

1. Introduction

iUDI Plate LACEseq unique dual index plate contains unique dual-indexed PCR primers for amplification of indexed Illumina®-compatible NGS libraries. These primers can be used in LACEseq kit that requires TruSeq™-Compatible Indexing Primers.

iUDI Plate LACEseq contains indexed PCR primers and offer up to 24 pair unique dual indexes for multiplexing up to 24 samples. The indexed PCR primers are supplied pre-dispensed in 96- wells PCR plate. Each dual index is provided in sufficient amounts for 5-10 reactions.

All indexes have been functionally validated to work with Illumina sequencing systems using two- or four- channel chemistry for base calling.

2. Product Catalog

Store all components at -20°C.

Product name	Cat. No.	Concentration	Volume per tube
iUDI Plate LACEseq set 1 – 16	#LS-UDI-001-16	20 µM total (10 µM each Primer)	10 µl
iUDI Plate LACEseq set 2 – 24	#LS-UDI-002-24	20 µM total (10 µM each Primer)	10 µl
iUDI Plate LACEseq set 3 – 24	#LS-UDI-003-24	20 µM total (10 µM each Primer)	10 µl
iUDI Plate LACEseq set 4 – 24	#LS-UDI-004-24	20 µM total (10 µM each Primer)	5 µl
iUDI Plate LACEseq set 5 – 24	#LS-UDI-005-24	20 µM total (10 µM each Primer)	5 µl
iUDI Plate LACEseq set 012A	#LS-UDI-012A-12	20 µM total (10 µM each Primer)	5 µl
iUDI Plate LACEseq set 012B	#LS-UDI-012B-12	20 µM total (10 µM each Primer)	5 µl
iUDI Plate LACEseq set 012C	#LS-UDI-012C-12	20 µM total (10 µM each Primer)	5 µl
iUDI Plate LACEseq set 012D	#LS-UDI-012D-12	20 µM total (10 µM each Primer)	5 µl

3. General Considerations

A. Best Practices

- It is not recommended to subject iUDI Plate LACEseq index plates to more than five freeze/thaw cycles.
- Prior to use, remove the lid. Thaw for 10 minutes at room temperature, then spin the plate to pellet contents at the bottom of the tubes. Ensure the plate show no visible condensation prior to opening. **Keep the plate on ice during use.**
- When preparing the indexing PCR, pierce the seal of the plate using a pipette tip, then directly pipet the required volume of your indexing primers. Always use a separate pipette tip for each well to avoid cross contamination of indexes. Seal again the plate after usage to avoid spillover.

B. Product Compatibility

The iUDI Plate LACEseq plate are designed for use with the LACEseq™ and PAGExt™ IMMAGINA products (Catalog no #LS-001 and Catalog no #KGE-002). Please refer to the LACEseq™ and PAGExt™ kit-specific user manual for instructions on using the indexed PCR primers provided in the iUDI Plate LACEseq plate. Primer Pairs are not methylated.

NOTE: No additional PCR reagents are provided with this index set. The required enzymes and buffers are provided with the respective main kit (LACEseq™ Library Prep Kit for Illumina).

C. Multiplexing and Index Pooling

It is important to select appropriate single indexes that are unique and meet the Illumina-recommended compatibility requirements. The indexes of the LACEseq iUDI primer pairs are color-balanced in sets of four (1-4, 5-8, 9-12, 13-16). Indexes within each group of four are fully color balanced and can be pooled for sequencing. Less than four samples can be multiplexed, but verify color balance before pooling. Do **not** pool libraries across a row.

We do not recommend multiplexing Immagina libraries with libraries from other vendors in the same sequencing lane. Though this is possible in principle, specific optimization of index combinations, library pooling conditions, and loading amounts may be required. Sequencing complex pools that include different library types at different lane shares may have unpredictable effects on sequencing run metrics, read quality, read outputs, and / or demultiplexing performance. Immagina assumes no responsibility for the altered performance of Immagina libraries sequenced in combination with external library types in the same lane (or run).

