Part No.:SOP-013-B02-189

User Manual

Version:4.0

MGIEasy Dual Barcode Exome Capture Accessory Kit

Cat. No.: 1000018647 (16 RXN)
1000018648 (96 RXN)
Kit Version: V1.0

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About the user manual

Manufacturer information

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Revision history

Manual version	Kit version	Date	Description
4.0	V1.0	Jul. 2024	• Update the manufacture information • Update the component names: Block 3 to UDB Block 3, Block 4 to UDB Block 4 • Update the manual style • Increase compatible kits and sequencing platforms
3.0	V1.0	Mar. 2022	Update the Manufacture LOGO
A2	V1.0	Mar. 2021	Update the combination of MGIEasy UDB Universal Library Prep Set
A1	V1.0	Jan. 2021	Update contact information
A0	V1.0	Mar. 2020	Initial release

Tips Please 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 Introduction

The MGIEasy Dual Barcode Exome Capture Accessory Kit offers high-quality reagents required to perform hybrid capture experiments using probes. The kit is specifically designed for the MGI high-throughput sequencing platform series and compatible with various DNA library preparation kits and commercial probes.

Tips

- Examples of combining MGIEasy Dual Barcode Exome Capture Accessory Kit with other products to give a complete library construction process required for hybridization-based target enrichment are listed in table below. Kit bundles provided by MGI are high performing.
- Select one from the three DNA library preparation kits in the following bundles, and choose one set from the two categories of probe and hybridization elution kits. Use these in combination with the capture auxiliary kit to perform hybrid capture experiments.

Table 1 The combination of kits for exome capture library construction

Combinat types	DNA library prep kit	Probes and reagents for capture	Accessory kit
1	MGIEasy Duplex UMI Universal DNA Library Prep Set	**RNA probe-related reagents:** MGIEasy Exome Capture V4 Probe Set MGIEasy Exome Capture V5 Probe Set Reagents or kits required by commercial probes for capture **DNA probe-related reagents:** MGIEasy Fast Hybridization and Wash Kit and other commercial probes for capture	MGIEasy Dual Barcode Exome Capture Accessory Kit
2	MGIEasy UDB Universal Library Prep Set		
3	MGIEasy Fast FS Library Prep V2.0 and MGIEasy Dual Barcode Circularization Kit		

1.2 Intended use

This kit provides adapter Blockers for MGISEQ/DNBSEQ platforms and PCR supplements after capture, collocated with commercial probe products of various vendors, e.g. Nimblegen, IDT, Agilent, MGI, and so on.

1.3 Applicable sequencing platforms

The prepared libraries are applicable to the following MGI sequencing platforms.

- DNBSEQ-G400 (PE100/PE150)
- DNBSEQ-T7RS (PE100/PE150)
- DNBSEQ-G99RS(PE150)

1.4 Components

This kit comes in two specifications: 16 RXN and 96 RXN. 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 2 MGIEasy Dual Barcode Exome Capture Accessory Kit (16 RXN) (Cat. No.: 1000018647)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy Dual Barcode Exome Capture Accessory Kit Cat. No.: 1000018647	Post-PCR Enzyme Mix	Blue	800 μL/tube × 1
	Dual Barcode PCR Primer Mix	Blue	96 μL/tube × 1
	UDB Block 3	Yellow	16 μL/tube × 1
	UDB Block 4	Yellow	16 μL/tube × 1

Table 3 MGIEasy Dual Barcode Exome Capture Accessory Kit (96 RXN) (Cat. No.: 1000018648)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy Dual Barcode Exome Capture Accessory Kit Cat. No.: 1000018648	Post-PCR Enzyme Mix	Blue	1200 μL/tube × 4
	Dual Barcode PCR Primer Mix	Blue	576 μL/tube × 1
	UDB Block 3	Yellow	96 μL/tube × 1
	UDB Block 4	Yellow	96 μL/tube × 1

1.5 Storage and transportation

MGIEasy Dual Barcode Exome Capture Accessory Kit
Storage temperature: -25 ℃ to -15 ℃
Transportation temperature: -80 ℃ to -15 ℃

