Part No.:SOP-013-B02-210

User Manual

Version:5.0

MGIEasy UDB Primers Adapter Kit

Cat. No.: 1000022800 (16 RXN)
1000022801 (Set A, 96 RXN)
1000022802 (Set B, 96 RXN)

Kit Version: V2.0

Leading Life Science Innovation

Research Use Only

About the user manual

MGI does not make any promise of this user manual, including (but not limited to) any special commercial purpose and any reasonable implied guarantee. MGI has taken measures to guarantee the correctness of this user manual. However, MGI is not responsible for any missing parts in the manual and reserves the right to revise the manual and the reagent to improve the reliability, performance, or design.

All the pictures in this user manual are schematic diagrams and are for reference only. The content of the pictures may be slightly different from the actual product or the actual layout.

Manufacturer information

Company	Shenzhen MGI Biological Electronic Technology Co., Ltd.
Address	2/F, Building 11, Beishan Industrial Zone, No.146, Beishan Road, Yantian Street, Yantian District, Shenzhen, 518083, P.R. China
Service hotline	(+86) 4000-688-114
Email	MGI-service@mgi-tech.com
Website	http://en.mgi-tech.com

Revision history

Manual version	Kit version	Date	Description
5.0	V2.0	Apr. 2024	• Update the manufacture information • Update the manual style
4.0	V2.0	Mar. 2022	Update Manufacturer LOGO
A2	V2.0	Sep. 2021	Update the kit version to V2.0
A1	V1.0	Jan. 2021	Update contact information
A0	V1.0	Oct. 2020	Initial release

Tips Download the latest version of the manual and use it with the corresponding kit.

Search for the manual by Cat. No. or product name from the following website:

https://en.mgi-tech.com/download/files.html

Contents

1 Product overview 1
1.1 Introduction 1
1.2 Intended use 1
1.3 Applicable sequencing platforms 1
1.4 Components 1
1.5 Storage and transportation 2
1.6 User-supplied materials 3
1.7 Precautions and warnings 3
2 Instruction 4
2.1 About the UDB Adapter and PCR Primer Mix 4
2.2 Barcode pooling guide 6

1 Product overview

1.1 Introduction

MGIEasy UDB Primers Adapter Kit is specially designed for constructing libraries for the MGI platform series and is suitable for multiple sample pooled sequencing. This kit provides the adapters and PCR primers required for the dual barcode libraries construction on both for DNBSEQ and MGISEQ sequencing platforms. Set A and Set B of the kit contain a total of 192 different PCR primers with unique barcodes for up to 192 samples pooled sequencing. With the double-checked barcode split method, the kit maximizes the uniformity and accuracy of sequencing data splitting.

1.2 Intended use

This kit is specifically designed to be used with the MGIEasy library prep kits for library construction. If you want to use this kit with library prep kits from other brands, contact MGI Technical Support for suggestions or pre-testing before preparing your formal experiment.

1.3 Applicable sequencing platforms

DNBSEQ/ MGISEQ high-throughput sequencing platform series.

1.4 Components

This kit comes in three specifications: 16 RXN, Set A 96 RXN (UDB 1-96), and Set B 96 RXN (UDB 97-192). For component details, refer to the following table.

Each kit contains an information card. Relevant manuals and SDS files can be downloaded from the MGI website provided on the information card.

Table 1 MGIEasy UDB Primers Adapter Kit (16 RXN) (Cat. No.: 1000022800)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy UDB Primers Adapter Kit Cat. No.: 1000022800	UDB Adapter (10 μM)	White	80 μL/tube × 1
	UDB PCR Primer Mix 57-64, 89-96 (20 μM)	Blue	12 μL/tube × 16

Table 2 MGIEasy UDB Primers Adapter Kit (Set A 96 RXN) (Cat. No.: 1000022801)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy UDB Primers Adapter Kit Cat. No.: 1000022801	UDB Adapter (10 μM)	White	480 μL/tube × 1
	UDB PCR Primer Mix 01-96 (20 μM)	-	12 μL/well × 96

