

NEBNext® Multiplex Oligos for Illumina® (Dual Index Primers Set 2)

NEB #E7780S

96 reactions

Version 3.0_6/24

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The NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2) Includes

The volumes provided are sufficient for preparation of up to 96 reactions (NEB #E7780S). Primers are supplied at 10 μM. All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (red) NEBNext Adaptor for Illumina
- (red) USER® Enzyme
- (white) NEBNext i509 Primer
- (white) NEBNext i510 Primer
- (white) NEBNext i511 Primer
- (white) NEBNext i512 Primer
- (white) NEBNext i513 Primer
- (white) NEBNext i514 Primer
- (white) NEBNext i515 Primer
- (white) NEBNext i516 Primer
- (green) NEBNext i713 Primer
- (green) NEBNext i714 Primer
- (green) NEBNext i715 Primer
- (green) NEBNext i716 Primer
- (green) NEBNext i717 Primer
- (green) NEBNext i718 Primer
- (green) NEBNext i719 Primer
- (green) NEBNext i720 Primer
- (green) NEBNext i721 Primer
- (green) NEBNext i722 Primer
- (green) NEBNext i723 Primer
- (green) NEBNext i724 Primer

Applications

The NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2) contains the adaptor and index primers that are ideally suited for multiplex sample preparation for next-generation sequencing on the Illumina platform. Each of these components must pass rigorous quality control standards and are lot controlled, both individually and as a set of reagents.

Lot Control: The lots provided in the NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2) 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 specific for each individual component.

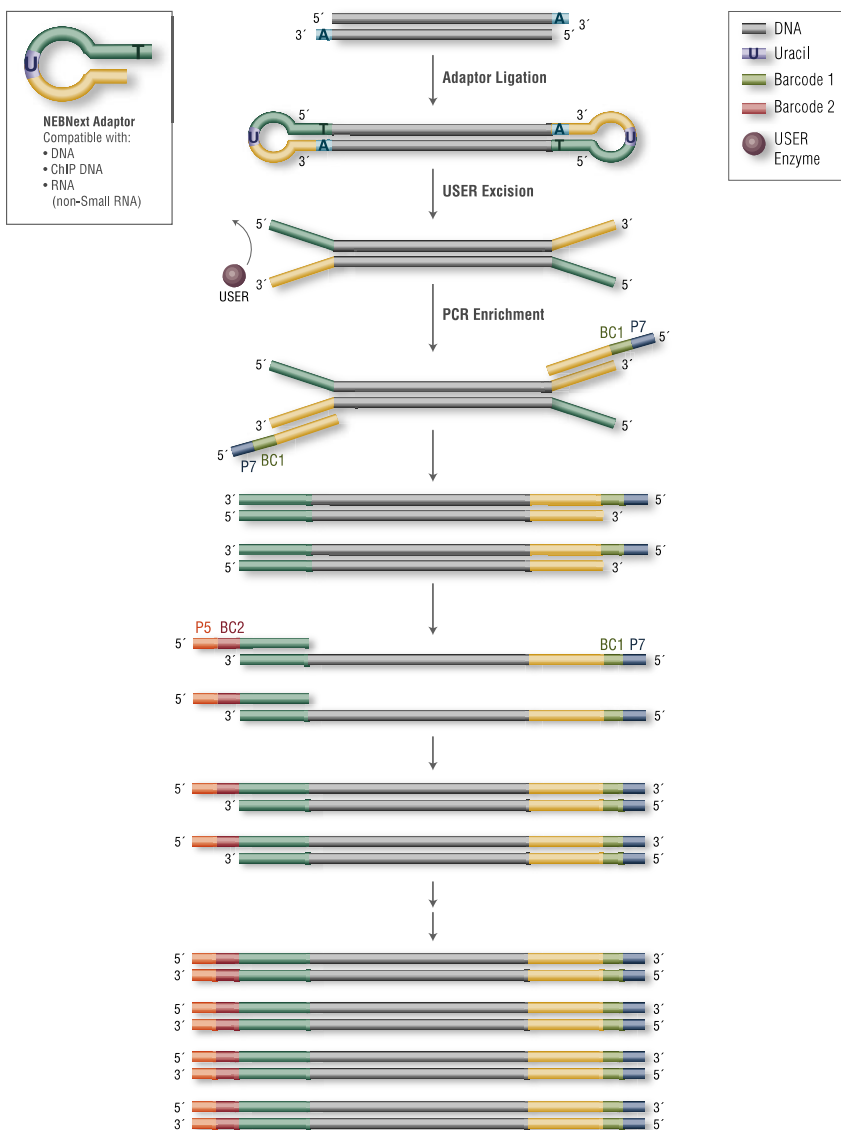
Functionally Validated: Each set of reagents is functionally validated together through construction and sequencing of genomic DNA libraries on the Illumina platform.

