

# LIBRARY PREPARATION

## NEBNext<sup>®</sup> Multiplex Oligos for Illumina<sup>®</sup> (Index Primers Set 2)

Instruction Manual

NEB #E7500S/L  
24/96 reactions  
Version 4.0 1/18



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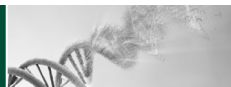
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## The NEBNext Multiplex Oligos for Illumina (Index Primers Set 2) Includes:

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*The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7500S) and 96 reactions (NEB #E7500L). All reagents should be stored at -20°C.*

NEBNext Adaptor for Illumina

USER Enzyme

NEBNext Universal PCR Primer for Illumina

NEBNext Index 13 Primer for Illumina

NEBNext Index 14 Primer for Illumina

NEBNext Index 15 Primer for Illumina

NEBNext Index 16 Primer for Illumina

NEBNext Index 18 Primer for Illumina

NEBNext Index 19 Primer for Illumina

NEBNext Index 20 Primer for Illumina

NEBNext Index 21 Primer for Illumina

NEBNext Index 22 Primer for Illumina

NEBNext Index 23 Primer for Illumina

NEBNext Index 25 Primer for Illumina

NEBNext Index 27 Primer for Illumina

*For the list of additional materials required, please check the manual for your NEBNext Library Prep Kit.*

## Applications:

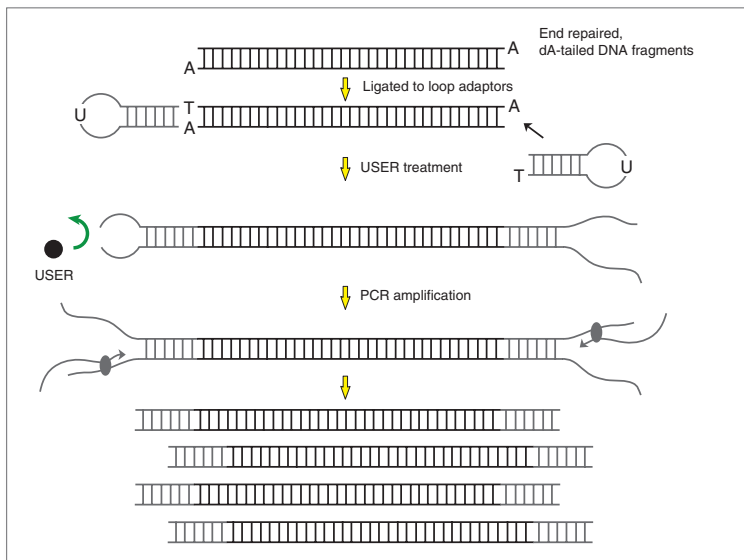
The NEBNext Multiplex Oligos for Illumina (Index Primers Set 2) contains adaptors and primers that are ideally suited for multiplex sample preparation for next-generation sequencing on the Illumina platform (Illumina, Inc.). Each of these components must pass rigorous quality control standards and is lot controlled, both individually and as a set of reagents.

**Lot Control:** The lots provided in the NEBNext Multiplex Oligos for Illumina (Index Primers Set 2) 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 of the components is functionally validated through construction and sequencing of genomic DNA libraries on the Illumina 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](mailto:OEM@neb.com) for further information.

Figure 1: Workflow.



## Please Refer to the Kit Specific Protocol for using the NEBNext Multiplex Oligos for Illumina:

The following kits are designed for use with the NEBNext Multiplex Oligos for Illumina:

- #E7645, NEBNext Ultra II DNA Library Prep Kit for Illumina
- #E7595, NEBNext Ultra II Ligation Module
- #E7420, NEBNext Ultra Directional RNA Library Prep Kit for Illumina
- #E7530, NEBNext Ultra RNA Library Prep Kit for Illumina
- #E7370, NEBNext Ultra DNA Library Prep Kit for Illumina
- #E7445, NEBNext Ultra Ligation Module
- #E6040, NEBNext DNA Library Prep Master Mix Set for Illumina
- #E6240, NEBNext ChIP-Seq Library Prep Master Mix Set for Illumina
- #E6056, NEBNext Quick Ligation Module
- #E7760, NEBNext Ultra II Directional RNA Library Prep Kit for Illumina
- #E7770, NEBNext Ultra II RNA Library Prep Kit for Illumina
- #E7765, NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads
- #E7775, NEBNext Ultra II RNA Library Prep with Sample Purification Beads
- #E7103, NEBNext Ultra II DNA Library Prep with Sample Purification Beads
- #E7805, NEBNext Ultra II FS DNA Library Prep Kit for Illumina
- #E6177, NEBNext Ultra II FS DNA Library Prep Kit with Sample Purification Beads

