INSTRUCTION MANUAL

NEBNext® Multiplex Oligos for Illumina® (Unique Dual Index UMI Adaptors DNA Sets 1-4)

NEB #E7395S/L, NEB #E7874S/L, NEB #E7876S/L, NEB #E7878S/L

96/384 reactions

Version 2.0_2/24

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Each set of NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Sets 1-4) includes

The volumes provided are sufficient for preparation of up to 96 reactions (S kits) and 384 reactions (L kits).
*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 of the same Set)

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

***** If the adaptor plate is thawed upon arrival, we recommend centrifuging the 96 well plate to collect the adaptor in the bottom of the well before re-freezing. If the plate arrived frozen, we recommend to store it at -20ºC right away and centrifuge the plate prior to the first use to avoid unnecessary freeze/ thaw cycles.

Overview

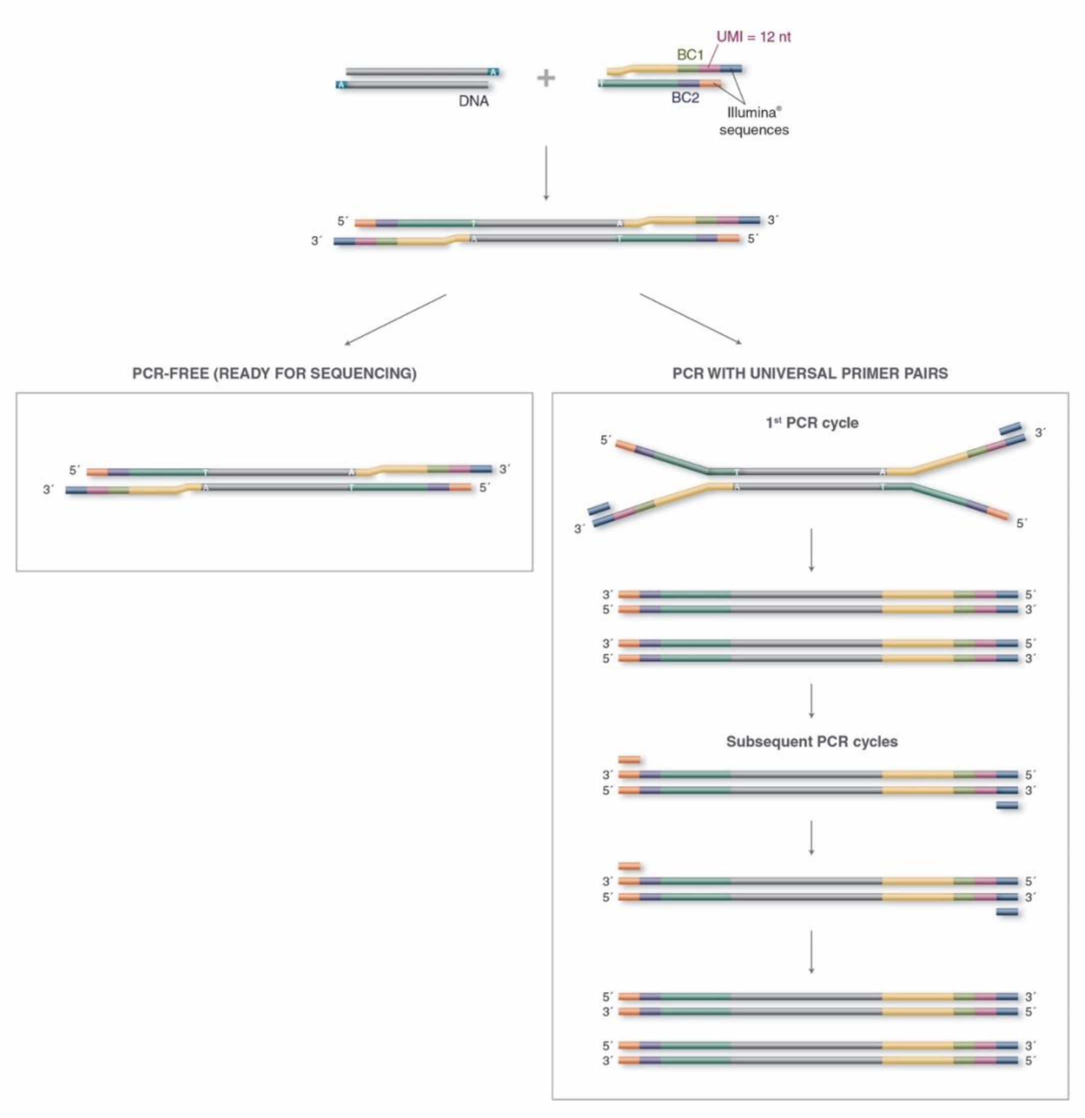
The NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Sets 1-4) contain index adaptors and PCR primers that are ideally suited for multiplex sample preparation for next-generation sequencing on the Illumina platform. NEBNext library prep reagents are different because each kit 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; this includes the adaptors and primers.

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 and ChIP DNA 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 12-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 library prep kits, and with other standard Illumina-compatible library preparation protocols that are based on TA single base overhang ligation, including PCR free workflows.

Figure 1. Workflow demonstrating the use of NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Sets 1-4).



Library Preparation Kits for use with NEBNext Unique Dual Index UMI Adaptors DNA Sets 1-4

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.**

For compatibility of NEBNext Multiplex Oligos please refer to the NEBNext Multiplex Oligos Selection Chart at **www.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

Kit Components

The NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1-4) are functionally validated through library preparation using the NEBNext Library Prep Kits and sequencing on Illumina platforms.

