# NEBNext® Multiplex Oligos for Illumina® (96 Unique Dual Index Primer Pairs Set 3)

NEB #E6444S/L

96/384 reactions

Version 4.0_7/22

## Table of Contents



## The NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 3) Includes

*The volumes provided are sufficient for preparation of up to 96 reactions (NEB #E6444S) and 384 reactions (NEB #E6444L). All reagents should be stored at –20°C.*

NEBNext Adaptor for Illumina

USER® Enzyme

NEBNext 96 Unique Dual Index Primer Pairs Plate (Set 3)
Each well contains a unique pair of Index Primers (S size contains 1 plate, L size contains 4 plates)

## Overview

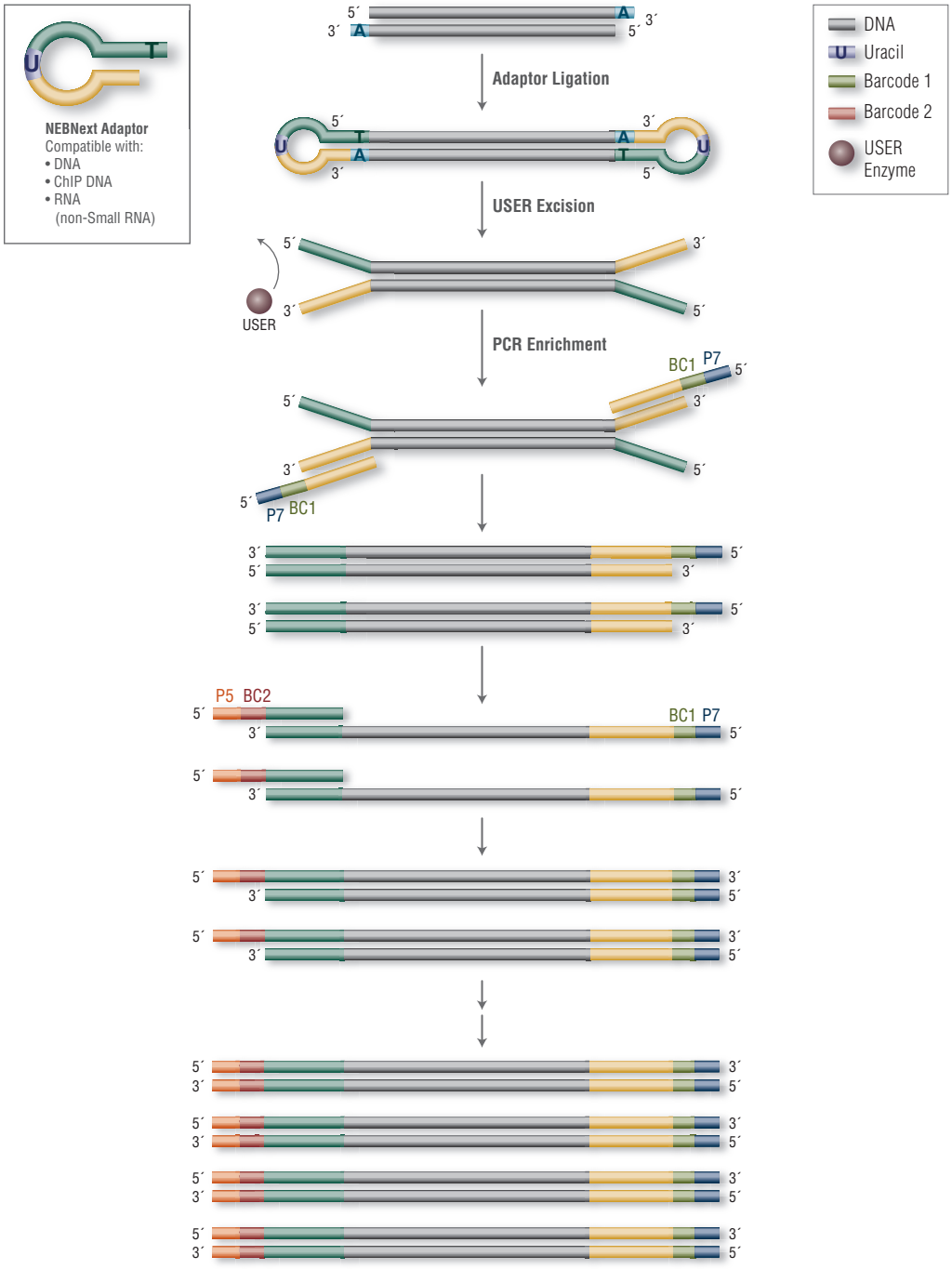
The NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 3) contains adaptors and primers that are ideally suited for multiplex sample preparation for next-generation sequencing on the Illumina platform (Illumina, Inc.). Each kit component must pass rigorous quality control standards, 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 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 96 8-base index primer pairs included in this kit are pre-mixed and 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.

