STOmics MGI

Part No.: H-020-000918-00

Cat No.	Product model
940-001886-00	G400 STO FCL PE75

Stereo-seq Visualization Reagent Set

DNBSEQ-G400RS

Instructions for Use

Version: 3.0

Leading Life Science Innovation

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CE Research Use Only

Wuhan MGI Tech Co., Ltd.

About the instructions for use

This instructions for use is applicable to DNBSEQ-G400RS Stereo-seq Visualization Reagent Set. The manual version is 3.0.

Figures in this Instructions for Use are for illustrative purpose only. The content might be slightly different from the device. For the most up-to-date details, refer to the device purchased.

StandardMPS sequencing reagents in modified form are available in Germany, UK, Sweden, and Switzerland.

Revision history

Version	Date	Description
3.0	January 20, 2025	Add FF V1.3 library sequencing
2.0	June 24, 2024	Update product name.
1.0	April 22, 2024	Initial release.

Visualization set

Catalog number	Name	Model	Version
940-001886-00	DNBSEQ-G400RS Stereo-seq Visualization Reagent Set	G400 STO FCL PE75	1.0

Contents

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Chapter 1 Introduction

This instructions for use describes how to perform sequencing using the DNBSEQ-G400RS Stereo-seq Visualization Reagent Set and includes instructions regarding sample preparation, flow cell preparation, sequencing kit storage, the sequencing protocol and device maintenance.

1.1 Applications

DNBSEQ-G400RS Stereo-seq Visualization Reagent Set is compatible with Stereo-seq FF V1.3 and Stereo-seq OMNI FFPE libraries constructed using the Stereo-seq 16 Barcode Library Preparation Kit (Cat. No.: 111KL160). This stereo-seq visualization reagent set is intended to be used for scientific research only and cannot be used for clinical diagnosis.

1.2 Sequencing technology

This stereo-seq visualization reagent set utilizes DNBSEQ technology. A sequencing run starts with the hybridization of a DNA anchor, then a fluorescent probe is attached to the DNA Nanoball (DNB) using combinatorial probe anchor sequencing (cPAS) chemistry. Finally, the high-resolution imaging system captures the fluorescent signal. After digital processing of the optical signal, the sequencer generates high-quality and accurate sequencing information.

1.3 Data analysis

During the sequencing run, the control software automatically runs basecalling analysis software and delivers raw sequencing data outputs for secondary analysis.

1.4 Sequencing read length

Sequencing read length determines the number of sequencing cycles for a given sequencing run. For example, a PE25+59 cycle run performs Read1 of 25 cycles and Read2 of 59 cycles for a total of 84 cycles. At the end of the insert sequencing run, an extra 10 cycles of barcode read can be performed if required.

Table 1 Sequencing cycle

Library type	Sequencing read length	Read1 read length	Read2 read length	Barcode read length	Total read length	Maximum cycles
OMNI FFPE	PE25+59	25	59	10	26+60+10	172
FF V1.3	PE50+100	50 (dark reaction cycle is from 26 to 40)	100	10	51+101+10	172

1.5 Sequencing and analysis times

Table 2 FCL Sequencing and analysis times for each read length (hr)

/	OMNI FFPE (PE25+59)	FF V1.3 (PE50+100)
Single flow cell	19.5	30.0
Dual flow cells	20.5	31.0
Data analysis (single flow cell)	0.3	0.4
Data analysis (dual flow cells)	0.6	0.8

Tips

- The sequencing time (single flow cell/dual flow cells) in the table above includes the time required from post-loading prime to sequencing completion. The data analysis time includes the time required for barcode demultiplexing (if **Split barcode** is selected) and FASTQ files output when sequencing is completed.
- The time in the table above is theoretical. The actual run time may vary among various sequencers.

1.6 Warnings and Cautions

- This product is for research use only. Please read the Instructions for Use carefully before use.
- Familiarize yourself with the precautions and operation methods of the various laboratory instruments before performing the experiment.
- If you accidentally splash reagents or waste liquids on your skin or into your eyes, immediately flush the affected area with large amounts of water and seek medical aid immediately.
- All the samples and waste materials should be disposed of according to relevant laws and regulations.
- This product is for one sequencing run only and cannot be reused.
- The components and packages are batched separately. Keep the components in the packages until use and do not remove them. Mixed use of reagent components from different batches of kits is not recommended.
- Do not use expired products.

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

2.1 Main components

Tips
- Mixed use of reagent components from different batches is not recommended.
- The components and packages are batched separately.
- Keep the components in the packages until use.

Table 3 DNBSEQ-G400RS Stereo-seq Visualization Reagent Set (G400 ST0 FCL PE75) Cat. No.: 940-001886-00

Component	Cap color	Spec&quantity	Storage temperature	Transportation temperature	Expiration date
Sequencing Flow cell	/	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	10 months
Low TE Buffer		300 μL×1 tube			
STO Make DNB Buffer		80 μL×1 tube			
Make DNB Enzyme Mix I (OS-V4.0)		160 μL×1 tube			
Make DNB Enzyme Mix II (OS-V4.0)		8 μL×1 tube			
Stop DNB Reaction Buffer		100 μL×1 tube			
DNB Load Buffer I		200 μL×1 tube			
DNB Load Buffer II		180 μL×1 tube			
Microcentrifuge Tube 0.5 mL (Empty)		1 tube			
dNTPs Mix		0.70 mL×1 tube			
dNTPs Mix II		0.96 mL×1 tube			
Sequencing Enzyme Mix II		2.00 mL×1 tube			
Inactive MDA Reagent		3.50 mL×1 tube			

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Expiration date
MDA Enzyme Mix II		0.50 mL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	10 months
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			

2.2 User-supplied equipment, reagents, and consumables

Tips
- Avoid making and loading DNBs using filtered pipette tips.
- It is highly recommended that pipettes and tips of the suggested brands and catalog numbers be used. Using other brands may yield negative results.

Table 4 User-supplied equipment and consumables

Type	Equipment and consumables	Recommended brand	Catalog number
Equipment	Qubit 4.0 Fluorometer	Thermo Fisher	Q33226
	Mini centrifuge	Major Laboratory Supplier (MLS)	/
	Vortex mixer	MLS	/
	Thermal cycler	Bio-Rad	/
	Pipette	Eppendorf	/
	2 ℃ to 8 ℃ refrigerator	MLS	/
	-25 ℃ to -15 ℃ freezer	MLS	/

Type	Equipment and consumables	Recommended brand	Catalog number
Reagent	Qubit ssDNA Assay Kit	Thermo Fisher	Q10212
	Qubit dsDNA HS Assay Kit	Thermo Fisher	Q32851/Q32854
	Power dust remover	MATIN	M-6318
	Sterile pipette tip (box)	AXYGEN	/
	200 µL Wide-Bore Pipette Tips	AXYGEN	T-205-WB-C
	Qubit Assay Tubes	Thermo Fisher	Q32856
	100%Tween-20	MLS	/
	5 M NaCl solution	MLS	/
	2 M NaOH solution	MLS	/
Consumables	0.2 mL PCR 8-tube strip	AXYGEN	/
	1.5 mL microcentrifuge tube	AXYGEN	MCT-150-C
	2.0 mL cryotube	SARSTEDT	72.609.003
	Ice bucket	MLS	/
	Electronic pipette	Labnet	FASTPETTEV-2
	Serological pipette	CORNING	/
	5 mL tube	SARSTEDT	60.558.001
	KimWipes™	VWR	/
	Portable DNB Loader	MGI	900-000217-00
	Sequencer Cleaning Cartridge	MGI	940-000622-00

Chapter 3 Sequencing workflow

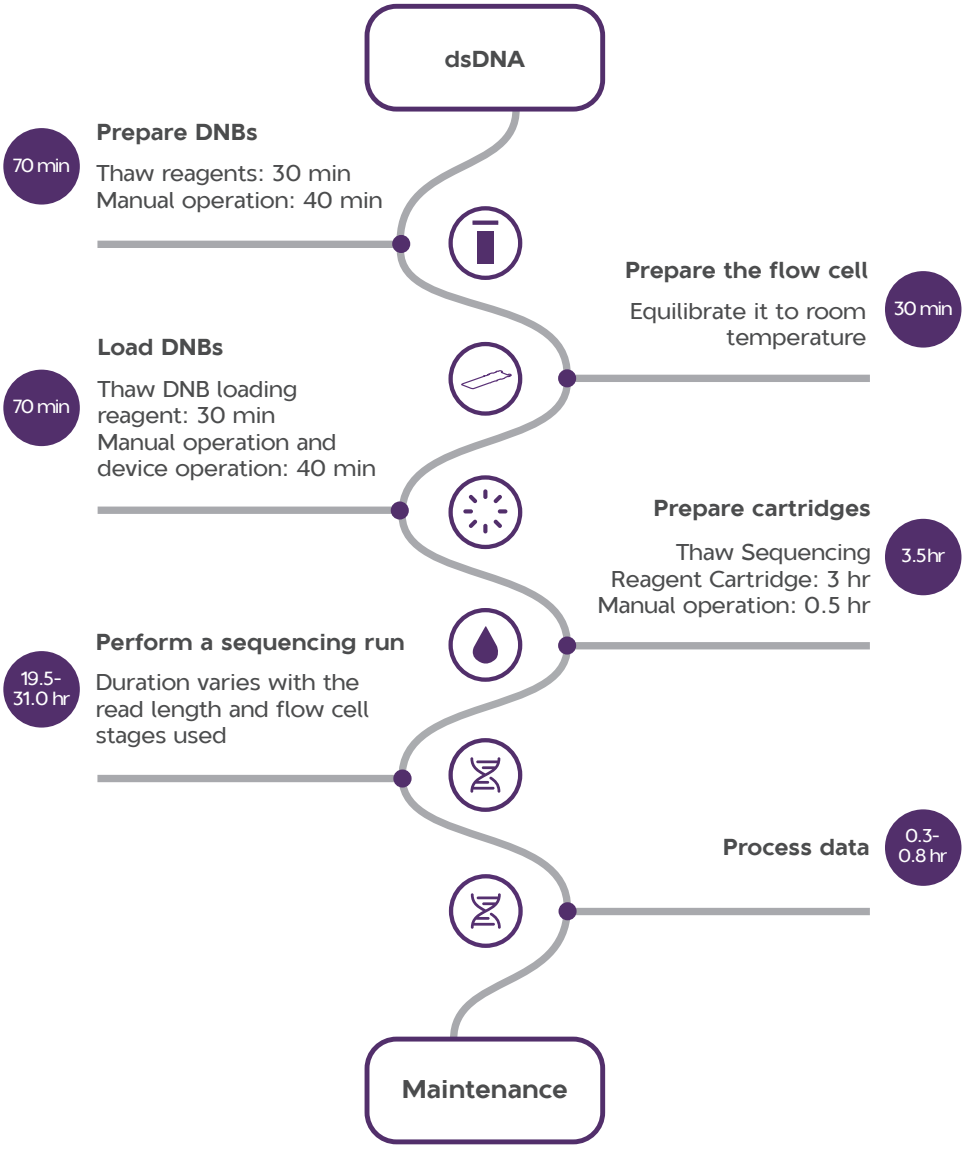

Figure 1 Sequencing workflow

Tips The manual operation duration mentioned above is for reference only. The actual duration may vary with your proficiency level.

