MGI 华大智造

MGIEasy

FS PCR-Free DNA Library Prep Set
User Manual

Cat. No.: 1000013454 (16 RXN), 1000013455 (96 RXN)

Kit Version: V1.1 (16 RXN), V1.2 (96 RXN)

Manual Version: A2

Revision History

Manual Version	Kit Version	Date	Description
A2	V1.1 (16 RXN), V1.2 (96 RXN)	May 2020	◆ Update kit version of 96 RXN to V1.2 ◆ Update the Specification of each components from MGIEasy FS PCR-Free DNA Library Prep Kit in Table 2.
A1	V1.1	Dec.2019	◆ Update kit version to V1.1. ◆ Reduce the minimum gDNA input to 50ng. ◆ Add WGA DNA to input DNA type. ◆ Add beads ratio for single beads purification in step 3.3.2. ◆ 1.3 Add DNBSEQ™ series sequencing platform. ◆ Change the reaction time in steps 3.2.2, 3.5.5, 3.8.3. ◆ Change the prep volume of En-TE buffer in step 3.1.2 (Table 6). ◆ Change the elute volume for low gDNA input in step 3.10.8. ◆ Change the QC criterion for quantification of the ligated product.
A0	V1.0	Mar. 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.mgitech.cn/download/files.html

Doc. #: B02-129-A2

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Chapter 1 Product Description

1.1 Introduction

MGIEasy FS PCR-Free DNA Library Prep Set is specifically designed for making WGS libraries without PCR for MGI High-throughput Sequencing Platforms. This library preparation set is optimized to convert 50 ng to 1000 ng gDNA into a customized library. This set incorporates a high-quality fragmentation enzyme and improved Adapter Ligation technology, which significantly increases library conversion rate. All reagents provided within this set have passed stringent quality control and functional verification procedures, ensuring performance stability and repeatability.

1.2 Application

This library preparation set is applicable for samples from common animals, plants, fungus, bacteria *etc.*, including humans (fresh tissue, cells, saliva), rice, candida glabrata and *E. coli*. The kit is also applicable for special DNA samples, such as WGA DNA. The fragmentation time should be titrated before library construction for optimal fragment size.

1.3 Platform Compatibility

Constructed libraries are compatible with:

BGISEQ-500RS (PE100)

MGISEQ-200RS, DNBSEQ-G50RS (PE100)

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

DNBSEQ-T7RS (PE100)

1.4 Contents

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

Each library preparation set consists of 3 modular kits of reagents, which contains enough material for the indicated numbers of reactions. Additional information regarding Cat. No., Components, and Specifications are listed below.

Table 1 MGIEasy FS PCR-Free DNA Library Prep Set V1.1 (16 RXN) (Cat. No: 1000013454)

Modules & Cat. No.	Components	Color Coded Screw Caps	Spec & Quantity
MGIEasy FS PCR-Free DNA Library Prep Kit V1.1 Cat. No. 1000013458	20x Elute Enhancer	Black	3 μL/ tube × 1 tube
	FS Buffer	Green	80 μL/ tube × 1 tube
	FS Enzyme Mix	Green	160 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 FS PCR-Free DNA Library Prep Set V1.2 (96 RXN) (Cat. No: 1000013455)

Modules & Cat. No.	Components	Color Coded Screw Caps	Spec & Quantity
MGIEasy FS PCR-Free DNA Library Prep Kit V1.2 Cat. No. 1000013459	20x Elute Enhancer	Black	20 μL/ tube × 1 tube
	FS Buffer	Green	640 μL/ tube × 1 tube
	FS Enzyme Mix	Green	1120 μL/ tube × 1 tube
	ER Buffer	Orange	896 μL/tube × 1 tube
	ER Enzyme Mix	Orange	352 μL/tube × 1 tube
	Ad-Lig Buffer	Red	1108 μL/tube × 2 tubes
	Ad Ligase	Red	560 μL/tube × 1 tube
	Ligation Enhancer	Brown	304μL/tube × 1 tube
	Cir Buffer	Purple	1456 μL/tube × 1 tube
	Cir Enzyme Mix	Purple	60 μL/tube × 1 tube
	Exo Buffer	White	282 μL/tube × 1 tube
	Exo Enzyme Mix	White	374 μL/tube × 1 tube
	Exo Stop Buffer	White	512 μL/tube × 1 tube
MGIEasy PF Adapters-96(Plate) Kit Cat. NO. 1000013461	DNA Adapters-96 plate	-	5 μL /tube × 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

