MGIEasy

Exome FS Library Prep Set User Manual

Cat No.: 1000009658 (16 RXN)

Kit Version: V2.1

Manual Version: B2

Revision History

Manual Version	Kit Version	Date	Description
B2	V2.1	2021.04	◆ Kit version V2.0 was upgraded to V2.1 ◆ Change composition of frag part (different article number) and fragmentation operation (volume, temperature, time) in step 3.1 ◆ Change the bead purification conditions in Step 3.2
B1	V2.0	2021.01	◆ Update contact information.
B0	V2.0	2019.09	◆ Kit version V1.0 was upgraded to V2.0 ◆ Change composition of frag part (different article number) and fragmentation operation (volume, temperature, time) ◆ Change the construction condition of PE150 library ◆ Delete some appendices and add the content to Chapter 3 ◆ Add DNBSEQ series sequencing platform
A0	V1.0	2019.07	◆ 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 Exome FS Library Prep Set is specifically designed for constructing human whole exome libraries for the MGI high-throughput sequencing platform series.

This library prep set is optimized to convert 50-400 ng of genomic DNA into a customized library and is compatible with various commercial probes for capture based on MGI sequencing platform. This set incorporates improved Adapter ligation technology and high-fidelity PCR enzymes, which significantly increase library yield and conversion rate. All reagents provided within this set have passed stringent quality control and functional verification procedures, ensuring performance stability and reproducibility.

⚠ **Note: If you are using MGI Exome V4 Probe or MGI Exome V5 Probe is used for capture, please use the corresponding regents from MGIEasy Exome Capture V4 probe Set or MGIEasy Exome Capture V5 probe Set and conduct the hybridization and capture process according to the user manual provided by the set.**

1.2 Application

This library prep set is applicable for samples derived from Human and provides supplement of the capture for whole exome collocated with Probes.

1.3 Platform Compatibility

Constructed libraries are compatible with

BGISEQ-500RS (PE50/PE100)
MGISEQ-2000RS (PE100/PE150)，DNBSEQ-G400RS (PE100/PE150)

1.4 Library Prep Kit Contents

Each Library Prep Set consists of a MGIEasy FS DNA Library Prep Set, and a MGIEasy Exome Capture Accessory Kit. Further information on Cat. No., Components and Specifications are listed below.

Table 1 MGIEasy FS DNA Library Prep Set (16 RXN) (Cat. No.: 1000006987)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy FS DNA Library Prep Kit Cat. No.: 1000005254	Frag BufferII	Green	160 μL/tube × 1 tube
	Frag EnzymeII	Green	80 μL/tube × 1 tube
	ERAT Buffer	Orange	114 μL/ tube × 1 tube
	ERAT Enzyme Mix	Orange	47 μL/ tube × 1 tube
	Ligation Buffer	Red	375 μL/ tube × 1 tube
	DNA Ligase	Red	26 μL/ tube × 1 tube
	PCR Enzyme Mix	Blue	400 μL/ tube ×1 tube
	PCR Primer Mix	Blue	96 μL/ tube × 1 tube
MGIEasy DNA Adapters-16 (Tube) Kit Cat. No.: 1000005284	DNA Adapters	White	10 μL/ tube ×16 tubes
MGIEasy DNA Clean Beads Cat. No.: 1000005278	DNA Clean Beads	White	8 mL/ tube × 1 tube
	TE Buffer	White	4 mL/ tube × 1 tube
MGIEasy Circularization Module Cat. No.: 1000005260	Splint Buffer	Purple	186 μL/ tube × 1 tube
	DNA Rapid Ligase	Purple	8 μL/ tube × 1 tube
	Digestion Buffer	White	23 μL/ tube × 1 tube
	Digestion Enzyme	White	42 μL/ tube × 1 tube
	Digestion Stop Buffer	White	120 μL/ tube × 1 tube

Table 2 MGIEasy Exome Capture Accessory Kit (16 RXN) (Cat. No.: 1000007743)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Exome Capture Accessory Kit Cat. No.: 1000007743	Post-PCR Enzyme Mix	Blue	800 μL/ tube × 1 tube
	PCR Primer Mix	Blue	96 μL/ tube × 1 tube
	Block 3	Yellow	16 μL/ tube × 1 tube
	Block 4	Yellow	160 μL/ tube × 1 tube

