MGIEasy

Universal DNA Library Prep Set User Manual

Cat. No.: 1000006985, 1000006986

Kit Version: V1.0

Manual Version: A1

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Contents

Chapter 1 Product Description

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 within this set have passed stringent quality control and functional verification procedures, ensuring performance stability and reproducibility.

1.2 Application

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

1.3 Sequencing Platform Compatibility

Constructed libraries are compatible with

BGISEQ-500RS (PE50/PE100/PE150)
MGISEQ-2000RS (PE100/PE150)
MGISEQ-200RS(PE100)

1.4 Contents

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

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

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

Table 2 MGIEasy Universal DNA Library Prep Set (96 RXN) (Cat. No.: 1000006986)

Modules & Cat. No.	Components	Caps Color	Spec & Quantity
MGIEasy Universal DNA Library Prep Kit Cat. No.: 1000005250	ERAT Buffer	Orange	682 μL/ tube × 1 tube
	ERAT Enzyme Mix	Orange	279 μL/ tube × 1 tube
	Ligation Buffer	Red	1124 μL/ tube × 2 tubes
	DNA Ligase	Red	154 μL/ tube ×1 tube
	PCR Enzyme Mix	Blue	1200 μL/ tube × 2 tubes
	PCR Primer Mix	Blue	576 μL/ tube × 1 tube
MGIEasy DNA Adapters-96 (Plate) Kit Cat. No.: 1000005282	DNA Adapters	——	10 μL/ well × 96 wells
MGIEasy DNA Clean Beads Cat. No.: 1000005279	DNA Clean Beads	White	50 mL/ tube × 1 tube
	TE Buffer	White	25 mL/ tube × 1 tube
MGIEasy Circularization Kit Cat. No.: 1000005260	Splint Buffer	Purple	186 μL/ tube × 1 tube
	DNA Rapid Ligase	Purple	8 μL/ tube × 1 tube
	Digestion Buffer	White	23 μL/ tube × 1 tube
	Digestion Enzyme	White	42 μL/ tube × 1 tube
	Digestion Stop Buffer	White	120 μL/ tube × 1 tube

1.5 Storage Conditions and Shelf Life

MGIEasy Universal DNA Library Prep Kit

- Storage Temperature: -25°C to -18°C.
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: transported on dry ice.

MGIEasy DNA Adapters Kit

- Storage Temperature: -25°C to -18°C.
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: transported on dry ice.

MGIEasy DNA Clean Beads

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

MGIEasy Circularization Kit

- Storage Temperature: -25°C to -18°C
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: transported on dry ice.

* Please ensure that an abundance of dry ice remains after transportation.

* Performance of products is guaranteed until the expiration date, under appropriate transport, storage, and usage conditions.

1.6 Equipment and Materials Required but not Provided

Table 3 Equipment and Materials Required but not Provided

Equipment	Covaris™ Focused-ultrasonicator (Thermo Fisher Scientific™) Vortex Mixer Desktop Centrifuge Pipets Thermocycler Magnetic rack DynaMagTM-2 (Thermo Fisher Scientific™, Cat. No. 12321D) or equivalent Qubit™ 3 Fluorometer (Thermo Fisher Scientific™, Cat. No. Q33216) Agilent 2100 Bioanalyzer (Agilent Technologies™, Cat. No. G2939AA) / LabChip® GX, GXII, GX Touch (PerkinElmer), or Fragment Analyzer™ (Advanced Analytical)
Reagents	Nuclease free water (NF water) (Ambion, Cat. No. AM9937) 100% Ethanol (Analytical Grade) Qubit® ssDNA Assay Kit (Invitrogen, Cat. No. Q10212) Qubit® dsDNA HS Assay Kit (Invitrogen, Cat. No. Q32854)/ Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Cat. No. P7589) High Sensitivity DNA Kits (Agilent Technologies™, Cat. No. 5067-4626) Agilent DNA 1000 Kit (Agilent, Cat. No. 5067-1504)
Consumables	Covaris AFA Tubes for use with Ultrasonicator Pipette Tips 1.5 mL centrifuge tubes (Axygen, Cat. No. MCT-150-C) 0.2 mL PCR tubes (Axygen, Cat. No. PCR-02-C) or 96-well plate (Axygen, Cat. No. PCR-96M2-HS-C) Qubit® Assay Tubes (Invitrogen, Cat. No. Q32856) or 0.5 mL Thin Wall PCR Tubes (Axygen, Cat. No. PCR-05-C)

