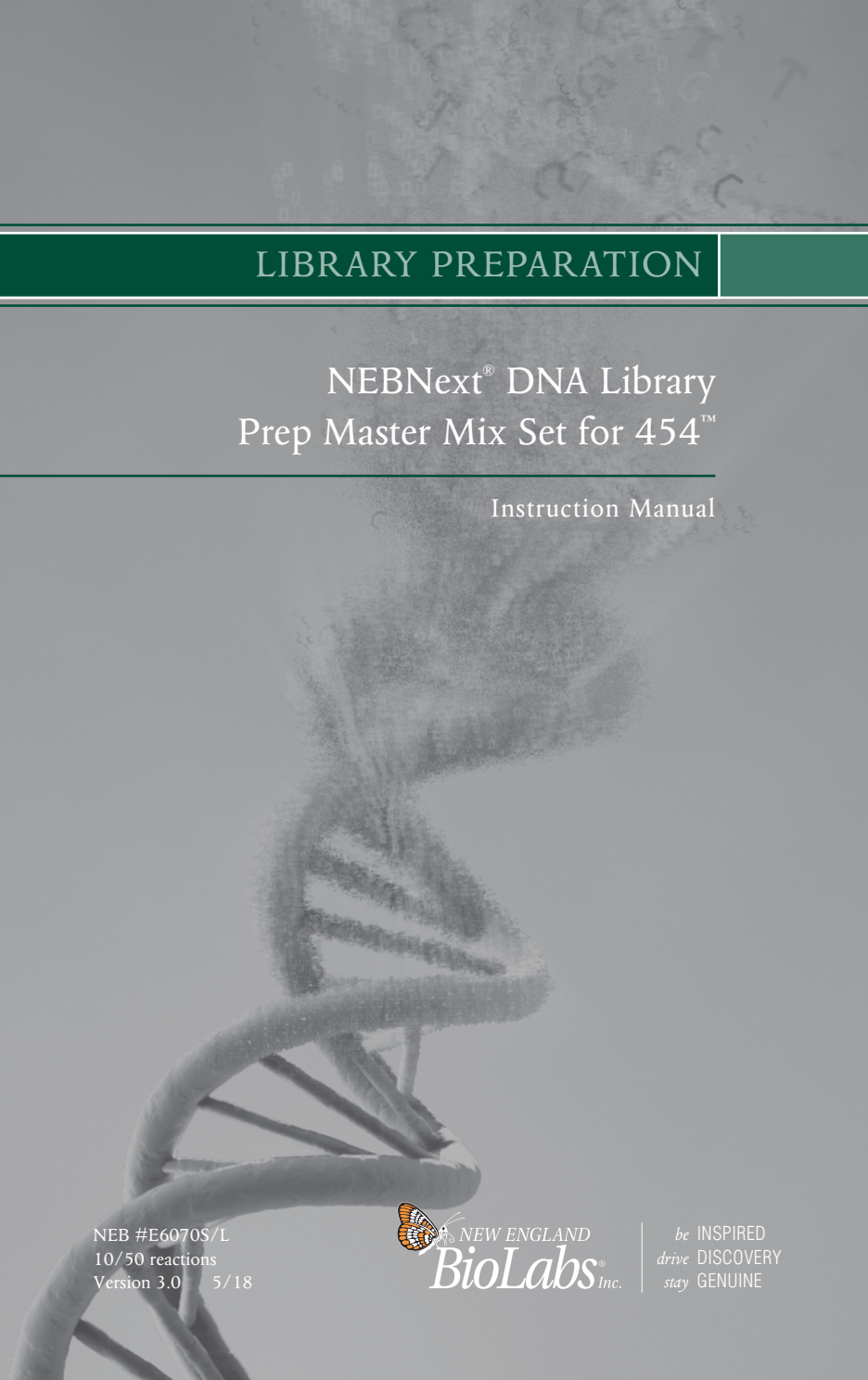
LIBRARY PREPARATION

NEBNext® DNA Library Prep Master Mix Set for 454™

Instruction Manual

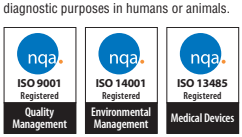


NEB #E6070S/L
10/50 reactions
Version 3.0 5/18

NEW ENGLAND BioLabs® Inc.

be INSPIRED
drive DISCOVERY
stay GENUINE

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.



