Part No.:H-940-000883-00-01

User Manual

Version:1.0

MGIEasy Fast PCR-FREE FS Library Prep Set

Cat. No.: 940-000886-00 (16 RXN)
940-000884-00 (96 RXN)
940-000882-00 (384 RXN)
Set Version: V2.0

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About the user manual

Manufacturer information

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Revision history

Manual version	Kit version	Date	Description
1.0	V2.0	June. 2023	Initial release

Tips Use the latest version of the manual and use it with the corresponding kit.

Contents

1 Product overview

1.1 Introduction

The MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 is designed to prepare WGS PCR-Free library for MGI high-throughput sequencing platforms. This library prep set is optimized to convert 25 ng to 900 ng genomic DNA (gDNA) into a customized library and uses high quality fast fragmentase to simplify the preparation process and significantly shorten the duration of DNA library preparation. All reagents provided in this set have passed strict quality control and functional verification procedures, ensuring stability and reproducibility.

Tips

- The MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 uses dual barcode adapters for library preparation. The constructed libraries (dsDNA, adapter-ligated libraries) can be combined with MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570) for a single stranded circular (ssCir) DNA libraries preparation and further for DNB preparation, or it can be combined with DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-DB, Cat. No.: 940-000036-00) or DNBSEQ Onestep DNB Make Reagent Kit (OS-DB, Cat. No.: 1000026466) for rapid DNB preparation.
- The MGIEasy Fast PCR-FREE FS Library Prep Module V2.0 in this set is campatible with the single barcode adapters MGIEasy PF Adapters-16(Tube) Kit (Cat. No.: 1000013460) or MGIEasy PF Adapters-96 (Plate) Kit (Cat. No.: 1000013461) to prepare single barcode library. The constructed libraries can be combined with MGIEasy Circularization Kit (Cat. No.: 1000005259) for ssCir and DNB preparation, or it can be combined with DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-SB, Cat. No.: 940-000035-00) for rapid DNB preparation. For detailed operation, refer to “Single barcode library preparation user manual” on page 46.

CAUTION ssCir library prepared by the Circularization Kit is compatible with all genetic sequencers and sequencing types. DNB prepared by the Onestep DNB Make Reagent Kit only is campatible with certain genetic sequencers and sequencing types.

1.2 Intended use

This library prep set is applicable to samples from common humans, animals, plants, fungi, bacteria, including humans (blood, saliva, oral swabs), mouse, *Arabidopsis*, rice, meta, *Ecoli*, and other types of samples. In addition, it is also used for long amplicon DNA fragments.

1.3 Applicable sequencing platform

Select the appropriate DNB preparation kit, sequencing platform, and sequencing type based on application requirements.

Table 1 Sequencing platform and sequencing type recommendation

Reagent kit	Sequencing platform and type	Recommended application scenarios
MGIEasy Dual Barcode Circularization Kit	DNBSEQ-G400RS (PE100/PE150) DNBSEQ-T7RS (PE100/PE150) DNBSEQ-T10x4RS (PE100/PE150) DNBSEQ-T20x2RS (PE100)	humans (blood, saliva, oral swabs), animals, plants, fungi, bacteria, and other types of samples
	DNBSEQ-G50RS (PE100)	Simple Genome (long amplicon DNA, Meta)
DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-DB)	DNBSEQ-G400RS (PE100/PE150) DNBSEQ-T7RS (PE100)	humans (blood, saliva, oral swabs), animals, plants, fungi, bacteria, and other types of samples
	DNBSEQ-E25RS (SE100)	Simple Genome (long amplicon DNA, Meta)
	DNBSEQ-G99RS (PE100) DNBSEQ-G50RS (PE100)	
DNBSEQ Onestep DNB Make Reagent Kit (OS-DB)	DNBSEQ-G99RS (PE100/SE100) DNBSEQ-G50RS (PE100/SE100)	Simple Genome (long amplicon DNA, Meta)

Tips Don't use the reagents in the sequencing kit for DNB preparation after DNB has been prepared by the DNBSEQ Onestep DNB Make Reagent Kit.

1.4 Components

This library prep set comes in three specifications: 16 RXN, 96 RXN and 384 RXN. Three separate boxes are included for each specification. For component details, refer to the following table.

Table 2 MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 (16 RXN) (Cat. No.: 940-000886-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Fast PCR-FREE FS Library Prep Module V2.0 Cat. No.: 940-000885-00	Fast FS Buffer II	Green	215 µL/tube x 1
	Fast FS Enzyme II	Green	105 µL/tube x 1
	Fast Ligation Buffer	Red	450 µL/tube x 1
	Ad Ligase	Red	100 µL/tube x 1
	Ligation Enhancer	Brown	55 µL/tube x 1
	20x Elute Enhancer	Black	7 µL/tube x 1
MGIEasy UDB PF Adapter Kit Cat. No.: 940-000018-00	UDB Adapters	Blue	5 µL/tube x 16
MGIEasy DNA Clean Beads Cat. No.: 940-001176-00	DNA Clean Beads	White	3.2 mL/tube x 1
	TE Buffer	White	3.2 mL/tube x 1

Table 3 MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 (96 RXN) (Cat. No.: 940-000884-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Fast PCR-FREE FS Library Prep Module V2.0 Cat. No.: 940-000883-00	Fast FS Buffer II	Green	1440 µL/tube x 1
	Fast FS Enzyme II	Green	660 µL/tube x 1
	Fast Ligation Buffer	Red	1440 µL/tube x 3
	Ad Ligase	Red	600 µL/tube x 1
	Ligation Enhancer	Brown	360 µL/tube x 1
	20x Elute Enhancer	Black	25 µL/tube x 1
	TE Buffer	White	4 mL/tube x 2
MGIEasy UDB PF Adapter Kit A Cat. No.: 940-000023-00	DNA Adapters-96 plate	-	5 µL/well x 96
MGIEasy DNA Clean Beads Cat. No.: 940-001174-00	DNA Clean Beads	White	15 mL/tube x 1
	TE Buffer	White	17 mL/tube x 1

Table 4 MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 (384 RXN) (Cat. No.: 940-000882-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Fast PCR-FREE FS Library Prep Module V2.0 x 4 Cat. No.: 940-000883-00	Fast FS Buffer II	Green	1440 µL/tube x 1
	Fast FS Enzyme II	Green	660 µL/tube x 1
	Fast Ligation Buffer	Red	1440 µL/tube x 3
	Ad Ligase	Red	600 µL/tube x 1
	Ligation Enhancer	Brown	360 µL/tube x 1
	20x Elute Enhancer	Black	25 µL/tube x 1
	TE Buffer	White	4 mL/tube x 2
MGIEasy UDB PF Adapter Kit A Cat. No.: 940-000023-00	UDB Adapters A	-	5 µL/well x 96
MGIEasy UDB PF Adapter Kit B Cat. No.: 940-000022-00	UDB Adapters B	-	5 µL/well x 96
MGIEasy UDB PF Adapter Kit C Cat. No.: 940-000025-00	UDB Adapters C	-	5 µL/well x 96
MGIEasy UDB PF Adapter Kit D Cat. No.: 940-000024-00	UDB Adapters D	-	5 µL/well x 96
MGIEasy DNA Clean Beads Cat. No.: 1000005279	DNA Clean Beads	White	50 mL/tube x 1
	TE Buffer	White	25 mL/tube x 1

1.5 Storage and transportation

Table 5 Kit storage and transportation

Modules	Cat. No.	Storage and transport temperature
MGIEasy Fast PCR-FREE FS Library Prep Module V2.0	940-000885-00	-25 ℃ to -15 ℃
MGIEasy Fast PCR-FREE FS Library Prep Module V2.0	940-000883-00	
MGIEasy UDB PF Adapter Kit	940-000018-00	
MGIEasy UDB PF Adapter Kit A	940-000023-00	
MGIEasy UDB PF Adapter Kit B	940-000022-00	
MGIEasy UDB PF Adapter Kit C	940-000025-00	
MGIEasy UDB PF Adapter Kit D	940-000024-00	
MGIEasy DNA Clean Beads	940-001176-00	2 ℃ to 8 ℃
MGIEasy DNA Clean Beads	940-001174-00	
MGIEasy DNA Clean Beads	1000005279	

Tips

- Production date and expiration date: refer to the label.
- With proper transport, storage, and use, all components can maintain complete activity within their shelf life.
- In MGIEasy Fast PCR-FREE FS Library Prep Module V2.0, TE Buffer should be stored at 2 ℃ to 8 ℃, 20x Elute Enhancer and Ligation Enhancer should be stored at room temperature, avoiding repeated freeze-thawing. The Ligation Enhancer should be stored away from light.

