

SAMPLE PREPARATION

NEBNext[®] DNA Library Prep Set for SOLiD[™]

Instruction Manual

NEB #E6060S/L
10/50 reactions

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NEBNext DNA Library Prep Set for SOLiD



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The Reagent Set Includes:

The volumes provided are sufficient for preparation of up to 10 reactions (NEB #E6060S) and 50 reactions (NEB #E6060L). (All reagents should be stored at -20°C):

- NEBNext End Repair Enzyme Mix **RII**
- NEBNext End Repair Reaction Buffer (10X)
- T4 DNA Ligase **RII**
- NEBNext Quick Ligation Reaction Buffer (5X)
- LongAmp Taq 2X Master Mix **RII**



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Applications:

The NEBNext DNA Library Prep Set for SOLiD contains enzymes and buffers in convenient master mix formulations 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 DNA 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.

Functional Validation: Each set of reagents is functionally validated together through construction and sequencing of a genomic DNA library on an SOLiD System V3 (Life Technologies, Inc.), and by construction of an expression library.

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: 1–5 µg of DNA Fragmented to 150–200 bp in ≤ 100 µl

End Repair of Fragmented DNA

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

Fragmented DNA	1–100 µl
NEBNext End Repair Reaction Buffer (10X)	20 µl
NEBNext End Repair Enzyme Mix	10 µl
Sterile H ₂ O	variable
Total volume	200 µl

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

Ligation of End Repaired DNA

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
Sterile H ₂ O	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 one column, elute in 50 µl of water or EB buffer.
4. Size select library fragments in the 200–230 bp range. Size selection can be performed using a number of methods including E-Gel® size select gels or standard 2% agarose gels.

PCR Amplification of Adaptor Ligated DNA

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

Adaptor ligated DNA (≤ 100 µl)	variable
Primer 1 (50 µM stock)	10 µl
Primer 2 (50 µM stock)	10 µl
LongAmp Taq 2X Master Mix	250 µl
Sterile H ₂ O	variable
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	95°C	15 sec	2–10*
Annealing	62°C	15 sec	
Extension	70°C	1 min	
Final Extension	70°C	5 min	1
Hold	4°C	∞	1

**Avoid over-amplification to optimize the number of unique molecules.*

Cycling suggestions:

FOR STARTING DNA QUANTITIES OF:	CYCLES
10 ng–100 ng	10 cycles of amplification
100 ng–1 µg	6–8
1 µg–2 µg	4–6
2 µg–5 µg	2–3

4. Purify sample on a DNA purification column, elute in 50 µl of water or EB buffer.

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 MgCl₂) 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 [³H]-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.

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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 MgCl₂

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 φX174 RF I 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 MgCl₂) 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.

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T4 DNA Ligase

#E6063A: 0.1 ml
#E6063AA: 0.5 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 φX174 RF I 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 MgCl₂) 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 [³H] DNA (10⁵ 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 10⁶ recombinant transformants per µg plasmid DNA.

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References:

- Engler, M. J. and Richardson, C. C. (1982). In P. D. Boyer (Ed.), *The Enzymes* Vol. 5, (p. 3). San Diego: Academic Press.
- 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 MgCl₂
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 φX174 RF I 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 MgCl₂) 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.

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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-SO₄

2 mM MgSO₄

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 φX174 RF I 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 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 MgCl₂) 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%.

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U.S. Patent No. 5,352,778



USA

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

CANADA

New England Biolabs, Ltd.
Telephone: (905) 665-4632
Toll Free: 1-800-387-1095
Fax: (905) 665-4635
Fax Toll Free: 1-800-563-3789
e-mail: info.ca@neb.com
www.neb.ca

CHINA, PEOPLE'S REPUBLIC

New England Biolabs (Beijing), Ltd.
Telephone: 010-82378265/82378266
Fax: 010-82378262
e-mail: info@neb-china.com
www.neb-china.com

FRANCE

New England Biolabs France
Free Call: 0800-100-632
Free Fax: 0800-100-610
e-mail: info.fr@neb.com
www.neb-online.fr

GERMANY & AUSTRIA

New England Biolabs GmbH
Telephone: +49/(0)69/305 23140
Free Call: 0800/246 5227 (Germany)
Free Call: 00800/246 52277 (Austria)
Fax: +49/(0)69/305 23149
Free Fax: 0800/246 5229 (Germany)
e-mail: info.de@neb.com
www.neb-online.de

JAPAN

New England Biolabs Japan, Inc.
Telephone: +81 (0)3 5669 6191
Fax: +81 (0)3 5669 6192
e-mail: info@neb-japan.com
www.nebj.jp

SINGAPORE

New England Biolabs Pte. Ltd.
Telephone: +65 6776 0903
Fax: +65 6778 9228
e-mail: sales.sg@neb.com
www.neb.sg

UNITED KINGDOM

New England Biolabs (UK) Ltd.
Telephone: (01462) 420616
Call Free: 0800 318486
Fax: (01462) 421057
Fax Free: 0800 435682
e-mail: info.uk@neb.com
www.neb.uk.com

