MGIEasy

FS DNA Library Prep Set User Manual

Cat. No.: 1000006987, 1000006988, 1000017572

Kit Version: V2.0

Manual Version: B1

Revision History

Manual Version	Kit Version	Date	Description
B1	V2.0	2019.09	◆ 1.3Add DNBSEQ series sequencing platform ◆ 1.4Add a new set and its contents. ◆ Add appendix C pathogen sample library construction protocol
B0	V2.0	2019.07	◆ Kit version V1.0 was upgraded to V2.0 ◆ Change composition of frag part (different article number) and fragmentation operation (volume, temperature, time) ◆ Change the construction condition of PE150 library and low input DNA library ◆ Delete some appendices and add the content to Chapter 3
A1	V1.0	2019.07	◆ Update appendix G Protocol
A0	V1.0	2019.03	◆ Initial release.

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

Contents

Chapter 1 Product Description

1.1 Introduction

The MGIEasy FS DNA Library Prep Set is specifically designed for WGS library construction for MGI high-throughput sequencing platforms. This library prep set is optimized to convert 5-400 ng genomic DNA into a customized library and uses advanced Adapter Ligation technology and High-fidelity PCR Enzymes to 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.

1.2 Application

This library prep set is applicable for samples from all common animals, plants, fungus, bacteria, etc., including human, mice, rice, Arabidopsis, yeast, E. coli, Metagenomics. Stable performance across all such sample types is expected. **(Pathogen sample library preparation protocol can see Appendix C).**

1.3 Platform Compatibility

Constructed libraries are compatible with

BGISEQ-500RS (PE100)

MGISEQ-2000RS (PE100/PE150)、DNBSEQ-G400RS (PE100/PE150)

MGISEQ-200RS (SE100), DNBSEQ-G50RS (SE100)

1.4 Contents

Each Library Prep Set consists of 4 modular kits of reagents sufficient for the indicated numbers of reactions. Further information on Cat. No., Components and Specifications is listed below:

Table 1 MGIEasy FS DNA Library Prep Set (Cat. No: 1000006987)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy FS DNA Library Prep Kit Cat. No.: 1000005254 Configuration: 16 RXN	Frag Buffer	Green	32 μL/tube× 1 tube
	Frag Enzyme	Green	64 μL/tube× 1 tube
	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 Configuration: 16 x 10 μL	DNA Adapters	White	10 μL/tube× 16 tubes
MGIEasy DNA Clean Beads Cat. No.: 1000005278 Configuration: 8 mL	DNA Clean Beads	White	8 mL/tube× 1 tube
	TE Buffer	White	4 mL/tube× 1 tube
MGIEasy Circularization Module Cat. No.: 1000005260 Configuration: 16 RXN	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 FS DNA Library Prep Set (Cat. No: 1000006988)

Modules & Cat. No.	Components	Caps Color	Spec & Quantity
MGIEasy FS DNA Library Prep Kit Cat. No.: 1000005256 Configuration: 96 RXN	Frag Buffer	Green	192 μL/tube× 1 tube
	Frag Enzyme	Green	384 μL/tube× 1 tube
	ERAT Buffer	Orange	682 μL/tube× 1 tube
	ERAT Enzyme Mix	Orange	279 μL/tube× 1 tube
	Ligation Buffer	Red	1124 μL/tube× 2 tubes
	DNA Ligase	Red	154 μL/tube× 1 tube
	PCR Enzyme Mix	Blue	1200 μL/tube× 2 tubes
	PCR Primer Mix	Blue	576 μL/tube× 1 tube
MGIEasy DNA Adapters-96 (Plate) Kit Cat. No.: 1000005282 Configuration: 96 x 10 μL	DNA Adapters	___	10 μL/well× 96 wells
MGIEasy DNA Clean Beads Cat. No.: 1000005279 Configuration: 50 mL	DNA Clean Beads	White	50 mL/tube× 1 tube
	TE Buffer	White	25 mL/tube× 1 tube
MGIEasy Circularization Module Cat. No.: 1000005260 Configuration: 16 RXN	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 FS DNA Library Prep Set (Cat. No: 1000017572)

Modules & Cat. No.	Components	Caps Color	Spec & Quantity
MGIEasy FS DNA Library Prep Kit Cat. No.: 1000005256 Configuration: 96 RXN	Frag Buffer	Green	192 μL/tube× 1 tube
	Frag Enzyme	Green	384 μL/tube× 1 tube
	ERAT Buffer	Orange	682 μL/tube× 1 tube
	ERAT Enzyme Mix	Orange	279 μL/tube× 1 tube
	Ligation Buffer	Red	1124 μL/tube× 2 tubes
	DNA Ligase	Red	154 μL/tube× 1 tube
	PCR Enzyme Mix	Blue	1200 μL/tube× 2 tubes
	PCR Primer Mix	Blue	576 μL/tube× 1 tube
MGIEasy DNA Adapters-96 (Plate) Kit Cat. No.: 1000005282 Configuration: 96 x 10 μL	DNA Adapters	——	10 μL/well× 96 wells
MGIEasy DNA Clean Beads Cat. No.: 1000005279 Configuration: 50 mL	DNA Clean Beads	White	50 mL/tube× 1 tube
	TE Buffer	White	25 mL/tube× 1 tube
MGIEasy Circularization Module Cat. No.: 1000017573 Configuration: 96 RXN	Splint Buffer	Purple	1114 μL/tube× 1 tube
	DNA Rapid Ligase	Purple	48 μL/tube× 1 tube
	Digestion Buffer	White	135 μL/tube× 1 tube
	Digestion Enzyme	White	250 μL/tube× 1 tube
	Digestion Stop Buffer	White	720 μL/tube× 1 tube

