

DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set User Manual



Sequencing set catalog number, name and version:

- 1000016941, DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE50), V3.1
- 1000016943, DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE100), V3.1
- 1000016950, DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE100), V3.1
- 1000016952, DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150), V3.1
- 1000016946, DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE400), V3.1
- 1000016955, DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE200), V3.1
- 1000016998, DNBSEQ-G400RS High-throughput Sequencing Set (Small RNA FCL SE50), V3.1
- 1000016978, DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS SE100), V1.0
- 1000016980, DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE100), V1.0
- 1000016982, DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE150), V1.0

Primer kit catalog number, name and version:

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

User manual version: A2

Revision History

Version	Date	Summary of change
A2	May 2020	<ul style="list-style-type: none">• Added the product information of High-throughput Rapid Sequencing Set (FCS).• Changed the Storage Temperature of flow cell to -25°C --15°C and updated the information of flow cell preparation before DNB loading.• Updated the sequencing time.• Updated the catalog number, kit version and Spec & Quantity of CPAS Barcode Primer 3 Reagent Kit.
A1	December 2019	<ul style="list-style-type: none">• Updated the equations used to calculate library input.• Updated the information about adding and mixing dNTPs and enzyme in the chapter of "Prepare the sequencing cartridge".• Added the solution for crystal precipitation in DNB Load Buffer II.• Added information for "Dark green crystals in well No.10" and "Library amount less than 40 fmol" in the chapter of "Troubleshooting".• Moved the chapter "Sequencing Sets and Consumables Required but not Provided" to the front of the chapter "Sequencing Workflow".• Added the section of "Attention".• Added the "Revision History".
A0	August 2019	<ul style="list-style-type: none">• The first version.

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1 Introduction

This manual describes how to perform sequencing using the DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set and includes instructions regarding sample preparation, Flow Cell preparation, sequencing kit storage, the 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 high accuracy 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 will determine 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 index 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
SE400	400	--	10	400+10	420
PE100	100	100	10	200+10	220
PE150	150	150	10	300+10	320
PE200	200	200	10	400+10	420

1.5 Sequencing Time

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

Time (hours)	SE50	SE100	SE400	PE100	PE150	PE200
Single flow cell	12.0	22.0	104.0	42.5	61.5	100.0
Dual flow cell	13.0	23.0	104.0	43.5	62.5	102.0
Data analysis (single flow cell)	0.5	0.8	2.5	1.3	1.8	2.5
Data analysis (dual flow cell)	1.0	1.5	5.0	2.5	3.5	5.0

Table 1-3: FCS Sequencing time for each read length (hours)

Time (hours)	SE100	PE100	PE150
Single flow cell	12.5	24.9	35.4
Dual flow cell	12.7	25.0	35.6
Data analysis (single flow cell)	0.4	0.5	0.6
Data analysis (dual flow cell)	0.7	1.0	1.2

Notes:

- ① The sequencing time (Single flow cell/Dual flow cell) 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: DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE50)

Catalog number: 1000016941

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985	Sequencing Flow Cell	1 EA	-25°C -- -15°C
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE50) Catalog number: 1000016940	Low TE Buffer	300 µL×1 tube	-25°C -- -15°C
	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	
	dNTPs Mix	0.80 mL×1tube	
	dNTPs Mix II	0.70 mL×1tube	
	Sequencing Enzyme Mix	1.60 mL×1tube	
	Sequencing Reagent Cartridge	1 EA	
Transparent sealing film	2 sheets		

Table 2-2: DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE100)

Catalog number: 1000016943

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985	Sequencing Flow Cell	1 EA	-25°C -- -15°C
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE100) Catalog number: 1000016942	Low TE Buffer	300 μ L \times 1tube	-25°C -- -15°C
	Make DNB Buffer	100 μ L \times 1tube	
	Make DNB Enzyme Mix I	200 μ L \times 1tube	
	Make DNB Enzyme Mix II (LC)	25 μ L \times 1tube	
	Stop DNB Reaction Buffer	100 μ L \times 1tube	
	DNB Load Buffer I	200 μ L \times 1tube	
	DNB Load Buffer II	200 μ L \times 1tube	
	Micro Tube 0.5mL (Empty)	1 tube	
	dNTPs Mix	1.20 mL \times 1tube	
	dNTPs Mix II	1.00 mL \times 1tube	
	Sequencing Enzyme Mix	2.30 mL \times 1tube	
	Sequencing Reagent Cartridge	1 EA	
Transparent sealing film	2 sheets		

Table 2-3: DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE400)

