# **INSTRUCTION MANUAL**



# NEBNext® Multiplex Oligos for Illumina® (Unique Dual Index UMI Adaptors RNA Set 1)

NEB #E7416S/L

96/384 reactions Version 4.0 2/24

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# The NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors RNA Set 1) Includes

The volumes provided are sufficient for preparation of up to 96 reactions (NEB #E7416S) and 384 reactions (NEB #E7416L). All reagents should be stored at  $-20^{\circ}$ C.\*

- NEBNext Primer Mix
- NEBNext UMI Adaptor Dilution Buffer
- NEBNext UMI RNA Adaptor Plate
  - Each well contains a unique dual index UMI adaptor (S size contains 1 plate, L size contains 4 plates)

#### 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 -20°C right away and centrifuge the plate prior to the first use to avoid unnecessary freeze/ thaw cycles.

#### **Overview**

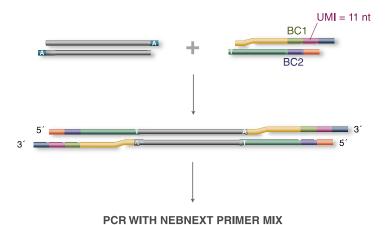
The NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors RNA Set 1) contains adaptors and primers that are ideally suited for multiplex sample preparation for next-generation sequencing on the Illumina platform (Illumina, Inc.). Each kit component must pass rigorous quality control standards, and for each new lot the entire set of reagents is functionally validated together by construction and sequencing of indexed libraries on an Illumina sequencing platform.

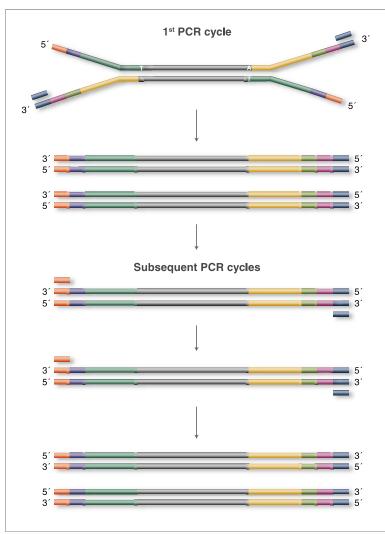
Where larger volumes, customized or bulk packaging are required, we encourage consultation with the Customized Solutions team at NEB. Please complete the NEB Custom Contact Form at <a href="www.neb.com/CustomContactForm">www.neb.com/CustomContactForm</a> to learn more.

#### Workflow

Designed for use in library prep for cDNA and RNA (but not Small RNA), the NEBNext Unique Dual Index UMI Adaptors enable high-efficiency adaptor ligation and high library yields. These adaptors contain all necessary sequences for sequencing on the Illumina platform and sample pooling prior to PCR amplification. The incorporation of a 12-base unique molecular identifier (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 analysis. 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 products, and with other standard Illumina-compatible library preparation protocols that are based on TA single base overhang ligation.

Figure 1. Workflow demonstrating the use of NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors RNA Set 1).





# Library Preparation Kits for use with NEBNext Unique Dual Index UMI Adaptors RNA

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

# 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.
- 1.1A.3. Thaw the NEBNext UMI RNA Adaptor Plate for 10-15 minutes on ice.
- 1.1A.4. Remove the hard plastic plate cover. If necessary centrifuge the plate  $(280 \times g \text{ for } \sim 1 \text{ min})$  to collect all of the adaptor at the bottom of each well.
- 1.1A.5. Orient the NEBNext UMI RNA 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 adaptor s. 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.

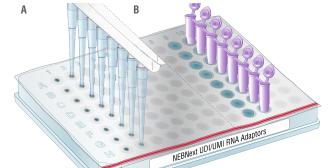


Figure 1.1. NEBNext UMI RNA Adaptor Plate

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

- 1.1B.1. Thaw the NEBNext UMI RNA Adaptor Plate for 10-15 minutes on ice.
- 1.1B.2. Remove the hard plastic plate cover. If necessary, centrifuge the plate  $(280 \times g \text{ for } \sim 1 \text{ min})$  to collect all of the adaptor at the bottom of each well.
- 1.1B.3. Orient the NEBNext UMI RNA 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: 96 Reaction Kit**



#### 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 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 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 <a href="mailto:info@neb.com">info@neb.com</a>.

Low Plex pooling options shown here are only for Illumina four channel chemistry.

