Part No.:SOP-013-B02-108

User Manual

Version:8.0

MGIEasy FS DNA Library Prep Set

Cat. No.: 1000006987, 1000006988
1000017572

Set Version: V2.1

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Revision history

Manual version	Set version	Date	Description
8.0	V2.1	Apr. 2024	• Update the manufacture information • Update the manual style
B4	V2.1	Jan. 2022	• Updated the protocol of step 3.1 • Update the risk when treating Bronchoalveolar lavage fluid/sputum samples
B3	V2.0	Apr. 2021	• Kit version V2.0 was upgraded to V2.1 • Change composition of frag part (different article number) and fragmentation operation (volume, temperature, time) in step 3.1 • Change the bead purification conditions in Step 3.2
B2	V2.0	Jan. 2021	Update contact information
B1	V2.0	Sep. 2019	• 1.3 Add DNBSEQ series sequencing platform • 1.4 Add a new set and its contents • Add appendix C pathogen sample library construction protocol
B0	V2.0	Jul. 2019	• 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	Jul. 2019	Update appendix G Protocol
A0	V1.0	Mar. 2019	Initial release

Tips Please download the latest version of the manual and use it with the corresponding kit.

Search for the manual by Cat. No. or product name from the following website.

https://en.mgi-tech.com/download/files.html

Contents

1 Product overview

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 in this set have passed strict quality control and functional verification procedures, ensuring stability and reproducibility.

1.2 Intended use

This library prep set is applicable to samples from human, animals (including but not limited to rat, and mouse), plants (including but not limited to rice and *Arabidopsis*), bacteria (including but not limited to *E. coli*), fungi, and other microbial species. Stable performance across all such sample types is expected.

1.3 Applicable sequencing platforms

The prepared libraries are applicable to the following sequencing platforms.

- BGISEQ-500RS (PE100)
- MGISEQ-2000RS (PE100/PE150), DNBSEQ-G400RS (PE100/PE150)
- MGISEQ-200RS (SE100), DNBSEQ-G50RS (SE100)

1.4 Components

This library prep set comes in two specifications: 16 RXN, 96 RXN, and 96 RXN. Four separate boxes are included for each specification. For component details, refer to the following table.

Each library prep set contains an information card. Relevant manuals and SDS files can be downloaded from the MGI website provided on the information card.

Table 1 MGIEasy FS DNA Library Prep Set (16 RXN) (Cat. No: 1000006987)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy FS DNA Library Prep Kit Cat. No.: 1000005254 Configuration: 16 RXN	Frag Buffer II	Green	160 μL/tube × 1
	Frag Enzyme II	Green	80 μL/tube × 1
	ERAT Buffer	Orange	114 μL/tube × 1
	ERAT Enzyme Mix	Orange	47 μL/tube × 1
	Ligation Buffer	Red	375 μL/tube × 1
	DNA Ligase	Red	26 μL/tube × 1
	PCR Enzyme Mix	Blue	400 μL/tube × 1
	PCR Primer Mix	Blue	96 μL/tube × 1
MGIEasy DNA Adapters-16 (Tube) Kit Cat. No.: 1000005284 Configuration: 16 x 10 μL	DNA Adapters	White	10 μL/tube × 16
MGIEasy DNA Clean Beads Cat. No.: 1000005278 Configuration: 8 mL	DNA Clean Beads	White	8 mL/tube × 1
	TE Buffer	White	4 mL/tube × 1
MGIEasy Circularization Module Cat. No.: 1000005260 Configuration: 16 RXN	Splint Buffer	Purple	186 μL/tube × 1
	DNA Rapid Ligase	Purple	8 μL/tube × 1
	Digestion Buffer	White	23 μL/tube × 1
	Digestion Enzyme	White	42 μL/tube × 1
	Digestion Stop Buffer	White	120 μL/tube × 1

Table 2 MGIEasy FS DNA Library Prep Set (96 RXN) (Cat. No: 1000006988)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy FS DNA Library Prep Kit Cat. No.: 1000005256 Configuration: 96 RXN	Frag Buffer II	Green	960 µL/tube × 1
	Frag Enzyme II	Green	480 µL/tube × 1
	ERAT Buffer	Orange	682 µL/tube × 1
	ERAT Enzyme Mix	Orange	279 µL/tube × 1
	Ligation Buffer	Red	1124 µL/tube × 1
	DNA Ligase	Red	154 µL/tube × 1
	PCR Enzyme Mix	Blue	1200 µL/tube × 1
	PCR Primer Mix	Blue	576 µL/tube × 1
MGIEasy DNA Adapters-96 (Plate) Kit Cat. No.: 1000005282 Configuration: 96 x 10 µL	DNA Adapters	---	10 µL/well × 96
MGIEasy DNA Clean Beads Cat. No.: 1000005279 Configuration: 50 mL	DNA Clean Beads	White	50 mL/tube × 1
	TE Buffer	White	25 mL/tube × 1
MGIEasy Circularization Module Cat. No.: 1000005260 Configuration: 16 RXN	Splint Buffer	Purple	186 µL/tube × 1
	DNA Rapid Ligase	Purple	8 µL/tube × 1
	Digestion Buffer	White	23 µL/tube × 1
	Digestion Enzyme	White	42 µL/tube × 1
	Digestion Stop Buffer	White	120 µL/tube × 1

Table 3 MGIEasy FS DNA Library Prep Set (96 RXN) (Cat. No: 1000017572)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy FS DNA Library Prep Kit Cat. No.: 1000005256 Configuration: 96 RXN	Frag Buffer II	Green	960 µL/tube × 1
	Frag Enzyme II	Green	480 µL/tube × 1
	ERAT Buffer	Orange	682 µL/tube × 1

Item & Cat. No.	Component	Cap color	Spec & Quantity
	ERAT Enzyme Mix	Orange	279 µL/tube × 1
	Ligation Buffer	Red	1124 µL/tube × 1
	DNA Ligase	Red	154 µL/tube × 1
	PCR Enzyme Mix	Blue	1200 µL/tube × 1
	PCR Primer Mix	Blue	576 µL/tube × 1
MGIEasy DNA Adapters-96 (Plate) Kit Cat. No.: 1000005282 Configuration: 96 x 10 µL	DNA Adapters	---	10 µL/well × 96
MGIEasy DNA Clean Beads Cat. No.: 1000005279 Configuration: 50 mL	DNA Clean Beads	White	50 mL/tube × 1
	TE Buffer	White	25 mL/tube × 1
MGIEasy Circularization Module Cat. No.: 1000017573 Configuration: 96 RXN	Splint Buffer	Purple	1114 µL/tube × 1
	DNA Rapid Ligase	Purple	48 µL/tube × 1
	Digestion Buffer	White	135 µL/tube × 1
	Digestion Enzyme	White	250 µL/tube × 1
	Digestion Stop Buffer	White	720 µL/tube × 1

