NEBNext® Multiplex Small RNA Library Prep Set for Illumina® Set 1, Set 2, Index Primers 1–48 and Multiplex Compatible

NEB #E7300S/L, E7580S/L, E7560S, E7330S/L

24/96 reactions

Version 8.0_10/20

Table of Contents

The NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1) Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7300S) and 96 reactions (NEB #E7300L). All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (green) NEBNext 3′ Ligation Reaction Buffer (2X)
- (green) NEBNext 3′ Ligation Enzyme Mix
- (green) NEBNext 3′ SR Adaptor for Ilumina
- (yellow) NEBNext 5′ SR Adaptor for Illumina
- (yellow) NEBNext 5′ Ligation Reaction Buffer (10X)
- (yellow) NEBNext 5′ Ligation Enzyme Mix
- (pink) NEBNext SR RT Primer for Illumina
- (red) NEBNext First Strand Synthesis Reaction Buffer
- (red) ProtoScript II Reverse Transcriptase
- (red) Murine RNase Inhibitor
- (blue) LongAmp *Taq* 2X Master Mix

- (blue) NEBNext SR Primer for Illumina
- (blue) NEBNext Index 1 Primer for Illumina
- (blue) NEBNext Index 2 Primer for Illumina
- (blue) NEBNext Index 3 Primer for Illumina
- (blue) NEBNext Index 4 Primer for Illumina
- (blue) NEBNext Index 5 Primer for Illumina
- (blue) NEBNext Index 6 Primer for Illumina
- (blue) NEBNext Index 7 Primer for Illumina
- (blue) NEBNext Index 8 Primer for Illumina
- (blue) NEBNext Index 9 Primer for Illumina
- (blue) NEBNext Index 10 Primer for Illumina
- (blue) NEBNext Index 11 Primer for Illumina
- (blue) NEBNext Index 12 Primer for Illumina
- (orange) Gel Loading Dye, Blue (6X)
- (orange) Quick-Load pBR322 DNA-MspI Digest
- (white) DNA Gel Elution Buffer, 1X
- (white) Linear Acrylamide (10 mg/ml)
- (white) TE Buffer
- (white) Nuclease-free Water

For kit components of other NEBNext Small RNA Library Prep Sets for Illumina, check the appropriate pages in this manual.

The NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2) Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7580S) and 96 reactions (NEB #E7580L). All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (green) NEBNext 3′ Ligation Reaction Buffer (2X)
- (green) NEBNext 3′ Ligation Enzyme Mix
- (green) NEBNext 3′ SR Adaptor for Ilumina
- (yellow) NEBNext 5′ SR Adaptor for Illumina
- (yellow) NEBNext 5′ Ligation Reaction Buffer (10X)
- (yellow) NEBNext 5′ Ligation Enzyme Mix
- (pink) NEBNext SR RT Primer for Illumina
- (red) NEBNext First Strand Synthesis Reaction Buffer
- (red) ProtoScript II Reverse Transcriptase
- (red) Murine RNase Inhibitor
- (blue) LongAmp *Taq* 2X Master Mix
- (blue) NEBNext SR Primer for Illumina
- (blue) NEBNext Index 13 Primer for Illumina
- (blue) NEBNext Index 14 Primer for Illumina
- (blue) NEBNext Index 15 Primer for Illumina
- (blue) NEBNext Index 16 Primer for Illumina
- (blue) NEBNext Index 17 Primer for Illumina
- (blue) NEBNext Index 18 Primer for Illumina
- (blue) NEBNext Index 19 Primer for Illumina
- (blue) NEBNext Index 20 Primer for Illumina

- (blue) NEBNext Index 21 Primer for Illumina
- (blue) NEBNext Index 22 Primer for Illumina
- (blue) NEBNext Index 23 Primer for Illumina
- (blue) NEBNext Index 24 Primer for Illumina
- (orange) Gel Loading Dye, Blue (6X)
- (orange) Quick-Load pBR322 DNA-MspI Digest
- (white) DNA Gel Elution Buffer, 1X
- (white) Linear Acrylamide (10 mg/ml)
- (white) TE Buffer
- (white) Nuclease-free Water

For kit components of other NEBNext Small RNA Library Prep Sets for Illumina, check the appropriate pages in this manual.

The NEBNext Multiplex Small RNA Prep Kit for Illumina (Index Primers 1-48) Includes

The volumes provided are sufficient for preparation of up to 96 reactions (NEB #E7560S). All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (green) NEBNext 3′ Ligation Reaction Buffer (2X)
- (green) NEBNext 3′ Ligation Enzyme Mix
- (green) NEBNext 3′ SR Adaptor for Illumina
- (yellow) NEBNext 5′ SR Adaptor for Illumina
- (yellow) NEBNext 5′ Ligation Reaction Buffer (10X)
- (yellow) NEBNext 5′ Ligation Enzyme Mix
- (pink) NEBNext SR RT Primer for Illumina
- (red) NEBNext First Strand Synthesis Reaction Buffer
- (red) ProtoScript II Reverse Transcriptase
- (red) Murine RNase Inhibitor
- (blue) LongAmp *Taq* 2X Master Mix
- (blue) NEBNext SR Primer for Illumina
- (blue) NEBNext Index 1 Primer for Illumina
- (blue) NEBNext Index 2 Primer for Illumina
- (blue) NEBNext Index 3 Primer for Illumina
- (blue) NEBNext Index 4 Primer for Illumina
- (blue) NEBNext Index 5 Primer for Illumina
- (blue) NEBNext Index 6 Primer for Illumina
- (blue) NEBNext Index 7 Primer for Illumina
- (blue) NEBNext Index 8 Primer for Illumina
- (blue) NEBNext Index 9 Primer for Illumina
- (blue) NEBNext Index 10 Primer for Illumina
- (blue) NEBNext Index 11 Primer for Illumina
- (blue) NEBNext Index 12 Primer for Illumina
- (blue) NEBNext Index 13 Primer for Illumina
- (blue) NEBNext Index 14 Primer for Illumina
- (blue) NEBNext Index 15 Primer for Illumina
- (blue) NEBNext Index 16 Primer for Illumina

- (blue) NEBNext Index 17 Primer for Illumina
- (blue) NEBNext Index 18 Primer for Illumina
- (blue) NEBNext Index 19 Primer for Illumina
- (blue) NEBNext Index 20 Primer for Illumina
- (blue) NEBNext Index 21 Primer for Illumina
- (blue) NEBNext Index 22 Primer for Illumina
- (blue) NEBNext Index 23 Primer for Illumina
- (blue) NEBNext Index 24 Primer for Illumina
- (blue) NEBNext Index 25 Primer for Illumina
- (blue) NEBNext Index 26 Primer for Illumina
- (blue) NEBNext Index 27 Primer for Illumina
- (blue) NEBNext Index 28 Primer for Illumina
- (blue) NEBNext Index 29 Primer for Illumina
- (blue) NEBNext Index 30 Primer for Illumina
- (blue) NEBNext Index 31 Primer for Illumina
- (blue) NEBNext Index 32 Primer for Illumina
- (blue) NEBNext Index 33 Primer for Illumina
- (blue) NEBNext Index 34 Primer for Illumina
- (blue) NEBNext Index 35 Primer for Illumina
- (blue) NEBNext Index 36 Primer for Illumina
- (blue) NEBNext Index 37 Primer for Illumina
- (blue) NEBNext Index 38 Primer for Illumina
- (blue) NEBNext Index 39 Primer for Illumina
- (blue) NEBNext Index 40 Primer for Illumina
- (blue) NEBNext Index 41 Primer for Illumina
- (blue) NEBNext Index 42 Primer for Illumina
- (blue) NEBNext Index 43 Primer for Illumina
- (blue) NEBNext Index 44 Primer for Illumina
- (blue) NEBNext Index 45 Primer for Illumina
- (blue) NEBNext Index 46 Primer for Illumina
- (blue) NEBNext Index 47 Primer for Illumina
- (blue) NEBNext Index 48 Primer for Illumina
- (orange) Gel Loading Dye, Blue (6X)
- (orange) Quick-Load pBR322 DNA-MspI Digest
- (white) DNA Gel Elution Buffer, 1X
- (white) Linear Acrylamide (10 mg/ml)
- (white) TE Buffer
- (white) Nuclease-free Water

For kit components of other NEBNext Small RNA Library Prep Sets for Illumina, check the appropriate pages in this manual.

The NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible) Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7330S) and 96 reactions (NEB #E7330L). Please note that only one index primer is included in this kit. Libraries made with Index 1 cannot be pooled and run together. If index primers are required, kits (NEB #E7300), (NEB #E7580) or (NEB #E7560) should be ordered instead. All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (green) NEBNext 3′ Ligation Reaction Buffer (2X)
- (green) NEBNext 3′ Ligation Enzyme Mix
- (green) NEBNext 3′ SR Adaptor for Ilumina
- (yellow) NEBNext 5′ SR Adaptor for Illumina
- (yellow) NEBNext 5′ Ligation Reaction Buffer (10X)
- (yellow) NEBNext 5′ Ligation Enzyme Mix
- (pink) NEBNext SR RT Primer for Illumina
- (red) NEBNext First Strand Synthesis Reaction Buffer
- (red) ProtoScript II Reverse Transcriptase
- (red) Murine RNase Inhibitor
- (blue) LongAmp *Taq* 2X Master Mix
- (blue) NEBNext SR Primer for Illumina
- (blue) NEBNext Index 1 Primer for Illumina
- (orange) Gel Loading Dye, Blue (6X)
- (orange) Quick-Load pBR322 DNA-MspI Digest
- (white) DNA Gel Elution Buffer, 1X
- (white) Linear Acrylamide (10 mg/ml)
- (white) TE Buffer
- (white) Nuclease-free Water

For kit components of other NEBNext Small RNA Library Prep Sets for Illumina, check the appropriate pages in this manual.

