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A Hologic Company

D-Plex Unique Dual Indexes Module

Unique dual indexes with UMI for D-Plex RNA-seq kits

Cat. No. C05030021 (Set A: 24 UDIs, 24 rxns)
C05030022 (Set B: 24 UDIs, 24 rxns)
C05030023 (Set C: 24 UDIs, 24 rxns)
C05030024 (Set D: 24 UDIs, 24 rxns)

USER GUIDE

Version 2 09_2022

Please read D-Plex RNA-seq manual carefully before starting your experiment

Summary

Introduction

The Diagenode D-Plex Unique Dual Indexes are an essential piece of the D-Plex suite of library preparation kits for RNA sequencing. These unique dual indexes (UDI) were designed and validated for use in library preparation workflow using the **D-Plex technology**. The modules are compatible with the following D-Plex RNA-seq Kits for **Illumina sequencing**:

- D-Plex Small RNA-seq Kit (Cat. No. C05030001)
- D-Plex Total RNA-seq Kit (Cat. No. C05030031)
- D-Plex mRNA-seq Kit (Cat. No. C05030033)

The D-Plex technology utilizes the innovative **capture and amplification by tailing and switching**, a ligation-free method for library preparation and offers key advantages such as:

- **Low to ultra-low input** capability
- Ease of use in a **one day, one tube protocol**
- **High performance** and **library complexity**

Each D-Plex Unique Dual Indexes module – Set A, B, C or D – includes 24 primer pairs with **unique dual barcodes** (unique i5 and i7 indexes) for library multiplexing up to 24. Four sets – Set A, B, C and D – can be used simultaneously for library **multiplexing up to 96**. The use of UDI is highly recommended to mitigate errors introduced by read misassignment, including index hopping frequently observed with patterned flow cells such as Illumina's NovaSeq system.

Materials

Table 1. D-Plex Unique Dual Indexes Sequence – Set A (1-24)

D-Plex Primer UDI #	PCR Reverse Primer	PCR Forward Primer	
	i7 Bases for sample sheet	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	CCGCGGTT	AGCGCTAG	CTAGCGCT
2	TTATAACC	GATATCGA	TCGATATC
3	GGACTTGG	CGCAGACG	CGTCTGCG
4	AAGTCCAA	TATGAGTA	TACTCATA
5	ATCCACTG	AGGTGCGT	ACGCACCT
6	GCTTGTCA	GAACATAC	GTATGTTC
7	CAAGCTAG	ACATAGCG	CGCTATGT
8	TGGATCGA	GTGCGATA	TATCGCAC
9	AGTTCAGG	CAACAGA	TCTGTTGG
10	GACCTGAA	TTGGTGAG	CTCACCAA
11	TCTCTACT	CGCGGTTC	GAACCGCG
12	CTCTCGTC	TATAACCT	AGGTTATA
13	CCAAGTCT	AAGGATGA	TCATCCTT
14	TTGGACTC	GGAAGCAG	CTGCTTCC
15	GGCTTAAG	TCGTGACC	GGTCACGA
16	AATCCGGA	CTACAGTT	AACTGTAG
17	TAATACAG	ATATTCAC	GTGAATAT
18	CGGCGTGA	GCGCCTGT	ACAGGCGC
19	ATGTAAGT	ACTCTATG	CATAGAGT
20	GCACGGAC	GTCTCGCA	TGCGAGAC
21	GGTACCTT	AAGACGTC	GACGTCTT
22	AACGTTCC	GGAGTACT	AGTACTCC
23	GCAGAATT	ACCGGCCA	TGGCCGGT
24	ATGAGGCC	GTTAATTG	CAATTAAC

Table 2. D-Plex Unique Dual Indexes Sequence – Set B (25-48)

D-Plex Primer UDI #	PCR Reverse Primer	PCR Forward Primer	
	i7 Bases for sample sheet	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
25	ACTAAGAT	AACCGCGG	CCGCGGTT
26	GTCGGAGC	GGTTATAA	TTATAACC
27	CTTGGTAT	CCAAGTCC	GGACTTGG
28	TCCAACGC	TTGGACTT	AAGTCCAA
29	CCGTGAAG	CAGTGGAT	ATCCACTG
30	TTACAGGT	TGACAAGC	GCTTGTCA
31	GGCATTCT	CTAGCTTG	CAAGCTAG
32	AATGCCTC	TCGATCCA	TGGATCGA
33	TACCGAGG	CCTGAACT	AGTTCAGG
34	CGTTAGAA	TTCAGGTC	GACCTGAA
35	AGCCTCAT	AGTAGAGA	TCTCTACT
36	GATTCTGC	GACGAGAG	CTCTCGTC
37	TCGTAGTG	AGACTTGG	CCAAGTCT
38	CTACGACA	GAGTCCAA	TTGGACTC
39	TAAGTGGT	CTTAAGCC	GGCTTAAG
40	CGGACAAC	TCCGGATT	AATCCGGA
41	ATATGGAT	CTGTATTA	TAATACAG
42	GCGCAAGC	TCACGCCG	CGGCGTGA
43	AAGATACT	ACTTACAT	ATGTAAGT
44	GGAGCGTC	GTCCGTGC	GCACGGAC
45	ATGGCATG	AAGGTACC	GGTACCTT
46	GCAATGCA	GGAACGTT	AACGTTCC
47	GTTCCAAT	AATTCTGC	GCAGAATT
48	ACCTTGGC	GGCCTCAT	ATGAGGCC