1.6 User-supplied materials

Table 6 Order information for MGI products

Catalog number	Model	Name
1000020570	16 RXN	MGIEasy Dual Barcode Circularization Kit
1000018650	96 RXN	MGIEasy Dual Barcode Circularization Kit (Customized)
1000005259	16 RXN	MGIEasy Circularization Kit
1000017573	96 RXN	MGIEasy Circularization Kit (Customized)
940-000035-00	OS-SB, 4 RXN	DNBSEQ Onestep DNB Make Reagent Kit V2.0
940-000036-00	OS-DB, 4 RXN	DNBSEQ Onestep DNB Make Reagent Kit V2.0
1000026466	OS-DB, 4 RXN	DNBSEQ Onestep DNB Make Reagent Kit
1000013460	16 RXN	MGIEasy PF Adapters-16 (Tube) Kit
1000013461	96 RXN	MGIEasy PF Adapters-96 (Plate) Kit
1000005279	50 mL	MGIEasy DNA Clean Beads

Tips Prepare reagent kits based on application requirements.

Table 7 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	/
Desktop centrifuge	/
Pipettes	/
Thermocycler	/
96M Magnum Plate	ALPAQUA, Part A000400 (Recommended)
Qubit Fluorometer	Thermo Fisher (Cat. No.: Q33216)
Agilent 2100 Bioanalyzer or equivalent	Agilent Technologies (Cat. No.: G2939AA)

Table 8 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/Quant-iT	Invitrogen (Cat. No.: Q32854) or equivalent
Agilent High Sensitivity DNA Kit	Agilent (Cat. No.: 5067-4626) or equivalent

Reagent/consumable	Recommended brand
Pipette tips	/
1.5 mL tube	/
0.2 mL PCR tube or 96-well plate	Axygen (Cat. No.: PCR-02-C or PCR-96M2-HS-C) or equivalent
Qubit Assay Tubes or 0.5mL Thin Wall PCR Tubes	Invitrogen (Cat. No.: Q32856) or Axygen (Cat. No.: PCR-05-C) or equivalent

1.7 Precautions and warnings

- This product is for research use only, not for clinical diagnosis. Read this manual carefully before use.
- 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.
- To prevent yield loss, try to avoid transferring reaction product to a new tube for bead purification.
- Avoid skin and eye 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 Technical Support: **MGI-service@mgi-tech.com**.

1.8 Workflow

Section	Workflow	Total time	Hands-on time
3.1	Fragmention	27 min	2 min
3.2	Cleanup of fragmentation product	7 - 13 min	1 - 2 min
3.3	Adapter ligation	12 min	2 min
3.4	Cleanup of adapter-ligated product ⏸	23 - 36 min	5 - 10 min
3.5	QC of adapter-ligated product ⏸	4 min	2 min

Tips
- Total time: When sample input is more than 200 ng, the theoretical use time of one reaction. The time will be extended if the number of reactions increases.
- Hands-on time: The total required hands-on time in the process.
- ⏸ : The stop point.

The adapter-ligated product (dsDNA) can be converted to DNB in three ways:

1. Using the MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570) to convert dsDNA library to ssCir and prepare DNB by using the High-throughput Sequencing kit. Refer to “DNB preparation” on page 29.
2. Using the DNBSEQ One-Step DNB Prep Kit V2.0 (OS-DB, Cat. No.: 940-000036-00) to prepare DNB directly. Refer to “Onestep DNB preparation V2.0 (option 2)” on page 30.
3. Using the DNBSEQ One-Step DNB Prep Kit (OS-DB, Cat. No.: 1000026466) to prepare DNB directly. Refer to “Onestep DNB preparation V1.0 (option 3)” on page 33.

2 Sample preparation

2.1 Sample type

This library prep set is applicable to samples from common humans, animals, plants, fungi, bacteria, etc., including humans (blood, saliva, oral swabs), mouse, *Arabidopsis*, rice, meta, *Ecoli*, and other types of samples. In addition, it can be used for long amplicon DNA fragments.

Different types of samples should be performed a fragmentation demo test before library prepartion to achieve the best results.

2.2 Sample purity

It is strongly recommended to use high quality genomic DNA ($1.8 \leq OD_{260}/OD_{280} \leq 2.0$, $OD_{260}/OD_{230} \geq 1.7$) for fragmentation.

Tips If the sample purity does not satisfy the recommended standards, or enzyme inhibitors are present, there is a risk of low library yield.

2.3 Sample input

25 ng to 900 ng gDNA can be used for library preparation. If the amount of genomic DNA is sufficient, it recommend using a high input of genomic DNA for library preparation. Qubit or BMG is recommended for quantification of sample concentrations.

Table 9 Recommended sample input range

Sample type	Input range	Recommended input	Recommended concentration
Complex genome	50 - 900 ng	500 ng	≥ 12 ng/μL
Simple genome	25 - 900 ng	200 ng	≥ 4.5 ng/μL
Microbiome	25 - 900 ng	200 ng	≥ 4.5 ng/μL
Meta	25 - 900 ng	200 ng	≥ 4.5 ng/μL

2.4 Sample requirements

Since Fast FS Enzyme II is sensitive to the pH and component of the DNA storage buffer, it is recommended that you use TE Buffer (pH 8.0) for DNA dissolution.

If DNA is dissolved in other buffers, such as 10 mM Tris (pH 6.8-8.0), AE Buffer (pH 8.5), 0.1x TE (pH 8.0) or other special buffers, perform a demo fragmentation test by adjusting the incubation time of 30 ℃ in “Table 19 Fragmentation reaction conditions (Volume: 60 μL)” on page 16. The incubation time can be titrated from 7.5 to 15 min.

If the sample contains many impurities and inhibitors, it is recommended that you re-purify the sample DNA with 1.8x magnetic beads and elute it into TE Buffer (pH 8.0). After repurification, carry out fragmentation test.

2.5 Library insert size requirement

A narrow size distribution of fragmented DNA is preferable. Better sequencing quality can be obtained with a narrow size distribution, while a wide distribution results in lower quality.

- The recommended peak size of single-sided size selection library is 600 bp to 850 bp.
- The recommended peak size of double-sided size selection library is 400 bp to 600 bp.

Tips Do not pool double-sided size selection library and single-sided size selection library together for sequencing. The single-sided size selection library has a wider insert size distribution than the double-sided size selection library, resulting in lower sequencing quality.

3 Library preparation protocol

CAUTION The protocol in this chapter is only for dual barcode library preparation. For the protocol of single barcode library preparation, refer to "Single barcode library preparation user manual" on page 46.

25 ng to 900 ng gDNA sample can be quickly converted to adapter-ligated libraries by fragmentation, end repair, simple purification, adapter ligation, and purification. The adapter-ligated libraries can be covernted to ssCir libraries by MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570) and then for DNB preparation. Or it can be used for rapid DNB preparation by DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-DB, Cat. No.: 940-000036-00) or DNBSEQ Onestep DNB Make Reagent Kit (OS-DB, Cat. No.: 1000026466). Refer to the tables below for different options.

Table 10 Recommended instruction combined with MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570)

gDNA amount (N ng)	gDNA input (ng)	Size selection method
200 ≤ N ≤ 500	200-500 (all input)	single-sided size selection
200 < N < 900	500	single-sided size selection
N ≥ 900	900	double-sided size selection

Tips
- Do not pool double-sided size selection library and single-sided size selection library together for sequencing.
- If gDNA input is less than 200 ng, the yield of ssCir is usually insufficient for once sequencing. In this case, library pooling with other PCR-free libraries may be necessary.
- If gDNA input is less than 900 ng, there is a risk of low ssCir yields by double sided size selection purification.

Table 11 Recommended instruction combined with DNBSEQ Onestep DNB Make Reagent Kit (OS-DB, Cat. No.: 940-000036-00 or 1000026466)

gDNA amount (N ng)	gDNA input (ng)	Size selection method
25 ≤ N ≤ 200	25-200 (all input)	single-sided size selection
200 < N < 500	200	single-sided size selection
N ≥ 500	500	double-sided size selection

Tips
- Do not pool double-sided size selection library and single-sided size selection library together for sequencing.
- If gDNA input is less than 50 ng, the library yield is usually insufficient for once sequencing. In this case, library pooling with other PCR-free libraries may be necessary.
- If gDNA input is less than 500 ng, there is a risk of low yields by double sided size selection purification.

3.1 Reagent preparation

3.1.1 Preparation

Table 12 Preparing the reagents

Reagent	Requirement
Nuclease-Free Water	User-supplied; place at room temperature (RT), mix thoroughly.
TE Buffer	
20x 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 8 samples. Increase the preparation reagent volumes in proportion if there are more samples.

