MGIEasy

PCR-Free DNA Library Prep Set User Manual

Cat. No.:1000013452 (16 RXN) , 1000013453 (96 RXN)

Kit Version: V1.1

Manual Version: A2

For Research Use Only.

Revision History

Manual Version	Kit Version	Date	Description
A2	V1.1	Jan. 2021	◆ Update contact information.
A1	V1.1	Dec. 2019	◆ Update the kit version to V1.1 ◆ Update the manual to new style ◆ 1.3 Add DNBSEQ™ series sequencing platform ◆ Reduce the minimum gDNA input to 200ng ◆ Add the beads ratio for single beads purification as table 5 ◆ Add amplicons into input DNA type ◆ Change the QC criteria at ligation product quantitative step
A0	V1.0	Apr. 2019	◆ Initial release

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

Search manual by Cat. No. or product name from website:

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

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Chapter 1 Product Description

1.1 Introduction

The MGIEasy PCR-Free Library Prep Set is specifically designed for making WGS libraries for MGI High-throughput Sequencing Platforms. This library prep set is optimized to convert 80-200 ng size selected fragment DNA into a customized library. This set incorporates improved Adapter Ligation technology, which significantly increases 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 used for samples from common animals, plants, fungus, bacteria *etc.* including Humans (blood, saliva, fresh tissue), Mice, Rice, *E.coli,* Microbiomes and amplicons. Stable performance across all such sample types is expected.

1.3 Platform Compatibility

Constructed libraries are compatible with

BGISEQ-500RS(PE100)

MGISEQ-200RS, DNBSEQ-G50RS (PE100)

MGISEQ-2000RS, DNBSEQ-G400RS (PE100/PE150/PE200/SE400)

DNBSEQ-T7RS (PE100)

1.4 Contents

There are currently two variations for MGIEasy PCR-Free DNA Library Prep Set: 16 RXN and 96 RXN.

Each library prep set consists of 3 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 PCR-Free DNA Library Prep Set V1.1 (16 RXN) (Cat. No:1000013452)

Modules & Cat. No.	Components	Color Coded Screw Caps	Spec & Quantity
MGIEasy PCR-Free DNA Library Prep Kit V1.1 Cat. No. 1000013456	20x Elute Enhancer	Black	3 μL/tube × 1 tube
	ER Buffer	Orange	112 μL/tube × 1 tube
	ER Enzyme Mix	Orange	48 μL/tube × 1 tube
	Ad-Lig Buffer	Red	288 μL/tube × 1 tube
	Ad Ligase	Red	80 μL/tube × 1 tube
	Ligation Enhancer	Brown	32 μL/tube × 1 tube
	Cir Buffer	Purple	184 μL/tube × 1 tube
	Cir Enzyme Mix	Purple	8 μL/tube × 1 tube
	Exo Buffer	White	23 μL/tube × 1 tube
	Exo Enzyme Mix	White	42 μL/tube × 1 tube
	Exo Stop Buffer	White	48 μL/tube × 1 tube
MGIEasy PF Adapters-16(Tube) Kit Cat. NO. 1000013460	DNA Adapters	Colorless	5 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

Table 2 MGIEasy PCR-Free DNA Library Prep Set V1.1 (96 RXN) (Cat. No：1000013453)

Modules & Cat. No.	Components	Color Coded Screw Caps	Spec & Quantity
MGIEasy PCR-Free DNA Library Prep Kit V1.1 Cat. No. 1000013457	20x Elute Enhancer	Black	15 μL/tube × 1 tube
	ER Buffer	Orange	672 μL/tube × 1 tube
	ER Enzyme Mix	Orange	288 μL/tube × 1 tube
	Ad-Lig Buffer	Red	864 μL/tube × 2 tubes
	Ad Ligase	Red	480 μL/tube × 1 tube
	Ligation Enhancer	Brown	192 μL/tube × 1 tube
	Cir Buffer	Purple	1104 μL/tube × 1 tube
	Cir Enzyme Mix	Purple	48 μL/tube × 1 tube
	Exo Buffer	White	135 μL/tube × 1 tube
	Exo Enzyme Mix	White	250 μL/tube × 1 tube
	Exo Stop Buffer	White	288 μL/tube × 1 tube
MGIEasy PF Adapters-96(Plate) Kit Cat. NO. 1000013461	DNA Adapters-96 plate	_	5 μL /tube × 96 well
MGIEasy DNA Clean Beads Cat. No.:1000005279	DNA Clean Beads	White	50 mL/ tube × 1 tube
	TE Buffer	White	25 mL/ tube × 1 tube

1.5 Storage Conditions and Shelflife

MGIEasy PCR-Free DNA Library Prep Kit

- Storage Temperature: -25°C to -15°C.
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: transported on dry ice.
- The Ligation Enhancer needs to be stored at room temperature and away from light.
- The 20x Elute Enhancer and Exo Stop Buffer need to be stored at room temperature.

