Part No.:SOP-013-B02-119

User Manual

Version:6.0

MGIEasy Universal DNA Library Prep Set

Cat. No.: 1000006985, 1000006986
1000017571

Set Version: V1.0

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About the user manual

Manufacturer information

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Revision history

Manual version	Set version	Date	Description
6.0	V2.1	Apr. 2024	• Update the manufacturer information • Update the manual style
5.0	V1.0	Mar. 2022	Update Manufacturer LOGO
A3	V1.0	Jan. 2021	Update contact information
A2	V1.0	Sep. 2019	• 1.3 Add DNBSEQ series sequencing platform • 1.4 Add a new set and its contents
A1	V1.0	May. 2019	Update the adapter dilution ratio in section adapter ligation
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 Universal DNA Library Prep Set is specifically designed for creating WGS libraries for the MGI high-throughput sequencing platform series. This library prep set is optimized to convert 0.5-50 ng of fragmented DNA into a customized library. This set incorporates improved Adapter ligation technology and high-fidelity PCR enzymes, which 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 (blood, saliva, FFPE, and so on), animals (mice, and so on), plants (*A. thaliana*, *O. sativa*, and so on), bacteria (*E. coli*, and so on), fungi (yeast, and so on), and microorganisms. 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 (PE50/PE100/PE150)
- MGISEQ-2000RS (PE100/PE150), DNBSEQ-G400RS (PE100/PE150)
- MGISEQ-200RS (PE100), DNBSEQ-G50RS (PE100)

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 Universal DNA Library Prep Set (Cat. No.:1000006985)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy Universal DNA Library Prep Kit Cat. No.: 1000005248 Configuration: 16 RXN	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 Universal DNA Library Prep Set (Cat. No: 1000006986)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy Universal DNA Library Prep Kit Cat. No.: 1000005250 Configuration: 96 RXN	ERAT Buffer	Orange	682 μL/tube × 1
	ERAT Enzyme Mix	Orange	279 μL/tube × 1
	Ligation Buffer	Red	1124 μL/tube × 1

Item & Cat. No.	Component	Cap color	Spec & Quantity
	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 Universal DNA Library Prep Set (Cat. No: 1000017571)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy Universal DNA Library Prep Kit Cat. No.: 1000005250 Configuration: 96 RXN	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

Item & Cat. No.	Component	Cap color	Spec & Quantity
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 Universal 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
Covaris Focused-ultrasonicator, or equivalent	Thermo Fisher Scientific
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, LabChip GX, GXII, GX Touch, 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 dsDNA HS Assay Kit	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
Covaris AFA Tubes for use with Ultrasonicator	ThermoFisher Scientific
Pipette tips	/
1.5 mL tube	/
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 ℃ 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

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

Follow Section 2.2.2 magnetic beads size selection, Section 3.2 adapter ligation, and Section 3.4 PCR to adjust this protocol for different amounts of the initial DNA sample and different sizes of target DNA fragments.

Section	Workflow	Total time	Hands-on time
3.1	End repair	55 min	10 min
3.2	Adapter ligation	40 min	10 min
3.3	Cleanup of adapter-ligated ⏸	20 - 30 min	10 - 15 min
3.4	PCR	50 min	10 min
3.5	Cleanup of PCR product ⏸	30 min	10 - 15 min
3.6	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	45 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.
- ⏸ Stop point.

2 Sample preparation

2.1 Sample requirements

This library prep set is applicable to samples from human (blood, saliva, FFPE, and so on), animals (mice, and so on), plants (*A. thaliana*, *O. sativa*, and so on), bacteria (*E. coli*, and so on), fungi (yeast, and so on), and microorganisms.

It is strongly recommended to use high quality genomic DNA (gDNA) samples ($A_{260/280}$ = 1.8-2.0) for fragmentation.

2.2 DNA fragmentation and size selection

2.2.1 Fragmentation

- Fragment gDNA into sizes ranging from 100 - 700 bp, with the majority of DNA fragments between 250 - 300 bp.
- "Reaction conditions of DNA fragmentation" on page 26 lists fragmentation parameters for Covaris 55 µL series Ultrasonicators. For fragmentation of other sample volumes (15 µL, 130 µL, 200 µL, and so on), please visit Covaris' official website for detailed instructions.
- If you choose other fragmentation methods, we recommend doing trial runs to determine optimal parameters for obtaining recommended fragment sizes before getting started with this set.

