USER GUIDE

HOLOGIC®
Diagenode

Primer Indexes for MicroPlex v3

High Performance Library Preparation for Illumina® NGS Platforms

24 UDI - Set I (48 rxns)

Cat. No. C05010008

24 UDI - Set II (48 rxns)

Cat. No. C05010009

24 Dual Indexes (48 rxns)

Cat. No. C05010003

Content

Please read this manual carefully before starting your experiment

Introduction

Primer indexes for MicroPlex kits are designed for use with the MicroPlex Library Preparation Kit v3 to construct libraries for multiplexed sequencing on Illumina® sequencers.

The ***Unique Dual Indexes kits (UDI)*** provide combinations of barcodes where each barcode is uniquely attributed to one sample. This is a great tool to identify mistakes during index sequencing. A phenomenon known as index hopping can lead to misattribution of some reads to the wrong sample. This is particularly frequent with the NovaSeq6000, and so the use of Unique Dual-Indexing (UDI) is therefore highly recommended when using this sequencer.

The ***Dual Indexes Kit*** is dedicated to combinatorial dual-indexing.

All primer indexes kits are presented in Table 1.

Table 1. *Components supplied with the kit 24 SI for tagmented libraries with their indexes sequences*

Product	Cat. No.	Format	Concentration	Volume per well or tube
24 UDI for MicroPlex kit v3 (48 rxns) - Set I	C05010008	Individual Tubes	5 µM	10 µl
24 UDI for MicroPlex kit v3 (48 rxns) - Set II	C05010009	Individual Tubes	5 µM	10 µl
24 Dual indexes for MicroPlex Kit v3	C05010003	Individual Tubes	5 µM	10 µl

SHIPPING AND STORAGE: The above kits are shipped on dry ice. The kits should be stored at −20°C upon arrival. The Primer Indexes for the MicroPlex Kit v3 are designed for use with the following kit:

- MicroPlex Library Preparation Kit v3 x48 rxns (Cat. No. C05010001)

Please refer to the kit-specific user manual for instructions on using the indexed PCR primers provided in the Primer indexes for MicroPlex v3 kits.

Remarks before starting

General using information

No more than four freeze/thaw cycles are recommended for the Primer Indexes for MicroPlex Kit v3. Prior to use, remove the kit from the freezer and thaw for 10 min on the bench top. Spin in a tabletop centrifuge to collect contents at the bottom of the tubes or wells. Thoroughly wipe the tubes or index plate foil seal with 70% ethanol and allow it to dry.

Follow standard laboratory safety procedures and wear a suitable lab coat, protective goggles and disposable gloves to ensure personal safety as well as to limit potential cross contamination during the sample preparation and subsequent amplification reactions.

I. Unique dual indexes for MicroPlex v3

Unique Dual-Indexing kits for MicroPlex v3:

24 UDI for MicroPlex kit v3 (48 rxns) - Set I

Cat. No. C05010008

24 UDI for MicroPlex kit v3 (48 rxns) - Set II

Cat. No. C05010009

These kits contain indexed PCR primers carrying the “IDT for Illumina UD” index sequences and offer a total of 48 unique dual indexes for multiplexing of up to 48 samples. The indexed PCR primers are supplied pre-dispensed in two different sets of 24 individual tubes.

24 UDI for MicroPlex kit v3 (48 rxns) - Set I

Cat. No. C05010008

Unique Dual indexing reagents are pre-dispensed in 24 individual tubes, each containing a unique dual index combination of i7 and i5 index primers carrying the 8-nt dual index sequences shown in Table 6. Each tube contains sufficient volume for up to two uses.

