Part No.:SOP-013-B02-127

User Manual

Version:6.0

MGIEasy PCR-Free DNA Library Prep Set

Cat. No.: 1000013452 (16 RXN)
1000013453 (96 RXN)
Set Version: V1.1

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About the user manual

Manufacturer information

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Revision history

Manual version	Kit version	Date	Description
6.0	V1.1	Apr. 2024	Update Cat. No. of MGIEasy DNA Clean Beads in section 1.4
5.0	V1.1	Mar. 2024	• Update the manufacturer information • Update the manual style • Delete appendix Magnetic beads and cleanup and About adapter ligation
4.0	V1.1	Mar. 2022	Update Manufacturer LOGO
A2	V1.1	Jan. 2021	Update contact information
A1	V1.1	Dec. 2019	• Update the kit version to V1.1 • Update the manual to new style • 1.3 Add DNBSEQTM series sequencing platform • Reduce the minimum gDNA input to 200ng • Add the beads ratio for single beads purification • Add amplicons into input DNA type • Change the QC criteria at ligation product quantitative step
A0	V1.0	Apr. 2019	Initial release

Tips Please download the latest version of the manual and use it with the corresponding kit. Search for the manual by Cat. No. or product name from the following website:

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

Contents

1 Product description

1.1 Introduction

The MGIEasy PCR-Free Library Prep Set is specifically designed for making WGS libraries for MGI High-throughput Sequencing Platforms. This library prep set is optimized to convert 80-200 ng size selected fragment DNA into a customized library. This set incorporates improved Adapter Ligation technology, which significantly increases library yield and conversion rate. All reagents provided in this set have passed strict quality control and functional verification procedures, ensuring stability and reproducibility.

1.2 Intended use

This library prep set is applicable to samples from human (including but not limited to blood, saliva, and fresh tissue), animals (including but not limited to rat), plants (including but not limited to rice), bacteria (including but not limited to *E. coli*), fungi, microbiomes, amplicons, and other microbial species. Stable performance across all such sample types is expected.

1.3 Applicable sequencing platforms

The prepared libraries are applicable to the following sequencing platforms.

- BGISEQ-500RS (PE100)
- MGISEQ-200RS (PE100), DNBSEQ-G50 RS(PE100)
- MGISEQ-2000RS (PE100/PE150/PE200/SE400), DNBSEQ-G400RS (PE100/PE150/PE200/SE400)
- DNBSEQ-T7RS (PE100)

1.4 Components

This library prep set comes in two specifications: 16 RXN and 96 RXN. Three separate boxes are included for each specification. For component details, refer to the following table.

Each library prep set contains an information card. Relevant manuals and SDS files can be downloaded from the MGI website provided on the information card.

Table 1 MGIEasy PCR-Free DNA Library Prep Set V1.1 (16 RXN) (Cat. No.: 1000013452)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy PCR-Free DNA Library Prep Kit V1.1 Cat. No.: 1000013456	20 × Elute Enhancer	Black	3 μL/ tube × 1
	ER Buffer	Orange	112 μL/ tube × 1
	ER Enzyme Mix	Orange	48 μL/ tube × 1
	Ad-Lig Buffer	Red	288 μL/ tube × 1
	Ad Ligase	Red	80 μL/ tube × 1
	Ligation Enhancer	Brown	32 μL/ tube × 1
	Cir Buffer	Purple	184 μL/ tube × 1
	Cir Enzyme Mix	Purple	8 μL/ tube × 1
	Exo Buffer	White	23 μL/ tube× 1
	Exo Enzyme Mix	White	42 μL/ tube × 1
	Exo Stop Buffer	White	48 μL/ tube × 1
MGIEasy PF Adapters-16 (Tube) Kit Cat. No.: 1000013460	DNA Adapters	Colorless	5 μL/ tube × 16
MGIEasy DNA Clean Beads Cat. No.: 940-001596-00	DNA Clean Beads	White	8 mL/ tube × 1
	TE Buffer	White	4 mL/ tube × 1

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

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy PCR-Free DNA Library Prep Kit V1.1 Cat. No.: 1000013457	20 × Elute Enhancer	Black	15 μL/ tube × 1
	ER Buffer	Orange	672 μL/ tube × 1
	ER Enzyme Mix	Orange	288 μL/ tube × 1
	Ad-Lig Buffer	Red	864 μL/ tube × 1
	Ad Ligase	Red	480 μL/ tube × 1
	Ligation Enhancer	Brown	192 μL/ tube × 1
	Cir Buffer	Purple	1104 μL/ tube × 1
	Cir Enzyme Mix	Purple	48 μL/ tube × 1
	Exo Buffer	White	135 μL/ tube × 1
	Exo Enzyme Mix	White	250 μL/ tube × 1
	Exo Stop Buffer	White	288 μL/ tube × 1
MGIEasy PF Adapters-96 (Plate) Kit Cat. No.: 1000013461	DNA Adapters-96 plate	-	5 μL/ well × 96
MGIEasy DNA Clean Beads Cat. No.: 940-001594-00	DNA Clean Beads	White	50 mL/ tube × 1
	TE Buffer	White	25 mL/ tube × 1

1.5 Storage and transportation

Table 3 Kit storage and transportation temperatures

Item		Storage temperature	Transportation temperature
MGIEasy PCR-Free DNA Library Prep Kit	20 × Elute Enhancer	2 ℃ to 30 ℃	-80 ℃ to -15 ℃
	Exo Stop Buffer		
	Ligation Enhancer	2 ℃ to 30 ℃ Store away from light	

Item		Storage temperature	Transportation temperature
	Other reagents	-25 ℃ to -15 ℃	
MGIEasy PF Adapters-16 (Tube) Kit			
MGIEasy PF Adapters-96 (Plate) Kit			
MGIEasy DNA Clean Beads		2 ℃ to 8 ℃	2 ℃ to 8 ℃

Tips
- Production date and expiration date: refer to the label.
- For ice packs or dry ice shipments, ensure that there is enough ice or dry ice remaining after transportation.
- With proper transport, storage, and use, all components can maintain complete activity within their shelf life.
- In MGIEasy PCR-Free DNA Library Prep Kit, after the first use of 20× Elute Enhancer, Exo Stop Buffer, and Ligation Enhancer, store them at room temperature. Avoid repeated freeze-thawing. The Ligation Enhancer should be stored away from light.

