

NEBNext[®] Multiplex Oligos for Illumina[®] (96 Index Primers)

NEB #E6609S/L

96/384 reactions

Version 5.0_5/22

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The NEBNext Multiplex Oligos for Illumina (96 Index Primers) Includes

The volumes provided are sufficient for preparation of up to 96 reactions (NEB #E6609S) and 384 reactions (NEB #E6609L). All reagents should be stored at –20°C.

NEBNext Adaptor for Illumina

USER[®] Enzyme

NEBNext Index/Universal Primer Mix Plate

Each well contains the Universal PCR Primer plus one of the Index Primers

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

Applications

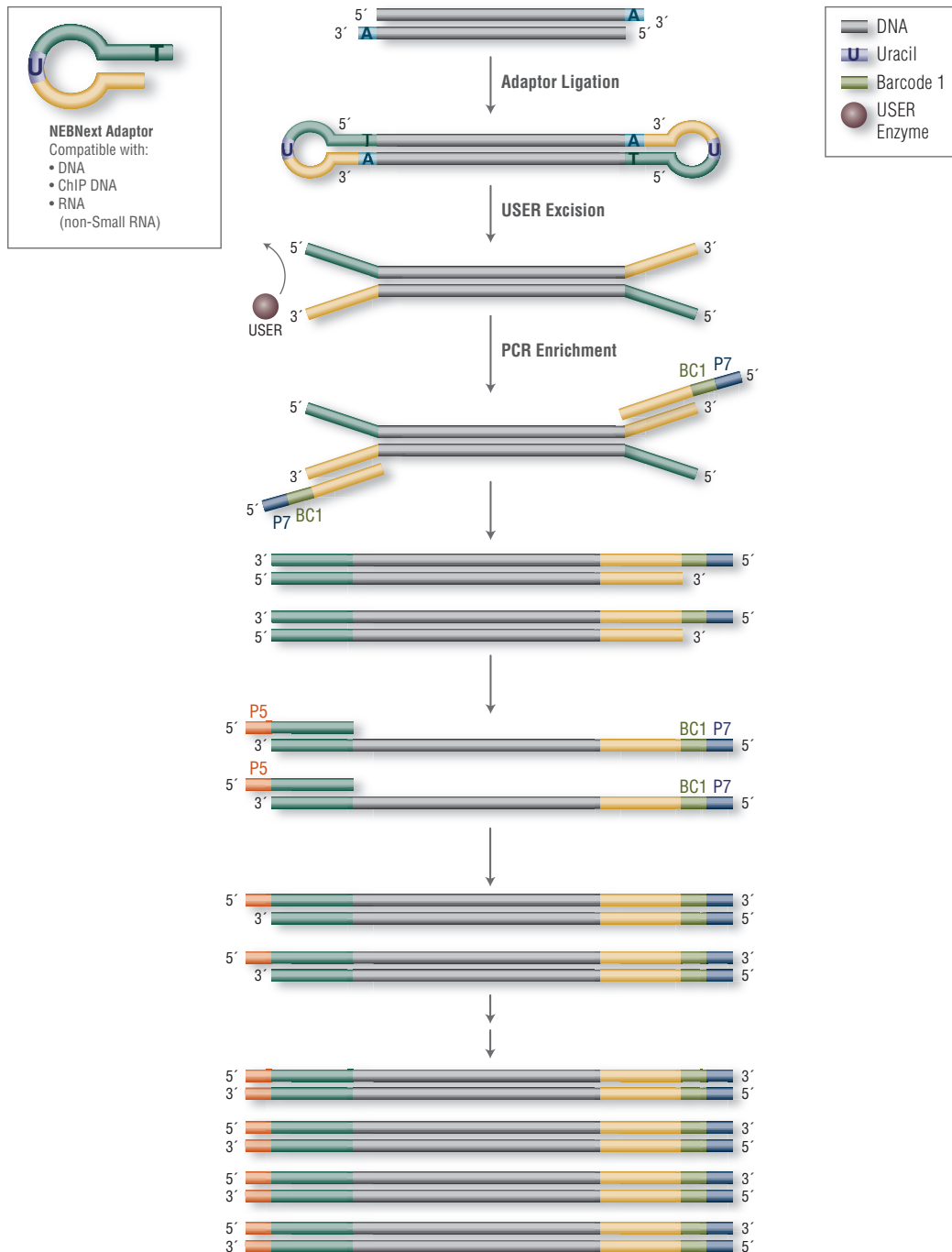
The NEBNext Multiplex Oligos for Illumina (96 Index Primers) contains adaptors and primers that are ideally suited for multiplex sample preparation for next-generation sequencing on the Illumina platform (Illumina, Inc.).

