diagenode

Innovating Epigenetics Solutions

D-Plex 24 DNBSEQ™ Barcodes

Barcodes modules for D-Plex Small RNA DNBSEQ™ Kit

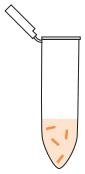
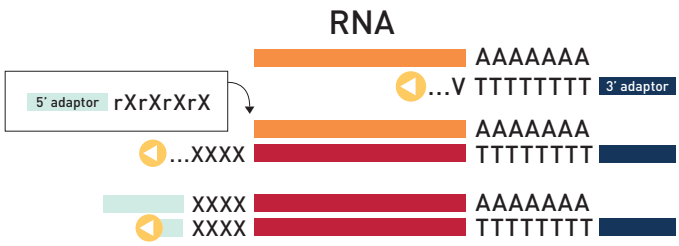
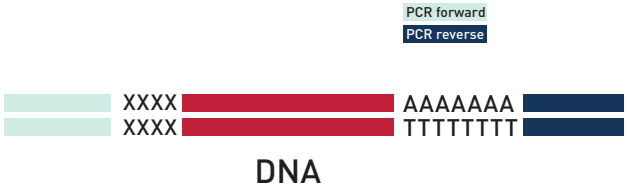
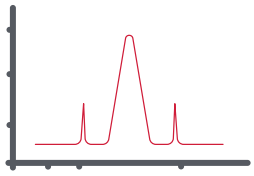
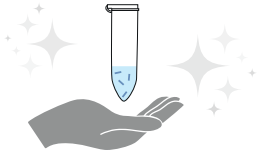
Cat. No. C05030060 (Set A: [4, 4, 8, 8])
C05030061 (Set B: [24])

USER GUIDE

V1 12_2020

Summary

Kit Method Overview

			Time	Pause
STEP 1		RNA sample preparation	20 min	❄
STEP 2		RNA dephosphorylation and tailing	1h30	
STEP 3		Reverse transcription	>2h	❄
STEP 4		PCR amplification	60 min	❄
STEP 5		Library purification		
STEP 6		Library clean-up and shaping		
STEP 7		Library control and quantification		

Introduction

The Diagenode D-Plex Small RNA DNBSEQ™ 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 double-stranded DNA libraries from RNA samples ready to be used for the DNA single-strand circularization step required for **DNBSEQ sequencing** on MGI sequencers.

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**
- Ease of use in a **one day, one tube protocol**
- **High library complexity**

The library preparation protocol works on either total intact RNA (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 circulating RNA **up to 100 ng** for total RNA.

The core of the technology relies on **ligation-free reactions** to attach the MGI adaptors to both ends of the library construct. Therefore, the results generated with the D-Plex Small RNA DNBSEQ kit will vastly differ from those produced with a ligase-based approach. For instance, the results generated with a 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.

Kit Materials

Table 1. D-Plex 24 DNBSEQ Barcode sequences – Set A [4, 4, 8, 8]

x-Barcode Set	D-Plex DNBSEQ Barcode #	PCR Reverse Primer sequence	Barcode
4	1	TGTGAGCCAAGGAGTTGATCGGACCTATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	TAGGTCCGAT
	2	TGTGAGCCAAGGAGTTGGATTCCGTCCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	GGACGGAATC
	3	TGTGAGCCAAGGAGTTGCGGCAGTAAGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	CTTACTGCCG
	4	TGTGAGCCAAGGAGTTGTCAATTAGGTTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	ACCTAATTGA
4	13	TGTGAGCCAAGGAGTTGCGGATTGCCGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	CGGCAATCCG
	14	TGTGAGCCAAGGAGTTGGAATCCTGATTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	ATCAGGATTC
	15	TGTGAGCCAAGGAGTTGTCTGGAATGATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	TCATTCCAGA
	16	TGTGAGCCAAGGAGTTGATCCAGCATCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	GATGCTGGAT
8	57	TGTGAGCCAAGGAGTTGTCCGTTGAATTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	ATTCAACGGA
	58	TGTGAGCCAAGGAGTTGCAGTACAGTTTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	AACTGTACTG
	59	TGTGAGCCAAGGAGTTGATTGAGGTACTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	GTACCTCAAT
	60	TGTGAGCCAAGGAGTTGATTAGAAGTCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	GACTTCTAAT
	61	TGTGAGCCAAGGAGTTGCAACGCTTCATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	TGAAGCGTTG
	62	TGTGAGCCAAGGAGTTGGGATCGCACGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	CGTGCGATCC
	63	TGTGAGCCAAGGAGTTGTGCCTTCCGATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	TCGGAAGGCA
	64	TGTGAGCCAAGGAGTTGGCGACATCGGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	CCGATGTCGC
8	65	TGTGAGCCAAGGAGTTGCATTCTAAGTTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	ACTTAGAATG
	66	TGTGAGCCAAGGAGTTGCAGGCTTGGATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	TCCAAGCCTG
	67	TGTGAGCCAAGGAGTTGATCATCGTCTTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	AGACGATGAT
	68	TGTGAGCCAAGGAGTTGGTCTTGTGAGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	CTCACAAGAC
	69	TGTGAGCCAAGGAGTTGAGTAGGAACGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	CGTTCCTACT
	70	TGTGAGCCAAGGAGTTGTCACAACCACTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	GTGGTTGTGA
	71	TGTGAGCCAAGGAGTTGGCAGGCCTTCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	GAAGGCCTGC
	72	TGTGAGCCAAGGAGTTGTGGCAAGCTATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	TAGCTTGCCA