1. Prepare the 1x Elute Enhancer according to the table below. Mix it by vortexing and centrifuge briefly. Store at room temperature before using. The shelf life of the 1 x Elute Enhancer is 7 days.

Table 13 1x Elute Enhancer

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

2. Prepare the En-TE according to the table below. Mix it by vortexing and centrifuge briefly. Store at 4 ℃ before using. The shelf life of the En-TE is 60 days.

Table 14 En-TE

Reagent	Volume
1x Elute Enhancer	3 μL
TE Buffer	1497 μL
Total	1500 μL

3. Prepare the En-Beads according to the table below. Mix it by vortexing and centrifuge briefly. Store at 4 ℃ before using. The shelf life of the En-Beads is 60 days.

Table 15 En-Beads

Reagent	Volume
1x Elute Enhancer	10 μL
DNA Clean Beads	990 μL
Total	1000 μL

3.2 Fragmentation

Tips The extent of fragmentation (size distribution of DNA fragments) is controlled by time and temperature. Therefore, ensure the accuracy of time and temperature during the reaction. Samples and enzyme mix should always be kept on ice.

3.2.1 Preparation

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

Table 16 Preparing the reagents

Reagent	Requirement
TE Buffer (pH 8.0)	User-supplied; place at RT.
Fast FS Buffer II	Thaw at RT, vortex, centrifuge briefly, and place on ice.
Fast FS Enzyme II	Keep on ice
80% ethanol	User-supplied; freshly prepared.
En-TE	Refer to 3.1.2, place at RT.
En-Beads	Refer to 3.1.2, and take out 30 min in advance to equilibrate to room temperature. Mix thoroughly by vortexing before use each use.

3.2.2 Fragmentation

1. Normalize gDNA referring to the table below. Based on the sample concentration, take the appropriate gDNA (recommended 25 ng - 900 ng) to a new 0.2 mL PCR tube. Add TE Buffer (pH 8.0) to make a total volume of **45 µL**. Place the normalized gDNA on ice.

Table 17 Normalization of gDNA dissolved in TE (pH 8.0)

Components	Volume
TE buffer (pH 8.0)	45-X µL
gDNA (25 ng - 900 ng)	X µL
Total	45 µL

Tips This enzyme is pH-sensitive. The normalization buffer should be the same as the DNA elution buffer.

2. Set and run the program (refer to "Table 19 Fragmentation reaction conditions (Volume: 60 µL)" on page 16). The thermocycler will perform the first step reaction described in table below and be kept at 4 ℃.
3. Mix Fast FS Enzyme II by inverting 10 times and flicking the bottom gently, ensure that no residual reagent is left at the bottom each time. Centrifuge briefly and place them on ice until use.

CAUTION
- DO NOT vortex the Fast FS Enzyme II. Strictly follow the manual instructions. Insufficient mixing would affect the fragmentation process.

4. According to the desired reaction number, prepare the fragmentation mixture in a 1.5 mL centrifuge tube on ice. Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 18 Fragmentation mixture

Reagent	Volume per reaction
Fast FS Buffer II	10 µL
Fast FS Enzyme II	5 µL
Total	15 µL

5. Add **15 µL of fragmentation mixture** to each sample tube from step 1 (volume: 45 µL). Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.
6. Place the PCR tube(s) into the thermocycler and skip the first step (4 ℃ Hold) to start the reaction.

Table 19 Fragmentation reaction conditions (Volume: 60 µL)

Temperature	Time
70 ℃ Heated lid	On
4 ℃	Hold
30 ℃	8.5 min
65 ℃	15 min
4 ℃	Hold

Tips The fragmentation condition above is suitable for human, animal, plant and bacterial gDNA. Fragment size range should be 100 bp - 3000 bp, with a peak size of 600 bp - 850 bp. If the sample types are not covered or the extent of fragmentation product can not satisfy your requirements, the incubation time at 30 ℃ can be shortened or extended for the best fragmentation results.

7. After reaction, centrifuge the tube(s) briefly and immediately proceed to the next step.

CAUTION DO NOT STOP AT THIS STEP.

Tips

- For the first fragmentation test, it is recommended that you take 10 μL of product from step 7 in section 3.2.2 for purification with 0.8 x magnetic beads and elute in 8 μL of En-TE. Take 1 μL of elute product for Agilent 2100 High Sensitivity test and ensure that the smear size is 100 bp - 3000 bp with the peak size between 600 bp - 850 bp. For example: 200 ng gDNA dissolved in TE Buffer (pH 8.0), when fragmentation time is 8.5 min, the size distribution of fragments is shown in Figure 1.
- If the peak size is too large or too small, it is recommended to re-adjust the incubation time at step 6 in section 3.2.2 (incubation 7.5 min - 15 min of 30 ℃ is recommended).

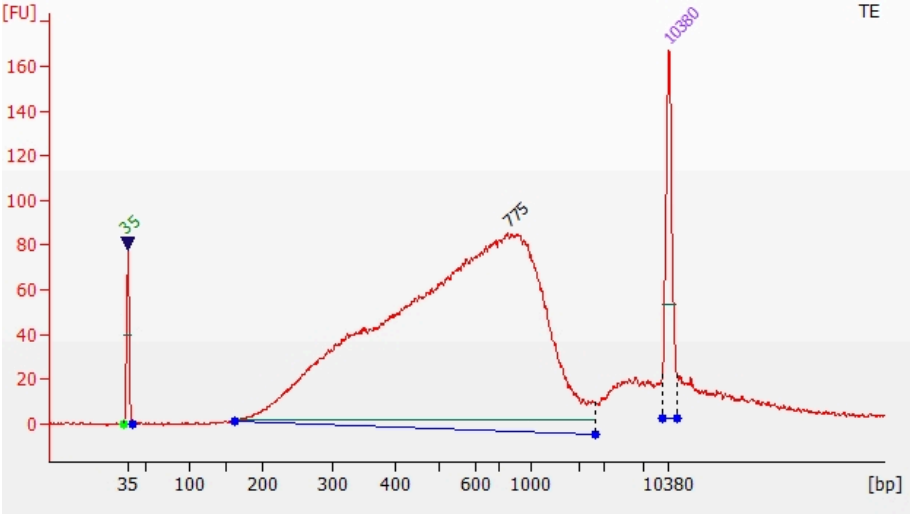

Figure 1 Agilent 2100 Bioanalyzer results of 0.8 x beads purification fragmentation product

3.3 Cleanup of fragmentation product

Tips Before starting the experiment, read "Library insert size requirement" on page 11 and "Magnetic beads and cleanup" on page 34 carefully. Select appropriate size selection method.

3.3.1 Single sided size selection (option 1)

Tips
- The peak size of the selected fragments is approximately 600 bp to 850 bp as shown in Figure 1.

3.3.1.1 Preparation

Table 20 Preparing the reagents

Reagent	Requirement
En-TE	Refer to 3.1.2, and place at RT.
En-Beads	Refer to 3.1.2, and take out 30 min in advance to equilibrate to RT. Mix thoroughly by vortexing before each use.

3.3.1.2 Size selection

1. Check the volume of the fragmentation product (from step 7 in section 3.2.2, volume: 60 µL). If the volume is less than 60 µL, add **EN-TE** to make a total volume of **60 µL**.
2. Mix the En-Beads thoroughly. Add **48 µL of En-Beads** to each sample tube. Mix with a vortexer until all beads are suspended.
3. Incubate at room temperature for 5 min.
4. 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 all the supernatant. If some liquid remains on the tube wall, centrifuge the tube(s) briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
5. Remove the tube(s) from the magnetic rack and add **47 µL of En-TE** to elute the DNA. Mix with a vortexer until all beads are suspended and centrifuge briefly.

CAUTION DO NOT STOP AT THIS step. Proceed to the section 3.4.

3.3.2 Double sided size selection (option 2)

Tips • The peak size of the selected fragments is approximately 400 bp to 600 bp.

3.3.2.1 Preparation

Table 21 Preparing the reagents

Reagent	Requirement
En-TE	Refer to 3.1.2, and place at RT.
En-Beads	Refer to 3.1.2, and take out 30 min in advance to equilibrate to RT. Mix thoroughly by vortexing before each use.

3.3.2.2 Size selection

1. Check the volume of the fragmentation product (from step 7 in section 3.2.2, volume: 60 μL). If the volume is less than 60 μL, add **EN-TE** to make total volume of **60 μL**.
2. Mix the En-Beads thoroughly. Add **32 μL of En-Beads** to each sample tube. Mix with a vortexer until all beads are suspended.
3. Incubate at room temperature for 5 min.
4. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer **92 μL of supernatant** to a new 0.2 mL PCR tube.

