SAMPLE PREPARATION

NEBNext® mRNA Library Prep Set for SOLiD™

Instruction Manual

NEB #E6114S/L
10/50 reactions

NEW ENGLAND BioLabs® Inc.
enabling technologies in the life sciences


Cloned at NEBiolabs


Recombinant Enzyme


Optimum Buffer


Requires BSA


Heat Inactivation

Table of Contents:

The Master Mix Includes:

The volumes provided are sufficient for preparation of up to 10 reactions (NEB #E6114S) and 50 reactions (NEB #E6114L). (All reagents should be stored at –20°C).

NEBNext RNA Fragmentation Buffer (10X)

NEBNext RNA Fragmentation Stop Solution (10X)

Linear Acrylamide (10 mg/ml)

Random Primers (3 µg/µl)

Murine RNase Inhibitor RR

NEBNext First Strand Synthesis Reaction Buffer (5X)

NEBNext Second Strand Synthesis Enzyme Mix

NEBNext Second Strand Synthesis Reaction Buffer (10X)

NEBNext End Repair Enzyme Mix RR

NEBNext End Repair Reaction Buffer (10X)

T4 DNA Ligase RR

NEBNext Quick Ligation Reaction Buffer (5X)

LongAmp® *Taq* 2X Master Mix RR

Nuclease-free water

Required Materials Not Included:

3 M Sodium Acetate, pH 5.5

100% Ethanol

70% Ethanol

SuperScript® II Reverse Transcriptase (Life Technologies, Inc.)

PCR Column Purification Kit (Qiagen or other)

DNA Gel Extraction Column Purification Kit

DNA Adaptors and Primers or Vector

Size Selection Materials [E-Gel® (Life Technologies, Inc.), Agarose Gel or AMPure XP Beads]

Applications:

The NEBNext mRNA Library Prep Set for SOLiD 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 Set for SOLiD 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 the SOLiD platform (Life Technologies, Inc.).

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:

Starting Material: Purified mRNA (50–250 ng)

mRNA Fragmentation Protocol

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

Purified mRNA	1–18 µl
10X RNA Fragmentation Reaction Buffer	2 µl
Nuclease-free Water	variable
Total volume	20 µl

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). Other types of mRNA may require optimizing incubation time to obtain desired fragment size distribution.
3. Transfer tube to ice.
4. Add 2 µl 10X RNA Fragmentation Stop Solution.

Clean Up Fragmented RNA Using RNeasy MinElute Spin Columns (Strongly Recommended)

1. Add 78 µl of the nuclease-free water to the 22 µl fragmented RNA from step 4. Purify sample using RNeasy® MinElute® Cleanup Kit (Qiagen #74204) following manufacturer instructions. Elute in 15.5 µl nuclease-free water. The recovered volume should be ~14.5 µl.

   Note: column purification removes short RNA Fragments and enriches the sample for RNA fragments longer than 200 nucleotides.

Alternatively, Clean Up Fragmented RNA Using Ethanol Precipitation

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

	Volume (µl)
Fragmented RNA from Step 4	22
3M Sodium Acetate, pH 5.5	2
Linear Acrylamide, 10 mg/ml	1–2
100% Ethanol	60
total volume	85–86

2. Incubate at –80°C or in a dry ice/methanol bath for 1 hour.
3. Centrifuge at 14,000 rpm for 25 minutes at 4°C in a microcentrifuge.
4. Carefully remove ethanol.

5. Wash pellet with 300 µl of freshly prepared 70% ethanol. Carefully pipette up and down the pellet. Make sure the pellet does not get stuck in the tip.
6. Centrifuge at 14,000 rpm for 5 minutes at 4°C in a microcentrifuge. Carefully remove all 70% ethanol.
7. Repeat steps 5 and 6 twice.

   Note: Insufficient pellet washing results in inhibition of the first strand cDNA synthesis due to carryover of magnesium and EDTA. If the Bioanalyzer traces of the mRNA fragments show a noisy baseline (Figure 2) repeat the ethanol precipitation step and pellet washing steps.
8. Air dry pellet for up to 10 minutes at room temperature (or longer if necessary) to remove residual ethanol.
9. Resuspend in 14.5 µl Nuclease-free Water.

Assess the Yield and the Size Distribution of the Fragmented mRNA.

Run 1 µl in the Agilent Bioanalyzer® 2100 (Agilent Technologies, Inc.) using a RNA Pico chip.

Note: Clean mRNA fragments with sufficient washing on the Bioanalyzer show a distinct band (Figure 1).

Figure 1: Relative size distribution of eukaryotic mRNA fragments as seen using the Bioanalyzer 2100.

