Part No.: H-020-000618-00

High-throughput Sequencing Set

DNBSEQ-E25RS

Instructions for Use

Version: 5.0

Leading Life Science Innovation

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CE Research Use Only

Qingdao MGI Tech Co., Ltd.

About the instructions for use

This instructions for use is applicable to DNBSEQ-E25RS High-throughput Sequencing set. The version of the instructions for use is 5.0, the set version is 1.0, and the version of the control software is 1.0.

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Revision history

Version	Date	Description
5.0	August 15, 2024	Modified the catalog number of the flow cell
4.0	May 23, 2024	• Modified the validity period of the flowcell, and reagent kits (FCL SE100/App-C FCL SE100) • Modified the thawing method of the conversion enzyme
3.0	February 7, 2024	• Modified the components • Modified the operation of making DNBs
2.0	September 25, 2023	• Added kit information and operation about App-C sequencing • Added the use of funnel • Updated the expression
1.0	April 13, 2023	Initial release

About the sequencing set

Cat. No.	Name	Model	Version
940-000573-00	DNBSEQ-E25RS High-throughput Sequencing Set	FCL SE100	1.0
940-000567-00	DNBSEQ-E25RS High-throughput Sequencing Set	FCL PE150	1.0
940-000569-00	DNBSEQ-E25RS High-throughput Sequencing Set	App-C FCL SE100	1.0
940-000574-00	DNBSEQ-E25RS High-throughput Sequencing Set	App-C FCL PE150	1.0

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Chapter 1 Introduction

This instructions for use describes how to perform sequencing by using the DNBSEQ-E25RS High-throughput Sequencing Set. It includes DNB (DNA Nanoball) making, flow cell preparation, components of sequencing reagent set, storage conditions and use methods, sequencing protocols, and device maintenance.

1.1 Intended use

This product is a universal reagent set for the sequencing of DNA or RNA libraries, and is used with the DNBSEQ-E25RS series genetic sequencer to complete high-throughput sequencing and obtain sample sequence information. This reagent set is for research use only, and cannot be used for clinical diagnosis.

1.2 Working principle

The device adopts the advanced DNB and the core technology of combinatorial probe-anchor synthesis (cPAS), and uses a flow cell in patterned array with the special functionalized surface. Each functionalized site of the flow cell contains a single DNB, and the functionalized sites are evenly arranged on the flow cell, ensuring that the optical signals of different Nanoballs cannot be interrupted by each other. Therefore, the accuracy of signal process is improved.

During sequencing, the DNBs and sequencing reagents are pumped into the sequencing flow cell through the fluidics component. Each DNB combines with different types of signal enzymes, which then reacts with signal reagents to generate self-luminescent signals. These signals are gathered by the signal gathering module and then transferred to digital signals that are transmitted to and processed by the computing module, so as to acquire the nucleotide sequence of the samples to be tested according to the type of self-luminescent signals and combinations.

1.3 Data analysis

During sequencing, the control software automatically runs the basecall software for analysis, and outputs the sequencing data to the designated location for secondary analysis.

1.4 Sequencing cycle and time

Sequencing read length determines the number of sequencing cycles for a given sequencing run. One sequencing cycle equates to one base pair of sequence data. For example, a PE150 cycle run performs reads of 150 cycles (2×150) for a total of 300 cycles or 300 bases sequenced. If required, an extra 8 or 10 cycles, or 16 or 20 cycles of barcode read can be performed to aid in identifying a specific library.

Table 1 Sequencing cycle and time

Sequencing read length	Read 1 read length	Read 2 read length	Total read length	Sequencing time (hours)	Maximum cycles
SE100	100	/	100+20	6.0	121
			100+10	5.5	
			100	5.0	
PE150	150	150	300+20	20.5	322
			300+10	20	
			300	19.5	
App-C FCL SE100	100	/	100+16	6.0	117
			100+8	5.5	
			100	5.0	
App-C FCL PE150	150	150	300+16	20.5	318
			300+8	20	
			300	19.5	

1.5 Warnings and precautions

- This product is for research use only. Please read the instructions for use of the product carefully before use.
- Before experiment, be sure to be familiar with and master the operation methods and precautions of various devices to be used.
- Direct contact with skin and eyes should be avoided for all samples and reagents. Do not swallow. Once it happens, immediately rinse with large amounts of water and go to hospital in time.

- All samples and wastes should be disposed of in accordance with relevant regulations.
- This product is disposable and shall not be reused.
- The components and packages are batched separately. Keep the components in the packages until use. It is not recommended to mixedly use reagents from different batches of reagent kits.
- Do not use expired products.

Chapter 2 Main components and user-supplied equipment, reagents and consumables

Tips
- It is not recommended to mixedly use reagents from different batches of reagent kits.
- The components and packages are batched separately.
- Keep the components in the packages until use.

Table 2 DNBSEQ-E25RS High-throughput Sequencing Set (FCL SE100) Cat. No.: 940-000573-00

Kit name	Component	Specifications	Storage temperature	Validity period	Transportation temperature
DNBSEQ-E25RS Sequencing Flow Cell Cat. No.: 930-000045-00	Flow cell	1 EA	10 ℃ to 25 ℃	10 months	10 ℃ to 25 ℃
DNBSEQ-E25RS High-throughput Sequencing kit (FCL SE100) Cat. No.: 940-000570-00	Low TE buffer	300 μL/tube×1	-25 ℃ to -15 ℃	9 months	-80 ℃ to -15 ℃
	Make DNB Buffer (OS-V2.0-DB)	80 μL/tube×1			
	Make DNB Buffer (OS-V2.0-SB)	80 μL/tube×1			
	Make DNB Enzyme Mix I (OS)	160 μL/tube×1			

Kit name	Component	Specifications	Storage temperature	Validity period	Transportation temperature
DNBSEQ-E25RS High-throughput Sequencing kit (FCL SE100) Cat. No.: 940-000570-00	Make DNB Enzyme Mix II (OS)	16 μL/tube×1	-25 ℃ to -15 ℃	9 months	-80 ℃ to -15 ℃
	Stop DNB Reaction Buffer	100 μL/tube×1			
	DNB Load Buffer II	120 μL/tube×1			
	Signal Protein 1	15 μL/tube×1			
	Signal Protein 2	10 μL/tube×1			
	Signal Protein Buffer	10 mL/tube×1			
	Sequencing Reagent Cartridge	1 EA			
	Waste container	1 EA			
	Funnel	1 EA			

Table 3 DNBSEQ-E25RS High-throughput Sequencing Set (FCL PE150) Cat. No.: 940-000567-00

Kit name	Component	Specifications	Storage temperature	Validity period	Transportation temperature
DNBSEQ-E25RS Sequencing Flow Cell Cat. No.: 930-000045-00	Flow cell	1 EA	10 ℃ to 25 ℃	10 months	10 ℃ to 25 ℃
DNBSEQ-E25RS High-throughput Sequencing kit (FCL PE150) Cat. No.: 940-000571-00	Low TE buffer	300 μL/tube×1	-25 ℃ to -15 ℃	6 months	-80 ℃ to -15 ℃
	Make DNB Buffer (OS-V2.0-DB)	80 μL/tube×1			

Kit name	Component	Specifications	Storage temperature	Validity period	Transportation temperature
DNBSEQ-E25RS High-throughput Sequencing kit (FCL PE150) Cat. No.: 940-000571-00	Make DNB Buffer (OS-V2.0-SB)	80 μL/tube×1	-25 ℃ to -15 ℃	6 months	-80 ℃ to -15 ℃
	Make DNB Enzyme Mix I (OS)	160 μL/tube×1			
	Make DNB Enzyme Mix II (OS)	16 μL/tube×1			
	Stop DNB Reaction Buffer	100 μL/tube×1			
	DNB Load Buffer II	120 μL/tube×1			
	Signal Protein 1	31.5 μL/tube×1			
	Signal Protein 2	21 μL/tube×1			
	Signal Protein Buffer	21 mL/tube×1			
	MDA T-Reagent	0.35 mL/ tube×1			
	MDA Enzyme Mix	0.05 mL/ tube×1			
	Sequencing Reagent Cartridge	1 EA			
	Waste container	1 EA			
	Funnel	1 EA			

Table 4 DNBSEQ-E25RS High-throughput Sequencing Set (App-C FCL SE100)
Cat. No.: 940-000569-00

