

## NEBNext® Multiplex Oligos for Illumina® (Index Primers Sets 1– 4)

NEB #E7335S/L, NEB #E7500S/L, NEB #E7710S/L, NEB #E7730S/L

24/96 reactions

Version 8.0\_6/24

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### The NEBNext Multiplex Oligos for Illumina (Index Primers Set 1) Includes:

*The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7335S) and 96 reactions (NEB #E7335L). All reagents should be stored at –20°C.*

NEBNext Adaptor for Illumina

USER® Enzyme

NEBNext Universal PCR Primer for Illumina

NEBNext Index 1 Primer for Illumina

NEBNext Index 2 Primer for Illumina

NEBNext Index 3 Primer for Illumina

NEBNext Index 4 Primer for Illumina

NEBNext Index 5 Primer for Illumina

NEBNext Index 6 Primer for Illumina

NEBNext Index 7 Primer for Illumina

NEBNext Index 8 Primer for Illumina

NEBNext Index 9 Primer for Illumina

NEBNext Index 10 Primer for Illumina

NEBNext Index 11 Primer for Illumina

NEBNext Index 12 Primer for Illumina

### 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® 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

### **The NEBNext Multiplex Oligos for Illumina (Index Primers Set 3) Includes:**

*The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7710S) and 96 reactions (NEB #E7710L). All reagents should be stored at -20°C.*

NEBNext Adaptor for Illumina  
USER® Enzyme  
NEBNext Universal PCR Primer for Illumina  
NEBNext Index 17 Primer for Illumina  
NEBNext Index 24 Primer for Illumina  
NEBNext Index 26 Primer for Illumina  
NEBNext Index 28 Primer for Illumina  
NEBNext Index 29 Primer for Illumina  
NEBNext Index 31 Primer for Illumina  
NEBNext Index 32 Primer for Illumina  
NEBNext Index 33 Primer for Illumina  
NEBNext Index 34 Primer for Illumina  
NEBNext Index 35 Primer for Illumina  
NEBNext Index 36 Primer for Illumina  
NEBNext Index 42 Primer for Illumina

### **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® 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 Sets 1–4) contain 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 Sets 1–4) 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.

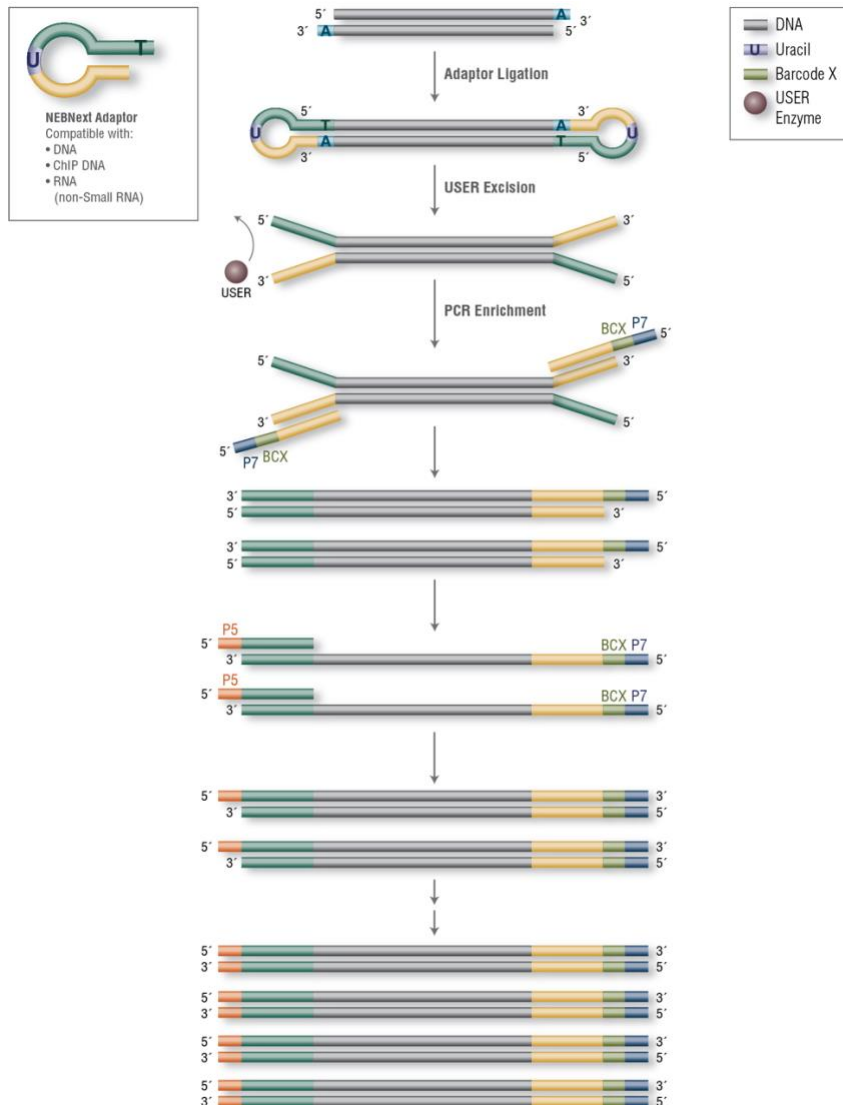
**Functionally Validated:** Each of the components is functionally validated through construction and sequencing of a genomic DNA libraries on the Illumina platform.