   Tips In this step, keep the supernatant and discard the beads.
5. Add **16 μL of En-Beads** to each sample tube (from step 4, volume: 92 μL). Mix with a vortexer until all beads are suspended.
6. Incubate at room temperature for 5 min. Centrifuge the tube(s) briefly.
7. Place the tube(s) on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant. If some liquid remains on the tube wall, centrifuge the tube(s) briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
8. Remove the tube(s) from the magnetic rack and add **47 μL of En-TE** to elute the DNA. Mix with a vortexer until all beads are suspended and centrifuge briefly.

CAUTION DO NOT STOP AT THIS STEP. Proceed to the section 3.4.

Tips In demo test, if you want to check the size distribution of double sided size product, it is recommended to take 10 μL of product from step 6 in section 3.2.2.2 and perform a purification and elute in 5 μL of En-TE. Perform the purification when the remaining products are at adapter ligation reaction. Take 1 μL of elute product for Agilent 2100 High Sensitivity test.

For example: 900 ng gDNA dissolved in TE Buffer (pH 8.0), when fragmentation time is 8.5 min, the size distribution of fragments is shown in Figure 2.

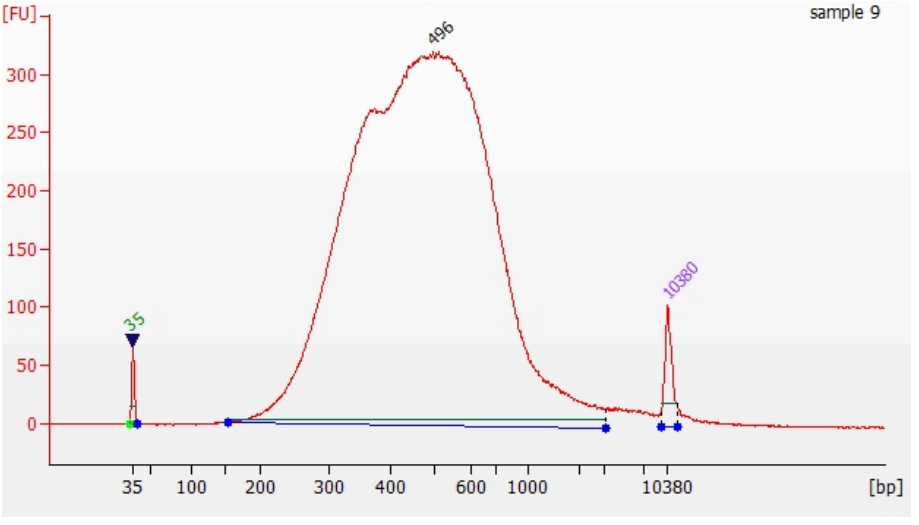

Figure 2 Agilent 2100 Bioanalyzer results of fragmentation product by double-sided size purification

3.4 Adapter ligation

Tips Barcode is in adapter, and read "Using adapters" on page 37 carefully before operation.

3.4.1 Preparation

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

Table 22 Preparing the reagents

Reagent	Requirement
MGIEasy Series UDB PF Adapter Kits	Thaw at RT, mix thoroughly, centrifuge briefly, and place on ice.
Fast Ligation Buffer	
Ad Ligase	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
Ligation Enhancer	Mix thoroughly, centrifuge briefly, and place at RT.

Tips
- Mix the adapter thoroughly before use. Adapters should not be mixed directly with the adapter ligation mixture.
- The Fast Ligation Buffer is highly viscous. Mix it thoroughly by vortexing 6 times (3 sec each) and centrifuge briefly.
- Mix Ad Ligase by inverting 10 times and flicking the bottom gently, ensure that no residual reagent is left at the bottom. Centrifuge briefly and place them on ice until use.
- After Ligation Enhancer is used for the first time, store it at 10 ℃ - 30 ℃ away from light.

3.4.2 Adapter ligation

1. Dilute the UDB PF Adapter with TE Buffer based on gDNA input.

Table 23 Relationship between UDB PF Adapter dilution ratio and gDNA input

gDNA Input (N ng)	Dilution Ratio of UDB PF Adapter	Volume after Dilution
500< N ≤ 900	No Dilution	3
200 ≤ N ≤ 500	1.5	3
100 ≤ N < 200	3	3
25 ≤ N< 100	10	3

2. Add **3 μL of UDB PF adapter** to the corresponding sample tube from step 5 in section 3.3.1.2 or step 8 in 3.3.2.2 (volume: 47 μL). Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.
3. According to the desired reaction number, prepare the adapter ligation mixture in a 1.5 mL centrifuge tube on ice. Vortex it 6 times (3 sec each), centrifuge briefly, and place on ice.

Table 24 Adapter ligation mixture

Reagent	Volume per reaction
Fast Ligation Buffer	23 μL
Ad Ligase	5 μL
Ligation Enhancer	2 μL
Total	30 μL

Tips It is recommended that you prepare the adapter ligation mixture while waiting for cleanup of fragmentation product, and place it on ice after preparation, and use it within 30 min.

4. Slowly pipette **30 μL of adapter ligation mixture** to each sample tube. Vortex it 6 times (3 sec each), centrifuge briefly, and place on ice.

Tips The adapter ligation mixture is highly viscous and slowly aspirated to ensure the volume is accurate.

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

Table 25 Adapter ligation reaction conditions (Volume: 80 μL)

Temperature	Time
30 ℃ Heated lid	On
25 ℃	10 min
4 ℃	Hold

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

CAUTION • DO NOT STOP AT THIS STEP. Proceed to the section 3.5.

3.5 Cleanup of adapter-ligated product

Tips Before starting the experiment, read "Magnetic beads and cleanup" on page 34 carefully.

3.5.1 Preparation

Table 26 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
En-TE	Refer to 3.1.2, and place at RT.
En-Beads	Refer to 3.1.2, and take out 30 min in advance to equilibrate to room temperature. Mix thoroughly by vortexing before each use.

3.5.2 Cleanup of adapter-ligated product

CAUTION If adapter-ligated libaries are prepared from 500 ng gDNA and double size selection, and converted to DNB by DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-DB, Cat. No.: 940-000036-00). Refer to "Cleanup1 of adapter-ligated ligation product" on page 36and "Cleanup2 of adapter-ligated ligation product" on page 36to perform cleanup of adapter-ligated product.

1. Add **20 μL of En-TE** to each sample tube (from step 6 in section 3.4.2, volume: 80 μL).
2. Mix the En-Beads thoroughly. Add **20 μL of En-Beads** to each sample tube. Mix with a vortexer until all beads are suspended.
3. Incubate the sample tube(s) at room temperature for 5 min.
4. 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 all the supernatant. 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.
5. Remove the tube(s) from the magnetic rack. Add **30 μL of En-TE** and **20 μL of En-Beads** to each sample tube. Mix with a vortexer until all beads are suspended.
6. Incubate the tube(s) at room temperature for 5 min.
7. 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.
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 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 tube(s) from the magnetic rack and add **51 μL of En-TE** to elute the DNA. Mix with a vortexer until all beads are suspended.

    Tips If gDNA input is less than 100 ng, add **15 μL of En-TE** for eluting in step 11.

12. Incubate the tube(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 **49 μL** of supernatant to a new 0.2 mL PCR tube.

    Tips If gDNA input is less than 100 ng, transfer **13 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 ℃.

3.6 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 more than 1 ng/μL, it is a qualified ligation product.
- If the concentration is between 0.8 ng/μL and 1 ng/μL, library preparation can still be attempted, but with a greater risk of failure.
- If the concentration is less than 0.8 ng/μL, do not continue with this sample.

Tips
- In order to avoid adapter contamination and attain a better uniformity of sequencing data, it is recommended to pool samples at DNB.

4 DNB preparation protocol

CAUTION The content in this chapter is only for double barcode library. For the DNB preparation and related reagents of single brcode adapter library, refer to "Single barcode library preparation user manual" on page 46.

The adapter-ligated libraries can be covernted to DNB in three ways.

- Combined with MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570) for ssCir preparation and further for DNB preparation.
- Combined with DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-DB, Cat. No.: 940-000036-00) for rapid DNB preparation.
- Combined with DNBSEQ Onestep DNB Make Reagent Kit (OS-DB, Cat. No.: 1000026466) for rapid DNB preparation.

4.1 Circularization and DNB preparation (option 1)

MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570, user-supplied) is required for ssCir preparation.

CAUTION Check the name and Cat. No. of the kit carefully before use.

4.1.1 Denaturation and single strand circularization

4.1.1.1 Preparation

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

Table 27 Preparing the reagents

Reagent	Requirement
Dual Barcode Splint Buffer	Thaw at RT, mix thoroughly, centrifuge briefly, and place on ice.
DNA Rapid Ligase	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
TE Buffer, pH 8.0	User-supplied; place at RT.