1.5 Storage Conditions and Shelf Life

MGIEasy FS 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

MGIEasy DNA Adapters Kit

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

MGIEasy Circularization Module

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

MGIEasy Exome Capture Accessory 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 Magnetic rack DynaMag™-2 (Thermo Fisher Scientific™, Cat. No. 12321D) or equivalent Qubit™ 3.0 Fluorometer (Thermo Fisher Scientific, Cat. No. Q33216) Agilent 2100 Bioanalyzer (Agilent Technologies™, Cat. No. G2939AA) Eppendorf Concentrator（Eppendorf, Cat. No. 5305000398） Thermomixer or water bath equipment Nutator or other nutating mixer/shaker Magnetic rack for 96-well plate (BioMag, Cat. No. BMB-96）or equivalent
Reagents	Nuclease free water (NF water) (Ambion, Cat. No. AM9937) 100% Ethanol (Analytical Grade) Qubit® ssDNA Assay Kit (Invitrogen, Cat. No. Q10212) Qubit® dsDNA HS Assay Kit (Invitrogen, Cat. No. Q32854) High Sensitivity DNA Kits (Agilent Technologies, Cat. No. 5067-4626) Agilent DNA 1000 Kit (Agilent Technologies, Cat. No. 5067-1504) Reagents or kits or beads required by commercial probes for capture
Consumables	Pipette Tips 1.5 mL centrifuge tubes (Axygen, Cat. No. MCT-150-C) 0.2 mL PCR tubes (Axygen, Cat. No. PCR-02-C) or 96-well plate (Axygen, Cat. No. PCR-96M2-HS-C) 2.0 mL centrifuge tubes (Axygen, Cat. No. MCT-200-C) or equivalent 8 Strip Domed Caps Fit 0.2 mL PCR Tube Strips（Axygen, Cat. No. PCR-02CP-C）or equivalent Qubit® Assay Tubes (Invitrogen, Cat. No. Q32856) or 0.5 mL Thin Wall PCR Tubes (Axygen, Cat. No. PCR-05-C) Filter Tips（Axygen, Cat. No. TF-100）or equivalent Clear Adhesive Film (ABI, Cat. No. 4306311) Blade or knife

1.7 Precautions and Warning

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

Chapter 2 Sample Preparation

2.1 Genomic DNA Type

This library preparation set is applicable for genomic DNA derived from human samples.

2.2 Genomic DNA Integrity

It is strongly recommended to use high quality genomic DNA (gDNA) samples (OD_{260}/OD_{280}=1.8~2.0 , OD_{260}/OD_{230} > 2.0) for fragmentation.

2.3 Genomic DNA Input

As the amount of genomic DNA decreases, the proportion of DNA fragments that successfully ligate Adapters will decrease. If the starting amount of genomic DNA is enough, it is recommended to use ≥200 ng input genomic DNA (concentration requirement: ≥15 ng/μL) for library construction to achieve optimal results.

2.4 Storage Condition of Genomic DNA

DNA storage buffers compatible with this kit include water, EB, 0.1×TE, buffer AE, TE and other common extraction and dissolution buffers.

In order to prevent the effect of too many inhibitors such as EDTA and EGTA on interrupting aging, it is recommended to dissolve in water, EB or 0.1×TE during sample extraction to ensure the consistency of interrupting results. If the DNA sample contains a high concentration of salt ions/proteins, the efficiency of DNA fragmentation may be affected.

- If other complex components (high salt ion/protein/bivalent cation/EDTA/ EGTA) are introduced into the DNA extraction process, it is recommended to use 2× beads for purification before fragmentation, and then elute with water, EB or 0.1×TE, with a recovery rate of about 90%. For precautions and purification procedures for DNA Clean Beads, please refer to Step 3.5 or Step 3.7 in Chapter 3.
- It is recommended to use 50 ng of non-precious DNA sample with the same extraction condition and the dissolved buffer for the fragmentation test, referring to step 3.1. Then assess the fragment size distribution of fragmentation products with Agilent 2100, in order to determine the 30°C incubation time in fragmentation step for achieving the optimum results.

Chapter 3 Library Construction Protocol

The DNA sample used in this Library Construction Protocol: 200 ng of human genomic DNA is fragmented with Frag Enzyme and the fragmented gDNA is selected with 36 μL 1st bead selection beads and 12 μL 2nd bead selection beads. After size selection, about 330 bp DNA fragments are obtained, and can be used for PE150 sequencing.

For different amounts of the initial DNA sample, please follow Table 7 in Step 3.2, and Table 12 in Step 3.6 to adjust this protocol.

3.1 Fragmentation

⚠ **Note: The performance of fragmentation (size distribution of DNA fragments) is controlled by incubation time and temperature. Therefore, please ensure the accuracy of time and temperature during the reaction. Samples and Frag Enzyme should always be kept on ice.**

⚠ **Note: The following fragmentation conditions are suitable to DNA dissolved in water, EB, 0.1×TE. Fragmented DNA between 100 bp-1000 bp, with a peak size of 300 bp-500 bp can be obtained, which is suitable for PE150 sequencing. If gDNA storage buffer is not listed above, please explore the interruption time of 30℃ by yourself.**

3.1.1. Transfer gDNA to a new 0.2 mL PCR tube. The volume should be less than or equal to **45 μL**. If the volume is less than 45 μL, add dilution buffer to bring the final volume to **45 μL** (see Table 4)

Table 4 Input DNA Dilution

Components	Volume
DNA	X μL
Dilution buffer	45-X μL
Total	45 μL

3.1.2. Take out Frag Enzyme II and Frag Buffer II from MGIEasy FS DNA Library Prep Kit. Mix Frag Enzyme II by inverting 10 times then centrifuge briefly and place on ice for use. DO NOT vortex Frag Enzyme II. Vortex Frag Buffer II 3 times (3s each) then centrifuge briefly and place on ice for use.

