INSTRUCTION MANUAL

NEBNext® Multiplex Oligos for Illumina® (Dual Index Primers Set 1)

NEB #E7600S

96 reactions

Version 6.0_6/24

Table of Contents

The NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1) Includes

The volumes provided are sufficient for preparation of up to 96 reactions (NEB #E7600S). 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 i501 Primer
- (white) NEBNext i502 Primer
- (white) NEBNext i503 Primer
- (white) NEBNext i504 Primer
- (white) NEBNext i505 Primer
- (white) NEBNext i506 Primer
- (white) NEBNext i507 Primer
- (white) NEBNext i508 Primer
- (orange) NEBNext i701 Primer
- (orange) NEBNext i702 Primer
- (orange) NEBNext i703 Primer
- (orange) NEBNext i704 Primer
- (orange) NEBNext i705 Primer
- (orange) NEBNext i706 Primer
- (orange) NEBNext i707 Primer
- (orange) NEBNext i708 Primer
- (orange) NEBNext i709 Primer
- (orange) NEBNext i710 Primer
- (orange) NEBNext i711 Primer
- (orange) NEBNext i712 Primer

Applications

The NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1) 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 1) 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.

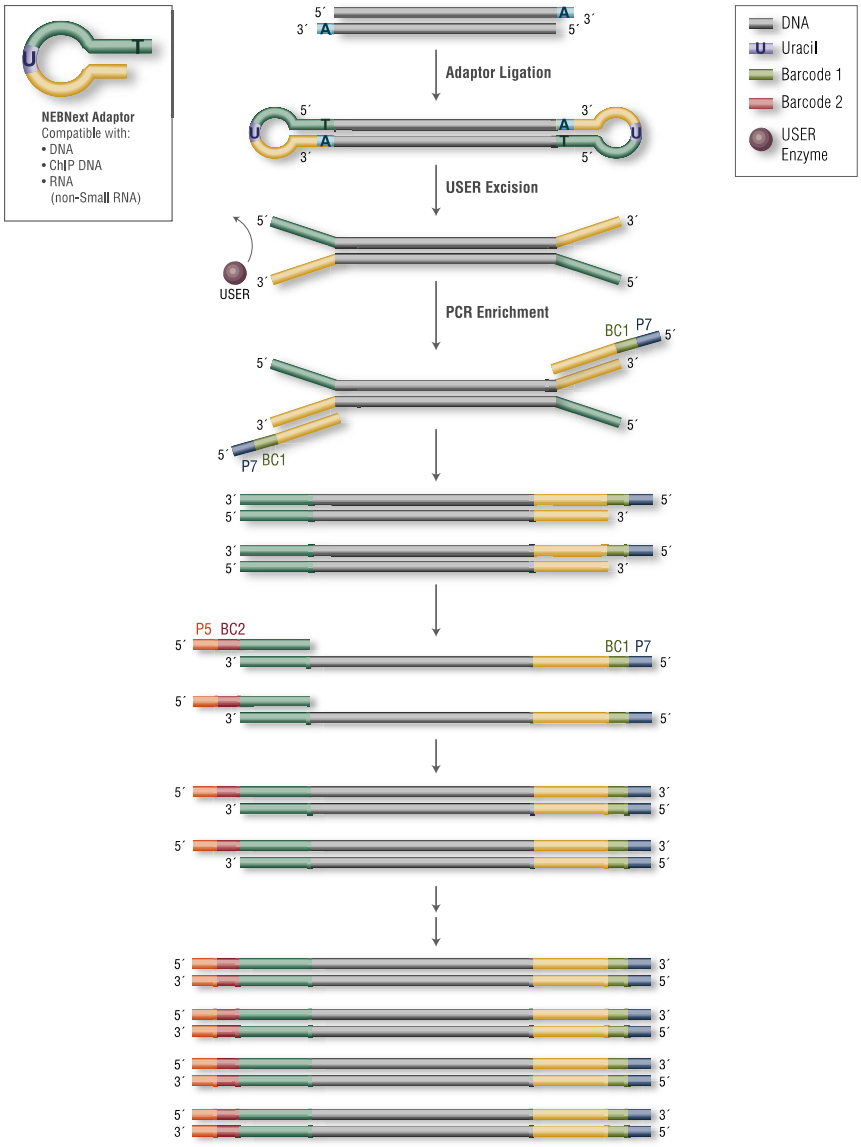
Functionally Validated: Each set of reagents is functionally validated together through construction and sequencing of genomic DNA libraries on the Illumina platform.