Kit name	Component	Specifications	Storage temperature	Validity period	Transportation temperature
DNBSEQ-E25RS Sequencing Flow Cell Cat. No.: 930-000045-00	Flow cell	1 EA	10 ℃ to 25 ℃	10 months	10 ℃ to 25 ℃
DNBSEQ-E25RS High-throughput Sequencing kit (App-C FCL SE100) Cat. No.: 940-000572-00	Low TE buffer	300 μL/tube×1	-25 ℃ to -15 ℃	9 months	-80 ℃ to -15 ℃
	Make DNB Buffer (OS-App-V4.0)	80 μL/tube×1			
	Conversion Enzyme	5 μL/tube×1			
	Make DNB Enzyme Mix I (OS)	160 μL/tube×1			
	Make DNB Enzyme Mix II (OS)	16 μL/tube×1			
	Stop DNB Reaction Buffer	100 μL/tube×1			
	DNB Load Buffer II	120 μL/tube×1			
	Signal Protein 1	15 μL/tube×1			
	Signal Protein 2	10 μL/tube×1			
	Signal Protein Buffer	10 mL/tube×1			
	Sequencing Reagent Cartridge	1 EA			
	Waste container	1 EA			
	Funnel	1 EA			

Table 5 DNBSEQ-E25RS High-throughput Sequencing Set (App-C FCL PE150)
Cat. No.: 940-000574-00

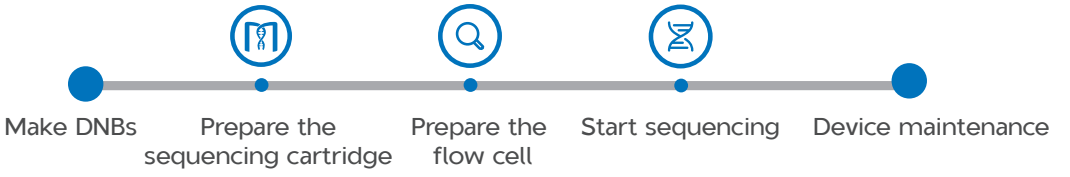
Kit name	Component	Specifications	Storage temperature	Validity period	Transportation temperature
DNBSEQ-E25RS Sequencing Flow Cell Cat. No.: 930-000045-00	Flow cell	1 EA	10 ℃ to 25 ℃	10 months	10 ℃ to 25 ℃
DNBSEQ-E25RS High-throughput Sequencing kit (App-C FCL PE150) Cat. No.: 940-000568-00	Low TE buffer	300 µL/tube×1	-25 ℃ to -15 ℃	6 months	-80 ℃ to -15 ℃
	Make DNB Buffer (OS-App-V4.0)	80 µL/tube×1			
	Conversion Enzyme	5 µL/tube×1			
	Make DNB Enzyme Mix I (OS)	160 µL/tube×1			
	Make DNB Enzyme Mix II (OS)	16 µL/tube×1			
	Stop DNB Reaction Buffer	100 µL/tube×1			
	DNB Load Buffer II	120 µL/tube×1			
	Signal Protein 1	31.5 µL/tube×1			
	Signal Protein 2	21 µL/tube×1			
	Signal Protein Buffer	21 mL/tube×1			
	MDA T-Reagent	0.35 mL/tube×1			
	MDA Enzyme Mix	0.05 mL/tube×1			

Kit name	Component	Specifications	Storage temperature	Validity period	Transportation temperature
DNBSEQ-E25RS High-throughput Sequencing kit (App-C FCL PE150) Cat. No.: 940-000568-00	Sequencing Reagent Cartridge	1 EA	-25 ℃ to -15 ℃	6 months	-80 ℃ to -15 ℃
	Waste container	1 EA			
	Funnel	1 EA			

Table 6 User-supplied equipment, reagent and consumables

Type	Name	Recommended brand	Cat. No.
Equipment	Qubit 4 fluorometer	Thermo Fisher	Q33226
	Mini centrifuge	None	None
	Vortex mixer	None	None
	PCR thermocycler	Bio-Rad	None
	Pipette	Eppendorf	None
	Pointed-tip tweezers	None	None
	2 ℃ to 8 ℃ refrigerator	None	None
	-25 ℃ to -15 ℃ freezer	None	None
Reagent	75% ethanol	None	None
	Qubit ssDNA Assay Kit	Thermo Fisher	Q10212
	Qubit dsDNA HS Assay Kit	Thermo Fisher	Q32854
Consumables	Dust-free paper	None	None
	Sterile tips, boxed	AXYGEN	None
	200 μL wide-bore tips (non-filtered)	MGI	091-000355-00
	Qubit Assay Tubes	Thermo Fisher	Q32856
	0.2 mL PCR 8-strip tube	AXYGEN	PCR-02-C
	1.5 mL centrifuge tube	AXYGEN	MCT-150-C
	Ice box	AXYGEN	None

Chapter 3 Sequencing workflow



Chapter 4 Making DNBs

4.1 Required library insert size

This kit is applicable to the library with MGI adapter and App library with TruSeq and Nextera adapters. The recommended library insert size ranges between 200 bp and 500 bp, with main band within ±100 bp.

If special requirements of library insert size are written in the instructions for use of the Library Prep kit, it shall prevail.

Table 7 Recommended library insert size

Model	Recommended insert size (bp)	Data output (GB/flow cell)
FCL SE100	200 to 400	About 2.5
FCL PE150	300 to 500	About 7.5
App-C FCL SE100	200 to 400	About 2
App-C FCL PE150	300 to 500	About 6

Tips
- Select sequencing kits according to the insert size.
- Average data output will vary with different library types and applications.

4.2 Required concentration and volume of library

- Required concentration
  - For the library with MGI adapter, the initial dsDNA library concentration is required to be no less than 2 fmol/μL.
  - For App library, the initial dsDNA library concentration is required to be no less than 5 fmol/μL.
  - If the library concentration is unknown, it is recommended to perform

dsDNA library quantitation (ng/μL) by using Qubit dsDNA HS Assay Kit and Qubit Fluorometer. Use the equation below to convert the concentration of the dsDNA library from ng/μL to fmol/μL:

$$c(\text{fmol}/\mu\text{L}) = \frac{\frac{10^6}{330\times 2}\times c(\text{ng}/\mu\text{L})}{N}$$

N represents the total library length including the adapter as determined by fragment size analysis. Typically, fragment size analysis is determined during library preparation. *c* represents the concentration.

- Required volume of library
  - For the sequencing with library with MGI adapter, making a DNB loading mixture needs 40 fmol of library.
  - For App-C sequencing, making a DNB loading mixture needs 100 fmol of library.

Tips
- The requirements of Make DNB kit instructions for use should prevail if any.
- The requirements of Library Prep kit instructions for use should prevail if any.

4.3 Making DNBs

Tips
- It is not recommended to mixedly use reagents from different batches of reagent kits.
- Do not use filtered pipette tips for making and loading DNBs. It is necessary to use the pipette and tips of recommended brands and catalog numbers.
- Each kit is sufficient to make DNBs for 4 sequencing runs.

4.3.1 Preparing DNB making reagents

Perform the following steps:

1. Place the DNA library on ice.
2. Take out the sequencing set according to different sequencing recipes:

Sequencing recipe	Kit name
SE100 sequencing	DNBSEQ-E25RS High-throughput Sequencing Set (FCL SE100)
PE150 sequencing	DNBSEQ-E25RS High-throughput Sequencing Set (FCL PE150)
App-C SE100 sequencing	DNBSEQ-E25RS High-throughput Sequencing Set (App-C FCL SE100)
App-C PE150 sequencing	DNBSEQ-E25RS High-throughput Sequencing Set (App-C FCL PE150)

3. Thaw Low TE Buffer, Make DNB Buffer (OS-V2.0-SB) or Make DNB Buffer (OS-V2.0-DB) or Make DNB Buffer (OS-App-V4.0), Make DNB Enzyme Mix I (OS) and Stop DNB Reaction Buffer by placing them on ice for 30 minutes.

   Tips
   - Make DNB Buffer (OS-V2.0-SB) is applicable to single-barcode library.
   - Make DNB Buffer (OS-V2.0-DB) is applicable to dual-barcode library.
   - Make DNB Buffer (OS-App-V4.0) is only applicable to App-C sequencing.

4. Use a vortex mixer to vortex them for 5 seconds. Centrifuge briefly and place them on ice until use.

4.3.2 Calculating required volume of dsDNA library

- If the initial input is 40 fmol, calculate the library input according to the following formula:

$$\text{Library input V}(\mu\text{L}) = \frac{\text{N(bp)} \times 330 \times 2 \times 40\ \text{fmol}}{\text{c(ng/}\mu\text{L)} \times 10^6}$$

- If the initial input is 100 fmol, calculate the library input according to the following formula:

$$\text{Library input V}(\mu\text{L}) = \frac{\text{N(bp)} \times 330 \times 2 \times 100\ \text{fmol}}{\text{c(ng/}\mu\text{L)} \times 10^6}$$

In the formula, *N* represents the total library length including the adapter as determined by fragment size analysis. *c* represents the library concentration. 330 represents the average molecular weight of base (330 g/mol).