Table 2. *Unique Dual Index sequences (Set I)*

i7 Index Name	i7 Bases for Sample Sheet	i5 Bases for Sample Sheet (MiSeq®, NovaSeq™, HiSeq® 2000/2500)	i5 Bases for Sample Sheet (MiniSeq™, NextSeq®, HiSeq® 3000/4000)
UDI 1	CCGCGGTT	AGCGCTAG	CTAGCGCT
UDI 2	TTATAACC	GATATCGA	TCGATATC
UDI 3	GGACTTGG	CGCAGACG	CGTCTGCG
UDI 4	AAGTCCAA	TATGAGTA	TACTCATA
UDI 5	ATCCACTG	AGGTGCGT	ACGCACCT
UDI 6	GCTTGTCA	GAACATAC	GTATGTTC
UDI 7	CAAGCTAG	ACATAGCG	CGCTATGT
UDI 8	TGGATCGA	GTGCGATA	TATCGCAC
UDI 9	AGTTCAGG	CCAACAGA	TCTGTTGG
UDI 10	GACCTGAA	TTGGTGAG	CTCACCAA
UDI 11	TCTCTACT	CGCGGTTC	GAACCGCG
UDI 12	CTCTCGTC	TATAACCT	AGGTTATA
UDI 13	CCAAGTCT	AAGGATGA	TCATCCTT
UDI 14	TTGGACTC	GGAAGCAG	CTGCTTCC
UDI 15	GGCTTAAG	TCGTGACC	GGTCACGA
UDI 16	AATCCGGA	CTACAGTT	AACTGTAG
UDI 17	TAATACAG	ATATTCAC	GTGAATAT
UDI 18	CGGCGTGA	GCGCCTGT	ACAGGCGC
UDI 19	ATGTAAGT	ACTCTATG	CATAGAGT
UDI 20	GCACGGAC	GTCTCGCA	TGCGAGAC
UDI 21	GGTACCTT	AAGACGTC	GACGTCTT
UDI 22	AACGTTCC	GGAGTACT	AGTACTCC
UDI 23	GCAGAATT	ACCGGCCA	TGGCCGGT
UDI 24	ATGAGGCC	GTTAATTG	CAATTAAC

Multiplexing and index pooling

It is very important to select appropriate dual indexes that are unique and meet the Illumina recommended compatibility requirements. For low- plex pooling guidelines, please refer to Illumina's Index Adapters Pooling Guide (1000000041074 v06) as shown in the table below.

Table 3. *Index pooling guidelines*

Plexity	Index combination
2 samples	UDI 1 – UDI 10 UDI 2 – UDI 9 UDI 3 – UDI 12 UDI 4 – UDI 11 UDI 5 – UDI 14 UDI 6 – UDI 13 UDI 7 – UDI 16 UDI 8 – UDI 15
3 samples	UDI 1 – UDI 2 – UDI 9 UDI 3 – UDI 4 – UDI 11 UDI 5 – UDI 6 - UDI 13 UDI 7 – UDI 8 – UDI 15
4 samples	UDI 1 – UDI 2 – UDI 9 – UDI 10 – UDI 17 – UDI 18 UDI 3 – UDI 4 – UDI 11 – UDI 12 – UDI 19 – UDI 20 UDI 5 – UDI 6 – UDI 13 – UDI 14 – UDI 21 – UDI 22 UDI 7 – UDI 8 – UDI 15 – UDI 16 – UDI 23 – UDI 24

24 UDI for MicroPlex v3 (48 rxns) - Set II

Cat. No. C05010009

Unique Dual indexing reagents are pre-dispensed in 24 individual tubes, each containing a unique dual index combination of i7 and i5 index primers carrying the 8-nt dual index sequences shown in Table 8. Each tube contains sufficient volume for up to two uses.

