# MGIEasy

## Exome Capture Accessory Kit User Manual

Cat No.: 1000007743 (16 RXN)

Kit Version: V1.0

Manual Version: A1

# Revision History

| Manual Version | Kit Version | Date | Description |
|---|---|---|---|
| A1 | V1.0 | Jan. 2021 | ◆ Update contact information. |
| A0 | V1.0 | Apr. 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.mgi-tech.com/download/files.html

# Contents

# Chapter 1 Product Description

## 1.1 Introduction

The MGIEasy 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.

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

Table 1 The combination of kits for exome capture library construction

| Combination types | DNA Library prep Kit | Probes and reagents for capture | Accessory kit |
|---|---|---|---|
| 1 | MGIEasy FS DNA Library Prep Set (1000006987) | MGIEasy Exome Capture V4 Probe Set (1000007745) | MGIEasy Exome Capture Accessory Kit (1000007743) |
| 2 | | MGIEasy Exome Capture V5 Probe Set (1000007746) | |
| 3 | | Reagents or kits required by commercial probes for capture | |
| 4 | MGIEasy Universal DNA Library Prep Set (1000006985) | MGIEasy Exome Capture V4 Probe Set (1000007745) | |
| 5 | | MGIEasy Exome Capture V5 Probe Set (1000007746) | |
| 6 | | Reagents or kits required by commercial probes for capture | |

## 1.2 Application

This kit provides adapter Blockers of MGISRQ/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

BGISEQ-500RS (PE100)
MGISEQ-2000RS (PE100/PE150), DNBSEQ-G400RS (PE100/PE150)

## 1.4 Contents

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

Table 2 MGIEasy Exome Capture Accessory Kit (16 RXN) (Cat. No.: 1000007743)

| Cat. No. | Components | Cap Color | Spec & Quantity |
|---|---|---|---|
| MGIEasy Exome Capture Accessory Kit Cat. No.: 1000007743 | Post-PCR Enzyme Mix | Blue | 800 μL/ tube × 1 tube |
| | PCR Primer Mix | Blue | 96 μL/ tube × 1 tube |
| | Block 3 | Yellow | 16 μL/ tube × 1 tube |
| | Block 4 | Yellow | 160 μL/ tube × 1 tube |

## 1.5 Storage Conditions and Shelf Life

MGIEasy Exome Capture Accessory Kit

- Storage Temperature: -25°C to -18°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<br>Desktop Centrifuge<br>Pipets<br>Thermocycler<br>Magnetic rack DynaMagTM-2 (Thermo Fisher Scientific™, Cat. No. 12321D) or equivalent<br>Magnetic rack for 96-well plate (BioMag, Cat. No. BMB-96）or equivalent<br>Eppendorf Concentrator（Eppendorf, Cat. No. 5305000398）<br>Thermomixer or water bath equipment<br>Nutator or other nutating mixer/shaker |
| Reagents | Nuclease free water (NF water) (Ambion, Cat. No. AM9937)<br>100% Ethanol (Analytical Grade)<br>Reagents or kits required by commercial probes for capture |
| Consumables | Pipette Tips<br>1.5 mL centrifuge tubes (Axygen, Cat. No. MCT-150-C)<br>0.2 mL PCR tubes (Axygen, Cat. No. PCR-02-C)<br>or 96-well plate (Axygen, Cat. No. PCR-96M2-HS-C)<br>2.0 mL centrifuge tubes (Axygen, Cat. No. MCT-200-C) or equivalent<br>8 Strip Domed Caps Fit 0.2 mL PCR Tube Strips（Axygen, Cat. No. PCR-02CP-C）or equivalent<br>Filter Tips（Axygen, Cat. No. TF-100）or equivalent<br>Clear Adhesive Film (ABI, Cat. No. 4306311)<br>Blade or knife<br>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@mgi-tech.com

# 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 FS DNA Library Prep Set or MGIEasy 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 library preparation kit.

