MGIEasy

Cell-free DNA Library Prep Set User manual

Cat.No.1000007037, 1000012701

Kit Version：V1.0

Manual Version：A0

MGI 华大智造

Contents:

Chapter1 Product Description

1.1 Introduction

The MGIEasy Cell-free DNA Library Prep Set is specifically designed for MGI high-throughput sequencing platforms. This library prep set is optimized to convert cell free DNA or 150-250bp fragmented DNA into a custom library. Combined with the MGIEasy Rapid Circularization Module, PCR products are converted into circularized single strand DNA (ssCirDNA) which can be sequenced on BGISEQ/MGISEQ sequencers. All reagents provided within this kit have passed stringent quality control and functional verification procedures, ensuring performance stability and reproducibility.

1.2 Applications

This library prep kit is used for cell-free DNA and 150-250bp fragmented DNA. The kit can be used for researching cell-free DNA and pathogen detection.

1.3 Platform Compatibility

Constructed libraries are compatible with both PE and SE sequencing on BGISEQ-500RS, MGISEQ-200RS, and MGISEQ-2000RS.

1.4 Contents

Information on Cat. No., Components and Specifications of the MGIEasy Cell-free DNA Library Prep Set is listed below:

Table 1 MGIEasy Cell-free DNA Library Prep Set (48 RXN) (Cat. No: 1000007037)

Modules & Cat. No.	Components	Color Coded Screw Caps	Spec & Quantity
MGIEasy Cell-free DNA Library Perp Kit (Box1) Cat. No: 1000003988	ERAT Buffer Mix	Colorless	200 μL/ tube × 3 tubes
	ERAT Enzyme Mix	Colorless	15 μL/ tube × 3 tubes
	Ligation Buffer Mix	Red	450 μL/ tube × 3 tubes
	Ligation Enzyme	Red	30 μL/ tube × 3 tubes
	Adapter Mix (Barcode 01-48)	Colorless	15 μL/ well × 48 wells
	PCR Enzyme Mix	Blue	475 μL/ tube × 3 tubes
	PCR Primer Mix	Blue	80 μL/ tube × 3 tubes
	DNA Control	Yellow	15 μL/ tube × 1 tube
MGIEasy Cell-free DNA Library Prep Kit (Box 2) Cat. No: 1000003988	Purification Beads	White	1800 μL/ tube × 3 tubes
	Elution Buffer	White	1800 μL/ tube × 3 tubes
MGIEasy Rapid Circularization Module Cat. No: 1000005258	Splint Buffer	Purple	225 μL/ tube × 1 tube
	DNA Rapid Ligase	Purple	10 μL/ tube × 1 tube

Table 2 MGIEasy Cell-free DNA Library Prep Set (96 RXN) (Cat. No: 1000012701)

Modules & Cat. No.	Components	Color Coded Screw Caps	Spec & Quantity
MGIEasy Cell-free DNA Library Perp Kit (Box1) Cat. No: 1000012700	ERAT Buffer Mix	Colorless	200 μL/ tube × 3 tubes
	ERAT Enzyme Mix	Colorless	15 μL/ tube × 3 tubes
	Ligation Buffer Mix	Red	450 μL/ tube × 3 tubes
	Ligation Enzyme	Red	30 μL/ tube × 3 tubes
	PCR Enzyme Mix	Blue	475 μL/ tube × 3 tubes
	PCR Primer Mix	Blue	80 μL/ tube × 3 tubes
	DNA Control	Yellow	10 μL/ tube × 1 tubes
MGIEasy Cell-free DNA Library Perp Kit (Box2) Cat. No: 1000012700	DNA Adapters-96 (1pmol/μL)	Colorless	10 μL/ tube × 96 wells
MGIEasy Cell-free DNA Library Prep Kit (Box 3) Cat. No: 1000012700	Purification Beads	White	1800 μL/ tube × 3 tubes
	Elution Buffer	White	1800 μL/ tube × 3 tubes
MGIEasy Rapid Circularization Module Cat. No: 1000005258	Splint Buffer	Purple	225 μL/ tube × 1 tube
	DNA Rapid Ligase	Purple	10 μL/ tube × 1 tube