1.7 Precautions and Warnings

- Instructions provided in this manual are intended for general use only, and may require optimization for specific applications. We recommend adjusting according to the experimental design, sample types, sequencing application, and other equipment.
- Remove the reagents from storage beforehand, and prepare them for use: For enzymes, centrifuge briefly and place on ice until further use. For other reagents, first thaw at room temperature and invert several times to mix properly, then centrifuge briefly and place on ice until further use.
- To prevent cross-contamination, we recommend using filtered pipette tips. Use a new tip each time for pipetting different solutions.
- We recommend using thermocyclers with heated lids for reactions. Preheat to reaction temperature before use.
- Improper handling of samples and reagents may contribute to aerosol contamination of PCR Products and may decrease the accuracy of results. Therefore, we recommend physically separating two working areas in the laboratory for PCR reaction preparation and PCR product cleanup, respectively. Use designated equipment for each area and cleaning regularly to ensure a sterile working environment. (Use 0.5% Sodium Hypochlorite or 10% Bleach to clean working environment)
- If you have other questions, please contact MGI technical support MGI-service@genomics.cn

Chapter 2 Sample Preparation

2.1 Sample Requirement

This library preparation set is applicable for samples from all common animals, plants, fungus, bacteria, etc., including human, mouse, rice, Arabidopsis, yeast, E. coli, Metagenomics. It is strongly recommended to use high quality genomic DNA (gDNA) samples (A260/A280=1.8-2.0) for fragmentation.

2.2 DNA Fragmentation and Size Selection

2.2.1 Fragmentation

- Fragment gDNA into sizes between 100-700 bp, with the majority of DNA fragments between 250-300 bp.
- Appendix A lists fragmentation parameters for Covaris 55 μL series Ultrasonicators. For fragmentation of other sample volumes (15 μL, 130 μL, 200 μL, etc.), please visit Covaris' official website for detailed instructions.
- If 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** (see Table 4). Gel extraction methods can also be used for the same purpose.

Table 4 Magnetic Bead Selection Steps for a 100 μL Sample to Target Specific Fragment Sizes

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

- In Appendix B and C, 500 ng gDNA is fragmented (80 μL), If the DNA volume post-fragmentation is less than 80 μL, add TE buffer to reach a final volume of 80 μL. Then, the 64 μL 1st bead selection and 16 μL 2nd bead selection are performed before end repair, which provides the selected fragment size of 280 bp.

  The DNA sample loss during beads selection is approximately 60%-95%. For important samples, you may retrieve the beads from the 1st beads selection process and wash twice with 80% ethanol. Air-dry the bead pellet, elute DNA with TE Buffer, and store the elution product at -20°C as a backup.

2.3 Sample DNA Quantitation and Quality Control

- Sample DNA amount refers to the amount of DNA input that is used in the end repair process. This set is compatible with sample DNA amounts between 0.5-50 ng in less than **40 μL**.

- Try to ensure a narrow distribution of DNA fragment size. A narrow distribution results in higher quality of sequencing. A wide distribution lowers sequencing quality.
- This library prep set supports a range of fragment sizes (see Table 4). Sequencing quality may slightly decrease with increasing fragment sizes. Please use an appropriate insert size for library construction based on your sequencing strategy. 150 bp inserts are recommended for PE50 sequencing. 250-300 bp inserts are recommended for PE100 sequencing.

⚠ **Note: It is not recommended to pool fragments of different lengths for multiplex sequencing.**

Chapter 3 Library Construction Protocol

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

Follow Table 4 in Chapter 2.1 Sample Requirement, Table 20 in Appendix D, and Table 23 Appendix E to adjust this protocol for different amounts of the initial DNA sample and different sizes of target DNA fragments.

3.1 End Repair and A-tailing

3.1.1 Transfer an appropriate amount of sample (recommended: 50 ng) to a new 0.2 mL PCR tube and add TE Buffer for a final volume of **40 μL**. Place the tube(s) on ice.

3.1.2 Prepare the end repair and A-tailing mixture in a new 0.2 mL PCR tube on ice (see Table 5).

Table 5 End Repair and A-tailing Mixture

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

3.1.3 Transfer 10 μL of the end repair mixture to the 0.2 mL PCR tube from step 3.1.1. Vortex 3 times (3 s each) and briefly centrifuge to collect the solution at the bottom of the tube.