Catalog number: 1000016946

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985	Sequencing Flow Cell	1 EA	-25°C --15°C
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE400) Catalog number: 1000016944	Low TE Buffer	300 µL×1 tube	-25°C --15°C
	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	
	dNTPs Mix	4.10 mL×1tube	
	dNTPs Mix II	12.20 mL×1tube	
	Sequencing Enzyme Mix	8.30 mL×1tube	
	Wash Buffer For Sequencing	2.90 mL×1tube	
	Sequencing Reagent Cartridge	1 EA	
Transparent sealing film	6 sheets		
DNBSEQ-G400RS High-throughput Sequencing Refill Kit (FCL SE400) Catalog number: 1000016945	Sequencing Reagent V4.0	200.0 mL×1bottle	-25°C --15°C
	Regeneration Reagent	200.0 mL×1bottle	
	Image Reagent V2.0	200.0 mL×1bottle	
	Wash Reagent 1 V2.0	200.0 mL×1bottle	
	Wash Reagent 2	200.0 mL×1bottle	

Table 2-4: DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE100)

Catalog number: 1000016950

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985	Sequencing Flow Cell	1 EA	-25°C -- -15°C
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL PE100) Catalog number: 1000016949	Low TE Buffer	300 μ L \times 1tube	-25°C -- -15°C
	Make DNB Buffer	100 μ L \times 1tube	
	Make DNB Enzyme Mix I	200 μ L \times 1tube	
	Make DNB Enzyme Mix II (LC)	25 μ L \times 1tube	
	Stop DNB Reaction Buffer	100 μ L \times 1tube	
	DNB Load Buffer I	200 μ L \times 1tube	
	DNB Load Buffer II	200 μ L \times 1tube	
	Micro Tube 0.5mL (Empty)	1 tube	
	dNTPs Mix	1.90 mL \times 1tube	
	dNTPs Mix II	1.60 mL \times 1tube	
	Sequencing Enzyme Mix	3.60 mL \times 1tube	
	MDA Reagent	3.50 mL \times 1tube	
	MDA Enzyme Mix	0.60 mL \times 1tube	
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 2-5: DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150)

Catalog number: 1000016952

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985	Sequencing Flow Cell	1 EA	-25°C --15°C
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL PE150) Catalog number: 1000016951	Low TE Buffer	300 µL×1tube	-25°C --15°C
	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	
	dNTPs Mix	1.30 mL×2tube	
	dNTPs Mix II	1.15 mL×2tube	
	Sequencing Enzyme Mix	4.80 mL×1tube	
	MDA Reagent	3.50 mL×1tube	
	MDA Enzyme Mix	0.60mL×1tube	
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 2-6: DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE200)

Catalog number: 1000016955

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985	Sequencing Flow Cell	1 EA	-25°C --15°C
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL PE200) Catalog number: 1000016953	Low TE Buffer	300 µL×1tube	-25°C --15°C
	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	
	dNTPs Mix	3.20 mL×2tube	
	dNTPs Mix II	8.30 mL×2tube	
	Sequencing Enzyme Mix	6.10 mL×1tube	
	MDA Reagent	3.50 mL×1tube	
	MDA Enzyme Mix	0.60mL×1tube	
Sequencing Reagent Cartridge	1 EA		
transparent sealing film	5 sheets		
DNBSEQ-G400RS High-throughput Sequencing Refill Kit (FCL PE200) Catalog number: 1000016954	Regeneration Reagent	200.0 mL×1bottle	-25°C --15°C
	Wash Reagent 1 V2.0	200.0 mL×1bottle	
	Wash Reagent 2	200.0 mL×1bottle	

Table 2-7: DNBSEQ-G400RS High-throughput Sequencing Set (Small RNA FCL SE50)

Catalog number: 1000016998

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985	Sequencing Flow Cell	1 EA	-25°C --15°C
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE50) Catalog number: 1000016940	Low TE Buffer	300 µL×1 tube	-25°C --15°C
	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	
	dNTPs Mix	0.80 mL×1tube	
	dNTPs Mix II	0.7 mL×1 tube	
	Sequencing Enzyme Mix	1.60 mL×1 tube	
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		
MGEasy Wash Buffer For Small RNA Sequencing Catalog number: 1000006387	Wash Buffer For Small RNA Sequencing	1.60 mL×3 tube	2°C -8°C

Table 2-8: DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS SE100)

Catalog number: 1000016978

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987	Sequencing Flow Cell	1 EA	-25°C -- -15°C
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS SE100) Catalog number: 1000016977	Low TE Buffer	300 µL×1tube	-25°C -- -15°C
	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	
	dNTPs Mix	0.90 mL×1tube	
	dNTPs Mix II	1.70 mL×1tubes	
	Sequencing Enzyme Mix	1.90 mL×1tube	
	Sequencing Reagent Cartridge	1 EA	
Transparent sealing film	2 sheets		

Table 2-9: DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE100)

Catalog number: 1000016980

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987	Sequencing Flow Cell	1 EA	-25°C -- -15°C
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE100) Catalog number: 1000016979	Low TE Buffer	300 µL×1tube	-25°C -- -15°C
	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	
	dNTPs Mix	1.50 mL×1tube	
	dNTPs Mix II	1.50 mL×2tubes	
	Sequencing Enzyme Mix	3.10 mL×1tube	
	MDA Reagent	3.50 mL×1tube	
	MDA Enzyme Mix	0.60 mL×1tube	
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 2-10: DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE150)