4.3.3 Making DNBs

Perform the following steps:

1. (Optional) For App-C sequencing, take out Conversion Enzyme.
2. Take out a 0.2 mL 8-strip tube or PCR tubes and prepare the Make DNB reaction mixture 1 according to the following table:

Table 8 Make DNB reaction mixture 1 (library with MGI adapter)

Component	Volume (μL)
Low TE Buffer	20-V
Make DNB Buffer	20
dsDNA library	V
Total volume	40

Tips
- The type of Make DNB Buffer depends on the buffer taken out in *Preparing DNB making reagents on Page 10*.
- *V* stands for the library input calculated in *Calculating required volume of dsDNA library on Page 11*.

Table 9 Make DNB reaction mixture 1 (App library)

Component	Volume (μL)
Low TE Buffer	20-V
Make DNB Buffer	20
dsDNA library	V
Conversion Enzyme	0.5
Total volume	40.5

Tips
- The type of Make DNB Buffer depends on the buffer taken out in *Preparing DNB making reagents on Page 10*.
- *V* stands for the library input calculated in *Calculating required volume of dsDNA library on Page 11*.

3. Mix Make DNB reaction mixture 1 thoroughly by vortexing, centrifuge it for 5 seconds by using a mini centrifuge, and place the tube into a thermal cycler to start reaction according to the following condition:

Table 10 Primer hybridization reaction condition (library with MGI adapter)

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

Table 11 Primer hybridization reaction condition (App library)

Temperature	Time
105 ℃ (Heated lid)	On
37 ℃	5 min
95 ℃	3 min
40 ℃	3 min
4 ℃	Hold

4. Place Make DNB Enzyme Mix II (OS) on ice, centrifuge briefly for 5 seconds and then place it on ice until use.

   Tips
   - Do not place Make DNB Enzyme Mix II (OS) at room temperature.
   - Do not hold the tube to avoid enzyme inactivation caused by high temperature.

5. Take the tube out of the thermal cycler when the temperature reaches 4 ℃. Centrifuge briefly for 5 seconds and place the tube on ice. Add the following reagents to the tube:

Table 12 Make DNB reaction mixture 2

Component	Volume (μL)
Make DNB Enzyme Mix I (OS)	40
Make DNB Enzyme Mix II (OS)	4

6. Mix the tube gently by vortexing, centrifuge for 5 seconds by using a mini centrifuge, place it into a thermal cycler, and start the reaction according to the following condition:

   Tips
   - It is recommended to preheat the heated lid to the required temperature before DNB reaction, because it might take some time for the lid temperature adjustment.
   - It is recommended to set the temperature of the heated lid to 35 ℃ or as close as possible to 35 ℃.

Table 13 Rolling circle replication condition

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

7. Take the tube out of the thermal cycler when the temperature reaches 4 ℃ , add 20 μL of Stop DNB Reaction Buffer to the tube, and use a wide-bore, non-filtered pipette tip to pipette up and down 8 times to mix the reagent gently.

   Tips
   - Be sure to use a wide-bore tip to pipette for 8 times to mix the tube gently. Do not centrifuge, vortex or pipette vigorously.
   - Store DNBs at 2 ℃ to 8 ℃ and perform sequencing within 48 hours.

4.4 Quantifying DNBs

Perform the following steps:

When DNB making is completed, use Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify 2 μL of DNBs.

Tips It is recommended to quantify in batches for large amounts of DNBs to avoid inaccurate quantification due to fluorescence quenching.

- For the library with MGI adapter, the concentration should be between 4 ng/μL and 40 ng/μL. If not, refer to *DNB concentration does not meet requirements on Page 34* for details.
- For App library, the concentration should be no less than 6 ng/μL. If not, refer to *DNB concentration does not meet requirements on Page 34* for details.

Chapter 5 Preparing the reagent cartridge

Tips

- For SE sequencing, prepare the reagent cartridge according to steps 1 to 10 and step 12.
- For PE sequencing, prepare the reagent cartridge according to steps 1 to 12.
- Signal Protein 1, Signal Protein 2 and Signal Protein Buffer are provided in different tubes and are packaged together with the Sequencing Reagent Cartridge. Before the sequencing run starts, an appropriate amount of Signal Protein 1, Signal Protein 2 and Signal Protein Buffer needs to be mixed together and added into the MSP well (MSP, Mixture of Signal Protein) of the reagent cartridge.
- If you perform PE sequencing, an appropriate amount of MDA Enzyme Mix and MDA T-Reagent needs to be mixed together and added into the MDA well (MDA, Multiple Displacement Amplification).

Perform the following steps:

1. Place the reagent cartridge upright with the label facing upwards.

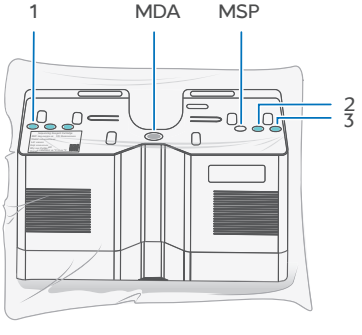


Figure 1 Placing the reagent cartridge upright

2. Thaw the reagent cartridge and Signal Protein Buffer according to the following table.

Model	Method	
	In a refrigerator at 2 °C to 8 °C (hours)	At room temperature from 15 °C to 25 °C (hours)
FCL SE100	6	3.5 to 4.5
FCL PE150	10	4.5 to 5
App-C FCL SE100	6	3.5 to 4.5
App-C FCL PE150	10	4.5 to 5

Tips
- The reagent cartridge is completely thawed when there is no sound of cracked ice during shaking.
- Keep other components at -25 ℃ to -15 ℃ if the reagent cartridge is thawed overnight.
- Do not thaw the reagent cartridge in a water bath.

3. Thaw Signal Protein 1 and Signal Protein 2 by placing them on ice for about 10 minutes. Thaw DNB Load Buffer II on ice until use.
4. After thawing, check whether there is ice in the cartridge by shaking the cartridge. If there is sound of cracked ice, place the reagent cartridge at room temperature until no ice exists and use dust-free paper to remove condensation from the surface of the reagent cartridge.

Tips
- Do not use the cartridge and transfer it to the designated container immediately when its packaging bulges or breaks, or when the reagents instead of condensation are leaking out of it.
- The reagents are leaking out of the reagent cartridge when the liquid is in color or leaks out of the bottom, the bottom cover in particular, of the reagent cartridge and when the amount of liquid is large enough to moistens the whole bottom part of the reagent cartridge.
- The flowing liquid is condensation when it exists on the sides or corners of the cartridge. Use dust-free paper to remove it.

5. Hold the two sides of the cartridge with two hands. Invert it 20 times and gently tap it on a flat surface 10 times. Invert it 10 times and gently tap it on a flat surface 10 times again.

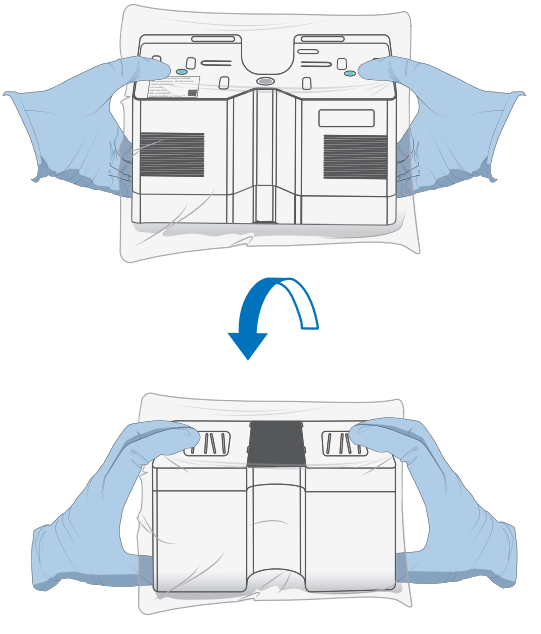


Figure 2 Inverting the reagent cartridge

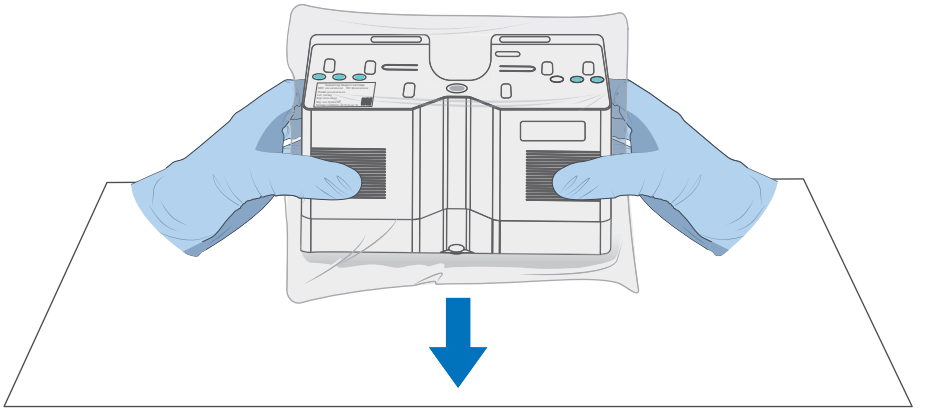


Figure 3 Tapping the reagent cartridge on a flat surface

6. Hold the reagent cartridge upright and swing downward 10 times to bring the reagents on the side of the walls to the bottom of their respective wells. Cut the outer packaging and remove it.
7. Use a vortex mixer to mix the Signal Protein 1 and Signal Protein 2 for 5 seconds. Centrifuge them briefly for 4 to 5 seconds and place them on ice until use.
8. According to the following table, add an appropriate amount of Signal Protein 1 and Signal Protein 2 to the tube containing the Signal Protein Buffer to make the Signal Protein Mixture.