1.5 Storage Conditions and Shelf-life

MGIEasy FS 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.
- Ligation Enhancer needs to be stored at room temperature and away from light exposure.
- 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 in 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	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 Horizontal electrophoresis tank Gel Imager Gel Electrophoresis apparatus
Reagents	Nuclease free water (Ambion™, Cat. No. AM9937) 1x TE Buffer，pH 8.0 (Ambion™, Cat. No. AM9858) 0.5 M EDTA (LONZA 51234) 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) REGULAR AGAROSE G-10 (BIOWEST, CB005-100G) GelStain (10000x) (TRANSGEN, Cat. No. #GS101-01)
Consumables	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 Warnings

- 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, mix well by inversion, then centrifuge briefly and place on ice until further use. For other buffer mixes, thaw at room temperature, mix well by vortex, then centrifuge briefly and place on ice until further use.
- CAUTION: During Step 3.2 Enzymatic Fragmentation step, the reaction mixture shall not be prepared by vortex.
- To prevent cross contamination, we recommend using pippet tips with filters. 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 reaction product to a new tube for bead purification especially in Exo Digestion Product purification step. We recommend adding the En-Beads directly to the reaction tube for product purification.
- If you have any question, please contact MGI technical support MGI-service@genomics.cn

Chapter 2 Sample Preparation

2.1 Sample Requirement

- This library prep set is suitable for samples from common animals, plants, fungus, bacteria etc. This includes Humans (fresh tissue, cells, saliva), Rice, Candida glabrata, E. coli, WGA DNA. It is strongly recommended to use high quality genomic DNA (OD_{260}/OD_{280}=1.8-2.0, $0D_{260}/OD_{230}$>2.0) for fragmentation.
- Since FS Enzyme Mix is sensitive to DNA storage buffer, we recommend using 1x TE buffer for dissolution of DNA. If other buffers (H_2O, EB, 0.1xTE) are present, refer to Table 8/11 for adjustment. If other special buffers are present, please adjust the concentration of EDTA to 0.7 mM (in 50 µL Fragmentation Mixture) by diluting 35mM EDTA solution.
- Any residual impurities (E.g. metal chelators or other salts) in the gDNA sample may adversely affect the efficiency of the fragmentation step and the fragmented size.

2.2 Library Insert Size Requirement

- A narrow distribution of fragmented DNA is preferable. Better sequencing quality is obtained with a narrow size distribution, while a wide distribution results in lower quality. A library with a peak of insert size distribution between 350-475 bp is recommended for PE100 sequencing. DNA fragments with a mean size between 400-660 bp is recommended for PE150 sequencing.

⚠ **Note: If the peak size is lower than 330 bp, the adaptor input amount will be insufficient, which will affect the ligation efficiency. If the peak size is higher than 660 bp, the sequencing quality will decrease.**

Chapter 3 Library Construction Protocol

The brief scheme of **Library Construction Protocol** using this kit is as following: 50-1000 ng of gDNA is fragmentated by Enzyme Fragmentation, a quantity less than 200ng DNA fragments is obtained and ready for further library construction. The recommend strategy for different gDNA amount is listed in Table4.