1.5 Storage Conditions and Shelf Life

MGIEasy FS DNA Library Prep Kit

- Storage Temperature: -25°C to -15°C
- Production Date and Expiration Date: refer to the label
- Transport Conditions: transported on dry ice

MGIEasy DNA Adapters Kit

- Storage Temperature: -25°C to -15°C
- Production Date and Expiration Date: refer to the label
- Transport Conditions: transported on dry ice

MGIEasy DNA Clean Beads

- Storage Temperature: 2°C to 8°C
- Production Date and Expiration Date: refer to the label
- Transport Conditions: transported with ice packs

MGIEasy Circularization Kit

- Storage Temperature: -25°C to -15°C
- Production Date and Expiration Date: refer to the label
- Transport Conditions: transported on dry ice

* 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	Vortex Mixer
	Desktop Centrifuge
	Pipets
	Thermocycler
	Magnetic rack DynaMag™-2 (Thermo Fisher Scientific™, Cat. No. 12321D) or equivalent
	Qubit™ 3 Fluorometer (Thermo Fisher Scientific™, Cat. No. Q33216)
	Agilent 2100 Bioanalyzer (Agilent Technologies™, Cat. No. G2939AA)/LabChip® GX, GXII, GX Touch (PerkinElmer), or Fragment Analyzer™ (Advanced Analytical)
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)/Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Cat. No. P7589)
	High Sensitivity DNA Kits (Agilent Technologies™, Cat. No. 5067-4626)
	Agilent DNA 1000 Kit (Agilent, Cat. No. 5067-1504)
Consumables	Pipette Tips
	1.5 mL centrifuge tubes (Axygen, Cat. No. MCT-150-C)
	0.2 mL PCR tubes (Axygen, Cat. No. PCR-02-C) or 96-well plate (Axygen, Cat. No. PCR-96M2-HS-C)
	Qubit® Assay Tubes (Invitrogen, Cat. No. Q32856) or 0.5 mL Thin Wall PCR Tubes (Axygen, Cat. No. PCR-05-C)

1.7 Precautions and Warnings

- This product is for research use only, not for clinical diagnosis. Please read this manual carefully before use.
- Before the experiment, please be familiar with the operation methods and precautions of various instruments to be used.
- 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, mix by inverting then centrifuge briefly and place on ice for use. For other reagents, first thaw at room temperature and vortex 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)
- All samples and reagents should avoid direct contact with the skin and eyes. Do not swallow.
- All samples and wastes should be treated as contaminants in accordance with relevant regulations.
- If you have other questions, please contact MGI technical support MGI-service@genomics.cn

Chapter 2 Sample Preparation

2.1 Genomic DNA Type

This library preparation set is applicable for samples from all common animals, plants, fungus, bacteria, etc., including human, mouse, rice, Arabidopsis, yeast, E. coli and Metagenomics.

2.2 Genomic DNA integrity

It is strongly recommended to use high quality genomic DNA (OD_{260}/OD_{280}=1.8 ~ 2.0, OD_{260}/OD_{230} > 2.0) for fragmentation.

2.3 Genomic DNA Input

As the amount of genomic DNA decreases, the proportion of DNA fragments that successfully ligate Adapters will decrease. If the starting amount of genomic DNA is enough, it is recommended to use high input genomic DNA for library construction to achieve optimal results. The recommendations for different processes are shown in Table 5 below.

Table 5 Recommended Sample Starting Amount

Sample Type	Input Range	Recommended Input	Recommended Concentration
Complex genome	50-400 ng	200 ng	≥15 ng/μL
Simple genome	5-400 ng	100 ng	≥7.2 ng/μL
Microbiome	5-400 ng	100 ng	≥7.2 ng/μL
Meta	5-400 ng	100 ng	≥7.2 ng/μL

2.4 Storage Conditions for Genomic DNA

DNA storage buffers compatible with this kit include: water, EB, 0.1×TE, buffer AE, TE and other common extraction and dissolution buffers. Different EDTA contents require different interruption times (see Table 6).

In order to prevent the effect of too many inhibitors such as EDTA and EGTA on interrupting aging, it is recommended to dissolve in water, EB or 0.1×TE during sample extraction to ensure the consistency of interrupting results. If the DNA sample contains a high concentration of salt ions/proteins, the efficiency of DNA fragmentation may be affected.

- If other complex components (high salt ion/protein/bivalent cation/EDTA/ EGTA) are introduced into the DNA extraction process, it is recommended to use 2× beads for purification before fragmentation, and then elute with water, EB or 0.1×TE, with a recovery rate of about 90%. For precautions and purification procedures for DNA Clean Beads, please refer to Step 3.5 or Step 3.7 in Chapter 3.
- It is recommended to use 50 ng of non-precious DNA with the same extraction condition and the dissolved buffer for the fragmentation test. Refer to step 3.1. Assess the fragment size distribution of purified PCR products with Agilent 2100, then shorten or extend the 32℃ incubation time to achieve optimum results.