Where larger volumes, customized or bulk packaging are required, we encourage consultation with the Customized Solutions team at NEB. Please complete the NEB Custom Contact Form at [www.neb.com/CustomContactForm](http://www.neb.com/CustomContactForm) to learn more.

## Workflow Overview

Designed for use in library prep for DNA, ChIP DNA and RNA (but not Small RNA), the NEBNext non indexed Adaptors enable high-efficiency adaptor ligation and high library yields, with minimized adaptor-dimer formation. Incorporating a novel hairpin loop structure, the NEBNext Adaptor ligates with increased efficiency to end-repaired, dA-tailed DNA. The loop contains a U, which is removed by treatment with USER Enzyme (a combination of UDG and Endo VIII), to open up the loop and make it available as a substrate for PCR. During PCR, barcodes can be incorporated by use of the NEBNext index primers, thereby enabling multiplexing. NEBNext Oligos can be used with NEBNext products, and with other standard Illumina-compatible library preparation protocols, except PCR free workflows.

**Figure 1. Workflow demonstrating the use of NEBNext Multiplex Oligos for Illumina (Index Primers Sets 1–4)**



Please refer to the NEBNext Multiplex Oligos Selection Chart at [www.neb.com/oligos](http://www.neb.com/oligos) for a list of compatible applications.

## 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

## Index Sequence Files

For a link to download a sample sheet with the index sequences for use with the Illumina Experiment Manager (IEM) please go to our FAQs or Usage Guidelines tab on the relevant product page on [www.neb.com](http://www.neb.com) for each set:

[www.neb.com/E7335](http://www.neb.com/E7335) – NEBNext Multiplex Oligos for Illumina (Index Primers Set 1) (NEB #E7335)

[www.neb.com/E7500](http://www.neb.com/E7500) – NEBNext Multiplex Oligos for Illumina (Index Primers Set 2) (NEB #E7500)

[www.neb.com/E7710](http://www.neb.com/E7710) – NEBNext Multiplex Oligos for Illumina (Index Primers Set 3) (NEB #E7710)

[www.neb.com/E7730](http://www.neb.com/E7730) – NEBNext Multiplex Oligos for Illumina (Index Primers Set 4) (NEB #E7730)

**Note: Multiple sets can be pooled together for up to 48 samples on some Illumina sequencing instrument types.**

## Index Pooling Guidelines Within Each Set

### For all HiSeq<sup>®</sup>/MiSeq<sup>®</sup> sequencers:

Illumina uses four channel chemistry with a red laser/LED to sequence bases A and C and a green laser/LED to sequence bases G and T. For each cycle, both the red and the green channel need to be read to ensure proper image registration (i.e. A or C must be in each cycle, and G or T must be in each cycle). If this color balance is not maintained, sequencing the index read could fail. The following tables list some valid combinations (up to 8-plex) for each Set that can be sequenced together. For combinations > 8 choose any column and add any plex combinations as needed.

### For the NovaSeq<sup>®</sup>6000/ NextSeq<sup>®</sup>/MiniSeq<sup>®</sup>:

Utilize red/ green or blue/ green 2 color chemistry, valid index combinations must include some indices that do not start with GG in the first two cycles. [See Illumina document Document # 1000000041074 v12.](#)

### For the NovaSeq<sup>®</sup>X and X Plus:

Utilize blue/ green 2 color chemistry, valid index combinations must include some indices that do not start with GG in the first two cycles.

**Please note: for Illumina Sequencing instruments using patterned flow cells (for example NovaSeq, NextSeq 1000/ 2000, Iseq) Unique Dual Indexing is recommended. Please see [Illumina Index Hopping White Paper](#).**

**Low Plex pooling options in this manual are only for Illumina four channel red/ green chemistry.**

## NEBNext Adaptors and Primers Set 1 for Illumina

For sample sheets please see NEB.com, E7335 Product Page, "Protocols, Manuals and Usage Guidelines" Tab, [Usage Guidelines](#).

