Part No.: SOP-013-B01-082

DNBSEQ-G400RS

High-throughput (Rapid) Sequencing Set

User Manual | Version: 7.0

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

Address 2: Building 24, Stage 3.1, BioLake Accelerator, No.388, 2nd Gaoxin Road, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China

Address 3: Main Building and Second floor of No. 11 Building, Beishan Industrial Zone, Yantian District, 518083 Shenzhen, Guangdong, P.R.China

Address 4: Lidostas parks, Marupes pag., Marupes nov., LV-2167

E-mail: MGI-service@genomics.cn

Website: en.mgi-tech.com

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Latvia MGI Tech SIA
MGI Tech Co., Ltd.
Wuhan MGI Tech Co., Ltd.

About the user manual

This user manual is applicable to DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set. The manual edition is 7.0.

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Revision history

Version	Date	Description
7.0	March 2022	Added the product information in Latvia
6.0	February 2022	• Changed the logo • Changed the transport temperature below -15 ℃ to -80 ℃ ~ -15 ℃ • Updated the device maintenance
5.0	October 2021	• Added the product information of FCS PE300 • Changed instructions for reagent tank preparation of FCL PE200
4.0	December 2020	• 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.
3.0	May 2020	• Added the product information of High-throughput Rapid Sequencing Set (FCS). • Changed the Storage Temperature of Flow Cell to -25 ℃ ~ -15 ℃ 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.

Version	Date	Description
2.0	December 2019	• 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
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
940-000238-00	DNBSEQ-G400RS High-throughput Sequencing Set (LV SM FCL PE150)	V1.0
1000020834	cPAS Barcode Primer 3 Reagent Kit	V2.0
1000014048	cPAS Barcode Primer 4 Reagent Kit	V1.0

NOTE

- 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 the control software version of sequencer to be 1.5.0.1283, and the software version of BaseCall to be V1.2.0.164.

Contents

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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 Data analysis

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

1.4 Sequencing read length

Sequencing read length determines the number of sequencing cycles for a given sequencing run. For example, a PE150 cycle run performs reads of 150 cycles (2×150) for a total of 300 cycles. At the end of the insert sequencing run, an extra 10 cycles of index 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	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
PE300	300	300	10	600+10	620

1.5 Sequencing time and analysis time

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 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 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 Cell	12.7	25.0	35.6	96.3
Data analysis (Single Flow Cell)	0.4	0.5	0.6	2
Data analysis (Dual Flow Cell)	0.7	1.0	1.2	4

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

1.6 Attention

- This product is for research use only, please read the manual carefully before use.
- Make sure 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 happened.
- All the samples and waste materials should be disposed of according to relevant laws and regulations.
- This product is for one sequencing run only and cannot be reused.
- Do not use expired products.

Chapter 2 Sequencing sets and self-prepared consumables

2.1 List of sequencing set components

Table 4 DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE50)
Catalog number: 1000016941

Component	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985			
Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE50) Catalog number:1000016940			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
Make DNB Buffer	100 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II (LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5 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 5 DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE100)
Catalog number: 1000016943

Component	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985			
Sequencing Flow Cell	1 EA	-25 ℃ to-15 ℃	-80 ℃ to-15 ℃
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE100) Catalog number: 1000016942			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
Make DNB Buffer	100 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II (LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5 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 6 DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE400)
Catalog number: 1000016946

Component	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985			
Sequencing Flow Cell	1 EA	-25 ℃ to-15 ℃	-80 ℃ to-15 ℃
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE400) Catalog number: 1000016944			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
Make DNB Buffer	100 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II (LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5 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 7 DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE100)
Catalog number: 1000016950

Component	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985			
Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Sequencing Kit(FCL PE100) Catalog number: 1000016949			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
Make DNB Buffer	100 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II (LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5 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	0.60 mL×1 tube		
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 8 DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150)
Catalog number: 1000016952

Component	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985			
Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Sequencing Kit(FCL PE150) Catalog number: 1000016951			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
Make DNB Buffer	100 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II (LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5 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	0.60 mL×1 tube		
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 9 DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE200)
Catalog number: 940-000151-00

Component	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985			
Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Sequencing Kit(FCL PE200) Catalog number: 940-000153-00			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
Make DNB Buffer	100 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II (LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5 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 10 DNBSEQ-G400RS High-throughput Sequencing Set (Small RNA FCL SE50) Catalog number: 1000016998