MGIEasy PF Adapters-16 (Tube) Kit

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

MGIEasy PF Adapters-96 (Plate) Kit

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

MGIEasy DNA Clean Beads

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

* 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 (ThermoFisher Scientific™) Vortex Mixer Desktop Centrifuge Pipets Thermocycler 96M Magnum™ Plate (ALPAQUA, Part#A000400) recommended Magnetic rack DynaMag™-2 (ThermoFisher Scientific™, Cat. No. 12321D) or equivalent Qubit™ 3 Fluorometer (ThermoFisher Scientific™, Cat. No. Q33216) Agilent 2100 Bioanalyzer (Agilent Technologies™, Cat. No. G2939AA) or equivalent
Reagents	Nuclease free water (Ambion™, Cat. No. AM9937) TE Buffer, pH 8.0 (Ambion™, Cat. No. AM9858) 100% Ethanol (Analytical Grade) Qubit™ ssDNA Assay Kit (ThermoFisher Scientific™, Cat. No. Q10212) Qubit™ dsDNA HS Assay Kit (ThermoFisher Scientific™, Cat. No. Q32854) High Sensitivity DNA Analysis Kits (Agilent Technologies™, Cat. No. 5067-4626) Agilent DNA 1000 Kit (Agilent Technologies™, Cat. No. 5067-1504)
Consumables	Covaris AFA Tubes for use with Ultrasonicator (ThermoFisher Scientific™) Pipette Tips 1.5 mL MaxyClear Snaplock Microcentrifuge Tube (Axygen™,Cat. No. MCT-150-C) or equivalent Axygen™ 0.2 mL Thin Wall PCR Tubes (Axygen™, Cat. No. PCR-02-C) or Axygen™ 96-well Polypropylene PCR Microplate (Axygen™, Cat. No. PCR-96M2-HS-C) Qubit™ Assay Tubes (ThermoFisher Scientific™, Cat. No. Q32856) or Axygen™ 0.5 mL Thin Wall PCR Tubes (Axygen™, Cat. No. PCR-05-C)

1.7 Precautions and Warning

- Instructions provided in this manual are intended for general use only, and it may require optimization for specific applications. We recommend adjusting the steps and volumes according to the experimental design, sample types, sequencing application, and other equipment restrictions.
- Remove the reagents from storage beforehand, and prepare them for use: For enzymes, mix well by vortex for one second, then centrifuge briefly and place on ice until further use. For other reagents, first thaw at room temperature and mix well by vortex. Finally, 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.
- To prevent yield loss, try to avoid transferring the reaction product to a new tube for beads purification especially in Exo Digestion Product purification step. Adding the En-Beads directly to the reaction tube for product purification is recommended.
- If you have any question, please contact MGI technical support MGI-service@mgi-tech.com

Chapter 2 Sample Preparation Overview

2.1 Sample Requirement

This library prep set is suit for samples from all common animals, plants, fungus, bacteria etc. including Humans (blood, saliva, fresh tissue), Mice, Rice, *E. coli*, Microbiomes and amplification products. It is strongly recommended to use 1000 ng of high-quality genomic DNA (OD_{260}/OD_{280}=1.8 - 2.0, OD_{A260}/OD_{230}>2.0) for fragmentation.

2.2 DNA Fragmentation and Size Selection

2.2.1 Fragmentation

- Fragmentated gDNA into sizes ranging from 150 bp to 1000 bp, and select for a target peak fragment size range of 300-500 bp.
- For gDNA fragmentation, please visit Covaris's official website for detailed instructions.

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.
- If the gDNA amount of sample is enough, we recommend using **500-1000 ng** for fragmentation followed by a **two-step bead selection process** for Size Selection (see Table 4).

Table 4 Two-step bead selection process:
75 μL Sample of the Theoretical Majority of DNA Fragments Using Magnetic Beads Selection

Target Peak Fragment Size (bp)	350	400	560
1st Bead Selection (μL)	50	45	41.25
2nd Bead Selection (μL)	15	15	15
Sequencing Strategy	PE100/PE150	PE100/PE150	PE200/SE400

⚠ **Note: The selection conditions of Table 4 are used for reference. For different samples, the target peak fragment size may have a ±50 bp deviation.**

- If the sample is rare and the gDNA amount is lower than 500 ng, we recommend attempting to use **200-500 ng** for fragmentation followed by **one-step bead selection process** for Size Selection (see Table 5).

