Part No.:H-940-001195-00-01

User Manual

Version:1.0

MGIEasy Fast FS Library Prep Set

Cat. No.: 940-001193-00 (16 RXN)
940-001194-00 (96 RXN)
940-001196-00 (192 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	December 2023	Initial release

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

Contents

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1 Product overview

1.1 Introduction

The MGIEasy Fast FS Library Prep Set V2.0 is designed to prepare WGS libraries for MGI high-throughput sequencing platforms. This library prep set is optimized to convert 1 ng - 1000 ng genomic DNA (gDNA) into a customized library using high-quality fast fragmentase to simplify the operation process, significantly shortening the DNA library preparation duration. All reagents provided in this set have passed strict quality control and functional verification procedures, ensuring stability and reproducibility.

The MGIEasy Fast FS Library Prep Set V2.0 uses dual barcode for library preparation. It is recommended to prepare 8 or more samples with this set due to the adapter design. For fewer than 8 samples, please contact MGI Technical Support. The constructed libraries (PCR dsDNA) can be used with the MGIEasy Dual Barcode Circularization Kit (not included in this set. Cat. No.: 1000020570) to create single-strand circular (ssCir) DNA libraries for subsequent DNB preparation. After DNB preparation, it can be directly sequenced on DNBSEQ high-throughput sequencing platforms. Or it can be combined with DNBSEQ Onestep DNB Make Reagent Kit (OS-DB) (not included in this set. Cat. No.: 1000026466) or DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-DB) (not included in this set. Cat. No.: 940-000036-00) for rapid DNB preparation.

1.2 Intended use

This library prep set is applicable to samples from human (including but not limited to blood, saliva, and oral swabs), animals (including but not limited to rat ,and mouse), plants (including but not limited to A. thaliana, and O. sativa), bacteria (including but not limited to E. coli), fungi, and other microbial species.

1.3 Applicable sequencing platforms

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

Table 1 Sequencing platform and sequencing type recommendation

Reagent kit	Sequencing platform and type
MGIEasy Dual Barcode Circularization Kit	All MGI sequencing platform
DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-DB)	DNBSEQ-G400RS, DNBSEQ-T7RS (Except PE150), DNBSEQ-E25
DNBSEQ Onestep DNB Make Reagent Kit (OS-DB)	DNBSEQ-G99, MGISEQ-200RS

Tips Do not use the reagents in the sequencing set for DNB preparation if DNBs have been prepared using the DNBSEQ Onestep DNB Make Reagent Kit.

1.4 Components

This library prep set comes in three specifications: 16 RXN, 96 RXN and 192 RXN. Refer to Table 2, 3, and 4 for component information.

Table 2 MGIEasy Fast FS Library Prep Set V2.0 (16 RXN) (Cat. No.: 940-001193-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Fast FS Library Prep Module V2.0 Cat. No.: 940-001197-00	Fast FS Buffer II	Green	215 μL/tube × 1
	Fast FS Enzyme II	Green	105 μL/tube × 1
	Fast Ligation Buffer	Red	450 μL/tube × 1
	Ad Ligase	Red	100 μL/tube × 1
	Ligation Enhancer	Brown	55 μL/tube × 1
	20x Elute Enhancer	Black	7 μL/tube × 1
	PCR Enzyme Mix	Blue	460 μL/tube × 1
MGIEasy UDB Primers Adapter Kit Cat. No.: 1000022800	UDB Adapter	White	80 μL/tube × 1
	UDB PCR Primer Mix-57-64, 89-96	Blue	12 μL/tube × 16
MGIEasy DNA Clean Beads Cat. No.: 940-001176-00	DNA Clean Beads	White	3.2 mL/tube × 1
	TE Buffer	White	3.2 mL/tube × 1

Table 3 MGIEasy Fast FS Library Prep Set V2.0 (96 RXN) (Cat. No.: 940-001194-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Fast FS Library Prep Module V2.0 Cat. No.: 940-001195-00	Fast FS Buffer II	Green	1440 μL/tube × 1
	Fast FS Enzyme II	Green	660 μL/tube × 1
	Fast Ligation Buffer	Red	1440 μL/tube × 3
	Ad Ligase	Red	600 μL/tube × 1
	Ligation Enhancer	Brown	360 μL/tube × 1
	20x Elute Enhancer	Black	25 μL/tube × 1
	PCR Enzyme Mix	Blue	1400 μL/tube × 2
MGIEasy UDB Primers Adapter Kit B Cat. No.: 1000022802	UDB Adapter	White	480 μL/tue × 1
	UDB PCR Primer Mix-97-192	/	12 μL/well × 96
MGIEasy DNA Clean Beads Cat. No.: 940-001174-00	DNA Clean Beads	White	15 mL/tube × 1
	TE Buffer	White	17 mL/tube × 1

