



Part No.: SOP-013-B01-082

High-throughput (Rapid) Sequencing Set

DNBSEQ-G400RS

Instruction For Use

Version: 10.0

Leading Life Science Innovation

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Research Use
Only

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About the instructions for use

This instructions for use is applicable to DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set. The manual edition is 10.0.

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Revision history

Version	Date	Description
10.0	May 2024	<ul style="list-style-type: none">Updated table 17
9.0	January 2024	<ul style="list-style-type: none">Updated the information about manufacturer and address in the Appendix.Updated the information about the user manual.Updated the Sequencing time and analysis time.Changed MDA Enzyme Mix to MDA Enzyme Mix II except FCL PE200 and FCS PE300.
8.0	August 2022	Changed the catalog number of FCL PE150/FCL PE200/FCL SE400 Sequencing Flow Cell
7.0	March 2022	Added the product information in Latvia
6.0	February 2022	<ul style="list-style-type: none">Changed the logoChanged the transport temperature below -15 °C to -80 °C ~ -15 °CUpdated the device maintenance
5.0	October 2021	<ul style="list-style-type: none">Added the product information of FCS PE300Changed instructions for reagent tank preparation of FCL PE200
4.0	December 2020	<ul style="list-style-type: none">Updated the logo, website and email address of MGI.Added the temperature for transportation.Updated the document template.Updated the part of sequencing reagent cartridge figures.Cancelled the Refill Kit of FCL SE400.

Version	Date	Description
3.0	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.
2.0	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".
1.0	August 2019	Initial release.

Sequencing set

Catalog number	Name	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

Catalog number	Name	Version
1000016952	DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150)	V3.1
1000016946	DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE400)	V3.1
940-000151-00	DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE200)	V3.2
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
940-000152-00	DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE300)	V1.0
1000020834	cPAS Barcode Primer 3 Reagent Kit	V2.0
1000014048	cPAS Barcode Primer 4 Reagent Kit	V1.0



- Tips**
- DNBSEQ-G400RS High-throughput Sequencing Set (LV SM FCL PE150) (Catalog number: 940-000238-00) is only manufactured in Latvia.
 - The DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE300) requires version 1.5.0.1283 and above of the control software, requires version 1.4.0.257 and above of the BaseCall.
 - The DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE400) requires version V1.7.1.03 and above of the script.

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Chapter 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 accurate sequencing information.

1.3 Sample requirements

This sequencing set is applicable to MGI Adapter library and App-A library. After being converted from the third-party library by the MGIEasy Universal Library Conversion Kit (App-A), the APP-A library is applicable to the MGI sequencing platforms.

1.4 Data analysis

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

1.5 Sequencing read length

Sequencing read length determines the number of sequencing cycles for a given sequencing run. For example, a PE150 cycle run performs reads of 150 cycles (2×150) for a total of 300 cycles. At the end of the insert sequencing run, an extra 10 cycles of barcode read can be performed, if required.

Table 1 Sequencing cycle

Sequencing read length	Read1 read length	Read2 read length	Barcode read length	Total read length	Maximum cycles
SE50	50	--	10	50+10	71
SE100	100	--	10	100+10	121
SE400	400	--	10	400+10	421
PE100	100	100	10	200+10	222
PE150	150	150	10	300+10	322
PE200	200	200	10	400+10	422
PE300	300	300	10	600+10	622



Tips • To ensure sequencing quality, when Read1 and Read2 sequencing is complete, the sequencer will automatically perform one more cycles for correction. For example, for PE100 dual barcode sequencing, Read1 length is 100, Read2 length is 100, barcode read length is 10 and dual barcode read length is 10, plus 1 correction cycle for Read1 and 1 correction cycle for Read2 (barcode does not require correction). The total cycle number of this sequencing is 222.

- PE means Pair-end sequencing; SE means Single-end sequencing.

1.6 Sequencing time and analysis time



- Tips**
- Table2 and Table3 show the time for ECR 3.0
 - Table4 and Table5 show the time for ECR 6.0
 - Table6 and Table7.show the time for ECR 7.0

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

/	SE50	SE100	SE400	PE100	PE150	PE200
Single Flow Cell	12.0	22.0	104.0	42.5	61.5	100.0
Dual Flow Cells	13.0	23.0	104.0	43.5	62.5	102.0
Write fastq (Single Flow Cell)	0.5	0.8	2.5	1.3	1.8	2.5
Write fastq (Dual Flow Cells)	1.0	1.5	5.0	2.5	3.5	5.0

Table 3 FCS Sequencing time and analysis time for each read length (hours)

/	SE100	PE100	PE150	PE300
Single Flow Cell	12.5	24.9	35.4	95.5
Dual Flow Cells	12.7	25.0	35.6	96.3
Write fastq (Single Flow Cell)	0.4	0.5	0.6	2
Write fastq (Dual Flow Cells)	0.7	1.0	1.2	4

Table 4 FCL Sequencing time and analysis time for each read length (hours)

/	SE50	SE100	SE400	PE100	PE150	PE200
Single Flow Cell	12.0	22.0	104.0	34.0	50.0	100.0
Dual Flow Cells	13.0	23.0	104.0	35.0	51.0	102.0
Write fastq (Single Flow Cell)	0.5	0.8	2.5	1.3	1.8	2.5
Write fastq (Dual Flow Cells)	1.0	1.5	5.0	2.5	3.5	5.0

Table 5 FCS Sequencing time and analysis time for each read length (hours)

/	SE100	PE100	PE150	PE300
Single Flow Cell	12.5	24.9	35.4	95.5
Dual Flow Cells	12.7	25.0	35.6	96.3
Write fastq (Single Flow Cell)	0.4	0.5	0.6	2
Write fastq (Dual Flow Cells)	0.7	1.0	1.2	4

Table 6 FCL Sequencing time and analysis time for each read length (hours)

/	SE50	SE100	SE400	PE100	PE150	PE200
Single Flow Cell	12.0	22.0	104.0	32.6	46.0	100.0
Dual Flow Cells	13.0	23.0	104.0	33.6	47.0	102.0
Write fastq (Single Flow Cell)	0.25	0.4	1.25	0.65	0.9	1.25
Write fastq (Dual Flow Cells)	0.5	0.75	2.5	1.25	1.8	2.5

Table 7 FCS Sequencing time and analysis time for each read length (hours)

/	SE100	PE100	PE150	PE300
Single Flow Cell	12.5	21.4	29.4	95.5
Dual Flow Cells	12.7	21.5	29.5	96.3
Write fastq (Single Flow Cell)	0.15	0.25	0.3	1
Write fastq (Dual Flow Cells)	0.3	0.5	0.6	2



- Tips**
- The sequencing time (Single Flow Cell/Dual Flow Cells) in the table above includes the time required from post loading prime to sequencing completion. The Write fastq time includes the time required for barcode demultiplexing (if **Split barcode** is selected) and FASTQ files output when sequencing is completed.
 - The time in the table above is theoretical. The actual run time may vary among various sequencers.

1.7 Attention

- This product is for research use only. Please read the manual carefully before use.
- Ensure that you are familiar with the SOP&Attention of all the laboratory apparatus to be used.
- Avoid direct skin and eye contact with any samples and reagents. Don't swallow. Please wash with plenty of water immediately and go to the hospital if this happens..
- All the samples and waste materials should be disposed of according to relevant laws and regulations.
- This product is for one sequencing run only and cannot be reused.
- The components and packages are batched separately. Keep the components in the packages until use and do not remove them. Mixed use of reagent components from different batches is not recommended.
- Do not use expired products.

Chapter 2 List of sequencing sets and self-prepared consumables

2.1 List of sequencing set components

Table 8 DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE50)
Catalog number: 1000016941












Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985				
Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE50) Catalog number: 1000016940				
Low TE Buffer		300 µL×1 tube	-25 °C to -15 °C	-80 °C to -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 0.5 mL (Empty)		1 tube		
dNTPs Mix		0.80 mL×1 tube		
dNTPs Mix II		0.70 mL×1 tube		
Sequencing Enzyme Mix		1.60 mL×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 9 DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE100)
Catalog number: 1000016943












Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985				
Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE100) Catalog number: 1000016942				
Low TE Buffer		300 µL × 1 tube	-25 °C to -15 °C	-80 °C to -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 0.5 mL (Empty)		1 tube		
dNTPs Mix		1.20 mL × 1 tube		
dNTPs Mix II		1.00 mL × 1 tube		
Sequencing Enzyme Mix		2.30 mL × 1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 10 DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE400)
Catalog number: 1000016946













Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 940-000625-00				
Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE400) Catalog number: 1000016944				
Low TE Buffer		300 µL×1 tube	-25 °C to -15 °C	-80 °C to -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 0.5 mL (Empty)		1 tube		
dNTPs Mix		4.10 mL×1 tube		
dNTPs Mix II		12.20 mL×1 tube		
Sequencing Enzyme Mix		8.30 mL×1 tube		
Wash Buffer For Sequencing		2.90 mL×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 11 DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE100)
Catalog number: 1000016950











Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985				
Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit(FCL PE100) Catalog number: 1000016949				
Low TE Buffer		300 µL×1 tube	-25 °C to -15 °C	-80 °C to -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 0.5 mL (Empty)		1 tube		
dNTPs Mix		1.90 mL×1 tube		
dNTPs Mix II		1.60 mL×1 tube		
Sequencing Enzyme Mix		3.60 mL×1 tube		
MDA Reagent		3.50 mL×1 tube		
MDA Enzyme Mix II		0.50 mL×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 12 DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150)
Catalog number: 1000016952