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 to learn more.

Workflow

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. The 8-base index primers included in this kit are supplied in tubes with spare caps. 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 (Dual Index Primers).



Library Preparation Kits for use with NEBNext Multiplex Oligos for Illumina (Dual Index Primers Sets 1 and 2)

Please refer to the kit specific **library preparation kit manual** for using the NEBNext Multiplex Oligos for Illumina **for additional required materials that are not included.**

Please refer to the NEBNext Multiplex Oligos Selection Chart at www.neb.com/oligos for a list of compatible applications.

Please note: for Illumina Sequencing instruments using patterned flow cells Unique Dual Indexing is recommended. Please see [Illumina Index Hopping White Paper](#).

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 for each set:

www.neb.com/E7600 – NEBNext® Multiplex Oligos for Illumina® (Dual Index Primers Set 1) (NEB #E7600)

www.neb.com/E7780 – NEBNext® Multiplex Oligos for Illumina® (Dual Index Primers Set 2) (NEB #E7780)

Note: Multiple sets can be pooled together for up to 384 samples on some Illumina sequencing instrument types.

Section 1 Setting up the PCR Reaction

Symbols



This caution sign signifies a step in the protocol that has multiple paths leading to the same end point but is dependent on a user variable, like the number of samples to be processed.

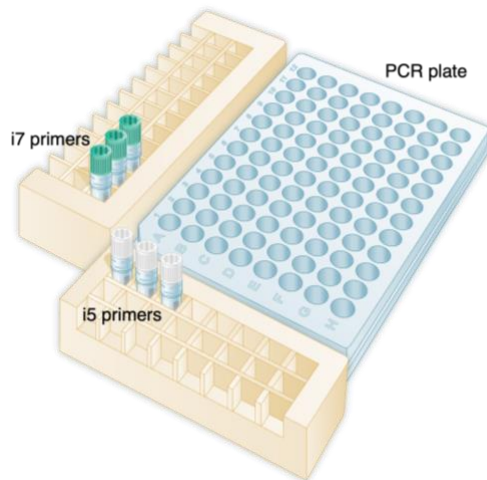
1.1. PCR Amplification



For < 96 samples, follow the protocol in Section 1.1A. For 96 samples, follow the protocol in Section 1.1B.

1.1A Setting up the PCR reactions (< 96 samples)

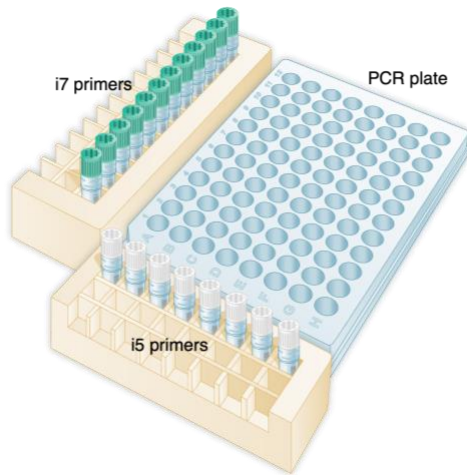
Note: We recommend using a PCR work-up rack such as the TruSeq® Index Plate Fixture (Illumina #FC-130-1005) to assist in properly combining the index primers during the PCR amplification step. Alternatively, 96-well deep well plates can be used and aligned against a PCR plate as in the diagram below.



