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Primer indexes for tagmented libraries

Cat. No. C01011033 – 8 SI for tagmented libraries
C01011032 – 24 SI for tagmented libraries
C01011035 – 8 UDI for tagmented libraries
C01011034 – 24 UDI for tagmented libraries – Set I
C01011036 - 24 UDI for tagmented libraries – Set II
C01011037 – 24 UDI for tagmented libraries – Set III

USER GUIDE

Version 6 06_2023

Please read this manual carefully
before starting your experiment

Contents

Introduction

The primer indexes for tagmented libraries are PCR primers targeting the Nextera sequencing adaptors, previously incorporated in the libraries by tagmentation. The structure of final library is shown in figure 1.

A – Single index libraries:

5’AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG – insert – CTGTCTCTTATACACATCTCCGAGCCCACGAGAC[i7index]ATCTCGTATGCCGTCTTCTGCTTG 3’

A – Dual index libraries:

5’ AATGATACGGCGACCACCGAGATCTACAC[i5 index]TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG – insert – CTGTCTCTTATACACATCTCCGAGCCCACGAGAC[i7 index]ATCTCGTATGCCGTCTTCTGCTTG 3

Figure1. Structure of tagmented libraries after final amplification with Diagenode primer indexes. A) Single-index libraries. B) Dual-index libraries.

Each primer index is baring a unique index (in red in figure 1) in order to identify each library before pooling them for sequencing in the same lane. To give flexibility in the experimental design and answer different needs several products are available:

- **SI for tagmented libraries** (Cat. No. C01011033)
- **24 SI for tagmented libraries** (Cat. No. C01011032)
- **8 UDI for tagmented libraries** (Cat. No. C01011035)
- **24 UDI for tagmented libraries – Set I** (Cat. No. C01011034)
- **24 UDI for tagmented libraries – Set II** (Cat. No. C01011036)
- **24 UDI for tagmented libraries – Set III** (Cat. No. C01011037)

They are all compatible with any Nextera-based libraries such as the one generated with ChIPmentation, ATAC-seq or CUT&Tag technologies.

8 SI for tagmented libraries

Cat. No. C01011033
The 8 SI for tagmented libraries includes 8 primer pairs for single indexing allowing the multiplexing of up to 8 samples for sequencing on Illumina platforms.

This set of indexes is compatible with any Nextera-based libraries such as the one generated with ChIPmentation, ATAC-seq or CUT&Tag technologies. This product is compatible with the following Diagenode's products: µChIPmentation Kit for Histones (Cat. No. C01011012), ATAC-seq kit (Cat. No. C01080001), pA-Tn5 (Cat. No. C01070001 and C01070002), iDeal CUT&Tag for Histones kit (Cat. No. C01070020).

The primer indexes for tagmented libraries contain 8-nucleotide long i7 indexes.

Table 1. Components supplied with the kit 8 SI for tagmented libraries with their indexes sequences

Component	i7 Index Sequence	Cap color	Volume
Primer pair SI 1	TAAGGCGA	transparent	24 µl
Primer pair SI 2	CGTACTAG	none	9 µl
Primer pair SI 3	AGGCAGAA	none	9 µl
Primer pair SI 4	TCCTGAGC	none	9 µl
Primer pair SI 5	GGACTCCT	none	9 µl
Primer pair SI 6	TAGGCATG	none	9 µl
Primer pair SI 7	CTCTCTAC	none	9 µl
Primer pair SI 8	CAGAGAGG	none	9 µl

NOTE: Upon receipt, store the components at the indicated temperatures.

Multiplexing and index pooling

When multiplexing less than 5 samples together the choice of the index is important to ensure a good quality sequencing. We recommend the following combinations when low multiplexing of libraries is required:

Multiplexing level	Index combination
2 samples	Index SI 1 - Index SI 2 Index SI 2 - Index SI 4
3 samples	Index SI 1 - Index SI 2 - Index SI 4 Index SI 3 - Index SI 5 - Index SI 6 2-plex option with any other index
4 samples	Index SI 1 – Index SI 2 – Index SI 3 – Index SI 4 3-plex option with any other index

For 5 - 8-plex pools use 4-plex options with any other available indexes.