Catalog number: 1000016982

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987	Sequencing Flow Cell	1 EA	-25°C --15°C
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE150) Catalog number: 1000016981	Low TE Buffer	300 μ L \times 1tube	-25°C --15°C
	Make DNB Buffer	100 μ L \times 1tube	
	Make DNB Enzyme Mix I	200 μ L \times 1tube	
	Make DNB Enzyme Mix II (LC)	25 μ L \times 1tube	
	Stop DNB Reaction Buffer	100 μ L \times 1tube	
	DNB Load Buffer I	200 μ L \times 1tube	
	DNB Load Buffer II	200 μ L \times 1tube	
	Micro Tube 0.5mL (Empty)	1 tube	
	dNTPs Mix	2.00 mL \times 1tube	
	dNTPs Mix II	2.00 mL \times 2tubes	
	Sequencing Enzyme Mix	4.80 mL \times 1tube	
	MDA Reagent	3.50 mL \times 1tube	
	MDA Enzyme Mix	0.60 mL \times 1tube	
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 2-1: CPAS Barcode Primer 3 Reagent Kit Catalog number: 1000020834

Product	Sequencing Kit	Component	Spec & Quantity	Storage Temperature
Primer for double barcode sequencing (Pair End Sequencing use only)	CPAS Barcode Primer 3 Reagent Kit Catalog number: 1000020834	1 μ M AD153 Barcode Primer 3 Catalog number: 1000020833	3.50 mL \times 1tube	-25 $^{\circ}$ C -- -15 $^{\circ}$ C

2.2 Equipment and Consumables Required but not Provided

Table 2-12: 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°C refrigerator	MLS	/
-25°C --15°C freezer	MLS	/
Qubit® ssDNA Assay Kit	Thermo Fisher	Q10212
Power Dust remover	MATIN	M-6318
Sterile pipette tip(box)	XYGEN	/
200µL Wide-Bore Pipette Tips	XYGEN	T-205-WB-C
Qubit Assay Tubes	Thermo Fisher	Q32856
100%Tween-20	MLS	/
5M NaCl solution	MLS	/
2M NaOH solution	MLS	/
0.2mL PCR 8-tube strip	XYGEN	/
1.5mL Eppendorf	XYGEN	MCT-150-C
Ice rack	MLS	/
Electronic pipette	Labnet	FASTPETTEV-2
Serological pipet	CORNING	/
5 mL Tube	SARSTEDT	60.558.001
10 mL Tube	SARSTEDT	60.551.001
15 mL Tube	SARSTEDT	60.732.001
25 mL Tube	SARSTEDT	60.9922.243

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



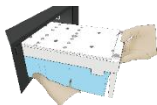
DNB loading: load the DNB onto sequencing flow cell



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



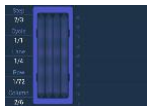
Load the Flow Cell: place the Flow Cell on the stage of the sequencer



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 20-800 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 4-1: Recommended insert size

Sequencing Kit*	Suggested insert distribution (bp)	Data output (Gb/lane) **
FCL SE50	50-230	18.7-22.5
FCL SE100	200-400	37.5-45.0
FCL SE400	400-600	150.0-180.0
FCL PE100	200-400	75.0-90.0
FCL PE150	300-500	112.5-135.0
FCL PE200	400-600	150-180.0
Small RNA FCL SE50	20-60	/
FCS SE100	200-400	-27.5
FCS PE100	200-400	-55.0
FCS PE150	300-500	-82.5

* 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 requirement is subject to the corresponding library preparation kit user manual. For general libraries, the ssDNA library concentration should be ≥ 2 fmol/ μ L and each Make DNB reaction requires 40 fmol library. For small RNA libraries, the ssDNA library concentration should be ≥ 3 fmol/ μ L and each Make DNB reaction requires 60 fmol library.

If the library concentration is unknown, it is recommended to perform ssDNA library quantitation (ng/ μ L) using Qubit® ssDNA Assay Kit and Qubit® Fluorometer. Use the following equation to convert the concentration of the ssDNA library from ng/ μ L to fmol/ μ L.

$$\text{Concentration (fmol}/\mu\text{L)} = 3030 * \text{Concentration (ng}/\mu\text{L)} / N$$

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, 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

Each DNBSEQ-G400RS sequencing flow cell contains 4 lanes and each DNBSEQ-G400RS rapid sequencing flow cell contains 2 lanes. DNB can be loaded onto the flow cell using the sequencer, the MGIDL-200RS or the MGIDL-200H.

- When using the sequencer to load DNB, all lanes in the flow cell must be the same DNB. Each lane requires 50 μ L DNB.
- When using the MGIDL-200RS to load DNB, different DNB can be loaded onto different lane. Each lane requires 50 μ L DNB.
- When using the MGIDL-200H to load DNB, different DNB can be loaded onto different lane. Each lane requires 25 μ L DNB.

