MGIEasy

Exome Universal Library Prep Set User Manual

Cat No.: 1000009657

Kit Version: V1.0

Manual Version: A1

Revision History

Manual Version	Kit Version	Date	Description
A1	V1.0	Oct. 2019	◆ Add DNBSEQ series sequencing platform ◆ Add Appendix G for FFPE DNA
A0	V1.0	May. 2019	◆ Initial release.

Note: Please download the latest version of the manual to use the corresponding kit.

Contents

Chapter 1 Product Description ..... - 1 -
1.1 Introduction ..... - 1 -
1.2 Application ..... - 1 -
1.3 Platform Compatibility ..... - 1 -
1.4 Contents ..... - 2 -
1.5 Storage Conditions and Shelf Life ..... - 3 -
1.6 Equipment and Materials Required but not Provided ..... - 4 -
1.7 Precautions and Warning ..... - 5 -
Chapter 2 Sample Preparation ..... - 6 -
2.1 Sample Requirement ..... - 6 -
2.2 DNA Fragmentation and Size Selection ..... - 6 -
2.3 Sample DNA Quantitation and Quality Control ..... - 7 -
Chapter 3 Library Construction Protocol ..... - 8 -
3.1 End Repair and A-tailing ..... - 8 -
3.2 Adapter Ligation ..... - 9 -
3.3 Cleanup of Adapter-Ligated DNA ..... - 9 -
3.4 PCR Amplification ..... - 10 -
3.5 Cleanup of PCR Product ..... - 11 -
3.6 Quality Control of PCR Product ..... - 12 -
3.7 Pre-Hybridization Preparation ..... - 13 -
3.8 Hybridization and Capture ..... - 15 -
3.9 Post-Capture PCR ..... - 15 -
3.10 Cleanup of Post-Capture PCR Product and Quantification ..... - 16 -
3.11 Denaturation ..... - 17 -
3.12 Single Strand Circularization ..... - 18 -
3.13 Enzymatic Digestion ..... - 18 -
3.14 Cleanup of Enzymatic Digestion Product ..... - 19 -
3.15 Quality Control of Enzymatic Digestion Product ..... - 20 -
Appendix ..... - 21 -
Appendix A Reaction Conditions of DNA Fragmentation ..... - 21 -
Appendix B Magnetic Beads and Cleanup Procedures ..... - 23 -
Appendix C Magnetic Beads Size Selection ..... - 24 -
Appendix D The Combination Barcode Adapters Strategies ..... - 26 -
Appendix E PCR Amplification ..... - 29 -

Appendix F Conversion between DNA Molecular Mass and Number of Moles ..... - 29 -
Appendix G Protocol Modifications for FFPE-derived gDNA Samples ..... - 30 -

Chapter 1 Product Description

1.1 Introduction

The MGIEasy Exome Universal Library Prep Set is specifically designed for constructing human whole exome libraries for the MGI high-throughput sequencing platform series.

This library prep set is optimized to convert 10-50 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. 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 and provides supplement of the capture for whole exome collocated with Probes.

1.3 Platform Compatibility

Constructed libraries are compatible with

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

1.4 Contents

Each Library Prep Set consists of a MGIEasy Universal DNA Library Prep Set, and a MGIEasy Exome Capture Accessory Kit. Further information on Cat. No., Components and Specifications are listed below.

Table 1 MGIEasy Universal DNA Library Prep Set (16 RXN) (Cat. No.: 1000006985)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Universal DNA Library Prep Kit Cat. No.: 1000005248	ERAT Buffer	Orange	114 μL/ tube × 1 tube
	ERAT Enzyme Mix	Orange	47 μL/ tube × 1 tube
	Ligation Buffer	Red	375 μL/ tube × 1 tube
	DNA Ligase	Red	26 μL/ tube × 1 tube
	PCR Enzyme Mix	Blue	400 μL/ tube ×1 tube
	PCR Primer Mix	Blue	96 μL/ tube × 1 tube
MGIEasy DNA Adapters-16 (Tube) Kit Cat. No.: 1000005284	DNA Adapters	White	10 μL/ tube ×16 tubes
MGIEasy DNA Clean Beads Cat. No.: 1000005278	DNA Clean Beads	White	8 mL/ tube × 1 tube
	TE Buffer	White	4 mL/ tube × 1 tube
MGIEasy Circularization Module Cat. No.: 1000005260	Splint Buffer	Purple	186 μL/ tube × 1 tube
	DNA Rapid Ligase	Purple	8 μL/ tube × 1 tube
	Digestion Buffer	White	23 μL/ tube × 1 tube
	Digestion Enzyme	White	42 μL/ tube × 1 tube
	Digestion Stop Buffer	White	120 μL/ tube × 1 tube