2.2.2 Magnetic beads size selection

- DNA fragmentation results in a wide distribution of fragment sizes. Size selection is usually required to ensure uniformity of the library.
- We recommend to use magnetic beads for size selection as table below. Gel extraction methods can also be used for the same purpose.

Table 8 Double size selection process: 100 μL sample of the theoretical majority of DNA fragments using magnetic beads selection

Target peak fragment size (bp)	180	230	280	335	420	550
1st beads selection (μL)	100	90	80	70	60	50
2nd beads selection (μL)	50	20	20	20	20	20

- **Example**: For a peak size of 280 bp: Fragment 500 ng gDNA (80 μL). If the DNA volume after fragmentation is less than 80 μL, add TE Buffer to reach a final volume of 80 μL. Then, perform a double size selection process with a 64 μL 1st beads selection followed by a 16 μL 2nd beads selection before End Repair, which provides the selected fragment size of 280 bp. For details, refer to "Magnetic beads size selection" on page 30.

2.3 Size selected DNA quantification and quality control

- Size selected DNA amount refers to the amount of DNA input that is used for the end repair process. This set is compatible with size selected DNA amounts between 0.5-50 ng in less than 40 μL.
- Try to ensure a narrow distribution of DNA fragment sizes. A narrow distribution results in a higher quality of sequencing. A wide distribution lowers sequencing quality.
- This library prep set supports a range of fragment sizes (see Table 8). Sequencing quality may slightly decrease with increasing fragment sizes. Use the appropriate insert size for library construction based on your planned sequencing strategies: A peak between 350-450 bp is recommended for PE100/PE150 sequencing, and the distribution around the peak should be near ± 200 bp.

  CAUTION It is not recommended to pool fragments of different lengths for multiplex sequencing.

- Any residual impurities (for example, metal chelators or other salts) in selected DNA fragments may adversely affect the efficiency of the End Repair process.

3 Library preparation protocol

3.1 End repair

Tips Preheat the thermocycler to reaction temperature in advance if the thermocycler heat up slowly.

3.1.1 Preparation

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

Table 9 Preparing the reagents

Reagent	Requirement
TE Buffer	User-supplied; place at room temperature (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.

3.1.2 End repair

1. Add an appropriate amount of sample (recommended 50 ng) into a new 0.2 mL PCR tube and add TE Buffer to make a total volume of 40 μL. Place the tube(s) on ice.
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 10 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 and 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 11 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.

CAUTION
- 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.

3.2 Adapter ligation

CAUTION Before operation, carefully read "Using adapters" on page 31.

Adapter quality as well as quantity directly affects the efficiency and quality of the library construction. Refer to the following table and the actual amount of genomic DNA to determine the corresponding times of adapter dilution. When the adapter needs to be diluted, use the TE Buffer in the kit.

Table 12 Recommended adapter input according to the amount of sample DNA (280 bp)

DNA Sample (ng)	Adapter : DNA (Ratio)	MGI Adapter Dilution Ratio	MGI Adapter Input after Dilution (μL)
50	200 : 1	No dilution	5
25	200 : 1	2	5
10	200 : 1	5	5
5	200 : 1	10	5
2.5	200 : 1	15	5
1	200 : 1	45	5
0.5	200 : 1	80	5

Increasing Adapter input may increase the library yield to a certain extent, especially when DNA sample ≤ 25 ng.

If it is necessary to optimize the efficiency of library construction, you may try increasing Adapter input (within the range of 2-10 times) as table above.

3.2.1 Preparation

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

Table 13 Preparing the reagents

Reagent	Requirement
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 use. Do not directly add the adapters into the adapter ligation mixture.
- The Ligation Buffer is highly viscous. Mix it by vortexing 6 times (3 sec each), and centrifuge briefly before use. When pipette the Ligation Buffer, slowly aspirate and pipette, to ensure that the volume is accurate.

3.2.2 Adapter ligation

1. Add **5 μL of adapter(s)** to the corresponding sample tube (from step 5 in section 3.1.2). Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.
2. According to the desired reaction number, prepare the adapter ligation mixture in a 0.2 mL PCR tube on ice. Vortex it 6 times (3 sec each), centrifuge briefly, and place on ice.

Table 14 Adapter ligation mixture

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

3. Slowly pipette 25 μL of adapter ligation mixture to each sample tube and vortex 6 times (3 sec each). Centrifuge the tube(s) 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 that the volume is accurate.

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

Table 15 Adapter ligation reaction conditions (Volume: 80 μL)

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

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

Stop point Adapter-ligated product can be stored at -20 ℃ for no more than 16 hr.