Table 3 MGIEasy UDB Primers Adapter Kit (Set B 96 RXN) (Cat. No.: 1000022802)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy UDB Primers Adapter Kit Cat. No.: 1000022802	UDB Adapter (10 μM)	White	480 μL/tube × 1
	UDB PCR Primer Mix 97-192 (20 μM)	-	12 μL/well × 96

1.5 Storage and transportation

Table 4 Kit storage and transportation temperatures

Kit	Storage temperature	Transportation temperature
MGIEasy UDB Primers Adapter Kit	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
MGIEasy UDB Primers Adapter Kit A		
MGIEasy UDB Primers Adapter Kit B		

Tips
- Production date and expiration date: refer to the label.
- For dry ice shipments, ensure that there is enough dry ice remaining after transportation.

- With proper transport, storage, and use, all components can maintain complete activity within their shelf life.

1.6 User-supplied materials

The order information for MGI products is provided in the following table. For information on other equipment and consumables, refer to the appropriate library preparation kit manual or the user-supplied materials.

Table 5 Order information for MGI products

Catalog number	Model	Name
1000020570	16 RXN	MGIEasy Dual Barcode Circularization Kit

1.7 Precautions and warnings

- This product is for research use only, not for in vitro diagnosis. Please read this manual carefully before use.
- This kit is designed for dual-barcoded library construction and is not suitable for other applications such as single-barcoded library construction.
- After constructing the library with this kit, use the MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570) to single-strand circularize the library.
- MGI dual-barcoded libraries can be sequenced with either single-barcoded sequencing or dual-barcoded sequencing. Please refer to the corresponding single-barcoded/dual-barcoded sequencing manual for specific instructions.
- UDB Adapter is double stranded. To prevent structural changes, such as denaturation, which might affect performance. Do not place the adapters above 30℃.
- Before use, mix the reagents well and centrifuge to collect the liquid at the bottom of the tubes or plates.
- It is recommended that you use pipette tips with filters to prevent cross-contamination. Use a new tip each time for pipetting different solutions or samples.
- For tube reagent, carefully open the tube cap to prevent spills or cross-contamination and close the cap immediately after use.
- For 96-well plate reagent, spray 75% alcohol and wipe the surface of the aluminum film of the plate with absorbent paper. The aluminum film is penetrable and do not touch the surface of the aluminum film with sharp objects. Pierce the aluminum film to pipette solutions for first-time use. Change tips when pipetting different solutions to avoid contamination. After use, transfer the remaining reagents to 1.5 mL centrifuge tube(s) or 0.2 mL PCR tube(s). Label the tubes clearly and store them at -20 ℃.
- Avoid skin and eyes contact with samples and reagents. Do not eat or drink the samples and reagents. In case of contact with skin and eyes, rinse immediately with plenty of water and seek medical advice.
- Conform to the local law and regulations when disposing of all samples and reagents.
- If you have questions, contact Technical Support: **MGI-service@mgi-tech.com**.

2 Instruction

2.1 About the UDB Adapter and PCR Primer Mix

Tips For detailed sequence information of each barcode, please reach out to our Technical Support team at: **MGI-service@mgi-tech.com**.

- In kits with different specifications, the UDB Adapter is the same, while the UDB PCR Primer Mix is different. Each UDB PCR Primer Mix contains two primers (barcode 1 and barcode 2, see Figure 1), corresponding to 288 unique dual-barcode combinations.
- The adapter ligation and PCR amplification process is shown in Figure 2.