**Figure 1. Workflow demonstrating the use of NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs).**



## Please Refer to the Kit Specific Protocol for using the NEBNext Multiplex Oligos for Illumina

For compatibility of NEBNext Multiplex Oligos please refer to the NEBNext Multiplex Oligos Selection Chart at **neb.com/oligos**

## 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

## Section 1
## Setting up the PCR Reactions

**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 amount of input DNA.*

### 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)

1.1A.1. Determine the number of libraries that will be amplified and pooled for subsequent sequencing.

1.1A.2. Ensure that you choose a valid combination of barcode primers based on color balance guidelines in Section 2.

1.1A.3. Thaw the 96 Unique Dual Index Primers Plate for 10-15 minutes at room temperature.

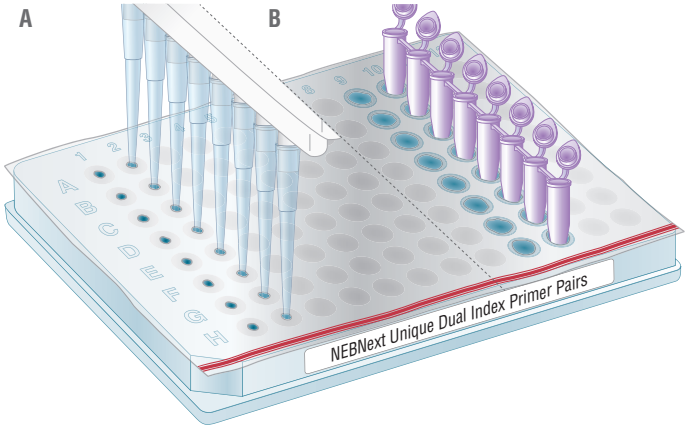
1.1A.4. Remove the hard plastic plate cover. Mix briefly by vortexing and then centrifuge the plate (280 × *g* for ~1 min) to collect all of the primer at the bottom of each well.

1.1A.5. Orient the 96 Unique Dual Index Primers Plate Set 3 as indicated in Figure 1.1 (red stripe towards the user). With a pipette tip, pierce the desired well(s) (Figure 1.1A) and transfer the volume of primer mix required for the PCR reaction to the PCR plate/tubes (see specific library construction manual for protocol). It is important to change pipette tips before piercing a new well to avoid cross contamination of indexed primers. Alternatively, the wells can be pierced using the bottom of clean PCR strip tubes (see Figure 1.1B) prior to pipetting the primer mix. Use a new, clean strip tube for each new well to be pierced.

*Note: Each well contains a unique pair of index primers. There is enough primer in each well for one PCR reaction. Do not reuse primer if the seal has been previously pierced to avoid contamination with other indexed primers.*

1.1A.6. Proceed with the PCR reaction according to the specific library construction manual.

**Figure 1.1. NEBNext Unique Dual Index Pairs Plate Set 3**




### 1.1B Setting up the PCR reactions (96 samples)

1.1B.1. Thaw the 96 Unique Dual Index Primer Pairs plate for 10-15 minutes at room temperature.

1.1B.2. Remove the hard plastic plate cover. Mix briefly by vortexing and then centrifuge the plate (280 × *g* for ~1 min) to collect all of the primer at the bottom of each well.

1.1B.3. Orient the 96 Unique Dual Index Primer Pairs plate as indicated in Figure 1.1 (red stripe towards the user). With a pipette tip, pierce the wells (Figure 1.1A) and transfer the volume of primer mix required for the PCR reaction to the PCR plate (see specific library construction manual for protocol). It is important to change pipette tips before piercing a new well to avoid cross contamination of indexed primers. Alternatively, the wells can be pierced using the bottom of clean PCR strip tubes (see Figure 1.1B) prior to pipetting the primer mix. Use a new, clean strip tube for each new well to be pierced.

