. **Identification and remediation of biases in the activity of RNA ligases in small-RNA deep sequencing**. Nucl. Acids Res. (2011) 39 (21):e141

Materials

MATERIALS

- 96 well PCR Plate Non-skirted **Phenix Research** **Catalog #**MPS-499
- Agencourt AMPure XP **Beckman Coulter** **Catalog #**A63880
- Magnetic Stand -96 **Life Technologies** **Catalog #**AM10027
- Sterile disposable pestles **Fisher Scientific** **Catalog #**K749521-1500
- 6% TBE PAGE gels (1.0 mm) **Life Technologies** **Catalog #** EC6265BOX
- 0.45µm, 2 mL Spin-X Centrifuge tube **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**CLS8162
- Magnetic stand for micrcentrifuge tubes **Life Technologies** **Catalog #**12321D
- NEXTflex Small RNA-Seq Kit v2 **Bioo Scientific** **Catalog #**5132-03

STEP MATERIALS

- Agencourt AMPure XP **Beckman Coulter** **Catalog #**A63880
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Protocol materials

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- Magnetic stand for micrcentrifuge tubes **Life Technologies** **Catalog #**12321D

Safety warnings

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.

• This kit contains a single Barcoded Primer. To enable multiplexing, please use the appopriate combination of NEXTflex™ Small RNA Barcodes during the PCR Amplification step.

• Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).

• RNA sample quality may vary between preparations. It is the user's responsibility to optimize the initial RNA input amount to obtain desired PCR bands for gel excision and sequencing. Refer to the Starting Material section for additional information.

• Vortex and micro centrifuge each component prior to use, to ensure material has not lodged in the cap or the side of the tube.

• Do not remove AIR Ligase or T4 RNA Ligase 1 enzymes from -20°C until immediately before use and return to -20°C immediately after use.

• Some total RNA extraction and purification methods may not efficiently isolate small RNAs. Users should verify that their extraction and purification method also isolates small RNAs.

Reagent Preparation

1 Vortex and micro centrifuge each component prior to use, to ensure material has not lodged in the cap or the side of the tube.

2 Add 18 mL of molecular biology-grade water to each bottle of Elution Buffer (10X) to make a 1X Elution Buffer. Check box on bottle to show water has been added.

NEXTflex™ 4N Adenylated Adapter Ligation

3 Allow 50% PEG to come to room temperature before use.

Note

Materials for "NEXTflex™ 4N Adenylated Adapter Ligation" section.

Bioo Scientific Supplied
RED CAP - 3' NEXTflex™ 4N Adenylated Adapter, AIR™ Ligase, 50% PEG, NEXTflex™ RNase Inhibitor
WHITE CAP - Nuclease-free Water

User Supplied
RNA (1-10 µg total RNA or small RNA isolated from total RNA) in 4 µL Nuclease-free Water
96 well PCR plate
Thermocycler
Ice

4 For each reaction combine the following in a 96 well PCR plate:

	4 µL	RNA (in Nuclease-free Water)
	1 µL	3′ NEXTflex™ 4N Adenylated Adapter

5 Heat at 70°C for 2 minutes then immediately place on ice.
00:02:00

6 Add the following components to each well and mix well:

	1 µL	AIR™ Ligase
	1 µL	AIR™ Ligase Buffer
	2.5 µL	50% PEG (Note: 50% PEG is very viscous, please pipette carefully)

	0.5 µL	RNase Inhibitor

7 Incubate at 22°C for 2 hours in a thermocycler. For ligations to 2′ O-methylated small RNAs, such as those found in plants, incubate at 16°C overnight.

02:00:00

Excess 3′ Adapter Removal

8 Add 10 µL of Nuclease-free Water to each sample and mix by pipetting.

Note

Materials for 'Excess 3′ Adapter Removal' section

Bioo Scientific Supplied
RED CAP - Adapter Depletion Solution
YELLOW CAP - Reusupension Buffer
WHITE CAP - Nuclease-free Water

User Supplied
Agencourt AMPure XP Magnetic Beads (room temperature)
Isopropanol
80% Ethanol, freshly prepared
Magnetic Stand
***10 µL of 3′ NEXTflex™ 4N Adenylated Adapter Ligated RNA (from section 'NEXTflex™ 4N Adenylated Adapter Ligation')**

9 Add 6 µL of Adapter Depletion Solution and mix well by pipetting.

6 µL

Agencourt AMPure XP **Beckman Coulter** **Catalog #**A63880

10 Add 40 µL of AMPure XP beads and 60 µL of ispropanol and mix well by pipetting.

11 Incubate for 5 minutes.

00:05:00

12 Magnetize beads until solution is clear.

13 Remove and discard supernatant.

14 Wash #1: Add 180 µL of freshly prepared 80% ethanol, incubate for 30 seconds, and remove with a P200 or P300 set to 200 µL.

