# MGIEasy

## Duplex UMI Universal Library Prep Set

## User Manual

Cat No.: 1000018643（16RXN） 1000018644(96RXN)

Kit Version: V1.0

Manual Version: 3.0

# Revision History

| Manual Version | Kit Version | Date | Description |
|---|---|---|---|
| 3.0 | V1.0 | Mar. 2022 | ◆ Update Manufacturer LOGO. |
| A1 | V1.0 | Jan. 2021 | ◆ Update contact information. |
| 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.mgi-tech.com/download/files.html

# Contents

# Chapter 1 Product Description

## 1.1 Introduction

The MGIEasy Duplex UMI Universal Library Prep Set is specifically designed for constructing libraries for the MGI high-throughput sequencing platform series.

This library prep set is optimized to convert 10-1000 ng of fragmented DNA into a customized library and is compatible with various commercial probes for capture based on MGI sequencing platform. This set incorporates improved adapter ligation technology and high-fidelity PCR enzymes, which significantly increase library yield and conversion rate. Specifically, when sample multiplexing is performing for sequencing, the dual barcode adapter design can help mitigate barcode contamination and hopping, which effectively reduces barcode swapping within samples. In addition, dual UMI tags in the adapters can be used to analyze the original molecules in the samples in order to reduce artifacts introduced during sample preparation, target enrichment, PCR amplification, and sequencing. All reagents provided within this set have passed stringent quality control and functional verification procedures, ensuring performance stability and reproducibility.

⚠ **Note: If MGI Exome V4 Probe or MGI Exome V5 Probe is used for capture, please refer to its protocol details during library preparation and capture.**

## 1.2 Application

This library prep set is used for samples derived from Human, including gDNA, cfDNA, FFPE derived DNA etc., and provides supplement of the capture for whole exome collocated with Probes.

## 1.3 Platform Compatibility

Constructed libraries are compatible with MGISEQ-2000RS/DNBSEQ-G400RS/DNBSEQ-T7RS (PE100/PE150)

## 1.4 Contents

Two types of library preparation sets are available for 16 reactions (Cat. No.: 1000018643) and 96 reactions (Cat. No.: 1000018644). Each Set consists of four modular kits of reagents. Further information on Cat. No. Components and Specifications are listed below.

Table 1 MGIEasy Duplex UMI Universal Library Prep Set (16 RXN) (Cat. No.: 1000018643)

| Modules & Cat. No. | Components | Cap Color | Spec & Quantity |
|---|---|---|---|
| MGIEasy Universal DNA Library Prep Module(16RXN)<br>Cat. No.: 1000019376 | ERAT Buffer | Orange | 96 μL/ tube × 1 tube |
| | ERAT Enzyme Mix | Orange | 72 μL/ tube × 1 tube |
| | Ligation Buffer | Red | 336 μL/ tube × 1 tube |
| | DNA Ligase | Red | 64 μL/ tube × 1 tube |
| | PCR Enzyme Mix | Blue | 800 μL/ tube ×1 tube |
| MGIEasy Duplex UMI Adapters Kit (16RXN)<br>Cat. No.: 1000018645 | Dual UMI_Adapter Mix | White | 80 μL/ tube ×1 tube |
| | UDB PCR Primer Mix-57-64，89-96 | White | 12 μL/ tube ×16 tubes |
| MGIEasy DNA Clean Beads<br>Cat. No.: 1000005278 | DNA Clean Beads | White | 8 mL/ tube × 1 tube |
| | TE Buffer | White | 4 mL/ tube × 1 tube |
| MGIEasy Dual Barcode Circularization Module(16RXN)<br>Cat. No.: 1000018649 | Dual Barcode Splint Buffer | Purple | 223 μL/ tube × 1 tube |
| | DNA Rapid Ligase | Purple | 10 μL/ tube × 1 tube |
| | Digestion Buffer | White | 27 μL/ tube × 1 tube |
| | Digestion Enzyme | White | 50 μL/ tube × 1 tube |
| | Digestion Stop Buffer | White | 144 μL/ tube × 1 tube |

Table 2 MGIEasy Duplex UMI Universal Library Prep Set (96 RXN) (Cat. No.: 1000018644)

| Modules & Cat. No. | Components | Cap Color | Spec & Quantity |
|---|---|---|---|
| MGIEasy Universal DNA Library Prep Module(96RXN)<br>Cat. No.: 1000019377 | ERAT Buffer | Orange | 576 μL/ tube × 1 tube |
| | ERAT Enzyme Mix | Orange | 432 μL/ tube × 1 tube |
| | Ligation Buffer | Red | 1008 μL/ tube × 2 tube |
| | DNA Ligase | Red | 384 μL/ tube × 1 tube |
| | PCR Enzyme Mix | Blue | 1600 μL/ tube ×3 tube |
| MGIEasy Duplex UMI Adapters Kit(96RXN)<br>Cat. No.: 1000018645 | Dual UMI Adapter Mix | White | 480 μL/ tube ×1 tube |
| | UDB PCR Primer Mix-01-96 | White | 12 μL/ tube ×96 well |
| MGIEasy DNA Clean Beads<br>Cat. No.: 1000005278 | DNA Clean Beads | White | 50 mL/ tube × 1 tube |
| | TE Buffer | White | 25 mL/ tube × 1 tube |
| MGIEasy Dual Barcode Circularization Module(16RXN)<br>Cat. No.: 1000018649 | Dual Barcode Splint Buffer | Purple | 223 μL/ tube × 1 tube |
| | DNA Rapid Ligase | Purple | 10 μL/ tube × 1 tube |
| | Digestion Buffer | White | 27 μL/ tube × 1 tube |
| | Digestion Enzyme | White | 50 μL/ tube × 1 tube |
| | Digestion Stop Buffer | White | 144 μL/ tube × 1 tube |

## 1.5 Storage Conditions and Shelf Life

MGIEasy Universal DNA Library Prep Module

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

MGIEasy Duplex UMI Adapters Kit

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

MGIEasy Dual Barcode Circularization Module

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

MGIEasy DNA Clean Beads

- Storage Temperature: 2°C to 8°C
- Transport Conditions: transported with ice packs

