MGI

MGIEasy

UDB Universal Library Prep Set User Manual

Cat No.: 1000022803 (16RXN)

1000022804 (96RXN) 1000022805 (192RXN)

Kit Version: V1.0

Manual Version: A1

Revision History

Manual Version	Kit Version	Date	Description
A1	V1.0	Jan. 2021	◆ Update contact information.
A0	V1.0	Oct. 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

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Chapter 1 Product Description

1.1 Introduction

The MGIEasy UDB Universal Library Prep Set is specifically designed for constructing libraries for MGI sequencing platform. This library prep set is optimized to convert 1-1000 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, which helps mitigate barcode contamination and hopping, effectively reduces barcode swapping within samples. All reagents provided within this set have passed stringent quality control and functional verification procedures, ensuring performance stability and reproducibility.

1.2 Application

This library prep set is applicable for samples from all common animals, plants, fungus, bacteria, etc., including human（including gDNA, cfDNA, FFPE derived DNA etc.）, mice, rice, Arabidopsis, yeast, E. coli, Metagenomics. Stable performance across all such sample types is expected.

1.3 Platform Compatibility

DNBSEQ Sequence platform

1.4 Contents

There are three specifications available for multiplexing option: 16 reactions (Cat. No.: 1000022803) ,96 reactions (Cat. No.: 1000022804) and 192 reactions (Cat. No.: 1000022805). Each Set consists of several modules. Further information are listed below.

Table 1 MGIEasy UDB Universal Library Prep Set (16 RXN) (Cat. No.: 1000022803)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Universal DNA Library Prep Module Cat. No.: 1000019376 Specification: 16RXN	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 tub
MGIEasy UDB Primers Adapter Kit Cat. No.: 1000022800 Specification: 16RXN	UDB Adapter	White	80 μL/ tube ×1 tube
	UDB PCR Primer Mix-57-64，89-96	Blue	12 μL/ tube ×16 tubes
MGIEasy DNA Clean Beads Cat. No.: 1000005278 Specification: 16RXN	DNA Clean Beads	White	8 mL/ tube × 1 tube
	TE Buffer	White	4 mL/ tube × 1 tube
MGIEasy Dual Barcode Circularization Module Cat. No.: 1000018649 Specification: 16RXN	Dual Barcode 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 UDB Universal Library Prep Set (96 RXN) (Cat. No.: 1000022804)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Universal DNA Library Prep Module Cat. No.: 1000019377 Specification: 96RXN	ERAT Buffer	Orange	576 μL/ tube × 1 tube
	ERAT Enzyme Mix	Orange	432 μL/ tube × 1 tube
	Ligation Buffer	Red	2016 μL/ tube × 1 tube
	DNA Ligase	Red	384 μL/ tube × 1 tube
	PCR Enzyme Mix	Blue	1200 μL/ tube ×4tube
MGIEasy UDB Primers Adapter Kit B Cat. No.: 1000022802 Specification: 96RXN	UDB Adapter	White	480 μL/ tube ×1 tube
	UDB PCR Primer Mix-97-192	——	12 μL/ tube ×96 well
MGIEasy DNA Clean Beads Cat. No.: 1000005279 Specification: 96RXN	DNA Clean Beads	White	50 mL/ tube × 1 tube
	TE Buffer	White	25 mL/ tube × 1 tube
MGIEasy Dual Barcode Circularization Module Cat. No.: 1000018649 Specification: 16RXN	Dual Barcode 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 3 MGIEasy UDB Universal Library Prep Set (192 RXN) (Cat. No.: 1000022805)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Universal DNA Library Prep Module Cat. No.: 1000019377 Specification: 96RXN***2**	ERAT Buffer	Orange	576 μL/ tube × 1 tube
	ERAT Enzyme Mix	Orange	432 μL/ tube × 1 tube
	Ligation Buffer	Red	2016 μL/ tube × 1 tube
	DNA Ligase	Red	384 μL/ tube × 1 tube
	PCR Enzyme Mix	Blue	1200 μL/ tube ×4tube
MGIEasy UDB Primers Adapter Kit A Cat. No.: 1000022801 Specification: 96RXN	UDB Adapter	White	480 μL/ tube ×1 tube
	UDB PCR Primer Mix-01-96	——	12 μL/ tube ×96 well
MGIEasy UDB Primers Adapter Kit B (96RXN) Cat. No.: 1000022802 Specification: 96RXN	UDB Adapter	White	480 μL/ tube ×1 tube
	UDB PCR Primer Mix-97-192	——	12 μL/ tube ×96 well
MGIEasy DNA Clean Beads Cat. No.: 1000005279 Specification: 96RXN	DNA Clean Beads	White	50 mL/ tube × 1 tube
	TE Buffer	White	25 mL/ tube × 1 tube
MGIEasy Dual Barcode Circularization Module Cat. No.: 1000018649 Specification: 16RXN***2**	Dual Barcode 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

