MGI 华大智造

MGIEasy

Dual Barcode Exome Capture Accessory Kit User Manual

Cat No.: 1000018647 (16 RXN),1000018648 (96 RXN)

Kit Version: V1.0

Manual Version: A0

Revision History

Manual Version	Kit Version	Date	Description
A0	V1.0	Mar. 2020	• Initial release.

Note: Please download the latest version of the manual and use it with the corresponding kit.

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

https://en.mgitech.cn/download/files.html

Doc. #：B02-189-A0

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Chapter 1 Product Description

1.1 Introduction

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

⚠ **Note: Examples of combining MGIEasy Dual Barcode Exome Capture Accessory Kit with other products to give a complete process required for library construction based on hybridization-based target enrichment are listed in Table 1. Bundle of kits provided by MGI work with high performance.**

Table 1 The combination of kits for exome capture library construction

	DNA Library prep Kit	Probes and reagents for capture	Supplementary Kit
1	MGIEasy Duplex UMI Universal DNA Library Prep Set (1000018643/1000018644)	MGIEasy Exome Capture V4 Probe Set (1000007745)	MGIEasy Dual Barcode Exome Capture Accessory Kit (1000018647, 1000018648)
2		MGIEasy Exome Capture V5 Probe Set (1000007746)	
3		Reagents or kits required by commercial probes for capture	

1.2 Application

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

1.3 Platform Compatibility

Constructed libraries are compatible with MGISEQ/DNBSEQ (PE100/PE150)

1.4 Contents

MGIEasy Dual Barcode Exome Capture Accessory Kit includes 16 RXN and 96 RXN. Further information on Cat. No., Components and Specifications are listed below.

Table 2 MGIEasy Dual Barcode Exome Capture Accessory Kit (16 RXN) (Cat. No.: 1000018647)

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

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

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

1.5 Storage Conditions and Shelf Life

MGIEasy Dual Barcode Exome Capture Accessory Kit

- Storage Temperature: -25°C to -15°C
- Transport Conditions: transported on dry ice

* Production Date and Expiration Date: refer to the label

* Please ensure that an abundance of dry ice remains after transportation.

* Performance of products is guaranteed until the expiration date, under appropriate transport, storage, and usage conditions.

1.6 Equipment and Materials Required but not Provided

Table 3 Equipment and Materials Required but not Provided

Equipment	Vortex Mixer Desktop Centrifuge Pipets Thermocycler Magnetic rack DynaMagTM-2 (Thermo Fisher Scientific™, Cat. No. 12321D) or equivalent Magnetic rack for 96-well plate (BioMag, Cat. No. BMB-96）or equivalent Eppendorf Concentrator（Eppendorf, Cat. No. 5305000398） Thermomixer or water bath equipment Nutator or other nutating mixer/shaker
Reagents	Nuclease free water (NF water) (Ambion, Cat. No. AM9937) 100% Ethanol (Analytical Grade) Reagents or kits required by commercial probes for capture
Consumables	Pipette Tips 1.5 mL centrifuge tubes (Axygen, Cat. No. MCT-150-C) 0.2 mL PCR tubes (Axygen, Cat. No. PCR-02-C) or 96-well plate (Axygen, Cat. No. PCR-96M2-HS-C) 2.0 mL centrifuge tubes (Axygen, Cat. No. MCT-200-C) or equivalent 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

- Instructions provided in this manual are intended for general use only and may require optimization for specific applications. We recommend adjusting according to the experimental design, sample types, sequencing application, and other equipment.
- Remove the reagents from storage beforehand and prepare them for use: For enzymes, centrifuge briefly and place on ice until further use. For other reagents, first thaw at room temperature and invert several times to mix properly, then centrifuge briefly and place on ice until further use.
- To prevent cross-contamination, we recommend using filtered pipette tips. Use a new tip each time for pipetting different solutions.
- We recommend using thermocyclers with heated lids for reactions. Preheat to reaction temperature before use.
- Improper handling of samples and reagents may contribute to aerosol contamination of PCR Products and may decrease the accuracy of results. Therefore, we recommend physically separating two working areas in the laboratory for PCR reaction preparation and PCR product cleanup, respectively. Use designated equipment for each area and clean regularly to ensure a sterile working environment. (Use 0.5% Sodium Hypochlorite or 10% Bleach to clean working environment)
- If you have other questions, please contact MGI technical support: MGI-service@genomics.cn

Chapter 2 Sample Preparation

2.1 Sample Preparation

The samples used for hybridization and capture are libraries of PCR products which can be prepared by MGIEasy Duplex UMI Universal DNA Library Prep Set.

2.2 Sample Quantitation and Quality Control

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

2.3 Reagents Preparation

Before hybridization and capture experiments, take out Block 3 and Block 4, and allow them to be thawed at room temperature or on ice for later use. Conduct the hybridization and capture according to Step 3.2. Block 3 and Block 4 are designed exclusively for the MGISEQ /DNBSEQ platform. Use Block 3 and Block 4 to replace reagents applicable for other platform's adaptor sequences.

After hybridization and capture, take out the Post-PCR Enzyme Mix/PCR Primer Mix, thaw them at room temperature and keep them on ice for later use. Conduct the Post-Capture PCR according to Step 3.3.

Chapter 3 Library Construction Protocol

Note: If you are using MGI Exome V4 Probe or MGI Exome V5 Probe, then you need to use the corresponding regents 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.