3.1.4 Place the 0.2 mL PCR tube from step 3.1.3 into the thermocycler and run the program in Table 6:

Table 6 End Repair and A-tailing Reaction Conditions

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

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

⚠ **Warning: DO NOT STOP AT THIS STEP. Please continue to step 3.2.**

3.2 Adapter Ligation

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

3.2.1 Please refer to the instructions for MGIEasy DNA Adapters (see Appendix D). Add 5 μL of MGIEasy DNA Adapters to the PCR tube from step 3.1.5. Vortex 3 times (3 s each) and briefly centrifuge to collect solution at the bottom of the tube.

3.2.2 Prepare the Adapter ligation mixture in a new 0.2 mL PCR tube on ice (see Table 7).

Table 7 Adapter Ligation Mixture

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

3.2.3 Pipette slowly to transfer 25 μL of Adapter ligation mixture to the PCR tube from step 3.2.1. Vortex 6 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.2.4 Place the 0.2 mL PCR tube from step 3.2.3 into the thermocycler and run the program in Table 8.

Table 8 Adapter Ligation Reaction Conditions

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

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

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

✔ **Stop Point: Adapter-ligated DNA can be stored at -20°C for NO more than 16 hours.**

3.3 Cleanup of Adapter-Ligated DNA

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

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

3.3.2 Transfer 50 μL DNA Clean Beads to the centrifuge tube from step 3.2.6. Pipette up and down at least 10

times to mix thoroughly. Ensure that all of the liquid and beads are fully dispensed from the pipette tip into the tube before proceeding.

3.3.3 Incubate at room temperature for 5 minutes.

3.3.4 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.3.5 Keep the tube on the Magnetic Separation Rack and add 200 μL of freshly prepared 80% ethanol to each tube to wash the beads and the walls of the tube. Incubate for 30 seconds, then carefully remove and discard the supernatant.

3.3.6 Repeat step 3.3.5 once and remove all of the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom of the centrifuge tube, separate the beads magnetically, and remove remaining liquid using a small volume pipette.

3.3.7 Keep the centrifuge tube on the Magnetic Separation Rack with the lid open, and air-dry beads at room temperature until no wetness (reflectiveness) is observed but before the pellet begins to crack.

3.3.8 Remove the centrifuge tube from the Magnetic Separation Rack and add 40 μL of TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

⚠ **Note: When using 50ng DNA, use 40μL TE Buffer for elution, using 19μL for PCR reaction; When using less than 50ng DNA, we recommend to use 21μL TE Buffer for elution, use 19μL for PCR.**

3.3.9 Incubate at room temperature for 5 minutes.

3.3.10 Centrifuge briefly and place the centrifuge tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 38 μL of supernatant to a new 1.5 mL centrifuge tube.

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

3.4 PCR Amplification

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

3.4.1 Transfer 19 μL of purified Adapter-ligated DNA sample into a new 0.2 mL PCR tube.

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

Table 9 PCR Amplification Mixture

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

3.4.3 Transfer 31 μL of PCR amplification mixture to the PCR tube from step 3.4.1. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.4.4 Place the PCR tube from step 3.4.3 into the thermocycler and run the program in Table 10.

Table 10 PCR Amplification Reaction Conditions

Temperature	Time	Cycles
Heated lid	on	
95°C	3 min	1 cycle
98°C	20 s	
60°C	15 s	7 cycles
72°C	30 s	
72°C	10 min	1 cycle
4°C	Hold	

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

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

3.5 Cleanup of PCR Product

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

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

3.5.2 Transfer 50 μL of DNA Clean Beads to the centrifuge tube from step 3.4.6. Pipette up and down at least 10 times to mix thoroughly. Ensure that all of the liquid and beads are fully dispensed from the pipette tip into the centrifuge tube before proceeding.

3.5.3 Incubate at room temperature for 5 minutes.

3.5.4 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.5.5 Keep the tube on the Magnetic Separation Rack and add 200 µL of freshly prepared 80% ethanol to wash the beads and the walls of the tube. Incubate for 30 seconds and carefully remove and discard the supernatant.

3.5.6 Repeat step 3.5.5 once, remove all of the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom. Separate the beads magnetically and remove remaining liquid using a small volume pipette.

3.5.7 Keep the centrifuge tube on the Magnetic Separation Rack with the lid open, and air-dry beads at room temperature until no wetness (reflectiveness) is observed but before the pellet begins to crack.

3.5.8 Remove the centrifuge tube from the Magnetic Separation Rack and add 32 µL of TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.5.9 Incubate at room temperature for 5 minutes.