**Table 2.1. Index Pooling Guidelines** 

PLEX	WELL POSITION
< 4	Not recommended
	A6, B6, C6, D6
	A12, B12, C12, D12
	B6, C6, D6, E6
4	B12, C12, D12, E12
4	C1, D1, E1, F1
	C7, D7, E7, F7
	E4, F4, G4, H4
	E10, F10, G10, H10
	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
5	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

# **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<sup>®</sup>, HiSeq<sup>®</sup> 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 <u>Guidelines for reverse complementing i5 sequences for demultiplexing - Illumina Knowledge</u>.

# Good and Bad Examples for Pooling and Color Balancing

Table 2.2. 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:

											BAl	D												
WELL	]	EXPI	ECTI	ED i7	IND	EX F	REAI	)	EXPECTED i5 INDEX READ															
POSITION										F		VARI ORK			D			REV		SE CO			ENT	
E8	Т	Α	Т	G	G	С	Α	С	Т	Т	G	С	G	Α	G	Α	Т	С	Т	С	G	С	Α	Α
F8	G	Α	Α	Т	C	Α	C	С	G	Α	Α	C	G	A	A	G	С	Т	Т	C	G	Т	Т	C
G8	G	Т	A	A	G	G	Т	G	С	G	A	Α	Т	Т	G	C	G	C	A	Α	Т	Т	C	G
Н8	С	G	Α	G	A	G	Α	Α	G	G	Α	A	G	A	G	Α	Т	C	Т	C	Т	Т	C	C
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	✓	✓	✓	✓	✓	✓	X	X	✓	✓	✓
A1	Т	Т	Α	С	С	G	Α	С	С	G	Α	Α	Т	Α	С	G	C	G	Т	Α	Т	Т	G	G
B1	Т	C	G	Т	C	Т	G	Α	G	Т	С	C	Т	Т	G	Α	Т	C	A	Α	G	G	Α	С
C1	Т	Т	С	С	Α	G	G	Т	С	Α	G	Т	G	C	Т	Т	Α	A	G	C	Α	C	Т	G
D1	Т	Α	C	G	G	Т	С	Т	Т	C	C	Α	Т	Т	G	C	G	C	Α	Α	Т	G	G	A
	x	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓

											GOC	D												
WELL	I	EXPI	ECTI	ED i7	IND	EX R	EAL	)	EXPECTED i5 INDEX READ															
POSITION										F	-	VAR ORK			D			REV			OMP XFLO		ENT	
C1	Т	Т	С	С	Α	G	G	Т	С	Α	G	Т	G	С	Т	Т	Α	Α	G	С	Α	С	G	G
D1	Т	A	C	G	G	Т	C	Т	Т	C	C	A	Т	Т	G	C	G	С	A	A	Т	G	G	Α
E1	A	A	G	A	С	C	G	Т	G	Т	C	G	A	Т	Т	G	С	A	A	Т	С	G	A	С
F1	С	Α	G	G	Т	Т	С	Α	Α	Т	Α	A	C	G	C	C	G	G	C	G	Т	Т	Α	Т
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	1																							
A12	С	G	G	С	Α	Т	Т	Α	G	Т	С	Α	G	Т	С	Α	Т	G	Α	С	Т	G	С	С
B12	С	A	С	G	С	A	A	Т	С	C	Т	Т	С	C	A	Т	Α	Т	G	G	Α	A	G	G
C12	G	G	A	A	Т	G	Т	C	A	G	G	A	A	C	A	С	G	Т	G	Т	Т	С	С	Т
D12	Т	G	G	Т	G	A	A	G	С	Т	Т	Α	C	A	G	C	G	С	Т	G	Т	A	A	G
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

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.

											GOO	OD												
WELL	1	EXPI	ECTE	E <b>D i7</b>	IND	EX R	REAL	)					]	EXP	ECTI	ED i5	IND	EX R	REAI	)				
POSITION										F		VAR ORK	_ ~ _		D			REV		SE CO		LEM W	ENT	
A12	С	G	G	С	Α	Т	Т	Α	G	Т	С	Α	G	Т	С	Α	Т	G	Α	С	Т	G	С	С
B12	С	A	C	G	С	A	Α	Т	С	C	Т	Т	С	C	Α	Т	Α	Т	G	G	A	A	G	G
C12	G	G	Α	A	Т	G	Т	С	Α	G	G	Α	A	C	Α	С	G	Т	G	Т	Т	С	С	Т
D12	Т	G	G	Т	G	A	A	G	С	Т	Т	A	С	A	G	C	G	С	Т	G	Т	A	A	G
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

