DNBSEQ-G400RS CoolMPS High-throughput Sequencing Set User Manual

Catalog number, name and version:

1000017992, CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS FCL SE50)

1000016933, CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS FCL SE100)

1000016935, CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS FCL PE100)

1000019478, CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS Small RNA FCL SE50)

Set version: V1.0

1000020834, CPAS Barcode Primer 3 Reagent Kit, V2.0

1000020835, High-Throughput Single-End Sequencing Primer Kit (App-A), V2.0

1000020832, High-Throughput Pair-End Sequencing Primer Kit (App-A), V2.0

1000014047, High-Throughput Barcode Primer 3 Reagent Kit (App-A), V1.0

User manual version: A0

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Revision History

Version	Date	Summary of change
A0	May 2020	• The first version.

Table of Contents

1 Introduction

This manual describes how to perform sequencing using the CoolMPS High-throughput Sequencing Set and includes instructions regarding sample preparation, Flow Cell preparation, sequencing kit storage, sequencing protocols and device maintenance.

1.1 Applications

CoolMPS High-throughput 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 the CoolMPS sequencing technology to determine the base sequence carried by a DNA Nanoball (DNB) loaded on a sequencing flow cell. The test procedure is divided into three parts: Make DNB, DNB loading, and sequencing. DNBs are prepared by rolling circle amplification using the reagents provided in the sequencing set, and then the DNB is loaded onto the sequencing flow cell. During the sequencing process, dNTPs with unlabeled bases and 3'hydroxyl groups that can be reversibly terminated (Cold dNTPs) are added. These Cold dNTPs are incorporated into the sequencing strand by DNA polymerase. Base calling is achieved by specific binding of fluorescently labeled antibodies. The high-resolution imaging system collects the optical signals, and then the sample sequences are obtained after the optical signals are digitized. Finally, the 3'hydroxyl groups on the Cold dNTPs are recovered and antibodies are removed from the sequencing strand, leading to the next cycle.

1.3 Data Analysis

During the sequencing run, the control software automatically operates base calling analysis software and delivers raw sequencing data outputs for secondary analysis.

1.4 Sequencing Read Length

Sequencing read length will determine the number of sequencing cycles for a given sequencing run. For example, a PE100 cycle run performs reads of 100 cycles (2 × 100) for a total of 200 cycles. At the end of the insert sequencing run, an extra 10 cycles of barcode read can be performed, if required.

Table 1-1: Sequencing cycle

Sequencing read length	Read 1 read length	Read 2 read length	Barcode read length	Total read length	Maximum cycles
SE50	50	--	10	50+10	70
SE100	100	--	10	100+10	120
PE100	100	100	10	200+10	220

1.5 Sequencing Time

Table 1-2: Sequencing time for each read length (hours)

Time (hours)	SE50	SE100	PE100
Sequencing time	16.2	28.6	55.0
Data analysis (Dual flow cell)	0.3	0.6	1.3
Data analysis (Single flow cell)	0.6	1.2	2.6

Notes:

① The sequencing time 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.

② The time in the table above is theoretical and actual run time may vary among various sequencing instruments.

1.6 Attention

1) This product is restricted for research use only, please read the manual carefully before use.
2) Make sure that you are familiar with the SOP & Attention of all the laboratory apparatus to be used.
3) 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 when this happened.
4) All the samples and waste materials should be disposed according to relevant laws and regulations.
5) This product is for one sequencing run only and cannot be reused.
6) Do not use expired products.

2 Sequencing Sets and Consumables Required but not Provided

2.1 List of sequencing set components

Table 2-1: CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS FCL SE50)
Catalog number: 1000017992

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985	Sequencing Flow Cell	1 EA	-25℃--15℃
CoolMPS High-throughput Sequencing Kit (DNBSEQ-G400RS FCL SE50) Catalog number: 1000017991	Low TE Buffer	300 μL×1 tube	-25℃--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（Empty）	1 tube	
	Dye Mix	0.80 mL×1tube	
	dNTPs Mix II	1.00 mL×2tube	
	Sequencing Enzyme Mix II	2.70 mL×1tube	
	Sequencing Reagent Cartridge	1 EA	
	Transparent sealing film	2 sheets	

Table 2-2: CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS FCL SE100)
Catalog number: 1000016933

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985	Sequencing Flow Cell	1 EA	-25℃~-15℃
CoolMPS High-throughput Sequencing Kit DNBSEQ-G400RS FCL SE100) Catalog number: 1000016934	Low TE Buffer	300 μL×1tube	-25℃~-15℃
	Make DNB Buffer	100 μL×1tube	
	Make DNB Enzyme Mix I	200 μL×1tube	
	Make DNB Enzyme Mix II（LC）	25 μL×1tube	
	Stop DNB Reaction Buffer	100 μL×1tube	
	DNB Load Buffer I	200 μL×1tube	
	DNB Load Buffer II	200 μL×1tube	
	Micro Tube 0.5mL（Empty）	1 tube	
	Dye Mix	1.20 mL×1tube	
	dNTPs Mix II	3.30 mL×1tube	
	Sequencing Enzyme Mix II	4.40 mL×1tube	
	Sequencing Reagent Cartridge	1 EA	
	Transparent sealing film	2 sheets	

Table 2-3: CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS FCL PE100)
Catalog number: 1000016935

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985	Sequencing Flow Cell	1 EA	-25℃~-15℃
CoolMPS High-throughput Sequencing Kit (DNBSEQ-G400RS FCL PE100) Catalog number: 1000016936	Low TE Buffer	300 μL×1tube	-25℃~-15℃
	Make DNB Buffer	100 μL×1tube	
	Make DNB Enzyme Mix I	200 μL×1tube	
	Make DNB Enzyme Mix II（LC）	25 μL×1tube	
	Stop DNB Reaction Buffer	100 μL×1tube	
	DNB Load Buffer I	200 μL×1tube	
	DNB Load Buffer II	200 μL×1tube	
	Micro Tube 0.5mL（Empty）	1 tube	
	Dye Mix	1.35 mL×2tube	
	dNTPs Mix II	2.95 mL×2tube	
	Sequencing Enzyme Mix II	7.90 mL×1tube	
	MDA Reagent	3.50 mL×1tube	
	MDA Enzyme Mix II	0.50 mL×1tube	
	Sequencing Reagent Cartridge	1 EA	
	Transparent sealing film	2 sheets	

Table 2-4: CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS Small RNA FCL SE50)
Catalog number: 1000019478