Component	Spec&quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985			
Sequencing Flow Cell	1 EA	-25 ℃ to-15 ℃	-80 ℃ to-15 ℃
DNBSEQ-G400RS High-throughput Sequencing Kit(FCL SE50) Catalog number: 1000016940			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
Make DNB Buffer	100 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II (LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5 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 ℃ to 8 ℃	2 ℃ to 8 ℃

Table 11 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS SE100) Catalog number: 1000016978

Component	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987			
Sequencing Flow Cell	1 EA	-25 ℃ to-15 ℃	-80 ℃ to-15 ℃
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS SE100) Catalog number: 1000016977			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
Make DNB Buffer	100 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II (LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5 mL (Empty)	1 tube		
dNTPs Mix	0.90 mL×1tube		
dNTPs Mix II	1.70 mL×1 tubes		
Sequencing Enzyme Mix	1.90 mL×1 tube		
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 12 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE100) Catalog number: 1000016980

Component	Spec&quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987			
Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE100) Catalog number: 1000016979			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
Make DNB Buffer	100 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II (LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5 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	0.60 mL×1 tube		
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 13 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE150) Catalog number: 1000016982

Component	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987			
Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE150) Catalog number: 1000016981			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
Make DNB Buffer	100 μL×1 tube		
Make DNB Enzyme Mix I	200 μL×1 tube		
Make DNB Enzyme Mix II (LC)	25 μL×1 tube		
Stop DNB Reaction Buffer	100 μL×1 tube		
DNB Load Buffer I	200 μL×1 tube		
DNB Load Buffer II	200 μL×1 tube		
Micro Tube 0.5 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	0.60 mL×1 tube		
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 14 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE300) Catalog number: 940-000152-00

Component	Spec&quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number: 1000016987			
Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE300) Catalog number: 940-000154-00			
Low TE Buffer	300 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
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 15 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 μM AD153 Barcode Primer 3	3.50 mL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃

Table 16 DNBSEQ-G400RS High-throughput Sequencing Set (LV SM FCL PE150) Catalog number: 940-000238-00

Component	Spec&quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell (LV FCL) Catalog number: 940-000240-00			
Sequencing Flow Cell	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ-G400RS High-throughput Sequencing Kit (LV SM FCL PE150) Catalog number: 940-000239-00			
Low TE Buffer	300 μL×1 tube		
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	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
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	0.60 mL×1 tube		
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

Table 17 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 μM AD153 Barcode Primer 4	3.50 mL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃

2.2 Self-prepared equipment and consumables

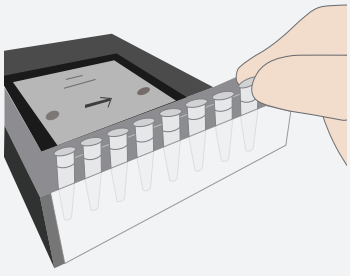
Table 18 Self-prepared equipment and consumables

Equipment and consumables	Recommended brand	Catalog number
Qubit 3.0 Fluorometer	Thermo Fisher	Q33216
Mini centrifuge	Major Laboratory Supplier (MLS)	/
Vortex mixer	MLS	/
Thermal cycler	Bio-Rad	/
Pipette	Eppendorf	/
2 ℃ to 8 ℃ refrigerator	MLS	/
-25 ℃ to -15 ℃ refrigerator	MLS	/
Qubit ssDNA Assay Kit	Thermo Fisher	Q10212
Power dust remover	MATIN	M-6318
Sterile pipette tip(box)	AXYGEN	/
200 µL Wide-Bore Pipette Tips	AXYGEN	T-205-WB-C
Qubit Assay Tubes	Thermo Fisher	Q32856
100%Tween-20	MLS	/
5 M NaCl solution	MLS	/
2 M NaOH solution	MLS	/
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	/
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

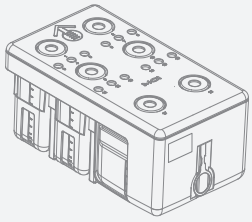
Chapter 3 Sequencing workflow

Making DNB: use DNB preparation kit for making DNB.