Chapter 4 Preparing DNBs

4.1 Recommended library insert size

The stereo-seq visualization reagent set is compatible with libraries constructed using the Stereo-seq OMNI Transcriptomics Set for FFPE or the Stereo-seq Transcriptomics Sets for FF V1.3. The recommended size distribution of OMNI FFPE inserts ranges between 150 bp and 1000 bp, with the main insert size fragment centered within ±100 bp. The recommended size distribution of FF V1.3 inserts ranges between 200 bp and 600 bp, with the main insert size fragment centered within ±100 bp.

Tips
- Average data output will vary with library types and applications.
- If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.

Table 5 Recommended insert size

Library type	Read length	Recommended library insert distribution (bp)	Data output (M/lane)
OMNI FFPE	PE25+59	150 to 1000	~320
FF V1.3	PE50+100	200 to 600	~320

4.2 Library concentration and amount requirement

Table 6 Library requirement

Libraries	library concentration
OMNI FFPE dsDNA	≥ 3 ng/μL
FF V1.3 dsDNA	≥ 20 fmol/μL

Tips

- If the library concentration is unknown, it is recommended to perform dsDNA library quantitation C (ng/μL) using Qubit dsDNA Assay Kit and Qubit 4.0 Fluorometer.
- Use the equation below to convert the concentration of the ssDNA library from ng/μL to fmol/μL:

$$C(\text{fmol}/\mu\text{L}) = \frac{3030 \times C(\text{ng}/\mu\text{L})}{N \times 2}$$

  N represents the average number of basepairs within the DNA fragments (the lengths of which includes associated adapter sequences). C (ng/μL) represents the DNA library concentration.
- If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.

4.3 Making DNBs

Tips

- Mixed use of reagent components from different batches is not recommended.
- For transferring or mixing DNBs, use the wide-bore, non-filtered pipette tips.
- For preparing other reagents, use a proper pipette tip according to the instructions.

4.3.1 Preparing reagents for making DNBs

Perform the following steps:

1. Place the library on ice until use.
2. Take the following reagents out of the stereo-seq visualization reagent set and thaw the reagents at room temperature.

Table 7 Reagent preparation 1

Component	Cap color
Low TE Buffer	
STO Make DNB Buffer	
Stop DNB Reaction Buffer	

3. Take the Make DNB Enzyme Mix I (OS-V4.0) out of the stereo-seq visualization reagent set and thaw the reagent for approximately 30 min on ice.

Table 8 Reagent preparation 2

Component	Cap color
Make DNB Enzyme Mix I (OS-V4.0)	

4. After thawing, mix all reagents using a vortex mixer for 5 sec. Centrifuge briefly and then place it on ice until use.

Tips Mixed use of reagent components from different batches is not recommended.

4.3.2 Calculating the number of DNB reactions

- Using the sequencer to load DNBs:

  All lanes in the flow cell must be loaded with the same DNBs. Each lane requires 50 µL DNB.

- Using the MGIDL-200H to load DNBs:

  Different DNBs can be loaded into different lanes. Each lane requires 25 µL DNB.

Table 9 Required number of make DNB reactions for each flow cell

Flow cell type	Loading system	DNB volume (µL) / Lane	Make DNB reaction (µL)	Required number of make DNB reactions / flow cell
FCL	Sequencer	50	100	2
	MGIDL-200H	25	100	1 to 4

4.3.3 Calculate the required amount of dsDNA library

- The required volume of dsDNA library is determined by the required library amount and library concentration quantified in *4.2 Library concentration and amount requirement on Page 9*.

Tips
- If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.
- All samples should be considered potentially infectious and should be handled in accordance with the applicable national regulations.
- Calculate the required dsDNA library for each Make DNB reaction and fill it in as V according to *Table 11 Make DNB Reaction Mixture 1 on Page 12*.
- C1 represents the OMNI FFPE library concentration (ng/μL) in *4.2 Library concentration and amount requirement on Page 9*. C2 represents the FF V1.3 library concentration (fmol/μL) in *4.2 Library concentration and amount requirement on Page 9*.

Table 10 Required volume of dsDNA library

Library	Required dsDNA volume of 100 μL DNB reaction (μL)
OMNI FFPE dsDNA	V=60 ng/C1
FF V1.3 dsDNA	V=400 fmol/C2

4.3.4 Making DNBs

Perform the following steps:

1. Take out a 0.2 mL 8-tube strip or PCR tubes. Prepare Make DNB Reaction Mixture 1 according to the table below.

Tips
- The following table illustrates the volume used for one make DNB reaction. The required number of make DNB reactions is determined by the actual application as described in *4.3.2 Calculating the number of DNB reactions on Page 10*.
- V represents the volume of input library; refer to *4.2 Library concentration and amount requirement on Page 9*.

Table 11 Make DNB Reaction Mixture 1

Component	Cap color	Volume (μL)
Low TE Buffer		20-V
STO Make DNB Buffer		20
dsDNA library	/	V
Total Volume		40

2. Mix Make DNB Reaction Mixture 1 thoroughly by using a vortex mixer. Centrifuge it for 5 sec and place it on ice until use.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown in the table below:

Table 12 Primer hybridization reaction conditions

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

4. Take Make DNB Enzyme Mix II (OS-V4.0) out of the stereo-seq visualization reagent set and place it on ice. Centrifuge briefly for 5 sec by using a mini spinner and place it on ice.

   Tips
   - Do not keep Make DNB Enzyme Mix II (OS-V4.0) at room temperature.
   - Avoid holding the tube for a prolonged time.

5. Take the Make DNB Reaction Mixture 1 tube out of the thermal cycler when the temperature reaches 4 ℃ .
6. Centrifuge briefly for 5 sec, and then place the tube on ice. Prepare the Make DNB Reaction Mixture 2 according to the table below:

   Tips Do not discard the Make DNB Enzyme Mix II (OS-V4.0) after you complete this step; it will be used in DNB loading operations.

Table 13 Make DNB Reaction Mixture 2

Component	Cap color	Volume (µL)
Make DNB Enzyme Mix I (OS-V4.0)		40
Make DNB Enzyme Mix II (OS-V4.0)		2
Total Volume		42

7. Add all of the Make DNB Reaction Mixture 2 into the Make DNB Reaction Mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer, and centrifuge for 5 sec by using a mini spinner.
8. Place the tubes into the thermal cycler for the Rolling Circle Amplification (RCA) reaction. The conditions are shown in the table below.

Tips

- The reaction procedures for FF V1.3 library and FFPE library are different. Select the corresponding procedure according to actual needs.
- When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
- It is recommended to set the temperature of the heated lid to 35 °C or as close as possible to 35 °C .

- RCA conditions for FF V1.3 library:

Table 14 RCA conditions for FF V1.3 library

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

- RCA conditions for OMNI FFPE library:

Table 15 RCA conditions for OMNI FFPE library

Temperature	Time
35 °C (Heated lid)	On
30 °C	30 min
4 °C	Hold

9. When the temperature reaches 4 °C, immediately add 20 µL Stop DNB Reaction Buffer into the RCA reaction tube. Mix gently by pipetting 8 times by using a wide-bore, nonfiltered pipette tip.

   Tips
   - It is very important to mix DNBs gently by using a wide-bore, non-filtered pipette tip.
   - Do not centrifuge, vortex, or shake the tube.
   - Store the DNBs at 4 °C and perform sequencing within 48 hr (about 2 days).

10. Proceed to *4.4 Quantifying DNBs on Page 14*.

4.4 Quantifying DNBs

Perform the following steps:

1. When DNB production is complete, take 2 µL of DNBs, and use Qubit ssDNA Assay Kit and the Qubit Fluorometer to quantify the DNBs. The DNB concentration should be no less than 8 ng/µL. For details, refer to *Appendix 1 Instructions for using Qubit to quantify the DNBs on Page 50*.

   Tips
   - If the concentration is less than 8 ng/µL, refer to *10.1 What should I do if the DNB concentration is low? on Page 46* for details.
   - If there are more than 8 samples to quantify, it is recommended to quantify in batches to avoid inaccurate DNB quantification as the result of fluorescence quenching.

2. Proceed to *Chapter 5 Preparing the flow cell on Page 15*.

Chapter 5 Preparing the flow cell

Perform the following steps:

1. Remove the flow cell plastic package from the stereo-seq visualization reagent set.

   Tips Do not open the outer plastic package yet.

2. Place the flow cell at room temperature for 30 min to 24 hr.
3. Unwrap the outer package before use.

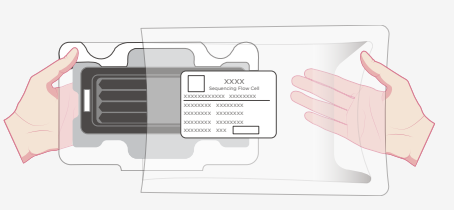

Figure 2 Unwrapping the outer plastic package

Tips
- If the flow cell will not be used within 24 hr after being placed in room temperate and the outer plastic package is intact, the flow cell can be returned to -25 ℃ to -15 ℃ for storage. But the switch between room temperature and -25 ℃ to -15 ℃ must not exceed 3 times.
- If the outer plastic package has been opened but the flow cell cannot be used immediately, store the flow cell at room temperature and use it within 24 hr. If storage exceed 24 hr, it is not recommended to use the flow cell.