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985	Sequencing Flow Cell	1 EA	-25℃~-15℃
CoolMPS High-throughput Sequencing Kit (DNBSEQ-G400RS FCL SE50) Catalog number: 1000017991	Low TE Buffer	300 μL×1 tube	-25℃~-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（Empty）	1 tube	
	Dye Mix	0.80 mL×1tube	
	dNTPs Mix II	1.00 mL×2tube	
	Sequencing Enzyme Mix II	2.70 mL×1tube	
	Sequencing Reagent Cartridge	1 EA	
	Transparent sealing film	2 sheets	
MGIEasy Wash Buffer For Small RNA Sequencing Catalog number: 1000006387	Wash Buffer For Small RNA Sequencing	1.60 mL×3tube	2℃-8℃

Table 2-5: CPAS Barcode Primer 3 Reagent Kit
Catalog number: 1000020834

Product	Sequencing Kit	Component	Spec & Quantity	Storage Temperature
Primer for dual barcode sequencing (Pair End Sequencing use only)	CPAS Barcode Primer 3 Reagent Kit Catalog number: 1000020834	1μM AD153 Barcode Primer 3 Catalog number: 1000020833	3.50 mL×1tube	-25℃~-15℃

Table 2-6: SE50 (App-A) single barcode sequencing reagent component

Product	Sequencing Kit	Component	Spec & Quantity	Storage Temperature
CoolMPS High-throughput Sequencing Set	CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS FCL SE50) Catalog number: 1000017992 (See Table 2-1 for details)			
Primers for App-A single barcode sequencing (Single End Sequencing)	High-Throughput Single-End Sequencing Primer Kit (App-A) Catalog number: 1000020835	App-A Make DNB Buffer	400 μL×1tube	-25℃~-15℃
		1μM App-A Insert Primer 1	2.20 mL×1tube	
		1μM App-A Barcode Primer 1	3.50 mL×1tube	

Table 2-7: SE100 (App-A) single barcode sequencing reagent component

Product	Sequencing Kit	Component	Spec & Quantity	Storage Temperature
CoolMPS High-throughput Sequencing Set	CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS FCL SE100) Catalog number: 1000016933 (See Table 2-2 for details)			
Primers for App-A single barcode sequencing (Single End Sequencing)	High-Throughput Single-End Sequencing Primer Kit (App-A) Catalog number: 1000020835	App-A Make DNB Buffer	400 μL×1tube	-25℃~-15℃
		1μM App-A Insert Primer 1	2.20 mL×1tube	
		1μM App-A Barcode Primer 1	3.50 mL×1tube	

Table 2-8: PE100 (App-A) single barcode sequencing reagent component

Product	Sequencing Kit	Component	Spec & Quantity	Storage Temperature
CoolMPS High-throughput Sequencing Set	CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS FCL PE100) Catalog number: 1000016935 (See Table 2-3 for details)			
Primers for App-A single barcode sequencing (Pair End Sequencing)	High-Throughput Pair-End Sequencing Primer Kit (App-A) Catalog number: 1000020832	App-A Make DNB Buffer	400 μL×1tube	-25℃~-15℃
		1μM App-A Insert Primer 1	2.20 mL×1tube	
		1μM App-A Insert Primer 2	4.20 mL×1tube	
		1μM App-A MDA Primer	4.20 mL×1tube	
		1μM App-A Barcode Primer 2	3.50 mL×1tube	

Table 2-9: PE100 (App-A) dual barcode sequencing reagent component

Product	Sequencing Kit	Component	Spec & Quantity	Storage Temperature
CoolMPS High-throughput Sequencing Set	CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS FCL PE100) Catalog number: 1000016935 (See Table 2-3 for details)			
Primers for App-A dual barcode sequencing (Pair End Sequencing)	High-Throughput Pair-End Sequencing Primer Kit (App-A) Catalog number: 1000020832	App-A Make DNB Buffer	400 μL×1tube	-25℃--15℃
		1μM App-A Insert Primer 1	2.20 mL×1tube	
		1μM App-A Insert Primer 2	4.20 mL×1tube	
		1μM App-A MDA Primer	4.20 mL×1tube	
		1μM App-A Barcode Primer 2	3.50 mL×1tube	
	High-Throughput Barcode Primer 3 Reagent Kit (App-A) Catalog number: 1000014047	1μM App-A Barcode Primer 3	3.50 mL×1tube	-25℃--15℃

2.2 Equipment and Consumables Required but not Provided

Table 2-10: Equipment and consumables Required but not Provided

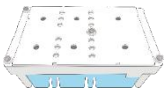
Equipment and consumables	Recommended brand	Catalog number
Qubit® 3.0 Fluorometer	Thermofisher	Q33216
Mini centrifuge	Major Laboratory Supplier (MLS)	/
Vortex mixer	MLS	/
PCR machine	Bio-Rad	/
Pipette	Eppendorf	/
2-8℃ refrigerator	MLS	/
-25--15℃ freezer	MLS	/
Qubit® ssDNA Assay Kit	Thermo Fisher	Q10212
Power Dust remover	MATIN	M-6318
Lint-free paper	MLS	/
Sterile pipette tip(box)	AXYGEN	/
200μL Wide-Bore Pipette Tips	AXYGEN	T-205-WB-C
Qubit Assay Tubes	Thermo Fisher	Q32856
100%Tween-20	MLS	/
5M NaCl solution	MLS	/
2M NaOH solution	MLS	/
75% Ethanol	MLS	/
0.2mL PCR 8-tube strip	AXYGEN	/
1.5mL Eppendorf	AXYGEN	MCT-150-C
Ice rack	MLS	/
Electronic pipette	Labnet	FASTPETTEV-2
Serological pipet	CORNING	/
10 mL Tube	SARSTEDT	60.551.001
15 mL Tube	SARSTEDT	60.732.001

3 Sequencing Workflow

Make DNB: use DNB preparation kit for making DNB

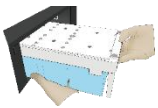
Prepare a new Flow Cell： remove the Flow Cell from package and inspect to ensure the Flow Cell is intact

DNB loading: load the DNB onto sequencing flow cell

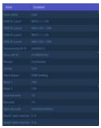


Prepare a new reagent kit： inspect and thaw the reagent cartridge and then load and mix the required reagents

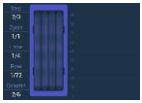
Load the Flow Cell: place the Flow Cell on the stage of the sequencer



Load the reagent kit into the sequencer



Start 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

4 Make DNB

4.1 Insert Size Recommendation

This sequencing set is compatible with the libraries prepared by MGI Library Prep Kits.

Recommended library insert size:

The size distribution of inserts should be between 20-600 bp, with the main band centered within ±100bp. If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.