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

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

3.6 Quality Control of PCR Product

Quantify the purified PCR products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit. The required yield for PCR products is ≥ 1 pmol. See Table 11 for the corresponding yield for different size fragments of PCR products. For pooled sequencing, please follow instructions provided by MGIEasy DNA Adapters User Manual. Detailed information shows how to plan your sample pooling (see Appendix D). Quantify your Adapter-ligated samples before pooling. The total yield after pooling should be 1 pmol, with a total volume ≤ 48 µL.

Table 11 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	170
230	314	210
280	364	240
335	419	280
420	504	330

Assess the fragment size distribution of purified PCR products with electrophoresis based equipment such as Bioanalyzer, Tapestation (Agilent Technologies), LabChip® GX, GXII, GX Touch (PerkinElmer), or Fragment

Analyzer™ (Advanced Analytical). The Agilent 2100 Bioanalyzer fragment size distribution results of the purified PCR Products, the final size distribution of purified PCR products for PE100 is 330-400bp, for PE150 is 480-520bp.

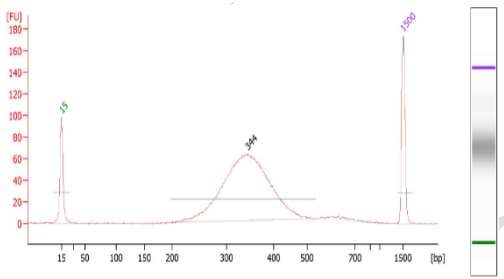

Figure 1 Agilent 2100 Bioanalyzer Fragment Size Distribution Results of the Purified PCR Product (PE100)

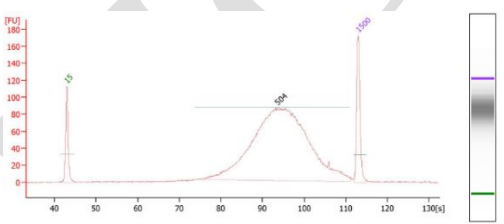

Figure 2 Agilent 2100 Bioanalyzer Fragment Size Distribution Results of the Purified PCR Product (PE150)

⚠ **Note: If the library will be delivered to a service lab for sequencing, please stop here.**

If the library will be sequenced in your lab, please go to step 3.7.

3.7 Denaturation

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

3.7.1 According to the measured PCR product size, measured concentration of sample, and Formula 1 in

Appendix F, calculate the volume needed for 1 pmol of PCR product sample. Transfer that volume of PCR Product to a new 0.2 mL PCR Tube. Add TE Buffer for a final volume of 48 µL.

3.7.2 Place the PCR tube from step 3.7.1 into the thermocycler and run the program in Table 12:

Table 12 Denaturation Reaction Conditions

Temperature	Time
Heated lid	On
95°C	3 min

3.7.3 After the reaction is complete, immediately place the PCR tube on ice for 2 minutes, then centrifuge briefly.

3.8 Single Strand Circularization

3.8.1 Prepare the single strand circularization reaction mixture in a new 0.2 mL PCR tube on ice (see Table 13).

Table 13 Single Strand Circularization Mixture

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

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

3.8.3 Place the PCR tube into the thermocycler and run the program in Table 14.

Table 14 Single Strand DNA Circularization Reaction Conditions

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

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

3.9 Enzymatic Digestion

3.9.1 Prepare the following enzymatic digestion mixture (see Table 15) in a new 0.2 mL PCR tube on ice during the reaction in step 3.8.3.

Table 15 Enzymatic Digestion Mixture

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

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

3.9.3 Place the PCR tube from step 3.9.2 into the thermocycler and run the program in Table 16.

Table 16 Enzymatic Digestion Reaction Conditions

Temperature	Time
Heated lid	On
37°C	30 min

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

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

3.10 Cleanup of Enzymatic Digestion Product

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

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

3.10.2 Transfer 170 μL of DNA Clean Beads to the Enzymatic Digestion product from step 3.9.5. Gently pipette at least 10 times to mix thoroughly. Ensure that all of the solution and beads are fully dispensed from the tip into the tube.

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

3.10.4 Centrifuge briefly and place the 1.5 mL tube on the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant using a pipette.

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

3.10.6 Repeat step 3.10.5 once. Remove all of the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom. Separate the beads magnetically, then

remove remaining liquid using a small volume pipette.

3.10.7 Keep the 1.5 mL centrifuge tube on the Magnetic Separation Rack with the lid open, and air-dry beads until no wetness (reflectiveness) is observed but before the pellet cracks.

3.10.8 Remove the 1.5 mL centrifuge tube from the Magnetic Separation Rack and add **30 μL of** TE Buffer to elute the DNA. Gently pipette up and down at least 10 times to mix thoroughly or until the beads are fully resuspended.