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 UDB Primers Adapter 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 4 Equipment and Materials Required but not Provided

Equipment	Covaris™ Focused-ultrasonicator (Thermo Fisher Scientific™)
	Vortex Mixer
	Desktop Centrifuge
	Pipette
	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)
Reagents	Nuclease free water (NF water) (Ambion, Cat. No. AM9937)
	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*
	MGIEasy Dual Barcode Exome Capture Accessory Kit（1000018647/1000018648）*
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
	Qubit® Assay Tubes（Invitrogen，Cat. No. Q32856）

* Only when the Library Prep Set is used for liquid hybird capture.

1.7 Precautions and Warnings

- This product is for research use only, not for clinical diagnosis. Please read this manual carefully before use.
- 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, Upside down several times, 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)
- Before the experiment, please be familiar with and master the operation method and matters needing attention of all kinds of instruments.
- All samples and reagents should avoid direct contact with the skin and eyes. Do not swallow. If this happens, rinse immediately with plenty of water and go to the hospital immediately.
- All samples and wastes shall be disposed of in accordance with relevant regulations.
- 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 samples like animals, plants, fungus, bacteria, etc., including human, mouse, rice, Arabidopsis, yeast, E. coli and Metagenomics. 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. 350 bp.
- 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 other fragmentation methods are used, trial runs are recommended to determine optimal parameters for obtaining recommended fragment sizes.

2.2.2 Size Selection

- DNA fragmentation results in a wide distribution of fragment sizes. Size selection with magnetic beads is recommended to ensure the uniformity of libraries (see Table 5). Gel extraction methods can also be used instead.

Table 5 Size Selection Guidelines for 100 μL fragmented samples

Fragment size peak (bp)	180	230	280	300	350	420
Library size peak (bp)	310	360	410	465	510	550
Beads added in 1st round (μL)	100	90	80	70	60	55
Beads added in 2nd round (μL)	50	20	20	20	20	15

- Appendix C presents an example when 500 ng gDNA is fragmented and the fragment size peak of which is narrowed to 350 bp after "60 μL+20 μL" size selection in 100 μL volume.
- The loss rate during bead selection is approximately 60%~95%. For rare samples, Retrieve the beads from the 1st round and wash twice with 80% ethanol. Air dry the beads, elute DNA with TE Buffer, and store the elution product at -20°C as a backup.

2.3 DNA Quantification and Quality Control

- DNA quantification refers to quantifying and controlling the amount of DNA input that is used in the End Repair process. These sets are compatible with DNA amounts between 1-1000 ng in less than 40 μL.

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

- Ensure a narrow distribution of DNA fragment size. Narrower distribution results in higher quality of sequencing. A wide distribution lowers sequencing quality.
- These Library Prep. Sets support libraries with different fragment sizes (see Table 5). Sequencing quality may be slightly decreased with fragment sizes being decreased. Please use an appropriate insert size for library construction based on your sequencing strategy.

⚠ **Note: It is not recommended to pool libraries with different isert size together for sequencing.**

Chapter 3 Library Construction Protocol

The example shows that 500 ng gDNA (100 μL) is fragmented with the Covaris, and the fragmented gDNA is selected with 60 μL 1st bead selection and a 20 μL 2nd bead selection beads. After size selection, about 50 ng of 350 bp DNA fragments are obtained.