*Note: Each well contains a unique pair of index primers. There is enough primer in each well for one PCR reaction. Do not reuse primer if the seal has been previously pierced to avoid contamination with other indexed primers.*

1.1B.4. Proceed with the PCR reaction according to the specific library construction manual.

## Section 2
## Index Pooling Guidelines: 96 Reaction Kit

**For a link to download a sample sheet with the index sequences for use with the Illumina Experiment Manager (IEM) please visit the "Usage Guidelines" sub tab located under the "protocols, manuals and usage" tab on the E6444 product page.**

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. See Table 2.1 for examples of Good and Bad Index combinations.

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. See Table 2.2 for examples of Good and Bad Index combinations.

The barcoded primers are organized on the plate such that including the primers in rows A and B from any column will produce a color balanced pool. For example, if preparing 2 libraries, choose primer wells A1 and B1. For pools containing 3-8 libraries, add any other primers from that column. For pools containing more than 8 libraries, choose any column and add any other primers as needed.

***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 Strand 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).

**Table 2.1. lists each index sequence 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. See below for examples of Good and Bad index combinations based on HiSeq/MiSeq guidelines:**

| GOOD | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WELL POSITION | EXPECTED i7 INDEX READ | | | | | | | | EXPECTED i5 INDEX READ | | | | | | | | | | | | | | | | |
| | | | | | | | | | FORWARD STRAND WORKFLOW* | | | | | | | | REVERSE STRAND WORKFLOW* | | | | | | | | |
| A1 | T | G | T | C | G | T | A | G | A | A | A | G | C | T | A | A | T | T | A | G | C | T | T | T | |
| B1 | C | A | A | T | C | A | T | A | T | G | G | A | G | A | T | T | A | A | T | C | T | C | C | A | |
| C1 | G | T | T | C | T | T | A | T | A | A | T | T | A | G | A | C | G | T | C | T | A | A | T | T | |
| D1 | G | A | T | G | C | G | A | C | A | C | T | T | T | G | G | G | C | C | C | A | A | A | G | T | |
| | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | |

| BAD | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WELL POSITION | EXPECTED i7 INDEX READ | | | | | | | | EXPECTED i5 INDEX READ | | | | | | | | | | | | | | | | |
| | | | | | | | | | FORWARD STRAND WORKFLOW | | | | | | | | REVERSE STRAND WORKFLOW | | | | | | | | |
| E1 | G | A | A | G | A | G | G | G | C | G | G | A | C | G | G | A | T | C | C | G | T | C | C | G | |
| F1 | T | A | G | T | A | A | T | C | G | C | A | G | A | G | C | C | G | G | C | T | C | T | G | C | |
| G1 | G | T | G | T | G | G | A | G | G | C | A | T | G | A | T | C | G | A | T | C | A | T | G | C | |
| H1 | A | C | G | T | T | G | T | A | T | C | G | A | C | C | T | A | T | A | G | G | T | C | G | A | |
| | ✓ | ✓ | ✓ | X | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | X | X | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

The index primer sequences, for different Illumina sequencer input sheets are indicated in Table 2.3.

**Table 2.2. 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. Listed here are some examples of good (signal in at least one channel for the first 2 cycles) and bad (the index read begins with GG) index combinations.**

| GOOD | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WELL POSITION | EXPECTED i7 INDEX READ | | | | | | | | EXPECTED i5 INDEX READ | | | | | | | | | | | | | | | |
| | | | | | | | | | FORWARD STRAND WORKFLOW | | | | | | | | REVERSE STRAND WORKFLOW | | | | | | | |
| A1 | T | G | T | C | G | T | A | G | A | A | A | G | C | T | A | A | T | T | A | G | C | T | T | T |
| B1 | C | A | A | T | C | A | T | A | T | G | G | A | G | A | T | T | A | A | T | C | T | C | C | A |
| C1 | G | T | T | C | T | T | A | T | A | A | T | T | A | G | A | C | G | T | C | T | A | A | T | T |
| | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

| BAD | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WELL POSITION | EXPECTED i7 INDEX READ | | | | | | | | EXPECTED i5 INDEX READ | | | | | | | | | | | | | | | |
| | | | | | | | | | FORWARD STRAND WORKFLOW | | | | | | | | REVERSE STRAND WORKFLOW | | | | | | | |
| D9 | C | A | G | C | A | G | G | G | A | T | G | C | T | C | C | C | G | G | G | A | G | C | A | T |
| G9 | C | G | A | T | A | C | A | T | C | G | A | G | A | A | C | C | G | G | T | T | C | T | C | G |
| D11 | C | T | C | A | C | G | T | C | T | A | T | T | T | A | C | C | G | G | T | A | A | A | T | A |
| | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | X | X | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