Chapter 3 Library Construction Protocol

⚠ **Note: The degree of fragmentation (size distribution of DNA fragments) is controlled by time and temperature. Therefore, please ensure the accuracy of time and temperature during the reaction. Samples and frag enzyme should be kept on ice at all times.**

⚠ **Note: The following fragmentation conditions are suitable to DNA dissolved in water, EB, 0.1×TE. Fragment size should be between 100 bp-1000 bp, with a peak size of 300bp-500bp - suitable for PE150 sequencing. If the genomic DNA storage buffer is not listed above, refer to Table 6 conditions to extend the 32°C incubation time to achieve the optimum results.**

3.1 Fragmentation

3.1.1 Select the appropriate input depending on the sample type (see Table 5). Select the appropriate dilution buffer and 32°C incubation time based on to the genomic DNA storage buffer (see Table 6):

Table 6 Recommended Fagmentation Conditions

gDNA Storage buffer	Dilution buffer	32°C incubation time	
		250 bp（PE100）	350 bp（PE150）
Water / EB / 0.1×TE (0-0.1 mM EDTA)	NF water	7-8 min	4-5 min
Buffer AE (0.5 mM EDTA)	Buffer AE / 0.5×TE	14-16 min	10-12 min
1×TE (1 mM EDTA)	1×TE	25-30 min	16-20 min

3.1.2 Transfer genomic DNA to a new 0.2 mL PCR tube. The volume should be less than or equal to **14 μL**. If the volume is less than 14 μL, add dilution buffer to bring the final volume to **14 μL**:

Table 7 Input DNA Dilution

Components	Volume
DNA	X μL
Dilution buffer	14-X μL
Total	14 μL

3.1.3 Mix Frag Enzyme by inverting 10 times then centrifuge briefly and place on ice for use. DO NOT vortex Frag Enzyme. Mix Frag Buffer by vortexing 3 times (3s each) then centrifuge briefly and place on ice for use.

3.1.4 Prepare the fragmentation mixture on ice (see Table 8). Pipette up and down at least 10 times to mix thoroughly. (DO NOT vortex)

Table 8 Fragmentation Mixture

Components	Volume
Frag Buffer	2 μL
Frag Enzyme	4 μL
Total	6 μL

3.1.5 Transfer 6 μL of the fragmentation mixture to the 0.2 mL PCR tube from step 3.1.2. Pipette at least 10 times to mix thoroughly and briefly centrifuge to collect the solution at the bottom of the tube.

3.1.6 Set and run the following program on the thermocycler (see Table 9). Make sure the thermocycler has cooled to 4°C.Place the 0.2 mL PCR tube from step 3.1.5 into the thermocycler and skip the 4°C Hold step to start the reaction at 32°C.

Table 9 Fragmentation Reaction Conditions

Temperature	Time
Heated lid	On
4°C	Hold
32°C	5 min
65°C	30 min
4°C	Hold

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

3.1.8 Add 31 μL of TE Buffer and transfer 50 μL of the solution to a new 1.5 mL centrifuge tube. Immediately add DNA Clean Beads for size selection.

3.1.9 The remaining samples can be reserved for size distribution detection.

⚠ **Note: For the first fragmentation test, it is recommended to take 1μL product from Step 3.1.9 and run Agilent 2100 Bioanalyzer (High Sensitivity DNA Kits). The normal PE150 fragment size should be between 100 bp-1000 bp, with a peak size of 300bp-500bp (see Figure 1). Titrate the 32°C incubation time if the peak size is too large or too small.**

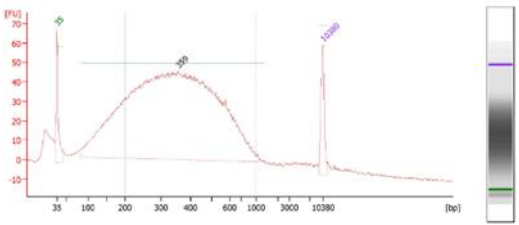

Figure 1 Agilent 2100 Bioanalyzer Fragment Size Distribution Results of the fragmentation Product

3.2 Size Selection/ Cleanup of Fragmentation Product（Alternative）

⚠ **Note: Please read Appendix A before you begin.**

⚠ **Note: After fragmentation, DNA has a wide size distribution, and it is usually necessary to conduct fragment screening to control the concentration of the final library fragments. We recommend performing Size Selection (see Step 3.2.1) when input DNA≥100 ng and perform Cleanup (see Step 3.2.2) when the sample input is low (≤50 ng) or high degradation (such as FFPE sample).**

Table 10 Recommended Purification Conditions after Fragmentation

Input gDNA	Operation after fragmentation	PE100 Beads Volume	PE150 Beads Volume
400 ng	Size Selection	40 μL+10 μL	30 μL+10 μL
200 ng	Size Selection	40 μL+10 μL	30 μL+10 μL
100 ng	Size Selection	40 μL+10 μL	30 μL+10 μL
50 ng	Purification	45 μL	40 μL
25 ng	Purification	45 μL	40 μL
10 ng	Purification	45 μL	40 μL
5 ng	Purification	45 μL	40 μL

3.2.1 Size Selection (Option 1)

The following steps used 30 μL + 10 μL beads to obtain the fragmentation products with the main peak≈350 bp, which is applicable for PE150. For other schemes, please refer to Table 10.