Table 4. *Unique Dual Index sequences (Set II)*

i7 Index Name	i7 Bases for Sample Sheet	i5 Bases for Sample Sheet (MiSeq®, NovaSeq™, HiSeq® 2000/2500)	i5 Bases for Sample Sheet (MiniSeq™, NextSeq®, HiSeq® 3000/4000)
UDI 25	ACTAAGAT	AACCGCGG	CCGCGGTT
UDI 26	GTCGGAGC	GGTTATAA	TTATAACC
UDI 27	CTTGGTAT	CCAAGTCC	GGACTTGG
UDI 28	TCCAACGC	TTGGACTT	AAGTCCAA
UDI 29	CCGTGAAG	CAGTGGAT	ATCCACTG
UDI 30	TTACAGGA	TGACAAGC	GCTTCTCA
UDI 31	GGCATTCT	CTAGCTTG	CAAGCTAG
UDI 32	AATGCCTC	TCGATCCA	TGGATCGA
UDI 33	TACCGAGG	CCTGAACT	AGTTCAGG
UDI 34	CGTTAGAA	TTCAGGTC	GACCTGAA
UDI 35	AGCCTCAT	AGTAGAGA	TCTCTACT
UDI 36	GATTCTGC	GACGAGAG	CTCTCGTC
UDI 37	TCGTAGTG	AGACTTGG	CCAAGTCT
UDI 38	CTACGACA	GAGTCCAA	TTGGACTC
UDI 39	TAAGTGGT	CTTAAGCC	GGCTTAAG
UDI 40	CGGACAAC	TCCGGATT	AATCCGGA
UDI 41	ATATGGAT	CTGTATTA	TAATACAG
UDI 42	GCGCAAGC	TCACGCCG	CGGCGTGA
UDI 43	AAGATACT	ACTTACAT	ATGTAAGT
UDI 44	GGAGCGTC	GTCCGTGC	GCACGGAC
UDI 45	ATGGCATG	AAGGTACC	GGTACCTT
UDI 46	GCAATGCA	GGAACGTT	AACGTTCC
UDI 47	GTTCCAAT	AATTCTGC	GCAGAATT
UDI 48	ACCTTGGC	GGCCTCAT	ATGAGGCC

Multiplexing and index pooling

It is very important to select appropriate dual indexes that are unique and meet the Illumina recommended compatibility requirements. For low- plex pooling guidelines, please refer to Illumina's Index Adapters Pooling Guide (1000000041074 v06) as shown in the table below.

Table 5. *Unique Dual Index sequences (Set II)*

Plexity	Index combination
2 samples	UDI 33 – UDI 42 UDI 34 – UDI 41 UDI 35 – UDI 44 UDI 36 – UDI 43 UDI 37 – UDI 46 UDI 38 – UDI 45 UDI 39 – UDI 48 UDI 40 – UDI 47
3 samples	UDI 33 – UDI 34 – UDI 41 UDI 35 – UDI 36 – UDI 43 UDI 37 – UDI 38 - UDI 45 UDI 39 – UDI 40 – UDI 47
4 samples	UDI 33 – UDI 34 – UDI 41 – UDI 42 UDI 35 – UDI 36 – UDI 43 – UDI 44 UDI 37 – UDI 38 - UDI 45 – UDI 46 UDI 39 – UDI 40 – UDI 47 – UDI 48
5 samples	UDI 25 – UDI 26 – UDI 33 – UDI 34 – UDI 41 UDI 27 – UDI 28 – UDI 35 – UDI 36 – UDI 43 UDI 29 – UDI 30 – UDI 37 – UDI 38 – UDI 45 UDI 31 – UDI 32 – UDI 39 – UDI 40 – UDI 47
6 samples	UDI 25 – UDI 26 – UDI 33 – UDI 34 – UDI 41 – UDI 42 UDI 27 – UDI 28 – UDI 35 – UDI 36 – UDI 43 – UDI 44 UDI 29 – UDI 30 – UDI 37 – UDI 38 – UDI 45 – UDI 46 UDI 31 – UDI 32 – UDI 39 – UDI 40 – UDI 47 – UDI 48

II. Dual indexes for MicroPlex v3

24 Dual indexes for MicroPlex Kit v3

Cat. No. C05010003

Dual indexing reagents are pre-dispensed in 24 individual tubes, each containing a unique combination of i7 and i5 index primers (Table 6) carrying the 8-nt dual index sequences shown in Table 7 and 8. Each tube contains sufficient volume for up to two uses.

Table 6. *Combination of i7 and i5 indexes in the 24 Dual indexes for MicroPlex Kit v3 (Cat. No. C05010003)*