											BA	D												
WELL	I	EXPE	ECTI	ED i7	IND	EX R	EAI	)					]	EXPI	ECTI	ED i5	IND	EX F	REAL	)				
POSITION										F		VARI ORK			D			REV		E CO			ENT	
C12	G	G	Α	Α	Т	G	Т	С	Α	G	G	Α	Α	С	Α	С	G	Т	G	Т	Т	С	С	Т
E12	G	G	A	C	A	Т	С	Α	Т	A	С	C	Т	G	С	Α	Т	G	С	Α	G	G	Т	A
F12	G	G	Т	G	Т	A	С	A	Α	G	A	С	G	С	Т	A	Т	A	G	С	G	Т	C	Т
G11	G	G	Т	Т	G	A	A	С	Т	С	С	A	С	G	Т	Т	Α	A	С	G	Т	G	G	Α
	X	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓

# **Section 3 Index Sequences**

**Table 3.1 Index Sequences** 

WELL	EXPECTED	i7 INDEX READ		EXPECTED i5 INDEX	K READ
POSITION	i7 INDEX ID		i5 INDEX ID	FORWARD STRAND WORKFLOW	REVERSE COMPLEMENT WORKFLOW
A1	S 7 6 2	TTACCGAC	S 5 1 2	CGAATACG	CGTATTCG
B1	S 7 1 3	TCGTCTGA	S 5 8 6	GTCCTTGA	TCAAGGAC
C1	S 7 3 6	TTCCAGGT	S 5 4 3	CAGTGCTT	AAGCACTG
D1	S 7 0 9	TACGGTCT	S 5 7 5	TCCATTGC	GCAATGGA
E1	S 7 3 2	AAGACCGT	S 5 5 0	GTCGATTG	CAATCGAC
F1	S 7 7 4	CAGGTTCA	S 5 0 6	ATAACGCC	GGCGTTAT
G1	S 7 4 7	TAGGAGCT	S 5 2 4	GCCTTAAC	GTTAAGGC
H1	S 7 9 4	TACTCCAG	S 5 9 0	GGTATAGG	CCTATACC
A2	S 7 2 9	AGTGACCT	S 5 9 1	TCTAGGAG	CTCCTAGA
B2	S 7 7 7	AGCCTATC	S 5 2 6	TGCGTAAC	GTTACGCA
C2	S 7 7 2	TCATCTCC	S 5 6 7	CTTGCTAG	CTAGCAAG
D2	S 7 2 5	CCAGTATC	S 5 3 8	AGCGAGAT	ATCTCGCT
E2	S 7 5 5	TTGCGAGA	S 5 6 6	TATGGCAC	GTGCCATA
F2	S 7 6 0	GAACGAAG	S 5 1 1	GAATCACC	GGTGATTC
G2	S 7 1 6	CGAATTGC	S 5 5 9	GTAAGGTG	CACCTTAC
H2	S 7 0 8	GGAAGAGA	S 5 2 1	CGAGAGAA	TTCTCTCG
A3	S 7 0 2	TCGGATTC	S 5 2 3	CGCAACTA	TAGTTGCG
В3	S 7 9 6	CTGTACCA	S 5 0 7	CACAGACT	AGTCTGTG
C3	S 7 5 7	GAGAGTAC	S 5 4 5	TGGAAGCA	TGCTTCCA
D3	S 7 8 3	TCTACGCA	S 5 4 6	CAATAGCC	GGCTATTG
E3	S 7 2 2	GCAATTCC	S 5 7 8	CTCGAACA	TGTTCGAG
F3	S 7 1 0	CTCAGAAG	S 5 8 1	GGCAAGTT	AACTTGCC
G3	S 7 7 0	GTCCTAAG	S 5 4 0	AGCTACCA	TGGTAGCT
Н3	S 7 3 4	GCGTTAGA	S 5 9 2	CAGCATAC	GTATGCTG
A4	S 7 6 3	CAAGGTAC	S 5 0 5	CGTATCTC	GAGATACG
B4	S 7 9 7	AGACCTTG	S 5 0 1	TTACGTGC	GCACGTAA
C4	S 7 3 5	GTCGTTAC	S 5 5 4	AGCTAAGC	GCTTAGCT
D4	S 7 2 7	GTAACCGA	S 5 9 8	AAGACACC	GGTGTCTT
E4	S 7 4 2	GAATCCGT	S 5 5 1	CAACTCCA	TGGAGTTG
F4	S 7 9 5	CATGAGCA	S 5 1 7	GATCTTGC	GCAAGATC
G4	S 7 4 9	CTTAGGAC	S 5 6 5	CTTCACTG	CAGTGAAG
H4	S 7 7 3	ATCTGACC	S 5 9 3	CTCGACTT	AAGTCGAG
A5	S769	TCCTCATG	S 5 1 9	GTACACCT	AGGTGTAC
B5	S 7 5 2	AGGATAGC	S 5 4 4	CCAAGGTT	AACCTTGG
C5	S 7 0 4	GGAGGAAT	S 5 8 5	GAACGGTT	AACCGTTC
D5	S 7 1 5	GACGTCAT	S 5 1 8	CCAGTTGA	TCAACTGG
E5	S 7 5 3	CCGCTTAA	S 5 4 8	GTCATCGT	ACGATGAC
F5	S 7 5 8	GACGAACT	S 5 6 8	CAATGCGA	TCGCATTG
G5	S 7 8 4	TCCACGTT	S 5 4 1	GGTTGAAC	GTTCAACC
Н5	S 7 1 4	AACCAGAG	S 5 2 0	CTTCGGTT	AACCGAAG