3.2.1.1 Take DNA Clean Beads out of the refrigerator and allow 30 minutes for the solution to warm to room temperature. Vortex and mix thoroughly before use.

3.2.1.2 Transfer 30 μL of DNA Clean Beads to the 1.5 mL centrifuge tube containing 50 μL of fragmentation product from step 3.1.8. Pipette up and down at least 10 times or vortex to mix thoroughly.

3.2.1.3 Incubate at room temperature for 5 minutes.

3.2.1.4 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.**

3.2.1.5 Transfer 10 μL of DNA Clean Beads to the centrifuge tube with 80 μL of supernatant from step 3.2.1.4. Pipette at least 10 times to mix thoroughly.

3.2.1.6 Incubate at room temperature for 5 minutes.

3.2.1.7 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 pipette.

3.2.1.8 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.

3.2.1.9 Repeat step 3.2.1.8 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 of the centrifuge tube, separate the beads magnetically, and then remove any remaining liquid using a small volume pipette.

3.2.1.10 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.

3.2.1.11 Remove the centrifuge tube from the Magnetic Separation Rack and add 42 μL of TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.2.1.12 Incubate at room temperature for 5 minutes.

3.2.1.13 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully transfer 40 μL of supernatant to a new 0.2 mL PCR tube.

3.2.1.14 Quantify the Size selection products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit.

3.2.2 Cleanup of Fragmentation Product (Option 2)

The following steps used 40 μL beads to obtain the fragmentation products with the main peak≈350 bp, which is applicable for PE150. For other schemes, please refer to Table 10.

3.2.2.1 Take DNA Clean Beads out of the refrigerator and allow 30 minutes for the solution to warm to room temperature. Vortex and mix thoroughly before use.

3.2.2.2 Transfer 40 μL of DNA Clean Beads to the 1.5 mL centrifuge tube containing 50 μL of fragmentation product from step 3.1.8. Pipette up and down at least 10 times or vortex to mix thoroughly.

3.2.2.3 Incubate at room temperature for 5 minutes.

3.2.2.4 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 pipette.

3.2.2.5 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.

3.2.2.6 Repeat step 3.2.2.5 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 of the centrifuge tube, separate the beads magnetically, and then remove any remaining liquid using a small volume pipette.

3.2.2.7 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.

3.2.2.8 Remove the centrifuge tube from the Magnetic Separation Rack and add 42 μL of TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.2.2.9 Incubate at room temperature for 5 minutes.

3.2.2.10 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully transfer 40 μL of supernatant to a new 0.2 mL PCR tube.

3.2.2.11 Quantify the purified fragmentation products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit.

✔ **Stopping Point: Size selection or purified fragmentation products can be stored at -20°C.**

3.3 End Repair and A-tailing

3.3.1 Transfer ≤100 ng of fragmentation product to a new 0.2 mL PCR tube. Add TE Buffer for a total volume of 40 μL.

3.3.2 Prepare the end repair and A-tailing mixture on ice (see Table 11).

Table 11 End Repair and A-tailing Mixture

Components	Volume
ERAT Buffer	7.1 μL
ERAT Enzyme Mix	2.9 μL
Total	10 μL

3.3.3 Transfer 10 μL of the end repair and A-tailing mixture to the 0.2 mL PCR tube from step 3.3.1. Vortex 3 times (3s each) and briefly centrifuge to collect the solution at the bottom of the tube.

3.3.4 Place the 0.2 mL PCR tube from step 3.3.3 into the thermocycler and run the program in Table 12.

Table 12 End Repair and A-tailing Reaction Conditions

Temperature	Time
Heated lid	On
37°C	30 min
65°C	15 min
4°C	Hold

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

⚠ **Warning: DO NOT STOP AT THIS STEP. Please continue to step 3.4.**

3.4 Adapter Ligation

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

⚠ **Note 2: Adapter quality as well as quantity directly effects the efficiency and quality of the library construction. Please refer to Table 13 for adapter dilution ratio. Please dilute the adapters with TE Buffer if necessary.**

Table 13 Recommended Adapter Input According to the Amount of DNA Sample

DNA Sample (ng)	MGI Adapter Dilution Ratio	MGI Adapter Input after dilution (μL)
400	No dilution	5
200	No dilution	5
100	No dilution	5
50	No dilution	5
25	2	5
10	5	5
5	10	5

⚠ **Note: Increasing Adapter input may increase the library yield to a certain extent, especially when DNA sample ≤50 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).**

3.4.1 Please refer to the instructions for MGIEasy DNA Adapters (see Appendix B) and Table 13. Add 5 μL of MGIEasy DNA Adapters (diluent) to the PCR tube from step 3.3.5. Vortex 3 times (3s each) and briefly centrifuge to collect the solution at the bottom of the tube.

3.4.2 Prepare the Adapter ligation mixture on ice (see Table 14).

Table 14 Adapter Ligation Mixture

Components	Volume
Ligation Buffer	23.4 μL
DNA Ligase	1.6 μL
Total	25 μL

3.4.3 Pipette slowly and transfer 25 μL of Adapter ligation mixture to the 0.2 mL PCR tube from step 3.4.1. Vortex 6 times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube.

