LIBRARY PREPARATION

NEBNext® DNA Library Prep Master Mix Set for 454™

Instruction Manual

NEB #E6070S/L
10/50 reactions
Version 3.0      5/18
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The Reagent Set Includes:

The volumes provided are sufficient for preparation of up to 10 reactions (NEB #E6070S) and 50 reactions (NEB #E6070L).

**Box 1: Store at –20°C**

- NEBNext End Repair Enzyme Mix
- NEBNext End Repair Reaction Buffer (10X)
- Quick T4 DNA Ligase
- NEBNext Quick Ligation Reaction Buffer (5X)
- Bst DNA Polymerase, Large Fragment
- NEBNext Adaptor Fill-in Reaction Buffer (10X)
- Molecular Biology Grade Water

**Box 2: Store at 4°C**

- Hydrophilic Streptavidin Magnetic Beads (4 mg/ml)
- NEBNext Bead Binding Buffer (2X)
- NEBNext Bead Wash Buffer (1X)
Applications:

The NEBNext DNA Library Prep Master Mix Set for 454 contains enzymes and buffers in convenient master mix formulations that are ideally suited for sample preparation for next-generation sequencing (1), and for preparation of single stranded DNA for use in high density hybridization arrays (2) or for genomic subtraction hybridization methods (3). Each of these components must pass rigorous quality control standards and are lot controlled.

Lot Control: The lots provided in the NEBNext DNA Library Prep Master Mix Set for 454 undergo standard enzyme activity and quality control assays, and also meet stringent criteria in the additional quality controls listed on each individual component page.

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.

References:


Protocols:

NEBNext End Repair Module Protocol
Starting Material: 1–5 µg of DNA Fragmented to 100–1000 bp in ≤ 85 µl

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragmented DNA</td>
<td>1–85 µl</td>
</tr>
<tr>
<td>NEBNext End Repair Reaction Buffer (10X)</td>
<td>10 µl</td>
</tr>
<tr>
<td>NEBNext End Repair Enzyme Mix</td>
<td>5 µl</td>
</tr>
<tr>
<td>Sterile H$_2$O for a final volume of 100 µl</td>
<td>variable</td>
</tr>
<tr>
<td>total volume</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

2. Incubate in a thermal cycler for 30 minutes at 20°C.
3. Purify DNA sample on one column and elute in 30 µl of sterile dH$_2$O or elution buffer.

NEBNext Quick Ligation Module Protocol

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>End Repaired, Blunt or dA-Tailed DNA</td>
<td>30 µl</td>
</tr>
<tr>
<td>Quick Ligation Reaction Buffer (5X)</td>
<td>10 µl</td>
</tr>
<tr>
<td>DNA Adaptors</td>
<td>5 µl</td>
</tr>
<tr>
<td>(not provided please use adaptors appropriate to specific application)</td>
<td></td>
</tr>
<tr>
<td>Quick T4 DNA Ligase</td>
<td>5 µl</td>
</tr>
<tr>
<td>total volume</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

2. Incubate in a thermal cycler for 15 minutes at 20°C.
3. Purify DNA sample on one column and elute in 25 µl of sterile dH$_2$O or elution buffer.

NEBNext Fill-in and ssDNA Isolation Module Protocol

*Recommended: Removal of small fragments using Agencourt AMPure® Beads (Beckman Coulter, Inc.) or gel size selection.*

1. Transfer 50 µl of Hydrophilic Streptavidin Magnetic Beads to a 1.5 ml tube.
2. Using a magnet, pellet the beads and remove the buffer
3. Wash beads twice with 100 µl of 2X Bead Binding Buffer, pelleting the beads with a magnet to remove the buffer after each wash.
5. Add 25 µl adapter-ligated DNA fragments to the beads.
6. Vortex and place on a tube rotator at room temperature for 20 minutes.
7. Using a magnet, wash the beads twice with 100 µl (1X) Bead Wash Buffer, pelleting the beads with a magnet to remove the buffer after each wash.
8. Mix the following components in a separate sterile microfuge tube:
   - Molecular Biology Grade Water 42 µl
   - Adapter Fill-in Reaction Buffer 5 µl
   - Bst DNA Polymerase, Large Fragment 3 µl

9. Transfer the 50 µl Fill-in Reaction Mix to the beads.

10. Vortex lightly and incubate at 37°C for 20 minutes.

11. Wash beads twice with 100 µl of 1X Bead Wash Buffer, pelleting the beads with a magnet to remove the buffer after each wash.