4. Take the flow cell out of the inner package and inspect it to ensure that the flow cell is intact and free of debris.

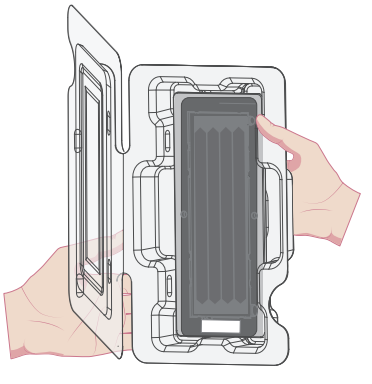

Figure 3 Inspecting the flow cell

Chapter 6 Loading DNBs

6.1 Loading DNBs by sequencer

6.1.1 Preparing reagents

Perform the following steps:

1. Remove DNB Load Buffer II from storage and thaw it on ice for approximately 30 min.
2. After thawing, mix it by using a vortex mixer for 5 sec, centrifuge briefly by using a mini spinner, and place it on ice until use.

   Tips If crystal precipitation is visible in DNB Load Buffer II, vigorously mix the reagent for 2 min by using a vortex mixer to dissolve the precipitation before use.
3. Take a 0.5 mL microcentrifuge tube out of the stereo-seq visualization reagent set and add the following reagents:

Table 16 DNB loading mixture 1

Component	Cap color	Volume (μL)
DNB Load Buffer II		64
Make DNB Enzyme Mix II (OS-V4.0)		2
DNB	/	200
Total Volume		266

4. Combine the components and mix them by gently pipetting 8 times using a wide-bore tip. Place the mixture at 4 ℃ until use.

   Tips
   - Do not centrifuge, vortex, or shake the tube.
   - Prepare a fresh DNB loading mix immediately (within 10 min) before the sequencing run.
   - Each flow cell requires 266 μL DNB loading mixture 1.

6.1.2 Loading DNBs

Perform the following steps:

1. Open the reagent compartment door.

2. Gently lift the DNB loading needle with one hand, remove the cleaning reagent tube with the other hand, load the sample tube prepared in *6.1.1 Preparing reagents on Page 16*, and then slowly lower the DNB loading needle until the tip reaches the bottom of the tube.

   Tips Perform this step if you load DNBs by the sequencer, if not, use an empty tube.

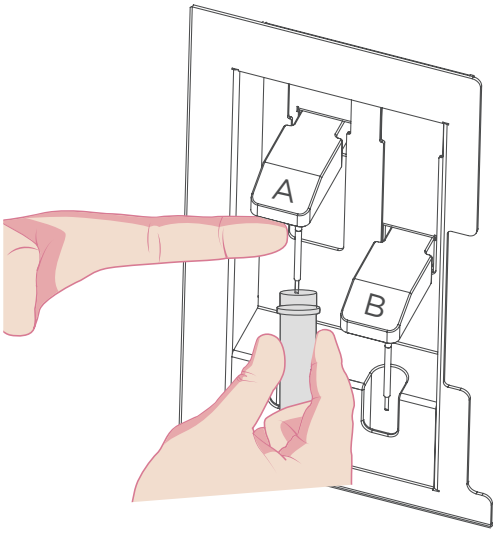

Figure 4 Loading the DNB tube

3. Close the reagent compartment door.
4. Select the DNB loading box in the DNB ID entry interface. After you prepare the Sequencing Reagent Cartridge, perform the sequencing run according to *8.4 Loading the Sequencing Reagent Cartridge on Page 30*.

6.2 Loading DNBs by MGIDL-200H

6.2.1 Preparing reagents

Perform the following steps:

1. Take out a new PCR 8-tube strip and add the reagents listed in the table below.

Table 17 DNB loading mixture 2

Component	Cap color	Volume (µL)
DNB Load Buffer II		8
Make DNB Enzyme Mix II (OS-V4.0)		0.25
DNB	/	25
Total Volume		33.25

2. Combine the components and mix them by gently pipetting 8 times using a wide-bore tip. Place the mixture at 4 °C until use.

   Tips
   - Do not centrifuge, vortex, or shake the tube.
   - Each lane requires at least 30 µL of DNB loading mixture 2.
   - Prepare a fresh DNB loading mixture 2 immediately before the sequencing run.

3. Install the sealing gasket and flow cell.
4. Aspirate 30 µL of DNB loading mixture 2 with a pipette (make sure there are no bubbles) and insert the wide bore tip into the fluidics inlet.

   Tips
   - Do not press the control button of the pipette after inserting the tip into the fluidics inlet.
   - To prevent bubbles from entering the fluidics inlet, do not move the wide-bore tip or flow cell while you are loading the DNB mixture.

6.2.2 Loading DNBs

Tips
- Ensure that the DL-200H is properly maintained.
- Ensure that the sealing gasket of the DL-200H is clean and properly maintained.

Perform the following steps:

1. Install the sealing gasket and the flow cell.

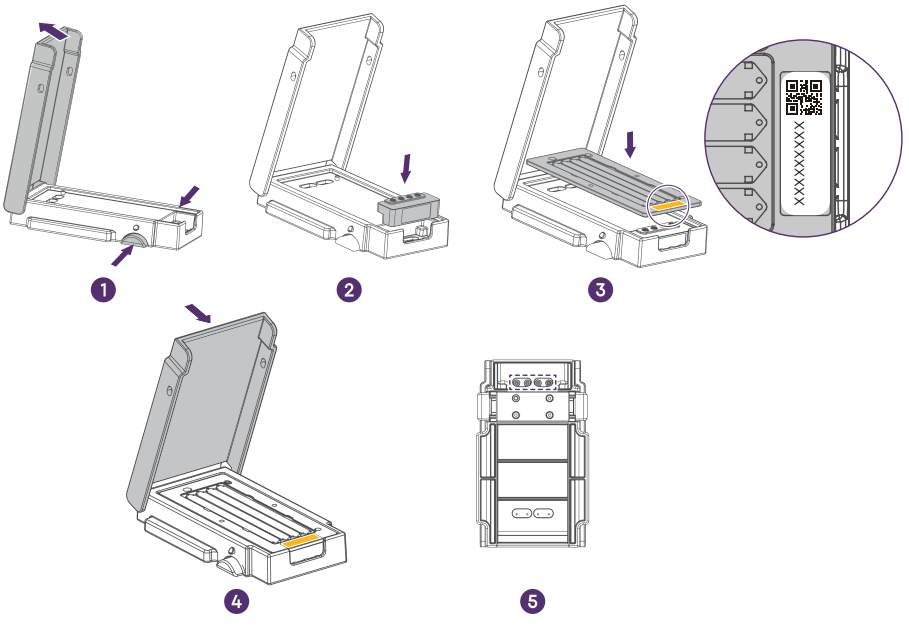

Figure 5 Installing sealing gasket and flow cell

1) Press the latches and open the cover.
2) Place a clean sealing gasket into the groove and ensure that the gasket surface is even.
3) Align the holes of the flow cell with the alignment pins of the device and then place the flow cell on it.

   Tips Ensure that the label of the flow cell is facing upward and in the same position as the sealing gasket.

4) Close the cover and ensure that the cover is securely closed.
5) Place the back of the DL-200H facing upward, and check whether the fluidics inlets align with the holes of the sealing gasket and ensure that the holes are clean.

2. Load DNBs by using the DL-200H.

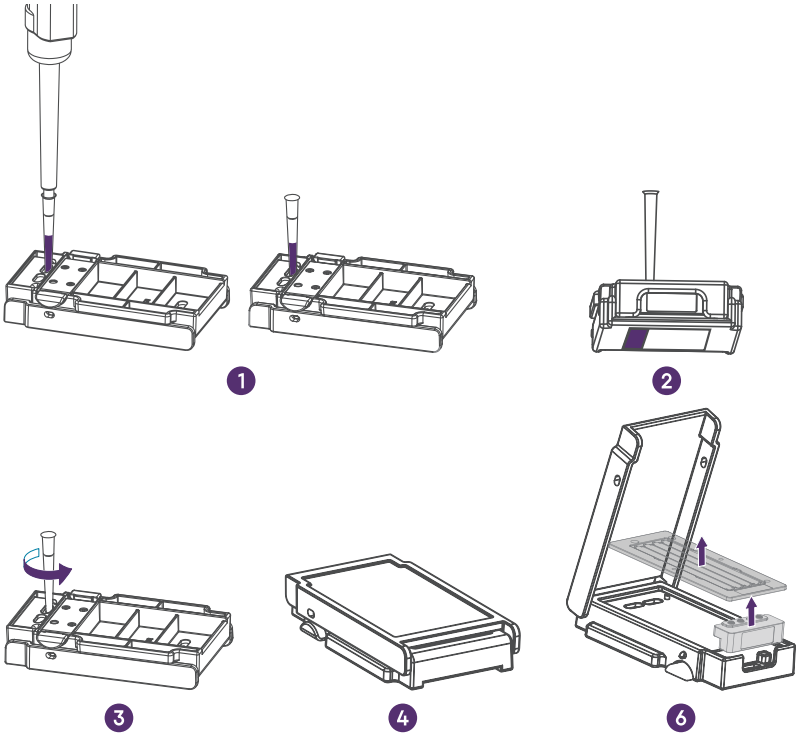

Figure 6 Loading DNBs by using the DL-200H

1) Place the DL-200H on the laboratory bench with the back facing you. Aspirate 30 µL of DNB loading mixture 2 with a wide-bore, non-filtered pipette tip and insert the tip into the fluidics inlet. Eject the tip from the pipette. DNBs will automatically flow into the flow cell.

   Tips Do not touch or move the tip when ejecting the tip. Doing so may introduce bubbles into the flow cell.

2) Keep the DL-200H parallel to the bench and keep the back facing upward. Hold up the device vertically to check whether the DNBs flow into the flow cell.

   Tips
   - During observation, do not tilt the DL-200H. Doing so may cause liquid leakage or even biological contamination.
   - If DNBs do not flow into the lane, slightly press the top of the pipette tip until DNBs start to flow.

3) Ensure that all DNBs flow into the flow cell. Hold the device and rotate the tip counterclockwise to remove it.

4) Repeat steps 1) to 3) to load the DNBs to the remaining lanes. Ensure that you load DNBs to the 4 lanes of the flow cell in ascending order, as shown in the figure below:

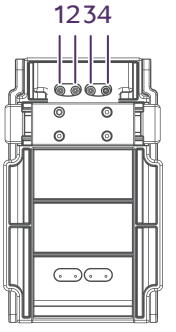

Figure 7 Lane order of DNB loading

5) Place the DL-200H on the bench with the front facing upward and wait 30 min for the DNB loading process.