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

Table 4 Order information for MGI products

Name	Model	Catalog number
MGIEasy Duplex UMI Universal DNA Library Prep Set	16 RXN	1000018643
	96 RXN	1000018644
MGIEasy UDB Universal Library Prep Set	16 RXN	1000022803
	96 RXN	1000022804
	192 RXN	1000022805
MGIEasy Exome Capture V4 Probe Set	16 RXN	940-000186-00
MGIEasy Exome Capture V5 Probe Set	16 RXN	940-000187-00
MGIEasy Fast FS Library PrepV2.0	16 RXN	940-001193-00
	96 RXN	940-001194-00
	192 RXN	940-001196-00
MGIEasy Fast Hybridization and Wash Kit	16 RXN	940-001974-00
	96 RXN	940-001973-00
MGIEasy Dual Barcode Circularization Kit	16 RXN	1000020570
MGIEasy DNA Clean Beads	50 mL	1000005279

Table 5 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	/
Desktop centrifuge	/
Pipettes	/

Equipment	Recommended brand
Thermocycler	/
Magnetic rack DynaMag -2, or equivalent	Thermo Fisher Scientific, Cat. No. 12321D
Eppendorf Concentrator	Eppendorf, Cat. No. 5305000398
Thermomixer or water bath equipment	/
Nutator or other nutating mixer/shaker	/

Table 6 Recommended reagent/consumable list

Reagent/consumable	Recommended brand
Nuclease Free (NF) water	Ambion, Cat. No. AM9937, or equivalent
100% Ethanol (Analytical Grade)	/
Reagents or kits required by commercial probes for capture	MGI, Nimblegen, IDT, Agilent, and so on.
Pipette tips	/
1.5 mL tube	/
0.2 mL PCR tube or 96-well plate	/
2.0 mL centrifuge tubes	/
8 Strip Domed Caps Fit 0.2 mL PCR Tube Strips	Axygen, Cat. No. PCR-02CP-C, or equivalent
Filter Tips	Axygen, Cat. No. TF-100, or equivalent
Clear Adhesive Film	ABI, Cat. No. 4306311
Blade or knife	/
Consumables required by commercial probes for capture	/

1.7 Precautions and warnings

- This product is for research use only, not for in vitro diagnosis. Please read this manual carefully before use.
- Familiarize yourself with the precautions and operation methods of various instruments before performing the experiment.
- This manual aims to provide a standard protocol. Changes can be made for different applications, but changes must be tested prior to starting the protocol.
- 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.

- It is recommended that you use the thermocyclers with heated lids for reactions. Preheat the thermocyclers to reaction temperature before use. If the thermocycler does not allow for lid temperature adjustments, the preset lid temperature of 105 ℃ is sufficient.
- Aerosol contamination may cause inaccurate results. It is recommended that you prepare separate working areas in the laboratory for PCR reaction preparation, PCR reaction, and PCR product cleanup. Use designated equipment for each area and clean the area regularly to ensure a sterile working environment (use 0.5% Sodium Hypochlorite or 10% bleach to clean the working area).
- 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 law and regulations when disposing of all samples and reagents.
- If you have questions, contact Technical Support: **MGI-service@mgi-tech.com**.

1.8 Workflow

Section	Workflow	Total time	Hands-on time
3.1	Sample preparation before capture	2 hr - 6 hr	/
3.2	Hybridization and capture	4 hr - 28 hr	/
3.3	Post-capture PCR	50 min	10 min
3.4	Cleanup of PCR product ⏸	30 - 40 min	20 - 30 min
3.5	QC of PCR product ⏸	15 - 60 min	10 - 20 min

Tips

- Total time: The theoretical use time of 8 reactions. The time will be extended if the number of reactions increases.
- Hands-on time: The total required hands-on time in the process.
- ⏸ Stop point.

2 Sample preparation

2.1 Sample requirements

The sample refers to the dual barcode library (PCR purified product) prepared using the MGIEasy series library preparation kit before hybridization.

2.2 Sample Quantitation and Quality Control

The quantitation and fragment size distribution of purified PCR products can be assessed according to the *QC of PCR Products* steps in user manuals provide by library preparation kit.

3 Protocol

3.1 Sample preparation before capture

Tips
- If you are using the MGI Exome V4 Probe or MGI Exome V5 Probe, you need to use the corresponding reagents from MGIEasy Exome Capture V4 Probe Set or MGIEasy Exome Capture V5 Probe Set and conduct the hybridization and capture according to the user manual provided by the set.
- If you are using other commercial probes for hybridization, you need to perform the hybridization and capture according to their instruction and replace the reagents that designed for other platform's adapter sequences with UDB Block 3 and UDB Block 4 from MGIEasy Dual Barcode Exome Capture Accessory Kit.