1.5 Storage Conditions and Shelf Life

MGIEasy Cell-free DNA Library Prep Kit (48 RXN) (Cat. No：1000003988) Box 1

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

MGIEasy Cell-free DNA Library Prep Kit (48 RXN) (Cat. No：1000003988) Box 2

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

MGIEasy Rapid Circularization Module (Cat. No：1000005258)

- Storage Temperature: -25°C to -15°C
- Production Date and Expiry Date: refer to the label.

MGIEasy Cell-free DNA Library Prep Kit (96 RXN) (Cat. No: 1000012700) Box 1

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

MGIEasy Cell-free DNA Library Prep Kit (96 RXN) (Cat. No: 1000012700) Box 2

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

MGIEasy Cell-free DNA Library Prep Kit (96 RXN) (Cat. No: 1000012700) Box 3

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

MGIEasy Rapid Circularization Module (Cat. No：1000005258)

- Storage Temperature: -25°C to -15°C
- Production Date and Expiry Date: refer to the label
- Transport Conditions: transport in dry ice

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

* Performance of products are guaranteed until the expiration date when appropriate transport, storage and usage conditions are met.

1.6 Equipment and Materials Required but not Provided

Table 3 Equipment and Materials Required but not Provided

Equipment	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)
Reagents	100% Ethanol (Analytical Grade)
	Qubit™ ssDNA Assay Kit (Thermo Fisher Scientific™, Cat. No. Q10212)
	Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific™, Cat. No. Q32854)
	High Sensitivity DNA Analysis Kits（Agilent Technologies™, Cat. No. 5067-4626）
	MGISEQ or BGISEQ High-throughput Sequencing Set
Consumables	Pipette Tips
	1.5 mL EP tube，1.5 mL MaxyClear Snaplock Microcentrifuge Tube（Axygen™,Cat. No. MCT-150-C）or equivalent
	Non-stick RNase-Free, 1.5 mL Microcentrifuge Tubes (Ambion™, Cat. No. AM12450)
	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 (Thermo Fisher 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 may require further adjustments to optimize performance. We recommend adjusting and accounting for the experimental design, sample characteristics, sequencing application and other equipment for optimization.
- Retrieve the reagents from storage before use, and prepare them for use: For enzymes, centrifuge briefly and place on ice for further use. For other reagents, first defrost at room temperature and invert several times to mix properly. Finally, centrifuge briefly and place on ice for further use.
- When preparing mixtures and working solutions, we recommend pipetting up and down at least 10 times to mix

thoroughly. Note that vigorous shaking may cause a decrease in library yield.

- 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 negatively affect experimental accuracy. As such, we recommend physically separating two work areas in the laboratory for PCR reaction preparation and PCR product cleanup. Use designated equipment for each area and clean regularly to ensure a sterile working environment. (Use 0.5% Sodium Hypochlorite or 10% Bleach to clean the work environment)
- If you have other questions, please contact MGI technical support MGI-service@genomics.cn

Chapter 2 Sample Preparation

2.1 Sample Requirements

It is recommended to use cell-free DNA extracted from plasma collected with EDTA as anticoagulant. DNA extracted from plasma with heparin blood collection tubes is incompatible with the cell-free DNA Library Prep Set. It is also recommended that to start with 200 μL of plasma.

The kit can also be used for the library preparation of the fragmented DNA. 2 ng~6 ng fragmented DNA (150-250bp in size) are recommended. Double-strand DNA quantitation kits such as Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit are recommended to quantify the sample in accordance with the instructions of the relevant kit user manuals.

Chapter 3 Library Construction Protocol

3.1 Reagent Preparation

Take out the necessary reagents from the set, briefly centrifuge, and then place on ice. Thaw the buffers at room temperature before use, vortex and centrifuge, then place on ice. Place molecular-grade water and elution buffer at room temperature. Precipitate may appear after the buffer is thawed, but this will not affect the buffer's function. Before use, please vortex and mix the buffer until the precipitate disappears.