Required Materials Not Included

- 3 M Sodium Acetate, pH 5.5
- 100% Ethanol
- 80% Ethanol
- Corning®, Costar®, Spin-X® Centrifuge Tube Filters (Cellulose Acetate Filters) (Sigma Aldrich # CLS8162)
- Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)
- Size Selection Materials:

  for gel size selection:

  6% Novex® TBE PAGE gel 1.0 mM

  10-well (Life Technologies, Inc. #EC6265BOX)

  SYBR® Gold Nucleic Acid Gel Stain (Life Technologies, Inc. #S-11494)

  RNase-free Disposable Pellet Pestles® (Kimble Kontes Asset Management, Inc. #749521-1590)

  Dry Ice/Methanol Bath or –80°C freezer

  for bead selection:

  Agencourt® AMPure® XP Beads (Beckman Coulter, Inc. #A63881)

  for Pippin Prep™ selection:

  3% Agarose Dye Free Gel (Sage Science #CDP 3010)
- Bioanalyzer® (Agilent® Technologies, Inc.)

Overview

The NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1) contains the adaptors, primers, enzymes and buffers required to convert small RNAs into indexed libraries for next generation sequencing on the Illumina platform. The novel workflow has been optimized to minimized adaptor-dimers, while producing high-yield, high-diversity libraries.

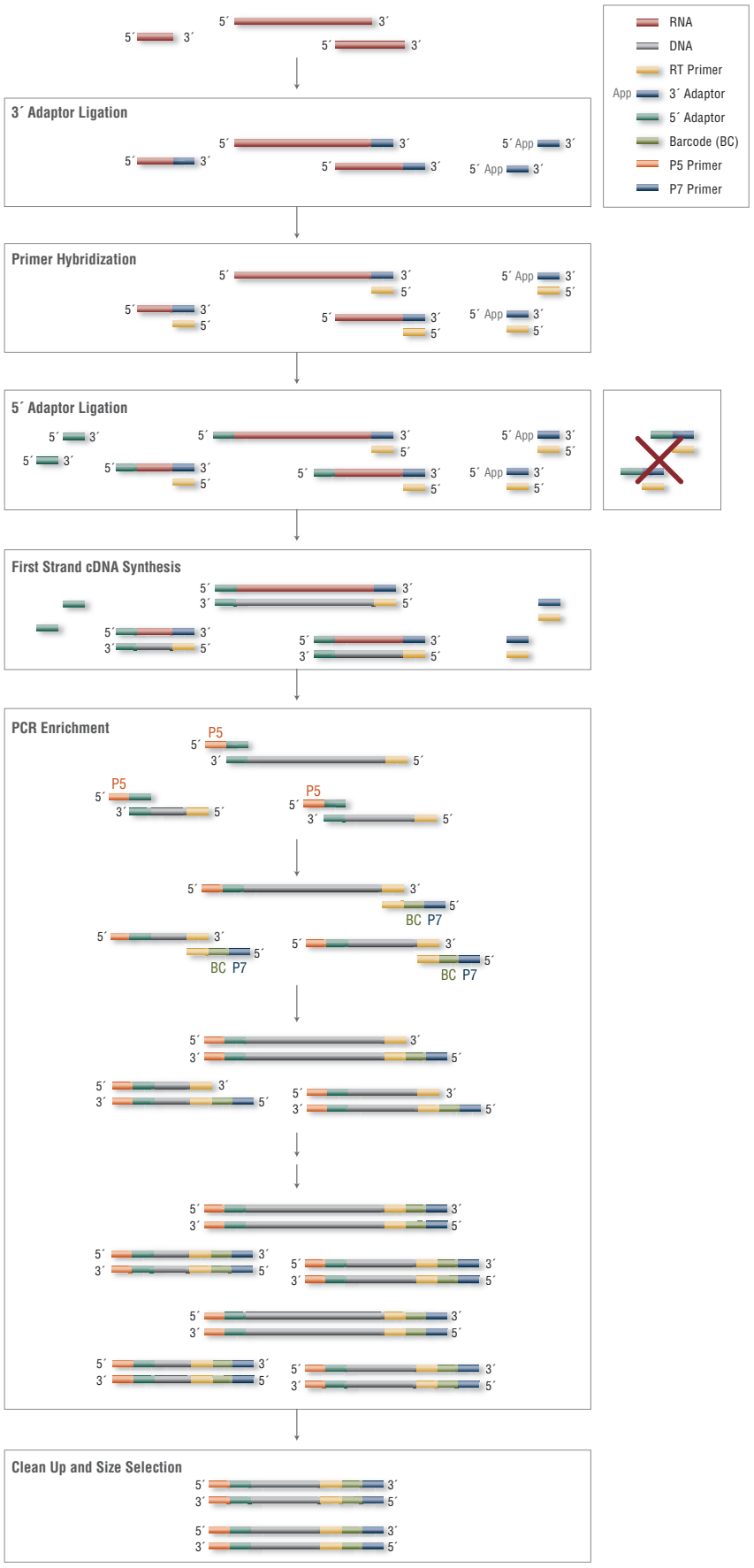
Each kit component must pass rigorous quality control standards, and each set of reagents is functionally validated together by construction and sequencing of indexed small RNA libraries on the Illumina sequencing platform.

For larger volume requirements, customized and bulk packaging is available by purchasing through the OEM/Bulks department at NEB. Please contact OEM@neb.com for further information.

RNA Sample Quality: This kit was optimized using high quality human RNA (First Choice® Human Brain Reference RNA from Life Technologies, Inc. #AM7962). High Quality total RNA (RNA Integrity Number (RIN) > 7) should be used as starting material whenever possible. The quality and quantity of your sample should be assessed, for example by use of the Agilent 2100 Bioanalyzer, using an Agilent RNA 6000 Nano Chip.

Multiplex Small RNA Library Prep Workflow

This kit includes a novel protocol that results in higher yields and lower adaptor-dimer contamination.



Protocols

Please refer to revision history for a summary of protocol updates

Symbols

This is a point where you can safely stop the protocol and store the samples prior to proceeding to the next step in the protocol.

This caution sign signifies a step in the protocol that has two paths leading to the same end point but is dependent on a user variable, like the type of RNA input.

●

Colored bullets indicate the cap color of the reagent to be added.

Libraries prepared by this method are compatible with Illumina paired-end flow cells.

Starting Material: 100 ng–1 µg total RNA. Small RNA fragments should have a 5′ phosphate and 3′ OH to ligate and must be free of ATP.

1. Ligate the 3′ SR Adaptor

Note: For total RNA inputs closer to 100 ng, dilute the ● (green) 3′ SR Adaptor for Illumina 1:2 (For example: 1 µl of 3′ SR adaptor and 1 µl nuclease-free water) in nuclease-free water. For total RNA inputs closer to 1 µg, do not further dilute the adaptor.

Adaptor dilutions may need to be optimized further.

1.1. Mix the following components in a sterile nuclease-free PCR tube. It is ok to premix the reagents. Use immediately.

COMPONENT	VOLUME
Input RNA	1–6 µl
● (green) 3′ SR Adaptor for Illumina	1 µl
Nuclease-free Water	Variable
Total Volume	7 µl

1.2. Incubate in a preheated thermal cycler for 2 minutes at 70°C. Transfer tube to ice.

1.3. Add and mix the following components. It is ok to premix the reagents. Use immediately.

COMPONENT	VOLUME
● (green) 3′ Ligation Reaction Buffer	10 µl
● (green) 3′ Ligation Enzyme Mix	3 µl
Total Volume	20 µl

1.4. Incubate for 1 hour at 25°C in a thermal cycler.

Note: Longer incubation times and reduced temperatures (18 hours; 16°C) increase ligation efficiency of methylated RNAs such as piwi-interacting RNAs (piRNAs) (if present in the sample). However, some concatamerization products might be formed.

2. Hybridize the Reverse Transcription Primer

This step is important to prevent adaptor-dimer formation. The SR RT Primer hybridizes to the excess of 3′ SR Adaptor (that remains free after the 3′ ligation reaction) and transforms the single stranded DNA adaptor into a double-stranded DNA molecule. dsDNAs are not substrates for ligation mediated by T4 RNA Ligase 1 and therefore do not ligate to the 5′ SR Adaptor in the subsequent ligation step.

Note: For total RNA inputs closer to 100 ng, dilute the ● (pink) SR RT Primer for Illumina 1:2 in nuclease free water.

For total RNA inputs closer to 1 µg do not dilute the primer. Depending on the small RNA quantity and quality of your sample additional dilution optimization may be required.

2.1. Add and mix the following components to the ligation mixture from Step 1.4 and mix well. It is ok to premix the reagents.

