

# LIBRARY PREPARATION

## NEBNext<sup>®</sup> mRNA Library Prep Master Mix Set for Illumina<sup>®</sup>

Instruction Manual

NEB #E6110S/L  
12/60 reactions

 NEW ENGLAND  
*BioLabs*<sup>®</sup> Inc.

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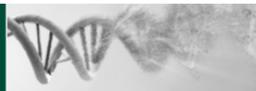
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## The Master Mix Set Includes:

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*The volumes provided are sufficient for preparation of up to 12 reactions (NEB #E6110S) and 60 reactions (NEB #E6110L). (All reagents should be stored at -20°C).*

- (white) NEBNext RNA Fragmentation Buffer (10X)
- (white) NEBNext RNA Fragmentation Stop Solution (10X)
- (pink) Random Primers (3 µg/µl)
- (pink) Murine RNase Inhibitor **RII**
- (pink) NEBNext First Strand Synthesis Reaction Buffer (5X)
- (pink) ProtoScript II Reverse Transcriptase
- (orange) NEBNext Second Strand Synthesis Enzyme Mix
- (orange) NEBNext Second Strand Synthesis Reaction Buffer (10X)
- (green) NEBNext End Repair Enzyme Mix **RII**
- (green) NEBNext End Repair Reaction Buffer (10X)
- (yellow) Klenow Fragment (3'→5' exo<sup>-</sup>)
- (yellow) NEBNext dA-Tailing Reaction Buffer (10X)
- (red) Quick T4 DNA Ligase **RII**
- (red) NEBNext Quick Ligation Reaction Buffer (5X)
- Nuclease-free water
- (blue) NEBNext Q5 Hot Start HiFi PCR Master Mix

## Required Materials Not Included:

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80% Ethanol (freshly prepared)

0.1X TE, pH 8.0

10 mM Tris-HCl, pH 7.5–8.0 (optional)

NEBNext Singleplex or Multiplex Oligos for Illumina (NEB #E7350, #E7335, #E7500, #E7710, #E7730, #E6609 or #E7600)

Agencourt<sup>®</sup> AMPure<sup>®</sup> XP Beads (Beckman Coulter, Inc., #A63881)

Agencourt RNAClean XP Beads (Beckman Coulter, Inc., #A63987)

Agilent Bioanalyzer

## Applications:

The NEBNext mRNA Library Prep Master Mix Set for Illumina contains enzymes and buffers that are ideally suited for sample preparation for next-generation sequencing, and for preparation of expression libraries. Each of these components must pass rigorous quality control standards and are Lot Controlled, both individually and as a set of reagents.

**Lot Control:** The lots provided in the NEBNext mRNA Library Prep Master Mix Set for Illumina are managed separately and are qualified by additional functional validation. Individual reagents undergo standard enzyme activity and quality control assays, and also meet stringent criteria in the additional quality controls listed on each individual component page.

**Functionally Validated:** Each set of reagents is functionally validated together through construction and sequencing of a transcriptome library on an 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.

# Protocols:

## Symbols

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

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

**Starting Material:** Purified mRNA (50–250 ng)

### 1.1 mRNA Fragmentation Protocol

1. Mix the following components in a sterile PCR tube:

Purified mRNA	1–18 $\mu$ l
○ (white) 10X RNA Fragmentation Reaction Buffer	2 $\mu$ l
Nuclease-free Water	variable
<hr/>	
Total volume	20 $\mu$ l

2. Incubate in a preheated thermal cycler for 5 minutes at 94°C. This is the optimal condition for eukaryotic mRNA to generate 200 nucleotide RNA fragments (see Figure 1.1). Other types of mRNA may require optimizing incubation time to obtain desired fragment size distribution.
3. Transfer tube to ice.
4. Add 2  $\mu$ l ○ (white) 10X RNA Fragmentation Stop Solution.

### 1.2 RNA Purification after RNA Fragmentation using Agencourt RNAClean XP

1. Add 28  $\mu$ l of the nuclease-free water to the 22  $\mu$ l fragmented RNA from Step 4 of the mRNA Fragmentation Protocol.
2. Add 2.2X (110  $\mu$ l) Agencourt RNAClean XP Beads and mix by pipetting up and down.
3. Incubate samples on ice for 15 minutes.
4. Place the tube on an appropriate magnetic rack to separate beads from the supernatant.
5. When the solution is clear (about 5 minutes), discard the supernatant.
6. Add 200  $\mu$ l of freshly prepared 80% ethanol to the tube while in the magnetic rack. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant

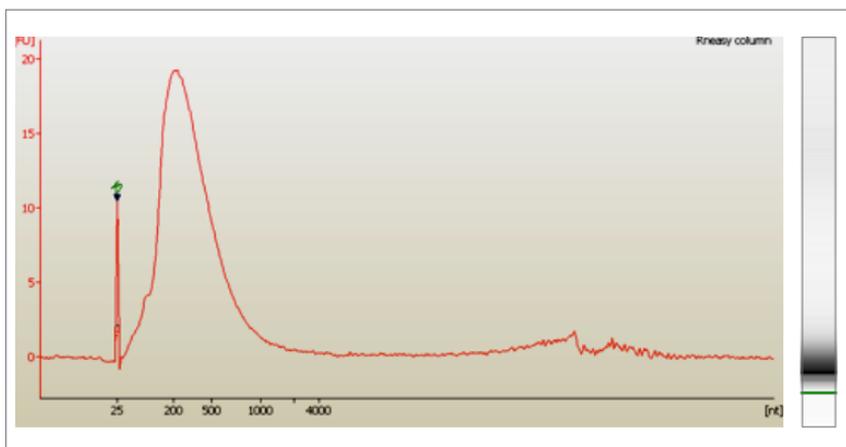
7. Repeat Step 6 once for a total of 2 washes.
8. Briefly spin the tube, and put the tube back in the magnetic rack.
9. Completely remove the residual ethanol, and air dry the beads.
10. Remove the tube from the magnetic rack. Elute RNA from the beads with 15  $\mu$ l nuclease-free water.
11. Mix well by pipetting up and down, and put the tube in the magnetic rack until the solution is clear.
12. Use 1  $\mu$ l of the supernatant to QC the fragment size distribution on an Agilent Bioanalyzer RNA pico chip (Section 1.3).
13. Transfer 13.5  $\mu$ l of the supernatant to a clean PCR tube.
14. Place the sample on ice and proceed with first strand cDNA synthesis in Section 1.4.