Table 4 MGIEasy Fast FS Library Prep Set V2.0 (192 RXN) (Cat. No.: 940-001196-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Fast FS Library Prep Module V2.0 x 2 Cat. No.: 940-001195-00	Fast FS Buffer II	Green	1440 µL/tube × 1
	Fast FS Enzyme II	Green	660 µL/tube × 1
	Fast Ligation Buffer	Red	1440 µL/tube × 3
	Ad Ligase	Red	600 µL/tube × 1
	Ligation Enhancer	Brown	360 µL/tube × 1
	20x Elute Enhancer	Black	25 µL/tube × 1
	PCR Enzyme Mix	Blue	1400 µL/tube × 2
MGIEasy UDB Primers Adapter Kit A Cat. No.: 1000022801	UDB Adapter	White	480 µL/tube × 1
	UDB PCR Primer Mix-01-96	/	12 µL/well × 96
MGIEasy UDB Primers Adapter Kit B Cat. No.: 1000022802	UDB Adapter	White	480 µL/tube × 1
	UDB PCR Primer Mix-97-192	/	12 µL/well × 96
MGIEasy DNA Clean Beads x 2 Cat. No.: 940-001174-00	DNA Clean Beads	White	15 mL/tube × 1
	TE Buffer	White	17 mL/tube × 1

1.5 Storage and transportation

Table 5 Kit storage and transportation

Modules	Cat. No.	Storage temperature	Transport temperature
MGIEasy Fast FS Library Prep Module V2.0	940-001197-00	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
MGIEasy Fast FS Library Prep Module V2.0	940-001195-00		
MGIEasy UDB Primers Adapter Kit	1000022800		
MGIEasy UDB Primers Adapter Kit A	1000022801		
MGIEasy UDB Primers Adapter Kit B	1000022802		
MGIEasy DNA Clean Beads	940-001176-00	2 ℃ to 8 ℃	2 ℃ to 8 ℃
MGIEasy DNA Clean Beads	940-001174-00		

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 lives.
- In MGIEasy Fast FS Library Prep Module V2.0, 20x Elute Enhancer and Ligation Enhancer should be stored at room temperature. Avoid 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
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

Tips Prepare reagent kits based on application requirements.

Table 7 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	General lab supplier
Desktop centrifuge	General lab supplier
Pipettes	General lab supplier
Thermocycler	General lab supplier
96M Magnum Plate	ALPAQUA, Part A000400 (Recommended)
Qubit® Fluorometer 2.0 or higher	Thermo Fisher (Cat. No.: Q33216), or equivalent
Agilent 2100 Bioanalyzer or Tape station	Agilent Technologies (Cat. No.: G2939AA), Tape station, or equivalent

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)	Fisher BioReagents™ (Cat. No.: BP2818500), or equivalent
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 or Tape station High sensitive DNA kit	Agilent (Cat. No.: 5067-4626), or equivalent
Pipette tips	Axygen, or equivalent
1.5 mL tube	Axygen, or equivalent
0.2 mL PCR tube or 96-well plate	Axygen (Cat. No.: PCR-02-C or PCR-96M2-HS-C), or equivalent

Reagent/Consumable	Recommended brand
Qubit Assay Tubes or 0.5 mL Thin Wall PCR Tubes	Invitrogen (Cat. No.: Q32856) or Axygen (Cat. No.: PCR-05-C), or equivalent

1.7 Precautions

1.7.1 MGIEasy UDB Primers Adapter Kit Instructions

This set is designed to construct dual barcode libraries only, and the barcode sequences are designed to be located on the UDB PCR Primer sequences. When multiple samples are mixed for sequencing, barcodes can be used to accurately attribute the sequencing results to the appropriate samples. To meet the requirements for batch processing of library construction and multiplex sequencing, the best primer combinations were selected based on the principle of balanced base composition.

Based on the principles of balanced base composition, primers must be used in specific groups. Follow the instructions below to use the primers in the proper combinations. UDB Primers Adapter Reagent Kits have three specifications, depending on the number of reactions.

Table 9 The MGIEasy UDB Primers Adapter of the MGIEasy Fast FS Library Prep Set

Model	Reagent Kits	Note
16 RXN	MGIEasy UDB Primers Adapter Kit	2 groups, each with 8 barcodes
96 RXN	MGIEasy UDB Primers Adapter Kit B	12 groups, each with 8 barcodes
192 RXN	MGIEasy UDB Primers Adapter Kit A	12 groups, each with 8 barcodes
	MGIEasy UDB Primers Adapter Kit B	12 groups, each with 8 barcodes

For specific Barcode coding information refer to “MGIEasy UDB Primers Kit’s barcode number and sequence information” on page 35.

1.7.1.1 Note for UDB Adapter and UDB PCR Primer Mix

- UDB Adapter is double-stranded. To prevent structure changes that might affect performance, such as denaturation, do not place the adapters in an area that exceeds 30 ℃.
- The UDB Adapter and UDB PCR Primer Mix must be mixed and centrifuged before being used to collect any liquid at the bottom of the tube or plate.
- For tubes, gently remove the cap to prevent liquid from spilling and cross-contamination. Cover the tube immediately after use.
- For 96-well plates, spray 75% alcohol and wipe the surface of the aluminum film of the plate with absorbent wipes. The aluminum film is penetrable. Do not touch the surface of the aluminum film with sharp objects. Pierce the aluminum film to pipette solutions for first-time use. After use, separately transfer the remaining reagents to 1.5 mL centrifuge tube(s) or 0.2 mL PCR tube(s). Label the tubes clearly, and store them at -20 ℃.
- To prevent cross contamination, change tips when pipetting different solutions.

1.7.1.2 UDB PCR Primer Mix pooling guide

It is recommended to optimize the base balance by planning UDB PCR Primer with diverse sequences when pooling libraries across DNBSEQ systems. Pooling combines at least eight libraries to sequence in one lane.