NEB #E7395S Set 1 Table of Components

NEB #	CONCENTRATION	PRODUCT	VOLUME
E7396A	20 µM	NEBNext UMI DNA Adaptor Plate Set 1	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 Set 1 Table of Components

NEB #	CONCENTRATION	PRODUCT	VOLUME
E7396A	20 µM	NEBNext UMI DNA Adaptor Plate Set 1	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

NEB #E7874S Set 2 Table of Components

NEB #	CONCENTRATION	PRODUCT	VOLUME
E7875A	20 µM	NEBNext UMI DNA Adaptor Plate Set 2	1 plate (2.5 µl/well)
E7397A	40µM (Total)	NEBNext PCR Primer Mix	0.48 ml
E7398A		NEBNext UMI Adaptor Dilution Buffer	5 ml

NEB #E7874L Set 2 Table of Components

NEB #	CONCENTRATION	PRODUCT	VOLUME
E7875AA	20 µM	NEBNext UMI DNA Adaptor Plate Set 2	4 plates (2.5 µl/well)
E7397AA	40µM (Total)	NEBNext PCR Primer Mix	2 x 0.96 ml
E7398AA		NEBNext UMI Adaptor Dilution Buffer	20 ml

NEB #E7876S Set 3 Table of Components

NEB #	CONCENTRATION	PRODUCT	VOLUME
E7877A	20 µM	NEBNext UMI DNA Adaptor Plate Set 3	1 plate (2.5 µl/well)
E7397A	40µM (Total)	NEBNext PCR Primer Mix	0.48 ml
E7398A		NEBNext UMI Adaptor Dilution Buffer	5 ml

NEB #E7876L Set 3 Table of Components

NEB #	CONCENTRATION	PRODUCT	VOLUME
E7877AA	20 µM	NEBNext UMI DNA Adaptor Plate Set 3	4 plates (2.5 µl/well)
E7397AA	40µM (Total)	NEBNext PCR Primer Mix	2 x 0.96 ml
E7398AA		NEBNext UMI Adaptor Dilution Buffer	20 ml

NEB #E7878S Set 4 Table of Components

NEB #	CONCENTRATION	PRODUCT	VOLUME
E7879A	20 µM	NEBNext UMI DNA Adaptor Plate Set 4	1 plate (2.5 µl/well)
E7397A	40 µM (Total)	NEBNext PCR Primer Mix	0.48 ml
E7398A		NEBNext UMI Adaptor Dilution Buffer	5 ml

NEB #E7878L Set 4 Table of Components

NEB #	CONCENTRATION	PRODUCT	VOLUME
E7879AA	20 µM	NEBNext UMI DNA Adaptor Plate Set 4	4 plates (2.5 µl/well)
E7397AA	40 µM (Total)	NEBNext PCR Primer Mix	2 x 0.96 ml
E7398AA		NEBNext UMI Adaptor Dilution Buffer	20 ml

Section 1

Setting up the Ligation 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 number of samples to be processed.

1.1. Ligation

⚠

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 ligation reactions (< 96 samples)

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

1.1A.2. Ensure that you choose a valid combination of barcode adaptors based on color balance guidelines in Section 2 and 3.

1.1A.3. Thaw the NEBNext UMI DNA Adaptor Plate for 10–15 minutes on ice.

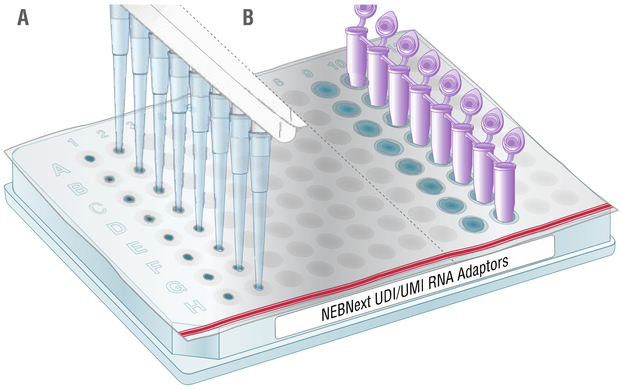
1.1A.4. Remove the hard plastic plate cover. Centrifuge the plate (280 × *g* for ~1 min) if necessary to collect all of the adaptor at the bottom of each well.

1.1A.5. Orient the NEBNext UMI DNA Adaptor Plate 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 adaptor mix required for the ligation reaction to the ligation 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 adaptors. Alternatively, the wells can be pierced using the bottom of clean PCR strip tubes (see Figure 1.1B) prior to pipetting the adaptor mix. Use a new, clean strip tube for each new well to be pierced.

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

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

Figure 1.1. NEBNext UMI DNA Adaptor Plate



1.1B Setting up the ligation reactions (96 samples)

1.1B.1. Thaw the NEBNext UMI DNA Adaptor Plate for 10-15 minutes on ice.

1.1B.2. Remove the hard plastic plate cover. Centrifuge the plate (280 × *g* for ~1 min) if necessary to collect all of the adaptor at the bottom of each well.

1.1B.3. Orient the NEBNext UMI DNA Adaptor 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 adaptor required for the ligation reaction to a 96 well 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 adaptors. Alternatively, the wells can be pierced using the bottom of clean PCR strip tubes (see Figure 1.1B) prior to pipetting the adaptor mix. Use a new, clean strip tube for each new well to be pierced.

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

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

Section 2
Index Pooling Guidelines

Index Pooling Guidelines Within Each Set

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 Chemistry and imaging on MiSeq - Illumina Knowledge

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.