**Table 2.3. Lists each index sequence 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.**

| | EXPECTED i7 INDEX READ | | EXPECTED i5 INDEX READ | | |
|---|---|---|---|---|---|
| WELL POSITION | i7 INDEX ID | | i5 INDEX ID | FORWARD STRAND WORKFLOW | REVERSE STRAND WORKFLOW |
| A1 | 7-197 | TGTCGTAG | 5-245 | AAAGCTAA | TTAGCTTT |
| B1 | 7-198 | CAATCATA | 5-246 | TGGAGATT | AATCTCCA |
| C1 | 7-199 | GTTCTTAT | 5-247 | AATTAGAC | GTCTAATT |
| D1 | 7-200 | GATGCGAC | 5-248 | ACTTTGGG | CCCAAAGT |
| E1 | 7-201 | GAAGAGGG | 5-249 | CGGACGGA | TCCGTCCG |
| F1 | 7-202 | TAGTAATC | 5-250 | GCAGAGCC | GGCTCTGC |
| G1 | 7-203 | GTGTGGAG | 5-251 | GCATGATC | GATCATGC |
| H1 | 7-204 | ACGTTGTA | 5-252 | TCGACCTA | TAGGTCGA |
| A2 | 7-205 | GCGCTAAT | 5-253 | ACCCTGAC | GTCAGGGT |
| B2 | 7-206 | AGAGCTGC | 5-254 | GTTGAAGG | CCTTCAAC |
| C2 | 7-207 | CATACTTA | 5-255 | GCACGGGA | TCCCGTGC |
| D2 | 7-208 | TTGCACCG | 5-256 | CGTATAAA | TTTATACG |
| E2 | 7-209 | GCGGGATA | 5-257 | AGAGACGG | CCGTCTCT |
| F2 | 7-210 | GAAGTGAA | 5-258 | TACAAGTC | GACTTGTA |
| G2 | 7-211 | CTGTTTAC | 5-259 | TGAATCTT | AAGATTCA |
| H2 | 7-212 | GAGCACTC | 5-260 | GCAACTTG | CAAGTTGC |
| A3 | 7-213 | TTGTTGCA | 5-261 | ACGACGTC | GACGTCGT |
| B3 | 7-214 | CCACACTT | 5-262 | GTATGACG | CGTCATAC |
| C3 | 7-215 | CCCGTTTG | 5-263 | TACAGCAA | TTGCTGTA |
| D3 | 7-216 | ATGCTCCC | 5-264 | CAGCAGGG | CCCTGCTG |
| E3 | 7-217 | GCTCAATA | 5-265 | GATAAATG | CATTTATC |
| F3 | 7-218 | GTAGTTCG | 5-266 | GCATCAAG | CTTGATGC |
| G3 | 7-219 | CGAGAACC | 5-267 | CGATACAT | ATGTATCG |
| H3 | 7-220 | GCCATGTA | 5-268 | AACCCTAT | ATAGGGTT |
| A4 | 7-221 | TTTCTCTA | 5-269 | ACGTCGAG | CTCGACGT |
| B4 | 7-222 | CCAGCGAT | 5-270 | TGACTAGA | TCTAGTCA |
| C4 | 7-223 | TGGGAGTG | 5-271 | TAGACGGG | CCCGTCTA |
| D4 | 7-224 | CCCTCGTA | 5-272 | CTCTTCTA | TAGAAGAG |
| E4 | 7-225 | CGATATGG | 5-273 | TACGTCCC | GGGACGTA |
| F4 | 7-226 | TTGTGCCC | 5-274 | GATGGAAA | TTTCCATC |
| G4 | 7-227 | TGTCCTCT | 5-275 | GTTCGTCG | CGACGAAC |
| H4 | 7-228 | GTATAGTC | 5-276 | GAGACCAA | TTGGTCTC |
| A5 | 7-229 | TTTGGGAT | 5-277 | ACGTGAAC | GTTCACGT |
| B5 | 7-230 | CACCAAGC | 5-278 | TTCCCTTT | AAAGGGAA |
| C5 | 7-231 | CGGAGAGG | 5-279 | GACGCTCG | CGAGCGTC |
| D5 | 7-232 | TATTTACC | 5-280 | CTCACGTC | GACGTGAG |
| E5 | 7-233 | TATATGGA | 5-281 | CTGCCAAG | CTTGGCAG |
| F5 | 7-234 | GTTAACAT | 5-282 | ACGCCGCA | TGCGGCGT |
| G5 | 7-235 | CGTCTTGG | 5-283 | CGCCAGTC | GACTGGCG |
| H5 | 7-236 | CGTAGCGA | 5-284 | CTAAACAA | TTGTTTAG |