Table 2. D-Plex 24 DNBSEQ Barcode sequences – Set B [24]

x-Barcode Set	D-Plex DNBSEQ Barcode #	PCR Reverse Primer sequence	Barcode
24	25	TGTGAGCCAAGGAGTTGTTGTCCTCTATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	TAGAGGACAA
	26	TGTGAGCCAAGGAGTTGATTCGCTAGGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	CCTAGCGAAT
	28	TGTGAGCCAAGGAGTTGACAGCTCAGCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	GCTGAGCTGT
	29	TGTGAGCCAAGGAGTTGTATCTAGGTTTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	AACCTAGATA
	30	TGTGAGCCAAGGAGTTGGAGATGGCAATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	TTGCCATCTC
	32	TGTGAGCCAAGGAGTTGGCCGATAGCGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	CGCTATCGGC
	33	TGTGAGCCAAGGAGTTGCCATCGTTGCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	GCAACGATGG
	34	TGTGAGCCAAGGAGTTGTGAACGATTATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	TAATCGTTCA
	35	TGTGAGCCAAGGAGTTGTAGAGCGAACTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	GTTCGCTCTA
	36	TGTGAGCCAAGGAGTTGATGTGTGAGATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	TCTCACACAT
	37	TGTGAGCCAAGGAGTTGATCCTAACAGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	CTGTTAGGAT
	38	TGTGAGCCAAGGAGTTGCGCGTCTGCGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	CGCAGACGCG
	39	TGTGAGCCAAGGAGTTGGATGATCCTTTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	AAGGATCATC
	49	TGTGAGCCAAGGAGTTGCTGCGTACATTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	ATGTACGCAG
	50	TGTGAGCCAAGGAGTTGATCTCATTAATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	TTAATGAGAT
	51	TGTGAGCCAAGGAGTTGAAGTGGCGCATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	TGCGCCACTT
	52	TGTGAGCCAAGGAGTTGGGCCTTAATGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	CATTAAGGCC
	53	TGTGAGCCAAGGAGTTGTCTGAGGCGGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	CCGCCTCAGA
	55	TGTGAGCCAAGGAGTTGGATAACCGGCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	GCCGGTTATC
	56	TGTGAGCCAAGGAGTTGTCAATATTCCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	GGAATATTGA
	114	TGTGAGCCAAGGAGTTGCTGACTCTGGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	CCAGAGTCAG
	115	TGTGAGCCAAGGAGTTGACTGCCTGTTTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	AACAGGCAGT
	116	TGTGAGCCAAGGAGTTGGTCATGGAGCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	GCTCCATGAC
	117	TGTGAGCCAAGGAGTTGGGATAGACATTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT	ATGTCTATCC

Table 3. Kit content

Reagent	Quantity	Storage temperature
D-Plex Forward Primer DNBSEQ (FP) (x1)	240 µl	-20°C/4°F
D-Plex Reverse Primer DNBSEQ (RP) (x24)	30 µl each	-20°C/4°F

Multiplexing Advices

The listed PCR Reverse Primers in Tables 1 and 2 are bearing the MGIEasy barcodes that can be used for library multiplexing up to 48. In case of a multiplexing scenario, it is then recommended to follow MGI's library pooling guidelines that are summarized in Table 4.