3.1.3. Prepare the fragmentation mixture on ice (see Table 5). Pipette up and down at least 10 times to mix thoroughly. (DO NOT vortex). Centrifuge briefly and place on ice for use.

Table 5 Fragmentation Mixture

Components	Volume
Frag Buffer II	10 μL
Frag Enzyme II	5 μL
Total	15 μL

3.1.4. Transfer 15 μL of the fragmentation mixture to the 0.2 mL PCR tube from step 3.1.1. Pipette at least 10 times to mix thoroughly and briefly centrifuge to collect the solution at the bottom of the tube.

3.1.5. Set and run the following program on the thermocycler (see Table 6). Make sure the thermocycler has cooled to 4°C. Place the 0.2 mL PCR tube from step 3.1.4 into the thermocycler and skip the '4°C Hold' step to start the reaction at 30°C.

Table 6 Fragmentation Reaction Conditions

Temperature	Time
Heated lid	On
4°C	Hold
30°C	8 min
65°C	15 min
4°C	Hold

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

⚠ **Note: For the first fragmentation test, it is recommended to take 3-5 ng samples from the 3.1.6 sample for 1.8x DNA Clean Beads purification (Elute the sample with 5 μ L TE Buffer, refer to Step 3.2.2) and run Agilent 2100 BioAnalyzer (High Sensitivity DNA Kits). The normal PE150 fragment size should be between 100 bp-1000 bp, with a peak size of 300 bp-500 bp (see Figure 1). Titrate the 32°C incubation time if the peak size is too large or too small.**

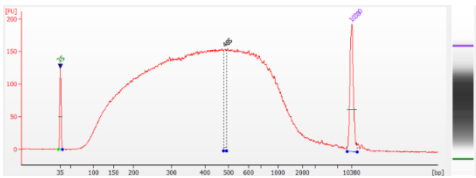

Figure 1 Agilent 2100 Bioanalyzer Fragment Size Distribution Results of the fragmentation Product (1.8x DNA Clean Beads purification)

3.2 Size Selection/ Cleanup of Fragmentation Product （Alternative）

⚠ **Note 1: Please read Appendix A carefully before you begin.**

Note 2 : DNA Clean Beads are included in 'MGIEasy DNA Clean Beads'.

Note 3: After fragmentation, DNA has a wide size distribution, and it is usually necessary to conduct fragment screening to control the range of the final library fragment size. We recommend performing Size Selection (see Step 3.2.1) when input DNA>100 ng and perform Cleanup (see Step 3.2.2) when the sample input is low (≤100 ng) or highly degradely (such as FFPE sample). Table 7 lists the recommended operation steps for different conditions.

Table 7 Recommended Purification Conditions after Fragmentation

Input gDNA	Operation after fragmentation	PE150 Beads Volume
>100 ng	Size Selection	36 μL+12 μL
50-100 ng	Cleanup	60 μL

3.2.1 Size Selection (Option 1)

The following steps used 36 μL + 12 μL beads to obtain the fragmentation products with the main peak≈330 bp, which is applicable for PE150. For other schemes, please refer to Table 7.

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

3.2.1.2 Transfer 36 μL of DNA Clean Beads to the 1.5 mL centrifuge tube containing 60 μL of fragmentation product from step 3.1.6. Pipette up and down at least 10 times or vortex to mix thoroughly.

3.2.1.3 Incubate at room temperature for 5 minutes.

3.2.1.4 Centrifuge briefly and place the tube onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Then, carefully transfer the supernatant to a new 1.5 mL centrifuge tube.

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

3.2.1.5 Transfer 12 μL of DNA Clean Beads to the centrifuge tube with 96 μL of supernatant from step 3.2.1.4. Pipette at least 10 times to mix thoroughly.

3.2.1.6 Incubate at room temperature for 5 minutes.

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

3.2.1.8 Keep the centrifuge tube on the Magnetic Separation Rack and add 200 μL of freshly prepared 80% ethanol to wash the beads and the walls of the tube. Carefully remove and discard the supernatant.

3.2.1.9 Repeat step 3.2.1.8 once. Remove all the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom of the centrifuge tube, separate the beads magnetically, and then remove any remaining liquid using a small volume pipette.

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

3.2.1.11 Remove the centrifuge tube from the Magnetic Separation Rack and add 43 μL of TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.2.1.12 Incubate at room temperature for 5 minutes.

3.2.1.13 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully transfer 41 μL of supernatant to a new 0.2 mL PCR tube.

3.2.1.14 Quantify the Size selection products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iT PicoGreen® dsDNA Assay Kit.