| WELL POSITION | EXPECTED i7 INDEX READ | | EXPECTED i5 INDEX READ | | |
|---|---|---|---|---|---|
| | i7 INDEX ID | | i5 INDEX ID | FORWARD STRAND WORKFLOW | REVERSE STRAND WORKFLOW |
| A6 | 7-237 | TAGTCACA | 5-285 | TATACCTC | GAGGTATA |
| B6 | 7-238 | AGAAGTGG | 5-286 | CTCTTGAT | ATCAAGAG |
| C6 | 7-239 | CGTGGATT | 5-287 | ACTCTTAG | CTAAGAGT |
| D6 | 7-240 | GTAGATGC | 5-288 | GAGCAACA | TGTTGCTC |
| E6 | 7-241 | TACCGCTC | 5-289 | CAGTGACG | CGTCACTG |
| F6 | 7-242 | CGAACCAC | 5-290 | AAGATTGA | TCAATCTT |
| G6 | 7-243 | TATTGTTC | 5-291 | GTGTGTTT | AAACACAC |
| H6 | 7-244 | GTTGTGTG | 5-292 | CGTCCGAC | GTCGGACG |
| A7 | 7-245 | AAAGCTAA | 5-197 | TGTCGTAG | CTACGACA |
| B7 | 7-246 | TGGAGATT | 5-198 | CAATCATA | TATGATTG |
| C7 | 7-247 | AATTAGAC | 5-199 | GTTCTTAT | ATAAGAAC |
| D7 | 7-248 | ACTTTGGG | 5-200 | GATGCGAC | GTCGCATC |
| E7 | 7-249 | CGGACGGA | 5-201 | GAAGAGGG | CCCTCTTC |
| F7 | 7-250 | GCAGAGCC | 5-202 | TAGTAATC | GATTACTA |
| G7 | 7-251 | GCATGATC | 5-203 | GTGTGGAG | CTCCACAC |
| H7 | 7-252 | TCGACCTA | 5-204 | ACGTTGTA | TACAACGT |
| A8 | 7-253 | ACCCTGAC | 5-205 | GCGCTAAT | ATTAGCGC |
| B8 | 7-254 | GTTGAAGG | 5-206 | AGAGCTGC | GCAGCTCT |
| C8 | 7-255 | GCACGGGA | 5-207 | CATACTTA | TAAGTATG |
| D8 | 7-256 | CGTATAAA | 5-208 | TTGCACCG | CGGTGCAA |
| E8 | 7-257 | AGAGACGG | 5-209 | GCGGGATA | TATCCCGC |
| F8 | 7-258 | TACAAGTC | 5-210 | GAAGTGAA | TTCACTTC |
| G8 | 7-259 | TGAATCTT | 5-211 | CTGTTTAC | GTAAACAG |
| H8 | 7-260 | GCAACTTG | 5-212 | GAGCACTC | GAGTGCTC |
| A9 | 7-261 | ACGACGTC | 5-213 | TTGTTGCA | TGCAACAA |
| B9 | 7-262 | GTATGACG | 5-214 | CCACACTT | AAGTGTGG |
| C9 | 7-263 | TACAGCAA | 5-215 | CCCGTTTG | CAAACGGG |
| D9 | 7-264 | CAGCAGGG | 5-216 | ATGCTCCC | GGGAGCAT |
| E9 | 7-265 | GATAAATG | 5-217 | GCTCAATA | TATTGAGC |
| F9 | 7-266 | GCATCAAG | 5-218 | GTAGTTCG | CGAACTAC |
| G9 | 7-267 | CGATACAT | 5-219 | CGAGAACC | GGTTCTCG |
| H9 | 7-268 | AACCCTAT | 5-220 | GCCATGTA | TACATGGC |
| A10 | 7-269 | ACGTCGAG | 5-221 | TTTCTCTA | TAGAGAAA |
| B10 | 7-270 | TGACTAGA | 5-222 | CCAGCGAT | ATCGCTGG |
| C10 | 7-271 | TAGACGGG | 5-223 | TGGGAGTG | CACTCCCA |
| D10 | 7-272 | CTCTTCTA | 5-224 | CCCTCGTA | TACGAGGG |
| E10 | 7-273 | TACGTCCC | 5-225 | CGATATGG | CCATATCG |
| F10 | 7-274 | GATGGAAA | 5-226 | TTGTGCCC | GGGCACAA |
| G10 | 7-275 | GTTCGTCG | 5-227 | TGTCCTCT | AGAGGACA |
| H10 | 7-276 | GAGACCAA | 5-228 | GTATAGTC | GACTATAC |