* 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 | Covaris™ Focused-ultrasonicator (Thermo Fisher Scientific™)<br>Vortex Mixer<br>Desktop Centrifuge<br>Pipets<br>Thermocycler<br>Magnetic rack DynaMag™-2 (Thermo Fisher Scientific™, Cat. No. 12321D) or equivalent<br>Qubit™ 3.0 Fluorometer (Thermo Fisher Scientific™, Cat. No. Q33216)<br>Agilent 2100 Bioanalyzer (Agilent Technologies™, Cat. No. G2939AA) |
| Reagents | Nuclease free water (NF water) (Ambion, Cat. No. AM9937)<br>100% Ethanol (Analytical Grade)<br>Qubit® ssDNA Assay Kit (Invitrogen, Cat. No. Q10212)<br>Qubit® dsDNA HS Assay Kit (Invitrogen, Cat. No. Q32854)<br>High Sensitivity DNA Kits (Agilent Technologies™, Cat. No. 5067-4626)<br>Agilent DNA 1000 Kit (Agilent Technologies™, Cat. No. 5067-1504)<br>MGIEasy Duplex UMI Adapters Kit (MGI, Cat. No. 1000018645/1000018646)<br>Reagents or kits or beads required by commercial probes for capture<br>MGIEasy Dual Barcode Exome Capture Accessory Kit (1000018647/1000018648) |
| Consumables | Covaris AFA Tubes for use with Ultrasonicator<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 |

## 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 Requirement

This library preparation set is applicable for genomic DNA derived from human samples (including blood, fresh or frozen tissues, FFPE derived samples and cfDNA). It is strongly recommended to use high quality genomic DNA (gDNA) samples ($A_{260}/A_{280}$=1.8-2.0) for fragmentation.

## 2.2 DNA Fragmentation and Size Selection

### 2.2.1 Fragmentation

- Fragment gDNA into sizes between 100-700 bp, with most fragments within the recommended size range: for PE100 recommend approx. 280 bp, for PE150 recommend approx. 330 bp.
- For the MGI Exome V5 probe, it is strongly recommended to set the insert main size around 280bp, please refer to the corresponding protocols for the details
- Appendix A lists fragmentation parameters for Covaris 55 μL series Ultrasonicators. For fragmentation of other sample volumes (15 μL, 130 μL, 200 μL, etc.), please visit Covaris' official website for detailed instructions.
- If you choose other fragmentation methods, we recommend doing trial runs to determine optimal parameters for obtaining recommended fragment sizes before getting started with this set.

### 2.2.2 Magnetic Beads Size Selection

- DNA fragmentation results in a wide distribution of fragment sizes. Size selection is usually required to ensure uniformity of the library. We recommended using magnetic beads for Size Selection (see Table 3), please make sure the total volume is 80 μL after fragmentation. Gel extraction methods can also be used for the same purpose.

⚠ **Note: If FFPE derived samples are fragmented, you can skip size selection for reducing sample loss. Modifications of protocol for FFPE samples are summarized in Appendix G**

Table 4 Magnetic Bead Selection Steps for an 80 μL Sample to Target Specific Fragment Sizes

| Target peak fragment size (bp) | 180 | 230 | 280 | 335 | 420 |
|---|---|---|---|---|---|
| Library size peak (bp) | 329 | 379 | 429 | 484 | 569 |
| 1st Bead Selection (μL) | 80 | 72 | 64 | 56 | 48 |
| 2nd Bead Selection (μL) | 40 | 16 | 16 | 16 | 16 |

- In Appendix B and C, 500 ng gDNA is fragmented (80 μL). If the DNA volume post-fragmentation is less than 80 μL, add TE buffer to reach a final volume of 80 μL. Then, the 64 μL 1st bead selection and 16 μL 2nd bead selection are performed before end repair, which provides the selected fragment size of 280 bp.

- The DNA sample loss during bead selection is approximately 60%-95%. For important samples, you may retrieve the beads from the 1st bead selection process and wash twice with 80% ethanol. Air dry the bead pellet, elute DNA with TE Buffer, and store the elution product at -20°C as a backup.

⚠ **Note: If MGI Exome V4 Probe or MGI Exome V5 Probe is used for capture, please refer to its protocol details during library preparation and capture.**

## 2.3 Sample DNA Quantitation and Quality Control

- Sample DNA amount refers to the amount of DNA input that is used in the end repair process. This set is compatible with sample DNA amounts between 10-1000 ng in less than **40 μL**.

⚠ **Note: For FFPE samples, 50-1000 ng gDNA amount in less than 40 μL is recommended. See Appendix G for more details of modification according to different qualities of FFPE samples.**

- Try to ensure a narrow distribution of DNA fragment size. A narrow distribution results in higher quality of sequencing. A wide distribution lowers sequencing quality.
- This library prep set supports a range of fragment sizes (see Table 3). Sequencing quality may slightly decrease with increasing fragment sizes. Please use an appropriate insert size for library construction based on your sequencing strategy. The recommended size for inserts is around 280 bp for PE100 sequencing and around 330 bp for PE150 sequencing.

⚠ **Note: It is not recommended to pool fragments of different lengths for multiplex sequencing.**

# Chapter 3 Library Construction Protocol

The DNA sample used in this Library Construction Protocol: 500 ng of gDNA (80 μL) is fragmented with the Covaris, and the fragmented gDNA is selected with 64 μL 1st bead selection and a 16 μL 2nd bead selection beads. After size selection, about 50 ng of 280 bp DNA fragments are obtained.

Follow Table 3 in Chapter 2 Sample Requirement, Table 21 in Appendix D, and Table 23 in Appendix E to adjust this protocol for different amounts of the initial DNA sample and different sizes of target DNA fragments.

⚠ **Note: If FFPE samples are using, please read Appendix G carefully including a summary of modifications for FFPE samples before you begin. If cfDNA samples are using, please skip steps of fragmentation and directly start from step 3.1.**

## 3.1 End Repair and A-tailing

3.1.1 Transfer an appropriate amount of sample (recommended: 50 ng) to a new 0.2 mL PCR tube and add TE Buffer for a total volume of **40 μL**. Place the tube on ice.

3.1.2 Take out MGIEasy Universal DNA Library Prep Module. Prepare the end repair and A-tailing mixture in a new 0.2 mL PCR tube on ice (see Table 5).