3.3 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.

3.3.1 Preparation

Table 16 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.

3.3.2 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 6 in section 3.2.2), 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 40 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.

   Tips
   - When using 50 ng DNA, use 40 µL TE Buffer for elution, using 19 µL for PCR reaction;
   - When using less than 50 ng DNA, we recommend to use 21 µL TE Buffer for elution, and transfer 19 µL to PCR tube in step 9 for PCR reaction.

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 38 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 °C.

3.4 PCR

The number of PCR cycles should be strictly controlled.

- Insufficient cycles may lead to a reduced library yield.
- Excessive cycles may also lead to adverse effects such as over amplification, an increase in bias, PCR duplicates, chimeric sequences, and accumulated mutations.

The indicated table shows the number of PCR cycles required to yield 300 ng and 1 µg of library from 0.5-500 ng high quality DNA sample (280 bp). For lower quality or longer DNA fragments, PCR cycles should be increased appropriately to generate sufficient yield.

Table 17 PCR cycles required to yield 300 ng and 1 µg libraries

DNA Sample (ng)	PCR cycles required for corresponding yield	
-	300 ng	1 µg
0.5	14-16	16-17
1	11-13	15-16
2.5	11-13	15-16
5	9-11	13-15
10	8-10	11-13
25	6-8	9-11
50 (take half of sample for PCR)	6-8	9-11

3.4.1 Preparation

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

Table 18 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.

3.4.2 PCR

1. Transfer 19 μL of purified Adapter-ligated DNA sample into a new 0.2 mL PCR tube.
2. According to the desired reaction number, prepare the PCR mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing, centrifuge briefly, and place on ice.

Table 19 PCR mixture

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

3. Add 31 μL of PCR mixture to each sample tube (from step 1). Vortex the tube(s) 3 times (3 sec each) and centrifuge briefly to collect the liquid to the bottom of the tube.
4. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 20 PCR reaction conditions (Volume: 50 μL)

Temperature	Time	Cycles
105 ℃ Heated lid	On	-
95 ℃	3 min	1
98 ℃	20 sec	7* see Table 17
60 ℃	15 sec	
72 ℃	30 sec	
72 ℃	10 min	1
4 ℃	Hold	-

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

3.5 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.

3.5.1 Preparation

Table 21 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.

3.5.2 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 5 in section 3.4.2). 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 °C.

3.6 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.

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

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

Mass corresponding to 1 pmol PCR product (ng) = PCR product peak size (bp) × 0.66

Table 22 The corresponding yield in 1 pmol for PCR products with different fragment sizes

Insert size (bp)	PCR product size (bp)	Corresponding yield in 1 pmol (ng)
180	264	175
230	314	208
280	364	241
335	419	277
420	504	333

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

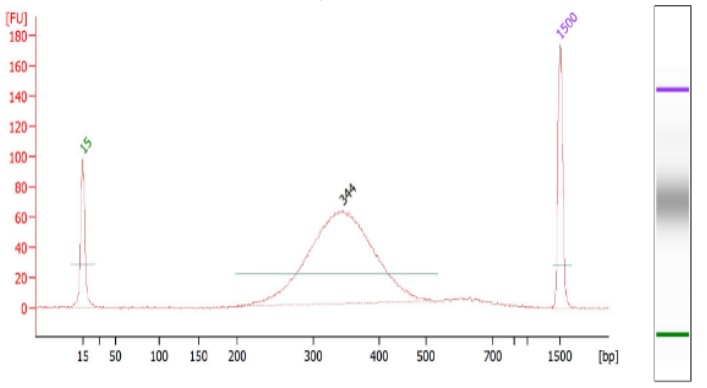

Figure 1 Agilent 2100 bioanalyzer fragment size distribution results of the purified PCR product (280 bp)

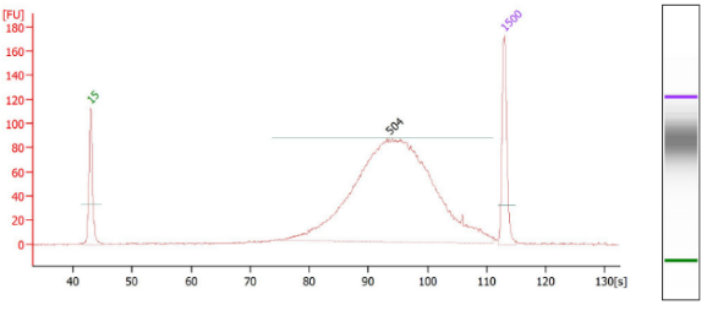

Figure 2 Agilent 2100 bioanalyzer fragment size distribution results of the purified PCR product (420 bp)

- For 1 sample sequencing in 1 lane, directly proceed to *Circularization and digestion* reaction.
- For multiple samples pooled sequencing, refer to section Pooling.