### 1.3 Assess the Yield and the Size Distribution of the Fragmented mRNA

Run 1  $\mu$ l in the Agilent Bioanalyzer<sup>®</sup> 2100 (Agilent Technologies, Inc.) using a RNA Pico chip.

**Note: Clean mRNA fragments show a distinct band on the Bioanalyzer (Figure 1.1).**

Figure 1.1: Relative size distribution of eukaryotic mRNA fragments as seen using the Bioanalyzer 2100.



## 1.4 First Strand cDNA Synthesis

- Mix the following components in a sterile PCR tube:

Fragmented mRNA	13.5 $\mu$ l
● (pink) Random Primers	1 $\mu$ l
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Total volume	14.5 $\mu$ l
- Incubate in a preheated thermal cycler for 5 minutes at 65°C.
- Spin tube briefly and place on ice
- To the fragmented mRNA and Random Primers add:

● (pink) 5X First Strand Synthesis Reaction Buffer	4 $\mu$ l
● (pink) Murine RNase Inhibitor	0.5 $\mu$ l
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Total volume	19 $\mu$ l
- Incubate in a preheated thermal cycler for 2 minutes at 25°C.
- Add 1  $\mu$ l ● ProtoScript II Reverse Transcriptase to the reaction.
- Incubate sample as follows with the heated lid set to 105°C:  
10 minutes at 25°C  
50 minutes at 42°C  
15 minutes at 70°C  
Hold at 4°C
- Place the tube on ice.

## 1.5 Second Strand cDNA Synthesis

- Add the following reagents to the First Strand Synthesis reaction:

Nuclease-free Water	48 $\mu$ l
● (orange) 10X Second Strand Synthesis Reaction Buffer	8 $\mu$ l
● (orange) Second Strand Synthesis Enzyme Mix	4 $\mu$ l
<hr/>	
Total volume	80 $\mu$ l
- Mix thoroughly by gentle pipetting.
- Incubate in a thermal cycler for 2.5 hours at 16°C.

**Note: If you need to stop at this point in the protocol after the 2.5 hours incubation at 16°C, samples can be left in the thermal cycler overnight at 4°C.**

## 1.6 Purify the double-stranded cDNA using 1.8X Agencourt AMPure XP Beads

1. Vortex AMPure XP Beads to resuspend.
2. Add 1.8X (144  $\mu$ l) of resuspended AMPure XP Beads to the second strand synthesis reaction (~80  $\mu$ l). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. 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.
5. Add 200  $\mu$ l of freshly prepared 80% ethanol to the tube while in the magnetic stand. Mix by pipetting up and down. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5 once.
7. Air dry the beads for 5 minutes while the tube is on the magnetic stand with lid open.

**Caution: Do not overdry the beads. This may result in lower recovery of DNA target.**

8. Remove the tube from the magnet. Elute the DNA target from the beads into 55  $\mu$ l 0.1X TE or 10 mM Tris-HCl. Mix well on a vortex mixer or by pipetting up and down, and incubate for 2 minutes at room temperature.
9. Put the tube in the magnetic stand until the solution is clear. Remove supernatant (50  $\mu$ l) and transfer to a clean 1.5 ml LoBind® (Eppendorf AG) tube.

## 1.7 End Repair of cDNA Library

1. Mix the following components in a sterile 1.5 ml microcentrifuge tube:

Purified double-stranded cDNA	50 $\mu$ l
Nuclease-free Water	35 $\mu$ l
● (green) NEBNext End Repair Reaction Buffer	10 $\mu$ l
● (green) NEBNext End Repair Enzyme Mix	5 $\mu$ l
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Total volume	100 $\mu$ l

2. Incubate in a heat block for 30 minutes at 20°C.

## 1.8 Purify the end-repaired cDNA using 1.8X Agencourt AMPure XP Beads. Note: X refers to original sample volume.

1. Vortex AMPure XP Beads to resuspend.
2. Add 1.8X (180  $\mu$ l) of resuspended AMPure XP Beads to the end-repaired DNA (~100  $\mu$ l). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. 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.
5. Add 200  $\mu$ l of freshly prepared 80% ethanol to the tube while in the magnetic stand. Mix by pipetting up and down. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5 once.
7. Air dry the beads for 5 minutes while the tube is on the magnetic stand with lid open.

**Caution: Do not overdry the beads. This may result in lower recovery of DNA target.**

8. Remove the tube from the magnet. Elute the DNA target from the beads into 46  $\mu$ l 0.1X TE or 10 mM Tris-HCl. Mix well on a vortex mixer or by pipetting up and down, and incubate for 2 minutes at room temperature.
9. Put the tube in the magnetic stand until the solution is clear. Remove supernatant (42  $\mu$ l) and transfer to a clean 1.5 ml LoBind tube.

## 1.9 dA-Tailing of cDNA Library

1. Mix the following components in a sterile 1.5 ml microcentrifuge tube:

Purified, End Repaired cDNA	42 $\mu$ l
● (yellow) 10X NEBNext dA-Tailing Reaction Buffer	5 $\mu$ l
● (yellow) Klenow Fragment (3' $\rightarrow$ 5' exo <sup>-</sup> )	3 $\mu$ l
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Total volume	50 $\mu$ l
2. Incubate in a heat block for 30 minutes at 37°C with the heated lid set to 42°C.

## 1.10 Purify the dA-Tailed DNA using 1.8X Agencourt AMPure XP Beads

1. Vortex AMPure XP Beads to resuspend.
2. Add 1.8X (90  $\mu$ l) of resuspended AMPure XP Beads to the dA tailed DNA (~50  $\mu$ l). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. 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.
5. Add 200  $\mu$ l of freshly prepared 80% ethanol to the tube while in the magnetic stand. Mix by pipetting up and down. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5 once.
7. Air dry the beads for 5 minutes while the tube is on the magnetic stand with the lid open.

**Caution: Do not overdry the beads. This may result in lower recovery of DNA target.**

8. Remove the tube from the magnet. Elute the DNA target from the beads into 42  $\mu$ l 0.1X TE or 10 mM Tris-HCl. Mix well on a vortex mixer or by pipetting up and down, and incubate for 2 minutes at room temperature.
9. Put the tube in the magnetic stand until the solution is clear. Remove supernatant (38  $\mu$ l) and transfer to a clean 1.5 ml LoBind tube.

