Part No.: H-T-042

DNBSEQ-G400RS

High-throughput Rapid Sequencing Set (G400 SM Integration)

User Manual | Version: 1.0

Address 1: Building B13, No.818, Gaoxin Avenue, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China

Address 2: 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@genomics.cn

Website: en.mgi-tech.com

Research Use Only

Wuhan MGI Tech Co., Ltd.

About the user manual

This user manual is applicable to DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM Integration). The manual version is 1.0.

Figures in this user manual are all illustrations. The contents might be slightly different from the kit, please refer to the kit purchased.

Unless otherwise informed, all sequencers and sequencing reagents are not available in Germany, USA, Spain, UK, Hong Kong, Sweden, Belgium and Italy.

Revision history

Version	Date	Description
1.0	February 2022	Initial release

Sequencing set

Catalog number	Name	Version
940-000233-00	DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM DB FCS SE100)	1.0
940-000232-00	DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM DB FCS PE100)	1.0
940-000231-00	DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM DB FCS PE150)	1.0
940-000230-00	DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM App-A FCS SE100)	1.0
940-000229-00	DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM App-A DB FCS PE100)	1.0
940-000228-00	DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM App-A DB FCS PE150)	1.0

Contents

---This page is intentionally left blank.---

Chapter 1 Introduction

This manual describes how to perform sequencing by using DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM Integration). It includes instructions regarding sample preparation, flow cell preparation, sequencing kit storage, sequencing protocol, and device maintenance.

1.1 Applications

DNBSEQ-G400RS High-throughput Rapid Sequencing Set is specifically designed for DNA or RNA sequencing on DNBSEQ-G400RS. This sequencing set is intended to be used for scientific research only and cannot be used for clinical diagnosis.

1.2 Sequencing technology

This sequencing 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 highly accurate sequencing information.

1.3 Data analysis

During the sequencing run, the control software automatically operates 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 PE150 cycle run performs reads of 150 cycles (2×150) for a total of 300 cycles. At the end of the insert sequencing run, an extra 10 cycles of barcode read and 10 cycles of dual barcode read can be performed, if required.

Table 1 Sequencing cycle

Sequencing read length	Read 1 read length	Read 2 read length	Barcode read length	Dual Barcode read length	Total read length	Maximum cycles
G400 SM App-A FCS SE100	100	/	10	/	100+10	120
G400 SM DB FCS PE150	150	150	10	10	300+20	320

1.5 Sequencing time and analysis time

Table 2 Sequencing time and analysis time for each read length (hours)

Type	Read length	Sequencing time	Analysis time
Single flow cell	SE100	13.5	0.5
	PE100	25.9	0.6
	PE150	36.4	0.7
Dual flow cell	SE100	13.7	0.9
	PE100	26.0	1.2
	PE150	36.6	1.4

NOTE
- The sequencing time in the table above is 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. Actual run time may vary among various sequencers.

1.6 Attention

- This product is for research use only. Please read the manual carefully before use.
- Ensure that you are familiar with the SOP&Attention of all the laboratory apparatus to be used.
- Avoid direct skin and eye contact with any samples and reagents. Don't swallow. Please wash with plenty of water immediately and go to the hospital if this happens.
- 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.
- Do not use expired products.

Chapter 2 Sequencing sets and Self-prepared Consumables

2.1 List of sequencing set components

Table 3 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM DB FCS SE100) Catalog number: 940-000233-00

Component	Spec&quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987			
Rapid Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (G400 SM DB FCS SE100) Catalog number:940-000221-00			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
Make DNB Buffer	100 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II(LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5mL	1 tube		
dNTPs Mix	0.90 mL×1 tube		
dNTPs Mix II	1.70 mL×1 tube		
Sequencing Enzyme Mix	1.90 mL×1 tube		
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 4 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM DB FCS PE100) Catalog number: 940-000232-00

Component	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987			
Rapid Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (G400 SM DB FCS PE100) Catalog number: 940-000220-00			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
Make DNB Buffer	100 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II(LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5mL	1 tube		
dNTPs Mix	1.50 mL×1 tube		
dNTPs Mix II	1.50 mL×2 tube		
Sequencing Enzyme Mix	3.10 mL×1 tube		
MDA Reagent	3.50 mL×1 tube		
MDA Enzyme Mix	0.60 mL×1 tube		
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 5 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM DB FCS PE150) Catalog number: 940-000231-00

Component	Spec&quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987			
Rapid Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (G400 SM DB FCS PE150) Catalog number: 940-000219-00			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
Make DNB Buffer	100 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II(LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5mL	1 tube		
dNTPs Mix	2.00 mL×1 tube		
dNTPs Mix II	2.00 mL×2 tube		
Sequencing Enzyme Mix	4.80 mL×1 tube		
MDA Reagent	3.50 mL×1 tube		
MDA Enzyme Mix	0.60 mL×1 tube		
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 6 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM App-A FCS SE100) Catalog number: 940-000230-00

Component	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987			
Rapid Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (G400 SM App-A FCS SE100) Catalog number: 940-000218-00			
Low TE Buffer	300 µL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
App-A Make DNB Buffer	80 µL×1 tube		
Make DNB Enzyme Mix I	200 µL×1 tube		
Make DNB Enzyme Mix II(LC)	25 µL×1 tube		
Stop DNB Reaction Buffer	100 µL×1 tube		
DNB Load Buffer I	200 µL×1 tube		
DNB Load Buffer II	200 µL×1 tube		
Micro Tube 0.5mL	1 tube		
dNTPs Mix	0.90 mL×1 tube		
dNTPs Mix II	1.70 mL×1 tube		
Sequencing Enzyme Mix	1.90 mL×1 tube		
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 7 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM App-A DB FCS PE100) Catalog number: 940-000229-00

Component	Spec&quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987			
Rapid Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (G400 SM App-A DB FCS PE100) Catalog number: 940-000217-00			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
App-A Make DNB Buffer	80 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II(LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5mL	1 tube		
dNTPs Mix	1.50 mL×1 tube		
dNTPs Mix II	1.50 mL×2 tube		
Sequencing Enzyme Mix	3.10 mL×1 tube		
MDA Reagent	3.50 mL×1 tube		
MDA Enzyme Mix	0.60 mL×1 tube		
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 8 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (G400 SM App-A DB FCS PE150) Catalog number: 940-000228-00

Component	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987			
Rapid Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (G400 SM App-A DB FCS PE150) Catalog number: 940-000216-00			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
App-A Make DNB Buffer	80 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II(LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5mL	1 tube		
dNTPs Mix	2.00 mL×1 tube		
dNTPs Mix II	2.00 mL×2 tube		
Sequencing Enzyme Mix	4.80 mL×1 tube		
MDA Reagent	3.50 mL×1 tube		
MDA Enzyme Mix	0.60 mL×1 tube		
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

2.2 Self-prepared equipment and consumables

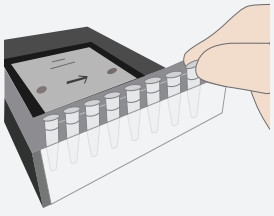
Table 9 Self-prepared equipment and consumables

Equipment and consumables	Recommended brand	Catalog number
Qubit 3.0 fluorometer	Thermo Fisher	Q33216
Mini centrifuge	Major Laboratory Supplier (MLS)	/
Vortex mixer	MLS	/
Thermal Cycler	Bio-Rad	/
Pipette	Eppendorf	/
2 ℃ to 8 ℃ refrigerator	MLS	/
-25 ℃ to -15 ℃ refrigerator	MLS	/
Qubit ssDNA Assay Kit	Thermo Fisher	Q10212
Power dust remover	MATIN	M-6318
Sterile pipette tip(box)	AXYGEN	/
200μL Wide-Bore Pipette Tips	AXYGEN	T-205-WB-C
Qubit Assay Tubes	Thermo Fisher	Q32856
100% Tween-20	BBI	A600560-0500
100% ProClin 300	SIGMA	48912-U
5 M NaCl solution	SIGMA	S5150-4L
2 M NaOH solution	Aladdin	S128511-1L
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 box	MLS	/
5 mL Tube	SARSTEDT	60.558.001
15 mL Tube	SARSTEDT	60.732.001
25 mL Tube	SARSTEDT	60.9922.243

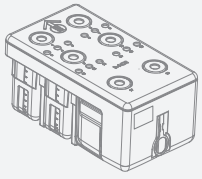
Chapter 3 Sequencing workflow

Making DNB: use DNB preparation kit for making DNB.

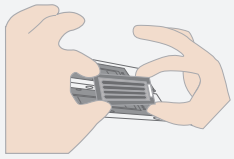
Preparing a new flow cell: take out the flow cell from package and inspect to ensure the flow cell is intact.



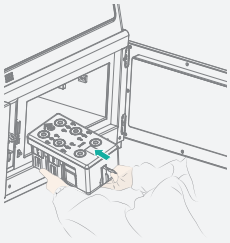
DNB loading: load the DNB into sequencing flow cell.



