Part No.:H-940-002049-00

User Manual

Version:1.0

MGIEasy Large-scale PCR-Free FS Library Prep Set for Low-pass WGS

Cat. No.: 940-002162-00 (96 RXN)
940-002049-00 (384 RXN)
Kit Version: V1.0

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About the user manual

Manufacturer information

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Revision history

Manual version	Kit version	Date	Description
1.0	V1.0	Aug. 2024	Initial release

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

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

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1 Product overview

1.1 Introduction

The MGIEasy Large-scale PCR-Free FS Library Prep Set for Low-pass WGS is designed to prepare WGS PCR-Free libraries for large-scale samples.

This library prep set can quickly convert 100 ~ 400 ng of genomic DNA (gDNA) into a customized library dedicated to the MGI high-throughput sequencing platforms. The reagent in this module is loaded on the 96-well plate, which facilitates the accelerated preparation of large-scale PCR-FREE libraries. All reagents provided in this set have passed strict quality control and functional verification procedures, ensuring stability and reproducibility.

Tips

- The MGIEasy Large-scale PCR-Free FS Library Prep Set for Low-pass WGS 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 DNA (ssCirDNA) libraries preparation and further for DNB preparation, or it can be combined with DNBSEQ Onestep DNB Make Reagent Kit (OS-DB, Cat. No.: 1000026466) for rapid DNB preparation.
- The MGIEasy Large-scale PCR-Free Library Prep Module for Low-pass WGS (Plate) in this set is campatible with the single barcode adapters 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 ssCirDNA and further for DNB preparation, or it can be combined with DNBSEQ Onestep DNB Make Reagent Kit (OS-SB, Cat. No.: 1000020563) for rapid DNB preparation.

1.2 Application

This library prep set is applicable to gDNA samples from sources including, but not limited to, humans and animals tissue/blood/saliva, plant leaves.

1.3 Applicable sequencing platforms

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

Table 1 DNB make reagent Sequencing platform and sequencing type recommendation

Reagent kit	Sequencing platform and type	Recommended application scenarios
DNB make reagent included in the sequencing set, with MGIEasy Dual Barcode Circularization Kit	DNBSEQ-G400RS (PE100/PE150) DNBSEQ-T7RS (PE100/PE150) DNBSEQ-G99RS (PE150)	Human, animal, plant, and other types of sample
DNBSEQ Onestep DNB Make Reagent Kit (OS-DB)	DNBSEQ-G400RS (PE100/PE150) DNBSEQ-T7RS (PE100) DNBSEQ-G99RS (PE150)	Human, animal, plant, and other types of sample

Tips

- After obtaining ssCirDNA using the MGIEasy Dual Carcode Cyclization Kit, the DNB can be prepared using the DNB preparation reagents in the sequencing kit, and the obtained DNB is compatible with all genetic sequencers and sequencing types listed in this table.
- The DNB prepared using DNBSEQ one-step DNB Make Reagent Kit (OS-DB) V1 is only campatible with certain genetic sequencers and sequencing types, and is not suitable for DNBSEQ-T7RS PE150 sequencing recipe, please choose carefully.

1.4 Components

The MGIEasy Large-scale PCR-Free FS Library Prep Set for Low-pass WGS comes in two specifications: 96 RXN and 384 RXN. For component details, refer to the following table.

Table 2 MGIEasy Large-scale PCR-Free FS Library Prep Set for Low-pass WGS (96 RXN) (Cat. No.: 940-002162-00)

Modules & Cat. No.	Components	Spec & Quantity
MGIEasy Large-scale PCR-Free Library Prep Module for Low-pass WGS (Plate) (96 RXN) Cat. No.: 950-000100-00	FE Reaction Mix	30 μL/well × 24
	Ligation Reaction Mix	70 μL/well × 24
	Stop Buffer	40 μL/well × 24
MGIEasy UDB PF Adapter Kit A Cat. No.: 940-000023-00	UDB Adapters A	5 μL/well × 96
MGIEasy DNA Clean Beads Cat. No.: 940-001176-00	DNA Clean Beads	3.2 mL/tube × 1
	TE Buffer	3.2 mL/tube × 1

CAUTION The MGIEasy Large-scale PCR-Free FS Library Prep Set for Low-pass WGS (Plate) (96 RXN) contains one plate of Library Prep Reagent, which consists of 3 components: FE Reaction Mix at columns 1 to 3, Ligation Reaction Mix at columns 5 to 7, and Stop buffer at columns 9 to 11.