## 1.11 Adaptor Ligation of cDNA Library

1. Mix the following components in a sterile 1.5 ml microcentrifuge tube:

Purified, dA-Tailed cDNA	38 $\mu$ l
● (red) 5X NEBNext Quick Ligation Reaction Buffer	10 $\mu$ l
● (red) NEBNext Adaptor (15 $\mu$ M)*	1 $\mu$ l
● (red) Quick T4 DNA Ligase	1 $\mu$ l
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Total volume	50 $\mu$ l

\* The NEBNext adaptor is provided in NEBNext Singleplex (NEB #E7350) or Multiplex (NEB #E7335, #E7500, #E7710, #E7730, #E6609, #E7600) Oligos for Illumina.

2. Incubate 15 minutes at room temperature.
3. Add 3  $\mu$ l of ●USER™ enzyme, mix by pipetting up and down, and incubate at 37°C for 15 minutes.

**NOTE: This step is only for use with NEBNext adaptors only. USER enzyme can be found in the NEBNext Singleplex ((NEB #E7350) or Multiplex (NEB #E7335, #E7500, #E7710, #E7730, #E6609, #E7600) Oligos for Illumina.**



***A precipitate can form upon thawing of the NEBNext Q5 Hot Start HiFi PCR Master Mix. To ensure optimal performance, place the master mix at room temperature while performing cleanup of adaptor-ligated DNA. Once thawed, gently mix by inverting the tube several times.***

## **1.12 Cleanup of Adaptor-Ligated DNA**

1. Vortex AMPure XP Beads to resuspend.
2. Add 90  $\mu$ l of resuspended AMPure XP Beads to the ligation reaction (~53  $\mu$ l). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. 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.
5. Add 200  $\mu$ l of freshly prepared 80% ethanol to the tube while in the magnetic stand. Mix by pipetting up and down. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5 once.
7. Air dry the beads for 5 minutes while the tube is on the magnetic stand with lid open.

**Caution: Do not overdry the beads. This may result in lower recovery of DNA target.**

8. Remove the tube/plate from the magnet. Elute the DNA target by adding 155  $\mu$ l of 10 mM Tris-HCl or 0.1 X TE to the beads for bead-based size selection.

**Note: For size selection using E-Gel size select gels, elute the DNA into 20  $\mu$ l 0.1X TE or 10 mM Tris-HCl.**

9. Mix well on a vortex mixer or by pipetting up and down and incubate for 2 minutes at room temperature.
10. Put the tube/PCR plate in the magnetic stand until the solution is clear. Remove supernatant (150  $\mu$ l) and transfer to a clean 1.5 ml LoBind tube.

### 1.13 Size Selection of Adaptor-ligated DNA using Agencourt AMPure XP Beads



*The following size selection protocol is for libraries with 200 bp inserts only. For libraries with larger fragment inserts, please optimize bead: DNA ratio accordingly.*

**Note: X refers to the original sample volume of 150  $\mu$ l.**

1. Add 135  $\mu$ l (0.9X) resuspended AMPure XP Beads to 150  $\mu$ l eluted DNA from step 9. Mix well on a vortex mixer or by pipetting up and down at least 10 times.
2. Incubate for 5 minutes at room temperature.
3. 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 to a new tube (**Caution: do not discard the supernatant**). Discard beads that contain the large fragments.
4. Add 30  $\mu$ l (0.2X) resuspended AMPure XP Beads to the supernatant, mix well and incubate for 5 minutes at room temperature.
5. Put 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**).
6. Add 200  $\mu$ l 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.
7. Repeat Step 6 once.
8. Briefly spin the tube, and put the tube back in the magnetic stand.
9. Completely remove the residual ethanol, and air dry beads for 5 minutes while the tube is on the magnetic stand with lid open.

**Caution: Do not overdry the beads. This may result in lower recovery of DNA target.**

10. Remove the tube/plate from the magnet. Elute the DNA target from the beads by adding 22  $\mu$ l of 10 mM Tris-HCl or 0.1X TE.
11. Mix well on a vortex mixer or by pipetting up and down and incubate for 2 minutes at room temperature.
12. Put the tube/PCR plate in the magnetic stand until the solution is clear. Transfer 20  $\mu$ l of the supernatant to a clean PCR tube, and proceed to PCR enrichment.

Adaptor ligated DNA can also be size selected using a E-Gel size select gel. After size selection, purify DNA sample on one QIAquick column and elute in 22  $\mu$ l of nuclease free water.

## 1.14 PCR Enrichment of Adaptor Ligated DNA



Follow Section 1.14A if you are using the following oligos (10  $\mu$ M primer):

NEBNext Singleplex Oligos for Illumina (NEB #E7350)

NEBNext Multiplex Oligos for Illumina (Set 1, NEB #E7335)

NEBNext Multiplex Oligos for Illumina (Set 2, NEB #E7500)

NEBNext Multiplex Oligos for Illumina (Set 3, NEB #E7710)

NEBNext Multiplex Oligos for Illumina (Set 4, NEB #E7730)

NEBNext Multiplex Oligos for Illumina (Dual Index Primers, NEB #E7600)

Follow Section 1.14B if you are using NEBNext Multiplex Oligos for Illumina (96 Index Primers, NEB #E6609).

### 1.14A PCR Enrich Adaptor Ligated cDNA Library

1. Mix the following components in sterile strip tubes:

Size Selected cDNA	20 $\mu$ l
● (blue) Index Primer/i7 Primer*, **	2.5 $\mu$ l
● (blue) Universal PCR Primer/i5 Primer*, ***	2.5 $\mu$ l
● (blue) NEBNext Q5 Hot Start HiFi PCR Master Mix	25 $\mu$ l
<hr/>	
Total volume	50 $\mu$ l

\* The primers are provided in NEBNext Singleplex (NEB #E7350) or Multiplex (NEB #E7335, #E7500, #E7710, #E7730, #E7600) Oligos for Illumina. For use with Dual Index Primers (NEB #E7600), look at the NEB #E7600 manual for valid barcode combinations and tips for setting up the PCR reaction.

\*\* For use with NEBNext Multiplex Oligos (NEB #E7335, #E7500, #E7710, #E7730) use only one index primer per PCR reaction. For use with Dual Index Primers (NEB #E7600) use only one i7 Primer per reaction.

\*\*\* For use with Dual Index Primers (NEB #E7600) use only one i5 Primer per reaction.