⚠ **Note: Read appendix A carefully to plan your barcode strategy.**

3.2 End Repair and A tailing

3.2.1 Transfer the DNA extracted from 200 μL plasma or 2 ng~6 ng fragmented DNA(150-200bp) in size into a new PCR tube. Add enough molecular grade water to the tube to bring the total volume to 40 μL. Mix the tube thoroughly and centrifuge briefly.

⚠ **Note: Sample Volume (μL) = 2 ng to 6 ng / concentration of DNA (ng/μL)**

3.2.2 Prepare the ERAT reaction mixture in a new microcentrifuge tube on ice in accordance with Table 4.

Table 4 ERAT Reaction Mixture

Components	Volume
ERAT Buffer Mix	9.6 μL
ERAT Enzyme Mix	0.4 μL
Total	10 μL

3.2.3 Add 10 μL of the ERAT reaction mixture to the PCR tube prepared in step 3.2.1, mix thoroughly by vortexing, centrifuge briefly, and place the tube in the PCR thermocycler for incubation at 37°C for 10 min, 65°C for 15 min, and cool the solution to 4°C. Then take out the tube and briefly centrifuge the tube.

3.3 Adapter Ligation

⚠ **Note：Please refer to appendix A for detailed adapter combination instructions.**

3.3.1 Prepare the ligation reaction mixture in a new microcentrifuge tube in accordance with Table 5.

Table 5 Ligation Reaction Mixture

Components	Volume
Ligation Buffer	24 μL
Ligation Enzyme	1 μL
Total	25 μL

3.3.2 Add 5 μL of adapter mixture and 25 μL of ligation reaction mixture to the PCR tube prepared in step 3.2.3. Mix thoroughly by vortexing, centrifuge briefly and place the tube in the PCR thermocycler for incubation at 23°C for 20 min. Afterwards, take out the tube, centrifuge briefly and transfer the solution to a new microcentrifuge tube.

⚠ **Note: Please perform step 3.4 immediately after step 3.3. Otherwise, the yield may decrease.**

3.4 Cleanup of Adapter-ligated DNA

⚠ **Note: Read appendix B carefully before the purification.**

3.4.1 Take out Purification Beads from the refrigerator and incubate at room temperature for at least 30 min. Vortex and mix thoroughly before use.

3.4.2 Transfer 40 μL of Purification Beads to the tube from step 3.3.2. Mix by pipetting up and down at least 10 times. Ensure that all of the liquid is fully dispensed from the pipette tip into the tube before proceeding.

3.4.3 Incubate the tube at room temperature for 5 min.

3.4.4 Centrifuge briefly and place the tube onto a magnetic separation rack for 2-5 min until the supernatant is clear. With the tube on the magnetic separation rack, remove and discard the supernatant.

3.4.5 With the tube on the magnetic separation rack, add 200 μL of freshly prepared 80% Ethanol to wash the beads and the sides of the tube. Incubate for 30s then carefully remove and discard the supernatant.

3.4.6 Repeat step 3.4.5 once, remove all of the supernatant from the tube without disrupting the beads. You may centrifuge briefly to collect all remaining supernatant at the bottom of the tube. Separate magnetically, then remove remaining supernatant using a small volume pipette.

3.4.7 Keep the tube on the magnetic separation rack with the lid open, air-dry the beads until no wetness (reflectiveness) is observed. Take care not to over-dry beads (cracks can be observed on the pellet).

3.4.8 Remove the tube from the magnetic separation rack, add 23 μL of Elution Buffer and pipette up and down at least 10 times to re-suspend the beads.

3.4.9 Incubate the tube at room temperature for 5 min.

3.4.10 Centrifuge briefly, then place the tube back onto the magnetic separation rack for 2-5 min until the supernatant is clear. Transfer 21 μL of supernatant to a new 1.5 mL EP tube.