Table 3. D-Plex Unique Dual Indexes Sequence – Set C (49-72)

D-Plex Primer UDI #	PCR Reverse Primer	PCR Forward Primer	
	i7 Bases for sample sheet	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
49	ATATCTCG	ATCTTAGT	ACTAAGAT
50	GCGCTCTA	GCTCCGAC	GTCGGAGC
51	AACAGGTT	ATACCAAG	CTTGGTAT
52	GGTGAACC	GCGTTGGA	TCCAACGC
53	CAACAATG	CTTCACGG	CCGTGAAG
54	TGGTGGCA	TCCTGTAA	TTACAGGA
55	AGGCAGAG	AGAATGCC	GGCATTCT
56	GAATGAGA	GAGGCATT	AATGCCTC
57	TGCGGCGT	CCTCGGTA	TACCGAGG
58	CATAATAC	TTCTAACG	CGTTAGAA
59	GATCTATC	ATGAGGCT	AGCCTCAT
60	AGCTCGCT	GCAGAATC	GATTCTGC
61	CGGAACTG	CACTACGA	TCGTAGTG
62	TAAGGTCA	TGTCGTAG	CTACGACA
63	TTGCCTAG	ACCACTTA	TAAGTGGT
64	CCATTCGA	GTTGTCCG	CGGACAAC
65	ACACTAAG	ATCCATAT	ATATGGAT
66	GTGTCGGA	GCTTGCGC	GCGCAAGC
67	TTCCTGTT	AGTATCTT	AAGATACT
68	CCTTCACC	GACGCTCC	GGAGCGTC
69	GCCACAGG	CATGCCAT	ATGGCATG
70	ATTGTGAA	TGCATTGC	GCAATGCA
71	ACTCGTGT	ATTGGAAC	GTTCCAAT
72	GTCTACAC	GCCAAGGT	ACCTTGGC

Table 4. D-Plex Unique Dual Indexes Sequence – Set D (73-96)

D-Plex Primer UDI #	PCR Reverse Primer	PCR Forward Primer	
	i7 Bases for sample sheet	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
73	CAATTAAC	CGAGATAT	ATATCTCG
74	TGGCCGGT	TAGAGCGC	GCGCTCTA
75	AGTACTCC	AACCTGTT	AACAGGTT
76	GACGTCTT	GGTTCACC	GGTGAACC
77	TGCGAGAC	CATTGTTG	CAACAATG
78	CATAGAGT	TGCCACCA	TGGTGGCA
79	ACAGGCGC	CTCTGCCT	AGGCAGAG
80	GTGAATAT	TCTCATTC	GAATGAGA
81	AACTGTAG	ACGCCGCA	TGCGGCGT
82	GGTCACGA	GTATTATG	CATAATAC
83	CTGCTTCC	GATAGATC	GATCTATC
84	TCATCCTT	AGCGAGCT	AGCTCGCT
85	AGGTTATA	CAGTTCCG	CGGAACTG
86	GAACCGCG	TGACCTTA	TAAGGTCA
87	CTCACCAA	CTAGGCAA	TTGCCTAG
88	TCTGTTGG	TCGAATGG	CCATTCGA
89	TATCGCAC	CTTAGTGT	ACACTAAG
90	CGCTATGT	TCCGACAC	GTGTCGGA
91	GTATGTTC	AACAGGAA	TTCCTGTT
92	ACGCACCT	GGTGAAGG	CCTTCACC
93	TACTCATA	CCTGTGGC	GCCACAGG
94	CGTCTGCG	TTCACAAT	ATTGTGAA
95	TCGATATC	ACACGAGT	ACTCGTGT
96	CTAGCGCT	GTGTAGAC	GTCTACAC

Table 5: Module Content

Component	Cap color	Quantity	Storage temperature
D-Plex Primer UDI (x24)	Black	20 µl each (= 1 rxn/UDI)	-20°C/-4°F

Multiplexing Advices

The D-Plex PCR primers in Tables 1 to 4 bear the TruSeq (Illumina) HT adapters that can be used for library multiplexing up to 96.

In case of a multiplexing scenario, we recommend to follow the numerical order of the indexes and submit the D-Plex libraries as TruSeq HT libraries to your sequencing provider. For example, if 8 samples must be multiplexed together, one can simply select a sub-set of 8 indexes following a consecutive order from the D-Plex Unique Dual Indexes module – Set A or from the D-Plex Unique Dual Index module – Set B, C or D.

Related Products

Product	Reference
D-Plex Small RNA-seq Kit	C05030001
D-Plex Total RNA-seq Kit	C05030031
D-Plex mRNA-seq Kit	C05030033

Revision history

Version	Date of modification	Description of modifications
Version 2 09_2022	Septembre 2022	Addition of the information related to the Sets C & D
Version 1 03_2021	March 2021	Creation of the manual

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