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A Hologic Company

D-Plex Single Indexes Module

Indexes modules for D-Plex Small RNA-seq Kit

Cat. No. C05030010 (Set A: 24 indexes, 24 rxns)

C05030011 (Set B: 24 indexes, 24 rxns)

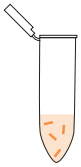
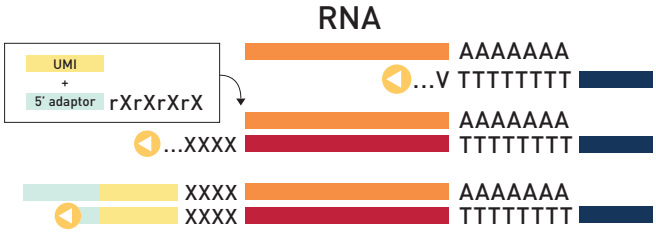
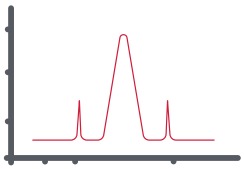
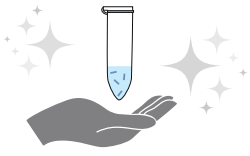
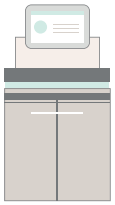
USER GUIDE

Version 2 03_2021

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

Summary

Kit Method Overview

			Time	Pause
STEP 1		RNA sample preparation	5 min	❄
STEP 2		RNA tailing	45 min	
STEP 3		Reverse transcription with template switching	>2h	❄
STEP 4		PCR amplification	45 min	❄
STEP 5		Library purification		
STEP 6		Library clean-up and shaping		
STEP 7		Library quality control and quantification		

Introduction

The Diagenode D-Plex Small RNA-seq Library Preparation Kit is a tool designed for the study of the **small non-coding transcriptome**. The present kit incorporates the unique **D-Plex technology** to generate small RNA libraries for **Illumina sequencing**.

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:

- **Ultra-low input** capability of the library preparation
- Ease of use in a **one day, one tube protocol**
- **Higher library complexity** than most of other available library preparation kits for small RNA-sequencing

The library preparation protocol works on either total intact RNAs (RIN≥8) extracted and purified from a given sample or a small RNA fraction (<200nt), that might very well represent the circulating content of a **liquid biopsy-type of sample** (blood serum and plasma). The input requirements of the method are flexible and allow the user to perform the method within a wide range of RNA quantities going **from 10 pg** of small RNAs (<200nt) or circulating RNAs **up to 100 ng** of total RNAs.

The core of the technology relies on **ligation-free reactions** to attach the Illumina adaptors to both ends of the library construct. Therefore, the results generated with the D-Plex Small RNA-seq kit will vastly differ from those produced with ligase-based approach. For instance, the results generated with the D-Plex kit will encompass a **vast spectrum of small non-coding RNAs** (miRNAs, snoRNAs, snRNAs, piRNAs) whereas a ligase-based approach will enrich the sequencing library in 5'-P – 3'-OH RNAs, mainly mature miRNAs.

Diagenode therefore recommends having a **clear understanding of the scientific question** being asked in a given experiment before proceeding to a small RNA-seq library preparation as the choice of technology will strongly impact the end result.

For optimal workflow flexibility, the library preparation is available in both **unique dual index (UDI)** and **single index (SI)** configurations. The D-Plex Unique Dual Indexes Modules (C05030021 and C05030022) and the D-Plex Single Indexes Modules (C05030010 and C05030011) are available separately from the library preparation kit, providing PCR primers 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.

An important addition to the D-Plex set of features is the use of **unique molecular identifiers (UMI)** to each transcript incorporated in the library. Given this new addition, it is now possible to exclude PCR duplicates from a set of reads, thus improving the transcript expression quantification.

Materials

Table 1: D-Plex Small RNA-seq Single Indexes Sequence - Set A (1-24)

D-Plex SI Reverse Primer Index #	PCR reverse primer sequence	Index
1	CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	ATCACG
2	CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CGATGT
3	CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TTAGGC
4	CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TGACCA
5	CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	ACAGTG
6	CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	GCCAAT
7	CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CAGATC
8	CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	ACTTGA
9	CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	GATCAG
10	CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TAGCTT
11	CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	GGCTAC
12	CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CTTGTA
13	CAAGCAGAAGACGGCATACGAGATTTGACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	AGTCAA
14	CAAGCAGAAGACGGCATACGAGATGGAACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	AGTTCC
15	CAAGCAGAAGACGGCATACGAGATTGACATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	ATGTCA
16	CAAGCAGAAGACGGCATACGAGATGGACGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CCGTCC
17	CAAGCAGAAGACGGCATACGAGATCTCTACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	GTAGAG
18	CAAGCAGAAGACGGCATACGAGATGCGGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	GTCCGC
19	CAAGCAGAAGACGGCATACGAGATTTTCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	GTGAAA
20	CAAGCAGAAGACGGCATACGAGATGGCCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	GTGGCC
21	CAAGCAGAAGACGGCATACGAGATCGAAACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	GTTTCG
22	CAAGCAGAAGACGGCATACGAGATCGTACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CGTACG
23	CAAGCAGAAGACGGCATACGAGATCCACTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	GAGTGG
24	CAAGCAGAAGACGGCATACGAGATGCTACCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	GGTAGC