00:00:30

Note

IMPORTANT: Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.

15 Wash #2: Add 180 µL of freshly prepared 80% ethanol, incubate for 30 seconds, and remove with a P200 or P300 set to 200 µL.

00:00:30

Note

IMPORTANT: Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.

16 Incubate sample for 3 minutes. After one minute, remove all residual liquid that may have collected at the bottom of the well.

00:03:00

17 Remove plate from magnetic stand and resuspend bead pellet in 22 µL of Resuspenion Buffer by pipetting volume up and down. Ensure that beads are completely resuspended.

18 Incubate 2 minutes. During incubation, add 6 µL of Adapter Depletion Solution to a new, empty well.

00:02:00

19 Magnetize sample until solution appears clear.

20 Transfer 20 µL of supernatant to the well containing 6 µL Adapter Depletion Solution and mix well by pipette.

21 Add 40 µL of AMPure XP beads.

40 µL

Agencourt AMPure XP **Beckman Coulter Catalog #**A63880

22 Add 60 µL of isopropanol and mix well by pipetting.

23 Incubate for 5 minutes.
00:05:00

24 Magnetize sample until solution appears clear.

25 Remove and discard supernatant.

26 Wash #1: Add 180 µL of freshly prepared 80% ethanol, incubate for 30 seconds, and remove with a P200 or P300 set to 200 µL.
00:00:30

Note

IMPORTANT: Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.

27 Wash #2: Add 180 µL of freshly prepared 80% ethanol, incubate for 30 seconds, and remove with a P200 or P300 set to 200 µL.
00:00:30

Note

IMPORTANT: Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.

28 Incubate sample for 3 minutes. After one minute, remove all residual liquid that may have collected at the bottom of the well.
00:03:00

29 Remove plate from magnetic stand and resuspend bead pellet in 12 µL of Nuclease-free Water by pipetting volume up and down. Ensure that beads are completely resuspended.

30 Incubate for 2 minutes.
00:02:00

31 Magnetize sample until solution appears clear.

32 Transfer 11 µL of supernatant to a new well.

5′ NEXTflex™ 4N Adapter Ligation

33 For each reaction, add 1.5 µL of the 5′ NEXTflex™ 4N Adapter and heat at 70°C for 2 minutes, then immediately place on ice.

00:02:00

Note

Materials for section "5′ NEXTflex™ 4N Adapter Ligation"

Bioo Scientific Supplied
PURPLE CAP - 5′ NEXTflex™ 4N Adapter, ATP, T4 RNA Ligase 1, RNase Inhibitor
RED CAP - AIR™ Ligase Buffer, 50% PEG (warm to room temperature before use)

User Supplied
Heatblock or thermocycler
96-well PCR plate or PCR tubes
Ice
***11 µL of 3′ NEXTflex™ 4N Adenylated Adapter Ligated RNA (from section "Excess 3′ Adapter Removal")**

34 For each reaction, combine the following components in a well of a 96-well PCR plate and mix thoroughly:

	12.5 µL	3′ NEXTflex™ 4N Adapter Ligated RNA & 5′ NEXTflex™ 4N Adapter
	1.5 µL	AIR™ Ligase Buffer
	1.5 µL	ATP
	1.5 µL	T4 RNA Ligase 1
	3.5 µL	50% PEG (Note: 50% PEG is very viscous, please pipette carefully)
	0.5 µL	RNase Inhibitor

35 Incubate at 20°C for 1 hour in a thermocycler.

01:00:00

Reverse Transcription-First Strand Synthesis

36 Add 1 µL NEXTflex™ RT primer to each sample. Heat at 70°C for 2 minutes then immediately place on ice.

00:02:00

Note

Materials for section "Reverse Transcription-First Strand Synthesis"