2. PCR cycling conditions:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	10 seconds	1
Denaturation	98°C	10 seconds	10–12*
Annealing/Extension	65°C	75 seconds	
Final Extension	65°C	5 minutes	1
Hold	4°C	∞	

\* The number of PCR cycles should be adjusted based on mRNA input. If 50 ng of purified mRNA is the starting input, it is recommended to perform 12 cycles of PCR.

3. Proceed to Purify using Ampure XP Beads in Section 1.15

### 1.14B PCR Enrich Adaptor Ligated cDNA Library

1. Mix the following components in sterile strip tubes:

Size Selected cDNA	20 µl
● (blue) Index/Universal Primer Mix*	5 µl
● (blue) NEBNext Q5 Hot Start HiFi PCR Master Mix	25 µl
<hr/>	
Total volume	50 µl

\* The primers are provided in NEBNext Multiplex Oligos for Illumina, NEB #E6609. Please refer to the NEB #E6609 manual for valid barcode combinations and tips for setting up PCR reactions.

2. PCR cycling conditions:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	10 seconds	1
Denaturation	98°C	10 seconds	10–12*
Annealing/Extension	65°C	75 seconds	
Final Extension	65°C	5 minutes	1
Hold	4°C	∞	

\* The number of PCR cycles should be adjusted based on mRNA input. If 50 ng of purified mRNA is the starting input, it is recommended to perform 12 cycles of PCR.

3. Proceed to Purify using Ampure XP Beads in Section 1.15

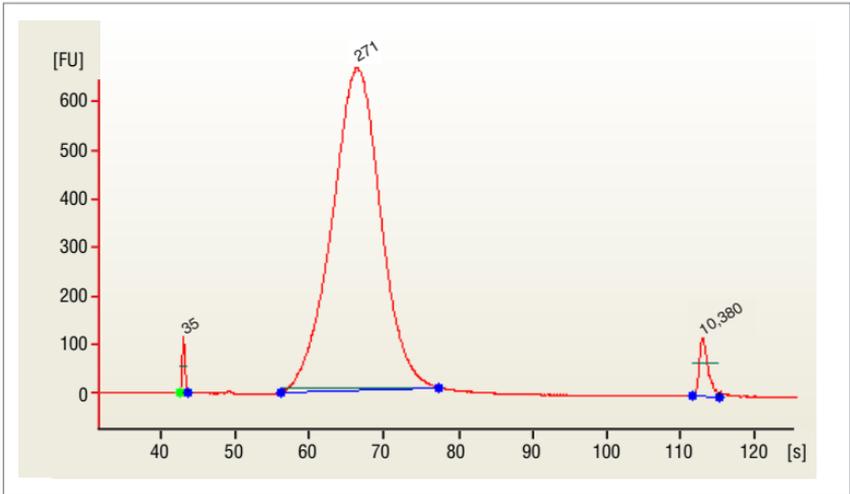
## 1.15 Purify using AMPure XP Beads

1. Vortex AMPure XP Beads to resuspend.
2. Add 60  $\mu\text{l}$  (1.2X) of resuspended AMPure XP Beads to the PCR reaction (~ 50  $\mu\text{l}$ ). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. 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.
5. Add 200  $\mu\text{l}$  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.
6. Repeat Step 5 once.
7. Air dry the beads for 5 minutes while the tube is on the magnetic stand with the lid open. **Caution: Do not overdry the beads. This may result in lower recover of DNA target.**
8. Remove the tube from the magnet. Elute the DNA target from the beads into 23  $\mu\text{l}$  0.1X TE Buffer. Mix well on a vortex mixer or by pipetting up and down, and incubate for 2 minutes at room temperature. Put the tube in the magnet stand until the solution is clear.
9. Transfer 20  $\mu\text{l}$  of the supernatant to a clean 1.5 ml LoBind tube. Libraries can be stored at  $-20^{\circ}\text{C}$ .

## 1.16 Assess Library Quality on a Bioanalyzer (High Sensitivity Chip)

Check that the electropherogram shows a narrow distribution with a peak size approximately 270 bp.

Figure 1.2: Example of mRNA Library size distribution on a Bioanalyzer.



# NEBNext RNA Fragmentation Buffer

#E6101A: 0.048 ml

Concentration: 10X

#E6101AA: 0.240 ml

Store at -20°C or 4°C

## 1X NEBNext RNA Fragmentation Buffer:

40 mM Tris-OAc

100 mM KOAc

30 mM Mg(OAc)<sub>2</sub>

pH 8.3 @ 25°C

## Quality Control Assays

**16-Hour Incubation:** 50 µl reactions containing 1X RNA Fragmentation Buffer and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing 1X RNA Fragmentation Buffer and 1 µg of T3 DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**Endonuclease Activity:** Incubation of a 10 µl reaction containing 1X RNA Fragmentation Buffer with 1 µg of φX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

**Phosphatase Activity:** Incubation of 1X RNA Fragmentation Buffer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub>) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Lot Controlled**

# NEBNext RNA Fragmentation Stop Solution

#E6102A: 0.048 ml

Concentration: 10X

#E6102AA: 0.240 ml

Store at -20°C

**1X NEBNext RNA Fragmentation Stop Solution:**

50 mM EDTA

## Quality Control Assays

**16-Hour Incubation:** 50 µl reactions containing 1X RNA Fragmentation Stop Solution and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing 1X RNA Fragmentation Stop Solution and 1 µg of T3 DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**Endonuclease Activity:** Incubation of a 10 µl reaction containing 1X RNA Fragmentation Stop Solution with 1 µg of φX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

**RNase Activity:** Incubation of a 10 µl reaction containing 1X RNA Fragmentation Stop Solution with 40 ng of RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by gel electrophoresis.

**Phosphatase Activity:** Incubation of 1X RNA Fragmentation Stop Solution in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub>) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Lot Controlled**

## Random Primers

#E6104A: 15  $\mu$ l

#E6104AA: 60  $\mu$ l

**Store at  $-20^{\circ}\text{C}$**

**Description:** This mixture of random hexanucleotides is used to prime DNA synthesis *in vitro* along multiple sites of template RNA.