Table 3 MGIEasy Large-scale PCR-Free FS Library Prep Set for Low-pass WGS (384 RXN) (Cat. No.: 940-002049-00)

Modules & Cat. No.	Components	Spec & Quantity
MGIEasy Large-scale PCR-Free Library Prep Module for Low-pass WGS (Plate) (384 RXN) Cat. No.: 950-000099-00	FE Reaction Mix	30 μL/well × 96
	Ligation Reaction Mix	70 μL/well × 96
	Stop Buffer	40 μL/well × 96
MGIEasy UDB PF Adapter Kit A Cat. No.: 940-000023-00	UDB Adapters A	5 μL/well × 96
MGIEasy UDB PF Adapter Kit B Cat. No.: 940-000022-00	UDB Adapters B	5 μL/well × 96
MGIEasy UDB PF Adapter Kit C Cat. No.: 940-000025-00	UDB Adapters C	5 μL/well × 96
MGIEasy UDB PF Adapter Kit D Cat. No.: 940-000024-00	UDB Adapters D	5 μL/well × 96
MGIEasy DNA Clean Beads Cat. No.: 940-001176-00	DNA Clean Beads	3.2 mL/tube × 1
	TE Buffer	3.2 mL/tube × 1

1.5 Storage and transportation

Table 4 Kit storage and transportation

Modules	Cat. No.	Storage temperature	Transport temperature
MGIEasy Large-scale PCR-Free Library Prep Module for Low-pass WGS (Plate) (96 RXN)	950-000100-00	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
MGIEasy Large-scale PCR-Free Library Prep Module for Low-pass WGS (Plate) (384 RXN)	950-000099-00		
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 ℃	2 ℃ to 8 ℃

Tips
- Production date and expiration date: refer to the label.
- For ice packs or dry ice shipments, ensure that there is enough ice or dry ice remaining after transportation.
- With proper transport, storage, and use, all components can maintain complete activity within their shelf life.

1.6 Material supplied by user

Table 5 Order information for MGI products

Catalog number	Model	Name
1000020570	16 RXN	MGIEasy Dual Barcode Circularization Kit
1000026466	OS-DB, 4 RXN	DNBSEQ Onestep DNB Make Reagent Kit

Tips Select one of the the reagent kit based on the application requirement.

Table 6 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	/
Mini centrifuge for 0.2 mL tube, 1.5 mL tube	/
Desktop centrifuge for 96-well plate	
Pipettes	/
Thermocycler	/
96-well Magnetic Rack	ALPAQUA(Cat. No.: A000400, Recommended) or equivalent
Qubit Fluorometer	Thermo Fisher (Cat. No.: Q33216)
Agilent 2100 Bioanalyzer	Agilent Technologies (Cat. No.: G2939AA) or equivalent

Table 7 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
Pipette tips	/
1.5 mL centrifuge tube	/
0.2 mL PCR tube	Axygen (Cat. No.: PCR-02-C)
0.2mL 96-well PCR plate	Axygen (Cat. No.: PCR-96M2-HS-C)
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

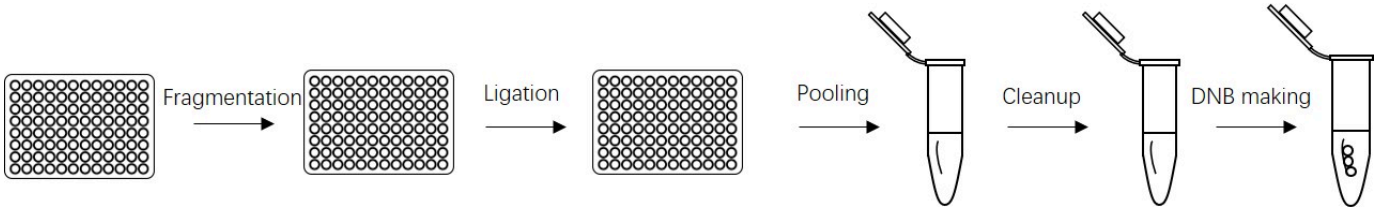

Figure 1 Workflow of MGIEasy Large-scale PCR-Free FS Library Prep Set for Low-pass WGS