Table 4-1: Recommended insert size

Sequencing Kit*	Suggested insert distribution (bp)	Data output (Gb/lane) **
DNBSEQ-G400RS FCL SE50	50-230	18.7-22.5
DNBSEQ-G400RS FCL SE100	200-400	37.5-45.0
DNBSEQ-G400RS FCL PE100	200-400	75.0-90.0
DNBSEQ-G400RS Small RNA FCL SE50	20-60	/

* Consider the insert size and the required data output when selecting sequencing kits.

**Average data output will vary with different library type and applications.

4.2 Library concentration and amount requirement

Library requirements are subject to the corresponding library preparation kit user manual. For general libraries, the ssDNA library concentration should be ≥ 2 fmol/μL and each Make DNB reaction requires 40 fmol library. For small RNA libraries and App-A libraries, the ssDNA library concentration should be ≥ 3 fmol/μL and each Make DNB reaction requires 60 fmol library.

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 following equation to convert the concentration of the ssDNA library from ng/μL to fmol/μL.

Concentration (fmol/μL) = 3030 * Concentration (ng/μL) / N

N represents the number of nucleotides (total library length including the adaptor).

If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.

4.3 Make DNB

4.3.1 Prepare reagents for DNB making

Place the library on ice until use. Remove Make DNB Buffer (for App-A libraries, use App-A Make DNB Buffer), Low TE Buffer and Stop DNB Reaction Buffer from storage and thaw reagents at room temperature. Thaw Make DNB Enzyme Mix I for approximately 0.5 hours on ice. After thawing, mix reagents using a vortex mixer for 5 seconds. Centrifuge briefly and place on ice until use.

① **Note**:

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

4.3.2 select the DNB loader

The sequencing flow cell contains 4 lanes. DNBs can be loaded onto the flow cell using the sequencer, the MGIDL-200RS, or the MGIDL-200H.

- When using the sequencer to load DNBs, all 4 lanes must be the same DNB. Each lane requires 50 μL DNBs.
- When using the MGIDL-200RS to load DNBs, 4 different DNBs can be loaded onto 4 different lane. Each lane requires 50 μL DNBs.
- When using the MGIDL-200H to load DNBs, 4 different DNBs can be loaded onto 4 different lane. Each lane requires 25 μL DNBs.

Table 4-2：The required number of make DNB reactions for each 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	2
MGIDL-200RS	50	100	2-4
MGIDL-200H	25	100	1-4

4.3.3 Calculate 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. The volume of each Make DNB reaction is 100 μL and the required library input for each Make DNB reaction is calculated as followed:

ssDNA library input (μL) = 40 fmol / library concentration (fmol/μL)

Note:

For Small RNA libraries and App-A libraries, ssDNA library input (μL) = 60 fmol / library concentration (fmol/μL)

If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.

Calculate the required ssDNA library for each Make DNB reaction and fill it in Table 4-3 as V.

① **Note**:

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

4.3.4 Make DNB

- Take a 0.2 mL 8-tube strip or PCR tubes. Prepare reaction mix following Table 4-3 below.

Table 4-3：Make DNB reaction mix 1

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

Note：For App-A libraries, use App-A Make DNB Buffer.

- V represents variable sample volume as determined in section 4.3.3. Mix gently by vortexing and centrifuge for 5 seconds using a mini centrifuge. Place the mix into a PCR machine and start the primer hybridization reaction. PCR machine settings are shown in the table below:

Table 4-4：Primer hybridization reaction condition

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

- Remove the Make DNB Enzyme Mix II (LC) from storage and place on ice. Centrifuge briefly for 5 s and hold on ice.

① **Note：**

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

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

Table 4-5：Make DNB reaction mix 2

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

- Add all the Make DNB reaction mix 2 into Make DNB reaction mix 1. Mix gently by vortexing, centrifuge for 5 s using a mini centrifuge, and place the tubes into the PCR machine for the next reaction. The conditions are shown in the table below:

Table 4-6：Rolling circle amplification conditions

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

- Immediately add 20 μL Stop DNB Reaction Buffer once the temperature reaches 4℃. Mix gently by pipetting 5-8 times using a wide bore tip. Do not vortex, shake the tube, or pipette vigorously.

ⓘ **Note：**

It is very important to mix DNB gently using a wide bore pipette tip. Do not centrifuge, vortex, or shake the tube. Store DNB at 4℃ and perform sequencing within 48 hours.

Notes:

① As some PCR machines 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 PCR machines, pre-heating of the heated lid is required to ensure the heated lid is at working temperature during the DNB reaction.

② It is recommended to set the temperature of the heated lid to 35℃ or the temperature closest to 35℃.

4.4 Quantify DNB

When the make DNB is complete, take 2 μL DNB and use Qubit® ssDNA Assay Kit and Qubit® Fluorometer to quantify the DNB. Sequencing requires a minimum DNB concentration of 8 ng/μL. If the

concentration is lower than 8 ng/μL, see "10.1 Low DNB Concentration" for details.

Notes:

① If the number of samples is large, it is recommended to quantify in batches to avoid inaccurate DNB quantification due to fluorescence quenching.

② If the concentration exceeds 40 ng/μL, the DNBs should be diluted to 20 ng/μL with DNB Load Buffer I before loading.

③ Store DNB at 4℃ and perform sequencing within 48 hours.

5 Prepare a Flow Cell

Take the flow cell out of -25℃ ~ -15℃ storage and remove the flow cell form the box. DO NOT open the outer plastic package at this moment. Place the flow cell at room temperature for at least 60 min (do not exceed 24 hours).

Unwrap the outer package before use and start DNB loading.

Note:

① If the flow cell can not 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℃ ~ -15℃ for storage. But the switch between room temperature and -25℃ ~ -15℃ must not exceed 3 times.

② If the outer plastic package has been opened but the flow cell can not 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 5-1: Unwrap the outer package

Remove the Flow Cell from the inner package and inspect to ensure the flow cell is intact.

Figure 5-2: Inspect the Flow Cell

6 DNB Loading

Remove DNB Load Buffer II from storage and thaw reagents on ice for approximately 0.5 hours. After thawing, mix reagents using a vortex mixer for 5 seconds, centrifuge briefly, and place on ice until use. If crystal precipitation is found in DNB Load Buffer II, vigorously mix the reagent with 1-2 min of continuous vortexing to re-dissolve the precipitate before use.

6.1 Sequencer DNB loading

- Take a 0.5 mL microfuge tube and add the following reagents (see table 6-1 below).

Table 6-1: DNB loading mix 1 (for sequencer loading)

Component	Volume (μL)
DNB Load Buffer II	64
Make DNB Enzyme Mix II (LC)	2
DNB	200
Total Volume	266

- Combine components and mix by gently pipetting 5-8 times using a wide bore tip. Do not centrifuge, vortex, or shake the tube. Place the mixture at 4℃ until use.