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 Overview

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 primers included in this kit are pre-mixed with the universal primer 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 Index Primers).



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

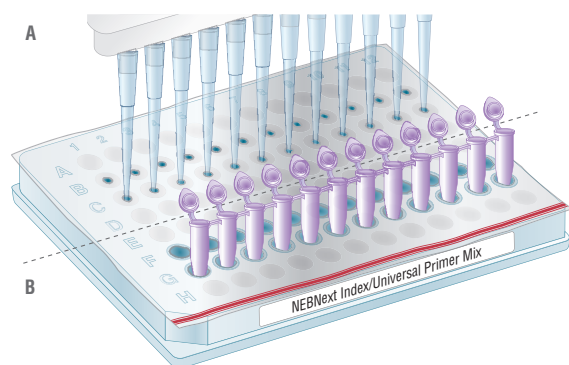


For < 96 samples, follow the protocol in Section 1.1A. For 96 samples, follow the protocol in Section 1.1B.

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 NEBNext Index/Universal Primer Mix plate for 10-15 minutes at room temperature.
- 1.1A.4. Remove the hard plastic plate cover. Briefly centrifuge the plate ($280 \times g$ for ~1 min) to collect all of the primer at the bottom of each well.
- 1.1A.5. Orient the NEBNext Index/Universal Primer Mix 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 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 the Universal Primer and the Index Primer. 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 Index/Universal Primer Mix Plate



1.1B Setting up the PCR reactions (96 samples)

- 1.1B.1. Thaw the NEBNext Index/Universal Primer Mix plate for 10-15 minutes at room temperature.
- 1.1B.2. Remove the hard plastic plate cover. Briefly centrifuge the plate ($280 \times g$ for ~1 min) to collect all of the primer at the bottom of each well.
- 1.1B.3. Orient the NEBNext Index/Universal Primer 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 the Universal Primer and the Index Primer. 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



For sample sheets please see NEB.com, E6609 Product Page, "Protocols, Manuals and Usage Guidelines" Tab, [Usage Guidelines](#)

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. Table 2.1 lists some valid combinations (up to 8-plex) that can be sequenced together. For combinations > 8 choose any column and add any plex combinations as needed.

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. Use Table 2.1 for some suggested combinations.

Table 2.1.

PLEX	WELL POSITION
2	P18-B6 and P22-B10 P37-D1 and P42-D6 P52-E4 and P76-G4 P68-F8 and P95-H11
3	P4-A4, P11-A11, and P12-A12 P15-B3, P22-B10, and P24-B12 P25-C1, P31-C7, and P33-C9 P37-D1, P42-D6, and P48-D12 P49-E1, P54-E6, and P55-E7 P64-F4, P69-F9, and P71-F11 P49-E1, P54-E6, and P55-E7 P64-F4, P69-F9, and P71-F11 P76-G4, P77-G5, and P83-G11 P87-H3, P93-H9, and P94-H10
4	P1-A1, P2-A2, P3-A3, and P4-A4 P5-A5, P6-A6, P8-A8, and P10-A10 P13-B1, P14-B2, P15-B3, and P16-B4 P17-B5, P18-B6, P19-B7, and P20-B8 P25-C1, P26-C2, P27-C3, and P30-C6 P28-C4, P29-C5, P32-C8, and P35-C11 P37-D1, P38-D2, P39-D3, and P40-D4 P45-D9, P46-D10, P47-D11, and P48-D12 P49-E1, P50-E2, P51-E3, and P52-E4 P56-E8, P58-E10, P59-E11, and P60-E12 P61-F1, P62-F2, P63-F3, and P69-F9 P64-F4, P65-F5, P66-F6, and P67-F7 P73-G1, P74-G2, P75-G3, and P76-G4 P80-G8, P82-G10, P83-G11, and P84-G12 P85-H1, P86-H2, P87-H3, and P89-H5 P91-H7, P94-H10, P95-H11, and P96-H12
5-7	Any 4 plex combination with any other primers
8	Any column