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 940-000625-00				
Sequencing Flow Cell		1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit(FCL PE150) Catalog number: 1000016951				
Low TE Buffer		300 µL×1 tube	-25 °C to -15 °C	-80 °C to -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 0.5 mL (Empty)		1 tube		
dNTPs Mix		1.30 mL×2 tubes		
dNTPs Mix II		1.15 mL×2 tubes		
Sequencing Enzyme Mix		4.80 mL×1 tube		
MDA Reagent		3.50 mL×1 tube		
MDA Enzyme Mix II		0.50 mL×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 13 DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE200)
Catalog number: 940-000151-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 940-000625-00				
Sequencing Flow Cell		1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit(FCL PE200) Catalog number: 940-000153-00				
Low TE Buffer		300 µL×1 tube	-25 °C to -15 °C	-80 °C to -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 0.5 mL (Empty)		1 tube		
dNTPs Mix		3.80 mL×1 tube		
dNTPs Mix II		2.85 mL×2 tubes		
Sequencing Enzyme Mix		7.60 mL×1 tube		
MDA Reagent		3.50 mL×1 tube		
MDA Enzyme Mix		0.60 mL×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 14 DNBSEQ-G400RS High-throughput Sequencing Set (Small RNA FCL SE50)
Catalog number: 1000016998

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985				
Sequencing Flow Cell		1 EA	-25 °C to-15 °C	-80 °C to-15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit(FCL SE50) Catalog number: 1000016940				
Low TE Buffer		300 μL×1 tube	 -25 °C to -15 °C	 -80 °C to -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 0.5 mL (Empty)		1 tube		
dNTPs Mix		0.80 mL×1 tube		
dNTPs Mix II		0.70 mL×1 tube		
Sequencing Enzyme Mix		1.60 mL×1 tube		
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×3 tubes	2 °C to 8 °C	2 °C to 8 °C

Table 15 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS SE100)
Catalog number: 1000016978












Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987				
Sequencing Flow Cell		1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS SE100) Catalog number: 1000016977				
Low TE Buffer		300 µL × 1 tube	-25 °C to -15 °C	-80 °C to -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 0.5 mL (Empty)		1 tube		
dNTPs Mix		0.90 mL × 1 tube		
dNTPs Mix II		1.70 mL × 1 tube		
Sequencing Enzyme Mix		1.90 mL × 1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 16 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE100)
Catalog number: 1000016980

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987				
Sequencing Flow Cell		1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE100) Catalog number: 1000016979				
Low TE Buffer		300 µL×1 tube	-25 °C to -15 °C	-80 °C to -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 0.5 mL (Empty)		1 tube		
dNTPs Mix		1.50 mL×1 tube		
dNTPs Mix II		1.50 mL×2 tubes		
Sequencing Enzyme Mix		3.10 mL×1 tube		
MDA Reagent		3.50 mL×1 tube		
MDA Enzyme Mix II		0.50 mL×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 17 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE150)
Catalog number: 1000016982

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987				
Sequencing Flow Cell		1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE150) Catalog number: 1000016981				
Low TE Buffer		300 µL×1 tube	-25 °C to -15 °C	-80 °C to -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 0.5 mL (Empty)		1 tube		
dNTPs Mix		2.00 mL×1 tube		
dNTPs Mix II		2.00 mL×2 tubes		
Sequencing Enzyme Mix		4.80 mL×1 tube		
MDA Reagent		3.50 mL×1 tube		
MDA Enzyme Mix II		0.50 mL×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 18 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE300)
Catalog number: 940-000152-00

Component	Cap color	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987					
Sequencing Flow Cell			1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE300) Catalog number: 940-000154-00					
Low TE Buffer			300 µL×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
Make DNB Buffer			100 µL×1 tube		
Make DNB rapid Enzyme Mix II			160 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 IV			200 µL×1 tube		
Micro Tube 0.5 mL (Empty)			1 tube		
dNTPs Mix			3.80 mL×1 tube		
dNTPs Mix II			2.85 mL×2 tubes		
Sequencing Enzyme Mix			7.60 mL×1 tube		
MDA Reagent			3.50 mL×1 tube		
MDA Enzyme Mix			0.60 mL×1 tube		
Sequencing Reagent Cartridge	/		1 EA		
Transparent sealing film	/		2 sheets		

Table 19 CPAS Barcode Primer 3 Reagent Kit Catalog number: 1000020834

Component	Spe & quantity	Storage temperature	Transportation temperature
Primer for dual barcode sequencing (Pair End Sequencing use only)			
1 μ M AD153 Barcode Primer 3	3.50 mL \times 1 tube	-25 $^{\circ}$ C to -15 $^{\circ}$ C	-80 $^{\circ}$ C to -15 $^{\circ}$ C

Table 20 CPAS Barcode Primer 4 Reagent Kit Catalog number: 1000014048

Component	Spe & quantity	Storage temperature	Transportation temperature
Primer for dual barcode sequencing (Single End Sequencing use only)			
1 μ M AD153 Barcode Primer 4	3.50 mL \times 1 tube	-25 $^{\circ}$ C to -15 $^{\circ}$ C	-80 $^{\circ}$ C to -15 $^{\circ}$ C

\

2.2 Self-prepared equipment and consumables



- Tips** • Avoid making and loading DNBs by the pipette tips with filter. It is necessary to use the pipette tips with recommended brands and catalog number.

Table 21 Self-prepared equipment and consumables

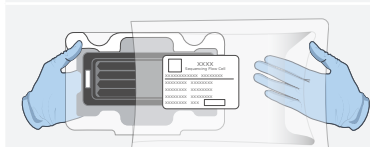
Equipment and consumables	Recommended brand	Catalog number
Qubit 4.0 Fluorometer	Thermo Fisher	Q33226
Mini centrifuge	Major Laboratory Supplier (MLS)	/
Vortex mixer	MLS	/
Thermal cycler	Bio-Rad	/
Pipette	Eppendorf	/
2 °C to 8 °C refrigerator	MLS	/
-25 °C to -15 °C freezer	MLS	/
Qubit ssDNA Assay Kit	Thermo Fisher	Q10212
Power dust remover	MATIN	M-6318
Sterile pipette tip(box)	AXYGEN	/
200 uL wide-bore,non-filtered pipette tips	MGI	091-000355-00
200 uL wide-bore,non-filtered pipette tips	AXYGEN	T-205-WB-C
Qubit Assay Tubes	Thermo Fisher	Q32856
100%Tween-20	MLS	/
5 M NaCl solution	MLS	/
2 M NaOH solution	MLS	/
0.2 mL PCR 8-tube strip	AXYGEN	/
ProClin 300	SIGMA	48912-U
1.5 mL Microcentrifuge tube	AXYGEN	MCT-150-C
2.0 mL cryotube	SARSTEDT	72.609.003
Ice box	MLS	/

Equipment and consumables	Recommended brand	Catalog number
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

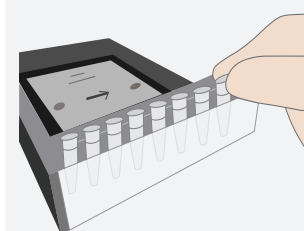
Chapter 3 Sequencing workflow



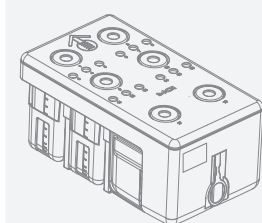
Making DNB: use DNB preparation reagents for making DNB.



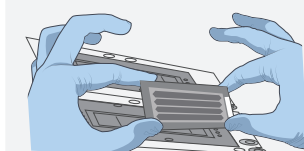
Preparing a new Flow Cell: take out the Flow Cell from package and inspect to ensure the Flow Cell is intact.



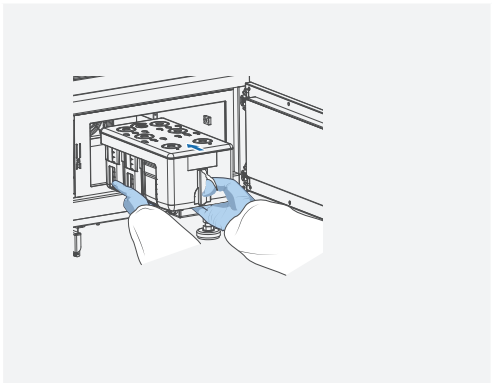
DNB loading: load the DNB onto sequencing Flow Cell.



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



Loading the Flow Cell: place the Flow Cell on the stage of the sequencer.



Loading the reagent cartridge into the sequencer.

Status: Preparing

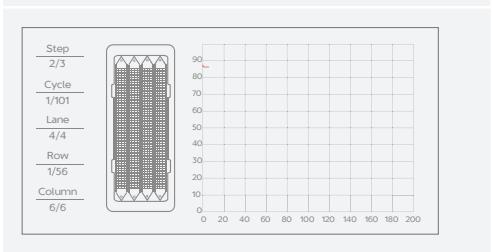
xxx kPa20.0°C

Item	Content
User name	XXXXXXXX
DNB ID Lane 1	XXXXXXXX
DNB ID Lane 2	XXXXXXXX
DNB ID Lane 3	XXXXXXXX
DNB ID Lane 4	XXXXXXXX
Sequencing cartridge ID	XXXXXXXX
Flow cell ID	XXXXXXXX
Recipe	

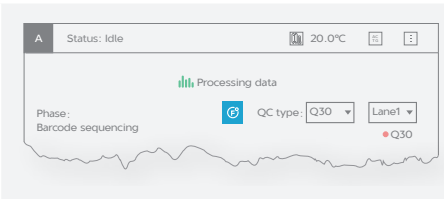
Previous

Start

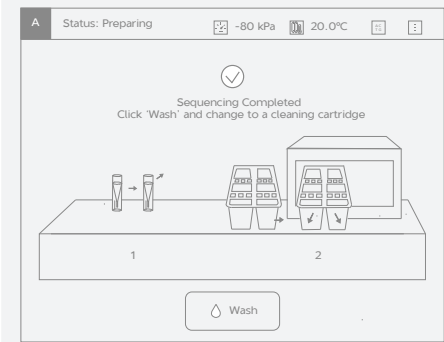
Starting sequencing: follow the user manual to enter sequencing information and start the run.



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



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



Device maintenance: perform device maintenance when sequencing is completed.

Chapter 4 Making DNB

4.1 Insert size recommendation

- 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 any special requirements or specifications for the library preparation kit, then the requirements of the kit should be followed.