Order	Process	Total time	Hands-on time
1	Fragmentation	40 min	10 min
2	Adapter ligation	25 min	10 min
3	Adding stop buffer and ligation product pooling ⏸	10 min	10 min
4	Cleanup of ligation product ⏸	25 min	25 min
5.1	Circularization and DNB making (Option 1)	1 h 30 min	40 min
5.2	Onestep DNB making (Option 2)	40 min	10 min

Tips

- The library preparation process consists of five steps: fragmentation, adapter ligation, pooling ligation product with equal volume, cleanup of ligation product, and DNB making (choose one from the circularization scheme and the one-step DNB scheme).
- Total time: refers to the theoretical time for 96 reactions, and the time will change according to the number of samples used.
- Hands-on time: it specifies the total manual operation time of the process.
- ⏸ : Stop point.

2 Sample Prep

2.1 Sample Requirements

2.1.1 Sample type

This library prep set is applicable to gDNA samples from sources including, but not limited to, human and animal tissue/blood/saliva, plant leaf.

2.1.2 Sample purity

It is recommended to use the sample gDNA with size (or gel band) ≥ 10kb, OD260/280 ≥ 1.8, 260/230 ≥ 1.7.

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

2.1.3 Sample input

This library prep set has a recommended input as 200 ng gDNA. Qubit or BMG is recommended for quantification of sample concentrations.

2.2 Sample Storage Condition

It is recommended to use TE Buffer (pH 8.0) to dissolve gDNA to avoid affecting the fragmentation reaction.

If there are impurities and enzyme inhibitors in the sample, it is recommended to purify the sample DNA with 1.8 x volume of magnetic beads and then dissolve it in TE buffer (pH 8.0) before use.

3 Library Prep

3.1 Fragmentation

Tips The extent of fragmentation (size distribution of DNA fragments) is controlled by reaction time under 30 ℃ temperature. Make sure the time and temperature during the fragmentation reaction are accurate. Samples and enzyme mix should be always on ice during preparation.

3.1.1 Preparation

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

Table 8 Preparing the reagents

Reagent	Requirement
TE Buffer (pH 8.0)	User-supplied; place at RT
FE Reaction Mix (384 RXN)	Thaw on ice; vortex 3 times (3 s each), centrifuge briefly; place on ice
Or, FE Reaction Mix (Library Prep Reagent plate, column 1 to 3) (96 RXN)	Thaw on ice; vortex 3 times (3 s each) , centrifuge briefly; place on ice

Tips
- Select the appropriate reagent specifications. When using the 384 RXN kit, remove the individual FE Reaction Mix plate for use; when using the 96 RXN kit, remove the Library Prep Reagent plate (FE Reaction Mix is in columns 1 to 3) for use.
- It is recommended to perform 96 x n (n is an integer and n ≥ 1) reactions per batch of experiments.

3.1.2 Fragmentation reaction

1. Normalize gDNA referring to the table below. Based on the sample concentration, transfer 200 ng gDNA to the well of a new 96 well PCR plate. Add TE Buffer (pH 8.0) to make a total volume of 22.5 μL. After sealing the plate, vortex the plate 3 times (3 s each), centrifuge briefly, and place on ice.

Table 9 Normalization of gDNA

Components	Volume
TE Buffer (pH 8.0)	22.5-X μL
gDNA (200 ng)	X μL
Total	22.5 μL

Tips
- Calculate the volume X required for 200 ng gDNA based on the sample concentration.
- Since the fragmentation enzyme is sensitive to the pH value of the DNA dissolution buffer, make sure to use the TE Buffer (pH 8.0) in DNA normalization so that different types of samples are fragmented under the same pH environment.

2. Set and run the program referring to "Table 10 Fragmentation reaction conditions (Volume: 30 μL)". The thermocycler will perform the first step reaction and be kept at 4 ℃.
3. Add **7.5 μL FE Reaction Mix** to the normalized gDNA plate(s) (step 1, volume: 22.5 μL). Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.
4. Place the PCR plate(s) into the thermocycler and skip the first step (4 ℃ Hold) to start the reaction.

Table 10 Fragmentation reaction conditions (Volume: 30 μL)

Temperature	Time
80 ℃ Heated lid	On
4 ℃	Hold
30 ℃	8 min 30 s
72 ℃	20 min
4 ℃	Hold

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

CAUTION Do not stop at this step. Proceed to section adapter ligation.