3.7 Pooling (option)

- **Purified PCR products pooling**

CAUTION
- Before pooling, carefully read "Using adapters" on page 31 .
- Do not pool PCR products with different insert size distributions in the same lane.

Quantify the purified PCR products before pooling. The total yield after pooling should be 1 pmol, with a total volume ≤ 48 μL.

Calculate the percentage of the required amount of sequencing data for samples on the same lane. Refer to Formula 1 in Section 3.6 and Formula 2 below to calculate the required mass of each sample. Formula 3 shows the calculation of sample volume.

Formula 2Calculation of each sample mass before pooling

Sample mass (ng)= Mass corresponding to 1 pmol PCR product (ng) × Ratio of sample data (%)

Formula 3Calculation of sample volume

$$\text{Sample volume (μL)} = \frac{\text{Sample mass (ng)}}{\text{Sample concentration (ng/μL)}}$$

For example: For 4 samples (belong to 280 bp insert size libraries, 84 bp adapter) pooled sequencing. The PCR products should have a total mass of 241 ng and be equal to a total yield of 1 pmol.

1. Calculation the mass of each sample.

- The expected amount of sequencingdata for each sample is the same. The ratio of each sample sequencing data is 25%. Referring to Formula 2, the required mass of each PCR sample is 241 ng × 25% = 60.25 ng.
- The expected amount of sequencing data for each sample is different. The ratios of sequencing data for samples 1-4 are 20%, 20%, 30%, and 30%. Referring to Formula 2, the required mass of sample 1 is 48.2 ng. Calculate the mass of samples 2 to 4 in the same way.

2. The concentration of sample 1 is 10 ng/μL. Refer to Formula 3 and the required volume is "A μL". Calculate the volume of samples 2 to 4 in the same way.
3. Transfer A μL of sample 1 into a new 0.2 mL PCR tube.
4. Add other samples into the same PCR tube.
5. Add TE Buffer to make a total volume of 48 μL.

Table 23 Multiple samples pooling (each sample volume should be at least 1 μL)

Name	Volume
Sample 1	A μL
Sample 2	B μL
Sample 3	C μL
Sample 4	D μL
TE Buffer	48 - (A+B+C+D) μL
Total	48 μL

Tips The volumes of A, B, C, and D should each be ≥ 1 μL.

Follow one of the two methods to pool the samples when the required volume of a sample is less than 1 μL. Method 1 is recommended.

Method 1: Increase the volume of all samples by Z (Z > 1) times. After mixing the samples, take 1/Z of the total volume W μL. Add TE Buffer to make a total volume of 48 μL.

Table 24 Samples mixture: All samples volume increase by Z times

Name	Volume
Sample 1	A × Z μL
Sample 2	B × Z μL
Sample 3	C × Z μL
Sample 4	D × Z μL
Total	W μL

Table 25 Method 1: Multiple samples pooling

Name	Volume
Samples mixture	(W ÷ Z) μL
TE Buffer	48 - (W ÷ Z) μL
Total	48 μL

Tips If necessary, quantify the samples mixture and calculate a new volume X μL (1 pmol). Replace "(W ÷ Z) μL" with "X μL".

Method 2: Dilutes a high concentration sample by Y (Y > 1) times if the required volume is less than 1 μL. Quantify the diluted sample and calculate a new volume. Pool the diluted sample with other samples.

For example: The required volume of sample 3 is < 1 μL. It needs to be diluted by Y times.

Table 26 Diluted sample: Dilute the high concentration sample by Y times

Name	Volume
Sample 3	5 μL
TE Buffer	5Y - 5 μL
Total	5Y μL

Tips The adding volume of high concentration sample is recommended to be more than 5 μL.

Quantify the diluted sample. Refer to Formula 3 to calculate a new volume "E μL". Pool the diluted sample with other samples. Add TE Buffer to make a total volume of 48 μL.

Table 27 Method 2: Multiple samples pooling

Name	Volume
Sample 1	A μL
Sample 2	B μL
Sample 4	D μL
Diluted sample 3	E μL
TE Buffer	48 - (A+B+D) - E μL
Total	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, 2, and 3.