The sections 3.1-3.5 are standard experimental procedures using the NimbleGen SeqCap EZ as an example.

1. Prepare the PCR product library according to the instructions of the corresponding MGIEasy series library preparation kit.
2. For Single-Plex capture, prepare hybridization product separately for each PCR product. For Multiple-Plex capture, refer to the instructions of UDB PCR Primer Mix for detailed information about samples pooling. Mix PCR products to the required input by SeqCap EZ Library SR User's Guide.

3.2 Hybridization and capture

3.2.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 7 Preparing the reagents

Reagent	Requirement
MGI Exome V4 Probe or MGI Exome V5 Probe or RSS_SeqCap_EZ or other commercial probes	User-supplied

Reagent	Requirement
UDB Block 3	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.
UDB Block 4	

3.2.2 Block replacement notes

Following Chapter 5 Step 3 in the SeqCap EZ Library SR User Guide, change SeqCap HE Universal Oligo and SeqCap HE Index 2/4/6/8 Oligo in Step 4 to UDB Block 3 and UDB Block 4. Refer to the table below for the Usage information of UDB Block 3 and UDB Block 4.

Tips
- If the usage volume of UDB Block 3 and UDB Block 4 is larger than the volume of the reagents to be replaced in the commercial probe, it is required/strongly recommended to add these two reagents before sample concentration step.
- for example, 'SeqCap EZ Library SR User's Guide' requires performing the concentration step to reduce the mixture volume after adding the Multiplex Hybridization Enhancing Oligo Pool to the sample.

Table 8 Recommended usages of UDB Block3 and UDB Block4 for different commercial probes

Commercial probes	UDB Block 3 usage (volume)	UDB Block 4 usage (volume)	Reagents that need to be replaced in the kits
MGI Exome V4 Probe	1 μL	1 μL	N/A
MGI Exome V5 Probe	1 μL	1 μL	N/A
Kits with SureSelect series probes (SureSelect Human All Exon V6 etc.)	1 μL	1 μL	SureSelect Indexing Block #3
SeqCap EZ Human Exome Probes v3.0	1 μL	1 μL	SeqCap HE Universal Oligo
			SeqCap HE Index 2 Oligo
			SeqCap HE Index 4 Oligo
			SeqCap HE Index 6 Oligo
			SeqCap HE Index 8 Oligo
xGen Exome Research Panel	1 μL	1 μL	xGen Universal Blocking Oligo (1)
			xGen Universal Blocking Oligo (2)
			xGen Universal Blocking Oligo (3)

3.2.3 Hybridization and capture

Conduct the Hybridization capture and elution following Chapter 5-6 of the SeqCap EZ Library SR User Guide. Any reagents that are not mentioned here should be used as required in the probe user manual.

Tips After elution, the total volume of the sample solution (including beads) should be 44 μL in the next post-capture PCR step.

- If the volume is less than 44 μL in other commercial probe after elution, you need to add NF water to make a final volume of 44 μL.
- If the volume is larger than 44 μL after elution, you need to reduce the usage volume of the elution buffer.

3.3 Post-Capture PCR

3.3.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 9 Preparing the reagents

Reagent	Requirement
Post-PCR Enzyme Mix	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
Dual Barcode PCR Primer Mix	Thaw at RT, mix by vortexing, centrifuge briefly, and place at RT.

3.3.2 Post-Capture PCR

1. According to the desired reaction number, prepare the PCR mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing, centrifuge briefly, and place on ice.

Table 10 Post-capture PCR mixture

Reagent	Volume per reaction
Post-PCR Enzyme Mix	50 μL
Dual Barcode PCR Primer Mix	6 μL
Total	56 μL

2. Add 56 μL of the post-capture PCR mixture into each sample tube (44 μL sample with beads) . Mix well and centrifuge briefly to collect the solution at the bottom of the tube.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 11 Post-capture PCR reaction conditions

Temperature	Time	Cycles
105 ℃ Heated lid	On	-
95 ℃	3 min	1
98 ℃	20 sec	X
60 ℃	15 sec	
72 ℃	30 sec	
72 ℃	10 min	1
4 ℃	Hold	-

Tips The number of Post-PCR cycles is recommended in the table below, in this condition as an example, the 'X' should be 12.