- 1.1A.1. Ensure that a valid combination of i7 and i5 primers is used. See Appendix A to verify that correct primer combinations have been selected.
- 1.1A.2. Mix briefly by vortexing and then centrifuge to collect all of the primer at the bottom of each tube.
- 1.1A.3. Arrange the index primers in the Index Plate Fixture as follows:
 - a. Arrange the ● (green) i7 primers in increasing order horizontally, so that the lowest number i7 index primer is in column 1, second lowest number i7 index primer is in column 2, etc.
 - b. Arrange the ○ (white) i5 primers in increasing order vertically, so that the lowest number i5 index primer is in row A, second lowest number i5 index primer is in row B, etc.
 - c. Record their positions on the PCR setup template (see Appendix B).
- 1.1A.4. Using a multichannel pipette, add desired volume of ○ (white) i5 primers to every column (as needed) of the PCR plate.
It is critical to change tips between columns to avoid cross-contamination.
- 1.1A.5. Discard the original i5 white caps and apply new caps to avoid index cross-contamination.
- 1.1A.6. Using a multichannel pipette, add desired volume of ● (green) i7 primers to every row (as needed) of the PCR plate.
It is critical to change tips between rows to avoid cross-contamination.
- 1.1A.7. Discard the original i7 green caps and apply new caps to avoid index cross-contamination.
- 1.1A.8. Proceed with the PCR reaction according to the specific library construction manual.

1.1B Setting up the PCR reactions (96 samples)

Note: We recommend using a PCR work-up rack such as the TruSeq Index Plate Fixture (Illumina #FC-130-1005) to assist in properly combining the index primers during the PCR amplification step. Alternatively, 96-well deep well plates can be used and aligned against a PCR plate as in the diagram below.



- 1.1B.1. Mix briefly by vortexing and then centrifuge to collect all of the primer at the bottom of each tube.
- 1.1B.2. Arrange the index primers in the Index Plate Fixture as follows:
 - a. Arrange ● (green) i7 primers in increasing order horizontally, so that i713 is in column 1, i714 is in column 2, i715 is in column 3, etc.
 - b. Arrange the ○ (white) i5 primers in increasing order vertically, so that i509 is in row A, i510 is in row B, i511 is in row C, etc.
 - c. Record their positions on the PCR setup template (see Appendix B).
- 1.1B.3. Using a multichannel pipette, add desired volume of ○ (white) i5 primers to every column of the PCR plate.
It is critical to change tips between columns to avoid cross-contamination.
- 1.1B.4. Discard the original i5 white caps and apply new caps to avoid index cross-contamination.
- 1.1B.5. Using a multichannel pipette, add desired volume of ● (green) i7 primers to every row of the PCR plate.
It is critical to change tips between rows to avoid cross-contamination.
- 1.1B.6. Discard the original i7 green caps and apply new caps to avoid index cross-contamination.
- 1.1B.7. Proceed with the PCR reaction according to the specific library construction manual.

Section 2

Appendix A: Principle for Use and Pooling Guide

Index Pooling Guidelines Within Each Set

Four Channel Chemistry Color Balancing

For all HiSeq®/MiSeq® 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®6000/ NextSeq®/MiniSeq®:

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®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. For additional NovaSeq X and X Plus color balancing guidelines please contact NEB technical support at info@neb.com

Low Plex pooling options shown here are only for Illumina four channel chemistry.