Table 5 One-step bead selection process:

75 μL Sample of the Theoretical Majority of DNA Fragments Using Magnetic Beads Selection

Target Peak Fragment Size (bp)	400	560
Bead Volume (μL)	60	52.5
Sequencing Strategy	PE100/PE150	PE200/SE400

⚠ **Note: The library constructed with 200ng gDNA usually meets once sequencing. As the insert size range of one-step bead selection process is broader than two-step bead selection process, the sequencing quality and effective sequencing reads will decrease slightly.**

- For detailed steps, seen the example in Appendix B, which provide two examples for purification

  Example 1: For a peak size of 400 bp: Fragmentated 1000 ng gDNA (80 μL). If the DNA volume after fragmentation is less than 75 μL, add En-TE buffer to reach a final volume of 75 μL. Then, perform a two-step bead selection process with a 45 μL 1st beads selection followed by a 15 μL 2cd beads selection before End Repair, which provides the selected fragment size of 400 bp.

  Example 2: For a peak size of 400 bp: Fragmentated 200 ng gDNA (80 μL). If the DNA volume after fragmentation is less than 75 μL, add En-TE buffer to reach a final volume of 75 μL. Then, perform a bead purification process with a 60 μL beads selection before End Repair, which provides the selected fragment size of 400 bp.

- In two-step bead selection process, the DNA sample loss during bead selection is approximately 60%-90%. So we recommend that you may retrieve the beads from the 1st Bead Selection process, then wash twice with 80% ethanol; Air-dry the beads pellet, elute DNA with En-TE Buffer, and store as a backup.

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 80-200 ng in a volume of 40 μL or less than. If the size selected DNA amount is 60-80 ng, library preparation can be attempted, but with the risk of failure. Otherwise, do not proceed.
- Try to ensure a narrow distribution of DNA fragment sizes. 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 and 5). Sequencing quality may slightly decrease with increasing fragment sizes. Please 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.
- Any residual impurities (E.g.: metal chelators or other salts) in selected DNA fragments may adversely affect the efficiency of the End Repair process.

Chapter 3 Library Construction Protocol

The brief scheme of **Library Construction Protocol** using this kit is as following: 80-200 ng of DNA fragments construct library through end-repair, ligation and Single Strand Circularization.

3.1 Reagent Prep

3.1.1 Prepare the 1x Elute Enhancer in a sterile centrifuge tube according to Table 6. Store at room temperature before use. The shelf life of the 1x Elute Enhancer is 7 days.

Table 6 1x Elute Enhancer

Components	Volume
20x Elute Enhancer	1 μL
Nuclease-Free Water	19 μL
Total	20 μL

3.1.2 Prepare the En-TE buffer in a sterile centrifuge tube according to Table 7. Store at 4°C before use，and the shelf life of the En-TE buffer is 7 days.

Table 7 En-TE buffer

Components	Volume
1x Elute Enhancer	2 μL
1x TE Buffer	998 μL
Total	1000 μL

3.1.3 Prepare the En-Beads in a sterile microfuge tube according to Table 8. Store at 4°C before use. The shelf life of the En-Beads is 7 days.

Table 8 En-Beads

Components	Volume
1x Elute Enhancer	15 μL
DNA Clean Beads	1485 μL
Total	1500 μL

⚠ **Note: The preparation volume of reagents listed above is enough for six samples. If there are more samples, you can increase the preparation reagent volumes in proportion.**

3.2 End Repair and A-tailing

3.2.1 Transfer an appropriate amount of sample (80-200 ng recommended) to a new 0.2 mL PCR tube and add En-TE Buffer for a total volume of 40 μL.

3.2.2 Prepare the End Repair and A-tailing Reaction Mixture on ice (see Table 9):

Table 9 End Repair and A-tailing Reaction Mixture

Components	Volume
ER Buffer	7 μL
ER Enzyme Mix	3 μL
Total	10 μL

3.2.3 Transfer 10 μL of the End Repair Reaction Mixture into the 0.2 mL PCR tube from 3.2.1. Vortex 3 times (3 s each) and briefly centrifuge 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 10. And the total reaction volume is 50 μL.

Table 10 End Repair and A-tailing Reaction Conditions

Temperature	Time
Heated Lid (70°C)	On
14°C	15 min
37°C	25 min
65°C	15 min
4°C	Hold

⚠ **Note: Preheat the thermocycler to reaction temperature before use.**

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

⚠ **Note: DO NOT STOP AT THIS STEP. Please continue to step 3.3.**

3.3 Adapter Ligation

⚠ **Note: Please read Appendix C and D carefully before you begin.**

3.3.1 Please refer to the instructions for MGIEasy PF Adapters (see Appendix C). Add 5 μL of MGIEasy PF Adapters to the PCR tube from step 3.2.5.

