

LIBRARY PREPARATION

NEBNext[®] Multiplex Oligos for Illumina[®] (Index Primers Set 3)

Instruction Manual

NEB #E7710S/L
24/96 reactions
Version 2.0 1/18



be INSPIRED
drive DISCOVERY
stay GENUINE

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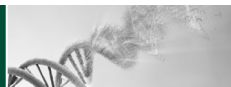


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The NEBNext Multiplex Oligos for Illumina (Index Primers Set 3) Includes:

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

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

Applications:

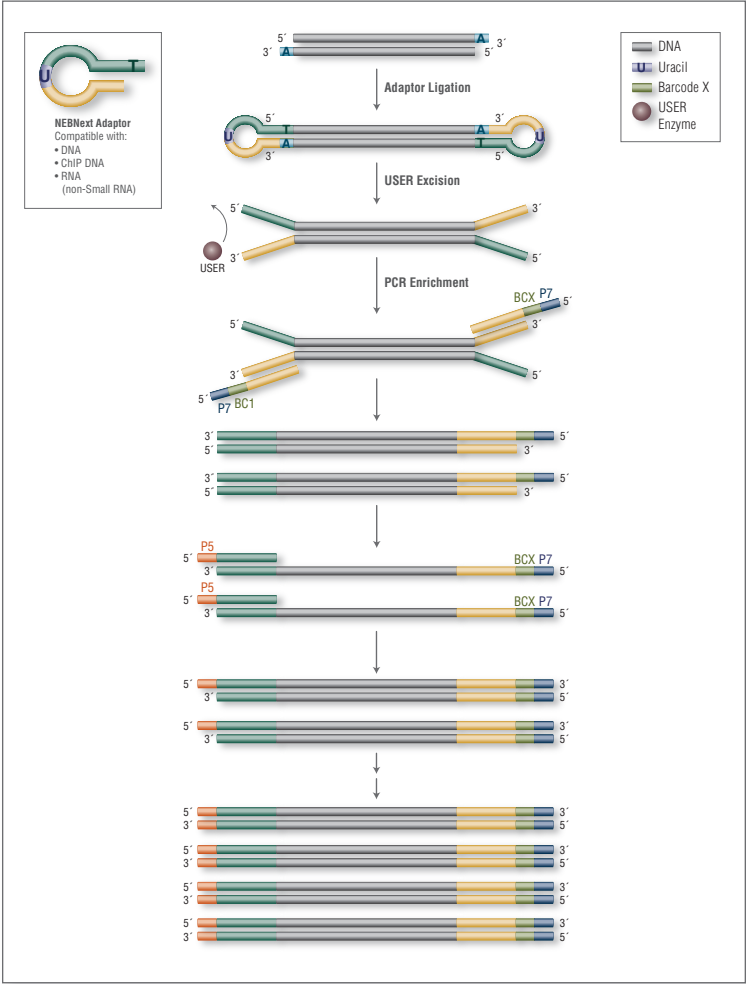
The NEBNext Multiplex Oligos for Illumina (Index Primers Set 3) contains adaptors and primers that are ideally suited for multiplex sample preparation for next-generation sequencing on the Illumina platform (Illumina, Inc.). Each of these components must pass rigorous quality control standards and is 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 3) 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 demonstrating the use of NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)



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 17 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGAT CTCTAC GTGACTGG AGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GTAGAG
NEBNext Index 24 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGAT GCTACC GTGACTG GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GGTAGC
NEBNext Index 26 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGAT GCTCAT GTGACTG GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	ATGAGC
NEBNext Index 28 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGAT CTTTTG GTGACTGG AGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CAAAAG
NEBNext Index 29 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGAT TAGTTG GTGACTG GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CAACTA
NEBNext Index 31 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGAT ATCGTG GTGACTG GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CACGAT
NEBNext Index 32 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGAT TGAGTG GTGACTG GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CACTCA
NEBNext Index 33 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGAT CGCCTG GTGACTG GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CAGGCG
NEBNext Index 34 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGAT GCCATG GTGACTG GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CATGGC
NEBNext Index 35 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGAT AAAATG GTGACTG GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CATTTT
NEBNext Index 36 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGAT TGTTGG GTGACTG GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CCAACA
NEBNext Index 42 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGAT CGATTAG TGACTGG AGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	TAATCG
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 #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

(other combinations are possible for pooling > 2 samples)

Kit Components

NEB #E7710S 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
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

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
E7585AA	NEBNext Index 17 Primer for Illumina	0.04 ml
E7592AA	NEBNext Index 24 Primer for Illumina	0.04 ml
E7713AA	NEBNext Index 26 Primer for Illumina	0.04 ml
E7714AA	NEBNext Index 28 Primer for Illumina	0.04 ml
E7715AA	NEBNext Index 29 Primer for Illumina	0.04 ml
E7717AA	NEBNext Index 31 Primer for Illumina	0.04 ml
E7718AA	NEBNext Index 32 Primer for Illumina	0.04 ml
E7719AA	NEBNext Index 33 Primer for Illumina	0.04 ml
E7720AA	NEBNext Index 34 Primer for Illumina	0.04 ml
E7721AA	NEBNext Index 35 Primer for Illumina	0.04 ml
E7722AA	NEBNext Index 36 Primer for Illumina	0.04 ml
E7736AA	NEBNext Index 42 Primer for Illumina	0.04 ml

Revision History:

REVISION #	DESCRIPTION	DATE
1.0	N/A	6/16
1.1	Updated sequences for NEBNext Index Primers 17 and 24.	8/16
2.0	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



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