

## NEBNext<sup>®</sup> Multiplex Oligos for Illumina<sup>®</sup> (Index Primers Set 4)

NEB #E7730S/L

24/96 reactions

Version 6.0\_6/22

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### The NEBNext Multiplex Oligos for Illumina (Index Primers Set 4) Includes:

*The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7730S) and 96 reactions (NEB #E7730L). All reagents should be stored at -20°C.*

NEBNext Adaptor for Illumina

USER<sup>®</sup> Enzyme

NEBNext Universal PCR Primer for Illumina

NEBNext Index 30 Primer for Illumina

NEBNext Index 37 Primer for Illumina

NEBNext Index 38 Primer for Illumina

NEBNext Index 39 Primer for Illumina

NEBNext Index 40 Primer for Illumina

NEBNext Index 41 Primer for Illumina

NEBNext Index 43 Primer for Illumina

NEBNext Index 44 Primer for Illumina

NEBNext Index 45 Primer for Illumina

NEBNext Index 46 Primer for Illumina

NEBNext Index 47 Primer for Illumina

NEBNext Index 48 Primer for Illumina

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

### Applications

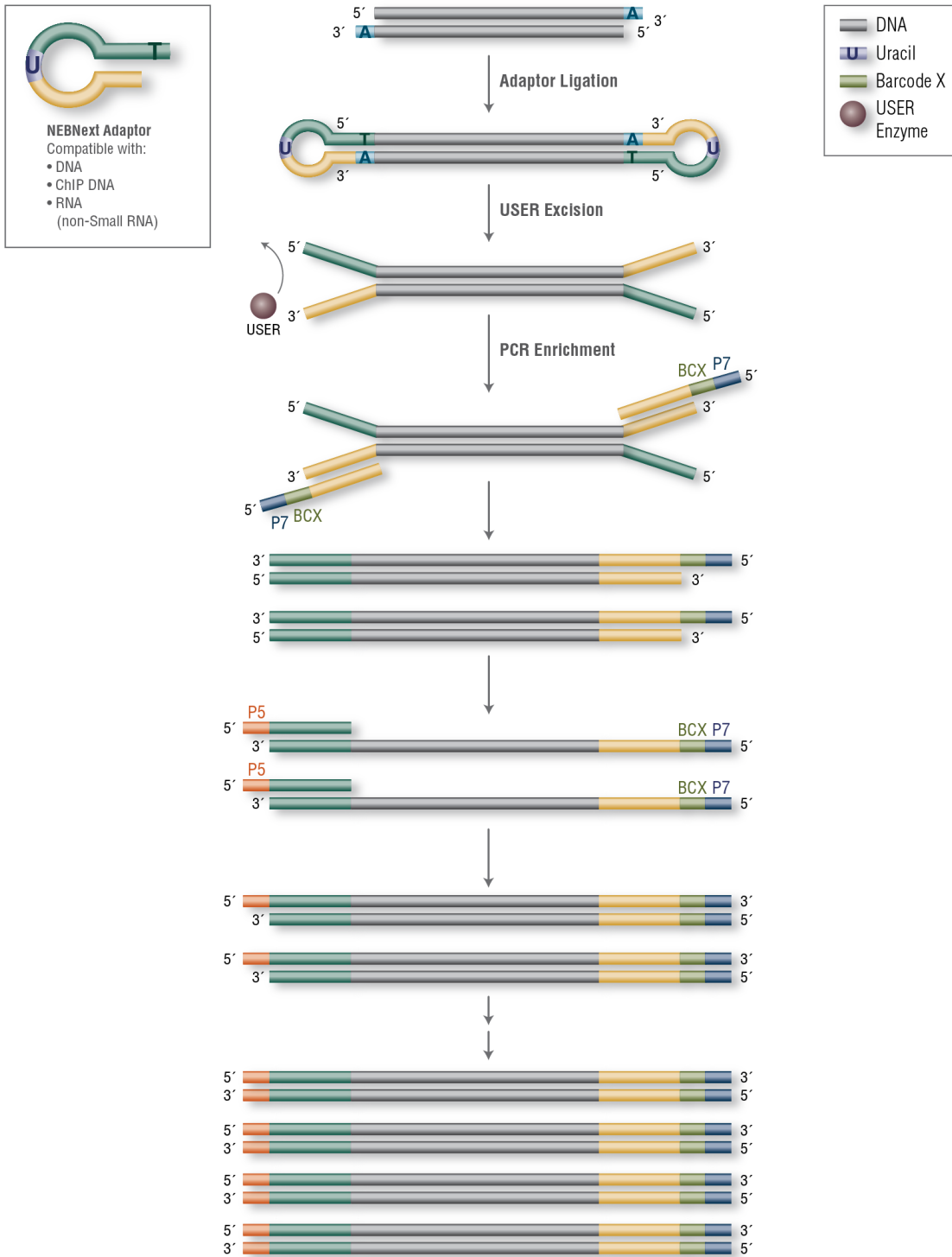
The NEBNext Multiplex Oligos for Illumina (Index Primers Set 4) contains adaptors and primers that are ideally suited for multiplex sample preparation for next generation sequencing on the Illumina platform (Illumina, Inc.). Each of these components must pass rigorous quality control standards and is 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 4) 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 4)**