⚠ **Note: Ligation Buffer is very viscous. It must be mixed thoroughly.**

3.4.4 Place the PCR tube from step 3.4.3 into the thermocycler and run the program in Table 15.

Table 15 Adapter Ligation Reaction Conditions

Temperature	Time
Heated lid	On
23°C	30 min
4°C	Hold

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

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

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

3.5 Adapter-Ligated DNA Cleanup

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

3.5.1 Take DNA Clean Beads out of the refrigerator and allow 30 minutes for the solution to warm to room temperature. Vortex and mix thoroughly before use.

3.5.2 Transfer 50 μL of DNA Clean Beads to the centrifuge tube from step 3.4.6. Pipette up and down at least 10 times or vortex to mix thoroughly.

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 of the 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.

3.5.7 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 21 μL of 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 and place the centrifuge tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 19 μL of supernatant to a new 0.2 mL PCR tube.

✔ **Stop Point: After cleanup, Adapter-ligated DNA can be stored at -20°C**

3.6 PCR Amplification

⚠ **Note: The number of PCR cycles must be strictly controlled. Insufficient cycles may lead a lower**

library yield. Excessive cycles may also lead to adverse effects such as over amplification, an increase in bias, PCR duplicates, chimeric sequences, or accumulated mutations. Table 16 shows the number of PCR cycles required to yield 300 ng and 1μg of PCR product from 5-400 ng of high-quality gDNA. When the quality of gDNA is poor and consists of a longer fragment, PCR cycles should be increased appropriately for sufficient yield.

Table 16 PCR Cycles Required to Yield 300 ng and 1 μg Products

Genomic DNA input (ng)	Operation after fragmentation	PCR Cycles required for corresponding yield	
		300 ng	1 μg
400 ng	Size Selection	3-4	6-7
200 ng	Size Selection	5-6	7-8
100 ng	Size Selection	8-9	9-10
50 ng	Purification	6-7	8-9
25 ng	Purification	7-8	9-11
10 ng	Purification	8-9	10-12
5 ng	Purification	9-10	11-13

3.6.1 Prepare the PCR amplification mixture on ice (see Table 17).

Table 17 PCR Amplification Mixture

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

3.6.2 Transfer 31 μL of PCR amplification mixture to the PCR tube from step 3.5.10. Vortex 3 times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.6.3 Place the PCR tube from step 3.6.2 into the thermocycler and run the program in Table 18. Please refer to Table 16 to adjust the number of cycles with different DNA input.

Table 18 PCR Amplification Reaction Conditions

Temperature	Time	Cycles
Heated lid	on	
95°C	3 min	1 cycle
98°C	20 s	
60°C	15 s	3-12 cycles
72°C	30 s	
72°C	10 min	1 cycle
4°C	Hold	

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

3.6.5 Transfer all of the solution to a new 1.5 mL centrifuge tube.

3.7 Cleanup of PCR Product

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

3.7.1 Take DNA Clean Beads out of the refrigerator and allow 30 minutes for the solution to warm to room temperature. Vortex and mix thoroughly before use.

3.7.2 Transfer 50 μL of DNA Clean Beads to the centrifuge tube from step 3.6.5. Pipette up and down at least 10 times or vortex to mix thoroughly.

3.7.3 Incubate at room temperature for 5 minutes.

3.7.4 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.

3.7.5 Keep the 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. Incubate for 30 seconds and carefully remove and discard the supernatant.

3.7.6 Repeat step 3.7.5 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, and remove any remaining liquid using a small volume pipette.

3.7.7 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.

3.7.8 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.

3.7.9 Incubate at room temperature for 5 minutes.

3.7.10 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 of supernatant to a new 1.5 mL centrifuge tube.

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

3.8 Quality Control of PCR Product

3.8.1 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 ≥ 1 pmol. Please refer to Formula 1 to calculate the amount of DNA needed. For example, for 384 bp PCR product the yield should reach 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 B). Quantify your Adapter-ligated samples before pooling. The total yield after pooling should be 1 pmol, with a total volume ≤ 48 μL.

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

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

3.8.2 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).

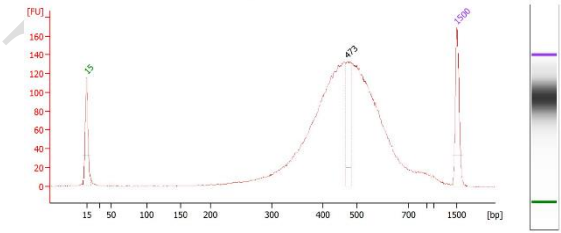

Figure 2 Agilent 2100 Bioanalyzer Fragment Size Distribution Results of the Purified PCR Product

3.9 Denaturation

3.9.1 According to the PCR product size, transfer 1 pmol of PCR product to a new 0.2 mL PCR tube. Add TE Buffer for a total volume of 48 µL.

3.9.2 Place the 0.2 mL PCR tube from step 3.9.1 into the thermocycler and run the program in Table 19.

Table 19 Denaturation Reaction Conditions

Temperature	Time
Heated lid	On
95°C	3 min

3.9.3 After the reaction is completed, immediately place the 0.2 mL PCR tube on ice for 2 minutes, then centrifuge briefly.

3.10 Single Strand Circularization

3.10.1 Prepare the single strand circularization mixture on ice (see Table 20).

Table 20 Single Strand Circularization Mixture

Components	Volume
Splint Buffer	11.6 µL
DNA Rapid Ligase	0.5 µL
Total	12.1 µL

3.10.2 Transfer 12.1 µL single strand circularization mixture to the 0.2 mL PCR tube from step 3.9.3. Vortex 3 times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.10.3 Place the PCR tube into the thermocycler and run the program in Table 21.