The following three application scenarios are predefined for the recommended method of selecting UDB PCR Primer.

1. The sequencing data output requirement is the same for all samples in one lane. Choose the UDB PCR Primer combinations in Table 10.

Tips Here X means positive integer. For example: 8X = 8 multiplied by X, which means there are 8X samples.

Table 10 UDB PCR Primer Mix Pooling Guide

Sample/ lane	Instruction (Example)
8X	Add 1 UDB PCR Primer per sample, from X set of 8 UDB PCR Primers (X column total) For example: X is equal to 1. The selected UDB PCR Primer is 57-64. Add UDB PCR Primer 57 to sample 1, UDB PCR Primer 58 to sample 2, … UDB PCR Primer 64 to sample 8
8X+1	Add X set of 8 UDB PCR Primers + 1 random well of UDB PCR Primer
8X+2	Add X set of 8 UDB PCR Primers + 2 random wells of UDB PCR Primers
8X+3	Add X set of 8 UDB PCR Primers + 3 random wells of UDB PCR Primers
8X+4	Add X set of 8 UDB PCR Primers + 4 random wells of UDB PCR Primers
8X+5	Add X set of 8 UDB PCR Primers + 5 random wells of UDB PCR Primers
8X+6	Add X set of 8 UDB PCR Primers + 6 random wells of UDB PCR Primers
8X+7	Add X set of 8 UDB PCR Primers + 7 random wells of UDB PCR Primers

2. Under exceptional circumstances (for example, insufficient reagents for a well), when it cannot meet the requirement of at least one balanced barcode combination for standard pooling, or if 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 contentis not less than 12.5% and is not more than 62.5%in single sequencing position in the same lane.

Table 11 Balanced 8 UDB PCR Primer pooling strategy (8 UDB PCR Primer from one entire column)

	Position of base in adapter sequence									
	Base 1	Base 2	Base 3	Base 4	Base 5	Base 6	Base 7	Base 8	Base 9	Base 10
Primer 1	A	G	G	A	C	G	T	A	G	A
Primer 2	C	T	G	A	A	C	C	G	A	A
Primer 3	G	A	A	C	G	T	G	T	C	G
Primer 4	T	C	C	G	T	G	A	C	T	C
Primer 5	A	A	T	T	C	A	C	T	G	T
Primer 6	C	C	T	G	A	A	G	G	A	T
Primer 7	T	T	C	C	T	T	A	C	T	G
Primer 8	G	G	A	T	G	C	T	A	C	C
Signal % per base	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0

Table 12 Unbalanced 9 UDB PCR Primer pooling strategy (UDB PCR Primer from different columns)

	Position of base in adapter sequence									
	Base 1	Base 2	Base 3	Base 4	Base 5	Base 6	Base 7	Base 8	Base 9	Base 10
Primer 1	A	G	G	A	C	G	T	A	G	T
Primer 2	A	C	G	A	A	G	G	T	C	C
Primer 3	G	A	A	C	G	T	G	T	C	G
Primer 4	T	C	C	G	T	G	A	C	T	C
Primer 5	A	A	T	T	C	A	C	T	G	T
Primer 6	G	C	T	G	A	A	G	G	A	T
Primer 7	T	G	C	C	T	T	A	C	T	G
Primer 8	G	G	A	T	G	A	T	A	C	C
Primer 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

1.7.2 Other 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 to use pipette tips with filters to prevent cross-contamination. Use a new tip each time for pipetting different solutions or samples.
- It is recommended to 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. 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**.

2 Sample preparation

2.1 Sample type

This library prep set is applicable to samples from human (including but not limited to blood, saliva, and oral swabs), animals (including but not limited to rat ,and mouse), plants (including but not limited to A. thaliana, and O. sativa), bacteria (including but not limited to E. coli), fungi, and other microbial species. Different types of samples should be performed in a fragmentation demo test before library preparation 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. If the sample purity does not satisfy the recommended standards, or if enzyme inhibitors are present, there is a risk of low library yield. Because Fast FS Enzyme II is sensitive to pH and components of DNA storage buffer, it is recommended to use TE Buffer (pH 8.0) for DNA dissolution.

Tips

- 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 21 on page 15.
- If the sample contains many impurities and inhibitors, it is recommended to re-purify the sample DNA with 1.8x magnetic beads and elute it with TE Buffer (pH 8.0). After repurification, perform a demo fragmentation test by adjusting the incubation time to 30 ℃ in Table 21 on page 15.

2.3 Sample input

1 ng - 1000 ng gDNA can be used for library preparation. If the amount of genomic DNA is sufficient, it is recommended to use a high input of genomic DNA for library preparation. Qubit or BMG is recommended for the quantification of sample concentrations.

3 Library preparation protocol

3.1 Workflow

Table 13 Time consumption of workflow

Workflow	Hands-on time (one reaction)	Actual time taken (one reaction)
Fragmentation	2 min	31 min
Cleanup of fragmentation product	1 - 2 min	7 - 13 min
Adapter ligation	2 min	12 min
Cleanup of adapter-ligated product ⏸	5 min	18 min
PCR	2 min	30 min
Cleanup of PCR product ⏸	5 min	18 min
QC of PCR product ⏸	2 min	4 min
Total	19 -20 min	120 - 126min

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

The PCR product can be converted to DNB in three ways:

1. Use the MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570) to convert dsDNA library product to ssCir and prepare DNBs by using the relevant High-throughput Sequencing kit. Refer to “Circularization and DNB preparation (option 1)” on page 27.
2. Use the DNBSEQ One-Step DNB Prep Kit V2.0 (OS-DB) (Cat. No.: 940-000036-00) to prepare DNBs directly. Refer to “Onestep DNB preparation V2.0 (option 2)” on page 32.
3. Use the DNBSEQ One-Step DNB Prep Kit (OS-DB) (Cat. No.: 1000026466) to prepare DNBs directly. Refer to “Onestep DNB preparation V1.0 (option 3)” on page 34.