Table 2.2 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 examples below:

GOOD																	
PRIMER	INDEX SEQUENCE							PRIMER	INDEX SEQUENCE								
P1-A1	T	T	A	C	C	G	A	C	P41-D5	G	A	C	G	T	C	A	T
P2-A2	A	G	T	G	A	C	C	T	P42-D6	C	T	T	A	C	A	G	C
P3-A3	T	C	G	G	A	T	T	C	P43-D7	T	C	C	A	T	T	G	C
P4-A4	C	A	A	G	G	T	A	C	P44-D8	A	G	C	G	A	G	A	T
	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓

BAD																	
PRIMER	INDEX SEQUENCE							PRIMER	INDEX SEQUENCE								
P9-A9	C	G	C	A	A	C	T	A	P56-E8	T	A	T	G	G	C	A	C
P10-A10	C	G	T	A	T	C	T	C	P57-E9	C	T	C	G	A	A	C	A
P11-A11	G	T	A	C	A	C	C	T	P58-E10	C	A	A	C	T	C	C	A
P12-A12	C	G	G	C	A	T	T	A	P59-E11	G	T	C	A	T	C	G	T
	✓	X	✓	X	✓	✓	✓	✓		✓	✓	✓	✓	✓	X	✓	✓

Table 2.3 Index Sequences (Color coded based on HiSeq/MiSeq guidelines)

INDEX PRIMER	INDEX SEQUENCE READ							
P1-A1	T	T	A	C	C	G	A	C
P2-A2	A	G	T	G	A	C	C	T
P3-A3	T	C	G	G	A	T	T	C
P4-A4	C	A	A	G	G	T	A	C
P5-A5	T	C	C	T	C	A	T	G
P6-A6	G	T	C	A	G	T	C	A
P7-A7	C	G	A	A	T	A	C	G
P8-A8	T	C	T	A	G	G	A	G
P9-A9	C	G	C	A	A	C	T	A
P10-A10	C	G	T	A	T	C	T	C
P11-A11	G	T	A	C	A	C	C	T
P12-A12	C	G	G	C	A	T	T	A
P13-B1	T	C	G	T	C	T	G	A
P14-B2	A	G	C	C	T	A	T	C
P15-B3	C	T	G	T	A	C	C	A
P16-B4	A	G	A	C	C	T	T	G
P17-B5	A	G	G	A	T	A	G	C
P18-B6	C	C	T	T	C	C	A	T

INDEX PRIMER	INDEX SEQUENCE READ							
P19-B7	G	T	C	C	T	T	G	A
P20-B8	T	G	C	G	T	A	A	C
P21-B9	C	A	C	A	G	A	C	T
P22-B10	T	T	A	C	G	T	G	C
P23-B11	C	C	A	A	G	G	T	T
P24-B12	C	A	C	G	C	A	A	T
P25-C1	T	T	C	C	A	G	G	T
P26-C2	T	C	A	T	C	T	C	C
P27-C3	G	A	G	A	G	T	A	C
P28-C4	G	T	C	G	T	T	A	C
P29-C5	G	G	A	G	G	A	A	T
P30-C6	A	G	G	A	A	C	A	C
P31-C7	C	A	G	T	G	C	T	T
P32-C8	C	T	T	G	C	T	A	G
P33-C9	T	G	G	A	A	G	C	A
P34-C10	A	G	C	T	A	A	G	C
P35-C11	G	A	A	C	G	G	T	T
P36-C12	G	G	A	A	T	G	T	C
P37-D1	T	A	C	G	G	T	C	T
P38-D2	C	C	A	G	T	A	T	C
P39-D3	T	C	T	A	C	G	C	A
P40-D4	G	T	A	A	C	C	G	A
P41-D5	G	A	C	G	T	C	A	T
P42-D6	C	T	T	A	C	A	G	C
P43-D7	T	C	C	A	T	T	G	C
P44-D8	A	G	C	G	A	G	A	T
P45-D9	C	A	A	T	A	G	C	C
P46-D10	A	A	G	A	C	A	C	C
P47-D11	C	C	A	G	T	T	G	A
P48-D12	T	G	G	T	G	A	A	G