	FCL SE100	FCL PE150	App-C FCL SE100	App-C FCL PE150
Signal Protein 1	15 µL	31.5 µL	15 µL	31.5 µL
Signal Protein 2	10 µL	21 µL	10 µL	21 µL
Signal Protein Buffer	10 mL	21 mL	10 mL	21 mL

9. Screw the cover of the tube containing the Signal Protein Mixture and invert it 10 to 15 times to mix thoroughly. To avoid bubble formation, do not vortex the mixture vigorously.

10. Place the reagent cartridge on a flat surface as the following figure. Place the funnel over the MSP well and add the Signal Protein Mixture into the MSP well.

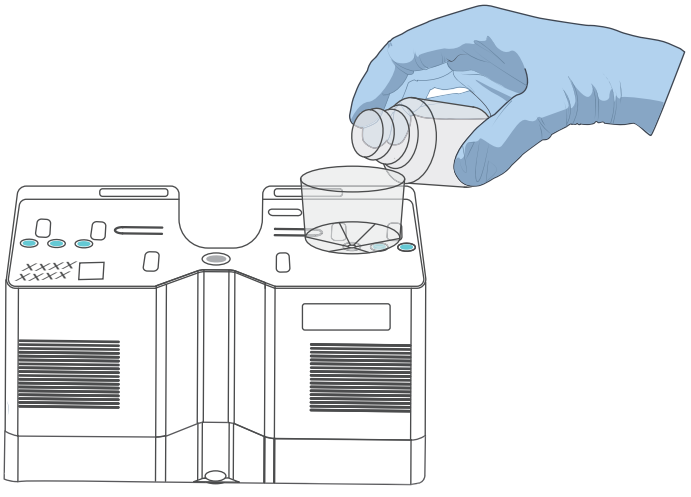


Figure 4 Adding the Signal Protein Mixture into MSP well

11. (Optional) For PE sequencing, perform the following steps:

    1) Take out MDA T-Reagent and MDA Enzyme Mix.
    2) Invert the MDA Enzyme Mix to mix it and then centrifuge it briefly.
    3) Transfer 50 µL of MDA Enzyme Mix to the tube containing MDA T-Reagent to make MDA mixture. Pipette the reagent 10 to 15 times to mix it without vortexing vigorously to prevent bubbles from forming.
    4) Use a clean tip to pierce the MDA well and transfer the MDA mixture into the MDA well. Do not vortex it vigorously in the process to prevent bubble formation.

    Tips The reagent cartridge with the MDA mixture should be loaded as soon as possible. Failure to do so might affect the sequencing quality.

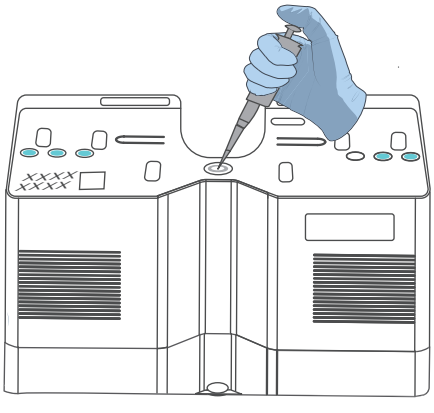


Figure 5 Adding MDA mixture into MDA well

12. Place the reagent cartridge on a flat surface as the following figure. Use the pointed-tip tweezers to remove the stoppers in wells No.1, No.2, and No.3.

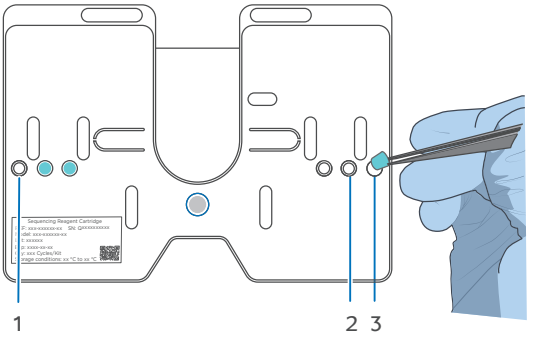


Figure 6 Removing the stoppers in wells No.1, No.2 and No.3

Chapter 6 Preparing the flow cell

Perform the following steps:

1. Take the flow cell box out of storage and remove the flow cell from the box.

   Tips Do not open the outer plastic package at this moment.

2. Unwrap the outer plastic package before use and use it within 24 hours.

Chapter 7 Starting sequencing

7.1 Logging in

Perform the following steps:

1. Ensure that the main unit and computing module are connected and the network cable between the main unit and the computing module is available.

   Tips The computing module should be kept on.

2. Power on the genetic sequencer, and the login interface is displayed.
3. Enter the username and password, and click **Log in**.

   Tips Two types accounts are supported by the control software: administrator account and user account.

Account type	Default username	Default password
Administrator account	admin	123456
User account	user	123

7.2 Selecting a recipe

Perform the following steps:

1. Select to enter the customization interface.

   When entering the customization interface, the reagent compartment starts initialization. The compartment door opens automatically and the rack slides out.

   Tips Do not close the compartment door manually before sequencing starts.

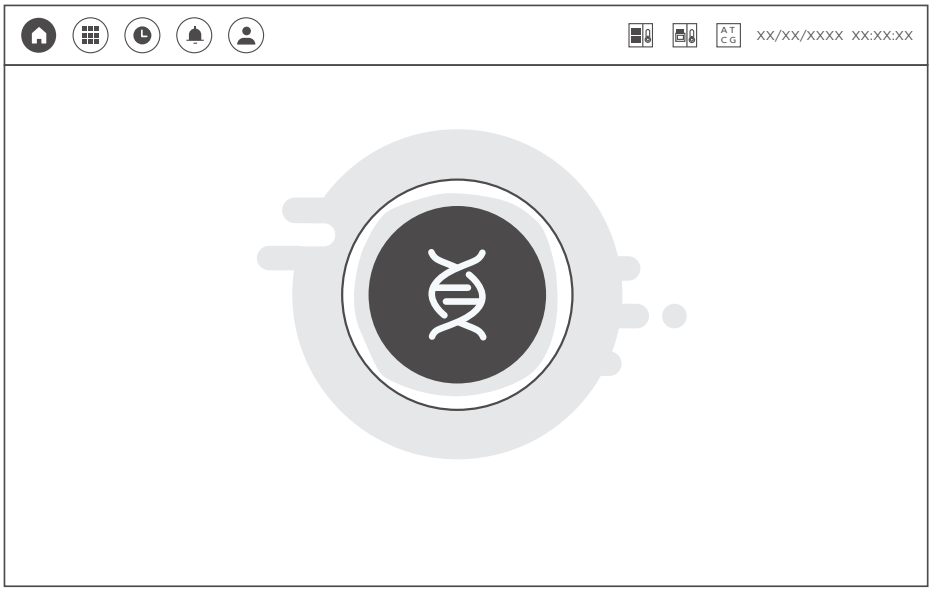


Figure 7 Main interface

2. Select the **Recipe** list and select a recipe.

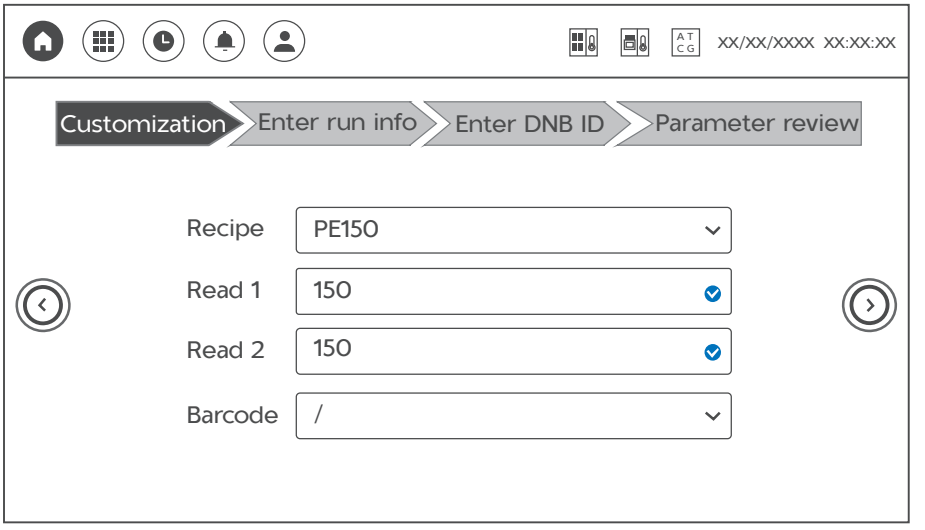


Figure 8 Selecting a recipe

3. Select the **Read 1** box and enter the read length of read 1 with the on-screen keyboard.

   Tips For any recipe, the entered read length cannot be greater than the largest read length the recipe can support. Reduce the read length manually if required.