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

3.5 PCR Amplification

3.5.1 Prepare the PCR reaction mixture in a new microcentrifuge tube in accordance with Table 6.

Table 6 PCR Reaction Mixture

Components	Volume
PCR Enzyme Mix	25 μL
PCR Primer Mix	4 μL
Total	29 μL

3.5.2 Add 29 μL of PCR reaction mixture to the PCR tube prepared in step 3.4.10, mix thoroughly by vortexing and set the reaction program on the PCR thermocycler in accordance with Table 7 and then run the program.

Table 7 PCR Amplification Reaction Conditions

Temperature	Time	Cycles
Heated Lid	on	
98°C	2 min	1 cycle
98°C	15 s	
56°C	15 s	12 cycles
72°C	30 s	
72°C	5 min	1 cycle
4°C	Hold	

3.5.3 After the program is complete, briefly centrifuge the tube.

3.6 Cleanup of PCR product

⚠ **Note: Read appendix B carefully before the purification.**

3.6.1 Take out the Purification Beads from the refrigerator and incubate at room temperature for at least 30 min beforehand. Vortex and mix thoroughly before use.

3.6.2 Transfer 50 μL Purification Beads to the tube from step 3.5.3. Mix by pipetting up and down at least 10 times. Ensure that all of the liquid is fully dispensed from the pipette tip into the tube before proceeding.

3.6.3 Incubate the tube at room temperature for 5 min.

3.6.4 Centrifuge briefly and place the tube onto a magnetic separation rack for 2-5 min until the supernatant is clear. With the tube on the magnetic separation rack, remove and discard the supernatant.

3.6.5 With the tube on the magnetic separation rack, add 200 μL of freshly prepared 80% ethanol to wash the

beads and the sides of the tube. Incubate for 30s then carefully remove and discard the supernatant.

3.6.6 Repeat step 3.6.5 once, remove all supernatant from the tube without disrupting the beads. You may centrifuge briefly to collect all remaining supernatant at the bottom of the tube, separate magnetically, then remove remaining supernatant using a small volume pipette.

3.6.7 Keep the tube on the magnetic separation rack with the lid open, air-dry the beads until no wetness (reflectiveness) is observed. Take care not to over-dry beads (cracks can be observed on the pellet).

3.6.8 Remove the tube from the magnetic separation rack, add 32 μL of Elution Buffer and pipette up and down at least 10 times to re-suspend the beads.

3.6.9 Incubate the tube at room temperature for 5 min.

3.6.10 Centrifuge briefly, then place the tube back onto the magnetic separation rack for 2-5 min until the supernatant is clear. Transfer 30 μL of supernatant to a new 1.5 mL EP tube.

✓Stopping Point: After cleanup, PCR product can be stored at -20°C.

3.7 Quality Control of PCR Products and DNA circulation

3.7.1 Quantitate the PCR products with dsDNA Fluorescence Assay Kits such as: Qubit™ dsDNA HS Assay Kit. The required yield for PCR products is ≥ 2 ng/μL.

3.7.2 If more than one samples are pooled for sequencing, please read the detailed information in Appendix A carefully before pooling the sample. Based on the expected throughput of each lane of the DNA sequencing chip from BGISEQ-500/MGISEQ-2000、MGISEQ-200 and required data for analyzing for each cell-free DNA sample, estimate the number of samples (N) can be pooled together.

⚠ **Note: The DNA input of each library (ng) = 185 /N , (for cell-free DNA, 185 ng is about 1.1 pmol)**

⚠ **Note: The volume of each library (μL) = the DNA input of each library (ng) / concentration of the library (ng/μL)**

⚠ **Note: It is strongly suggested that the minimum volume of each PCR library to be pooled is 1 uL.**

3.7.3 Transfer 168 ng of pooled PCR product into a 200 μL PCR tube. Add enough Elution Buffer to the tube to reach a total volume of 48 μL. Mix by vortexing and centrifuge briefly.

