



APPLIED GENOMICS

#NOVA-5132-31  
#NOVA-5132-32  
#NOVA-5132-41  
#NOVA-5132-42  
#NOVA-5132-43  
#NOVA-5132-44

AUTOMATED ON THE:  
SCICLONE<sup>®</sup> G3 NGSX WORKSTATION  
ZEPHYR<sup>®</sup> G3 NGS WORKSTATION



Compatible with  
Illumina<sup>®</sup> platforms

# NEXTFLEX<sup>®</sup>

## Small RNA-Seq Kit v4 with UDIs

KIT CONTAINS: 8, 48 or 96 UDIs

**This product is for research use only.  
Not for use in diagnostic procedures.**

This manual is proprietary to PerkinElmer and intended only for customer use in connection with the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose without the prior written consent of PerkinElmer. Follow the protocol included with the kit.

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# NEXTFLEX® Small RNA-Seq Kit v4 with UDIs

NOVA-5132-31, NOVA-5132-32, NOVA-5132-41,  
NOVA-5132-42, NOVA-5132-43, NOVA-5132-44

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## ///// Product Overview

The NEXTFLEX® Small RNA-Seq Kit v4 with UDIs can be used to prepare small RNA libraries from total RNA or purified small RNA. The NEXTFLEX® Small RNA-Seq Kit v4 with UDIs is designed to greatly reduce formation of adapter-dimer product in small RNA-seq library preparation, allowing completely gel-free library preparation from typical input amounts. This kit is designed to greatly reduce sequence bias in small RNA sequencing library construction, allowing more accurate identification and quantification of microRNAs, piRNAs, and other small RNAs.

Sequencing libraries generated by this kit contain Unique Dual Indices (UDIs), which are designed to specifically address the index-hopping phenomenon associated with Illumina platforms utilizing a patterned flow cell. These UDIs prevent mis-assigned reads from appearing in final datasets, allowing for the highest assurance of data integrity. These indexes work on Illumina® instruments such as the MiSeq®, HiSeq® 2000/2500, MiniSeq® any NextSeq® and NovaSeq®.

## ///// Kit Overview

This kit can be used to generate 8, 48, 96 small RNA libraries. The kit contains the necessary reagents to process the user's purified RNA sample through library preparation.

## ///// Kit Contents, Storage & Shelf Life

The NEXTFLEX® Small RNA-Seq Kit v4 with UDIs contain enough material to prepare 8, 48, 96 RNA samples for Illumina-compatible next-generation sequencing. The shelf life of all reagents is 6 months when stored properly. All components can safely be stored at -20°C, except: NEXTFLEX® Adapter Depletion Solution, Resuspension Buffer, and Nuclease-free Water, which can be stored at room temperature, and NEXTFLEX® Cleanup Beads, which should be stored at 4°C.