4. Select the **Read 2** box and enter the read length of read 2 with the on-screen keyboard.

   Tips
   - For single-end recipes, the read 2 box cannot be edited.
   - For paired-end recipes, such as PE150, read 1 and read 2 are 150 by default.
   - Reduce the read length manually if required.

5. Select the **Barcode** list to select the required barcode recipe.

   Four optional barcode recipes are shown as below:

Item	Description
MGI UDBA	Supports dual-barcode sequencing. Compatible with MGI UDB Primers Adapter Kit A.
MGI PFA	Supports dual-barcode sequencing. Compatible with MGI UDB PF Adapter Kit A.
MGI Single	Supports single-barcode sequencing. Compatible with MGI single-barcode Adapter Kit.
/	No barcode sequencing.

If customizing barcode recipe is required, refer to *DNBSEQ-E25RS&DNBSEQ-E25ARS Genetic Sequencer User Manual*.

6. Ensure that the required recipe is selected, and select  .

   If you select  , a message appears stating that **Are you sure you want to quit? Loaded consumables shall be discarded once offloaded**.

   - To cancel the sequencing, select **Yes**. The device returns to the main interface and starts to offload.
   - To continue the sequencing, select **No**.

7.3 Loading the flow cell, reagent cartridge and waste container

Perform the following steps:

1. Enter information for the **Flow cell ID, Throughput** and **Expiration date** boxes on the left by scanning the QR code on the plastic package of the sequencing flow cell or manually with the on-screen keyboard.

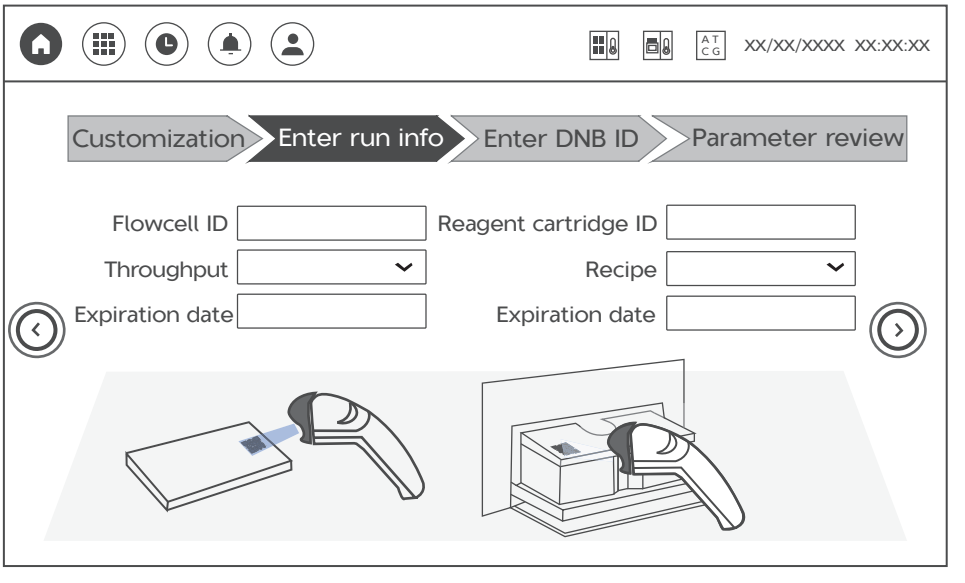


Figure 9 Scanning the QR code of the flow cell

2. Open the package of the flow cell to check whether the flow cell is intact and whether the scanned ID is the same as the ID on the flow cell.

   Tips Do not touch small wells in the flow cell when the flow cell is being held to prevent contamination.

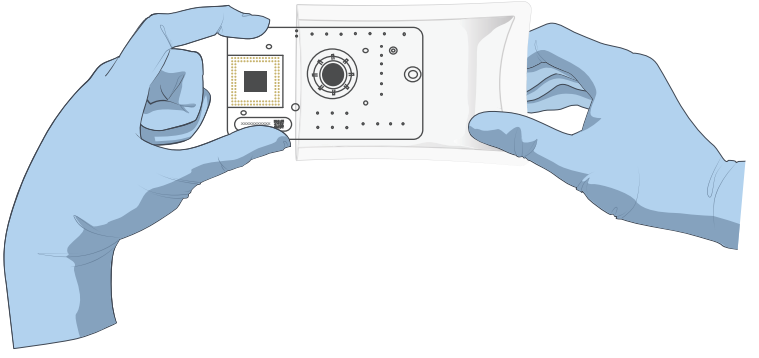


Figure 10 Checking the flow cell

3. After the rack pops up, pinch the rotary valve in the flow cell and align the wells in the flow cell correspondingly with the positioning columns on the rack to install the flow cell onto the rack.

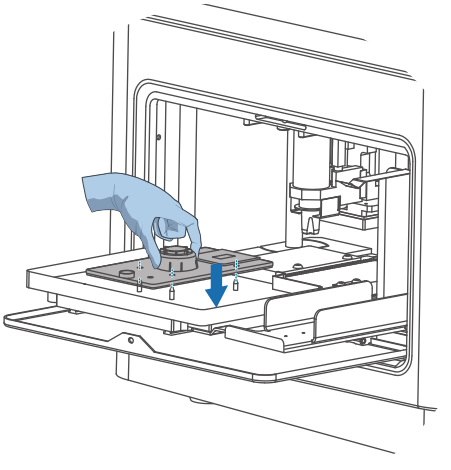


Figure 11 Placing the flow cell

4. Enter information for the **Reagent cartridge ID, Recipe** and **Expiration date** boxes on the right by scanning the QR code on the reagent cartridge or manually with the on-screen keyboard.

   Tips When selecting the recipe, select **PE150** for PE sequencing and **SE100** for SE sequencing.

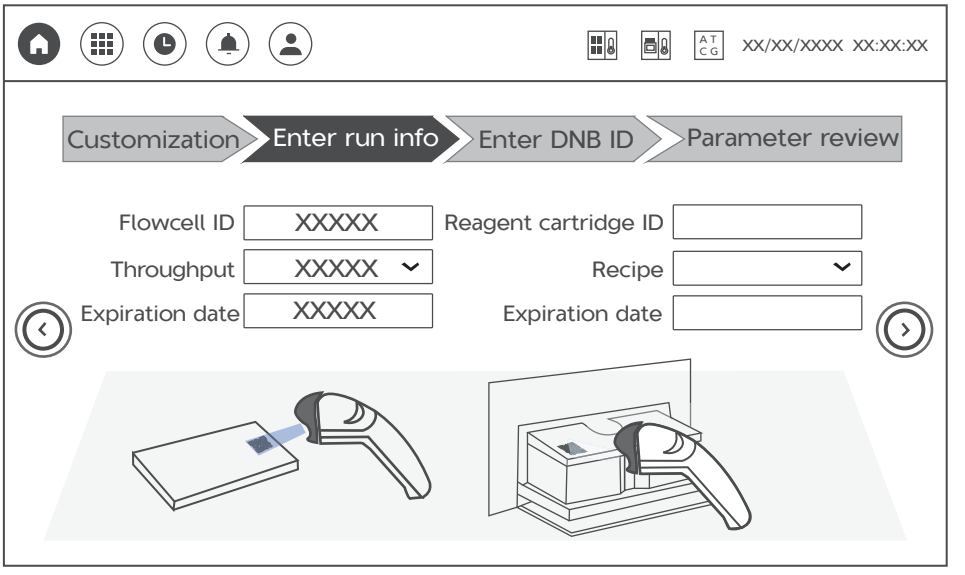


Figure 12 Scanning the QR code of the reagent cartridge

5. Slowly and carefully remove the bottom cover in the middle of the reagent cartridge and ensure that 21 rubber stoppers are present in the wells on the bottom side of the reagent cartridge.