3.7.4 Incubate the pooled DNA from step 3.7.3 at 95°C for 5 min on a PCR thermocycler and chill the PCR tubes on the ice.

3.7.5 Prepare the PCR reaction mixture in a new microcentrifuge tube in accordance with Table 8.

Table 8 Circularization Reaction Mixture

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

3.7.6 Add 12.1 μL of the circularization reaction mixture into each of the PCR tubes from step 3.7.4. Mix by vortexing and incubate the sample at 37°Cfor 30 min on the PCR thermocycler.

3.7.7 Centrifuge briefly. The reaction products can be used immediately for DNB making or kept at -20°C.

✓**Stopping Point: Circularized DNA can be stored at -20°C.**

3.8 DNB making and Sequencing

About 20 μL of circularized ssDNA product are used to prepare DNA nanoballs (DNBs) and sequencing.

Please follow the protocol described in 'MGISEQ High-throughput Sequencing Instructure Manual" for DNB making and sequencing. The available sequencing kits including:

a) SE50: MGISEQ-2000RS **High-throughput Sequencing Set (SE50) Instruction Manual, PN: 1000003703.**

b) PE100: MGISEQ-2000RS **High-throughput Sequencing Set (PE100) Instruction Manual, PN: 1000002873.**

c) PE150: MGISEQ-2000RS **High-throughput Sequencing Set (PE150) Instruction Manual, PN: 1000003981.**

d) PE50: MGISEQ-200RS **High-throughput Sequencing Set (PE50) Instruction Manual, PN: 1000005228.**

e) PE100: MGISEQ-200RS **High-throughput Sequencing Set (PE100) Instruction Manual, PN: 1000005229.**

Appendix

Appendix A Adapters instruction

A-1 Adapters Mix (Barcode 01-48) Instruction

4 sets of 4 Adapters: Column 1 (01-04, 05-08,09-12,13-16)

4 sets of 8 Adapters: Column 2-9 (17-24、25-32、33-40、41-48)

- If the sequencing data output requirement is the same for all samples in a lane, please refer to Table 9 below to organize your barcode Adapter combinations.

Table 9 Adapter Mix (Barcode 01-48) 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 sample 1-4 and 5.
6	For samples 1-4, use the method for (4 samples/lane) above. For sample 5-6, use the method for (2 sample/lane) above. Note that you should use different Adapter sets for sample 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 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 (1≤n≤5,x=1-8, Total 9-48)	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 to 8n, separate samples into groups of 8, and use the method for (8 samples/lane) above. 3) For samples 8n+1 to 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).

A-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.

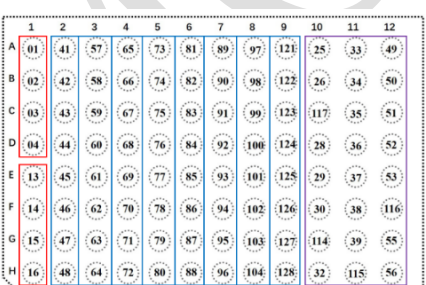

Figure 1 MGIEasy DNA Adapters-96 (Plate) Adapters Layout and Combination Instructions

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

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 1)

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

- If the sequencing data output requirement is the same for all samples in a lane, please refer to the Table 10 below to organize your barcode Adapter combinations.

Table 10 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 sample 1-4 and 5.
6	For samples 1-4, use the method for (4 samples/lane) above. For sample 5-6, use the method for (2 sample/lane) above. Note that you should use different Adapter sets for sample 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 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 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 B Purification Beads and Cleanup Procedures

Before You Use

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

Operation Notes

- If the sample volume decreases due to evaporation during incubation, add additional elution buffer to 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 min. Consider the different magnetic strength of your specific Separation Plate / Rack, and allow enough time for the solution to turn completely clear.
- Avoid touching the beads with pipette tips when pipetting. 2-3 μL of fluids can be left in the tube to avoid contact. In 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. 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 wash of beads with ethanol, try to remove all of the liquid from 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 min 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 Elution 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.

MGI Wechat

■ Contact Information

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