Kit Contents	Cap Color	Amount (8, 48, 96)	Storage Temp.
NEXTFLEX® 3' Adenylated Adapter v4	<b>RED CAP</b>	8/48/96 µL	-20°C
NEXTFLEX® 3' Ligation Buffer v4	<b>RED CAP</b>	100/600/1,200 µL	-20°C
NEXTFLEX® 3' Ligation Enzyme Mix	<b>RED CAP</b>	12/72/144 µL	-20°C
NEXTFLEX® Adapter Depletion Solution	<b>RED CAP</b>	320/(2)960/4,000 µL	Room Temp.
NEXTFLEX® tRNA/YRNA Blockers v4	<b>RED CAP</b>	8/48/96 µL	-20°C
NEXTFLEX® Adapter Inactivation Mix v4	<b>PINK CAP</b>	32/192/384 µL	-20°C
NEXTFLEX® 5' Adapter v4	<b>LIGHT PURPLE CAP</b>	8/48/96 µL	-20°C
NEXTFLEX® 5' Ligation Buffer v4	<b>LIGHT PURPLE CAP</b>	24/144/288 µL	-20°C
NEXTFLEX® 5' Ligation Enzyme Mix	<b>LIGHT PURPLE CAP</b>	16/96/192 µL	-20°C
NEXTFLEX® RT Enzyme Mix	<b>BLUE CAP</b>	16/96/192 µL	-20°C
NEXTFLEX® RT Buffer v4	<b>BLUE CAP</b>	56/336/672 µL	-20°C
NEXTFLEX® RT Primer v4	<b>BLUE CAP</b>	8/48/96 µL	-20°C
NEXTFLEX® UDI Barcoded Primer Mix v4* (6.25 µM, 4 µL per well)		1 Plate	-20°C
NEXTFLEX® Small RNA PCR Master Mix v4	<b>GREEN CAP</b>	48/288/576 µL	-20°C
Resuspension Buffer	<b>YELLOW CAP</b>	1/1/(2)1 mL	Room Temp.
Nuclease-free Water	<b>WHITE CAP</b>	1/(2)1.5/10 mL	Room Temp.
microRNA Control	<b>CLEAR CAP</b>	10/(2)10/(2)10 µL	-20°C
NEXTFLEX® Cleanup Beads	<b>WHITE CAP BOTTLE</b>	1/5/10 mL	4°C

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### //// Required Materials Not Provided

- 1 ng - 2 µg total RNA or purified small RNA from 1-10 µg total RNA in up to 5 µL Nuclease-free Water
- Isopropanol
- 80% Ethanol
- 2, 10, 20, 200 and 1000 µL pipettes
- RNase-free pipette tips
- Microcentrifuge
- 96 well PCR Plate Non-skirted (Phenix Research, Cat # MPS-499) or similar
- Thin-wall nuclease-free PCR tubes
- Thermal Cycler
- Vortex
- Magnetic Stand -96 (Ambion, Cat # AM10027) or similar

### //// Revision History

Version	Date	Description
V22.04	April 2022	Early Technology Release
V22.06	June 2022	Product Launch

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## //// Warnings and Precautions

We strongly recommend 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 us at [NGS@perkinelmer.com](mailto:NGS@perkinelmer.com).

- Do not use the kit past the expiration date.
- 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 purification and sequencing. Refer to the Starting Material section for additional information.
- Vortex and centrifuge each component prior to use. To ensure material has not lodged in the cap or side of the tube, centrifuge in a microcentrifuge at >12,000 x g for 5 seconds.
- Do not remove NEXTFLEX® 3' Ligation Enzyme Mix or NEXTFLEX® 5' Ligation Enzyme Mix 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 methods also isolate small RNAs.
- Do not freeze NEXTFLEX® Cleanup Beads. NEXTFLEX® Cleanup Beads should be stored at 4°C and brought to room temperature before use.