Notes:

① Prepare a fresh DNB loading mix immediately before the sequencing run.

② Each flow cell requires 266 μL DNB loading mix.

6.2 MGIDL-200RS DNB loading

- Take a new PCR 8-tube strip and add the following reagents (see table 6-2 below)

Table 6-2：DNB loading mix 2 (for MGIDL-200RS loading)

Component	Volume（μL）
DNB Load Buffer II	16
Make DNB Enzyme Mix II（LC）	0.5
DNB	50
Total volume	66.5

- Combine components and mix by gently pipetting 5-8 times using a wide bore tip. Do not centrifuge, vortex, or shake the tube. Place the mixture at 4℃ until use.
- Please refer to the "MGIDL-200RS User Manual" for details on loading operation.

Notes:

① Each lane requires at least 66.5 μL of DNB loading mix.

② Before DNB loading, perform a wash as described in the MGIDL-200RS User Manual.

③ Place the tubes containing DNB loading mix in the labeled positions of MGIDL-200RS (see Figure 6-1).

④ Press the flow cell attachment button. Holding the flow cells by edges, 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 desired loading recipe from the drop-down list and start DNB loading.

⑥ After DNB loading, remove the flow cell and place it in a container (such as a PE glove or a plastic bag) at room temperature for 30 min, then immediately place it on the sequencer for use.

Figure 6-1: Place the loading samples

6.3 MGIDL-200H DNB loading

- Take a new PCR 8-tube strip and add the following reagents (see table 6-3 below)

Table 6-3: DNB loading mix 3 (for MGIDL-200H loading)

Component	Volume (μL)
DNB Load Buffer II	8
Make DNB Enzyme Mix II (LC)	0.25
DNB	25
Total volume	33.25

- Combine components and mix by gently pipetting 5-8 times using a wide bore tip. Do not centrifuge, vortex, or shake the tube. Place the mixture at 4℃ until use.
- Please refer to the "MGIDL-200H Portable DNB Loader Quick Start Guide" for details on loading operation.

Notes:

① Each lane requires 30 μL of DNB loading mix.

② Before DNB loading, clean the device as described in the MGIDL-200H Quick Start Guide.

③ Install the sealing gasket and flow cell. Aspirate 30 μL DNB loading mix with a pipette and insert the wide bore tip into the fluidics inlet (see Figure 6-2). Do not press the control button of the pipette after inserting the tip into the fluidics inlet. Eject the tip from the pipette and the DNB loading mix will automatically flow into the flow cell.

④ After DNB loading, rotate the tips counterclockwise to remove them. Place the device on the bench with the front upward for 30 min before use.

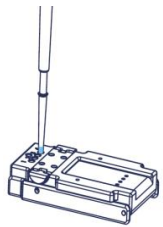


Figure 6-2：Load samples using MGIDL-200H

① **Note：**

Do not move the flow cell during loading procedure. After being placed at room temperature, the flow cell should be used immediately.

7 Prepare the sequencing cartridge

Remove the Sequencing Reagent Cartridge from storage. Thaw in a room temperature water bath until completely thawed (or thaw in 2-8℃ fridge one day in advance). Store at 2-8℃ storage until use. The flow cell can be removed from -25--15℃ and place at room temperature at this point. Invert the cartridge 3 times to mix before use. Vigorously shake the cartridge in all directions 10-20 times until no visible layers can be seen in the cartridge, especially for reagents in well No.9 and No.10.

Note:

① 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" in this manual for details.

② After being removed form -25--15℃, the flow cell must be placed at room temperature for at least 60 min (and not more than 24 hours) before DNB loading.

Open the cartridge cover and wipe any water condensation with lint-free paper (see Figure 7-1). Well positions are shown in Figure 7-2.

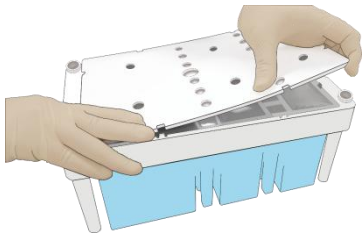


Figure 7-1: Open and clean the kit

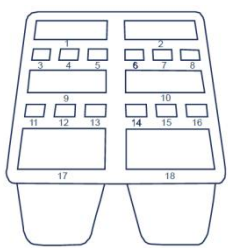


Figure 7-2: Well position

Remove Dye Mix and dNTPs Mix II from -20℃ storage 1h in advance and thaw at room temperature. Store at 4℃ until use. Mix dNTPs Mix II using a vortex mixer for 5 seconds and centrifuge briefly before use. Invert the Dye Mix 4-6 times before use.

① **Note:**

Do not mix the Dye Mix using a vortex mixer.

Remove Sequencing Enzyme Mix II from -20℃ storage and place on ice until use. Invert Sequencing Enzyme Mix II 4-6 times before use.

7.1 Prepare the SE50 and SE100 sequencing cartridge

- Pierce the seal at the edge of well No.1 and No.2 (see Figure 7-2) to make a hole around 2 cm in diameter using 1 mL sterile tip (see Figure 7-3):

Figure 7-3: Pierce the seal of SE50 or SE100 cartridge

- Take a pipette with the appropriate volume range and add Dye Mix into well No.1 following the table below:

Table 7-1: SE50 and SE100 sequencing cartridge well No.1 reagent loading

Sequencing kit	Dye Mix loading volume
SE50	0.800 mL
SE100	1.200 mL

- Take a pipette with the appropriate volume range and add reagents following the table below. First, add dNTPs Mix II into a new 10 mL sterile tube, then add Sequencing Enzyme Mix II into the dNTPs Mix II in the same tube. Invert the tube 4-6 times to mix the reagents in the tube before adding all of them into well No.2:

Table 7-2: SE50 and SE100 sequencing cartridge well No.2 reagent loading

Sequencing kit	dNTPs Mix II loading volume	Sequencing Enzyme Mix II loading volume
SE50	2.000 mL	2.700 mL
SE100	3.300 mL	4.400 mL

- Seal the loading wells of well No.1 and No.2 with the transparent sealing film. Do not cover the

center of the well to avoid blocking the sampling needle:

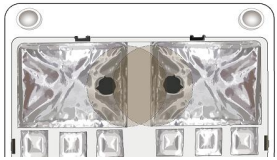


Figure 7-4：Seal the loading wells of SE50 or SE100 cartridge

- Place the cartridge horizontally on the table and hold both sides of the cartridge with both hands (see Figure7-5). Shake it clockwise 10-20 times, and then counterclockwise 10-20 times, until the reagent color in well No.1 is uniform. Make sure that you see the vortex to ensure reagents are fully mixed. The SE50 or SE100 sequencing cartridge is now ready for use.