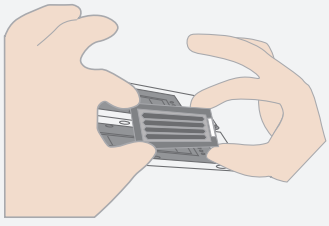
Preparing a new Flow Cell: take out the Flow Cell from package and inspect to ensure the Flow Cell is intact.



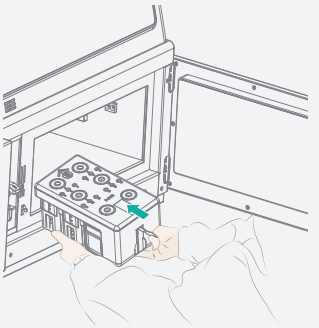
DNB loading: load the DNB into sequencing Flow Cell.



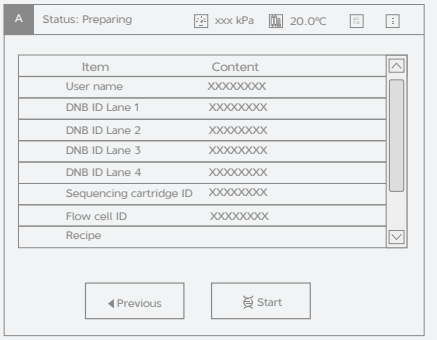
Preparing a new reagent cartridge: inspect and thaw the reagent cartridge and then load and mix the necessary reagents.



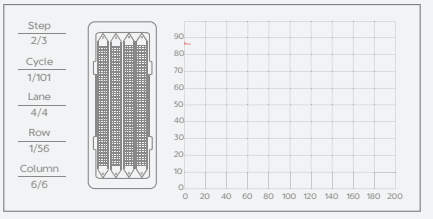
Loading the Flow Cell: place the Flow Cell on the stage of the sequencer.



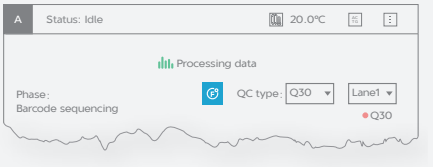
Loading the reagent cartridge into the sequencer.



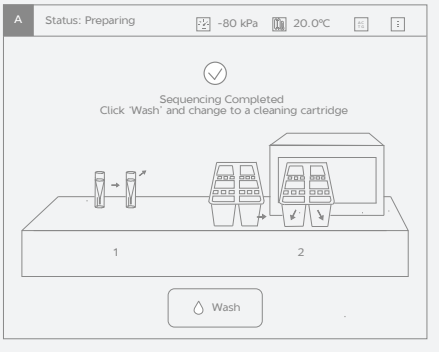
Starting sequencing: follow the instructions to enter sequencing information and start the run.



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



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



Device maintenance: perform device maintenance when sequencing is completed.

Chapter 4 Making 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 19 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

NOTE
- 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 20 Library requirement

Libraries	library concentration (bp)
general libraries	≥ 2 fmol/μL
small RNA libraries	≥ 3 fmol/μL
PCR free	≥ 3.75 fmol/μL

NOTE
- If the library concentration is unknown, it is recommended to perform ssDNA library quantitation (ng/μL) using Qubit ssDNA Assay Kit and Qubit 3.0 Fluorometer. Use the equation below to convert the concentration of the ssDNA library from ng/μL to fmol/μL:

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

4.3 Making DNB

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 20*

Scheme 2: Make DNB of other, see *4.3.2 Making DNB of other on Page 25*

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.

NOTE
- Mixed use of reagent components from different batches is strictly prohibited.
- PE300 uses Make DNB rapid Enzyme Mix II, do not use it wrong.

4.3.1.2 Selecting the DNB loader

Each DNBSEQ-G400RS rapid sequencing Flow Cell contains 2 lanes. DNB can be loaded into 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 45μL DNB.

- When using the MGIDL-200RS to load DNB

  Different DNBs can be loaded into different lane. Each lane requires 45 μL DNB.

- When using the MGIDL-200H to load DNB

  Different DNBs can be loaded into different lane. Each lane requires 22.5 μL DNB.

Table 21 The required number of make DNB reactions for each DNBSEQ-G400RS rapid Flow Cell

Loading system	DNB loading volume (μL)/Lane	Make DNB reaction (μL)	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 *4.2 Library concentration and amount requirement on Page 20*.