   Tips If rubber stoppers fall off the cartridge or onto the inside of the bottom cover, refer to *Solution for rubber stoppers that fall off or tilt on Page 34*.

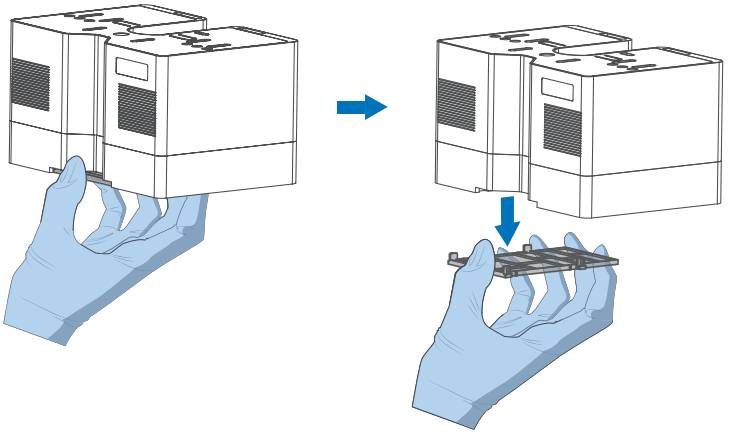


Figure 13 Taking off the bottom cover

6. Align the reagent cartridge with the positioning columns on the rack and place it over the flow cell. Keep the reagent cartridge horizontal in the process.

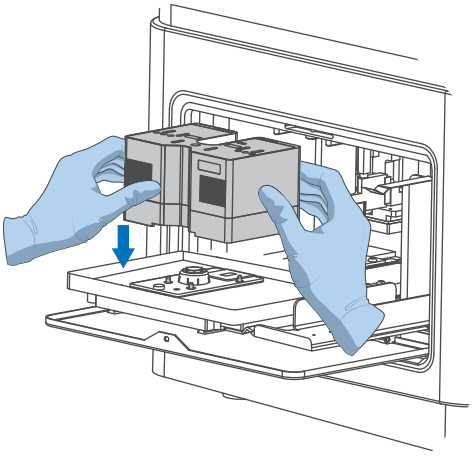


Figure 14 Placing the reagent cartridge

Tips If the reagent cartridge is placed for the second time, ensure that the rubber stoppers are present on the reagent cartridge.

7. Ensure that the cover of the waster container is open, place the waste container on the rack according to the direction shown in the figure below and ensure that it fits into the bent metal clip.

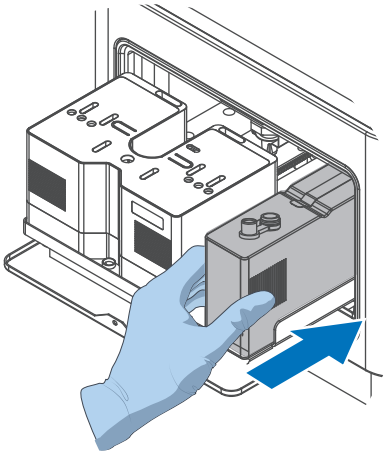


Figure 15 Placing the waste container

Tips The waste container is in place when the positioning clamp exactly aligns with the groove of the waste container.

8. After placement, click ⓞ >**Yes**. The rack automatically retracts into the compartment and the device presses the components tightly. Click ⓞ to enter the next step.

CAUTION Do not manually close the compartment door.

7.4 Loading DNBs

Perform the following steps:

1. Enter information for the **DNB ID** box by scanning the QR code on the sample tube or manually with the on-screen keyboard.

CAUTION Returning to the customization interface and selecting to cancel sequencing will cause the loaded reagent cartridge and flow cell to become inoperative.

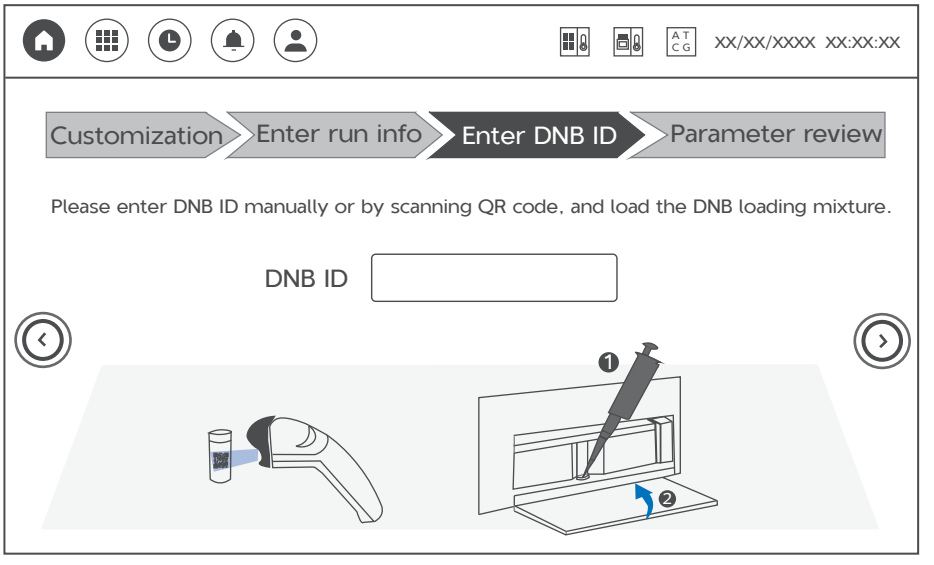


Figure 16 Entering DNB ID

2. Prepare the DNB loading mixture.

   1) Use a vortex mixer to mix DNB Load Buffer II thoroughly for 5 seconds, centrifuge it briefly. Place it on ice until use.

   Tips If crystals appear in DNB Load Buffer II, vortex it vigorously by using a vortex mixer for 1 to 2 minutes until the precipitation dissolves. Centrifuge it briefly before use.

   2) Add 34 μL of DNB Load Buffer II to the PCR tubes containing DNBs to make the DNB loading mixture.

   Tips The DNB loading mixture should be used immediately after preparation.

Table 14 DNB loading mixture

Component	Volume (µL)
DNB Load Buffer II	34
DNB	102
Total volume	136

3) Use a wide-bore, non-filtered pipette tip to pipette up and down 8 times to mix the DNB loading mixture gently.

Tips Do not centrifuge, vortex, or shake the tube.

3. Add all the DNB loading mixture into the DNB loading well by using a wide-bore pipette tip. Ensure that no bubbles exist in the well and then go to the next step.

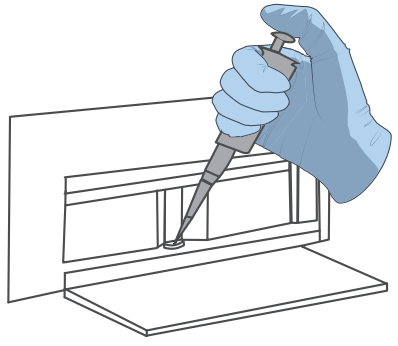


Figure 17 Adding DNB loading mixture

Tips To prevent bubbles formation, put the wide-bore pipette tip against the inner wall of the DNB loading well to ensure that the mixture flows into the well slowly.

4. Push the compartment door back to close it.
5. Click ⊙ to enter the next step.

7.5 Reviewing parameters

Check all the information.

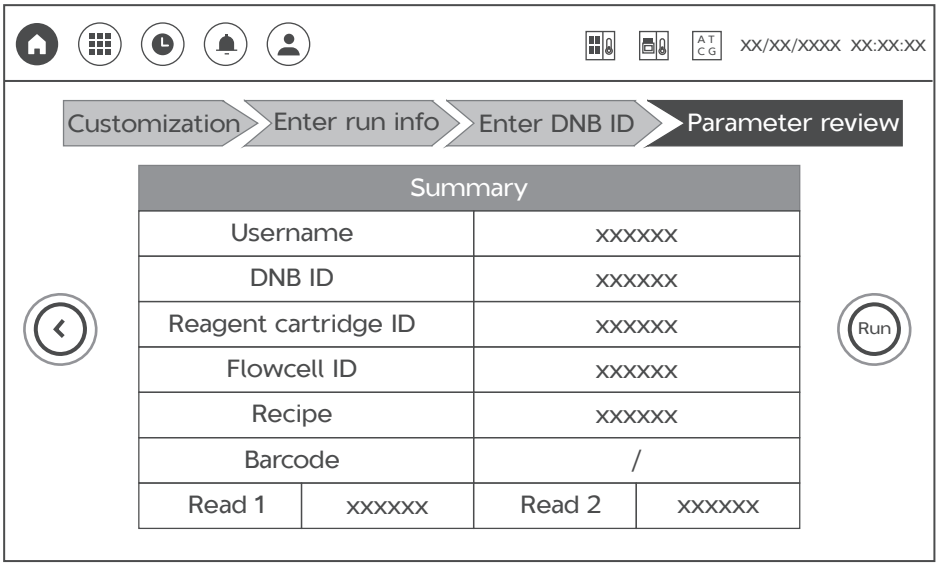


Figure 18 Reviewing parameters

- If the information is incorrect, select ‹ to return to the previous interface to modify the information.