POSITION	WELL	EXPECTED	o i7 INDEX READ		EXPECTED i5 INDEX	READ
B6         8779         CCTTCCAT         \$589         CACGCAAT         ATTGCGTG           C6         \$788         AGGAACAC         \$587         GGAATGTC         GACATTCC           D6         \$739         CTTACAGC         \$576         GGACATCA         TGATGTCC           E6         \$728         AGACGCTA         \$576         GGACATCA         TGATGTCC           P6         \$728         AGACGCTA         \$582         GGTGTACA         TGATGTCC           G6         \$780         CAACACAG         \$530         GATAGCCA         TGGCTATC           H6         \$761         GTACCACA         \$533         CCACAACA         TGTTGTGG           A7         \$712         CGAATACG         \$553         TTACCGAC         TGTGGTAA           B7         \$786         GTCCTTGA         \$531         TCGTCTGA         TCAGACGA           C7         \$743         CAGTGCTT         \$536         TTCCAGGT         ACCTGGAA           C7         \$743         CAGTGCTT         \$536         TTCCAGGT         ACCGTCTT           E7         \$750         GTCGATTG         \$539         TACGGTCT         ACGGTCTT           F7         \$760         ATAACGCC         \$574         C	POSITION				1	
C6         S788         AGGAACAC         S587         GGAATGTC         GACATTCC           D6         8739         CTTACAGC         S503         TGGTGAAG         CTTCACCA           E6         8737         TACCTGCA         S576         GGACATCA         TGATGTCC           F6         8788         AGACGCTA         S582         GGTGTACA         TGGCTATC           H6         8761         GTACCACA         S533         CCACAACA         TGGTTGG           H6         8761         GTACCACA         S533         CCACAACA         TGTTGTGG           H7         8712         CGAATACG         S562         TTACCGAC         GTCGGTAA           B7         8786         GTCCTTGA         S513         TCCAGGT         ACCTGGAA           D7         8775         TCCATTGC         S509         TACGGTCT         AGACCGTA           E7         8750         GTCGATTG         S532         AAGACCGT         AGCTCTT           F7         8706         ATAACGCC         S574         CAGGTCA         TGCTCTA           H7         8790         GGTATAGG         S594         TACTCCAG         CTGGAGTA           A8         8791         TCTAGGAG         S529         AGTGAC	A6	S 7 7 1	GTCAGTCA	S 5 3 1	CGGCATTA	TAATGCCG
D6         S739         CTTACAGC         S503         TGGTGAAG         CTTCACCA           E6         S337         TACCTGCA         S576         GGACATCA         TGATGTCC           F6         S728         AGACGCTA         S582         GGTTACA         TGTACCACC           G6         S780         CAACACAG         S530         GATAGCA         TGGTATC           H6         S761         GTACCACA         S533         CCACAACA         TGTTGTGG           A7         S712         CGAATACG         S562         TTACCGAC         GTCGGTAA           B7         S786         GTCCTTGA         S513         TCCTTGA         TCAGACGA           C7         S743         CAGTGCTT         S536         TTCCAGGT         ACCTGGAA           D7         S775         TCCATTGC         S509         TACGGTT         ACCGGTAA           E7         S750         GTCGATTG         S532         AAGACCGT         ACGGTCTT           F7         S706         ATAACGCC         S574         CAGGTTCA         TGAACCTG           G7         S724         GCCTTAAC         S547         TAGCGACT         AGCTCCTA           B8         S726         TGCGTAAC         S577         AGCCT	В6	S779	CCTTCCAT	S 5 8 9	CACGCAAT	ATTGCGTG
E6         8737         TACCTGCA         8576         GGACATCA         TGATGTCC           F6         8728         AGACGCTA         8582         GGTGTACA         TGTACACC           G6         8780         CAACACAG         8530         GATAGCCA         TGGCTATC           H6         8761         GTACCACA         8533         CCACAACA         TGTGTGG           A7         8712         CGAATACG         8562         TTACCGAC         GTCGGTAA           B7         8786         GTCCTTGA         8513         TCCAGGT         ACCTGGAA           C7         8743         CAGTGCTT         8536         TTCCAGGT         ACCTGGAA           D7         8775         TCCATTGC         8559         TACGGTCT         ACGGTCTT           E7         8750         GTCGATTG         8532         AAGACCGT         ACGGTCTT           F7         8706         ATAACGCC         8574         CAGGTCTA         TGAACCTG           G7         8724         GCCTTAAC         8547         TAGGACT         AGCTCCTA           B8         8791         TCTAGGAG         8529         AGTACCT         AGGTCACT           C8         8767         CTTGCTAG         8577         AGCCT	C6	S 7 8 8	AGGAACAC	S 5 8 7	GGAATGTC	GACATTCC
F6	D6	S739	CTTACAGC	S 5 0 3	TGGTGAAG	CTTCACCA
G6         S780         CAACACAG         S530         GATAGCCA         TGGCTATC           H6         S761         GTACCACA         S533         CCACAACA         TGTTGTGG           A7         S712         CGAATACG         S562         TTACCGAC         GTCGTAA           B7         S786         GTCCTTGA         S513         TCGTCTGA         TCAGACGA           C7         S743         CAGTGCTT         S536         TTCCAGGT         ACCTGGAA           D7         S775         TCCATTGC         S509         TACGGTCT         AGACCGTA           E7         S750         GTCGATTG         S532         AAGACCGT         ACGGTCTT           E7         S706         ATAACGCC         S574         CAGGTTCA         TGAACCTG           G7         S724         GCCTTAAC         S547         TAGGAGCT         AGCTCCTA           H7         S790         GGTATAGG         S594         TACTCCAG         CTGGAGTA           A8         S791         TCTAGGAG         S529         AGTGACCT         AGCTCACT           B8         S726         TGCGTAAC         S577         AGCCTATC         GATAGGCT           C8         S767         CTTGCTAG         S572         TC	E6	S 7 3 7	TACCTGCA	S 5 7 6	GGACATCA	TGATGTCC