Table 5 End Repair and A-tailing Mixture

| Components | Volume |
|---|---|
| ERAT Buffer | 6 μL |
| ERAT Enzyme Mix | 4.5 μL |
| Total | 10.5 μL |

3.1.3 Transfer 10 μL of the end repair mixture to the 0.2 mL PCR tube from step 3.1.1. Vortex 3 times (3 s each) and briefly centrifuge to collect the solution at the bottom of the tube.

3.1.4 Place the 0.2 mL PCR tube from step 3.1.3 into the thermocycler and run the program in Table 6.

Table 6 End Repair and A-tailing Reaction Conditions

| Temperature | Time |
|---|---|
| Heated lid(105°C) | On |
| 20°C | 30 min |
| 65°C | 15 min |
| 4°C | Hold |

3.1.5 Briefly centrifuge to collect the solution at the bottom of the tube.

⚠ **Warning: DO NOT STOP AT THIS STEP. Please continue to step 3.2.**

## 3.2 Adapter Ligation

⚠ **Note: Please read Appendix D carefully before you begin.**

3.2.1 Take out MGIEasy Duplex UMI Adapters Kit. Add 5 μL of Dual UMI_Adapter Mix to each PCR tube from step 3.1.5. Vortex 3 times (3 s each) and briefly centrifuge to collect solution at the bottom of the tube.

3.2.2 Prepare the Adapter ligation mixture in a new 0.2 mL PCR tube on ice (see Table 7).

Table 7 Adapter Ligation Mixture

| Components | Volume |
|---|---|
| Ligation Buffer | 21 μL |
| DNA Ligase | 4 μL |
| Total | 25 μL |

3.2.3 Pipette slowly to transfer 25 μL of Adapter ligation mixture to the PCR tube from step 3.2.1. Vortex 6 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

⚠ **Note: Due to the viscosity of the Adapter ligation mixture, please pipette slowly and ensure that the correct amount has been used.**

3.2.4 Place the 0.2 mL PCR tube from step 3.2.3 into the thermocycler and run the program in Table 8.

Table 8 Adapter Ligation Reaction Conditions

| Temperature | Time |
|---|---|
| Heated lid(50°C) | On |
| 23°C | 30 min |
| 4°C | Hold |

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

3.2.6 Add 20 μL TE Buffer, for a total volume of 100 μL, and transfer all of the solution to a new 1.5 mL centrifuge tube.

✓ **Stopping Point: Adapter-ligated DNA can be stored at -20°C for a maximum of 16 hours.**

### 3.3 Cleanup of Adapter-Ligated DNA

⚠ **Note: Please read Appendix B carefully before you begin.**

3.3.1 Please refer to the instructions in Appendix B. Take out DNA Clean Beads from refrigerator and bring to room temperature for 30 minutes beforehand. Vortex and mix thoroughly before use.

⚠ **Note: DNA Clean Beads are included in 'MGIEasy DNA Clean Beads'.**

3.3.2 Transfer 50 μL DNA Clean Beads to the centrifuge tube from step 3.2.6. Pipette up and down at least 10 times to mix thoroughly. Ensure that the solution and beads are fully dispensed from the pipette tip into the tube before proceeding.

3.3.3 Incubate at room temperature for 5 minutes.

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

3.3.5 Keep the tube on the Magnetic Separation Rack and add 200 μL freshly prepared 80% ethanol to wash the beads and the walls of the tube. Incubate for 30 seconds, then carefully remove and discard the supernatant.

3.3.6 Repeat step 3.3.4 once, remove all liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom of the centrifuge tube, separate the beads magnetically and then remove any remaining liquid using a small volume pipette.

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

3.3.8 Remove the centrifuge tube from the Magnetic Separation Rack and add **40 μL** TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.3.9 Incubate at room temperature for 5 minutes.

3.3.10 Centrifuge briefly and place the centrifuge tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer **38 μL** supernatant to a new 0.2 mL PCR tube.

✓ **Stopping Point: After cleanup, Adapter-ligated DNA can be stored at -20°C.**

## 3.4 PCR Amplification

⚠ **Note: Please read Appendix D-2, E and G carefully before you begin.**

3.4.1 Add 50 μL PCR Enzyme Mix to each PCR tube from 3.3.10 on ice.

3.4.2 Please refer to the UDB PCR Primer Mix instructions in Appendix D-2 for Barcode choosing. Add 12 μL of UDB PCR Primer Mix to each tube from 3.4.1 to give the total volume of 100 μL. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube

3.4.3 Place the PCR tube from step 3.4.2 into the thermocycler and run the program in Table 9.

Table 9 Reaction Conditions for PCR Amplification

| Temperature | Time | Cycles |
|---|---|---|
| Heated lid(105°C) | on | |
| 95°C | 3 min | 1 cycle |
| 98°C | 20 s | 8-10 cycles |
| 60°C | 15 s | |
| 72°C | 30 s | |
| 72°C | 10 min | 1 cycle |
| 4°C | Hold | |

⚠ **Note: The cycle number of Table 9 is set for DNA input of 50ng following the standard protocol. Please refer to the recommended PCR cycle in Appendix E Table 23 for different DNA input amount, Appendix G for FFPE samples.**

3.4.4 Centrifuge briefly to collect the solution at the bottom of the tube. Transfer all the solution to a new 1.5 mL centrifuge tube.

## 3.5 Cleanup of PCR Product

⚠ **Note: Please read Appendix B carefully before you begin.**

3.5.1 Take out DNA Clean Beads from refrigerator and bring to room temperature for 30 minutes beforehand. Vortex and mix thoroughly before use.

3.5.2 Transfer 100 μL DNA Clean Beads to the centrifuge tube from step 3.4.4. Pipette up and down at least 10 times to mix thoroughly. Ensure that all liquid and beads are expelled from the pipette tip into the centrifuge tube before proceeding.

3.5.3 Incubate at room temperature for 5 minutes.

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

3.5.5 Keep the tube on the Magnetic Separation Rack and add 200 μL freshly prepared 80% ethanol to wash the beads and the walls of the tube. Incubate for 30 seconds, then carefully remove and discard the supernatant.

3.5.6 Repeat step 3.5.5 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 any remaining liquid using a small volume pipette.

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

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

3.5.9 Incubate at room temperature for 5 minutes.

3.5.10 Centrifuge briefly then place the centrifuge tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer **30 μL** supernatant to a new 1.5 mL centrifuge tube.

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

## 3.6 Quality Control of PCR Product

3.6.1 Quantify the purified PCR products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit. The required yield for PCR products is the input amount requirement from the commercial probes used for capture.