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](#)

The Principle of Combinatorial Dual Index Primers

The combinatorial dual index primer strategy utilizes two 8 base indices within each primer pair; i7 primers contain indices that are adjacent to the P7 sequence; i5 primers contain indices that are adjacent to the P5 sequence. Combinatorial dual indexing is enabled by adding a different index to both ends of a sample to be sequenced. Up to 96 different samples can be uniquely indexed by combining each of the 12 i7 primers with each of the 8 i5 primers. In combinatorial dual indexing the combinations are unique, but each primer is used several times; as opposed to Unique Dual Indexing, where each i7 and each i5 is used only once in a pool. Similarly, < 96 samples can be indexed by combining i7 primers with i5 primers as follows:

$$N = \text{Number of samples} = X(i7) * Y(i5) + \text{other primers as needed}$$

Examples:

1. For N = 12 samples

Option 1: 4 (i7) * 3 (i5)

From the i7 primers, choose a valid set of 4. From the i5 primers choose a valid set of 3. Use each i7 primer with each i5 primer to form 12 primer pairs for PCR amplification of 12 libraries. When setting up the sequencing run, select “Dual Index” and choose the indices used for each sample.

Option 2: 3 (i7) * 4 (i5)

From the i7 primers, choose a valid set of 3. From the i5 primers choose a valid set of 4. Use each i7 primer with each i5 primer to form 12 primer pairs for PCR amplification of 12 libraries. When setting up the sequencing run, select “Dual Index” and choose the indices used for each sample.

Option 3: 12 (i7) * 1 (i5)

Use all 12 i7 primers. Use any i5 primer. Use each i7 primer with the i5 primer to form 12 primer pairs for PCR amplification of 12 libraries. When setting up the sequencing run, select “Single Index”, and choose the i7 index used for each sample.

1. For N = 26 samples

Option 1: 6 (i7) * 4 (i5) + 2 (i5)

From the i7 primers, choose a valid set of 4 and add any other two i7 primers, for a total of 6 primers. From the i5 primers choose a valid set of 4 and add any other two i5 primers, for a total of 6 primers. Use each i7 primer with four of the i5 primers to form 24 primer pairs. Use any of the six i7 primers with the remaining two i5 primers to form 2 primer pairs. This will give you a total of 26 primer pairs for PCR amplification of 26 libraries. When setting up the sequencing run, select “Dual Index” and choose the indices used for each sample.

Option 2: 6 (i7) * 5 (i5)

From the i7 primers, choose a valid set of 4 and add any other two i7 primers, for a total of 6 primers. From the i5 primers choose a valid set of 4 and add any other one i5 primer. Use each i7 primer with each i5 primer to form 30 primer pairs for PCR amplification. Use 26 of the 30 primer pairs to amplify 26 libraries. When setting up the sequencing run, select “Dual Index” and choose the indices used for each sample.

Low Plexity Pooling Guidelines

CAUTION: Sufficient primers are provided to generate 96 different samples if each i5 primer is used only once with each i7 primer.

If using subsets of i5 and i7 primers multiple times, you may have to readjust primer pairs to be able to generate 96 samples.

Low Plex pooling options shown here are only for Illumina four channel chemistry.

Table 2.1 Pooling: 2–12 libraries; Sequencing Workflow: Single Index (Select "1" Index Reads in the Illumina Experiment Manager).

PLEX	i7 PRIMERS	i5 PRIMERS
2	i713 and i721 i714 and i724 i715 and i716 i717 and i719 i718 and i720 i722 and i723	Any i5 Primer
3	i714, i715 and i716 i715, i716 and i717 i717, i718 and i719 i718, i719 and i720 i721, i722 and i723 i722, i723 and i724	Any i5 Primer
4	i713, i714, i715 and i716 i715, i716, i717 and i718 i717, i718, i719 and i720 i718, i719, i720 and i721 i720, i721, i722 and i723 i721, i722, i723 and i724	Any i5 Primer
5–12	Any valid i7 4-plex with any other i7 Primers	Any i5 Primer

Table 2.2 Pooling: 6+ libraries; Sequencing Workflow: Dual Index
(Select "2" Index Reads in the Illumina Experiment Manager).