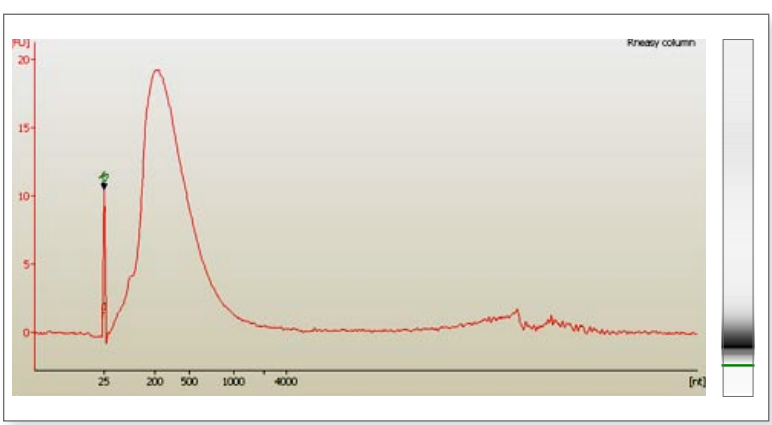
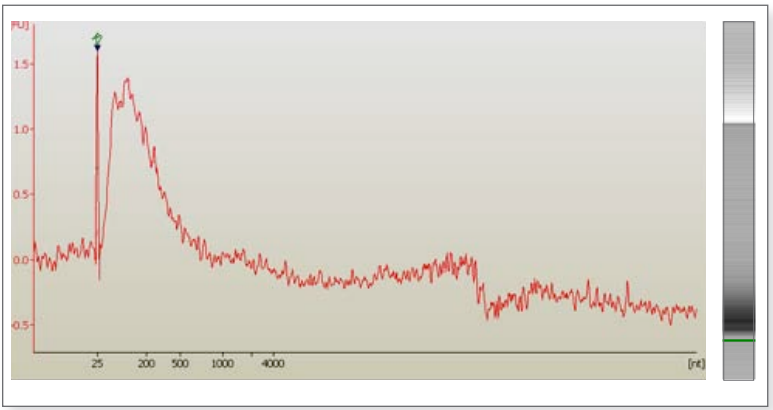


Figure 2: Bioanalyzer traces of mRNA Fragments with insufficient washing show a noisy baseline.



First Strand cDNA Synthesis

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

Fragmented mRNA	13.5 µl
Random Primers	1 µl
Total volume	14.5 µl

2. Incubate in a preheated thermal cycler for 5 minutes at 65°C.
3. Spin tube briefly and place on ice
4. To the fragmented mRNA and Random Primers add:

5X First Strand Synthesis Reaction Buffer	4 µl
Murine RNase Inhibitor	0.5 µl
Total volume	19 µl

5. Incubate in a preheated thermal cycler for 2 minutes at 25°C.
6. Add 1 µl SuperScript II Reverse Transcriptase to the reaction.
7. Incubate sample as follows:
   10 minutes at 25°C
   50 minutes at 42°C
   15 minutes at 70°C
   Hold at 4°C
8. Place the tube on ice.

Second Strand cDNA Synthesis

1. Add the following to the First Strand Synthesis reaction:

Nuclease-free water	48 µl
10X Second Strand Synthesis Reaction Buffer	8 µl
Second Strand Synthesis Enzyme Mix	4 µl
Total volume	80 µl

3. Mix thoroughly by gentle pipetting.
4. Incubate in a thermal cycler for 2.5 hours at 16°C.
5. Purify cDNA using a PCR Column Purification Kit, purifying the sample on a QIAquick Column and elute in 50 µl Nuclease-free water or elution buffer.

End Repair cDNA Library

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

Purified double stranded cDNA	50 µl
NEBNext End Repair Reaction Buffer (10X)	20 µl
NEBNext End Repair Enzyme Mix	10 µl
Nuclease-free water	120 µl
Total volume	200 µl

2. Incubate in a thermal cycler for 30 minutes at 20°C.
3. Purify DNA sample on a QIAquick Column, elute in 50 µl of water or EB Buffer.

Adaptor Ligation of cDNA Library

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

End Repaired, Blunt DNA	50 µl
NEBNext Quick Ligation Reaction Buffer (5X)	40 µl
P1 Adaptor (50 µM)*	variable
P2 Adaptor (50 µM)*	variable
T4 DNA Ligase	10 µl
Nuclease-free water	variable
Total volume	200 µl

**Adaptors are not included, use adaptors appropriate to specific application. Adjust adaptor concentration to obtain a final adaptor to DNA ratio of 30:1.*

2. Incubate in a thermal cycler for 15 minutes at 20°C.
3. Purify DNA sample on a QIAquick column, elute in 30 µl water or EB Buffer.

