

LIBRARY PREPARATION

NEBNext[®] Multiplex Oligos for Illumina[®] (Index Primers Set 1)

Instruction Manual

NEB #E7335S/L
24/96 reactions
Version 5.0 4/18



be INSPIRED
drive DISCOVERY
stay GENUINE

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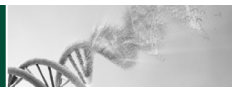


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The NEBNext Multiplex Oligos for Illumina (Index Primers Set 1) Includes:

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

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

Applications:

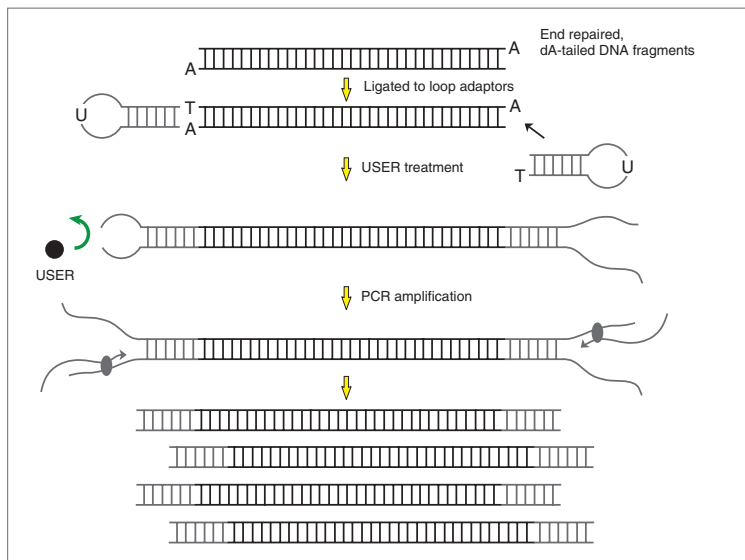
The NEBNext Multiplex Oligos for Illumina (Index Primers Set 1) contains adaptors and primers that are ideally suited for multiplex sample preparation for next-generation sequencing on the Illumina platform (Illumina, Inc.). Each of these components must pass rigorous quality control standards and is 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 1) 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 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 1 Primer for Illumina	5'-CAAGCAGAAGACGGCATAACGAGAT CGTGAT GTGACTG GAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	ATCACC
NEBNext Index 2 Primer for Illumina	5'-CAAGCAGAAGACGGCATAACGAGAT ACATCGG GTGACTG GAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	CGATGT
NEBNext Index 3 Primer for Illumina	5'-CAAGCAGAAGACGGCATAACGAGAT GCCTAAG GTGACTG GAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	TTAGGC
NEBNext Index 4 Primer for Illumina	5'-CAAGCAGAAGACGGCATAACGAGAT TGGTCAG GTGACTG GAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	TGACCA
NEBNext Index 5 Primer for Illumina	5'-CAAGCAGAAGACGGCATAACGAGAT CACTGT GTGACTG GAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	ACAGTG
NEBNext Index 6 Primer for Illumina	5'-CAAGCAGAAGACGGCATAACGAGAT ATTGGC GTGACTG GAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	GCCAAT
NEBNext Index 7 Primer for Illumina	5'-CAAGCAGAAGACGGCATAACGAGAT GATCTG GTGACTG GAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	CAGATC
NEBNext Index 8 Primer for Illumina	5'-CAAGCAGAAGACGGCATAACGAGAT TCAAGT GTGACTG GAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	ACTTGA
NEBNext Index 9 Primer for Illumina	5'-CAAGCAGAAGACGGCATAACGAGAT CTGATC GTGACTG GAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	GATCAG
NEBNext Index 10 Primer for Illumina	5'-CAAGCAGAAGACGGCATAACGAGAT AAGCTA GTGACTG GAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	TAGCTT
NEBNext Index 11 Primer for Illumina	5'-CAAGCAGAAGACGGCATAACGAGAT GTAGCC GTGACTG GAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	GGCTAC
NEBNext Index 12 Primer for Illumina	5'-CAAGCAGAAGACGGCATAACGAGAT TACAAG GTGACTG GAGTTCAGACGTGTGCTCTCCGATC-s-T-3'	CTTGTA
NEBNext Adaptor for Illumina	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	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 indices:

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

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

Revision History:

REVISION #	DESCRIPTION	DATE
3.0	Change Index Primer and Universal Primer concentration from 25 μ M to 10 μ M. All part #'s have changed. Concentrations of Universal PCR Primer and Index Primers have changed from 25 μ M to 10 μ M.	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



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