  ⚠ CAUTION Returning to the customization interface and selecting ‹ to cancel sequencing will cause the loaded reagent cartridge and flow cell to become inoperative.

- If the information is correct, ensure that the computing module is connected and the compartment door is closed. Select Run > **Yes** to start sequencing.

7.6 Starting sequencing

CAUTION

- Before sequencing, check whether the compartment door is closed. If it is open, ensure that no barrier is in the door and close the door.
- During sequencing, do not operate the door to avoid affecting the sequencing result.
- Do not impact or move the device. Remove vibration-producing equipment around the device during sequencing. Otherwise, inaccurate results or even damages to the device might occur.
- Pay attention to the icons, status indicators, and pop-up dialog boxes on the screen. In the event that abnormalities occur, check the problematic parts according to the prompts. If problems persist, contact the technical support.

When sequencing starts, the DNB loading interface is displayed.

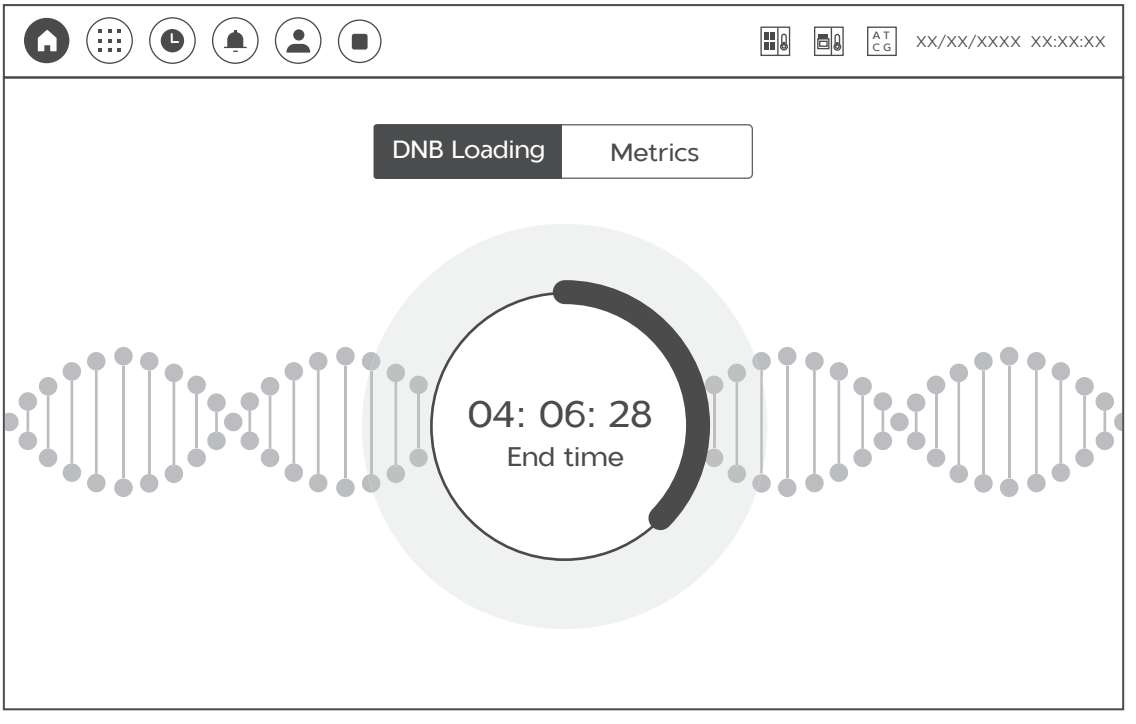


Figure 19 DNB loading interface

The following table describes the controls in the interface:

Item	Description
■	Select to end sequencing in advance. ⚠ CAUTION Sequencing cannot be resumed after ending, so please operate with care.
DNB loading	Displays the current sequencing phase, such as **DNB loading** and **progress (current cycles/total cycles)**.
Progress	Displays the sequencing progress as a percentage.
Time remaining	Displays the remaining time of sequencing. Select to view **End time**.
End time	Displays the estimated ending time of sequencing. Select to view **Time remaining**.
Metrics	Displays sequencing quality. In **Metrics**, select the drop-down list of **Sequencing metrics** and select the required indicator to view the graph.

After the first cycle is completed, the first base report is displayed.

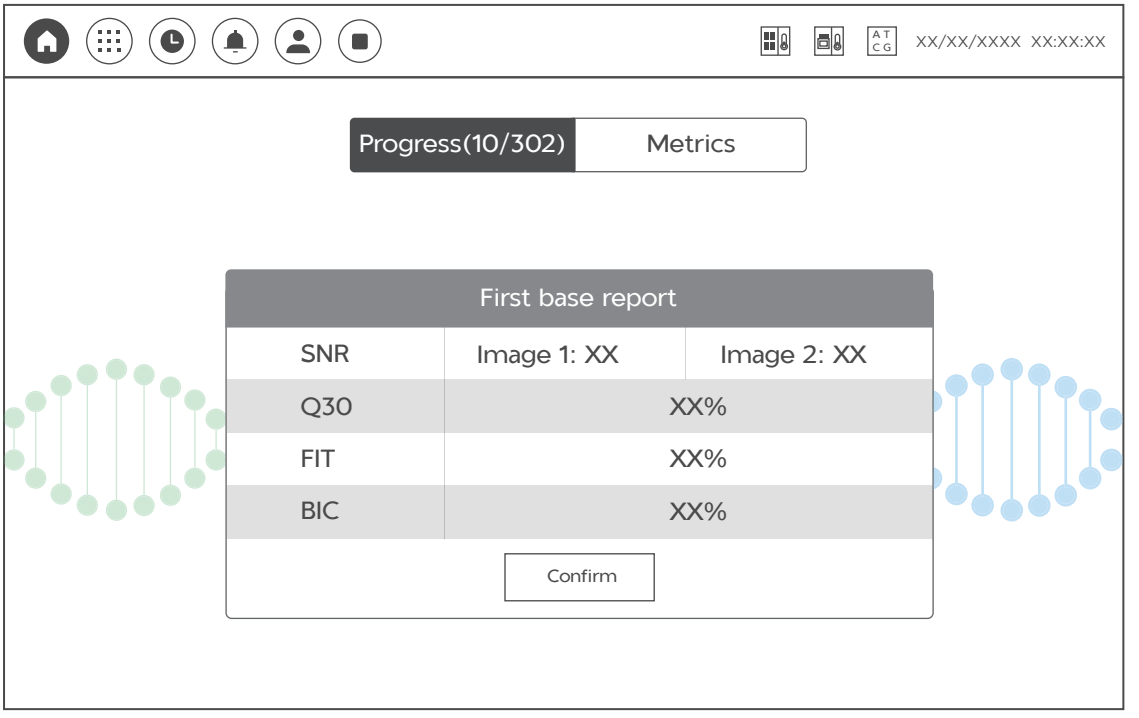


Figure 20 First base report

Tips After tapping **Confirm**, the first base report cannot be viewed again.

After all cycles are completed, wait for the report as prompted.