**Sequence:** 5' d(N<sup>6</sup>) 3' [N=A,C,G,T]

**Phosphorylated:** No.

### Quality Control Assays

**16-Hour Incubation:** 50  $\mu$ l reactions containing 1  $\mu$ l Random Primers and 1  $\mu$ g of HindIII digested Lambda DNA incubated for 16 hours at  $37^{\circ}\text{C}$  results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50  $\mu$ l reactions containing 1  $\mu$ l Random Primers and 1  $\mu$ g of T3 DNA incubated for 16 hours at  $37^{\circ}\text{C}$  results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**Endonuclease Activity:** Incubation of a 10  $\mu$ l reaction containing 1  $\mu$ l Random Primers with 1  $\mu$ g of  $\phi$ X174 RF I supercoiled DNA for 4 hours at  $37^{\circ}\text{C}$  results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

**RNase Activity:** Incubation of a 10  $\mu$ l reaction containing 1  $\mu$ l Random Primers with 40 ng of RNA transcript for 16 hours at  $37^{\circ}\text{C}$  resulted in no detectable degradation of RNA as determined by gel electrophoresis.

**Phosphatase Activity:** Incubation of 1  $\mu$ l Random Primers in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM  $\text{MgCl}_2$ ) containing 2.5 mM *p*-nitrophenyl phosphate at  $37^{\circ}\text{C}$  for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Lot Controlled**

# Murine RNase Inhibitor

#E6105A: 15  $\mu$ l

#E6105AA: 30  $\mu$ l

Store at  $-20^{\circ}\text{C}$

**Description:** Murine RNase inhibitor is a 50 kDa recombinant protein of murine origin. The inhibitor specifically inhibits RNases A, B and C. It inhibits RNases by binding noncovalently in a 1:1 ratio with high affinity. It is not effective against RNase 1, RNase T1, S1 Nuclease, RNase H or RNase from *Aspergillus*. In addition, no inhibition of polymerase activity is observed when RNase Inhibitor is used with *Taq* DNA Polymerase, AMV or M-MuLV Reverse Transcriptases, or Phage RNA Polymerases (SP6, T7, or T3).

Recombinant murine RNase inhibitor does not contain the pair of cysteines identified in the human version that is very sensitive to oxidation, which causes inactivation of the inhibitor (1). As a result, murine RNase inhibitor has significantly improved resistance to oxidation compared to the human/porcine RNase inhibitors, even under conditions where the DTT concentration is low. Therefore, it is advantageous to use murine RNase inhibitor in reactions where high concentration DTT is adverse to the reaction (eg. Real-time RT-PCR).

**Source:** An *E. coli* strain that carries the Ribonuclease Inhibitor gene from mouse.

**Supplied in:** 20 mM HEPES-KOH, 50 mM KCl, 8 mM DTT and 50% glycerol.

## Quality Control Assays

**16-Hour Incubation:** 50  $\mu$ l reactions containing a minimum of 40 units of Murine RNase Inhibitor and 1  $\mu$ g of HindIII digested Lambda DNA incubated for 16 hours at  $37^{\circ}\text{C}$  results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50  $\mu$ l reactions containing a minimum of 40 units of Murine RNase Inhibitor and 1  $\mu$ g of T3 DNA incubated for 16 hours at  $37^{\circ}\text{C}$  results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**Exonuclease Activity:** Incubation of a 50  $\mu$ l reaction containing 200 units of Murine RNase Inhibitor with 1  $\mu$ g of a mixture of single and double-stranded [ $^3\text{H}$ ] *E. coli* DNA ( $20^6$  cpm/ $\mu$ g) for 4 hours at  $37^{\circ}\text{C}$  released  $< 0.5\%$  of the total radioactivity.

**Latent RNase Assay:** Heating the Murine RNase Inhibitor for 20 minutes at  $65^{\circ}\text{C}$ , followed by incubation of a 10  $\mu$ l reaction containing 40 units of RNase Inhibitor with 40 ng of RNA transcript for 4 hours at  $37^{\circ}\text{C}$  resulted in no detectable degradation of RNA as determined by gel electrophoresis.

**RNase Activity:** Incubation of a 10  $\mu$ l reaction containing 40 units of Murine RNase Inhibitor with 40 ng of RNA transcript for 16 hours at  $37^{\circ}\text{C}$  resulted in no detectable degradation of RNA as determined by gel electrophoresis.

**Endonuclease Activity:** Incubation of a 10  $\mu$ l reaction containing 40 units of Murine RNase Inhibitor with 1  $\mu$ g of  $\phi$ X174 RF I supercoiled DNA for 4 hours at  $37^{\circ}\text{C}$  results in  $< 10\%$  conversion to RF II (nicked molecules) as determined by gel electrophoresis.

**Phosphatase Activity:** Incubation of a minimum of 40 units of Murine RNase Inhibitor in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM  $\text{MgCl}_2$ ) containing 2.5 mM *p*-nitrophenyl phosphate at  $37^{\circ}\text{C}$  for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Lot Controlled**

**References:**

1. Kim, B.M. et al. (1999). *Protein Science*, 8, 430–434.

# NEBNext First Strand Synthesis Reaction Buffer

#E6106A: 0.048 ml

Concentration: 5X

#E6106AA: 0.240 ml

Store at -20°C

## 1X NEBNext First Strand Synthesis Reaction Buffer:

50 mM Tris-Acetate

75 mM KOAc

3.1 mM Mg(OAc)<sub>2</sub>

0.5 mM dNTPs each

pH 8.3 @ 25°C

## Quality Control Assays

**16-Hour Incubation:** 50 µl reactions containing 1X First Strand Synthesis Reaction Buffer and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing 1X First Strand Synthesis Reaction Buffer and 1 µg of T3 DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**Endonuclease Activity:** Incubation of a 10 µl reaction containing 1X First Strand Synthesis Reaction Buffer with 1 µg of φX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

**RNase Activity:** Incubation of a 10 µl reaction containing 1X First Strand Synthesis Reaction Buffer with 40 ng of RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by gel electrophoresis.

**Phosphatase Activity:** Incubation of 1X First Strand Synthesis Reaction Buffer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub>) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Lot Controlled**

# NEBNext Second Strand Synthesis Enzyme Mix

#E6107A: 0.048 ml

#E6107AA: 0.240 ml

Store at -20°C

**Description:** NEBNext Second Strand Synthesis Enzyme Mix is optimized to convert 10–100 ng of short single-stranded cDNAs to double-stranded cDNAs with Random Primers and NEBNext Second Strand Synthesis Reaction Buffer.