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

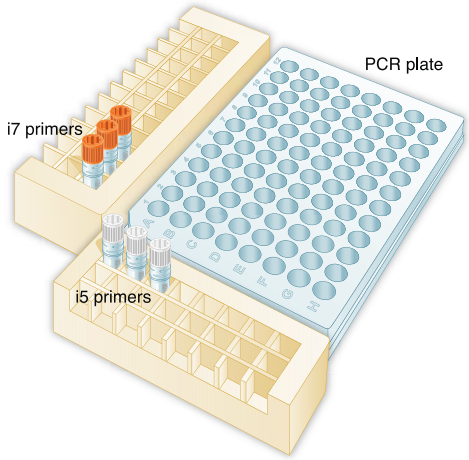
This caution sign signifies a step in the protocol that has multiple paths leading to the same end point but is dependent on a user variable, like the number of samples to be processed.

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 • (orange) 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.

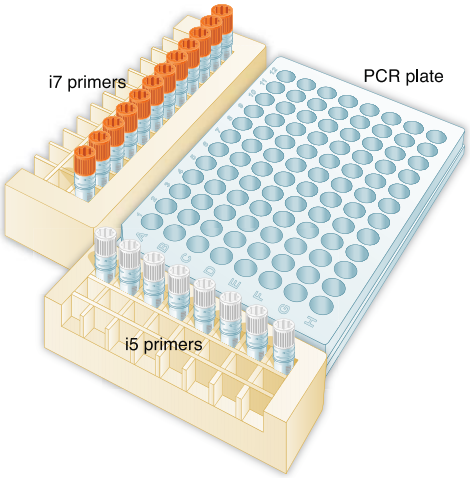
1.1A.6. Using a multichannel pipette, add desired volume of • (orange) i7 primers to every row (as needed) of the PCR plate. **It is critical to change tips between rows to avoid cross-contamination.**

1.1A.7. Discard the original i7 orange 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 • (orange) i7 primers in increasing order horizontally, so that i701 is in column 1, i702 is in column 2, i703 is in column 3, etc.

b. Arrange the ○ (white) i5 primers in increasing order vertically, so that i501 is in row A, i502 is in row B, i503 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 • (orange) 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 orange 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 NovaSeqX 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 = Number of samples = X(i7) * Y(i5) + 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: 6 (i7) * 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 2. 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 4: 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	i701 and i702 i703 and i704 i705 and i706 i707 and i708 i709 and i710 i711 and i712	Any i5 Primer
3	i701, i702 and i703 i703, i704 and i705 i705, i706 and i707 i707, i708 and i709 i709, i710 and i711	Any i5 Primer
4	i701, i702, i703 and i704 i703, i704, i705 and i706 i705, i706, i707 and i708 i707, i708, i709 and i710 i709, i710, i711 and i712	Any i5 Primer
5–12	Any valid i7 4-plex with any other i7 Primers	Any i5 Primer

Table 2.2 Pooling: 7+ libraries; Sequencing Workflow: Dual Index (Select "2" Index Reads in the Illumina Experiment Manager).