# NEBNext Adaptors and Primers for Illumina

PRODUCT	INDEX PRIMER SEQUENCE	EXPECTED INDEX PRIMER SEQUENCE READ
NEBNext Index 13 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATT <b>GTGACT</b> GTGACT GGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	AGTCAA
NEBNext Index 14 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATAC <b>GGAACT</b> GTGAC TGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	AGTTCC
NEBNext Index 15 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATT <b>TGACAT</b> GTGACT GGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	ATGTCA
NEBNext Index 16 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATGC <b>CGGACGG</b> GTGAC TGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CCGTCC
NEBNext Index 18 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATGT <b>GCGGAC</b> GTGAC TGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GTCCGC
NEBNext Index 19 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATCG <b>TTTACG</b> GTGACT GGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GTGAAA
NEBNext Index 20 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATA <b>AGGCAC</b> GTGAC TGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GTGGCC
NEBNext Index 21 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATT <b>CGAAAC</b> GTGAC TGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GTTTCG
NEBNext Index 22 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATT <b>CGTACG</b> GTGACT GGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CGTACG
NEBNext Index 23 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATAT <b>CCACTC</b> GTGACT GGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GAGTGG
NEBNext Index 25 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATAT <b>ATCAGT</b> GTGACT GGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	ACTGAT
NEBNext Index 27 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATA <b>AGGAAT</b> GTGACT GGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	ATTCTT
NEBNext Adaptor for Illumina	5'-/5Phos/GAT CGG AAG AGC ACA CGT CTG AAC TCC AGT CUA CAC TCT TTC CCT ACA CGA CGC TCT TCC GAT C-s-T-3'	N/A
NEBNext Universal PCR Primer for Illumina	5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC-s-T-3'	N/A

Where -s- indicates phosphorothioate bond.

Note: If fewer than 12 indexes are used in a lane for sequencing, it is recommended to use the following indices:

Pool of 2 samples: Index #18 and 25

Pool of 3 samples: Index #13, 18 and 23

Pool of 4 samples: Index #16, 18, 20 and 23

# Kit Components

## NEB #E7500S Table of Components

NEB #	PRODUCT	VOLUME
E7337A	NEBNext Adaptor for Illumina	0.24 ml
E7338A	USER Enzyme	0.072 ml
E6861A	NEBNext Universal PCR Primer for Illumina	0.12 ml
E6862A	NEBNext Index 13 Primer for Illumina	0.010 ml
E6863A	NEBNext Index 14 Primer for Illumina	0.010 ml
E6864A	NEBNext Index 15 Primer for Illumina	0.010 ml
E7511A	NEBNext Index 16 Primer for Illumina	0.010 ml
E6866A	NEBNext Index 18 Primer for Illumina	0.010 ml
E6867A	NEBNext Index 19 Primer for Illumina	0.010 ml
E6868A	NEBNext Index 20 Primer for Illumina	0.010 ml
E6869A	NEBNext Index 21 Primer for Illumina	0.010 ml
E6870A	NEBNext Index 22 Primer for Illumina	0.010 ml
E6871A	NEBNext Index 23 Primer for Illumina	0.010 ml
E6872A	NEBNext Index 25 Primer for Illumina	0.010 ml
E6873A	NEBNext Index 27 Primer for Illumina	0.010 ml

## NEB #E7500L Table of Components

NEB #	PRODUCT	VOLUME
E7337AA	NEBNext Adaptor for Illumina	0.96 ml
E7338AA	USER Enzyme	0.288 ml
E6861AA	NEBNext Universal PCR Primer for Illumina	0.48 ml
E6862AA	NEBNext Index 13 Primer for Illumina	0.04 ml
E6863AA	NEBNext Index 14 Primer for Illumina	0.04 ml
E6864AA	NEBNext Index 15 Primer for Illumina	0.04 ml
E7511AA	NEBNext Index 16 Primer for Illumina	0.04 ml
E6866AA	NEBNext Index 18 Primer for Illumina	0.04 ml
E6867AA	NEBNext Index 19 Primer for Illumina	0.04 ml
E6868AA	NEBNext Index 20 Primer for Illumina	0.04 ml
E6869AA	NEBNext Index 21 Primer for Illumina	0.04 ml
E6870AA	NEBNext Index 22 Primer for Illumina	0.04 ml
E6871AA	NEBNext Index 23 Primer for Illumina	0.04 ml
E6872AA	NEBNext Index 25 Primer for Illumina	0.04 ml
E6873AA	NEBNext Index 27 Primer for Illumina	0.04 ml

## Revision History:

REVISION #	DESCRIPTION	DATE
1.0	N/A	1/13
2.0	Change Index Primer and Universal Primer concentration from 25 $\mu$ M to 10 $\mu$ M. All part #'s have changed. Concentrations of Universal PCR Primer and Index Primers have changed from 25 $\mu$ M to 10 $\mu$ M.	1/15
3.0	Switched out Index 16 Primer with a new sequence.	10/15
3.1	Update "Required Materials Not Included for DNA or ChIP Libraries" and "Required Materials Not Included for RNA Libraries". Update the list of kits using the NEBNext Multiplex Oligos for Illumina.	4/16
4.0	Delete "Required Materials Not Included for DNA or ChIP Libraries" and "Required Materials Not Included for RNA Libraries" and replace with guidance note. Update the list of kits using the NEBNext Multiplex Oligos for Illumina. Note, Pool of 4 Samples: Index# 13, 14, 16 and 18, replace with 16, 18, 20 and 23. DNA sequence for NEBNext Adaptor for Illumina has been corrected. Change part number for NEBNext Index 16 Primer for Illumina and add 2 additional bases to the sequence. Create "Kit Component – Table of Components" for small and large size kits. Change Index Primer sequences table to include adaptor, and NEBNext Universal PCR Primer and rename it as NEBNext Adaptors and Primers for Illumina. Delete individual component information pages. Updated the functionally validated statement on page 2.	1/18





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