Table 4 Recommend strategy for different gDNA amounts

gDNA amount（N）	gDNA input	Size selection method for fragment DNA
N>1000ng	1000ng	Two step beads purification
1000ng≥N≥800ng	800-1000ng（fully used）	Two step beads purification
800ng>N>200ng	200ng	One step beads purification
200ng≥N≥50ng	50-200ng（fully used）	One step beads purification

⚠ **Note：The one step beads purification method will result in lower sequence reads, as the range of insert size is wider than the two step beads purification method. 500-800ng gDNA also can be used as shearing input followed by the two step beads purification for size selection. However, it has a risk of low yield.**

⚠ **Note: Library construction with 50-200ng input gDNA results in low yeild of ssCir. Usually it is insufficient for a sequencing run, which can be pooled and sequenced with other PCR-free libraries.**

3.1 Reagent Prep

3.1.1 Prepare the 1x Elute Enhancer according to Table 5 in a sterile centrifuge tube, and store at room temperature until use. The shelf life of the 1x Elute Enhancer is 7 days.

Table 5 1× 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 according to Table 6 in a sterile centrifuge tube, and store at 4°C until use. The shelf life of the En-TE buffer is 7 days.

Table 6 En-TE buffer

Components	Volume
1x Elute Enhancer	2.4 μL
1x TE Buffer	1197.6 μL
Total	1200 μL

3.1.3 Prepare the En-Beads according to Table 7 in a sterile microfuge tube, and store at 4°C until use. The shelf life of the En-Beads is 7 days.

Table 7 En-Beads

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

⚠ **Note: The preparation volume of reagents in Table 6 - Table 7 is enough for 6 samples. If there are more samples, you can increase the preparation reagent volumes in proportion.**

3.1.4 Prepare the 35 mM EDTA according to Table 8 in a sterile centrifuge tube, and store at room temperature. The shelf life of 35 mM EDTA is 7 days.

Table 8 35 mM EDTA

Components	Volume
0.5 M EDTA	3.5 μL
Nuclease-Free Water	46.5 μL
Total	50 μL

⚠ **Note: The preparation volume of reagents in Table 8 is enough for 50 samples which are not dissolved with TE (e.g. H_2O, 10 mM Tris or 0.1xTE). If there are more samples, you can increase the preparation reagent volumes in proportion. If the gDNA sample is dissolved in 1xTE buffer, there is no need to prepare Table 8 reagents and no need to normalize genomic DNA according to Table 11.**

3.2 Fragmentation

Note: The following fragmentation conditions are suitable for Human blood, Saliva, fresh tissue, plant, or bacterial gDNA. The kit is also suitable for WGA DNA. Fragment size should be between 150-1000 bp, with a peak size of 300-500 bp. If the sample is not listed above, refer to the following conditions to shorten or extend the 32 °C incubation time to achieve the optimum results.

3.2.1 Take out FS Buffer and FS Enzyme Mix in advance. Mix FS Enzyme Mix by pipetting up and down for 5-10 times. DO NOT vortex FS Enzyme Mix. Mix FS Buffer by vortex.

3.2.2 Set and run the following program on the thermocycler (see Table 9). The total reaction volume is 50 μL. The thermocycler will perform the first step reaction described in Table 9 and be kept at 4°C until step 3.2.6.

Table 9 The Fragmentation Condition

Temperature	Time
Heated Lid (70°C)	On
4°C	Hold
32°C	16 min
65°C	15 min
4°C	Hold

3.2.3 In a new 0.2 mL PCR tube, normalize gDNA to a total volume of 35 μL based on Qubit® dsDNA HS Assay Kit results (see Table 10, Table 11). Vortex 3 times (3 s each), centrifuge briefly, then place on ice.

Table 10 Normalization of gDNA Dissolved in 1xTE

Components	Volume
1xTE	35-X μL
gDNA (50-1000 ng)	X μL
Total	35 μL

Table 11 Normalization of gDNA Dissolved in Different Buffer (H_2O, 10 mM Tris and 0.1xTE)

Components	Volume
Nuclease-Free Water	34-X μL
gDNA (50-1000 ng)	X μL
35 mM EDTA	1 μL
Total	35 μL

⚠ **Note: Please strictly follow the dissolution buffer type for gDNA normalization. If other special buffers are present, please adjust the concentration of EDTA to 0.7 mM (in 50 μL Fragmentation Mixture) by diluting 35 mM EDTA solution.**