## 2.3 Reagents Preparation

Before hybridization and capture experiments, take out Block 3 and Block 4, and allow them to thaw 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 instructions and replace the reagents designed for the other platform's adaptor sequences with Block 3 and Block 4 from MGIEasy 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 | 10 μL | N/A |
| MGI Exome V5 Probe | 1 μL | 10 μL | N/A |
| Kits with SureSelect series probes (SureSelect Human All Exon V6 etc.) | 1 μL | 10 μL | SureSelect Indexing Block #3 |
| SeqCap® EZ Human Exome Probes v3.0 | 1 μL | 10 μL | SeqCap HE Universal Oligo;<br>SeqCap HE Index 2 Oligo;<br>SeqCap HE Index 4 Oligo;<br>SeqCap HE Index 6 Oligo;<br>SeqCap HE Index 8 Oligo |
| xGen Exome Research Panel | 1 μL | 10 μL | xGen® Universal Blocking Oligo (1);<br>xGen® Universal Blocking Oligo (2);<br>xGen® Universal Blocking Oligo (3) |

⚠ **Note: Recommended Post-Capture PCR cycles for different commercial probes are list 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 user manual of MGIEasy FS DNA Library Prep Set or MGIEasy Universal DNA Library Prep Set. According to the sample input required for SeqCap EZ hybridization, amplify samples under recommended cycles to obtain sufficient yield.

3.1.2 For Single-Plex capture, prepare PCR product separately for each hybridization reaction. For Multiple-Plex capture, please follow Appendix A for detailed information about sample pooling, 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 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 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, '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.)**

3.2.2 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 requi red 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, then you need to add NF water to make the final sample volume 44 μL. 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 |
| 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 recommended number of Post-PCR cycles can be found in Table 5.**

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 use AMPure® XP (Beckman Coulter, Cat. No. A63882) as 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.

3.4.11 Quantify the purified post-capture PCR products with dsDNA Fluorescence Assay Kits such as the Qubit® dsDNA HS Assay Kit or the Quant-iT™ PicoGreen® dsDNA Assay Kit. The desired yield for each PCR products is ≥1 pmol. Please refer to Formula 1 to calculate the yield. For example, the desired yield for the fragmented DNA with a peak fragment size of 300 bp (Post-

hybridization PCR products with a peak fragment size of 384 bp) should be ≥250 ng. For pooling sequencing, please follow instructions provided by MGIEasy DNA Adapters User Manual. See Appendix A for detailed information on planning sample pooling. Quantify your post-captured PCR samples before pooling. The total yield after pooling should be ≥1 pmol, with a total volume ≤48 μL.

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

$$\text{Mass (ng) corresponding to 1 pmol PCR Products} = \frac{\text{DNA Fragement Size (bp)}}{1000\ \text{bp}} \times 660\ \text{ng}$$

**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 Exome Universal Library Prep Set', or step '3.15 Denaturation' from 'MGIEasy Exome FS Library Prep Set' to finish the library construction. If the library will be sequenced on other platforms, please refer to the requirements of that platform.**

# Appendix

## Appendix A The Combination Barcode Adapters Strategies

- This set includes a MGIEasy DNA Adapters-16 (Tube) Kit. This kit was developed to meet requirements for batch processing of library construction and multiplex sequencing. We selected the best adapter combination based on the principle of balanced base composition. However, the number of Barcode Adapters are not always continuous. For optimal performance, please carefully read instructions in Appendix A-1.
- Our Adapters are double stranded. Please do not incubate above room temperature to avoid structural changes such as denaturation, which might affect performance. Before use, please centrifuge to collect liquid at the bottom of tubes. Gently remove the cap to prevent spills and cross-contamination. Mix Adapters with a pipette before you use. Remember to close the cap immediately after use.
- Adapters from other MGI Library Prep Kits (number 501-596) are designed differently and are incompatible for mixed use. Mixed use will cause errors in barcode demultiplexing in data analysis procedures.