Follow Table 5, Table 8 and Table 11 to adjust this protocol for different amounts of the initial DNA sample and different sizes of target DNA fragments.

⚠ **Note: If dealing with FFPE samples, please read Appendix E which includes a summary of modifications for FFPE samples carefully before you begin. If dealing with cfDNA samplessed, skip the fragmentation step 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 **39.5 μL.** Place the tube on ice.

3.1.2 Take out the MGIEasy Universal DNA Library Prep Module. Prepare the End Repair and A-tailing Mixture on ice (see Table 6).

Table 6 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 10.5 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 centrifuge briefly to collect the solution at the bottom of the tube.

3.1.4 Place the 0.2 mL PCR tube into the thermocycler and run the program in Table 7.

Table 7 End Repair and A-tailing Program

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 with step 3.2.**

3.2 Adapter Ligation

⚠ **Note: Please read Appendix D-1 carefully before you begin.**

⚠ **Adapter quality as well as quantity effects the efficiency and quality of the library construction directly. Read table 8 for the link between DNA input and adapter dilution. Dilute the adapters with TE Buffer if necessary.**

Table 8 Recommended Adapter Input According to the Amount of DNA Sample

DNA Sample (ng)	MGI Adapter Dilution Ratio	MGI Adapter Input after Dilution (μL)
≥ 50	No dilution	5
25	2	5
10	5	5
5	10	5
2.5	15	5
1	45	5

⚠ **Increased Adapter amount may increase the library yield to a certain extent, especially when DNA sample ≤25 ng. If there is a need to optimize the efficiency of library construction, it is optional to increase Adapter input (within the range of 2-10 times).**

3.2.1 Take out MGIEasy UDB Primers Adapter Kit. Add 5 μL of UDB Adapter 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 on ice (see Table 9).

Table 9 Adapter Ligation Mixture

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

3.2.3 Pipette slowly then 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, the mixture should be pipetted slowly and the amount should be ensured.**

3.2.4 Place the 0.2 mL PCR tube from step 3.2.3 into the thermocycler and run the program in Table 10.

Table 10 Adapter Ligation Program

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

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 3.3.5 once, remove all liquid from the tube without disrupting the beads. Optionally , 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: The number of PCR cycles must be strictly controlled. Insufficient cycles may lead to a lower library yield. Excessive cycles may also lead to adverse effects such as over amplification, increased bias, PCR duplicates, chimeric sequences, or accumulated mutations. Table 11 shows the number of PCR cycles required to yield 500 ng or 1µg PCR product from 1-1000 ng high-quality gDNA. When the quality of gDNA is poor or consists of a longer fragment, PCR cycles should be increased appropriately for sufficient yield.**

Table 11 PCR Cycles Required to Yield 500 ng and 1 µg Libraries

DNA Sample (ng)	PCR Cycles required for corresponding yield	
	500 ng	1 µg
1	12-13	13-15
2.5	11-12	12-14
5	10-11	11-13
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

⚠ **Note: Please read Appendix D-2, D-3 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 pooling guide in Appendix D-3 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.

Table 12 PCR Mixture

Components	Volume
Ligation product	38 μL
PCR Enzyme Mix	50 μL
UDB PCR Primer mix	12 μL
Total	100 μL

3.4.3 Place the PCR tube into the thermocycler and run the program in Table 13.

Table 13 PCR Program

Temperature	Time	Cycles
Heated lid(105°C)	on	
95°C	3 min	1 cycle
98°C	20 s	
60°C	15 s	see Table 11
72°C	30 s	
72°C	10 min	1 cycle
4°C	Hold	

Note: Please refer to the recommended PCR cycle in Table 11 for different DNA input amount, Table 27 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 MGIEasy DNA Clean Beads 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. Optionally, 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. The formula 1 shows the calculation of the mass in (ng) that corresponds to 1 pmol of dsDNA sample with varying fragment sizes.