PLEX	i7 PRIMERS	i5 PRIMERS
7–12	Any 3 plex combination from Table 2.1 with any other i7 primer (as needed)	i501 and i502 i503 and i504 i505 and i506 i507 and i508
Greater than 12	Any 4 plex combination from the Table 2.1 with any other i7 primer (as needed)	i501, i502 and any other i5 primer (as needed) i503, i504 and any other i5 primer (as needed) i505, i506 and any other i5 primer (as needed) i507, i508 and any other i5 primer (as needed)

To determine possible combinations for low plex pooling, please also see https://www.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 1	A	T	T	A	C	T	C	G	i5-PCR Index 3	C	C	T	A	T	C	C	T	A	G	G	A	T	A	G	G
i7-PCR Index 2	T	C	C	G	G	A	G	A	i5-PCR Index 4	G	G	C	T	C	T	G	A	T	C	A	G	A	G	C	C
i7-PCR Index 3	C	G	C	T	C	A	T	T	i5-PCR Index 5	A	G	G	C	G	A	A	G	T	A	A	G	A	T	T	A
i7-PCR Index 4	G	A	G	A	T	T	C	C	i5-PCR Index 6	T	A	A	T	C	T	T	A	T	A	A	T	C	T	T	A
	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table 3.2

BAD																					
i7 PRIMERS									i5 PRIMERS	FORWARD STRAND WORKFLOW*								REVERSE COMPLEMENT WORKFLOW*							
i7-PCR Index 1	A	T	T	A	C	T	C	G	i5-PCR Index 2	A	T	A	G	A	G	G	C	G	C	C	T	C	T	A	T
i7-PCR Index 2	T	C	C	G	G	A	G	A	i5-PCR Index 4	G	G	C	T	C	T	G	A	T	C	A	G	A	G	C	C
i7-PCR Index 3	C	G	C	T	C	A	T	T	i5-PCR Index 6	T	A	A	T	C	T	G	A	T	A	A	G	A	T	T	A
i7-PCR Index 4	G	A	G	A	T	T	C	C	i5-PCR Index 8	G	T	A	C	T	G	A	C	G	T	C	A	G	T	A	C
	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	X	✓	✓	X	✓	X	X	✓	X	✓	✓	X	✓	✓