3.3.2 Vortex 3 times (3 s each) and briefly centrifuge to collect solution at the bottom of the tube.

3.3.3 Prepare the Adapter Ligation Reaction Mixture on ice (see Table 11):

Table 11 Adapter Ligation Reaction Mixture

Components	Volume
Ad-Lig Buffer	18 μL
Ad Ligase	5 μL
Ligation Enhancer	2 μL
Total	25 μL

⚠ **Note: Ad-Lig Buffer is very viscous. It must be mixed thoroughly before use.**

3.3.4 Pipette slowly to transfer 25 μL of Adapter Ligation Reaction Mixture into the 0.2 mL PCR tube from step 3.3.1. Vortex 6 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.3.5 Place the 0.2 mL PCR tube from step 3.3.3 into the thermocycler and run the program in Table 12. And the total reaction volume is 80 μL.

Table 12 Adapter Ligation Reaction Conditions

Temperature	Time
Heated Lid (30°C)	On
25°C	30 min
4°C	Hold

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

3.3.7 Add 20 μL of En-TE Buffer for a total volume of 100 μL.

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

3.4 Cleanup of Adapter-ligated DNA

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

3.4.1 Remove En-Beads from refrigerator and bring it to room temperature for 30 minutes beforehand. Mix by vortex thoroughly before use.

3.4.2 Transfer 50 μL En-Beads to the tube from step 3.3.7. Pipette up and down at least 10 times to mix thoroughly, and ensure that all of the liquid and beads are expelled from the pipette tip into the tube before proceeding; or mix by vortex thoroughly instead of pipette mixing.

3.4.3 Incubate at room temperature for 10 minutes.

3.4.4 Centrifuge briefly, then place the tube onto a Magnetic Separation Rack for 2-5 min until the liquid become clear. Carefully remove and discard the supernatant with a pipette.

3.4.5 Keep the tube on the Magnetic Separation Rack and add 160 μL of freshly prepared 80% ethanol to wash the beads and the walls of the tube. Reverse the 0.2 mL PCR tube on magnetic stand twice and then carefully remove and discard the supernatant.

3.4.6 Repeat step 3.4.5 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 tube, separate magnetically and then remove remaining liquid using a small volume pipette.

3.4.7 Keep the tube on the Magnetic Separation Rack with the lid open, and air-dry beads at room temperature until no wetness is observed. Do not over-dry beads (the pellet will begin to crack).

3.4.8 Remove the tube from the Magnetic Separation Rack, and add 50 μL of En-TE Buffer to elute the DNA. Gently pipette up and down at least 10 times to mix thoroughly; or mix by vortex thoroughly.

3.4.9 Incubate at room temperature for 5 minutes.

3.4.10 Centrifuge briefly, then place the tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 49 μL of supernatant to a new 0.2 mL PCR tube.

3.4.11 Take 1 μL of supernatant to quantify the concentration with Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit. If the concentration is >1.2 ng/μL, then you can continue to the next step of library construction; if the concentration between 0.8-1.2 ng/μL, library preparation can still be attempted, but with the risk of failure; and if the concentration

is <0.8 ng/μL, we don't recommend continuing with this sample.

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

3.5 Denaturation

3.5.1 Place the 0.2 mL PCR tube from step 3.4.10 into the thermocycler and run the program in Table 13. And the total reaction volume is 50 μL.

Table 13 Denaturation Reaction Conditions

Temperature	Time
Heated Lid (**100°C**)	On
95°C	3 min
4°C	10 min

⚠ **Note: There is another Denaturation Reaction Conditions: 95°C 3min (Heated Lid 100°C), and then quickly on ice 2min, then continue to step 3.5.2.**

3.5.2 Then centrifuge briefly and immediately continue to the next step.

3.6 Single Strand Circularization

3.6.1 Prepare the Circularization Reaction Mixture on ice (see Table 14).

Table 14 Circularization Reaction Mixture

Components	Volume
Cir Buffer	11.5 μL
Cir Enzyme Mix	0.5 μL
Total	12 μL

3.6.2 Transfer 12 μL of Circularization Reaction Mixture into the PCR tube from step 3.5.2. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.6.3 Place the PCR tube into the thermocycler and run the program in Table 15. And the total reaction volume is 60 μL.