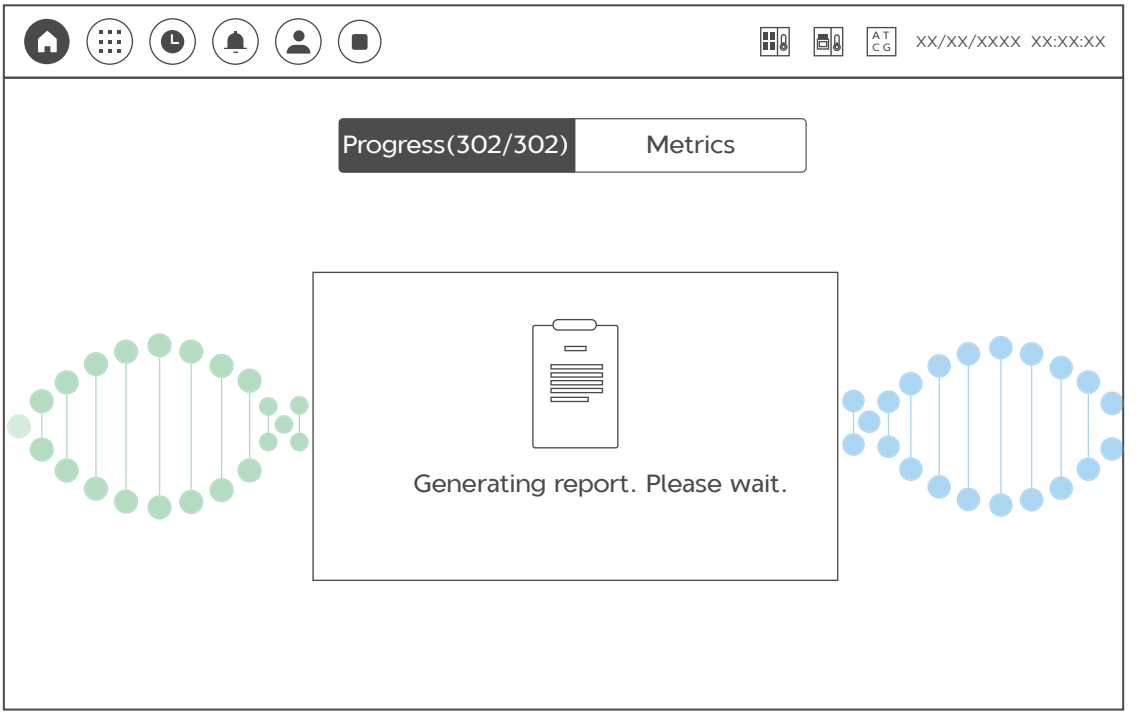


Figure 21 Report generation interface

- If the report is generated, the report is displayed automatically. Close the report and select to enter the sequencing completion interface.
- If no report is generated, and **Task exception** are displayed. Select to enter the sequencing completion interface. Check the possible causes of the failure.
  - If failure results from the device problem, contact the technical support to maintain it.
  - If failure results from improper operation (for example, the computing module is disconnected), perform the sequencing run again by following the required procedure.

After the following figure is displayed, the sequencing is completed.

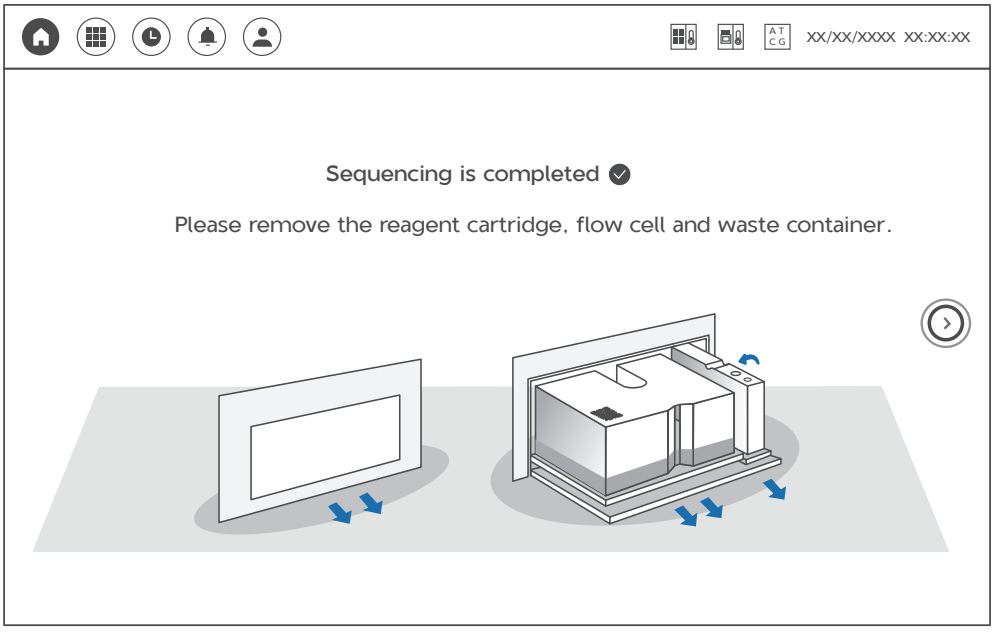


Figure 22 Sequencing completion interface

7.7 Removing the reagent cartridge, flow cell and waste container

CAUTION

- When removing the flow cell and the reagent cartridge after sequencing, ensure that the flow cell is firmly attached to the reagent cartridge and keep the reagent cartridge horizontal to prevent waste spills causing biological contamination.
- After sequencing and before shutting down the sequencer, check whether the reagent cartridge and waste container are removed to avoid damages to components.

Perform the following steps:

1. Remove the flow cell and the reagent cartridge. In the process, ensure that the flow cell is firmly attached to the reagent cartridge and keep the reagent cartridge horizontal to prevent waste spills causing biological contamination.

   Tips After removing the reagent cartridge and flow cell, transfer them to a container as soon as possible because reagents may leak out of them.

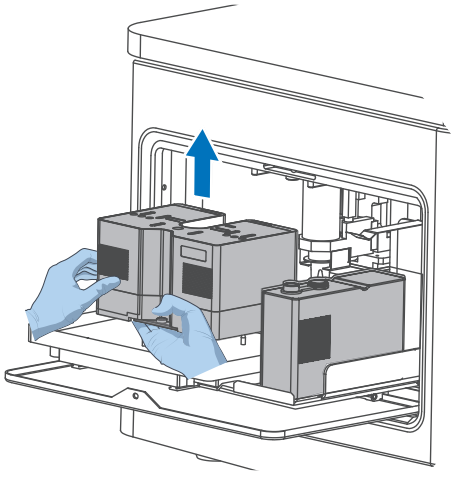


Figure 23 Removing the flow cell and reagent cartridge

2. Close the cover of the waste container, slightly raise up the waste container and remove it from the rack. Transfer it to the designated container.

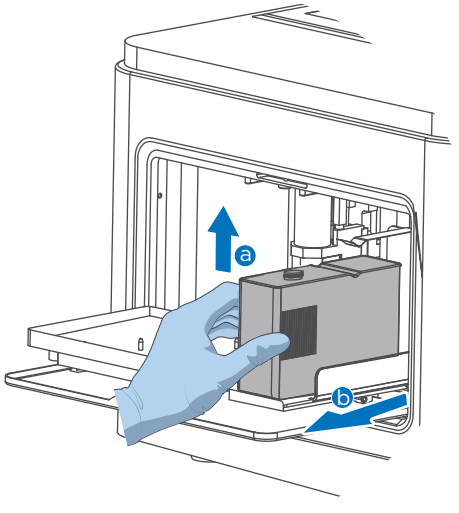


Figure 24 Removing the waste container

3. Push the compartment door back to close it.
4. Click  to return to the main interface.
5. Dispose of the tube, flow cell, and reagent cartridge in accordance with local regulations and safety standards of your laboratory.

Chapter 8 Device maintenance

After each sequencing, the device starts to wash automatically.

The wash is used to clear remaining reagents in the pipelines to avoid crystals blocking the pipelines.

Chapter 9 Troubleshooting

9.1 DNB concentration does not meet requirements

When the DNB concentration does not meet requirements specified in *Quantifying DNBs on Page 14*, try the steps below:

1. Check whether the reagent kit has expired.
2. Check whether the libraries meet the requirements.
3. Make DNBs again.

   If the DNB concentration still does not meet the requirements after a new preparation, contact the technical support.

9.2 Temporary storage of the open or thawed set

- If the set has been thawed, but it cannot be used in time, it can go through up to one freeze-thaw cycle.
- If a reagent cartridge has been thawed, but it cannot be used in time, store it in a refrigerator at 2 ℃ to 8 ℃ and use it within 24 hours. Before use, mix the reagent cartridge according to *Preparing the reagent cartridge on Page 15*.
- If a reagent cartridge has been thawed and the signal protein mixture has been added into the cartridge, but it cannot be used in time, store it at room temperature and use it within 2 hours.
- If a reagent cartridge has been thawed and the MDA mixture has been added into the cartridge, but it cannot be used in time, store it at room temperature and use it within 2 hours.

9.3 Solution for rubber stoppers that fall off or tilt

When the rubber stopper at the bottom of the reagent cartridge falls off or tilts, but the reagent does not leak out of the cartridge, try the steps below:

1. Keep the reagent cartridge horizontal.

2. Check the well whose rubber stopper falls off or tilts.
3. Use a pair of pointed-tip tweezers to hold the rubber stopper, align it with the well and tighten the rubber stopper.
4. Tighten other rubber stoppers that do not come off or tilt.

Appendix 1 Manufacturer information

Manufacturer	Qingdao MGI Tech Co., Ltd.
Address	Building 4, No. 2, Hengyunshan Road, Qingdao Area, Pilot Free Trade Zone, Shandong, China.
E-mail	MGI-service@mgi-tech.com
Website	www.mgi-tech.com

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