24 SI for tagmented libraries

Cat. No. C01011032

The 24 SI for tagmented libraries includes 24 primer pairs for single indexing allowing the multiplexing of up to 24 samples for sequencing on Illumina platforms.

This set of indexes is compatible with any Nextera-based libraries such as the one generated with ChIPmentation, ATAC-seq or CUT&Tag technologies. This product is compatible with the following Diagenode's products: µChIPmentation Kit for Histones (Cat. No. C01011011), ChIPmentation Kit for Histones (Cat. No. C01011009), ATAC-seq kit (Cat. No. C01080002), pA-Tn5 (Cat. No. C01070001 and C01070002), iDeal CUT&Tag for Histones kit (Cat. No. C01070020).

The primer indexes for tagmented libraries contain 8-nucleotide long i7 indexes.

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

Component	i7 Index Sequence	Cap color	Volume	Storage
Primer pair SI 1	TAAGGCGA	transparent	24 µl	-20°C
Primer pair SI 2	CGTACTAG	none	9 µl	-20°C
Primer pair SI 3	AGGCAGAA	none	9 µl	-20°C
Primer pair SI 4	TCCTGAGC	none	9 µl	-20°C
Primer pair SI 5	GGACTCCT	none	9 µl	-20°C
Primer pair SI 6	TAGGCATG	none	9 µl	-20°C
Primer pair SI 7	CTCTCTAC	none	9 µl	-20°C
Primer pair SI 8	CAGAGAGG	none	9 µl	-20°C
Primer pair SI 9	GCTACGCT	none	9 µl	-20°C
Primer pair SI 10	CGAGGCTG	none	9 µl	-20°C
Primer pair SI 11	AAGAGGCA	none	9 µl	-20°C
Primer pair SI 12	GTAGAGGA	none	9 µl	-20°C
Primer pair SI 13	GTCGTGAT	none	9 µl	-20°C
Primer pair SI 14	ACCACTGT	none	9 µl	-20°C
Primer pair SI 15	TGGATCTG	none	9 µl	-20°C
Primer pair SI 16	CCGTTTGT	none	9 µl	-20°C
Primer pair SI 17	TGCTGGGT	none	9 µl	-20°C
Primer pair SI 18	GAGGGGTT	none	9 µl	-20°C
Primer pair SI 19	AGGTTGGG	none	9 µl	-20°C

Primer pair SI 20	GTGTGGTG	none	9 µl	-20°C
Primer pair SI 21	TGGGTTTC	none	9 µl	-20°C
Primer pair SI 22	TGGTCACA	none	9 µl	-20°C
Primer pair SI 23	TTGACCCT	none	9 µl	-20°C
Primer pair SI 24	CCACTCCT	none	9 µl	-20°C

NOTE: Upon receipt, store the components at the indicated temperatures.

Multiplexing and index pooling

When multiplexing less than 5 samples together the choice of the index is important to ensure a good quality sequencing. We recommend the following combinations when low multiplexing of libraries is required:

Multiplexing level	Index combination
2 samples	Index SI 1 - Index SI 2 Index SI 2 - Index SI 4
3 samples	Index SI 1 - Index SI 2 - Index SI 4 Index SI 3 - Index SI 5 - Index SI 6 Index SI 10 - Index SI 11 - Index SI 12 Index SI 13 - Index SI 14 - Index SI 22 2-plex option with any other index
4 samples	Index SI 8 – Index SI 9 – Index SI 11 – Index SI 12 Index SI 8 - Index SI 12 – Index SI 21 – Index SI 24 Index SI 1 – Index SI 2 - Index SI 3 – Index SI 4 Index SI 4 - Index SI 7 - Index SI 19 - Index SI 23 3-plex option with any other index

For 5 - 11-plex pools use 4-plex options with any other available indexes. If a higher multiplexing degree is required (> 12), any combination is possible regardless of the index chosen.