3.2 Reagent preparation

3.2.1 Preparation

Table 14 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	

3.2.2 Operation

CAUTION The preparation volume of reagents listed below 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 following table. Mix it by vortexing, and centrifuge briefly. Store at room temperature before using. The shelf life of the 1x Elute Enhancer is 7 days.

Table 15 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 following table. Mix it by vortexing, and centrifuge briefly. Store at 4 ℃ before using. The shelf life of the En-TE is 60 days.

Table 16 En-TE

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

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

Table 17 En-Beads

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

3.3 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.

3.3.1 Preparation

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

Table 18 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; place on ice
Fast FS Enzyme II	Keep on ice
80% ethanol	User-supplied; freshly prepared
En-TE	Refer to Table 16; place at RT
En-Beads	Refer to Table 17; allow 30 min to equilibrate to RT before use; mix thoroughly by vortexing before each use

3.3.2 Fragmentation

1. Normalize gDNA. Refer to the following table. Based on the sample concentration, transfer the appropriate gDNA (recommended 1 ng - 1000 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 19 Normalization of gDNA dissolved in TE (pH 8.0)

Components	Volume
TE Buffer (pH 8.0)	45-X µL
gDNA (1 ng - 1000 ng)	X µL
Total	45 µL

Tips It is recommended that the normalization buffer should be the same as DNA elution buffer.

2. Set the thermal cycler program according to Table 21 on page 15. Run the program to allow the reaction block to cool to 4 ℃. Hold the program at this step until the fragmentation mixture has been prepared and added to the sample.
3. Mix the Fast FS Enzyme II by inverting 10 times and flicking the bottom of the tube(s) gently. Ensure that no residual reagent is left at the bottom each time. Centrifuge briefly, and place it on ice until use.

CAUTION DO NOT vortex the Fast FS Enzyme II. Strictly follow the manual instructions. Insufficient mixing will 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 20 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 tube(s) into the thermocycler. Skip the first step (4 ℃ Hold) to start the reaction.

Table 21 Fragmentation reaction conditions (Volume: 60 µL)

Temperature	Time
70 ℃ Heated lid	On
4 ℃	Hold
30 ℃	Refer to Table 22
65 ℃	15 min
4 ℃	Hold

The appropriate incubation time at 30 ℃ is shown in the following table.

Table 22 The incubation time for different gDNA input

gDNA input	Incubation time	Size selection method
1000 ng	12 min	Double size selection
500 ng	12 min	Double size selection
200 ng	12 min	Single size selection
100 ng	13 min	Single size selection
50 ng	16 min	Single size selection
25 ng	16 min	Single size selection
10 ng	18 min	Single size selection
5 ng	20 min	Single size selection
1 ng	22 min	Single size selection

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

CAUTION DO NOT STOP AT THIS STEP.

3.4 Cleanup of fragmentation product

Tips Select the appropriate fragment screening method according to "Table 22 The incubation time for different gDNA input" on page 16. Choose single size selection or double size selection of beads according to different gDNA inputs. The PCR products from this library construction process range from 300 bp - 2000 bp.

- When single size selection is selected, the peak size of the single size product is approximately 500 bp - 750 bp.
- When double size selection is selected, the peak size of the double size product is approximately 450 bp - 550 bp.

3.4.1 Single size selection (option 1)

3.4.1.1 Preparation

Table 23 Preparing the reagents

Reagent	Requirement
En-TE	Refer to Table 16; place at RT
En-Beads	Refer to Table 17; allow 30 min to equilibrate to RT before use; mix thoroughly by vortexing before each use

3.4.1.2 Size selection

1. Check the volume of the fragmentation product (from step 7 in section 3.3.2). 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 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 45 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 section 3.5.

3.4.2 Double size selection (option 2)

3.4.2.1 Preparation

Table 24 Preparing the reagents

Reagent	Requirement
En-TE	Refer to Table 16; place at RT
En-Beads	Refer to Table 17; allow 30 min to equilibrate to RT before use; mix thoroughly by vortexing before each use

3.4.2.2 Size selection

1. Check the volume of the fragmentation product (from step 7 in section 3.3.2). 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 36 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 96 μL of supernatant to a new 0.2 mL PCR tube.

   Tips In this step, keep the supernatant and discard the beads.

5. Add 12 μL of En-Beads to each sample tube (from step 4, volume: 96 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 45 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 section 3.5.

3.5 Adapter ligation

Tips Barcodes are in the primers. Read "Precautions" on page 7 carefully before operation.