3.2 Adapter ligation

Tips Barcode sequences are designed to be located on the adapter, please read appendix "About the UDB PF Adapters " on page 25carefully before operation.

3.2.1 Preparation

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

Table 11 Preparing the reagents

Reagent	Requirement
UDB PF Adapter Kits Series	Thaw at RT, mix thoroughly, centrifuge briefly, and place on ice.
Ligation Reaction Mix (384 RXN)	Thaw on ice; vortex 3 times (3 s each) , centrifuge briefly; place on ice
Or, Ligation Reaction Mix (Library Prep Reagent plate, column 5 to 7) (96 RXN)	Thaw on ice; vortex 3 times (3 s each) , centrifuge briefly; place on ice

Tips
- Mix the adapter thoroughly before use. Adapters should not be mixed directly with the adapter ligation mixture.
- Select the appropriate reagent specifications. When using the 384 RXN kit, remove the individual Ligation Reaction Mix plate for use; when using the 96 RXN kit, remove the Library Prep Reagent plate (Ligation Reaction Mix is in columns 5 to 7) for use.
- Ligation Reaction Mix is a viscous solution, mix it by pipetting it 10 times before use. When aspirating and emptying, pipette slowly to ensure that the correct amount of liquid is added.

3.2.2 Adapter ligation reaction

1. Add **3 µL of UDB PF Adapter** to the corresponding well of PCR plate(s) (step 5 in section 3.1.2, volume: 30 µL). Vortex it 3 times (3 s each), centrifuge briefly, and place on ice.
2. Add **17.5 µL of Ligation Reaction Mix** into each well of PCR plate(s), pipette slowly to mix the reaction mixture 10 times. Seal the plate(s), centrifuge briefly, and place on ice.
3. Place the PCR plate(s) into the thermocycler. Run the program with the following conditions.

Table 12 Adapter ligation reaction conditions (Volume: 50.5 μL)

Temperature	Time
30 ℃ Heated lid	On
25 ℃	15 min
4 ℃	Hold

Tips If the library yield is low, the reaction time at 25 ℃ can be extended to 30 min.

4. When the program is completed, place the PCR plate(s) on ice.

CAUTION Do not stop at this step. Proceed to section Adding stop buffer and pooling the product.

3.3 Adding stop buffer and pooling the product

3.3.1 Preparation

Table 13 Preparing the reagents

Reagent	Requirement
Stop Buffer (384RXN)	Thaw at RT, mix thoroughly, centrifuge briefly, and place on ice.
Or, Stop Buffer (Library Prep Reagent plate, column 9 to 11) (96 RXN)	Thaw at RT, mix thoroughly, centrifuge briefly, and place on ice.

Tips
- Select the appropriate reagent specifications. When using the 384 RXN kit, remove the individual Stop Buffer plate for use; when using the 96 RXN kit, remove the Library Prep Reagent plate (Stop Buffer is in columns 9 to 11) for use.

3.3.2 Stop ligation reaction

1. Add **10 μL Stop Buffer** to the corresponding well of PCR plate(s) (step 4 in section 3.2.2, volume: 50.5 μL). After sealing the plate, vortex the plate 3 times (3 s each), centrifuge briefly, and place on RT.

Stop point After adding Stop Buffer, the product can be stored in -25 ℃ to -15 ℃ freezer.

3.3.3 Pooling the product with equal volume

1. Determine the pooling solution referring to appendix “UDB PF Adapters pooling guide” on page 28. Determine the number of pooling samples based on the expected amount of sequencing data and the amount of data required for each sample. It is recommended to

pool 48 or 96 x n (n ≥ 1) ligation products with equal volume to a new 1.5 mL centrifuge tube. The pooling product should have a total volume ≥ 240 μL.

Stop point The pooling product can be stored in -25 ℃ to -15 ℃ freezer.

3.4 Cleanup of ligation product

Tips Before starting the experiment, read "Magnetic beads and cleanup" on page 23 carefully.

3.4.1 Preparation

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

3.4.2 Cleanup of ligation product

1. Add **240 μL of post-ligation pooling product** (section 3.3.3) to a new 1.5 mL centrifuge tube.
2. Add **60 μL TE Buffer** to each sample tube (volume: 300 μL).
3. Mix the DNA Clean Beads thoroughly. Add **72 μL of DNA Clean Beads** to each sample tube. Mix with a vortexer until all beads are suspended.
4. Incubate the sample tube(s) at room temperature for 5 min.
5. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 min until the liquid is clear. Carefully transfer the supernatant to new 1.5 mL centrifuge tube(s).