1.5 Storage and transportation

Table 4 Kit storage and transportation temperatures

Item	Storage temperature	Transportation temperature
MGIEasy FS DNA Library Prep Kit	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
MGIEasy DNA Adapters-16 (Tube) Kit		
MGIEasy DNA Adapters-96 (Plate) Kit		
MGIEasy Circularization Module		
MGIEasy DNA Clean Beads	2 ℃ to 8 ℃	

Tips • Production date and expiration date: refer to the label.

- For ice packs or dry ice shipments, ensure that there is enough ice or dry ice remaining after transportation.
- With proper transport, storage, and use, all components can maintain complete activity within their shelf life.

1.6 User-supplied materials

Table 5 Order information for MGI products

Catalog number	Model	Name
1000005279	50 mL	MGIEasy DNA Clean Beads

Table 6 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	/
Desktop centrifuge	/
Pipettes	/
Thermocycler	/
Magnetic rack DynaMag -2 or equivalent	Thermo Fisher Scientific, Cat. No. 12321D
Qubit Fluorometer or equivalent	Thermo Fisher, Cat. No. Q33216
Agilent 2100 Bioanalyzer or equivalent	Agilent Technologies , Cat. No. G2939AA

Table 7 Recommended reagent/consumable list

Reagent/consumable	Recommended brand
Nuclease free (NF) water	Ambion, Cat. No. AM9937 or equivalent
TE Buffer, pH 8.0	Ambion, Cat. No. AM9858 or equivalent
100% Ethanol (Analytical Grade)	/
Qubit ssDNA Assay Kit	Invitrogen, Cat. No. Q10212, or equivalent
Qubit dsDNA HS Assay Kit/Quant-iT	Invitrogen, Cat. No. Q32854) or equivalent
PicoGreen dsDNA Assay Kit	Invitrogen, Cat. No. P7589
Agilent High Sensitivity DNA Kit	Agilent, Cat. No. 5067-4626, or equivalent
Agilent DNA 1000 Kit	Agilent, Cat. No. 5067-1504, or equivalent
RECOVEREASE DNA ISOLATION KIT	Agilent Technologies, Cat No. 720203
Pipette tips	/
1.5 mL tube	/

Reagent/consumable	Recommended brand
0.2 mL PCR tube or 96-well plate	/
Qubit Assay Tubes or 0.5mL Thin Wall PCR Tubes	Invitrogen or Axygen or equivalent

1.7 Precautions and warnings

- This product is for research use only, not for in vitro diagnosis. Please read this manual carefully before use.
- Familiarize yourself with the precautions and operation methods of various instruments before performing the experiment.
- This manual aims to provide a standard protocol. Changes can be made for different applications, but changes must be tested prior to starting the protocol.
- It is recommended that you use pipette tips with filters to prevent cross-contamination. Use a new tip each time for pipetting different solutions or samples.
- It is recommended that you use the thermocyclers with heated lids for reactions. Preheat the thermocyclers to reaction temperature before use. If the thermocycler does not allow for lid temperature adjustments, the preset lid temperature of 105 °C is sufficient.
- Aerosol contamination may cause inaccurate results. It is recommended that you prepare separate working areas in the laboratory for PCR reaction preparation, PCR reaction, and PCR product cleanup. Use designated equipment for each area and clean the area regularly to ensure a sterile working environment (use 0.5% Sodium Hypochlorite or 10% bleach to clean the working area).
- Avoid skin and eyes contact with samples and reagents. Do not eat or drink the samples and reagents. In case of contact with skin and eyes, rinse immediately with plenty of water and seek medical advice.
- Conform to the law and regulations when disposing of all samples and reagents.
- If you have questions, contact Technical Support: **MGI-service@mgi-tech.com**.

1.8 Workflow

Section	Workflow	Total time	Hands-on time
3.1	Fragmentation	40 - 50 min	10 - 15 min
3.2	Magnetic beads size selection	30 - 60 min	15 - 25 min
3.3	End repair	55 min	5 - 10 min
3.4	Adapter ligation	40 min	10 min
3.5	Cleanup of adapter-ligated 	30 min	10 - 15 min
3.6	PCR	50 min	10 min

Section	Workflow	Total time	Hands-on time
3.7	Cleanup of PCR product ⏸	30 min	10~15 min
3.8	QC of PCR product ⏸	15 - 60 min	10 - 20 min
4.1	Denaturation and single strand circularization	45 - 50 min	15 min
4.2	Digestion	35 - 40 min	10 min
4.3	Cleanup of digestion product ⏸	40 - 50 min	10 - 15 min
4.4	QC of digestion product ⏸	15 - 20 min	10 - 15 min

Tips

- Total time: The theoretical use time of 8 reactions. The time will be extended if the number of reactions increases.
- Hands-on time: The total required hands-on time in the process.
- ⏸ : The stop point.

2 Sample preparation

2.1 Genomic DNA Type

This library prep set is applicable to samples from human, animals (including but not limited to rat, and mouse), plants (including but not limited to rice and *Arabidopsis*), bacteria (including but not limited to *E. coli*), fungi, and other microbial species.

Fragmentation may fail when treating samples from Bronchoalveolar lavage fluid/sputum.

2.2 Genomic DNA integrity

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

Table 8 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.

To prevent the effect of too many inhibitors such as EDTA and EGTA on the interruption of 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, refer to Appendix 5.1 or Section 3.5 or Section 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 incubation time of 30 ℃ to achieve optimum results.

3 Library preparation protocol

3.1 Fragmentation

Tips
- 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 always be kept on ice.
- 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 300 bp-500 bp suitable for PE150 sequencing. If the genomic DNA storage buffer is not listed above, please explore the interruption time of 30 ℃ by yourself.

3.1.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 9 Preparing the reagents

Reagent	Requirement
Dilution Buffer	User-supplied; place at room temperature (RT).
Frag Buffer II	Thaw at RT, mix well, centrifuge briefly, and place on ice.
Frag Enzyme II	Keep on ice.