PRODUCT	INDEX PRIMER SEQUENCE	EXPECTED INDEX PRIMER SEQUENCE READ
NEBNext Index 1 Primer for Illumina (10 µM)	5'- CAAGCAGAAGACGGCATAACGAGAT <b>CGTGAT</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGA TC-s-T-3'	ATCACG
NEBNext Index 2 Primer for Illumina (10 µM)	5'- CAAGCAGAAGACGGCATAACGAGAT <b>ACATCG</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGA TC-s-T-3'	CGATGT
NEBNext Index 3 Primer for Illumina (10 µM)	5'- CAAGCAGAAGACGGCATAACGAGAT <b>GCCTAA</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGA TC-s-T-3'	TTAGGC
NEBNext Index 4 Primer for Illumina (10 µM)	5'- CAAGCAGAAGACGGCATAACGAGAT <b>TGGTCA</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGA TC-s-T-3'	TGACCA
NEBNext Index 5 Primer for Illumina (10 µM)	5'- CAAGCAGAAGACGGCATAACGAGAT <b>CACTGT</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGA TC-s-T-3'	ACAGTG
NEBNext Index 6 Primer for Illumina (10 µM)	5'- CAAGCAGAAGACGGCATAACGAGAT <b>ATTGGC</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGA TC-s-T-3'	GCCAAT
NEBNext Index 7 Primer for Illumina (10 µM)	5'- CAAGCAGAAGACGGCATAACGAGAT <b>GATCTG</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGA TC-s-T-3'	CAGATC
NEBNext Index 8 Primer for Illumina (10 µM)	5'- CAAGCAGAAGACGGCATAACGAGAT <b>TCAAGT</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGA TC-s-T-3'	ACTTGA
NEBNext Index 9 Primer for Illumina (10 µM)	5'- CAAGCAGAAGACGGCATAACGAGAT <b>CTGATC</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGA TC-s-T-3'	GATCAG
NEBNext Index 10 Primer for Illumina (10 µM)	5'- CAAGCAGAAGACGGCATAACGAGAT <b>AAGCTA</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGA TC-s-T-3'	TAGCTT
NEBNext Index 11 Primer for Illumina (10 µM)	5'- CAAGCAGAAGACGGCATAACGAGAT <b>GTAGCC</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGA TC-s-T-3'	GGCTAC
NEBNext Index 12 Primer for Illumina (10 µM)	5'- CAAGCAGAAGACGGCATAACGAGAT <b>TACAAG</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGA TC-s-T-3'	CTTGTA
NEBNext Adaptor for Illumina (15 µM)	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'	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 7 indexes are used in a lane for sequencing, it is recommended to use the following indexes:

Pool of 2 samples: Index #6 and 12

Pool of 3 samples: Index #4, 6 and 12

Pool of 6 samples: Index #2, 4, 5, 6, 7 and 12

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

## NEBNext Adaptors and Primers Set 2 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 PRIMER SEQUENCE READ
NEBNext Index 13 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATACGAGATTG <b><u>TTGACT</u></b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	AGTCAA
NEBNext Index 14 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATACGAGATAC <b><u>GGA</u></b> ACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	AGTTCC
NEBNext Index 15 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATACGAGATT <b><u>CTGACAT</u></b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	ATGTCA
NEBNext Index 16 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATACGAGATGC <b><u>GGACGG</u></b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CCGTCC
NEBNext Index 18 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATACGAGATGT <b><u>GCGGAC</u></b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GTCCGC
NEBNext Index 19 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATACGAGATCG <b><u>TTTCAC</u></b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GTGAAA
NEBNext Index 20 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATACGAGATAA <b><u>GGCCAC</u></b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GTGGCC
NEBNext Index 21 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATACGAGATT <b><u>CGAAAC</u></b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GTTTCG
NEBNext Index 22 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATACGAGATTA <b><u>CGTACG</u></b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CGTACG
NEBNext Index 23 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATACGAGATAT <b><u>CCACTC</u></b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GAGTGG
NEBNext Index 25 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATACGAGATAT <b><u>ATCAGT</u></b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	ACTGAT
NEBNext Index 27 Primer for Illumina (10 μM)	5'-CAAGCAGAAGACGGCATACGAGATAA <b><u>AGGAAT</u></b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-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>.

## NEBNext Adaptors and Primers Set 3 for Illumina

For sample sheets please see NEB.com, E7710 Product Page, "Protocols, Manuals and Usage Guidelines" Tab, [Usage Guidelines](#).