Table 15 Circularization Reaction Conditions

Temperature	Time
Heated Lid (**42°C**)	On
37°C	30 min
4°C	Hold

3.6.4 Briefly centrifuge, then place the PCR tube on ice. Continue to the next step immediately.

3.7 Exo Digestion

3.7.1 Prepare the Exo Digestion Reaction Mixture (see Table 16) on ice during the reaction in step 3.6.3.

Table16 Exo Digestion Reaction Mixture

Components	Volume
Exo Buffer	1.4 μL
Exo Enzyme Mix	2.6 μL
Total	4 μL

3.7.2 Transfer 4 μL of Exo Digestion Reaction Mixture into the PCR tube from step 3.6.4. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.7.3 Place the 0.2 mL PCR tube from step 3.7.2 into the thermocycler and run the program in Table 17. And the total reaction volume is 64 μL.

Table 17 Exo Digestion Reaction Conditions

Temperature	Time
Heated Lid (42°C)	On
37°C	30 min
4°C	Hold

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

3.7.5 Add 3 μL Exo Stop Buffer to the PCR tube from step 3.7.4. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.8 Cleanup of Exo Digestion Product

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

3.8.1 Remove En-Beads from refrigerator and bring it to room temperature for 30 minutes beforehand. Vortex and mix thoroughly before use.

3.8.2 Transfer 120 μL of En-Beads to Exo Digestion product from step 3.7.5. Gently pipette up and down at least 10 times to mix thoroughly and ensure that all of the solution and beads are expelled from the tip into the tube; or mix by vortex thoroughly.

3.8.3 Incubate at room temperature for 10 minutes.

3.8.4 Centrifuge briefly, then place the tube onto a Magnetic Separation Rack for 2-5 min until the liquid becomes clears. Carefully remove and discard the supernatant using a pipette.

3.8.5 Keep the tube on the Magnetic Separation Rack, and add 160 μL of freshly prepared 80% ethanol to wash the beads and the walls of the tube. Reverse the 0.2 mL PCR tube on magnetic stand twice and then carefully remove and discard the supernatant.

3.8.6 Repeat step 3.8.5 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.

3.8.7 Keep the tube on the Magnetic Separation Rack with the lid open, and air-dry beads at room temperature until no wetness is observed. Do not over-dry beads (the pellet will begin to crack).

3.8.8 Remove the tube from the Magnetic Separation Rack, and add 25 μL of En-TE Buffer to elute the DNA. Gently pipette up and down at least 10 times to mix thoroughly; or mix by vortex thoroughly.

3.8.9 Incubate at room temperature for 10 minutes.

3.8.10 Centrifuge briefly then place the tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 24 μL of supernatant to a new 0.2 mL or a new 1.5 mL centrifuge tube. Take care not to disturb the beads.

✔ **Stopping Point: Purified Digestion Products can be stored at -20°C.**

3.9 Quality Control of Digestion Product

3.9.1 Quantify the purified Exo Digestion Products with QubitTM ssDNA Assay Kit.

3.9.2 The final yield should be ≥ 75 fmol (enough for one sequencing run). Please refer to Table 18 or formula 1 in Appendix E for your calculations.

3.9.3 Sequencing requires a single strand circle input is 75 fmol/lane. If you plan to pool multiple samples in one lane for sequencing, you can pool the single strand circles of different samples by certain mole ratio at this step. The barcodes used in the pooled samples should strictly adhere to the instructions for MGIEasy PF Adapters (see Appendix C). And the mole ratio is based on your required sequencing data of each sample being pooled.

⚠ **Note: The insert size and the size range will affect sequencing quality and effective sequencing reads, so it will take risk to pool fragments of different peak size and different size range (eg. pool one-step bead selection fragments and two-step bead selection fragments together) for sequencing in the same lane.**

Table 18 Corresponding Molecular Weight equal to 75 fmol Circularized ssDNA for Different Selected Fragment Size

Target Peak Fragment Size (bp)	Circularized ssDNA size (bp)	Corresponding Molecular Weight equal to 75 fmol of Circularized ssDNA (ng)
350	434	10.7
400	484	12.0
560	644	16.0

Appendix

Appendix A Magnetic Beads and Cleanup Procedures

For magnetic bead-bead purification, we recommend using DNA Clean Beads included in the MGIEasy DNA Clean Beads Kit (MGI, Cat. No. 1000005278 or 1000005279). If you use 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 at room temperature for 30 min beforehand. Vortex and mix thoroughly before use.
- Vortex or pipette up and down to ensure that the beads are thoroughly mixed before each use.
- The volume of the beads directly determines the lower limit of fragment size that can be purified.