Dual Indexing Reagent	i7 Index	i5 index
Dual Indexing Reagent 1	N701	S505
Dual Indexing Reagent 2	N702	S505
Dual Indexing Reagent 3	N703	S505
Dual Indexing Reagent 4	N704	S505
Dual Indexing Reagent 5	N705	S505
Dual Indexing Reagent 6	N706	S505
Dual Indexing Reagent 7	N701	S506
Dual Indexing Reagent 8	N702	S506
Dual Indexing Reagent 9	N703	S506
Dual Indexing Reagent 10	N704	S506
Dual Indexing Reagent 11	N705	S506
Dual Indexing Reagent 12	N706	S506
Dual Indexing Reagent 13	N701	S508
Dual Indexing Reagent 14	N702	S508
Dual Indexing Reagent 15	N703	S508
Dual Indexing Reagent 16	N704	S508
Dual Indexing Reagent 17	N705	S508
Dual Indexing Reagent 18	N706	S508
Dual Indexing Reagent 19	N701	S517
Dual Indexing Reagent 20	N702	S517
Dual Indexing Reagent 21	N703	S517
Dual Indexing Reagent 22	N704	S517
Dual Indexing Reagent 23	N705	S517
Dual Indexing Reagent 24	N706	S517

Table 7. *Sequences of i7 index primers*

i7 Index Name	i7 Bases for Sample Sheet
N701	TAAGGCGA
N702	CGTACTAG
N703	AGGCAGAA
N704	TCCTGAGC
N705	GGACTCCT
N706	TAGGCATG
N707	CTCTCTAC
N710	CGAGGCTG
N711	AAGAGGCA
N712	GTAGAGGA
N714	GCTCATGA
N715	ATCTCAGG
N716	ACTCGCTA
N718	GGAGCTAC
N719	GCGTAGTA
N720	CGGAGCCT
N721	TACGCTGC
N722	ATGCGCAG
N723	TAGCGCTC
N724	ACTGAGCG
N726	CCTAAGAC
N727	CGATCAGT
N728	TGCAGCTA
N729	TCGACGTC

Table 8. *Sequences of i5 index primers*

i5 Index Name	i5 Bases for Sample Sheet (MiSeq®, NovaSeq, HiSeq® 2000/2500)	i5 Bases for Sample Sheet (MiniSeq™, NextSeq®, HiSeq 3000/4000)
S502	CTCTCTAT	ATAGAGAG
S503	TATCCTCT	AGAGGATA
S505	GTAAGGAG	CTCCTTAC
S506	ACTGCATA	TATGCAGT
S507	AAGGAGTA	TACTCCTT
S508	CTAAGCCT	AGGCTTAG
S510	CGTCTAAT	ATTAGACG
S511	TCTCTCCG	CGGAGAGA
S513	TCGACTAG	CTAGTCGA
S515	TTCTAGCT	AGCTAGAA
S516	CCTAGAGT	ACTCTAGG
S517	GCGTAAGA	TCTTACGC
S518	CTATTAAG	CTTAATAG
S520	AAGGCTAT	ATAGCCTT
S521	GAGCCTTA	TAAGGCTC
S522	TTATGCGA	TCGCATAA

Multiplexing and index pooling

It is very important to select appropriate dual indexes that are unique and meet the Illumina recommended compatibility requirements. For low-plex (2- to 12-plex) pooling guidelines, please refer to Illumina's Index Adapters Pooling Guide (1000000041074 v06) as shown in the table below.

Table 9. *Index pooling guidelines*

Plexity	Index 1 (i7) Adapters	Index 2 (i5) Adapters
2 - 6	At least 2 unique i7 adapters	At least two unique i5 adapters
7 - 12	One of the following combinations: N701, N702, N704 and any other i7 adapter N703, N705, N706 and any other i7 adapter	One of the following combinations: • S503 and S504 • S505 and S506
More than 12	N701–N706 and any other i7 adapter	One of the following combinations: • S503, S504, and any other i5 adapter • S505, S506, and any other i5 adapter

Related Products

Product	Cat. No.
iDeal ChIP-seq kit for Transcription Factors	C01010055
Auto iDeal ChIP-seq Kit for Transcription Factors	C01010172
iDeal ChIP-Seq Kit for Histones	C01010051
Auto iDeal ChIP-seq Kit for Histones	C01010171
True MicroChIP-seq kit	C01010132
Auto True MicroChIP Kit	C01010140
iDeal FFPE Kit	C01010190
Universal Plant ChIP-seq Kit	C01010152
Auto Universal Plant ChIP-seq kit	C01010153
MicroPlex Library Preparation Kit v3 x48 rxns	C05010001
Bioruptor Pico	B01060010

Revision history

Version	Date of modification	Description of modifications
Version 3 03_2025	March 2025	Manual rebranding + Remove obsolete references.

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