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

Modules & 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 Universal DNA Library Prep Kit

- Storage Temperature: -25°C to -18°C
- Transport Conditions: transported on dry ice

MGIEasy DNA Adapters Kit

- Storage Temperature: -25°C to -18°C
- Transport Conditions: transported on dry ice

MGIEasy Circularization Module

- Storage Temperature: -25°C to -18°C
- Transport Conditions: transported on dry ice

MGIEasy Exome Capture Accessory Kit

- Storage Temperature: -25°C to -18°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™)
	Vortex Mixer
	Desktop Centrifuge
	Pipets
	Thermocycler
	Magnetic rack DynaMag™-2 (Thermo Fisher Scientific™, Cat. No. 12321D) or equivalent
	Qubit™ 3.0 Fluorometer (Thermo Fisher Scientific™, Cat. No. Q33216)
	Agilent 2100 Bioanalyzer (Agilent Technologies™, Cat. No. G2939AA)
	Eppendorf Concentrator（Eppendorf, Cat. No. 5305000398）
	Thermomixer or water bath equipment
	Nutator or other nutating mixer/shaker
	Magnetic rack for 96-well plate (BioMag, Cat. No. BMB-96）or equivalent
Reagents	Nuclease free water (NF water) (Ambion, Cat. No. AM9937)
	100% Ethanol (Analytical Grade)
	Qubit® ssDNA Assay Kit (Invitrogen, Cat. No. Q10212)
	Qubit® dsDNA HS Assay Kit (Invitrogen, Cat. No. Q32854)
	High Sensitivity DNA Kits (Agilent Technologies™, Cat. No. 5067-4626)
	Agilent DNA 1000 Kit (Agilent Technologies™, Cat. No. 5067-1504)
	Reagents or kits or beads required by commercial probes for capture
Consumables	Covaris AFA Tubes for use with Ultrasonicator
	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
	Qubit® Assay Tubes (Invitrogen, Cat. No. Q32856)
	0.5 mL Thin Wall PCR Tubes (Axygen, Cat. No. PCR-05-C)
	Filter Tips（Axygen, Cat. No. TF-100）or equivalent
	Clear Adhesive Film (ABI, Cat. No. 4306311)
	Blade or knife

1.7 Precautions and Warning

- 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 Requirement

This library preparation set is applicable for genomic DNA derived from human samples (including blood, fresh or frozen tissues and FFPE derived samples). 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 the majority of 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 4). Gel extraction methods can also be used for the same purpose.

⚠ **Note: If FFPE derived samples are fragmented, you can skip this step of 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
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 10-50 ng in less than **40 μL**.

⚠ **Note: For FFPE samples, 50-200 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 4). 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 ranges for inserts are 250-300 bp for PE100 sequencing and 300-350 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 4 in Chapter 2 Sample Requirement, Table 22 in Appendix D, and Table 24 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.**

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 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	7.1 μL
ERAT Enzyme Mix	2.9 μL
Total	10 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	On
37°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 Please refer to the instructions for MGIEasy DNA Adapters (see Appendix D). Add 5 μL of MGIEasy DNA Adapters 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	23.4 μL
DNA Ligase	1.6 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	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 21 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 19 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 E and G carefully before you begin.**

3.4.1 Prepare the PCR amplification mixture on ice (see Table 9).

Table 9 PCR Amplification Mixture

Components	Volume
PCR Enzyme Mix	25 μL
PCR Primer Mix	6 μL
Total	31 μL

3.4.2 Transfer 31 μL of PCR amplification mixture to the PCR tube from step 3.3.10. 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 following program in Table 10.