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

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 shows the detection results of Agilent 2100 Bioanalyzer:

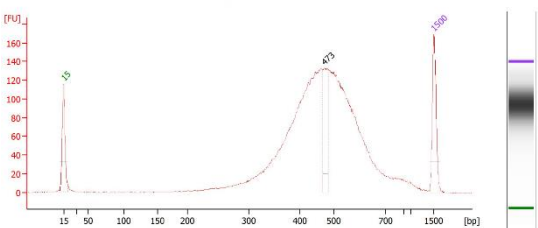

Figure 1 Agilent 2100 Bioanalyzer Fragment Size Distribution Results of the Purified PCR Product

⚠ **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.

3.7 Denaturation

3.7.1 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.

3.7.2 Place the PCR tube(s) into the thermocycler and run the program in Table 14.

Table 14 Denaturation Program

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

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

3.8 Single Strand Circularization

3.8.1 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

3.8.2 Transfer 12.1 μL of single strand circularization mixture into each PCR tube from step 3.7.3. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube(s).

3.8.3 Place the PCR tube(s) into the thermocycler and run the program in Table 16:

Table 16 Single Strand DNA Circularization Program

Temperature	Time
Heated lid(50°C)	On
37°C	10 min
4°C	Hold

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

3.9 Enzymatic Digestion

3.9.1 Prepare the following Enzymatic Digestion Mixture (see Table 17) in a new 0.2 mL PCR tube on ice during the reaction in step 3.8.3.

Table 17 Enzymatic Digestion Mixture

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

3.9.2 Transfer 4 μL of enzymatic digestion mixture into each PCR tube from step 3.8.4. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube(s).

3.9.3 Place the PCR tube(s) into the thermocycler and run the following program in Table 18.

Table 18 Enzymatic Digestion Program

Temperature	Time
Heated lid(105°C)	On
37°C	10 min
4°C	Hold

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

3.9.5 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.

3.10 Cleanup of Enzymatic Digestion Product

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

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

3.10.2 Transfer 170 μL of DNA Clean Beads to each tube containing Enzymatic Digestion product from step 3.9.5. Gently pipette 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.

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

3.10.4 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 with a pipette.

3.10.5 Keep the tube on the Magnetic Separation Rack, add **500 µL** of 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.

3.10.6 Repeat step 3.10.5 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.

3.10.7 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.

3.10.8 Remove the centrifuge tube from the Magnetic Separation Rack and add **22 µL of** TE Buffer 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 and place the centrifuge tube back onto 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.**

3.11 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 482 bp in Bioanalyzer (corresponding to the insert fragment peak around 350 bp) is 318 ng, the final yield after enzymatic digestion should be more than 22 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 into sizes between 100-700 bp.

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) 						
	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) 	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, It is recommended using DNA Clean Beads included in the MGIEasy DNA Clean Beads (MGI, Cat. No. 1000005278) or AMPure® XP (Agencourt, Cat. No. A63882). If Magnetic Beads from other sources are used, the cleanup conditions should be re-optimized.

Before 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. Optionally, 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 60 μL 1st bead selection and a 20 μL 2nd bead selection to target a 280 bp size fragment from fragmented DNA (100 μL). To select different fragment sizes, please refer to Table 5 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 60 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 20 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 UDB Primers Adapter Kit Instructions

MGIEasy UDB Universal Library Prep Sets (16 RXN) provides primers in tubes, 96RXN provides primers in plates, and 192RXN provides two plates of primers. This kit was developed to meet requirements for batch processing of library construction and multiplex sequencing. The maximum 192-well of UDB PCR Primer Mix and one tube of UDB Adapter Mix are provided, which allows for 192 samples of multiplex sequencing.

D-1 Note for UDB Adapter

- UDB Adapter 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 UDB Adapter to collect liquid at the bottom of tubes. Mix Adapters with a pipette before you use. Remember to close the cap immediately after use.