Table 12 Post-capture PCR cycles for different commercial probes

Commercial probe	PCR cycles
MGI Exome V4 Probe	12
MGI Exome V5 Probe	12
SeqCap EZ Human Exome Probes v3.0	12
xGen Exome Research Panel	6 (12 pool)-10 (1 pool)
SureSelect series probes(SureSelect Human All Exon V6 etc.)	12

4. When the program is completed, centrifuge the tube(s) briefly.
5. Place the tube(s) on a magnetic rack for 2 to 5 min until the liquid becomes clear. Transfer **100 µL** of supernatant to a new 1.5 mL centrifuge tube (one tube per reaction).

3.4 Cleanup of post-capture PCR product

Tips
- For use with MGIEasy DNA Clean Beads (User-supplied). If you use the magnetic beads from other brands, optimize the cleanup conditions before getting started.
- Do not disturb or pipette the beads when adding reagents or transferring supernatant. If you accidentally disturb or pipette the beads, pipette the solution and beads back into the tube and restart the separation process.

3.4.1 Preparation

Table 13 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.

Reagent	Requirement
TE Buffer	Place at RT.
DNA Clean Beads	Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

3.4.2 Cleanup of post-capture PCR product

1. Mix the DNA Clean Beads thoroughly. Add 100 µL of DNA Clean Beads to each sample tube (from step 5 in section 3.3.2). Gently pipette at least 10 times until all beads are suspended. Ensure that all of the solution and beads in the tip are transferred into the tube after mixing. Or, mix with a vortexer.
2. Incubate the sample(s) at room temperature for 5 min.
3. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant.
4. While keeping the tube(s) on the magnetic rack, add 200 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
5. Repeat step 4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
6. Keep the tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

   Tips Over-drying the beads will result in reduced yield.

7. Remove the tube(s) from the magnetic rack and add 32 µL of TE Buffer to elute the DNA. Gently pipette the liquid at least 10 times until all beads are suspended. Or, mix with a vortexer.
8. Incubate the sample(s) at room temperature for 5 min.
9. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 30 µL of supernatant to a new 1.5 mL centrifuge tube.

   Stop point After cleanup, products can be stored at -20 ℃.

3.5 QC of post-capture PCR product

- **dsDNA fluorescence quantification method**: Quantify the purified PCR products with dsDNA fluorescence assay kits and instructions.

Table 14 Different QC methods and standards for library

Method	Equipment/Reagent	Standard
dsDNA fluorescence quantification method	Qubit dsDNA HS Assay Kit, Quant-iT PicoGreen dsDNA Assay Kit	Yield for PCR products: ≥ 1 pmol

Refer to the formula below to calculate the mass (in ng) that corresponds to 1 pmol of dsDNA sample with varying fragment sizes.

Formula 1Conversion between 1 pmol of dsDNA sample and mass in ng

Mass corresponding to 1 pmol PCR product (ng) = PCR product peak size (bp) × 0.66

For example, the desired yield for the fragmented DNA with a insert size of 300 bp (post-capture PCR products with a peak size of 432 bp; the length of UDB Primers Mix is 132 bp) should be ≥ 286 ng.

Table 15 The corresponding yield in 1 pmol for PCR products with different fragment sizes

Insert size (bp)	PCR product size /post-capture PCR product size (bp)	Corresponding yield in 1 pmol (ng)
150	282	187
200	332	220
250	382	253
300	432	286
350	482	319
400	532	352
450	582	385
500	632	418

- If the library will be sequenced on other platforms, please refer to the requirements of that platform.
- For subsequent circularization steps, please refer to the *MGIEasy Dual Barcode Circularization Kit User Manual* for ssDNA library preparation or the'Circularization Digestion' section of the MGIEasy Series Library Preparation Kit Manual for ssDNA library preparation.
- For pooled sequencing, please follow instructions provided by *MGIEasy UDB Primers Adapter Kit User Manual*. Detailed information shows how to plan your sample pooling. Quantify your post-capture PCR products before pooling. The total yield after pooling should be 1 pmol, with a total volume ≤ 48 μL.