3.2.4 Prepare the Fragmentation Mixture on ice (see Table 12). Mix the solution of Table 12 by pipetting over 10 times **(Do not vortex)**, and place the mixture on ice after centrifuge briefly:

Table12 The Fragmentation Mixture

Components	Volume
FS Buffer	5 μL
FS Enzyme Mix	10 μL
Total	15 μL

3.2.5 Transfer 15 μL of Fragmentation Mixture to the 0.2 mL PCR tube from step 3.2.3. Transfer a 50 μL pipette to 40 μL and mix the solution by pipetting 10 times **(Do not vortex)**. Place the mixture on ice after centrifuge briefly to collect the solution at the bottom of the tube.

3.2.6 Make sure the thermocycler has cooled to 4℃ (see step 3.2.2). Place the Fragmentation Mixture from 3.2.5 into the thermocycler and skip the 4℃ Hold step to start the reaction at 32°C.

3.2.7 Centrifuge briefly to collect solution at the bottom of the tube. Add 30 μL En-TE Buffer to a total volume of 80 μL. Vortex 3 times (3 s each), centrifuge briefly, then place the mixture on ice.

⚠ **Note: For the first fragmentation test, it is recommended to take 5 μL product from 80μL mixture in Step 3.2.7 and run on a 1.5% agarose gel to ensure the smear size is between 150-1000 bp (Figure 1), with the peak size between 300-500 bp. If the size is too large or too small, titrate the 32℃-incubation time from Table 9. For samples which cannot reach the ideal fragmentation size by incubation time titration, we recommend re-purifying the sample DNA with 1.5x magnetic beads and eluting into Nuclease-Free Water. After re-purification, re-titrate the incubation time (3-10 min is recommended).**

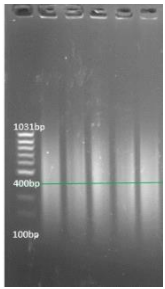

Figure 1 The 1.5% agarose gel test of Fragmentation product

3.3 Clean Up of Fragmentation Products.

DNA fragmentation results in a wide distribution of fragment sizes. Size selection is usually required to ensure uniformity of the library. A two-step magnetic beads size selection (Following Step 3.3.1) is recommended, if the DNA input is higher than 800 ng (see table 13). If the DNA input is less than 800 ng, One-step bead purification (Following Step 3.3.2) is recommended.

Less than 200 ng purified fragmented DNA should be used for End Repair and A-tailing. If the fragmented DNA is less than 40 ng, library preparation may have the risk of failure.

Table 13 Two-step bead selection process:

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

Main Fragment Size of Selected Fragment DNA (bp)	350	475
1st Bead Selection (μL)	50.3	45
2nd Bead Selection (μL)	15.0	11.3
Sequencing Strategy	PE100/PE150	PE100/PE150

⚠ **Note: The selection conditions of Table 13 are used for reference. For different samples, the fragment of the selected main band may have a ±50 bp deviation.**

3.3.1 Two-step Magnetic Beads Size Selection

3.3.1.1 Remove DNA Clean Beads from the refrigerator and let stand at room temperature for 30 minutes before the experiment. Vortex to mix thoroughly before use.

3.3.1.2 Prepare the En-Beads, refer to Table 7 for detailed conditions.

3.3.1.3 Transfer 75 μL Fragmentation Products to a new 0.2 mL PCR tube. Add En-TE Buffer to a final volume of 75 μL if the volume is less than 75 μL.

3.3.1.4 Transfer 45 μL En-Beads to the tube with 75 μL Fragmentation Products. Pipette up and down at least 10 times to mix thoroughly. Ensure that all liquid and beads are expelled from the pipette tip into the tube before proceeding.

3.3.1.5 Incubate at room temperature for 10 minutes.

3.3.1.6 Centrifuge briefly, and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid clears. Carefully transfer the supernatant to a new 0.2 mL PCR tube.

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

3.3.1.7 Transfer 11.3 μL En-Beads to the tube from step 3.3.1.6 containing 120 μL supernatant. Pipette at least 10 times to mix thoroughly.