⚠ **Note: The recovery rate after size selection is around 10%~20%. For example, following the standard protocol, 200 ng gDNA input for fragmentation, then use 36 μL + 12 μL beads for size selection, in total 20 ~40 ng yield of fragmentation products can be obtained. If the recovery rate is too low, it is possible due to too many losses during size selection, then the yield of PCR products and data performance could be affected.**

3.2.2 Cleanup (Option 2)

The following steps used 60 μL beads to obtain the fragmentation products with the main peak≈330 bp, which is applicable for PE150. For other schemes, please refer to Table 7.

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

3.2.2.2 Transfer 60 μL of DNA Clean Beads to the 1.5 mL centrifuge tube containing 60 μL of

fragmentation product from step 3.1.6. Pipette up and down at least 10 times or vortex to mix thoroughly.

3.2.2.3 Incubate at room temperature for 5 minutes.

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

3.2.2.5 Keep the centrifuge tube on the Magnetic Separation Rack and add 200 μL of freshly prepared 80% ethanol to wash the beads and the walls of the tube. Carefully remove and discard the supernatant.

3.2.2.6 Repeat step 3.2.2.5 once. Remove all the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom of the centrifuge tube, separate the beads magnetically, and then remove any remaining liquid using a small volume pipette.

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

3.2.2.8 Remove the centrifuge tube from the Magnetic Separation Rack and add 43 μL of TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.2.2.9 Incubate at room temperature for 5 minutes.

3.2.2.10 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully transfer 41 μL of supernatant to a new 0.2 mL PCR tube.

3.2.2.11 Quantify the purified fragmentation products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iT PicoGreen® dsDNA Assay Kit.

⚠ **Note: The recovery rate after cleanup is around 40%-60%. If the recovery rate is too low, it is possible due to too many losses during size selection, then the yield of PCR products and data performance could be affected.**

✔ **Stopping Point: Fragmentation products after Size selection or Cleanup can be stored at -20°C.**

3.3 End Repair and A-tailing

3.3.1 Based on the quantification result of fragmentation products after Size selection or Cleanup, if the yield ≤100 ng, then transfer total products to a new 0.2 mL PCR tube for next step; If the yield >100 ng, it is recommended to transfer 100 ng products to a new 0.2 mL PCR tube for next step. The total volume should be less than 40 μL, add TE Buffer for a total volume of 40 μL.

3.3.2 Prepare the end repair and A-tailing mixture on ice (see Table 8).

Table 8 End Repair and A-tailing Mixture

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

3.3.3 Transfer 10 μL of the end repair mixture to the 0.2 mL PCR tube from step 3.3.1. Vortex 3 times (3 s each) and briefly centrifuge to collect the solution at the bottom of the tube.

3.3.4 Place the 0.2 mL PCR tube from step 3.3.3 into the thermocycler and run the program in Table 9.

Table 9 End Repair and A-tailing Reaction Conditions

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

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

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

3.4 Adapter Ligation

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

3.4.1 Please refer to the instructions for MGIEasy DNA Adapters (see Appendix B). Add 5 μL of MGIEasy DNA Adapters to each PCR tube from step 3.3.5. Vortex 3 times (3 s each) and briefly centrifuge to collect solution at the bottom of the tube.

3.4.2 Prepare the Adapter ligation mixture in a new 0.2 mL PCR tube on ice (see Table 10).

Table 10 Adapter Ligation Mixture

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

3.4.3 Pipette slowly to transfer 25 μL of Adapter ligation mixture to the PCR tube from step 3.4.1. Vortex 6 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

⚠ **Note: Due to the viscosity of the Adapter ligation mixture, please pipette slowly and ensure that the correct amount has been used.**

3.4.4 Place the 0.2 mL PCR tube from step 3.4.3 into the thermocycler and run the program in Table 11.

Table 11 Adapter Ligation Reaction Conditions

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

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

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

✔ **Stopping Point: Adapter-ligated DNA can be stored at -20°C for a maximum of 16 hours.**

3.5 Cleanup of Adapter-Ligated DNA

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

3.5.1 Take out DNA Clean Beads from refrigerator and bring to room temperature for 30 minutes beforehand. Vortex and mix thoroughly before use.

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

3.5.3 Incubate at room temperature for 5 minutes.

3.5.4 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the

liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.5.5 Keep the tube on the Magnetic Separation Rack and add 200 µL 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.

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

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

3.5.8 Remove the centrifuge tube from the Magnetic Separation Rack and add 21 µL TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.5.9 Incubate at room temperature for 5 minutes.

3.5.10 Centrifuge briefly and place the centrifuge tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 19 µL supernatant to a new 0.2 mL PCR tube.