PLEX	i7 PRIMERS	i5 PRIMERS
6–12	Any 2 plex combination from Table 2.1 with any other i7 primer(s) (as needed)	i509, i511 and i513 i510, i511 and i513 i511, i513 and i514 i511, i513 and i515 i511, i515 and i516
Greater than 12	Any 3 plex combination from the Table 2.1 with any other i7 primer(s) (as needed)	i509, i510, i511, i513 and any other i5 primer (as needed) i509, i511, i512, i513 and any other i5 primer (as needed) i509, i511, i514, i515 and any other i5 primer (as needed) i510, i511, i515, i516 and any other i5 primer (as needed) i511, i513, i515, i516 and any other i5 primer (as needed)

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

***Forward Strand Workflow** for the following instruments: NovaSeq 6000 with v1.0 reagents kits, MiniSeq with rapid reagent kits, MiSeq®, HiSeq® 2000/2500 (pair-end flow cell), HiSeq 3000/4000 (single-read flow cell).

***Reverse Complement Workflow** for the following instruments: iSeq 100, MiniSeq with standard reagent kits, NextSeq Systems, NovaSeq 6000 with v1.5 reagent kits, HiSeq 2000/5000 (single-read flow cell), HiSeq 3000/4000 (paired-end flow cell).

See Illumina Document “Indexed Sequencing Overview” 15057455 and Illumina Guidelines for reverse complementing i5 sequences” for demultiplexing Illumina Knowledge Article #1800 [Guidelines for reverse complementing i5 sequences for demultiplexing - Illumina Knowledge](#).

Tables 2.1 and 2.2 above do not include an extensive list of all valid index combinations. Please check the sequences of each index to be used to ensure that you will have signal in both the red and green channels for every cycle. See example below:

Table 3.1

GOOD			
i7 PRIMERS	i5 PRIMERS	FORWARD STRAND WORKFLOW*	REVERSE COMPLEMENT WORKFLOW*
i7-PCR Index 13 T T C C T C C T	i5-PCR Index 9	T T G C T T G C	G C A A G C A A
i7-PCR Index 14 T G C T T G C T	i5-PCR Index 10	G A G A G G T T	A A C C T C C
i7-PCR Index 15 G G T G A T G A	i5-PCR Index 11	A C C T G G T T	A A C C A G G T
i7-PCR Index 16 A A C C T A C G	i5-PCR Index 13	C G G A A C A A	T T G T T C C G
✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓		✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓	✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓

Table 3.2

BAD			
i7 PRIMERS	i5 PRIMERS	FORWARD STRAND WORKFLOW*	REVERSE COMPLEMENT WORKFLOW*
i7-PCR Index 13 T T C C T C C T	i5-PCR Index 9	T T G C T T G C	G C A A G C A A
i7-PCR Index 14 T G C T T G C T	i5-PCR Index 10	G A G A G G T T	A A C C T C T C
i7-PCR Index 15 G G T G A T G A	i5-PCR Index 12	A A G C G G A A	T T C C G C T T
i7-PCR Index 16 A A C C T A C G	i5-PCR Index 14	G G T A A G C T	A G C T T A C C
✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓		✓ ✓ X X ✓ X ✓ ✓	✓ ✓ X ✓ X X ✓ ✓

Two Color Chemistry Color Balancing

NovaSeq 6000, NextSeq (500, 550, 1000 and 2000) and MiniSeq use red/ green or blue/ green 2 color chemistry to simplify nucleotide detection. See [Sequencing Chemistry \(illumina.com\)](https://illumina.com/sequencing-chemistry) Illumina Document # 1000000041074 v12 . 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. Within the NEB #E7780 and NEB #E7600 NEBNext Multiplex Oligos for Illumina kits there are few such combinations, but we wanted to include this information here in case of combining these kits with oligos from other index kits.