Note: If you are using other commercial probes for hybridization, then you need to perform the hybridization and capture according to their instruction and replace the reagents that designed for other platform's adaptor sequences with Block 3 and Block 4 from MGIEasy Dual Barcode Exome Capture Accessory Kit. Recommended usages of Block 3 and Block 4 for different commercial probes are listed below:

Table 4 Recommended usages of Block3 and Block4 for different commercial probes

Commercial probes	Block 3 usage(volume)	Block 4 usage(volume)	Reagents that need to be replaced in the kits
MGI Exome V4 Probe	1 μL	1μL	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)

⚠ **Note: Recommended Post-Capture PCR cycles for different commercial probes are listed below:**

Table 5 Post-Capture PCR cycles for different commercial probes

Commercial probe	PCR cycles
MGI Exome V4 Probe	12 or 13
MGI Exome V5 Probe	12 or 13
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

The following steps 3.1-3.4 are standard experimental procedures using the NimbleGen® SeqCap EZ as an example.

3.1 Sample Preparation before Capture

3.1.1 Prepare libraries of PCR products following the MGIEasy Duplex UMI Universal DNA Library Prep Set user manual. According to the sample input required for SeqCap EZ hybridization, amplify samples under recommended cycles to obtain enough yields.

3.1.2 If Single-Plex capture is introduced, prepare PCR product separately for each hybridization reaction. If Multiple-Plex capture is introduced, please follow detailed information showing how to plan your sample pooling (see Appendix A), and then mix PCR products to the required input by SeqCap EZ Library SR User's Guide.

3.2 Hybridization and Capture

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

⚠ **Note: If the usage volume of Block 3 and 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, in 'SeqCap EZ Library SR User's Guide', it requires that perform the concentration step to reduce the mixture volume after adding the Multiplex Hybridization Enhancing Oligo Pool to the sample.)**

3.2.2 Conduct the Hybridization capture and elution referring to Chapter 5-6 of the SeqCap EZ Library SR User's Guide. Any reagents that are not mentioned here should be used as required

in the probe user manual.

⚠ **Note: After elution, the total volume of the sample solution (including beads) should be 44 ul in the next post-capture PCR step. If the volume is less than 44 μL in other commercial probe after elution. You need to make the final sample volume up to 44 μL with NF water. If the volume is larger than 44 μL after elution, then you need to reduce the usage volume of the elution buffer.**

3.3 Post-Capture PCR

3.3.1 Prepare the Post-capture PCR mixture on ice (see Table 6).

Table 6 Post-capture PCR Mixture

Components	Volume
Post-PCR Enzyme Mix	50 μL
Dual Barcode PCR Primer Mix	6 μL
Total	56 μL

3.3.2 Transfer 56 μL of the Post-capture PCR mixture into each of the captured sample solutions (including beads) from the step 3.2.2 and centrifuge briefly to collect the solution at the bottom of the tube.

3.3.3 Place the PCR tube(s) from step 3.3.2 into the thermocycler and run the program described in Table 7.

Table 7 Post-capture PCR Reaction Conditions

Temperature	Time	Cycles
Heated lid	on	
95°C	3 min	1 cycle
98°C	20 s	
60°C	15 s	X cycles
72°C	30 s	
72°C	10 min	1 cycle
4°C	Hold	

⚠ **Note: The number of Post-PCR cycles is recommended in Table 5, in this condition as an example, the 'X' should be 12.**

3.3.4 Centrifuge briefly to collect the solution at the bottom of the tube.

3.3.5 Place the tube(s) onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 100 µL supernatant from each tube to a new 1.5 mL Microcentrifuge tube.

3.4 Cleanup of Post-Capture PCR Product and Quantification

3.4.1 Take out DNA Clean Beads from the refrigerator and allow 30 minutes to bring the beads to room temperature. Vortex and mix thoroughly before use.

⚠ **Note: DNA Clean Beads are included in 'MGIEasy DNA Clean Beads' (MGI, Cat. No. 1000005278). Or AMPure® XP (Beckman Coulter, Cat. No. A63882) is an alternative.**

3.4.2 Transfer 100 µL DNA Clean Beads to each centrifuge tube from step 3.3.5. Pipette up and down at least 10 times to mix thoroughly. Ensure that the liquid and beads are fully dispensed from the pipette tip into the centrifuge tube before proceeding.

3.4.3 Incubate at room temperature for 5 minutes.

3.4.4 Centrifuge briefly and place the tube(s) onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.4.5 Keep the tube(s) on the Magnetic Separation Rack and add 200 µL of freshly prepared 80% ethanol to each tube to wash the beads and the walls of the tube. Incubate for 30 seconds and carefully remove and discard the supernatant.

3.4.6 Repeat step 3.4.5 once, remove all liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom, separate the beads magnetically, and remove remaining liquid using a small volume pipette.

3.4.7 Keep the centrifuge tube(s) on the Magnetic Separation Rack with the lid open, and air-dry the beads at room temperature until no wetness (reflectiveness) is observed, but before the pellet begins to crack.

3.4.8 Remove the centrifuge tube(s) from the Magnetic Separation Rack and add **32 µL** TE Buffer to each tube to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.4.9 Incubate at room temperature for 5 minutes.

3.4.10 Centrifuge briefly, then place the centrifuge tube(s) back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer **30 µL** supernatant from each tube to a different new 1.5 mL centrifuge tube.

✔ **Stopping Point: After cleanup, purified PCR Products can be stored at -20°C.**

⚠ **Note: If the library will be sequenced on MGISEQ/DNBSEQ platform, please refer to the step**

'3.13 Denaturation' from 'MGIEasy Duplex UMI Universal Library Prep Set' to finish the library construction. If the library will be sequenced on other platforms, please refer to the requirement according to the platform.

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