Table 4-2: The required number of make DNB reactions for each DNBSEQ-G400RS flow cell

Flow cell type	Loading system	DNB loading volume (μ L)/Lane	Make DNB reaction (μ L)	The required number of make DNB reaction /flow cell
FCL	Sequencer	50	100	2
	MGIDL-200RS	50	100	2-4
	MGIDL-200H	25	100	1-4
FCS	Sequencer	50	100	
	MGIDL-200RS	50	100	1-2
	MGIDL-200H	25	100	

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 μ L and the required library input for each Make DNB reaction is calculated as followed:

ssDNA library input (μ L) = 40 fmol / library concentration (fmol/ μ L)

Note:

For Small RNA libraries, ssDNA library input (μ L) = 60 fmol / library concentration (fmol/ μ L)

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	20
Total Volume	40

- 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°C)	On
95°C	1 min
65°C	1 min
40°C	1 min
4°C	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°C. 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 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 4-6: Rolling circle amplification conditions

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

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°C or the temperature closest to 35°C.
- Immediately add 20 µL Stop DNB Reaction Buffer once the temperature reaches 4 °C. Mix gently by pipetting 5-8 times using a wide bore tip. Do not vortex, shake the tube or pipette vigorously.

① **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°C and perform sequencing within 48 hours.

4.4 Quantify DNB

When the make DNB is completed, take 2 μL DNB, 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°C and perform sequencing within 48 hours.

5 Prepare a Flow Cell

- Take the flow cell out of -25°C - -15°C storage and remove the flow cell from 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 temperature and the outer plastics package is intact, the flow cell can be placed back in -25°C - -15°C for storage. But the switch between room temperature and -25°C - -15°C 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	FCL Volume (μL)	FCS Volume (μL)
DNB Load Buffer II	64	32
Make DNB Enzyme Mix II (LC)	2	1
DNB	200	100
Total Volume	266	133

- 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°C until use.

Notes:

- ① Prepare a fresh DNB loading mix immediately before the sequencing run.
- ② Each sequencing flow cell (FCL) requires 266 μL DNB loading mix and each rapid sequencing flow

cell (FCS) requires 133 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	FCL Volume (μL)	FCS Volume (μL)
DNB Load Buffer II	16	16
Make DNB Enzyme Mix II (LC)	0.5	0.5
DNB	50	50
Total volume	66.5	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°C 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, 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 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 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	FCL Volume (μL)	FCS Volume (μL)
DNB Load Buffer II	8	8
Make DNB Enzyme Mix II (LC)	0.25	0.25
DNB	25	25
Total volume	33.25	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°C 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.

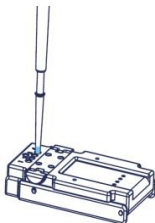


Figure 6-2: Load samples using MGIDL-200H

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 and the High-throughput Sequencing Refill Kit (only for SE400 and PE200 sequencing) from storage. Thaw in a room temperature water bath until completely thawed (or thaw in 2-8°C fridge one day in advance). Store at 2-8°C storage until use. The flow cell can be removed from -25--15°C and place at room temperature at this point. Invert the cartridge 3 times to mix before use. Shake the cartridge violently in all directions for 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 from -25--15°C, 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 position is shown in Figure 7-2.

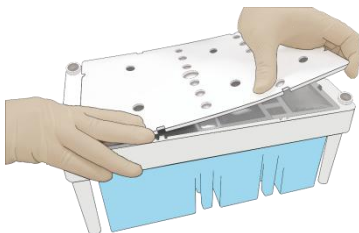


Figure 7-1: Open and clean the kit

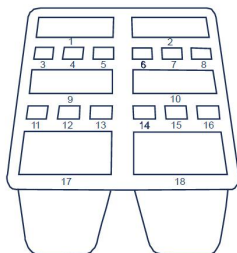


Figure 7-2: Well position

- Remove dNTPs Mix and dNTPs Mix II from $-20\text{ }^{\circ}\text{C}$ storage 1h in advance and thaw at room temperature. Store at 4°C until use. Mix the reagents using a vortex mixer for 5 seconds and centrifuge briefly before use.
- Remove Sequencing Enzyme Mix from $-20\text{ }^{\circ}\text{C}$ storage and place on ice until use. Invert Sequencing Enzyme Mix 4-6 times before use.

7.1 Prepare the FCL SE50 and FCL 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 FCL SE50 or FCL SE100 cartridge

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

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

Sequencing kit	dNTPs Mix loading volume	Sequencing Enzyme Mix loading volume
FCL SE50	0.700 mL	0.700 mL
FCL SE100	1.100 mL	1.100 mL

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

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

Sequencing kit	dNTPs Mix II loading volume	Sequencing Enzyme Mix loading volume
FCL SE50	0.600 mL	0.600 mL
FCL SE100	0.900 mL	0.900 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:

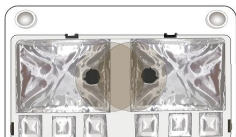


Figure 7-4: Seal the loading wells of FCL SE50 or FCL SE100 cartridge

- Place the cartridge horizontally on the table, 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 FCL SE50 or FCL SE100 sequencing cartridge is now ready for use.