2.1 Index Pooling Guidelines for NEBNext Multiplex Oligos 96 Unique Dual Index UMI Adaptors DNA Set 1 (NEB #E7395)

Table 2.1 Index Pooling Guidelines for DNA Set 1 (NEB #E7395)

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

2.2 Index Pooling Guidelines for NEBNext Multiplex Oligos 96 Unique Dual Index UMI Adaptors DNA Set 2 (NEB #E7874)

Table 2.2 Index Pooling Guidelines for DNA Set 2 (NEB #E7874)

PLEX	WELL POSITION
2	A1, B1 A2, B2 A3, B3 A4, B4 (for additional combinations, confirm color balance according to examples in Table 3.2)
3	A1, B1, C1 A2, B2, C2 A3, B3, C3 A4, B4, C4 (for additional combinations, confirm color balance according to examples in Table 3.2)
4	A1, B1, C1, D1 A2, B2, C2, D2 A3, B3, C3, D3 A4, B4, C4, D4 A2, B2, G2, H2 A3, B3, G3, H3 A6, F6, G6, H6 A8, E8, F8, G8 B9, E9, F9, G9 A12, B12, C12, E12
5	A1, B1, C1, D1, E1 A2, B2, C2, D2, E2 A3, B3, C3, D3, E3 A4, B4, C4, D4, E4 A2, B2, C2, G2, H2 A3, B3, C3, G3, H3 A6, E6, F6, G6, H6
	A8, E8, F8, G8, H8 A9, B9, E9, F9, G9 A12, B12, C12, D12, E12
6-7	Any 5 plex plus 1-2 adjacent wells from the same column
8	Any column

2.3 Index Pooling Guidelines for NEBNext Multiplex Oligos 96 Unique Dual Index UMI Adaptors DNA Set 3 (NEB #E7876)

Table 2.3. Index Pooling Guidelines for DNA Set 3 (NEB #E7876)

PLEX	WELL POSITION
2	A1, B1 A2, B2 A3, B3 A4, B4 (for additional combinations, confirm color balance according to examples in Table 3.3)
3	A1, B1, C1 A2, B2, C2 A3, B3, C3 A4, B4, C4 (for additional combinations, confirm color balance according to examples in Table 3.3)
4	A1, B1, C1, D1 A2, B2, C2, D2 A3, B3, C3, D3 A4, B4, C4, D4 A2, B2, G2, H2 A3, B3, G3, H3 A6, F6, G6, H6 A8, E8, F8, G8 B9, E9, F9, G9 A12, B12, C12, E12
5	A1, B1, C1, D1, E1 A2, B2, C2, D2, E2 A3, B3, C3, D3, E3 A4, B4, C4, D4, E4 A2, B2, C2, G2, H2 A3, B3, C3, G3, H3 A6, E6, F6, G6, H6 A8, E8, F8, G8, H8 A9, B9, E9, F9, G9 A12, B12, C12, D12, E12
6-7	Any 5 plex plus 1-2 adjacent wells from the same column
8	Any column

2.4 Index Pooling Guidelines for NEBNext Multiplex Oligos 96 Unique Dual Index UMI Adaptors DNA Set 4 (NEB #E7878)

Table 2.4. Index Pooling Guidelines for DNA Set 4 (NEB #E7878)

PLEX	WELL POSITION
2	A1, B1 A2, B2 A3, B3 A4, B4 (for additional combinations, confirm color balance according to examples in Table 3.4)
3	A1, B1, C1 A2, B2, C2 A3, B3, C3 A4, B4, C4 (for additional combinations, confirm color balance according to examples in Table 3.4)
4	A1, B1, C1, D1 A2, B2, C2, D2 A3, B3, C3, D3 A4, B4, C4, D4 A2, B2, G2, H2 A3, B3, G3, H3 A6, F6, G6, H6 A8, E8, F8, G8 B9, E9, F9, G9 A12, B12, C12, E12
5	A1, B1, C1, D1, E1 A2, B2, C2, D2, E2 A3, B3, C3, D3, E3 A4, B4, C4, D4, E4 A2, B2, C2, G2, H2 A3, B3, C3, G3, H3 A6, E6, F6, G6, H6 A8, E8, F8, G8, H8 A9, B9, E9, F9, G9 A12, B12, C12, D12, E12
6-7	Any 5 plex plus 1-2 adjacent wells from the same column
8	Any column

Good and Bad Examples for Pooling and Color Balancing

Four Channel Chemistry Color Balancing

***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.

Listed below are index sequences color coded to correspond to the four color chemistry 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 four color chemistry guidelines:

GOOD																								
WELL POSITION (E7395)	**EXPECTED i7 INDEX READ**								**EXPECTED i5 INDEX READ**															
									Forward Strand Workflow*								**Reverse Complement Workflow***							
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
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

BAD																								
WELL POSITION (E7395)	**EXPECTED i7 INDEX READ**								**EXPECTED i5 INDEX READ**															
									Forward Strand Workflow*								**Reverse Complement Workflow***							
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	✓	✓	✓	✓

* The index adaptor sequences for different Illumina sequencer input sheets are indicated in Section 3.

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. 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 (E7395)	**EXPECTED i7 INDEX READ**								**EXPECTED i5 INDEX READ**															
									Forward Strand Workflow*								**Reverse Complement Workflow***							
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 (E7395)	**EXPECTED i7 INDEX READ**								**EXPECTED i5 INDEX READ**															
									Forward Strand Workflow*								**Reverse Complement Workflow***							
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	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Section 3
Index Sequences

Table 3.1. Index Sequences for Set 1 (NEB #E7395).