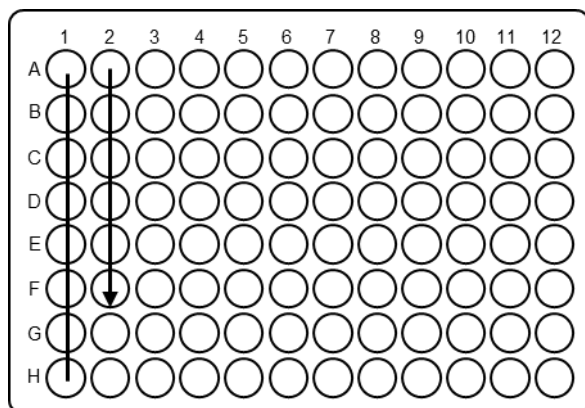


Figure 1. Index map and multiplexing strategy for the iUDI Plate LACEseq plates. We recommend pooling indexes down a column in group of four. Do not pool libraries between rows.

4. iUDI LACEseq Plates Unique Dual Index Sequences

Set 1. In the iUDI LACEseq Plate **iUDI Plate LACEseq set 1 – 16, Cat. Number #LS-UDI-001-16**, the unique dual indexes (LU1-LU16) are 8-nt long i5 and i7 dual index sequences (Table I).

IMMAGINA index name	Well position	i5 index (HiSeq® 2000/2500 MiSeq®, NextSeq2000, NovaSeq®6000 v1.0)	i5 index (NextSeq®500/550, iSeq, MiniSeq, HiSeq 3000/4000/X, NextSeq 2000 NovaSeq®6000 v1.5)	i7 index (all Illumina systems)
LU1	A1	ATATGCGC	GCGCATAT	CTGATCGT
LU2	B1	TGGTACAG	CTGTACCA	ACTCTCGA
LU3	C1	AACCGTTC	GAACGGTT	TGAGCTAG
LU4	D1	TAACCGGT	ACCGGTTA	GAGACGAT
LU5	E1	GAACATCG	CGATGTTC	CTTGTCTGA
LU6	F1	CCTTGTAG	CTACAAGG	TTCCAAGG
LU7	G1	TCAGGCTT	AAGCCTGA	CGCATGAT
LU8	H1	GTTCTCGT	ACGAGAAC	ACGGAACA
LU9	A2	AGAACGAG	CTCGTTCT	CGGCTAAT
LU10	B2	TGCTTCCA	TGGAAGCA	ATCGATCG
LU11	C2	CTTCGACT	AGTCGAAG	GCAAGATC
LU12	D2	CACCTGTT	AACAGGTG	GCTATCCT
LU13	E2	ATCACACG	CGTGTGAT	TACGCTAC
LU14	F2	CCGTAAGA	TCTTACGG	TGGACTCT
LU15	G2	TACGCCTT	AAGGCGTA	AGAGTAGC
LU16	H2	CGACGTTA	TAACGTCG	ATCCAGAG

Table I. iUDI Plate LACEseq Unique Dual Index Sequences Cat. Number #LS-UDI-001-16

Set 2. In the iUDI LACEseq Plate **iUDI Plate LACEseq set 2 – 24, Cat. Number #LS-UDI-002-24**, the unique dual indexes (Lv2U1-Lv2U24) are 8-nt long i5 and i7 dual index sequences (Table II).