Bioo Scientific Supplied
BLUE CAP - NEXTflex™ RT primer, 10X M-MuLV buffer, M-MuLV Reverse Transcriptase, dNTPs
WHITE CAP - Nuclease-free Water

User Supplied
96 well PCR Plate
Thermocycler
***5' and 3' NEXTflex™ Adapter Ligated RNA (21 µL) (from section "5' NEXTflex™ 4N Adapter Ligation")**

37 For each sample, add the following components and mix well.

	4 µL	10X M-MuLV Buffer
	4 µL	dNTPs
	8 µL	Nuclease-free Water
	2 µL	M-MuLV Reverse Transcriptase

38 Incubate in a thermocycler at 44°C for 1 hour followed by 90°C for 10 minutes. The procedure may be safely stopped at this step and samples stored at -20°C.

Bead Cleanup

39 Add 10 µL of Adapter Depletion Solution to each sample and mix well by pipette

Note

Materials for section "Bead Cleanup"

Bioo Scientific Supplied
RED CAP - Adapter Depletion Solution

User Supplied
Agencourt AMPure XP Magnetic Beads (room temperature)
Isopropanol
80% Ethanol, freshly prepared
Magnetic Stand
***40 µL of First Strand Synthesis product (from section "Reverse Transcription-First Strand Synthesis")**

40 Add 40 µL of AMPure XP beads and 90 µL of ispropanol and mix well by pipette.

40 µL

Agencourt AMPure XP **Beckman Coulter Catalog #**A63880

41 Incubate for 5 minutes.

00:05:00

42 Magnetize sample until solution is clear.

43 Remove and discard supernatant.

44 Wash #1: Add 180 µL of freshly prepared 80% ethanol, incubate for 30 seconds, and remove with a P200 or P300 set to 200 µL.

00:00:30

Note

IMPORTANT: Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.

45 Wash #2: Add 180 µL of freshly prepared 80% ethanol, incubate for 30 seconds, and remove with a P200 or P300 set to 200 µL.

00:00:30

Note

IMPORTANT: Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.

46 Incubate sample for 3 minutes. After one minute, remove any residual liquid that may have collected at the bottom of the well.

00:03:00

47 Remove plate from magnetic stand and resuspend bead pellet in 19 µL of Nuclease-free Water by pipetting. Ensure that beads are completely resuspended.

48 Incubate for 2 minutes.

00:02:00

49 Magnetize until solution is clear.

50 Transfer 18 µL of supernatant to a new well.

PCR Amplification

51 For each PCR reaction add the following to the 18 µL purified First Strand Synthesis product (from section "Bead Cleanup"):

	5 µL	5X DuroTaq Master Mix
	1 µL	NEXTflex™ Barcode Primer 1 or Barcoded Primer from NEXTflex™ Small RNA Barcodes Kit
	1 µL	NEXTflex™ Universal Primer

Note

Materials

Bioo Scientific Supplied
GREEN CAP - NEXTflex™ Barcode Primer 1, NEXTflex™ Universal Primer, 5X DuroTaq Master Mix

User Supplied
(Optional) NEXTflex™ Barcode Primer (NEXTflex™ Small RNA Barcodes: 513305, 513306, 513307, or 513308)
Thermocycler
***18 µL Purified First Strand Synthesis product (from section "Bead Cleanup")**

52 Cycle as follows: (Make sure thermocycler is above 80°C before placing samples on block)

2 min	95°C	
20 sec	95°C	
30 sec	60°C	Repeat 12 -18 cycles
15 sec	72°C	
2 min	72°C	

Gel Electrophoresis

53 Add 5 µL of 6X Gel Loading Dye to each PCR product and mix well

Note

Materials

Bioo Scientific Supplied
ORANGE CAP - 6X Gel Loading Dye, Ready to Load Low MW Ladder

User Supplied
6% TBE-PAGE Gel
1X TBE Buffer
Nucleic acid stain such as SYBR Gold (Invitrogen)
UV transilluminator or other visualization tool
Clean razor or scalpel
Nuclease-free 1.7 mL microcentrifuge tube
***PCR Product (25 µL) (from section "PCR Amplification")**

54 Load purified PCR products onto a 6% TBE-PAGE gel. We recommend leaving 1-2 lanes between samples prepared with the same barcode primer to avoid cross contamination. Samples prepared with different barcodes and that will be sequenced together may be run in adjacent lanes.