## **NEBNext Second Strand Synthesis Enzyme Mix:**

6,000 units/ml DNA Polymerase I (*E. coli*)

5,000 units/ml RNase H

25,000 units/ml *E. coli* DNA Ligase

**Supplied in:** 10 mM Tris-HCl (pH 7.5 @ 25°C), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA and 50% glycerol.

## Quality Control Assays

**SDS-PAGE Purity:** SDS-PAGE analysis of this enzyme indicates > 95% enzyme purity.

**Endonuclease Activity:** Incubation of a 10 µl reaction containing 1 µl Second Strand Synthesis Enzyme Mix with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

**Phosphatase Activity:** Incubation of a minimum of 1 µl Second Strand Synthesis Enzyme Mix in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub>) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Functional Activity:** One unit of the *E. coli* DNA Ligase ligated 50% of HindIII fragments of λ DNA (5' DNA termini concentration of 0.12 µM, 300 µg/ml) in a total reaction volume of 20 µl in 30 minutes at 16°C in 1X *E. coli* DNA Ligase Reaction Buffer. One unit of *E. coli* DNA Polymerase I incorporated 10 nmol of dNTP into acid-insoluble material in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X EcoPol Reaction Buffer with 33 µM dNTPs including [<sup>3</sup>H]-dTTP and 70 µg/ml denatured herring sperm DNA. Incubation of 50 units of RNase H with 1 µg sonicated and denatured [<sup>3</sup>H]-DNA (10<sup>5</sup> cpm/µg) for 30 minutes at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

**Lot Controlled**

# NEBNext Second Strand Synthesis Reaction Buffer

#E6108A: 0.096 ml

Concentration: 10X

#E6108AA: 0.480 ml

Store at -20°C

## 1X NEBNext Second Strand Synthesis Reaction Buffer:

20 mM Tris-HCl

12 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>

5 mM MgCl<sub>2</sub>

0.16 mM β-NAD

0.19 mM dNTPs each

pH 7.4 @ 25°C

## Quality Control Assays

**16-Hour Incubation:** 50 µl reactions containing 1X NEBNext Second Strand Synthesis Reaction Buffer and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing 1X Second Strand Synthesis Reaction Buffer and 1 µg of T3 DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**Endonuclease Activity:** Incubation of a 10 µl reaction containing 1X NEBNext Second Strand Synthesis Reaction Buffer with 1 µg of φX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

**RNase Activity:** Incubation of a 10 µl reaction containing 1X NEBNext Second Strand Synthesis Reaction Buffer with 40 ng of RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by gel electrophoresis.

**Phosphatase Activity:** Incubation of 1X NEBNext Second Strand Synthesis Reaction Buffer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub>) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Lot Controlled**

# NEBNext End Repair Enzyme Mix

#E6041A: 0.06 ml

#E6041AA: 0.300 ml



Store at -20°C

**Description:** NEBNext End Repair Enzyme Mix is optimized to convert 1 to 5 µg of fragmented to repaired DNA having 5'-phosphorylated, blunt ends.

## NEBNext End Repair Enzyme Mix:

T4 Polynucleotide Kinase

T4 DNA Polymerase

## Storage Conditions:

10 mM Tris-HCl

100 mM KCl

1 mM DTT

0.1 mM EDTA

50% Glycerol

0.1% Triton X-100

pH 7.4 @ 25°C

## Quality Control Assays

**SDS-PAGE Purity:** SDS-PAGE analyses of each individual enzyme indicates > 95% enzyme purity.

**Endonuclease Activity:** Incubation of a minimum of 10 µl of this enzyme mix with 1 µg of φX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

**Phosphatase Activity:** Incubation of a minimum of 10 µl of this enzyme mix in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub>) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Functional Activity (Nucleotide Incorporation):** 0.2 µl of this enzyme mix incorporates 10 nmol of dNTP into acid-precipitable material in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X T4 DNA Polymerase Reaction Buffer with 33 µM dNTPs including [<sup>3</sup>H]-dTTP, 70 µg/ml denatured herring sperm DNA and 50 µg/ml BSA.

**Functional Activity (Nucleotide Incorporation and Phosphorylation):** 5 µl of this enzyme mix repairs and phosphorylates the ends of > 95% of 10 µg of DNA fragments containing both 3' and 5' overhangs within 30 minutes at 20°C in 1X End Repair Buffer, as determined by capillary electrophoresis.

**Lot Controlled**

# NEBNext End Repair Reaction Buffer

#E6042A: 0.12 ml

Concentration: 10X

#E6042AA: 0.6 ml

Store at -20°C

## 1X NEBNext End Repair Reaction Buffer:

50 mM Tris-HCl

10 mM MgCl<sub>2</sub>

10 mM DTT

1 mM ATP

0.4 mM dATP

0.4 mM dCTP

0.4 mM dGTP

0.4 mM dTTP

pH 7.5 @ 25°C

## Quality Control Assays

**16-Hour Incubation:** 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**Endonuclease Activity:** Incubation of this reaction buffer at a 1X concentration with 1 µg of φX174 RF I DNA for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

**RNase Activity:** Incubation of this reaction buffer at 1X concentration with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

**Phosphatase Activity:** Incubation of this reaction buffer at a 1X concentration in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub>) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Lot Controlled**

# Klenow Fragment (3' → 5' exo<sup>-</sup>)

#E6044A: 0.036 ml

#E6044AA: 0.18 ml



**Store at -20°C**

**Description:** Klenow Fragment (3' → 5' exo<sup>-</sup>) is an N-terminal truncation of DNA Polymerase I which retains polymerase activity, but lacks 5' → 3' exonuclease activity. Mutations (D355A, E357A) abolish the 3' → 5' exonuclease activity (1). Klenow Fragment (3' → 5' exo<sup>-</sup>) with dA-Tailing buffer can be used to add a dAMP to the 3' end at a blunt DNA fragment (2).

**Source:** An *E. coli* strain containing a plasmid with a fragment of the *E. coli* polA (D355A, E357A) gene starting at codon 324.