3.10.9 Incubate at room temperature for 10 minutes.

3.10.10 Centrifuge briefly and place the 1.5 mL centrifuge tube back on the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer **27 μL of** supernatant to a new 1.5 mL centrifuge tube. Take care to not disturb the beads.

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

3.11 Quality Control of Enzymatic Digestion Product

Quantify the purified Enzymatic Digestion products with Qubit® ssDNA Assay Kit. The final Enzymatic Digestion products(ssDNA, ng) / input products of PCR (dsDNA, ng) should be ≥ 7%. Please refer to Table 17 or Formula 1 in Appendix F for your calculations.

Table 17 The Corresponding Yield in 1 pmol for Different PCR Product Size (Circularized ssDNA)

Insert Size (bp)	PCR Product Size (bp)	Corresponding Yield in 1 pmol (ng)
250	334	≧15.4
280	364	≧16.8
300	384	≧17.5

Appendix

Appendix A Reaction Conditions of DNA Fragmentation

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

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

Table 18 Fragmentation Parameters of Covaris S220 for Target BP peaks between 150 and 550 bp (55 µL of Sample Volume)

	Vessel	microTUBE-50 AFA Fiber-Screw-Cap (PN 520166) 						
	Sample Volume	55 µL						
S220	Holder	S-Series Holder microTUBE-50 Screw-Cap (PN 500492)						
	Water Level	10						
	Temperature (℃)	7						
	Target BP (Peak)	150	200	250	300	350	400	550
	Peak Incident Power (W)	100	75	75	75	75	75	50
	Duty Factor	30%	25%	20%	20%	15%	10%	10%
	Cycles per Burst	1000	1000	1000	1000	1000	1000	1000
	Treatment Time (s)	150	95	65	45	45	55	50

Table 19 The Fragmentation Parameters of Covaris Series from 150 to 550 bp (55 µL of Sample Volume)

	Vessel	MicroTUBE-50 Screw-Cap (PN 520166) 	8 microTUBE-50 AFA Fiber Strip V2 (PN 520174) 8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240) 	96 microTUBE-50 AFA Fiber Plate (PN 520168) 96 microTUBE-50 AFA Fiber Plate Thin Foil (PN 520232) 
	Sample Volume	55 µL		
E220	Racks	Rack 24 Place microTUBE Screw-Cap (PN 500308)	Rack 12 Place 8 microTUBE Strip (PN 500444)	No Rack needed

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

Appendix B Magnetic Beads and Cleanup Procedures

For magnetic bead-based purification, we recommend using DNA Clean Beads included in the MGIEasy DNA Clean Beads (MGI, Cat. No.940-200073-00 or 940-200074-00) or AMPure® XP (Agencourt, Cat. No. A63882). If you choose Magnetic Beads from other sources, please optimize the cleanup conditions before getting started.

Before You Use

- To ensure capture efficiency of the Magnetic Beads, remove beads from 4°C refrigerator storage, and equilibrate to room temperature (expected time: 30 minutes). Vortex and mix thoroughly before use.
- Vortex or pipette up and down to ensure that the beads are thoroughly mixed before each use.
- The volume of the beads determines the lower limit of fragment size that can be purified.

Operation Notes

- If the sample volume decreases due to evaporation during incubation, add additional TE buffer to reach the designated volume before using the beads to purify. It ensures that the correct ratio for the beads is used.
- In the magnetic separation step, please allow the solution to become completely clear before removing the supernatant. This process takes approximately 2-3 minutes. Consider the different magnetic strength of your specific Separation Plate / Rack, and allow enough time for the solution to turn completely clear.
- Avoid contacting the beads with pipette tips when pipetting. 2-3 μL of fluids can be left in the tube to avoid contact. In the event of contact between the beads and the pipette tip, expel all of the solution and beads back into the tube and restart the separation process.
- Use freshly prepared 80% ethanol (at room temperature) to wash the beads twice. Keep the centrifuge tube on the Magnetic Separation Rack when washing with ethanol. Do not shake or disturb the beads in any way.
- After the 2nd bead wash with ethanol, try to remove all of the liquid within the tube. You may centrifuge briefly to collect any remaining liquid at the bottom. Separate beads magnetically and remove the remaining liquid by using a small volume pipette.
- After washing twice with ethanol, air-dry the beads at room temperature. Drying takes approximately 2-5 minutes depending on your specific lab environment. Watch closely until the pellet appears sufficiently dry with a matte appearance, then continue to the elution step with TE Buffer.
- During the elution step, do not touch the beads with the pipette tips when removing the supernatant. Contamination in DNA by the beads may affect subsequent purification. Therefore, the total volume of TE buffer and the beads should be 2 uL more than the volume of the supernatant.