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         AGCCTACT         GATAGGCT           C8         S767         CTTGGTAG         S572         TCATCTCC         GATACTGG           C8         S761         TATGGCAC         S555         T	F6	S 7 2 8	AGACGCTA	S 5 8 2	GGTGTACA	TGTACACC
A7         8712         CGAATACG         8562         TTACCGAC         GTCGGTAA           B7         8786         GTCCTTGA         8513         TCGTCTGA         TCAGACGA           C7         8743         CAGTGCTT         8536         TTCCAGGT         ACCTGGAA           D7         8775         TCCATTGC         8509         TACGGTCT         AGACCGTA           E7         8750         GTCGATTG         8532         AAGACCGT         ACGGTCTT           F7         8706         ATAACGCC         8574         CAGGTTCA         TGAACCTGA           G7         8724         GCCTTAAC         8547         TAGGAGCT         AGCTCCTA           H7         8790         GGTATAGG         8594         TACTCCAG         CTGGAGTA           A8         8791         TCTAGGAG         8529         AGTGACCT         AGGTCACT           B8         8726         TCTGCTAG         8577         AGCCTATC         GATAGGCT           C8         8767         CTTGCTAG         8572         TCATCTCC         GGAGATGA           B8         8766         TATGGCAC         8555         TTGCGAGA         TCTCGCAA           F8         8711         GAATCACC         8560	G6	S 7 8 0	CAACACAG	S 5 3 0	GATAGCCA	TGGCTATC
B7         S786         GTCCTTGA         S513         TCGTCTGA         TCAGAGGA           C7         S743         CAGTGCTT         S536         TTCCAGGT         ACCTGGAA           D7         S775         TCCATTGC         S509         TACGGTCT         AGACCGTA           E7         S750         GTCGATTG         S532         AAGACCGT         ACGGTCTTA           F7         S706         ATAACGCC         S574         CAGGTTCA         TGAACCTG           G7         S724         GCCTTAAC         S547         TAGGAGCT         AGCTCCTA           H7         S790         GGTATAGG         S594         TACTCCAG         CTGGAGTA           A8         S791         TCTAGGAG         S594         TACTCCAG         CTGGAGTA           A8         S791         TCTAGGAG         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	Н6	S 7 6 1	GTACCACA	S 5 3 3	CCACAACA	TGTTGTGG
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         TTGCGAA         TCTCGCAA           F8         S711         GAATCACC         S560         GAACGAAG         CTTCTTC           G8         S759         GTAACGAG         CTCTTCTC	A7	S 7 1 2	CGAATACG	S 5 6 2	TTACCGAC	GTCGGTAA
D7         8775         TCCATTGC         8509         TACGGTCT         AGACCGTA           E7         8750         GTCGATTG         8532         AAGACCGT         ACGGTCT           F7         8706         ATAACGCC         8574         CAGGTTCA         TGAACCTG           G7         8724         GCCTTAAC         8547         TAGGAGCT         AGCTCCTA           H7         8790         GGTATAGG         8594         TACTCCAG         CTGGAGTA           A8         8791         TCTAGGAG         8529         AGTGACCT         AGGTCACT           B8         8726         TGCGTAAC         8577         AGCCTATC         GATAGGCT           C8         8767         CTTGCTAG         8577         AGCCTATC         GGAAGGCT           C8         8766         TATGGCAC         8557         TCACTCC         GGAAGTGA           E8         8766         TATGGCAC         8555         TTGCGAGA         TCTCGCAA           F8         8711         GAATCACC         8560         GAACGAAG         CTTCGTTC           G8         8759         GTAAGGTG         8516         CGAATTGC         GCAATTCG           H8         8721         CGAGAGAA         8502         TCG	В7	S 7 8 6	GTCCTTGA	S 5 1 3	TCGTCTGA	TCAGACGA
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         GAACGAGA         CTTCGTTC           G8         S759         GTAAGGTG         S516         CGAATTGC         GCAATTCG           H8         S721         CGAGAGAA         S508         GGAAGGA         TCTCTTCC           A9         S723         CGCAACTA         S506         CT	C7	S 7 4 3	CAGTGCTT	S 5 3 6	TTCCAGGT	ACCTGGAA
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           G8         S721         CGAAGAA         S508         GGAAGAG         TCTCTTCC           A9         S723         CGCAACTA         S502         TCGGATC         GAATCCGA           C9         S745         TGGAAGCA         S557         GAGA	D7	S 7 7 5	TCCATTGC	S 5 0 9	TACGGTCT	AGACCGTA
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         CTTCGTC           G8         S759         GTAAGGTG         S516         CGAATTGC         GCAATTCG           H8         S721         CGAGAGAA         S508         GGAAGAGA         TCTCTTCC           A9         S723         CGCAACTA         S502         TCGGATC         GAATCCGA           B9         S707         CACAGACT         S596         CTGACCA         TGCGTACA           C9         S745         TGGAAGCA         S557         GAGA	E7	S 7 5 0	GTCGATTG	S 5 3 2	AAGACCGT	ACGGTCTT
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         TGGAACA         S557         GAGAGTAC         GTACTCTC           D9         S746         CAATAGCC         S583         TC	F7	S 7 0 6	ATAACGCC	S 5 7 4	CAGGTTCA	TGAACCTG
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         S781         GGCAAGTT         S510         C	G7	S 7 2 4	GCCTTAAC	S 5 4 7	TAGGAGCT	AGCTCCTA