IMMAGINA index name	Well position	i5 index (HiSeq® 2000/2500 MiSeq®, NextSeq2000, NovaSeq®6000 v1.0)	i5 index (NextSeq®500/550, iSeq, MiniSeq, HiSeq 3000/4000/X, NextSeq 2000 NovaSeq®6000 v1.5)	i7 index (all Illumina systems)
Lv2U1	A1	AGAGTCCA	TGGACTCT	GTCATCGT
Lv2U2	B1	GCTCAGTT	AACTGAGC	AAGGCGTA
Lv2U3	C1	CAGGTTCA	TGAACCTG	GAACCTTC
Lv2U4	D1	GAACGGTT	AACCGTTC	ACCTCTTC
Lv2U5	E1	ACTACGGT	ACCGTAGT	GTCGATTG
Lv2U6	F1	GTCTGAGT	ACTCAGAC	CATACGGA
Lv2U7	G1	ACGCAGTA	TACTGCGT	ATCTGACC
Lv2U8	H1	GTAGCGTA	TACGCTAC	GACCGATA
Lv2U9	A2	AACGTAGC	GCTACGTT	CAGGATGT
Lv2U10	B2	TATGCGGT	ACCGCATA	GATGGAGT
Lv2U11	C2	AGAAGGAC	GTCCTTCT	CTGCCATA
Lv2U12	D2	ATGAGTGC	GCACTCAT	AGGTAGGA
Lv2U13	E2	GGAATGTC	GACATTCC	ATAGTCGG
Lv2U14	F2	CTCGTTCT	AGAACGAG	TCCGATCA
Lv2U15	G2	CCTTAGGT	ACCTAAGG	CTCTTGTC
Lv2U16	H2	GACTACGA	TCGTAGTC	GAGGCATT
Lv2U17	A3	AGGCAATG	CATTGCCT	CGCAACTA
Lv2U18	B3	ATCCGTTG	CAACGGAT	CGAATACG
Lv2U19	C3	TTCGGCTA	TAGCCGAA	GGCATTCT
Lv2U20	D3	ACGTATGG	CCATACGT	ATGTGGAC
Lv2U21	E3	CGGAGTAT	ATACTCCG	TTCCAGGT
Lv2U22	F3	TCCAAGT	CAGTTGGA	AGACATGC
Lv2U23	G3	ACAGCAAG	CTTGCTGT	GGACATCA
Lv2U24	H3	TTGAGCTC	GAGCTCAA	AGCGTGTA

Table II. iUDI Plate LACEseq Unique Dual Index Sequences Cat. Number #LS-UDI-002-24

Set 3. In the iUDI LACEseq Plate **iUDI Plate LACEseq set 3 – 24, Cat. Number #LS-UDI-003-24**, the unique dual indexes (Lv3U1-Lv3U24) are 8-nt long i5 and i7 dual index sequences (Table III).

IMMAGINA index name	Well position	i5 index (HiSeq® 2000/2500 MiSeq®, NextSeq2000, NovaSeq®6000 v1.0)	i5 index (NextSeq®500/550, iSeq, MiniSeq, HiSeq 3000/4000/X, NextSeq 2000 NovaSeq®6000 v1.5)	i7 index (all Illumina systems)
Lv3U1	A1	CCGGAATA	TATTCCGG	GGTACTTC
Lv3U2	B1	CGATTCTG	CAGAATCG	AGAGCAGA
Lv3U3	C1	GCCTATGT	ACATAGGC	TAGGAGCT
Lv3U4	D1	CGTGTGAT	ATCACACG	CCTCGTTA
Lv3U5	E1	AGACGCTA	TAGCGTCT	AGATCGTC
Lv3U6	F1	GGATGTAG	CTACATCC	CGGATCAA
Lv3U7	G1	ACCGAATG	CATTCGGT	TCGTGCAT
Lv3U8	H1	GGAGGAAT	ATTCCTCC	CAGCATAC
Lv3U9	A2	CATCCAAG	CTTGGATG	GCTTCACA
Lv3U10	B2	TGCAAGAC	GTCTTGCA	AAGACGAG
Lv3U11	C2	AGTCAGGT	ACCTGACT	CTTCGGTT
Lv3U12	D2	GATTGTCC	GGACAATC	AAGCATCG
Lv3U13	E2	CTGACTAC	GTAGTCAG	CTCAAGCT
Lv3U14	F2	AAGCGACT	AGTCGCTT	ACAGTGAC
Lv3U15	G2	TCCTGGTA	TACCAGGA	CGCTACAT
Lv3U16	H2	AAGTCCTC	GAGGACTT	GCCTTCTT
Lv3U17	A3	AGCCTATC	GATAGGCT	ACGTCGTT
Lv3U18	B3	GACACAGT	ACTGTGTC	TACGGTCT
Lv3U19	C3	CTGTACCA	TGGTACAG	GAAGATCC
Lv3U20	D3	TACTCCAG	CTGGAGTA	CGTCTAAC
Lv3U21	E3	ACAGAGGT	ACCTCTGT	CTAAGACC
Lv3U22	F3	GGTAACGT	ACGTTACC	GCATAACG
Lv3U23	G3	ATGGCGAT	ATCGCCAT	CCTAACAG
Lv3U24	H3	TCACCTAG	CTAGGTGA	ACGGACTT