| WELL POSITION | EXPECTED i7 INDEX READ | | EXPECTED i5 INDEX READ | | |
|---|---|---|---|---|---|
| | i7 INDEX ID | | i5 INDEX ID | FORWARD STRAND WORKFLOW | REVERSE STRAND WORKFLOW |
| A11 | 7-277 | ACGTGAAC | 5-229 | TTTGGGAT | ATCCCAAA |
| B11 | 7-278 | TTCCCTTT | 5-230 | CACCAAGC | GCTTGGTG |
| C11 | 7-279 | GACGCTCG | 5-231 | CGGAGAGG | CCTCTCCG |
| D11 | 7-280 | CTCACGTC | 5-232 | TATTTACC | GGTAAATA |
| E11 | 7-281 | CTGCCAAG | 5-233 | TATATGGA | TCCATATA |
| F11 | 7-282 | ACGCCGCA | 5-234 | GTTAACAT | ATGTTAAC |
| G11 | 7-283 | CGCCAGTC | 5-235 | CGTCTTGG | CCAAGACG |
| H11 | 7-284 | CTAAACAA | 5-236 | CGTAGCGA | TCGCTACG |
| A12 | 7-285 | TATACCTC | 5-237 | TAGTCACA | TGTGACTA |
| B12 | 7-286 | CTCTTGAT | 5-238 | AGAAGTGG | CCACTTCT |
| C12 | 7-287 | ACTCTTAG | 5-239 | CGTGGATT | AATCCACG |
| D12 | 7-288 | GAGCAACA | 5-240 | GTAGATGC | GCATCTAC |
| E12 | 7-289 | CAGTGACG | 5-241 | TACCGCTC | GAGCGGTA |
| F12 | 7-290 | AAGATTGA | 5-242 | CGAACCAC | GTGGTTCG |
| G12 | 7-291 | GTGTGTTT | 5-243 | TATTGTTC | GAACAATA |
| H12 | 7-292 | CGTCCGAC | 5-244 | GTTGTGTG | CACACAAC |

## Kit Components

The NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 3) are functionally validated through library preparation using the NEBNext Library Prep Kits and sequencing on the Illumina platforms.

### NEB #E6444S Table of Components

| NEB # | CONCENTRATION | PRODUCT | VOLUME |
|---|---|---|---|
| E6612A | 15 µM | NEBNext Adaptor for Illumina | 0.96 ml |
| E6610A | | USER Enzyme | 0.288 ml |
| E6445A | 5 µM each | NEBNext 96 Unique Dual Index Primer Pairs Plate (Set 3) | 1 plate (10 µl/well) |

### NEB #E6444L Table of Components

| NEB # | CONCENTRATION | PRODUCT | VOLUME |
|---|---|---|---|
| E6612A | 15 µM | NEBNext Adaptor for Illumina | 4 x 0.96 ml |
| E6610AA | | USER Enzyme | 2 x 0.576 ml |
| E6445A | 5 µM each | NEBNext 96 Unique Dual Index Primer Pairs Plate (Set 3) | 4 plates (10 µl/well) |

Note :
For the NEBNext Adaptor for Illumina sequence, please see NEBNext Multiplex Oligos for Illumina (Index Primers Set 1), NEB #E7335, Manual.

## Revision History

| REVISION # | DESCRIPTION | DATE |
|---|---|---|
| 1.0 | N/A | 5/20 |
| 2.0 | Update concentration of E6445A in Table of Components | 7/20 |
| 3.0 | Updating tables to have the most current Illumina instrument information and removed HiSeqX. | 2/21 |
| 4.0 | Update Protocol and Tables | 7/22 |