1.6 User-supplied materials

Table 4 User-supplied equipment list

Equipment	Recommended brand
Covaris Focused ultrasonicator	ThermoFisher Scientific or equivalent
Vortex mixer	/
Desktop centrifuge	/
Pipettes	/
Thermocycler	/
Magnetic rack DynaMag -2 or equivalent	Thermo Fisher Scientific, Cat. No. 12321D
Qubit Fluorometer or equivalent	Thermo Fisher, Cat. No. Q33216
Agilent 2100 Bioanalyzer or equivalent	Agilent Technologies , Cat. No. G2939AA

Table 5 Recommended reagent/consumable list

Reagent/consumable	Recommended brand
Nuclease free (NF) water	Ambion, Cat. No. AM9937 or equivalent
TE Buffer, pH 8.0	Ambion, Cat. No. AM9858 or equivalent
100% Ethanol (Analytical Grade)	/
Qubit ssDNA Assay Kit	Invitrogen, Cat. No. Q10212, or equivalent
Qubit dsDNA HS Assay Kit	Invitrogen, Cat. No. Q32854) or equivalent

Reagent/consumable	Recommended brand
Agilent High Sensitivity DNA Kit	Agilent, Cat. No. 5067-4626, or equivalent
Agilent DNA 1000 Kit	Agilent, Cat. No. 5067-1504, or equivalent
Covaris AFA Tubes for use with Ultrasonicator	ThermoFisher Scientific
Pipette tips	/
1.5 mL tube	/
0.2 mL PCR tube or 96-well plate	/
Qubit Assay Tubes or 0.5mL Thin Wall PCR Tubes	Invitrogen or Axygen or equivalent

1.7 Precautions and warnings

- This product is for research use only, not for in vitro diagnosis. Please read this manual carefully before use.
- To prevent yield loss, try to avoid transferring the reaction product to a new tube for beads purification especially in Exo Digestion Product purification step. Adding the En-Beads directly to the reaction tube for product purification is recommended.
- Familiarize yourself with the precautions and operation methods of various instruments before performing the experiment.
- This manual aims to provide a standard protocol. Changes can be made for different applications, but changes must be tested prior to starting the protocol.
- It is recommended that you use pipette tips with filters to prevent cross-contamination. Use a new tip each time for pipetting different solutions or samples.
- It is recommended that you use the thermocyclers with heated lids for reactions. Preheat the thermocyclers to reaction temperature before use. If the thermocycler does not allow for lid temperature adjustments, the preset lid temperature of 105 ℃ is sufficient.
- Aerosol contamination may cause inaccurate results. It is recommended that you prepare separate working areas in the laboratory for PCR reaction preparation, and PCR reaction and PCR product cleanup. Use designated equipment for each area and clean the area regularly to ensure a sterile working environment (use 0.5% Sodium Hypochlorite or 10% Bleach to clean the working area).
- Avoid skin and eyes contact with samples and reagents. Do not eat or drink the samples and reagents. In case of contact with skin and eyes, rinse immediately with plenty of water and seek medical advice.
- Conform to the law and regulations when disposing of all samples and reagents.
- If you have questions, contact the MGI technical support: **MGI-service@mgi-tech.com**

1.8 Workflow

Section	Workflow	Total time	Hands-on time
3.1	Reagent prep	10 min	10 min
3.2	End repair	60 - 70 min	5- 10 min
3.3	Adapter ligation	40 min	10 min
3.4	Cleanup of adapter-ligated product ⏸	35 - 40 min	10 - 15 min
3.5	QC of ligation product ⏸	15 - 60 min	10 - 20 min
4.1	Denaturation and single strand circularization	45 min	15 min
4.2	Exo digestion	40 min	10 min
4.3	Cleanup of exo digestion product ⏸	40 min	10 - 15 min
4.4	QC of digestion product ⏸	15 - 20 min	10 - 15 min

Tips

- Total time: The theoretical use time of 8 reactions. The time will be extended if the number of reactions increases.
- Hands-on time: The total required time for manual operation in the process.
- ⏸ : The stop point.

2 Sample preparation

2.1 Sample requirements

This library prep set is applicable to samples from human (including but not limited to blood, saliva, and fresh tissue), animals (including but not limited to rat), plants (including but not limited to rice), bacteria (including but not limited to E. coli), fungi, microbiomes, amplicons, and other microbial species.

It is strongly recommended that you use 1000 ng of high-quality genomic DNA ($OD_{260/280}$ = 1.8 - 2.0, $OD_{A260/230}$ > 2.0) for fragmentation.

2.2 DNA fragmentation and size selection

2.2.1 Fragmentation

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

2.2.2 Magnetic beads size selection

- DNA fragmentation results in a wide distribution of fragment sizes. Size selection is usually required to ensure uniformity of the library.
- If the amount of gDNA is sufficient, we recommend using 500-1000 ng for fragmentation and follow double size selection process as table below to select different main fragment size.

Table 6 Double size selection process: 75 μL sample of the theoretical majority of DNA fragments using magnetic beads selection

Target peak fragment size (bp)	350	400	560
1st beads selection (μL)	50	45	41.25
2nd beads selection (μL)	15	15	15
Sequencing strategy	PE100/PE150	PE100/PE150	PE200/SE400

Tips The selection conditions of table above are used for reference. For different samples, the target peak fragment size may have a ± 50 bp deviation.