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

6. Add 30 μL DNA Clean Beads to the tube(s) containing the supernatant, mix with a vortexer until all beads are suspended.
7. Incubate the sample tube(s) at room temperature for 5 min.
8. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 min until the liquid is clear. Carefully remove and discard the supernatant.
9. 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 s. Carefully remove and discard the supernatant.

10. Repeat step 9. 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.
11. 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.
12. Remove the tube(s) from the magnetic rack and add **52 μL of TE Buffer** to elute the DNA. Mix with a vortexer until all beads are suspended.
13. Incubate the tube(s) at room temperature for 5 min.
14. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 min until the liquid is clear. Carefully transfer **50 μL** of supernatant to new 0.2 mL PCR tube(s).

Stop point After cleanup, the post-ligation purification product(s) can be stored in -25 ℃ to -15 ℃ freezer.

3.5 QC of ligation product

Use 2 μL of post-ligation purification product to quantify the concentration with Qubit dsDNA HS Assay Kit or Quant-iT PicoGreen dsDNA Assay Kit.

- For circularization, digestion and standard DNB preparation, the concentration of post-ligation purification product is required to be ≥ 2.0 ng/μL.
- For one-step DNB preparation, the concentration of post-ligation purification product is only required to be ≥ 1.2 ng/μL.

4 DNB Make

4.1 Circularization and DNB making (option 1)

The library prepared by MGIEasy Large-scale PCR-Free FS Library Prep Set for Low-pass WGS is paired with MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570, user-supplied). The circularization input is 100 ng ~ 200 ng. The DNB can be prepared using the reagent in sequncing set after ssCirDNA 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 in the refrigerator immediately after use.

Table 14 Preparing the reagents

Reagent	Requirement
TE Buffer (pH 8.0)	User-supplied; place at RT.
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.

4.1.1.2 Denaturation

1. Based on the purified ligation product concentration, pipette 200 ng product (If the yield is less than 200 ng, pipette 48 μL) into a new 0.2 mL PCR tube, add TE Buffer (pH 8.0) to make a total volume of **48 μL**. Mix it thoroughly, and centrifuge briefly.

Tips Minimum allowable input is 100 ng. In the range of 100 ng ~ 200 ng, more yield of ssCirDNA will be obtained with more ligation product.

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

Table 15 Denaturation reaction conditions (Volume: 48 μL)

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

3. After the 95 ℃ reaction is finished, immediately remove the PCR tube(s) and place on ice for 2 min, then 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 s each), centrifuge briefly, and place on ice.

Table 16 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, volume: 48 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 17 Single strand DNA circularization reaction conditions (Volume: 60 μL)

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

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

4.1.2 Digestion

4.1.2.1 Preparation

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

Table 18 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 reaction

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 s each), centrifuge briefly, and place on ice.

Table 19 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 20 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 s each), centrifuge briefly, and place on ice.

CAUTION Do not stop at this step. Proceed to the section Cleanup of digestion product.

4.1.3 Cleanup of digestion product

Tips Before starting the experiment, read "Magnetic beads and cleanup" on page 23carefully.

4.1.3.1 Preparation

Table 21 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 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.
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 (ssCirDNA) can be stored in -25 °C to -15 °C freezer for 30 days.

4.1.4 QC of digestion product

Quantify the ssCirDNA with Qubit ssDNA Assay Kit. The final yields are required more than 16 ng.

4.1.5 DNB preparation

Refer to *DNBSEQ-G99RS High-throughput Sequencing Set User Manual*, *DNBSEQ-G400RS Highthroughput (Rapid) Sequencing Set User Manual*, or *DNBSEQ-T7RS High-throughput Sequencing Set User Manual* to prepare DNB. Use 16 ng ssCirDNA for DNB preparation.

4.2 Onestep DNB making (option 2)

DNBSEQ Onestep DNB Make Reagent Kit (OS-DB, Cat. No.: 1000026466) is compatible with the library prepared by this set for rapid DNB preparation.