Figure 1 The UDB Adapter and UDB PCR Primer Mix structure sketch map

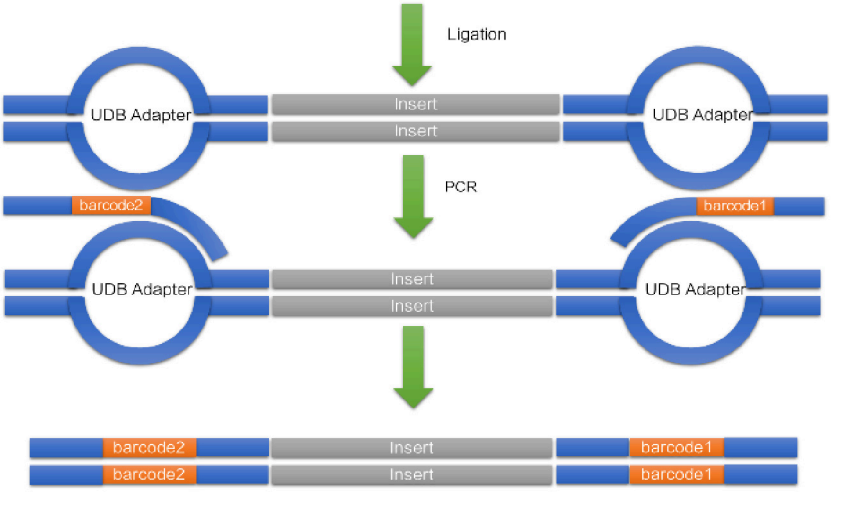

Figure 2 The UDB Adapter and UDB PCR Primer Mix library preparation process

- There are 16 tubes of UDB PCR Primer Mix in the 16 RXN kit, one UDB PCR Primer Mix per tube, 8 tubes as a group: UDB PCR Primer Mix 57 to 64 and UDB PCR Primer Mix 89 to 96.

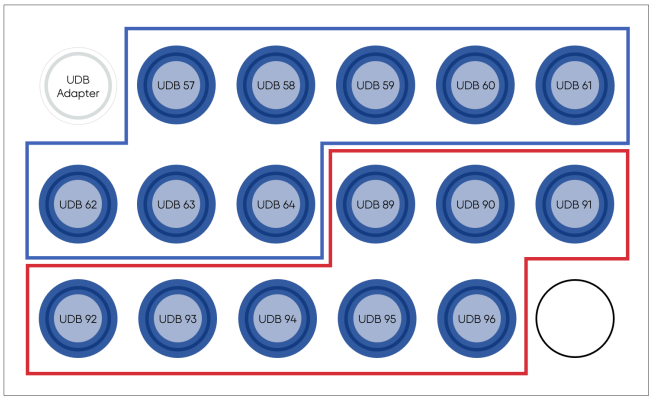

Figure 3 The UDB Adapter and PCR Primer Mix layout

- There is 1 plate of UDB PCR Primer Mix in Set A, Set B, and Set C. Each plate contains 96 UDB PCR Primer Mix, and one column is a group. The tables below show the layout of the well positions and the number of the UDB PCR Primer Mix in Set A, Set B, and Set C.

Tips The 8- and 12-column Barcode numbers of Set A overlap with those of the 16 RXN kit. The base sequences are the same and cannot be sequenced in the same lane.

Table 6 Set A UDB PCR Primer Mix layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDB1	UDB9	UDB17	UDB25	UDB33	UDB41	UDB49	**UDB57**	UDB65	UDB73	UDB81	**UDB89**
B	UDB2	UDB10	UDB18	UDB26	UDB34	UDB42	UDB50	**UDB58**	UDB66	UDB74	UDB82	**UDB90**
C	UDB3	UDB11	UDB19	UDB27	UDB35	UDB43	UDB51	**UDB59**	UDB67	UDB75	UDB83	**UDB91**
D	UDB4	UDB12	UDB20	UDB28	UDB36	UDB44	UDB52	**UDB60**	UDB68	UDB76	UDB84	**UDB92**
E	UDB5	UDB13	UDB21	UDB29	UDB37	UDB45	UDB53	**UDB61**	UDB69	UDB77	UDB85	**UDB93**
F	UDB6	UDB14	UDB22	UDB30	UDB38	UDB46	UDB54	**UDB62**	UDB70	UDB78	UDB86	**UDB94**
G	UDB7	UDB15	UDB23	UDB31	UDB39	UDB47	UDB55	**UDB63**	UDB71	UDB79	UDB87	**UDB95**
H	UDB8	UDB16	UDB24	UDB32	UDB40	UDB48	UDB56	**UDB64**	UDB72	UDB80	UDB88	**UDB96**

