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NEBNext® Multiplex Oligos for Illumina® (Unique Dual Index UMI Adaptors DNA Set 1)

NEB #E7395S/L

96/384 reactions

Version 1.0_3/20

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The NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1) Includes

The volumes provided are sufficient for preparation of up to 96 reactions (NEB #E7395S) and 384 reactions (NEB #E7395L).
All reagents should be stored at –20°C.

NEBNext Primer Mix

NEBNext UMI Adaptor Dilution Buffer

NEBNext UMI DNA Adaptor Plate
  Each well contains a unique dual index UMI adaptor (S size contains 1 plate, L size contains 4 plates)

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

Overview

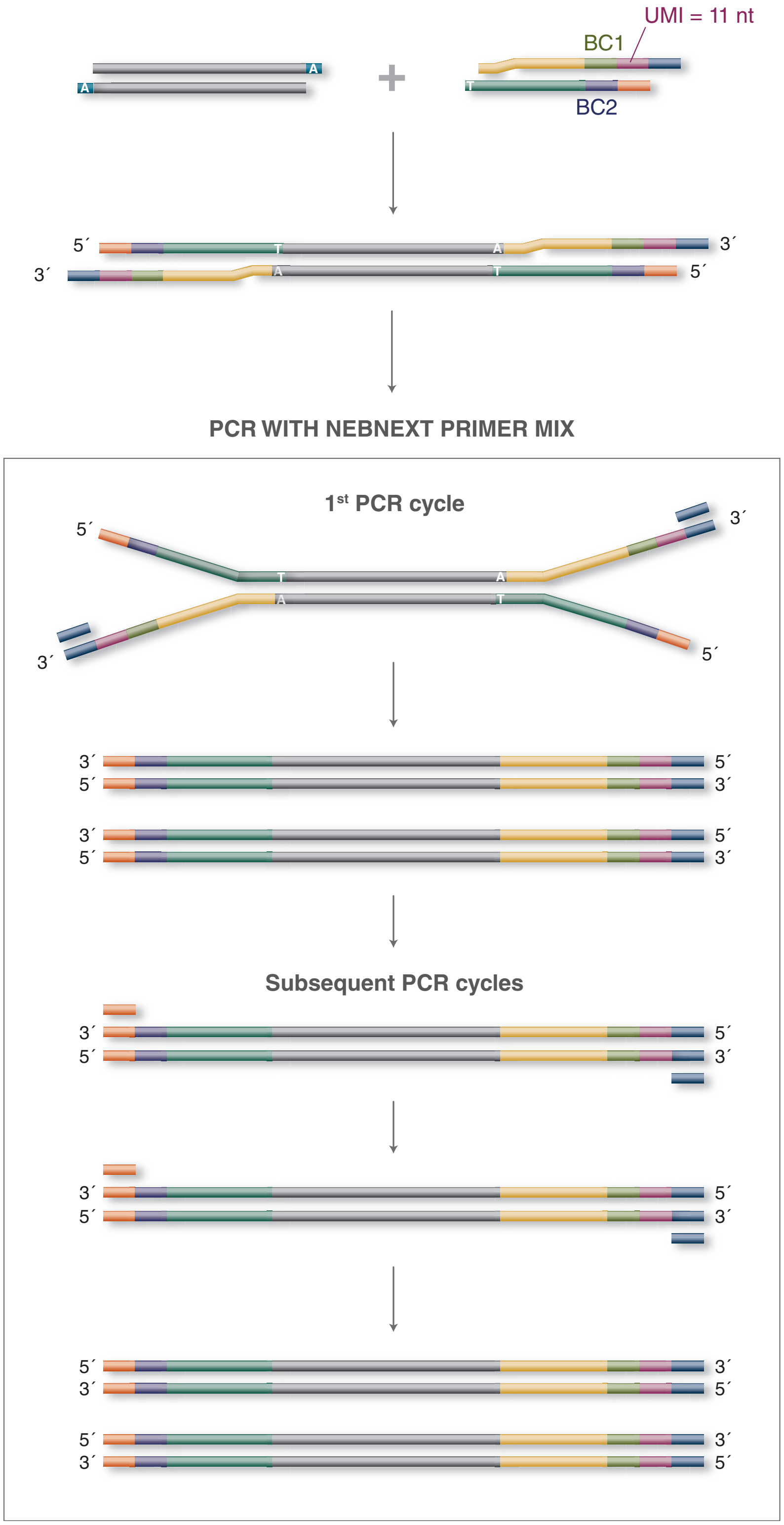
The NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1) contains adaptors and primers that are ideally suited for multiplex sample preparation for next-generation sequencing on the Illumina platform (Illumina, Inc.). Each kit component must pass rigorous quality control standards, and for each new lot the entire set of reagents is functionally validated together by construction and sequencing of indexed libraries on an Illumina sequencing 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.