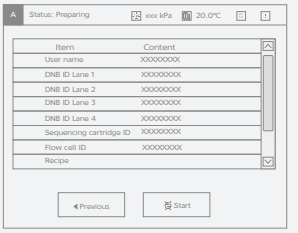
Preparing a new reagent cartridge: inspect and thaw the reagent cartridge and then load and mix the necessary reagents.



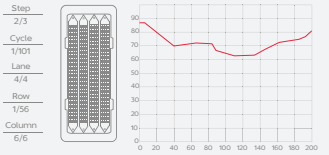
Loading the flow cell: place the flow cell on the stage of the sequencer.



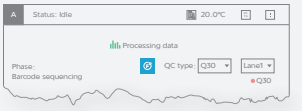
Loading the reagent cartridge into the sequencer.



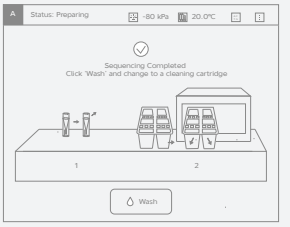
Starting sequencing: follow the instructions to enter sequencing information and start the run.



Sequencing: monitor the sequencing run from the control software interface.



Data analysis: the sequencer will automatically split barcode (if Split barcode is selected) and output FASTQ files when sequencing is completed.



Device maintenance: perform device maintenance when sequencing is completed.

Chapter 4 Preparing DNB

4.1 Insert size recommendation

- This kit is applicable to the dual barcode library constructed by the MGIEasy dual terminal independent barcode general library preparation kit provided by MGI, or the ssDNA product transformed by the Illumina Truseq library combined with the adapter constructed by the MGIEasy general library conversion kit (the App-A libraries).
- Recommended library insert size: The size distribution of inserts should be between 20 bp and 800 bp, with the main band centered within ±100 bp. If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.

Table 10 Recommended insert size

Product model	Suggested insert distribution (bp)	Data output (Gb/lane)
G400 SM DB FCS SE100	200 ~ 400	~ 27.5
G400 SM App-A FCS SE100		
G400 SM DB FCS PE100		~ 55.0
G400 SM App-A DB FCS PE100		
G400 SM DB FCS PE150	300 ~ 500	~ 82.5
G400 SM App-A DB FCS PE150		

NOTE
- Consider the insert size and the required data output when selecting sequencing kits.
- Average data output varies with the library type and applications.

4.2 Library concentration and amount requirement

Table 11 Library requirement

Libraries	library concentration
General libraries	≥2 fmol/μL
App-A libraries	≥3 fmol/μL

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

  C (fmol/μL)=3030×C (ng/μL)/N, N represents the number of nucleotides (total library length including the adapter).
- If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.

4.3 Making DNB

4.3.1 Preparing reagents for DNB making

NOTE Mixed use of reagent components from different batches is strictly prohibited.

Perform the steps below:

1. Take out the library, Low TE buffer, Make DNB Buffer/App-A Make DNB Buffer and Stop DNB Reaction Buffer from storage and thaw them at room temperature.
2. Take out Make DNB Enzyme Mix I and thaw the reagnet for approximately 0.5 hours on ice.
3. After thawing, mix reagents by using a vortex mixer for 5 seconds. Centrifuge briefly and place them on ice until use.

4.3.2 selecting the DNB loader

Each DNBSEQ-G400RS Rapid Sequencing Flow Cell contains 2 lanes. DNB can be loaded onto the flow cell using the three ways:

- Sequencer

  2 lanes in the flow cell must be the same DNB. Each lane requires 50 μL DNB.

- MGIDL-200RS

  Different DNBs can be loaded onto different 2 lanes. Each lane requires 50 μL DNB.

- MGIDL-200H

  Different DNBs can be loaded onto different 2 lanes Each lane requires 25 μL DNB.

Table 12 The required number of make DNB reactions for each DNBSEQ-G400RS flow cell

Loading system	DNB loading volume (μL)/lane	Make DNB reaction (μL)	The required number of make DNB reaction/flow cell
Sequencer	50	100	1 to 2
MGIDL-200RS	50	100	1 to 2
MGIDL-200H	25	100	1 to 2

4.3.3 Calculating the required amount of ssDNA library

- The required volume of ssDNA library is determined by the required library amount (fmol) and library concentration quantified in *4.2 Library concentration and amount requirement on Page 14*.

Table 13 The volume of ssDNA

Library	The volume of 100 µL DNB reaction (µL)
General libraries	V=40 fmol/C
App-A libraries	V=60 fmol/C

NOTE All samples should be considered potentially infectious and should be handled in accordance with relevant national regulations.

- Calculate the required ssDNA library for each Make DNB reaction and fill it in *Table 14 on Page 16* as V.

4.3.4 Making DNB

Perform the steps below:

1. Take a 0.2 mL 8-tube strip or PCR tubes. Prepare reaction mix following the table below.

Table 14 Make DNB reaction mix 1

Component	Volume (µL)
Low TE Buffer	20-V
Make DNB Buffer/App-A Make DNB Buffer	20
ssDNA libraries	V
Total Volume	40

NOTE
- For general library, using the Make DNB Buffer.
- For App-A library, using the App-A Make DNB Buffer.

2. Mix gently by vortexing and centrifuge for 5 seconds by using a mini centrifuge.
3. Place the mix into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown in the table below.

Table 15 Primer hybridization reaction condition

Temperature	Time
Heated lid (105 °C)	On
95 °C	1 min
65 °C	1 min
40 °C	1 min
4 °C	Hold

4. Take out the Make DNB Enzyme Mix II (LC) from storage. Centrifuge briefly for 5 seconds and hold on ice.

   NOTE
   - Do not place Make DNB Enzyme Mix II (LC) at room temperature.
   - Avoid holding the tube for a prolonged time.

5. Take the PCR tube out of the thermal cycler when the temperature reaches 4 °C .
6. Centrifuge briefly for 5 seconds, place the tube on ice and prepare the Make DNB reaction mix 2 as shown in the table below.

Table 16 Make DNB reaction mix 2

Component	Volume (µL)
Make DNB Enzyme Mix I	40
Make DNB Enzyme Mix II (LC)	4.0

7. Add all the Make DNB reaction mix 2 into the Make DNB reaction 1.
8. Mix gently by vortexing, centrifuge for 5 seconds by using a mini centrifuge.
9. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below.

Table 17 Rolling circle amplification conditions

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

NOTE
- As some thermal cyclers are slow in temperature adjustment. When the heated lid is being heated or cooled, the sample block may remain at room temperature and the procedure is not performed. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at working temperature during the DNB reaction.
- It is recommended to set the temperature of the heated lid to 35 °C or as close as possible to 35 °C .

10. Immediately add 20.0 µL Stop DNB Reaction Buffer once the temperature reaches 4 °C . Mix gently by pipetting 5 to 8 times by using a wide bore tip.

NOTE
- It is very important to mix DNB gently by using a wide bore pipette tip.
- Do not centrifuge, vortex, or shake the tube.

4.4 Quantifying DNB

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

NOTE
- Sequencing requires a minimum DNB concentration of 8 ng/µL. If the concentration is lower than 8 ng/µL, make a new DNB preparation.
- If the concentration exceeds 40 ng/µL, the DNBs should be diluted to 20 ng/µL by DNB Load Buffer I before loading.
- If there are too many samples in a single test, it is recommended to quantify in batches to avoid inaccurate DNB quantification due to fluorescence quenching.
- Store DNB at 4 °C and perform sequencing within 48 hours.

Chapter 5 Preparing a flow cell

Perform the steps below:

1. Take the flow cell out of storage.

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

2. Place the flow cell at room temperature for at least 60 min but not exceeding 24 hours.

3. Unwrap the outer package before use and start DNB loading.

   NOTE
   - If the flow cell cannot be used within 24 hours after being placed in room temperate and the outer plastics package is intact, the flow cell can be placed back in -25 ℃ to -15 ℃ for storage. But the switch between room temperature and -25 ℃ to -15 ℃ cannot exceed 3 times.
   - When the outer plastic package is open but the flow cell cannot be used immediately, store the flow cell at room temperature and use within 24 hours. If exceed 24 hours, it is not recommended to use the flow cell.

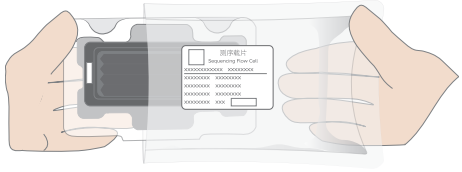


Figure 1 Unwrapping the outer package

4. Take out the flow cell from the inner package and inspect to ensure the flow cell is intact.

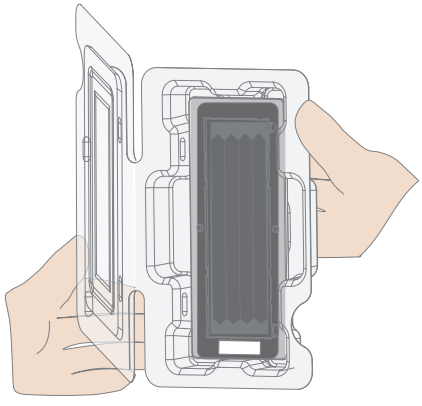


Figure 2 Inspecting the flow cell

Chapter 6 DNB loading

Perform the steps below:

1. Take out DNB Load Buffer II from storage and thaw reagents on ice for approximately 0.5 hours.
2. After thawing, mix reagents by using a vortex mixer for 5 seconds, centrifuge briefly and place on ice until use.