COMPONENT	VOLUME
Nuclease-free Water	4.5 µl
● (pink) SR RT Primer for Illumina	1 µl
Total Volume	25.5 µl

2.2. Place in a thermal cycler with heated lid set to > 85°C and run the following program:

5 minutes at 75°C
15 minutes at 37°C
15 minutes at 25°C
Hold at 4°C

3. Ligate the 5′ SR Adaptor

3.1. With 5 minutes remaining, resuspend the ● (yellow) 5′ SR adaptor in 120 µl of nuclease free water.

Note: For total RNA inputs closer to 100 ng, additionally dilute the ● (yellow) 5′ SR Adaptor for Illumina 1:2 in nuclease free water. For total RNA inputs closer to 1 µg do not dilute the adaptor further.

3.2. Aliquot the ● (yellow) 5′ SR Adaptor into a separate, nuclease-free 200 µl PCR tube, for the number of samples in the experiment plus an excess of 10%.

3.3. Incubate the adaptor in the thermal cycler at 70°C for 2 minutes and then immediately place the tube on ice. Keep the tube on ice and use the denatured adaptor within 30 minutes of denaturation.

Note: Store the remaining resuspended 5′ SR adaptor at –80°C. Denature aliquots before use. Please minimize freeze/thaw cycles. If only a few libraries are to be made at a time, the 5′ SR adaptor could be aliquoted.

3.4. Add and mix the following components to the ligation mixture from Step 2.2 and mix well. Do not premix reagents.

COMPONENT	VOLUME
● (yellow) 5′ SR Adaptor for Illumina (denatured)	1 µl
● (yellow) 5′ Ligation Reaction Buffer (10X)	1 µl
● (yellow) 5′ Ligation Enzyme Mix	2.5 µl
Total Volume	30 µl

3.5. Incubate for 1 hour at 25°C in a thermal cycler.

4. Perform Reverse Transcription

4.1. Mix the following components in a sterile, nuclease-free tube. It is ok to premix the reagents. Use immediately.

COMPONENT	VOLUME
Adaptor Ligated RNA from Step 3.5	30 µl
● (red) First Strand Synthesis Reaction	8 µl
● (red) Murine RNase Inhibitor	1 µl
● (red) ProtoScript II Reverse Transcriptase	1 µl
Total Volume	40 µl

4.2. Incubate for 60 minutes at 50°C.

4.3. Immediately proceed to PCR amplification.

Safe Stopping Point: If you do not plan to proceed immediately to PCR amplification, then heat inactivate the RT reaction at 70°C for 15 minutes. Samples can be safely stored at –15°C to –25°C.

5. Perform PCR Amplification

5.1. Add and mix the following components to the RT reaction mix from Step 4.2 and mix well:

COMPONENT	VOLUME
• (blue) LongAmp *Taq* 2X Master Mix	50 µl
• (blue) SR Primer for Illumina	2.5 µl
• (blue) Index (X) Primer*	2.5 µl
Nuclease free water	5 µl
Total Volume now should be	100 µl

**Note: The NEBNext Multiplex Small RNA Library Prep Set for Illumina Set 1 contains 1–12 PCR primers, Set 2 contains 23–24 PCR primers, kit index primers 1–48 PCR primer, each with a different index. For each reaction, only one of the 12 PCR primer indices is used during the PCR step.*

PCR Cycling conditions:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	94°C	30 seconds	1
Denaturation	94°C	15 seconds	12–15*
Annealing	62°C	30 seconds	
Extension	70°C	15 seconds	
Final Extension	70°C	5 minutes	1
Hold	4°C	∞	

*Amplification conditions may vary based on RNA input amount, tissue, and species. This protocol was optimized using 1 µg of total RNA from human brain and 12 PCR cycles. The number of PCR cycles may need to be adjusted if clear and distinct bands are not observed in the gel image. For 100 ng total RNA input run 15 cycles of PCR. For samples containing high amounts of small RNA, less than 12 cycles may be appropriate.

Safe Stopping Point: It is safe to store the library at -20°C after PCR. Avoid leaving the sample at 4°C overnight if possible.

6. Quality Control

Note: There are several different methods for performing size selection. It is recommended to choose the appropriate method based on the QC check of the library using the Bioanalyzer. Size selection using AMPure XP Beads does not remove small fragments. If you perform the QC check and your sample contains Adaptor dimer (127 bp peak) or excess primers (70-80 bp) it is recommended to use gel or Pippin Prep for size selection.

6A. QC Check and Size Selection using 6% PolyAcrylamide Gel

6A.1. Purify the PCR amplified cDNA construct (100 µl) using a Monarch PCR & DNA Kit.

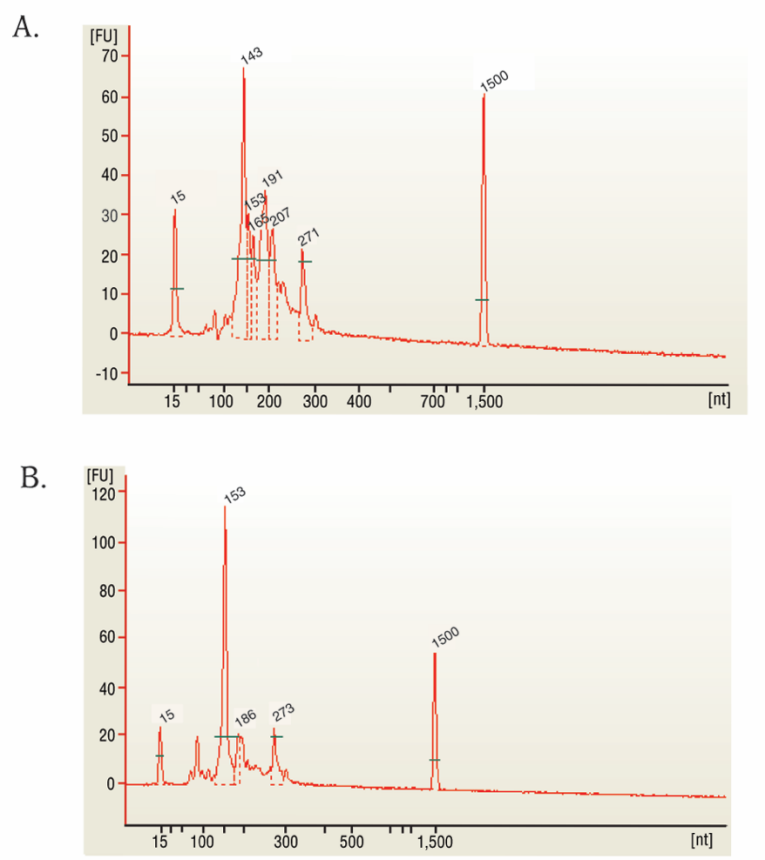
IMPORTANT: Use the 7:1 ratio of binding buffer:sample.Discard the flow through after each centrifugation step.

6A.2. Elute amplified DNA in 27.5 µl Nuclease-free Water.

Safe Stopping Point: It is safe to store the library at -20°C.

6A.3. Load 1 µl of the purified PCR reaction on the Bioanalyzer using a DNA 1000 chip according to the manufacturer's instructions (Figure 1).

Figure 1. Typical results from (A) human brain and (B) rat testis total RNA libraries before size selection.



The 143 and 153 bp bands correspond to miRNAs and piRNAs, respectively. The bands on the Bionalyzer electropherograms resolve in sizes ~ 6-8 nucleotides larger than sizes observed on PAGE gels and can shift from sample to sample due to an incorrect identification of the marker by the bioanalyzer software. miRNA peak should be ~ 143-146 bp.

6A.4. Mix the purified PCR product (25 µl) with 5 µl of Gel Loading Dye, Blue (6X).

Note: Vortex the Gel Loading Dye, Blue throughly to mix well before using.

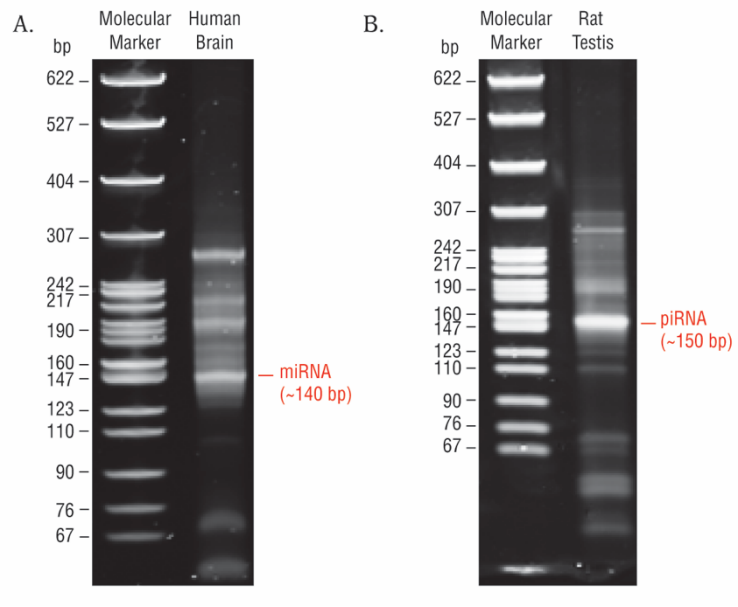
6A.5. Load 5 µl of Quick-Load pBR322 DNA-MspI Digest in one well on the 6% PAGE 10-well gel.

6A.6. Load two wells with 15 µl each of mixed amplified cDNA construct and loading dye on the 6% PAGE 10-well gel.