3.5.1 Preparation

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

Table 25 Preparing the reagents

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

3.5.2 Adapter ligation

1. Dilute the UDB Adapter with TE Buffer (pH 8.0) based on gDNA input.

Table 26 Recommended adapter usage and dilutions for different amounts of gDNA input

gDNA input (*N* ng)	Dilution of UDB Adapter	Volume after dilution
50< N ≤ 1000	No Dilution	5 μL
25	2 x	5 μL
10	5 x	5 μL
5	10 x	5 μL
1	50 x	5 μL

2. Add 5 μL of UDB Adapter to the corresponding sample tube (from step 5 in section 3.4.1.2 or step 8 in section 3.4.2.2, volume: 45 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 27 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 to prepare the adapter ligation mixture while waiting for cleanup of fragmentation product. 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. Pipette slowly and carefully.

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

Table 28 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 section 3.6.

3.6 Cleanup of adapter-ligated product

3.6.1 Preparation

Table 29 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared
En-TE	Refer to Table 16; place at RT
En-Beads	Refer to Table 17; allow 30 min to equilibrate to RT before use; mix thoroughly by vortexing before each use

3.6.2 Cleanup of adapter-ligated product

1. Add 22 μL of En-TE to each sample tube (from step 6 in section 3.5.2, 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. 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 20 μL of En-TE to elute the DNA. Mix with a vortexer until all beads are suspended.
9. Incubate the tube(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 19 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 °C.

3.7 PCR

3.7.1 Preparation

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

Table 30 Preparing the reagents

Reagent	Requirement
PCR Enzyme Mix	Thaw at RT; mix by vortexing; centrifuge briefly; place on ice
UDB PCR Primer Mix	Thaw at RT; mix by vortexing; centrifuge briefly; place at RT

3.7.2 PCR

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

Table 31 PCR mixture

Reagent	Volume per reaction
PCR Enzyme Mix	25 μL
UDB PCR Primer Mix	6 μL
Total	31 μL

2. Add 31 μL of PCR mixture to each sample tube (from step 10 in section 3.6.2). Vortex the tube(s) 3 times (3 sec each) and centrifuge briefly to collect the liquid to the bottom of the tube.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 32 PCR reaction conditions (Volume: 50 μL)

Temperature	Time	Cycles
105 ℃ Heated lid	On	-
95 ℃	3 min	1
98 ℃	20 sec	Refer to Table 33
60 ℃	15 sec	
72 ℃	30 sec	
72 ℃	10 min	1
4 ℃	Hold	-

The number of PCR cycles is shown in the following table.

Table 33 PCR cycles required to yield 300 ng, 600 ng, and 1000 ng of libraries

gDNA input	PCR cycles required for corresponding yield		
	300 ng	600 ng	1000 ng
1000 ng	/	3	4 ~ 5
500 ng	3	4 ~ 5	5 ~ 6
200 ng	3	4 ~ 5	5 ~ 6
100 ng	4 ~ 5	5 ~ 6	6 ~ 7
50 ng	5 ~ 6	6 ~ 7	7 ~ 8
25 ng	6 ~ 7	7 ~ 8	8 ~ 9
10 ng	7 ~ 8	8 ~ 9	9 ~ 10
5 ng	8 ~ 10	9 ~ 11	/
1 ng	11 ~ 12	12 ~ 14	/

Tips The number of PCR cycles should be strictly controlled.

- Insufficient cycles may lead to a reduced library yield.
- Excessive cycles may also lead to adverse effects such as over amplification, an increase in bias, PCR duplicates, chimeric sequences, and accumulated mutations.

The indicated table shows the number of PCR cycles required to yield 300 ng, 600 ng, and 1000 ng of libraries from 1-1000 ng of high-quality gDNA sample. For lower quality, longer DNA fragments, or if the recommended cycle number fails to achieve the ideal yield, PCR cycles should be increased appropriately to generate sufficient yield.

4. When the program is completed, centrifuge the tube(s) briefly.

3.8 Cleanup of PCR product

3.8.1 Preparation

Table 34 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
En-TE	Refer to Table 16; place at RT.
En-Beads	Refer to Table 17; allow 30 min to equilibrate to RT before use; mix thoroughly by vortexing before each use.

3.8.2 Cleanup of PCR product

1. Mix the En-Beads thoroughly. Add 38 μL of En-Beads to each sample tube. Mix with a vortexer until all beads are suspended.
2. Incubate the sample tube(s) at room temperature for 5 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 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.
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 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 32 μL of En-TE to elute the DNA. Mix with a vortexer until all beads are suspended.
8. Incubate the tube(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 30 μL of supernatant to a new 0.2 mL PCR tube.

   Stop point After cleanup, the PCR product can be stored at -20 °C.

3.9 QC of PCR product

- **dsDNA fluorescence quantification method**: Quantify the purified PCR products with dsDNA fluorescence assay kits and instructions.
- **Electrophoresis method**: Assess the size range of purified PCR products with electrophoresis based equipment and instructions.