Figure 7-5：Mix reagents after loading

7.2 Prepare the PE100 sequencing cartridge

- Pierce the seal at the edge of well No.1 and No.2 (see Figure 7-2) to make a hole around 2 cm in diameter using 1 mL sterile tip (see Figure 7-6):

Figure 7-6: Pierce the seal of PE100 cartridge

- Take a pipette with the appropriate volume range and add Dye Mix into well No.1 following the table below:

Table 7-3：PE100 sequencing cartridge well No.1 reagent loading

Sequencing kit	Dye Mix loading volume
PE100	2.700 mL

- 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 II into the dNTPs Mix II in the same tube. Invert the tube 4-6 times to mix the reagents in the tube before adding all of them into well No.2:

Table 7-4：PE100 and PE150 sequencing cartridge well No.2 reagent loading

Sequencing kit	dNTPs Mix II loading volume	Sequencing Enzyme Mix II loading volume
PE100	5.900 mL	7.900 mL

- Seal the loading wells of well No.1 and No.2 with the transparent sealing film. Do not cover the center of the well to avoid blocking the sampling needle:

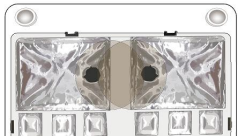


Figure 7-7: Seal the loading wells of PE100 cartridge

- Place the cartridge horizontally on the table and hold both sides of the cartridge with both hands (see Figure7-5). Shake it clockwise 10-20 times, and then counterclockwise 10-20 times, until the reagent color in well No.1 is uniform. Make sure that you see the vortex to ensure reagents are fully mixed.

- Pierce the seal of well No.15 using 1 mL sterile tip. Add 500 μL of MDA Enzyme Mix II to the MDA Reagent tube with a 1 mL pipette. Invert the tube for 4-6 times to mix the reagents, then add the mixture to well No.15. When adding the mixture, make sure there are no bubbles at the bottom of the tube. The PE100 sequencing cartridge is now ready for use.

ⓘ **Note:**

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

7.3 Prepare the SE50 (Small RNA) sequencing cartridge

- Remove the Wash Buffer For Small RNA Sequencing from storage and thaw at room temperature.

- Pierce the seal at the edge of well No.1 and No.2 (see Figure 7-2) to make a hole around 2 cm in diameter using 1 mL sterile tip (see Figure 7-8):

Figure 7-8: Pierce the seal of SE50 cartridge

- Take a pipette with the appropriate volume range and add Dye Mix into well No.1 following the table below:

Table 7-5: SE50 sequencing cartridge well No.1 reagent loading

Sequencing kit	Dye Mix loading volume
SE50	0.800 mL

- Take a pipette with the appropriate volume range and add reagents following the table below. First, add dNTPs Mix II into a new 10 mL sterile tube, then add Sequencing Enzyme Mix II into the dNTPs Mix II in the same tube. Invert the tube 4-6 times to mix the reagents in the tube before adding all of them into well No.2:

Table 7-6: SE50 sequencing cartridge well No.2 reagent loading

Sequencing kit	dNTPs Mix II loading volume	Sequencing Enzyme Mix II loading volume
SE50	2.000 mL	2.700 mL

- Seal the loading wells of well No.1 and No.2 with the transparent sealing film. Do not cover the center of the well to avoid blocking the sampling needle:

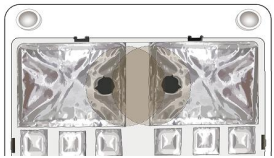


Figure 7-9: Seal the loading wells of SE50 cartridge

- Place the cartridge horizontally on the table and hold both sides of the cartridge with both hands (see Figure7-5). Shake it clockwise 10-20 times, and then counterclockwise 10-20 times, until the reagent color in well No.1 is uniform. Make sure that you see the vortex to ensure reagents are fully mixed.

- Mix the Wash Buffer For Small RNA Sequencing using a vortex mixer for 5 seconds and centrifuge briefly before use. Pierce the seal of well No.7 then add 4.50 mL of the Wash Buffer For Small RNA Sequencing. When adding the reagent, make sure there are no bubbles at the bottom of the tube. The SE50 (Small RNA) sequencing cartridge is now ready for use.

⚠ **Note:**

Wash Buffer For Small RNA Sequencing contains highly concentrated formamide which may have potential reproductive toxicity. Avoid breathing steam and wear protective gloves/protective clothing/protective eye mask/protective mask when using. The waste reagent must be discarded according to local and national regulations.

7.4 Dual barcode sequencing

For dual barcode sequencing, perform the following steps after preparing the PE sequencing cartridge:

- Remove the CPAS Barcode Primer 3 (for Pair End Sequencing only) from the CPAS Barcode Primer 3 Reagent Kit (Catalog number: 1000020834) and thaw at room temperature. Mix the CPAS Barcode Primer 3 using a vortex mixer for 5 seconds and centrifuge briefly before use.

- Pierce the seal of well No.4 using a sterile tip, then add 2.90 mL of the CPAS Barcode Primer 3. When adding the reagent, make sure there are no bubbles at the bottom of the tube.

7.5 App-A library sequencing

7.5.1 Sequencing cartridge preparation step 1

- Prepare the sequencing cartridge following "7 Prepare the sequencing cartridge" in this manual.

7.5.2 Sequencing cartridge preparation step 2

7.5.2.1 Pair End Sequencing

- Remove 1μM App-A Insert Primer 1, 1μM App-A Insert Primer 2, 1μM App-A MDA Primer and 1μM App-A Barcode Primer 2 from storage and thaw at room temperature for 1 hour. After thawing, mix reagents using a vortex mixer for 5 seconds, centrifuge briefly and place back on ice.

- Pierce the seal of well No.3, No.6, No.7 and No.8 with a sterile tip, remove and discard the reagents inside using 5 mL electronic serological pipette. Add the following reagents to corresponding wells, make sure that there are no bubbles at the bottom:

Table 7-7: App-A pair end primer loading

Well No.	Reagent Name	Loading volume mL
3	1μM App-A Insert Primer 1,	2.20
6	1μM App-A Barcode Primer 2	2.90
7	1μM App-A MDA Primer	3.10
8	1μM App-A Insert Primer 2	3.30

Note:

Perform the following steps if dual barcode sequencing of pair end sequencing is required:

① Remove App-A Barcode Primer 3 from the High-Throughput Barcode Primer 3 Reagent Kit (App-A) (Catalog number: 1000014047) and thaw at room temperature for 1 hour. After thawing, mix reagents using a vortex mixer for 5 seconds, centrifuge briefly and place back on ice.