CAUTION The onestep DNB is compatible with following sequencine recipe: DNBSEQ-G400RS (PE100/PE150), DNBSEQ-T7RS (PE100) and DNBSEQ-G99RS (PE150), but not compatible with DNBSEQ-T7RS (PE150).

4.2.1 Preparation

1. Samples: place the dsDNA library from step 14 in section 3.4.2 on ice. Calculate the required library volume.
2. Mix the reagents before using and store the remaining reagents immediately after use.

Table 22 Preparing the reagents

Reagent	Requirement
Make DNB Buffer (OS-DB)	Thaw at RT, mix thoroughly, centrifuge briefly, and place on ice.
Low TE Buffer	
Make DNB Enzyme Mix I (OS)	Flick and/or invert the tube gently, 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.

4.2.2 Making Onestep DNB

1. Based on the ligation product concentration, add appropriate dsDNA library (24ng) to a new 0.2 mL PCR tube. Add the following reagents into the tube(s) on ice. Vortex it 3 times (3 s each), centrifuge briefly, and place on ice.

Table 23 DNB making system 1

Reagent	Volume per reaction
dsDNA library	V μL
Low TE Buffer	20-V μL
Make DNB Buffer (OS-DB)	20 μL
Total	40 μL

Tips Calculate the volume V required for 24 ng of library based on the concentration of the ligation product.

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

Table 24 DNB reaction condition 1 (Volume: 40 μL)

Temperature	Time
105 ℃ Heated lid	On
95 ℃	3 min
40 ℃	3 min
4 ℃	Hold

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

Table 25 DNB making system 2

Reagent	Volume per reaction
Make DNB Enzyme Mix I (OS)	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 s each), centrifuge briefly, and place on ice.
5. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 26 DNB reaction condition 2 (Volume: 84 μL)

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

CAUTION For DNBSEQ-G99RS/DNBSEQ-G99ARS platforms, the reaction time for 30 ℃ should be set to 20 min in DNB reaction condition 2.

Tips
- For certain thermocyclers from some manufacturers, the heated lids may take an extended period of time to reach operating temperatures. For this type of thermocycler, 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 8 times with a **wide-bore tip**. After mixing, store the DNBs 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 DNBs vigorously.

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

- If the DNB concentration is less than 6 ng/μL, it is not qualified, please prepare DNB again.
- If the DNB concentration is between 6 ng/μL and 8 ng/μL, it can be used to sequencing, and there is a risk of low sequencing quality.
- If the DNB concentration is greater than 8 ng/μL, proceed to sequencing.


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

5 Appendix

5.1 Magnetic beads and cleanup

It is recommended that you use DNA Clean Beads included in the MGIEasy DNA Clean Beads to purify the libraries. If 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 at leat 30 min in advance to allow the beads to equilibrate to room temperature. Equilibrating to room temperature ensures that 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 a 96-well magnetic plate holder or a similar 0.2 mL magnetic rack during the separation process. The yield may lose about 20% if transferring the liquid into a 1.5 mL centrifuge tube and separating with a 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

- 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.

- 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.
- 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.
- To avoid touching or removing the magnetic beads, leave 2 to 3 μL of liquid in the tube. Pipette all of the solution and beads back into the tube and restart the separation process if necessary.

Ethanol wash

- Wash the beads with freshly prepared room temperature 80% ethanol. Sufficient ethanol should be added to immerse the beads entirely.
- Keep the centrifuge tube(s) on the magnetic rack during washing. Do not shake or disturb the beads while washing.
- Carefully remove all remaining ethanol after washing two more times. 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

- 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, affecting subsequent reactions.
  - The surface of the magnetic beads is reflective: Indicates insufficient drying and easily causes anhydrous ethanol residues, affecting subsequent reactions.
- Air-drying takes approximately 3 to 5 min at room temperature. Different indoor temperatures and humidities of labs may affect the drying time.

Elution

- Elute the DNA with the TE Buffer or En-TE prepared from section 3.1.
- The elution volume of En-TE should be 2 μL more than the pipetting volume of the supernatant. This will allow you to avoid touching or pipetting the magnetic beads.

5.2 About the UDB PF Adapters

Tips If you want the sequence information of each Barcode, you can contact MGI technical support (email: **MGI-service@mgi-tech.com**)

This kit is designed for the construction of dual barcode libraries only, and the barcode sequences are designed to be located on the adapter sequences. When multiple samples are mixed together for sequencing, barcodes can be used to accurately attribute the sequencing results to the appropriate samples.