3.3.1.8 Incubate at room temperature for 10 minutes.

3.3.1.9 Centrifuge briefly, then place the tube onto a Magnetic Separation Rack for at least 5 minutes until the liquid clears. Carefully remove and discard the supernatant with pipette.

3.3.1.10 Keep the tube on the Magnetic Separation Rack, and add 160 μL freshly prepared 80% Ethanol to wash the beads and the walls of the tube. Incubate for 30 seconds, then carefully remove and discard the supernatant.

3.3.1.11 Repeat step 3.3.1.10 and try to remove all liquid from the tube.

3.3.1.12 Keep the tube on the Magnetic Separation Rack with the lid open, and air dry the beads at room temperature until no wetness is observed. Do not over-dry the beads (cracks can be observed on pellet).

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

3.3.1.14 Incubate at room temperature for 5 minutes.

3.3.1.15 Centrifuge briefly then place the tube back onto the Magnetic Separation Rack for 5 minutes until the liquid clears. Transfer 44 μL supernatant to a new 0.2 mL PCR tube.

3.3.1.16 Take 2 μL eluted product for quantification using either Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit.

Note: The DNA sample loss during bead selection can be as high as 60%-95%. For important samples, you may retrieve the beads from the first 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. For the first size selection of the sample, it is recommended to take 2 μL of step 3.3.1.15 eluted product for Agilent 2100 High Sensitivity test （Figure 2）, to make sure the peak size of the selected fragments about 475 bp (May have a ±50 bp deviation) .

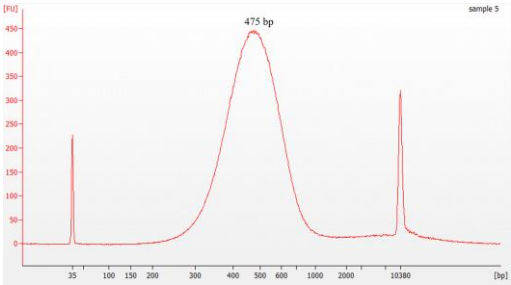

Figure 2 Agilent 2100 Bioanalyzer Results of beads purification Product with two-step bead selection process

3.3.2 Cleanup of Fragmentated DNA

Note: Please read Appendix A carefully before you begin.

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

3.3.2.2 Prepare the En-Beads refer to Table 7 in Chapter 3 for detailed conditions.

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

3.3.2.4 Transfer 60 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 the liquid and the beads are fully expelled from the pipette tip into the tube before proceeding; or mix by vortex thoroughly.

3.3.2.5 Incubate at room temperature for 10 min.

3.3.2.6 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.

3.3.2.7 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.

3.3.2.8 Repeat step 3.3.2.7 and try to remove all 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.

3.3.2.9 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).

3.3.2.10 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.

3.3.2.11 Incubate at room temperature for 5 min.

3.3.2.12 Centrifuge briefly then place the tube back onto the Magnetic Separation Rack until the liquid clears. Transfer 44 μL supernatant to a new 0.2 mL PCR tube.

3.3.2.13 Take 2 μL of 3.3.2.12 eluted product for quantification using either Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit.

⚠ **Note: For different samples, the fragment of the selected main band may have a ±140 bp deviation. The main band means the peak size of bead selected DNA with Agilent 2100 High Sensitivity test, and the main band in the sequencing results will be reduced.**

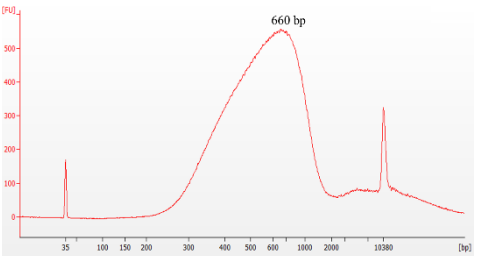

Figure 3 Agilent 2100 Bioanalyzer results of beads purification product with one-step bead selection process

3.4 End Repair and A-tailing

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

3.4.2 Prepare the End Repair and A-tailing Reaction Mixture on ice (see Table 14):

Table 14 End Repair and A-tailing Reaction Mixture

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

3.4.3 Transfer 10 μL of the End Repair Reaction Mixture to the 0.2 mL PCR tube from step 3.4.1. Vortex 3 times (3 s each) and briefly centrifuge to collect the solution at the bottom of the tube.