8 UDI for tagmented libraries

Cat. No. C01011035

The 8 UDI (Unique dual indexes) for tagmented libraries provides combinations of barcodes where each barcode is uniquely attributed to one sample. This is a great tool to identify mistakes during index sequencing. Indeed, 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 thus the use of Unique Dual-Indexing (UDI) is therefore highly recommended when using this sequencer.

The 8 UDI for tagmented libraries includes 8 primer pairs for unique dual indexing allowing the multiplexing of up to 8 samples for sequencing on Illumina platforms.

This set of indexes is compatible with any Nextera-based libraries such as the one generated with ChIPmentation, ATAC-seq or CUT&Tag technologies. This product is compatible with the following Diagenode’s products: µChIPmentation Kit for Histones (Cat. No. C01011011), ChIPmentation Kit for Histones (Cat. No. C01011009), ATAC-seq kit (Cat. No. C01080002), pA-Tn5 (Cat. No. C01070001 and C01070002), iDeal CUT&Tag for Histones kit (Cat. No. C01070020).

The primer indexes for tagmented libraries contain 8-nucleotide long i7 and i5 indexes.

Table 1. Components supplied with the kit 8 UDI for tagmented libraries with their indexes sequences.
Concentration: 10 µM
Quantity in the tube: 12 µl (Primer pair UDI 1); 5 µl (Primer pair UDI 2 to UDI 8)
Storage: -20°C

Primer Pair UDI #	i(7) = reverse primer		i(5) = forward primer		
	sequence as in primer	sequence as read in sequencing	sequence as in primer	sequence as read in sequencing	
				i5 bases for sample sheet Forward Strand Workflow NovaSeq 6000 v1.0, MiSeq, HiSeq 2000/2500	i5 bases for sample sheet Reverse Strand Workflow NovaSeq 6000 v1.5, iSeq, MiniSeq, NextSeq, HiSeq 3000/4000
1	AACCGCGG	CCGCGGTT	AGCGCTAG	AGCGCTAG	CTAGCGCT
2	GGTTATAA	TTATAACC	GATATCGA	GATATCGA	TCGATATC
3	CCAAGTCC	GGACTTGG	CGCAGACG	CGCAGACG	CGTCTGCG
4	TTGGACTT	AAGTCCAA	TATGAGTA	TATGAGTA	TACTCATA
5	CAGTGGAT	ATCCACTG	AGGTGCGT	AGGTGCGT	ACGCACCT
6	TGACAAGC	GCTTGTCA	GAACATAC	GAACATAC	GTATGTTC
7	CTAGCTTG	CAAGCTAG	ACATAGCG	ACATAGCG	CGCTATGT
8	TCGATCCA	TGGATCGA	GTGCGATA	GTGCGATA	TATCGCAC

NOTE: Upon receipt, store the components at the indicated temperatures.

Multiplexing and index pooling

When multiplexing less than 5 samples together the choice of the index is important to ensure a good quality sequencing. We recommend the following combinations when low multiplexing of libraries is required:

Multiplexing level	Index combination
2 samples	UDI 1 - UDI 2 UDI 1 – UDI 8 UDI 2 – UDI 7 UDI 3 – UDI 4 UDI 5 – UDI 6 UDI 7 – UDI 8
3 samples	UDI 1 – UDI 2 – UDI 7 UDI 1 – UDI 2 – UDI 8 UDI 1 – UDI 7 – UDI 8 UDI 2 – UDI 7 – UDI 8 UDI 5 – UDI 6 – UDI 7 UDI 5 – UDI 6 – UDI 8 UDI 5 – UDI 7 – UDI 8 UDI 6 – UDI 7 – UDI 8
4 samples	3-plex option with any other index

For 5 - 8-plex pools use 4-plex options with any other available indexes.

24 UDI for tagmented libraries – Set I to III

Cat. No. C01011034, C01011036, C01011037

The 24 UDI (Unique dual indexes) for tagmented libraries – Set I, Set II and Set III provide combinations of barcodes where each barcode is uniquely attributed to one sample. This is a great tool to identify mistakes during index sequencing. Indeed, 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 thus the use of Unique Dual-Indexing (UDI) is therefore highly recommended when using this sequencer.