### ////// NEXTFLEX® Small RNA Sample Preparation Flow Chart

#### NEXTFLEX® Small RNA Sample Preparation Flow Chart

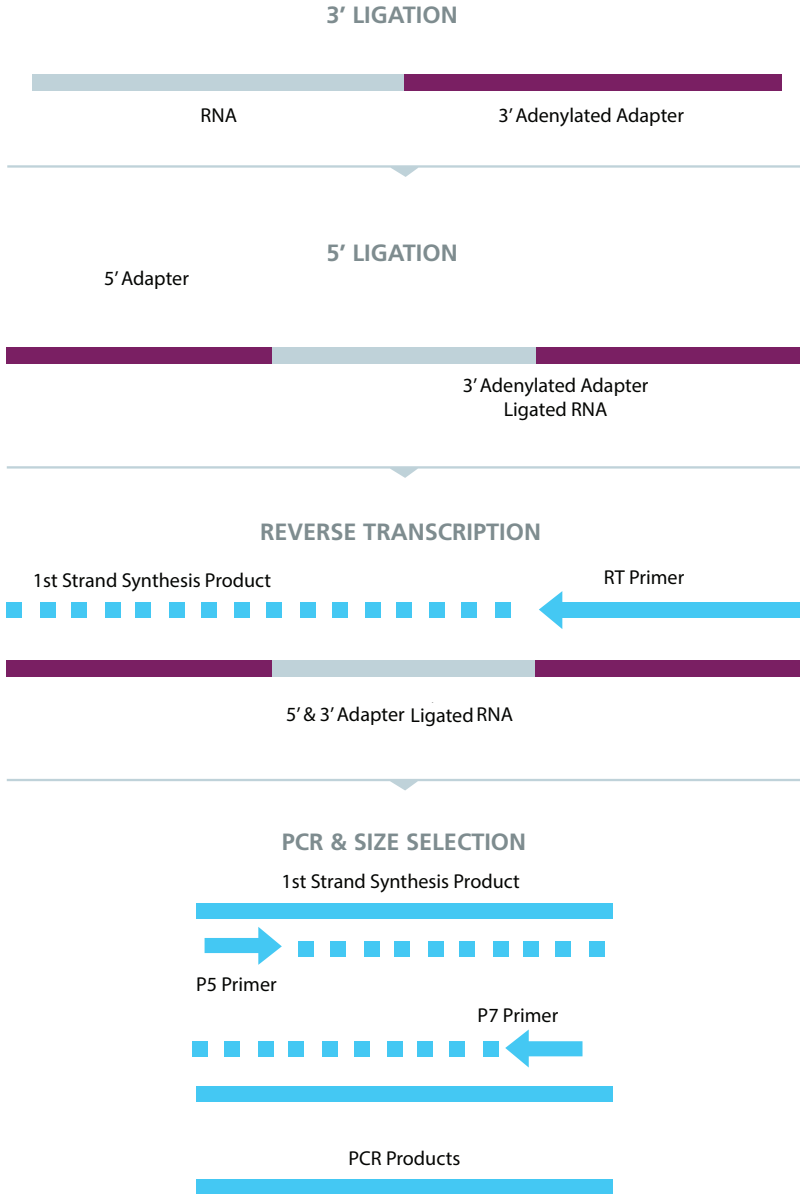


Figure 1. Sample flow chart.





## //// Starting Material

The NEXTFLEX® Small RNA-Seq Kit v4 with UDIs has been optimized and validated using total RNA (1 ng - 2 µg), purified small RNA (from 1 - 10 µg total RNA), and a synthetic miRNA pool (≥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. We recommend running total RNA on a 1 - 2% agarose gel or examining its integrity using an Agilent Bioanalyzer. High quality total RNA preparations should have a 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 a LabChip® assay or similar.

For very poor-quality samples, diluting the NEXTFLEX® 3' Adenylated adapter and the NEXTFLEX® 5' Adapter 1/4 with nuclease-free water can help reduce adapter dimer formation. See Table 1 below for more details. The NEXTFLEX® Small RNA-Seq™ Kit v4 with UDIs is compatible with cell-free RNA, such as RNA isolated from plasma. Users who wish to deplete the abundant tRNA fragments and YRNA fragments found in many types of cell-free RNA should use the NEXTFLEX® tRNA/YRNA Blockers (included in kit). When working with cell-free RNA, it is recommended that users input 4 µl of extracted RNA, or 5 µl if not using tRNA/YRNA Blockers.

If the user is performing the procedure for the first time, we recommend using the microRNA Control included in the kit. 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 µL of the microRNA Control in STEP A instead of their small RNA sample and expect to observe a strong ~165 bp PCR product following 15 cycles of PCR. 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 -20°C. For a total RNA positive control, human brain total RNA (Ambion catalog number AM7962 or similar) is recommended.

**Table 1.** Guidelines for different input amounts. Some optimizations may be required.

Input Amount	PCR Cycles
1 µg	12
100 ng	15
10 ng	18
1 ng	22*
Plasma/Serum	22*
miRNA Control	15

\*When diluting adapters by 1/4, use 24 PCR cycles.