B8         \$726         TGCGTAAC         \$577         AGCCTATC         GATAGGCT           C8         \$767         CTTGCTAG         \$572         TCATCTCC         GGAGATGA           D8         \$738         AGCGAGAT         \$525         CCAGTATC         GATACTGG           E8         \$766         TATGGCAC         \$555         TTGCGAGA         TCTCGCAA           F8         \$711         GAATCACC         \$560         GAACGAAG         CTTCGTTC           G8         \$759         GTAAGGTG         \$516         CGAATTGC         GCAATTCG           H8         \$721         CGAGAGAA         \$508         GGAAGAG         TCTCTTCC           A9         \$723         CGCAACTA         \$502         TCGGATC         GAATCCGA           B9         \$707         CACAGACT         \$596         CTGTACCA         TGGTACAG           C9         \$745         TGGAAGCA         \$557         GAGAGTAC         GTACTCTC           D9         \$746         CAATAGCC         \$583         TCTACGCA         TGCGTAGA           E9         \$778         CTCGAACA         \$522         GCAATTCC         GGAATTGC           F9         \$781         GGCAAGTT         \$510         CTC	H7	S 7 9 0	GGTATAGG	S 5 9 4	TACTCCAG	CTGGAGTA
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         TCGGATC         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         CTTCGAGA           H9         S792         CAGCATAC         S534         GC	A8	S 7 9 1	TCTAGGAG	S 5 2 9	AGTGACCT	AGGTCACT
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         TCTCTCC           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         CTTAACGC           H9         S792         CAGCATAC         S534         GC	В8	S 7 2 6	TGCGTAAC	S 5 7 7	AGCCTATC	GATAGGCT
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         CTTAAGGC           H9         S792         CAGCATAC         S534         GCGTTAGA         TCTAACGC           A10         S705         CTATCTC         S563         C	C8	S 7 6 7	CTTGCTAG	S 5 7 2	TCATCTCC	GGAGATGA
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         CTTAACGC           H9         S792         CAGCATAC         S534         GCGTTAGA         TCTAACGC           A10         S705         CGTATCTC         S563         CAAGGTAC         GTACCTG           B10         S701         TTACGTG         S535         G	D8	S 7 3 8	AGCGAGAT	S 5 2 5	CCAGTATC	GATACTGG
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         S527 <t< td=""><td>E8</td><td>S 7 6 6</td><td>TATGGCAC</td><td>S 5 5 5</td><td>TTGCGAGA</td><td>TCTCGCAA</td></t<>	E8	S 7 6 6	TATGGCAC	S 5 5 5	TTGCGAGA	TCTCGCAA
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         <	F8	S 7 1 1	GAATCACC	S 5 6 0	GAACGAAG	CTTCGTTC
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           B10         S798         AAGACACC         S527         GTAACCGA         TCGGTTAC           E10         S751         CAACTCCA         S542	G8	S 7 5 9	GTAAGGTG	S 5 1 6	CGAATTGC	GCAATTCG
B9         \$707         CACAGACT         \$596         CTGTACCA         TGGTACAG           C9         \$745         TGGAAGCA         \$557         GAGAGTAC         GTACTCTC           D9         \$746         CAATAGCC         \$583         TCTACGCA         TGCGTAGA           E9         \$778         CTCGAACA         \$522         GCAATTCC         GGAATTGC           F9         \$781         GGCAAGTT         \$510         CTCAGAAG         CTTCTGAG           G9         \$740         AGCTACCA         \$570         GTCCTAAG         CTTAGGAC           H9         \$792         CAGCATAC         \$534         GCGTTAGA         TCTAACGC           A10         \$705         CGTATCTC         \$563         CAAGGTAC         GTACCTTG           B10         \$701         TTACGTGC         \$597         AGACCTTG         CAAGGTCT           C10         \$754         AGCTAAGC         \$535         GTCGTTAC         GTAACGAC           D10         \$798         AAGACACC         \$527         GTAACCGA         TCGGTTAC           E10         \$751         CAACTCCA         \$542         GAATCCGT         ACGGATTC           F10         \$717         GATCTTGC         \$595	Н8	S 7 2 1	CGAGAGAA	S 5 0 8	GGAAGAGA	TCTCTTCC
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	A9	S 7 2 3	CGCAACTA	S 5 0 2	TCGGATTC	GAATCCGA
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	B9	S 7 0 7	CACAGACT	S 5 9 6	CTGTACCA	TGGTACAG
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	C9	S 7 4 5	TGGAAGCA	S 5 5 7	GAGAGTAC	GTACTCTC
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	D9	S 7 4 6	CAATAGCC	S 5 8 3	TCTACGCA	TGCGTAGA
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	E9	S 7 7 8	CTCGAACA	S 5 2 2	GCAATTCC	GGAATTGC
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	F9	S 7 8 1	GGCAAGTT	S 5 1 0	CTCAGAAG	CTTCTGAG
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	G9	S 7 4 0	AGCTACCA	S 5 7 0	GTCCTAAG	CTTAGGAC
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	Н9	S 7 9 2	CAGCATAC	S 5 3 4	GCGTTAGA	TCTAACGC
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	A10	S 7 0 5	CGTATCTC	S 5 6 3	CAAGGTAC	GTACCTTG
D10         S798         AAGACACC         S527         GTAACCGA         TCGGTTAC           E10         S751         CAACTCCA         S542         GAATCCGT         ACGGATTC           F10         S717         GATCTTGC         S595         CATGAGCA         TGCTCATG           G10         S765         CTTCACTG         S549         CTTAGGAC         GTCCTAAG	B10	S 7 0 1	TTACGTGC	S 5 9 7	AGACCTTG	CAAGGTCT
E10 S751 CAACTCCA S542 GAATCCGT ACGGATTC F10 S717 GATCTTGC S595 CATGAGCA TGCTCATG G10 S765 CTTCACTG S549 CTTAGGAC GTCCTAAG	C10	S 7 5 4	AGCTAAGC	S 5 3 5	GTCGTTAC	GTAACGAC
F10 S717 GATCTTGC S595 CATGAGCA TGCTCATG G10 S765 CTTCACTG S549 CTTAGGAC GTCCTAAG	D10	S 7 9 8	AAGACACC	S 5 2 7	GTAACCGA	TCGGTTAC
G10 S765 CTTCACTG S549 CTTAGGAC GTCCTAAG	E10	S 7 5 1	CAACTCCA	S 5 4 2	GAATCCGT	ACGGATTC
	F10	S 7 1 7	GATCTTGC	S 5 9 5	CATGAGCA	TGCTCATG
H10 S793 CTCGACTT S573 ATCTGACC GGTCAGAT	G10	S 7 6 5	CTTCACTG	S 5 4 9	CTTAGGAC	GTCCTAAG
	H10	S 7 9 3	CTCGACTT	S 5 7 3	ATCTGACC	GGTCAGAT