Table III. iUDI Plate LACEseq **Unique Dual Index Sequences Cat. Number #LS-UDI-003-24**

Set 4. In the iUDI LACEseq Plate **iUDI Plate LACEseq set 4 – 24, Cat. Number #LS-UDI-004-24**, the unique dual indexes (1-24) are 8-nt long i5 and i7 dual index sequences (Table IV).

IMMAGINA index name	i5 index (HiSeq® 2000/2500 MiSeq®, NextSeq2000, NovaSeq®6000 v1.0)	i5 index (NextSeq®500/550, iSeq, MiniSeq, HiSeq 3000/4000/X, NextSeq 2000 NovaSeq®6000 v1.5)	i7 index (all Illumina systems)
1	TCGTCTGA	TCAGACGA	CAGTGCTT
2	AGATACGG	CCGTATCT	TAGCCATG
3	CCGCTTAA	TTAAGCGG	ACATGGAG
4	AGCCGTAA	TTACGGCT	GCAATTCC
5	CCACATTG	CAATGTGG	AACCGTGT
6	GCAATGAG	CTCATTGC	CTACAAGG
7	CGCCTTAT	ATAAGGCG	ACCTTCGA
8	AACCAGAG	CTCTGGTT	GGAACATG
9	CACCAGTT	AACTGGTG	CCAGTATC
10	TTGCGAGA	TCTCGCAA	AACAGTCC
11	ACAAGCTC	GAGCTTGT	TCGGATTC
12	GATAGCCA	TGGCTATC	CAACGAGT
13	CAGAACTG	CAGTTCTG	CGCGTATT
14	CAGATCCT	AGGATCTG	TGGTATCC
15	ATCCTTCC	GGAAGGAT	CAAGGTAC
16	AGAAGCCT	AGGCTTCT	ACGGTACA
17	CCTTCCAT	ATGGAAGG	TCCACGTT
18	TAGAACGC	GCGTTCTA	ACCTCAGT
19	AACAGCGA	TCGCTGTT	ACACGAGA
20	ACCGGTTA	TAACCGGT	CACTGTAG
21	GATCAGAC	GTCTGATC	GCGTTAGA
22	CACGTCTA	TAGACGTG	AACGCCTT
23	TCGAGAGT	ACTCTCGA	ACCATGTC
24	ATACTGGC	GCCAGTAT	GATCTTGC

Table IV. iUDI Plate LACEseq **Unique Dual Index Sequences Cat. Number #LS-UDI-004-24**

Set 5. In the iUDI LACEseq Plate **iUDI Plate LACEseq set 5 – 24, Cat. Number #LS-UDI-005-24**, the unique dual indexes (25-48) are 8-nt long i5 and i7 dual index sequences (Table V).