D-2 Note for UDB PCR Primer Mix

- The MGI dual-barcode library can be used for both single-barcode sequencing and dual-barcode sequencing. Please refer to the corresponding single-barcode/dual-barcode sequencing user manual.
- Before use, please centrifuge to collect liquid at the bottom of tubes or plates.
- For tubes, gently remove the cap to prevent liquid from spilling and cross-contamination. Remember to cover the tube immediately after use. For 96-well Plate, note that the sealing film is penetrable, and the surface of the sealing film should not touch sharp objects; Before use, spray 75% alcohol and wipe the surface of sealing film with absorbent paper.
- Note that when using different PCR Primer mixes, replace the pipette tips to avoid cross-contamination.
- 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-57_ UDB PCR Primer Mix-64 and UDB PCR Primer Mix-89_ UDB PCR Primer Mix—96 separately.

  For Plate: 96 UDB PCR Primer Mix per plate, 8 wells of each column are preset as a balanced dual barcode combination. The detailed layout is as follows.

Table 21 Set A barcode layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDB 1	UDB 9	UDB 17	UDB 25	UDB 33	UDB 41	UDB 49	UDB 57	UDB 65	UDB 73	UDB 81	UDB 89
B	UDB 2	UDB 10	UDB 18	UDB 26	UDB 34	UDB 42	UDB 50	UDB 58	UDB 66	UDB 74	UDB 82	UDB 90
C	UDB 3	UDB 11	UDB 19	UDB 27	UDB 35	UDB 43	UDB 51	UDB 59	UDB 67	UDB 75	UDB 83	UDB 91
D	UDB 4	UDB 12	UDB 20	UDB 28	UDB 36	UDB 44	UDB 52	UDB 60	UDB 68	UDB 76	UDB 84	UDB 92
E	UDB 5	UDB 13	UDB 21	UDB 29	UDB 37	UDB 45	UDB 53	UDB 61	UDB 69	UDB 77	UDB 85	UDB 93
F	UDB 6	UDB 14	UDB 22	UDB 30	UDB 38	UDB 46	UDB 54	UDB 62	UDB 70	UDB 78	UDB 86	UDB 94
G	UDB 7	UDB 15	UDB 23	UDB 31	UDB 39	UDB 47	UDB 55	UDB 63	UDB 71	UDB 79	UDB 87	UDB 95
H	UDB 8	UDB 16	UDB 24	UDB 32	UDB 40	UDB 48	UDB 56	UDB 64	UDB 72	UDB 80	UDB 88	UDB 96

Table 22 Set B barcode layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDB 97	UDB 105	UDB 113	UDB 121	UDB 129	UDB 137	UDB 145	UDB 153	UDB 161	UDB 169	UDB 177	UDB 185
B	UDB 98	UDB 106	UDB 114	UDB 122	UDB 130	UDB 138	UDB 146	UDB 154	UDB 162	UDB 170	UDB 178	UDB 186
C	UDB 99	UDB 107	UDB 115	UDB 123	UDB 131	UDB 139	UDB 147	UDB 155	UDB 163	UDB 171	UDB 179	UDB 187
D	UDB 100	UDB 108	UDB 116	UDB 124	UDB 132	UDB 140	UDB 148	UDB 156	UDB 164	UDB 172	UDB 180	UDB 188
E	UDB 101	UDB 109	UDB 117	UDB 125	UDB 133	UDB 141	UDB 149	UDB 157	UDB 165	UDB 173	UDB 181	UDB 189
F	UDB 102	UDB 110	UDB 118	UDB 126	UDB 134	UDB 142	UDB 150	UDB 158	UDB 166	UDB 174	UDB 182	UDB 190
G	UDB 103	UDB 111	UDB 119	UDB 127	UDB 135	UDB 143	UDB 151	UDB 159	UDB 167	UDB 175	UDB 183	UDB 191
H	UDB 104	UDB 112	UDB 120	UDB 128	UDB 136	UDB 144	UDB 152	UDB 160	UDB 168	UDB 176	UDB 184	UDB 192

D-3 Barcode Pooling Guide

- It is recommended to optimize base balance by planning dual barcode with diverse sequences when pooling libraries across DNBSEQ systems. Pooling combines at least eight libraries to sequence in one lane. Eight wells of each column are preset as a balanced dual barcode combination. Use this guide as a reference to plan X-plex pooling (X≥8) strategies showed in Table 23.