- If the sample is rare and the gDNA amount is less than 500 ng, we recommend attempting to use 200-500 ng for fragmentation and follow single size selection process to select different main fragment size.

Table 7 Single size selection process: 75 μL sample of the theoretical majority of DNA fragments using magnetic beads selection

Target peak fragment size (bp)	400	560
1st beads selection (μL)	60	52.5
Sequencing strategy	PE100/PE150	PE200/SE400

Tips
- The selection conditions of table above are used for reference. For different samples, the target peak fragment size may have a ± 50 bp deviation.
- The final ssCir yield can be sequencing once if ues 200ng gDNA for fragmentation. As the insert size range of single size selection process is broader than double size selection process, the sequencing quality and effective sequencing reads will decrease slightly.

- **Example 1**: For a peak size of 400 bp: Fragment 1000 ng gDNA (80 μL). If the DNA volume after fragmentation is less than 75 μL, add En-TE buffer to reach a final volume of 75 μL. Then, perform a double size selection process with a 45 μL 1st beads selection followed by a 15 μL 2nd beads selection before End Repair, which provides the selected fragment size of 400 bp.
- **Example 2**: For a peak size of 400 bp: Fragment 200 ng gDNA (80 μL). If the DNA volume after fragmentation is less than 75 μL, add En-TE buffer to reach a final volume of 75 μL. Then, perform a bead purification process with a 60 μL beads selection before End Repair, which provides the selected fragment size of 400 bp.

Tips For detailed steps, refer to "Magnetic beads size selection" on page 21.

2.3 Size selected DNA quantification and quality control

- Size selected DNA amount refers to the amount of DNA input that is used for the End Repair process. This set is compatible with size selected DNA amounts between 80 - 200 ng in a volume of 40 μL or less. If the size selected DNA amount is 60 - 80 ng, library preparation can be attempted, but with a greater risk of failure. Do not proceed with End Repair if the amount of DNA is below 60 ng.
- Try to ensure a narrow distribution of DNA fragment sizes. A narrow distribution results in a higher quality of sequencing. A wide distribution lowers sequencing quality.

- This library prep set supports a range of fragment sizes (see Table 6 and 7). Sequencing quality may slightly decrease with increasing fragment sizes. Please use the appropriate insert size for library construction based on your planned sequencing strategies. A peak between 350 - 450 bp is recommended for PE100/PE150 sequencing, and the distribution around the peak should be near ± 200 bp.
- Any residual impurities (for example, metal chelators or other salts) in selected DNA fragments may adversely affect the efficiency of the End Repair process.

3 Library preparation protocol

3.1 Reagent prep

3.1.1 Preparation

Table 8 Preparing the reagents

Reagent	Requirement
Nuclease-Free Water	User-supplied; place at room temperature (RT), mix well.
TE Buffer	
20 × Elute Enhancer	Place at RT, mix thoroughly.
DNA Clean Beads	

3.1.2 Operation

CAUTION The preparation volume of reagents listed blow is enough for 6 samples. Increase the preparation reagent volumes in proportion if there are more samples.

1. Prepare the 1x Elute Enhancer in a 1.5 mL sterile centrifuge tube. Mix the tube by vortexing and centrifuge briefly. Store at room temperature before using. The shelf life of the 1x Elute Enhancer is 7 days.

Table 9 1x Elute Enhancer

Reagent	Volume per reaction
20 × Elute Enhancer	1 μL
Nuclease-Free Water	19 μL
Total	20 μL

2. Prepare the En-TE in a 1.5 mL sterile centrifuge tube. Mix the tube by vortexing. Store at 4 ℃ before using. The shelf life of the En-TE is 7 days.

Table 10 En-TE

Reagent	Volume per reaction
1 × Elute Enhancer	2 μL
TE Buffer	998 μL
Total	1000 μL

3. Prepare the En-Beads in a 1.5 mL sterile centrifuge tube. Mix the tube by vortexing. Store at 4 ℃ before using. The shelf life of the En-Beads is 7 days.

Table 11 En-Beads

Reagent	Volume per reaction
1 × Elute Enhancer	15 μL
DNA Clean Beads	1485 μL
Total	1500 μL

3.2 End repair

Tips Preheat the thermocycler to reaction temperature in advance if the thermocycler heat up slowly.

3.2.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 12 Preparing the reagents

Reagent	Requirement
En-TE	Refer to 3.1 Reagent prep, place at RT.
ER Buffer	Thaw at RT, mix well, centrifuge briefly, and place on ice.
ER Enzyme Mix	Centrifuge briefly and place on ice.

3.2.2 End repair

1. Add an appropriate amount of sample (recommended 80-200 ng) into a new 0.2 mL PCR tube. Add En-TE to make a total volume of 40 μL.
2. According to the desired reaction number, prepare the end repair mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing, centrifuge briefly, and place on ice.

Table 13 End repair mixture

Reagent	Volume per reaction
ER Buffer	7 μL
ER Enzyme Mix	3 μL
Total	10 μL

3. Add 10 μL of end repair mixture to each sample tube. Vortex 3 times (3 sec each), centrifuge briefly, and place on ice.
4. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 14 End repair reaction conditions (Volume: 50 μL)

Temperature	Time
70 ℃ Heated lid	On
14 ℃	15 min
37 ℃	25 min
65 ℃	15 min
4 ℃	Hold

5. When the program is completed, centrifuge the PCR tube(s) briefly to collect the liquid to the bottom of the tube.

WARNING
- Do not stop at this step. Please proceed to next reaction.
- If the operation stops here, store the end repair product(s) at –20 ℃ overnight with a risk of 20% decrease in yield.