WELL POSITION	EXPECTED i7 INDEX READ		EXPECTED i5 INDEX READ		
	i7 Index ID		i5 Index ID	Forward Strand Workflow*	Reverse Complement Workflow*
A1	S762	TTACCGAC	S512	CGAATACG	CGTATTCG
B1	S713	TCGTCTGA	S586	GTCCTTGA	TCAAGGAC
C1	S736	TTCCAGGT	S543	CAGTGCTT	AAGCACTG
D1	S709	TACGGTCT	S575	TCCATTGC	GCAATGGA
E1	S732	AAGACCGT	S550	GTCGATTG	CAATCGAC
F1	S774	CAGGTTCA	S506	ATAACGCC	GGCGTTAT
G1	S747	TAGGAGCT	S524	GCCTTAAC	GTTAAGGC
H1	S794	TACTCCAG	S590	GGTATAGG	CCTATACC
A2	S729	AGTGACCT	S591	TCTAGGAG	CTCCTAGA
B2	S777	AGCCTATC	S526	TGCGTAAC	GTTACGCA
C2	S772	TCATCTCC	S567	CTTGCTAG	CTAGCAAG
D2	S725	CCAGTATC	S538	AGCGAGAT	ATCTCGCT
E2	S755	TTGCGAGA	S566	TATGGCAC	GTGCCATA
F2	S760	GAACGAAG	S511	GAATCACC	GGTGATTC
G2	S716	CGAATTGC	S559	GTAAGGTG	CACCTTAC
H2	S708	GGAAGAGA	S521	CGAGAGAA	TTCTCTCG
A3	S702	TCGGATTC	S523	CGCAACTA	TAGTTGCG
B3	S796	CTGTACCA	S507	CACAGACT	AGTCTGTG
C3	S757	GAGAGTAC	S545	TGGAAGCA	TGCTTCCA
D3	S783	TCTACGCA	S546	CAATAGCC	GGCTATTG
E3	S722	GCAATTCC	S578	CTCGAACA	TGTTCGAG
F3	S710	CTCAGAAG	S581	GGCAAGTT	AACTTGCC
G3	S770	GTCCTAAG	S540	AGCTACCA	TGGTAGCT
H3	S734	GCGTTAGA	S592	CAGCATAC	GTATGCTG
A4	S763	CAAGGTAC	S505	CGTATCTC	GAGATACG
B4	S797	AGACCTTG	S501	TTACGTGC	GCACGTAA
C4	S735	GTCGTTAC	S554	AGCTAAGC	GCTTAGCT
D4	S727	GTAACCGA	S598	AAGACACC	GGTGTCTT
E4	S742	GAATCCGT	S551	CAACTCCA	TGGAGTTG
F4	S795	CATGAGCA	S517	GATCTTGC	GCAAGATC
G4	S749	CTTAGGAC	S565	CTTCACTG	CAGTGAAG
H4	S773	ATCTGACC	S593	CTCGACTT	AAGTCGAG
A5	S769	TCCTCATG	S519	GTACACCT	AGGTGTAC
B5	S752	AGGATAGC	S544	CCAAGGTT	AACCTTGG
C5	S704	GGAGGAAT	S585	GAACGGTT	AACCGTTC
D5	S715	GACGTCAT	S518	CCAGTTGA	TCAACTGG
E5	S753	CCGCTTAA	S548	GTCATCGT	ACGATGAC
F5	S758	GACGAACT	S568	CAATGCGA	TCGCATTG
G5	S784	TCCACGTT	S541	GGTTGAAC	GTTCAACC
H5	S714	AACCAGAG	S520	CTTCGGTT	AACCGAAG

	EXPECTED i7 INDEX READ		EXPECTED i5 INDEX READ		
WELL POSITION	i7 Index ID		i5 Index ID	Forward Strand Workflow*	Reverse Complement Workflow*
A6	S771	GTCAGTCA	S531	CGGCATTA	TAATGCCG
B6	S779	CCTTCCAT	S589	CACGCAAT	ATTGCGTG
C6	S788	AGGAACAC	S587	GGAATGTC	GACATTCC
D6	S739	CTTACAGC	S503	TGGTGAAG	CTTCACCA
E6	S737	TACCTGCA	S576	GGACATCA	TGATGTCC
F6	S728	AGACGCTA	S582	GGTGTACA	TGTACACC
G6	S780	CAACACAG	S530	GATAGCCA	TGGCTATC
H6	S761	GTACCACA	S533	CCACAACA	TGTTGTGG
A7	S712	CGAATACG	S562	TTACCGAC	GTCGGTAA
B7	S786	GTCCTTGA	S513	TCGTCTGA	TCAGACGA
C7	S743	CAGTGCTT	S536	TTCCAGGT	ACCTGGAA
D7	S775	TCCATTGC	S509	TACGGTCT	AGACCGTA
E7	S750	GTCGATTG	S532	AAGACCGT	ACGGTCTT
F7	S706	ATAACGCC	S574	CAGGTTCA	TGAACCTG
G7	S724	GCCTTAAC	S547	TAGGAGCT	AGCTCCTA
H7	S790	GGTATAGG	S594	TACTCCAG	CTGGAGTA
A8	S791	TCTAGGAG	S529	AGTGACCT	AGGTCACT
B8	S726	TGCGTAAC	S577	AGCCTATC	GATAGGCT
C8	S767	CTTGCTAG	S572	TCATCTCC	GGAGATGA
D8	S738	AGCGAGAT	S525	CCAGTATC	GATACTGG
E8	S766	TATGGCAC	S555	TTGCGAGA	TCTCGCAA
F8	S711	GAATCACC	S560	GAACGAAG	CTTCGTTC
G8	S759	GTAAGGTG	S516	CGAATTGC	GCAATTCG
H8	S721	CGAGAGAA	S508	GGAAGAGA	TCTCTTCC
A9	S723	CGCAACTA	S502	TCGGATTC	GAATCCGA
B9	S707	CACAGACT	S596	CTGTACCA	TGGTACAG
C9	S745	TGGAAGCA	S557	GAGAGTAC	GTACTCTC
D9	S746	CAATAGCC	S583	TCTACGCA	TGCGTAGA
E9	S778	CTCGAACA	S522	GCAATTCC	GGAATTGC
F9	S781	GGCAAGTT	S510	CTCAGAAG	CTTCTGAG
G9	S740	AGCTACCA	S570	GTCCTAAG	CTTAGGAC
H9	S792	CAGCATAC	S534	GCGTTAGA	TCTAACGC
A10	S705	CGTATCTC	S563	CAAGGTAC	GTACCTTG
B10	S701	TTACGTGC	S597	AGACCTTG	CAAGGTCT
C10	S754	AGCTAAGC	S535	GTCGTTAC	GTAACGAC
D10	S798	AAGACACC	S527	GTAACCGA	TCGGTTAC
E10	S751	CAACTCCA	S542	GAATCCGT	ACGGATTC
F10	S717	GATCTTGC	S595	CATGAGCA	TGCTCATG
G10	S765	CTTCACTG	S549	CTTAGGAC	GTCCTAAG
H10	S793	CTCGACTT	S573	ATCTGACC	GGTCAGAT