Each set of 24 UDI for tagmented libraries includes 24 primer pairs for unique dual indexing allowing the multiplexing of up to 24 samples (and up to 72 samples for the 3 sets) for sequencing on Illumina platforms.

These sets of indexes are compatible with any Nextera-based libraries such as the one generated with ChIPmentation, ATAC-seq or CUT&Tag technologies. These products are compatible with the following Diagenode's products: µChIPmentation Kit for Histones (Cat. No. C01011011), ChIPmentation Kit for Histones (Cat. No. C01011009), TAG kit for ChIPmentation (Cat. No. C01011030), ATAC-seq kit (Cat. No. C01080002), pA-Tn5 (Cat. No. C01070001 and C01070002), iDeal CUT&Tag for Histones kit (Cat. No. C01070020).

The primer indexes for tagmented libraries contain 8-nucleotide long i7 and i5 indexes.

Table 1. Components supplied with the 24 UDI for tagmented libraries - Set I and their indexes sequences

Concentration: 10 µM
Quantity in the tube: 12 µl (Primer pair UDI 1); 5 µl (Primer pair UDI 2 to UDI 26)
Storage: -20°C

	i(7) = reverse primer		i(5) = forward primer		
				sequence as read in sequencing	
Primer Pair UDI #	sequence as in primer	sequence as read in sequencing	sequence as in primer	i5 bases for sample sheet Forward Strand Workflow NovaSeq 6000 v1.0, MiSeq, HiSeq 2000/2500	i5 bases for sample sheet Reverse Strand Workflow NovaSeq 6000 v1.5, iSeq, MiniSeq, NextSeq, HiSeq 3000/4000
1	AACCGCGG	CCGCGGTT	AGCGCTAG	AGCGCTAG	CTAGCGCT
2	GGTTATAA	TTATAACC	GATATCGA	GATATCGA	TCGATATC
3	CCAAGTCC	GGACTTGG	CGCAGACG	CGCAGACG	CGTCTGCG
4	TTGGACTT	AAGTCCAA	TATGAGTA	TATGAGTA	TACTCATA
5	CAGTGGAT	ATCCACTG	AGGTGCGT	AGGTGCGT	ACGCACCT
6	TGACAAGC	GCTTGTCA	GAACATAC	GAACATAC	GTATGTTC
7	CTAGCTTG	CAAGCTAG	ACATAGCG	ACATAGCG	CGCTATGT
8	TCGATCCA	TGGATCGA	GTGCGATA	GTGCGATA	TATCGCAC
9	CCTGAACT	AGTTCAGG	CCAACAGA	CCAACAGA	TCTGTTGG
10	TTCAGGTC	GACCTGAA	TTGGTGAG	TTGGTGAG	CTCACCAA
11	AGTAGAGA	TCTCTACT	CGCGGTTC	CGCGGTTC	GAACCGCG
12	GACGAGAG	CTCTCGTC	TATAACCT	TATAACCT	AGGTTATA
13	AGACTTGG	CCAAGTCT	AAGGATGA	AAGGATGA	TCATCCTT
14	GAGTCCAA	TTGGACTC	GGAAGCAG	GGAAGCAG	CTGCTTCC
15	CTTAAGCC	GGCTTAAG	TCGTGACC	TCGTGACC	GGTCACGA
16	TCCGGATT	AATCCGGA	CTACAGTT	CTACAGTT	AACTGTAG
17	CTGTATTA	TAATACAG	ATATTCAC	ATATTCAC	GTGAATAT
19	ACTTACAT	ATGTAAGT	ACTCTATG	ACTCTATG	CATAGAGT
20	GTCCGTGC	GCACGGAC	GTCTCGCA	GTCTCGCA	TGCGAGAC
22	GGAACGTT	AACGTTCC	GGAGTACT	GGAGTACT	AGTACTCC
23	AATTCTGC	GCAGAATT	ACCGGCCA	ACCGGCCA	TGGCCGGT
24	GGCCTCAT	ATGAGGCC	GTTAATTG	GTTAATTG	CAATTAAC
25	ATCTTAGT	ACTAAGAT	AACCGCGG	AACCGCGG	CCGCGGTT
26	GCTCCGAC	GTCGGAGC	GGTTATAA	GGTTATAA	TTATAACC