NOTE If crystal precipitation is found in DNB Load Buffer II, vigorously mix the reagent by vortexing for 1 to 2 minutes to re-dissolve the precipitate before use.

6.1 Sequencer DNB loading

Perform the steps below:

1. Take out the 0.5 mL microfuge tube and add the reagents according to the table below.

Table 18 DNB loading mix 1

Component	Volume (µL)
DNB Load Buffer II	32.0
DNB	100.0
Make DNB Enzyme Mix II (LC)	1.0
Total Volume	133.0

2. Combine components and mix by using a wide bore tip to gently pipette 5 to 8 times. Place the mixture at 4 ℃ until use.

NOTE
- Do not centrifuge, vortex, or shake the tube.
- Each rapid sequencing flow cell(FCS) requires 133 µL DNB loading mix.
- Prepare a fresh DNB loading mix immediately before the sequencing run.

6.2 MGIDL-200RS DNB loading

NOTE Refer to the *MGIDL-200RS DNB Loader User Manual* for details on loading operation. Before DNB loading, perform a wash as described in the manual.

Perform the steps below:

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

Table 19 DNB loading mix 2

Component	Volume (μL)
DNB Load Buffer II	16.0
DNB	50.0
Make DNB Enzyme Mix II (LC)	0.5
Total Volume	66.5

2. Combine components and mix them by using a wide bore tip to gently pipette 5 to 8 times. Place the mixture at 4 ℃ until use.

NOTE
- Do not centrifuge, vortex, or shake the tube.
- Each lane requires at least 66.5 μL of DNB loading mix.

3. Place the tubes containing DNB loading mix in the labeled positions of MGIDL-200RS.

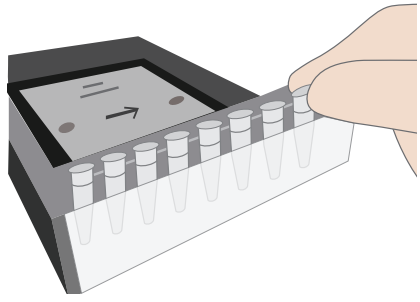


Figure 3 Placing the loading samples

4. Press the flow cell attachment button, hold the flow cells by edges and align the holes on the flow cells with the locating pins on the flow cell stages. Press the left and right sides of the flow cell on the stage at the same time to ensure that the flow cells are securely seated on the stage.
5. Select the loading recipe from the drop-down list and start DNB loading.
6. After DNB loading, take out the flow cell and place it in a PE glove or a plastic bag at room temperature for 30 minutes, then immediately place it on the sequencer for use.

6.3 MGIDL-200H DNB loading

NOTE Refer to the *MGIDL-200H Portable DNB Loader Quick Start Guide* for details on loading operation. Before DNB loading, clean the device as described in the *guide*.

Perform the steps below:

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

Table 20 DNB loading mix 3

Component	Volume (µL)
DNB Load Buffer II	8.00
DNB	25.00
Make DNB Enzyme Mix II (LC)	0.25
Total volume	33.25

2. Combine components and mix by using a wide bore tip to gently pipette 5 to 8 times. Place the mixture at 4 °C until use.

   NOTE Each lane requires 30 µL of DNB loading mix.

3. Install the sealing gasket and flow cell.

4. Aspirate 30 μL DNB loading mix with a pipette and insert the wide bore tip into the fluidics inlet.

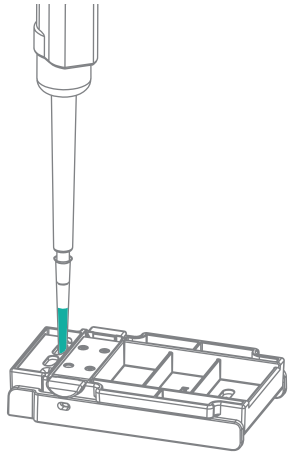


Figure 4 Loading samples by using MGIDL-200H

NOTE
- Do not press the control button of the pipette after inserting the tip into the fluidics inlet.
- Do not move the flow cell during loading.

5. Eject the tip from the pipette and the DNB loading mix will automatically flow into the flow cell.
6. After DNB loading, rotate the tips counterclockwise to take out them.
7. Place the device on the bench with the front upward for 30 minutes before use.

Chapter 7 Preparing the sequencing reagent cartridge

Perform the steps below:

1. Take out the Sequencing Reagent Cartridge from storage.Thaw them in water bath at room temperature until being completely thawed (or thaw in 2 ℃ to 8 ℃ fridge one day in advance). Store at 2 ℃ to 8 ℃ storage until use.
2. Invert the cartridge 3 times to mix before use.
3. Shake the cartridge vigorously in all directions for 10 to 20 times and ensure that reagents are fully mixed.

NOTE If dark green crystals appear in well No.10, it is precipitation of raw materials of the reagent in well No.10. This is a normal phenomenon. When the cartridge is thawed, mix the reagents in the cartridge well and the crystals will dissolve. Sequencing quality will not be affected. See *10.8 Dark green crystals in well No.10 on Page 55* in this manual for details.

4. Wipe condensate water on the cartridge cover and well around with lint-free paper. Well positions are shown in the figure below.

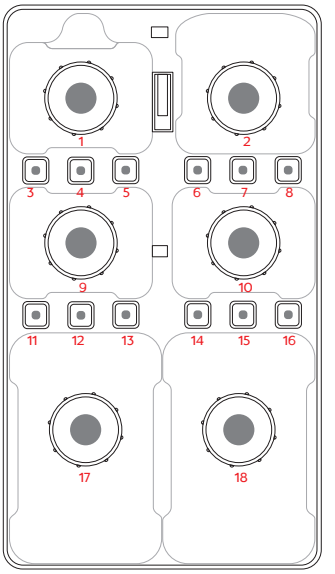


Figure 5 Sequencing Reagent Cartridge well position

5. Take out dNTPs Mix and dNTPs Mix II from -20 ℃ storage 1 hour in advance and thaw them at room temperature. Store at 4 ℃ until use.
6. Mix the reagents by using a vortex mixer for 5 seconds and centrifuge briefly before use.
7. Take out Sequencing Enzyme Mix from -20 ℃ storage and place on ice until use. Invert Sequencing Enzyme Mix 4 to 6 times before use.

7.1 Preparing the DB FCS SE100/ App-A FCS SE100 sequencing cartridge

Perform the steps below:

1. Pierce the seal in the center of well No.1 and No.2 to make a hole around 2 cm in diameter using 1 mL sterile tip.

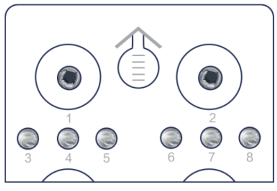


Figure 6 Piercing the seal on the cartridge

2. Take a pipette with the appropriate volume range and add reagents following the table below. First, add dNTPs Mix into a new 5 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube.

Table 21 Sequencing cartridge well No.1 reagent loading

Sequencing kit	dNTPs Mix loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
G400 SM DB FCS SE100	0.800	0.800
G400 SM App-A FCS SE100	0.800	0.800

3. Invert the tube for 4 to 6 times to mix the reagents in the tube before adding all of them into well No.1.

NOTE When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.

4. Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix II into a new 5 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix II in the same tube.

Table 22 Sequencing cartridge well No.2 reagent loading

Sequencing kit	dNTPs Mix II loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
G400 SM DB FCS SE100	1.600	0.800
G400 SM App-A FCS SE100	1.600	0.800

5. Invert the tube for 4 to 6 times to mix the reagents in the tube before adding all of them into well No.2.

   NOTE When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.

6. Seal the loading wells of well No.1 and No.2 with the transparent sealing film.

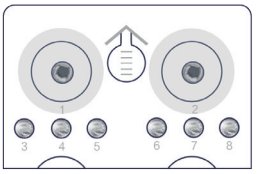


Figure 7 Sealing the loading wells of the cartridge

7. Press the film with thumb around the round cap. Ensure to seal tightly and no bubbles exist between the film and cap. Ensure the reagents would not overflow from the cartridge.

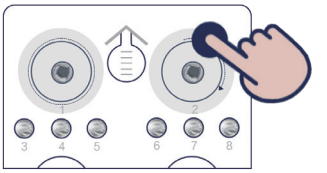


Figure 8 Sealing the loading wells tightly of the cartridge

8. Lift the cartridge horizontally, hold both sides of the cartridge with both hands. Shake it clockwise 10 to 20 times, and then counterclockwise 10 to 20 times. Ensure that reagents are fully mixed.