	EXPECTED i7 INDEX READ		EXPECTED i5 INDEX READ		
WELL POSITION	**i7 Index ID**		**i5 Index ID**	**Forward Strand Workflow***	**Reverse Complement Workflow***
A11	S719	GTACACCT	S569	TCCTCATG	CATGAGGA
B11	S744	CCAAGGTT	S552	AGGATAGC	GCTATCCT
C11	S785	GAACGGTT	S504	GGAGGAAT	ATTCCTCC
D11	S718	CCAGTTGA	S515	GACGTCAT	ATGACGTC
E11	S748	GTCATCGT	S553	CCGCTTAA	TTAAGCGG
F11	S768	CAATGCGA	S558	GACGAACT	AGTTCGTC
G11	S741	GGTTGAAC	S584	TCCACGTT	AACGTGGA
H11	S720	CTTCGGTT	S514	AACCAGAG	CTCTGGTT
A12	S731	CGGCATTA	S571	GTCAGTCA	TGACTGAC
B12	S789	CACGCAAT	S579	CCTTCCAT	ATGGAAGG
C12	S787	GGAATGTC	S588	AGGAACAC	GTGTTCCT
D12	S703	TGGTGAAG	S539	CTTACAGC	GCTGTAAG
E12	S776	GGACATCA	S537	TACCTGCA	TGCAGGTA
F12	S782	GGTGTACA	S528	AGACGCTA	TAGCGTCT
G12	S730	GATAGCCA	S580	CAACACAG	CTGTGTTG
H12	S733	CCACAACA	S561	GTACCACA	TGTGGTAC

Table 3.2. Index Sequences for Set 2 (NEB #E7874).

WELL POSITION	EXPECTED i7 INDEX READ		EXPECTED i5 INDEX READ		
	i7 Index ID		i5 Index ID	Forward Strand Workflow*	Reverse Complement Workflow*
A1	P7126	CACTGTAG	P5134	AAGCGACT	AGTCGCTT
B1	P7148	GTGCACGA	P5136	TGATAGGC	GCCTATCA
C1	P7133	ATGTTCCT	P5107	TCAGCGCC	GGCGCTGA
D1	P7141	CATTATGG	P5108	AGTCACAT	ATGTGACT
E1	P7142	TCTTGTTT	P5109	CCTTTCAC	GTGAAAGG
F1	P7143	GGCTTACT	P5111	CTTTCCCT	AGGGAAAG
G1	P7146	ACGATATG	P5117	GACAATTC	GAATTGTC
H1	P7152	ATCCGCAG	P5119	ACACGACT	AGTCGTGT
A2	P7134	AAGCGACT	P5135	ACGAATCC	GGATTCGT
B2	P7136	TGATAGGC	P5170	GTCTGAGT	ACTCAGAC
C2	P7153	AACACCAC	P5122	GGTGTGAG	CTCACACC
D2	P7154	ACCTCTTC	P5124	CTTGCATA	TATGCAAG
E2	P7155	GTCCGATC	P5125	GCCAATCC	GGATTGGC
F2	P7157	GAGGACCA	P5129	ATGCCGGT	ACCGGCAT
G2	P7158	CGCTCTTA	P5137	CATACCGT	ACGGTATG
H2	P7159	CTGAGCTC	P5138	ATCAGAGC	GCTCTGAT
A3	P7135	ACGAATCC	P5127	ATTACCCA	TGGGTAAT
B3	P7170	GTCTGAGT	P5169	GACTTGTG	CACAAGTC
C3	P7160	CCTAAACT	P5139	ACGAGGAG	CTCCTCGT
D3	P7162	TGTCACAC	P5140	TAATCTCG	CGAGATTA
E3	P7165	GATATGAA	P5144	TACGGCAG	CTGCCGTA
F3	P7166	AAGTGTGG	P5145	TGCCCATC	GATGGGCA
G3	P7174	GTTGGCGT	P5147	CAGCAGTC	GACTGCTG
H3	P7176	TAGCTGGC	P5149	TACCGGCT	AGCCGGTA
A4	P7127	ATTACCCA	P5126	CACTGTAG	CTACAGTG
B4	P7169	GACTTGTG	P5148	GTGCACGA	TCGTGCAC
C4	P7177	CAGGTAAG	P5150	CTCGAAAT	ATTTCGAG
D4	P7181	AAGGAGAC	P5151	CTCACAAC	GTTGTGAG
E4	P7182	AGTCAGGT	P5156	GTAACCAC	GTGGTTAC
F4	P7184	ACCGTAAG	P5161	CATATCCA	TGGATATG
G4	P7185	TATGACGT	P5163	CGCTAATC	GATTAGCG
H4	P7186	TTGGGTAC	P5164	CTTCCAAC	GTTGGAAG
A5	P7101	TTCAATAG	P5115	TCCCACGA	TCGTGGGA
B5	P7116	GTTTGCTC	P5132	ACCAACAG	CTGTTGGT
C5	P7187	AGAAGCCT	P5167	GTCAGTAT	ATACTGAC
D5	P7188	CTAGGTTG	P5168	ATTCGAGC	GCTCGAAT
E5	P7190	TGTGTCAG	P5171	CACCTGTA	TACAGGTG
F5	P7191	AGAACCAG	P5172	CCGACTCT	AGAGTCGG
G5	P7192	ATTGGACA	P5173	TTGCTGGA	TCCAGCAA
H5	P7385	ACCCGTTG	P5175	CAGCTTCG	CGAAGCTG