Table 22 The volume of ssDNA library

Library	The volume of 90 μL DNB reaction (μL)	The volume of 45 μL DNB reaction (μL)
Normal library	V=40 fmol/C	V=20 fmol/C
PCR free	V=75 fmol/C	V=37.5 fmol/C

NOTE
- 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.

- Calculate the required ssDNA library for each Make DNB reaction and fill it in *Table 23 Make DNB reaction mix 1 on Page 22* 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 23 Make DNB reaction mix 1

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

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

Table 24 Primer hybridization reaction condition

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

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

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

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

Table 25 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

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

Table 26 Rolling circle amplification conditions

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

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

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

Table 27 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

NOTE
- 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 ℃ and perform sequencing within 48 hours.

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 reagnet 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.

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

4.3.2.2 Selecting the DNB loader

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 into different lanes. Each lane requires 50 μL DNB.

- When using the MGIDL-200H to load DNB

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

Table 28 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

Flow Cell type	Loading system	DNB volume (μL) / Lane	Make DNB reaction (μL)	The required number of make DNB reaction / Flow Cell
FCS	Sequencer	50	100	1-2
	MGIDL-200RS	50	100	
	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 *4.2 Library concentration and amount requirement on Page 20*.

Table 29 The required amount of ssDNA library

Library	The volume of 90 μL DNB reaction (μL)
Normal library	V=40 fmol / C
Small RNA libraries	V=60 fmol / C

NOTE
- 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.

- Calculate the required ssDNA library for each Make DNB reaction and fill it in *Table 30 Make DNB reaction mix 1 on Page 27* as V.

4.3.2.4 Making DNB

Perform the steps below:

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

Table 30 Make DNB reaction mix 1

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

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

Table 31 Primer hybridization reaction condition

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

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

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

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

Table 32 Make DNB reaction mix 2

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

7. 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.
8. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

Table 33 Rolling circle amplification conditions

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

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

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

NOTE
- It is very important to mix DNB gently using a wide bore pipette tip.Do not centrifuge, vortex, or shake the tube.
- Store DNB at 4 ℃ 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 3.0 Fluorometer to quantify the DNB.

Table 34 DNB concentration standard

Type	Normal library	Application library
FCS PE300	≥10 ng/µL	≥8 ng/µL
The Others	≥8 ng/µL	≥8 ng/µL

NOTE
- If the concentration is lower than 8 ng/μL, see *10.1Low 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 ng/μL, the DNBs should be diluted to 20 ng/μL according to the table below:

Table 35 DNB dilution scheme

Type	Component	Storage conditions	Storage time
FCS PE300	Low TE Buffer	4 ℃	≤4 h
The Others	DNB Load Buffer I	4 ℃	≤48 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.

   NOTE 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.

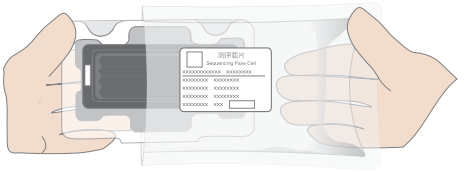


Figure 1 Unwrapping the outer package

NOTE
- 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 ℃ to -15 ℃ for storage. But the switch between room temperature and -25 ℃ to -15 ℃ must not exceed 3 times.
- If the outer plastic package has been opened but the Flow Cell 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.

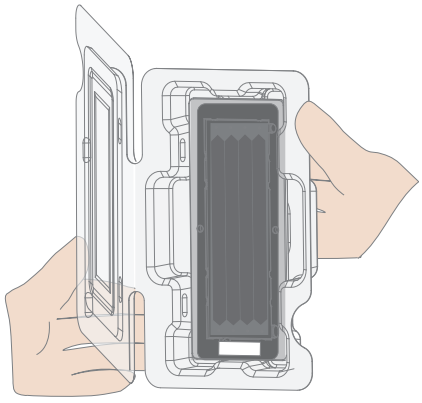


Figure 2 Inspecting the Flow Cell

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 31* 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 34 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 36 DNB loading mix 1 (For sequencer loading)

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

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.