WELL	EXPECTE	D i7 INDEX READ		EXPECTED i5 INDEX	READ
POSITION	i7 INDEX ID		i5 INDEX ID	FORWARD STRAND WORKFLOW	REVERSE COMPLEMENT WORKFLOW
A11	S719	GTACACCT	S 5 6 9	TCCTCATG	CATGAGGA
B11	S 7 4 4	CCAAGGTT	S 5 5 2	AGGATAGC	GCTATCCT
C11	S 7 8 5	GAACGGTT	S 5 0 4	GGAGGAAT	ATTCCTCC
D11	S 7 1 8	CCAGTTGA	S 5 1 5	GACGTCAT	ATGACGTC
E11	S 7 4 8	GTCATCGT	S 5 5 3	CCGCTTAA	TTAAGCGG
F11	S 7 6 8	CAATGCGA	S 5 5 8	GACGAACT	AGTTCGTC
G11	S 7 4 1	GGTTGAAC	S 5 8 4	TCCACGTT	AACGTGGA
H11	S 7 2 0	CTTCGGTT	S 5 1 4	AACCAGAG	CTCTGGTT
A12	S 7 3 1	CGGCATTA	S 5 7 1	GTCAGTCA	TGACTGAC
B12	S 7 8 9	CACGCAAT	S 5 7 9	CCTTCCAT	ATGGAAGG
C12	S 7 8 7	GGAATGTC	S 5 8 8	AGGAACAC	GTGTTCCT
D12	S 7 0 3	TGGTGAAG	S 5 3 9	CTTACAGC	GCTGTAAG
E12	S 7 7 6	GGACATCA	S 5 3 7	TACCTGCA	TGCAGGTA
F12	S 7 8 2	GGTGTACA	S 5 2 8	AGACGCTA	TAGCGTCT
G12	S 7 3 0	GATAGCCA	S 5 8 0	CAACACAG	CTGTGTTG
H12	S 7 3 3	CCACAACA	S 5 6 1	GTACCACA	TGTGGTAC