② Pierce the seal of well No.4 with a sterile tip, add 2.90 mL 1μM App-A Barcode Primer 3 to well No.4. Make sure that there are no bubbles at the bottom.

7.5.2.2 Single End Sequencing

- Remove 1μM App-A Insert Primer 1 and 1μM App-A Barcode Primer 1 from storage and thaw at room temperature for 1 hour. After thawing, mix reagents using a vortex mixer for 5 seconds, centrifuge briefly and place back on ice.
- Pierce the seal of well No.3 and No.5 with a sterile tip, remove and discard the reagents inside using 5 mL electronic serological pipette. Add the following reagents to corresponding wells:

Table 7-8: App-A single end primer loading

Well No.	Reagent Name	Loading volume mL
3	1μM App-A Insert Primer 1	2.20
5	1μM App-A Barcode Primer 1	2.90

8 Sequencing

8.1 Enter the main interface

- Enter the user name "user" and password "123", click "Log in" to enter the main interface.

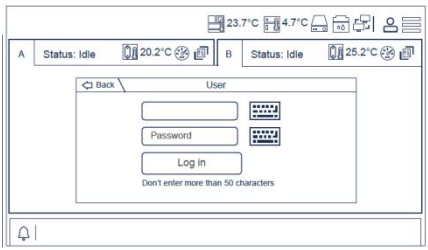


Figure 8-1: Log-in interface

- See the interface below, click the "Sequence":

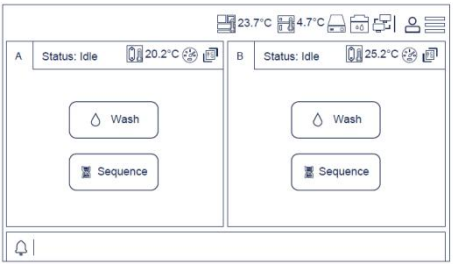


Figure 8-2: Main interface

- Click on "CoolMPS" if applicable and enter the following interface.

8.2 Load the DNBs

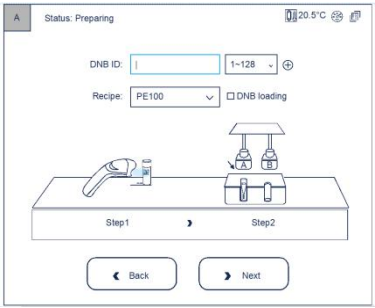


Figure 8-3: DNBs loading interface

- Click on the ⊕ on the right of the "DNB ID" and the four lane information will appear.

Figure 8-4: DNBs and information selection interface

- Move the cursor to the blank area next to the "DNB ID" and enter the library name or number.
- Pull the drop-down menu on the left of ⊕ and select the barcode sequence of different lanes.

- When using the sequencer to load DNB, open the reagent compartment door, gently lift the sampling needle with one hand, 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.
  Note: perform this step if using the sequencer to load the DNB. If not, skip this step.

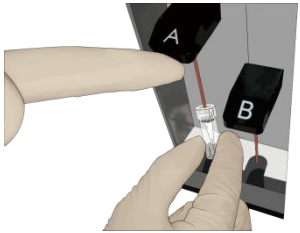


Figure 8-5：Load the DNBs tube

- Close the reagent compartment door.

8.3 Select sequencing parameters

- Select the sequencing recipe in the "Recipe" drop-down menu. There are one-click sequencing run (PE150, SE50, etc.) and user-customized run (Customize).

Note:

For Small RNA sequencing, choose recipe "Customize".

For dual barcode sequencing, choose recipe "Customize".

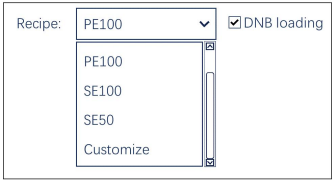


Figure 8-6：Select sequencing solutions

- If you choose one-click sequencing and DNBs will be loaded on the sequencer, check the "DNB loading" box (see Figure 8-6). Otherwise leave it blank and then go to step 8.4. If you choose "Customize", continue performing the following steps.

- In the beginning, please select a step to start the sequencing run.

Figure 8-7: Select the step to start sequencing

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

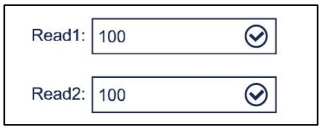


Figure 8-8: Choose the read length

Note:

For Small RNA sequencing, enter 50 for read 1 and leave the read 2 blank. Click the "V"before "Start phase" in Figure 8-7 to enter the second page of Customize. Check "Small RNA" (see Figure 8-9).

Figure 8-9: Select Small RNA

- Select the barcode length. For dual barcode sequencing, fill in the length of the Dual barcode. Leave the Dual barcode blank if it is a single barcode sequencing run.

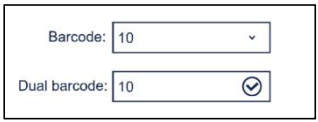


Figure 8-10：Select the barcode length

- Select the lanes for barcode demultiplexing.

Figure 8-11：Barcode demultiplexing on different lanes

- Select the dark reaction for any position of read length in read 1 or 2. If dark reactions are not required, leave the table below blank.
  Dark reaction: only chemical reaction without optical information capture.

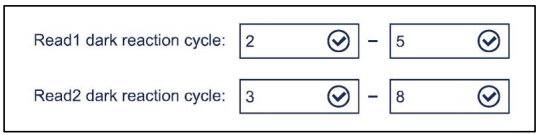


Figure 8-12：Select the dark reaction

- Click "Confirm"

8.4 Load the reagent cartridge

- Move the cursor to the "Reagent cartridge ID" blank, enter the cartridge information manually or use the barcode scanner to scan the cartridge barcode.

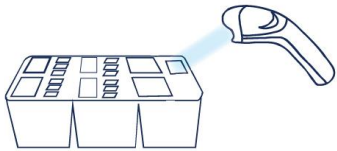


Figure 8-13：Reagent cartridge information entry interface

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

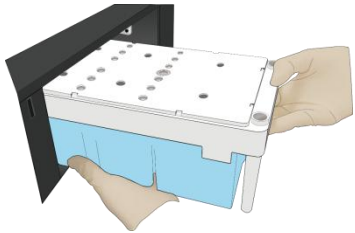


Figure 8-14：Remove cleaning cartridge

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

Figure 8-15：Maintain the reagent compartment

- Hold the handle of the reagent kit with one hand and place the other hand underneath for support. Slide the new kit into the compartment following the direction printed on the cover until it stops. Check that the reagent kit is in the correct position and close the reagent compartment door.