Table 7 Set B UDB PCR Primer Mix layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDB97	UDB105	UDB113	UDB121	UDB129	UDB137	UDB145	UDB153	UDB161	UDB169	UDB177	UDB185
B	UDB98	UDB106	UDB114	UDB122	UDB130	UDB138	UDB146	UDB154	UDB162	UDB170	UDB178	UDB186
C	UDB99	UDB107	UDB115	UDB123	UDB131	UDB139	UDB147	UDB155	UDB163	UDB171	UDB179	UDB187
D	UDB100	UDB108	UDB116	UDB124	UDB132	UDB140	UDB148	UDB156	UDB164	UDB172	UDB180	UDB188
E	UDB101	UDB109	UDB117	UDB125	UDB133	UDB141	UDB149	UDB157	UDB165	UDB173	UDB181	UDB189
F	UDB102	UDB110	UDB118	UDB126	UDB134	UDB142	UDB150	UDB158	UDB166	UDB174	UDB182	UDB190

	1	2	3	4	5	6	7	8	9	10	11	12
G	UDB103	UDB111	UDB119	UDB127	UDB135	UDB143	UDB151	UDB159	UDB167	UDB175	UDB183	UDB191
H	UDB104	UDB112	UDB120	UDB128	UDB136	UDB144	UDB152	UDB160	UDB168	UDB176	UDB184	UDB192

2.2 Barcode pooling guide

It is recommended that you optimize the base balance by planning UDB Adapter with diverse sequences when pooling libraries across DNBSEQ systems. Pooling combines at least eight libraries to sequence in one lane.

The following three application scenarios are predefined for the recommended method of selecting UDB Adapter.

1. The sequencing data output requirement is the same for all samples in one lane. Choose the UDB Adapter combinations in the table below.

Tips Here X means positive integer. For example: 8X = 8 multiplied by X , which means there are 8X samples.

Table 8 UDB Adapter pooling guide

Sample/ lane	Instruction (Example)
8X	Add 1 UDB Adapter per sample, from X set of 8 UDB Adapters (X column total). For example: X is equal to 1. The selected UDB Adapter is 393-400. Add adapter 393 to sample 1, adapter 394 to sample 2, … adapter 400 to sample 8.
8X+1	Add X set of 8 UDB Adapters + 1 random well of UDB Adapter
8X+2	Add X set of 8 UDB Adapters + 2 random wells of UDB Adapters
8X+3	Add X set of 8 UDB Adapters + 3 random wells of UDB Adapters
8X+4	Add X set of 8 UDB Adapters + 4 random wells of UDB Adapters
8X+5	Add X set of 8 UDB Adapters + 5 random wells of UDB Adapters
8X+6	Add X set of 8 UDB Adapters + 6 random wells of UDB Adapters
8X+7	Add X set of 8 UDB Adapters + 7 random wells of UDB Adapters