- Pay attention when opening/ closing the lids of centrifuge tubes on the Separation Rack. Strong vibrations may cause sample loss by spilling liquid or beads from the tubes. Secure the tubes before opening the lids.

Appendix C Magnetic Beads Size Selection

The following example uses 64 μL1st bead selection and a 16 μL 2nd bead selection to target a 280 bp size fragment from fragmented DNA (80 μL).

To select different fragment sizes, please refer to Table 3 in Chapter 2 for detailed conditions.

Protocol

1. Take DNA Clean Beads out of refrigerator, and allow 30 minutes for the solution to come to room temperature. Vortex and mix thoroughly before use.
2. Transfer all fragmentation products to a new 1.5 mL centrifuge tube. Add TE Buffer for a final volume of 80 μL.
3. Transfer 64 μL of DNA Clean Beads to the 1.5 mL centrifuge tube containing 80 μL fragmentation products. Pipette up and down at least 10 times to mix thoroughly. Ensure that the liquid and the beads are fully dispensed from the pipette tip into the tube before proceeding.
4. Incubate at room temperature for 5 minutes.
5. Centrifuge briefly and place the tube onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Then, carefully transfer the supernatant to a new 1.5 mL centrifuge tube.

⚠ **Note: Retain the Supernatant and discard the Beads.**

6. Transfer 16 μL of DNA Clean Beads to the centrifuge tube with 144 uL supernatant. Pipette at least 10 times to mix thoroughly.
7. Incubate at room temperature for 5 minutes.
8. Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.
9. Keep the centrifuge tube on the Magnetic Separation Rack and add 200 μL of freshly prepared 80% ethanol to wash the beads and the walls of the tube. Carefully remove and discard the supernatant.
10. Repeat step 9 and try to remove all of the liquid from the tube.
11. Keep the centrifuge tube on the Magnetic Separation Rack with the lid open, and air-dry the beads until they no longer appear shiny but before the bead pellet starts to crack.
12. Remove the centrifuge tube from the Magnetic Separation Rack and add 32 μL of TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.
13. Incubate at room temperature for 5 minutes.

14. Centrifuge briefly then place the centrifuge tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 30 μL of supernatant to a new 1.5 mL centrifuge tube.

Appendix D The Combination Barcode Adapters Strategies

- We currently offer two product specifications of Adapter Reagent Kit based on the number of reactions, the MGIEasy DNA Adapters-16 (Tube) Kit and MGIEasy DNA Adapters-96 (Plate) Kit. Both kits were developed to meet requirements for batch processing of library construction and multiplex sequencing. We selected the best Adapter combination based on the principle of balanced base composition. However, the number of Barcode Adapters are not always continuous. For optimal performance, please carefully read instructions in Appendix D-1 and D-2. Please note that Adapters from the two kits contain overlapping Barcodes, and cannot be sequenced in the same lane.
- Our Adapters are double stranded. Please do not incubate above room temperature to avoid structural changes such as denaturation, which might affect performance.
- Before use, please centrifuge to collect liquid at the bottom of tubes or plates. Gently remove the cap to prevent spills and cross-contamination. Mix Adapters with a pipette before you use. Remember to close the cap immediately after use. For MGIEasy DNA Adapters-96 (Plate) Kit, pierce the film to pipette solutions for first use. After use, please transfer the remaining reagents to individual 1.5 mL tubes or 0.2 mL PCR tubes, label and store at -20°C.
- Adapters from other MGI Library Prep Kits (number 501-596) are designed differently and are incompatible for mixed use. Mixed use will cause errors in barcode demultiplexing in data analysis procedures.
- Adapter quality as well as quantity directly affects the efficiency and quality of the library construction. We recommend the Adapter and sample DNA ratio to be between 10:1 – 200:1. An excessive input of Adapters may cause Adapter dimers; whereas insufficient input may cause lower library yield and lower efficiency of library construction.

Table 20 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 there is a need to optimize the efficiency of library construction, you may try increasing Adapter input (within the range of 2-10 times).

D-1 MGIEasy DNA Adapters-16 (Tube) Kit Instruction

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

2 sets of 4 Adapters: (01-04) and (13-16)

1 set of 8 Adapters: (97-104)

If sequencing data output requirement is the same for all samples in one lane, please refer to Table 21 below to choose your Barcode Adapter combinations.