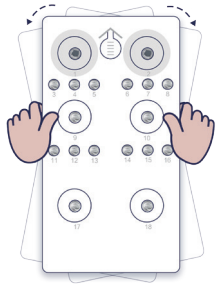


Figure 9 Mixing reagents after loading

9. Take out the seal of loading wells from the cartridge carefully after fully mixing.

NOTE
- Do not use the wasted seals again.
- Ensure that well No.1 and No.2 are clean to avoid cross contamination.

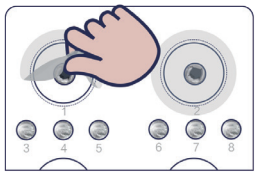


Figure 10 Removing the seal from the cartridge after mixing

10. The G400 SM DB FCS SE100/G400 SM App-A FCS SE100 sequencing cartridge is now ready for use.

7.2 Preparing the DB FCS PE100/DB FCS PE150/App-A DB FCS PE100/ App-A DB FCS PE150 sequencing cartridge

Perform the steps below:

1. Pierce the seal in the center of well No.1 and No.2 to make a hole around 2 cm in diameter using 1 mL sterile tip.

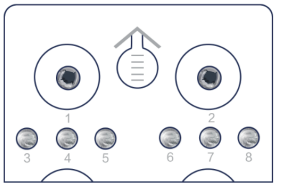


Figure 11 Piercing the seal on the cartridge

2. Take a pipette with the appropriate volume range and add reagents following the table below. First, add dNTPs Mix into a new 5 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube.

Table 23 Sequencing cartridge well No.1 reagent loading

Sequencing kit	dNTPs Mix loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
G400 SM DB FCS PE100	1.400	1.400
G400 SM DB FCS PE150	1.900	1.900
G400 SM App-A DB FCS PE100	1.400	1.400
G400 SM App-A DB FCS PE150	1.900	1.900

3. Invert the tube for 4 to 6 times to mix the reagents in the tube before adding all of them into well No.1.

NOTE When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.

4. Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix II into a new 15 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix II in the same tube.

Table 24 Sequencing cartridge well No.2 reagent loading

Sequencing kit	dNTPs Mix II loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
G400 SM DB FCS PE100	2.800	1.400
G400 SM DB FCS PE150	3.800	1.900
G400 SM App-A DB FCS PE100	2.800	1.400
G400 SM App-A DB FCS PE150	3.800	1.900

5. Invert the tube for 4 to 6 times to mix the reagents in the tube before adding all of them into well No.2.

NOTE When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.

6. Seal the loading wells of well No.1, and No.2 with the transparent sealing film.

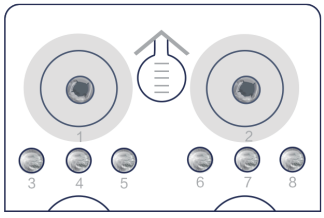


Figure 12 Sealing the loading wells of the cartridge

7. Press the film with thumb around the round cap. Ensure to seal tightly and no bubbles exist between the film and cap. Ensure the reagents would not overflow from the cartridge.

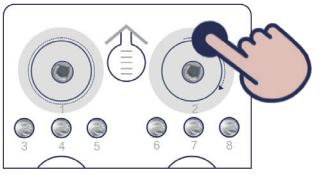


Figure 13 Sealing the loading wells tightly of the cartridge

8. Lift the cartridge horizontally, hold both sides of the cartridge with both hands. Shake it clockwise 10 to 20 times, and then counterclockwise 10 to 20 times, until the reagent color in well No.1 is uniform. Ensure the reagents are fully mixed.

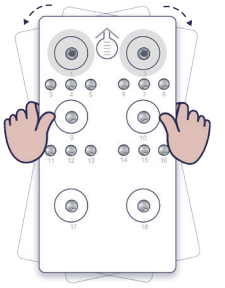


Figure 14 Mixing reagents after loading

9. Pierce the seal of well No.15 using 1 mL sterile tip.
10. Add 500 µL of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette.

NOTE When using MDA Enzyme Mix, do not touch the wall of the tube to prevent influencing the enzyme activity.

11. Invert the tube for 4 to 6 times to mix the reagents,
12. Add the mixture to well No.15. When adding the mixture, ensure there are no bubbles at the bottom of the tube.

NOTE When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.

13. Take out the seal of loading wells from the cartridge carefully after fully mixing.

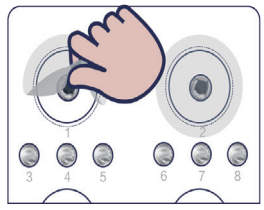


Figure 15 Removing the seal from cartridge after mixing

NOTE
- Do not use the wasted seals again.
- Ensure that well No.1 and No.2 are clean to avoid cross contamination.

14. The G400 SM DB FCS PE100/G400 SM DB FCS PE150/G400 SM
15. App-A DB FCS PE100/G400 SM App-A DB FCS PE150 sequencing cartridge is now ready for use.

Chapter 8 Sequencing

8.1 Entering the main interface

NOTE Verify that the barcode list is available in the sequencer before starting sequencing.

Perform the steps below:

1. Input the user name "user" and password "123", tap **Log in**.

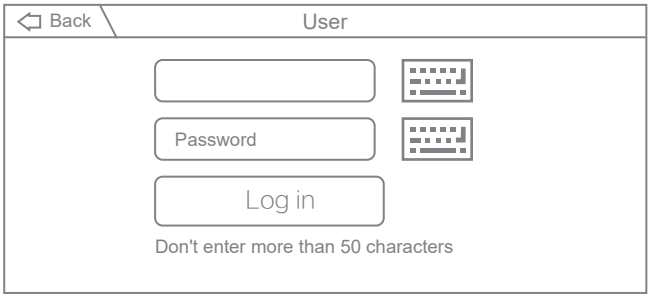


Figure 16 Log-in interface

2. Enter the main interface.

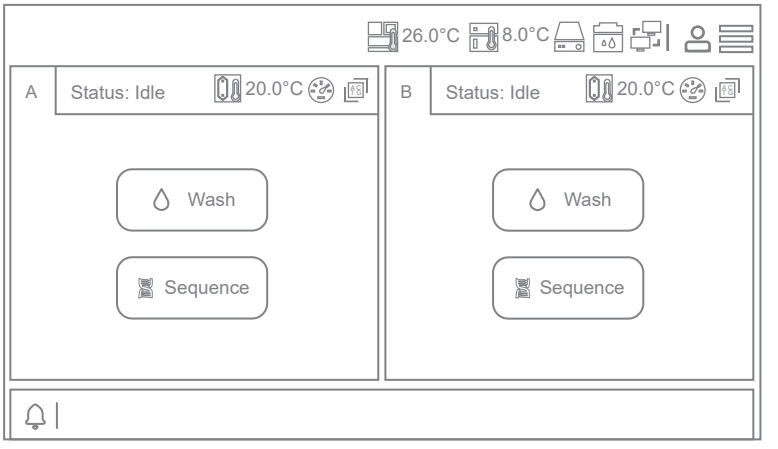


Figure 17 Main interface

8.2 Loading the DNBs

Perform the steps below:

1. Tap **Sequence** in the interface to enter the interface below.

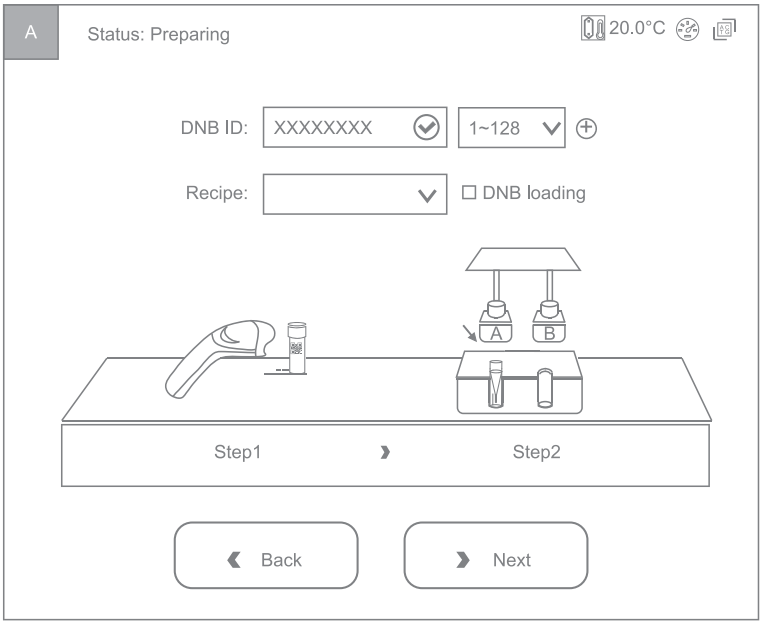


Figure 18 DNB loading interface

2. Tap ⊕ on the right of **DNB ID** and the lane information will appear.

Figure 19 DNBs and information selection interface

NOTE Select 2 lanes for FCS.