Table 22 Recommended insert size

Product model	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	150.0–180.0
Small RNA FCL SE50	20–60	/
FCS SE100	200–400	~ 27.5
FCS PE100	200–400	~ 55.0
FCS PE150	300–500	~ 82.5
FCS PE300	400–700	~ 90



- Tips**
- 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

Table 23 Library requirement

Libraries	library concentration (bp)
general libraries	$\geq 2 \text{ fmol}/\mu\text{L}$
small RNA libraries	$\geq 3 \text{ fmol}/\mu\text{L}$
PCR free	$\geq 3.75 \text{ fmol}/\mu\text{L}$



- Tips**
- If the library concentration is unknown, it is recommended to perform ssDNA library quantitation ($\text{ng}/\mu\text{L}$) using Qubit ssDNA Assay Kit and Qubit 4.0 Fluorometer. Use the equation below to convert the concentration of the ssDNA library from $\text{ng}/\mu\text{L}$ to $\text{fmol}/\mu\text{L}$:

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

$$N$$
 represents the number of nucleotides (total library length including the adapter).
 - If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.

4.3 Making DNB

This section includes two make DNB schemes, available on demand:

Scheme 1: Make DNB of FCS PE300, see *4.3.1 Making DNB of FCS PE300 on Page 24*

Scheme 2: Make DNB of other, see *4.3.2 Making DNB of other on Page 28*

4.3.1 Making DNB of FCS PE300

4.3.1.1 Prepare reagents for DNB making

Perform the steps below:

1. Place the library on ice until use.
2. Take out Make DNB Buffer, Low TE Buffer and Stop DNB Reaction Buffer from storage and thaw reagents at room temperature.
3. Thaw Make DNB rapid Enzyme Mix II for approximately 0.5 hour on ice.
4. After thawing, mix reagents using a vortex mixer for 5 seconds. Centrifuge briefly and place on ice until use.



Tips Make DNB rapid Enzyme Mix II should be used for PE300.

4.3.1.2 Calculating the number of DNB reaction system

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 45 μ L DNB.
- When using the MGIDL-200RS to load DNB
Different DNBs can be loaded onto different lane. Each lane requires 45 μ L DNB.
- When using the MGIDL-200H to load DNB
Different DNBs can be loaded onto different lane. Each lane requires 22.5 μ L DNB.

Table 24 The required number of make DNB reactions for each DNBSEQ-G400RS rapid Flow Cell

Loading system	DNB loading volume (μ L)/ Lane	Make DNB reaction (μ L)	The required number of make DNB reaction / Flow Cell
Sequencer	45	90	1
MGIDL-200RS	45	90	1
MGIDL-200H	22.5	90	1

4.3.1.3 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 section 4.2 *Library concentration and amount requirement on Page 24*.



- Tips**
- If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.
 - All samples should be considered potentially infectious and should be handled in accordance with relevant national regulations.

Table 25 The volume of ssDNA library

Library	The volume of 90 μ L DNB reaction (μ L)	The volume of 45 μ L DNB reaction (μ L)
Normal library	$V=40 \text{ fmol/C}$	$V=20 \text{ fmol/C}$
PCR free	$V=75 \text{ fmol/C}$	$V=37.5 \text{ fmol/C}$

- Calculate the required ssDNA library for each Make DNB reaction and fill it in *Table 26 Make DNB reaction mix 1 on Page 26 as V.*

4.3.1.4 Making DNB

Perform the steps below:

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

Table 26 Make DNB reaction mix 1

Component	The volume of 90 μ L DNB reaction (μ L)	The volume of 45 μ L DNB reaction (μ L)
Low TE Buffer	$20 - V$	$10 - V$
Make DNB Buffer	20	10
ssDNA libraries	V	V
Total Volume	40	20

2. Mix reagents using a vortex mixer for 5 seconds, centrifuge briefly by a mini centrifuge.
3. Place the mix into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown in the table below:

Table 27 Primer hybridization reaction condition

Temperature	Time
Heated lid (105 $^{\circ}$ C)	On
95 $^{\circ}$ C	1 min
65 $^{\circ}$ C	1 min

Temperature	Time
40 °C	1 min
4 °C	Hold

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



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

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

Table 28 Make DNB reaction mix 2

Component	The volume of 90 µL DNB reaction (µL)	The volume of 45 µL DNB reaction (µL)
Make DNB rapid Enzyme Mix II	40	20
Make DNB Enzyme Mix II (LC)	1.6	0.8

- Add all the Make DNB reaction mix 2 into the Make DNB reaction 1.
- Mix reagents using a vortex mixer for 5 seconds, centrifuge briefly by a mini centrifuge.
- Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:



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

Table 29 Rolling circle amplification conditions

Temperature	Time
Heated lid (35 °C)	On
30 °C	15 min
4 °C	Hold

10. Immediately add Stop DNB Reaction Buffer once the temperature reaches 4 °C ,The volume of Stop DNB Reaction Buffer according to table below.



- Tips**
- Keep DNB on ice during the entire operation to prevent DNB from performing secondary amplification.
 - It is very important to mix DNB gently using a wide bore pipette tip. Do not vortex, shake the tube or pipette vigorously.
 - Store DNB at 4 °C and perform sequencing within 48 hours.

Table 30 Make DNB reaction mix 2

Component	The volume of 90 µL DNB reaction (µL)	The volume of 45 µL DNB reaction (µL)
Stop DNB Reaction Buffer	10	5

4.3.2 Making DNB of other

4.3.2.1 Preparing reagents for DNB making

Perform the steps below:

1. Place the library on ice until use.
2. Take out Make DNB Buffer, Low TE Buffer and Stop DNB Reaction Buffer from storage and thaw reagents at room temperature.
3. Take out Make DNB Enzyme Mix I and thaw the reagent for approximately 0.5 hour on ice.
4. After thawing, mix reagents using a vortex mixer for 5 seconds. Centrifuge briefly and place on ice until use.

4.3.2.2 Calculating the number of DNB reaction system

Each DNBSEQ-G400RS sequencing Flow Cell contains 4 lanes and each DNBSEQ-G400RS rapid sequencing Flow Cell contains 2 lanes.

- When using the sequencer to load DNB
All lanes in the Flow Cell must be the same DNB. Each lane requires 50 µL DNB.
- When using the MGIDL-200RS to load DNB
Different DNBs can be loaded onto different lanes. Each lane requires 50 µL DNB.
- When using the MGIDL-200H to load DNB

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

Table 31 The required number of make DNB reactions for each DNBSEQ-G400RS Flow Cell

Flow Cell type	Loading system	DNB volume (μL) / Lane	Make DNB reaction (μL)	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	1
	MGIDL-200RS	50	100	1-2
	MGIDL-200H	25	100	

4.3.2.3 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 section 4.2 *Library concentration and amount requirement on Page 24*.



- Tips**
- If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.
 - All samples should be considered potentially infectious and should be handled in accordance with relevant national regulations.

Table 32 The required amount of ssDNA library

Library	The volume of 90 μL DNB reaction (μL)
Normal library	$V=40 \text{ fmol} / C$
Small RNA libraries	$V=60 \text{ fmol} / C$
PCR free	$V=75 \text{ fmol} / C$

- Calculate the required ssDNA library for each Make DNB reaction and fill it in *Table 33 Make DNB reaction mix 1 on Page 30* as V .

4.3.2.4 Making DNB

Perform the steps below:

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

Table 33 Make DNB reaction mix 1

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

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

Table 34 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

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



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

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

Table 35 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 seconds using a mini centrifuge.
- Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:



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

Table 36 Rolling circle amplification conditions

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

9. Immediately add 20 µL Stop DNB Reaction Buffer once the temperature reaches 4 °C. Mix gently by pipetting 5 to 8 times using a wide bore tip.

-  **Tips**
- It is very important to mix DNB gently using a wide-bore, non-filtered pipette tips. Do not centrifuge, vortex, or shake the tube.
 - Store DNB at 4 °C and perform sequencing within 48 hours.


4.4 Quantifying DNB

Perform the steps below:

1. When DNB making is completed, take 2 μL DNB, use Qubit ssDNA Assay Kit and Qubit 4.0 Fluorometer to quantify the DNB.

Table 37 DNB concentration standard

Type	Application library
FCS PE300	$\geq 8 \text{ ng}/\mu\text{L}$
The Others	$\geq 8 \text{ ng}/\mu\text{L}$

 **Tips** • If the concentration is lower than $8 \text{ ng}/\mu\text{L}$, see *10.1 Low DNB concentration on Page 68* for details.

- If there are too many samples in a single test, it is recommended to quantify in batches to avoid inaccurate DNB quantification due to fluorescence quenching.

2. If the concentration exceeds $40 \text{ ng}/\mu\text{L}$, the DNBs should be diluted to $20 \text{ ng}/\mu\text{L}$ according to the table below:

Table 38 DNB dilution scheme

Type	Component	Storage conditions	Storage time
FCS PE300	Low TE Buffer	4°C	$\leq 4 \text{ h}$
The Others	DNB Load Buffer I	4°C	$\leq 48 \text{ h}$

Chapter 5 Preparing a Flow Cell

Perform the steps below:

1. Take the Flow Cell out of storage and take out the Flow Cell form the box.

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

2. Balance the Flow Cell at room temperature for 60 minutes to 24 hours.
3. Unwrap the outer package before use and start DNB loading.

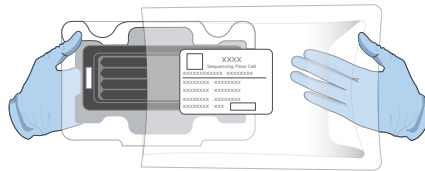



Figure 1 Unwrapping the outer package

-  **Tips**
- 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 °C to -15 °C for storage. But the switch between room temperature and -25 °C to -15 °C must not exceed 3 times.
 - 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.