Table 23 Dual barcode Pooling Guide

Plexity	Combinations
8X	X entire column
8X+1	X entire column + 1 random well
8X+2	X entire column + 2 random well
8X+3	X entire column + 3 random well
8X+4	X entire column + 4 random well
8X+5	X entire column + 5 random well
8X+6	X entire column + 6 random well
8X+7	X entire column + 7 random well

- Under exceptional circumstance (for example, one well of barcode missed), when it cannot meet the requirement of at least one balanced barcode combination for standard pooling or the required data amount of each library pooled is not equal, make sure to determine the pooling strategy by calculating the content of each base in each sequencing cycle. It is necessary to ensure that each base content is not less than 12.5% and not more than 62.5% in single sequencing position in the same lane. (see table 24 and table 25)

Table 24 perfect balanced 8 barcode Pooling strategy（8 barcode from one entire column）

Sample 1	A	G	G	A	C	G	T	A	G	A
Sample 2	C	T	G	A	A	C	C	G	A	A
Sample 3	G	A	A	C	G	T	G	T	C	G
Sample 4	T	C	C	G	T	G	A	C	T	C
Sample 5	A	A	T	T	C	A	C	T	G	T
Sample 6	C	C	T	G	A	A	G	G	A	T
Sample 7	T	T	C	C	T	T	A	C	T	G
Sample 8	G	G	A	T	G	C	T	A	C	C
Signal%	**25.0**	**25.0**	**25.0**	**25.0**	**25.0**	**25.0**	**25.0**	**25.0**	**25.0**	**25.0**

Table 25 unacceptable 9 barcode Pooling strategy（barcodes from different column）

Sample 1	A	G	G	A	C	G	T	A	G	T
Sample 2	A	C	G	A	A	G	G	T	C	C
Sample 3	G	A	A	C	G	T	G	T	C	G
Sample 4	T	C	C	G	T	G	A	C	T	C
Sample 5	A	A	T	T	C	A	C	T	G	T
Sample 6	G	C	T	G	A	A	G	G	A	T
Sample 7	T	G	C	C	T	T	A	C	T	G
Sample 8	G	G	A	T	G	A	T	A	C	C
Sample 9	G	A	C	G	G	T	C	G	A	G
A signal%	33.3	33.3	22.2	22.2	22.2	33.3	22.2	22.2	22.2	0
T signal%	22.2	0	22.2	22.2	22.2	33.3	22.2	33.3	22.2	33.3
C signal%	0	33.3	33.3	22.2	22.2	0	22.2	22.2	33.3	33.3
G signal%	44.4	33.3	22.2	33.3	33.3	33.3	33.3	22.2	22.2	33.3

Appendix E 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.

E-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).

E-2 Recommendation of Input and Fragmentation for FFPE DNA

This set is compatible with 50-1000 ng input amount of FFPE gDNA. It is recommended to adjust the treatment time of fragmentation according to DNA integrity (Table 26).

Table 26 Recommendation of DNA Shearing for FFPE DNA

Shearing Parameter	Non-FFPE DNA	FFPE DNA	
		Main band>13000 bp or Q score >0.9	Smear band<13000 bp or Q score <0.9
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.**

E-3 Protocol Modifications for FFPE DNA

Protocol modifications based on Chapter 3 that should be applied to FFPE samples are summarized in Table 27.

Table 27 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
DNA Fragmentation and Size Selection	Size selection	No selection	No selection	No selection
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
PCR Amplification (for 500 ng PCR yield)	See table 11	Same as Non-FFPE DNA	Increase 1-2 cycles	Increase 2-3 cycles

Appendix F Sequencing Instructions

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.

MGI provides a preset dual barcode split list, which is distinguished according to the sequencer ECR version and SE/PE sequencing. If you use SE sequencing, you need to import the SE special dual barcode list of the corresponding software version.If you use PE sequencing, the PE special dual Barcode list needs to be imported.

If you use Barcode primer combinations other than the 192 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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