6) Open the cover and take out the flow cell and the sealing gasket.

3. After the DNB loading process is completed, immediately take the flow cell out and transfer it to the sequencer for sequencing. After you have prepared the Sequencing Reagent Cartridge, perform the sequencing run according to *Chapter 8 Performing a sequencing run on Page 27*.

Chapter 7 Preparing the Sequencing Reagent Cartridge

The sequencing enzyme mix and dNTP mixes are provided in different tubes and are packaged together with the Sequencing Reagent Cartridge. Before the sequencing run can be started, an appropriate amount of sequencing enzyme mix and dNTP mixes must be added to well No. 1 and well No. 2 of Sequencing Reagent Cartridge. Furthermore, MDA Enzyme Mix (MDA: Multiple Displacement Amplification) must be added to well No. 15. If prepared reagent cartridges are not used immediately, refer to *10.3 What rules should I follow if I need to store a reagent kit temporarily? on Page 48*.

Perform the following steps:

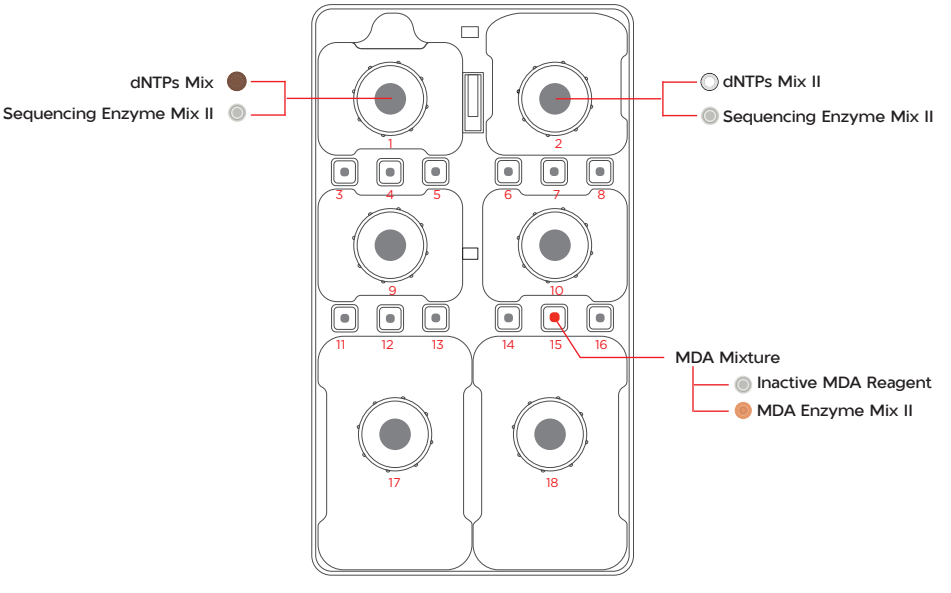

Figure 8 Well position

1. Remove the Sequencing Reagent Cartridge from storage.
2. Thaw in a water bath at room temperature until completely thawed (or thaw in a 2 ℃ to 8 ℃ refrigerator 1 to 2 days in advance). The approximate time to thaw is listed in the following table. Store it in a 2 ℃ to 8 ℃ refrigerator until use.

   Tips After removing the flow cell from -25 ℃ to -15 ℃, the flow cell must be placed at room temperature for at least 30 min and not longer than 24 hr before DNB loading.

Table 18 Approximate thaw times for various sequencing kits

Model	Method		
	Water bath at room temperature (hr)	Refrigerator at 2 °C to 8 °C overnight then water bath at room temperature (hr)	Refrigerator at 2 °C to 8 °C (hr)
G400 STO FCL PE75	3	1.5	36

3. Invert the cartridge 3 times to mix before use.
4. Shake the cartridge vigorously up and down 20 times. And shake the cartridge vigorously clockwise 20 times, and then counterclockwise 20 times. Ensure that the reagents are fully mixed.
5. Wipe away any water condensation on the cartridge cover and well surround with a KimWipes tissue.

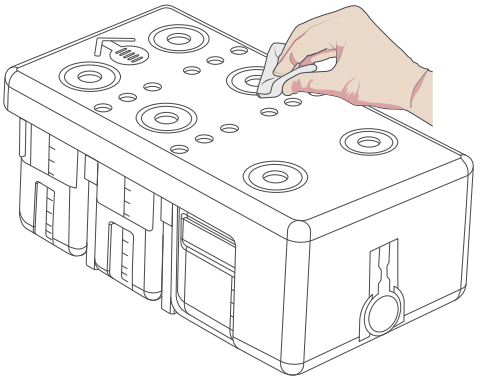

Figure 9 Wiping cartridge cover

6. Remove dNTPs Mix and dNTPs Mix II from -25 °C to -15 °C storage 1 hr in advance and thaw at room temperature. Store at 2 °C to 8 °C until use.
7. Remove the Sequencing Enzyme Mix II from -25 °C to -15 °C storage and place it on ice until use.
8. Remove the Inactive MDA Reagent from storage and place it on ice until use.

Well positions are shown in the figure below:

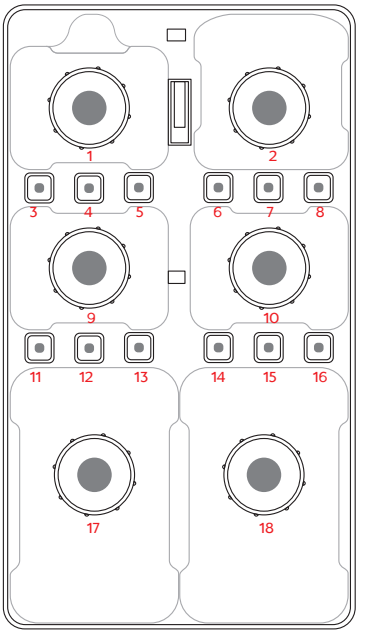

Figure 10 Well positions

9. Pierce the seals in the center of wells No. 1 and No. 2 to make a hole approximately 2 cm in diameter using a 1 mL sterile pipette tip.

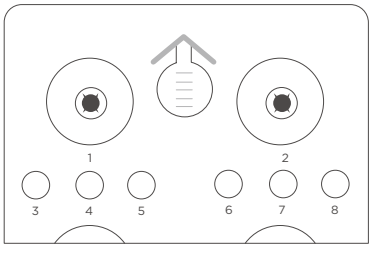

Figure 11 Piercing the seal of cartridge

10. Use a pipette with an appropriate volume range. Add dNTPs Mix into a new 5 mL sterile tube, and then add Sequencing Enzyme Mix II into the dNTPs Mix in the same tube according to *Table 19 Reagent preparation for well No. 1 on Page 25*.

Tips

- Mix the dNTPs Mix using a vortex mixer for 5 sec and centrifuge briefly before use.
- Invert the Sequencing Enzyme Mix II 6 times before use.

Table 19 Reagent preparation for well No. 1

Model	Volume (mL)	
	dNTPs Mix	Sequencing Enzyme Mix II
G400 STO FCL PE75	0.700	1.400

11. Invert the tube 6 times to mix the reagents in the tube before adding the mix into well No. 1.

    Tips When transferring the mixture, pipette carefully to prevent the mixture from spilling out of the reagent tube.

12. Take out a pipette with the appropriate volume range and add reagents according to *Table 20 Reagent preparation for well No. 2 on Page 25*. Add dNTPs Mix II into a new 5 mL sterile tube, and then add Sequencing Enzyme Mix II into the dNTPs Mix II in the same tube.

    Tips
    - Mix the dNTPs Mix II by using a vortex mixer for 5 sec and centrifuge briefly before use.
    - Invert the Sequencing Enzyme Mix II 6 times before use.

Table 20 Reagent preparation for well No. 2

Model	Volume (mL)	
	dNTPs Mix II	Sequencing Enzyme Mix II
G400 STO FCL PE75	0.960	0.600

13. Invert the tube 6 times to mix the reagents in the tube before adding the mix into well No. 2.

    Tips When transferring the mixture, pipette carefully to prevent the mixture from spilling out of the reagent tube.

14. Seal loading wells No. 1 and No. 2 with transparent sealing films.

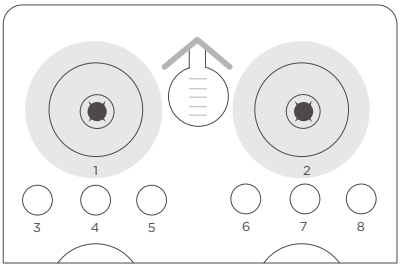

Figure 12 Seal the loading wells of cartridge

15. Press the film around the well with your finger. Ensure that the well is tightly sealed and that no air bubbles exist between the film and the cartridge surface so that the reagents will not flow over the cartridge.

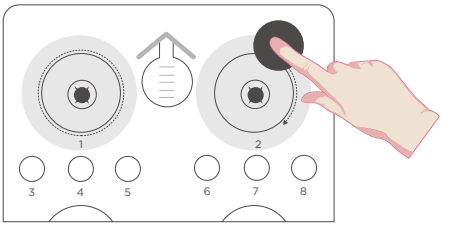

Figure 13 Seal the loading wells of the cartridge tightly

16. Lift the cartridge horizontally and hold both sides of the cartridge with both hands. Shake the cartridge 20 times in a clockwise and counterclockwise direction. Ensure that the reagents are fully mixed.

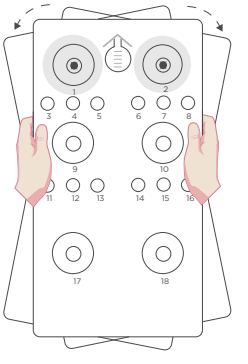

Figure 14 Mixing reagents after loading

17. Carefully remove the seals from the loading wells after fully mixing.

Tips
- Do not reuse the used sealing film.
- To prevent cross-contamination, ensure that the surface around wells No. 1 and No. 2 is clean.

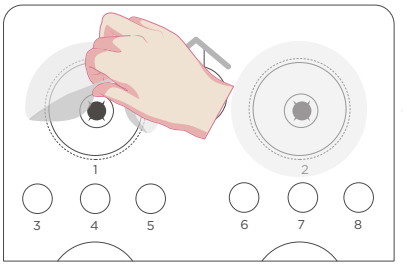

Figure 15 Removing the seal from the cartridge

18. Gently tap the cartridge on the bench to reduce air bubbles in the reagents.
19. Pierce the seal of well No. 15 by using a 1 mL sterile pipette tip.