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

3.6 PCR Amplification

⚠ **Note: The number of PCR cycles must be strictly controlled. Insufficient cycles may lead a lower library yield. Excessive cycles may also lead to adverse effects such as over amplification, an increase in bias, PCR duplicates, chimeric sequences, or accumulated mutations. Table 12 shows the number of PCR cycles required to yield 500 ng and 1 µg of PCR product from 50-400 ng of high-quality gDNA. When the quality of gDNA is poor and consists of a longer fragment, PCR cycles should be increased appropriately for desired yield.**

Table 12 PCR Cycles Required to Yield 300 ng and 1 µg Products

Genomic DNA input (ng)	Operation after fragmentation	PCR Cycles required for corresponding yield	
		500 ng	1 µg
400 ng	Size Selection	4-6	6-8
200 ng	Size Selection	5-7	7-9
100 ng	Cleanup	5-7	7-9
50 ng	Cleanup	7-9	9-11

3.6.1 Prepare the PCR amplification mixture in a new PCR tube on ice (see Table 13).

Table 13 PCR Amplification Mixture

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

3.6.2 Transfer 31 μL of PCR amplification mixture to the PCR tube from step 3.5.10. 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 from step 3.6.2 into the thermocycler and run the following program in Table 14.

Table 14 Reaction Conditions for PCR Amplification

Temperature	Time	Cycles
Heated lid	on	
95°C	3 min	1 cycle
98°C	20 s	
60°C	15 s	8 cycles
72°C	30 s	
72°C	10 min	1 cycle
4°C	Hold	

Note: The cycle number above table is for standard protocol with 200 ng gDNA input, refer to Table 12 to adjust cycles for different input.

3.6.4 Centrifuge briefly to collect the solution at the bottom of the tube. Transfer all of the solution to a new 1.5 mL centrifuge tube.

3.7 Cleanup of PCR Product

Note: Please read Appendix A carefully before you begin.

3.7.1 Take out DNA Clean Beads from refrigerator and bring to room temperature for 30 minutes beforehand. Vortex and mix thoroughly before use.

3.7.2 Transfer 50 μL DNA Clean Beads to the centrifuge tube from step 3.6.4. 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 centrifuge tube before proceeding.

3.7.3 Incubate at room temperature for 5 minutes.

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

3.7.5 Keep the tube on the Magnetic Separation Rack and add 200 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.

3.7.6 Repeat step 3.7.5 once, remove all liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom, separate the beads magnetically, and remove any remaining liquid using a small volume pipette.

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

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

3.7.9 Incubate at room temperature for 5 minutes.

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

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

3.8 Quality Control of PCR Product

3.8.1 Quantify the purified PCR products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iT PicoGreen® dsDNA Assay Kit. The required yield for PCR products is ≥1000 ng.

3.8.2 Assess the fragment size distribution of purified PCR products with electrophoresis-based equipment such as Bioanalyzer, Tapestation (Agilent Technologies), LabChip® GX, GXII, GX Touch (PerkinElmer), or Fragment Analyzer (Advanced Analytical). Figure 2 shows the final size distribution of purified PCR products obtained by the standard library construction protocol.

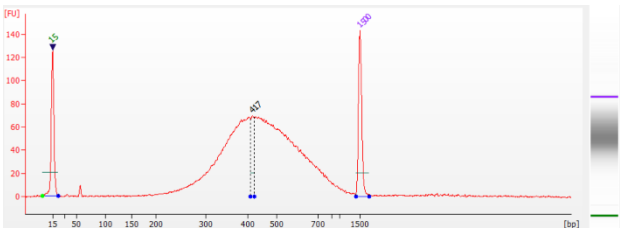

Figure 2 Agilent 2100 Bioanalyzer Fragment Size Distribution Results of the Purified PCR Product

3.9 Pre-Hybridization Preparation

- Before hybridization, take out the Block 3 and Block 4 from the MGIEasy Exome Capture Accessory Kit, and allow them to be thawed at room temperature or on ice for later use. Conduct the hybridization and capture process according to the user manual from the chosen probe kit. Block 3 and Block 4 are designed exclusively for the MGISEQ/DNBSEQ platform. Use Block 3 and Block 4 to replace reagents applicable for other platform's adaptor sequences.
- After hybridization and capture, take out the Post-PCR Enzyme Mix/PCR Primer Mix from the MGIEasy Exome Capture Accessory Kit, thaw them at room temperature and keep them on ice for later use. Conduct the Post-Capture PCR process according to Step 3.11.

⚠ **Note: if you are using MGI Exome V4 Probe or MGI Exome V5 Probe, then you need to use the corresponding regents from MGIEasy Exome Capture V4 probe Set or MGIEasy Exome Capture V5 probe Set and conduct the hybridization and capture process according to the user manual provided by the set.**

⚠ **Note: if you are using other commercial probes for hybridization, then you need to perform the hybridization and capture process according to their instruction and replace the reagents that designed for other platform's adaptor sequences with Block 3 and Block 4 from MGIEasy Exome Capture Accessory Kit. Recommended usages of Block 3 and Block 4 for different commercial probes are listed below:**

Table 15 Recommended usages of Block 3 and Block 4 for different commercial probes