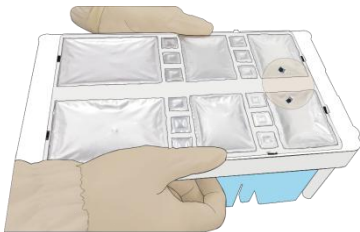


Figure 7-5: Mix reagents after loading

7.2 Prepare the FCL SE400 sequencing cartridge

- Remove the Wash Buffer For Sequencing from storage and thaw at room temperature.
- Pierce the seal at the edge of well No.1, No.2, No.7, No.9, No.10, No.17 and No.18 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 FCL SE400 cartridge

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix into a new 10 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.1:

Table 7-3: FCL SE400 sequencing cartridge well No.1 reagent loading

Sequencing kit	dNTPs Mix loading volume	Sequencing Enzyme Mix loading volume
FCL SE400	4.000 mL	4.000 mL

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix II into a new 20 mL or 25 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix II in that tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.2:

Table 7-4: FCL SE400 sequencing cartridge well No.2 reagent loading

Sequencing kit	dNTPs Mix II loading volume	Sequencing Enzyme Mix loading volume
FCL SE400	12.00 mL	4.000 mL

- Invert to mix the refill reagents 3 times and mix the Wash Buffer For Sequencing using a vortex mixer for 5 seconds. Take a pipette with the appropriate volume range and add reagents following the table below:

Table 7-5: FCL SE400 refill reagent loading

Sequencing kit	Well No.	Reagent name	Loading volume mL
FCL SE400	1	Sequencing Reagent V4.0	100.0
	2	Sequencing Reagent V4.0	100.0
	17	Wash Reagent 1 V2.0	200.0
	18	Wash Reagent 2	200.0
	10	Image Reagent V2.0	200.0
	9	Regeneration Reagent	200.0
	7	Wash Buffer For Sequencing	2.700

① **Note:**

- ① Please add the refill reagents in strict accordance with the order shown in the table.
- ② After adding one reagent, please change your gloves before adding the next reagent.

⚠ **Note:**

Wash Buffer For 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.

- Seal the loading wells of well No.1, No.2, No.9, No.10, No.17 and No.18 with the transparent sealing film. Do not cover the center of the well to avoid blocking the sampling needle (see Figure 7-7):



Table 7-7: Seal the loading wells of FCL SE400 cartridge

- Place the cartridge horizontally on the table, hold both sides of the cartridge with both hands (see Figure 7-5). 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 FCL SE400 sequencing cartridge is now ready for use.

7.3 Prepare the FCL PE100 and FCL PE150 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 7-8):



Figure 7-8: Pierce the seal of FCL PE100 or FCL PE150 cartridge

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix into a new 10 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.1:

Table 7-6: FCL PE100 and FCL PE150 sequencing cartridge well No.1 reagent loading

Sequencing kit	dNTPs Mix loading volume	Sequencing Enzyme Mix loading volume
FCL PE100	1.800 mL	1.800 mL
FCL PE150	2.400 mL	2.400 mL

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

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

Sequencing kit	dNTPs Mix II loading volume	Sequencing Enzyme Mix loading volume
FCL PE100	1.500 mL	1.500 mL
FCL PE150	2.100 mL	2.100 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:

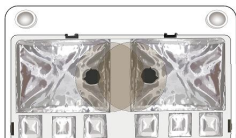


Figure 7-9: Seal the loading wells of FCL PE100 or FCL PE150 cartridge

- Place the cartridge horizontally on the table, 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 μ L of MDA Enzyme Mix 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 FCL PE100 or FCL PE150 sequencing cartridge is now ready for use.

① **Note:**

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

7.4 Prepare the FCL PE200 sequencing cartridge

- Pierce the seal at the edge of well No.1, No.2, No.9, No.17 and No.18 to make a hole around 2 cm in diameter using 1 mL sterile tip (see Figure 7-10):



Figure 7-10: Pierce the seal of FCL PE200 cartridge

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix into a new 10 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube. Invert the tube for 4–6 times to mix the reagents in the tube before adding all of them into well No.1:

Table 7-8: FCL PE200 sequencing cartridge well No.1 reagent loading

Sequencing kit	dNTPs Mix loading volume	Sequencing Enzyme Mix loading volume
FCL PE200	3.100 mL	3.100 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 into the dNTPs Mix II in the same tube. Invert the tube for 4–6 times to mix the reagents in the tube before adding all of them into well No.2:

Table 7-9: FCL PE200 sequencing cartridge well No.2 reagent loading

Sequencing kit	dNTPs Mix II loading volume	Sequencing Enzyme Mix loading volume
FCL PE200	8.100 mL	2.700 mL

- Invert to mix the refill reagents 3 times to mix. Take a pipette with the appropriate volume range and add reagents following the table below:

Table 7-10: FCL PE200 refill reagent loading

Sequencing kit	Well No.	Reagent name	Loading volume mL
FCL PE200	17	Wash Reagent 1 V2.0	200.0
	18	Wash Reagent 2	200.0
	9	Regeneration Reagent	200.0

① **Note:**

- ① Please add the refill reagents in strict accordance with the order shown in the table.
- ② After adding one reagent, please change your gloves before adding the next reagent.