3. Move the cursor to the DNB ID box and enter the library name or number.

4. Pull the drop-down menu on the left of ⊕ and select the barcode sequence of different lanes.

5. When using the sequencer to load DNB, open the reagent compartment door, gently lift the sampling needle with one hand, and remove the cleaning reagent tube with the other hand. Load the sample tube, then slowly lower the sampling needle until the tip reaches the bottom of the tube.

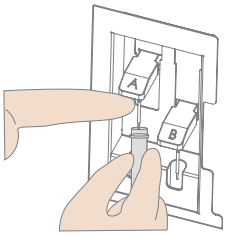


Figure 20 Loading the DNBs tube

NOTE Perform this step if using the sequencer to load the DNB, if not, Place an empty tube.

6. Close the reagent compartment door.

8.3 Selecting the sequencing parameters

Perform the steps below:

1. Select the sequencing recipe in the **Recipe** drop-down menu. Choose **Customize**,

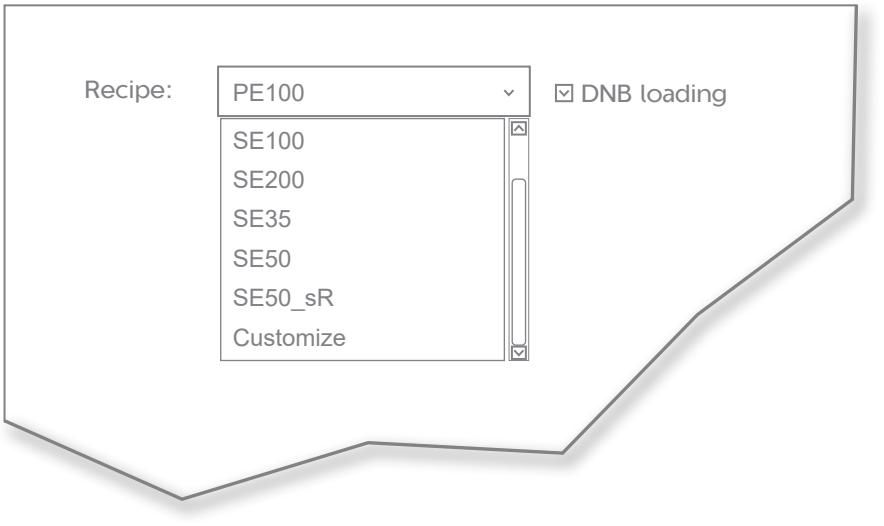


Figure 21 Selecting sequencing solutions

2. In the beginning, please select a step to start the sequencing run:If sequencer DNB loading,choose **DNB loading**; If others, choose **Post loading**.

Figure 22 Selecting the step to start sequencing

3. Select the read length. For example, with PE100 enter 100 for read 1 and 100 for read 2.

Figure 23 Choosing the read length

4. Select the barcode length. For dual barcode sequencing, fill in the length of Barcode and Dual barcode. Leave the Dual barcode blank if it is a Single Barcode sequencing run.

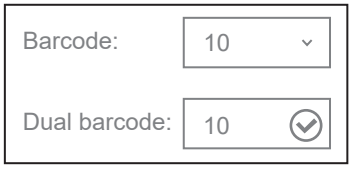


Figure 24 Selecting the barcode length

5. Select the lane for barcode demultiplexing.

Figure 25 Barcode demultiplexing on different lanes

6. Select the dark reaction for any position of read length in read 1 or 2. If dark reaction is not required, leave the table below blank.

Figure 26 Selecting the dark reaction

NOTE Dark reaction means there is only chemical reaction without capturing optical information.

7. Tap **Confirm**.

8.4 Loading the reagent cartridge

Perform the steps below:

1. Move the cursor to the **Reagent cartridge ID**, enter the cartridge information manually or use the barcode scanner to scan the cartridge barcode.

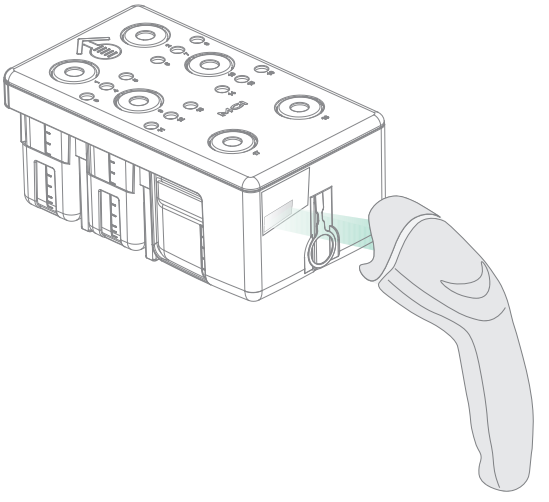


Figure 27 Reagent cartridge information entry interface

2. Open the reagent compartment door. Hold the handle of the cleaning cartridge 1 with one hand, place the other hand underneath the cartridge for support, and slowly take out cleaning cartridge from the compartment.

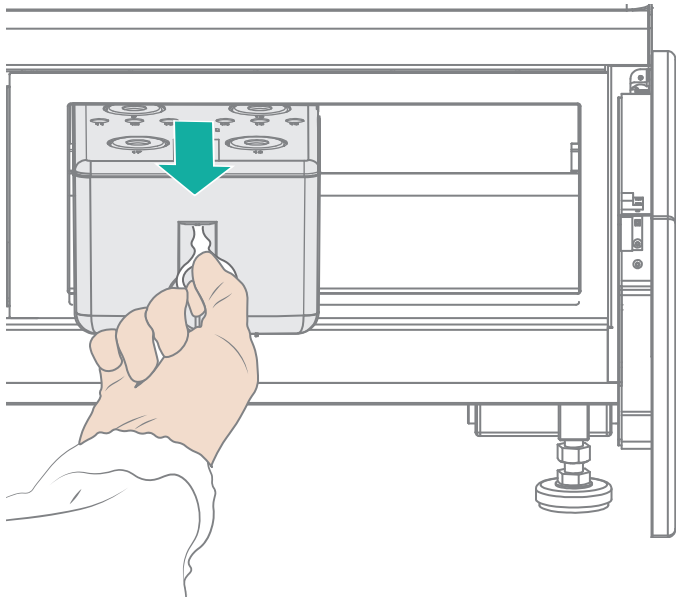


Figure 28 Removing cleaning cartridge

3. Moisten dust-free paper or a dust-free cloth with the laboratory-grade water and use it to wipe the bottom and sides of the compartment to keep it clean and dry.

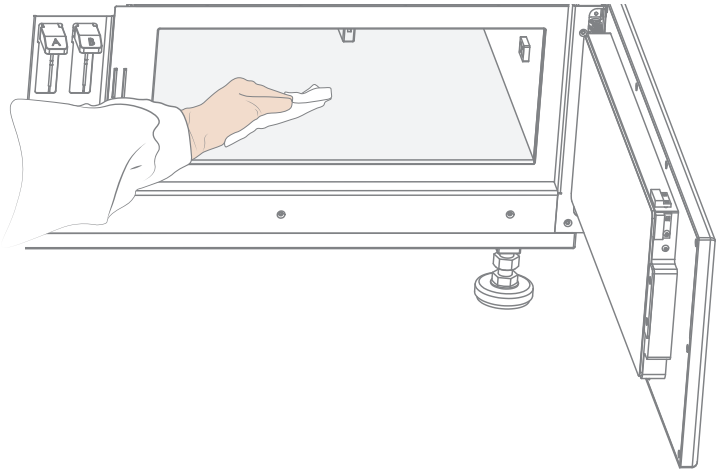


Figure 29 Maintaining the reagent compartment

4. Hold the handle of the reagent kit with one hand and place the other hand underneath for support.

5. Slide the new kit into the compartment following the direction printed on the cover until it stops.

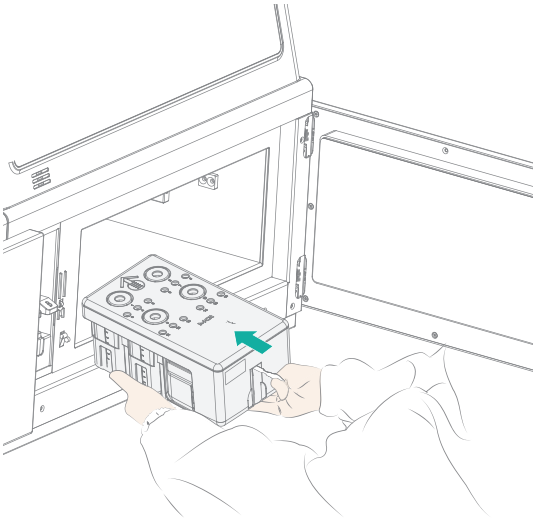


Figure 30 Sliding the new reagent cartridge into the reagent compartment

6. Check that the reagent kit is in the correct position and close the reagent compartment door.

8.5 Loading the flow cell

Perform the steps below:

1. Open the flow cell compartment door,
2. Press both sides of the flow cell used for washing, and press the flow cell attachment button with the other hand.
3. After the vacuum is released, take out the flow cell for washing from the stage.

