diagenode

Innovating Epigenetics Solutions

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)

USER GUIDE

Version 1 03_2021

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 or Set B – includes 24 primer pairs with **unique dual barcodes** (unique i5 and i7 indexes) for library multiplexing up to 24. Both sets – Set A and Set B – can be used simultaneously for library **multiplexing up to 48**. 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.

These UDI also include a **unique molecular identifier (UMI)** sequence for more accurate profiling of the transcriptome. The use of UMI enables the identification and removal of PCR errors or duplicates from amplified libraries, thus improving the transcript expression quantification.

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	CCAACAGA	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

(*) = phosphorothioate bond

Table 2: D-Plex Unique Dual Index 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

(*) = phosphorothioate bond

Table 3: Module content

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

Multiplexing Advices

The D-Plex PCR primers in Table 1 and 2 bear the TruSeq (Illumina) HT adapters that can be used for library **multiplexing up to 48**.

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 have to 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.

Related Products

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

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