WELL POSITION	EXPECTED i7 INDEX READ		EXPECTED i5 INDEX READ		
	i7 Index ID		i5 Index ID	Forward Strand Workflow*	Reverse Complement Workflow*
A6	P7105	ACCGGAGT	P5114	AAGGAAGG	CCTTCCTT
B6	P7118	CTTGACGA	P5131	GCACACAA	TTGTGTGC
C6	P7998	GCCACGAC	P5178	CCTCGGGT	ACCCGAGG
D6	P7099	TCTGGAAC	P5179	TAGCACCT	AGGTGCTA
E6	P7100	CACTAGAC	P5180	TGAGGACT	AGTCCTCA
F6	P7102	TTGCGTTA	P5183	TTCCCGAA	TTCGGGAA
G6	P7103	CCTATGCA	P5189	GAGTCGAT	ATCGACTC
H6	P7104	CAACCGAG	P5997	TACCTGTG	CACAGGTA
A7	P7106	TGTTCGCC	P5113	AGGTAGGA	TCCTACCT
B7	P7121	ACAAGGCA	P5130	TCGCGCAA	TTGCGCGA
C7	P7107	TCAGCGCC	P5133	ATGTTCCT	AGGAACAT
D7	P7108	AGTCACAT	P5141	CATTATGG	CCATAATG
E7	P7109	CCTTTCAC	P5142	TCTTGTTT	AAACAAGA
F7	P7111	CTTTCCCT	P5143	GGCTTACT	AGTAAGCC
G7	P7117	GACAATTC	P5146	ACGATATG	CATATCGT
H7	P7119	ACACGACT	P5152	ATCCGCAG	CTGCGGAT
A8	P7110	CCTGTCAA	P5112	ATGGCTGT	ACAGCCAT
B8	P7123	CCATCCGC	P5128	AAGGCGTA	TACGCCTT
C8	P7122	GGTGTGAG	P5153	AACACCAC	GTGGTGTT
D8	P7124	CTTGCATA	P5154	ACCTCTTC	GAAGAGGT
E8	P7125	GCCAATCC	P5155	GTCCGATC	GATCGGAC
F8	P7129	ATGCCGGT	P5157	GAGGACCA	TGGTCCTC
G8	P7137	CATACCGT	P5158	CGCTCTTA	TAAGAGCG
H8	P7138	ATCAGAGC	P5159	CTGAGCTC	GAGCTCAG
A9	P7112	ATGGCTGT	P5110	CCTGTCAA	TTGACAGG
B9	P7128	AAGGCGTA	P5123	CCATCCGC	GCGGATGG
C9	P7139	ACGAGGAG	P5160	CCTAAACT	AGTTTAGG
D9	P7140	TAATCTCG	P5162	TGTCACAC	GTGTGACA
E9	P7144	TACGGCAG	P5165	GATATGAA	TTCATATC
F9	P7145	TGCCCATC	P5166	AAGTGTGG	CCACACTT
G9	P7147	CAGCAGTC	P5174	GTTGGCGT	ACGCCAAC
H9	P7149	TACCGGCT	P5176	TAGCTGGC	GCCAGCTA
A10	P7113	AGGTAGGA	P5106	TGTTCGCC	GGCGAACA
B10	P7130	TCGCGCAA	P5121	ACAAGGCA	TGCCTTGT
C10	P7150	CTCGAAAT	P5177	CAGGTAAG	CTTACCTG
D10	P7151	CTCACAAC	P5181	AAGGAGAC	GTCTCCTT
E10	P7156	GTAACCAC	P5182	AGTCAGGT	ACCTGACT
F10	P7161	CATATCCA	P5184	ACCGTAAG	CTTACGGT
G10	P7163	CGCTAATC	P5185	TATGACGT	ACGTCATA
H10	P7164	CTTCCAAC	P5186	TTGGGTAC	GTACCCAA