NOTE
- 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

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

Table 37 DNB loading mix 2

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

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

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

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

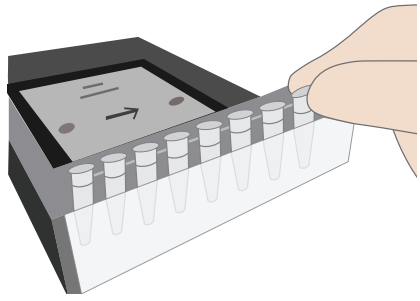


Figure 3 Placing the loading samples

4. Press the Flow Cell attachment button, hold the Flow Cells by edges and align the holes on the Flow Cells with the locating pins on the Flow Cell stages. Press the left and right sides of the Flow Cell on the stage at the same time to ensure that the Flow Cells are securely seated on the stage.
5. Select the 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 room temperature.

   NOTE After DNB loading, place it at room temperature for 60-90 minutes, then immediately place it on the sequencer for use,don't exceed 90 minutes.

6.1.3 MGIDL-200H DNB loading

NOTE
- 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 38 DNB loading mix 3 (for MGIDL-200H loading)

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 °C until use.

NOTE
- 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.

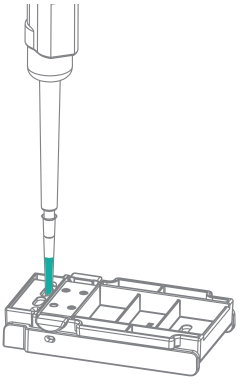


Figure 4 Loading samples using MGIDL-200H

NOTE
- 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.

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

NOTE About PE300 flow cell, it is recommended to place it on the MGIDL-200H to incubation 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 39 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

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

NOTE
- 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

NOTE
- 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 40 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 ℃ until use.

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

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

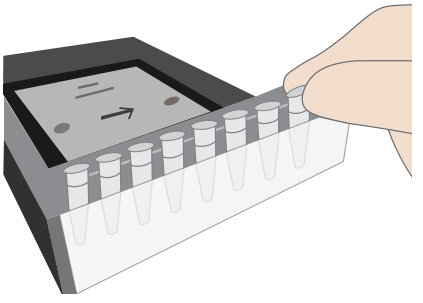


Figure 5 Placing the loading samples

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

NOTE
- 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 (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

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.

NOTE
- 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.

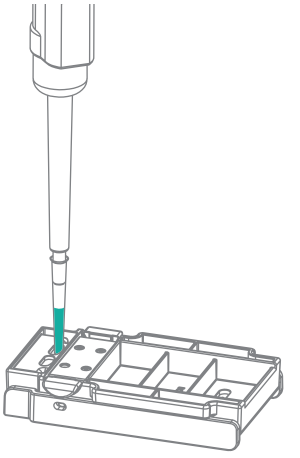


Figure 6 Loading samples using MGIDL-200H

NOTE
- 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.

5. Eject the tip from the pipette and the DNB loading mix will automatically flow into the Flow Cell.
6. After DNB loading, rotate the tips counterclockwise to take out them. 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 with room temperature until completely thawed (or thaw in 2 ℃ to 8 ℃ fridge one day in advance). Store at 2 ℃ to 8 ℃ storage until use.

   NOTE After being taked out form -25 ℃ to -15 ℃ , 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 for 10 to 20 times and making sure reagents are fully mixed.

   NOTE If dark green crystals appear in well No.10, it is precipitation of raw materials of the reagent in well No.10. This is a normal phenomenon. When the cartridge is thawed, mix the reagents in the cartridge well and the crystals will dissolve. Sequencing quality will not be affected. See *10.8 Dark green crystals in well No.10 on Page 71* in this manual for details.

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

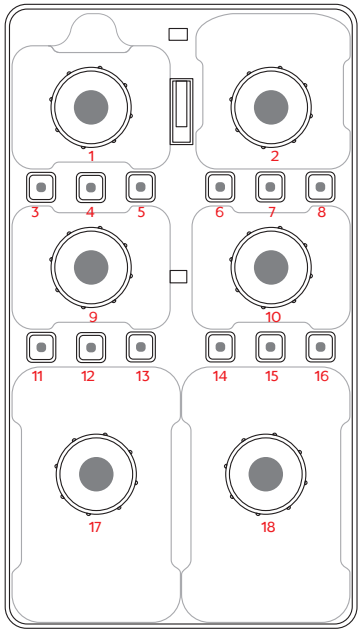


Figure 7 Well position

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

7.1 Preparing FCL SE50/FCL SE100/ FCS SE100 sequencing cartridge

Perform the steps below:

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

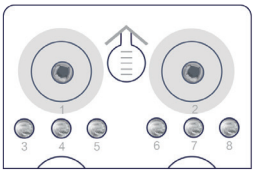


Figure 8 Piercing the seal of FCL SE50/FCL SE100 /FCS SE100 cartridge

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

Table 42 FCL SE50/FCL SE100 /FCS SE100 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 SE100	1.100	1.100
FCS SE100	0.800	0.800

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

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

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

Table 43 FCL SE50/FCL SE100 /FCS SE100 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 SE100	0.900	0.900
FCS SE100	1.600	0.800

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

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

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

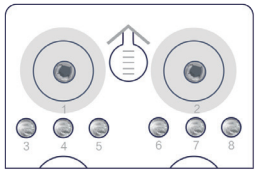


Figure 9 Seal the loading wells of FCL SE50/FCL SE100 /FCS SE100 cartridge

7. Press the film with thumb 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.

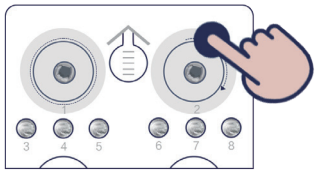


Figure 10 Seal the loading wells tightly of FCL SE50/FCL SE100 /FCS SE100 cartridge

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

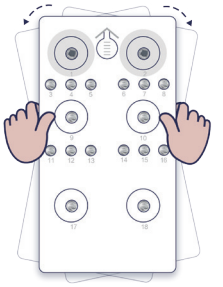


Figure 11 Mixing reagents after loading

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

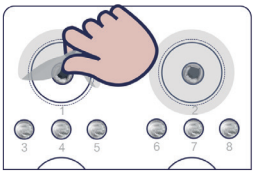


Figure 12 Removing the seal from FCL SE50/FCL SE100 /FCS SE100 cartridge after mixing

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

7.2 Preparing the FCL PE100/FCL PE150/ FCL PE200/FCS PE100/ FCS PE150/ FCS PE300 sequencing cartridge

Perform the steps below:

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

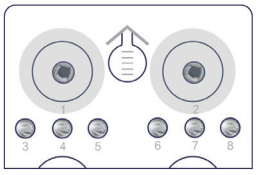


Figure 13 Piercing the seal of FCL PE100/FCL PE150/FCL PE200/FCS PE100/ FCS PE150/FCS PE300 cartridge

2. Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix into a new 5 mL or 10 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube.

Table 44 FCL PE100/FCL PE150/FCL PE200/FCS PE100/FCS PE150/FCS PE300 sequencing cartridge well No.1 reagent loading

Product model	dNTPs Mix loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
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

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

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

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

Table 45 FCL PE100/FCL PE150/FCL PE200/FCS PE100/FCS PE150/FCS PE300 sequencing cartridge well No.2 reagent loading

Product model	dNTPs Mix II loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
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

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

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

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

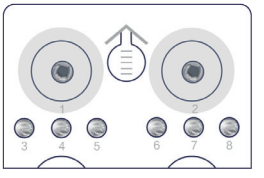


Figure 14 Sealing the loading wells of FCL FCL PE100/FCL PE150/FCL PE200/FCS PE100/FCS PE150/FCS PE300 cartridge

7. Press the film with thumb 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.

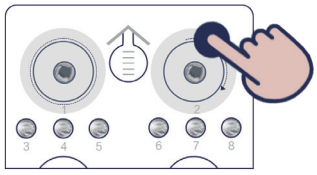


Figure 15 Sealing the loading wells of FCL PE100/FCL PE150/FCL PE200/FCS PE100/FCS PE150/FCS PE300 cartridge

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

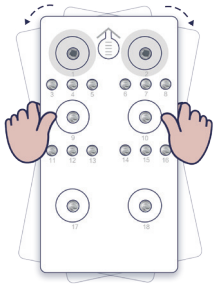


Figure 16 Mixing reagents after loading

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

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

11. Invert the tube for 4 to 6 times to mix the reagents. Pierce the seal of well No.15 using 1 mL sterile tip, then add the mixture to well No.15. When adding the mixture, making sure there are no bubbles at the bottom of the tube.