Commercial probes	Block 3 usage (volume)	Block 4 usage (volume)	Reagents that need to be replaced in the kits
MGI Exome V4 Probe	1 μL	10 μL	None
MGI Exome V5 Probe	1 μL	10 μL	None
SureSelect series probes (SureSelect Human All Exon V6 etc.)	1 μL	10 μL	SureSelect Indexing Block #3
SeqCap® EZ Human Exome Probes v3.0	1 μL	10 μL	SeqCap HE Universal Oligo; SeqCap HE Index 2 Oligo; SeqCap HE Index 4 Oligo; SeqCap HE Index 6 Oligo; SeqCap HE Index 8 Oligo
xGen Exome Research Panel	1 μL	10 μL	xGen® Universal Blocking Oligo (1); xGen® Universal Blocking Oligo (2); xGen® Universal Blocking Oligo (3)

⚠ **Note: Recommended Post-Capture PCR cycles for different commercial probes are list below:**

Table 16 Post-Capture PCR cycles for different commercial kit

Commercial probe	PCR cycles
MGI Exome V4 Probe	12
MGI Exome V5 Probe	12
SeqCap EZ Human Exome Probes v3.0	12
xGen Exome Research Panel	6 (12 pool)-10 (1 pool)
SureSelect series probes (SureSelect Human All Exon V6 etc.)	12

The following steps 3.9-3.11 are standard experimental procedures using the NimbleGen® SeqCap EZ as an example.

3.9.1 According to the sample input required for hybridization, amplify the samples by PCR with recommended cycles in Table 12. Select the correct the adapters for constructing the library according to Appendix B. Then perform the hybridization with the required PCR product input according to requirement in SeqCap EZ Library SR User's Guide.

3.10 Hybridization and Capture

3.10.1 Following the Chapter 5 Step.3 in SeqCap EZ Library SR User's Guide, change SeqCap HE Universal Oligo and SeqCap HE Index 2/4/6/8 Oligo in Step 4 to Block 3 and Block 4 in the MGIEasy Exome Capture Accessory Kit. Refer to Table 15 for the Usage information of Block 3 and Block 4.

⚠ **Note: if the usage volume of Block 3 and Block 4 is larger than the volume of the reagents to be replaced in the commercial probe, it is required/strongly recommended to add these two reagents before sample concentration step (for example, in 'SeqCap EZ Library SR User's Guide', it requires that perform the concentration step to reduce the mixture volume after adding the Multiplex Hybridization Enhancing Oligo Pool to the sample.)**

3.10.2 Conduct the Hybridization capture and elution referring to SeqCap EZ Library SR User's Guide Chapter 5-6. any reagents that are not mentioned here should be used as required in the probe user manual.

⚠ **Note: After elution, the total volume of the sample solution (including beads) should be 44 μL in the next post capture PCR step. If the volume is less than 44 μL in other commercial probe after elution. You need to make the final sample volume up to 44 μL with NF water. If the volume is larger than 44 μL after elution, then you need to reduce the usage volume of the elution buffer.**

3.11 Post-Capture PCR

3.11.1 Take out the MGIEasy Exome Capture Accessory Kit and prepare the Post-capture PCR mixture on ice (see Table 17).

Table 17 Post- capture PCR Mixture

Components	Volume
Post-PCR Enzyme Mix	50 μL
PCR Primer Mix	6 μL
Total	56 μL

3.11.2 Transfer 56 μL of the Post-capture PCR mixture into each of the captured sample solutions (including beads) from the step 3.10.2 and centrifuge briefly to collect the solution at the bottom of the tube.

3.11.3 Place the PCR tube(s) from step 3.11.2 into the thermocycler and run the program in Table 18.

Table 18 Post-capture PCR Reaction Conditions

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

⚠ **Note: The number of Post-PCR cycles is recommended in Table 16, in this condition as an example, the 'X' should be 12.**

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

3.11.5 Place the tube(s) onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 100 µL supernatant from each tube to a new 1.5 mL Microcentrifuge tube.

3.12 Cleanup of Post-Capture PCR Product and Quantification

3.12.1 Take out DNA Clean Beads from the refrigerator and allow 30 minutes to bring the beads to room temperature. Vortex and mix thoroughly before use.

3.12.2 Transfer 100 µL DNA Clean Beads to each centrifuge tube from step 3.11.5. Pipette up and down at least 10 times to mix thoroughly. Ensure that the liquid and beads are fully dispensed from the pipette tip into the centrifuge tube before proceeding.

3.12.3 Incubate at room temperature for 5 minutes.

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

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

3.12.6 Repeat step 3.12.5 once, remove all liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom, separate the beads magnetically, and remove remaining liquid using a small volume pipette.

3.12.7 Keep the centrifuge tube(s) on the Magnetic Separation Rack with the lid open, and air dry the

beads at room temperature until no wetness (reflectiveness) is observed but before the pellet begins to crack.

3.12.8 Remove the centrifuge tube(s) from the Magnetic Separation Rack and add **32 μL** TE Buffer to each tube to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.12.9 Incubate at room temperature for 5 minutes.

3.12.10 Centrifuge briefly, then place the centrifuge tube(s) back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer **30 μL** supernatant from each tube to a different new 1.5 mL centrifuge tube.