PRODUCT	INDEX PRIMER SEQUENCE	EXPECTED INDEX PRIMER SEQUENCE READ
NEBNext Index 17 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATACGAGAT <b>CTCTAC</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GTAGAG
NEBNext Index 24 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATACGAGAT <b>GCTACC</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GGTAGC
NEBNext Index 26 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATACGAGAT <b>GCTCAT</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	ATGAGC
NEBNext Index 28 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATACGAGAT <b>CTTTTG</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CAAAAG
NEBNext Index 29 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATACGAGAT <b>TAGTTG</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CAACTA
NEBNext Index 31 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATACGAGAT <b>ATCGTG</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CACGAT
NEBNext Index 32 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATACGAGAT <b>TGAGTG</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CACTCA
NEBNext Index 33 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATACGAGAT <b>CGCCTG</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CAGGCG
NEBNext Index 34 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATACGAGAT <b>GCCATG</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CATGGC
NEBNext Index 35 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATACGAGAT <b>AAAATG</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CATTTT
NEBNext Index 36 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATACGAGAT <b>TGTTGG</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CCAACA
NEBNext Index 42 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATACGAGAT <b>CGATTA</b> GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	TAATCG
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 #24 and 31; Index #26 and 42

Pool of 3 samples: Index #24, 32 and 33; Index #24, 34 and 42

Pool of 4 samples: Index #24, 32, 35 and 36; Index #17, 26, 36 and 42

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

## NEBNext Adaptors and Primers Set 4 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>CCGGT</b> GTGACTGGAGTTCAGACGTGTGCTC TTCCGATC-s-T-3'	CACCGG
NEBNext Index 37 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>ATTCCG</b> GTGACTGGAGTTCAGACGTGTGCTC TTCCGATC-s-T-3'	CGGAAT
NEBNext Index 38 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>AGCTAG</b> GTGACTGGAGTTCAGACGTGTGCTC TTCCGATC-s-T-3'	CTAGCT
NEBNext Index 39 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>GTATAG</b> GTGACTGGAGTTCAGACGTGTGCTC TTCCGATC-s-T-3'	CTATAC
NEBNext Index 40 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>TGGATCAC</b> GTGACTGGAGTTCAGACGTGTGCTC TCTTCCGATC-s-T-3'	GTGATC
NEBNext Index 41 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>GTCGTC</b> GTGACTGGAGTTCAGACGTGTGCTC TTCCGATC-s-T-3'	GACGAC
NEBNext Index 43 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>GCTGTA</b> GTGACTGGAGTTCAGACGTGTGCTC TTCCGATC-s-T-3'	TACAGC
NEBNext Index 44 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>ATTATA</b> GTGACTGGAGTTCAGACGTGTGCTC TTCCGATC-s-T-3'	TATAAT
NEBNext Index 45 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>GAATGA</b> GTGACTGGAGTTCAGACGTGTGCTC TTCCGATC-s-T-3'	TCATTC
NEBNext Index 46 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>TCGGGA</b> GTGACTGGAGTTCAGACGTGTGCTC TTCCGATC-s-T-3'	TCCCGA
NEBNext Index 47 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>CTTCGA</b> GTGACTGGAGTTCAGACGTGTGCTC TTCCGATC-s-T-3'	TCGAAG
NEBNext Index 48 Primer for Illumina (10 µM)	5'-CAAGCAGAAGACGGCATAACGAGAT <b>TGCCGA</b> GTGACTGGAGTTCAGACGTGTGCTC TTCCGATC-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 #E7335S 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
E7311A	NEBNext Index 1 Primer for Illumina	0.010 ml
E7312A	NEBNext Index 2 Primer for Illumina	0.010 ml
E7313A	NEBNext Index 3 Primer for Illumina	0.010 ml
E7314A	NEBNext Index 4 Primer for Illumina	0.010 ml
E7315A	NEBNext Index 5 Primer for Illumina	0.010 ml
E7316A	NEBNext Index 6 Primer for Illumina	0.010 ml
E7317A	NEBNext Index 7 Primer for Illumina	0.010 ml
E7318A	NEBNext Index 8 Primer for Illumina	0.010 ml
E7319A	NEBNext Index 9 Primer for Illumina	0.010 ml
E7320A	NEBNext Index 10 Primer for Illumina	0.010 ml
E7321A	NEBNext Index 11 Primer for Illumina	0.010 ml
E7322A	NEBNext Index 12 Primer for Illumina	0.010 ml