6A.7. Run the gel for 1 hour at 120 V or until the blue dye reaches the bottom of the gel. Do not let the blue dye exit the gel.

6A.8. Remove the gel from the apparatus and stain the gel with SYBR Gold nucleic acid gel stain in a clean container for 2–3 minutes and view the gel on a UV transiluminator (Figure 2).

Figure 2.



Shows typical results from Human Brain (A) and Rat Testis (B) Total RNA libraries.
The 140 and 150 bp bands correspond to miRNAs (21 nt) and piRNAs (30 nt), respectively.

6A.9. The 140 and 150 nucleotide bands correspond to adapter-ligated constructs derived from the 21 and 30 nucleotide RNA fragments, respectively. For miRNAs, isolate the bands corresponding to ~140 bp. For piRNAs, isolate the band corresponding to ~150 bp. For other small RNA, the band size may be different.

6A.10. Place the two gel slices from the same sample in one 1.5 ml tube and crush the gel slices with the RNase-free Disposable Pellet Pestles and then soak in 250 µl DNA Gel Elution buffer (1X).

6A.11. Rotate end-to-end for at least 2 hours at room temperature.

6A.12. Transfer the eluate and the gel debris to the top of a gel filtration column (for example Corning®, Costar®, Spin-X® Centrifuge Tube Filters (Cellulose Acetate Filters) (Sigma Aldrich # CLS8162).

6A.13. Centrifuge the filter for 2 min at > 13,200 rpm.

6A.14. Recover eluate and add 1 µl Linear Acrylamide, 25 µl 3M sodium acetate, pH 5.5 and 750 µl of 100% ethanol.

6A.15. Vortex well.

6A.16. Precipitate in a dry ice/methanol bath or at –80°C for at least 30 minutes.

6A.17. Spin in a microcentrifuge @ > 14,000 x g for 30 minutes at 4°C.

6A.18. Remove the supernatant taking care not to disturb the pellet.

6A.19. Wash the pellet with 80% ethanol by vortexing vigorously.

6A.20. Spin in a microcentrifuge @ > 14,000 x g for 30 minutes at 4°C.

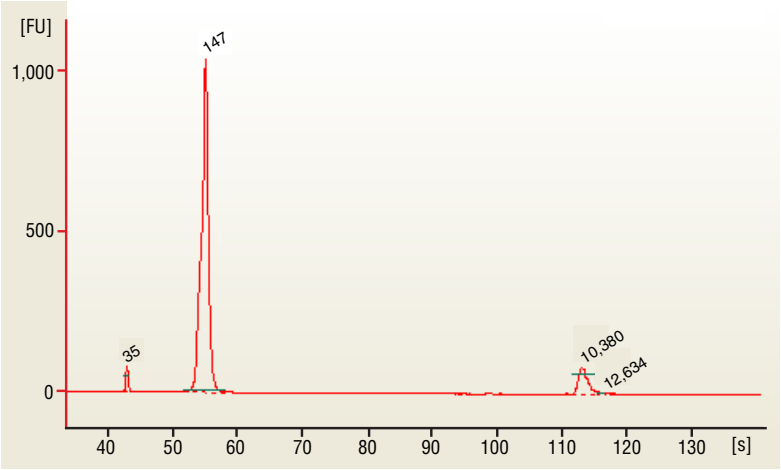
6A.21. Air dry pellet for up to 10 minutes at room temperature to remove residual ethanol.

6A.22. Resuspend pellet in 12 µl TE Buffer.

6A.23. Load 1 µl of the size selected purified library on a 2100 Bioanalyzer using a DNA 1000 or High Sensitivity DNA chip according to the manufacturer's instructions (Figure 3).

6A.24. Check the size, purity, and concentration of the sample.

Figure 3. Electropherogram trace of the gel size selected purified library from human brain total RNA.



6B. QC Check and Size Selection Using Pippin Prep

Size selection of the Small RNA library (147 bp) can done on Pippin Prep instrument using the 3% Agarose, dye free gel with internal standards (Sage Science # CDP3010).

Note: There are several different methods for performing size selection. It is recommended to choose the appropriate method based on the QC check of the library using the Bioanalyzer. Size selection using AMPure XP Beads does not remove small fragments. If you perform the QC check and your sample contains Adaptor dimer (127 bp peak) or excess primers (70-80 bp) it is recommended to use gel or Pippin Prep for size selection.

6B.1. Purify the PCR amplified cDNA construct (100 µl) using a Monarch PCR & DNA Cleanup Kit.

IMPORTANT: Use the 7:1 ratio of binding buffer:sample.Discard the flow through after each centrifugation step.

6B.2. Elute amplified DNA in 32 µl nuclease-free water.

Safe Stopping Point: It is safe to store the library at -20°C after PCR cleanup.

It is recommended to QC your library before performing size selection:

6B.3. Load 1 µl of the purified PCR reaction on the Bioanalyzer using a DNA 1000 chip according to the manufacturer's instructions (Figure 1). miRNA library should appear as a peak at 147 bp peak (that correspond for 21 nucleotide insert).

Program the protocol for size selection on Pippin Prep Instrument as follows:

6B.4. In the Pippin Prep software, go to the Protocol Editor Tab.

6B.5. Click “Cassette” folder, and select “3% DF Marker P”.

6B.6. Select the collection mode as “Range” and enter the size selection parameters as follow: BP start (105) and the BP end (155). BP Range Flag should indicate “broad”. *Note: This protocol is optimized to select for 147–149 bp peak. When targeting other small RNA these settings may have to be adjusted.*

6B.7. Click the “Use of Internal Standards” button.

6B.8. Make sure the “Ref Lane” values match the lane numbers.

6B.9. Press “Save As” and name and save the protocol.

Prepare sample for size selection as follows:

6B.10. Bring loading solution to room temperature

6B.11. For each sample, combine 30 µl sample with 10 µl of DNA marker P (labeled P).

6B.12. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.

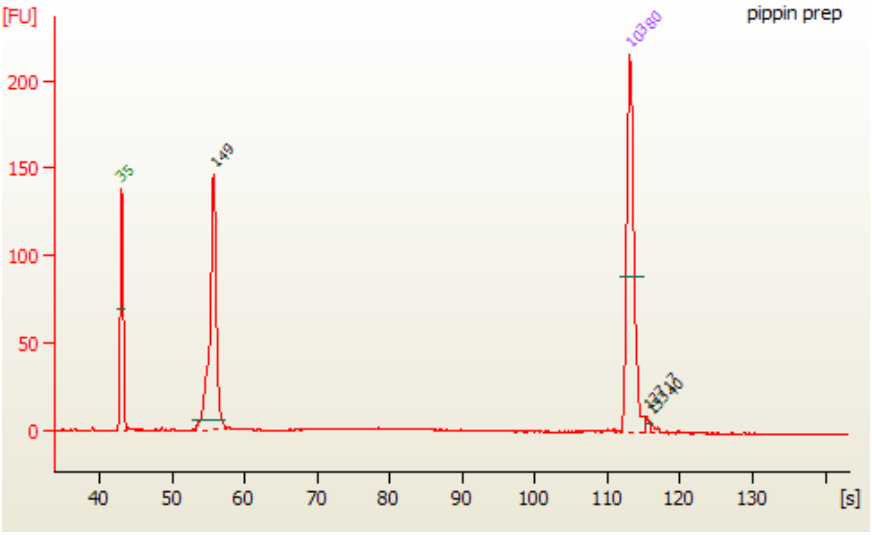
6B.13. Load 40 µl (DNA plus marker) on one well of the 3% agarose cassette.

6B.14. Run the program with the settings indicated above.

6B.15. After sample has been eluted, collect 40 µl sample from elution well. Run 1 µl in a Bioanalzyer using the high sensitivity chip.

Note: If the Ethidium Bromide free cassettes was used, no purification is required before running sample on the bioanalyzer.

Figure 4. Electropherogram trace of Pippin Prep size selected library from human brain total RNA.



6C. QC Check and Size Selection using AMPure XP Beads

Note: Bead size selection is only recommended for samples showing no primer dimer and no adaptor dimer on Bioanalyzer. It will be suitable to remove peaks > 150 bp. If fragments larger than 150 bp are abundant, two rounds of bead size selection may be necessary to completely eliminate the high molecular weight fragments.

There are several different methods for performing size selection. It is recommended to choose the appropriate method based on the QC check of the library using Bioanalyzer.

6C.1. Purify the PCR amplified cDNA construct (100 µl) using a Monarch PCR & DNA Kit.

IMPORTANT: Use the 7:1 ratio of binding buffer:sample.Discard the flow through after each centrifugation step.

6C.2. Elute amplified DNA in 27.5 µl Nuclease-free Water.

Safe Stopping Point: It is safe to store the library at -20°C after PCR cleanup.

6C.3. Load 1 µl of the purified PCR reaction on the Bioanalyzer using a DNA 1000 chip according to the manufacturer's instructions (Figure 1).

6C.4. To the purified PCR reaction (25 µl), add 32.5 µl (1.3X) of resuspended AMPure XP beads and mix well on a vortex mixer or by pipetting up and down at least 10 times.

6C.5. Incubate for 5 minutes at room temperature.

6C.6. Place the tube on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully transfer the supernatant (57.5 µl) to a new tube **(Caution: do not discard the supernatant)**. Discard beads that contain the large DNA fragments.