3.6.2 Assess the fragment size distribution of purified PCR products with electrophoresis-based equipment such as Bioanalyzer, Tapestation (Agilent Technologies), LabChip® GX, GXII, GX Touch (PerkinElmer), or Fragment Analyzer™ (Advanced Analytical). Figure 1 shows the final size distribution is 250 bp~450 bp.

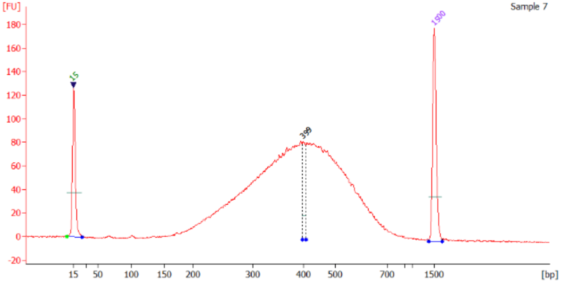


Figure 1 Agilent 2100 Bioanalyzer Fragment Size Distribution Results of the Purified PCR Product

## 3.7 Pre-Hybridization Preparation

- Before hybridization, take out Block3 and Block4 from the MGIEasy Dual Barcode Exome Capture Accessory Kit, and allow them to thaw at room temperature or on ice for later use. Conduct the hybridization and capture process according to the user manual from the chosen probe kit. 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 from the MGIEasy Dual Barcode Exome Capture Accessory Kit, thaw them at room temperature, and keep them on ice for later use. Conduct the Post-Capture PCR process according to Step 3.9.

⚠ **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 process 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 process 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 10 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 | 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;<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 | 1 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 11 Post-Capture PCR cycles for different commercial kit

| Commercial probe | PCR cycles |
|---|---|
| MGI Exome V4 Probe | 12-13 |
| MGI Exome V5 Probe | 12-13 |
| SeqCap EZ Human Exome Probes v3.0 | 12-13 |
| xGen Exome Research Panel | 6 (12 pool)-10 (1 pool) |
| SureSelect series probes (SureSelect Human All Exon V6 etc.) | 12-13 |

**The following steps 3.7-3.9 are standard experimental procedures using the NimbleGen® SeqCap EZ as an example.**

3.7.1. According to the sample input required for hybridization, amplify the samples by PCR with recommended cycles in Table 23. Select the correct the adapters for constructing the library according to Appendix D. Then perform the hybridization with the required PCR product input according to requirement in SeqCap EZ Library SR User's Guide.

## 3.8 Hybridization and Capture

3.8.1. Following Chapter 5 Step 3 inSeqCap 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 in the MGIEasy Dual Barcode Exome Capture Accessory Kit. Refer to Table 10 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.)**

3.8.2. Conduct the Hybridization capture and elution by referring to SeqCap EZ Library SR User's Guide Chapter 5-6. 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.**

## 3.9 Post-Capture PCR

3.9.1. Take out the MGIEasy Dual Barcode Exome Capture Accessory Kit and prepare the Post-capture PCR mixture on ice (see Table 12).

Table 12 Post-capture PCR Mixture

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

3.9.2. Transfer 56 μL of the Post-capture PCR mixture into each of the captured sample solutions (including beads) from the step 3.8.2 and centrifuge briefly to collect the solution at the bottom of the tube.

3.9.3. Place the PCR tube(s) from step 3.9.2 into the thermocycler and run the program described in Table 13.

Table 13 Post-capture PCR Reaction Conditions

| Temperature | Time | Cycles |
|---|---|---|
| Heated lid(105°C) | on | |
| 95°C | 3 min | 1 cycle |
| 98°C | 20 s | X cycles |
| 60°C | 15 s | |
| 72°C | 30 s | |
| 72°C | 10 min | 1 cycle |
| 4°C | Hold | |

⚠ **Note: The recommended number of Post-PCR cycles is in Table 12, in this condition as an example, the 'X' should be 12 or 13.**

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

3.9.5. 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.

## 3.10 Cleanup of Post-Capture PCR Product and Quantification

3.10.1. 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.

3.10.2. Transfer 100 μL DNA Clean Beads to each centrifuge tube from step 3.9.5. 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.

3.10.3. Incubate at room temperature for 5 minutes.

3.10.4. 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.

3.10.5. 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.

3.10.6. Repeat step 3.10.5 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.

3.10.7. 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.

3.10.8. 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.

3.10.9. Incubate at room temperature for 5 minutes.

3.10.10. 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.

3.10.11. 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. For a single post-capture PCR product for one sequencing reaction, the desired yield for PCR products is ≥ 1 pmol. Please refer to Appendix F 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 429 bp) should be ≥ 250 ng. For pooled sequencing, please follow instructions provided by MGIEasy DNA Adapters User Manual. Detailed information shows how to plan your sample pooling (see Appendix D). Quantify your post-captured PCR samples before pooling. The total yield after pooling should be 1 pmol, with a total volume ≤ 48 μL.

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

⚠ **Note: If the library will be delivered to a service lab for sequencing, please stop here. If the library will be sequenced in your lab, please go to step 3.11 to 3.15.**

## 3.11 Denaturation

⚠ **Note: Please read Appendix F carefully before you begin**

3.11.1. According to the PCR product size and Formula 1 in Appendix F, transfer 1 pmol of each PCR Product to a new 0.2 mL PCR Tube. Add TE Buffer for a total volume of 48 μL.

3.11.2. Place the PCR tube(s) from step 3.11.1 into the thermocycler and run the program in Table 14.

Table 14 Denaturation Reaction Conditions

| Temperature | Time |
|---|---|
| Heated lid(105°C) | On |
| 95°C | 3 min |

3.11.3. After the reaction is complete, immediately place the PCR tube(s) on ice for 2 minutes, and centrifuge briefly.

## 3.12 Single Strand Circularization

3.12.1. Take out the MGIEasy Dual Barcode Circularization Module and prepare the single strand circularization reaction mixture in a new 0.2 mL PCR tube on ice (see Table 15).

Table 15 Single Strand Circularization Mixture

| Components | Volume |
|---|---|
| Dual Barcode Splint Buffer | 11.6 μL |
| DNA Rapid Ligase | 0.5 μL |
| Total | 12.1 μL |

3.12.2. Transfer 12.1 μL of single strand circularization mixture into each PCR tube from step 3.11.3. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube(s).

