INSTRUCTION MANUAL

NEBNext® Primers for Epigenetics (Unique Dual Index Primer Pairs)

NEB #E3392S, #E3404S

24, 96 reactions

Version 1.0_12/23

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The NEBNext Primers for Epigenetics includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E3392S) or 96 reactions (NEB #E3404S). All reagents should be stored at –20°C.

NEBNext *Primers for Epigenetics (Unique Dual Index Set 2B) NEB #E3392S:*
NEBNext Primers for Epigenetics (Unique Dual Index Set 2B) Plate

NEBNext *Primers for Epigenetics (Unique Dual Index Set 3) NEB #E3404S:*
NEBNext Primers for Epigenetics (Unique Dual Index Set 3) Plate

Applications

The NEBNext Primers for Epigenetics contain unique dual index primers that are needed to make E5hmC-seq™ libraries. These oligos have been optimized for use with the NEBNext Enzymatic 5hmC-seq (E5hmC-seq) Kit (NEB #E3350).

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 of indexed libraries and then sequenced on an Illumina® sequencing platform.

For larger volume requirements, customized and bulk packaging is available by purchasing through the Customized Solutions team at NEB. Please contact custom@neb.com for further information.

Workflow Overview

During PCR, indexes are incorporated using the Unique Dual Index Primer Pairs provided in this kit. The Unique Dual Index Primers enable multiplexing and have been designed to minimize index hopping. The 24-reaction kit (NEB #E3392S) is supplied with a plate containing 24 wells with premixed i5 and i7, 8-base index primers. For the 96-reaction kit (NEB #E3404S), each well of a 96-well plate contains an i5 and i7 primer pair. The 24-reaction and 96-reaction primers are supplied with a pierceable foil seal for easy, single use. The index sequences from the 24-reaction kit (NEB #E3392S) and the 96-reaction kit (NEB #E3404S) are compatible, and can be combined to multiplex up to 120 reactions.

Please refer to the Enzymatic 5hmC-seq Kit Manual (NEB #E3350) for using the NEBNext Primers for Epigenetics.

Section 1
Setting up PCR Reactions

Symbols

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This caution sign signifies a step in the protocol that has two paths leading to the same end point.

1.1. Setting up PCR reactions (24-reaction kit, NEB #E3392S or 96-reaction kit NEB #E3404S)

1.1.1. Determine the number of libraries that will be amplified and pooled for subsequent sequencing.

1.1.2. Ensure that a valid combination of index primers is chosen based on color balance guidelines in Section 2A for NEB #E3392S or Section 2B for NEB #E3404S.

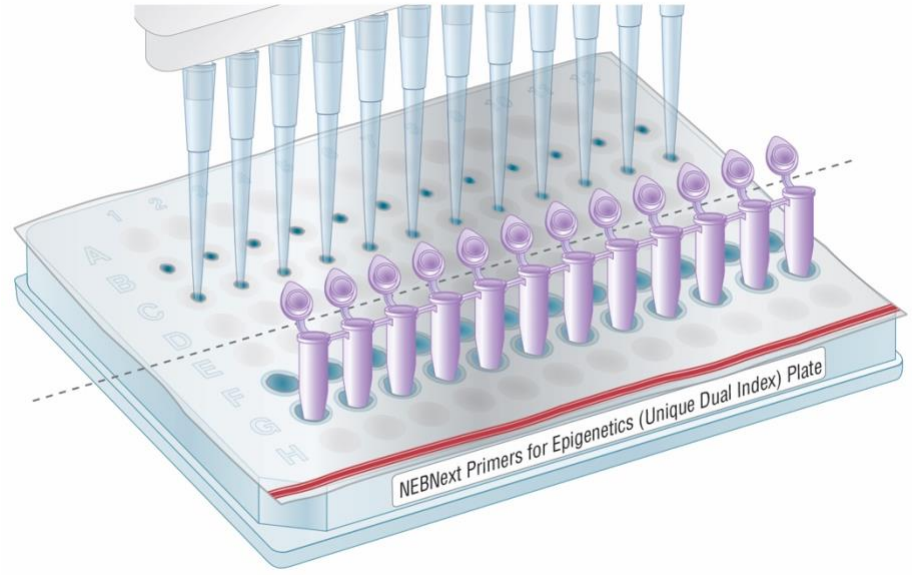
1.1.3. Thaw the NEBNext Primers for Epigenetics (Unique Dual Index) Plate for 10 minutes at room temperature.