6C.7. Add 92.5 µl (3.7X) of resuspended AMPure XP beads to the supernatant (57.5 µl), mix well and incubate for 5 minutes at room temperature.

6C.8. Place the tube on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets **(Caution: do not discard beads)**.

6C.9. Add 200 µl of freshly prepared 80% ethanol to the tube while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.

6C.10. Repeat Step 6C.9. once.

6C.11. Briefly spin the tube, and put the tube back in the magnetic stand.

6C.12. Completely remove the residual ethanol, and air dry beads for up to 10 minutes while the tube is on the magnetic stand with lid open.

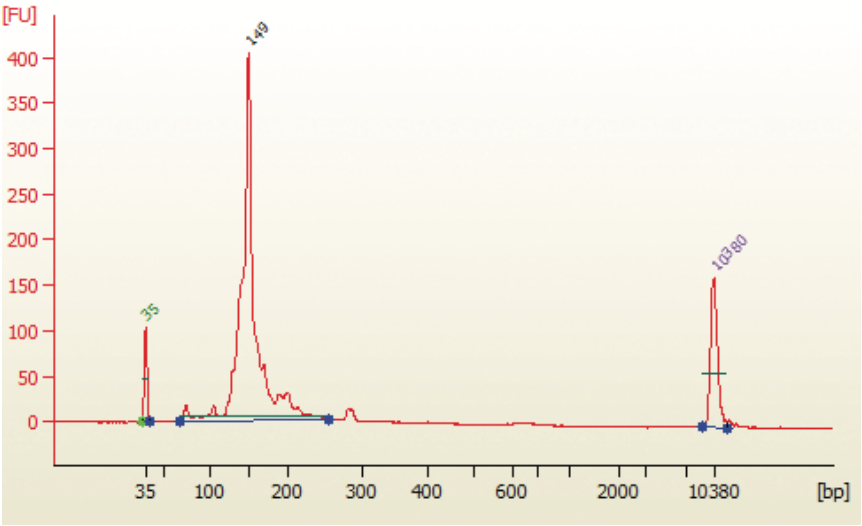
Caution: Do not overdry the beads, which may result in lower recovery of the DNA target. Elute the sample when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack, they are too dry.

6C.13. Elute the DNA target from the beads with 15 µl nuclease-free water. Mix well on a vortex mixer or by pipetting up and down, incubate for 2 minutes and put the tube in the magnetic stand until the solution is clear.

6C.14. Transfer the supernatant to a clean PCR tube.

6C.15. Run 1 µl on the Bioanalyzer High Sensitivity chip. Check peak distribution and concentration of the small RNA library.

Figure 5. Electropherogram trace of the bead size selected purified library from human brain total RNA.



NEBNext Adaptors and Primers for Illumina (NEB #E7300)

PRODUCT	INDEX PRIMER SEQUENCE	EXPECTED INDEX PRIMER SEQUENCE READ
NEBNext SR Primer for Illumina (10 µM)	5´-AATGATACGGCGACCACCGAGATCTACACGTTCAGAGTTCTACAGTCCG*A-3´	N/A
NEBNext SR RT Primer for Illumina	5´-AGACGTGTGCTCTTCCGATCT-3´	N/A
NEBNext Index 1 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ATCACG
NEBNext Index 2 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CGATGT
NEBNext Index 3 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TTAGGC
NEBNext Index 4 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TGACCA
NEBNext Index 5 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ACAGTG
NEBNext Index 6 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GCCAAT
NEBNext Index 7 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CAGATC
NEBNext Index 8 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ACTTGA
NEBNext Index 9 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GATCAG
NEBNext Index 10 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TAGCTT
NEBNext Index 11 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GGCTAC
NEBNext Index 12 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CTTGTA
NEBNext 3´ SR Adaptor for Illumina	5´-rAppAGATCGGAAGAGCACACGTCT-NH_2-3´	N/A
NEBNext 5´ SR Adaptor for Illumina	5´- rGrUrUrCrArGrArGrUrUrCrUrArCrArGrUrCrCrGrArCrGrArUrC-3´	N/A

Where * indicates phosphorothioate bond.

Note: If fewer than 12 indexes are used in a lane for sequencing, it is recommended to use the following combinations:

Pool of 2 samples: Index #6 and 12
Pool of 3 samples: Index #4, 6 and 12
Pool of 6 samples: Index #2, 4, 5, 6, 7 and 12

NEBNext Adaptors and Primers for Illumina (NEB #E7580)

PRODUCT	INDEX PRIMER SEQUENCE	EXPECTED INDEX PRIMER SEQUENCE READ
NEBNext Index 13 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATTTGACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	AGTCAA
NEBNext Index 14 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATGGAACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	AGTTCC
NEBNext Index 15 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATTGACATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ATGTCA
NEBNext Index 16 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATGGACGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CCGTCC
NEBNext Index 17 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATCTCTACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GTAGAG
NEBNext Index 18 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATGCGGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GTCCGC
NEBNext Index 19 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATTTTCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GTGAAA
NEBNext Index 20 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATGGCCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GTGGCC
NEBNext Index 21 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATCGAAACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GTTTCG
NEBNext Index 22 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATCGTACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CGTACG
NEBNext Index 23 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATCCACTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GAGTGG
NEBNext Index 24 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATGCTACCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GGTAGC
NEBNext 3´ SR Adaptor for Illumina	5´-rAppAGATCGGAAGAGCACACGTCT-NH_2-3´	N/A
NEBNext 5´ SR Adaptor for Illumina	5´-rGrUrUrCrArGrArGrUrUrCrUrArCrArGrUrCrCrGrArCrGrArUrC-3´	N/A

Where * indicates phosphorothioate bond.

Note: If fewer than 12 indexes are used in a lane for sequencing, it is recommended to use the following combinations:

Pool of 3 samples: Index #13, 18 and 23
Pool of 4 samples: Index #13, 14, 16 and 18

NEBNext Adaptors and Primers for Illumina (NEB #E7560)

NEBNext Index 1–48 Primers for Illumina

PRODUCT	INDEX PRIMER SEQUENCE	EXPECTED INDEX PRIMER SEQUENCE READ
NEBNext Index 1 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**CGTGAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ATCACG
NEBNext Index 2 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**ACATCG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CGATGT
NEBNext Index 3 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**GCCTAA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TTAGGC
NEBNext Index 4 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**TGGTCA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TGACCA
NEBNext Index 5 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT-**CACTGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ACAGTG
NEBNext Index 6 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**ATTGGC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GCCAAT
NEBNext Index 7 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**GATCTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CAGATC
NEBNext Index 8 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**TCAAGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ACTTGA
NEBNext Index 9 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**CTGATC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GATCAG
NEBNext Index 10 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT-**AAGCTA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TAGCTT
NEBNext Index 11 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**GTAGCC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GGCTAC
NEBNext Index 12 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**TACAAG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CTTGTA
NEBNext Index 13 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**TTGACT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	AGTCAA
NEBNext Index 14 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**GGAACT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	AGTTCC
NEBNext Index 15 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**TGACAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ATGTCA
NEBNext Index 16 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**GGACGG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CCGTCC
NEBNext Index 17 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**CTCTAC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GTAGAG
NEBNext Index 18 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**GCGGAC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GTCCGC
NEBNext Index 19 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**TTTCAC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GTGAAA
NEBNext Index 20 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**GGCCAC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GTGGCC

NEBNext Index 21 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**CGAAAC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GTTTCG
NEBNext Index 22 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**CGTACG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CGTACG
NEBNext Index 23 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**CCACTC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GAGTGG
NEBNext Index 24 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**GCTACC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GGTAGC
NEBNext Index 25 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**ATCAGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ACTGAT
NEBNext Index 26 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**GCTCAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ATGAGC
NEBNext Index 27 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**AGGAAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ATTCCT
NEBNext Index 28 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**CTTTTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CAAAAG
NEBNext Index 29 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**TAGTTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CAACTA
NEBNext Index 30 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**CCGGTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CACCGG
NEBNext Index 31 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**ATCGTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CACGAT
NEBNext Index 32 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**TGAGTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CACTCA
NEBNext Index 33 Primer for Illumina (10 µM)	5´- CAAGCAGAAGACGGCATACGAGAT**CGCCTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CAGGCG
NEBNext Index 34 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**GCCATG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CATGGC
NEBNext Index 35 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**AAAATG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CATTTT
NEBNext Index 36 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**TGTTGG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CCAACA
NEBNext Index 37 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**ATTCCG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CGGAAT
NEBNext Index 38 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**AGCTAG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CTAGCT
NEBNext Index 39 Primer for Illumina (10 µM)	5´- CAAGCAGAAGACGGCATACGAGAT**GTATAG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CTATAC
NEBNext Index 40 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**TCTGAG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CTCAGA
NEBNext Index 41 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**GTCGTC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GACGAC
NEBNext Index 42 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**CGATTA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TAATCG
NEBNext Index 43 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**GCTGTA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TACAGC

NEBNext Index 44 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**ATTATA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TATAAT
NEBNext Index 45 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**GAATGA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TCATTC
NEBNext Index 46 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**TCGGGA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TCCCGA
NEBNext Index 47 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**CTTCGA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TCGAAG
NEBNext Index 48 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGAT**TGCCGA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TCGGCA
NEBNext 3´ SR Adaptor for Illumina	5´-rAppAGATCGGAAGAGCACACGTCT-NH2-3´	N/A
NEBNext 5´ SR Adaptor for Illumina	5´-rGrUrUrCrArGrArGrUrUrCrUrArCrArGrUrCrCrGrArCrGrArUrC -3´	N/A
NEBNext SR RT Primer for Illumina	5´-AGACGTGTGCTCTTCCGATCT-3´	N/A
NEBNext SR Primer for Illumina	5´-AATGATACGGCGACCACCGAGATCTACACGTTCAGAGTTCTACAGTCCG-s-A-3´	N/A

Where * indicates phosphorothioate bond.