12. Prepare Melt Solution:
   - 10 N NaOH 125 µl
   - Water 9.875 ml

13. Prepare Neutralization Solution in a 1.5 ml tube:
   - 3 M sodium acetate, pH 5.2 10 µl
   - Column binding buffer with pH indicator 500 µl

14. Add 50 µl of Melt Solution to the beads.

15. Vortex well, pellet the beads with a magnet.

16. Carefully transfer the Melt Solution containing the ssDNA Fragment Library to a 1.5 ml tube containing the Neutralization Solution.

17. Repeat steps 14–16, adding the second round of Melt Solution containing the ssDNA Fragment Library to the same 1.5 ml tube containing the Neutralization Solution and the first round of Melt Solution and ssDNA Fragments. Adjust pH if necessary by adding an additional 5 µl of 3 M sodium acetate.

18. Purify DNA on one column without adding any additional column binding buffer. Wash column twice with column wash buffer to remove all residual salts. Elute in 25 µl of sterile dH₂O or elution buffer.
### NEB #E6070S Table of Components

<table>
<thead>
<tr>
<th>NEB #</th>
<th>PRODUCT</th>
<th>VOLUME</th>
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<tbody>
<tr>
<td>E6041A</td>
<td>NEBNext End Repair Enzyme Mix</td>
<td>0.06 ml</td>
</tr>
<tr>
<td>E6042A</td>
<td>NEBNext End Repair Reaction Buffer</td>
<td>0.12 ml</td>
</tr>
<tr>
<td>E6047A</td>
<td>Quick T4 DNA Ligase</td>
<td>0.06 ml</td>
</tr>
<tr>
<td>E6048A</td>
<td>NEBNext Quick Ligation Reaction Buffer</td>
<td>0.12 ml</td>
</tr>
<tr>
<td>E6030A</td>
<td><em>Bst</em> DNA Polymerase, Large Fragment</td>
<td>0.03 ml</td>
</tr>
<tr>
<td>E6035A</td>
<td>NEBNext Adaptor Fill-in Reaction Buffer</td>
<td>0.05 ml</td>
</tr>
<tr>
<td>E6031A</td>
<td>Molecular Biology Grade Water</td>
<td>1 ml</td>
</tr>
<tr>
<td>E6032A</td>
<td>Hydrophilic Streptavidin Magnetic Beads</td>
<td>0.5 ml</td>
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<tr>
<td>E6034A</td>
<td>NEBNext Bead Binding Buffer</td>
<td>2.25 ml</td>
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<tr>
<td>E6033A</td>
<td>NEBNext Bead Wash Buffer</td>
<td>4.0 ml</td>
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### NEB #E6070L Table of Components

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<td>E6047AA</td>
<td>Quick T4 DNA Ligase</td>
<td>0.3 ml</td>
</tr>
<tr>
<td>E6048AA</td>
<td>NEBNext Quick Ligation Reaction Buffer</td>
<td>0.6 ml</td>
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<tr>
<td>E6030AA</td>
<td><em>Bst</em> DNA Polymerase, Large Fragment</td>
<td>0.15 ml</td>
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<tr>
<td>E6035AA</td>
<td>NEBNext Adaptor Fill-in Reaction Buffer</td>
<td>0.25 ml</td>
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<tr>
<td>E6031AA</td>
<td>Molecular Biology Grade Water</td>
<td>5 ml</td>
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<tr>
<td>E6032AA</td>
<td>Hydrophilic Streptavidin Magnetic Beads</td>
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<tr>
<td>E6034AA</td>
<td>NEBNext Bead Binding Buffer</td>
<td>11.25 ml</td>
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<tr>
<td>E6033AA</td>
<td>NEBNext Bead Wash Buffer</td>
<td>20.0 ml</td>
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## Revision History:

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<td>2.2</td>
<td>Updated Applications text.</td>
<td>3/14</td>
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<tr>
<td>3.0</td>
<td>Create &quot;Kit Component – Table of Components&quot; for small and large size kits.</td>
<td>5/18</td>
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DNA CLONING
DNA AMPLIFICATION & PCR
EPIGENETICS
RNA ANALYSIS
LIBRARY PREP FOR NEXT GEN SEQUENCING
PROTEIN EXPRESSION & ANALYSIS
CELLULAR ANALYSIS

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
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
Fax Free: 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.jp@neb.com
www.nebj.jp

SINGAPORE
New England Biolabs Pte. Ltd.
Telephone: +65 638 59623
Fax: +65 638 59617
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