**Supplied in:** 25 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

## Quality Control Assays

**SDS-PAGE Purity:** SDS-PAGE analysis of this enzyme indicates > 95% enzyme purity.

**16-Hour Incubation:** 50 µl reactions containing a minimum of 5 units of this enzyme and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing a minimum of 5 units of this enzyme and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**Endonuclease Activity:** Incubation of a minimum of 50 units of this enzyme with 1 µg of ϕX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

**Phosphatase Activity:** Incubation of a minimum of 50 units of this enzyme in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub>) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**RNase Activity:** Incubation of a minimum of 5 units of this enzyme with 40 ng of a FAM- labeled RNA transcript for 16 hours at 37°C results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

**Exonuclease Activity:** Incubation of a minimum of 200 units of this enzyme with 1 µg sonicated [<sup>3</sup>H] DNA (10<sup>5</sup> cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer releases < 0.1% radioactivity.

**3' → 5' Exonuclease Activity:** Incubation of a minimum of 50 units of enzyme in 20 µl of a 10 nM solution of a fluorescent 5'-FAM labeled oligonucleotide for 30 minutes at 37°C yields no detectable 3' → 5' degradation as determined by capillary electrophoresis.

**Functional Activity (Nucleotide Incorporation):** One unit of this enzyme incorporates 10 nmol of dNTP into acid-precipitable material in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 2 with 33 µM dNTPs including [<sup>3</sup>H]-dTTP, 70 µg/ml denatured herring sperm DNA and 50 µg/ml BSA.

## **References:**

1. Derbyshire, V. et al. (1988) *Science*, 240, 199–201.
2. Clark, J.M. et al. (1987) *J. Mol. Biol.* 198(1); 123–127.

# NEBNext dA-Tailing Reaction Buffer

#E6045A: 0.06 ml

Concentration: 10X

#E6045AA: 0.3 ml

Store at -20°C

## 1X NEBNext dA-Tailing Reaction Buffer:

10 mM Tris-HCl

10 mM MgCl<sub>2</sub>

50 mM NaCl

1 mM DTT

0.2 mM dATP

pH 7.9 @ 25°C

## Quality Control Assays

**16-Hour Incubation:** 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**Endonuclease Activity:** Incubation of this reaction buffer at a 1X concentration with 1 µg of φX174 RF I DNA for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

**RNase Activity:** Incubation of this reaction buffer at 1X concentration with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

**Phosphatase Activity:** Incubation of this reaction buffer at a 1X concentration in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub>) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Lot Controlled**

# Quick T4 DNA Ligase

#E6011A: 0.015 ml

#E6011AA: 0.06 ml



Store at -20°C

Source: Purified from *E. coli* C600 pCl857 pPLc28 lig8 (2).

## Quality Control Assays

**SDS-PAGE Purity:** SDS-PAGE analysis of this enzyme indicates > 95% enzyme purity.

**16-Hour Incubation:** 50 µl reactions containing a minimum of 2,000 units of this enzyme and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing a minimum of 2,000 units of this enzyme and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**Endonuclease Activity:** Incubation of a minimum of 3,200 units of this enzyme with 1 µg of ϕX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

**Phosphatase Activity:** Incubation of a minimum of 20,000 units of this enzyme in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub>) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**RNase Activity:** Incubation of a minimum of 2,000 units of this enzyme with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

**Exonuclease Activity:** Incubation of a minimum of 3,200 units of this enzyme with 1 µg sonicated [<sup>3</sup>H] DNA (10<sup>5</sup> cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer releases < 0.1% radioactivity.

**Functional Activity (Blunt End Ligation):** 50 µl reactions containing a 0.5 µl Quick T4 DNA Ligase, 18 µg HaeIII digested ϕX174 and 1X T4 DNA Ligase Buffer incubated at 16°C for 7.5 min results in > 95% of fragments ligated as determined by agarose gel electrophoresis.

**Functional Activity (Cohesive End Ligation):** 20 µl reactions containing 0.5 µl Quick T4 DNA Ligase, 12 µg HindIII digested lambda DNA and 1X T4 DNA Ligase Buffer incubated at 37°C overnight results in > 95% of fragments ligated as determined by agarose gel electrophoresis. Redigestion of the ligated products, 50 µl reactions containing 6 µg of the ligated fragments, 40 units HindIII, and 1X NEBuffer 2 incubated at 37°C for 2 hours, results in no detectable undigested fragments as determined by agarose gel electrophoresis.

**Functional Activity (Adaptor Ligation):** 50 µl reactions containing 0.125 µl Quick T4 DNA Ligase, 8 nmol 12 bp adaptor, and 1X T4 DNA Ligase Buffer incubated at 16°C overnight results in no detectable unligated adaptor as determined by agarose gel electrophoresis.

**Functional Activity (Transformation):** After a five-minute ligation of linearized, dephosphorylated LITMUS™ 28 (containing either blunt [EcoRV] or cohesive [HindIII] ends) and a mixture of compatible insert fragments, transformation into chemically competent *E. coli* DH-5 alpha cells yields a minimum of 1 x 10<sup>6</sup> recombinant transformants per µg plasmid DNA.

## Lot Controlled

### References:

1. Engler, M. J. and Richardson, C. C. (1982). In P. D. Boyer (Ed.), *The Enzymes* Vol. 5, (p. 3). San Diego: Academic Press.
2. Remaut, E., Tsao, H. and Fiers, W. (1983) *Gene*, 22, 103-113.

# ProtoScript II Reverse Transcriptase

#E6014A: 0.015 ml

Concentration: 200,000 U/ml

#E6014AA: 0.06 ml

Store at  $-20^{\circ}\text{C}$

**Description:** ProtoScript II Reverse Transcriptase is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild type M-MuLV. The enzyme is active up to  $50^{\circ}\text{C}$ , providing higher specificity, higher yield of cDNA and more full-length cDNA product up to 12 kb.

**Source:** The gene encoding a mutant M-MuLV Reverse Transcriptase (RNase H<sup>-</sup>) is expressed in *E. coli* and purified to near homogeneity.

**Supplied in:** 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% (v/v) IGEPAL<sup>®</sup> CA-630, 50% (v/v) glycerol

## Quality Control Assays

**16-Hour Incubation:** A 50  $\mu\text{l}$  reaction containing 1  $\mu\text{g}$  of  $\phi\text{X174}$  DNA and 100 units of ProtoScript II Reverse Transcriptase incubated for 16 hours at  $37^{\circ}\text{C}$  resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Exonuclease Activity:** Incubation of a 50  $\mu\text{l}$  reaction containing 100 units of ProtoScript II Reverse Transcriptase with 1  $\mu\text{g}$  of a mixture of single and double-stranded [ $^3\text{H}$ ] *E. coli* DNA ( $10^5$  cpm/ $\mu\text{g}$ ) for 4 hours at  $37^{\circ}\text{C}$  released < 0.2% of the total radioactivity.