Table 35 Different QC methods and standards for library

Method	Equipment/Reagent
dsDNA fluorescence quantification method	Qubit dsDNA HS Assay Kit, Quant-iT PicoGreen dsDNA Assay Kit, or equivalent
Electrophoresis method	Tapestation (Agilent Technologies), Bioanalyzer, LabChip GX, GXII, GX Touch (PerkinElmer), Fragment Analyzer (Advanced Analytical), or equivalent

1. If the MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570) is used, the required yield for PCR products is ≥ 300 ng. For pooled sequencing, please follow pooling instructions provided by the MGIEasy UDB Primers Adapter Kit. After quantification, different PCR products were mixed. The total yield after pooling should be ≥ 300 ng, with a concentration of ≥ 6.25 ng/μL.
2. If the DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-DB) (Cat. No.: 940-000036-00) is used, the PCR product required for each DNB preparation is 45 ng. For pooled sequencing, please follow instructions provided by MGIEasy UDB Primers Adapter Kit. After quantification, different PCR products were mixed. The total yield after pooling should be ≥ 45 ng, with a concentration of ≥ 2.25 ng/μL.
3. The Agilent 2100 Bioanalyzer can be used to analyze the fragment distribution of PCR-purified products. The PCR products from this library construction process range from 300 to 2000 bp, with bead single size selection for peaks of 500 to 750 bp, and bead double size selection for peaks of 450 to 550 bp.

Tips
- Double barcode PCR adapter length plus primer length is 131 bp
- Single barcode PCR adapter length plus primer length is 84 bp.

The Figure 1 and Figure 2 shows the Agilent 2100 Bioanalyzer detection results of purified PCR products.

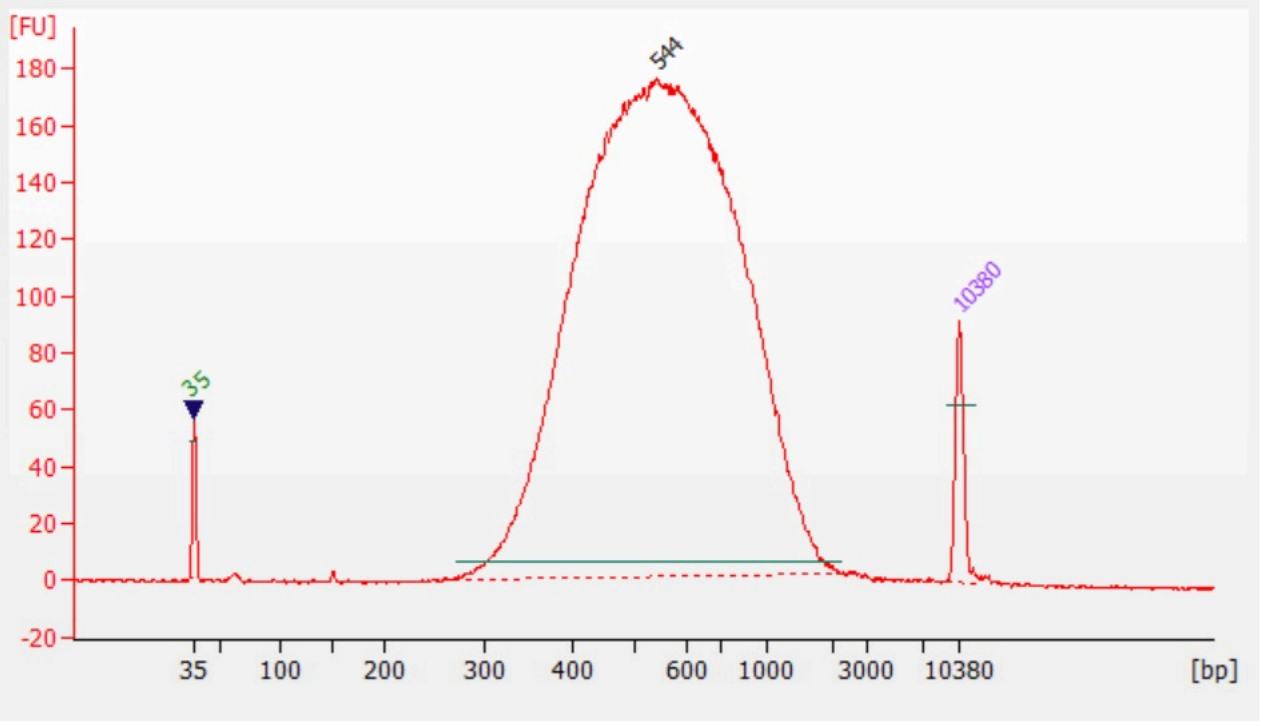

Figure 1 Agilent 2100 Bioanalyzer Image of PCR Products with 200 ng of genome input

