

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

NEB #E7500S/L

24/96 reactions

Version 6.0\_6/22

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

*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<sup>®</sup> 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

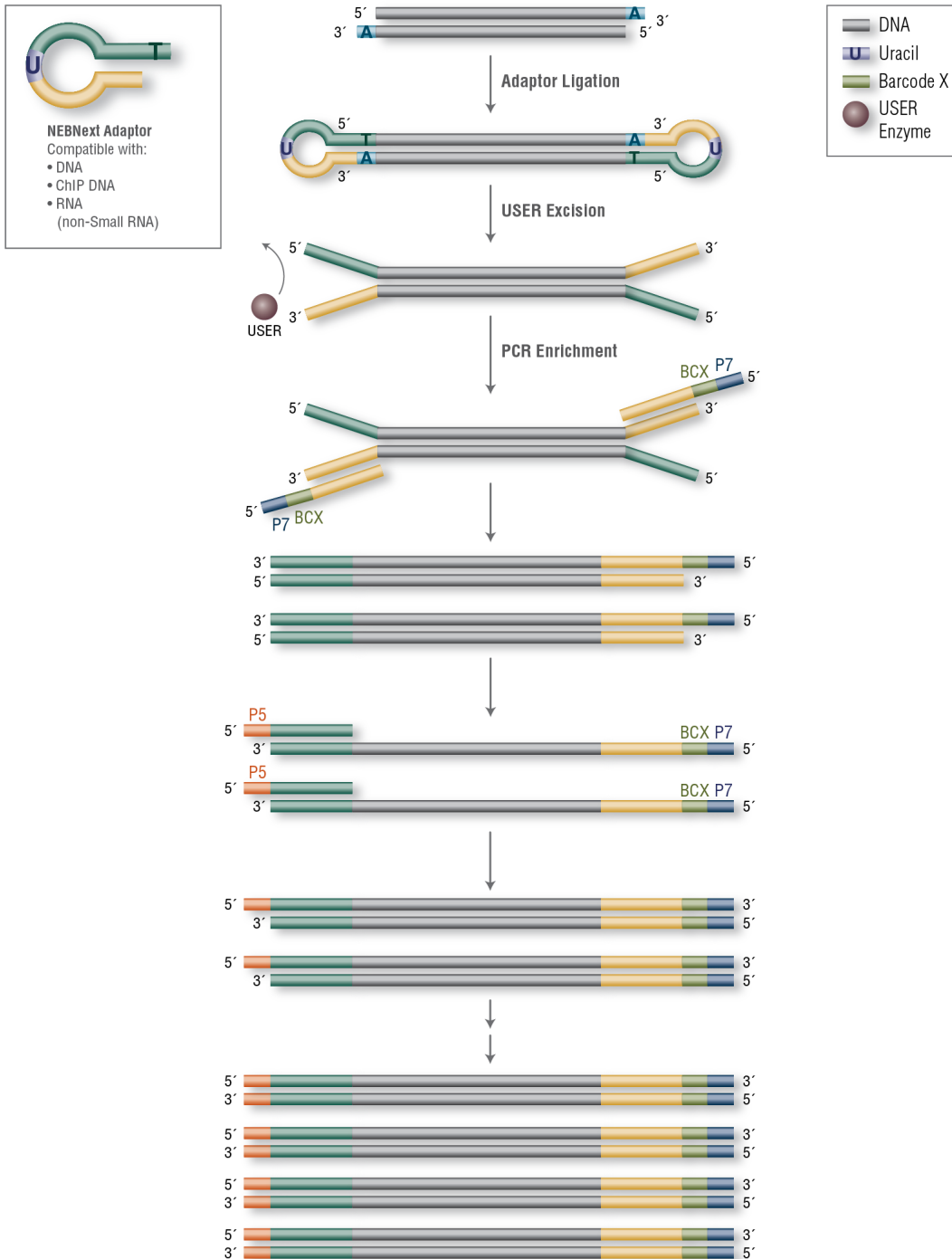
The NEBNext Multiplex Oligos for Illumina (Index Primers Set 2) contains adaptors and primers that are ideally suited for multiplex sample preparation for next generation sequencing on the Illumina platform (Illumina, Inc.). Each of these components must pass rigorous quality control standards and is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

**Figure 1. Workflow demonstrating the use of NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)**



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

For compatibility of NEBNext Multiplex Oligos please refer to the NEBNext Multiplex Oligos Selection Chart at [neb.com/oligos](http://neb.com/oligos)

### NEBNext Adaptor for Illumina Overview

NEBNext Adaptor for Illumina sequence:

5'-/5Phos/GAT CGG AAG AGC ACA CGT CTG AAC TCC AGT CdUA CAC TCT TTC CCT ACA CGA CGC TCT TCC GAT C-s-T-3'

The following sequences are used for adaptor trimming of NEBNext adaptors for Illumina.

Read 1 AGATCGGAAGAGCACACGTCTGAACTCCAGTCA

Read 2 AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

## NEBNext Adaptors and Primers for Illumina

For sample sheets please see NEB.com, E7500 Product Page, "Protocols, Manuals and Usage Guidelines" Tab, [Usage Guidelines](#)

INDEX PRIMER	INDEX PRIMER SEQUENCE	EXPECTED INDEX SEQUENCE READ
NEBNext Index 13 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGATTG <b>TTGACT</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	AGTCAA
NEBNext Index 14 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGATAC <b>GGAAGT</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	AGTTCC
NEBNext Index 15 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGATT <b>TGACAT</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	ATGTCA
NEBNext Index 16 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGATGC <b>GGACGG</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	CCGTCC
NEBNext Index 18 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGATGT <b>GCGGAC</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	GTCCGC
NEBNext Index 19 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGATCG <b>TTTCACT</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	GTGAAA
NEBNext Index 20 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGATAA <b>GGCCAC</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	GTGGCC
NEBNext Index 21 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGATT <b>CGAAAC</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	GTTTCG
NEBNext Index 22 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGATT <b>CGTACG</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	CGTACG
NEBNext Index 23 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGATAT <b>CCACTC</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	GAGTGG
NEBNext Index 25 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGATAT <b>ATCAGT</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	ACTGAT
NEBNext Index 27 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGATAA <b>AGGAAT</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	ATTCTT
NEBNext Adaptor for Illumina (15 µM)	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 (10 µM)	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 indexes:

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

To determine possible combinations for low plex pooling, please also see <https://indexoligo.neb.com/>

## 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	0.120 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
5.0	Update to new manual format	1/20
6.0	Updated adaptors and primers table for Illumina	6/22

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