3.12.3. Place the PCR tube(s) from step 3.12.2 into the thermocycler and run the program in Table 16:

Table 16 Single Strand DNA Circularization Reaction Conditions

| Temperature | Time |
|---|---|
| Heated lid(50°C) | On |
| 37°C | 30 min |
| 4°C | Hold |

3.12.4. After the reaction is complete, immediately place the tube(s) on ice for the next reaction.

## 3.13 Enzymatic Digestion

3.13.1. Prepare the following enzymatic digestion mixture (see Table 17) in a new 0.2 mL PCR tube on ice during the reaction in step 3.12.3.

Table 17 Enzymatic Digestion Mixture

| Components | Volume |
|---|---|
| Digestion Buffer | 1.4 μL |
| Digestion Enzyme | 2.6 μL |
| Total | 4.0 μL |

3.13.2. Transfer 4 μL of enzymatic digestion mixture into each PCR tube from step 3.12.4. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube(s).

3.13.3. Place the PCR tube(s) from step 3.13.2 into the thermocycler and run the following program in Table 18.

Table 18 Enzymatic Digestion Reaction Conditions

| Temperature | Time |
|---|---|
| Heated lid(105°C) | On |
| 37°C | 30 min |
| 4°C | Hold |

3.13.4. After the reaction is complete, centrifuge briefly to collect the solution at the bottom of the tube(s).

3.13.5. Immediately add 7.5 μL of Digestion Stop Buffer to each PCR tube. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube(s). Transfer all of the solution from each tube into a separate new 1.5 mL centrifuge tube.

## 3.14 Cleanup of Enzymatic Digestion Product

⚠ **Note: Please read Appendix B carefully before you begin.**

3.14.1. Take out DNA Clean Beads from the refrigerator and allow 30 minutes for the solution to come to room temperature. Vortex and mix thoroughly before use.

3.14.2. Transfer 170 μL of DNA Clean Beads to each tube containing Enzymatic Digestion product from step 3.13.5. Gently pipette at least 10 times to mix thoroughly. Ensure that the solution and beads are fully dispensed from the tip into the tube.

3.14.3. Incubate at room temperature for **10 minutes.**

3.14.4. Centrifuge briefly and place each 1.5 mL tube on the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant using a pipette.

3.14.5. Keep the 1.5 mL tube(s) on the Magnetic Separation Rack, add 500 μL of freshly prepared 80% ethanol to each tube without disturbing the beads. Incubate for 30 seconds. Carefully remove and discard the supernatant.

3.14.6. Repeat step 3.14.5 once. Remove all the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom, separate the beads magnetically, then remove remaining liquid using a small volume pipette.

3.14.7. Keep the 1.5 mL centrifuge tube(s) on the Magnetic Separation Rack with the lid open, and air-dry beads until no wetness (reflectiveness) is observed but before the pellet begins to crack.

3.14.8. Remove the 1.5 mL centrifuge tube(s) from the Magnetic Separation Rack and add **22 μL of** TE Buffer each tube to elute the DNA. Gently pipette up and down at least 10 times to mix thoroughly.

3.14.9. Incubate at room temperature for 10 minutes.

3.14.10. Centrifuge briefly. Place the 1.5 mL centrifuge tube(s) back on the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer **20 μL** supernatant from each tube to a separate new 1.5 mL centrifuge tube. Be careful to not disturb the beads.

✓ Stopping Point: Purified Enzymatic Digestion products can be stored at -20°C for one month.

## 3.15 Quality Control of Enzymatic Digestion Product

Quantify the purified Enzymatic Digestion products with Qubit® ssDNA Assay Kit. The final Enzymatic Digestion products (ssDNA, ng)/ input products of PCR (dsDNA, ng) should be ≥ 7%.

For example, if the input of PCR product used for the circularization with the band peak around 429 bp in Bioanalyzer (corresponding to the insert fragment peak around 280 bp) is 250 ng, the final yield after enzymatic digestion should be more than 17.2 ng.

# Appendix

## Appendix A Reaction Conditions of DNA Fragmentation

The following table shows the Fragmentation parameters of 55 µL sample with Covaris series models. The information is from the Covaris official website and for reference only.

Please follow the parameters below to fragment gDNA (including blood, fresh or frozen tissues) into sizes between 100-700 bp. For FFPE derived DNA samples, it is recommended to slightly reduce treatment time according to the DNA quality, please refer to Appendix G for more details on FFPE sample.

Table 19 Fragmentation Parameters of Covaris S220 for Target BP peaks between 150 and 550 bp

(55 µL of Sample Volume)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Vessel | microTUBE-50 AFA Fiber-Screw-Cap (PN 520166)<br> | | | | | | |
| | Sample Volume | 55 µL | | | | | | |
| S220 | Holder | S-Series Holder microTUBE-50 Screw-Cap (PN 500492) | | | | | | |
| | Water Level | 10 | | | | | | |
| | Temperature (°C) | 7 | | | | | | |
| | Target BP (Peak) | 150 | 200 | 250 | 300 | 350 | 400 | 550 |
| | Peak Incident Power (W) | 100 | 75 | 75 | 75 | 75 | 75 | 50 |
| | Duty Factor | 30% | 25% | 20% | 20% | 15% | 10% | 10% |
| | Cycles per Burst | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| | Treatment Time (s) | 150 | 95 | 65 | 45 | 45 | 55 | 50 |

Table 20 The Fragmentation Parameters of Covaris Series for Target BP peaks between 150 and 550 bp

(55 µL of Sample Volume)

| | | | | |
|---|---|---|---|---|
| | Vessel | MicroTUBE-50 Screw-Cap (PN 520166)<br> | 8 microTUBE-50 AFA Fiber Strip V2 (PN 520174)<br>8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240)<br> | 96 microTUBE-50 AFA Fiber Plate (PN 520168)<br>96 microTUBE-50 AFA Fiber Plate Thin Foil (PN 520232)<br> |
| | Sample Volume | 55 µL | | |