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## //////Reagent Preparation

1. Vortex and centrifuge each component prior to use, to ensure material has not lodged in the cap or the side of the tube.
2. Allow NEXTFLEX® Cleanup Beads to come to room temperature and vortex the beads until liquid appears homogenous before every use
3. Due to the viscosity of certain materials, attempting to prepare more than the stated number of reactions may result in a shortage of materials. All NEXTFLEX® enzyme components must be centrifuged at 600xg for 5 seconds before opening the tube(s). Adapters/barcoded primers supplied in a 96-well plate must be centrifuged at 280xg for 1 minute before removing the plate seal. Adapters/barcoded primers supplied in plates must be centrifuged at 600xg for 5 seconds before using.

### ! IMPORTANT - PLEASE READ

The NEXTFLEX® Small RNA Sequencing Kit v4 protocol requires 6-7 hours for completion. Approximate times to complete each step and safe Stopping Points are noted in the manual; however, careful planning and time management are important for efficient and successful small RNA library preparation. If performing the protocol for the first time, we highly recommend preparing a library with the included microRNA control.



30 MIN



TOTAL

1.5 HR

## STEP A: NEXTFLEX® 3' Adenylyated Adapter Ligation

### MATERIALS

- **RED CAP** - NEXTFLEX® 3' Adenylyated Adapter
- **RED CAP** - NEXTFLEX® 3' Ligation Buffer
- **RED CAP** - NEXTFLEX® 3' Ligation Enzyme Mix
- **RED CAP** - NEXTFLEX® tRNA/YRNA Blockers
- **WHITE CAP** - Nuclease-free Water

### User Supplied

- RNA (1 ng - 2 µg total RNA or small RNA isolated from total RNA) in up to 5 µL Nuclease- Free Water
- 96-well PCR Plate
- Adhesive PCR Plate Seal
- Thermal Cycler
- Ice

- ! 1. **NOTE:** Be sure to mix the following reaction until visibly homogenous by brief vortexing. For each sample, combine the following reagents on ice in a nuclease- free 96-well PCR plate. It is recommended to combine these reagents as a master mix if processing multiple samples:

_ µL	RNA
_ µL	Nuclease-Free Water
1 µL	NEXTFLEX® tRNA/YRNA Blockers (optional)
1 µL	NEXTFLEX® 3' Adenylyated Adapter
12.5 µL	NEXTFLEX® 3' Ligation Buffer
1.5 µL	NEXTFLEX® 3' Ligation Enzyme Mix
<hr/>	
20 µL	TOTAL

2. Mix viscous reaction by vortexing for at least 3 seconds until homogenized.
3. Incubate at 25°C for 1 hour in a thermal cycler. Hold at 4°C for at least 5 minutes. For ligations to 2'-O-methylated small RNAs, such as those found in plants, incubate at 16°C overnight.
4. Proceed immediately to Step B: Adapter Inactivation.

## STEP B: Excess 3' Adapter Removal



10 MIN

TOTAL



15 MIN

### MATERIALS

- **PINK CAP** - NEXTFLEX® Adapter Inactivation Buffer

#### User Supplied

- 96 well PCR plate
- Adhesive PCR Plate Seal
- Thermal cycler
- Ice
- **20 µL of NEXTFLEX® 3' Adapter Ligated RNA (from Step A)**

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

20 µL	NEXTFLEX® 3' Adapter Ligated RNA (from Step A)
4 µL	NEXTFLEX® Adapter Inactivation Mix
24 µL	TOTAL

2. Mix viscous reaction by vortexing for at least 3 seconds until homogenized.

3. Incubate as follows:

2 min	70°C
5 min	4°C
HOLD	4°C

4. Proceed immediately to Step C: NEXTFLEX® 5' Adapter Ligation.

STEP A

STEP B

STEP C

STEP D

STEP E

STEP F

STEP G

## STEP C: NEXTFLEX® 5' Adapter Ligation



### MATERIALS

- **LIGHT PURPLE CAP** - NEXTFLEX® 5' Adapter
- **LIGHT PURPLE CAP** - NEXTFLEX® 5' Ligation Buffer
- **LIGHT PURPLE CAP** - NEXTFLEX® Ligation Enzyme Mix