IMMAGINA index name	i5 index (HiSeq® 2000/2500 MiSeq®, NextSeq2000, NovaSeq®6000 v1.0)	i5 index (NextSeq®500/550, iSeq, MiniSeq, HiSeq 3000/4000/X, NextSeq 2000 NovaSeq®6000 v1.5)	i7 index (all Illumina systems)
25	GGTTGAAC	GTTCAACC	CCGTAACT
26	CCTCGAAT	ATTCGAGG	AACAAGGC
27	TGGCTACA	TGTAGCCA	TTGCAACG
28	CAGGTAAG	CTTACCTG	CAATCAGG
29	GTAAGCAC	GTGCTTAC	GTACACCT
30	AACACGCT	AGCGTGTT	CGAGTTAG
31	TTACCGAC	GTCGGTAA	ACAGGCAT
32	ACCGCTAT	ATAGCGGT	GTTCCATG
33	CATGAGCA	TGCTCATG	CACGATTC
34	TGACCGTT	AACGGTCA	CTCTCAGA
35	ACACTCTG	CAGAGTGT	GAACGAAG
36	GAGCAATC	GATTGCTC	CATCACGT
37	CAATGCGA	TCGCATTG	CTCGGTAA
38	CAACTTGG	CCAAGTTG	CCAAGTAG
39	TCTAGGAG	CTCCTAGA	GGTGTACA
40	CCAAGGTT	AACCTTGG	CCTGTCAA
41	CTGGTCAT	ATGACCAG	ACAACGTG
42	ACCATAGG	CCTATGGT	TGGACCAT
43	GCCTTAAC	GTTAAGGC	CACATGGT
44	TATGGCAC	GTGCCATA	AACTTGCC
45	ACGAATCC	GGATTTCGT	CCTCATCT
46	TCACTCGA	TCGAGTGA	AGTACACG
47	CAGACGTT	AACGTCTG	CCTACCTA
48	GTCAACAG	CTGTTGAC	TCAGTAGG

Table V. iUDI Plate LACEseq **Unique Dual Index Sequences Cat. Number #LS-UDI-005-24**

Set 012A-12. In the iUDI LACEseq Plate **iUDI Plate LACEseq set 012A – 12, Cat. Number #LS-UDI-012A-12**, the unique dual indexes (1-12) are 8-nt long i5 and i7 dual index sequences (Table VI).

IMMAGINA index name	i5 index (HiSeq® 2000/2500 MiSeq®, NextSeq2000, NovaSeq®6000 v1.0)	i5 index (NextSeq®500/550, iSeq, MiniSeq, HiSeq 3000/4000/X, NextSeq 2000 NovaSeq®6000 v1.5)	i7 index (all Illumina systems)
1	TCGTCTGA	TCAGACGA	CAGTGCTT
2	AGATACGG	CCGTATCT	TAGCCATG
3	CCGCTTAA	TTAAGCGG	ACATGGAG
4	AGCCGTAA	TTACGGCT	GCAATTCC
5	CCACATTG	CAATGTGG	AACCGTGT
6	GCAATGAG	CTCATTGC	CTACAAGG
7	CGCCTTAT	ATAAGGCG	ACCTTCGA
8	AACCAGAG	CTCTGGTT	GGAACATG
9	CACCAGTT	AACTGGTG	CCAGTATC
10	TTGCGAGA	TCTCGCAA	AACAGTCC
11	ACAAGCTC	GAGCTTGT	TCGGATTC
12	GATAGCCA	TGGCTATC	CAACGAGT

Table VI. *iUDI Plate LACEseq Unique Dual Index Sequences Cat. Number #LS-UDI-012A-12*

Set 012B-12. In the iUDI LACEseq Plate **iUDI Plate LACEseq set 012B – 12, Cat. Number #LS-UDI-012B-12**, the unique dual indexes (13-24) are 8-nt long i5 and i7 dual index sequences (Table VII).