Table 2. Components supplied with the 24 UDI for tagmented libraries - Set II and their indexes sequences

Concentration: 10 µM
Quantity in the tube: 5 µl
Storage: -20°C

Primer Pair UDI #	i(7) = reverse primer		i(5) = forward primer		
	sequence as in primer	sequence as read in sequencing	sequence as in primer	sequence as read in sequencing	
				i5 bases for sample sheet Forward Strand Workflow NovaSeq 6000 v1.0, MiSeq, HiSeq 2000/2500	i5 bases for sample sheet Reverse Strand Workflow NovaSeq 6000 v1.5, iSeq, MiniSeq, NextSeq, HiSeq 3000/4000
28	GCGTTGGA	TCCAACGC	TTGGACTT	TTGGACTT	AAGTCCAA
29	CTTCACGG	CCGTGAAG	CAGTGGAT	CAGTGGAT	ATCCACTG
30	TCCTGTAA	TTACAGGA	TGACAAGC	TGACAAGC	GCTTGTCA
31	AGAATGCC	GGCATTCT	CTAGCTTG	CTAGCTTG	CAAGCTAG
32	GAGGCATT	AATGCCTC	TCGATCCA	TCGATCCA	TGGATCGA
33	CCTCGGTA	TACCGAGG	CCTGAACT	CCTGAACT	AGTTCAGG
35	ATGAGGCT	AGCCTCAT	AGTAGAGA	AGTAGAGA	TCTCTACT
36	GCAGAATC	GATTCTGC	GACGAGAG	GACGAGAG	CTCTCGTC
37	CACTACGA	TCGTAGTG	AGACTTGG	AGACTTGG	CCAAGTCT
38	TGTCGTAG	CTACGACA	GAGTCCAA	GAGTCCAA	TTGGACTC
40	GTTGTCCG	CGGACAAC	TCCGGATT	TCCGGATT	AATCCGGA
41	ATCCATAT	ATATGGAT	CTGTATTA	CTGTATTA	TAATACAG
42	GCTTGCGC	GCGCAAGC	TCACGCCG	TCACGCCG	CGGCGTGA
45	CATGCCAT	ATGGCATG	AAGGTACC	AAGGTACC	GGTACCTT
46	TGCATTGC	GCAATGCA	GGAACGTT	GGAACGTT	AACGTTCC
47	ATTGGAAC	GTTCCAAT	AATTCTGC	AATTCTGC	GCAGAATT
48	GCCAAGGT	ACCTTGGC	GGCCTCAT	GGCCTCAT	ATGAGGCC
49	CGAGATAT	ATATCTCG	ATCTTAGT	ATCTTAGT	ACTAAGAT
51	AACCTGTT	AACAGGTT	ATACCAAG	ATACCAAG	CTTGGTAT
52	GGTTCACC	GGTGAACC	GCGTTGGA	GCGTTGGA	TCCAACGC
53	CATTGTTG	CAACAATG	CTTCACGG	CTTCACGG	CCGTGAAG
54	TGCCACCA	TGGTGGCA	TCCTGTAA	TCCTGTAA	TTACAGGA
56	TCTCATTC	GAATGAGA	GAGGCATT	GAGGCATT	AATGCCTC
57	ACGCCGCA	TGCGGCGT	CCTCGGTA	CCTCGGTA	TACCGAGG

Table 3. Components supplied with the 24 UDI for tagmented libraries - Set III and their indexes sequences