4.1.1.2 Denaturation

1. Pipette 100 ng - 300 ng adapter-ligated product (from step 13 in section 3.5.2) into a new 0.2 mL PCR tube. If the volume is less than 48 µL, add TE Buffer to make a total volume of **48 µL**.

Tips In the range of 100 ng -300 ng, more yield of ssCir will be attained with more adapter-ligated product.

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

Table 28 Denaturation reaction conditions (Volume: 48 µL)

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

3. After the reaction, centrifuge the tube briefly and place on ice.

4.1.1.3 Single strand circularization

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

Table 29 Circularization reaction mixture

Reagent	Volume per reaction
Dual Barcode Splint Buffer	11.5 µL
DNA Rapid Ligase	0.5 µL
Total	12 µL

2. Add **12 µL of circularization reaction mixture** to each sample tube (from step 3 in section 4.1.1.2). Vortex it 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 30 Single strand DNA circularization reaction conditions (Volume: 60 µL)

Temperature	Time
42 ℃ Heated lid	On
37 ℃	10 min
4 ℃	Hold

Tips Prepare the "Table 32 Digestion mixture" on page 27 in advance of this step.

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

4.1.2 Digestion

4.1.2.1 Preparation

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

Table 31 Preparing the reagents

Reagent	Requirement
Digestion Buffer	Thaw at RT, mix thoroughly, centrifuge briefly, and place on ice.
Digestion Enzyme	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
Digestion Stop Buffer	Thaw at RT, mix thoroughly, centrifuge briefly, and place at RT.

4.1.2.2 Digestion

1. According to the desired reaction number, prepare the 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 32 Digestion mixture

Reagent	Volume per reaction
Digestion Buffer	1.4 μL
Digestion Enzyme	2.6 μL
Total	4.0 μL

2. Add **4 μL of digestion mixture** to each sample tube (from step 4 in section 4.1.1.3, volume: 60 μL).Vortex it 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 33 Digestion reaction conditions (Volume: 64 μL)

Temperature	Time
42 ℃ Heated lid	On
37 ℃	10 min
4 ℃	Hold

4. When the program is completed, centrifuge the tube briefly and immediately add **7.5 μL of Digestion Stop Buffer** to each sample tube. Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.

Tips DO NOT STOP AT THIS STEP. Proceed to the section 4.1.3.

4.1.3 Cleanup of digestion product

Tips Before starting the experiment, read "Magnetic beads and cleanup" on page 34 carefully.

4.1.3.1 Preparation

Table 34 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
TE Buffer, pH 8.0	User-supplied; place at RT.
DNA Clean Beads	User-supplied; take out 30 min in advance to equilibrate to RT. Mix thoroughly by vortexing before each use.

4.1.3.2 Cleanup of digestion product

1. Mix the DNA Clean Beads thoroughly. Add **130 µL of DNA Clean Beads** to each sample tube (from step 4 in section 4.1.2.2, volume: 71.5 µL). Mix with a vortexer until all beads are suspended.
2. Incubate at room temperature for 5 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 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 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 TE Buffer** to elute the DNA. Mix with a vortexer until all beads are suspended.
8. Incubate at room temperature for 5 min.
9. Centrifuge the tube 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 1.5 mL centrifuge tube.

   Stop point After cleanup, the digestion product(s) (ssCir) can be stored at -20 ℃ for one month.

4.1.4 QC of digestion product

Quantify the ssCir with Qubit ssDNA Assay Kit. The final yields should be more than 50 fmol (about 10 ng). Refer to the formula below to calculate the mass of 50 fmol ssCir:

Formula 1 Conversion between fmol and mass in ng of circular ssDNA

$$50\ \text{fmol ssDNA (ng)} = 0.05 \times \frac{\text{DNA fragement peak size (bp)} + \text{the length of adapter (bp)}}{1000\ \text{bp}} \times 330\ \text{ng}$$

Tips In MGIEasy Series Adapter Kits, the length of dual barcode PCR-Free adapter is 131 bp, the length of single barcode PCR-Free adapter is 84 bp.

4.1.5 DNB preparation

Refer to MGISEQ-200RS High-throughput (Rapid) Sequencing Set User Manual, DNBSEQ-G400RS Highthroughput (Rapid) Sequencing Set User Manual, or DNBSEQ-T7RS High-throughput Sequencing Set User Manual to prepare DNB. 50 fmol (about 10 ng) ssCir was required for DNB preparation.

If multiple ssCir libraries need to be pooled, it is recommended that you mix them based on their molar ratio.The molar ratio of pooled ssCir depends on the expected data volume ratio of the different samples by the customers. However, the barcode corresponding to the pooled sample must comply with “UDB Barcode Pooling Guide” on page 40.

Tips
- The insert size and the size range affect sequencing quality and amount of effective sequencing reads. Therefore, it has a risk of a decrease of sequencing quality and effective sequencing reads, when pooling libraries with different insert sizes or prepared by different purification methods(e.g. pooling single size selection purification products with double size selection purification products for sequencing).
- If mixed sequencing is necessary, it is recommended to pool the PCR-free libraries that have the similar insert size and the size range.

4.2 Onestep DNB preparation V2.0 (option 2)

DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-DB, Cat. No.: 940-000036-00) is required for rapid DNB preparation. According to the different size selection methods, select the correct input of DNB preparation as shown in the table below.

Table 35 Relationship of DNBSEQ Onestep DNB Make Reagent Kit V2.0 and Size selection method

Size selection method	Single-sided size selection	Double-sided size selection
Input of DNB preparation (ng)	20	16

Tips Library Pooling Considerations.

- If adapter-ligated product library is inaccurately quantified or have impurities that inhibit enzymatic reaction, there is a risk of failure or abnormal concentrations in DNB preparation.
- When pooling libraries from same type of sample and same gDNA input, refer to "UDB Barcode Pooling Guide" on page 40. Libraries can be pooled by certain mass ratio based on the desired ratio of sequencing data output.
- Pooling library from different types of sample or different gDNA input is not recommended as it could potentially result in non-uniformed data split across different barcode.

4.2.1 Preparation

- Samples: Place the product from step 13 in section 3.5.2 on ice. Calculate the required library volume.
- Mix the reagents before using and store the remaining reagents immediately after use.

Table 36 Preparing the reagents

Reagent	Requirement
Make DNB Buffer (OS-V2.0-DB)	Thaw at RT, mix thoroughly, centrifuge briefly, and place on ice.
Molecular Grade Water	
Make DNB Enzyme Mix I (OS-V2.0)	Centrifuge briefly and place on ice.
Make DNB Enzyme Mix II (OS)	
Stop DNB Reaction Buffer	Thaw at RT, mix thoroughly, centrifuge briefly, and place on ice.
Qubit ssDNA Assay Kit	User-supplied.

4.2.2 Operation

1. Based on the adapter-ligated dsDNA library concentration, add appropriate dsDNA library to a new 0.2 ml 8-strip or PCR tube. Add the following reagents into the tube(s) on ice. Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 37 DNB Making System 1

Reagent	Volume per reaction
dsDNA library	V μL
Molecular Grade Water	20-V μL
Make DNB Buffer (OS-V2.0-DB)	20 μL
Total	40 μL

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

Table 38 DNB Reaction Condition 1 (Volume: 40 μL)

Temperature	Time
105 ℃ Heated lid	On
95 ℃	3 min
57 ℃	3 min
4 ℃	Hold

3. According to the desired reaction number, prepare the DNB Making System 2 on ice. Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 39 DNB Making System 2

Reagent	Volume per reaction
Make DNB Enzyme Mix I (OS-V2.0)	40 μL
Make DNB Enzyme Mix II (OS)	4 μL
Total	44 μL

4. Add **44 μL of DNB Making System 2** to each sample tube (from step 2 , volume: 40 μL). Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.
5. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 40 DNB Reaction Condition 2 (Volume: 84 μL)

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

Tips
- For certain PCR instruments from some manufacturers, it takes extended time to achieve desired temperature of the heated lids. For this type of PCR instrument, preheat the lid in advance to ensure that the lid remains at working temperature during the reaction.
- The temperature of heated lids is suggested to be 35 ℃, or as close as possible to the lowest temperature of 35 ℃.

6. When the program is completed, immediately add **20 μL of Stop DNB Reaction Buffer** to each sample tube. Mix gently by pipetting 5 to 8 times with a wide-bore tip. After mixing, store samples at 4 ℃ for later use (use within 48 hours).

CAUTION DNB must be pipetted gently with a wide-bore pipette tip. Do not centrifuge, vortex, shake, or pipette DNB vigorously.