<table>
<tr><td rowspan="2">E220</td><td>Racks</td><td colspan="3">Rack 24 Place microTUBE Screw-Cap (PN 500308)</td><td colspan="3">Rack 12 Place 8 microTUBE Strip (PN 500444)</td><td colspan="3">No Rack needed</td></tr>
<tr><td>Plate Definitions</td><td colspan="3">"E220_500308 Rack 24 Place microTUBE- 50 Screw-Cap +6.5mm offset"</td><td colspan="3">"E220_500444 Rack 12 Place 8 microTUBE-50 Strip V2 -10mm offset"</td><td colspan="3">"E220_520168 96 microTUBE-50 Plate -10.5mm offset" "E220_520232 96 microTUBE-50 Plate Thin Foil -10.5mm offset"</td></tr>
<tr><td rowspan="2">E220 evolution</td><td>Racks</td><td colspan="3">Rack E220e 4 Place microTUBE Screw Cap (PN 500432) Rack E220e 8 microTUBE Strip V2 (PN 500437) Non Compatible</td><td colspan="3">Rack E220e 4 Place microTUBE Screw Cap (PN 500432) Rack E220e 8 microTUBE Strip V2 (PN 500437) Non Compatible</td><td colspan="3">Rack E220e 4 Place microTUBE Screw Cap (PN 500432) Rack E220e 8 microTUBE Strip V2 (PN 500437) Non Compatible</td></tr>
<tr><td>Plate Definitions</td><td colspan="3">"500432 E220e 4 microTUBE-50 Screw Cap -8.32mm offset" "500437 E220e 8 microTUBE- 50 Strip V2 -10mm offset" N/A</td><td colspan="3">"500432 E220e 4 microTUBE-50 Screw Cap -8.32mm offset" "500437 E220e 8 microTUBE- 50 Strip V2 -10mm offset" N/A</td><td colspan="3">"500432 E220e 4 microTUBE-50 Screw Cap -8.32mm offset" "500437 E220e 8 microTUBE- 50 Strip V2 -10mm offset" N/A</td></tr>
<tr><td rowspan="5">All</td><td>Temperature (°C)</td><td colspan="9">7</td></tr>
<tr><td>Water Level</td><td colspan="3">6</td><td colspan="3">-2</td><td colspan="3">0</td></tr>
<tr><td>Intensifier (PN 500141)</td><td colspan="3">Yes</td><td colspan="3">Yes</td><td colspan="3">Yes</td></tr>
<tr><td>Y-dithering</td><td colspan="3">No</td><td colspan="3">No</td><td colspan="3">Yes (0.5 mm Y-dither at 10 mm/s)</td></tr>
<tr><td>Target BP (Peak)</td><td>150</td><td>200</td><td colspan="2">250</td><td>300</td><td colspan="2">350</td><td>400</td><td>550</td></tr>
<tr><td rowspan="4">Screw-Cap</td><td>Peak Incident Power (W)</td><td>100</td><td>75</td><td colspan="2">75</td><td>75</td><td colspan="2">75</td><td>75</td><td>30</td></tr>
<tr><td>Duty Factor</td><td>30%</td><td>20%</td><td colspan="2">20%</td><td>20%</td><td colspan="2">20%</td><td>10%</td><td>10%</td></tr>
<tr><td>Cycles per Burst</td><td>1000</td><td>1000</td><td colspan="2">1000</td><td>1000</td><td colspan="2">1000</td><td>1000</td><td>1000</td></tr>
<tr><td>Treatment Time (s)</td><td>130</td><td>95</td><td colspan="2">62</td><td>40</td><td colspan="2">30</td><td>50</td><td>70</td></tr>
<tr><td rowspan="4">8-Strip</td><td>Peak Incident Power (W)</td><td>75</td><td>75</td><td colspan="2">75</td><td>75</td><td colspan="2">75</td><td>75</td><td>50</td></tr>
<tr><td>Duty Factor</td><td>15%</td><td>15%</td><td colspan="2">20%</td><td>20%</td><td colspan="2">20%</td><td>10%</td><td>10%</td></tr>
<tr><td>Cycles per Burst</td><td>500</td><td>500</td><td colspan="2">1000</td><td>1000</td><td colspan="2">1000</td><td>1000</td><td>1000</td></tr>
<tr><td>Treatment Time (s)</td><td>360</td><td>155</td><td colspan="2">75</td><td>45</td><td colspan="2">35</td><td>52</td><td>50</td></tr>
<tr><td rowspan="4">Plate</td><td>Peak Incident Power (W)</td><td>100</td><td>100</td><td colspan="2">75</td><td>75</td><td colspan="2">75</td><td>75</td><td>75</td></tr>
<tr><td>Duty Factor</td><td>30%</td><td>30%</td><td colspan="2">20%</td><td>20%</td><td colspan="2">20%</td><td>10%</td><td>10%</td></tr>
<tr><td>Cycles per Burst</td><td>1000</td><td>1000</td><td colspan="2">1000</td><td>1000</td><td colspan="2">1000</td><td>1000</td><td>1000</td></tr>
<tr><td>Treatment Time (s)</td><td>145</td><td>90</td><td colspan="2">70</td><td>49</td><td colspan="2">34</td><td>50</td><td>32</td></tr>
</table>

## Appendix B Magnetic Beads and Cleanup Procedures

For magnetic bead-based purification, we recommend using DNA Clean Beads included in the MGIEasy DNA Clean Beads (MGI, Cat. No. 1000005278) or AMPure® XP (Agencourt, Cat. No. A63882). If you choose Magnetic Beads from other sources, please optimize the cleanup conditions before getting started.

### Before You Use

- To ensure capture efficiency of the Magnetic Beads, remove beads from 4°C refrigerator storage and equilibrate to room temperature for 30 minutes beforehand. Vortex and mix thoroughly before use.
- Vortex or pipette up and down to ensure that the beads are thoroughly mixed before each use.
- The volume of the beads determines the lower limit of fragment size that can be purified.

### Operation Notes

- If the sample volume decreases due to evaporation during incubation, add additional TE buffer to reach the designated volume before using the beads to purify. It ensures that the correct ratio for the beads is used.
- In the magnetic separation step, please allow the solution to become completely clear before removing the supernatant. This process takes approximately 2-3 minutes. Consider the different magnetic strength of your specific Separation Plate / Rack and allow enough time for the solution to turn completely clear.
- Avoid contacting the beads with pipette tips when pipetting. 2-3 μL of fluids can be left in the tube to avoid contact. In the event of contact between the beads and the pipette tip, expel all of the solution and beads back into the tube and restart the separation process.
- Use freshly prepared 80% ethanol (at room temperature) to wash the beads. Keep the centrifuge tube on the Magnetic Separation Rack when washing with ethanol. Do not shake or disturb the beads in any way.
- After the 2nd bead wash with ethanol, try to remove all liquid from within the tube. You may centrifuge briefly to collect any remaining liquid at the bottom. Separate the beads magnetically and remove any remaining liquid by using a small volume pipette.
- After washing twice with ethanol, air-dry the beads at room temperature. Drying takes approximately 2-5 minutes depending on your specific lab environment. Watch closely until the pellet appears sufficiently dry with a matte appearance, then continue to the elution step with TE

Buffer.

