

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 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
iUDI Plate LACEseq set Z1-12	#LS-UDI-012Z1-12	20 µM total (10 µM each Primer)	5 µl
iUDI Plate LACEseq set Z13-24	#LS-UDI-012Z13-24	20 µM total (10 µM each Primer)	5 µl
iUDI Plate LACEseq set Z25-36	#LS-UDI-012Z25-36	20 µM total (10 µM each Primer)	5 µl
iUDI Plate LACEseq set Z37-48	#LS-UDI-012Z37-48	20 µM total (10 µM each Primer)	5 µl

Table I. iUDI Plate LACEseq **Unique Dual Index sets**.

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 #LS001_12 and Catalog no #KGE00-12). 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).

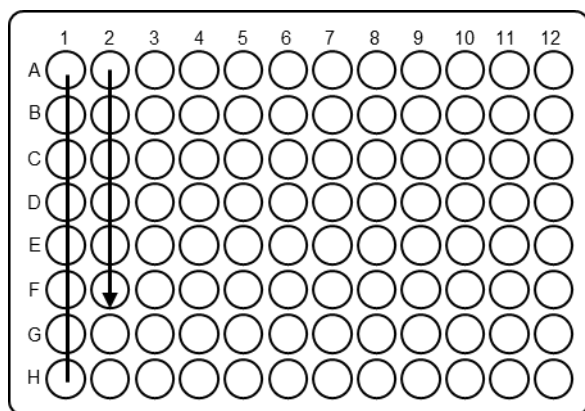


Figure 1. Index map and multiplexing strategy for the UDI Plate LACEseq plates. We recommend pooling indexes down a column in group of four. Do not pool libraries between rows.

4. iUDI LACEseq Plates Unique Dual Index Sequences

Set 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 II).

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 II. 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 III).

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 III. 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 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)
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	GTCCATG
33	CATGAGCA	TGCTCATG	CACGATTC
34	TGACCGTT	AACGGTCA	CTCTCAGA
35	ACACTCTG	CAGAGTGT	GAACGAAG
36	GAGCAATC	GATTGCTC	CATCACGT

Table IV. *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 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)
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 V. iUDI Plate LACEseq **Unique Dual Index Sequences Cat. Number # LS-UDI-012D-12**

Set Z1-12. In the iUDI LACEseq Plate **iUDI Plate LACEseq set Z1-12, Cat. Number #LS-UDI-012Z1-12**, the unique dual indexes (z1-z12) are 10-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)
Z1	ATGCGCTTAT	ATAAGCGCAT	CTTCGCAACT
Z2	CACTTCACCA	TGGTGAAGTG	CGTCAAGACG
Z3	GGTATAGGGG	CCCCTATACC	TGTCACACTG
Z4	GCAATTCCGC	GCGGAATTGC	AGCCGTAAAG
Z5	AACCGTGTA	TTACACGGTT	CCACATTGCC
Z6	CTTCACTGCT	AGCAGTGAAG	ACGTCCAAAC
Z7	ACTGCACTAC	GTAGTGCAGT	CTCGAACACT
Z8	GGAACATGGG	CCCATGTTCC	AACCAGAGAA
Z9	CCAGTATCCC	GGGATACTGG	CACCAGTTCA
Z10	AACAGTCCAA	TTGGACTGTT	TTGCGAGATT
Z11	TCGGATTCTC	GAGAATCCGA	ACAAGCTCAC
Z12	CAACGAGTCA	TGACTCGTTG	GATAGCCAGA

Table VI. iUDI Plate LACEseq Unique Dual Index Sequences Cat. Number #LS-UDI-012Z1-12

Set Z13-24. In the iUDI LACEseq Plate **iUDI Plate LACEseq set Z13-24, Cat. Number #LS-UDI-012Z13-24**, the unique dual indexes (z13-z24) are 10-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)
Z13	CGCGTATTCG	CGAATACGCG	CAGAACTGCA
Z14	TGGTATCCTG	CAGGATACCA	CAGATCCTCA
Z15	CAAGGTACCA	TGGTACCTTG	ATCCTTCCAT
Z16	ACGGTACAAC	GTTGTACCGT	AGAAGCCTAG
Z17	TCCACGTTTC	GAAACGTGGA	CCTTCCATCC
Z18	ACCTCAGTAC	GTACTGAGGT	TAGAACGCTA
Z19	ACACGAGAAC	GTTCTCGTGT	AACAGCGAAA
Z20	CACTGTAGCA	TGCTACAGTG	ACCGGTTAAC
Z21	GCGTTAGAGC	GCTCTAACGC	GATCAGACGA
Z22	AACGCCTTAA	TTAAGGCGTT	CACGTCTACA
Z23	ACCATGTCAC	GTGACATGGT	TCGAGAGTTC
Z24	GATCTTGCGA	TCGCAAGATC	ATACTGGCAT

Table VII. iUDI Plate LACEseq Unique Dual Index Sequences Cat. Number #LS-UDI-012Z13-24

Set Z25-36. In the iUDI LACEseq Plate **iUDI Plate LACEseq set Z25-36, Cat. Number #LS-UDI-012Z25-36**, the unique dual indexes (z25-z36) are 10-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)
Z25	CCGTAACTCC	GGAGTTACGG	GGTTGAACGG
Z26	AACAAGGCAA	TTGCCTTGTT	CCTCGAATCC
Z27	TTGCAACGTT	AACGTTGCAA	TGGCTACATG
Z28	CAATCAGGCA	TGCCTGATTG	CAGGTAAGCA
Z29	GTACACCTGT	ACAGGTGTAC	GTAAGCACGT
Z30	CGAGTTAGCG	CGCTAACTCG	AACACGCTAA
Z31	ACAGGCATAC	GTATGCCTGT	TTACCGACTT
Z32	GTTCCATGGT	ACCATGGAAC	ACCGCTATAC
Z33	CACGATTCCA	TGGAATCGTG	CATGAGCACA
Z34	CTCTCAGACT	AGTCTGAGAG	TGACCGTTTG
Z35	GAACGAAGGA	TCCTTCGTTC	ACACTCTGAC
Z36	CATCACGTCA	TGACGTGATG	GAGCAATCGA

Table VIII. iUDI Plate LACEseq Unique Dual Index Sequences Cat. Number #LS-UDI-012Z25-36

Set Z37-48. In the iUDI LACEseq Plate **iUDI Plate LACEseq set Z37-48, Cat. Number #LS-UDI-012Z37-48**, the unique dual indexes (z37-z48) are 10-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)
Z37	CTCGGTA ACT	AGTTACCGAG	CAATGCGACA
Z38	CCAAGTAGCC	GGCTACTTGG	CAACTTGGCA
Z39	GGTGTACAGG	CCTGTACACC	TCTAGGAGTC
Z40	CCTGTCAACC	GGTTGACAGG	CCAAGGTTCC
Z41	ACAACGTGAC	GTCACGTTGT	CTGGTCATCT
Z42	TGGACCATTG	CAATGGTCCA	ACCATAGGAC
Z43	CACATGGTCA	TGACCATGTG	GCCTTAACGC
Z44	AACTTGCCAA	TTGGCAAGTT	TATGGCACTA
Z45	CCTCATCTCC	GGAGATGAGG	ACGAATCCAC
Z46	AGTACACGAG	CTCGTGTACT	TCACTCGATC
Z47	CCTACCTACC	GGTAGGTAGG	CAGACGTTCA
Z48	TCAGTAGGTC	GACCTACTGA	GTCAACAGGT

Table IX. iUDI Plate LACEseq Unique Dual Index Sequences Cat. Number #LS-UDI-012Z37-48

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