3.4.4 Place the 0.2 mL PCR tube from step 3.4.3 into the thermocycler and run the program in Table 15. And the total reaction volume is 50 μL.

Table 15 The Reaction Conditions of the End Repair and A-tailing

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.**

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

⚠ **Warning: DO NOT STOP AT THIS STEP. Please continue to step 3.5.**

3.5 Adapter Ligation

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

3.5.1 Refer to the instructions for MGIEasy PF Adapters (see Appendix B). Add 5 μL of MGIEasy PF Adapters to the PCR tube from step 3.4.5.

3.5.2 Vortex 3 times (3 s each), then briefly centrifuge to collect solution at the bottom of the tube.

3.5.3 Prepare the Adapter Ligation Reaction Mixture on ice (see Table 16):

Table 16 Adapter Ligation Reaction Mixture

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

3.5.4 Pipette slowly and transfer 25 μL of Adapter Ligation Reaction Mixture to the 0.2 mL PCR tube from step 3.5.2. Vortex 6 times (3 s each), then centrifuge briefly to collect the solution at the bottom of the tube.

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

3.5.5 Place the 0.2 mL PCR tube from step 3.5.4 into the thermocycler and run the following program in Table 17. And the total reaction volume is 80 μL.

Table 17 The Reaction Conditions of Adapter Ligation

Temperature	Time
Heated Lid (30°C)	On
25°C	10 min
4°C	Hold

⚠ **Note：The ligation incubation time for 25°C can extend into 30min for improving ssCir output as needed.**

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

3.5.7 Add 20 μL En-TE Buffer to a total volume of 100 μL.

⚠ **Warning: DO NOT STOP AT THIS STEP. Please continue to step 3.6.**

3.6 Cleanup of Adapter-ligated DNA

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

3.6.1 Remove En-Beads from the refrigerator and bring to room temperature for 30 minutes beforehand. Mix thoroughly by vortexing before use.

3.6.2 Transfer 50 μL En-Beads to the tube in step 3.5.7. Mix thoroughly by vortexing or by pipetting up and down at least 10 times. Ensure that all liquid and beads are expelled from the pipette tip into the tube before proceeding.

3.6.3 Incubate at room temperature for 10 minutes.

3.6.4 Centrifuge briefly, then place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid clears. Carefully remove and discard the supernatant with a pipette.

3.6.5 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. Incubate for 30 seconds, then carefully remove and discard the supernatant once the liquid clears.

3.6.6 Repeat step 3.6.5 once, remove all liquid from the tube without disturbing the beads. Centrifuge briefly to collect any remaining liquid to the bottom, separate magnetically, then remove any remaining liquid using a small volume pipette.

3.6.7 Keep the tube on the Magnetic Separation Rack with the lid open, and air-dry the beads at room temperature until no wetness is observed. Do not over-dry beads (cracks can be observed

on pellet).

3.6.8 Remove the tube from the Magnetic Separation Rack, add 50 µL En-TE Buffer to elute the DNA. Gently mix by vortexing or by pipetting up and down at least 10 times.

3.6.9 Incubate at room temperature for 5 minutes.

3.6.10 Centrifuge briefly, then place the tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid clears. Transfer 48 µL supernatant to a new 0.2 mL PCR tube.

3.6.11 Take 1 µL supernatant for quantification using Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit. If the concentration is >1.2 ng/µL, proceed to the next step. If the concentration is between 0.8-1.2 ng/µL, attmpt to the next step, but with the risk of library failure. If the concentration is < 0.8 ng/µL, it is not recommended to proceed to the next step.

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

3.7 Denaturation

3.7.1 Place the 0.2 mL PCR tube from step 3.6.10 into the thermocycler and run the program in Table 18. And the total reaction volume is 50 µL.