There are two specifications of UDB PF Adapter Reagent Kits, available for purchase based on the number of reactions required:

- The 96 RXN is paired with the MGIEasy UDB PF Adapter Kit A (Cat. No.: 940-000023-00), which provides a total of 1 plate of adapters to support simultaneous mixed sequencing of 96 samples.
- The 384 RXN is paired with the MGIEasy UDB PF Adapter Kit A (Cat. No.: 940-000023-00), MGIEasy UDB PF Adapter Kit B (Cat. No.: 940-000022-00), MGIEasy UDB PF Adapter Kit C (Cat. No.: 940-000025-00) and MGIEasy UDB PF Adapter Kit D (Cat. No.: 940-000024-00), which provide a total of 4 panels of adapters to support simultaneous mixed sequencing of up to 384 samples.

Tips The MGIEasy Large-scale PCR-Free Library Prep Module for Low-pass WGS can be also paired with single barcode adapters of MGIEasy PF Adapters-96 (Plate) Kit (Cat. No.: 1000013461), available for purchase as needed. Please select the correct usage rules according to the adapter type.

5.2.1 Instructions for UDB PF Adapter Kit A/B/C/D (96 RXN)

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

Table 27 UDB PF Adapter Kit A (96 RXN) 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 28 UDB PF Adapter Kit B (96 RXN) 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 29 UDB PF Adapter Kit C (96 RXN) 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 30 UDB PF Adapter Kit D (96 RXN) 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

5.2.2 UDB PF Adapters pooling guide

It is recommended that you optimize the base balance by planning UDB PF Adapter with diverse sequences when pooling libraries across DNBSEQ systems. Pooling combines at least eight libraries to sequence in one lane. The following application scenarios are predefined for the recommended method of selecting UDB PF Adapter.

Table 31 UDB PF Adapter pooling guide

Sample/ lane	Instruction (Example)
8X	Add 1 UDB PF Adapter per sample, from X set of 8 UDB PF Adapters (X column total).
8X+1	Add X set of 8 UDB PF Adapters + 1 random well of UDB PF Adapter
8X+2	Add X set of 8 UDB PF Adapters + 2 random wells of UDB PF Adapters
8X+3	Add X set of 8 UDB PF Adapters + 3 random wells of UDB PF Adapters
8X+4	Add X set of 8 UDB PF Adapters + 4 random wells of UDB PF Adapters
8X+5	Add X set of 8 UDB PF Adapters + 5 random wells of UDB PF Adapters
8X+6	Add X set of 8 UDB PF Adapters + 6 random wells of UDB PF Adapters
8X+7	Add X set of 8 UDB PF Adapters + 7 random wells of UDB PF Adapters

For UDB PF Adapters Kit C/D, it is recommended to pool the entire plate in groups of 96.

Under exceptional circumstances (for example, insufficient reagents for a well), when the requirement of at least one balanced UDB PF Adapter combination for standard pooling cannot be met, or if the required data amount of each library pooled is not equal, make 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 32 Balanced 8 UDB PF Adapter Pooling strategy (8 UDB PF Adapter 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
Adapter 1	A	G	G	A	C	G	T	A	G	A
Adapter 2	C	T	G	A	A	C	C	G	A	A
Adapter 3	G	A	A	C	G	T	G	T	C	G
Adapter 4	T	C	C	G	T	G	A	C	T	C
Adapter 5	A	A	T	T	C	A	C	T	G	T
Adapter 6	C	C	T	G	A	A	G	G	A	T
Adapter 7	T	T	C	C	T	T	A	C	T	G
Adapter 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 33 Unbalanced 9 UDB PF Adapter Pooling strategy (UDB PF Adapters 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
Adapter 1	A	G	G	A	C	G	T	A	G	T
Adapter 2	A	C	G	A	A	G	G	T	C	C
Adapter 3	G	A	A	C	G	T	G	T	C	G
Adapter 4	T	C	C	G	T	G	A	C	T	C
Adapter 5	A	A	T	T	C	A	C	T	G	T
Adapter 6	G	C	T	G	A	A	G	G	A	T
Adapter 7	T	G	C	C	T	T	A	C	T	G
Adapter 8	G	G	A	T	G	A	T	A	C	C
Adapter 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

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