Workflow

Designed for use in library prep for DNA, ChIP DNA and RNA (but not Small RNA), the NEBNext Unique Dual Index UMI (Unique Molecular Identifier) Adaptors enable high-efficiency adaptor ligation and high library yields. These adaptors contain all necessary sequences for sequencing on the Illumina platform, and are compatible with PCR-free applications and sample pooling prior to PCR amplification. The incorporation of a 11 base UMI allows 1) accurate identification and removal of duplicate reads, and 2) consensus sequence building and error correction, ideally suited for accurate analysis of quantitative NGS data. The 96 8-base unique dual index UMI adaptors included in this kit are packaged in a single-use 96-well plate with a pierceable foil seal. NEBNext Oligos can be used with NEBNext products, and with other standard Illumina-compatible library preparation protocols that are based on TA single base overhang ligation.

Figure 1. Workflow demonstrating the use of NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1).



Library Preparation Kits for use with NEBNext Unique Dual Index UMI Adaptors DNA Set 1

Please refer to the kit specific protocol. The following kits are designed for use with the NEBNext Multiplex Oligos for Illumina:

- #E7805, NEBNext Ultra™ II FS DNA Library Prep Kit for Illumina
- #E6177, NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads
- #E7645, NEBNext Ultra II DNA Library Prep Kit for Illumina
- #E7103, NEBNext Ultra II DNA Library Prep with Sample Purification Beads
- #E7595, NEBNext Ultra II Ligation Module

Please refer to the library preparation kit manual for additional required materials that are not included.

Index Pooling Guidelines: 96 Reaction Kit

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For a link to download a sample sheet with the index sequences for use with the Illumina Experiment Manager (IEM) please go to our FAQ's; or Usage Guidelines tab on www.neb.com/E7395 – NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1) (NEB #E7395).

For all HiSeq®/MiSeq® sequencers, Illumina uses 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. Table 1 lists some valid combinations (up to 8-plex) that can be sequenced together. For combinations > 8 choose any column and add any plex combinations as needed.

For the NovaSeq®/NextSeq®/MiniSeq® which utilize 2 color chemistry, valid index combinations must include some indices that do not start with GG in the first two cycles. Use Table 1 for some suggested combinations.

Table 1. Index Pooling Guidelines

PLEX	WELL POSITION
< 4	Not recommended
4	A6, B6, C6, D6 A12, B12, C12, D12 B6, C6, D6, E6 B12, C12, D12, E12 C1, D1, E1, F1 C7, D7, E7, F7 E4, F4, G4, H4 E10, F10, G10, H10
5	A1, B1, C1, D1, E1 A6, B6, C6, D6, E6 A7, B7, C7, D7, E7 A12, B12, C12, D12, E12 B1, C1, D1, E1, F1 B6, C6, D6, E6, F6 B7, C7, D7, E7, F7 B12, C12, D12, E12, F12 C1, D1, E1, F1, G1 C2, D2, E2, F2, G2 C4, D4, E4, F4, G4 C7, D7, E7, F7, G7 C8, D8, E8, F8, G8 C10, D10, E10, F10, G10 D4, E4, F4, G4, H4 D10, E10, F10, G10, H10
6-7	Any 5 plex plus 1-2 adjacent wells from the same column
8	Any column

Table 2. Examples of "good" and "bad" index combinations based on HiSeq/MiSeq guidelines. Each index sequence is color coded to correspond to the red/green channel. For combinations of valid indices, ensure that you will have signal in both the red and green channels in each cycle.