4. Take out the Flow Cell from the inner package and inspect to ensure the Flow Cell is intact.

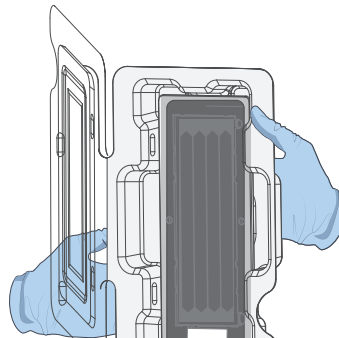


Figure 2 Inspecting the Flow Cell

Chapter 6 DNB loading

Perform the following steps according to different situations:

- For FCS PE300:
 - 1) Take out DNB Load Buffer IV from storage and thaw reagents on ice for approximately 0.5 hour.
 - 2) After thawing, mix reagents using a vortex mixer for 5 seconds, centrifuge briefly and place on ice until use.
 - 3) If crystal precipitation is found in DNB Load Buffer II, vigorously mix the reagent with 1-2 minutes of continuous vortexing to re-dissolve the precipitate before use.
 - 4) Go to 6.1 *DNB loading for FCS PE300 on Page 34* for DNB loading.
- For others:
 - 1) Take out DNB Load Buffer II from storage and thaw reagents on ice for approximately 0.5 hour.
 - 2) After thawing, mix reagents using a vortex mixer for 5 seconds, centrifuge briefly and place on ice until use.
 - 3) If crystal precipitation is found in DNB Load Buffer II, vigorously mix the reagent with 1-2 minutes of continuous vortexing to re-dissolve the precipitate before use.
 - 4) Go to 6.2 *Loading DNB for other reads on Page 37* for DNB loading.

6.1 FCS PE300 DNB loading

6.1.1 Sequencer DNB loading

Perform the steps below:

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

Table 39 DNB loading mix 1

Component	Volume (μL)
DNB Load Buffer IV	45
DNB	90
Total volume	135

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



- Tips**
- Do not centrifuge, vortex, or shake the tube.
 - Prepare a fresh DNB loading mix immediately before the sequencing run.
 - Each sequencing Flow Cell (FCS) requires 135 µL DNB loading mix.

6.1.2 MGIDL-200RS DNB loading



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

Perform the steps below:

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

Table 40 DNB loading mix 2

Component	FCS PE300 Volume (µL)
DNB loading buffer IV	22.5
DNB	45
Total volume	67.5

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



- Tips**
- Do not centrifuge, vortex, or shake the tube.
 - Each lane requires at least 66.5 µL of DNB loading mix.
- Place the tubes containing DNB loading mix in the labeled positions of MGIDL-200RS.

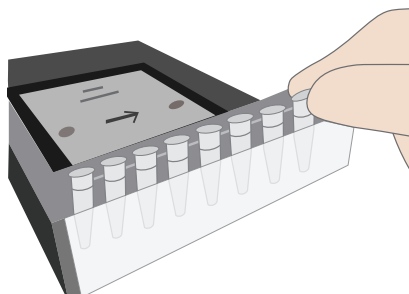




Figure 3 Placing the loading samples

4. Press the Flow Cell attachment button, hold the Flow Cells by the 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, take out the Flow Cell and place it in a PE glove or a plastic bag at $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ for 60–90 minutes, then immediately place it on the sequencer for use.

 **Tips** Do not exceed 90 minutes.

6.1.3 MGIDL-200H DNB loading

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


Performing the steps below:

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

Table 41 DNB loading mix 3

Component	Volume (μL)
DNB loading buffer IV	11.5
DNB	22.5
Total volume	34

2. Combine components and mix by gently pipetting 5 to 8 times using a wide bore tip. Place the mixture at $4\text{ }^{\circ}\text{C}$ until use.

-  **Tips**
- Do not centrifuge, vortex, or shake the tube.
 - Each lane requires at least 30 μL of DNB loading mix.

3. Install the sealing gasket and Flow Cell.
4. Aspirate 30 μL DNB loading mix with a pipette (make sure there are no bubbles) and insert the wide bore tip into the fluidics inlet.

- Tips**
- Do not press the control button of the pipette after inserting the tip into the fluidics inlet.
 - During loading DNB, do not move the wide bore tip or Flow Cell to prevent bubbles from entering.

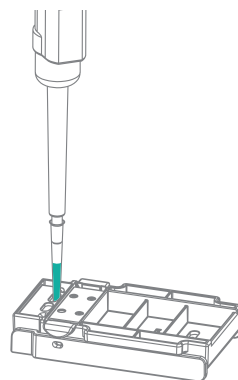


Figure 4 Loading samples using MGIDL-200H

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

- Tips** For PE300 flow cell, it is recommended to place it on the MGIDL-200H to incubation at 25 °C \pm 2 °C for 60–90 minutes, don't exceed 90 minutes.

6.2 Other DNB loading

6.2.1 Sequencer DNB loading


Perform the steps below:

1. Take a 0.5 mL microfuge tube and add the following reagents:


Table 42 DNB loading mix 1

Component	FCL Volume (μ L)	FCS Volume (μ L)
DNB Load Buffer II	64	32
Make DNB Enzyme Mix II (LC)	2	1
DNB	200	100
Total Volume	266	133

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

-  **Tips**
- Do not centrifuge, vortex, or shake the tube.
 - Prepare a fresh DNB loading mix immediately before the sequencing run.
 - Each sequencing Flow Cell (FCL) requires 266 µL DNB loading mix and each rapid sequencing Flow Cell(FCS) requires 133 µL DNB loading mix.

6.2.2 MGIDL-200RS DNB loading

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


Perform the steps below:

1. Take a new PCR 8-tube strip and add the following reagents

Table 43 DNB loading mix 2

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

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

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

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

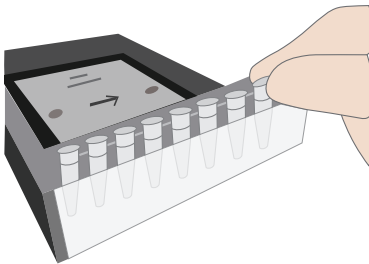



Figure 5 Placing the loading samples

4. Press the Flow Cell attachment button, hold the Flow Cells by edges and align the holes on the Flow Cells with the locating pins on the Flow Cell stages. Press the left and right sides of the Flow Cell on the stage at the same time to ensure that the Flow Cells are securely seated on the stage.
5. Select the 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 minutes, then immediately place it on the sequencer for use.

6.2.3 MGIDL-200H DNB loading

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


Performing the steps below:

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


Table 44 DNB loading mix 3

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

2. Combine components and mix by gently pipetting 5 to 8 times using a wide-bore, non-filtered pipette tips. Place the mixture at 4 °C until use.

-  **Tips**
- Do not centrifuge, vortex, or shake the tube.
 - Each lane requires at least 30 μL of DNB loading mix.

3. Install the sealing gasket and Flow Cell.
4. Aspirate 30 μL DNB loading mix with a pipette (make sure there are no bubbles) and insert the wide bore tip into the fluidics inlet.

-  **Tips**
- Do not press the control button of the pipette after inserting the tip into the fluidics inlet.
 - During loading DNB, do not move the wide bore tip or Flow Cell to prevent bubbles from entering.

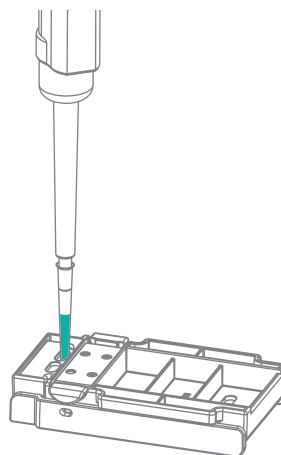



Figure 6 Loading samples using MGIDL-200H

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


Chapter 7 Preparing the sequencing reagent cartridge

Perform the steps below:

1. Take out the Sequencing Reagent Cartridge from storage.
2. Thaw them in water bath at room temperature until completely thawed (or thaw in a 2 °C to 8 °C fridge one day in advance). Store at 2 °C to 8 °C fridge until use.

-  **Tips** After being taken out from -25 °C to -15 °C, the Flow Cell must be placed at room temperature for at least 60 minutes and not more than 24 hours before DNB loading.

3. Invert the cartridge 3 times to mix before use.
4. Shake the cartridge violently in all directions 10 to 20 times and making sure reagents are fully mixed.

 **Tips** If dark green crystals appear in well No.10, it is precipitation of raw materials of the reagent in well No.10. This is a normal phenomenon. When the cartridge is thawed, mix the reagents in the cartridge well and the crystals will dissolve. Sequencing quality will not be affected. See *10.8 Dark green crystals in well No.10 on Page 70* in this manual for details.

5. Wipe any water condensation on the cartridge cover and well surround with lint-free paper. Well positions are shown in the figure below.

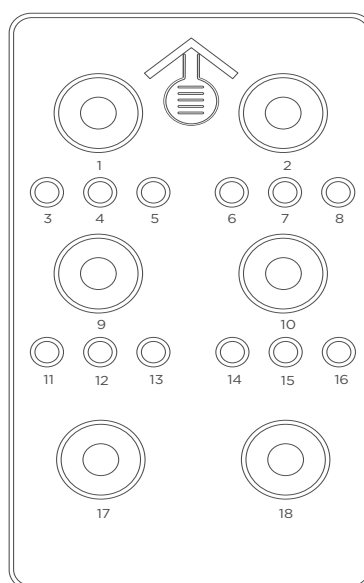


Figure 7 Well positions

6. Take out dNTPs Mix and dNTPs Mix II from -20 °C storage 1 hour in advance and thaw at room temperature. Store at 2 °C to 8 °C until use.
7. Take out Sequencing Enzyme Mix from -20 °C storage and place on ice until use.
8. Perform the following steps according to different situations:
 - If perform PE sequencing, take out MDA Reagent from storage and place on ice until use.
 - If perform Small RNA Sequencing or FCL SE400 sequencing. Take out the Wash Buffer For Small RNA Sequencing or Wash Buffer For Sequencing from storage and thaw at room temperature. Store at 2 °C to 8 °C until use.
 - If perform dual barcode sequencing of SE. Take out the 1 μ M AD153 Barcode Primer 4 from the cPAS Barcode Primer 4 Reagent Kit and thaw at room temperature. Store at 2 °C to 8 °C until use.

- If perform dual barcode sequencing of PE. Take out the 1μM AD153 Barcode Primer 3 from the cPAS Barcode Primer 3 Reagent Kit and thaw at room temperature. Store at 2 °C to 8 °C until use.
9. Pierce the seal in the center of well No.1 and No.2 to make a hole around 2 cm in diameter using a 1 mL sterile tip.