**RNase Activity:** Incubation of a 10  $\mu\text{l}$  reaction containing 100 units of ProtoScript II Reverse Transcriptase with 40 ng of RNA transcripts for 2 hours at  $37^{\circ}\text{C}$  resulted in no detectable degradation of the RNA as determined by gel electrophoresis.

**Phosphatase Activity:** Incubation of 100 units of ProtoScript II Reverse Transcriptase in protein phosphatase assay buffer containing 2.5 mM *p*-nitrophenyl phosphate at  $37^{\circ}\text{C}$  for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Protein Purity (SDS-PAGE):** ProtoScript II Reverse Transcriptase is > 95% pure as determined by SDS PAGE analysis using Coomassie blue detection.

**Lot Controlled**

# NEBNext Quick Ligation Reaction Buffer

#E6048A: 0.12 ml

Concentration: 5X

#E6048AA: 0.6 ml

Store at -20°C

## 1X NEBNext Quick Ligation Reaction Buffer:

66 mM Tris-HCl

10 mM MgCl<sub>2</sub>

1 mM dithiothreitol

1 mM ATP

6% Polyethylene glycol (PEG 6000)

pH 7.6 @ 25°C

## Quality Control Assays

**16-Hour Incubation:** 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**Endonuclease Activity:** Incubation of this reaction buffer at a 1X concentration with 1 µg of φX174 RF I DNA for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

**RNase Activity:** Incubation of this reaction buffer at 1X concentration with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

**Phosphatase Activity:** Incubation of this reaction buffer at a 1X concentration in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub>) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Lot Controlled**

## Nuclease-free Water

#E6109A: 4 ml

#E6109AA: 20 ml

Store at  $-20^{\circ}\text{C}$  or  $4^{\circ}\text{C}$

**Description:** Nuclease-free Water is free of detectable DNA and RNA nucleases and phosphatases and suitable for use in DNA and RNA applications.

### Quality Control Assays

**16-Hour Incubation:** 50  $\mu\text{l}$  reactions containing Nuclease-free Water and 1  $\mu\text{g}$  of HindIII digested Lambda DNA incubated for 16 hours at  $37^{\circ}\text{C}$  results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50  $\mu\text{l}$  reactions containing Nuclease-free Water and 1  $\mu\text{g}$  of T3 DNA incubated for 16 hours at  $37^{\circ}\text{C}$  results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**Endonuclease Activity:** Incubation of a 10  $\mu\text{l}$  reaction containing Nuclease-free Water with 1  $\mu\text{g}$  of  $\phi\text{X174}$  RF I supercoiled DNA for 4 hours at  $37^{\circ}\text{C}$  results in  $< 10\%$  conversion to RF II (nicked molecules) as determined by gel electrophoresis.

**RNase Activity:** Incubation of a 10  $\mu\text{l}$  reaction containing Nuclease-free Water with 40 ng of RNA transcript for 16 hours at  $37^{\circ}\text{C}$  resulted in no detectable degradation of RNA as determined by gel electrophoresis.

**Phosphatase Activity:** Incubation of 1X Second Strand Synthesis Reaction Buffer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM  $\text{MgCl}_2$ ) containing 2.5 mM *p*-nitrophenyl phosphate at  $37^{\circ}\text{C}$  for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Lot Controlled**

# NEBNext Q5 Hot Start HiFi PCR Master Mix

**E6630A: 0.3 ml**

**Concentration: 2X**

**E6630AA: 0.75 ml (2 vials provided)**

**Store at -20°C**

**Description:** The NEBNext Q5 Hot Start HiFi PCR Master Mix is specifically optimized for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries, regardless of GC content. The polymerase component of the master mix, Q5 High-Fidelity DNA Polymerase, is a novel thermostable DNA polymerase that possesses 3'→5' exonuclease activity, and is fused to a processivity-enhancing Sso7d domain. Q5 also has an ultra-low error rate (> 100-fold lower than that of *Taq* DNA Polymerase and ~12-fold lower than that of *Pyrococcus furiosus* (Pfu) DNA Polymerase). The buffer component of the master mix has been optimized for robust amplification, even with GC-rich amplicons and offers enhanced compatibility with a variety of beads used in typical NGS workflows. These features make the NEBNext Q5 Hot Start HiFi PCR Master Mix ideal for NGS library construction. This convenient 2X master mix contains dNTPs, Mg<sup>++</sup> and a proprietary buffer, and requires only the addition of primers and DNA template for robust amplification. The inclusion of the hot start aptamer allows convenient room temperature reaction set up.

## Quality Control Assays

**16-Hour Incubation:** A 50 µl reaction containing NEBNext Q5 Hot Start HiFi PCR Master Mix and 1 µg of HindIII digested λ DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing NEBNext Q5 Hot Start HiFi PCR Master Mix and 1 µg of T3 DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**Phosphatase Activity:** Incubation of NEBNext Q5 Hot Start HiFi PCR Master Mix in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub>) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Functional Activity (Multiplex PCR, Bead Inhibition):** 30 cycles of PCR amplification of 20 ng genomic DNA with and without carboxylated magnetic beads in a 50 µl reaction containing 0.5 µM 4-plex primer mix and 1X NEBNext Q5 Hot Start HiFi PCR Master Mix result in the four expected amplicons and no inhibition of amplification in the presence of the beads.

## **Lot Controlled**

This product is covered by one or more Patents.

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## Revision History:

Revision #	Description
4.0	Include protocol for use with NEBNext Q5 Hot Start HiFi PCR Master Mix. Include protocol for changes in concentration of NEBNext Singleplex and Multiplex Oligos for Illumina. Changed all AMPure Bead drying times after ethanol washes to 5 minutes. Changed all AMPure Bead elutions to 0.1X TE or 10 mM Tris-HCl. Added 2 minute incubation after eluting DNA from AMPure beads. Removed protocol for clean up of fragmented RNA using ethanol precipitation. Removed linear acrylamide from kit.
5.0	Remove protocol for use with NEBNext High-Fidelity 2X PCR Master Mix. Include protocol for use with NEBNext Multiplex Oligos (96 Index Primers, NEB #E6609).
6.0	Protocol updated to include NEB #E7710 and NEB #E7730. Section C in the PCR setup step was removed because all of the 25 $\mu$ M primers are now expired.



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