- Seal the loading wells of well No.1, No.2, No.9, No.17 and No.18 with the transparent sealing film. Do not cover the center of the well to avoid blocking the sampling needle (see Figure 7-11):



Table 7-11: Seal the loading wells of FCL PE200 cartridge

- Place the cartridge horizontally on the table, 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 SE400 sequencing cartridge is now ready to be used.
- Pierce the seal of well No.15 using 1 mL sterile tip. Add 500 μ L of MDA Enzyme Mix 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 PE200 sequencing cartridge is now ready for use.

① **Note:**

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

7.5 Prepare the FCL 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 7-12):



Figure 7-12: Pierce the seal of FCL SE50 cartridge

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

Table 7-11: FCL SE50 sequencing cartridge well No.1 reagent loading

Sequencing kit	dNTPs Mix loading volume	Sequencing Enzyme Mix loading volume
FCL SE50	0.700 mL	0.700 mL

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix II into a new 5 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix II in the same tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.2:

Table 7-12: FCL SE50 sequencing cartridge well No.2 reagent loading

Sequencing kit	dNTPs Mix II loading volume	Sequencing Enzyme Mix loading volume
FCL SE50	0.600 mL	0.600 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:

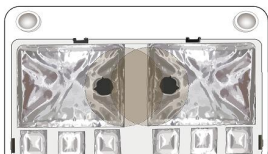


Figure 7-13: Seal the loading wells of FCL SE50 cartridge

- Place the cartridge horizontally on the table, hold both sides of the cartridge with both hands (see Figure 7-5). 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 FCL 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.

7.6 Prepare the FCS 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 7-14):



Figure 7-14: Pierce the FCS SE100 cartridge

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

Table 7-13: FCS SE100 sequencing cartridge well No.1 reagent loading

Sequencing kit	dNTPs Mix loading volume	Sequencing Enzyme Mix loading volume
FCS SE100	0.800 mL	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 5 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix II in the same tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.2:

Table 7-14: FCS SE100 sequencing cartridge well No.2 reagent loading

Sequencing kit	dNTPs Mix II loading volume	Sequencing Enzyme Mix loading volume
FCS SE100	1.600 mL	0.800 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 7-15: Seal the loading wells of FCS SE100 cartridge

- Place the cartridge horizontally on the table, hold both sides of the cartridge with both hands

(see Figure 7-5). 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 FCS SE100 sequencing cartridge is now ready for use.

7.7 Prepare the FCS PE100 and FCS PE150 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 7-16):



Figure 7-16: Pierce the seal of FCS PE100 or FCS PE150 cartridge

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

Table 7-15: FCS PE100 and FCS PE150 sequencing cartridge well No.1 reagent loading

Sequencing kit	dNTPs Mix loading volume	Sequencing Enzyme Mix loading volume
FCS PE100	1.400 mL	1.400 mL
FCS PE150	1.900 mL	1.900 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 into the dNTPs Mix II in the same tube. Invert the tube for 4–6 times to mix the reagents in the tube before adding all of them into well No.2:

Table 7-16: FCS PE100 and FCS PE150 sequencing cartridge well No.2 reagent loading

Sequencing kit	dNTPs Mix II loading volume	Sequencing Enzyme Mix loading volume
FCS PE100	2.800 mL	1.400 mL
FCS PE150	3.800 mL	1.900 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:

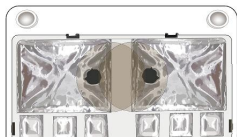


Figure 7-17: Seal the loading wells of FCS PE100 or FCS PE150 cartridge

- Place the cartridge horizontally on the table, 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 μ L of MDA Enzyme Mix 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 FCS PE100 or FCS PE150 sequencing cartridge is now ready for use.

① **Note:**

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

7.8 Dual barcode sequencing

For dual barcode sequencing, perform the following steps after finishing the preparation of PE sequencing cartridge:

- Remove the CPAS Barcode Primer 3 (for Pair End Sequencing only) from the CPAS Barcode Primer 3 Reagent Kit 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.

8 Sequencing

8.1 Enter the main interface

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

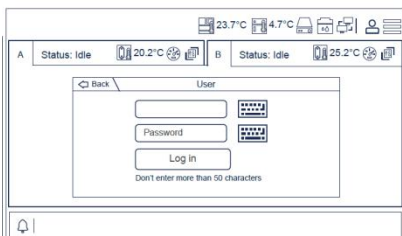


Figure 8-1: Log-in interface

- See the interface below.