CAUTION Mix the Frag Enzyme II by inverting 10 times and flicking the bottom gently, ensure that no residual reagent is left at the bottom. Centrifuge briefly and place them on ice until use. DO NOT vortex the Frag Enzyme II.

3.1.2 Fragmentation

1. Add 45 μL of genomic DNA to a new 0.2 mL PCR tube. Add dilution buffer to make a total volume of 45 μL if the fragmentation gDNA volume not enough. Place the tube(s) on ice.

Table 10 Normalization of gDNA

Reagent	Volume
gDNA	X μL
Dilution buffer	45 - X μL
Total	45 μL

2. Set and run the program as table below. The thermocycler will perform the first step reaction described in table below and be kept at 4 ℃.

Table 11 Fragmentation reaction conditions (Volume: 60 μL)

Temperature	Time
70 ℃ Heated lid	On
4 ℃	Hold
30 ℃	8 min
65 ℃	15 min
4 ℃	Hold

3. Mix the Frag Enzyme II by inverting 10 times and flicking the bottom gently, ensure that no residual reagent is left at the bottom. Centrifuge briefly and place on ice.

Tips DO NOT vortex the Frag Enzyme II. Insufficient mixing would affect the fragmentation process.

4. According to the desired reaction number, prepare the fragmentation mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 12 Fragmentation mixtuer

Reagent	Volume per reaction
Frag Buffer II	10 μL
Frag Enzyme II	5 μL
Total	15 μL

5. Add 15 μL of fragmentation mixture to each sample tube. Mix by vortexing 3 times (3 sec each), centrifuge briefly, and place on ice.
6. Make sure the thermocycler has cooled to 4 ℃. Place the PCR tube(s) into the thermocycler, and skip the 4 ℃ Hold step to start the reaction at 30 ℃.

7. When the program is completed, centrifuge the PCR tube(s) briefly and place on ice.

Tips
- For the first fragmentation test, it is recommended to take 3~5 ng fragmentation product for 1.8x DNA Clean Beads purification (Elute the sample with 5μL TE Buffer, refer to section 3.2.2 step 8 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 300 bp-500 bp. Titrate the 30 ℃ incubation time if the peak size is too large or too small.

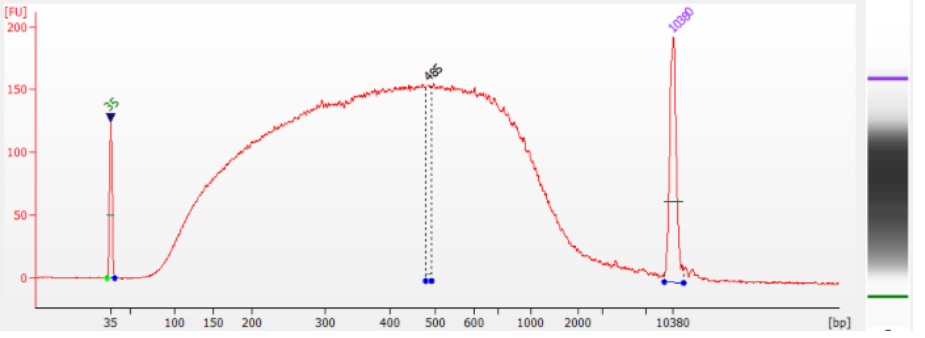

Figure 1 Agilent 2100 Bioanalyzer fragment size distribution results of the fragmentation product

3.2 Magnetic beads size selection

Tips Select one of the two methods based on your needs.

- After fragmentation, the DNA has a wide size distribution and it is usually necessary to perform a fragment screen to control the concentration of the final library fragments.
- Select the double size selection when the input DNA is >100 ng.
- Select the single size selection when the input sample is small (≤100 ng) or highly degraded (e.g. FFPE samples).

Table 13 Recommended purification conditions after fragmentation

Input gDNA	Operation after fragmentation	PE150 beads volume
400 ng	Double size selection	36 μL+12 μL
200 ng	Double size selection	36 μL+12 μL
100 ng	Single size selection	48 μL
50 ng	Single size selection	48 μL
25 ng	Single size selection	48 μL
10 ng	Single size selection	48 μL
5 ng	Single size selection	48 μL

3.2.1 Double size selection

Tips
- Using a 36 μL beads selection step followed by a 12 μL beads selection step to target a peak size of 330 bp from 60 μL of fragmentated gDNA, which is applicable for PE150.
- The DNA sample may loss approximately 60% to 90% in this process. If the sample is rare, it is recommended to recycle the DNA which adsorb on the 1st beads. Process the step 8 to 13 to recycle the DNA and store the eluted DNA as a backup.
- Do not disturb or pipette the beads when adding reagents or transferring supernatant. If you accidentally disturb or pipette the beads, pipette the solution and beads back into the tube and restart the separation process.

3.2.1.1 Preparation

Table 14 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
TE Buffer	Place at RT.
DNA clean beads	Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

3.2.1.2 Double size selection

Tips Transfer all liquid to a new 1.5 mL centrifuge tube (one tube per reaction) in advance if using a 1.5 mL centrifuge tube and a corresponding magnetic rack for purification.

1. Check the volume of the fragmentation product (from step 7 in section 3.1.2). If the volume is less than 60 μL, add TE Buffer to make a total volume of 60 μL.
2. Mix the DNA Clean Beads thoroughly. Add 36 μL of DNA Clean Beads to each sample tube. Gently pipette at least 10 times until all beads are suspended. Ensure all of the solution and beads in the tip are transferred into the tube after mixing. Or, mix with a vortexer.
3. Incubate the sample(s) at room temperature for 5 min.

   Tips In the next step, keep the supernatant and discard the beads. If necessary, recycle the DNA on that beads.

4. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 94 μL of supernatant to a new PCR tube or centrifuge tube.
5. Add 12 μL of DNA Clean Beads to each sample tube (contain 94 μL of supernatant). Gently pipette at least 10 times until all beads are suspended. Or mix with a vortexer.
6. Incubate the sample(s) at room temperature for 5 min.
7. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant.
8. While keeping the centrifuge tube(s) on the magnetic rack, add 200 μL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.

9. Repeat step 8. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
10. Keep the tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

    Tips Over-drying the beads will result in reduced yield.