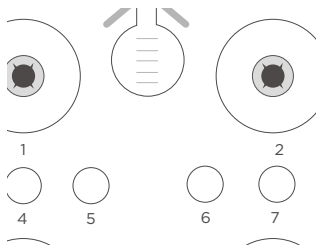


Figure 8 Piercing the seal of cartridge


10. Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix into a new 5 mL/15 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube.
-  **Tips**
- Mix the dNTPs Mix using a vortex mixer for 5 seconds and centrifuge briefly before use.
 - Invert Sequencing Enzyme Mix 4 to 6 times before use.

Table 45 Sequencing cartridge well No.1 reagent loading

Product model	dNTPs Mix loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
FCL SE50	0.700	0.700
FCL sRNA SE50	0.700	0.700
FCL SE100	1.100	1.100
FCL SE400	4.000	4.000
FCS SE100	0.800	0.800
FCL PE100	1.800	1.800
FCL PE150	2.400	2.400
FCL PE200	3.800	3.800
FCS PE100	1.400	1.400
FCS PE150	1.900	1.900
FCS PE300	3.800	3.800

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



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

12. 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/15 mL/25 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix II in the same tube.



- Tips**
- Mix the dNTPs Mix II using a vortex mixer for 5 seconds and centrifuge briefly before use.
 - Invert Sequencing Enzyme Mix 4 to 6 times before use.

Table 46 Sequencing cartridge well No.2 reagent loading

Product model	dNTPs Mix II loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
FCL SE50	0.600	0.600
FCL sRNA SE50	0.600	0.600
FCL SE100	0.900	0.900
FCL SE400	12.000	4.000
FCS SE100	1.600	0.800
FCL PE100	1.500	1.500
FCL PE150	2.100	2.100
FCL PE200	5.700	3.800
FCS PE100	2.800	1.400
FCS PE150	3.800	1.900
FCS PE300	5.700	3.800

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



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

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

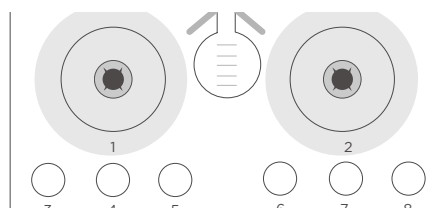


Figure 9 Seal the loading wells of cartridge

15. Press the film with finger around the round cap. Make sure to seal tightly and no bubbles between the film and cap. Ensure the reagents would not overflow from the cartridge.

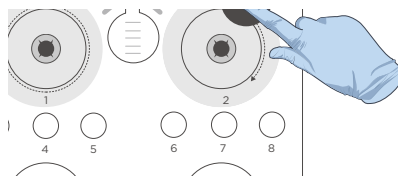


Figure 10 Seal the loading wells of cartridge tightly

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

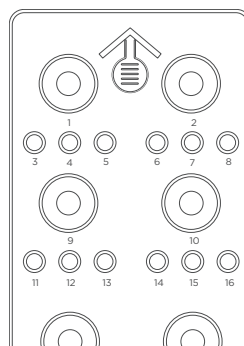


Figure 11 Mixing reagents after loading

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



- Tips**
- Do not use the wasted seals again.
 - Make sure the well No.1 and No.2 clean around avoiding a cross contamination.

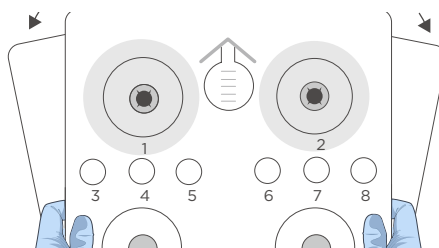


Figure 12 Removing the seal from cartridge

18. Gently tap the cartridge on the bench to reduce air bubbles in the reagents.

- Tips**
- The FCL SE50/FCL SE100/FCS SE100 sequencing cartridge for single barcode is now ready for use.
 - For next step, refer to *Chapter 8 Sequencing on Page 47*.

19. Perform the following steps according to different situations:

- Tips** Wash Buffer For Small RNA Sequencing Wash Buffer For Sequencing contains highly concentrated formamide which may have potential reproductive toxicity. Avoid breathing vapor 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.

■ For FCL SE50 (Small RNA)



- 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.

- Tips**
- The FCL SE50(Small RNA) sequencing cartridge for single barcode is now ready for use.
 - For next step, refer to *Chapter 8 Sequencing on Page 47*.


■ For FCL SE400

- Mix the Wash Buffer For Sequencing using a vortex mixer for 5 seconds and centrifuge briefly before use.
- Pierce the seal of well No.7 then add 2.70 mL of the Wash Buffer For Sequencing. When adding the reagent, make sure there are no bubbles at the bottom of the tube.


- Tips**
- The FCL SE400 sequencing cartridge for single barcode is now ready for use.
 - For next step, refer to *Chapter 8 Sequencing on Page 47*.

- For PE sequencing
 - a. Pierce the seal of well No.15 using a 1 mL sterile tip.
 - b. Perform the following steps according to different situations: For FCS PE300 and FCL PE200, adding 500 μ L of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette. For others, adding 500 μ L of MDA Enzyme Mix II to the MDA Reagent tube with a 1 mL pipette.
 -  **Tips** When using MDA Enzyme Mix or MDA Enzyme Mix II, do not touch the wall of the tube to prevent influencing the enzyme activity.
 - c. Invert the tube 4 to 6 times to mix the reagents.
 - d. Add the mixture to well No.15. When adding the mixture, making sure there are no bubbles at the bottom of the tube.
 -  **Tips**
 - When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.
 - The FCL PE100/FCL PE150/FCL PE200/FCS PE100/FCS PE150/FCS PE300 sequencing cartridge for single barcode is now ready for use.
 - For next step, refer to *Chapter 8 Sequencing on Page 47*.

20. Perform the following steps according to different situations:

- For dual barcode sequencing of SE, perform the following steps after finishing the preparation of SE sequencing cartridge for single barcode:
 - a. Take out the 1 μ M AD153 Barcode Primer 4 from the cPAS Barcode Primer 4 Reagent Kit and thaw at room temperature.
 - b. Mix the 1 μ M AD153 Barcode Primer 4 using a vortex mixer for 5 seconds and centrifuge briefly before use.
 - c. Pierce the seal of well No.4 using a sterile tip, then add 2.90 mL of the 1 μ M AD153 Barcode Primer 4. When adding the reagent, make sure there are no bubbles at the bottom of the tube.
 -  **Tips**
 - The FCL SE50/FCL SE50(Small RNA)/FCL SE100/FCL SE400/FCS SE100 sequencing cartridge for dual barcode is now ready for use.
 - For next step, refer to *Chapter 8 Sequencing on Page 47*.
- For dual barcode sequencing of PE, perform the following steps after finishing the preparation of PE sequencing cartridge for single barcode:
 - a. Take out the 1 μ M AD153 Barcode Primer 3 from the cPAS Barcode Primer 3 Reagent Kit and thaw at room temperature.
 - b. Mix the 1 μ M AD153 Barcode Primer 3 using a vortex mixer for 5 seconds and centrifuge briefly before use.

- c. Pierce the seal of well No.4 using a sterile tip, then add 2.90 mL of the 1 μ M AD153 Barcode Primer 3. When adding the reagent, make sure there are no bubbles at the bottom of the tube.

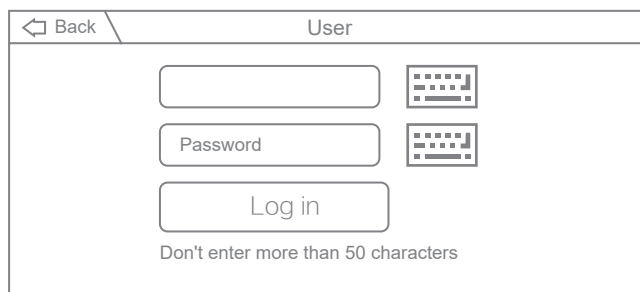
 **Tips** The FCL PE100/FCL PE150/FCL PE200/FCS PE100/FCS PE150/FCS PE300 sequencing cartridge for dual barcode is now ready for use.

Chapter 8 Sequencing

8.1 Entering the main interface

Perform the steps below:

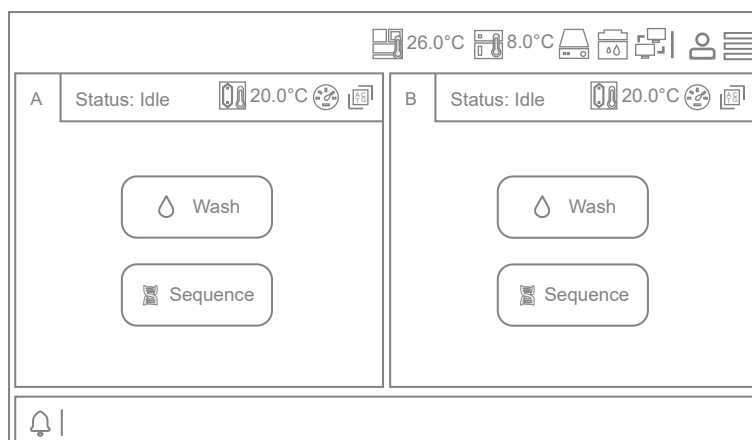
1. Enter the user name "user" and password "password123", click **Log in** to enter the main interface.



The screenshot shows a log-in screen with a title bar containing a back arrow and the word "User". Below the title bar are three input fields: the first is empty, the second is labeled "Password", and the third is labeled "Log in". To the right of each input field is a small icon of a barcode. Below the "Log in" button, there is a note: "Don't enter more than 50 characters".

Figure 13 Log-in interface

2. The main interface is as below:



The screenshot shows the main interface with a top status bar displaying various icons and temperatures: 26.0°C, 8.0°C, and 20.0°C. Below the status bar are two main panels, A and B. Each panel has a "Status: Idle" indicator, a temperature display of 20.0°C, and two buttons: "Wash" and "Sequence". At the bottom of the interface is a notification bell icon.