NEB #E7335L 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
E7311AA	NEBNext Index 1 Primer for Illumina	0.040 ml
E7312AA	NEBNext Index 2 Primer for Illumina	0.040 ml
E7313AA	NEBNext Index 3 Primer for Illumina	0.040 ml
E7314AA	NEBNext Index 4 Primer for Illumina	0.040 ml
E7315AA	NEBNext Index 5 Primer for Illumina	0.040 ml
E7316AA	NEBNext Index 6 Primer for Illumina	0.040 ml
E7317AA	NEBNext Index 7 Primer for Illumina	0.040 ml
E7318AA	NEBNext Index 8 Primer for Illumina	0.040 ml
E7319AA	NEBNext Index 9 Primer for Illumina	0.040 ml
E7320AA	NEBNext Index 10 Primer for Illumina	0.040 ml
E7321AA	NEBNext Index 11 Primer for Illumina	0.040 ml
E7322AA	NEBNext Index 12 Primer for Illumina	0.040 ml

### NEB #E7500S Table of Components

<b>NEB #</b>	<b>PRODUCT</b>	<b>VOLUME</b>
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

<b>NEB #</b>	<b>PRODUCT</b>	<b>VOLUME</b>
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

### NEB #E7710S Table of Components

<b>NEB #</b>	<b>PRODUCT</b>	<b>VOLUME</b>
E7337A	NEBNext Adaptor for Illumina	0.24 ml
E7338A	USER Enzyme	0.072 ml
E6861A	NEBNext Universal PCR Primer for Illumina	0.120 ml
E7585A	NEBNext Index 17 Primer for Illumina	0.010 ml
E7592A	NEBNext Index 24 Primer for Illumina	0.010 ml
E7713A	NEBNext Index 26 Primer for Illumina	0.010 ml
E7714A	NEBNext Index 28 Primer for Illumina	0.010 ml
E7715A	NEBNext Index 29 Primer for Illumina	0.010 ml
E7717A	NEBNext Index 31 Primer for Illumina	0.010 ml
E7718A	NEBNext Index 32 Primer for Illumina	0.010 ml
E7719A	NEBNext Index 33 Primer for Illumina	0.010 ml
E7720A	NEBNext Index 34 Primer for Illumina	0.010 ml
E7721A	NEBNext Index 35 Primer for Illumina	0.010 ml
E7722A	NEBNext Index 36 Primer for Illumina	0.010 ml
E7736A	NEBNext Index 42 Primer for Illumina	0.010 ml

### NEB #E7710L Table of Components

<b>NEB #</b>	<b>PRODUCT</b>	<b>VOLUME</b>
E7337AA	NEBNext Adaptor for Illumina	0.96 ml
E7338AA	USER Enzyme	0.288 ml
E6861AA	NEBNext Universal PCR Primer for Illumina	0.480 ml
E7585AA	NEBNext Index 17 Primer for Illumina	0.040 ml
E7592AA	NEBNext Index 24 Primer for Illumina	0.040 ml
E7713AA	NEBNext Index 26 Primer for Illumina	0.040 ml
E7714AA	NEBNext Index 28 Primer for Illumina	0.040 ml
E7715AA	NEBNext Index 29 Primer for Illumina	0.040 ml
E7717AA	NEBNext Index 31 Primer for Illumina	0.040 ml
E7718AA	NEBNext Index 32 Primer for Illumina	0.040 ml
E7719AA	NEBNext Index 33 Primer for Illumina	0.040 ml
E7720AA	NEBNext Index 34 Primer for Illumina	0.040 ml
E7721AA	NEBNext Index 35 Primer for Illumina	0.040 ml
E7722AA	NEBNext Index 36 Primer for Illumina	0.040 ml
E7736AA	NEBNext Index 42 Primer for Illumina	0.040 ml

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
3.0	Change Index Primer and Universal Primer concentration from 25 µM to 10 µM. All part #'s have changed. Concentrations of Universal PCR Primer and Index Primers have changed from 25 µM to 10 µM.	1/15
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	DNA sequence for NEBNext Adaptor for Illumina has been corrected. Delete "Required Materials Not Included for DNA or ChIP Libraries" and "Required Materials Not Included for RNA Libraries" and replace with guidance note. Kit specific protocol list has been updated. 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	Adjusted NEBNext Adaptor for Illumina Table Index Primer Sequence. Adjusted volumes on Kit Components Table of Components for "S" Sizes.	4/18
6.0	Updated to new manual format.	1/20
7.0	Updated adaptors and primers table for Illumina.	6/22
8.0	Combined manual for all 4 sets. Also updated header and footer with new logo, as well as updated legal footnote.	6/24

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