NEBNext Adaptors and Primers for Illumina (#E7330)

PRODUCT	INDEX PRIMER SEQUENCE	EXPECTED INDEX PRIMER SEQUENCE READ
NEBNext SR Primer for Illumina (10 µM)	5´-AATGATACGGCGACCACCGAGATCTACACGTTCAGAGTTCTACAGTCCG*A-3´	N/A
NEBNext SR RT Primer for Illumina	5´-AGACGTGTGCTCTTCCGATCT-3´	N/A
NEBNext Index 1 Primer for Illumina (10 µM)	5´-CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ATCACG
NEBNext 3´ SR Adaptor for Illumina	5´-rAppAGATCGGAAGAGCACACGTCT-NH_2-3´	N/A
NEBNext 5´ SR Adaptor for Illumina	5´- rGrUrUrCrArGrArGrUrUrCrUrArCrArGrUrCrCrGrArCrGrArUrC-3´	N/A

Where * indicates phosphorothioate bond.

Kit Components

NEB #E7300S Table of Components

NEB #	PRODUCT	VOLUME
E7301A	NEBNext 3′ Ligation Reaction Buffer	0.24 ml
E7288A	NEBNext 3′ Ligation Enzyme Mix	0.072 ml
E7332A	NEBNext 3′ SR Adaptor for Illumina	0.024 ml
E7328A	NEBNext 5′ SR Adaptor for Illumina	1350 pmol
E7304A	NEBNext 5′ Ligation Reaction Buffer	0.024 ml
E7305A	NEBNext 5′ Ligation Enzyme Mix	0.06 ml
E7333A	NEBNext SR RT Primer for Illumina	0.024 ml
E7334A	NEBNext First Strand Synthesis Reaction Buffer	0.192 ml
E7355A	ProtoScript II Reverse Transcriptase	0.024 ml
E7308A	Murine RNase Inhibitor	0.024 ml
E7309A	LongAmp *Taq* 2X Master Mix	1.2 ml
E7310A	NEBNext SR Primer for Illumina	0.060 ml
E6138A	Gel Loading Dye, Blue	0.2 ml
E7323A	Quick-Load pBR322 MspI-DNA Digest	0.24 ml
E7324A	DNA Gel Elution Buffer	12 ml
E7325A	Linear Acrylamide	0.048 ml
E7326A	TE Buffer	0.48 ml
E7327A	Nuclease-free Water	5.0 ml
E7311A	NEBNext Index 1 Primer for Illumina	0.010 ml
E7312A	NEBNext Index 2 Primer for Illumina	0.010 ml
E7313A	NEBNext Index 3 Primer for Illumina	0.010 ml
E7314A	NEBNext Index 4 Primer for Illumina	0.010 ml
E7315A	NEBNext Index 5 Primer for Illumina	0.010 ml
E7316A	NEBNext Index 6 Primer for Illumina	0.010 ml
E7317A	NEBNext Index 7 Primer for Illumina	0.010 ml
E7318A	NEBNext Index 8 Primer for Illumina	0.010 ml
E7319A	NEBNext Index 9 Primer for Illumina	0.010 ml
E7320A	NEBNext Index 10 Primer for Illumina	0.010 ml
E7321A	NEBNext Index 11 Primer for Illumina	0.010 ml
E7322A	NEBNext Index 12 Primer for Illumina	0.010 ml

Kit Components

NEB #E7300L Table of Components

NEB	PRODUCT	VOLUME
E7301AA	NEBNext 3′ Ligation Reaction Buffer	0.96 ml
E7288AA	NEBNext 3′ Ligation Enzyme Mix	0.288 ml
E7332AA	NEBNext 3′ SR Adaptor for Illumina	0.096 ml
E7328A	NEBNext 5′ SR Adaptor for Illumina	1350 pmol
E7304AA	NEBNext 5′ Ligation Reaction Buffer	0.096 ml
E7305AA	NEBNext 5′ Ligation Enzyme Mix	0.24 ml
E7333AA	NEBNext SR RT Primer for Illumina	0.096 ml
E7334AA	NEBNext First Strand Synthesis Reaction Buffer	0.768 ml
E7355AA	ProtoScript II Reverse Transcriptase	0.096 ml
E7308AA	Murine RNase Inhibitor	0.096 ml
E7309AA	LongAmp *Taq* 2X Master Mix	4.8 ml
E7310AA	NEBNext SR Primer for Illumina	0.240 ml
E6138AA	Gel Loading Dye, Blue	1 ml
E7323AA	Quick-Load pBR322 MspI-DNA Digest	0.96 ml
E7324AA	DNA Gel Elution Buffer	48 ml
E7325AA	Linear Acrylamide	0.192 ml
E7326AA	TE Buffer	1.92 ml
E7327AA	Nuclease-free Water	20.0 ml
E7311AA	NEBNext Index 1 Primer for Illumina	0.040 ml
E7312AA	NEBNext Index 2 Primer for Illumina	0.040 ml
E7313AA	NEBNext Index 3 Primer for Illumina	0.040 ml
E7314AA	NEBNext Index 4 Primer for Illumina	0.040 ml
E7315AA	NEBNext Index 5 Primer for Illumina	0.040 ml
E7316AA	NEBNext Index 6 Primer for Illumina	0.040 ml
E7317AA	NEBNext Index 7 Primer for Illumina	0.040 ml
E7318AA	NEBNext Index 8 Primer for Illumina	0.040 ml
E7319AA	NEBNext Index 9 Primer for Illumina	0.040 ml
E7320AA	NEBNext Index 10 Primer for Illumina	0.040 ml
E7321AA	NEBNext Index 11 Primer for Illumina	0.040 ml
E7322AA	NEBNext Index 12 Primer for Illumina	0.040 ml

Kit Components

NEB #E7580S Table of Components

NEB	PRODUCT	VOLUME
E7301A	NEBNext 3′ Ligation Reaction Buffer (2X)	0.24 ml
E7288A	NEBNext 3′ Ligation Enzyme Mix	0.072 ml
E7332A	NEBNext 3′ SR Adaptor for Illumina	0.024 ml
E7328A	NEBNext 5′ SR Adaptor for Illumina	1350 pmol
E7304A	NEBNext 5′ Ligation Reaction Buffer (10X)	0.024 ml
E7305A	NEBNext 5′ Ligation Enzyme Mix	0.06 ml
E7333A	NEBNext SR RT Primer for Illumina	0.024 ml
E7334A	NEBNext First Strand Synthesis Reaction Buffer	0.192 ml
E7355A	ProtoScript® II Reverse Transcriptase	0.024 ml
E7308A	Murine RNase Inhibitor	0.024 ml
E7309A	LongAmp® *Taq* 2X Master Mix	1.2 ml
E7310A	NEBNext SR Primer for Illumina	0.060 ml
E6138A	Gel Loading Dye, Blue (6X)	0.2 ml
E7323A	Quick-Load® pBR322 DNA-MspI Digest	0.24 ml
E7324A	DNA Gel Elution Buffer, 1X	12 ml
E7325A	Linear Acrylamide (10 mg/ml)	0.048 ml
E7326A	TE Buffer	0.48 ml
E7327A	Nuclease-free Water	5.0 ml
E7581A	NEBNext Index 13 Primer for Illumina	0.010 ml
E7582A	NEBNext Index 14 Primer for Illumina	0.010 ml
E7583A	NEBNext Index 15 Primer for Illumina	0.010 ml
E7584A	NEBNext Index 16 Primer for Illumina	0.010 ml
E7585A	NEBNext Index 17 Primer for Illumina	0.010 ml
E7586A	NEBNext Index 18 Primer for Illumina	0.010 ml
E7587A	NEBNext Index 19 Primer for Illumina	0.010 ml
E7588A	NEBNext Index 20 Primer for Illumina	0.010 ml
E7589A	NEBNext Index 21 Primer for Illumina	0.010 ml
E7590A	NEBNext Index 22 Primer for Illumina	0.010 ml
E7591A	NEBNext Index 23 Primer for Illumina	0.010 ml
E7592A	NEBNext Index 24 Primer for Illumina	0.010 ml