11. Remove the centrifuge tube(s) from the magnetic rack and add 43 µL of TE Buffer to elute the DNA. Gently pipette the liquid at least 10 times until all beads are suspended. Or, mix with a vortexer.
12. Incubate the sample(s) at room temperature for 5 min.
13. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 41 µL of supernatant to a new 0.2 mL PCR tube.
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.

    Stop point Products can be stored at -20 ℃.

3.2.2 Single size selection

Tips
- Using a 48 µL beads selection step to target a peak size of 330 bp from 60 µL of fragmentated gDNA, which is applicable for PE150.
- Do not disturb or pipette the beads when adding reagents or transferring supernatant. If you accidentally disturb or pipette the beads, pipette the solution and beads back into the tube and restart the separation process.

3.2.2.1 Preparation

Table 15 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
TE Buffer	Place at RT.
DNA clean beads	Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

3.2.2.2 Single size selection

Tips Transfer all liquid to a new 1.5 mL centrifuge tube (one tube per reaction) in advance if using the 1.5 mL centrifuge tube and a corresponding magnetic rack for purification.

1. Check the volume of the fragmentation product (from step 7 in section 3.1.2). If the volume is less than 60 µL, add TE Buffer to make a total volume of 60 µL.

2. Mix the DNA Clean Beads thoroughly. Add 48 µL of DNA Clean Beads to each sample tube. Gently pipette at least 10 times until all beads are suspended. Or, mix with a vortexer.
3. Incubate the sample(s) at room temperature for 5 min.
4. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant.
5. While keeping the PCR tube(s) on the magnetic rack, add 200 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
6. Repeat step 5. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
7. Keep the centrifuge tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

   Tips Over-drying the beads will result in reduced yield.

8. Remove the PCR tube(s) from the magnetic rack and add 43 µL of TE Buffer to elute the DNA. Gently pipette the liquid at least 10 times until all beads are suspended. Or, mix with a vortexer.
9. Incubate the sample(s) at room temperature for 5 min.
10. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 41 µL of supernatant to a new 0.2 mL PCR tube.
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.

    Stop point Products can be stored at -20 ℃.

3.3 End repair

3.3.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 16 Preparing the reagents

Reagent	Requirement
TE Buffer	User-supplied; place at RT.
ERAT Buffer	Thaw at RT, mix well, centrifuge briefly, and place on ice.
ERAT Enzyme Mix	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.

3.3.2 End repair

1. Based on the quantification result of fragmentation products after size selection, if the yield ≤100 ng, transfer total products to a new 0.2 mL PCR tube for next step; If the yield >100 ng, it is recommended to transfer 100 ng products to a new 0.2 mL PCR tube for next step. The total volume should be less than 40 μL, add TE Buffer for a total volume of 40 μL.
2. According to the desired reaction number, prepare the end repair mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing, centrifuge briefly, and place on ice.

Table 17 End repair mixture

Reagent	Volume per reaction
ERAT Buffer	7.1 μL
ERAT Enzyme Mix	2.9 μL
Total	10 μL

3. Add 10 μL of end repair mixture to each sample tube (from step 12 in 3.2.1.2 or step 9 in 3.2.2.2). Vortex 3 times (3 sec each) to mix well. Centrifuge the tube(s) briefly and place on ice.
4. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 18 End repair reaction conditions (Volume: 50 μL)

Temperature	Time
70 ℃ Heated lid	On
37 ℃	30 min
65 ℃	15 min
4 ℃	Hold

5. When the program is completed, centrifuge the PCR tube(s) briefly to collect the liquid to the bottom of the tube.

WARNING
- Do not stop at this step. Please proceed to next reaction.
- If the operation stops here, store the end repair product(s) at –20 ℃ overnight with a risk of 20% decrease in yield.

3.4 Adapter ligation

Tips
- Before operation, carefully read “Using adapters” on page 29.
- Adapter quality as well as quantity directly effects the efficiency and quality of the library construction. Refer to table below for adapter dilution ratio. Dilute the adapters with TE Buffer if necessary.

- 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).

Table 19 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

3.4.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 20 Preparing the reagents

Reagent	Requirement
TE Buffer	User-supplied; place on RT.
Ligation Buffer	Thaw at RT, mix well, centrifuge briefly, and place on ice.
DNA Ligase	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
Adapters	Mix thoroughly, centrifuge briefly, and place on ice.

Tips
- Mix the adapter(s) well before using. Adapters should not be mixed directly with the adapter ligation mixture.
- The Ligation Buffer is highly viscous. Mix it by vortexing 6 times (3 sec each) and centrifuge briefly. When pipetting the Ligation Buffer, slowly aspirate to ensure that the volume is accurate.

3.4.2 Adapter ligation

1. Based on the input amount of the sample, dilute the adapters to the corresponding dilution ratio using TE Buffer. Mix it well by vortexing 3 times (3 sec each), centrifuge briefly, and place on ice.
2. **Add 5 μL of adapters or diluted adapters** to the corresponding sample tube (from step 5 in 3.3.2). Vortex the tube(s) 3 times (3 sec each), centrifuge briefly, and place on ice.

3. According to the desired reaction number, prepare the adapter ligation mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 21 Adapter ligation mixture

Reagent	Volume per reaction
Ligation Buffer	23.4 μL
DNA Ligase	1.6 μL
Total	25 μL

4. Slowly pipette 25 μL of adapter ligation mixture to each sample tube and vortex 6 times (3 sec each). Centrifuge briefly to collect the liquid to the bottom of the tube and place on ice.

Tips The adapter ligation mixture is highly viscous. Slowly aspirate to ensure the volume is accurate.

5. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 22 Adapter ligation reaction conditions (Volume: 80 μL)

Temperature	Time
30 ℃ Heated lid	On
23 ℃	30 min
4 ℃	Hold

6. When the program is completed, centrifuge the PCR tube(s) briefly and place on ice.
7. Add 20 μL of TE Buffer to make a total volume of 100 μL. Mix it well and centrifuge briefly.

Stop point The adapter-ligated DNA can be stored at -20 ℃ for no more than 16 hr.

3.5 Cleanup of adapter-ligated product

Tips Do not disturb or pipette the beads when adding reagents or transferring supernatant. If you accidentally disturb or pipette the beads, pipette the solution and beads back into the tube and restart the separation process.