Table 18 Denature 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 3.7.2.**

3.7.2 Centrifuge briefly and continue to the next step immediately.

3.8 Single Strand Circularization

3.8.1 Prepare the Circularization Reaction Mixture on ice (see Table 19)

Table 19 Circularization Reaction Mixture

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

3.8.2 Transfer 12 μL Circularization Reaction Mixture to the PCR tube from step 3.7.2. 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 20. And the total reaction volume is 60 μL.

Table 20 The Reaction Conditions of Single Strand DNA Circularization

Temperature	Time
Heated Lid (42°C)	On
37°C	10 min
4°C	Hold

3.8.4 Briefly centrifuge, and place the PCR tube on ice. Continue to the next step immediately.

3.9 Exo Digestion

3.9.1 Prepare the following Exo Reaction Mixture (see Table 21) on ice during the reaction from step 3.8.3.

Table 21 Exo Digestion Reaction Mixture

Components	Volume
Exo Buffer	1.4 μL
Exo Enzyme Mix	2.6 μL
Total	4.0 μL

3.9.2 Transfer 4 μL Exo Digestion Reaction 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 0.2 mL PCR tube from step 3.9.2 into the thermocycler and run the program in Table 22. And the total reaction volume is 64 μL.

Table 22 The Reaction Conditions of Exo Digestion

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

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

3.9.5 Add 3 μL Exo 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.

3.10 Cleanup of Exo Digestion Product

Note: Please read Appendix A carefully before you begin.

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

3.10.2 Transfer 120 μL En-Beads to the Exo Digestion product from step 3.9.5. 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.

3.10.3 Incubate at room temperature for 10 minutes.

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

3.10.5 Keep the tube on the Magnetic Separation Rack, and add 160 μL freshly prepared 80% ethanol to wash the beads and the walls of the tube. Incubate for 30 seconds, then carefully remove and discard the supernatant.

3.10.6 Repeat step 3.10.5 once. Remove all liquid from the tube without disrupting the beads. Centrifuge briefly to collect any remaining liquid to the bottom, separate the beads magnetically, then remove any remaining liquid using a small volume pipette.

3.10.7 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 (cracks can be observed on pellet).

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

Note：If the gDNA input is 50-100 ng, it is recommended to use 12 μL En-TE for DNA elution, and to collect 11 μL of the supernatant in step 3.10.10.

3.10.9 Incubate at room temperature for 10 minutes.

3.10.10 Centrifuge briefly then place the tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid clears. Transfer 24 μL 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.

3.11 Quality Control of Digestion Product

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

3.11.2 The final yields should be≥ 75, 60 and 30 fmol when using 200ng-1ug, 100ng-200ng and 50ng-100ng gDNA as input respectively. Please refer to Table 23 or formula 1 in Appendix E.

3.11.3 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 B). And the mole ratio is based on your required sequencing data of each sample being pooled.

⚠ **Note: The insert size and the size range affects sequencing quality and amount of effective sequencing reads. Therefore, it has a risk of a decrease of sequencing quality and effective sequencing reads, when pooling libraries with different insert sizes or by using different purification methods, eg. to pool one step bead purification products with two step bead purification products to sequence.**

Table 23 The Corresponding Molecular Weight equal to 75 fmol Circularized ssDNA for Different Selected Fragment Size

Peak Size of Selected Fragment (bp)	Circularized ssDNA Size (bp)	Corresponding Molecular Weight equal to 75 fmol Circularized ssDNA (ng)
330	364	9.0
350	384	9.5
400	434	10.7
450	484	12.0
475	509	12.6
500	534	13.2
600	634	15.7
660	694	17.2