Operation Notes

- If the sample volume decreased due to evaporation during incubation, add additional En-TE buffer to reach the designated volume. This ensures that the correct ratio for the beads is used. In the magnetic separation step, please allow the solution to become completely clear before removing the supernatant. This process takes approximately 2-3 minutes. Consider the different magnetic strength of your specific Separation Plate / Rack, and allow enough time for the solution to turn completely clear. And if you use Magnetic rack DynaMag™-2, you will need to transfer the product to a new 1.5 mL centrifuge tube, and that transfer will result in about 20% loss.
- Avoid touching the beads with pipette tips when pipetting. 2-3 μL of fluids can be left in the tube to avoid contact. In case of contact between the beads and pipette tip, expel all of the solution from the pipette tip into the tube and restart the separation process.
- Use freshly prepared 80% ethanol (at room temperature) to wash the beads. Keep the centrifuge tube on the Magnetic Separation Rack when washing. Do not shake or disturb the beads in any way.
- After the 2nd wash of beads with ethanol, try to remove all of the liquid within the tube. You may centrifuge briefly to collect any remaining liquid at the bottom, separate beads magnetically, and remove remaining liquid by using a small volume pipette.

- After washing twice with 80% ethanol, air-dry the beads at room temperature. Insufficient drying (observed by a reflective surface) will allow anhydrous Ethanol to deposit, affecting subsequent reactions. Over-drying (observed by the pellet cracking) may cause a reduction in yield. Drying takes approximately 5-10 min depending on your specific lab environment. Observe closely, until the pellet appears sufficiently dry with a matte appearance, then continue to the elution process with En-TE Buffer.
- Avoid contact between the pipette and the beads while removing the supernatant. Contamination from the beads may affect subsequent reactions. As such, the volume of the supernatant should be 2 µL less than the original elute containing beads.
- Take extra care when opening / closing the lids of tubes on the Separation Rack. Strong vibrations may cause sample loss via liquid or bead spillage. Secure the tubes well before tampering with the lids.

Appendix B Magnetic Beads Size Selection

Example 1: Using a 45 μL Bead selection step followed by a 15 μL Bead selection step to target a peak size of 400 bp from 75 μL of fragmentated gDNA. To select a different fragment size, please refer to Table 4 in Chapter 2 for detailed conditions.

1) Remove DNA Clean Beads from refrigerator and let it stand at room temperature for 30 min beforehand. Mix thoroughly vortex before use.
2) Prepare the En-Beads refer to Table 3 in Chapter 2 for detailed conditions.
3) Transfer all Fragmentation Products to a new 0.2 mL PCR tube. Add En-TE Buffer to reach a final volume of 75 μL.
4) Transfer 45 μL of En-Beads to the tube containing Fragmentation Products. Pipette up and down at least 10 times to mix thoroughly and ensure that all of the liquid and the beads are fully expelled from the pipette tip into the tube before proceeding; or mix by vortex thoroughly .
5) Incubate at room temperature for 10 min.
6) Centrifuge briefly, and place the tube onto a Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Then, carefully transfer the supernatant to a new 0.2 mL PCR tube.

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

7) Transfer 15 μL En-Beads to the tube from step 6 which contains 120 μL of supernatant. Pipette at least 10 times to mix thoroughly; or mix by vortex thoroughly.
8) Incubate at room temperature for 10 minutes.
9) Centrifuge briefly, then place the tube onto a Magnetic Separation Rack for more than 5 min until the liquid becomes clear. Carefully remove and discard the supernatant with pipette.
10) Keep the tube on the Magnetic Separation Rack, and add 160 μL of freshly prepared 80% ethanol to wash the beads and the walls of the tube. Reverse the 0.2 mL PCR tube on magnetic stand twice and then carefully remove and discard the supernatant.
11) Repeat step 10 and try to remove all of the liquid from the tube. You may centrifuge briefly to collect any remaining liquid at the bottom, separate magnetically and then remove remaining liquid using a small volume pipette.
12) Keep the tube on the Magnetic Separation Rack with the lid open, and air-dry beads at room temperature until no wetness is observed. Do not over-dry beads (the pellet will begin to crack).

13) Remove the tube from the Magnetic Separation Rack, and add 45 μL of En-TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly, or mix by vortex thoroughly.

14) Incubate at room temperature for 5 min.

15) Centrifuge briefly then place the tube back onto the Magnetic Separation Rack for 5 min until the liquid becomes clear. Transfer 43 μL of supernatant to a new 0.2 mL PCR tube.

16) Quantify the purified products with Qubit® dsDNA HS Assay Kit or Quant-iT PicoGreen dsDNA Assay Kit.

17) Take 1- 2 μL of supernatant for size distribution detection by Bioanalyzer, Tapestation (Agilent Technologies), LabChip® GX/GXII/GX Touch (PerkinElmer) or Fragment Analyzer (Advanced Analytical).