1.1.4. Remove the hard-plastic plate cover from the 96-well plate. Briefly centrifuge the plate (280 x g for ~1 minute) to collect all of the primer at the bottom of each well.

1.1.5. Orient the NEBNext Primers for Epigenetics (Unique Dual Index) Plate as indicated in Figure 1.1 (red stripe towards the user). With a pipette tip, pierce the desired well(s) (see Figure 1.1) and transfer 5 µl of primer mix required for the PCR reaction into the PCR plate/tubes. 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 1.1) prior to pipetting the primer mix. Use a new, clean strip tube for each new well to be pierced.

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

Figure 1.1. NEBNext Primers for Epigenetics (Unique Dual Index) Plate



1.1.6. Proceed with the PCR reaction according to the NEBNext Enzymatic 5hmC-seq (NEB #E3350) product manual.

Section 2A
Index Pooling Guidelines: NEBNext Primers for Epigenetics (Unique Dual Index Set 2B) NEB #E3392S

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 tab on www.neb.com/E3392 **– NEBNext Primers for Epigenetics (Unique Dual Index Set 2B) NEB #E3392S.**

For all HiSeq® and 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 1.1 for examples of Good and Bad Index combinations.

The NovaSeq®, NextSeq® and MiniSeq® sequencers utilize 2 color chemistry. Valid index combinations require that some indices do not start with GG in the first two cycles. See Table 1.2 for examples of Good and Bad Index combinations.

Forward Strand Workflow for the following instruments: NovaSeq 6000 with v1.0 reagent 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 (or greater), HiSeq 2000/5000 (single-read flow cell), HiSeq 3000/4000 (paired-end flow cell).

The index primer sequences, for different Illumina sequencer input sheets, are indicated in Table 1.3.

Table 1.1. 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 COMPLEMENT WORKFLOW							
A1	A	T	G	T	T	C	C	T	T	C	A	G	C	G	C	C	G	G	C	G	C	T	G	A
B1	A	A	C	A	C	C	A	C	G	G	T	G	T	G	A	G	C	T	C	A	C	A	C	C
H1	G	G	T	G	T	G	A	G	A	A	C	A	C	C	A	C	G	T	G	G	T	G	T	T
F2	A	C	C	T	C	T	T	C	C	T	T	G	C	A	T	A	T	A	T	G	C	A	A	G
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

BAD																									
WELL POSITION	EXPECTED i7 INDEX READ								EXPECTED i5 INDEX READ																
									FORWARD STRAND WORKFLOW								REVERSE COMPLEMENT WORKFLOW							
A1	A	T	G	T	T	C	C	T	T	C	A	G	C	G	C	C	G	G	C	G	C	T	G	A
B1	A	A	C	A	C	C	A	C	G	G	T	G	T	G	A	G	C	T	C	A	C	A	C	C
H1	G	G	T	G	T	G	A	G	A	A	C	A	C	C	A	C	G	T	G	G	T	G	T	T
G2	T	G	T	C	A	C	A	C	T	A	A	T	C	T	C	G	C	G	A	G	A	T	T	A
	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X	✓	✓	✓	✓	✓	✓

Table 1.2. 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. Note that there are no two-plex combinations in NEB #E3392S that start with GG in the first two cycles. An example of a single index that begins with GG is included for reference.

GOOD																									
WELL POSITION	EXPECTED i7 INDEX READ								EXPECTED i5 INDEX READ																
									FORWARD STRAND WORKFLOW								REVERSE COMPLEMENT WORKFLOW								
A1	A	T	G	T	T	C	C	T	A	A	A	G	C	T	A	A	T	T	A	G	C	T	T	T	
B1	A	A	C	A	C	C	A	C	G	G	T	G	T	G	A	G	C	T	C	A	C	A	C	C	
G2	T	G	T	C	A	C	A	C	T	A	A	T	C	T	C	G	C	G	A	G	A	T	T	A	
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	

BAD																									
WELL POSITION	EXPECTED i7 INDEX READ								EXPECTED i5 INDEX READ																
									FORWARD STRAND WORKFLOW								REVERSE COMPLEMENT WORKFLOW								
B1	A	A	C	A	C	C	A	C	G	G	T	G	T	G	A	G	C	T	C	A	C	A	C	C	
	✓	✓	✓	✓	✓	✓	✓	✓	X	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	

Table 1.3. 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.