Table 21 MGIEasy DNA Adapters-16 (Tube) Kit Instruction

Sample/lane	Instructions (Example)
1	Requires at least 1 set of Adapters: 1. Take a set of 4 Adapters (e.g. 01-04), mix 4 Adapters with equal volumes, then add the mixture to the sample. Or 2. Take a set of 8 Adapters (e.g. 97-104), mix 8 Adapters with equal volumes, then add the mixture to the sample.
2	Requires at least 1 set of Adapters: 1. Take a set of 4 Adapters (e.g. 01-04), mix Adapters with equal volumes in pairs to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 01 & 02, then add to sample 1; Mix 03 & 04, then add to sample 2) Or 2. Take a set of 8 Adapters (97-104), mix Adapters with equal volumes in groups of 4 to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 97-100, then add to sample 1; Mix 101-104, then add to sample 2)
3	Requires at least 2 sets of Adapters: For sample 1&2, use the method for (2 samples/lane) above. For sample 3, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for samples 1-2 and for sample 3.
4	Requires at least 1 set of Adapters: 1. Take a set of 4 Adapters (e.g. 01-04), add 1 Adapter to each sample in an equal volume. (e.g. Add Adapters 01, 02, 03, 04 to samples 1, 2, 3, 4, respectively.)

	Or 2. Take a set of 8 Adapters (97-104), mix Adapters with an equal volume in pairs to obtain 4 mixtures of equal volumes. Add 1 mixture to each sample. (e.g. Mix 97-98, 99-100, 101-102, 103-104, then add respectively to samples 1, 2, 3, 4.)
5	Requires at least 2 sets of Adapters: For samples 1-4, use the method for (4 samples/lane) above. For sample 5, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for samples 1-4 and for sample 5.
6	Requires at least 2 sets of Adapters: For samples 1-4, use the method for (4 samples/lane) above. For samples 5-6, use the method for (2 sample/lane) above. Note that you should use different Adapter sets for samples 1-4 and for samples 5-6.
7	Requires all 3 Adapter sets and follow these 3 steps: 1) For samples 1-4, use the method for (4 samples/lane) above (Use 1st Adapter set). 2) For samples 5-6, use the method for (2 samples/lane) above (Use 2nd Adapter set). 3) For sample 7, use the method for (1 sample/lane) above (Use 3rd Adapter set). You can add a single Adapter within the Adapter set. Or add the Adapter mix which is mixed from all Adapters within the Adapter set with an equal volume. Note that you should use different Adapter sets for samples 1-4, for samples 5-6 and for sample 7.
8	Requires at least 1 set of Adapters: 1. Take a set of 8 Adapters (97-104), respectively add 1 Adapter to each sample in an equal volume. Or 2. Take 2 sets of 4 Adapters (01-04 and 13-16), add 1 Adapter to each sample in an equal volume.

For situations in which sequencing data output requirements are different between samples, any sample with a data output of more than 20% for each lane must use a separate set of Adapters. For example, 9 samples are pooled into 1 lane, one of which requires 30% of the total data output. In this case, the other 8 samples may use Adapters (97-104), whereas the final sample must use a full Adapter set instead of using only a single Adapter (e.g. Adapter set (01-04) or (13-16)).

D-2 MGIEasy DNA Adapters-96 (Plate) Kit Instruction

Based on the principles of balanced base composition, Adapters must be used in specific groups. Please follow the instructions bellow to use Adapters in proper combination.

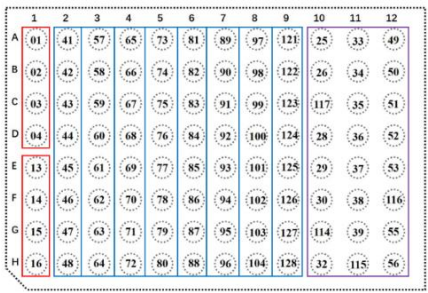

Figure 3 MGIEasy DNA Adapters-96 (Plate) Adapters Layout and Combination Instructions

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

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

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

If sequencing data output requirement is the same for all samples in a lane, please refer to the Table 22 below to organize your Barcode Adapter combinations:

Table 22 MGIEasy DNA Adapters-96 (Plate) Kit Instruction

Sample/lane	Instruction (Example)
1	1. Take a set of 4 Adapters (e.g. 01-04), mix 4 Adapters with equal volumes, then add the mixture to the sample. Or 2. Take a set of 8 Adapters (e.g. 41-48), mix 8 Adapters with equal volumes, then add the mixture to the sample.
2	1. Take a set of 4 Adapters (e.g. 01-04), mix Adapters with equal volumes in pairs to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 01 & 02, then add to sample 1; Mix 03 & 04, then add to sample 2) Or 2. Take a set of 8 Adapters (41-48), mix Adapters with equal volumes in groups of 4 to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 41-44, then add to sample 1; Mix