Section 3

Appendix B: PCR Setup Template

For each well, record: 1. DNA Sample Name _____

2. Index Primer Pairs _____

	1	2	3	4	5	6	7	8	9	10	11	12
A	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /
B	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /
C	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /
D	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /
E	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /
F	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /
G	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /
H	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /	1. _____ 2. /

NEBNext Adaptors and Primers for Illumina

For sample sheets please see NEB.com, E7780 Product Page, "Protocols, Manuals and Usage Guidelines" Tab, [NEBNext Multiplex Oligos for Illumina \(Dual Index Primers Set 2\)](#).

***Forward Strand Workflow** for the following instruments: NovaSeq 6000 with v1.0 reagents kits, MiniSeq with rapid reagent kits, MiSeq®, HiSeq® 2000/2500 (pair-end flow cell), HiSeq 3000/4000 (single-read flow cell).

***Reverse Complement Workflow** for the following instruments: iSeq 100, MiniSeq with standard reagent kits, NextSeq Systems, NovaSeq 6000 with v1.5 reagent kits, HiSeq 2000/5000 (single-read flow cell), HiSeq 3000/4000 (paired-end flow cell).

See Illumina Document "Indexed Sequencing Overview" 15057455 and Illumina Guidelines for reverse complementing i5 sequences" for demultiplexing Illumina Knowledge Article #1800 [Guidelines for reverse complementing i5 sequences for demultiplexing - Illumina Knowledge](#).

NEBNext i509 Primer–NEBNext i516 Primer

Description: 8 Index Primers (10 µM) are included for producing barcoded libraries.

NEB #	PRODUCT	INDEX PRIMER SEQUENCE	EXPECTED INDEX	
			Forward Strand Workflow*	Reverse Complement Workflow*
#E7781A	NEBNext i509 Primer	5'-AATGATACGGCGACCACCGAGATCTACAC TTGCTTGC ACACTCTTTCCC TACACGACGCTCTCCGATC*T-3'	TTGCTTGC	GCAAGCAA
#E7782A	NEBNext i510 Primer	5'-AATGATACGGCGACCACCGAGATCTACAC GAGAGGTT ACACTCTTTCCCT ACACGACGCTCTCCGATC*T-3'	GAGAGGTT	AACCTCTC
#E7783A	NEBNext i511 Primer	5'-AATGATACGGCGACCACCGAGATCTACAC ACCTGGTT ACACTCTTTCCC TACACGACGCTCTCCGATC*T-3'	ACCTGGTT	AACCAGGT
#E7784A	NEBNext i512 Primer	5'-AATGATACGGCGACCACCGAGATCTACAC AAGCGGAA ACACTCTTTCCC TACACGACGCTCTCCGATC*T-3'	AAGCGGAA	TTCCGCTT
#E7785A	NEBNext i513 Primer	5'-AATGATACGGCGACCACCGAGATCTACAC CGGAACAA ACACTCTTTCCC TACACGACGCTCTCCGATC*T-3'	CGGAACAA	TTGTCCG
#E7786A	NEBNext i514 Primer	5'-AATGATACGGCGACCACCGAGATCTACAC GGTAAGCT ACACTCTTTCCC TACACGACGCTCTCCGATC*T-3'	GGTAAGCT	AGCTTACC
#E7787A	NEBNext i515 Primer	5'-AATGATACGGCGACCACCGAGATCTACAC TGTGGCAT ACACTCTTTCCC TACACGACGCTCTCCGATC*T-3'	TGTGGCAT	ATGCCACA
#E7788A	NEBNext i516 Primer	5'-AATGATACGGCGACCACCGAGATCTACAC ACTACGGA ACACTCTTTCCC TACACGACGCTCTCCGATC*T-3'	ACTACGGA	TCCGTAGT

NEBNext i713 Primer–NEBNext i724 Primer

Description: 12 Index Primers (10 µM) are included for producing barcoded libraries.