**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, E7730 Product Page, "Protocols, Manuals and Usage Guidelines" Tab, [Usage Guidelines](#)

PRODUCT	INDEX PRIMER SEQUENCE	EXPECTED INDEX PRIMER SEQUENCE READ
NEBNext Index 30 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>CCGGTG</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	CACCGG
NEBNext Index 37 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>ATTCCGGT</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	CGGAAT
NEBNext Index 38 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>AGCTAG</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	CTAGCT
NEBNext Index 39 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>GTATAG</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	CTATAC
NEBNext Index 40 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>TGGATCAC</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	GTGATC
NEBNext Index 41 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>GTCTGT</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	GACGAC
NEBNext Index 43 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>GCTGTA</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	TACAGC
NEBNext Index 44 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>ATTATA</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	TATAAT
NEBNext Index 45 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>GAATGA</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	TCATTC
NEBNext Index 46 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>TCCGGG</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	TCCCGA
NEBNext Index 47 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>CTTCCA</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	TCGAAG
NEBNext Index 48 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>TGCCGA</b> GTGACTGGAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	TCGGCA
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 #37 and 45

Pool of 3 samples: Index #30, 38 and 48

Pool of 6 samples: Index #39, 43, 44, and 46

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

## Kit Components

NEB #E7730S 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
E7716A	NEBNext Index 30 Primer for Illumina	0.010 ml
E7731A	NEBNext Index 37 Primer for Illumina	0.010 ml
E7732A	NEBNext Index 38 Primer for Illumina	0.010 ml
E7733A	NEBNext Index 39 Primer for Illumina	0.010 ml
E7744A	NEBNext Index 40 Primer for Illumina	0.010 ml
E7735A	NEBNext Index 41 Primer for Illumina	0.010 ml
E7737A	NEBNext Index 43 Primer for Illumina	0.010 ml
E7738A	NEBNext Index 44 Primer for Illumina	0.010 ml
E7739A	NEBNext Index 45 Primer for Illumina	0.010 ml
E7740A	NEBNext Index 46 Primer for Illumina	0.010 ml
E7741A	NEBNext Index 47 Primer for Illumina	0.010 ml
E7742A	NEBNext Index 48 Primer for Illumina	0.010 ml

NEB #E7730L 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	0.480 ml
E7716AA	NEBNext Index 30 Primer for Illumina	0.040 ml
E7731AA	NEBNext Index 37 Primer for Illumina	0.040 ml
E7732AA	NEBNext Index 38 Primer for Illumina	0.040 ml
E7733AA	NEBNext Index 39 Primer for Illumina	0.040 ml
E7744AA	NEBNext Index 40 Primer for Illumina	0.040 ml
E7735AA	NEBNext Index 41 Primer for Illumina	0.040 ml
E7737AA	NEBNext Index 43 Primer for Illumina	0.040 ml
E7738AA	NEBNext Index 44 Primer for Illumina	0.040 ml
E7739AA	NEBNext Index 45 Primer for Illumina	0.040 ml
E7740AA	NEBNext Index 46 Primer for Illumina	0.040 ml
E7741AA	NEBNext Index 47 Primer for Illumina	0.040 ml
E7742AA	NEBNext Index 48 Primer for Illumina	0.040 ml

## Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	6/16
2.0	Part number and sequence replaced for NEBNext Index 40 Primer for Illumina	12/16
3.0	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. 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
4.0	Updated to new manual format.	1/20
5.0	Update adaptors and primers table	11/21
6.0	Updated adaptors and primers table for Illumina	6/22

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