# **Sequencing on the Illumina Platform**

Pool equal molar amounts of libraries for sequencing on the Illumina platforms using the cycles settings shown in the table below.

RUN SEGMENT	CYCLE NUMBER
Read 1	X defined by users
Index 1 (i7)	8 (without UMI)
	20 (with UMI)
Index 2 (i5)	8
Read 2	X defined by users

# **Index Sequence File**

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's tab on www.neb.com/E7416 or you can access the sample sheets by visiting the "Usage Guidelines" sub tab located under the "protocols, manuals and usage" tab on the E7416 product page.

# **Kit Components**

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

### NEB #E7416S Table of Components

NEB#	CONCENTRATION	PRODUCT	VOLUME
E7417A	1 μΜ	NEBNext UMI RNA Adaptor Plate	1 plate (5 μl/well)
E7397A	40 μM (Total)	NEBNext Primer Mix	0.48 ml
E7398A		NEBNext UMI Adaptor Dilution Buffer	5 ml

#### NEB #E7416L Table of Components

NEB#	CONCENTRATION	PRODUCT	VOLUME
E7417A	1 μΜ	NEBNext UMI RNA Adaptor Plate	4 plates (5 μl/well)
E7397AA	40 μM (Total)	NEBNext Primer Mix	2 x 0.96 ml
E7398AA		NEBNext UMI Adaptor Dilution Buffer	20 ml

# **Revision History**

REVISION #	DESCRIPTION	DATE
1.0	N/A	3/20
2.0	Updated tables to have the most current Illumina instrument information and removed HiSeqX.	3/21
3.0	Updated protocol.	8/22
4.0	Updated primer sequences, indexing pool guidelines, header/footer and legal footer.	2/24

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