20. Add 500 µL of MDA Enzyme Mix II to the Inactive MDA Reagent tube with a 1 mL pipette.

    Tips When using MDA Enzyme Mix II, do not touch the wall of the tube. The heat from your hands may affect the enzyme activity.

21. Invert the tube 6 times to mix the reagents.
22. Add all of the mixture to well No. 15. When adding the mixture, ensure that no bubbles appear at the bottom of the tube.
23. Gently tap the cartridge on the bench to reduce air bubbles in the reagents.

    Tips
    - When transferring the mixture, pipette carefully to prevent the mixture from spilling out of the reagent tube.
    - Proceed to *Chapter 8 Performing a sequencing run on Page 27.*

Chapter 8 Performing a sequencing run

8.1 Logging into the main interface

Perform the following steps:

1. Enter the user name and password, select **Log in** to go to the main interface.

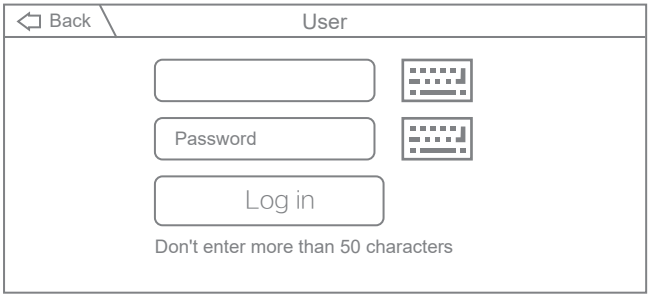

Figure 16 Login interface

2. The main interface is as follows:

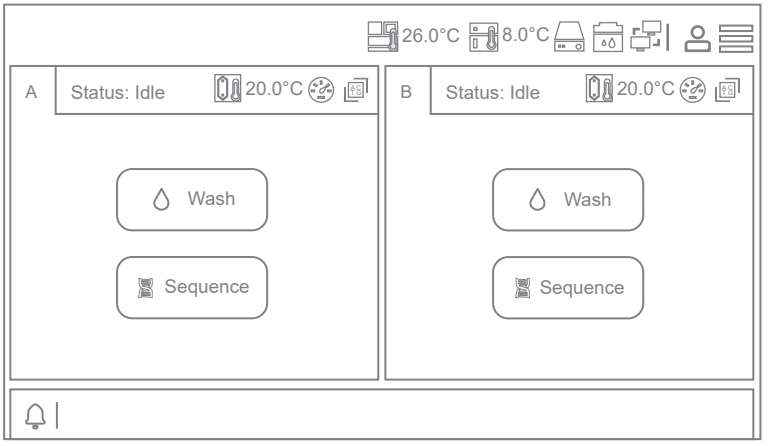

Figure 17 Main interface

8.2 Entering the DNB ID

Perform the following steps:

1. In the main interface, select **Sequence** to go to the DNB ID entry interface:

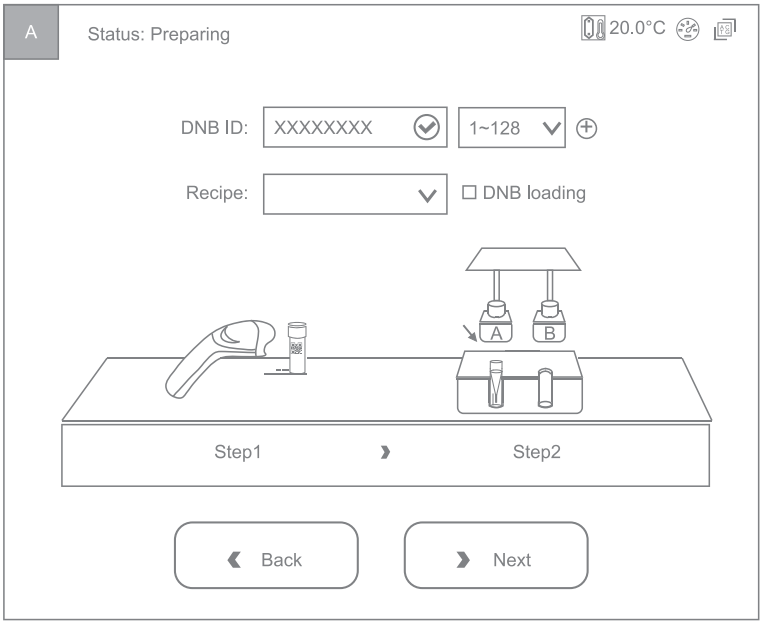

Figure 18 DNB ID entry interface

2. Select the DNB ID box, scan the QR code on the tube, or enter the DNB ID manually by using the on-screen keyboard.
3. Select a barcode range of different lanes from the list next to the DNB ID box.

4. Select ⊕ to the right of DNB ID and the lane information will appear.

   Tips Select 4 lanes for FCL.

Figure 19 DNB information selection interface

8.3 Selecting sequencing parameters

Perform the following steps:

1. Select STO_N_25+59+10 or STO_N_25+59_noBC recipe from the Recipe list for OMNI FFPE sequencing and select STO_T_50+100+10 or STO_T_50+100_noBC recipe from the Recipe list for FF V1.3 sequencing.

   Tips
   - For FF V1.3 sequencing: for barcode sequencing, select STO_T_50+100+10; otherwise select STO_T_50+100_noBC.
   - For OMNI FFPE sequencing: for barcode sequencing, select STO_N_25+59+10; otherwise select STO_N_25+59_noBC.

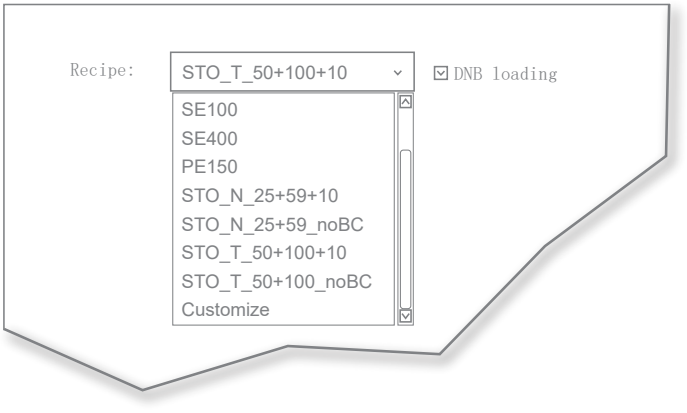

Figure 20 Selecting the sequencing recipe

2. If the DNBs are loaded by the sequencer, select DNB loading on the right of the Recipe list.

8.4 Loading the Sequencing Reagent Cartridge

Perform the following steps:

1. Select the Sequencing cartridge ID field, enter the cartridge ID manually or use the barcode scanner to scan the cartridge barcode at the lower-right corner of the Sequencing Reagent Cartridge label.

   Tips The 14-digit serial number (SN) contains information about the manufacturing site (the first digit) and the type (the second and third digits) of the reagent, the first 3 digits out of 14 are W05.

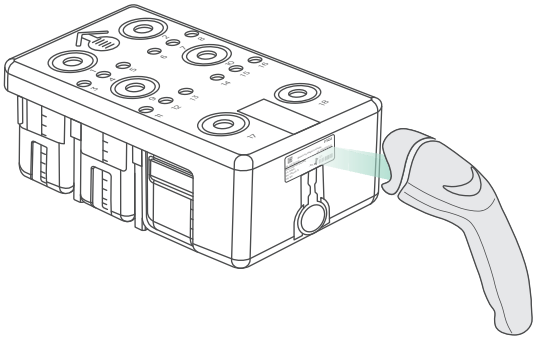

Figure 21 Scanning the Sequencing Reagent Cartridge ID

2. Open the reagent compartment door and slowly remove the cleaning cartridge from the compartment.

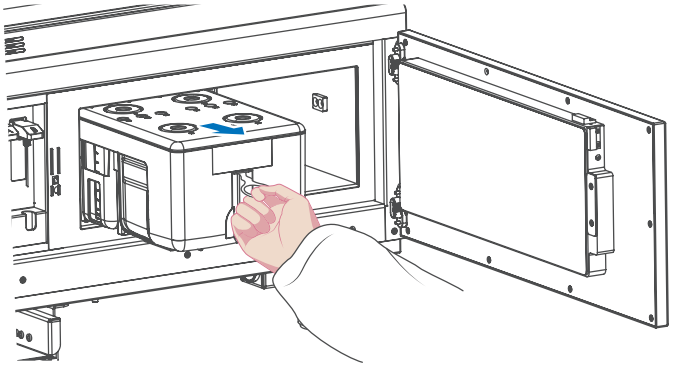

Figure 22 Removing the cleaning cartridge

3. Moisten a KimWipes tissue with laboratory-grade water and use it to wipe the bottom and sides of the compartment to keep it clean and dry.

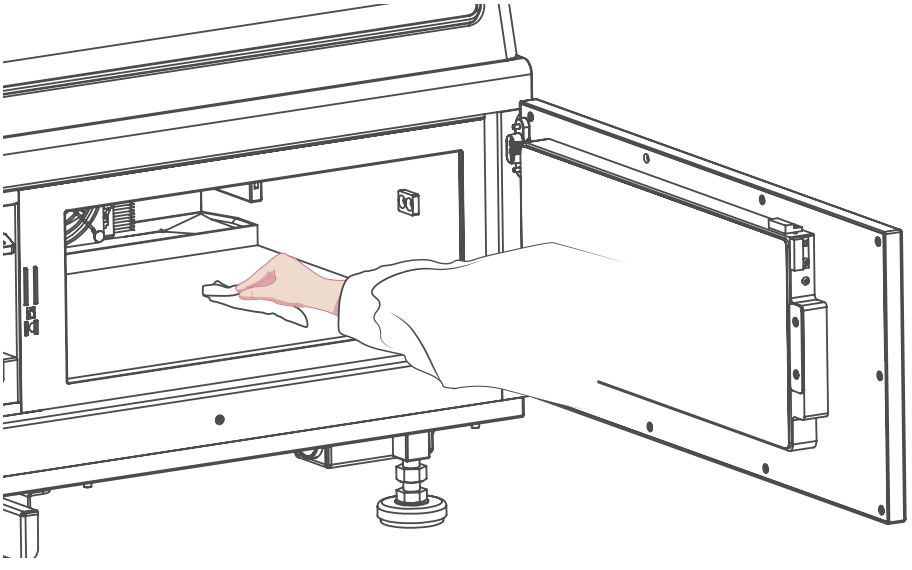

Figure 23 Cleaning the reagent compartment

4. Hold the handle of the new Sequencing Reagent Cartridge with one hand and place the other hand underneath for support.
5. Slide the cartridge into the compartment until it stops. Follow the instructions printed on the cover of the cartridge.