Table 4. Multiplexing recommendations for the D-Plex Small RNA DNBSEQ kit

Level of multiplexing	Instruction (Mixtures need to be prepared with equal volumes of each barcode to use them in a final volume of 10 µl for the PCR reaction)
1	**1.** Mix barcoded Reverse Primers of a 4-Barcode Set and give it to the sample. **2.** Mix barcoded Reverse Primers of a 8-Barcode Set and give it to the sample.
2	**1.** Prepare 2 different mixtures with 2 different barcoded Reverse Primers of a 4-Barcode Set and give one mixture to one sample. **2.** Prepare 2 different mixtures with 4 different barcoded Reverse Primers of a 8-Barcode Set and give one mixture to one sample.
3	For sample 1 & 2, use the method for 2-sample multiplexing above. For sample 3, use the method for 1-sample multiplexing above. **Note that you should use different Barcode Sets for samples 1, 2 and 3.**
4	**1.** Use a different barcoded Reverse Primers of a 4-Barcode Set for each sample. **2.** Prepare 4 different mixtures with 2 different barcoded Reverse Primers of a 8-Barcode Set and give one mixture to one sample.
5	For samples 1-4, use the method for 4-sample multiplexing above. For sample 5, use the method for 1-sample multiplexing above. **Note that you should use different Barcode Sets for samples 1-4 and 5.**
6	For samples 1-4, use the method for 4-sample multiplexing above. For samples 5-6, use the method for 2-sample multiplexing above. **Note that you should use different Barcode Sets for sample 1-4 and 5-6.**

Level of multiplexing	Instruction (Mixtures need to be prepared with equal volumes of each barcode to use them in a final volume of 10 µl for the PCR reaction)
7	**1.** For samples 1-4, use the method for 4-sample multiplexing above. (Use 1st Barcode Set). **2.** For samples 5-6, use the method for 2-sample multiplexing above. (Use 2nd Barcode Set). **3.** For sample 7, use the method for 1-sample multiplexing above. (Use 3rd Barcode set) **Note that you should use different Barcode Sets for samples 1-4, 5-6 and 7.**
8	Use a different barcoded Reverse Primers of a 8-Barcode Set for each sample.
8n+X (1≤n≤2; X=1-8; total 17-24)	**1.** For samples 1-8, use the method for 8-sample multiplexing above. **2.** For samples 9-8n, use the method for 8-sample multiplexing above. **3.** For samples 8n+1 - 8n+X, according to the value of X, use the methods above for X-sample multiplexing accordingly. Remember to use different Barcode Sets. **Note that you should use different Barcode sets for steps 1), 2) and 3).**
8n+X (3≤n≤5; X=1-8; total 25-48)	**1.** For samples 1-24 use a different barcoded Reverse Primer of the 24-Barcode Set for each sample. **2.** For samples 25-8n, separate the samples into groups of 8, and use the method for 8-sample multiplexing above. Remember to use different Barcode Sets. **3.** For samples 8n+1 - 8n+X, according to the value of X, use the methods above for X-sample multiplexing accordingly. Remember to use different Barcode Sets. **Note that you should use different Barcode Sets for steps 1), 2) and 3).**

Related products

Indexes Module Reference	Reference
D-Plex Small RNA DNBSEQ™ Kit	C05030059
DiaMag 0.2 mL tube magnetic rack	B04000001
DiaMag 1.5 mL tube magnetic rack	B04000003
MicroChIP DiaPure columns	C03040001

FOR RESEARCH USE ONLY.

Not intended for any animal or human therapeutic or diagnostic use.

NOTICE TO PURCHASER LIMITED LICENSE

The information provided herein is owned by Diagenode and/or its affiliates. Subject to the terms and conditions that govern your use of such products and information, Diagenode and/or its affiliates grant you a nonexclusive, nontransferable, non-sublicensable license to use such products and information only in accordance with the manuals and written instructions provided by Diagenode and/or its affiliates. You understand and agree that exceptas expressly set forth in the terms and conditions governing your use of such products, that no right or license to any patent or other intellectual property owned or licensable by Diagenode and/or its affiliates is conveyed or implied by providing these products. In particular, no right or license is conveyed or implied to use these products in combination with any product not provided or licensed to you by Diagenode and/or its affiliates for such use. Limited Use Label License: Research Use Only The purchase of this product conveys to the purchaser the limited, non-transferable right to use the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This productis for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact info@diagenode.com.

TRADEMARKS

The trademarks mentioned herein are the property of Diagenode or their respective owners. Bioanalyzer is a trademark of Agilent Technologies, Inc. Agencourt and AMPure are registered trademarks of Beckman Coulter, Inc. DNBSEQ is a trademark of Complete Genomics, Inc. M MGI is a trademark of MGI Tech Co., Ltd. SYBR is a trademark of Molecular Probes, Inc. MinElute is a trademark of Qiagen Group. Qubit and DNA AWAY are registered trademarks of Thermo Fisher Scientific.