INDEX PRIMER	INDEX SEQUENCE READ							
P49-E1	A	A	G	A	C	C	G	T
P50-E2	T	T	G	C	G	A	G	A
P51-E3	G	C	A	A	T	T	C	C
P52-E4	G	A	A	T	C	C	G	T
P53-E5	C	C	G	C	T	T	A	A
P54-E6	T	A	C	C	T	G	C	A
P55-E7	G	T	C	G	A	T	T	G
P56-E8	T	A	T	G	G	C	A	C
P57-E9	C	T	C	G	A	A	C	A
P58-E10	C	A	A	C	T	C	C	A
P59-E11	G	T	C	A	T	C	G	T
P60-E12	G	G	A	C	A	T	C	A
P61-F1	C	A	G	G	T	T	C	A
P62-F2	G	A	A	C	G	A	A	G
P63-F3	C	T	C	A	G	A	A	G
P64-F4	C	A	T	G	A	G	C	A
P65-F5	G	A	C	G	A	A	C	T
P66-F6	A	G	A	C	G	C	T	A
P67-F7	A	T	A	A	C	G	C	C
P68-F8	G	A	A	T	C	A	C	C
P69-F9	G	G	C	A	A	G	T	T
P70-F10	G	A	T	C	T	T	G	C
P71-F11	C	A	A	T	G	C	G	A
P72-F12	G	G	T	G	T	A	C	A
P73-G1	T	A	G	G	A	G	C	T
P74-G2	C	G	A	A	T	T	G	C
P75-G3	G	T	C	C	T	A	A	G
P76-G4	C	T	T	A	G	G	A	C
P77-G5	T	C	C	A	C	G	T	T
P78-G6	C	A	A	C	A	C	A	G
P79-G7	G	C	C	T	T	A	A	C

INDEX PRIMER	INDEX SEQUENCE READ							
P80-G8	G	T	A	A	G	G	T	G
P81-G9	A	G	C	T	A	C	C	A
P82-G10	C	T	T	C	A	C	T	G
P83-G11	G	G	T	T	G	A	A	C
P84-G12	G	A	T	A	G	C	C	A
P85-H1	T	A	C	T	C	C	A	G
P86-H2	G	G	A	A	G	A	G	A
P87-H3	G	C	G	T	T	A	G	A
P88-H4	A	T	C	T	G	A	C	C
P89-H5	A	A	C	C	A	G	A	G
P90-H6	G	T	A	C	C	A	C	A
P91-H7	G	G	T	A	T	A	G	G
P92-H8	C	G	A	G	A	G	A	A
P93-H9	C	A	G	C	A	T	A	C
P94-H10	C	T	C	G	A	C	T	T
P95-H11	C	T	T	C	G	G	T	T
P96-H12	C	C	A	C	A	A	C	A

NEBNext Adaptors and Primers for Illumina

For sample sheets please see NEB.com, E6609 Product Page, "Protocols, Manuals and Usage Guidelines" Tab, [Usage Guidelines](#)