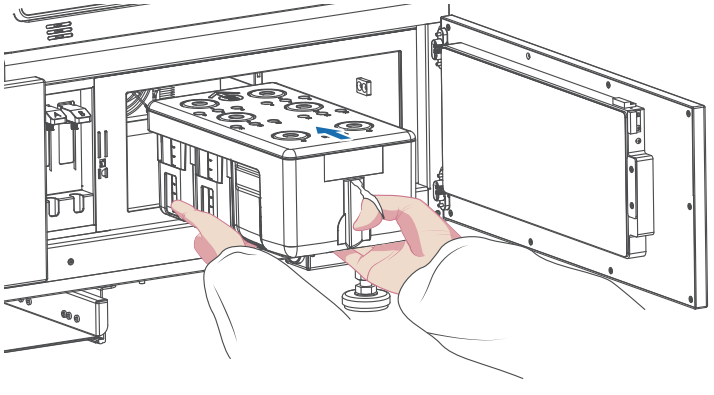

Figure 24 Sliding the new Sequencing Reagent Cartridge into the reagent compartment

6. Ensure that the cartridge is in the correct position and then close the reagent compartment door.

8.5 Loading the flow cell

Perform the following steps:

1. Open the flow cell compartment door.
2. Press both sides of the washing flow cell with one hand and press the flow cell attachment button with the other hand.
3. After the vacuum is released, remove the washing flow cell from the stage.
4. Use a canned air duster to remove the dust from the flow cell stage and the back of the flow cell.

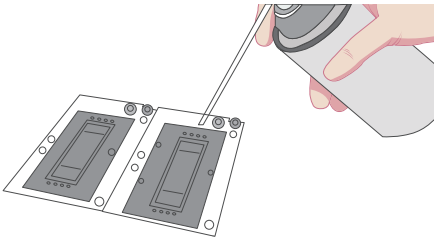

Figure 25 Cleaning the flow cell stage

Tips If there are impurities on the stage surface, gently wipe the surface with a wet KimWipe tissue to ensure that the flow cell can be held properly.

5. Take out a new flow cell or the loaded flow cell.
6. There are two alignment holes on the left side and one hole on the right side. The label is on the right. Hold the edges of the flow cell with both hands as shown in the figure below.

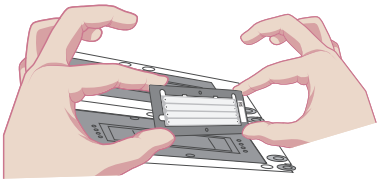

Figure 26 Loading the flow cell

7. Align the holes on the flow cell with the locating pins on the flow cell stage. Gently slide the flow cell at an angle of 45 degrees to the upper-left corner to keep the flow cell aligned with the pin.
8. Press the flow cell attachment button. Press the left and right sides of the flow cell on the stage at the same time to ensure that the flow cell is properly seated on the stage.

Tips The flow cell is fragile; handle it with caution.

9. Ensure that the negative pressure is within the range of -80 kPa to -99 kPa before continuing. If the negative pressure is abnormal, refer to *10.4 What should I do if abnormal negative pressure appears during flow cell attachment? on Page 48* in this manual for troubleshooting.
10. Use a canned air duster to remove the dust from the flow cell surface and then close the flow cell compartment door.

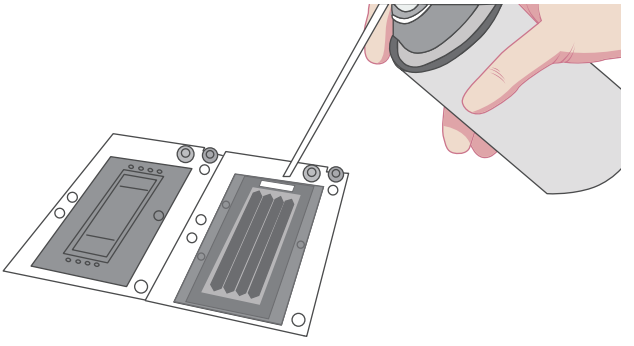

Figure 27 Cleaning the flow cell

11. Select Next, and then the flow cell ID can be entered with the barcode scanner. If automated entry does not work, and enter the ID manually in the Flow Cell ID box.

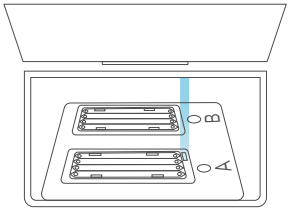

Figure 28 Scanning flow cell ID

12. Select **Next**.

8.6 Reviewing parameters

Review the run parameters to ensure that all information is correct.

Tips To ensure sequencing quality, when sequencing of Read1 and Read2 is completed, the sequencer will automatically perform another cycle for calibration. For FFPE sequencing, the length of Read1 is 25, the length of Read2 is 59, and the length of barcode is 10. Adding 1 correction cycle for Read1 and 1 correction cycle for Read2 (barcode does not need to be corrected), the total number of sequencing cycles is 96. For FF v1.3 sequencing, the length of Read1 is 50 (dark reaction cycle is from 26 to 40), the length of Read2 is 100, and the length of barcode is 10. Adding 1 correction cycle for Read1 and 1 correction cycle for Read2 (barcode does not need to be corrected), the total number of sequencing cycles is 162.

Item	Content
User name	user
DNB ID Lane 1	STO-1|1 ~ 128
DNB ID Lane 2	STO-2|1 ~ 128
DNB ID Lane 3	STO-3|1 ~128
DNB ID Lane 4	STO-4|1 ~ 128
Sequencing cartridge ID	W05000012
Flow cell ID	V300001234
Recipe	STO_N_25+59+10
Start phase	DNB Loading
Cycles	96

Figure 29 Reviewing information for OMNI FFPE

Item	Content
User name	user
DNB ID Lane 1	STO-1 | 1~ 128
DNB ID Lane 2	STO-2 | 1~ 128
DNB ID Lane 3	STO-3 | 1~128
DNB ID Lane 4	STO-4 | 1~ 128
Sequencing cartridge ID	W05000012
Flow cell ID	V300001234
Recipe	STO_T_50+100+10
Start phase	DNB Loading
Cycles	162

Figure 30 Reviewing information for FF V1.3

8.7 Starting sequencing

Perform the following steps:

1. After confirming that the information is correct, select **Start** and then select **Yes** when prompted to begin sequencing.

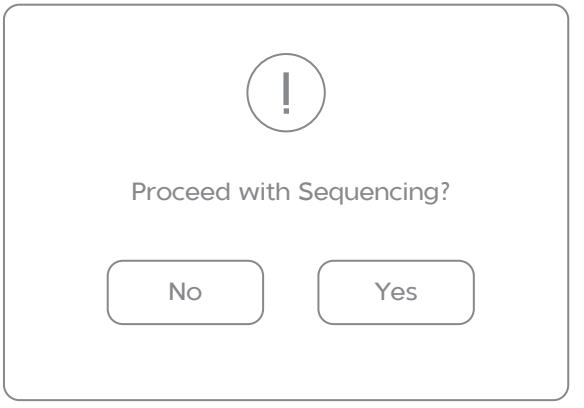

Figure 31 Confirming sequencing interface

2. When sequencing has started, immediately open the flow cell compartment door to inspect the flow cells, and ensure that the DNBs or reagents are flowing through the flow cell.
3. Sequencing is finished when the interface is idle or when processing data is displayed. Perform the wash process according to *Chapter 9 Device Maintenance on Page 36*.

8.8 Data access

Tips For detailed information, refer to *MGISEQ-2000&MGISEQ-2000RS Gene Sequencer Software Operation Guide*.

After sequencing is started, the sequencing results generated by the control software will appear in D drive.

- The data folder is named after the flow cell ID and contains primarily pictures and data generated during the instrument operation (such as metrics).
- The Results folder is named after the flow cell ID and contains primarily Bioinfo files and FASTQ files.

Chapter 9 Device Maintenance

9.1 Wash type instructions

Table 21 Wash type instructions

Wash protocol	Wash type	Cartridge type	Description
Pre-run wash	Regular (54 min)	Cleaning cartridge 1 (Laboratory-grade water)	• After an SE sequencing run. • It has been more than 24 h but less than 7 d since the last maintenance wash. • After the sequencer maintenance is performed by an engineer, which includes the replacement of accessories not exposed to reagents. • Other situations except for maintenance wash.