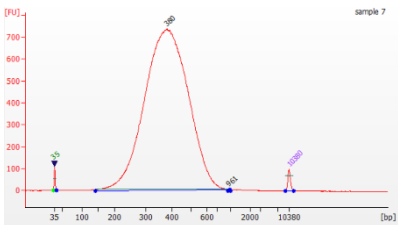

Figure 1 Agilent 2100 Bioanalyzer results of beads purification product

Example 2: Using a 60 μL Bead purification to target a peak size of 400 bp from 75 μL of fragmentated gDNA. To select a different fragment size, please refer to Table 5 in Chapter 2 for detailed conditions.

1) Remove DNA Clean Beads from refrigerator and let it stand at room temperature for 30 min beforehand. Mix thoroughly vortex before use.

2) Prepare the En-Beads refer to Table 3 in Chapter 2 for detailed conditions.

3) Transfer all Fragmentation Products to a new 0.2 mL PCR tube. Add En-TE Buffer to reach a final volume of 75 μL.

4) Transfer 45 μL of En-Beads to the tube containing Fragmentation Products. Pipette up and down at least 10 times to mix thoroughly and ensure that all of the liquid and the beads are fully expelled from the pipette tip into the tube before proceeding; or mix by vortex thoroughly.

5) Incubate at room temperature for 10 min.

6) Centrifuge briefly, and place the tube onto a Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Then, carefully remove and discard the supernatant with pipette.

7) Keep the tube on the Magnetic Separation Rack, and add 160 µL of freshly prepared 80% ethanol to wash the beads and the walls of the tube. Reverse the 0.2 mL PCR tube on magnetic stand twice and then carefully remove and discard the supernatant.

8) Repeat step 7 and try to remove all of the liquid from the tube. You may centrifuge briefly to collect any remaining liquid at the bottom, separate magnetically and then remove remaining liquid using a small volume pipette.

9) Keep the tube on the Magnetic Separation Rack with the lid open, and air-dry beads at room temperature until no wetness is observed. Do not over-dry beads (the pellet will begin to crack).

10) Remove the tube from the Magnetic Separation Rack, and add 45 µL of En-TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly, or mix by vortex thoroughly.

11) Incubate at room temperature for 5 min.

12) Centrifuge briefly then place the tube back onto the Magnetic Separation Rack for 5 min until the liquid becomes clear. Transfer 43 µL of supernatant to a new 0.2 mL PCR tube.

13) Quantify the purified products with Qubit® dsDNA HS Assay Kit or Quant-iT PicoGreen dsDNA Assay Kit.

14) Take 1- 2 µL of supernatant for size distribution detection by Bioanalyzer, Tapestation (Agilent Technologies), LabChip® GX/GXII/GX Touch (PerkinElmer) or Fragment Analyzer (Advanced Analytical).

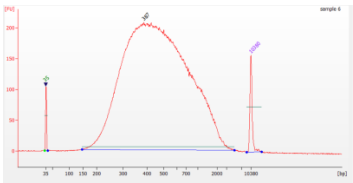

Figure 2 Agilent 2100 Bioanalyzer results of beads purification product

Appendix C Using Barcode Adapters

- We currently offer two product specifications of Adapter Reagent Kit based on the number of reactions, the MGIEasy PF Adapters-16 (Tube) Kit and MGIEasy PF Adapters-96 (Plate) Kit. Both kits were developed to meet requirements for batch processing library construction and Multiplex Sequencing. We selected the best adapter combination based on the principle of balanced base composition. However, not all Barcode Adapters combinations are compatible. For optimum performance, please refer to instructions in Appendix C-1 and Appendix C-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 the liquid at the bottom of the tubes or plates. Gently remove the cap / sealing film to prevent spills and cross-contamination; Mix the adapters by pipetting up and down before you use; Remember to close the cap immediately after use. For MGIEasy PF Adapters-96 (Plate) Kit, pierce the film to pipette the solution for the first use. After use, please transfer the remaining adapter volume to individual 0.2 mL PCR tube. Label and store at -20°C.
- Adapters from other MGI library Kits (numbered 501-596) are designed for library construction with amplification strategy and are incompatible with PCR-Free Kits.