Size Selection of cDNA Library

1. Run adaptor ligated cDNA library on 2% agarose gel or E-Gel and isolate desired cDNA fragment size from the gel (the 150 and 250 bp bands correspond to adaptor-ligated constructs derived from ~100 and 200 nucleotide RNA fragments, respectively).
2. Extract cDNA from the gel and purify the sample on a QIAquick Column and elute in 50 µl nuclease-free water or elution buffer.

PCR Amplification of Adaptor Ligated cDNA Library

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

Size Selected DNA	50 µl
Primer 1 (50 µM stock)	10 µl
Primer 2 (50 µM stock)	10 µl
LongAmp *Taq* 2X Master Mix	250 µl
Nuclease-free water	180 µl
Total volume	500 µl

2. Aliquot 125 µl into four PCR tubes.

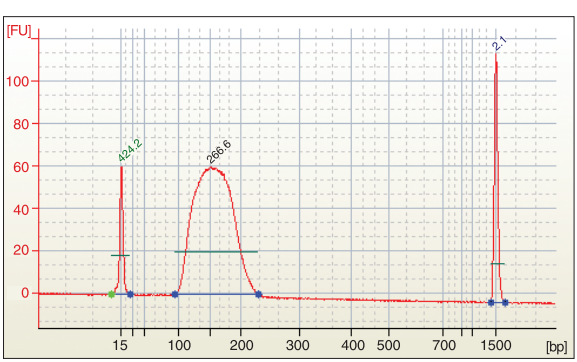
3. PCR cycling conditions

CYCLE STEP	TEMP	TIME	CYCLES
Nick Translation	72°C	20 min	1
Initial Denaturation	95°C	5 min	1
Denaturation Annealing Extension	95°C 62°C 70°C	15 sec 15 sec 1 min	10
Final Extension	70°C	5 min	1
Hold	4°C	∞	1

4. Purify DNA sample on a MinElute Column and elute in 10 µl nuclease-free water.

Perform the following quality control analysis on your sample library to quantify the DNA concentration.

5. Load 1 µl of the resuspended construct on an Agilent 2100 Bioanalyzer® (Agilent Technologies, Inc.) using a DNA specific chip such as the Agilent DNA-1000.



Final mRNA-Seq Library Bioanalyzer Trace

6. Check the size, purity and concentration of the sample. The final product should be a distinct band.*

* *A peak @ 60 bp indicates primer amplification. If you see a peak @ 60 bp perform a second round of size selection.*

NEBNext RNA Fragmentation Buffer

#E6139A: 0.04 ml **Concentration: 10X**
#E6139AA: 0.200 ml

Store at –20°C or 4°C

1X NEBNext RNA Fragmentation Buffer:
40 mM Tris-OAc
100 mM KOAc
30 mM $Mg(OAc)_2$
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 300 ng ϕX174 RF I plasmid for 4 hours at 37°C produced no 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 $MgCl_2$) 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

#E6140A: 0.04 ml **Concentration: 10X**
#E6140AA: 0.200 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 300 ng ϕX174 RF I plasmid for 4 hours at 37°C produced no 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 $MgCl_2$) 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

Linear Acrylamide

#E6141A: 20 µl **Concentration: 10 mg/ml**
#E6141AA: 100 µl

Store at –20°C or 4°C

1X Linear Acrylamide:
10 mg/ml Linear Acrylamide in sterile water

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µg Linear Acrylamide 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 1 µg Linear Acrylamide 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 1 µg Linear Acrylamide with 300 ng ϕX174 RF I plasmid for 4 hours at 37°C produced no nicked molecules as determined by gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 1 µg Linear Acrylamide 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 1 µg Linear Acrylamide in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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

#E6142A: 15 µl
#E6142AA: 50 µl

Store at –20°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^6) 3´ [N=A,C,G,T]

Phosphorylated: No.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µl Random Primers 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 1 µl Random Primers 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 1 µl Random Primers with 300 ng ϕX174 RF I plasmid or 4 hours at 37°C produced no nicked molecules as determined by gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 1 µl Random Primers 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 1 µl Random Primers in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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

Murine RNase Inhibitor

#E6143A: **15 µl**
#E6143AA: **25 µl**

Store at –20°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 µl reactions containing a minimum of 40 units of Murine RNase Inhibitor 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 40 units of Murine RNase Inhibitor 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.

Exonuclease Activity: Incubation of a 50 µl reaction containing 200 units of Murine RNase Inhibitor with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (20^5 cpm/µg) for 4 hours at 37°C released < 0.5% of the total radioactivity.