3.12.11 Quantify the purified post-capture PCR products with dsDNA Fluorescence Assay Kits such as the Qubit® dsDNA HS Assay Kit or the Quant-iT PicoGreen® dsDNA Assay Kit. The required yield for PCR products is ≥ 1 pmol. Please refer to Formula 1 below to calculate the yield. For example, the desired yield for the fragmented DNA with a peak fragment size of 350 bp (Post-hybridization PCR products with a peak fragment size of 430 bp) should be ≥280 ng. For pooled sequencing, please follow instructions provided by MGIEasy DNA Adapters User Manual. Detailed information shows how to plan your sample pooling (see Appendix B). Quantify your Adapter-ligated samples before pooling. The total yield after pooling should be 1 pmol, with a total volume ≤ 48 μL.

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

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

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

⚠ **Note: If the library will be delivered to a service lab for sequencing, please stop here. If the library will be sequenced in your lab, please go to step 3.11 to 3.15.**

3.13 Denaturation

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

3.13.1 According to the PCR product size and Formula 1, transfer 1 pmol of each PCR Product to a new 0.2 mL PCR Tube. Add TE Buffer for a total volume of 48 μL.

3.13.2 Place the PCR tube(s) from step 3.13.1 into the thermocycler and run the program in Table 19.

Table 19 Denaturation Reaction Conditions

Temperature	Time
Heated lid	On
95°C	3 min

3.13.3 After the reaction is complete, immediately place the PCR tube(s) on ice for 2 minutes, and centrifuge briefly.

3.14 Single Strand Circularization

3.14.1 Take out the MGIEasy Circularization Kit and prepare the single strand circularization reaction mixture in a new 0.2 mL PCR tube on ice (see Table 20).

Table 20 Single Strand Circularization Mixture

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

3.14.2 Transfer 12.1 μL of single strand circularization mixture into each PCR tube from step 3.13.3. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube(s).

3.14.3 Place the PCR tube(s) from step 3.14.2 into the thermocycler and run the program in Table 21

Table 21 Single Strand DNA Circularization Reaction Conditions

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

3.14.4 After the reaction is complete, immediately place the tube(s) on ice for the next reaction.

3.15 Enzymatic Digestion

3.15.1 Prepare the following enzymatic digestion mixture (see Table 22) in a new 0.2 mL PCR tube on ice during the reaction in step 3.14.3.

Table 22 Enzymatic Digestion Mixture

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

3.15.2 Transfer 4 µL of enzymatic digestion mixture into each PCR tube from step 3.14.4. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube(s).

3.15.3 Place the PCR tube(s) from step 3.15.2 into the thermocycler and run the program in Table 23.

Table 23 Enzymatic Digestion Reaction Conditions

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

3.15.4 After the reaction is complete, centrifuge briefly to collect the solution at the bottom of the tube(s).

3.15.5 Immediately add 7.5 µL of Digestion Stop Buffer to each PCR tube. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube(s). Transfer all of the solution from each tube into a separate new 1.5 mL centrifuge tube.

3.16 Cleanup of Enzymatic Digestion Product

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

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

3.16.2 Transfer 170 µL of DNA Clean Beads to each tube containing Enzymatic Digestion product from step 3.15.5. Gently pipette at least 10 times to mix thoroughly. Ensure that the solution and beads are fully dispensed from the tip into the tube.

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

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

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

3.16.6 Repeat step 3.16.5 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.

3.16.7 Keep the 1.5 mL centrifuge tube(s) on the Magnetic Separation Rack with the lid open, and air dry beads until no wetness (reflectiveness) is observed but before the pellet begins to crack.

3.16.8 Remove the 1.5 mL centrifuge tube(s) from the Magnetic Separation Rack and add **22 µL** of TE Buffer each tube to elute the DNA. Gently pipette up and down at least 10 times to mix thoroughly.

3.16.9 Incubate at room temperature for 10 minutes.

3.16.10 Centrifuge briefly. Place the 1.5 mL centrifuge tube(s) back on the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer **20 µL** supernatant from each tube to a separate new 1.5 mL centrifuge tube. Be careful to not disturb the beads.

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

3.17 Quality Control of Enzymatic Digestion Product

Quantify the purified Enzymatic Digestion products with Qubit® ssDNA Assay Kit. The final Enzymatic Digestion products (ssDNA, ng)/ input products of PCR (dsDNA, ng) should be ≥ 7%.

For example, if the input of PCR product used for the circularization with the band peak around 430 bp in Bioanalyzer (corresponding to the insert fragment peak around 350 bp) is 280 ng, the final yield after enzymatic digestion should be more than 19.5 ng.

Appendix

Appendix A Magnetic Beads and Cleanup Procedures

For bead-based purification, we recommend using DNA Clean Beads included in the MGIEasy DNA Clean Beads (MGI, Cat. No.1000005278 or 1000005279) or AMPure® XP (Agencourt, Cat. No. A63882). If you choose Magnetic Beads from other sources, please optimize the cleanup conditions before getting started.