3.3 Adapter ligation

Tips Before operation, carefully read “Using adapters” on page 24.

3.3.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 15 Preparing the reagents

Reagent	Requirement
Ligation Enhancer	mix well, centrifuge briefly, and place at RT.
Ad-Lig Buffer	Thaw at RT, mix well, centrifuge briefly, and place on ice.
Ad Ligase	Centrifuge briefly and place on ice.
Adapters	Mix thoroughly, centrifuge briefly, and place on ice.

Reagent	Requirement
En-TE	Refer to 3.1 Reagent prep, place at RT.

Tips
- Mix the adapter(s) well before use. Adapters should not be mixed directly with the adapter ligation mixture.
- The Ad-Lig Buffer is highly viscous. Mix it by vortexing 6 times (3 sec each) and centrifuge briefly. When pipetting the Ad-Lig Buffer, slowly aspirate to ensure that the volume is accurate.

3.3.2 Adapter ligation

1. Add **5 μL of adapter(s)** to the corresponding sample tube (from step 5 in section 3.2.2). Vortex the tube(s) 3 times (3 sec each), centrifuge briefly, and place on ice.
2. According to the desired reaction number, prepare the adapter ligation mixture in a 0.2 mL PCR tube on ice. Vortex it 6 times (3 sec each), centrifuge briefly, and place on ice.

Table 16 Adapter ligation mixture

Reagent	Volume per reaction
Ad-Lig Buffer	18 μL
Ad Ligase	5 μL
Ligation Enhancer	2 μL
Total	25 μL

3. Slowly pipette 25 μL of adapter ligation mixture to each sample tube and vortex 6 times (3 sec each). Centrifuge briefly to collect the liquid to the bottom of the tube and place on ice.

Tips The adapter ligation mixture is highly viscous. Slowly aspirate to ensure the volume is accurate.

4. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 17 Adapter ligation reaction conditions (Volume: 80 μL)

Temperature	Time
30 ℃ Heated lid	On
25 ℃	30 min
4 ℃	Hold

5. When the program is completed, centrifuge the PCR tube(s) briefly and place on ice.
6. Add 20 μL of En-TE to make a total volume of 100 μL. Mix it well by vortexing and centrifuge briefly.

CAUTION
- The yield may loss if transfer the liquid into 1.5 mL tube to cleanup. It is not recommended to transfer the liquid.

- Do not stop at this step. Please proceed to next reaction.

3.4 Cleanup of adapter-ligated product

Tips
- Do not disturb or pipette the beads when adding reagents or transferring supernatant. If you accidentally disturb or pipette the beads, pipette the solution and beads back into the tube and restart the separation process.
- 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. It is recommended that you use 50 µL of En-Beads for the cleanup.

3.4.1 Preparation

Table 18 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
En-TE	Refer to section 3.1. Place at RT.
En-Beads	Refer to section 3.1. Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

3.4.2 Cleanup of adapter-ligated product

1. Mix the En-Beads thoroughly. Add 50 µL of En-Beads to each sample tube (from step 6 in section 3.3.2). Gently pipette at least 10 times until all beads are suspended. Ensure that all of the solution and beads in the tip are transferred into the tube after mixing. Or, mix with a vortexer.
2. Incubate the sample(s) at room temperature for 10 min.
3. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant.
4. While keeping the PCR tube(s) on the magnetic rack, add 160 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
5. Repeat step 4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.

6. Keep the centrifuge tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

   Tips Over-drying the beads will result in reduced yield.

7. Remove the tube(s) from the magnetic rack and add 50 μL of En-TE to elute the DNA. Gently pipette the liquid at least 10 times until all beads are suspended. Or, mix with a vortexer.
8. Incubate the sample(s) at room temperature for 5 min.
9. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 49 μL of supernatant to a new 0.2 mL PCR tube.

   Stop point After cleanup, the adapter-ligated product(s) can be stored at -20 ℃.

3.5 QC of ligation product

Take 1 μL of supernatant to quantify the concentration with Qubit dsDNA HS Assay Kit or Quant-iT PicoGreen dsDNA Assay Kit.

- If the concentration is >1.2 ng/μL, it is a qualified ligation product.
- If the concentration is between 0.8-1.2 ng/μL, library preparation can still be attempted, but with a greater risk of failure.
- If the concentration is <0.8 ng/μL, do not continue with this sample.

CAUTION Do not mix the samples for multiple samples sequencing after cleanup of adapter-ligated product. The residual adapter dimer may cause the sample cross-contamination.

4 Circularization and digestion

4.1 Denaturation and single strand circularization

4.1.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 19 Preparing the reagents

Reagent	Requirement
Cir Buffer	Thaw at RT, mix well, centrifuge briefly, and place on ice.
Cir Enzyme Mix	Centrifuge briefly and place on ice.

4.1.2 Denaturation

1. Place the PCR tube(s) (from step 9 in section 3.4.2) into the thermocycler and run the program with the following conditions.

Table 20 Denaturation reaction conditions (Volume: 48 μL)

Temperature	Time
100 ℃ Heated lid	On
95 ℃	3 min
4 ℃	10 min

2. When the program is completed, centrifuge the PCR tube(s) briefly and place on ice.

4.1.3 Single strand circularization

1. According to the desired reaction number, prepare the circularization mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 21 Circularization mixture

Reagent	Volume per reaction
Cir Buffer	11.5 μL
Cir Enzyme Mix	0.5 μL
Total	12 μL

2. Add 12 μL of circularization mixture to each sample tube (from step 2 in section 4.1.2). Vortex the tube(s) 3 times (3 sec each), centrifuge briefly, and place on ice.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 22 Circularization reaction conditions (Volume: 60 μL)

Temperature	Time
42 ℃ Heated lid	On
37 ℃	30 min
4 ℃	Hold

4. When the program is completed, place the PCR tube(s) on ice, centrifuge briefly, and immediately proceed to the next step.