- During the elution step, do not touch the beads with the pipette tips when removing the supernatant. Contamination in DNA by the beads may affect subsequent purification. Therefore, the total volume of TE buffer and the beads should be 2 μL more than the volume of the supernatant.
- Pay attention when opening/ closing the lids of centrifuge tubes on the Separation Rack. Strong vibrations may cause sample loss by spilling liquid or beads from the tubes. Secure the tubes before opening the lids.

## Appendix C Magnetic Beads Size Selection

The following example uses a 64 μL 1st bead selection and a 16 μL 2nd bead selection to target a 280 bp size fragment from fragmented DNA (80 μL). To select different fragment sizes, please refer to Table 3 in Chapter 2 for detailed conditions.

### Protocol

1) Remove DNA Clean Beads from the refrigerator, allow 30 minutes for the solution to come to room temperature. Vortex and mix thoroughly before use.
2) Transfer all fragmentation products to a new 1.5 mL centrifuge tube. Add TE Buffer for a final volume of 100 μL.
3) Transfer 64 μL of DNA Clean Beads to the 1.5 mL centrifuge tube containing 100 μL of fragmentation product. Pipette up and down at least 10 times to mix thoroughly. Ensure that the liquid and the beads are fully dispensed from the pipette tip into the tube before proceeding.
4) Incubate at room temperature for 5 minutes.
5) Centrifuge briefly and place the tube onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Then, carefully transfer the supernatant to a new 1.5 mL centrifuge tube.

⚠ **Note: Retain the Supernatant and discard the Beads.**

6) Transfer 16 μL of DNA Clean Beads to the centrifuge tube with 144 μL supernatant. Pipette at least 10 times to mix thoroughly.
7) Incubate at room temperature for 5 minutes.
8) Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the

liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

9) Keep the centrifuge tube on the Magnetic Separation Rack and add 200 μL of freshly prepared 80% ethanol to wash the beads and the walls of the tube. Carefully remove and discard the supernatant.

10) Repeat step 9 and try to remove all of the liquid from the tube.

11) Keep the centrifuge tube on the Magnetic Separation Rack with the lid open, and air-dry the beads until they no longer appear shiny but before the bead pellet starts to crack.

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

13) Incubate at room temperature for 5 minutes.

14) Centrifuge briefly then place the centrifuge tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 30 μL of supernatant to a new 1.5 mL centrifuge tube.

## Appendix D MGIEasy Duplex UMI Adapters Kit Instructions

This 16RXN set provides MGIEasy Duplex UMI Adapters (Tube) Kit, 96RXN set provides MGIEasy Duplex UMI Adapters (Plate) Kit. This kit was developed to meet requirements for batch processing of library construction and multiplex sequencing. The dual barcode adapter design can help to effectively reduce barcode swapping within samples, and dual UMI tags are useful for low-frequency variant detection by reducing artifacts. The maximum 96-well of Dual Barcode PCR Primer Mix and one tube of Duplex UMI Adapter Mix are provided, which allows for 96 samples of multiplex sequencing. We selected the best Barcode combination based on the principle of balanced base composition. For optimal performance, please carefully read instructions in Appendix D-1.

### D-1 The usage of Dual UMI Adapter Mix

- Duplex UMI Adapter Mix 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 Duplex UMI Adapter Mix 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.
- Adapter quality as well as quantity directly affects the efficiency and quality of the library construction. We recommend the dilution ratio to be between 2 - 5. An excessive input of Adapters may cause Adapter dimers; whereas insufficient input may cause lower library yield and lower efficiency of library construction.

Table 21 Recommended Adapter Input According to the Amount of Sample DNA (280 bp)

| DNA Sample (ng) | MGI Adapter Dilution Ratio | MGI Adapter Input after Dilution (μL) |
|---|---|---|
| ≥ 50 | No dilution | 5 |
| 25 | 2 | 5 |
| 10 | 5 | 5 |

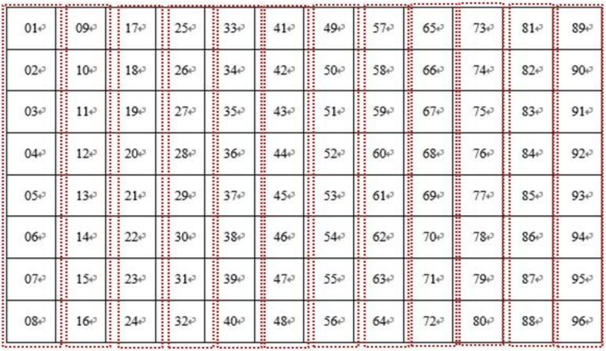
- Increasing Adapter input may increase the library yield to a certain extent, especially when the DNA sample ≤ 25 ng. If there is a need to optimize the efficiency of library construction, you may try increasing Adapter input (within the range of 2-10 times).

## D-2 The usage of UDB PCR Primer Mix (Tube/Plate)

Before use, please centrifuge to collect liquid at the bottom of tubes or plates. Gently remove the cap/sealing film to prevent liquid from spilling and cross-contamination. Remember to reseal the Primer Mix immediately after use. For 96-well Plate, if the seal film is contaminated, discard the old seal film and use a new one to reseal the 96-well plate. Based on the principles of balanced base composition, UDB PCR Primer Mix must be used in specific groups. Please follow the instructions below to use UDB PCR Primer Mix in proper combination:

For Tube: 2 sets of 8 UDB PCR Primers, which are UDB PCR Primer Mix-01_ UDB PCR Primer Mix-8 and UDB PCR Primer Mix-09_ UDB PCR Primer Mix—16 separately.