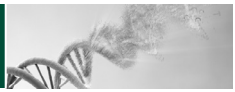


Table of Contents:

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:

1. Maricic, T. and S. Paabo (2009). "Optimization of 454 sequencing library preparation from small amounts of DNA permits sequence determination of both DNA strands." *Biotechniques,* 46, 51–52, 54–57.
2. Straus, D. and F.M. Ausubel (1990). "Genomic subtraction for cloning DNA corresponding to deletion mutations." *Proc. Natl. Acad. Sci. USA* 87, 1889–1893.
3. Zhou, X. and D.T. Wong (2007). "Single nucleotide polymorphism mapping array assay." *Methods Mol. Biol.* 396, 295–314.

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:

Fragmented DNA	1–85 µl
NEBNext End Repair Reaction Buffer (10X)	10 µl
NEBNext End Repair Enzyme Mix	5 µl
Sterile H_2O for a final volume of 100 µl	variable
total volume	100 µl

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_2O or elution buffer.

NEBNext Quick Ligation Module Protocol

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

End Repaired, Blunt or dA-Tailed DNA	30 µl
Quick Ligation Reaction Buffer (5X)	10 µl
DNA Adaptors (not provided please use adaptors appropriate to specific application)	5 µl
Quick T4 DNA Ligase	5 µl
total volume	50 µl

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_2O 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.
4. Resuspend beads in 25 µl of 2X Bead Binding Buffer.
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 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 dH_2O or elution buffer.

Kit Components

NEB #E6070S Table of Components

NEB #	PRODUCT	VOLUME
E6041A	NEBNext End Repair Enzyme Mix	0.06 ml
E6042A	NEBNext End Repair Reaction Buffer	0.12 ml
E6047A	Quick T4 DNA Ligase	0.06 ml
E6048A	NEBNext Quick Ligation Reaction Buffer	0.12 ml
E6030A	*Bst* DNA Polymerase, Large Fragment	0.03 ml
E6035A	NEBNext Adaptor Fill-in Reaction Buffer	0.05 ml
E6031A	Molecular Biology Grade Water	1 ml
E6032A	Hydrophilic Streptavidin Magnetic Beads	0.5 ml
E6034A	NEBNext Bead Binding Buffer	2.25 ml
E6033A	NEBNext Bead Wash Buffer	4.0 ml

NEB #E6070L Table of Components

NEB #	PRODUCT	VOLUME
E6041AA	NEBNext End Repair Enzyme Mix	0.3 ml
E6042AA	NEBNext End Repair Reaction Buffer	0.6 ml
E6047AA	Quick T4 DNA Ligase	0.3 ml
E6048AA	NEBNext Quick Ligation Reaction Buffer	0.6 ml
E6030AA	*Bst* DNA Polymerase, Large Fragment	0.15 ml
E6035AA	NEBNext Adaptor Fill-in Reaction Buffer	0.25 ml
E6031AA	Molecular Biology Grade Water	5 ml
E6032AA	Hydrophilic Streptavidin Magnetic Beads	2.5 ml
E6034AA	NEBNext Bead Binding Buffer	11.25 ml
E6033AA	NEBNext Bead Wash Buffer	20.0 ml

Revision History:

REVISION #	DESCRIPTION	DATE
2.2	Updated Applications text.	3/14
3.0	Create "Kit Component – Table of Components" for small and large size kits. Delete individual component information pages.	5/18

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