IMMAGINA index name	i5 index (HiSeq® 2000/2500 MiSeq®, NextSeq2000, NovaSeq®6000 v1.0)	i5 index (NextSeq®500/550, iSeq, MiniSeq, HiSeq 3000/4000/X, NextSeq 2000 NovaSeq®6000 v1.5)	i7 index (all Illumina systems)
13	CAGAACTG	CAGTTCTG	CGCGTATT
14	CAGATCCT	AGGATCTG	TGGTATCC
15	ATCCTTCC	GGAAGGAT	CAAGGTAC
16	AGAAGCCT	AGGCTTCT	ACGGTACA
17	CCTTCCAT	ATGGAAGG	TCCACGTT
18	TAGAACGC	GCGTTCTA	ACCTCAGT
19	AACAGCGA	TCGCTGTT	ACACGAGA
20	ACCGGTTA	TAACCGGT	CACTGTAG
21	GATCAGAC	GTCTGATC	GCGTTAGA
22	CACGTCTA	TAGACGTG	AACGCCTT
23	TCGAGAGT	ACTCTCGA	ACCATGTC
24	ATACTGGC	GCCAGTAT	GATCTTGC

Table VII. iUDI Plate LACEseq *Unique Dual Index Sequences* Cat. Number #LS-UDI-012B-12

Set 012C-12. In the iUDI LACEseq Plate **iUDI Plate LACEseq set 012C – 12, Cat. Number #LS-UDI-012C-12**, the unique dual indexes (25-36) are 8-nt long i5 and i7 dual index sequences (Table VIII).

IMMAGINA index name	i5 index (HiSeq® 2000/2500 MiSeq®, NextSeq2000, NovaSeq®6000 v1.0)	i5 index (NextSeq®500/550, iSeq, MiniSeq, HiSeq 3000/4000/X, NextSeq 2000 NovaSeq®6000 v1.5)	i7 index (all Illumina systems)
25	GGTTGAAC	GTTCAACC	CCGTAACT
26	CCTCGAAT	ATTCGAGG	AACAAGGC
27	TGGCTACA	TGTAGCCA	TTGCAACG
28	CAGGTAAG	CTTACCTG	CAATCAGG
29	GTAAGCAC	GTGCTTAC	GTACACCT
30	AACACGCT	AGCGTGTT	CGAGTTAG
31	TTACCGAC	GTCGGTAA	ACAGGCAT
32	ACCGCTAT	ATAGCGGT	GTTCCATG
33	CATGAGCA	TGCTCATG	CACGATTC
34	TGACCGTT	AACGGTCA	CTCTCAGA
35	ACACTCTG	CAGAGTGT	GAACGAAG
36	GAGCAATC	GATTGCTC	CATCACGT

Table VIII. iUDI Plate LACEseq Unique Dual Index Sequences Cat. Number #LS-UDI-012C-12

Set 012D-12. In the iUDI LACEseq Plate **iUDI Plate LACEseq set 012D – 12, Cat. Number #LS-UDI-012D-12**, the unique dual indexes (37-48) are 8-nt long i5 and i7 dual index sequences (Table IX).

IMMAGINA index name	i5 index (HiSeq® 2000/2500 MiSeq®, NextSeq2000, NovaSeq®6000 v1.0)	i5 index (NextSeq®500/550, iSeq, MiniSeq, HiSeq 3000/4000/X, NextSeq 2000 NovaSeq®6000 v1.5)	i7 index (all Illumina systems)
37	CAATGCGA	TCGCATTG	CTCGGTAA
38	CAACTTGG	CCAAGTTG	CCAAGTAG
39	TCTAGGAG	CTCCTAGA	GGTGTACA
40	CCAAGGTT	AACCTTGG	CCTGTCAA
41	CTGGTCAT	ATGACCAG	ACAACGTG
42	ACCATAGG	CCTATGGT	TGGACCAT
43	GCCTTAAC	GTTAAGGC	CACATGGT
44	TATGGCAC	GTGCCATA	AACTTGCC
45	ACGAATCC	GGATTCGT	CCTCATCT
46	TCACTCGA	TCGAGTGA	AGTACACG
47	CAGACGTT	AACGTCTG	CCTACCTA
48	GTCAACAG	CTGTTGAC	TCAGTAGG

Table IX. iUDI Plate LACEseq Unique Dual Index Sequences Cat. Number # LS-UDI-012D-12

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