4. Use dust remover to remove the dust on the stage and the back of the flow cell.

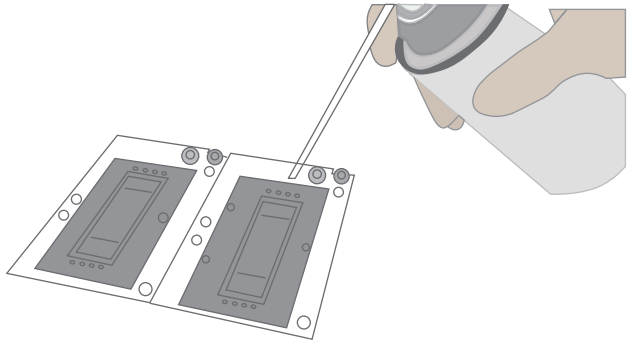


Figure 31 Cleaning the flow cell stage

NOTE If there are impurities on the stage surface, please gently wipe it with wet dust-free paper 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 flow cell by the edges with both hands.

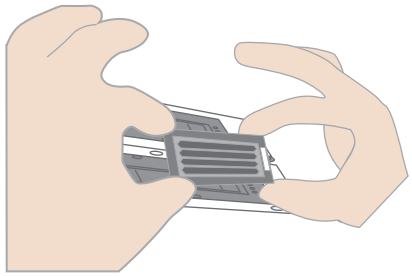


Figure 32 Loading the flow cell

7. Align the holes on the flow cell with the locating pins on the flow cell stage. Gently slide the flow cell at a 45-degree angle to the upper left corner (45-degree to the upper right corner when loading flow cell on MGIDL-200RS) 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 the flow cell is properly seated on the stage.

NOTE The flow cell is fragile, please use caution when handling the flow cell.

9. Ensure that the negative pressure is within the range from -80 to -99 kPa before continuing.

   NOTE If the negative pressure is abnormal, refer to *10.2 Abnormal negative pressure on Page 52* in this manual for troubleshooting.

10. Use a dust remover to remove the dust on the flow cell surface and close the flow cell compartment door.

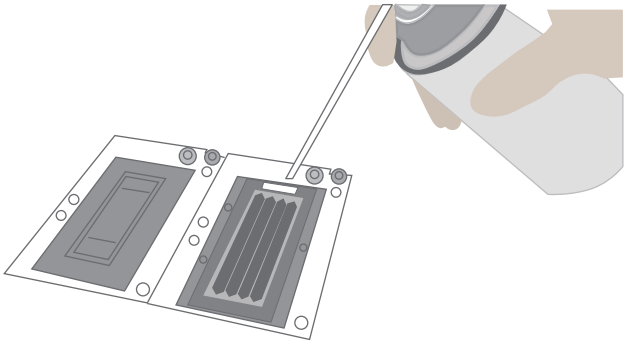


Figure 33 Cleaning the flow cell

11. Tap **Next**, The flow cell ID can be entered by using the barcode scanner. If automated entry does not work, move the cursor to the “Flow Cell ID” and enter the ID manually.

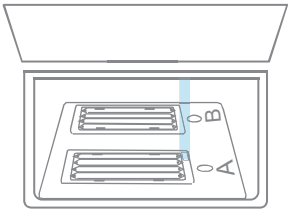


Figure 34 flow cell information entry interface

12. Tap **Next**.

8.6 Reviewing parameters

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

Item	Content
User name	user
DNB ID Lane 1	WGS | 1 ~ 128
DNB ID Lane 2	RNA | 501 ~ 596
DNB ID Lane 3	WGS | 1 ~128
DNB ID Lane 4	WGS | 1 ~ 128
Sequencing cartridge ID	AA000012
Flow cell ID	F300001234
Recipe	Customize
Start phase	DNB Loading
Cycles	222
Read 1	100
Read 2	100
Dual Barcode	10
Barcode	10
Split barcode	Yes | Yes | No | No
Read 1 dark reaction	2 ~ 5
Read 2 dark reaction	3 ~ 8

Figure 35 Reviewing information

NOTE To ensure sequencing quality, when read 1 and read 2 sequencing is completed, the sequencer will automatically perform another cycle for correction.

For example, for PE100 dual barcode sequencing, read length of read 1 is 100, read length of read 2 is 100, barcode read length is 10 and dual barcode read length is 10, plus 1 correction cycle for read 1 and 1 correction cycle for read 2 (barcode does not require correction), the total cycle number of the sequencing is 222.

8.7 Starting sequencing

Perform the steps below:

1. After confirming that the information is correct, tap **Start**.
2. The system will display the dialog box "Proceed with Sequencing?". Tap **Yes** to start sequencing (see the figure below).

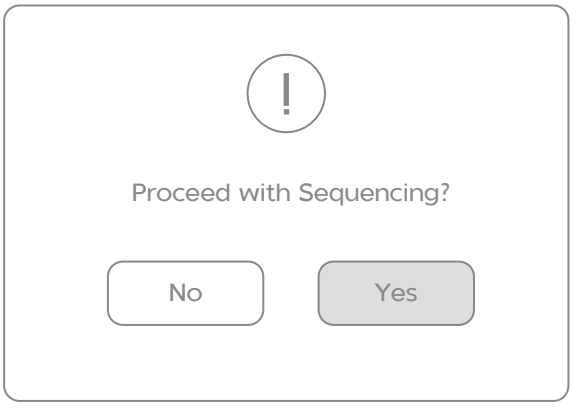


Figure 36 Confirming sequencing interface

3. Once sequencing has started, immediately open the flow cell compartment door to ensure that DNB (or reagents) is flowing through the flow cell.

Chapter 9 Device maintenance

9.1 Washing type

Table 25 Wash requirments

Wash type	Description	Time
Full wash 1	Maintenance wash 1 Regular wash	About 76 min
Full wash 2	Maintenance wash 2 Regular wash	About 62 min
Maintenance wash 1	To remove residual reagents and proteins in the pipeline, reducing the risk of blockage. Procedure: Cleaning cartridge 3 > Cleaning cartridge 2.	About 28 min
Maintenance wash 2	To remove residual reagents and proteins in the pipeline, reducing the risk of blockage. Procedure: Cleaning cartridge 4	About 14 min
Regular wash	To remove residual reagents, reducing the risk of cross-contamination. Procedure: Cleaning cartridge 1 > Air Prime	About 48 min

9.2 Washing instruction

When sequencing is completed, the device needs to be washed within 24 hours. When the following interface appears, please perform wash.

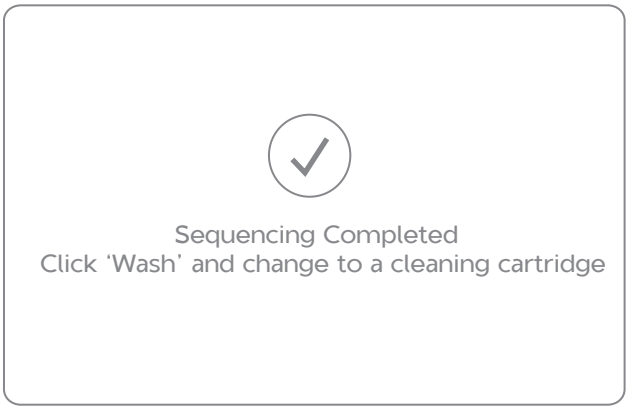


Figure 37 Wash interface

Table 26 Wash Instruction

Wash type	Description
Full wash	● A full wash is required if the sequencer is used for a PE run or a DNB loading/post-load. ● If the sequencer was left unused for more than 7 days after a full wash, please perform a full wash before use. ● If impurities are found on the flow cell, perform a full wash. ● After the replacement of pipelines, sample needles and other accessories exposed to reagents, please perform a full wash.
Regular wash	● A regular wash is sufficient for an SE run. ● If the device was left unused for more than 12 hours after a full wash, please perform a regular wash again before use. ● After the system maintenance performed by an engineer, please perform a regular wash.
Maintenance wash	If the sequencer is to be powered off for more than 7 days, perform a maintenance wash before being powered off and after being powered on.

Refer to the flow chart below for details.