Table 10 Reaction Conditions for PCR Amplification

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

⚠ **Note: The cycle number of 8 is set for DNA input of 50ng following the standard protocol. Please refer to the recommended PCR cycle in Appendix E Table 24 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 of 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 50 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 to reach the input amount requirement from commercial probe 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.

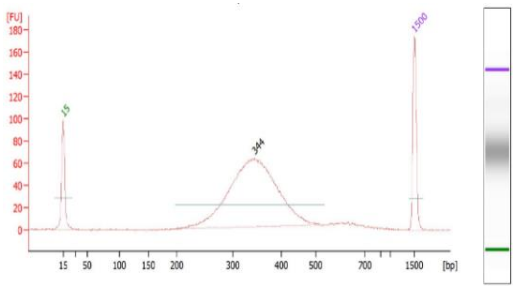

Figure 1 Agilent 2100 Bioanalyzer Fragment Size Distribution Results of the Purified PCR Product

3.7 Pre-Hybridization Preparation

- Before hybridization, take out the Block3 and Block4 from the MGIEasy Exome Capture Accessory Kit, and allow them to be thawed 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 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 Exome Capture Accessory Kit. Recommended usages of Block 3 and Block 4 for different commercial probes are listed below:**

Table 11 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	无
MGI Exome V5 Probe	1 μL	10 μL	无
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; 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	10 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 list below:**

Table 12 Post-Capture PCR cycles for different commercial kit

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

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 24. 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 the 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 Exome Capture Accessory Kit. Refer to Table 11 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 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 Exome Capture Accessory Kit and prepare the Post-capture PCR mixture on ice (see Table 13).

Table 13 Post-capture PCR Mixture

Components	Volume
Post-PCR Enzyme Mix	50 µL
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 14.

Table 14 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 12, in this condition as an example, the 'X' should be 12.**

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. If one 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 384 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 15.

Table 15 Denaturation Reaction Conditions

Temperature	Time
Heated lid	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 Circularization Kit and prepare the single strand circularization reaction mixture in a new 0.2 mL PCR tube on ice (see Table 16).

Table 16 Single Strand Circularization Mixture

Components	Volume
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 17:

Table 17 Single Strand DNA Circularization Reaction Conditions

Temperature	Time
Heated lid	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 18) in a new 0.2 mL PCR tube on ice during the reaction in step 3.12.3.

Table 18 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 19.

Table 19 Enzymatic Digestion Reaction Conditions

Temperature	Time
Heated lid	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 of 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 364 bp in Bioanalyzer (corresponding to the insert fragment peak around 280 bp) is 240 ng, the final yield after enzymatic digestion should be more than 16.8 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 20 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) 						
	Sample Volume	55 µL						
S220	Holder	S-Series Holder microTUBE-50 Screw-Cap (PN 500492)						
	Water Level	10						
	Temperature (℃)	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 21 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) 	8 microTUBE-50 AFA Fiber Strip V2 (PN 520174)  8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240)	96 microTUBE-50 AFA Fiber Plate (PN 520168)  96 microTUBE-50 AFA Fiber Plate Thin Foil (PN 520232)
	Sample Volume	55 µL		

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

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 4 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 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 D-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.
- 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 22 Recommended Adapter Input According to the Amount of Sample DNA (280 bp)

DNA Sample (ng)	MGI Adapter	MGI Adapter
	Dilution Ratio	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-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 23 below to choose your barcode adapter combinations.

Table 23 MGIEasy DNA Adapters-16 (Tube) Kit Instruction

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

	1) For samples 1-4, use the method for (4 samples/lane) above (Use 1st Adapter set). 2) For samples 5-6, use the method for (2 samples/lane) above (Use 2nd Adapter set). 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. 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: 1. Take a set of 8 Adapters (97-104), respectively add 1 Adapter to each sample in an equal volume. 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)).

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 10-50 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 sufficient yields.

Table 24 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	11-13
25	7-9	9-11
50	6-8	8-10

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.

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

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 25).

Table 25 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 26 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	10~50 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)	6-11 cycles	7-12 cycles	8-13 cycles	9-14 cycles

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