(*) = phosphorothioate bond

Table 2: D-Plex Small RNA-seq Single Indexes Sequence - Set B (25-48)

D-Plex SI Reverse Primer Index #	PCR reverse primer sequence	Index
25	CAAGCAGAAGACGGCATACGAGATATCAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	ACTGAT
26	CAAGCAGAAGACGGCATACGAGATGCTCATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	ATGAGC
27	CAAGCAGAAGACGGCATACGAGATAGGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	ATTCCT
28	CAAGCAGAAGACGGCATACGAGATCTTTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CAAAAG
29	CAAGCAGAAGACGGCATACGAGATTAGTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CAACTA
30	CAAGCAGAAGACGGCATACGAGATCCGGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CACCGG
31	CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CACGAT
32	CAAGCAGAAGACGGCATACGAGATTGAGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CACTCA
33	CAAGCAGAAGACGGCATACGAGATCGCCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CAGGCG
34	CAAGCAGAAGACGGCATACGAGATGCCATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CATGGC
35	CAAGCAGAAGACGGCATACGAGATAAAATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CATTTT
36	CAAGCAGAAGACGGCATACGAGATTGTTGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CCAACA
37	CAAGCAGAAGACGGCATACGAGATATTCCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CGGAAT
38	CAAGCAGAAGACGGCATACGAGATAGCTAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CTAGCT
39	CAAGCAGAAGACGGCATACGAGATGTATAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CTATAC
40	CAAGCAGAAGACGGCATACGAGATTCTGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CTCAGA
41	CAAGCAGAAGACGGCATACGAGATGTCGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	GACGAC
42	CAAGCAGAAGACGGCATACGAGATCGATTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TAATCG
43	CAAGCAGAAGACGGCATACGAGATGCTGTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TACAGC
44	CAAGCAGAAGACGGCATACGAGATATTATAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TATAAT
45	CAAGCAGAAGACGGCATACGAGATGAATGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TCATTC
46	CAAGCAGAAGACGGCATACGAGATTCGGGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TCCCGA
47	CAAGCAGAAGACGGCATACGAGATCTTCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TCGAAG
48	CAAGCAGAAGACGGCATACGAGATTGCCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TCGGCA

(*) = phosphorothioate bond

Table 3: Module content

Component	Cap color	Quantity	Storage
D-Plex Forward Primer FP (x1)	Blue	240 µl	-20°C/-4°F
D-Plex Reverse Primer Index RP (x24)	Blue	30 µl each	-20°C/-4°F
RT Primer H (RTPH)	Purple	24 µl	-20°C/-4°F
RT Primer M (RTPM)	Purple	24 µl	-20°C/-4°F
Small Template Switching Oligo (STSO)	Purple	48 µl	-20°C/-4°F

Important Notice

The RT Primer H, RT Primer M and Small Template Switching Oligo components included in the D-Plex Small RNA-seq kit (C05030001) are only suitable for unique dual index (UDI) library construction.

For single index (SI) library construction, the RT Primer H, RT Primer M and Small Template Switching Oligo components suitable for SI library constructions are included in the D-Plex Single Indexes modules (C05030010 and C05030011). You should use the components corresponding to the desired – UDI or SI – library construction.

Multiplexing Advices

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

In case of a multiplexing scenario, we recommend to follow Illumina's library pooling guidelines that are explained in Table 4 and submit the D-Plex libraries as TruSeq small RNA libraries to your sequencing provider.

Table 4: Multiplexing recommendations for the D-Plex Small RNA-seq SI library construction

Level of multiplexing	Option #	For index set A (#1-24)
2	1	RP#6 + RP#12
3	1	RP#1 + RP#3 + RP#7
	2	RP#2 + RP#4 + RP#8
	3	RP#16 + RP#17 + RP#18
	4	RP#13 + RP#17 + RP#23
	5	2-plex with any other index
4	1	RP#2 + RP#9 + RP#10 + RP#11
	2	RP#4 + RP#5 + RP#6 + RP#7
	3	3-plex with any other index
Level of multiplexing	**Option #**	**For index set B (#25-48)**
2	1	RP#37 + RP#45
3	1	RP#38 + RP#44 + RP#46
	2	RP#40 + RP#47 + RP#48
	3	2-plex with any other index
4	1	RP#37 + RP#39 + RP#42 + RP#43
	2	RP#37 + RP#38 + RP#45 + RP#46
	3	3-plex with any other index

Related Products

Product	Reference
D-Plex Small RNA-seq Kit	C05030001
D-Plex Unique Dual Indexes Module – Set A	C05030021
D-Plex Unique Dual Indexes Module – Set B	C05030022

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