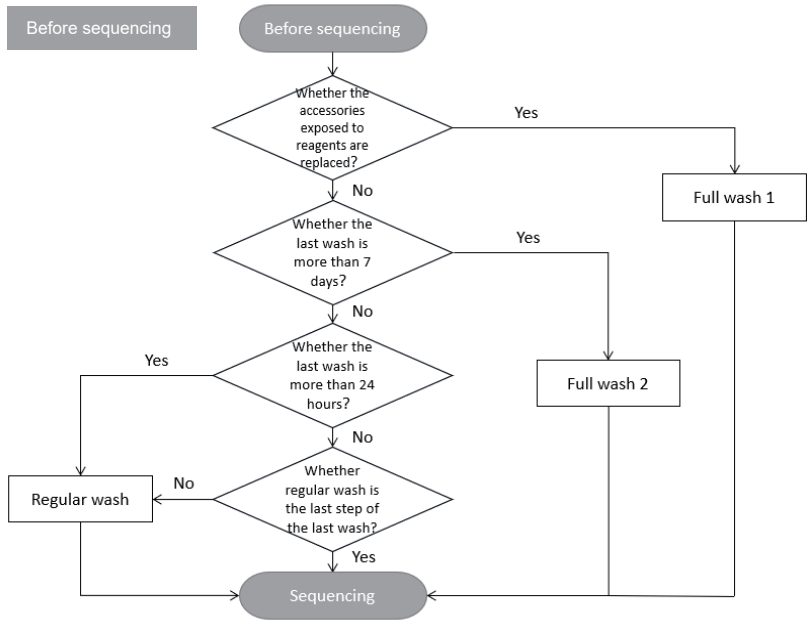


Figure 38 Wash instruction before sequencing

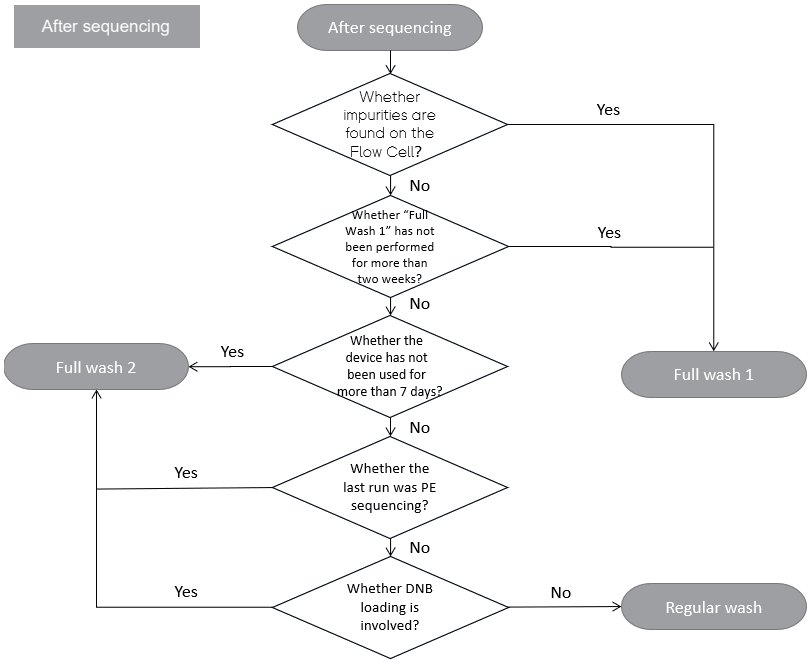


Figure 39 Wash instruction after sequencing

NOTE
- The function of full wash 1 includes that of full wash 2.
- Full wash 2 needs to be used with the script StandardMPS_V1.6.1.04 and above.

9.3 Preparing wash reagents

NOTE Wash reagents are valid for 28 days if being stored at 4 ℃ .

- Prepare wash reagent 1 following the table below.

Table 27 Wash reagent 1 preparation

Reagent	Volume
100% Tween-20	0.5 mL
Laboratory-grade water	999.5 mL

- Prepare wash reagent 2 following the table below.

Table 28 Wash reagent 2 preparation

Reagent	Weight/Volume
5M NaCl solution	200 mL
100% Tween-20	0.5 mL
Laboratory-grade water	799.5 mL

- Prepare wash reagent 3 following the table below.

Table 29 Wash reagent 3 preparation

Reagent	Volume
2M NaOH solution	50 mL
Laboratory-grade water	950 mL

- Prepare wash reagent 4 following the table below.

Table 30 Wash reagent 4 preparation

Reagent	Weight/Volume
100 % Tween-20	0.5 mL
100 % ProClin300	0.3 mL
Laboratory-grade water	999.2 mL

9.4 Washing cartridge

- An empty cleaning cartridge and washing flow cell for a full wash are provided together with the device.

- Wash the cleaning cartridge every time before refilling it with cleaning reagents. Replace cleaning cartridge after using 20 times or every half year.
- Used flow cells from previous runs can be used as washing flow cells. Each flow cell can be used for up to 20 full washes.

Table 31 Wash cleaning cartridge preparation

Cartridge name	2 mL cryotube	Large wells	No.15 well	Other small wells
Wash cleaning cartridge 1	More than 95% volume of laboratory-grade water			
Wash cleaning cartridge 2	1800 µL Wash reagent 3	50 mL Wash reagent 3	6 mL Wash reagent 3	6 mL Wash reagent 3
Wash cleaning cartridge 3	1800 µL Wash reagent 2	50 mL Wash reagent 1	6 mL Wash reagent 2	6 mL Wash reagent 1
Wash cleaning cartridge 4	1800 µL Wash reagent 4	50 mL Wash reagent 4	6 mL Wash reagent 4	6 mL Wash reagent 4

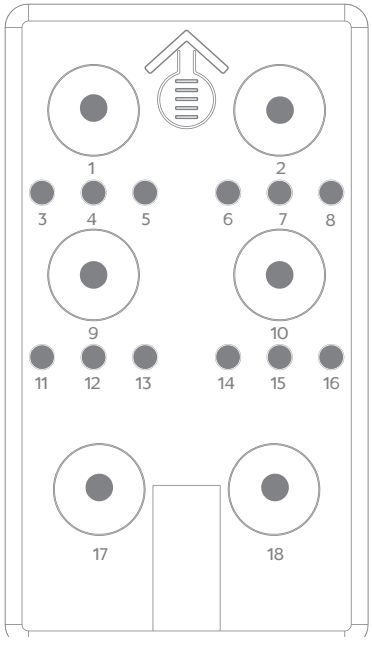


Figure 40 Wash cleaning cartridge well positio

NOTE
- 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.

9.5 Washing procedures

9.5.1 Regular wash

Perform the steps below:

1. Use cleaning cartridge 1. Open the reagent compartment door.
2. Hold the handle of the cleaning cartridge 1 with one hand and place the other hand underneath the cartridge 1 for support.
3. Slide it into the reagent compartment slowly following the direction printed on the cartridge cover until it stops.
4. Close the reagent compartment door.
5. Tap **Wash** on the interface.
6. Place the flow cell for washing.
7. Select **Regular** from the drop-down menu to start the regular wash which takes about 48 minutes.

Figure 41 Selecting the wash type

NOTE
- If you perform the regular wash only, observe the status of the washing flow cell in this step. If you see many bubbles, continue the wash. If not, stop the wash, replace the flow cell and start the wash.
- If you perform the regular wash after the maintenance wash, skip this step.

8. When the figure below appears on the interface, the regular wash ends.

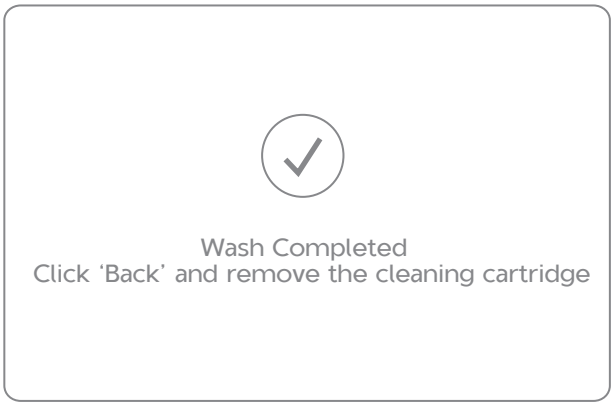


Figure 42 Regular wash end interface

9.5.2 Maintenance wash 1

Perform the steps below:

1. Use cleaning cartridge 3. Open the reagent compartment door.
2. Hold the handle of the cleaning cartridge 3 with one hand and place the other hand underneath for support.
3. Slide it to the reagent compartment slowly following the direction printed on the cartridge cover until it stops.
4. Close the reagent compartment door.
5. Tap **Wash** on the interface.
6. Place the flow cell for washing.
7. Select the **Maintenance** from the drop-down menu to start the maintenance wash which takes about 14 minutes.
8. Observe the status of flow cell for wash in this step. If you see many bubbles, continue the wash. If not, stop the wash, replace the flow cell and start the wash.

9. When the figure below appears on the interface, tap **Yes** and the sequencer will automatically lift the sampling needles.

Figure 43 Maintenance wash [1] end interface

10. Then open the compartment door and replace the cleaning cartridge.
11. Use cleaning cartridge 2 and continue the maintenance wash which takes around 14 minutes.
12. When the figure below appears on the interface, tap **No** to end the maintenance wash.