4.2 Exo digestion

4.2.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 23 Preparing the reagents

Reagent	Requirement
Exo Buffer	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.
Exo Enzyme Mix	Mix by flicking with a finger, centrifuge briefly, and place on ice.
Exo Stop Buffer	Thaw at RT, mix by vortexing, centrifuge briefly, and place at RT.

4.2.2 Exo digestion

1. According to the desired reaction number, prepare the exo digestion mixture in a 0.2 mL PCR tube on ice. Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 24 Exo digestion mixture

Reagent	Volume per reaction
Exo Buffer	1.4 μL
Exo Enzyme Mix	2.6 μL
Total	4.0 μL

2. Add 4 μL of exo digestion mixture to each sample tube (from step 4 in section 4.1.3). Vortex the tube(s) 3 times (3 sec each), centrifuge briefly, and place on ice.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 25 Exo digestion reaction conditions (Volume: 64 μL)

Temperature	Time
42 ℃ Heated lid	On
37 ℃	30 min
4 ℃	Hold

4. When the program is completed, centrifuge the tube(s) briefly. Immediately add **3 μL of Exo Stop Buffer** to each sample tube. Vortex the tube(s) 3 times (3 sec each) and centrifuge briefly.

CAUTION The yield may loss if transfer the liquid into 1.5 mL tube to cleanup. It is not recommended to transfer the liquid.

4.3 Cleanup of exo digestion product

Tips Do not disturb or pipette the beads when adding reagents or transferring supernatant. If you accidentally disturb or pipette the beads, pipette the solution and beads back into the tube and restart the separation process.

4.3.1 Preparation

Table 26 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
En-TE	Refer to section 3.1. Place at RT.

Reagent	Requirement
En-Beads	Refer to section 3.1. Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

4.3.2 Cleanup of exo digestion product

1. Mix the En-Beads thoroughly. Add 120 µL of En-Beads to each sample tube (from step 4 in section 4.2.2). Gently pipette at least 10 times until all beads are suspended. Ensure that all of the solution and beads in the tip are transferred into the tube after mixing. Or, mix with a vortexer.
2. Incubate the sample(s) at room temperature for 10 min.
3. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant.
4. While keeping the PCR tube(s) on the magnetic rack, add 160 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
5. Repeat step 4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
6. Keep the centrifuge tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

   Tips Over-drying the beads will result in reduced yield.

7. Remove the tube(s) from the magnetic rack and add 25 µL of En-TE to elute the DNA. Gently pipette the liquid at least 10 times until all beads are suspended. Or, mix with a vortexer.
8. Incubate the sample(s) at room temperature for 10 min.
9. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 24 µL of supernatant to a new 0.2 mL PCR tube.

   Stop point After cleanup, the exo digestion product(s) can be stored at -20 ℃.

4.4 QC of digestion product

Quantify the purified exo digestion product by following the instructions of the Qubit ssDNA Assay Kit.

- The yield of exo digestion product should be not less than 75 fmol.

Refer to the formula below to calculate the mass of 75 fmol ssCir.

Formula 1 Conversion between circular ssDNA fmol and mass in ng

$$75\ \text{fmol ssDNA (ng)} = 0.075 \times [\text{DNA fragment peak size (bp)} + 84\ \text{(bp)}] \times 0.33$$

Table 27 Corresponding Mass yield in 75 fmol circularized ssDNA for different selected fragment size

Target peak fragment size (bp)	Circularized ssDNA size (bp)	Corresponding mass yield in 75 fmol (ng)
350	434	10.7
400	484	12.0
560	644	16.0

Sequencing requires the single strand circle input is 75 fmol/lane.

For multiple samples sequencing in one lane, you can pool the single strand circles of different samples by a particular mole ratio at this step. The barcodes used in the pooled samples should strictly adhere to the instructions for MGIEasy PF Adapters (see "Using adapters" on page 24). The mole ratio is based on your required sequencing data of each sample being pooled.

CAUTION The insert size and the size range will affect sequencing quality and effective sequencing reads, ooling samples with different peak fragment sizes or size ranges (for example, pooling single size selection and double size selection samples) may significantly impact sequencing quality.

5 Appendix

5.1 Magnetic beads size selection

5.1.1 Double size selection

Tips
- Do not disturb or pipette the beads when adding reagents or transferring supernatant. If you accidentally disturb or pipette the beads, pipette the solution and beads back into the tube and restart the separation process.
- Using a 45 μL Beads selection step followed by a 15 μL Beads selection step to target a peak size of 400 bp from 75 μL of fragmentated gDNA.
- To select a different fragment size, refer to Table 6 in Chapter 2 for detailed conditions.
- The DNA sample may loss about 60%-90% in this process. As such, it is recommended to recycle the DNA which adsorb on the 1st beads. Process the step 8 to 13 to recycle the DNA and store the eluted DNA as a backup.

5.1.1.1 Preparation

Table 28 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied. freshly prepared.
En-TE	Refer to section 3.1. Place at RT.
En-Beads	Refer to section 3.1. Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

5.1.1.2 Double size selection

1. Add 75 μL fragmentation gDNA into a new 0.2 mL PCR tube. Add En-TE to make a total volume of 75 μL if the fragmentation gDNA volume is not enough.

2. Mix the En-Beads thoroughly. Add 45 μL of En-Beads to each sample tube and gently pipette at least 10 times until all beads are suspended. Ensure all of the solution and beads in the tip are transferred into the tube after mixing. Or, mix with a vortexer.
3. Incubate the sample(s) at room temperature for 10 min.

   Tips In the next step, keep the supernatant and discard the beads. If necessary, recycle the DNA on that beads.

4. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 118 μL of supernatant to a new 0.2 mL PCR tube.
5. Add 15 μL of En-Beads to each sample tube, and gently pipette at least 10 times until all beads are suspended. Or, mix with a vortexer.
6. Incubate the sample(s) at room temperature for 10 min.
7. Centrifuge the sample tube(s) briefly and place on the magnetic rack for more than 5 min until the liquid is clear. Carefully remove and discard the supernatant.
8. While keeping the PCR tube(s) on the magnetic rack, add 160 μL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
9. Repeat step 8. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
10. Keep the centrifuge tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

    Tips Over-drying the beads will result in reduced yield.

11. Remove the PCR tube(s) from the magnetic rack and add 45 μL of En-TE to elute the DNA. Gently pipette the liquid at least 10 times until all beads are suspended. Or, mix with a vortexer.
12. Incubate the sample(s) at room temperature for 5 min.
13. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 43 μL of supernatant to a new 0.2 mL PCR tube.
14. Take 1- 2 μL of supernatant for size distribution detection by Bioanalyzer, Tapestation (Agilent Technologies), LabChip GX/GXII/GX Touch (PerkinElmer) or Fragment Analyzer (Advanced Analytical).

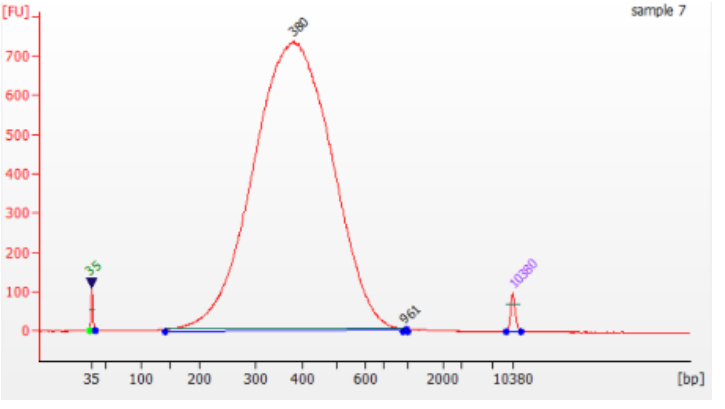

Figure 1 Agilent 2100 bioanalyzer results of beads purification product

5.1.2 Single size selection

Tips
- Using a 60 μL Beads purification to target a peak size of 400 bp from 75 μL of fragmentated gDNA.
- To select a different fragment size, please refer to Table 7 in Chapter 2 for detailed conditions.
- Do not disturb or pipette the beads when adding reagents or transferring supernatant. If you accidentally disturb or pipette the beads, pipette the solution and beads back into the tube and restart the separation process.

5.1.2.1 Preparation

Table 29 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied. Freshly prepared.
En-TE	Refer to section 3.1. Place at RT.
En-Beads	Refer to section 3.1. Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

5.1.2.2 Single size selection

1. Add 75 μL fragmentation gDNA into a new 0.2 mL PCR tube. Add En-TE to make a total volume of 75 μL if the fragmentation gDNA volume is not enough.
2. Mix the En-Beads thoroughly. Add 60 μL of En-Beads to each sample tube and gently pipette at least 10 times until all beads are suspended. Ensure all of the solution and beads in the tip are transferred into the tube after mixing. Or, mix with a vortexer.
3. Incubate the sample(s) at room temperature for 10 min.
4. Centrifuge the sample tube(s) briefly and place on the magnetic rack for more than 5 min until the liquid is clear. Carefully remove and discard the supernatant.
5. While keeping the PCR tube(s) on the magnetic rack, add 160 μL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
6. Repeat step 5. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
7. Keep the centrifuge tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

   Tips Over-drying the beads will result in reduced yield.

8. Remove the PCR tube(s) from the magnetic rack and add 45 μL of En-TE to elute the DNA. Gently pipette the liquid at least 10 times until all beads are suspended. Or, mix with a vortexer.

9. Incubate the sample(s) at room temperature for 5 min.
10. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 43 μL of supernatant to a new 0.2 mL PCR tube.
11. Take 1-2 μL of supernatant for size distribution detection by Bioanalyzer, Tapestation (Agilent Technologies), LabChip GX/GXII/GX Touch (PerkinElmer) or Fragment Analyzer (Advanced Analytical).

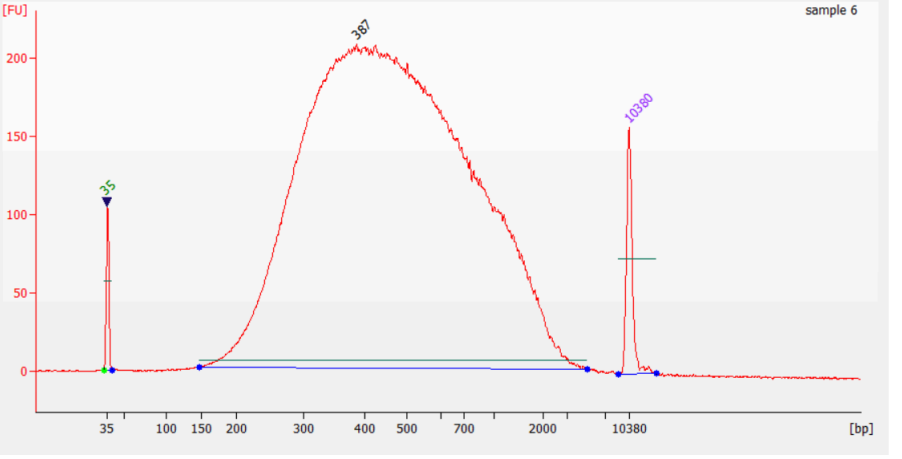

Figure 2 Agilent 2100 bioanalyzer results of beads purification product

5.2 Using adapters

MGI currently offers the Adapter Reagent Kits with two specifications based on the number of reactions: the MGIEasy PF Adapters-16 (Tube) Kit and MGIEasy PF Adapters-96 (Plate) Kit.