Kit Components

NEB #E7580L Table of Components

NEB	PRODUCT	VOLUME
E7301AA	NEBNext 3′ Ligation Reaction Buffer (2X)	0.96 ml
E7288AA	NEBNext 3′ Ligation Enzyme Mix	0.288 ml
E7332AA	NEBNext 3′ SR Adaptor for Illumina	0.096 ml
E7328A	NEBNext 5′ SR Adaptor for Illumina	1350 pmol
E7304AA	NEBNext 5′ Ligation Reaction Buffer (10X)	0.096 ml
E7305AA	NEBNext 5′ Ligation Enzyme Mix	0.24 ml
E7333AA	NEBNext SR RT Primer for Illumina	0.096 ml
E7334AA	NEBNext First Strand Synthesis Reaction Buffer	0.768 ml
E7355AA	ProtoScript® II Reverse Transcriptase	0.096 ml
E7308AA	Murine RNase Inhibitor	0.096 ml
E7309AA	LongAmp® *Taq* 2X Master Mix	4.8 ml
E7310AA	NEBNext SR Primer for Illumina	0.240 ml
E6138AA	Gel Loading Dye, Blue (6X)	1 ml
E7323AA	Quick-Load® pBR322 DNA-MspI Digest	0.96 ml
E7324AA	DNA Gel Elution Buffer, 1X	48 ml
E7325AA	Linear Acrylamide (10 mg/ml)	0.192 ml
E7326AA	TE Buffer	1.92 ml
E7327AA	Nuclease-free Water	20.0 ml
E7581AA	NEBNext Index 13 Primer for Illumina	0.040 ml
E7582AA	NEBNext Index 14 Primer for Illumina	0.040 ml
E7583AA	NEBNext Index 15 Primer for Illumina	0.040 ml
E7584AA	NEBNext Index 16 Primer for Illumina	0.040 ml
E7585AA	NEBNext Index 17 Primer for Illumina	0.040 ml
E7586AA	NEBNext Index 18 Primer for Illumina	0.040 ml
E7587AA	NEBNext Index 19 Primer for Illumina	0.040 ml
E7588AA	NEBNext Index 20 Primer for Illumina	0.040 ml
E7589AA	NEBNext Index 21 Primer for Illumina	0.040 ml
E7590AA	NEBNext Index 22 Primer for Illumina	0.040 ml
E7591AA	NEBNext Index 23 Primer for Illumina	0.040 ml
E7592AA	NEBNext Index 24 Primer for Illumina	0.040 ml

Kit Components

NEB #E7560S Table of Components

NEB	PRODUCT	VOLUME
E7301AA	NEBNext 3′ Ligation Reaction Buffer	0.96 ml
E7288AA	NEBNext 3′ Ligation Enzyme Mix	0.288 ml
E7332AA	NEBNext 3′ SR Adaptor for Illumina	0.096 ml
E7328A	NEBNext 5′ SR Adaptor for Illumina	1350
E7304AA	NEBNext 5′ Ligation Reaction Buffer	0.096 ml
E7305AA	NEBNext 5′ Ligation Enzyme Mix	0.24 ml
E7333AA	NEBNext SR RT Primer for Illumina	0.096 ml
E7334AA	NEBNext First Strand Synthesis Reaction	0.768 ml
E7355AA	ProtoScript II Reverse Transcriptase	0.096 ml
E7308AA	Murine RNase Inhibitor	0.096 ml
E7309AA	LongAmp *Taq* 2X Master Mix	4 x 1.2 ml
E7310AA	NEBNext SR Primer for Illumina	0.24 ml
E6138AA	Gel Loading Dye, Blue	1 ml
E7323AA	Quick-Load pBR322 MspI-DNA Digest	0.96 ml
E7324AA	DNA Gel Elution Buffer	48 ml
E7325AA	Linear Acrylamide	0.192 ml
E7326AA	TE Buffer	1.92 ml
E7327AA	Nuclease-free Water	20 ml
E7311A	NEBNext Index 1 Primer for Illumina	0.010 ml
E7312A	NEBNext Index 2 Primer for Illumina	0.010 ml
E7313A	NEBNext Index 3 Primer for Illumina	0.010 ml
E7314A	NEBNext Index 4 Primer for Illumina	0.010 ml
E7315A	NEBNext Index 5 Primer for Illumina	0.010 ml
E7316A	NEBNext Index 6 Primer for Illumina	0.010 ml
E7317A	NEBNext Index 7 Primer for Illumina	0.010 ml
E7318A	NEBNext Index 8 Primer for Illumina	0.010 ml
E7319A	NEBNext Index 9 Primer for Illumina	0.010 ml
E7320A	NEBNext Index 10 Primer for Illumina	0.010 ml
E7321A	NEBNext Index 11 Primer for Illumina	0.010 ml
E7322A	NEBNext Index 12 Primer for Illumina	0.010 ml
E7581A	NEBNext Index 13 Primer for Illumina	0.010 ml
E7582A	NEBNext Index 14 Primer for Illumina	0.010 ml
E7583A	NEBNext Index 15 Primer for Illumina	0.010 ml
E7584A	NEBNext Index 16 Primer for Illumina	0.010 ml
E7585A	NEBNext Index 17 Primer for Illumina	0.010 ml
E7586A	NEBNext Index 18 Primer for Illumina	0.010 ml
E7587A	NEBNext Index 19 Primer for Illumina	0.010 ml
E7588A	NEBNext Index 20 Primer for Illumina	0.010 ml
E7589A	NEBNext Index 21 Primer for Illumina	0.010 ml
E7590A	NEBNext Index 22 Primer for Illumina	0.010 ml
E7591A	NEBNext Index 23 Primer for Illumina	0.010 ml
E7592A	NEBNext Index 24 Primer for Illumina	0.010 ml
E7561A	NEBNext Index 25 Primer for Illumina	0.010 ml
E7713A	NEBNext Index 26 Primer for Illumina	0.010 ml
E7562A	NEBNext Index 27 Primer for Illumina	0.010 ml

E7714A	NEBNext Index 28 Primer for Illumina	0.010 ml
E7715A	NEBNext Index 29 Primer for Illumina	0.010 ml
E7716A	NEBNext Index 30 Primer for Illumina	0.010 ml
E7717A	NEBNext Index 31 Primer for Illumina	0.010 ml
E7718A	NEBNext Index 32 Primer for Illumina	0.010 ml
E7719A	NEBNext Index 33 Primer for Illumina	0.010 ml
E7720A	NEBNext Index 34 Primer for Illumina	0.010 ml
E7721A	NEBNext Index 35 Primer for Illumina	0.010 ml
E7722A	NEBNext Index 36 Primer for Illumina	0.010 ml
E7731A	NEBNext Index 37 Primer for Illumina	0.010 ml
E7732A	NEBNext Index 38 Primer for Illumina	0.010 ml
E7733A	NEBNext Index 39 Primer for Illumina	0.010 ml
E7734A	NEBNext Index 40 Primer for Illumina	0.010 ml
E7735A	NEBNext Index 41 Primer for Illumina	0.010 ml
E7736A	NEBNext Index 42 Primer for Illumina	0.010 ml
E7737A	NEBNext Index 43 Primer for Illumina	0.010 ml
E7738A	NEBNext Index 44 Primer for Illumina	0.010 ml
E7739A	NEBNext Index 45 Primer for Illumina	0.010 ml
E7740A	NEBNext Index 46 Primer for Illumina	0.010 ml
E7741A	NEBNext Index 47 Primer for Illumina	0.010 ml
E7742A	NEBNext Index 48 Primer for Illumina	0.010 ml

Kit Components

NEB #E7330S Table of Components

NEB	PRODUCT	VOLUME
E7301A	NEBNext 3′ Ligation Reaction Buffer	0.24 ml
E7288A	NEBNext 3′ Ligation Enzyme Mix	0.072 ml
E7332A	NEBNext 3′ SR Adaptor for Illumina	0.024 ml
E7328A	NEBNext 5′ SR Adaptor for Illumina	1350 pmol
E7304A	NEBNext 5′ Ligation Reaction Buffer	0.024 ml
E7305A	NEBNext 5′ Ligation Enzyme Mix	0.06 ml
E7333A	NEBNext SR RT Primer for Illumina	0.024 ml
E7334A	NEBNext First Strand Synthesis Reaction Buffer	0.192 ml
E7355A	ProtoScript II Reverse Transcriptase	0.024 ml
E7308A	Murine RNase Inhibitor	0.024 ml
E7309A	LongAmp *Taq* 2X Master Mix	1.2 ml
E7310A	NEBNext SR Primer for Illumina	0.060 ml
E6138A	Gel Loading Dye, Blue	0.2 ml
E7323A	Quick-Load pBR322 MspI-DNA Digest	0.24 ml
E7324A	DNA Gel Elution Buffer	12 ml
E7325A	Linear Acrylamide	0.048 ml
E7326A	TE Buffer	0.48 ml
E7327A	Nuclease-free Water	5.0 ml
E7329A	NEBNext Index 1 Primer for Illumina	0.12 ml

Kit Components

NEB #E7330L Table of Components

NEB	PRODUCT	VOLUME
E7301AA	NEBNext 3′ Ligation Reaction Buffer	0.96 ml
E7288AA	NEBNext 3′ Ligation Enzyme Mix	0.288 ml
E7332AA	NEBNext 3′ SR Adaptor for Illumina	0.096 ml
E7328A	NEBNext 5′ SR Adaptor for Illumina	1350 pmol
E7304AA	NEBNext 5′ Ligation Reaction Buffer	0.096 ml
E7305AA	NEBNext 5′ Ligation Enzyme Mix	0.24 ml
E7333AA	NEBNext SR RT Primer for Illumina	0.096 ml
E7334AA	NEBNext First Strand Synthesis Reaction Buffer	0.768 ml
E7355AA	ProtoScript II Reverse Transcriptase	0.096 ml
E7308AA	Murine RNase Inhibitor	0.096 ml
E7309AA	LongAmp *Taq* 2X Master Mix	4.8 ml
E7310AA	NEBNext SR Primer for Illumina	0.240 ml
E6138AA	Gel Loading Dye, Blue	1 ml
E7323AA	Quick-Load pBR322 MspI-DNA Digest	0.96 ml
E7324AA	DNA Gel Elution Buffer	48 ml
E7325AA	Linear Acrylamide	0.192 ml
E7326AA	TE Buffer	1.92 ml
E7327AA	Nuclease-free Water	20.0 ml
E7329AA	NEBNext Index 1 Primer for Illumina	0.48 ml

Checklist:

1. Ligate the 3′ SR Adaptor

[_] 1.0. Adaptor Dilution if < 100 ng total RNA input 1:2

[_] 1.1. Add Reagents to 1-6 µl sample:

[_] 1 µl SR Adaptor

[_] X µl nuclease free water

[_] 1.2. Mix and incubate at 70°C for 2 min. Transfer to ice.