### User Supplied

- 96 well PCR Plate
- Adhesive PCR Plate Seal
- Thermal Cycler
- Ice
- **24 µL of NEXTFLEX® 3' Adapter Ligated RNA (from Step B)**

- ! 1. **NOTE:** Be sure to mix the following reaction until visibly homogenous by brief vortexing. For each sample, combine the following reagents on ice in a nuclease-free 96 well PCR plate. It is recommended to combine these reagents as a master mix if processing multiple samples:

24 µL	NEXTFLEX® 3' Adapter Ligated RNA (from Step B)
1 µL	NEXTFLEX® 5' Adapter
3 µL	NEXTFLEX® 5' Ligation Buffer
2 µL	NEXTFLEX® 5' Ligation Enzyme Mix
<hr/>	
30 µL	TOTAL

- Mix reaction by vortexing for at least 3 seconds until homogenized.
- Incubate at 20°C for 60 minutes in a thermal cycler.

60 min	20°C
5 min	4°C
HOLD	4°C
- Proceed immediately to Step D: Reverse Transcription - First Strand Synthesis.

## STEP D: Reverse Transcription-First Strand Synthesis



20 MIN

TOTAL

1.5 HR

### MATERIALS

- **BLUE CAP** - NEXTFLEX® RT Buffer
- **BLUE CAP** - NEXTFLEX® RT Enzyme Mix
- **BLUE CAP** - NEXTFLEX® RT Primer

### User Supplied

- 96 well PCR plate
- Adhesive PCR Plate Seal
- Thermal cycler
- Ice
- **30 µL of 5' and 3' NEXTFLEX® Adapter Ligated RNA (from Step C)**

1. For each sample, combine the following reagents on ice in a nuclease-free 96 well PCR plate. It is recommended to combine these reagents as a master mix if processing multiple samples:

30 µL	5' and 3' NEXTFLEX® Adapter Ligated RNA (from Step C)
1 µL	NEXTFLEX® RT Primer
7 µL	NEXTFLEX® RT Buffer
2 µL	NEXTFLEX® RT Enzyme Mix
<hr/>	
40 µL	TOTAL

2. Mix reaction by vortexing for at least 3 seconds until homogenized.
3. Incubate as follows:

60 min	50°C
5 min	90°C
5 min	4°C
HOLD	4°C

4. Proceed immediately to Step E: Bead Cleanup.

**!** **STOPPING POINT:** Alternatively, the procedure may be stopped at this point with samples stored overnight at 4°C or up to one week at -20°C. To restart, thaw frozen samples on ice before proceeding to Step E: Bead Cleanup.

STEP A

STEP B

STEP C

STEP D

STEP E

STEP F

STEP G

## STEP E: Bead Cleanup



### MATERIALS

- **RED CAP** - NEXTFLEX® Adapter Depletion Solution
- **CLEAR CAP BOTTLE** - NEXTFLEX® Cleanup Beads
- **WHITE CAP** - Nuclease-Free Water