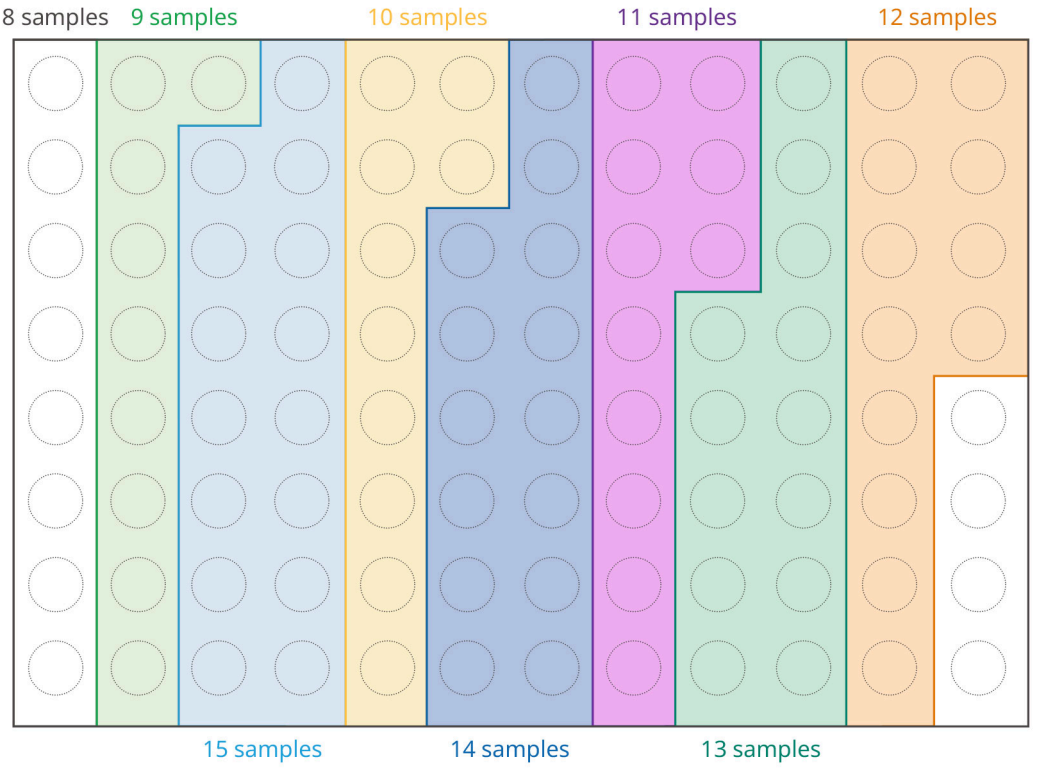

Figure 4 Dual barcode 8 to 15 samples pooling example

2. Under exceptional circumstances (for example, insufficient reagents for a well), when it cannot meet the requirement of at least one balanced UDB Adapter combination for standard pooling or if the required data amount of each library pooled is not equal, make sure to determine the pooling strategy by calculating the content of each base in each sequencing cycle. It is necessary to ensure that each base content **is not less than 12.5% and is not greater than 62.5%** in single sequencing position in the same lane.

Table 9 Balanced 8 UDB Adapter Pooling strategy (8 UDB Adapter from one entire column)

	Position of base in adapter sequence									
	Base 1	Base 2	Base 3	Base 4	Base 5	Base 6	Base 7	Base 8	Base 9	Base 10
Adapter 1	A	G	G	A	C	G	T	A	G	A
Adapter 2	C	T	G	A	A	C	C	G	A	A
Adapter 3	G	A	A	C	G	T	G	T	C	G
Adapter 4	T	C	C	G	T	G	A	C	T	C
Adapter 5	A	A	T	T	C	A	C	T	G	T
Adapter 6	C	C	T	G	A	A	G	G	A	T
Adapter 7	T	T	C	C	T	T	A	C	T	G
Adapter 8	G	G	A	T	G	C	T	A	C	C
Signal % per base	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0

Table 10 Unbalanced 9 UDB Adapter Pooling strategy (UDB Adapters from different columns)

	Position of base in adapter sequence									
	Base 1	Base 2	Base 3	Base 4	Base 5	Base 6	Base 7	Base 8	Base 9	Base 10
Adapter 1	A	G	G	A	C	G	T	A	G	T
Adapter 2	A	C	G	A	A	G	G	T	C	C
Adapter 3	G	A	A	C	G	T	G	T	C	G
Adapter 4	T	C	C	G	T	G	A	C	T	C
Adapter 5	A	A	T	T	C	A	C	T	G	T
Adapter 6	G	C	T	G	A	A	G	G	A	T
Adapter 7	T	G	C	C	T	T	A	C	T	G
Adapter 8	G	G	A	T	G	A	T	A	C	C
Adapter 9	G	A	C	G	G	T	C	G	A	G
A signal %	33.3	33.3	22.2	22.2	22.2	33.3	22.2	22.2	22.2	0
T signal %	22.2	0	22.2	22.2	22.2	33.3	22.2	33.3	22.2	33.3
C signal %	0	33.3	33.3	22.2	22.2	0	22.2	22.2	33.3	33.3
G signal %	44.4	33.3	22.2	33.3	33.3	33.3	33.3	22.2	22.2	33.3