Figure 14 Main interface

8.2 Loading the DNBs

Perform the steps below:

- 1. Click **Sequence** in the interface to enter the interface below:

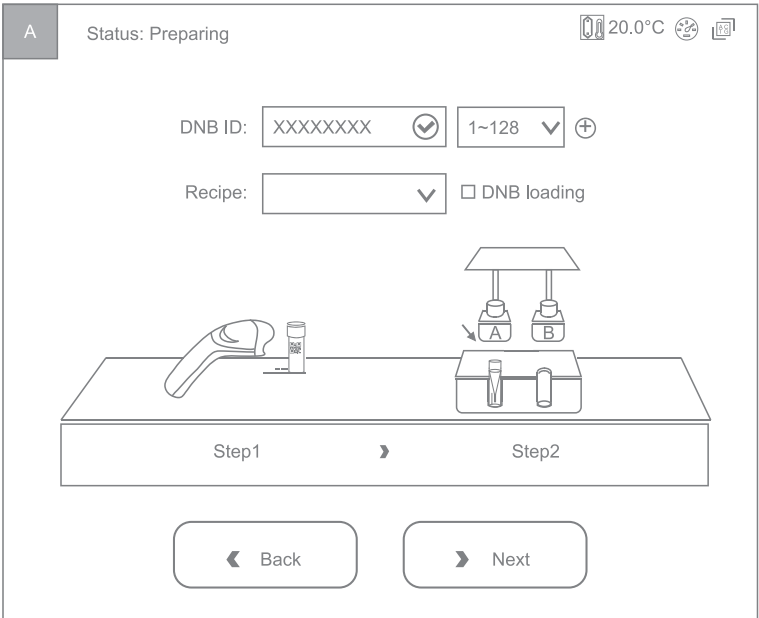


Figure 15 DNB loading interface

- 2. Click  on the right of **DNB ID** and the lane information will appear.

 **Tips** Select 4 lanes for FCL and 2 lanes for FCS.

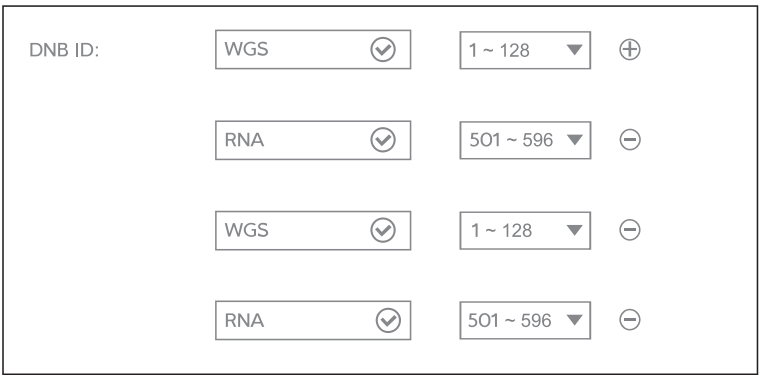




Figure 16 DNBs and information selection interface

- 3. Move the cursor to the DNB ID box and enter the library name or number.
- 4. Pull the drop-down menu on the left of  and select the barcode sequence of different lanes.

- 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.

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

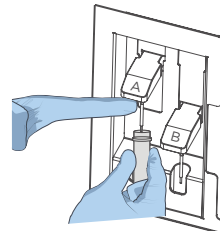


Figure 17 Loading the DNBs tube

- Close the reagent compartment door.

8.3 Selecting the sequencing parameters

Perform the steps below:

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

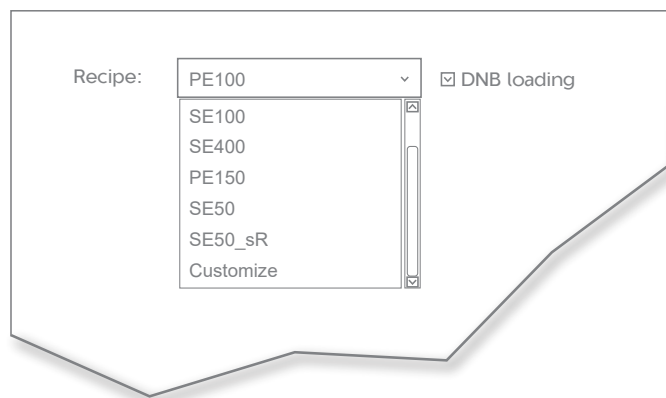



Figure 18 Selecting sequencing solutions

 **Tips** Sequencing recipe **SE50_sR** is for Small RNA sequencing. If for Dual Barcode sequencing, choose recipe **Customize**.

- 2. If you choose one-click sequencing and the DNB is loaded on the sequencer, check the “DNB loading”. Otherwise leave it blank and then go to *8.4 Loading the reagent cartridge on Page 51*. If you choose **Customize**, continue performing the steps below.
- 3. In the beginning, please select a step to start the sequencing run.

▼

Start phase: ☐ DNB loading ☒ Post loading ...
☐ Sequencing prime ☒ Prime ☐ Sequencing

Figure 19 Selecting the step to start sequencing

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

Read1: 100 ✓

Read2: 100 ✓

Figure 20 Selecting the read length

- 5. 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 21 Selecting the barcode length

- 6. Select the lane for barcode demultiplexing.

Split Barcode: ☒ Lane1 ☒ Lane2 ☒ Lane3 ☒ Lane4

Figure 22 Barcode demultiplexing on different lanes

7. 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:	2	✓	-	5	✓
Read2 dark reaction cycle:	3	✓	-	8	✓

Figure 23 Selecting the dark reaction

8. Click **Confirm**.

8.4 Loading the reagent cartridge

Perform the steps below:

1. Move the cursor to the **Reagent cartridge ID** blank, enter the cartridge information manually or use the barcode scanner to scan the cartridge barcode at the lower right corner of the reagent cartridge label.

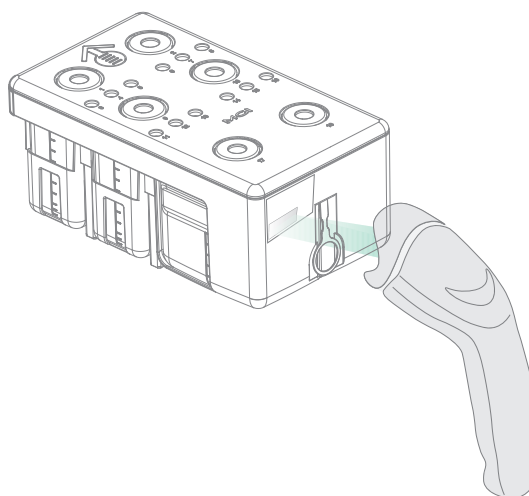


Figure 24 Reagent cartridge information entry interface

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

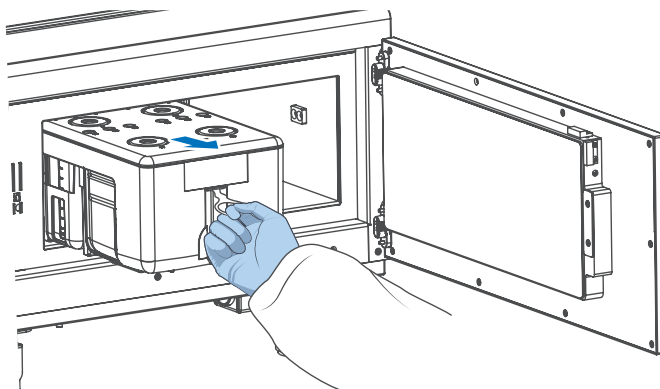


Figure 25 Removing cleaning cartridge

3. 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.

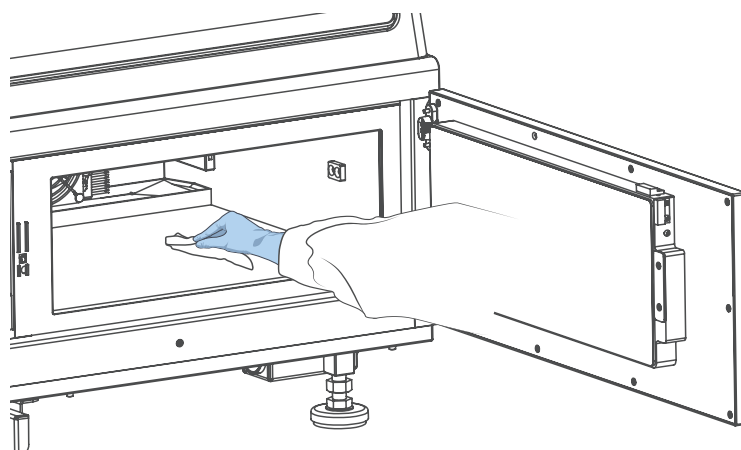


Figure 26 Maintaining the reagent compartment

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

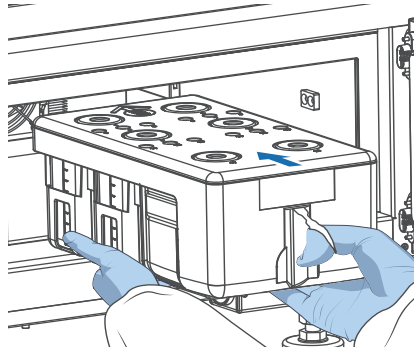


Figure 27 Sliding the new reagent cartridge into the reagent compartment

8.5 Loading the Flow Cell

Perform the steps below:

1. Open the Flow Cell compartment door,
2. Press both sides of the Flow Cell used for washing, and press the Flow Cell attachment button with the other hand.
3. After the vacuum is released, remove the Flow Cell for washing from the stage.
4. Use dust remover to remove the dust on the Flow Cell stage and the back of the Flow Cell.

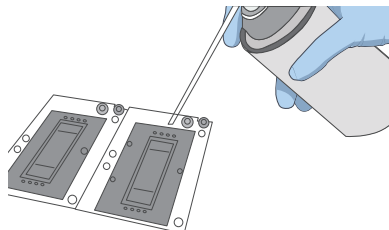



Figure 28 Cleaning the Flow Cell stage

 **Tips** If there are impurities on the stage surface, please gently wipe it with wet dust-free paper to ensure that the Flow Cell can be held properly.