4.1.1 Preparation

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

Table 28 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 29 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 30 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 31 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 32 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 33 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 34 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 35 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 section 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 centrifuge 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 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.

7. Remove the tube(s) from the magnetic rack and add 22 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 20 µL of supernatant to a new 1.5 mL centrifuge tube.

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

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, input 280 bp fragmentation product, and the main size of PCR product is 364 bp after reactions. Input 241 ng dsDNA for circularization and digestion, the yield of the ssDNA should be more than 16.9 ng.

Table 36 Yields of 1 pmol PCR products with different fragment sizes after reactions

Input size (bp)	PCR product size (bp)	Qualified yield (ng)
180	264	≧ 12.3
230	314	≧ 14.6
280	364	≧ 16.9
335	419	≧ 19.4
420	504	≧ 23.4

5 Appendix

5.1 Reaction conditions of DNA fragmentation

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

Follow the parameters below to fragment gDNA into sizes between 100 to 700 bp with the target peak fragment size between 250 to 300 bp.

Table 37 Fragmentation parameters of Covaris S220 for target BP peaks between 150 to 550 bp (55 μL sample volume)

	Vessel	microTUBE-50 AFA Fiber-Screw-Cap (PN 520166) 						
	Sample volume	55 μL						
S220	Holder	S-Series Holder microTUBE-50 Screw-Cap (PN 500492)						
	Water level	10						
	Temperatu (°C)	7						
	Target BP (Peak)	150	200	250	300	350	400	550
	Peak incident power (W)	100	75	75	75	75	75	50
	Duty factor	0.3	0.25	0.2	0.2	0.15	0.1	0.1
	Cycles per burst	1000	1000	1000	1000	1000	1000	1000

	Treatment time (s)	150	95	65	45	45	55	50

Table 38 The Fragmentation parameters of Covaris series from 150 to 550 bp (55 μL sample volume)

	Vessel	MicroTUBE-50 Screw-Cap(PN 520166)	"8 microTUBE-50 AFA Fiber Strip V2 (PN 520174)" "8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240)"	"96 microTUBE-50 AFA Fiber Plate (PN 520168)" "96 microTUBE-50 AFA Fiber Plate Thin Foil (PN 520232)"
				
	Sample volume	55 μL		
E220	Racks	Rack 24 Place microTUBE Screw-Cap (PN 500308)	Rack 12 Place 8 microTUBE Strip (PN 500444)	No Rack needed
	Plate definitions	"E220_500308 Rack 24 Place microTUBE- 50 Screw-Cap +6.5mm offset"	"E220_500444 Rack 12 Place 8 microTUBE-50 Strip V2 -10mm offset"	"E220_520168 96 microTUBE-50 Plate -10.5mm offset" "E220_520232 96 microTUBE-50 Plate Thin Foil -10.5mm offset"
E220 evolution	Racks	"Rack E220e 4 Place microTUBE Screw Cap (PN 500432)""Rack E220e 8 microTUBE Strip V2 (PN 500437) Non Compatible"	"Rack E220e 4 Place microTUBE Screw Cap (PN 500432)""Rack E220e 8 microTUBE Strip V2 (PN 500437) Non Compatible"	"Rack E220e 4 Place microTUBE Screw Cap (PN 500432)""Rack E220e 8 microTUBE Strip V2 (PN 500437) Non Compatible"
	Plate definitions	"500432 E220e 4 microTUBE-50 Screw Cap -8.32mm offset" "500437 E220e 8 microTUBE- 50 Strip V2 -10mm offset" N/A	"500432 E220e 4 microTUBE-50 Screw Cap -8.32mm offset" "500437 E220e 8 microTUBE- 50 Strip V2 -10mm offset" N/A	"500432 E220e 4 microTUBE-50 Screw Cap -8.32mm offset" "500437 E220e 8 microTUBE- 50 Strip V2 -10mm offset" N/A
All	Temperatu (℃)	7		
	Water level	6	-2	0
	Intensifier (PN 500141)	Yes	Yes	Yes
	Y-dithering	No	No	Yes (0.5 mm Y-dither at 10 mm/s)