C-1 MGIEasy PF Adapters-16 (Tube) Kit Instruction

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

This kit contains 16 Adapters separated into 3 sets:

- 2 sets of 4 Adapters: (01-04) and (13-16)
- 1 set of 8 Adapters: (97-104)

Assuming that the data output requirement is the same for each sample in a given lane, please refer to the Table 19 below to organize your Barcode Adapter combinations:

Table 19 MGIEasy PF Adapters-16 (Tube) Kit Instruction

Samples / lane	Instructions (Example)
1	Requires at least 1 set of Adapter:

	(1.)Take a set of 4 Adapters (01-04), mix equal volumes, then add to the sample. Or (2.) Take a set of 8 Adapters (97-104), mix equal volumes, then add to the sample. Or (3.) Take one barcode for one sample, if you don't need to sequence the barcode.
2	Requires at least 1 set of Adapter: (1.) Take a set of 4 Adapters (01-04), mix equal volumes in pairs to obtain 2 mixtures of equal volume. Add 1 mixture to each 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 equal volumes in groups of 4 to obtain 2 mixtures of equal volume. Add 1 mixture to each 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 sample 1,2 and 3.
4	Requires at least 1 set of Adapter: (1.) Take a set of 4 Adapters (01-04), add 1 Adapter for each sample in equal volumes. (e.g. Respectively add Adapters 01, 02, 03, 04 to samples 1, 2, 3, 4.) Or (2.) Take a set of 8 Adapters (97-104), mix equal volumes in pairs to obtain 4 mixtures of equal volume. 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 Adapter sets: For samples 1-4, use the method for (4 samples/lane) above. For sample 5, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for sample 1-4 and 5.
6	Requires at least 2 Adapter sets: 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 sample 1-4 and 5-6.
7	Requires all 3 Adapter sets, 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) Note that you should use different Adapter sets for sample 1-4, sample 5-6 and sample 7.

8	Requires at least 1 set of Adapter: (1.) Take a set of 8 Adapters (97-104), add 1 Adapter for each sample in equal volumes. Or (2.) Take 2 sets of 4 Adapters (01-04 and 13-16), add 1 Adapter for each sample in equal volumes.
8n+x (n=1, x=1-8, Total 9-16)	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. Note that you should use different Adapter sets for steps 1), 2) and 3).

For the situations in which the sequencing data output requirements are different among 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, and one sample 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)).

C-2 MGIEasy PF 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 i n proper combination.

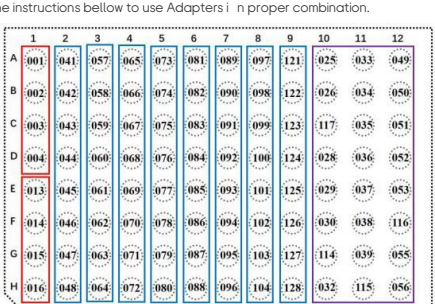

Figure 3 MGIEasy PF Adapters-96 (Plate) Kit Adapter Layout and Combination Instructions

This kit contains 96 Adapters separated into 11 sets:

- 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)

Assuming that the data output requirement is the same for each sample in a given lane, please refer to the Table 19 below to organize your Barcode Adapter combinations:

Table 20 MGIEasy PF Adapters-96 (Plate) Kit Instruction

Samples / lane	Instructions (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. Or 3. Take one barcode for one sample, if you don't need to sequence the barcode.
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 sample 1-4 and 5.

6	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 sample 1-4, sample 5-6 and sample 7.
7	Requires all 3 Adapter sets, 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 (3 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 sample 1-4, sample 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 the sequencing data output requirements are different among 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, and one sample 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 D Adapter Ligation

- The Adapter Reaction mixture contains a high concentration of PEG which increases the viscosity of the mixture. Please pipette slowly and ensure that the correct volume has been used.
- Due to the presence of PEG, the volume of beads required for the cleanup of Adapter-ligated DNA can be reduced. There is a risk of capturing Adapter dimers with a higher multiplier of beads. Therefore, we recommend using 50 μL of En-Beads for the cleanup.
- To avoid the sample cross-contamination caused by residual adapter dimmer，it is prohibit to pool the adapter ligation purified products for Single Strand Circularization.

Appendix E Conversion between DNA Molecular Mass and number of Moles

The yield for circularized ssDNA after cleanup must be above 75 fmol for one sequencing run. Please refer to Formula 1 to calculate the mass of 75 fmol sscir:

Formula 1 Conversion between Circular ssDNA fmol and Mass in ng:

$$ssDNA\ (ng) = 0.075 \times 330\ ng \times [DNA\ fragment\ peak\ size(bp) + 84(bp)] / 1000(bp)$$

Contact Us
Company: MGI Tech Co., Ltd
Address: 2/F, Building 11, Beishan Industrial Zone, Yantian District, Shenzhen, CHINA, 518083
Website: http://en.mgi-tech.com
Email: MGI-service@mgi-tech.com
Service Hotline: (+86) 4000-966-988

MGI Website

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