5. Take out a new Flow Cell or the loaded Flow Cell.
6. There are two alignment holes on the left side and one hole on the right side. The label is on the right. Hold the Flow Cell by the edges with both hands.

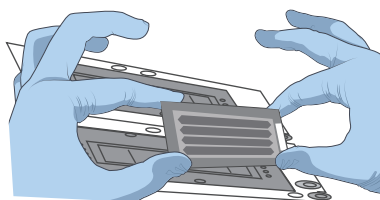



Figure 29 Loading the Flow Cell

7. Align the holes on the Flow Cell with the locating pins on the Flow Cell stage. Gently slide the Flow Cell at an angle of 45 degrees to the upper left corner (45 degrees to the upper right corner when loading the Flow Cell on the MGIDL-200RS) to keep the Flow Cell aligned with the pin.
8. Press the Flow Cell attachment button. Press the left and right sides of the Flow Cell on the stage at the same time to ensure the Flow Cell is properly seated on the stage.

 **Tips** The Flow Cell is fragile, please use caution when handling the Flow Cell.

9. Ensure that the negative pressure is within the range of -80 to -99 kPa before continuing. If the negative pressure is abnormal, refer to *10.2 Abnormal negative pressure on Page 68* in this manual for troubleshooting.
10. Use a dust remover to remove the dust on the Flow Cell surface and close the Flow Cell compartment door.

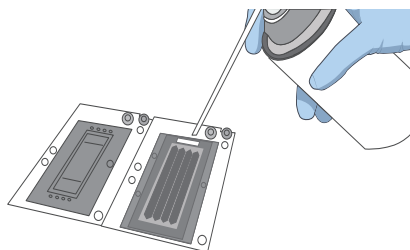


Figure 30 Cleaning the Flow Cell

11. Click **Next**. The flowcell ID can be entered through the barcode scanner; if automated entry does not work, move the cursor to the “Flow Cell ID” blank and enter the ID manually.

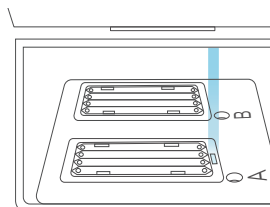



Figure 31 Flow Cell information entry interface

12. Click **Next**.

8.6 Reviewing parameters

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

 **Tips** To ensure sequencing quality, when read 1 and read 2 sequencing is complete, 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.

Item	Content
User name	user
DNB ID Lane 1	WGS 1 ~ 128
DNB ID Lane 2	RNA 501 ~ 596
DNB ID Lane 3	WGS 1 ~ 128
DNB ID Lane 4	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
Read 1 dark reaction	2 ~ 5
Read 2 dark reaction	3 ~ 8

Figure 32 Reviewing information

8.7 Starting sequencing

Perform the steps below:

1. After confirming that the information is correct, click **Start**.

2. The system will display the dialog box “Proceed with Sequencing?”. Click **Yes** to start sequencing.

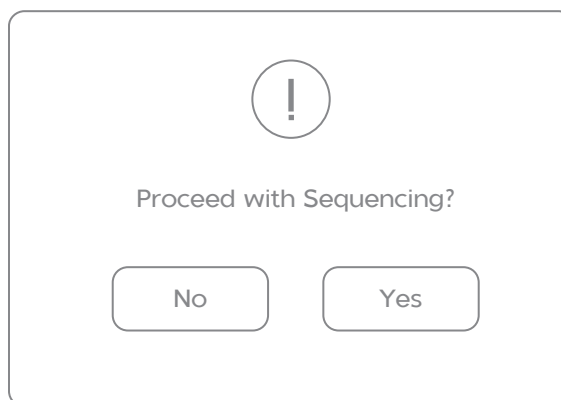



Figure 33 Confirming sequencing interface

3. Once sequencing has started, immediately open the Flow Cell compartment door to ensure that DNB or reagents are flowing through the Flow Cell.

8.8 Data access

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

After clicking to start sequencing, the sequencing results generated by the control software will appear in D drive.

- The data folder named after the flow cell ID, mainly contains pictures and data generated during the instrument operation (such as metrics).
- The Result folder named after the flow cell ID, mainly contains Bioinfo file and FASTQ file.

Chapter 9 Device Maintenance

9.1 Wash instruction

- When the interface below appears, please perform a wash.

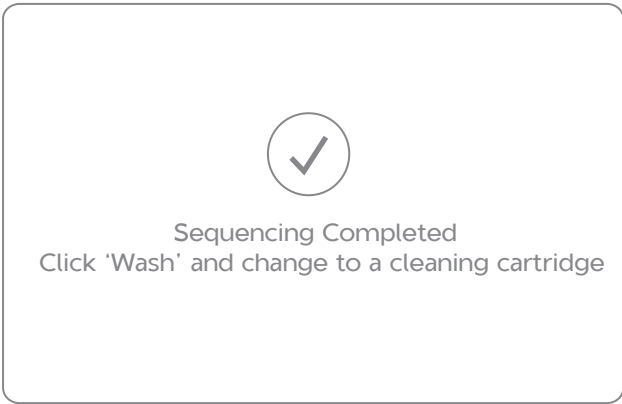


Figure 34 Wash interface

Table 47 Wash instruction

Wash type	Description
Full wash 1 or Full wash 2	<ul style="list-style-type: none">• A Full Wash 1 or Full Wash 2 is required if the sequencer is used for a PE run or a DNB loading/postload.• After the replacement of pipelines, sample needles and other accessories exposed to reagents, please perform a Full Wash 1 or Full Wash 2.• If the sequencer is to be powered off for more than 7 days, perform a Full Wash 1 or Full Wash 2 before being powered off and after being powered on.• If the sequencer was left unused for more than 7 days after a Full Wash 1 or Full Wash 2, please perform a Full Wash 1 or Full Wash 2 before use.• If impurities are found on the flow cell, perform a Full Wash 1 or Full Wash 2.

Wash type	Description
Regular wash	<ul style="list-style-type: none"> A Regular Wash is sufficient for an SE run. If the device was left unused for more than 12 hours after a Full Wash, please perform a Regular Wash again before use. After the system maintenance performed by an engineer, please perform a Regular Wash.
Maintenance wash	The sequencer is to be powered off for more than 7 days, perform a wash before being powered off and after being powered on.

- Refer to the flow chart below for details:



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

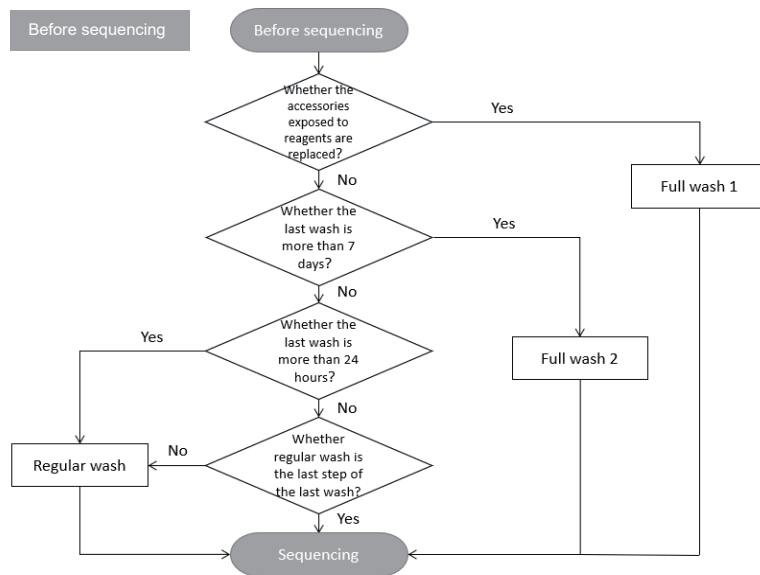


Figure 35 Wash instruction after sequencing

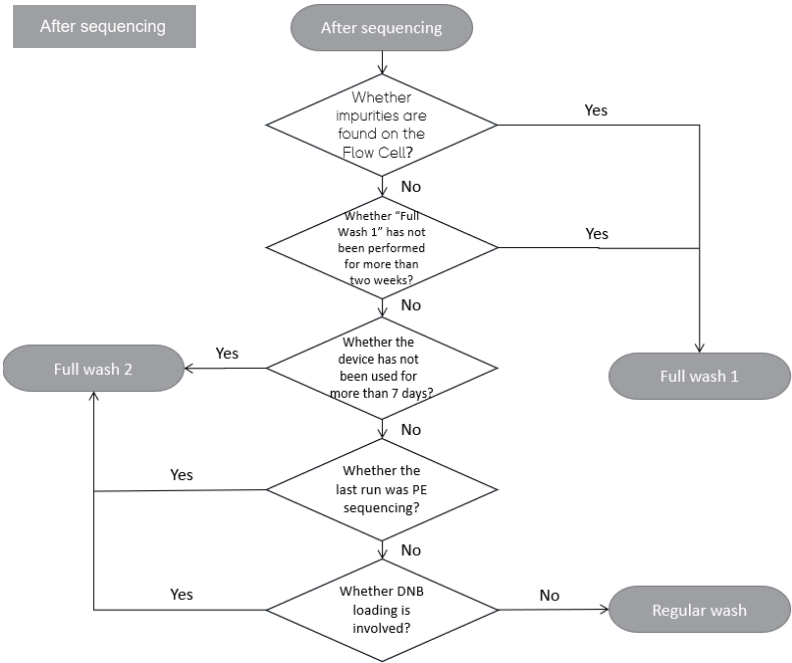



Figure 36 Wash instruction after sequencing

9.2 Wash type

Table 48 Wash requirments

Wash type	Description	Time
Regular wash	Procedure: Cleaning cartridge 1	About 48 min
Full wash 1	Procedure: Cleaning cartridge 3 > Cleaning cartridge 2 > Cleaning cartridge 1	About 76 min
Full wash 2	Procedure: Cleaning cartridge 4 > Cleaning cartridge 1	About 62 min

-  **Tips**
- Regular Wash: to remove residual reagents, reducing the risk of cross-contamination.
 - Full Wash 1 and Full Wash 2: to remove residual reagents and proteins in the pipeline, reducing the risk of blockage.