### User Supplied

- Isopropanol
  - 80% Ethanol, freshly prepared
  - Magnetic Stand
  - **40 µL of First Strand Synthesis product (from Step D)**
1. Add 40 µL NEXTFLEX® Adapter Depletion Solution and mix well by pipette.
  2. Add 40 µL of NEXTFLEX® Cleanup Beads and mix well by pipette.
  3. Add 90 µL of isopropanol and mix well by pipette.
  4. Incubate for 5 minutes.
  5. Magnetize sample for 5 minutes, or until solution appears clear.
  6. Remove and discard supernatant.
  7. Add 200 µL of freshly prepared 80% ethanol, incubate for 30 seconds, and remove all of the supernatant. Repeat this step for a total of 2 ethanol washes.
- ! **IMPORTANT:** Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.
8. Incubate sample for 3 minutes. After one minute, remove all residual liquid that may have collected at the bottom of the well.
  9. Remove plate from magnetic stand and resuspend bead pellet in 18 µL Nuclease-free Water by pipetting volume up and down. Ensure that beads are completely resuspended.
  10. Incubate for 2 minutes.
  11. Magnetize sample for 3 minutes or until solution appears clear.
  12. Transfer 16 µL of supernatant to a new well.
  13. Proceed immediately to Step F: PCR Amplification.

! **STOPPING POINT:** Alternatively, the procedure may be stopped at this point with samples stored overnight at -20°C. To restart, thaw frozen samples on ice before proceeding to Step F: PCR Amplification.

STEP A

STEP B

STEP C

STEP D

STEP E

STEP F

STEP G

## STEP F: PCR Amplification



15 MIN

TOTAL



40-60 MIN

### MATERIALS

- PLATE - NEXTFLEX® UDI Barcoded Primer Mix
- GREEN CAP - NEXTFLEX® Small RNA PCR Master Mix

### User Supplied

- 96 well PCR plate
- Adhesive PCR Plate Seal
- Thermal cycler
- Ice
- **18 µL Purified First Strand Synthesis Product (from Step E)**

1. For each sample, combine the following reagents on ice in a nuclease-free 96 well PCR plate. It is recommended to combine these reagents as a master mix if processing multiple samples:

16 µL	Purified First Strand Synthesis Product (From Step E)
4 µL	NEXTFLEX® UDI Barcoded Primer Mix (a different barcoded primer should be used for each sample that will be multiplexed for sequencing)
6 µL	NEXTFLEX® Small RNA PCR Master Mix
<hr/>	
26 µL	TOTAL

2. Cycle as follows:

30 sec	98°C	} (See Table 1 on page 9 in Starting Materials for recommendations)
10 sec	98°C	
20 sec	65°C	
15 sec	72°C	
2 min	72°C	

3. Proceed immediately to Step G: Size Selection & Cleanup.

! STOPPING POINT: Alternatively, the procedure may be stopped at this point with samples stored up to one week at -20°C. To restart, thaw frozen samples on ice before proceeding to Step G: Size Selection Cleanup

STEP A

STEP B

STEP C

STEP D

STEP E

STEP F

STEP G



## STEP G: Size Selection & Cleanup



45 MIN



TOTAL  
45 MIN

### MATERIALS

- **WHITE CAP** - Nuclease-Free Water
- **YELLOW CAP** - Resuspension Buffer
- **CLEAR CAP BOTTLE** - NEXTFLEX® Cleanup Beads

### User Supplied

- 80% Ethanol, freshly prepared
  - Magnetic Stand
  - **26  $\mu$ L of PCR Product (from Step F)**
1. Ensure the volume of all samples is 26  $\mu$ L. If less, add Nuclease-free Water to bring the entire volume up to 26  $\mu$ L.
  2. Add 34  $\mu$ L of NEXTFLEX® Cleanup Beads and mix well by pipetting.
  3. Incubate for 5 minutes.
  4. Magnetize sample for 5 minutes or until solution is clear.
  5. Transfer 56  $\mu$ L of supernatant to a new well. **DO NOT DISCARD SUPERNATANT**, this solution contains the amplified product. Take care to not transfer beads along with clear supernatant.
  6. Remove plate from magnetic stand.
  7. Add 13  $\mu$ L of NEXTFLEX® Cleanup Beads to each sample and mix well by pipette.
  8. Incubate for 5 minutes.
  9. Magnetize sample for 5 minutes, or until solution appears clear.
  10. Remove and discard supernatant.
  11. Add 200  $\mu$ L of freshly prepared 80% ethanol, incubate for 30 seconds, and remove all of the supernatant. Repeat this step for a total of 2 ethanol washes.  
**! IMPORTANT:** Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.
  12. Incubate sample for 3 minutes. After one minute, remove all residual liquid that may have collected at the bottom of the well.
  13. Remove plate from magnetic stand and resuspend bead pellet in 17  $\mu$ L of Resuspension Buffer by pipetting volume up and down. Ensure that beads are completely resuspended.
  14. Incubate for 2 minutes.
  15. Magnetize sample for 3 minutes or until solution appears clear.
  16. Transfer 15  $\mu$ L of supernatant to a new well or clean microcentrifuge tube. This is your sequencing library.
  17. Check the size distribution of the final library by LabChip® or equivalent and the concentration by Qubit dsDNA HS Assay (Life Technologies).