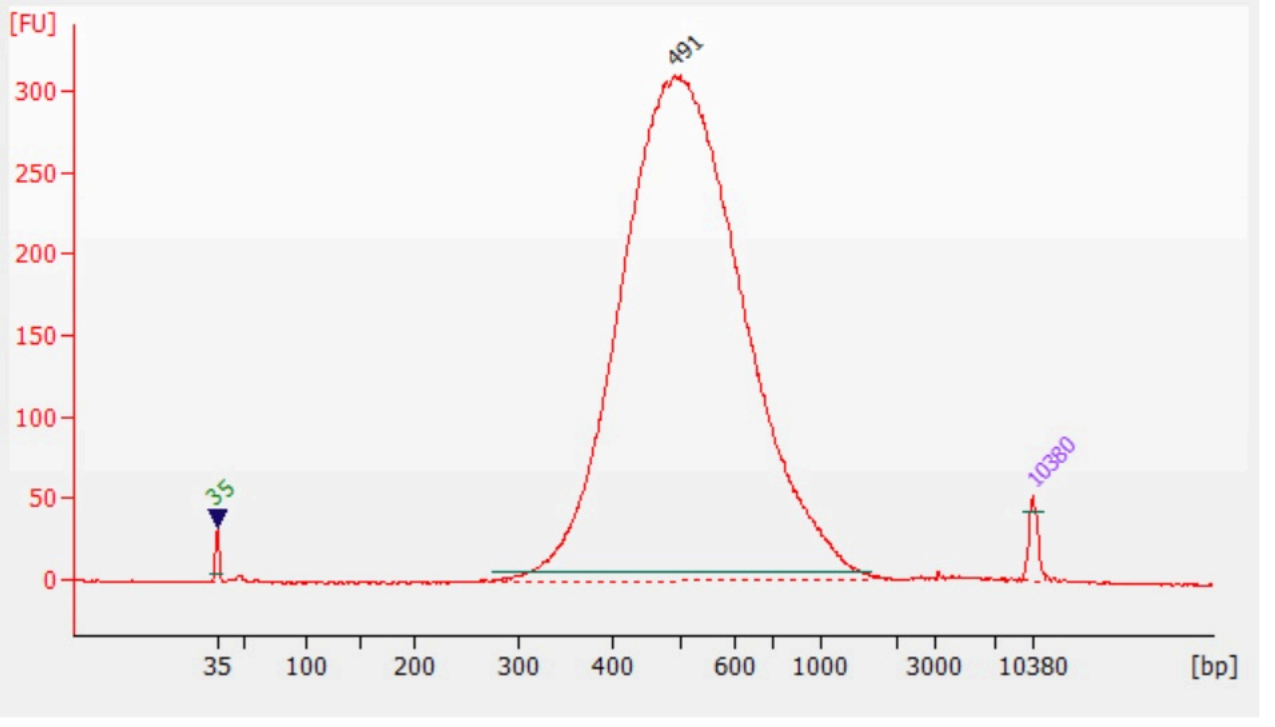

Figure 2 Agilent 2100 Bioanalyzer Image of PCR Products with 500 ng of genome input

4 DNB preparation protocol

The PCR product can be converted 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. Afterward, DNB preparation can be performed using the reagent kit appropriate for the preferred sequencing platform.

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

4.1.1 Denaturation and single-stranded circularization

4.1.1.1 Preparation

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

Table 36 Preparing the reagents

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

4.1.1.2 Denaturation

1. Based on the PCR products concentration, add 300 ng of PCR products (from step 9 in section 3.8.2) 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.
2. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 37 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-stranded 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 38 Circularization reaction mixture

Reagent	Volume per reaction
Dual Barcode Splint Buffer	11.6 μL
DNA Rapid Ligase	0.5 μL
Total	12.1 μL

2. Add 12.1 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 39 Single-stranded DNA circularization reaction conditions (Volume: 60 μL)

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

Tips Prepare the "Table 41 Digestion mixture" on page 29 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 40 Preparing the reagents

Reagent	Requirement
Digestion Buffer	Thaw at RT; mix thoroughly; centrifuge briefly; place on ice
Digestion Enzyme	Flick and/or invert the tube gently; centrifuge briefly; place on ice
Digestion Stop Buffer	Thaw at RT; mix thoroughly; centrifuge briefly; 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 41 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 then place on ice.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 42 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

4.1.3.1 Preparation

Table 43 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; allow 30 min to equilibrate to RT before use; 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 30 days.

4.1.4 QC of digestion product

Quantify the ssCir with Qubit ssDNA Assay Kit. The final Enzymatic Digestion products (ssDNA, ng) / input products of PCR (dsDNA, 300 ng) should be ≥ 7%.

4.1.5 DNB preparation

Prepare DNBs according to the user manual for the appropriate sequencing platform.

4.2 Onestep DNB preparation V2.0 (option 2)

The library prepared by MGIEasy Fast FS DNA Library Prep Set is used for rapid DNB preparation with DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-DB) (Cat. No.: 940-000036-00).

4.2.1 Preparation

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

Table 44 Preparing the reagents

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

4.2.2 Operation

1. Based on the PCR products concentration, add 45 ng of PCR products 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 45 DNB making system 1

Reagent	Volume per reaction
PCR products (45 ng)	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 46 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 47 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 48 DNB reaction condition 2 (Volume: 84 μL)

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

Tips
- Thermocyclers from some manufacturers take an extended time to achieve the desired temperature of the heated lids. For these types of thermocyclers, 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 the 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 violently.

7. Quantify the DNB with ssDNA Fluorescence Assay Kits, such as Qubit ssDNA Assay Kit. A volume of 2 μL DNB is suggested to be measured. The DNB is qualified when the concentration is more than 4 ng/μL.

4.3 Onestep DNB preparation V1.0 (option 3)

Based on the PCR products concentration, 30 ng PCR products (from step 9 in section 3.8.2) 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 "DNB reaction condition 2" is 20 min.