Table 21 Single Strand DNA Circularization Reaction Conditions

Temperature	Time
Heated lid	On
37°C	30 min
4°C	Hold

3.10.4 After the reaction is complete, immediately place the tube on ice for the next reaction.

3.11 Enzymatic Digestion

3.11.1 Prepare the following enzymatic digestion mixture (see Table 22) on ice during the reaction in step 3.10.3.

Table 22 Enzymatic Digestion Mixture

Components	Volume
Digestion Buffer	1.4 µL
Digestion Enzyme	2.6 µL
Total	4 µL

3.11.2 Transfer 4 µL of enzymatic digestion mixture into the PCR tube from step 3.10.4. Vortex 3 times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.11.3 Place the PCR tube from step 3.11.2 into the thermocycler and run the program in Table 23.

Table 23 Enzymatic Digestion Reaction Conditions

Temperature	Time
Heated lid	On
37°C	30 min
4°C	Hold

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

3.11.5 Add 7.5 µL Digestion Stop Buffer to the 0.2 mL PCR tube. Vortex 3 times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube. Transfer all of the solution into a new 1.5 mL centrifuge tube.

3.12 Enzymatic Digestion Product Cleanup

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

3.12.1 Take DNA Clean Beads out of the refrigerator and allow 30 minutes for the solution to warm to room temperature. Vortex and mix thoroughly before use.

3.12.2 Transfer 170 µL of DNA Clean Beads to the Enzymatic Digestion product from step 3.11.5. Gently pipette at least 10 times or vortex to mix thoroughly.

3.12.3 Incubate at room temperature for 10 minutes.

3.12.4 Centrifuge briefly and place the 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.

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

3.12.6 Repeat step 3.12.5 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 any remaining liquid using a small volume pipette.

3.12.7 Keep the 1.5 mL 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.

3.12.8 Remove the 1.5 mL centrifuge tube from the Magnetic Separation Rack and add 32 μL of TE Buffer to elute the DNA. Gently pipette up and down at least 10 times to mix thoroughly or until the beads are fully resuspended.

3.12.9 Incubate at room temperature for 10 minutes.

3.12.10 Centrifuge briefly and 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.

✔ **Stop Point: Purified Enzymatic Digestion products can be stored at -20°C for one month.**

3.13 Quality Control of Enzymatic Digestion Product

Quantitate the purified Enzymatic Digestion product with Qubit® ssDNA Assay Kit. The final Enzymatic Digestion products (ssDNA, ng) / input products of PCR (dsDNA, ng) should be ≥ 7%. For example, for 384 bp PCR product the final Enzymatic Digestion products should reach 17.5 ng.

Appendix

Appendix A Magnetic Beads and Cleanup Procedures

For bead-based purification, we recommend using DNA Clean Beads included in the MGIEasy DNA Clean Beads (MGI, Cat. No. 1000005278 or 1000005279) 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 before use. 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. This 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 touching the beads with pipette tips when pipetting. 2-3 μL of fluids can be left in the tube to avoid contact. In case 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 twice. Keep the centrifuge tube on the Magnetic Separation Rack when washing. Do not shake or disturb the beads in any way.
- After the 2nd wash of beads with ethanol, try to remove all of the liquid in the tube. You may centrifuge briefly to collect any remaining liquid at the bottom, separate beads magnetically, and remove the remaining liquid 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 of 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 B the Combination Barcode Adapters Strategies

- We currently offer two product specifications of the Adapter Reagent Kit based on the number of reactions: the MGIEasy DNA Adapters-16 (Tube) Kit and MGIEasy DNA Adapters-96 (Plate) Kit. Both kits were developed to meet requirements for batch processing of library construction and multiplex sequencing. We selected the best Adapter combinations based on the principle of balanced base composition. However, not all barcode adapter combinations are compatible. For optimal performance, please carefully read instructions in Appendix B-1 and B-2. Please note that Adapters from the two kits contain overlapping Barcodes and cannot be sequenced in the same lane.
- 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 or plates. Gently remove the cap/sealing film to prevent liquid from spilling and cross-contamination. Mix Adapters with a pipette before use. Remember to reseal the Adapters immediately after use. For Adapters-96 (Plate), if the seal film is contaminated, discard the old seal film and use a new one to reseal the 96-well plate.
- 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 during data analysis.

B-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 24 below to choose your barcode Adapter combinations.

Table 24 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 samples 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 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)).

B-2 MGIEasy DNA Adapters-96 (Plate) 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.

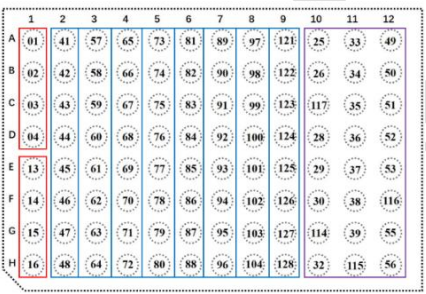

Figure 3 MGIEasy DNA Adapters-96 (Plate) Adapters Layout and Combination Instructions

2 sets of 4 Adapters: Column 1 (01-04, 13-16) (see the red box in Figure 3)

8 sets of 8 Adapters: Column 2-9 (41-48, 57-64, 65-72, 73-80, 81-88, 89-96, 97-104 and 121-128) (see the blue box in Figure 3)

1 set of 24 Adapters: Column 10-12 (see the purple box in Figure 3)

- If sequencing data output requirement is the same for all samples in a lane, please refer to the Table 25 below to organize your barcode Adapter combinations.