WELL POSITION	EXPECTED i7 INDEX READ		EXPECTED i5 INDEX READ		
	i7 Index ID		i5 Index ID	Forward Strand Workflow*	Reverse Complement Workflow*
A11	P7114	AAGGAAGG	P5105	ACCGGAGT	ACTCCGGT
B11	P7131	GCACACAA	P5118	CTTGACGA	TCGTCAAG
C11	P7167	GTCAGTAT	P5187	AGAAGCCT	AGGCTTCT
D11	P7168	ATTCGAGC	P5188	CTAGGTTG	CAACCTAG
E11	P7171	CACCTGTA	P5190	TGTGTCAG	CTGACACA
F11	P7172	CCGACTCT	P5191	AGAACCAG	CTGGTTCT
G11	P7173	TTGCTGGA	P5192	ATTGGACA	TGTCCAAT
H11	P7175	CAGCTTCG	P5385	ACCCGTTG	CAACGGGT
A12	P7115	TCCCACGA	P5101	TTCAATAG	CTATTGAA
B12	P7132	ACCAACAG	P5116	GTTTGCTC	GAGCAAAC
C12	P7178	CCTCGGGT	P5998	GCCACGAC	GTCGTGGC
D12	P7179	TAGCACCT	P5099	TCTGGAAC	GTTCCAGA
E12	P7180	TGAGGACT	P5100	CACTAGAC	GTCTAGTG
F12	P7183	TTCCCGAA	P5102	TTGCGTTA	TAACGCAA
G12	P7189	GAGTCGAT	P5103	CCTATGCA	TGCATAGG
H12	P7997	TACCTGTG	P5104	CAACCGAG	CTCGGTTG

Table 3.3. Index Sequences for Set 3 (NEB #E7876).

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

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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

Table 3.4. Index Sequences for Set 4 (NEB #E7878).

	EXPECTED i7 INDEX READ		EXPECTED i5 INDEX READ		
WELL POSITION	i7 Index ID		i5 Index ID	Forward Strand Workflow*	Reverse Complement Workflow*
A1	7-297	ACTTCTGC	5-345	AGTCCCGG	CCGGGACT
B1	7-298	TTAAGCAG	5-346	TCCTGGAC	GTCCAGGA
C1	7-299	ATCAAATC	5-347	CTACATGA	TCATGTAG
D1	7-300	TTTGAGTC	5-348	CCGGATAG	CTATCCGG
E1	7-301	AAATCCTC	5-349	AACCCGCC	GGCGGGTT
F1	7-302	TACAGATG	5-350	CGAACGTG	CACGTTCG
G1	7-303	TAAGCGCA	5-351	CCGTAGAA	TTCTACGG
H1	7-304	CAACGGAA	5-352	CATCTACT	AGTAGATG
A2	7-305	AGCCTGGA	5-353	AGTCTGCT	AGCAGACT
B2	7-306	GAGGACAG	5-354	GCCGAATC	GATTCGGC
C2	7-307	CTATCGAA	5-355	ACTATGAT	ATCATAGT
D2	7-308	TCACTAAC	5-356	CCCTATCT	AGATAGGG
E2	7-309	TCGATAAG	5-357	CGTTGTCC	GGACAACG
F2	7-310	CTTTATTC	5-358	TGGAACGG	CCGTTCCA
G2	7-311	CTGCCTTC	5-359	CCCTTCGG	CCGAAGGG
H2	7-312	ACAACCAA	5-360	TGTCCAAA	TTTGGACA
A3	7-313	GCAATGGG	5-361	AGTACAAG	CTTGTACT
B3	7-314	CTGGACAC	5-362	TACTGTGA	TCACAGTA
C3	7-315	AAGTATGC	5-363	CCGGAATT	AATTCCGG
D3	7-316	TCCGATGG	5-364	TCGCTCGG	CCGAGCGA
E3	7-317	GACAACGG	5-365	AGTGCGGA	TCCGCACT
F3	7-318	TAGCTTTA	5-366	GCTTCACA	TGTGAAGC
G3	7-319	AAACAGTC	5-367	CCGATCGT	ACGATCGG
H3	7-320	ACCTCACT	5-368	CCGTAAGC	GCTTACGG
A4	7-321	GACATTAA	5-369	AGTTGGAT	ATCCAACT
B4	7-322	ATGTACGT	5-370	TAACACGC	GCGTGTTA
C4	7-323	ATGACAAA	5-371	AGACTCAC	GTGAGTCT
D4	7-324	CCACCTAC	5-372	CAGAGTGT	ACACTCTG
E4	7-325	TGCTGTTG	5-373	ACATTACG	CGTAATGT
F4	7-326	CATTTCAG	5-374	ATTACTAC	GTAGTAAT
G4	7-327	CGTCCCTA	5-375	TGGACCCT	AGGGTCCA
H4	7-328	TACGATTA	5-376	CGCTTGCA	TGCAAGCG
A5	7-329	AAAGATCG	5-377	GTTGCCTT	AAGGCAAC
B5	7-330	GTGAGCTA	5-378	CCAATGAA	TTCATTGG
C5	7-331	AACGCGGG	5-379	ATCAGTTG	CAACTGAT
D5	7-332	CTTGTGCT	5-380	ACATACAC	GTGTATGT
E5	7-333	GCTTACCC	5-381	CTCCATAT	ATATGGAG
F5	7-334	AGTAAACA	5-382	GCTTAAGT	ACTTAAGC
G5	7-335	CGATGTAA	5-383	ACACGTAT	ATACGTGT
H5	7-336	CCTAGTCG	5-384	CAAGAAGT	ACTTCTTG