Wash protocol	Wash type	Cartridge type	Description
Maintenance wash	Maintenance (20 min)	Cleaning cartridge 3 (Tween-20)	• It has been more than 7 d since the last operation, and a pop-up dialog box indicates a wash. • When using the control software for the first time, updating to a new version, or manually clearing data resulting in no records, a pop-up dialog box indicates a wash. • After an PE sequencing run or DNB loading. • After the sequencer maintenance is performed by an engineer. This includes, but is not limited to the replacement of pipelines, sample needles and other accessories exposed to reagents. • If the sequencer is to be powered off for more than 7 d, a wash before being powered off and after being powered on is required. • When impurities are visible on the flow cell,
	Maintenance (20 min)	Cleaning cartridge 2 (NaOH)	
	Regular (54 min)	Cleaning cartridge 1 (Laboratory-grade water)	
DNBTube wash (Optional)	DNBTube (5 min)	Cleaning cartridge 1(Laboratory-grade water)	If you need an extra wash for the DNB loading tubes after pre-wash and maintenance wash.
	DNBTube (5 min)		
	DNBTube (5 min)		

9.2 Preparing wash reagents

Prepare the washing reagents according to table below:

Table 22 Washing reagent 1: 0.05% Tween-20

Reagent name	Volume (mL)	Final concentration
100% Tween-20	0.5	0.05%
Laboratory-grade water	999.5	/
Total volume	1000	
Validity period	1 month at 2 ℃ to 8 ℃	

Table 23 Washing reagent 2: 0.05% Tween-20+1 M NaCl

Reagent name	Volume (mL)	Final concentration
100% Tween-20	0.5	0.05%
5 M NaCl solution	200	1 M
Laboratory-grade water	799.5	/
Total volume	1000	
Validity period	1 month at 2 ℃ to 8 ℃	

Table 24 Washing reagent 3: 0.1 M NaOH

Reagent name	Volume (mL)	Final concentration
2 M NaOH	50	0.1 M
Laboratory-grade water	950	/
Total volume	1000	
Validity period	1 month at 2 ℃ to 8 ℃	

9.3 Preparing cleaning cartridges, DNB loading needle washing tubes, and washing flow cell

9.3.1 Preparing cleaning cartridges

Fill the cleaning cartridges with washing reagents according to the table below:

Table 25 Reagents and volume of the cleaning cartridges

Cleaning cartridges	Well position	Washing reagent	Volume (mL)
Cleaning cartridge 1	1, 9, 10	Laboratory-grade water	300
	2		380
	17, 18		700
	3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16 (all small wells)		9
Cleaning cartridge 2	1, 2, 9, 10, 17, 18	Washing reagent 3: 0.1 M NaOH	50
	3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16 (all small wells)		8.5

Cleaning cartridges	Well position	Washing reagent	Volume (mL)
Cleaning cartridge 3	1, 2, 9, 10, 17, 18	Washing reagent 1: 0.05% Tween-20	50
	3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 16 (all small wells, except 15)		8.5
	15 (small well)	Washing reagent 2: 0.05% Tween-20+1 M NaCl	8.5

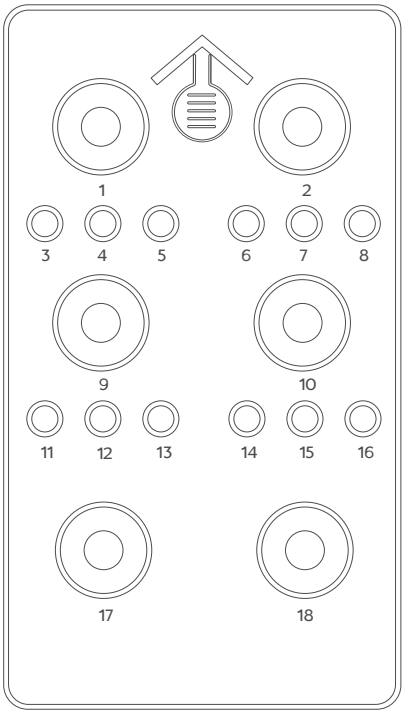

Figure 32 Top view of cleaning cartridge

Tips
- Large wells are No. 1, 2, 9, 10, 17, 18.
- Small wells are No. 3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16.

9.3.2 Preparing DNB loading needle washing tubes

Prepare the 2.0 mL sterile microcentrifuge tube for DNB loading needle washing according to table below:

Table 26 Reagents and volume of DNB loading needle washing tubes

DNB loading needle washing tubes	Washing reagent	Volume (mL)
DNB loading needle washing tube 1	Laboratory-grade water	1.8
DNB loading needle washing tube 2	Washing reagent 3: 0.1 M NaOH	1.8
DNB loading needle washing tube 3	Washing reagent 1: 0.05% Tween-20	1.8

9.3.3 Preparing the washing flow cell

A used flow cell without physical damage can be used as a washing flow cell.

Each washing flow cell, stored at room temperature or at a 2 ℃ to 8 ℃ refrigerator, can be reused 20 times.

9.4 Performing a wash

9.4.1 Selecting wash

When the sequencing run is completed, the device must be washed within 24 hr. When the following interface appears, select **Wash** and perform the wash procedures.

Figure 33 Wash instructions interface

9.4.2 Performing a pre-run wash (~54 min)

Perform the following steps:

1. Slowly insert cleaning cartridge 1 into the reagent compartment until it stops. Follow the instructions printed on the cartridge cover.
2. Put the DNB loading needle washing tube 1 (sterile microcentrifuge tube, 2.0 mL with 1.8 mL Laboratory-grade water) into the DNB loading position. Close the reagent compartment door.
3. Load the washing flow cell. Ensure that the washing flow cell is properly loaded. For details, refer to *8.5 Loading the flow cell on Page 32.*
4. Select **Wash** in the main interface. Select Regular from the Wash type list to start pre-run wash, which takes approximately 54 min.

Tips When you perform the wash, observe the status of the washing flow cell. If bubbles are observed, stop the wash, replace the flow cell, and re-start the wash. If no bubbles are visible, continue the wash.

Figure 34 Selecting the wash type

9.4.3 Performing a maintenance wash (~94 min)

Perform the following steps:

1. Insert the cleaning cartridge 3 into the reagent compartment until it stops. Follow the instructions printed on the cartridge cover.
2. Put DNB loading needle washing tube 3 (sterile microcentrifuge tube, 2.0 mL with 1.8 mL Tween-20) into the DNB loading position. Close the reagent compartment door.
3. Place the washing flow cell on the stage. Ensure that the washing flow cell is properly seated.

   For details, refer to *8.5 Loading the flow cell on Page 32*.
4. Select **Wash** in the wash instructions interface. Select Maintenance from the Wash type list to start the wash. The wash takes approximately 20 min.

Figure 35 Selecting Maintenance

5. When the interface appears as shown in the figure below, select **Yes** and the sequencer will automatically lift the sampling needles.

Figure 36 Maintenance wash [2] prompt

6. Insert the cleaning cartridge 2 into the reagent compartment until it stops. Follow the instructions printed on the cartridge cover.
7. Put DNB loading needle washing tube 2 (sterile microcentrifuge tube, 2.0 mL with 1.8 mL NaOH) into the DNB loading position. Close the reagent compartment door.
8. Select **Wash** in the wash instructions interface. Select Maintenance from the Wash type list to start the wash. The wash takes approximately 20 min.
9. When the interface appears as shown in the figure below, select **No** and the sequencer will automatically lift the sampling needles.

Figure 37 Maintenance wash [3] prompt

10. Insert the cleaning cartridge 1 into the reagent compartment until it stops. Follow the instructions printed on the cartridge cover.
11. Put the DNB loading needle washing tube 1 (Sterile Microcentrifuge tube, 2.0 mL with 1.8 mL Laboratory-grade water) into the DNB loading position.
12. Close the reagent compartment door.
13. Select **Wash** in the wash instructions interface. Select Regular from the Wash type list to start the wash. The wash takes approximately 54 min.

9.4.4 (Optional) Performing a DNBTube wash (~15 min)

Once the pre-run wash and maintenance wash are completed, if you need another wash for the DNB loading tubes, perform the following steps:

1. Slowly insert the Cleaning cartridge 1 into the reagent compartment by following the direction printed on the cartridge cover until it stops.
2. Place DNB loading needle washing tube 3 (2.0 mL sterile microcentrifuge tube with 1.8 mLTween-20) into the DNB tube rack. Close the reagent compartment door.
3. Place the washing flow cell on the stage. Ensure that the washing flow cell is properly seated.

   For details, refer to *8.5 Loading the flow cell on Page 32*.
4. Select **Wash** in the main interface. Select DNBTube from the Wash type list to start the wash. The wash takes approximately 5 min.

Figure 38 Selecting DNBTube

5. Select **Back** to return to the main interface.
6. Place the DNB loading needle washing tube 2 (2.0 mL sterile microcentrifuge tube with1.8 mLNaOH) into the DNB tube rack. Close the reagent compartment door.
7. Repeat steps 4 through 5.
8. Place DNB loading needle washing tube 1 (2.0 mL sterile microcentrifuge tube with1.8 mL Laboratory-grade water) into the DNB tube rack.
9. Select **Wash** in the main interface. Select DNBTube from the Wash type list to start the wash. The wash takes approximately 5 min.

Chapter 10 FAQs

If malfunctions occur during operation, the device sounds an alarm or a message is displayed on the screen. Follow the prompts to troubleshoot and solve the issue. If the problem persists after you try the recommended actions, contact a field service engineer.

10.1 What should I do if the DNB concentration is low?

When the DNB concentration is less than that specified in *4.4 Quantifying DNBs on Page 14*, perform the following steps:

- Check whether the DNB preparation kit has expired.
- Check whether the libraries meet the requirements.
- Make a new DNB preparation. If the DNB concentration still does not meet the requirements after a new sample preparation, contact a field service engineer.

10.2 What should I do if I forget to add reagent into well No. 15 for PE sequencing run?

MDA Enzyme is required to make the second strand template for PE sequencing. When preparing the Sequencing Reagent Cartridge, the appropriate amounts of MDA Enzyme Mix II and Inactive MDA Reagent must be added to well No. 15. If you mistakenly forget to add the reagent into well No. 15 before starting the sequencing run, this can be resolved by performing the following steps if the sequencing run is in the sequencing phase of Read1.

1. Pause the run: At any sequencing cycle within Read1, while sequencing is at Step 3/3, and the indicator is at well No. 17 or well No. 18, select  , and select **Yes** when you are prompted, as shown in the following two figures.

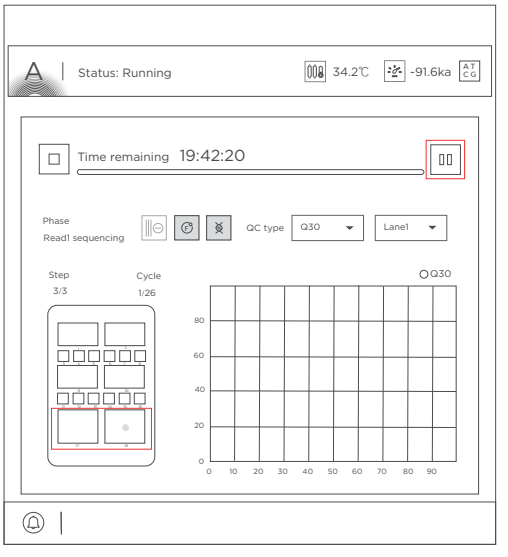

Figure 39 Selecting the sequencing stage to pause

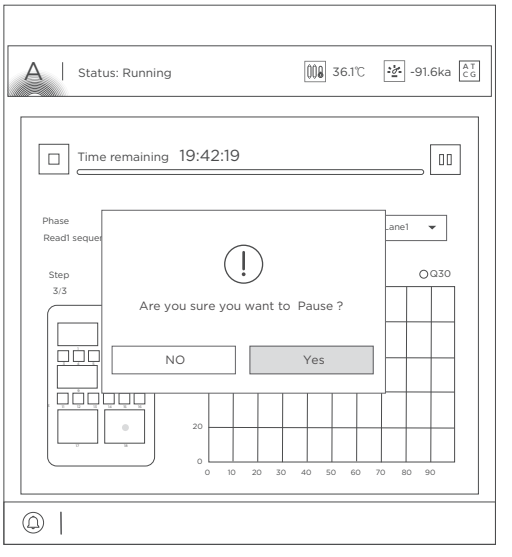

Figure 40 Confirming to pause the run

2. Lift the needle: Select  to lift the needle, and select **Yes** when you are prompted, as shown in the following two figures.