Table 25 MGIEasy DNA Adapters-96 (Plate) Kit Instruction

Sample/lane	Instruction (Example)
1	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. 41-48), mix 8 Adapters with equal volumes, then add the mixture

	to the sample.
2	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 (41-48), 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 41-44, then add to sample 1; Mix 45-48, then add to sample 2)
3	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 3.
4	1. Take a set of 4 Adapters (e.g. 01-04), add 1 Adapter to each sample in an equal volume. (e.g. Respectively add Adapters 01, 02, 03, 04 to samples 1, 2, 3, 4.) Or 2. Take a set of 8 Adapters (41-48), mix Adapters with equal volumes in pairs to obtain 4 mixtures of equal volumes. Add 1 mixture to each sample. (e.g. Mix 41-42, 43-44, 45-46, 47-48, then add respectively to samples 1, 2, 3, 4.)
5	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 sample 1-4 and 5.
6	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 5-6.
7	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) Note that you should use different Adapter sets for samples 1-4, samples 5-6 and sample 7.
8	Take a set of 8 Adapters (e.g. 41-48), add 1 Adapter to each sample in an equal volume.
8n+x (n=1,2, x=1-8, Total 9-24)	Follow these 3 steps: 1) For samples 1-8, use the method for (8 samples/lane) above. Or separate into 2 groups of 4 and use the method for (4 samples/lane) above for each group. 2) For samples 9-8n, separate samples into groups of 8, and use the method for (8 samples/lane) above. 3) For samples 8n+1 - 8n+X, according to the value of X, use the methods above for 1-8 sample/lane accordingly. Remember to use different Adapter sets. Note that you should use different Adapter sets for steps 1), 2) and 3).

8n+x (3≤n<11, x=1-8, Total 25-96)	Follow these 3 steps: 1) For samples 1-24, take a set of 24 Adapters and add 1 Adapter for each sample in an equal volume. 2) For samples 25-8n, separate the samples into groups of 8, and use the method for (8 samples/lane) above. 3) For samples 8n+1 - 8n+X, according to the value of X, use the methods above for 1-8 sample/lane accordingly. Remember to use different Adapter sets. Note that you should use different Adapter sets for steps 1), 2) and 3).

- For situations in which 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 C Pathogen Sample Library Construction Protocol

- MGIEasy FS DNA Library Prep Set is also applicable for pathogen samples. The sample types including alveolar lavage fluid, cerebrospinal fluid, sputum, blood. This library prep set is optimized to convert 5-100 ng genomics DNA into a customized library for MGI high-throughput sequencing platform series.
- It is strongly recommended to use high quality gDNA (OD260/OD280=1.8 ~ 2.0, OD260/OD230＞2.0) for fragmentation. The input 100 ng is recommended.
- The storage conditions for genomic DNA follow 2.4 of Chapter 2 Sample Preparation.
- Constructed libraries are compatible with:
  MGISEQ-200 (SE100)、DNBSEQ-G50 (SE100)

C-1 Fragmentation

⚠ **Note: The degree of fragmentation (size distribution of DNA fragments) is controlled by time and temperature. Therefore, please ensure the accuracy of time and temperature during the reaction. Samples and frag enzyme should be kept on ice at all times.**

⚠ **Note: The following fragmentation conditions are suitable to DNA dissolved in Buffer AE. The size of fragment product, after ligating the adaptor, should be between 200 bp-600 bp, with a peak size of 300bp-400bp - suitable for SE100 sequencing. If the genomic DNA storage buffer is not listed above, refer to Table 6 conditions to extend the 32°C incubation time to achieve the optimum results.**

C-1.1 Select the appropriate dilution buffer and 32°C incubation time based on to the genomic DNA storage buffer (see Table 26):

Table 26 Recommended Fragmentation Conditions

gDNA Storage buffer	Dilution buffer	32°C incubation time
		250 bp（PE100）
Water / EB / 0.1×TE (0-0.1 mM EDTA)	NF water	7-8 min
Buffer AE (0.5 mM EDTA)	Buffer AE / 0.5×TE	12-15 min
1×TE (1 mM EDTA)	1×TE	25-30 min

C-1.2 Transfer genomic DNA to a new 0.2 mL PCR tube. The volume should be less than or equal to 14 μL. If the volume is less than 14 μL, add dilution buffer to bring the final volume to 14 μL:

Table 27 Input DNA Dilution

Components	Volume
DNA	X µL
Dilution buffer	14-X µL
Total	14 µL

C-1.3 Mix Frag Enzyme by inverting 10 times then centrifuge briefly and place on ice for use. DO NOT vortex Frag Enzyme. Mix Frag Buffer by vortexing 3 times (3s each) then centrifuge briefly and place on ice for use.

C-1.4 Prepare the fragmentation mixture on ice (see Table 28). Pipette up and down at least 10 times to mix thoroughly. (DO NOT vortex)

Table 28 Fragmentation Mixture

Components	Volume
Frag Buffer	2 µL
Frag Enzyme	4 µL
Total	6 µL

C-1.5 Transfer 6 µL of the fragmentation mixture to the 0.2 mL PCR tube from step C-1.2. Pipette at least 10 times to mix thoroughly and briefly centrifuge to collect the solution at the bottom of the tube.