NEB #	PRODUCT	INDEX PRIMER SEQUENCE	EXPECTED INDEX READ
#E7789A	NEBNext i713 Primer	5'-CAAGCAGAAGACGGCATACGAGAT AGGAGGAA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	TTCCTCCT
#E7790A	NEBNext i714 Primer	5'-CAAGCAGAAGACGGCATACGAGAT AGCAAGCA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	TGCTTGCT
#E7791A	NEBNext i715 Primer	5'-CAAGCAGAAGACGGCATACGAGAT TCATCACCC GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	GGTGATGA
#E7792A	NEBNext i716 Primer	5'-CAAGCAGAAGACGGCATACGAGAT CGTAGGTT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	AACCTACG
#E7793A	NEBNext i717 Primer	5'-CAAGCAGAAGACGGCATACGAGAT TCAGATCC GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	GGATCTGA
#E7794A	NEBNext i718 Primer	5'-CAAGCAGAAGACGGCATACGAGAT CGTGATCA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	TGATCACG
#E7795A	NEBNext i719 Primer	5'-CAAGCAGAAGACGGCATACGAGAT AGTCGCTT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	AAGCGACT
#E7796A	NEBNext i720 Primer	5'-CAAGCAGAAGACGGCATACGAGAT GAACGCTT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	AAGCGTTC
#E7797A	NEBNext i721 Primer	5'-CAAGCAGAAGACGGCATACGAGAT TACGCCTT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	AAGGCGTA
#E7798A	NEBNext i722 Primer	5'-CAAGCAGAAGACGGCATACGAGAT CTCATCAG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	CTGATGAG
#E7799A	NEBNext i723 Primer	5'-CAAGCAGAAGACGGCATACGAGAT TCTTCTGCG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	GCAGAAGA
#E7800A	NEBNext i724 Primer	5'-CAAGCAGAAGACGGCATACGAGAT GCTGGATT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	AATCCAGC

Kit Components

The NEBNext Dual Index Primers Set 2 is functionally validated through library preparation using the NEBNext Library Prep Kits and sequencing on the Illumina platform.

NEB #E7780S Table of Components

NEB #	Product Name	Volume
E7601A	NEBNext Adaptor for Illumina*	0.96 ml
E7602A	USER Enzyme	0.288 ml
E7781A	NEBNext i509 Primer	0.060 ml
E7782A	NEBNext i510 Primer	0.060 ml
E7783A	NEBNext i511 Primer	0.060 ml
E7784A	NEBNext i512 Primer	0.060 ml
E7785A	NEBNext i513 Primer	0.060 ml
E7786A	NEBNext i514 Primer	0.060 ml
E7787A	NEBNext i515 Primer	0.060 ml
E7788A	NEBNext i516 Primer	0.060 ml
E7789A	NEBNext i713 Primer	0.040 ml
E7790A	NEBNext i714 Primer	0.040 ml
E7791A	NEBNext i715 Primer	0.040 ml
E7792A	NEBNext i716 Primer	0.040 ml
E7793A	NEBNext i717 Primer	0.040 ml
E7794A	NEBNext i718 Primer	0.040 ml
E7795A	NEBNext i719 Primer	0.040 ml
E7796A	NEBNext i720 Primer	0.040 ml
E7797A	NEBNext i721 Primer	0.040 ml
E7798A	NEBNext i722 Primer	0.040 ml
E7799A	NEBNext i723 Primer	0.040 ml
E7800A	NEBNext i724 Primer	0.040 ml

* 15 μ M Concentration

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	11/17
2.0	Updated manual format	8/22
3.0	Updated color balance information. Updated adaptor and index sequence sections. Also updated header and footer with new logo and updated legal footnote.	6/24

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