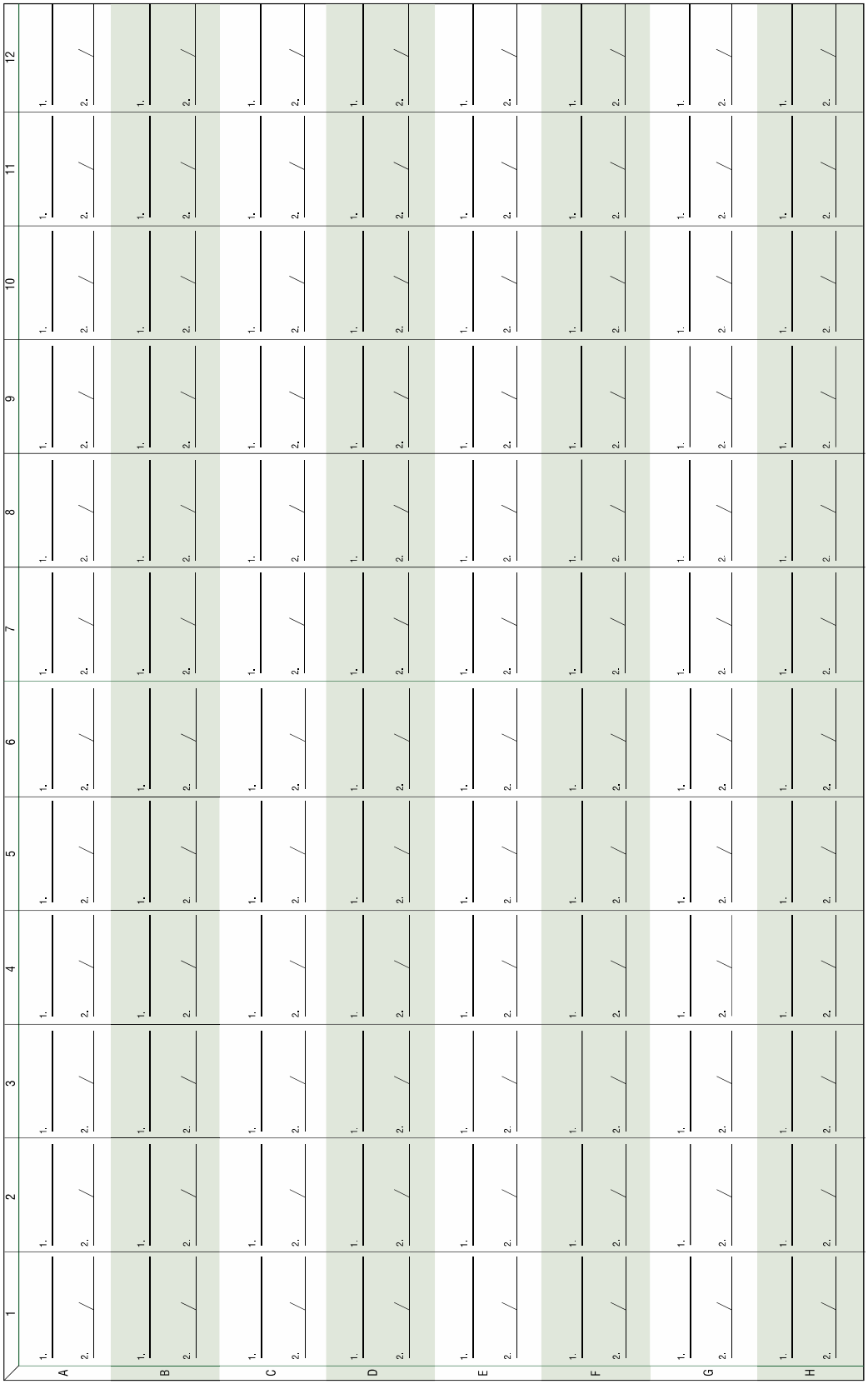
Two Color Chemistry Color Balancing

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) 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 _____



NEBNext Adaptors and Primers for Illumina

For sample sheets please see NEB.com, E7600 Product Page, "Protocols, Manuals and Usage Guidelines" Tab: NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1) | NEB

***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 i501 Primer–NEBNext i508 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*
#E7603A	**NEBNext i501 Primer**	5´-AATGATACGGCGACCACCGAGATCTACAC**TATAGCCT**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3´	TATAGCCT	AGGCTATA
#E7604A	**NEBNext i502 Primer**	5´-AATGATACGGCGACCACCGAGATCTACAC**ATAGAGGC**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3´	ATAGAGGC	GCCTCTAT
#E7605A	**NEBNext i503 Primer**	5´-AATGATACGGCGACCACCGAGATCTACAC**CCTATCCT**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3´	CCTATCCT	AGGATAGG
#E7606A	**NEBNext i504 Primer**	5´-AATGATACGGCGACCACCGAGATCTACAC**GGCTCTGA**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3´	GGCTCTGA	TCAGAGCC
#E7607A	**NEBNext i505 Primer**	5´-AATGATACGGCGACCACCGAGATCTACAC**AGGCGAAG**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3´	AGGCGAAG	CTTCGCCT
#E7608A	**NEBNext i506 Primer**	5´-AATGATACGGCGACCACCGAGATCTACAC**TAATCTTA**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3´	TAATCTTA	TAAGATTA
#E7609A	**NEBNext i507 Primer**	5´-AATGATACGGCGACCACCGAGATCTACAC**CAGGACGT**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3´	CAGGACGT	ACGTCCTG
#E7610A	**NEBNext i508 Primer**	5´-AATGATACGGCGACCACCGAGATCTACAC**GTACTGAC**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3´	GTACTGAC	GTCAGTAC