Both kits were developed to meet requirements for batch processing of library construction and multiplex sequencing. We selected the best adapter combination based on the principle of balanced base composition. However, the numbers of barcode adapters are not continuous. For optimal performance, read the instructions of Adapter Reagent Kits carefully before use.

- Adapters from the two kits contain overlapping barcodes, and cannot be sequenced in the same lane.
- Do not place the adapters above 30 ℃ to avoid structural changes such as denaturation, which might affect performance.
- Before use, mix the adapter(s) well and centrifuge to collect liquid at the bottom of tubes or plates. Wipe the surface of the tube cap or aluminum film with absorbent paper.
- For MGIEasy PF Adapters-16 (Tube) Kit, carefully open the tube cap to prevent spills or to prevent cross-contamination. Close the cap immediately after use.
- For MGIEasy PF Adapters-96 (Plate) Kit, pierce the aluminum film to pipette solutions for first use. During the process, remember to replace the tip to avoid contamination. After use, transfer the remaining reagents to individual 1.5 mL tube(s) or 0.2 mL PCR tube(s) , label them and store at -20 ℃.
- Adapters from other MGI library Kits are designed for library construction with amplification strategies and are incompatible with PCR-Free Kits.

5.2.1 Instructions for PF Adapters-16 (Tube)

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

- 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, choose the barcode adapter combinations in the table below.

CAUTION The number of the adapter should not be repeated between samples in one lane.

Table 30 Instructions for PF Adapters-16 (Tube)

Sample/lane	Instruction (Example)
1	• For a set of 4 adapters, add 4 adapters to each sample. For example: 01-04. Mix 4 adapters with equal volume and add the mixture to the sample. • Or, for a set of 8 adapters, add 8 adapters to each sample. For example: 97-104. Mix 8 adapters with equal volume and add the mixture to the sample. • Or, add one adapter to the sample, if you don't need to sequence the barcode. This method should not be used for samples pooling sequencing.
2	• For a set of 4 adapters, add 2 adapters to each sample. For example: 01-04. Mix 01 and 02 with equal volume and add the mixture to sample 1; Mix 03 and 04 with equal volume and add the mixture to sample 2. • Or, for a set of 8 adapters, add 4 adapters to each sample. For example: 97-104. Mix 97-100 with equal volume and add the mixture to sample 1; Mix 101-104 with equal volume and add the mixture to sample 2.
3	1. For samples 1 and 2, use the method for (2 samples/lane) above. 2. For sample 3, use the method for (1 sample/lane) above. Tips Use different adapter sets for samples 1, 2, and 3.
4	• For a set of 4 adapters, add 1 adapter to each sample. For example: 01-04. Add adapter 01, 02, 03, 04 to sample 1, 2, 3, 4, in that order. • Or, for a set of 8 adapters, add 2 adapters to each sample. For example: 97-104. Mix 97-98, 99-100, 101-102, and 103-104 with equal volume. Add the mixture to sample 1, 2, 3, 4, in that order.

Sample/lane	Instruction (Example)
5	• For samples 1-4, use the method for (4 samples/lane) above. • For sample 5, use the method for (1 sample/lane) above. Tips Use different adapter sets for samples 1-4 and for sample 5.
6	1. For samples 1-4, use the method for (4 samples/lane) above. 2. For samples 5-6, use the method for (2 sample/lane) above. Tips Use different adapter sets for samples 1-4 and for samples 5-6.
7	1. For samples 1-4, use the method for (4 samples/lane) above (Use the first adapter set). 2. For samples 5-6, use the method for (2 samples/lane) above (Use the second adapter set). 3. For sample 7, use the method for (1 sample/lane) above (Use the third adapter set). Tips Use different adapter sets for samples 1-4, for samples 5-6 and for sample 7.
8	• For a set of 8 adapters, add 1 adapter to each sample. For example: 97-104. Add adapters 97-104 to samples 1-8, in that order. • Or, for 2 sets of 4 adapters, add 1 adapter to each sample. For example: 01-04 and 13-16. Add 1 adapter to each sample.
8+x (x=1-8, Total 9-16)	Perform the following 3 steps: 1. For samples 1 to 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 X, according to the value of X, use the methods above for 1-8 sample/lane accordingly. Tips Use different adapter sets for steps 1 and 2.

For situations in which sequencing data output requirements are different among samples, any sample with a data output of more than 20% for each lane should use a separate set of adapters.

For example, 9 samples are pooled into one lane, one sample of which requires 30% of the total data output.

1. 8 samples may use adapter set (97-104).
2. The final sample should use a full adapter set instead of using only a single adapter. (For example: adapter set (01-04) or (13-16)).

5.2.2 Instructions for PF Adapters-96 (Plate)

Based on the principles of balanced base composition, adapters should be used in specific groups. Follow the instructions below to use the adapters in proper combinations.