C-1.6 Set and run the following program on the thermocycler (see Table 29). Make sure the thermocycler has cooled to 4°C. Place the 0.2 mL PCR tube from step C-1.5 into the thermocycler and skip the 4°C Hold step to start the reaction at 32°C.

Table 29 Fragmentation Reaction Conditions

Temperature	Time
Heated lid	On
4°C	Hold
32°C	12 min
65°C	30 min
4°C	Hold

C-1.7 Briefly centrifuge to collect the solution at the bottom of the tube. Add 20 µL of TE Buffer and pipette at least 10 times to mix thoroughly.

C-2 End Repair and A-tailing

Follow step 3.3 of chapter 3 Library Construction Protocol.

C-3 Adapter Ligation

Follow step 3.4 of chapter 3 Library Construction Protocol.

C-4 Adapter-Ligated DNA Cleanup

Follow step 3.5 of chapter 3 Library Construction Protocol.

C-5 PCR Amplification

⚠ **Note: The number of PCR cycles must be strictly controlled. Insufficient cycles may lead a lower library yield. Excessive cycles may also lead to adverse effects such as over amplification, an increase in bias, PCR duplicates, chimeric sequences, or accumulated mutations. Table 30 shows the number of PCR cycles required to yield 100 ng from 5-100 ng of high-quality gDNA. When the quality of gDNA is poor and consists of a longer fragment, PCR cycles should be increased appropriately for sufficient yield.**

Table 30 PCR Cycles Required to Yield 100 ng Products

Genomic DNA input（ng）	PCR Cycles required for corresponding yield 100 ng
100 ng	9
50 ng	11
25 ng	14
10 ng	16
5 ng	18

C-5.1 Prepare the PCR amplification mixture on ice (see Table 31).

Table 31 PCR Amplification Mixture

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

C-5.2 Transfer 31 μL of PCR amplification mixture to the PCR tube containing 19 μL ligation purified products. Vortex 3 times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube.

C-5.3 Place the PCR tube from step C-5.2 into the thermocycler and run the program in Table 32. Please refer to Table 30 to adjust the number of cycles with different DNA input.

Table 32 PCR Amplification Reaction Conditions

Temperature	Time	Cycles
Heated lid	on	
95°C	3 min	1 cycle
98°C	20 s	
60°C	15 s	9-18 cycles
72°C	30 s	
72°C	10 min	1 cycle
4°C	Hold	

C-5.4 Centrifuge briefly to collect the solution at the bottom of the tube.

C-5.5 Transfer all of the solution to a new 1.5 mL centrifuge tube.

C-6 Size Selection of PCR product

The following steps used 30 μL + 10 μL beads to obtain the PCR products with the main peak≈350 bp, which is applicable for SE100.

C-6.1 Take DNA Clean Beads out of the refrigerator and allow 30 minutes for the solution to warm to room temperature. Vortex and mix thoroughly before use.

C-6.2 Transfer 30 μL of DNA Clean Beads to the 1.5 mL centrifuge tube containing 50 μL of PCR product from step C-5.5. Pipette up and down at least 10 times or vortex to mix thoroughly.

C-6.3 Incubate at room temperature for 5 minutes.

C-6.4 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.**

C-6.5 Transfer 10 μL of DNA Clean Beads to the centrifuge tube with 80 μL of supernatant from step C-6.4. Pipette at least 10 times to mix thoroughly.

C-6.6 Incubate at room temperature for 5 minutes.

C-6.7 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 pipette.

C-6.8 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.

C-6.9 Repeat step C-6.8 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 of the centrifuge tube, separate the beads magnetically, and then remove any remaining liquid using a small volume pipette.

C-6.10 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.

C-6.11 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.

C-6.12 Incubate at room temperature for 5 minutes.

C-6.13 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully transfer 30 µL of supernatant to a new 1.5 mL centrifuge tube.

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

C-7 Quality Control of PCR product

Follow step 3.8 of Chapter 3 Library Construction Protocol.

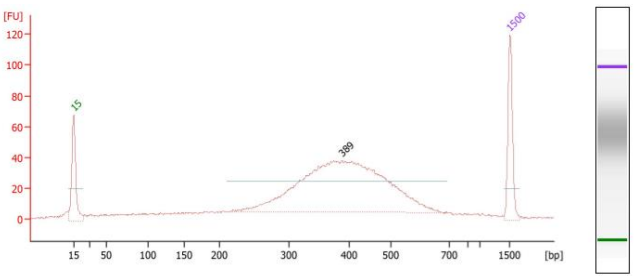

Figure 4 Agilent 2100 Bioanalyzer Fragment Size Distribution Results of the Purified PCR Product

C-8 Denaturation

Follow step 3.9 of Chapter 3 Library Construction Protocol.

C-9 Single Strand Circularization

Follow step 3.10 of Chapter 3 Library Construction Protocol.

C-10 Enzymatic Digestion

Follow step 3.11 of Chapter 3 Library Construction Protocol.

C-11 Enzymatic Digestion Product Cleanup

Follow step 3.12 of Chapter 3 Library Construction Protocol.

C-12 Quality Control of Enzymatic Digestion Product

Follow step 3.13 of Chapter 3 Library Construction Protocol.

■ Contact Information

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