Figure 44 Maintenance wash end interface

9.5.3 Full wash procedures

Perform the steps below:

1. Perfrom Maintenance wash 1,
2. Followed by Regular wash with a total time of around 76 min.

9.5.4 Maintenance wash 2

Perform the steps below:

1. Use cleaning cartridge 4. Open the reagent compartment door.
2. Hold the handle of the cleaning cartridge 4 with one hand and place the other hand underneath for support.
3. Slide it to the reagent compartment slowly following the direction printed on the cartridge cover until it stops.
4. Close the reagent compartment door.
5. Tap **Wash** on the interface.
6. Place the flow cell for washing.
7. Select the **Maintenance wash** from the drop-down menu to start the maintenance wash which takes about 14 minutes.
8. Observe the status of flow cell for wash in this step. If you see many bubbles, continue the wash. If not, stop the wash, replace the flow cell and start the wash.
9. When the interface appears as the figure below, tap **No** to end the maintenance wash.

Figure 45 Maintenance wash end interface

9.5.5 Full wash procedures 2

Perform the steps below:

1. Perfrom Maintenance wash 2.
2. Fllowed by Regular wash with a total time of around 62 min.

Chapter 10 Troubleshooting

10.1 Low DNB concentration

When DNB concentration is lower than 8 ng/μL, try the steps below:

- Check if the kit has expired.
- Check if the library meets the requirements.
- Make a new DNB preparation. You can order DNBSEQ DNB Make Reagent Kit (Catalog No. 1000016115 for general library, or Catalog No. 1000027738 for App-A library) to make new DNBs.

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

10.2 Abnormal negative pressure

When the negative pressure is shown in red, the negative pressure is abnormal, try the steps below:

- Gently wipe the stage surface with a damp lint-free paper or a lint-free cloth and blow the stage with a power dust remover and ensure no dust is left.
- Blow the back of the flow cell with a dust remover to ensure no dust is left.
- If the problem persists, please contact technical support.

10.3 Bubbles

If bubbles appear, try the steps below:

- Replace the used flow cell and inspect the pump.
- If the problem persists, please contact technical support.

10.4 Impurities

If impurities appear, try the steps below:

- Perform a full wash on MGIDL-200RS and the sequencer following the MGIDL-200RS User Manual and *9.5 Washing procedures on Page 48* in this manual.
- If the problem persists after a full wash, please contact technical support.

10.5 Pump fails

If liquids cannot be pumped into the flow cell, or large bubbles appear in the flow cell, try the steps below:

- MGIDL-200RS and the sequencer:
  1) Take out the flow cell,
  2) Check if there are impurities in sealing gasket and take out the dust with the dust remover.
  3) Place the flow cell following the instruction in *8.5 Loading the flow cell on Page 38* and start the pump again.
- Check if the sampling needles move properly.
  - ➢ If the sampling needles cannot move properly, restart sequencing software.
  - ➢ If the problem persists, please contact technical support.

10.6 Reagent kit storage

- If the kit has been thawed (including dNTPs) and cannot be used within 24 hours, it can be frozen and thawed at most once.
- If the kit has been thawed (including dNTPs) but cannot be used immediately,
  1) store it at 4 ℃ and use it within 24 hours.

2) Mix the reagents in the cartridge following instruction in *Chapter 7 Preparing the sequencing reagent cartridge on Page 23* before use.

- If dNTPs and enzyme have been added into the cartridge, i.e. the cartridge has been prepared but cannot be used immediately,

  1) Store it at 4 ℃ and use it within 24 hours.
  2) Mix the reagents in the cartridge following instruction in *Chapter 7 Preparing the sequencing reagent cartridge on Page 23* before use.

- If dNTPs and enzyme have been added into the cartridge, i.e. the cartridge has been prepared and the sampling needles have started aspiration, but the cartridge cannot be used in time,

  1) The cartridge must be sealed with foil or plastic wrap.
  2) Store the cartridge at 4 ℃ and use it within 24 hours.
  3) Gently mix the reagents in the cartridge before use. When mixing, be careful not to spill any reagent from the needle holes to avoid reagent contamination.

10.7 Post loading fails

If post loading fails, but prime step has been performed, in this condition please re-start from the post loading.

1) Start from *Chapter 8 Sequencing on Page 32* and re-load the flow cell.
2) When selecting *8.3 Selecting the sequencing parameters on Page 34*, choose program **Customize**.
3) Select **Post loading** and tap **...**.

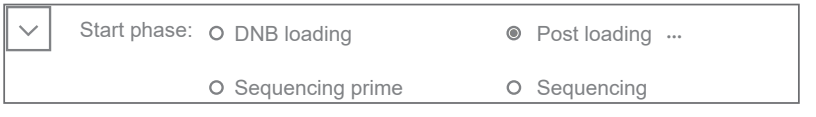


Figure 46 Selecting re-start Post loading 1

4) If starts from the Post loading prime, select **Prime** in the figure below. If starts from the step Post loading, don't select **Prime**.

Figure 47 Selecting re-start Post loading 2

5) Other steps please follow *Chapter 8 Sequencing on Page 32* in this manual.

10.8 Dark green crystals in well No.10

- If dark green crystals appear in well No.10, it is precipitation of raw materials of the reagent in well No.10. This is a normal phenomenon.
- When the cartridge is thawed, mix the reagents in the cartridge well and the crystals will dissolve. Sequencing quality will not be affected.

Figure 48 Dark green crystals in well No.10

10.9 Library amount less than 40 fmol/ App-A library amount less than 60 fmol

If the general library amount is less than 40 fmol but not less than 24 fmol, or the App-A library amount is less than 60 fmol but not less than 36 fmol, 60 μL Make DNB reaction can be tried. It must be noted that 60 μL Make DNB reaction may cause data loss and sequencing quality poorer than expectation. When the library amount is adequate, 100 μL Make DNB reaction is still required.

10.9.1 Calculate the required amount of ssDNA library

Perform the steps below:

1. The required volume of ssDNA library is determined by the required library amount (fmol) and library concentration quantified in *4.2 Library concentration and amount requirement on Page 14*.

2. The volume of each Make DNB reaction is 60 µL and the required library input for each Make DNB reaction is calculated as followed:

- For general library, ssDNA library input (µL)=24 fmol/library concentration (fmol/µL).
- For App-A library, ssDNA library input (µL)=36 fmol/library concentration (fmol/µL).

3. Calculate the required ssDNA library for each Make DNB reaction and fill it in *Table 32 Make DNB reaction mix 1 on Page 56.* as V.

10.9.2 Make DNB

Perform the steps below:

1. Take a 0.2 mL 8-tube strip or PCR tubes. Prepare reaction mix following the table below.

Table 32 Make DNB reaction mix 1

Component	Volume (µL)
ssDNA libraries	V
Low TE Buffer	12-V
Make DNB Buffer/App-A Make DNB Buffer	12
Total Volume	24

2. Mix gently by vortexing and centrifuge for 5 seconds using a mini centrifuge.
3. Place the mix into a PCR machine and start the primer hybridization reaction. PCR machine settings are shown in the table below.

Table 33 Primer hybridization reaction condition

Temperature	Time
Heated lid (105 ℃)	On
95 ℃	1 min
65 ℃	1 min
40 ℃	1 min
4 ℃	Hold

4. Take out the Make DNB Enzyme Mix II (LC) from storage and place on ice.

5. Centrifuge briefly for 5 seconds and hold on ice.

NOTE
- Do not place Make DNB Enzyme Mix II (LC) at room temperature
- Avoid holding the tube for a prolonged time.

6. Take the PCR tube out of the PCR machine when the temperature reaches 4 ℃ .
7. Centrifuge briefly for 5 seconds, place the tube on ice and prepare the Make DNB reaction mix 2.
8. Make DNB reaction mix 2.

Table 34 Make DNB reaction mix 2

Component	Volume (μL)
Make DNB Enzyme Mix I	24.0
Make DNB Enzyme Mix II (LC)	2.4

9. Add all the Make DNB reaction mix 2 into the Make DNB reaction 1.
10. Mix gently by vortexing, centrifuge for 5 seconds using a mini centrifuge.
11. Place the tubes into the PCR machine for the next reaction. The conditions are shown *in Table 32 Make DNB reaction mix 1 on Page 56*.

Table 35 Rolling circle amplification conditions

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

12. Immediately add 12 μL Stop DNB Reaction Buffer once the temperature reaches 4 ℃.
13. Mix gently by pipetting 5 to 8 times using a wide bore tip.

NOTE
- It is very important to mix DNB gently using a wide bore pipette tip.
- Do not vortex, shake the tube or pipette vigorously.

14. Store the DNB at 4 ℃ and perform sequencing within 48 hours. Proceed to *4.4 Quantifying DNB on Page 18*.

Appendix 1 Manufacturer

Manufacturer	Wuhan MGI Tech Co., Ltd.
Address	Building 24, Stage 3.1, BioLake Accelerator, No.388, 2nd Gaoxin Road, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China
	Building B13, No.818, Gaoxin Avenue, East Lake HighTech Development Zone, 430075, Wuhan, P.R.China
Service hotline	(+86)4000-966-988
E-mail	MGI-service@genomocs.cn
Website	en.mgi-tech.com