Before You Use

- To ensure capture efficiency of the Magnetic Beads, remove beads from 4°C refrigerator storage, and equilibrate to room temperature for 30 minutes 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 TE buffer to reach the designated volume before using the beads to purify. 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.
- 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 the pipette tip, expel all of the solution and beads back into the tube and restart the separation process.
- Use freshly prepared 80% ethanol (at room temperature) to wash the beads twice. Keep the centrifuge tube on the Magnetic Separation Rack when washing. 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 in the tube. You may centrifuge briefly to collect any remaining liquid at the bottom, separate beads magnetically, and remove the remaining liquid using a small volume pipette.
- After washing twice with ethanol, air-dry the beads at room temperature. Drying takes approximately 2-5 minutes depending on your specific lab environment. Watch closely until the pellet appears

sufficiently dry with a matte appearance, then continue to the elution step with TE Buffer.

- During the elution step, do not touch the beads with the pipette tips when removing the supernatant. Contamination of DNA by the beads may affect subsequent purification. Therefore, the total volume of TE buffer and the beads should be 2 μL more than the volume of the supernatant.
- Pay attention when opening/closing the lids of centrifuge tubes on the Separation Rack. Strong vibrations may cause sample loss by spilling liquid or beads from the tubes. Secure the tubes before opening the lids.

Appendix B The Combination Barcode Adapters Strategies

- This set includes a MGIEasy DNA Adapters-16 (Tube) Kit. This kit was developed to meet requirements for batch processing of library construction and multiplex sequencing. We selected the best adapter combination based on the principle of balanced base composition. However, the number of Barcode Adapters are not always continuous. For optimal performance, please carefully read instructions in Appendix B-1.
- Our Adapters are double stranded. Please do not incubate above room temperature to avoid structural changes such as denaturation, which might affect performance.
- Before use, please centrifuge to collect liquid at the bottom of tubes. Gently remove the cap to prevent spills and cross-contamination. Mix Adapters with a pipette before you use. Remember to close the cap immediately after use.
- Adapters from other MGI Library Prep Kits (number 501-596) are designed differently and are incompatible for mixed use. Mixed use will cause errors in barcode demultiplexing in data analysis procedures.

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

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

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

1 set of 8 Adapters: (97-104)

If the sequencing data output requirement is the same for all samples in one lane, please refer to Table 24 below to choose your barcode adapter combinations.

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

Sample(s)/lane	Instructions (Example)
1	Requires at least 1 set of Adapters: 1. Take a set of 4 Adapters (e.g. 01-04), mix 4 Adapters with equal volumes, then add the mixture to the sample. Or 2. Take a set of 8 Adapters (e.g. 97-104), mix 8 Adapters with equal volumes, then add the mixture to the sample.

2	Requires at least 1 set of Adapters: 1. Take a set of 4 Adapters (e.g. 01-04), mix Adapters with equal volumes in pairs to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 01 & 02, then add to sample 1; Mix 03 & 04, then add to sample 2) Or 2. Take a set of 8 Adapters (97-104), mix Adapters with equal volumes in groups of 4 to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 97-100, then add to sample 1; Mix 101-104, then add to sample 2)
3	Requires at least 2 sets of Adapters: For sample 1&2, use the method for (2 samples/lane) above. For sample 3, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for samples 1-2 and for sample 3.
4	Requires at least 1 set of Adapters: 1. Take a set of 4 Adapters (e.g. 01-04), add 1 Adapter to each sample in an equal volume. (e.g. Add Adapters 01, 02, 03, 04 to samples 1, 2, 3, 4, respectively.) Or 2. Take a set of 8 Adapters (97-104), mix Adapters with an equal volume in pairs to obtain 4 mixtures of equal volumes. Add 1 mixture to each sample. (e.g. Mix 97-98, 99-100, 101-102, 103-104, then add respectively to samples 1, 2, 3, 4.)
5	Requires at least 2 sets of Adapters: For samples 1-4, use the method for (4 samples/lane) above. For sample 5, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for samples 1-4 and for sample 5.
6	Requires at least 2 sets of Adapters: For samples 1-4, use the method for (4 samples/lane) above. For sample 5-6, use the method for (2 sample/lane) above. Note that you should use different Adapter sets for samples 1-4 and for samples 5-6.
7	Requires all 3 Adapter sets and follow these 3 steps: 1) For samples 1-4, use the method for (4 samples/lane) above (Use 1st Adapter set). 2) For samples 5-6, use the method for (2 samples/lane) above (Use 2nd Adapter set). 3) For sample 7, use the method for (1 sample/lane) above (Use 3rd Adapter set). You can add a single Adapter within the Adapter set. Or add the Adapter mix which is mixed from all Adapters within the Adapter set with an equal volume. Note that you should use different Adapter sets for samples 1-4, for samples 5-6 and for sample 7.

8	Requires at least 1 set of Adapters: 1. Take a set of 8 Adapters (97-104), respectively add 1 Adapter to each sample in an equal volume. Or 2. Take 2 sets of 4 Adapters (01-04 and 13-16), add 1 Adapter to each sample in an equal volume.

For situations in which the 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)).

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