BAD

WELL POSITION									EXPECTED i5 INDEX READ															
	EXPECTED i7 INDEX READ								MiSeq, HiSeq 2000/2500								HiSeq 3000/4000, HiSeq X							
E8	T	A	T	G	G	C	A	C	T	T	G	C	G	A	G	A	T	C	T	C	G	C	A	A
F8	G	A	A	T	C	A	C	C	G	A	A	C	G	A	A	G	C	T	T	C	G	T	T	C
G8	G	T	A	A	G	G	T	G	C	G	A	A	T	T	G	C	G	C	A	A	T	T	C	G
H8	C	G	A	G	A	G	A	A	G	G	A	A	G	A	G	A	T	C	T	C	T	T	C	C
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	✓	✓	✓	✓	✓	✓	X	X	✓	✓	✓
A1	T	T	A	C	C	G	A	C	C	G	A	A	T	A	C	G	C	G	T	A	T	T	G	G
B1	T	C	G	T	C	T	G	A	G	T	C	C	T	T	G	A	T	C	A	A	G	G	A	C
C1	T	T	C	C	A	G	G	T	C	A	G	T	G	C	T	T	A	A	G	C	A	C	T	G
D1	T	A	C	G	G	T	C	T	T	C	C	A	T	T	G	C	G	C	A	A	T	G	G	A
	X	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓

GOOD

WELL POSITION									EXPECTED i5 INDEX READ															
	EXPECTED i7 INDEX READ								MiSeq, HiSeq 2000/2500								HiSeq 3000/4000, HiSeq X							
C1	T	T	C	C	A	G	G	T	C	A	G	T	G	C	T	T	A	A	G	C	A	C	G	G
D1	T	A	C	G	G	T	C	T	T	C	C	A	T	T	G	C	G	C	A	A	T	G	G	A
E1	A	A	G	A	C	C	G	T	G	T	C	G	A	T	T	G	C	A	A	T	C	G	A	C
F1	C	A	G	G	T	T	C	A	A	T	A	A	C	G	C	C	G	G	C	G	T	T	A	T
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A12	C	G	G	C	A	T	T	A	G	T	C	A	G	T	C	A	T	G	A	C	T	G	C	C
B12	C	A	C	G	C	A	A	T	C	C	T	T	C	C	A	T	A	T	G	G	A	A	G	G
C12	G	G	A	A	T	G	T	C	A	G	G	A	A	C	A	C	G	T	G	T	T	C	C	T
D12	T	G	G	T	G	A	A	G	C	T	T	A	C	A	G	C	G	C	T	G	T	A	A	G
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

The index adaptor sequences for different Illumina sequencer input sheets are indicated in Table 4.

Table 3. Examples of "good" and "bad" index combinations based on NovaSeq, NextSeq and MiniSeq guidelines. NovaSeq, NextSeq and MiniSeq use 2 color channel sequencing to simplify nucleotide detection. Clusters only in red or green are interpreted as C or T, respectively. Clusters in both red and green are read as A, while unlabeled clusters are G bases. For multiplexing a small number of samples, make sure the final index pool contains some indices that do not start with GG in the first two cycles.

GOOD																								
WELL POSITION	EXPECTED i7 INDEX READ								EXPECTED i5 INDEX READ															
									NovaSeq								MiniSeq, NextSeq							
A12	C	G	G	C	A	T	T	A	G	T	C	A	G	T	C	A	T	G	A	C	T	G	C	C
B12	C	A	C	G	C	A	A	T	C	C	T	T	C	C	A	T	A	T	G	G	A	A	G	G
C12	G	G	A	A	T	G	T	C	A	G	G	A	A	C	A	C	G	T	G	T	T	C	C	T
D12	T	G	G	T	G	A	A	G	C	T	T	A	C	A	G	C	G	C	T	G	T	A	A	G
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

BAD																								
WELL POSITION	EXPECTED i7 INDEX READ								EXPECTED i5 INDEX READ															
									NovaSeq								MiniSeq, NextSeq							
C12	G	G	A	A	T	G	T	C	A	G	G	A	A	C	A	C	G	T	G	T	T	C	C	T
E12	G	G	A	C	A	T	C	A	T	A	C	C	T	G	C	A	T	G	C	A	G	G	T	A
F12	G	G	T	G	T	A	C	A	A	G	A	C	G	C	T	A	T	A	G	C	G	T	C	T
G11	G	G	T	T	G	A	A	C	T	C	C	A	C	G	T	T	A	A	C	G	T	G	G	A
	X	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Index Sequences

Table 4. Index Sequences (color coded based on HiSeq/MiSeq guidelines).