7. Quantify the DNB with ssDNA Fluorescence Assay Kits such as Qubit ssDNA Assay Kit.

- If DNB concentration is less than 6 ng/μL, prepare DNB again.
- If DNB concentration is between 6 ng/μL and 8 ng/μL, there is a risk of low sequencing results.
- If DNB concentration is more than 8 ng/μL, the DNB is qualified.

CAUTION A volume of 2 μL DNB is suggested to be measured. If the number of samples is large, it is recommended that they are quantified in batches. This helps to avoid inaccurate measurement of DNB concentration.

4.3 Onestep DNB preparation V1.0 (option 3)

20 ng adapter-ligated product (from step 13 in section 3.5.2) and DNBSEQ Onestep DNB Make Reagent Kit (OS-DB, Cat. No.: 1000026466) are required for rapid DNB preparation. Refer to H-T-007 4.0 DNBSEQ DNB Make Reagent Kit User Manual to prepare DNB.

⚠ CAUTION if the sequencer is DNBSEQ-G99RS, the incubation time of 30 ℃ in "Table 40 DNB Reaction Condition 2 (Volume: 84 μL)" on page 31 is **20 min**.

5 Appendix

5.1 Magnetic beads and cleanup

It is recommended that you use DNA Clean Beads included in the MGIEasy DNA Clean Beads (MGI, Cat. No.: 940-001176-00, Cat. No.: 940-001174-00 or Cat. No.: 1000005279) to purify the libraries. If the magnetic beads from other sources are used, optimize the cleanup conditions before getting started.

5.1.1 Before use

1. Remove the magnetic beads from the 4 ℃ refrigerator 30 min in advance to allow the beads to equilibrate to room temperature. Equilibrating to room temperature ensures the beads are at the expected capture efficiency.
2. Before each use, vortex or pipette the beads to ensure that they are thoroughly mixed.
3. The volume of the beads used during cleanup determines the lower size limit of the fragment that can be purified. A higher volume of beads used allows for selection of a smaller fragment size.

5.1.2 Operation notes

- Use 96-hole magnetic plate or other 0.2 mL magnetic rack during the separation process. The yield may loss about 20% if transfer the liquid into 1.5 mL centrifuge tube and separate with 1.5 mL magnetic rack.
- **Sample volume**: If the sample volume decreases (for example, from evaporation during incubation), add TE Buffer to reach the recommended sample volume. Purify the sample with the recommended volume of magnetic beads.
- **Uncapping**: Carefully open or close the tube cap while keeping the tube on the magnetic rack. Strong vibrations may cause sample loss from liquid or beads spilling out of the tubes. It is recommended that you hold the middle or lower part of the tube when opening the cap.

- **Removing the supernatant**

  1) Mix the sample and magnetic beads by vortexing. Place the tube on a magnetic rack for separation. Do not remove the supernatant until the solution is completely clear.

  2) The separation process takes approximately 2 to 3 min. Considering the difference in magnetism of magnetic racks or plates, leave enough time for the solution to become completely clear.

  3) Keep the centrifuge tube(s) on the magnetic rack when removing the supernatant. Place the tip on the tube wall that is away from the rack and bead pellet.

- **Ethanol wash**

  1) Wash the beads with freshly prepared room temperature 80% ethanol. Sufficient ethanol should be added to immerse the beads entirely.

  2) Keep the centrifuge tube(s) on the magnetic rack during washing. Do not shake or disturb the beads while washing.

  3) Carefully remove all remaining ethanol after washing twice. If liquid remains on the tube wall, centrifuge the tube briefly and separate the beads from the liquid on the magnetic rack. Remove all remaining liquid with a low-volume pipette.

- **Air-dry**

  1) After washing twice with 80% ethanol, air-dry the beads at room temperature.

- The surface of the magnetic beads is **cracking**: **Indicates over-drying and reduces the purification yield.**
- The surface of the magnetic beads is reflective: Indicates insufficient drying and easily causes anhydrous ethanol residues and affects subsequent reactions.
- The surface of the magnetic beads is reflective: Indicates insufficient drying and easily causes anhydrous ethanol residues and affects subsequent reactions.

  2) Air-drying takes approximately 3 to 5 min at room temperature. Different indoor temperatures and humidities of labs may affect the drying time.

- **Elution**

  1) Elute the DNA with the TE Buffer or En-TE prepared from section 3.1 reagent preparation.

  2) The elution volume of En-TE should be 2 μL more than the pipetting volume of the supernatant to avoid touching or pipetting the magnetic beads.

5.1.3 Cleanup1 of adapter-ligated ligation product

1. Add **20 μL of En-TE** to each sample tube (from step 6 in section 3.4.2, volume: 80 μL).
2. Mix the En-Beads thoroughly. Add **20 μL of En-Beads** to each sample tube. Mix with a vortexer until all beads are suspended.
3. Incubate the sample tube(s) at room temperature for 5 min.
4. 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.
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 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 tube(s) from the magnetic rack and add **32 μL of En-TE** to elute the DNA. Mix with a vortexer until all beads are suspended.
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 **30 μL** of supernatant to a new 0.2 mL PCR tube.

5.1.4 Cleanup2 of adapter-ligated ligation product

1. Check the volume of the product (for example, sample volume is **30 μL**)
2. Mix the En-Beads or DNA Clean Beads thoroughly. Add **0.7 x μL**(for example, **21 μL**) **of En-Beads or DNA Clean Beads** to each sample tube. Mix with a vortexer until all beads are suspended.
3. Incubate at room temperature for 5 min.
4. 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.
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 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 tube(s) from the magnetic rack and add **30 μL of En-TE** to elute the DNA. 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 **28 μL** of supernatant to a new 0.2 mL PCR tube.

    Tips Quantify the concentration of adapter-ligated product refer to "QC of ligation product" on page 24.

5.2 Using adapters

MGIEasy Fast PCR-FREE FS Library Prep Module V2.0 is suitable for UDB PF Adapter and PF adapter. If Adapter is UDB PF Adapter, refer to instructions in Appendix 5.2.1, 5.2.2 and 5.2.3. If Adapter is PF Adapter, refer to instructions in Appendix 5.2.4 and 5.2.5.

- There are three specifications of UDB PF Adapter Reagent Kit depending on the number of reactions: the MGIEasy UDB PF Adapter Kit (16 RXN), MGIEasy UDB PF Adapter Kit A (96 RXN) and MGIEasy UDB PF Adapter Kit A/B/C/D (384 RXN). Among the adapters from the three sets, adapters with same ID number share same sequence thus cannot be sequenced in the same lane.
- There are two specifications of PF Adapter Reagent Kit depending on the number of reactions: the MGIEasy PF Adapters-16 (Tube) Kit and MGIEasy PF Adapters-96 (Plate) Kit.
- Both kits were developed to meet requirements for batch processing of library construction and multiplex sequencing. We selected the best Adapter combinations based on the principle of balanced base composition. However, not all barcode adapter combinations are compatible. For optimal performance, carefully read the instructions. Adapters from the two kits contain overlapping Barcodes and cannot be sequenced in the same lane.
- All adapters are double stranded. To prevent structural changes, such as denaturation, which might affect performance, do not place the adapters above room temperature.
- Change tips when pipetting different adapters to avoid cross-contamination.
- Before use, centrifuge to collect liquid at the bottom of tubes or plates. Gently remove the cap/sealing film to prevent liquid from spilling and cross-contamination. Mix Adapters with a pipette before use. Remember to reseal the Adapters immediately after use. For Adapters-96 (Plate), if the seal film is contaminated, discard the old seal film and use a new one to reseal the 96-well plate.
- 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 during data analysis.

5.2.1 MGIEasy UDB PF Adapter Kit (16 RXN) Instruction

This kit contains 16 Adapters grouped into 2 sets:

- UDB Adapter-393 to UDB Adapter-400 (see the blue box in the figure below)
- UDB Adapter-401 to UDB Adapter-408 (see the red box in the figure below).

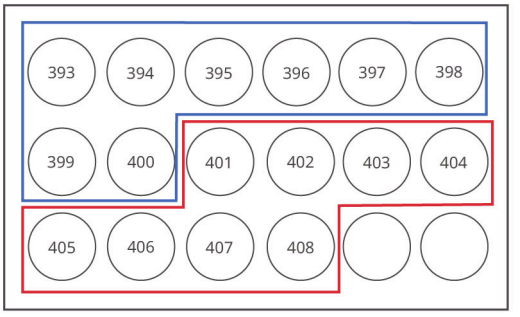

Figure 3 MGIEasy UDB PF Adapter Kit (16 RXN) Adapter Layout and Combination Instructions

5.2.2 MGIEasy UDB PF Adapter Kit A/B/C/D (96 RXN) Instruction

- Adapter Plate: There are four plates of UDB PF adapters in total. Each set of 8 adapters are balanced in base distribution.
- UDB Adapters A/B: 8 adapters within same column on the plate are grouped into same set and are balanced in base distribution. There are 12 rows on each plate and thus there are 12 sets of adapters on each plate.
- UDB Adapters C/D: All 96 adapters are within single set in terms of balanced base distribution, and this type of plate must be used in its entirety.