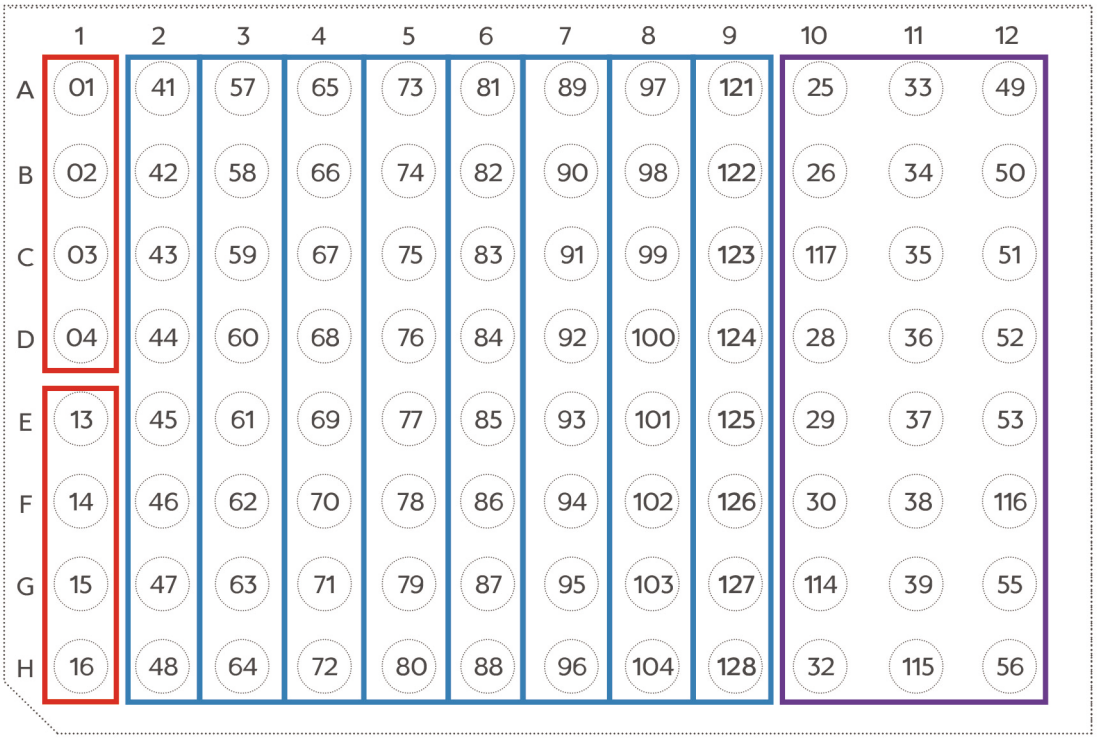

Figure 3 PF adapters-96 (plate) adapters layout and combination instructions

- 2 sets of 4 adapters: Column 1 (01-04, 13-16) (see the red box in the figure above)
- 8 sets of 8 adapters: Columns 2-9 (41-48, 57-64, 65-72, 73-80, 81-88, 89-96, 97-104, and 121-128) (see the blue box in the figure above)
- 1 set of 24 adapters: Columns 10-12 (see the purple box in the figure above)

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

CAUTION The number of the adapter should not be repeated between samples in one lane.

Table 31 Instructions for PF Adapters-96 (Plate)

Sample/lane	Instruction (Example)
1	• For a set of 4 adapters, add 4 adapters to each sample. For example: 01-04. Mix 4 adapters with equal volume and add the mixture to the sample. • Or, for a set of 8 adapters, add 8 adapters to each sample. For example: 41-48. Mix 8 adapters with equal volume and add the mixture to the sample. • Or, add one adapter to the sample, if you don't need to sequence the barcode. This method should not be used for samples pooling sequencing.

Sample/lane	Instruction (Example)
2	• For a set of 4 adapters, add 2 adapters to each sample. For example: 01-04. Mix 01 and 02 with equal volume and add the mixture to sample 1; Mix 03 and 04 with equal volume and add the mixture to sample 2. • Or, for a set of 8 adapters, add 4 adapters to each sample. For example: 41-48. Mix 41-44 with equal volume and add the mixture to sample 1; Mix 45-48 with equal volume and add the mixture to sample 2.
3	1. For samples 1 and 2, use the method for (2 samples/lane) above. 2. For sample 3, use the method for (1 sample/lane) above. Tips Use different adapter sets for samples 1, 2, and 3.
4	• For a set of 4 adapters, add 1 adapter to each sample. For example: 01-04. Add adapters 01, 02, 03, 04 to samples 1, 2, 3, 4, in that order. • Or, for a set of 8 adapters, add 2 adapters to each sample. For example: 41-48. Mix 41-42, 43-44, 45-46, and 47-48 with equal volume. Add the mixture to sample 1, 2, 3, 4, in that order.
5	1. For samples 1-4, use the method for (4 samples/lane) above. 2. For sample 5, use the method for (1 sample/lane) above. Tips Use different adapter sets for samples 1-4 and 5.
6	1. For samples 1-4, use the method for (4 samples/lane) above. 2. For samples 5-6, use the method for (2 sample/lane) above. Tips Use different adapter sets for samples 1-4 and 5-6.

Sample/lane	Instruction (Example)
7	1. For samples 1-4, use the method for (4 samples/lane) above. (Use the first adapter set) 2. For samples 5-6, use the method for (2 samples/lane) above. (Use the second adapter set) 3. For sample 7, use the method for (1 sample/lane) above. (Use the third adapter set) Tips Use different adapter sets for samples 1-4, samples 5-6, and sample 7.
8	• For a set of 8 adapters, add 1 adapter to each sample. For example: 41-48. Add adapters 41-48 to samples 1 - 8, in that order.
8n+x (n=1 or 2, x=1-8, total 9-24)	Perform the following 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. Tips Use different adapter sets for steps 1, 2, and 3.
8n+x (3≤n<11, x=1-8, total 25-96)	Perform the following 3 steps: 1. For samples 1-24, use a set of 24 adapters and add 1 adapter to each sample. 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. Tips Use different adapter sets for steps 1, 2, and 3.

For situations in which sequencing data output requirements are different among samples, any sample with a data output of more than 20% for each lane should use a separate set of adapters.

For example, 9 samples are pooled into one lane, one sample of which requires 30% of the total data output.

1. 8 samples may use adapters (41-48).
2. The final sample should use a full adapter set instead of using only a single adapter. (For example: adapter set (01-04) or (13-16)).