Latent RNase Assay: Heating the Murine RNase Inhibitor for 20 minutes at 65°C, followed by incubation of a 10 µl reaction containing 40 units of RNase Inhibitor with 40 ng of RNA transcript for 4 hours at 37°C resulted in no detectable degradation of RNA as determined by gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 40 units of Murine RNase Inhibitor with 40 ng of RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by gel electrophoresis.

Endonuclease Activity: Incubation of a 10 µl reaction containing 40 units of Murine RNase Inhibitor with 300 ng ϕX174 RF I plasmid for 4 hours at 37°C produced no 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 $MgCl_2$) 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

References:

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

NEBNext First Strand Synthesis Reaction Buffer

#E6144A: 40 µl **Concentration: 5X**
#E6144AA: 200 µl

Store at –20°C

1X NEBNext First Strand Synthesis Reaction Buffer:
50 mM Tris-Acetate
75 mM KOAc
3.1 mM $Mg(OAc)_2$
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 300 ng ϕX174 RF I plasmid for 4 hours at 37°C produced no 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 $MgCl_2$) 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

#E6145A: 0.04 ml
#E6145AA: 0.200 ml

Store at –20°C

Description: NEBNext Second Strand Synthesis Enzyme Mix is optimized to conver 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 300 ng ϕX174 RF I plasmid for 4 hours at 37°C produced no 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 $MgCl_2$) 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 [^{3}H]-dTTP and 70 µg/ml denatured herring sperm DNA. Incubation of 50 units of RNase H with 1 µg sonicated and denatured [^{3}H]-DNA (10^5 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

#E6147A: 0.08 ml **Concentration: 10X**
#E6147AA: 0.400 ml

Store at –20°C

1X NEBNext Second Strand Synthesis Reaction Buffer:
20 mM Tris-HCl
12 mM $(NH_4)_2SO_4$
5 mM $MgCl_2$
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 300 ng ϕX174 RF I plasmid for 4 hours at 37°C produced no 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 $MgCl_2$) 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

#E6061A: 0.1 ml
#E6061AA: 0.5 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:
10,000 units/ml T4 Polynucleotide Kinase
3,000 units/ml 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 $MgCl_2$) 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 [^{3}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

#E6062A: 0.2 ml **Concentration: 10X**
#E6062AA: 1 ml

Store at –20°C

1X NEBNext End Repair Reaction Buffer:
50 mM Tris-HCl
10 mM $MgCl_2$
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 $MgCl_2$) 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

T4 DNA Ligase

#E6063A: 0.1 ml
#E6063AA: 0.5 ml

Store at –20°C

Source: Purified from *E. coli* C600 pcI857 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 $MgCl_2$) 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 [^{3}H] DNA (10^5 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 T4 DNA Ligase, 18 µg HindIII 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 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 (Adapter Ligation): 50 µl reactions containing 0.125 µl T4 DNA Ligase, 8 nmol 12 bp adapter, and 1X T4 DNA Ligase Buffer incubated at 16°C overnight results in no detectable unligated adapter 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 10^6 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.

NEBNext Quick Ligation Reaction Buffer

#E6064A: 0.4 ml **Concentration: 5X**
#E6064AA: 2 ml

Store at –20°C

1X NEBNext Quick Ligation Reaction Buffer:
66 mM Tris-HCl
10 mM $MgCl_2$
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 $MgCl_2$) 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

LongAmp *Taq* 2X Master Mix

E6065A: 2.5 ml **Concentration: 2X**
E6065AA: 12.5 ml

Store at –20°C

1X LongAmp *Taq* Master Mix:
60 mM Tris-SO_4
2 mM $MgSO_4$
0.3 mM dNTPs
125 units/ml LongAmp *Taq* DNA Polymerase
20 mM ammonium sulfate
pH 9.0 @ 25°C

Quality Control Assays

Long Amplicon PCR: The master mix is tested for the ability to amplify a 40 kb amplicon from lambda DNA and a 30 kb fragment from human genomic DNA.

SDS-PAGE Purity: SDS-PAGE analyses of each individual 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 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 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 $MgCl_2$) 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.

HPLC: dNTP purity is determined by HPLC to be > 99%.

Lot Controlled

Nuclease-free Water

#E6109A: 4 ml
#E6109AA: 20 ml

Store at –20°C or 4°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 µl reactions containing Nuclease-free Water 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 Nuclease-free Water 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 Nuclease-free Water with 300 ng ϕX174 RF I plasmid for 4 hours at 37°C produced no nicked molecules as determined by gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing Nuclease-free Water 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 Nuclease-free Water Buffer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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

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3/12