Table 41 MGIEasy UDB PF Adapter Kit A (96 RXN) Adapter Layout

Adapter	1	2	3	4	5	6	7	8	9	10	11	12
A	385	393	401	409	417	425	433	441	449	457	465	473
B	386	394	402	410	418	426	434	442	450	458	466	474
C	387	395	403	411	419	427	435	443	451	459	467	475
D	388	396	404	412	420	428	436	444	452	460	468	476
E	389	397	405	413	421	429	437	445	453	461	469	477
F	390	398	406	414	422	430	438	446	454	462	470	478
G	391	399	407	415	423	431	439	447	455	463	471	479
H	392	400	408	416	424	432	440	448	456	464	472	480

Table 42 MGIEasy UDB PF Adapter Kit B (96 RXN) Adapter Layout

Adapter	1	2	3	4	5	6	7	8	9	10	11	12
A	481	489	497	505	513	521	529	537	545	553	561	569
B	482	490	498	506	514	522	530	538	546	554	562	570
C	483	491	499	507	515	523	531	539	547	555	563	571
D	484	492	500	508	516	524	532	540	548	556	564	572
E	485	493	501	509	517	525	533	541	549	557	565	573
F	486	494	502	510	518	526	534	542	550	558	566	574
G	487	495	503	511	519	527	535	543	551	559	567	575
H	488	496	504	512	520	528	536	544	552	560	568	576

Table 43 MGIEasy UDB PF Adapter Kit C (96 RXN) Adapter Layout

Adapter	1	2	3	4	5	6	7	8	9	10	11	12
A	577	585	593	601	609	617	625	633	641	649	657	665
B	578	586	594	602	610	618	626	634	642	650	658	666
C	579	587	595	603	611	619	627	635	643	651	659	667
D	580	588	596	604	612	620	628	636	644	652	660	668
E	581	589	597	605	613	621	629	637	645	653	661	669
F	582	590	598	606	614	622	630	638	646	654	662	670
G	583	591	599	607	615	623	631	639	647	655	663	671
H	584	592	600	608	616	624	632	640	648	656	664	672

Table 44 MGIEasy UDB PF Adapter Kit D (96 RXN) Adapter Layout

Adapter	1	2	3	4	5	6	7	8	9	10	11	12
A	673	681	689	697	705	713	721	729	737	745	753	761
B	674	682	690	698	706	714	722	730	738	746	754	762
C	675	683	691	699	707	715	723	731	739	747	755	763
D	676	684	692	700	708	716	724	732	740	748	756	764
E	677	685	693	701	709	717	725	733	741	749	757	765
F	678	686	694	702	710	718	726	734	742	750	758	766
G	679	687	695	703	711	719	727	735	743	751	759	767
H	680	688	696	704	712	720	728	736	744	752	760	768

5.2.3 UDB Barcode Pooling Guide

It is recommended to maintain base balance for dual barcode in each lane when sequencing on DNBSEQ or MGISEQ sequencer. **Eight wells of each column are preset as a balanced base dual barcode combinations**. When the samples data amount requirements are the same, follow the Double Barcode Pooling Guide in the table below for samples pooling, and it is suggested to pool at least 8 samples per lane.

Table 45 Dual barcode pooling guide

Plexity	Combinations
8X	X entire columns
8X+1	X entire columns + 1 random well
8X+2	X entire columns + 2 random wells
8X+3	X entire columns + 3 random wells
8X+4	X entire columns + 4 random wells
8X+5	X entire columns + 5 random wells
8X+6	X entire columns + 6 random wells
8X+7	X entire columns + 7 random wells

MGIEasy UDB PF Adapter Kit C/D shall be used in entirety of each plate.

Under exceptional circumstances (for example, one well of barcode missed), when it cannot meet the requirement of at least one balanced barcode combination for standard pooling or the required data amount of each library pooled is not equal, be sure to determine the pooling strategy by calculating the content of each base in each sequencing cycle. It is necessary to ensure that **each base content is not less than 12.5% and is not greater than 62.5%** in single sequencing position in the same lane.

Table 46 Perfect balanced 8 barcode Pooling strategy (8 barcode from one entire column)

Sample 1	A	G	G	A	C	G	T	A	G	A
Sample 2	C	T	G	A	A	C	C	G	A	A
Sample 3	G	A	A	C	G	T	G	T	C	G
Sample 4	T	C	C	G	T	G	A	C	T	C
Sample 5	A	A	T	T	C	A	C	T	G	T
Sample 6	C	C	T	G	A	A	G	G	A	T
Sample 7	T	T	C	C	T	T	A	C	T	G
Sample 8	G	G	A	T	G	C	T	A	C	C
Signal%	**25.0**	**25.0**	**25.0**	**25.0**	**25.0**	**25.0**	**25.0**	**25.0**	**25.0**	**25.0**

Table 47 Unacceptable 9 barcode Pooling strategy (barcodes from different column)

Sample 1	A	G	G	A	C	G	T	A	G	T
Sample 2	A	C	G	A	A	G	G	T	C	C
Sample 3	G	A	A	C	G	T	G	T	C	G
Sample 4	T	C	C	G	T	G	A	C	T	C
Sample 5	A	A	T	T	C	A	C	T	G	T
Sample 6	G	C	T	G	A	A	G	G	A	T
Sample 7	T	G	C	C	T	T	A	C	T	G
Sample 8	G	G	A	T	G	A	T	A	C	C
Sample 9	G	A	C	G	G	T	C	G	A	G
A signal%	33.3	33.3	22.2	22.2	22.2	33.3	22.2	22.2	22.2	**0**
T signal%	22.2	**0**	22.2	22.2	22.2	33.3	22.2	33.3	22.2	33.3
C signal%	**0**	33.3	33.3	22.2	22.2	**0**	22.2	22.2	33.3	33.3
G signal%	44.4	33.3	22.2	33.3	33.3	33.3	33.3	22.2	22.2	33.3

5.2.4 Instructions for PF Adapters-16 (Tube)

Based on the principles of balanced base composition, adapters must be used in specific groups. 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 adapters should not be repeated between samples in one lane.

Table 48 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.

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: 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.
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.

Sample/lane	Instruction (Example)
8+x (x=1-8, Total 9-16)	Perform the following 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. It is recommeded that you eight samples may use adapter set (97-104) and the 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)).

5.2.5 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.

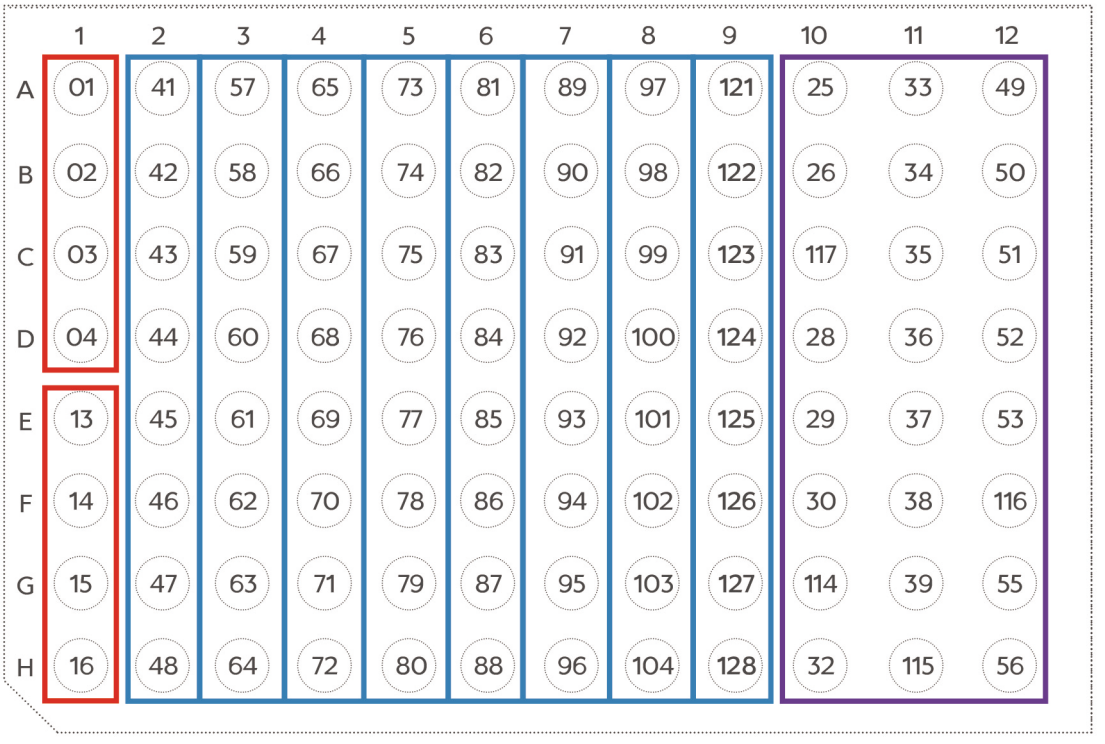

Figure 4 PF adapters-96 (plate) adapters layout and combination instructions

- 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, refer to the table below to organize your barcode adapter combinations.