WELL POSITION	EXPECTED i7 INDEX READ	EXPECTED i5 INDEX READ	
		NovaSeq, MiSeq, HiSeq 2000/2500 (PE Flow Cell), HiSeq 3000/4000 (Single Read Flow Cell)	NextSeq, MiSeq, HiSeq 2000/2500 (Single Read Flow Cell), HiSeq 3000/4000/ HiSeq X (PE Flow Cell)
A1	TTACCGAC	CGAATACG	CGTATTCG
B1	TCGTCTGA	GTCCTTGA	TCAAGGAC
C1	TTCCAGGT	CAGTGCTT	AAGCACTG
D1	TACGGTCT	TCCATTGC	GCAATGGA
E1	AAGACCGT	GTCGATTG	CAATCGAC
F1	CAGGTTCA	ATAACGCC	GGCGTTAT
G1	TAGGAGCT	GCCTTAAC	GTTAAGGC
H1	TACTCCAG	GGTATAGG	CCTATACC
A2	AGTGACCT	TCTAGGAG	CTCCTAGA
B2	AGCCTATC	TGCGTAAC	GTTACGCA
C2	TCATCTCC	CTTGCTAG	CTAGCAAG
D2	CCAGTATC	AGCGAGAT	ATCTCGCT
E2	TTGCGAGA	TATGGCAC	GTGCCATA
F2	GAACGAAG	GAATCACC	GGTGATTC
G2	CGAATTGC	GTAAGGTG	CACCTTAC
H2	GGAAGAGA	CGAGAGAA	TTCTCTCG
A3	TCGGATTC	CGCAACTA	TAGTTGCG
B3	CTGTACCA	CACAGACT	AGTCTGTG
C3	GAGAGTAC	TGGAAGCA	TGCTTCCA
D3	TCTACGCA	CAATAGCC	GGCTATTG
E3	GCAATTCC	CTCGAACA	TGTTCGAG
F3	CTCAGAAG	GGCAAGTT	AACTTGCC
G3	GTCCTAAG	AGCTACCA	TGGTAGCT
H3	GCGTTAGA	CAGCATAC	GTATGCTG
A4	CAAGGTAC	CGTATCTC	GAGATACG
B4	AGACCTTG	TTACGTGC	GCACGTAA
C4	GTCGTTAC	AGCTAAGC	GCTTAGCT
D4	GTAACCGA	AAGACACC	GGTGTCTT
E4	GAATCCGT	CAACTCCA	TGGAGTTG
F4	CATGAGCA	GATCTTGC	GCAAGATC
G4	CTTAGGAC	CTTCACTG	CAGTGAAG
H4	ATCTGACC	CTCGACTT	AAGTCGAG
A5	TCCTCATG	GTACACCT	AGGTGTAC
B5	AGGATAGC	CCAAGGTT	AACCTTGG
C5	GGAGGAAT	GAACGGTT	AACCGTTC
D5	GACGTCAT	CCAGTTGA	TCAACTGG
E5	CCGCTTAA	GTCATCGT	ACGATGAC
F5	GACGAACT	CAATGCGA	TCGCATTG
G5	TCCACGTT	GGTTGAAC	GTTCAACC
H5	AACCAGAG	CTTCGGTT	AACCGAAG

WELL POSITION	EXPECTED i7 INDEX READ	EXPECTED i5 INDEX READ	
		NovaSeq, MiSeq, HiSeq 2000/2500 (PE Flow Cell), HiSeq 3000/4000 (Single Read Flow Cell)	NextSeq, MiSeq, HiSeq 2000/2500 (Single Read Flow Cell), HiSeq 3000/4000/ HiSeq X (PE Flow Cell)
A6	GTCAGTCA	CGGCATTA	TAATGCCG
B6	CCTTCCAT	CACGCAAT	ATTGCGTG
C6	AGGAACAC	GGAATGTC	GACATTCC
D6	CTTACAGC	TGGTGAAG	CTTCACCA
E6	TACCTGCA	GGACATCA	TGATGTCC
F6	AGACGCTA	GGTGTACA	TGTACACC
G6	CAACACAG	GATAGCCA	TGGCTATC
H6	GTACCACA	CCACAACA	TGTTGTGG
A7	CGAATACG	TTACCGAC	GTCGGTAA
B7	GTCCTTGA	TCGTCTGA	TCAGACGA
C7	CAGTGCTT	TTCCAGGT	ACCTGGAA
D7	TCCATTGC	TACGGTCT	AGACCGTA
E7	GTCGATTG	AAGACCGT	ACGGTCTT
F7	ATAACGCC	CAGGTTCA	TGAACCTG
G7	GCCTTAAC	TAGGAGCT	AGCTCCTA
H7	GGTATAGG	TACTCCAG	CTGGAGTA
A8	TCTAGGAG	AGTGACCT	AGGTCACT
B8	TGCGTAAC	AGCCTATC	GATAGGCT
C8	CTTGCTAG	TCATCTCC	GGAGATGA
D8	AGCGAGAT	CCAGTATC	GATACTGG
E8	TATGGCAC	TTGCGAGA	TCTCGCAA
F8	GAATCACC	GAACGAAG	CTTCGTTC
G8	GTAAGGTG	CGAATTGC	GCAATTCG
H8	CGAGAGAA	GGAAGAGA	TCTCTTCC
A9	CGCAACTA	TCGGATTC	GAATCCGA
B9	CACAGACT	CTGTACCA	TGGTACAG
C9	TGGAAGCA	GAGAGTAC	GTACTCTC
D9	CAATAGCC	TCTACGCA	TGCGTAGA
E9	CTCGAACA	GCAATTCC	GGAATTGC
F9	GGCAAGTT	CTCAGAAG	CTTCTGAG
G9	AGCTACCA	GTCCTAAG	CTTAGGAC
H9	CAGCATAC	GCGTTAGA	TCTAACGC
A10	CGTATCTC	CAAGGTAC	GTACCTTG
B10	TTACGTGC	AGACCTTG	CAAGGTCT
C10	AGCTAAGC	GTCGTTAC	GTAACGAC
D10	AAGACACC	GTAACCGA	TCGGTTAC
E10	CAACTCCA	GAATCCGT	ACGGATTC
F10	GATCTTGC	CATGAGCA	TGCTCATG
G10	CTTCACTG	CTTAGGAC	GTCCTAAG
H10	CTCGACTT	ATCTGACC	GGTCAGAT