3.5.1 Preparation

Table 23 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
TE Buffer	Place at RT

Reagent	Requirement
DNA Clean Beads	Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

3.5.2 Cleanup of adapter-ligated product

Tips Transfer all liquid to a new 1.5 mL centrifuge tube (one tube per reaction) in advance if using a 1.5 mL centrifuge tube and a corresponding magnetic rack for purification.

1. Mix the DNA Clean Beads thoroughly. Add 50 µL of DNA Clean Beads to each sample tube (from step 7 in 3.4.2), and gently pipette at least 10 times until all beads are suspended. Ensure that all of the solution and beads in the tip are transferred into the tube after mixing. Or, mix with a vortexer.
2. Incubate the sample(s) at room temperature for 5 min.
3. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant.
4. While keeping the tube(s) on the magnetic rack, add 200 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
5. Repeat step 4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
6. Keep the tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

   Tips Over-drying the beads will result in reduced yield.

7. Remove the tube(s) from the magnetic rack and add 21 µL of TE Buffer to elute the DNA. Gently pipette the liquid at least 10 times until all beads are suspended. Or, mix with a vortexer.
8. Incubate the sample(s) at room temperature for 5 min.
9. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 19 µL of supernatant to a new 0.2 mL PCR tube.

   Stop point After cleanup, the adapter-ligated product(s) can be stored at -20 ℃.

3.6 PCR

Tips
- 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.
- As table below which shows the number of PCR cycles required to yield 300 ng and 1 µg 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 24 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	Double size selection	3-4	6-7
200 ng	Double size selection	5-6	7-8
100 ng	Single size selection	5-6	7-8
50 ng	Single size selection	6-7	8-9
25 ng	Single size selection	7-8	9-11
10 ng	Single size selection	8-9	10-12
5 ng	Single size selection	9-10	11-13

3.6.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 25 Preparing the reagents

Reagent	Requirement
PCR Enzyme Mix	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.
PCR Primer Mix	Thaw at RT, mix by vortexing, centrifuge briefly, and place at RT.

3.6.2 PCR

1. According to the desired reaction number, prepare the PCR mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 26 PCR mixture

Reagent	Volume per reaction
PCR Enzyme Mix	25 μL
PCR Primer Mix	6 μL
Total	31 μL

2. Add 31 μL of PCR mixture to each sample tube (from step 9 in 3.5.2). Vortex the tube(s) 3 times (3 sec each) and centrifuge briefly to collect the liquid to the bottom of the tube.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 27 PCR reaction conditions (Volume: 50 μL)

Temperature	Time	Cycles
105 ℃ Heated lid	On	-
95 ℃	3 min	1
98 ℃	20 sec	3-12 see Table 24
60 ℃	15 sec	
72 ℃	30 sec	
72 ℃	10 min	1
4 ℃	Hold	-

4. When the program is completed, centrifuge the tube(s) briefly.

3.7 Cleanup of PCR product

Tips Do not disturb or pipette the beads when adding reagents or transferring supernatant. If you accidentally disturb or pipette the beads, pipette the solution and beads back into the tube and restart the separation process.

3.7.1 Preparation

Table 28 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
TE Buffer	Place at RT.
DNA Clean Beads	Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

3.7.2 Cleanup of PCR product

Tips Transfer all liquid to a new 1.5 mL centrifuge tube (one tube per reaction) in advance if using a 1.5 mL centrifuge tube and a corresponding magnetic rack for purification.

1. Mix the beads thoroughly. Add 50 μL of DNA Clean Beads to each sample tube (from step 4 in section 3.6.2). Gently pipette at least 10 times until all beads are suspended. Ensure that all of the solution and beads in the tip are transferred into the tube after mixing. Or, mix with a vortexer.
2. Incubate the sample(s) at room temperature for 5 min.

3. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant.
4. While keeping the tube(s) on the magnetic rack, add 200 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
5. Repeat step 4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
6. Keep the tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

   Tips Over-drying the beads will result in reduced yield.

7. Remove the tube(s) from the magnetic rack and add 32 µL of TE Buffer to elute the DNA. Gently pipette the liquid at least 10 times until all beads are suspended. Or, mix with a vortexer.
8. Incubate the sample(s) at room temperature for 5 min.
9. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 30 µL of supernatant to a new 1.5 mL centrifuge tube.

   Stop point After cleanup, the PCR product(s) can be stored at -20 ℃.

3.8 QC of PCR product

- **dsDNA fluorescence quantification method**: Quantify the purified PCR products with dsDNA fluorescence assay kits and instructions.
- **Electrophoresis method**: Assess the size range of purified PCR products with electrophoresis based equipment and instructions.

Table 29 Different QC methods and standards for library

Method	Equipment/Reagent	Standard
dsDNA fluorescence quantification method	Qubit dsDNA HS Assay Kit, Quant-iT PicoGreen dsDNA Assay Kit	Yield for PCR products: ≥ 1 pmol
Electrophoresis method	Tapestation (Agilent Technologies), Bioanalyzer, LabChip GX, GXII, GX Touch (PerkinElmer), Fragment Analyzer (Advanced Analytical)	/

Refer to Formula 1 to calculate the amount of DNA needed. For example, for 384 bp PCR product the yield should reach 254 ng.

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

$$\text{Mass corresponding to 1 pmol PCR product (ng)} = \text{PCR product peak size (bp)} \times 0.66$$

- The following figure shows the Agilent 2100 Bioanalyzer detection results of purified PCR products.

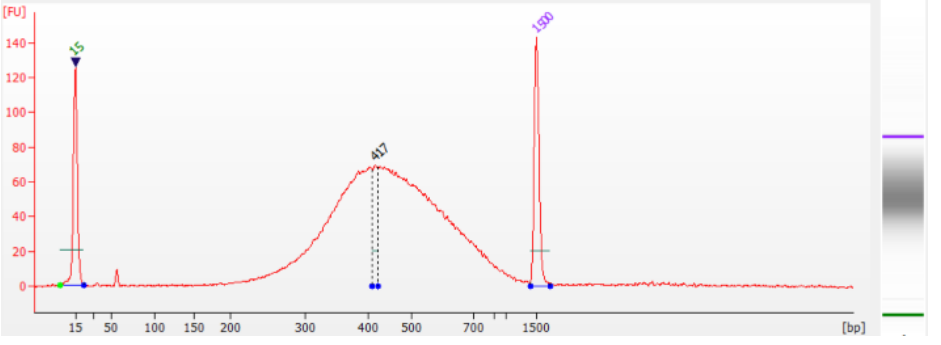

Figure 2 Agilent 2100 Bioanalyzer fragment size distribution results of the purified PCR product

- For multiple samples pooling in one land for sequencing, please follow the instructions provided by MGIEasy DNA Adapters Kitorrefer to Appendix 5.2. Detailed information shows how to plan samples pooling.
- Quantify the purified PCR products before pooling. The total yield after pooling should be 1 pmol, with a total volume ≤ 48 μL.