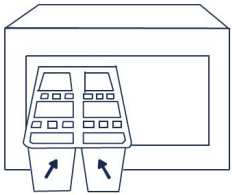


Figure 8-16：Slide the new reagent cartridge into the reagent compartment

8.5 Load the Flow Cell

- Open the flow cell compartment door, press both sides of the flow cell used for washing, and press the flow cell attachment button with the other hand. After the vacuum is released, remove the flow cell for washing from the stage.

- Use dust remover to remove the dust on the flow cell stage and the back of the flow cell. If there are impurities on the stage surface, please gently wipe it with wet lint-free paper to ensure that the flow cell can be held properly.

- Use the dust remover to remove the dust on the O-rings. Press each O-ring with lint-free paper moistened with 75% ethanol to remove the residual reagent and crystallization on the O-rings. Then wipe each O-ring in a counterclockwise direction and use the dust remover to blow-dry the

O-rings. Wait for 1 min before placing the flow cell.

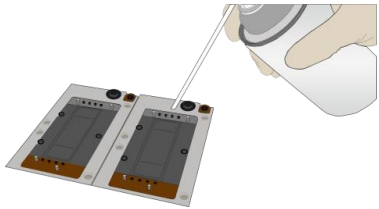


Figure 8-17：Clean the Flow Cell stage

- Take out a new flow cell or the loaded flow cell. 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.

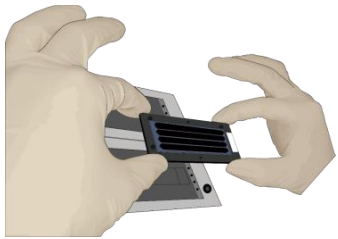


Figure 8-18：Load the Flow Cell

- Align the holes on the flow cell with the locating pins on the flow cell stage. Gently slide the flow cell at a 45° angle to the upper left corner (45° to the upper right corner when loading the flow cell on the MGIDL-200RS) to keep the flow cell aligned with the pin. 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.

- Ensure that the negative pressure is within the range of -80 ~ -99 kPa before continuing. If the negative pressure is abnormal, refer to "10.2 Abnormal negative pressure" in this manual for troubleshooting.

- Use a dust remover to remove the dust on the Flow Cell surface and close the Flow Cell compartment door.

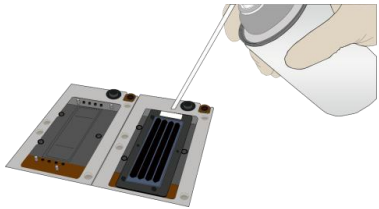


Figure 8-19：Clean the Flow Cell

- Click "Next", the device will automatically enter the Flow Cell ID; if automated entry does not work, move the cursor to the "Flow cell ID" blank and enter the ID manually.

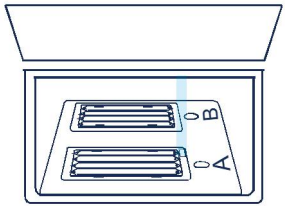


Figure 8-20：Flow Cell information entry interface

- Click "Next"

8.6 Review parameters

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

Item	Content
User name	user
DNB ID Lane1	WGS | 1~128
DNB ID Lane2	RNA | 501~596
DNB ID Lane3	WGS | 1~128
DNB ID Lane4	RNA | 501~596
Sequencing cartridge ID	AA000012
Flow cell ID	V300001234
Recipe	Customize
Start phase	DNB loading
Cycles	222
Read 1	100
Read 2	100
Dual Barcode	10
Barcode	10
Split barcode	Yes | Yes | Yes | Yes
Read1 dark reaction	2 - 5
Read2 dark reaction	3 - 8

Figure 8-21：Review information

Note:

To ensure sequencing quality, when read 1 and read 2 sequencing is completed, the sequencer will automatically perform one more cycle for correction. For example, for PE100 dual barcode sequencing, read 1 read length is 100, read 2 read length 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 Start sequencing

- After confirming that the information is correct, click "Start".

- The system will display the dialog box "Proceed with Sequencing?". Click "Yes" to start sequencing.

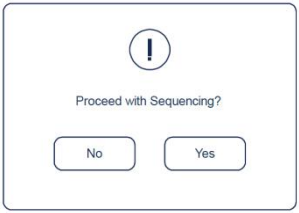


Figure 8-22: Confirm sequencing interface

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

9 Device Maintenance

9.1 Terminology and Definition

Table 9-1: Wash Solution

Wash type	Description	Time
Full wash	Step 1: Maintenance wash → Step 2: Regular wash.	~ 125 min
Maintenance wash	To remove residual reagents and proteins in the pipeline, reducing risk of blockage. Procedure: Cleaning cartridge 4 → Cleaning cartridge 3 → Cleaning cartridge 2	~ 75 min
Regular wash	To remove residual reagents, reducing risk of cross-contamination. Procedure: Cleaning cartridge 1 → Air Prime	~ 50 min

9.2 Wash instruction

- When the following interface appears, you can perform a wash.

Figure 9-1: Wash interface

- When the sequencing is completed, the device needs to be washed within 24 hours.
- A Full Wash is required if the sequencer was used for either A) a PE run or B) a DNB loading/post-load. A regular wash is sufficient for an SE run.
- After a full wash is completed, if the device has been idle for more than 12 hours, perform a regular wash again before use.
- After an engineer performs system maintenance, perform a regular wash.
- After replacing the tubing, sampling needles, or other accessories exposed to the reagents, perform a full wash.
- If the sequencer is to be powered off for more than 7 days, perform a maintenance wash before powering off and after powering on.
- If the sequencer has been idle for seven days or longer, perform a full wash prior to sequencing.
- If impurities are found on the Flow Cell, perform a full wash.

9.3 Prepare wash reagents

- Prepare 1M NaCl + 0.05% Tween-20 following the table below (valid for 28 days if stored at 4°C).

Table 9-2: Wash reagent preparation (1)

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

- Prepare 0.1M NaOH following the table below (valid for 28 days if stored at 4°C).

Table 9-3: Wash reagent preparation (2)

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

9.4 Wash the cleaning 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 20 uses 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.

- Wash cleaning cartridge 1: Take a clean cleaning cartridge and a 0.5 mL cryotube (for DNB loading tube washing), add laboratory-grade water to the cryotube and cleaning cartridge (all wells) to a final 90% volume and mark it as cleaning reagent cartridge 1.

- Wash cleaning cartridge 2: Take a clean cleaning cartridge and a 0.5 mL cryotube, add laboratory-grade water to the cryotube and cleaning cartridge (all wells) to a final 90% volume and mark it as cleaning reagent cartridge 2.

- Wash cleaning cartridge 3: Take a clean cleaning cartridge and a 0.5 mL cryotube, add 50 mL 0.1M NaOH into large wells, 6 mL 0.1M NaOH into small wells and 400 µL 0.1M NaOH to 0.5mL cryotube. Mark it as cleaning reagent cartridge 3.