5 Appendix

5.1 MGIEasy UDB Primers Kit's barcode number and sequence information

Tips For detailed sequence information of each barcode, please reach out to our Technical Support team at: **MGI-service@mgi-tech.com.**

5.1.1 Instructions for UDB Primers Kit (16 RXN)

Based on the principles of balanced base composition, UDB PCR Primer Mix must be used in specific groups. Follow the instructions to use UDB PCR Primer Mix in proper combination:

This kit contains 16 Adapters grouped into 2 sets:

- UDB PCR Primer Mix-57 to UDB PCR Primer Mix-64 (see the blue box in the Figure 3)
- UDB PCR Primer Mix-89 to UDB PCR Primer Mix-97 (see the red box in the Figure 3)

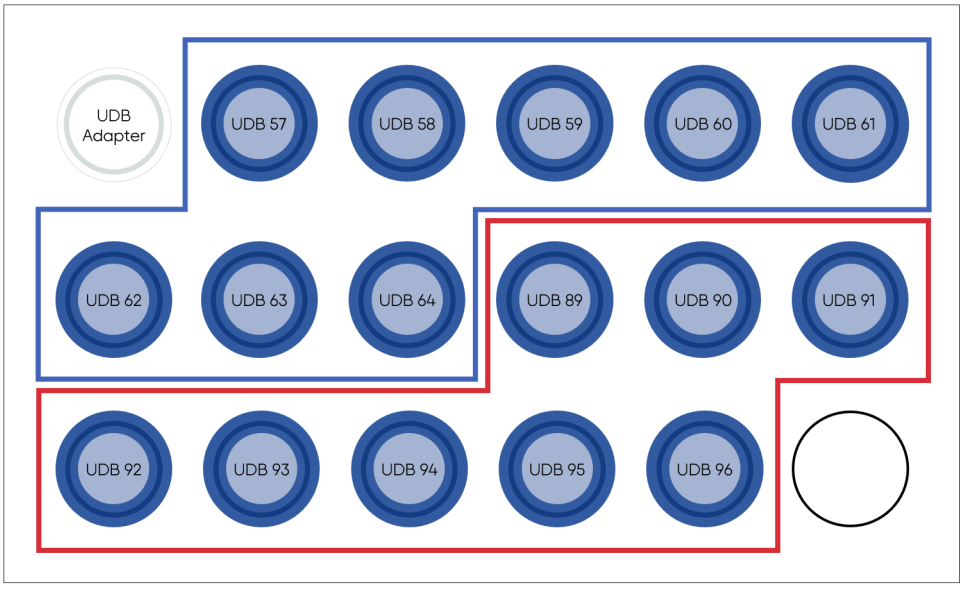

Figure 3 MGIEasy UDB Primers Adapter Kit (16 RXN) layout and combination instructions

5.1.2 Instructions for UDB Primers Adapter Kit A/B (96 RXN)

There is 1 plate of UDB PCR Primer Mix in Set A, and Set B. Each plate contains 96 UDB PCR Primer Mix, and 8 wells of each column are preset as a balanced dual barcode combination. Check the detailed layout in the following Table 49 and Table 50.

Tips UDB PCR Primer Mix in columns 8 and 12 of Set A, same as MGIEasy UDB Primers Adapter Kit (16 RXN).

Table 49 Set A barcode layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDB1	UDB9	UDB17	UDB25	UDB33	UDB41	UDB49	**UDB57**	UDB65	UDB73	UDB81	**UDB89**
B	UDB2	UDB10	UDB18	UDB26	UDB34	UDB42	UDB50	**UDB58**	UDB66	UDB74	UDB82	**UDB90**
C	UDB3	UDB11	UDB19	UDB27	UDB35	UDB43	UDB51	**UDB59**	UDB67	UDB75	UDB83	**UDB91**
D	UDB4	UDB12	UDB20	UDB28	UDB36	UDB44	UDB52	**UDB60**	UDB68	UDB76	UDB84	**UDB92**
E	UDB5	UDB13	UDB21	UDB29	UDB37	UDB45	UDB53	**UDB61**	UDB69	UDB77	UDB85	**UDB93**
F	UDB6	UDB14	UDB22	UDB30	UDB38	UDB46	UDB54	**UDB62**	UDB70	UDB78	UDB86	**UDB94**
G	UDB7	UDB15	UDB23	UDB31	UDB39	UDB47	UDB55	**UDB63**	UDB71	UDB79	UDB87	**UDB95**
H	UDB8	UDB16	UDB24	UDB32	UDB40	UDB48	UDB56	**UDB64**	UDB72	UDB80	UDB88	**UDB96**

Table 50 Set B barcode layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDB97	UDB105	UDB113	UDB121	UDB129	UDB137	UDB145	UDB153	UDB161	UDB169	UDB177	UDB185
B	UDB98	UDB106	UDB114	UDB122	UDB130	UDB138	UDB146	UDB154	UDB162	UDB170	UDB178	UDB186
C	UDB99	UDB107	UDB115	UDB123	UDB131	UDB139	UDB147	UDB155	UDB163	UDB171	UDB179	UDB187
D	UDB100	UDB108	UDB116	UDB124	UDB132	UDB140	UDB148	UDB156	UDB164	UDB172	UDB180	UDB188
E	UDB101	UDB109	UDB117	UDB125	UDB133	UDB141	UDB149	UDB157	UDB165	UDB173	UDB181	UDB189
F	UDB102	UDB110	UDB118	UDB126	UDB134	UDB142	UDB150	UDB158	UDB166	UDB174	UDB182	UDB190
G	UDB103	UDB111	UDB119	UDB127	UDB135	UDB143	UDB151	UDB159	UDB167	UDB175	UDB183	UDB191
H	UDB104	UDB112	UDB120	UDB128	UDB136	UDB144	UDB152	UDB160	UDB168	UDB176	UDB184	UDB192