4 Circularization and digestion

4.1 Denaturation and single strand circularization

Tips Calculate the required purified PCR product volume based on the main fragment size of purified PCR product, concentration of sample, and Formula 1.

4.1.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 30 Preparing the reagents

Reagent	Requirement
TE Buffer	User-supplied; place at RT.
Splint Buffer	Thaw at RT, mix well, centrifuge briefly, and place on ice.
DNA Rapid Ligase	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.

4.1.2 Denaturation

1. Add 1 pmol of PCR product into a new 0.2 mL PCR tube. Add TE Buffer to make a total volume of 48 µL.
2. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 31 Denaturation reaction conditions (Volume: 48 µL)

Temperature	Time
105 ℃ Heated lid	On
95 ℃	3 min

3. When the program is completed, immediately place the PCR tube(s) on ice for 2 min, centrifuge briefly, and place on ice.

4.1.3 Single strand circularization

1. According to the desired reaction number, prepare the single strand circularization mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 32 Single strand circularization mixture

Reagent	Volume per reaction
Splint Buffer	11.6 μL
DNA Rapid Ligase	0.5 μL
Total	12.1 μL

2. Add 12.1 μL of single strand circularization mixture to each sample tube (from step 3 in 4.1.2). Vortex the tube(s) 3 times (3 sec each), centrifuge briefly, and place on ice.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 33 Single strand circularization reaction conditions (Volume: 60.1 μL)

Temperature	Time
45 ℃ Heated lid	On
37 ℃	30 min
4 ℃	Hold

4. When the program is completed, place the PCR tube(s) on ice, centrifuge briefly, and immediately proceed to the next step.

4.2 Digestion

4.2.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 34 Preparing the reagents

Reagent	Requirement
Digestion Buffer	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.
Digestion Enzyme	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
Digestion Stop Buffer	Thaw at RT, mix by vortexing, centrifuge briefly, and place at RT.

4.2.2 Digestion

1. According to the desired reaction number, prepare the digestion mixture in a 0.2 mL PCR tube on ice. Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 35 Digestion mixture

Reagent	Volume per reaction
Digestion Buffer	1.4 μL
Digestion Enzyme	2.6 μL
Total	4.0 μL

2. Add 4 μL of digestion mixture to each sample tube (from step 4 in section 4.1.3). Vortex 3 times (3 sec each), centrifuge briefly, and place on ice.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 36 Digestion reaction conditions (Volume: 64.1 μL)

Temperature	Time
45 ℃ Heated lid	On
37 ℃	30 min
4 ℃	Hold

4. When the program is completed, centrifuge the tube(s) briefly. Immediately add **7.5 μL of Digestion Stop Buffer** to each sample tube.
5. Vortex the tube(s) 3 times (3 sec each) and centrifuge briefly. Transfer all liquid to a new 1.5 mL centrifuge tube (one tube per reaction).

4.3 Cleanup of digestion product

Tips Do not disturb or pipette the beads when adding reagents or transferring supernatant. If you accidentally disturb or pipette the beads, pipette the solution and beads back into the tube and restart the separation process.

4.3.1 Preparation

Table 37 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied. Freshly prepared.
TE Buffer	Place at RT.

Reagent	Requirement
DNA Clean Beads	Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

4.3.2 Cleanup of digestion product

1. Mix the DNA Clean Beads thoroughly. Add 170 µL of DNA Clean Beads to each sample tube (from step 5 in 4.2.2). Gently pipette at least 10 times until all beads are suspended. Ensure that all of the solution and beads in the tip are transferred into the tube after mixing. Or, mix with a vortexer.
2. Incubate the sample(s) at room temperature for 10 min.
3. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant.
4. While keeping the tube(s) on the magnetic rack, add 500 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
5. Repeat step 4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
6. Keep the tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

   Tips Over-drying the beads will result in reduced yield.

7. Remove the tube(s) from the magnetic rack and add 32 µL of TE Buffer to elute the DNA. Gently pipette the liquid at least 10 times until all beads are suspended. Or, mix with a vortexer.
8. Incubate the sample(s) at room temperature for 10 min.
9. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 30 µL of supernatant to a new 1.5 mL centrifuge tube or PCR tube.

   Stop point After cleanup, the digestion product(s) can be stored at -20 ℃.

4.4 QC of digestion product

Quantify the purified digestion product by following the instructions of the Qubit ssDNA Assay Kit.

- The yield of digestion product (ssDNA) / PCR input (dsDNA) should be not less than 7%.

For example, for a 384 bp PCR product, 254 ng of the product is used for circularization and digestion. The yield of the final product should be above 17.8 ng.

5 Appendix

5.1 Magnetic beads and cleanup

It is recommended to use the DNA Clean Beads included in the MGIEasy DNA Clean Beads to purify the libraries. If magnetic beads from other brands are used, optimize the cleanup conditions before getting started.

5.1.1 Before use

1. Remove the magnetic beads from the 4 ℃ refrigerator 30 min in advance to allow the beads to equilibrate to room temperature. Equilibrating to room temperature ensures that the beads are at the expected capture efficiency.
2. Before each use, vortex or pipette the beads to ensure that they are thoroughly mixed.
3. The volume of the beads determines the lower limit of fragment size that can be purified. The higher volume of beads indicates the lower limit of fragment size.

5.1.2 Operation notes

- Sample volume: If the sample volume decreases (for example, from evaporation during incubation), add TE Buffer to reach the recommended sample volume. Purify the sample with the recommended volume of magnetic beads.
- Uncapping: Carefully open or close the tube cap while keeping the tube on the magnetic rack. Strong vibrations may cause sample loss from liquid or beads spilling out of the tubes. It is recommended that you hold the middle or lower part of the tube when opening the cap.

Removing the supernatant

1. Mix the sample and magnetic beads by vortexing or gently pipetting. Place the tube on a magnetic rack for separation. Do not remove the supernatant until the solution is completely clear.
2. The separation process takes approximately 2 to 3 min. Considering the difference in magnetism of magnetic racks or plates, leave enough time for the solution to become completely clear.