For 96-well plate: 12 sets of 8 UDB PCR Primers. Figure 2 shows the instructions of proper combination.（one set in each red box）



| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 01 | 09 | 17 | 25 | 33 | 41 | 49 | 57 | 65 | 73 | 81 | 89 |
| 02 | 10 | 18 | 26 | 34 | 42 | 50 | 58 | 66 | 74 | 82 | 90 |
| 03 | 11 | 19 | 27 | 35 | 43 | 51 | 59 | 67 | 75 | 83 | 91 |
| 04 | 12 | 20 | 28 | 36 | 44 | 52 | 60 | 68 | 76 | 84 | 92 |
| 05 | 13 | 21 | 29 | 37 | 45 | 53 | 61 | 69 | 77 | 85 | 93 |
| 06 | 14 | 22 | 30 | 38 | 46 | 54 | 62 | 70 | 78 | 86 | 94 |
| 07 | 15 | 23 | 31 | 39 | 47 | 55 | 63 | 71 | 79 | 87 | 95 |
| 08 | 16 | 24 | 32 | 40 | 48 | 56 | 64 | 72 | 80 | 88 | 96 |

Figure 2 96 UBD PCR Primer Mix Layout and Combination Instruction

If the sequencing data output requirement is the same for all samples in one lane, please refer to Table 22 below to choose your barcode adapter combinations.

Table 22 MGIEasy DNA Adapters-16 (Tube) Kit Instruction

| Sample(s)/ lane | Instructions (Example) |
|---|---|
| ≥8, Multiples of 8 | Requires at least 1 set of UDB PCR Primers:<br>Take N set(s) of 8 UDB PCR Primers (N is multiple factor), respectively add one primer to each sample in an equal volume |
| ＞8，not Multiples of 8 | Requires at least 1 or more sets of UDB PCR Primers according to the numbers and choose other UDB PCR Primers from other set for the left samples. Respectively add one primer to each sample in an equal volume |

## Appendix E PCR Amplification

- The number of PCR cycles must be carefully controlled. Insufficient cycles may lead to the reduced yield of library.
- Table 24 shows the number of PCR cycles required to yield 500 ng and 1 μg of library from 10-1000 ng high quality DNA sample (280 bp). When DNA sample is of lower quality and consists of longer fragments, PCR cycles should be increased appropriately to generate enough yields.

Table 23 PCR Cycles Required to Yield 500 ng and 1 μg Libraries

| DNA Sample (ng) | PCR Cycles required for corresponding yield | |
|---|---|---|
| | 500 ng | 1 μg |
| 10 | 9-11 | 10-12 |
| 25 | 8-10 | 9-11 |
| 50 | 7-9 | 8-10 |
| 100 | 6-8 | 7-9 |
| 200 | 5-7 | 6-8 |
| 400 | 4-6 | 5-7 |
| 600 | 3-5 | 4-6 |
| 1000 | 3-4 | 3-5 |

## Appendix F Conversion between DNA Molecular Mass and Number of Moles

The formula 1 shows the calculation of the mass in (ng) that corresponds to 1 pmol of dsDNA sample with varying fragment sizes.

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

## Appendix G Protocol Modifications for FFPE-derived gDNA Samples

This Appendix summarizes the protocol modifications through library preparation to apply to FFPE-derived gDNA samples (short as FFPE DNA) based on DNA integrity.

### G-1 Methods for FFPE Sample Qualification

Considering that FFPE DNA has different degrees of degradation, which will affect the fragmentation and library yield. DNA integrity may be assessed using following two methods after extract:

Method 1: Using agarose gel electrophoresis to detect distribution of main band.

Method 2: Using commercial FFPE QC kit to assess Q score detected by qPCR, for example, KAPA Human Genomic DNA Quantification and QC Kits (KK9406).

### G-2 Recommendation of Input and Fragmentation for FFPE DNA

This set is compatible with 50~200 ng input amount of FFPE gDNA. It is recommended to adjust the treatment time of fragmentation according to DNA integrity (Table 24).

Table 24 Recommendation of DNA Shearing for FFPE DNA

| Shearing Parameter | Non-FFPE DNA | FFPE DNA | |
|---|---|---|---|
| | | Main band>13000 bp or Q score >0.8 | Smear band<13000 bp or Q score <0.8 |
| Treatment Time | Standard time (refer to Appendix A) | 66% of Standard time | 50% of Standard time |

⚠ **Note: The total volume for fragmentation should be less than 55 µL. It is important to take a small amount (eg. 20 ng) of FFPE gDNA to test the condition of fragmentation for desired size before you begin the library preparation.**

### G-3 Protocol Modifications for FFPE DNA

Protocol modifications based on Chapter 3 that should be applied to FFPE samples are summarized in Table 26.

Table 25 Summary of protocol modifications for FFPE samples

| Workflow Step | Non-FFPE DNA | FFPE DNA | | |
|---|---|---|---|---|
| | | Main band>13000 bp or Q score >0.8 | Smear band>500 bp or 0.5<Q score<0.8 | Smear band<500 bp or Q score<0.5 |
| 2.2 DNA Fragmentation and Size Selection | Size selection | No selection | No selection | No selection |
| 2.3 DNA input | 10~1000 ng fragmented DNA | Total of 40 μL product after fragmentation | Total of 40 μL product after fragmentation | Total of 40 μL product after fragmentation |
| 3.4 PCR Amplification (for 500 ng PCR yield) | standard cycles 3-11 | Same as Non-FFPE DNA | Increase 1-2 cycles | Increase 2-3 cycles |

## Appendix H Sequencing Instructions

### G-1 The settings for dark reaction

Two dark reactions are necessary for the sequencing of library constructed by MGIEasy Duplex UMI Universal Library Prep Set. Due to the specific design of duplex UMI adapters, definite bases are introduced to the final sequences of library, and we strongly recommend set cycle 6-7 bp as dark reaction during sequencing, in order to retain the quality; If not, the sequencing quality will be affected. For the operation details of dark reaction, please refer to DNBSEQ-G400RS High-throughput Sequencing Set User Manual.

### G-1 Dual Barcode Splitting

It is required that the sequencer software version for dual barcode machine splitting is ECR3.0 or ECR4.0, please check the software version of the sequencer before sequencing. If you use Barcode primer combinations other than the 96 UDB PCR Primer Mix provided by the kit for library sequencing, it needs to be based on the actual used Barcode combination to re-import the Barcode list. If the import step is omitted, the split will fail.

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