NEBNext i701 Primer–NEBNext i712 Primer

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

NEB #	PRODUCT	INDEX PRIMER SEQUENCE	EXPECTED INDEX READ
#E7611A	**NEBNext i701 Primer**	5´-CAAGCAGAAGACGGCATACGAGAT**CGAGTAAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ATTACTCG
#E7612A	**NEBNext i702 Primer**	5´-CAAGCAGAAGACGGCATACGAGAT**TCTCCGGA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TCCGGAGA
#E7613A	**NEBNext i703 Primer**	5´-CAAGCAGAAGACGGCATACGAGAT**AATGAGCG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CGCTCATT
#E7614A	**NEBNext i704 Primer**	5´-CAAGCAGAAGACGGCATACGAGAT**GGAATCTC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GAGATTCC
#E7615A	**NEBNext i705 Primer**	5´-CAAGCAGAAGACGGCATACGAGAT**TTCTGAAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	ATTCAGAA
#E7616A	**NEBNext i706 Primer**	5´-CAAGCAGAAGACGGCATACGAGAT**ACGAATTC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	GAATTCGT
#E7617A	**NEBNext i707 Primer**	5´-CAAGCAGAAGACGGCATACGAGAT**AGCTTCAG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CTGAAGCT
#E7618A	**NEBNext i708 Primer**	5´-CAAGCAGAAGACGGCATACGAGAT**GCGCATTA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TAATGCGC
#E7619A	**NEBNext i709 Primer**	5´-CAAGCAGAAGACGGCATACGAGAT**CATAGCCG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	CGGCTATG
#E7620A	**NEBNext i710 Primer**	5´-CAAGCAGAAGACGGCATACGAGAT**TTCGCGGA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TCCGCGAA
#E7621A	**NEBNext i711 Primer**	5´-CAAGCAGAAGACGGCATACGAGAT**GCGCGAGA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	TCTCGCGC
#E7622A	**NEBNext i712 Primer**	5´-CAAGCAGAAGACGGCATACGAGAT**CTATCGCT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3´	AGCGATAG

Kit Components

The NEBNext Dual Index Primers Set 1 is functionally validated through library preparation using the NEBNext Library Prep Kits and sequencing on the Illumina platform.

NEB #E7600S Table of Components

NEB #	PRODUCT NAME	VOLUME
E7601A	NEBNext Adaptor for Illumina*	0.96 ml
E7602A	USER Enzyme	0.288 ml
E7603A	NEBNext i501 Primer	0.060 ml
E7604A	NEBNext i502 Primer	0.060 ml
E7605A	NEBNext i503 Primer	0.060 ml
E7606A	NEBNext i504 Primer	0.060 ml
E7607A	NEBNext i505 Primer	0.060 ml
E7608A	NEBNext i506 Primer	0.060 ml
E7609A	NEBNext i507 Primer	0.060 ml
E7610A	NEBNext i508 Primer	0.060 ml
E7611A	NEBNext i701 Primer	0.040 ml
E7612A	NEBNext i702 Primer	0.040 ml
E7613A	NEBNext i703 Primer	0.040 ml
E7614A	NEBNext i704 Primer	0.040 ml
E7615A	NEBNext i705 Primer	0.040 ml
E7616A	NEBNext i706 Primer	0.040 ml
E7617A	NEBNext i707 Primer	0.040 ml
E7618A	NEBNext i708 Primer	0.040 ml
E7619A	NEBNext i709 Primer	0.040 ml
E7620A	NEBNext i710 Primer	0.040 ml
E7621A	NEBNext i711 Primer	0.040 ml
E7622A	NEBNext i712 Primer	0.040 ml

* 15 µM Concentration

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	
2.0	Removed kit specific protocols.	
2.1	Update "Required Materials Not Included for DNA or ChIP Libraries" and "Required Materials Not Included for RNA Libraries". Update the list of kits using the NEBNext Multiplex Oligos for Illumina. Insert new Step 1 to Chapter 1 Section 1.1B.	3/16
3.0	Corrected sequence for NEBNext Adaptor for Illumina. Created table of kit components in place of component pages. Placed edits to Chapter 1,2,3. Deleted required materials not included. Added list of kits for use with NEBNext Multiplex Oligos for Illumina.	11/17
4.0	Updated manual format.	1/20
5.0	Updated manual protocols.	7/22
6.0	Updated color balance information. Updated Adaptors and Primers section. Added new logo to header and footer and updated legal footnote.	6/24