STEP A

STEP B

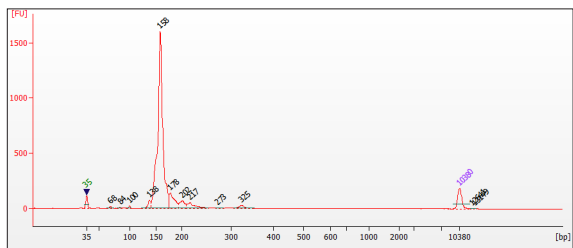
STEP C

STEP D

STEP E

STEP F

STEP G



*Figure 2.* Sample Bioanalyzer HS DNA traces from libraries created from 1 ng human brain total RNA.

## Oligonucleotide Sequences

NEXTFLEX® 3' Adenylated Adapter
5' rApp /TGGAATTCTCGGGTGCCAAGG/ 3SpC3/
NEXTFLEX® 5' Adapter
5' UCUUUCCCUACACGACGCUCUCCGAUCU
NEXTFLEX® RT Primer
5' CCTTGGCACCCGAGAATTCCA
UDI primer 1 – P7 (included in NEXTFLEX UDI Barcoded Primer Mix)
5' CAAGCAGAAGACGGCATACGAGATXXXXXXXX'GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
UDI primer 2 – P5 (included in NEXTFLEX UDI Barcoded Primer Mix)
5' AATGATACGGCGACCACCGAGATCTACACXXXXXXXX <sup>2</sup> ACACTCTTCCCTACACGACGCTCTCCGATCT

XXXXXXXX<sup>1</sup> denotes the P7 index region of the primer. The index sequences that are added to each barcoded library via PCR are listed below. Note: UDI primer 1 contains the reverse complement of the sequence listed under P7 Index. The final library, however, will contain the P7 Index listed below, and the sequencer will read the index as listed below.

XXXXXXXX<sup>2</sup> denotes the P5 index region of the primer. The index sequences that are added to each barcoded library via PCR are listed below.

The complete index sequences can be found under the Technical Resources tab at <https://perkinelmer-appliedgenomics.com/home/products/library-preparation-kits/small-rna-library-prep/nextflex-small-rna-seq-kit-v4/>

When entering index sequences for the Illumina® MiniSeq®, NextSeq®, HiSeq® 3000 or HiSeq® 4000 platforms, enter the P5 Index Reverse Complement. For all other Illumina® platforms, enter the P5 Index in the first column. For additional information, please email [NGS@perkinelmer.com](mailto:NGS@perkinelmer.com)

## NOVA-5132-32 (UDI 1-48)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41						
B	2	10	18	26	34	42						
C	3	11	19	27	35	43						
D	4	12	20	28	36	44						
E	5	13	21	29	37	45						
F	6	14	22	30	38	46						
G	7	15	23	31	39	47						
H	8	16	24	32	40	48						

## NOVA-5132-41 (UDI 1-96)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96



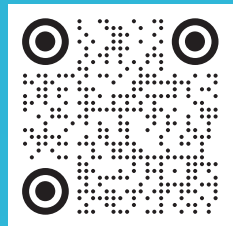


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