	Target BP (Peak)	150	200	250	300	350	400	550
Screw-Cap	Peak incident power (W)	100	75	75	75	75	75	30
	Duty factor	0.3	0.2	0.2	0.2	0.2	0.1	0.1
	Cycles per burst	1000	1000	1000	1000	1000	1000	1000
	Treatment time (s)	130	95	62	40	30	50	70
8-Strip	Peak incident power (W)	75	75	75	75	75	75	50
	Duty factor	0.15	0.15	0.2	0.2	0.2	0.1	0.1
	Cycles per burst	500	500	1000	1000	1000	1000	1000
	Treatment time (s)	360	155	75	45	35	52	50
Plate	Peak incident power (W)	100	100	75	75	75	75	75
	Duty factor	0.3	0.3	0.2	0.2	0.2	0.1	0.1
	Cycles per burst	1000	1000	1000	1000	1000	1000	1000
	Treatment time (s)	145	90	70	49	34	50	32

5.2 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.

5.2.1 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.

5.2.2 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.

5.3 Magnetic beads size selection

Tips
- In the following example, 64 μL(1st) + 16 μL(2nd) beads are used to select the fragmented product (80 μL), and the 280 bp Input DNA is finally obtained.
- To select different fragment sizes, refer to Table 8 in Chapter 2 for detailed conditions.
- The DNA sample may loss about 60%-95% in this process. As such, 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.

5.3.1 Preparation

For use with MGIEasy DNA Clean Beads (user-supplied). If you use the magnetic beads from other brands, optimize the cleanup conditions before getting started.

Table 39 Reagents preparation

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.

5.3.2 Double size selection

1. Transfer 80 μL of Fragmentation Products to a new 1.5 mL centrifuge tube. Add TE Buffer to make a final volume of 80 μL if the volume is less than 80 μL.
2. Mix the DNA Clean Beads thoroughly. Add 64 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 142 μL of supernatant to a new 1.5 mL centrifuge tube.
5. Add 16 μL of DNA Clean Beads to each sample tube (contain 142 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 tube(s) briefly and place on the magnetic rack for at least 5 min until the liquid is clear. Carefully remove and discard the supernatant.
8. 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.
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.

    Stop point Over-drying the beads will result in reduced yield.

11. 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. 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 30 μL of supernatant to a new 0.2 mL PCR tube.

5.4 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.

5.4.1 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 40 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).

5.4.2 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.

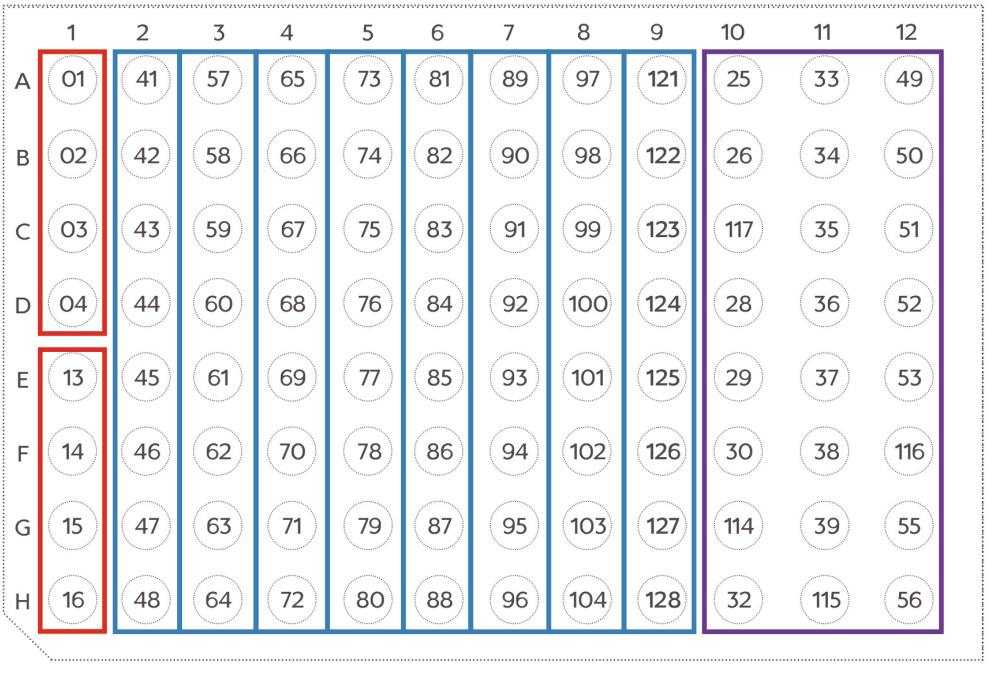

Figure 3 DNA adapters-96 (plate) adapters layout and combination instructions

- 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 41 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).