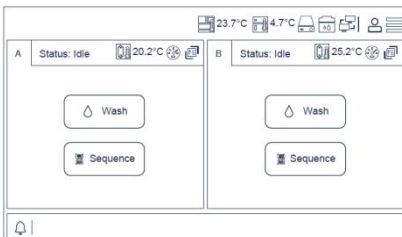


Figure 8-2: Main interface

8.2 Load the DNBs

- Click the "Sequence" option on the interface to enter the following interface:

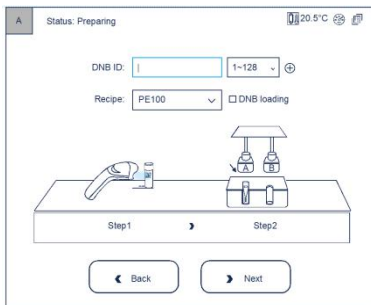


Figure 8-3: DNBs loading interface

- Click on the ⊕ on the right of the "DNB ID" and the lane information will appear.
Note: select 4 lanes for FCL and 2 lanes for FCS.

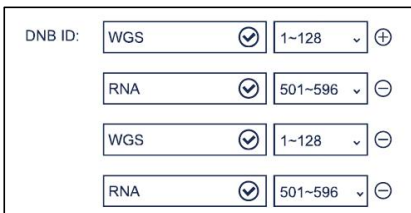


Figure 8-4: DNBs and information selection interface

- 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.

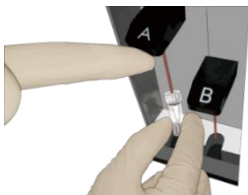


Figure 8-5: Load the DNBs tube

- 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:

Sequencing recipe "SE50_sR" is for Small RNA sequencing.

If for dual barcode sequencing, choose recipe "Customize"

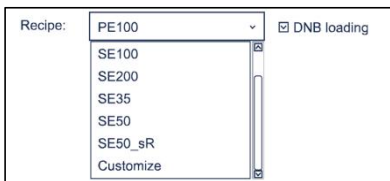
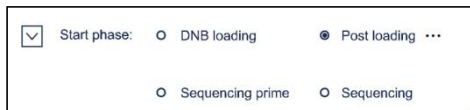


Figure 8-6: Select sequencing solutions

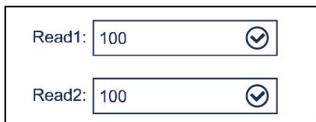
- If you choose one-click sequencing and the DNB is loaded on the sequencer, check the "DNB loading" (see Figure 8-6). Otherwise leave it blank and then go to step 8.4. If you choose "Customize", continue performing the following steps.
- In the beginning, please select a step to start the sequencing run.



Start phase: DNB loading Post loading ...
 Sequencing prime Sequencing

Figure 8-7: Select the step to start sequencing

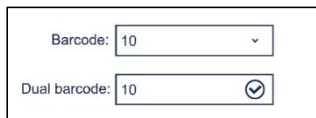
- Select the read length. For example, with PE100 enter 100 for read 1 and 100 for read 2.



Read1: 100 ✓
Read2: 100 ✓

Figure 8-8: Choose the read length

- 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.



Barcode: 10 ▾
Dual barcode: 10 ✓

Figure 8-9: Select the barcode length

- Select the lane for barcode demultiplexing.

Split barcode: <input checked="" type="checkbox"/> Lane1 <input checked="" type="checkbox"/> Lane2 <input checked="" type="checkbox"/> Lane3 <input checked="" type="checkbox"/> Lane4
--

Figure 8-10: Barcode demultiplexing on different lanes

- 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.

Dark reaction: only chemical reaction without optical information capture.

Read1 dark reaction cycle:	<input type="text" value="2"/> <input checked="" type="checkbox"/>	-	<input type="text" value="5"/> <input checked="" type="checkbox"/>
Read2 dark reaction cycle:	<input type="text" value="3"/> <input checked="" type="checkbox"/>	-	<input type="text" value="8"/> <input checked="" type="checkbox"/>

Figure 8-11: 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.

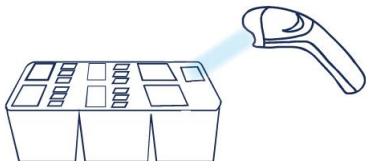


Figure 8-12: Reagent cartridge information entry interface

- 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 remove it from the compartment.

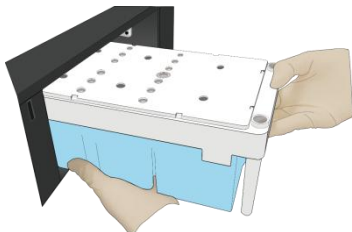


Figure 8-13: Remove cleaning cartridge

- Moisten dust-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 8-14: 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 8-15: 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 dust-free paper to ensure that the flow cell can be held properly.