INDEX PRIMER	INDEX PRIMER SEQUENCE	EXPECTED INDEX SEQUENCE READ
P1-A1	5'-CAAGCAGAAGACGGCATAACGAGAT GTCCGGTAA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	TTACCGAC
P2-A2	5'-CAAGCAGAAGACGGCATAACGAGAT AGGTCAC GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	AGTGACCT
P3-A3	5'-CAAGCAGAAGACGGCATAACGAGAT GAATCCGA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	TCGGATTC
P4-A4	5'-CAAGCAGAAGACGGCATAACGAGAT GTACCTTGG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	CAAGGTAC
P5-A5	5'-CAAGCAGAAGACGGCATAACGAGAT CATGAGGA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	TCCTCATG
P6-A6	5'-CAAGCAGAAGACGGCATAACGAGAT TGACTGAC GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	GTCAGTCA
P7-A7	5'-CAAGCAGAAGACGGCATAACGAGAT CGTATTCGG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	CGAATACG
P8-A8	5'-CAAGCAGAAGACGGCATAACGAGAT CTCCTAGA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	TCTAGGAG
P9-A9	5'-CAAGCAGAAGACGGCATAACGAGAT TAGTTGCCG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	CGCAACTA
P10-A10	5'-CAAGCAGAAGACGGCATAACGAGAT GAGATACG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	CGTATCTC
P11-A11	5'-CAAGCAGAAGACGGCATAACGAGAT AGGTGTAC GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	GTACACCT
P12-A12	5'-CAAGCAGAAGACGGCATAACGAGAT TAATGCCG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	CGGCATTA
P13-B1	5'-CAAGCAGAAGACGGCATAACGAGAT TCAGACGA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	TCGTCTGA
P14-B2	5'-CAAGCAGAAGACGGCATAACGAGAT GATAGGCT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	AGCCTATC
P15-B3	5'-CAAGCAGAAGACGGCATAACGAGAT TGGTACAG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	CTGTACCA
P16-B4	5'-CAAGCAGAAGACGGCATAACGAGAT CAAGGTCT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	AGACCTTG
P17-B5	5'-CAAGCAGAAGACGGCATAACGAGAT GCTATCCT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	AGGATAGC
P18-B6	5'-CAAGCAGAAGACGGCATAACGAGAT ATGGAAGG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	CCTTCCAT
P19-B7	5'-CAAGCAGAAGACGGCATAACGAGAT TCAAGGAC GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	GTCTTGA
P20-B8	5'-CAAGCAGAAGACGGCATAACGAGAT GTTACGCA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	TGCGTAAC
P21-B9	5'-CAAGCAGAAGACGGCATAACGAGAT AGTCTGTG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	CACAGACT
P22-B10	5'-CAAGCAGAAGACGGCATAACGAGAT GCACGTAA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	TTACGTGC
P23-B11	5'-CAAGCAGAAGACGGCATAACGAGAT AACCTTGG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	CCAAGGTT
P24-B12	5'-CAAGCAGAAGACGGCATAACGAGAT ATTGCGTG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	CACGCAAT
P25-C1	5'-CAAGCAGAAGACGGCATAACGAGAT ACCTGGAA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	TTCCAGGT
P26-C2	5'-CAAGCAGAAGACGGCATAACGAGAT GGAGATGA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	TCATCTCC
P27-C3	5'-CAAGCAGAAGACGGCATAACGAGAT GTACTCTC GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	GAGAGTAC
P28-C4	5'-CAAGCAGAAGACGGCATAACGAGAT GTAACGAC GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	GTGTTAC
P29-C5	5'-CAAGCAGAAGACGGCATAACGAGAT ATTCTCCG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	GGAGGAAT
P30-C6	5'-CAAGCAGAAGACGGCATAACGAGAT GTGTTCTT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	AGGAACAC
P31-C7	5'-CAAGCAGAAGACGGCATAACGAGAT AAGCACTG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3'	CAGTGCTT