6% TBE PAGE gels (1.0 mm) **Life Technologies Catalog #** EC6265BOX

55 In an adjacent lane load 10 µL of Ready to Load Low MW Ladder.

56 Run the gel with 1X TBE buffer at 200 V until the lower dye band is near the bottom of the gel (0.5-1 cm). The gel should run for approximately 30 minutes. Run times may vary depending on individual equipment.

00:30:00

57 Carefully remove the gel from the glass plates and stain with a nucleic acid stain such as SYBR Gold (Invitrogen) per manufacturer instructions.

58 Visualize gel bands on a UV transilluminator or other gel documentation instrument.

59 Using a clean razor cut out the ~150 bp band and place into clean 1.7 mL tube. Do not cut out the ~130 bp band; this is adapter dimer product. See Figure 2 in Guidelines for example. The ladder band at 200 bp is twice as intense as the other bands and can be used for orientation.

Nucleic Acid Elution and Purification

60 Briefly centrifuge the microcentrifuge tube containing the gel slice to collect the gel slice at the bottom of the tube.

Note

Materials

Bioo Scientific Supplied
YELLOW CAP - Resuspension Buffer
CLEAR CAP BOTTLE - 1X Elution Buffer (Dilute prior to use as described in the Reagent Preparation section)

User Supplied
Agencourt AMPure XP Magnetic Beads (room temperature)
Isopropanol
80% Ethanol
Nuclease-free 1.7 mL microcentrifuge tubes
Spin-X Centrifuge tube (Sigma)
Sterile disposable pestles (Fisher Cat # K749521-1500 or similar)
Magnetic stand for micrcentrifuge tubes (Life Technologies DynaMag™-2 or similar)
***Gel Slice (in 1.7 mL tube) (from section "Gel Electrophoresis")**

61 Crush the gel slice thoroughly with a disposable pestle. Leave the pestle in the tube.

Sterile disposable pestles **Fisher Scientific Catalog #**K749521-1500

62 Add 300 µL of Elution Buffer to each tube and then remove the pestle, ensuring that as much gel as possible has been washed from the pestle.

63 Let gel pieces soak at least 2 hours or overnight at room temperature with agitation. DO NOT incubate longer than overnight.

64 Pulse spin tubes to collect all eluate from wall and lid.

65 Carefully transfer the eluate (including crushed gel) to the top of a Spin-X Centrifuge tube (Sigma). Cutting the end off of a P1000 tip can help in transfer of larger gel pieces. Centrifuge the Spin-X tube at 16,000 x g for 2 minutes. Dispose of the spin filter.

00:02:00

0.45µm, 2 mL Spin-X Centrifuge tube **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**CLS8162

66 Add to each tube and mix well*:

	50 µL	AMPure XP Beads

	350 µL	Isopropanol

50 µL

Agencourt AMPure XP **Beckman Coulter Catalog #**A63880

67 Incubate at room temperature for 10 minutes. Agitation during this incubation may increase efficiency of recovery.

00:10:00

68 Magnetize sample until solution appears clear.

69 Wash #1: Carefully remove and discard the supernatant, add 950 µL 80% ethanol, incubate 30 seconds, and remove.

00:00:30

70 Wash #2: Carefully remove and discard the supernatant, add 950 µL 80% ethanol, incubate 30 seconds, and remove.

00:00:30

71 Dry sample for 3 minutes. After one minute, remove all residual liquid that may have collected at the bottom of the tube.

00:03:00

72 Remove plate from magnetic rack and resuspend bead pellet in 13 µL of Resuspension Buffer by pipetting volume up and down. Ensure that beads are completely resuspended and rehydrated.

73 Incubate for 2 minutes.

00:02:00

74 Magnetize for 5 minutes or until supernatant appears clear.

00:05:00

75 Transfer 12 µL of supernatant to a clean 1.7 mL tube. This is your sequencing library.

76 Check the size distribution and concentration of the final library by Bioanalyzer High Sensitivity DNA Assay (Agilent).

Note

If desired, ethanol precipiatation or other nucleic acid purification methods may be used for purification of supernatant after this step. For ethanol precipitation, it is recommended to transfer eluate from this step to a clean microcentrifuge tube, as pellets can be difficult to handle in the Spin-X tubes.