9.3 Preparing wash reagents

 **Tips** Validity period of cleaning reagents for 28 days if stored at 4 °C .

1. Prepare 0.05% Tween-20 following the table below.

Table 49 Wash reagent 1 preparation

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

2. Prepare 1 M NaCl+0.05% Tween-20 following the table below .

Table 50 Wash reagent 2 preparation

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

3. Prepare 0.1 M NaOH following the table below.

Table 51 Wash reagent 3 preparation

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

4. Prepare 0.05 % Tween-20+0.03 % ProClin300 following the table below.

Table 52 Wash reagent 4 preparation

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

9.4 Washing cartridge

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

- Wash the cleaning cartridge every time before refilling it with cleaning reagents. Replace cleaning cartridge after 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.

Table 53 Wash cleaning cartridge preparation

Cartridge name	2.0 mL cryotube	Large wells	No.15 well	Small wells
Cleaning cartridge 1	More than 95% volume of laboratory grade water			
Cleaning cartridge 2	1800 μ L Wash reagent 3	50 mL Wash reagent 3	6 mL Wash reagent 3	6 mL Wash reagent 3
Cleaning cartridge 3	1800 μ L Wash reagent 2	50 mL Wash reagent 1	6 mL Wash reagent 2	6 mL Wash reagent 1
Cleaning cartridge 4	1800 μ L Wash reagent 4	50 mL Wash reagent 4	6 mL Wash reagent 4	6 mL Wash reagent 4




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

9.5 Wash procedures

9.5.1 Regular wash

Perform the steps below:

1. 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.
2. Click the wash button on the interface.
3. Place the Flow Cell for washing.
4. Select regular wash from the drop-down menu to start the regular wash which takes about 48 minutes.

 **Tips** If perform the regular wash only, observe the status of the washing Flow Cell in this step. If many bubbles are observed, continue the wash. If not, stop the wash, replace the Flow Cell and re-start the wash.



Wash type: Regular ▾

Figure 37 Selecting the wash type

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

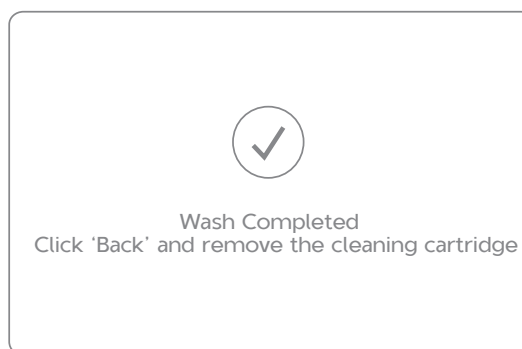


Figure 38 Regular wash end interface

9.5.2 Full wash 1

Perform the steps below:

1. Use cleaning cartridge 3. Open the reagent compartment door. Hold the handle of the cleaning cartridge 3 with one hand and place the other hand underneath 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.
2. Click the wash button on the interface.
3. Place the Flow Cell for washing.
4. Select the maintenance wash from the drop-down menu to start the maintenance wash which takes about 14 minutes.
5. Observe the status of the 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 re-start the wash.
6. When the figure below appears on the interface, click **Yes** and the sequencer will automatically lift the sampling needles. Then open the compartment door and replace the cleaning cartridge.



Figure 39 Maintenance wash [1] end interface

7. Use cleaning cartridge 2 and continue the maintenance wash which takes around 14 minutes.

8. When the figure below appears on the interface, click **No** to end the maintenance wash.



Figure 40 Maintenance wash end interface

9. 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.
10. Click the wash button on the interface.
11. Place the Flow Cell for washing.
12. Select regular wash from the drop-down menu to start the regular wash which takes about 48 minutes.



Figure 41 Selecting the wash type

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

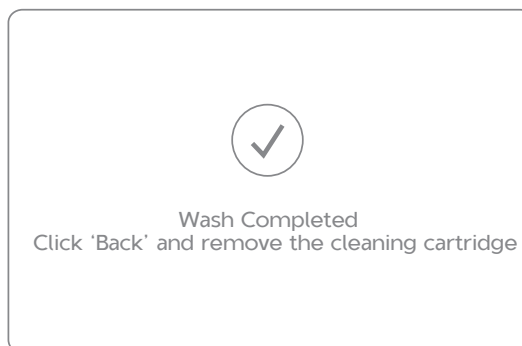


Figure 42 Regular wash end interface

9.5.3 Full wash 2

Perform the steps below:

1. 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 into the reagent compartment slowly following the direction printed on the cartridge cover until it stops. Close the reagent compartment door.
2. Click the wash button on the interface.
3. Place the Flow Cell for washing.
4. Select the maintenance wash from the drop-down menu to start the maintenance wash which takes about 14 minutes.
5. Observe the status of the 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 re-start the wash.

- When the interface appears as the figure below, click **No** to end the maintenance wash.



Figure 43 Maintenance wash end interface

- 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 48 minutes.



Figure 44 Selecting the wash type

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

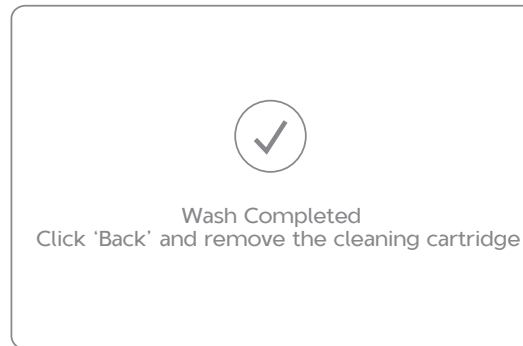


Figure 45 Regular wash end interface

Chapter 10 Troubleshooting

10.1 Low DNB concentration

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

- Check if the kit has expired.
- Check if the library meets the requirements.
- Make a new DNB preparation. You can order DNBSEQ DNB Make Reagent Kit (Catalog No. 1000016115) to make new DNBs. If the 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 steps below:

- Gently wipe the stage surface with a damp lint-free paper or a lint-free cloth and blow the stage with a power dust remover and ensure no dust is left.
- Blow the back of the Flow Cell with a dust remover to ensure no dust is left.
- If the problem persists, please contact a field service engineer.

10.3 Bubbles

If bubbles appear, try the steps below:

- Replace the used Flow Cell and inspect the pump.
- If the problem persists, please contact a field service engineer.

10.4 Impurities

If impurities appear, try the steps below:

- Perform a full wash on MGIDL-200RS and the sequencer following the MGIDL-200RS User Manual and *9.5 Wash procedures on Page 62* 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 onto the Flow Cell, or large bubbles appear in the Flow Cell, try the steps below:

- MGIDL-200RS and the sequencer: remove the Flow Cell, check if there are impurities in the sealing gasket and remove dust with the dust remover. Place the Flow Cell following the instruction in *8.5 Loading the Flow Cell on Page 53* 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 (no including dNTPs) and cannot be used within 24 hours, it can be frozen and thawed at most once.
- If a kit has been thawed (including dNTPs) but cannot be used immediately, store it at 2 °C to 8 °C. It is strongly recommended to use it within 24 hours. Mix the reagents in the cartridge following instruction in *Chapter 7 Preparing the sequencing reagent cartridge on Page 40* before use.
- If dNTPs and Sequencing Enzyme Mix have been added into the cartridge, i.e. the cartridge has been prepared but cannot be used immediately, store it at 2 °C to 8 °C and use it within 24 hours. Mix the reagents in the cartridge following instruction in *Chapter 7 Preparing the sequencing reagent cartridge on Page 40* before use.

- If dNTPs and Sequencing Enzyme Mix 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 2 °C to 8 °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 *Chapter 8 Sequencing on Page 47* and re-load the Flow Cell.
- When selecting *8.3 Selecting the sequencing parameters on Page 49*, choose program **Customize**.
- Select **Post loading** and click
- If starts from the Post loading prime, select **Prime** in the figure below. If starts from the step Post loading, don't select **Prime**.

<input checked="" type="checkbox"/> Start phase:	<input type="radio"/> DNB loading	<input checked="" type="radio"/> Post loading ...
		<input checked="" type="checkbox"/> Prime
	<input type="radio"/> Sequencing prime	<input type="radio"/> Sequencing

Figure 46 Selecting re-start Post loading

- Other steps please follow *Chapter 8 Sequencing on Page 47* in this manual.

10.8 Dark green crystals in well No.10

- If dark green crystals appear in well No.10, it is precipitation of raw materials of the reagent in well No.10. This is a normal phenomenon.

- When the cartridge is thawed, mix the reagents in the cartridge well and the crystals will dissolve. Sequencing quality will not be affected.



Figure 47 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 lower sequencing quality than expected. When the library amount is adequate, 100 μL Make DNB reaction is still required.

10.9.1 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 follows:

$$\text{ssDNA library input } V (\mu\text{L}) = 24 \text{ fmol} / \text{library concentration (fmol}/\mu\text{L})$$

- Calculate the required ssDNA library for each Make DNB reaction and fill it in the table below as V.

10.9.2 Making DNB

Perform the steps below:

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

Table 54 Make DNB reaction mix 1

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

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

Table 55 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 seconds and hold on ice.



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

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

Table 56 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 seconds using a mini centrifuge and place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

Table 57 Rolling circle amplification conditions

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

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



- Tips**
- It is very important to mix DNB gently using a wide-bore, non-filtered pipette tips.
 - Do not vortex, shake the tube or pipette vigorously.

8. Store the DNB at 4 °C and perform sequencing within 48 hours. Proceed to 4.4 *Quantifying DNB on Page 32*.

Appendix 1 Manufacturer

Manufacturer	Wuhan MGI Tech Co., Ltd.
	Latvia MGI Tech SIA
Address	Building B13, No.818, Gaoxin Avenue, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China
	Building 24, Stage 3.1, BioLake Accelerator, No.388, 2nd Gaoxin Road, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China
	"Lidostas parks", Marupes pag., Marupes nov., LV-2167,Latvia
E-mail	MGI-service@mgi-tech.com
Website	www.mgi-tech.com