Concentration: 10 µM
Quantity in the tube: 5 µl
Storage: -20°C

Primer Pair UDI #	i(7) = reverse primer		i(5) = forward primer		
	sequence as in primer	sequence as read in sequencing	sequence as in primer	sequence as read in sequencing	
				i5 bases for sample sheet Forward Strand Workflow NovaSeq 6000 v1.0, MiSeq, HiSeq 2000/2500	i5 bases for sample sheet Reverse Strand Workflow NovaSeq 6000 v1.5, iSeq, MiniSeq, NextSeq, HiSeq 3000/4000
59	GATAGATC	GATCTATC	ATGAGGCT	ATGAGGCT	AGCCTCAT
60	AGCGAGCT	AGCTCGCT	GCAGAATC	GCAGAATC	GATTCTGC
61	CAGTTCCG	CGGAACTG	CACTACGA	CACTACGA	TCGTAGTG
62	TGACCTTA	TAAGGTCA	TGTCGTAG	TGTCGTAG	CTACGACA
63	CTAGGCAA	TTGCCTAG	ACCACTTA	ACCACTTA	TAAGTGGT
64	TCGAATGG	CCATTCGA	GTTGTCCG	GTTGTCCG	CGGACAAC
65	CTTAGTGT	ACACTAAG	ATCCATAT	ATCCATAT	ATATGGAT
66	TCCGACAC	GTGTCGGA	GCTTGCGC	GCTTGCGC	GCGCAAGC
67	AACAGGAA	TTCCTGTT	AGTATCTT	AGTATCTT	AAGATACT
69	CCTGTGGC	GCCACAGG	CATGCCAT	CATGCCAT	ATGGCATG
70	TTCACAAT	ATTGTGAA	TGCATTGC	TGCATTGC	GCAATGCA
71	ACACGAGT	ACTCGTGT	ATTGGAAC	ATTGGAAC	GTTCCAAT
72	GTGTAGAC	GTCTACAC	GCCAAGGT	GCCAAGGT	ACCTTGGC
73	GTTAATTG	CAATTAAC	CGAGATAT	CGAGATAT	ATATCTCG
74	ACCGGCCA	TGGCCGGT	TAGAGCGC	TAGAGCGC	GCGCTCTA
75	GGAGTACT	AGTACTCC	AACCTGTT	AACCTGTT	AACAGGTT
76	AAGACGTC	GACGTCTT	GGTTCACC	GGTTCACC	GGTGAACC
77	GTCTCGCA	TGCGAGAC	CATTGTTG	CATTGTTG	CAACAATG
78	ACTCTATG	CATAGAGT	TGCCACCA	TGCCACCA	TGGTGGCA
79	GCGCCTGT	ACAGGCGC	CTCTGCCT	CTCTGCCT	AGGCAGAG
80	ATATTCAC	GTGAATAT	TCTCATTC	TCTCATTC	GAATGAGA
81	CTACAGTT	AACTGTAG	ACGCCGCA	ACGCCGCA	TGCGGCGT
82	TCGTGACC	GGTCACGA	GTATTATG	GTATTATG	CATAATAC
84	AAGGATGA	TCATCCTT	AGCGAGCT	AGCGAGCT	AGCTCGCT

NOTE: Upon receipt, store the components at the indicated temperatures.

Multiplexing and index pooling

When multiplexing less than 12 samples together the choice of the index is important to ensure a good quality sequencing. We recommend to follow Illumina's instructions (Illumina Index Adapter – Pooling guide, Document # 1000000041074 v11).

If a higher multiplexing degree is required (>12), any combination is possible regardless of the index chosen.

Related Products

Product	Reference
ChIPmentation Kit for Histones (24 rxns)	C01011009
µChIPmentation Kit for Histones (24 rxns)	C01011011
µChIPmentation Kit for Histones (8 rxns)	C01011012
TAG Kit for ChIPmentation (24 rxns)	C01011030
Tagmentase (loaded)	C01070012
Tagmentase	C01070010
Tagmentase Dilution Buffer	C01070011
ATAC-seq Kit	C01080002
iDeal CUT&Tag for Histones kit	C01070020
pA-Tn5 Transposase - loaded	C01070001
pA-Tn5 Transposase - unloaded	C01070002

Revision history

Version	Date of modification	Description of modifications
Version 6 06_2023	June 2023	Correction of the sequence - UDI 30.
Version 6 02_2023	February 2023	Modification of the tables with indexes sequences: presentation as “sequence as in primer” and as “sequence as read in sequencing”, page 11-16
Version 6 02_2022	February 2022	1. UDI for tagmented libraries set I – III: modification of the sets content 2. Removing the set IV of the UDI 3. Modification of the section “Multiplexing and index pooling” for UDI.

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