- Wash cleaning cartridge 4: Take a clean cleaning cartridge and a 0.5 mL cryotube, add 1M NaCl + 0.05% Tween-20 solution into large wells, 6 mL 1M NaCl + 0.05% Tween-20 solution into small wells, 400 µL 1M NaCl + 0.05% Tween-20 solution to 0.5mL cryotube. Mark it as cleaning reagent cartridge 4.

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 Wash procedures

9.5.1 Regular wash

- Use cleaning cartridge 1. Open the reagent compartment door. Hold the handle of the cleaning cartridge 1 with one hand and place the other hand underneath the cartridge 1 for support. Slide it into the reagent compartment slowly following the direction printed on the cartridge cover until it stops. Close the reagent compartment door.

- Click the wash button on the interface.

- Place the Flow Cell for washing.

- Select regular wash from the drop-down menu to start the regular wash which takes about 50 minutes.

- 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.

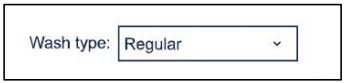


Figure 9-2: Select the wash type

- When the interface appears as the figure below, the regular wash ends.

Figure 9-3: Regular wash end interface

9.5.2 Maintenance wash

- Use cleaning cartridge 4. Open the reagent compartment door. Hold the handle of the cleaning cartridge 4 with one hand and place the other hand underneath for support. Slide it to the reagent compartment slowly following the direction printed on the cartridge cover until it stops. Close the reagent compartment door.

- Click the wash button on the interface.

- Place the flow cell for washing.

- Select the maintenance wash from the drop-down menu to start the maintenance wash which takes about 25 minutes.

- 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.

- When the interface appears as Figure 9-4, click "Yes" and the sequencer will automatically lift the sampling needles. Open the compartment door and replace the cleaning cartridge.

- Use cleaning cartridge 3 and continue the maintenance wash which takes around 25 minutes.

Figure 9-4：Maintenance wash [1] end interface

- When the interface appears as figure 9-5, click "Yes" and the sequencer will automatically lift the sampling needles. Then open the compartment door and replace the cleaning cartridge.

Figure 9-5：Maintenance wash [2] end interface

- Use cleaning cartridge 2 and continue the maintenance wash which takes around 25 minutes.

- When the interface appears as Figure 9-6, click "No" to end the maintenance wash.

Figure 9-6: Maintenance wash end interface

9.5.3 Full wash procedures

Step 1 – Maintenance wash, Step 2 – Regular wash. Total time is around 125 min.

10 Troubleshooting

10.1 Low DNB concentration

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

- 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) to make new DNBs. If DNB concentration still does not meet the requirements after a new sample preparation, please contact a field service engineer.

10.2 Abnormal negative pressure

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

- 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 a field service engineer.

10.3 Bubbles

- Replace the used flow cell and inspect the flow cell attachment pump (see "10.5 Pump fails").
- If the problem persists, please contact a field service engineer.

10.4 Impurities

- Perform a full wash on MGIDL-200RS and the sequencer following the MGIDL-200RS User Manual and "9.5 Wash procedures" in this manual.
- If the problem persists after a full wash, please contact a field service engineer.

10.5 Pump fails

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

- MGIDL-200RS and the sequencer: remove the flow cell, check if there are impurities in sealing gasket and remove the dust with the dust remover. Place the flow cell following the instruction in "8.5 Load the Flow Cell" 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 a field service engineer.

10.6 Reagent kit storage

- If the kit has been thawed (including Dye Mix and dNTPs Mix II) and cannot be used within 24 hours, it can be frozen and thawed at most once.
- If the kit has been thawed (including Dye Mix and dNTPs Mix II) but cannot be used immediately, store it at 4℃ and use it within 24 hours. Mix the reagents in the cartridge following instruction in "7. Prepare the sequencing cartridge" before use.
- If Dye Mix, dNTPs Mix II and enzyme have been added into the cartridge, i.e. the cartridge has been prepared but cannot be used immediately, store it at 4°C and use it within 24 hours. Mix the reagents in the cartridge following instruction in "7. Prepare the sequencing cartridge" before

use.

- If Dye Mix, dNTPs Mix II 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, the cartridge must be sealed with foil or plastic wrap. Store the cartridge at 4°C and use it within 24 hours. 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, please re-start from post loading.
- Start from "8 Sequencing" and re-load the flow cell.
- When selecting "8.3 Select sequencing parameters", choose the "Customize" program.
- Select "Post loading" and click "…".

Figure 10-1: Select re-start Post loading

- If starting from the Post loading prime, select "Prime" in Figure 10-1. If starting from the step Post loading, do not select "Prime".
- Other steps please follow "8 Sequencing" in this manual.

10.8 Dark green crystals in well No.10

- If dark green crystals appear in well No.10 (see Figure 10-2), 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.

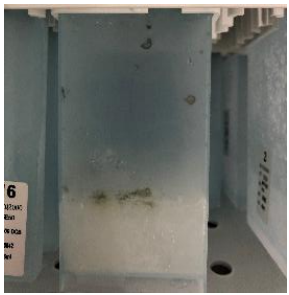


Figure 10-2: Dark green crystals in well No.10

10.9 Library amount less than 40 fmol

If the library amount is less than 40 fmol (but not less than 24 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.

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

  ssDNA library input (μL) = 24 fmol / library concentration (fmol/μL)

  Calculate the required ssDNA library for each Make DNB reaction and fill it in Table 10-1 as V.

- Make DNB

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

Table 10-1: Make DNB reaction mix 1

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

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

Table 10-2: Primer hybridization reaction condition

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

Remove the Make DNB Enzyme Mix II (LC) from storage and place on ice. Centrifuge briefly for 5 s and hold on ice.

ⓘ **Note:**

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

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

Table 10-3：Make DNB reaction mix 2

Component	Volume（μL）
Make DNB Enzyme Mix I	24
Make DNB Enzyme Mix II（LC）	2.4

Add all the Make DNB reaction mix 2 into the Make DNB reaction 1. Mix gently by vortexing, centrifuge for 5 s using a mini centrifuge and place the tubes into the PCR machine for the next reaction. The conditions are shown in the table below:

Table 10-4：Rolling circle amplification conditions

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

Immediately add 12 μL Stop DNB Reaction Buffer once the temperature reaches 4 ℃. Mix gently by pipetting 5-8 times using a wide bore tip. Do not vortex, shake the tube, or pipette vigorously.

ⓘ **Note：**

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

➢ Store the DNB at 4℃ and perform sequencing within 48 hours. Proceed to "4.4 Quantify DNB".

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