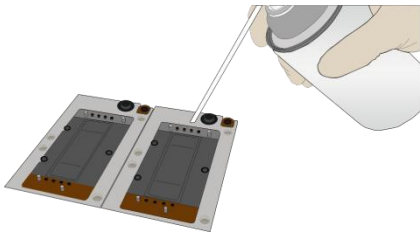


Figure 8-16: Clean the Flow Cell stage

- 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.

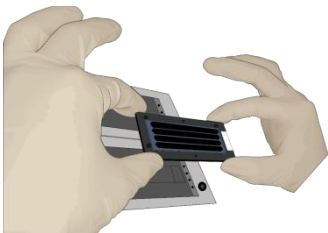


Figure 8-17: Load the Flow Cell

- 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.

① **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.

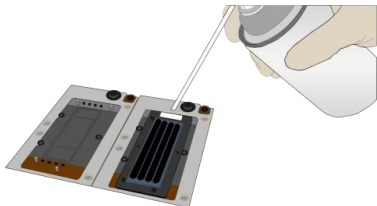


Figure 8-18: Clean the Flow Cell

- 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.

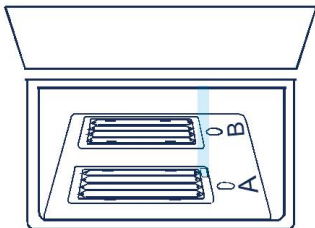


Figure 8-19: Flow Cell information entry interface

- 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 8-20: 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.

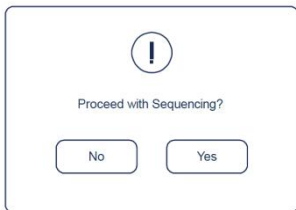


Figure 8-21: Confirm sequencing interface

- 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 0.05% Tween-20 following the table below (valid for 28 days if stored at 4°C)

Table 9-2: Wash reagents preparation (1)

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

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

Table 9-3: Wash reagent preparation (2)

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 9-4: Wash reagent preparation (3)

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 the 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 the 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 μ L 0.1M NaOH to 0.5mL cryotube. Mark it as the cleaning reagent cartridge 3.
- Wash cleaning cartridge 4: Take a clean cleaning cartridge and a 0.5 mL cryotube, add 50 mL 0.05% Tween-20 solution into large wells, 6 mL 1M NaCl + 0.05% Tween-20 solution to No.15 well, 400 μ L 1M NaCl + 0.05% Tween-20 solution to 0.5mL cryotube and 6 mL 0.05% Tween-20 solution to the rest of the wells. Mark it as the 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.

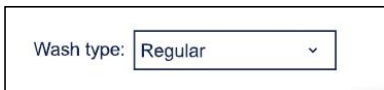


Figure 9-2: Select the wash type

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

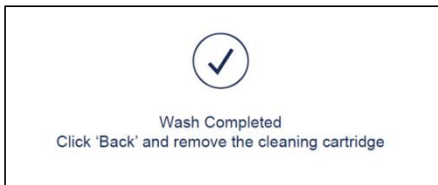


Figure 9-3: Regular wash end interface

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. Then 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/ μ L, 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 pump.
- 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 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, 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 dNTPs 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 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, 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, in this condition please re-start from the post loading.
- Start from "8 Sequencing" and re-load the flow cell.
- When selecting "8.3 Select sequencing parameters", choose programme "Customize".
- Select "Post loading" and click "...".

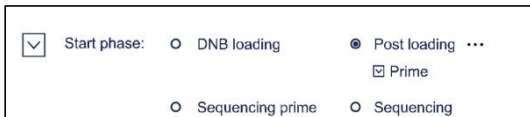


Figure 10-1: Select re-start Post loading

- If starts from the Post loading prime, select "Prime" in Figure 10-1. If starts from the step Post loading, don't 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.

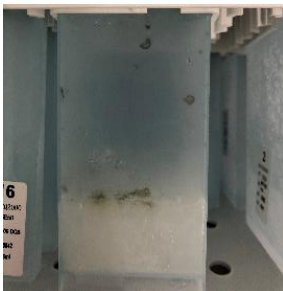


Figure 10-2: Dark green crystals in well No.10

10.9 Library amount less than 40 fmol

If the library amount is less than 40 fmol (but not less than 24 fmol), 60 μ L Make DNB reaction can be tried. It must be noted that 60 μ L Make DNB reaction may cause data loss and sequencing quality poorer than expectation. When the library amount is adequate, 100 μ L 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 μ L and the required library input for each Make DNB reaction is calculated as followed:
$$\text{ssDNA library input } (\mu\text{L}) = 24 \text{ fmol} / \text{library concentration (fmol}/\mu\text{L)}$$
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°C)	On
95°C	1 min
65°C	1 min
40°C	1 min
4°C	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°C. 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°C)	On
30°C	25 min
4°C	Hold

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

① **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°C and perform sequencing within 48 hours. Proceed to "4.4 Quantify DNB".



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