	45-48, then add to sample 2)
3	For sample 1&2, use the method for (2 samples/lane) above. For sample 3, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for sample 1,2 and 3.
4	1. Take a set of 4 Adapters (e.g. 01-04), add 1 Adapter to each sample in an equal volume. (e.g. Respectively add Adapters 01, 02, 03, 04 to samples 1, 2, 3, 4.) Or 2. Take a set of 8 Adapters (41-48), mix Adapters with equal volumes in pairs to obtain 4 mixtures of equal volumes. Add 1 mixture to each sample. (e.g. Mix 41-42, 43-44, 45-46, 47-48, then add respectively to samples 1, 2, 3, 4.)
5	For samples 1-4, use the method for (4 samples/lane) above. For sample 5, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for samples 1-4 and 5.
6	For samples 1-4, use the method for (4 samples/lane) above. For sample 5-6, use the method for (2 sample/lane) above. Note that you should use different Adapter sets for samples 1-4 and 5-6.
7	1) For samples 1-4, use the method for (4 samples/lane) above. (Use 1st Adapter set) 2) For samples 5-6, use the method for (2 samples/lane) above. (Use 2nd Adapter set) 3) For sample 7, use the method for (1 sample/lane) above. (Use 3rd Adapter set) Note that you should use different Adapter sets for samples 1-4, samples 5-6 and sample 7.
8	Take a set of 8 Adapters (e.g. 41-48), add 1 Adapter to each sample in an equal volume.
8n+x (n=1,2, x=1-8, Total 9-24)	Follow these 3 steps: 1) For samples 1-8, use the method for (8 samples/lane) above. Or separate into 2 groups of 4 and use the method for (4 samples/lane) above for each group. 2) For samples 9-8n, separate samples into groups of 8, and use the method for (8 samples/lane) above. 3) For samples 8n+1 - 8n+X, according to the value of X, use the methods above for 1-8 sample/lane accordingly. Remember to use different Adapter sets. Note that you should use different Adapter sets for steps 1), 2) and 3).
8n+x (3≤n<11, x=1-8, Total 25-96)	Follow these 3 steps: 1) For samples 1-24, take a set of 24 Adapters and add 1 Adapter for each sample in an equal volume. 2) For samples 25-8n, separate the samples into groups of 8, and use the method for (8 samples/lane) above. 3) For samples 8n+1 - 8n+X, according to the value of X, use the methods above for 1-8 sample/lane

	accordingly. Remember to use different Adapter sets. Note that you should use different Adapter sets for steps 1), 2) and 3).

For situations in which sequencing data output requirements are different between samples, any sample with a data output of more than 20% for each lane must use a separate set of Adapters. For example, 9 samples are pooled into 1 lane, one of which requires 30% of the total data output. In this case, the other 8 samples may use Adapters (97-104), whereas the final sample must use a full Adapter set instead of using only a single Adapter (e.g. Adapter set (01-04) or (13-16)).

Appendix E Adapter Ligation and PCR

- The Adapter reaction mixture contains a high concentration of PEG that increases the viscosity of the mixture. Please pipette slowly and ensure that the correct amount has been used.
- When using PEG, the beads required for the Adapter Ligation product cleanup can be reduced. There is a risk of capturing Adapter dimers with a higher multiplier of beads. Therefore, we recommend using 50 μL Beads for the cleanup.
- According to standard protocol: during the cleanup of Adapter-ligated DNA, if the DNA sample= 50 ng, use 40 μL TE Buffer to elute and take half of the product for PCR Amplification. However, if the DNA sample < 50 ng, we recommend eluting with 21 μL TE Buffer and using all the product for PCR Amplification to ensure library complexity.
- The number of PCR cycles must 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. Table 23 shows the number of PCR cycles required to yield 300 ng and 1ug of library from 0.5-500 ng high quality DNA sample (280 bp). For lower quality, longer DNA fragments, PCR cycles should be increased appropriately to generate a sufficient yield.

Table 23 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

Appendix F Conversion between DNA Molecular Mass and Number of Moles

- Formula 1 shows the calculation of the Mass (in ng) that corresponds to 1 pmol of a dsDNA sample with varying fragment sizes. Please refer to Formula 1 to calculate the amount of DNA needed.

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

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

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