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

Table 49 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.
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. It is recommeded that you eight samples may use adapter set (97-104) and the 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)).

5.3 Single barcode library preparation user manual

5.3.1 Kits combination of single barcode library preparation

The MGIEasy Fast PCR-FREE FS Library Prep Module V2.0 is also compatible with the MGIEasy PF Adapters-16 (Tube) Kit (Cat. No.: 1000013460, self-prepared) or MGIEasy PF Adapters-96 (Plate) Kit (Cat. No.: 1000013461, self-prepared) to prepare single barcode libraries. See the table below for the kit and component information required for single barcode library preparation.

Table 50 Information of combined kits for PF adapter (16 RXN)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Fast PCR-FREE FS Library Prep Module V2.0 Cat. No.: 940-000885-00	Fast FS Buffer II	Green	215 µL/tube x 1
	Fast FS Enzyme II	Green	105 µL/tube x 1
	Fast Ligation Buffer	Red	450 µL/tube x 1
	Ad Ligase	Red	100 µL/tube x 1
	Ligation Enhancer	Brown	55 µL/tube x 1
	20x Elute Enhancer	Black	7 µL/tube x 1
MGIEasy PF Adapters-16 (Tube) Kit Cat. No.: 1000013460	DNA Adapters	Colorless	5 µL/tube x 16
MGIEasy DNA Clean Beads Cat. No.: 940-001176-00	DNA Clean Beads	White	3.2 mL/tube x 1
	TE Buffer	White	3.2 mL/tube x 1

Table 51 Information of combined kits for PF adapter (96 RXN)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Fast PCR-FREE FS Library Prep Module V2.0 Cat. No.: 940-000883-00	Fast FS Buffer II	Green	1440 µL/tube x 1
	Fast FS Enzyme II	Green	660 µL/tube x 1
	Fast Ligation Buffer	Red	1440 µL/tube x 3
	Ad Ligase	Red	600 µL/tube x 1

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
	Ligation Enhancer	Brown	360 μL/tube x 1
	20x Elute Enhancer	Black	25 μL/tube x 1
	TE Buffer	White	4 mL/tube x 2
MGIEasy PF Adapters-96 (Plate) Kit Cat. No.: 1000013461	DNA Adapters-96 plate	-	5 μL x 96
MGIEasy DNA Clean Beads x 2 Cat. No.: 940-001174-00	DNA Clean Beads	White	15 mL/tube x 1
	TE Buffer	White	17 mL/tube x 1

5.3.2 Applicable sequencing platform of single barcode libraries

Select the appropriate DNB preparation kit, sequencing platform, and sequencing type based on application requirements.

Table 52 Sequencing platform and sequencing type recommendation

Reagent kit	Sequencing platform and type	Recommended application scenarios
MGIEasy Circularization Kit	DNBSEQ-G400 (PE100/PE150) DNBSEQ-T7RS (PE100/PE150) DNBSEQ-T10x4RS (PE100/PE150) DNBSEQ-T20x2RS (PE100)	humans (blood, saliva, oral swabs), animals, plants, fungi, bacteria, and other types of samples.
	DNBSEQ-G50RS (PE100)	Simple Genome (long amplicon DNA, Meta)
DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-SB)	DNBSEQ-G400RS (PE100/PE150) DNBSEQ-T7RS (PE100)	humans (blood, saliva, oral swabs), animals, plants, fungi, bacteria, and other types of samples.
	DNBSEQ-E25RS (SE100)	Simple Genome (long amplicon DNA, Meta)

5.3.3 Single barcode library preparation process

25 ng to 900 ng gDNA sample can be quickly converted to adapter-ligated libraries by fragmentation, end repair, simple purification, adapter ligation and purification. The adapter-ligated libraries can be covernted to ssCir libraries by MGIEasy Circularization Kit (Cat. No.: 1000005259) and then for DNB preparation. Or it can be used for rapid DNB preparation by

DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-DB, Cat. No.: 940-000035-00). Refer to the tables below for different options.

Table 53 Recommend instructions combined with MGIEasy Circularization Kit (Cat. No.: 1000005259)

gDNA amount (N ng)	gDNA input (ng)	Size selection method
200 ≤ N ≤ 500	200-500 (all input)	single-sided size selection
200 < N < 900	500	single-sided size selection
900 ≤ N	900	double-sided size selection

Tips
- Do not pool double-sided size selection library and single-sided size selection library together for sequencing.
- If gDNA input is less than 200 ng, the yield of ssCir is usually insufficient for once sequencing. In this case, library pooling with other PCR-free libraries may be necessary.
- If gDNA input is less than 900 ng, there is a risk of low ssCir yields by double sided size selection purification.

Table 54 Recommend instructions combined with DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-SB, Cat. No.: 940-000035-00)

gDNA amount (N ng)	gDNA input (ng)	Size selection method
25 ≤ N ≤ 200	25-200 (all input)	single-size selection
200 < N < 500	200	single-size selection
500 ≤ N	500	double-size selection

Tips
- Do not pool double-sided size selection library and single-sided size selection library together for sequencing.
- If gDNA input is less than 50 ng, the library yield is usually insufficient for once sequencing. In this case, library pooling with other PCR-free libraries may be necessary.
- If gDNA input is less than 500 ng, there is a risk of low yields by double sided size selection purification.

- **Instructions of single barcode library preparation:**

1. **Reagent Preparation**
   Same as "Reagent preparation" on page 13.
2. **Fragmentation**
   Same as "Fragmentation" on page 15.
3. **Cleanup of fragmentation product**
   Refer to "Cleanup of fragmentation product " on page 18.

- If single-sided size selection is selected for purification, refer to "Single sided size selection (option 1)" on page 18, where step 5 in section 3.3.1.2 is changed to: Remove the tube from the magnetic rack and add **45 μL of En-TE** to elute the DNA. Vortex to mix and centrifuge briefly.
- If double-sided size selection is selected for purification, refer to "Double sided size selection (option 2)" on page 19, where step 8 in section 3.3.2.2 is changed to: Remove the tube from the magnetic rack and add **45 μL of En-TE** to elute the DNA. Vortex to mix and centrifuge briefly.

4. **Adapter ligation**
   Refer to "Adapter ligation" on page 21. Step 1 in section 3.4.2 is changed to: Add **5 μL of adapter** to the corresponding sample tube from step 5 in section 3.3.1.2 or step 8 in section 3.3.2.2 (volume: 47 μL). Vortex it 3 times (3 s each), centrifuge briefly and place on ice.

Tips The single barcode adapter Kit is MGIEasy PF Adapters-16(Tube) Kit (Cat. No.: 1000013460) or MGIEasy PF Adapters-96(Plate) Kit (Cat. No.: 1000013461).

5. **Cleanup of adapter-ligated product**
   Refer to "Cleanup of adapter-ligated product" on page 22. It should be noted that step 1 and step 2 in Section 3.5.2 need to be changed as follows:

- Step 1 of 3.5.2 is changed to: Transfer **15 μL of En-TE** to each sample tube from step 5 in section 3.4.2 (volume 80 μL).
- Step 2 of 3.5.2 is changed to: Add **35 μL of En-Beads** to feach sample tube from step 5 in section 3.4.2. Mix by vortex thoroughly.

6. **QC of ligation product**
   Same as "QC of ligation product" on page 24.

- **Instruction of DNB preparation:**

- **Circularization and DNB preparation**
  The adapter-ligated library can be covernted to ssCir library by MGIEasy Circularization Kit (Cat. No.: 1000005259). Then DNB should be prepared using a sequencing reagent set that matches the sequencing platform. Refer to "Circularization and DNB preparation (option 1)" on page 25. Except for the change of the kit used, the operations remain unchanged.
- **Onestep DNB preparation V2.0**
  DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-SB, Cat. No.: 940-000035-00) is required for rapid DNB preparation. According to the different size selection methods, select the correct input of DNB preparation. Refer to "Onestep DNB preparation V2.0 (option 2)" on page 30. Except for the change of the kit used, the operations remain unchanged.