INDEX PRIMER	INDEX PRIMER SEQUENCE	EXPECTED INDEX SEQUENCE READ
P32-C8	5'-CAAGCAGAAGACGGCATAACGAGATCTAGCAAGGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CTTGCTAG
P33-C9	5'-CAAGCAGAAGACGGCATAACGAGATTGCTTCCAAGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	TGGAAGCA
P34-C10	5'-CAAGCAGAAGACGGCATAACGAGATGCTTAGCTGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	AGCTAAGC
P35-C11	5'-CAAGCAGAAGACGGCATAACGAGATAACCGTTCTGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GAACGGTT
P36-C12	5'-CAAGCAGAAGACGGCATAACGAGATGACATTCCGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GGAATGTC
P37-D1	5'-CAAGCAGAAGACGGCATAACGAGATAGACCGTAGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	TACGGTCT
P38-D2	5'-CAAGCAGAAGACGGCATAACGAGATGATACTGGGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CCAGTATC
P39-D3	5'-CAAGCAGAAGACGGCATAACGAGATTGCGTAGAGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	TCTACGCA
P40-D4	5'-CAAGCAGAAGACGGCATAACGAGATTGCGTTACGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GTAACCGA
P41-D5	5'-CAAGCAGAAGACGGCATAACGAGATATGACGCTCGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GACGTCAT
P42-D6	5'-CAAGCAGAAGACGGCATAACGAGATGCTGTAAGGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CTTACAGC
P43-D7	5'-CAAGCAGAAGACGGCATAACGAGATGCAATTGGAAGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	TCCATTGC
P44-D8	5'-CAAGCAGAAGACGGCATAACGAGATATCTCGCTGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	AGCGAGAT
P45-D9	5'-CAAGCAGAAGACGGCATAACGAGATGGCTATTGGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CAATAGCC
P46-D10	5'-CAAGCAGAAGACGGCATAACGAGATGGTGTCTTGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	AAGACACC
P47-D11	5'-CAAGCAGAAGACGGCATAACGAGATTCAACTGGGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CCAGTTGA
P48-D12	5'-CAAGCAGAAGACGGCATAACGAGATCTTCACCAAGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	TGGTGAAG
P49-E1	5'-CAAGCAGAAGACGGCATAACGAGATACGGTCTTGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	AAGACCGT
P50-E2	5'-CAAGCAGAAGACGGCATAACGAGATTCTCGCAAAGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	TTGCGAGA
P51-E3	5'-CAAGCAGAAGACGGCATAACGAGATGGAATTGCGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GCAATTCC
P52-E4	5'-CAAGCAGAAGACGGCATAACGAGATACGGATTCTGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GAATCCGT
P53-E5	5'-CAAGCAGAAGACGGCATAACGAGATTTAAGCGGGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CCGCTTAA
P54-E6	5'-CAAGCAGAAGACGGCATAACGAGATTGCAGGTAAGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	TACCTGCA
P55-E7	5'-CAAGCAGAAGACGGCATAACGAGATCAATCGACGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GTCGATTG
P56-E8	5'-CAAGCAGAAGACGGCATAACGAGATGTGCCATAAGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	TATGGCAC
P57-E9	5'-CAAGCAGAAGACGGCATAACGAGATTGTTTCGAGGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CTCGAACA
P58-E10	5'-CAAGCAGAAGACGGCATAACGAGATTGGAGTTGGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CAACTCCA
P59-E11	5'-CAAGCAGAAGACGGCATAACGAGATACGATGACGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GTCATCGT
P60-E12	5'-CAAGCAGAAGACGGCATAACGAGATTGATGTCCGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GGACATCA
P61-F1	5'-CAAGCAGAAGACGGCATAACGAGATTGAACCTGGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CAGGTTCA
P62-F2	5'-CAAGCAGAAGACGGCATAACGAGATCTTCGTTCTGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GAACGAAG
P63-F3	5'-CAAGCAGAAGACGGCATAACGAGATCTTCTGAGGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CTCAGAAG
P64-F4	5'-CAAGCAGAAGACGGCATAACGAGATTGCTCATGGTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CATGAGCA

INDEX PRIMER	INDEX PRIMER SEQUENCE	EXPECTED INDEX SEQUENCE READ
P65-F5	5'-CAAGCAGAAGACGGCATAACGAGAT AGTTGCTC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GACGAACT
P66-F6	5'-CAAGCAGAAGACGGCATAACGAGAT TAGCGTCT GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	AGACGCTA
P67-F7	5'-CAAGCAGAAGACGGCATAACGAGAT GGCGTTAT GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	ATAACGCC
P68-F8	5'-CAAGCAGAAGACGGCATAACGAGAT GGTGATTC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GAATCACC
P69-F9	5'-CAAGCAGAAGACGGCATAACGAGAT AACTTGCC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GGCAAGTT
P70-F10	5'-CAAGCAGAAGACGGCATAACGAGAT GCAAGATC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GATCTTGC
P71-F11	5'-CAAGCAGAAGACGGCATAACGAGAT TGCGATTG GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CAATGCCA
P72-F12	5'-CAAGCAGAAGACGGCATAACGAGAT TGTACACC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GGTGATCA
P73-G1	5'-CAAGCAGAAGACGGCATAACGAGAT AGCTCCTA GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	TAGGAGCT
P74-G2	5'-CAAGCAGAAGACGGCATAACGAGAT GCAATTCC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CGAATTGC
P75-G3	5'-CAAGCAGAAGACGGCATAACGAGAT CTTAGGAC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GTCCTAAG
P76-G4	5'-CAAGCAGAAGACGGCATAACGAGAT GTCCTAAG GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CTTAGGAC
P77-G5	5'-CAAGCAGAAGACGGCATAACGAGAT AACGTGGA GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	TCCACGTT
P78-G6	5'-CAAGCAGAAGACGGCATAACGAGAT CTGTGTTG GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CAACACAG
P79-G7	5'-CAAGCAGAAGACGGCATAACGAGAT GTTAAGGC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GCCTAAC
P80-G8	5'-CAAGCAGAAGACGGCATAACGAGAT CACCTTAC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GTAAGGTG
P81-G9	5'-CAAGCAGAAGACGGCATAACGAGAT TGGTAGCT GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	AGCTACCA
P82-G10	5'-CAAGCAGAAGACGGCATAACGAGAT CAGTGAAG GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CTTCACTG
P83-G11	5'-CAAGCAGAAGACGGCATAACGAGAT GTTCAACC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GGTTGAAC
P84-G12	5'-CAAGCAGAAGACGGCATAACGAGAT TGGCTATC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GATAGCCA
P85-H1	5'-CAAGCAGAAGACGGCATAACGAGAT CTGGAGTA GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	TACTCCAG
P86-H2	5'-CAAGCAGAAGACGGCATAACGAGAT TCTCTTCC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GGAAGAGA
P87-H3	5'-CAAGCAGAAGACGGCATAACGAGAT TCTAACGC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GCGTTAGA
P88-H4	5'-CAAGCAGAAGACGGCATAACGAGAT GGTCAGAT GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	ATCTGACC
P89-H5	5'-CAAGCAGAAGACGGCATAACGAGAT CTCTGGTT GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	AACCAGAG
P90-H6	5'-CAAGCAGAAGACGGCATAACGAGAT TGTGGTAC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GTACCACA
P91-H7	5'-CAAGCAGAAGACGGCATAACGAGAT CCTATACC GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	GGTATAGG
P92-H8	5'-CAAGCAGAAGACGGCATAACGAGAT TCTCTCGG GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CGAGAGAA
P93-H9	5'-CAAGCAGAAGACGGCATAACGAGAT GTATGCTG GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CAGCATA
P94-H10	5'-CAAGCAGAAGACGGCATAACGAGAT AAGTCGAG GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CTCGACTT
P95-H11	5'-CAAGCAGAAGACGGCATAACGAGAT AACCGAAG GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CTTCGGTT
P96-H12	5'-CAAGCAGAAGACGGCATAACGAGAT TGTTGTGG GTGACTGGAGTTCAGACGTGTGCTCTCCGATC*T-3'	CCACAACA