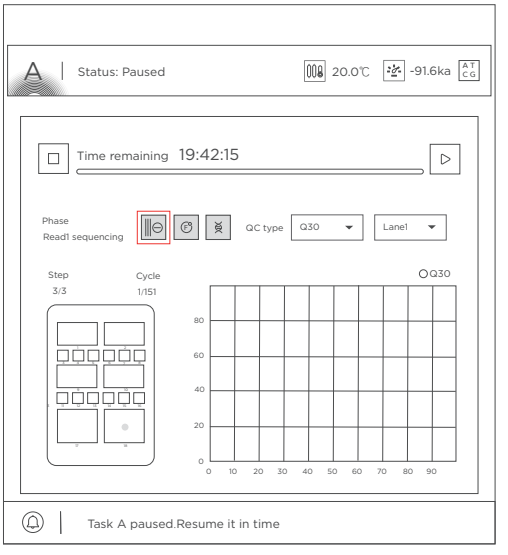

Figure 41 Selecting to lift the needle

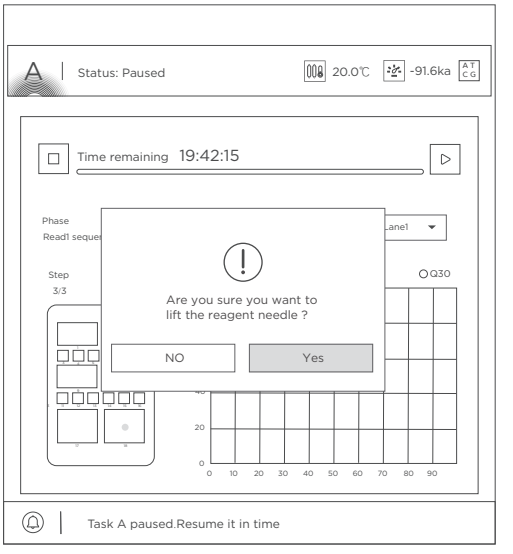

Figure 42 Confirming to lift the needle

3. Prepare the Sequencing Reagent Cartridge: Open the reagent compartment door and take out the Sequencing Reagent Cartridge. Add the appropriate amount of MDA Enzyme Mix II into Inactive MDA Reagent tube, mix well, mix well and add all of the mixture to well No. 15 according to *Chapter 7 Preparing the Sequencing Reagent Cartridge on Page 22*.
4. Resume the run: Put the cartridge back to the sequencer and close the reagent compartment door. Select ▷ to resume the run, and select **Yes** when you are prompted, as shown in the following two figures.

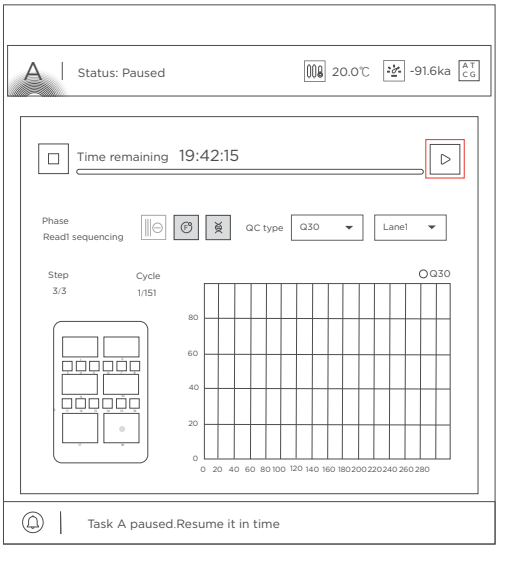

Figure 43 Selecting to resume the run

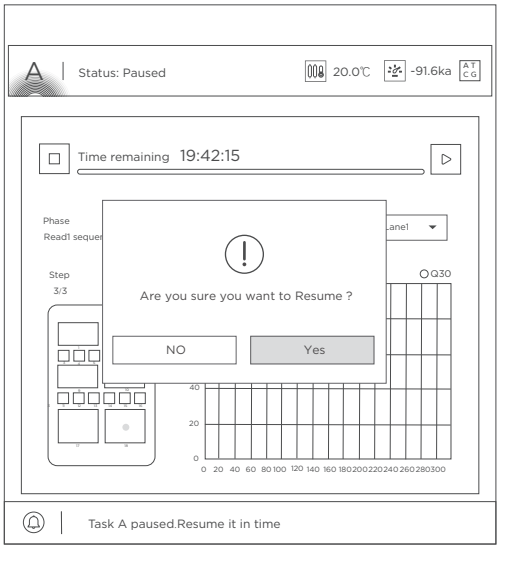

Figure 44 Confirming to resume the run

After the sequencing run is resumed, the sampling needles automatically move down. The sequencer continues to pump reagents into the flow cell. The Read1 sequencing phase continues.

10.3 What rules should I follow if I need to store a reagent kit temporarily?

- If a kit has been thawed (not including dNTPs) but cannot be used within 24 hr, it can be frozen and thawed at most one time.
- If a kit has been thawed (including dNTPs) but cannot be used immediately, store it at 2 ℃ to 8 ℃. It is strongly recommended to use it within 24 hr. Mix the reagents in the cartridge following the instructions in *Chapter 7 Preparing the Sequencing Reagent Cartridge on Page 22* before use.
- If dNTPs and Sequencing Enzyme Mix II have been added into the cartridge; that is, the cartridge has been prepared but cannot be used immediately, store it at 2 ℃ to 8 ℃ and use it within 24 hr. Mix the reagents in the cartridge following the instructions in *Chapter 7 Preparing the Sequencing Reagent Cartridge on Page 22* before use.
- If dNTPs and Sequencing Enzyme Mix II have been added into the cartridge; that is. the cartridge has been prepared and the needles have punctured the seal, but the cartridge cannot be used immediately, the cartridge must be sealed with foil or plastic wrap. Store the cartridge at 2 ℃ to 8 ℃ and use it within 24 hr. Gently mix the reagents in the cartridge before use. To prevent reagent contamination when mixing, be careful not to spill any reagent from the needle holes.

10.4 What should I do if abnormal negative pressure appears during flow cell attachment?

When the negative pressure is shown in red, the negative pressure is abnormal.

Perform the following steps:

- Gently wipe the stage surface of flow cell stage with a damp KimWipes tissue and remove dust from the stage with a canned air duster. Ensure that no dust is present on the flow cell stage.
- Remove dust from the back of the flow cell with a canned air duster to ensure that no dust is present.
- If the problem persists, contact a field service engineer.

10.5 What should I do if a pumping failure occurs during DNB loading and sequencing?

If liquids cannot be pumped onto the flow cell, or large bubbles appear in the flow cell, perform the following steps:

- The sequencer: remove the flow cell, check for impurities in the sealing gasket and remove any dust with a canned air duster. Inspect the pump. Insert a new flow cell by following the instructions in *8.5 Loading the flow cell on Page 32* and start the pump again.
- Confirm that the sampling needles are moving properly. If the sampling needles are not moving properly, restart the control software of the sequencer.
- If the problem persists, contact a field service engineer.

10.6 What should I do if impurities appear in the original sequencing image?

If impurities appear, perform the following steps:

- Moisten a KimWipes tissue with 75% ethanol and use it to wipe the sealing gaskets on the flow cell stage and perform a maintenance wash on the sequencer according to *9.4.3 Performing a maintenance wash (~94 min) on Page 43*.
- If the problem persists, contact a field service engineer.

Appendix 1 Instructions for using Qubit to quantify the DNBs

Tips
- Working solution should be used within 30 min following preparation.
- Avoid touching the wall of tapered detection tubes.
- Ensure that there are no bubbles in detection tubes.

Perform the following steps:

1. Prepare the Qubit working solution by diluting the Qubit ssDNA Reagent 1:200 in Qubit ssDNA Buffer. Use a clean plastic tube each time you prepare Qubit working solution. Do not mix the working solution in a glass container.

Tips The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit working solution, and each sample tube requires from 180 - 199 µL of Qubit working solution.

Prepare sufficient Qubit working solution to accommodate all standards and samples.

For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~ 200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit reagent plus 1990 µL of Qubit Buffer).

2. Add 190 µL of Qubit working solution to each tube used for standards.
3. Add 10 µL of each Qubit standard to the appropriate tube, then mix by vortexing 3 to 5 sec. Be careful not to create bubbles.
4. Set up the required number of 0.5-mL tubes for standards and samples. The Qubit ssDNA Assay requires 2 standards.

Tips
- Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit assay tubes (Cat. No.: Q32856) or Axygen PCR-05-C tubes (Part No.: 10011-830).
- The number of Qubit test tubes needed are the number of samples plus 2 standards tubes. For example, if you have 3 samples, you will need 5 tubes.

5. Label the tube lids. Do not label the side of tube.
6. Prepare the solutions used for standards and sample tests according to the table below.

Component	S1 (μL)	S2 (μL)	D1 (μL)	D2 (μL)	D3 (μL)
Working solution	190	190	198	198	198
S1 (0 ng/μL)	10	/	/	/	/
S2 (20 ng/μL)	/	10	/	/	/
Sample (μL)	/	/	2	2	2
Total volume	200	200	200	200	200

7. Mix tubes by using a vortex mixer, centrifuge briefly for 5 sec, then incubate at room temperature for 2 min.
8. Refer to the Qubit user manual for instructions on reading standards and samples. Follow the appropriate procedure for your instrument.

Appendix 2 Manufacturer

Manufacturer	Wuhan MGI Tech Co., Ltd.
	Building 24, Stage 3.1, BioLake Accelerator, No.388, 2nd Gaoxin Road, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China
E-mail	MGI-service@mgi-tech.com
Website	www.mgi-tech.com

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