WELL POSITION	**EXPECTED i7 INDEX READ**		**EXPECTED i5 INDEX READ**		
	i7 Index ID		**i5 Index ID**	**Forward Strand Workflow***	**Reverse Complement Workflow***
A6	7-337	AGTAGTAA	5-386	TATCAGTA	TACTGATA
B6	7-338	TACTAAGG	5-387	CGAGTCAG	CTGACTCG
C6	7-339	CATTCGGA	5-388	TTTCCATC	GATGGAAA
D6	7-340	AATCGTCA	5-389	CTTTAACT	AGTTAAAG
E6	7-341	GCTGATTT	5-390	GCCTCTAT	ATAGAGGC
F6	7-342	CGCGAAAG	5-391	CCTCCTTT	AAAGGAGG
G6	7-343	TTGCCACT	5-392	CAGTTCCC	GGGAACTG
H6	7-344	TTCGTGGA	5-393	TACCTTGT	ACAAGGTA
A7	7-345	AGTCCCGG	5-297	ACTTCTGC	GCAGAAGT
B7	7-346	TCCTGGAC	5-298	TTAAGCAG	CTGCTTAA
C7	7-347	CTACATGA	5-299	ATCAAATC	GATTTGAT
D7	7-348	CCGGATAG	5-300	TTTGAGTC	GACTCAAA
E7	7-349	AACCCGCC	5-301	AAATCCTC	GAGGATTT
F7	7-350	CGAACGTG	5-302	TACAGATG	CATCTGTA
G7	7-351	CCGTAGAA	5-303	TAAGCGCA	TGCGCTTA
H7	7-352	CATCTACT	5-304	CAACGGAA	TTCCGTTG
A8	7-353	AGTCTGCT	5-305	AGCCTGGA	TCCAGGCT
B8	7-354	GCCGAATC	5-306	GAGGACAG	CTGTCCTC
C8	7-355	ACTATGAT	5-307	CTATCGAA	TTCGATAG
D8	7-356	CCCTATCT	5-308	TCACTAAC	GTTAGTGA
E8	7-357	CGTTGTCC	5-309	TCGATAAG	CTTATCGA
F8	7-358	TGGAACGG	5-310	CTTTATTC	GAATAAAG
G8	7-359	CCCTTCGG	5-311	CTGCCTTC	GAAGGCAG
H8	7-360	TGTCCAAA	5-312	ACAACCAA	TTGGTTGT
A9	7-361	AGTACAAG	5-313	GCAATGGG	CCCATTGC
B9	7-362	TACTGTGA	5-314	CTGGACAC	GTGTCCAG
C9	7-363	CCGGAATT	5-315	AAGTATGC	GCATACTT
D9	7-364	TCGCTCGG	5-316	TCCGATGG	CCATCGGA
E9	7-365	AGTGCGGA	5-317	GACAACGG	CCGTTGTC
F9	7-366	GCTTCACA	5-318	TAGCTTTA	TAAAGCTA
G9	7-367	CCGATCGT	5-319	AAACAGTC	GACTGTTT
H9	7-368	CCGTAAGC	5-320	ACCTCACT	AGTGAGGT
A10	7-369	AGTTGGAT	5-321	GACATTAA	TTAATGTC
B10	7-370	TAACACGC	5-322	ATGTACGT	ACGTACAT
C10	7-371	AGACTCAC	5-323	ATGACAAA	TTTGTCAT
D10	7-372	CAGAGTGT	5-324	CCACCTAC	GTAGGTGG
E10	7-373	ACATTACG	5-325	TGCTGTTG	CAACAGCA
F10	7-374	ATTACTAC	5-326	CATTTCAG	CTGAAATG
G10	7-375	TGGACCCT	5-327	CGTCCCTA	TAGGGACG
H10	7-376	CGCTTGCA	5-328	TACGATTA	TAATCGTA

WELL POSITION	EXPECTED i7 INDEX READ		EXPECTED i5 INDEX READ		
	i7 Index ID		i5 Index ID	Forward Strand Workflow*	Reverse Complement Workflow*
A11	7-377	GTTGCCTT	5-329	AAAGATCG	CGATCTTT
B11	7-378	CCAATGAA	5-330	GTGAGCTA	TAGCTCAC
C11	7-379	ATCAGTTG	5-331	AACGCGGG	CCCGCGTT
D11	7-380	ACATACAC	5-332	CTTGTGCT	AGCACAAG
E11	7-381	CTCCATAT	5-333	GCTTACCC	GGGTAAGC
F11	7-382	GCTTAAGT	5-334	AGTAAACA	TGTTTACT
G11	7-383	ACACGTAT	5-335	CGATGTAA	TTACATCG
H11	7-384	CAAGAAGT	5-336	CCTAGTCG	CGACTAGG
A12	7-386	TATCAGTA	5-337	AGTAGTAA	TTACTACT
B12	7-387	CGAGTCAG	5-338	TACTAAGG	CCTTAGTA
C12	7-388	TTTCCATC	5-339	CATTCGGA	TCCGAATG
D12	7-389	CTTTAACT	5-340	AATCGTCA	TGACGATT
E12	7-390	GCCTCTAT	5-341	GCTGATTT	AAATCAGC
F12	7-391	CCTCCTTT	5-342	CGCGAAAG	CTTTCGCG
G12	7-392	CAGTTCCC	5-343	TTGCCACT	AGTGGCAA
H12	7-393	TACCTTGT	5-344	TTCGTGGA	TCCACGAA

Sequencing on the Illumina Platform

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

RUN SEGMENT	READ LENGTH	
	Without UMI	With UMI
Read 1	X defined by users	X defined by users
Index 1 (i7)	8 (without UMI)	20 (with UMI)
Index 2 (i5)	8	8
Read 2	X defined by user	X defined by user

Note: NEBNext Multiplex Oligos (Unique Dual Index UMI Adaptors DNA Sets 1 through 4) are available for pooling up to 384 samples on some Illumina sequencing instrument types. Each of the sets allows pooling of up to 96 samples and, when combined, they allow for a maximum of 384 samples to be pooled. The following sections give guidance pertaining to pooling fewer than eight samples.

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/E7395 – NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1) (NEB #E7395)

www.neb.com/E7874 – NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 2) (NEB #E7874)

www.neb.com/E7876 – NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 3) (NEB #E7876)

www.neb.com/E7878 – NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 4) (NEB #E7878)

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	5/23
2.0	Updated to add new section on setting up ligation reaction (Section 1). Also updates to index pooling guidelines and header/footer.	2/24