INDEX PRIMER	INDEX PRIMER SEQUENCE	EXPECTED INDEX SEQUENCE READ
NEBNext Adaptor for Illumina	5'-/5Phos/GAT CGG AAG AGC ACA CGT CTG AAC TCC AGT CdUA CAC TCT TTC CCT ACA CGA CGC TCT TCC GAT C*T-3'	N/A
NEBNext Universal PCR Primer for Illumina	5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC*T-3'	N/A

Where -* indicates phosphorothioate bond.

Kit Components

NEB #E6609S Table of Components

NEB #	CONCENTRATION	PRODUCT	VOLUME
E6612A	15 µM	NEBNext Adaptor for Illumina	0.96 ml
E6610A		USER Enzyme	0.288 ml
E6611A	5 µM each	NEBNext Index/Universal Primer Mix	1 plate (10 µl/well)

NEB #E6609L Table of Components

NEB #	CONCENTRATION	PRODUCT	VOLUME
E6612AA	15 µM	NEBNext Adaptor for Illumina	4 x 0.96 ml
E6610AA		USER Enzyme	2 x 0.576 ml
E6611AA	5 µM each	NEBNext Index/Universal Primer Mix	4 plates (10 µl/well)

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	3/16
1.1	Corrected note on page 6 and 7 from "Do not use reuse primer if the seal has been previously pierced to avoid contamination with other indexed primers." to "Do not reuse primer if the seal has been previously pierced to avoid contamination with other indexed primers."	4/16
1.2	Updated Figure 1.1 page 7.	5/16
1.3	Clarified primer concentration. Corrected primer sequences 5' end.	6/16
2.0	Delete "Required Materials Not Included" and replace with guidance note. Change Index Primer sequences table to include adaptor, and NEBNext Universal Primer and rename it as NEBNext Adaptors and Primers for Illumina. Update the list of kits using the NEBNext Multiplex Oligos for Illumina. Delete Materials Create "Kit Component – Table of Components" for small and large size kits. Delete individual component information pages.	4/18
3.0	Edited Applications text. Added a description for Table 2.1. Corrected NEBNext Adaptors and Primers Table, changed -s- to a *. Added concentration to Table of Components.	8/18
4.0	Update to new manual format	1/20
5.0	Updated Adaptors and Primers for Illumina	5/22

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