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 (MGI, Cat. No. 1000005278 or 1000005279). 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 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 decreases due to evaporation during incubation, add En-TE buffer to the designated volume during the beads purification step. This ensures that the correct ratio for the beads is used. During 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 or Rack, and allow enough time for the solution to turn completely clear. And if you use Magnetic rack DynaMagTM-2, you need transfer the product to a new 1.5 mL centrifuge tube. Doing so will result in roughly a 20% loss.
- Avoid touching the beads with pipette tips when pipetting. 2-3 μL of liquid can be left in the tube to avoid contact. In case of contact between the beads and pipette tip, expel all the solution to 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.
- After the 2nd wash of beads with ethanol, try to remove all liquid from the tube. You may centrifuge briefly to collect any remaining liquid to 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, which can affect

subsequent reactions. Over-drying (observed by cracking of pellet) may cause a reduction in yield. Drying takes approximately 5-10 min depending on your specific lab environment. Observe closely and wait until the pellet appears sufficiently dry with a matte appearance, then continue to the elution process with En-TE Buffer.

- Avoid the contact between the pipette and the beads while removing the supernatant. Contamination from the beads may affect subsequent reactions. The volume of the supernatant should be 2 μL less than the original elution 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 opening or closing lids.

Appendix B Using Barcode Adapters

- We currently offer two versions of the Adapter Reagent Kit depending 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 combinations based on base composition balance. However, the Barcode Adapters are not continuous. For optimum performance, please refer to instructions in Appendix B-1 and Appendix B-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 place above room temperature to avoid structural changes such as denaturation, which might affect performance. Before use, please centrifuge to collect liquid to the bottom of tubes or plates. Gently remove the cap / perforable membrane to prevent spills and cross-contamination; Mix the adapters by pipetting up and down before use; Remember to close the cap immediately after use. For MGIEasy PF Adapters-96 (Plate) Kit, if the perforable membrane is accidentally contaminated, it should be discarded immediately and the plate sealed again using PCR sealing machine.
- Adapters from other MGI library Kits (numbered 501-596) are designed for library construction with amplification strategies and are incompatible with PCR-Free Kits.

Appendix B-1 MGIEasy PF Adapters-16 (Tube) Kit Instruction

Based on the principle of balanced base composition, adapters must be used in specific groups. Please follow the instructions bellow to use Adapters with the 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 data output requirement is the same for all samples in a lane, please refer to the Table 24 below to organize your Barcode Adapter combinations:

Table 24 MGIEasy PF Adapters-16 (Tube) Kit Instruction

Samples /lane	Instructions (Example)
1	Requires at least 1 set of Adapters: (1.)Take a set of 4 Adapters (01-04), mix with equal volume, then add to the sample.

	Or (2.) Take a set of 8 Adapters (97-104), mix with equal volume, 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 Adapters: (1.) Take a set of 4 Adapters (01-04), mix with equal volume 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 with equal volume 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 samples 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 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 with equal volume 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 samples 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 samples 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 samples 1-4, samples 5-6 and sample 7.
8	Requires at least 1 set of Adapters:

	(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).

In cases that 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, 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-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 in proper combination.

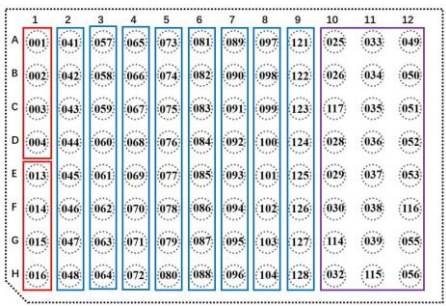

Figure 4 MGIEasy PF Adapters-96 (Plate) Kit Adapter Layout and Combination Instructions

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 4)
- 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 4)
- 1 set of 24 Adapters: Column 10-12 (see the purple box in Figure 4)

Assuming data output requirement is the same for all samples in a lane, please refer to the Table 25 below to organize your Barcode Adapter combinations:

Table 25 MGIEasy PF Adapters-96 (Plate) Kit Instruction

Sample /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).

In cases that 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, 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 C 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 amount 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 Beads for the cleanup.

Appendix D 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 × 330 ng × [DNA fragment peak size (bp) + 84 (bp) - 50 (bp)] /1000 (bp)

Note:

" + 84 (bp) " in the formula indicates the adapter length,

"- 50 (bp)" indicates that the DNA peak size will have a decrease of about 50 bp from the size selection to the circularized ssDNA library.

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