3. Keep the centrifuge tube(s) on the magnetic rack when removing the supernatant. Place the tip on the tube wall that is away from the rack and bead pellet.
4. To avoid touching or removing the magnetic beads, leave 2 to 3 μL of liquid in the tube. Pipette all of the solution and beads back into the tube and restart the separation process if necessary.

Wash

1. Wash the beads with freshly prepared room temperature 80% ethanol. Sufficient ethanol should be added to immerse the beads entirely.
2. Keep the centrifuge tube(s) on the magnetic rack during washing. Do not shake or disturb the beads while washing.
3. Remove all remaining ethanol after wash the beads two more times. If liquid remains on the tube wall, centrifuge the tube briefly and separate the beads from the liquid on the magnetic rack. Remove all remaining liquid with a low-volume pipette.

Air-dry

1. After both ethanol wash steps, air-dry the beads at room temperature.
   - The surface of the magnetic beads is **cracking**: **Indicates over-drying and reduces the purification yield.**
   - The surface of the magnetic beads is reflective: Indicates insufficient drying and easily causes anhydrous ethanol residues and affects subsequent reactions.
   - The surface of the magnetic beads is matte: Indicates that the beads are completely dry.
2. Air-drying takes approximately 3 to 5 min at room temperature. Different indoor temperatures and humidities of the air in labs may affect the drying time. Watch closely until the beads appear to have a matte appearance, which indicates complete drying.

Elution

1. Elute with the TE Buffer included with the kit.
2. The elution volume of TE buffer should be 2 μL more than the pipetting volume of the supernatant. This will allow you to avoid touching or pipetting the magnetic beads.

5.2 Using adapters

MGI currently offers the Adapter Reagent Kits with two specifications 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 combination based on the principle of balanced base composition. However, the numbers of barcode adapter are not continuous. For optimal performance, read the instructions of Adapter Reagent Kits carefully before use.

- Adapters from the two kits contain overlapping barcodes and cannot be sequenced in the same lane.
- All adapters are double stranded. To prevent structural changes, such as denaturation, which might affect performance, do not place the adapters above 30 ℃.
- Before use, mix the adapter(s) well and centrifuge to collect the liquid at the bottom of tubes or plates.
- Change tips when pipetting different adapters to prevent cross-contamination.

- For MGIEasy DNA Adapters-16 (Tube) Kit, carefully open the tube cap to prevent spills or cross-contamination and close the cap immediately after use.
- For the MGIEasy DNA Adapters-96 (Plate) Kit, spray 75% alcohol and wipe the surface of the aluminum film of the plate with absorbent paper. The aluminum film is penetrable and do not touch the surface of the aluminum film with sharp objects. Pierce the aluminum film to pipette solutions for first-time use. After use, separately transfer the remaining reagents to 1.5 mL centrifuge tube(s) or 0.2 mL PCR tube(s), label the tubes clearly, and store them at -20 ℃.
- Adapters from other MGI Library Prep Kits are designed differently and cannot be mixed with the adapters described here. Otherwise, errors will occur during barcode demultiplexing procedures on DNBSEQ platforms.

5.2.1 Instructions for DNA Adapters-16 (Tube)

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

- 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, choose the barcode adapter combinations in the table below.

⚠ CAUTION The number of the adapter should not be repeated between samples in one lane.

Table 38 Instructions for DNA Adapters-16 (Tube)

Sample/lane	Instruction (Example)
1	Requires at least 1 set of adapters: • For a set of 4 adapters, add 4 adapters to each sample. For example: 01-04. Mix 4 adapters with equal volume and add the mixture to the sample. • Or, for a set of 8 adapters, add 8 adapters to each sample. For example: 97-104. Mix 8 adapters with equal volume and add the mixture to the sample.
2	Requires at least 1 set of adapters: • For a set of 4 adapters, add 2 adapters to each sample. For example: 01-04. Mix 01 and 02 with equal volume and add the mixture to sample 1; Mix 03 and 04 with equal volume and add the mixture to sample 2. • Or, for a set of 8 adapters, add 4 adapters to each sample. For example: 97-104. Mix 97-100 with equal volume and add the mixture to sample 1; Mix 101-104 with equal volume and add the mixture to sample 2.

Sample/lane	Instruction (Example)
3	Requires at least 2 sets of Adapters: 1. For samples 1 and 2, use the method for (2 samples/lane) above. 2. For sample 3, use the method for (1 sample/lane) above. Tips Use different adapter sets for samples 1, 2, and 3.
4	Requires at least 1 set of adapters: • For a set of 4 adapters, add 1 adapter to each sample. For example: 01-04. Add adapter 01, 02, 03, 04 to sample 1, 2, 3, 4, in that order. • Or, for a set of 8 adapters, add 2 adapters to each sample. For example: 97-104. Mix 97-98, 99-100, 101-102, and 103-104 with equal volume. Add the mixture to sample 1, 2, 3, 4, in that order.
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. Tips Use different adapter sets for samples 1-4 and for sample 5.
6	Requires at least 2 sets of adapters: 1. For samples 1-4, use the method for (4 samples/lane) above. 2. For samples 5-6, use the method for (2 sample/lane) above. Tips Use different adapter sets for samples 1-4 and for samples 5-6.
7	Requires all 3 Adapter sets and follow 3 steps: 1. For samples 1-4, use the method for (4 samples/lane) above (use the first adapter set). 2. For samples 5-6, use the method for (2 samples/lane) above (use the second adapter set). 3. For sample 7, use the method for (1 sample/lane) above (use the third adapter set). ▪ 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. Tips Use different adapter sets for samples 1-4, for samples 5-6 and for sample 7.
8	Requires at least 1 set of adapters: • For a set of 8 adapters, add 1 adapter to each sample. For example: 97-104. Add adapters 97-104 to samples 1-8, in that order. • Or, for 2 sets of 4 adapters, add 1 adapter to each sample. For example: 01-04 and 13-16. Add 1 adapter to each sample.

Sample/lane	Instruction (Example)
8+x (x=1-8, Total 9-16)	Perform the following 3 steps: 1. For samples 1 to 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 X, according to the value of X, use the methods above for 1-8 sample/lane accordingly. Tips Use different adapter sets for steps 1 and 2.