### A-1 MGIEasy DNA Adapters-16 (Tube) Kit Instruction

Based on the principles of balanced base composition, adapters must be used in specific groups. Please follow the instructions below to use Adapters in proper combination:

2 sets of 4 Adapters: (01-04) and (13-16)

1 set of 8 Adapters: (97-104)

If the sequencing data output requirement is the same for all samples in one lane, please refer to Table 8 below to choose your barcode adapter combinations.

Table 8 MGIEasy DNA Adapters-16 (Tube) Kit Instruction

| Sample(s)/lane | Instructions (Example) |
|---|---|
| 1 | Requires at least 1 set of Adapters:<br>1. Take a set of 4 Adapters (e.g. 01-04), mix 4 Adapters with equal volumes, then add the mixture to the sample.<br>Or 2. Take a set of 8 Adapters (e.g. 97-104), mix 8 Adapters with equal volumes, then add the mixture to the sample. |

| | |
|---|---|
| 2 | Requires at least 1 set of Adapters:<br>1. Take a set of 4 Adapters (e.g. 01-04), mix Adapters with equal volumes in pairs to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 01 & 02, then add to sample 1; Mix 03 & 04, then add to sample 2)<br>Or 2. Take a set of 8 Adapters (97-104), mix Adapters with equal volumes in groups of 4 to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 97-100, then add to sample 1; Mix 101-104, then add to sample 2) |
| 3 | Requires at least 2 sets of Adapters:<br>For sample 1&2, use the method for (2 samples/lane) above. For sample 3, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for samples 1-2 and for sample 3. |
| 4 | Requires at least 1 set of Adapters:<br>1. Take a set of 4 Adapters (e.g. 01-04), add 1 Adapter to each sample in an equal volume. (e.g. Add Adapters 01, 02, 03, 04 to samples 1, 2, 3, 4, respectively.)<br>Or 2. Take a set of 8 Adapters (97-104), mix Adapters with an equal volume in pairs to obtain 4 mixtures of equal volumes. Add 1 mixture to each sample. (e.g. Mix 97-98, 99-100, 101-102, 103-104, then add respectively to samples 1, 2, 3, 4.) |
| 5 | Requires at least 2 sets of Adapters:<br>For samples 1-4, use the method for (4 samples/lane) above. For sample 5, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for samples 1-4 and for sample 5. |
| 6 | Requires at least 2 sets of Adapters:<br>For samples 1-4, use the method for (4 samples/lane) above. For sample 5-6, use the method for (2 sample/lane) above. Note that you should use different Adapter sets for samples 1-4 and for samples 5-6. |
| 7 | Requires all 3 Adapter sets and follow these 3 steps:<br>1) For samples 1-4, use the method for (4 samples/lane) above (Use 1st Adapter set).<br>2) For samples 5-6, use the method for (2 samples/lane) above (Use 2nd Adapter set).<br>3) For sample 7, use the method for (1 sample/lane) above (Use 3rd Adapter set). You can add a single Adapter within the Adapter set. Or add the Adapter mix which is mixed from all Adapters within the Adapter set with an equal volume.<br>Note that you should use different Adapter sets for samples 1-4, for samples 5-6 and for sample 7. |

| | |
|---|---|
| 8 | Requires at least 1 set of Adapters:<br>1. Take a set of 8 Adapters (97-104), respectively add 1 Adapter to each sample in an equal volume.<br>Or 2. Take 2 sets of 4 Adapters (01-04 and 13-16), add 1 Adapter to each sample in an equal volume. |

For situations in which the sequencing data output requirements are different between samples, any sample with a data output of more than 20% for each lane must use a separate set of Adapters. For example, 9 samples are pooled into 1 lane, one of which requires 30% of the total data output. In this case, the other 8 samples may use Adapters (97-104), whereas the final sample must use a full Adapter set instead of using only a single Adapter (e.g. Adapter set (01-04) or (13-16)).

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