[_] 1.3. Add Reagents

[_] 10 µl 3′ Ligation Reaction Buffer (2X)

[_] 3 µl 3′ Ligation Enzyme Mix

[_] 1.4. Mix and incubate at 25°C for 1 hour

2. Hybridize the Reverse Transcription Primer

[_] 2.0. Dilute adaptor if necessary

[_] 2.1. Add reagents to sample:

[_] 4.5 µl water

[_] 1 µl SR RT Primer

[_] 2.2. Mix and incubate 75°C for 5 min, 37°C for 15 min, 25°C for 15 min.

3. Ligate the 5′ SR Adaptor

[_] 3.1. Resuspend 5′ SR adaptor in 120 µl nuclease free water; dilute?

[_] 3.2. Aliquot.

[_] 3.3. Denature one aliquot 70°C for 2 min., then immediately put on ice

[_] 3.4. Add Reagents to Sample

[_] 1 µl 5′ SR adaptor

[_] 1 µl 5′ Ligation Reaction Buffer (10X)

[_] 2.5 µl 5′ Ligation Enzyme

[_] 3.5. Mix and incubate at 25°C for 1hr.

4. Perform Reverse Transcription

[_] 4.1. Add Reagents to Sample

[_] 8 µl First Strand Synthesis Reaction Buffer

[_] 1 µl Murine RNase Inhibitor

[_] 1 µl ProtoScript II Reverse Transcriptase

[_] 4.2. Mix and incubate at 50°C for 1 hour.

[_] 4.3. Immediately proceed to PCR or heat inactivate at 70°C for 15 min

5. Perform PCR Amplification

[_] 5.1. Add Reagents to Sample

[_] 50 µl LongAmp Taq 2X Master Mix

[_] 2.5 µl SR primer for Illumina

[_] 2.5 µl Index Primer

[_] 5 µl Nuclease-free water

[_] 5.2. Mix and thermal cycle (94°C 30 Sec, 12-15 cycles of 94°C 15 sec, 62°C 30 sec, 70°C 15 sec; 70°C for 5 min, 4°C hold)

6. Quality Control Check and Size Selection

6A. QC Check and Size Selection using 6% Poly Acrylamide Gel

[_] 6A.1 Purify the PCR using Monarch PCR & DNA Cleanup Kit

[_] 6A.2 Elute in 27.5 µl Nuclease-free Water

[_] 6A.3 Load 1 µl on a Bioanalyzer DNA 1000 Chip

[_] 6A.4 Vortex Gel Loading Dye well and mix 25 µl PCR product with 5 µl Gel Loading Dye

[_] 6A.5 Load 5 µl Quick-Load pBR322 DNA-MspI on one well

[_] 6A.6 Load two wells with each sample

[_] 6A.7 Run 1 hour 120V

[_] 6A.8 Stain with SYBR Gold 2-3 min and view

[_] 6A.9 Cut out appropriate bands

[_] 6A.10 Place gel in 1.5 ml tubes and soak in 250 µl gel elution buffer

[_] 6A.11 Rotate for at least 2 hours at RT

[_] 6A.12 Transfer eluate and gel to filter column

[_] 6A.13. Spin 2 min > 13,200 rpm

[_] 6A.14. Recover eluate, add

[_] 1 µl linear acrylamide

[_] 25 µl 3 M Sodium Acetate pH 5.5

[_] 750 µl 100% ethanol

[_] 6A.15. Vortex

[_] 6A.16. Precipitate for > 30 min

[_] 6A.17. Spin > 14,000 x g for 30 min at 4°C

[_] 6A.18. Remove supernatant

[_] 6A.19. Add 80% ethanol and vortex

[_] 6A.20. Spin > 14,000 x g for 30 min at 4°C

[_] 6A.21. Air dry pellet 10 min

[_] 6A.22. Resuspend pellet in 12 µl TE buffer

[_] 6A.23. Load 1 µl on Bioanalyzer

[_] 6A.24. Check size, concentration and purity

6B. QC Check and Size Selection Using Pippin Prep

[_] 6B.1. Purify the PCR using Monarch PCR and DNA Cleanup Kit

[_] 6B.2. Elute in 32 µl Nuclease-free Water

[_] 6B.3. Load 1 µl on a Bioanalyzer DNA 1000 Chip

[_] 6B.4. Go to protocol editor tab

[_] 6B.5. Click cassette folder and select 3% DF marker P

[_] 6B.6. Select collection mode as “Range”; BP Start (105) and BP End (155), range flag broad

[_] 6B.7. Use internal standards

[_] 6B.8. Check ref lane values match lane numbers

[_] 6B.9. Save protocol

[_] 6B.10. Warm loading solution to RT

[_] 6B.11. Combine 30 µl sample and 10 µl DNA Marker P

[_] 6B.12. Vortex and quick spin

[_] 6B.13. Load 40 µl sample in agarose cassette

[_] 6B.14. Run program

[_] 6B.15. Collect sample from elution well and load 1 µl on Bioanalyzer High Sensitivity Chip

6C. QC Check and Size Selection using AMPure XP or SPRIselect Beads

[_] 6C.1. Purify the PCR using Monarch PCR & DNA Cleanup Kit

[_] 6C.2. Elute in 27.5 µl Nuclease-free Water

[_] 6C.3. Load 1 µl on a Bioanalyzer DNA 1000 Chip

[_] 6C.4. Add 32.5 µl of beads to 25 µl sample and mix by pipetting 10 times

[_] 6C.5. Incubate 5 min

[_] 6C.6. Place tubes on magnet, separate, and transfer supernatant to a new tube (keep supernatant!)

[_] 6C.7. Add 92.5 µl of beads to the supernatant and mix by pipetting 10 times. Incubate 5 min

[_] 6C.8. Place tubes on magnet. Wait 5 min then remove the supernatant (keep the beads)

[_] 6C.9. On magnet add 200 µl 80% ethanol, wait 30 seconds and remove

[_] 6C.10. Repeat Step 6C.9 once

[_] 6C.11. Briefly spin tube and return to magnet

[_] 6C.12. Remove residual ethanol, air dry beads, do not overdry

[_] 6C.13. Off magnet add 15 µl Nuclease-free Water; mix by pipetting 10 times. Incubate 2 min; place tubes on magnet. Wait 5 min

[_] 6C.14. Transfer supernatant to a new tube

[_] 6C.15. Run 1 µl on a Bioanalyzer High Sensitivity Chip

Revision History

REVISION #	DESCRIPTION	DATE
2.0	Replaced M-MuLV Reverse Transcriptase RNase H⁻ with ProtoScript II Reverse Transcriptase.	
2.1	Formatted components with cap color information. Added Pippin Prep as an alternative method for size selection. Adding more recommendations on starting material and how to choose the method for size selection. Removed AMPure Bead Protocol that did not require the Qiagen column cleanup. Provided more clarification on how to choose size selection method based on Library QC. Added note to vortex loading dye prior to use. Added FAQ section to the manual.	
2.2	Updated marker settings for size selection using the Pippin Prep. Marker P is replacing Marker M.	
3.0	Change catalog number for NEBNext 3´ Ligation Enzyme Mix to NEB #E7288.	
4.0	Workflow diagram added. Protocol new numbering applied and text edits applied. New Checklist applied. FAQs deleted. Note added to NEBNext Index 1-12 Primers for Illumina. In Step 2.2 added heated lid temperature. Added where it is ok to premix reagents. Added Safe Stop after PCR and after PCR cleanup. Clarification of when it's ok to use AMPure XP Size Selection. Changed Pippen Prep Cassette Type and Marker to newly released product. Replaced Qiagen Cleanup kit with Monarch Cleanup Kit.	9/17
5.0	Change Index Primer sequences table to include adaptors and rename it as NEBNext Adaptors and Primers for Illumina. Create "Kit Component – Table of Components" for small and large size kits. Delete individual component information pages.	5/18
6.0	Combine all Small RNA: E7300, E7580, E7560 and E7330 to one manual	10/18
7.0	New manual format applied.	1/20
8.0	Update legal text	10/20

New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938-2723 Telephone: (978) 927-5054 Toll Free: (USA Orders) 1-800-632-5227 (USA Tech) 1-800-632-7799 Fax: (978) 921-1350 e-mail: info@neb.com