For situations in which sequencing data output requirements are different among samples, any sample with a data output of more than 20% for each lane should use a separate set of adapters.

For example, 9 samples are pooled into one lane, one sample of which requires 30% of the total data output.

1. 8 samples may use adapter set (97-104).
2. The final sample should use a full adapter set instead of using only a single adapter. For example: adapter set (01-04) or (13-16).

5.2.2 Instructions for DNA Adapters-96 (Plate)

Based on the principles of balanced base composition, adapters should be used in specific groups. Follow the instructions below to use the adapters in the proper combinations.

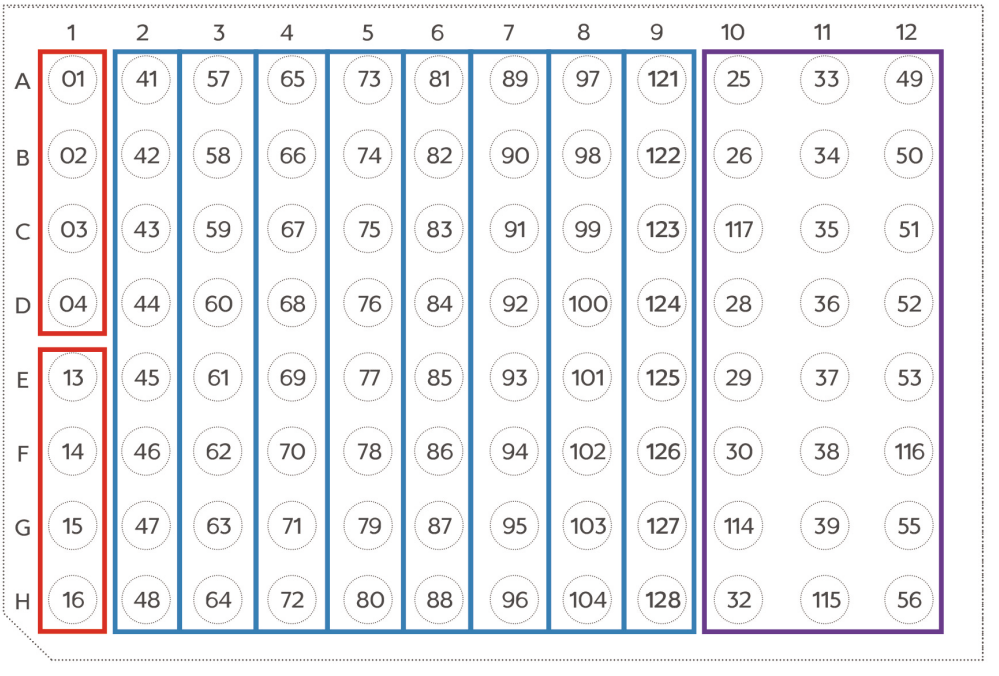

Figure 3 DNA adapters-96 (plate) adapters layout and combination instructions

- 2 sets of 4 adapters: Column 1 (01-04, 13-16) (see the red box in the figure above)
- 8 sets of 8 adapters: Columns 2-9 (41-48, 57-64, 65-72, 73-80, 81-88, 89-96, 97-104, and 121-128) (see the blue box in the figure above)
- 1 set of 24 adapters: Columns 10-12 (see the purple box in the figure above)

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

CAUTION The number of the adapter should not be repeated between samples in one lane.

Table 39 Instructions for DNA Adapters-96 (Plate)

Sample/lane	Instruction (Example)
1	• For a set of 4 adapters, add 4 adapters to each sample. For example: 01-04. Mix 4 adapters with equal volume and add the mixture to the sample. • Or, for a set of 8 adapters, add 8 adapters to each sample. For example: 41-48. Mix 8 adapters with equal volume and add the mixture to the sample.
2	• For a set of 4 adapters, add 2 adapters to each sample. For example: 01-04. Mix 01 and 02 with equal volume and add the mixture to sample 1; Mix 03 and 04 with equal volume and add the mixture to sample 2. • Or, for a set of 8 adapters, add 4 adapters to each sample. For example: 41-48. Mix 41-44 with equal volume and add the mixture to sample 1; Mix 45-48 with equal volume and add the mixture to sample 2.
3	1. For samples 1 and 2, use the method for (2 samples/lane) above. 2. For sample 3, use the method for (1 sample/lane) above. Tips Use different adapter sets for samples 1, 2, and 3.
4	• For a set of 4 adapters, add 1 adapter to each sample. For example: 01-04. Add adapters 01, 02, 03, 04 to samples 1, 2, 3, 4, in that order. • Or, for a set of 8 adapters, add 2 adapters to each sample. For example: 41-48. Mix 41-42, 43-44, 45-46, and 47-48 with equal volume. Add the mixture to sample 1, 2, 3, 4, in that order.
5	1. For samples 1-4, use the method for (4 samples/lane) above. 2. For sample 5, use the method for (1 sample/lane) above. Tips Use different adapter sets for samples 1-4 and 5.
6	1. For samples 1-4, use the method for (4 samples/lane) above. 2. For samples 5-6, use the method for (2 sample/lane) above. Tips Use different adapter sets for samples 1-4 and 5-6.

Sample/lane	Instruction (Example)
7	1. For samples 1-4, use the method for (4 samples/lane) above (use the first adapter set). 2. For samples 5-6, use the method for (2 samples/lane) above (use the second adapter set). 3. For sample 7, use the method for (1 sample/lane) above (use the third adapter set). Tips Use different adapter sets for samples 1-4, samples 5-6, and sample 7.
8	• For a set of 8 adapters, add 1 adapter to each sample. For example: 41-48. Add adapters 41-48 to samples 1 - 8, in that order.
8n+x (n=1 or 2, x=1-8, total 9-24)	Perform the following 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. Tips Use different adapter sets for steps 1, 2, and 3.
8n+x (3≤n<11, x=1-8, total 25-96)	Perform the following 3 steps: 1. For samples 1-24, use a set of 24 adapters and add 1 adapter to each sample. 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. Tips Use different adapter sets for steps 1, 2, and 3.

For situations in which sequencing data output requirements are different among samples, any sample with a data output of more than 20% for each lane should use a separate set of adapters.

For example, 9 samples are pooled into one lane, one sample of which requires 30% of the total data output.

1. 8 samples may use adapters (41-48).
2. The final sample should use a full adapter set instead of using only a single adapter. For example: adapter set (01-04) or (13-16).