WELL POSITION	EXPECTED i7 INDEX READ		EXPECTED i5 INDEX READ		
	i7 INDEX ID		i5 INDEX ID	FORWARD STRAND WORKFLOW	REVERSE COMPLEMENT WORKFLOW
A1	P7133	ATGTTCCT	P5107	TCAGCGCC	GGCGCTGA
B1	P7153	AACACCAC	P5122	GGTGTGAG	CTCACACC
C1	P7160	CCTAAACT	P5139	ACGAGGAG	CTCCTCGT
D1	P7177	CAGGTAAG	P5150	CTCGAAAT	ATTTCGAG
E1	P7187	AGAAGCCT	P5167	GTCAGTAT	ATACTGAC
F1	P7998	GCCACGAC	P5178	CCTCGGGT	ACCCGAGG
G1	P7107	TCAGCGCC	P5133	ATGTTCCT	AGGAACAT
H1	P7122	GGTGTGAG	P5153	AACACCAC	GTGGTGTT
A2	P7139	ACGAGGAG	P5160	CCTAAACT	AGTTTAGG
B2	P7150	CTCGAAAT	P5177	CAGGTAAG	CTTACCTG
C2	P7167	GTCAGTAT	P5187	AGAAGCCT	AGGCTTCT
D2	P7178	CCTCGGGT	P5998	GCCACGAC	GTCGTGGC
E2	P7141	CATTATGG	P5108	AGTCACAT	ATGTGACT
F2	P7154	ACCTCTTC	P5124	CTTGCATA	TATGCAAG
G2	P7162	TGTCACAC	P5140	TAATCTCG	CGAGATTA
H2	P7181	AAGGAGAC	P5151	CTCACAAC	GTTGTGAG
A3	P7188	CTAGGTTG	P5168	ATTCGAGC	GCTCGAAT
B3	P7099	TCTGGAAC	P5179	TAGCACCT	AGGTGCTA
C3	P7108	AGTCACAT	P5141	CATTATGG	CCATAATG
D3	P7124	CTTGCATA	P5154	ACCTCTTC	GAAGAGGT
E3	P7140	TAATCTCG	P5162	TGTCACAC	GTGTGACA
F3	P7151	CTCACAAC	P5181	AAGGAGAC	GTCTCCTT
G3	P7168	ATTCGAGC	P5188	CTAGGTTG	CAACCTAG
H3	P7179	TAGCACCT	P5099	TCTGGAAC	GTTCCAGA

Section 2B
Index Pooling Guidelines: NEBNext Primers for Epigenetics (Unique Dual Index Set 3) NEB #E3404S

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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 tab on www.neb.com/E3404 **– NEBNext Primers for Epigenetics (Unique Dual Index Set 3) NEB #E3404S.**

For all HiSeq and 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.

The NovaSeq, NextSeq and MiniSeq sequencers utilize 2 color chemistry. Valid index combinations require that some indices do not start with GG in the first two cycles. See Table 2.2 for examples of Good and Bad Index combinations.

Forward Strand Workflow for the following instruments: NovaSeq 6000 with v1.0 reagent 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 (or greater), HiSeq 2000/5000 (single-read flow cell), HiSeq 3000/4000 (paired-end flow cell).

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

Table 2.1. 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 COMPLEMENT 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 COMPLEMENT 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	✓	✓	✓	✓	✓	✓	✓

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 COMPLEMENT 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 COMPLEMENT 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. 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.

WELL POSITION	EXPECTED i7 INDEX READ		EXPECTED i5 INDEX READ		
	i7 INDEX ID		i5 INDEX ID	FORWARD STRAND WORKFLOW	REVERSE COMPLEMENT 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 COMPLEMENT 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 COMPLEMENT 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

NEB #E3392S Table of Components

NEB #	PRODUCT	VOLUME
E3399A	NEBNext Primers for Epigenetics (Unique Dual Index Set 2B) Plate	0.005 ml x 24

NEB #E3404S Table of Components

NEB#	PRODUCT	VOLUME
E3404A	NEBNext Primers for Epigenetics (Unique Dual Index Set 3) Plate	0.005 ml x 96

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	12/23

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