WELL POSITION	EXPECTED i7 INDEX READ	EXPECTED i5 INDEX READ	
		NovaSeq, MiSeq, HiSeq 2000/2500 (PE Flow Cell), HiSeq 3000/4000 (Single Read Flow Cell)	NextSeq, MiSeq, HiSeq 2000/2500 (Single Read Flow Cell), HiSeq 3000/4000/ HiSeq X (PE Flow Cell)
A11	GTACACCT	TCCTCATG	CATGAGGA
B11	CCAAGGTT	AGGATAGC	GCTATCCT
C11	GAACGGTT	GGAGGAAT	ATTCCTCC
D11	CCAGTTGA	GACGTCAT	ATGACGTC
E11	GTCATCGT	CCGCTTAA	TTAAGCGG
F11	CAATGCGA	GACGAACT	AGTTCGTC
G11	GGTTGAAC	TCCACGTT	AACGTGGA
H11	CTTCGGTT	AACCAGAG	CTCTGGTT
A12	CGGCATTA	GTCAGTCA	TGACTGAC
B12	CACGCAAT	CCTTCCAT	ATGGAAGG
C12	GGAATGTC	AGGAACAC	GTGTTCCT
D12	TGGTGAAG	CTTACAGC	GCTGTAAG
E12	GGACATCA	TACCTGCA	TGCAGGTA
F12	GGTGTACA	AGACGCTA	TAGCGTCT
G12	GATAGCCA	CAACACAG	CTGTGTTG
H12	CCACAACA	GTACCACA	TGTGGTAC

Sequencing on the Illumina Platform

Pool equal molar amounts of libraries for sequencing on the Illumina platforms using the cycle settings shown in the table below.

RUN SEGMENT	CYCLE NUMBER
Read 1	X defined by users
Index 1 (i7)	8 (without UMI)
	19 (with UMI)
Index 2 (i5)	8
Read 2	X defined by users

Kit Components

The NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1) are functionally validated through library preparation using the NEBNext Library Prep Kits and sequencing on the Illumina platforms.

NEB #E7395S Table of Components

NEB #	CONCENTRATION	PRODUCT	VOLUME
E7396A	20 µM	NEBNext UMI DNA Adaptor Plate	1 plate (2.5 µl/well)
E7397A	40 µM (Total)	NEBNext Primer Mix	0.48 ml
E7398A		NEBNext UMI Adaptor Dilution Buffer	5 ml

NEB #E7395L Table of Components

NEB #	CONCENTRATION	PRODUCT	VOLUME
E7396A	20 µM	NEBNext UMI DNA Adaptor Plate	4 plates (2.5 µl/well)
E7397AA	40 µM (Total)	NEBNext Primer Mix	2 x 0.96 ml
E7398AA		NEBNext UMI Adaptor Dilution Buffer	20 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	3/20

ISO 9001 Registered Quality Management ISO 14001 Registered Environmental Management ISO 13485 Registered Medical Devices

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