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

12. Removing the seal of loading wells from the cartridge carefully after fully mixing.

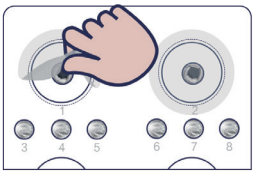


Figure 17 Removing the seal from FCL PE100/FCL PE150/FCL PE200/FCS PE100/FCS PE150/FCS PE300 cartridge after mixing

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

7.3 Preparing the FCL SE50 (Small RNA) /FCL SE400 sequencing cartridge

Perform the steps below:

1. Take out the Wash Buffer For Small RNA Sequencing or Wash Buffer For Sequencing from storage and thaw at room temperature.
2. 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.

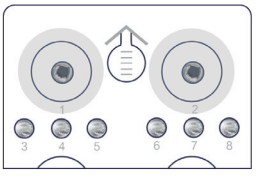


Figure 18 Piercing the seal of FCL SE50/FCL SE400 cartridge

3. Take a pipette with the appropriate volume range and add them into well No.1 following the table below. First add dNTPs Mix into a new 5 mL or 15 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube.

Table 46 FCL SE50/FCL SE400 sequencing cartridge well No.1 reagent loading

Product model	dNTPs Mix loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
FCL sRNA SE50	0.700	0.700
FCL SE400	4.000	4.000

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

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

5. Take a pipette with the appropriate volume range and add them into well No.2 following the table below.First add dNTPs Mix II into a new 5 mL or 25 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix II in the same tube.

Table 47 FCL SE50/FCL SE400 sequencing cartridge well No.2 reagent loading

Product model	dNTPs Mix loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
FCL sRNA SE50	0.600	0.600
FCL SE400	12.000	12.000

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

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

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

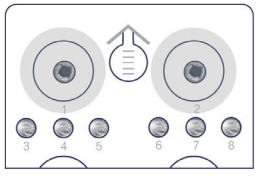


Figure 19 Sealing the loading wells of FCL sRNA SE50 cartridge

8. Press the film with thumb 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.

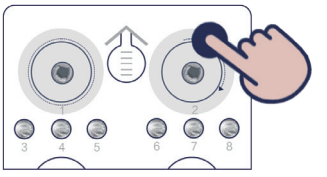


Figure 20 Sealing the loading wells of FCL sRNA SE50 cartridge

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

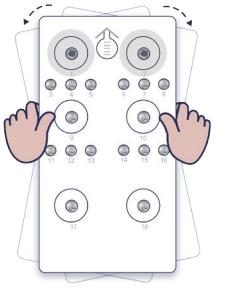


Figure 21 Mixing reagents after loading

10. Perform the following steps according to different situations:

    1) 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.
    2) 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.

NOTE Wash Buffer For Small RNA Sequencing 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.

11. Removing the seal of loading wells from the cartridge carefully after fully mixing.

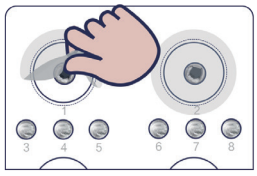


Figure 22 Removing the seal from cartridge after mixing

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

12. The sequencing cartridge is now ready for use.

7.4 Dual Barcode Sequencing

Perform the following steps according to different situations:

- For dual barcode sequencing of PE, perform the following steps after finishing the preparation of PE sequencing cartridge:
  1) Take out the 1μM AD153 Barcode Primer 3 from the cPAS Barcode Primer 3 Reagent Kit and thaw at room temperature.
  2) Mix the 1μM AD153 Barcode Primer 3 using a vortex mixer for 5 seconds and centrifuge briefly before use.
  3) Pierce the seal of well No.4 using a sterile tip, then add 2.90 mL of the 1μM AD153 Barcode Primer 3. When adding the reagent, make sure there are no bubbles at the bottom of the tube
- For dual barcode sequencingof SE, perform the following steps after finishing the preparation of SE sequencing cartridge:
  1) Take out the 1μM AD153 Barcode Primer 4 from the cPAS Barcode Primer 4 Reagent Kit and thaw at room temperature.

2) Mix the 1μM AD153 Barcode Primer 4 using a vortex mixer for 5 seconds and centrifuge briefly before use.
3) Pierce the seal of well No.4 using a sterile tip, then add 2.90 mL of the 1μM AD153 Barcode Primer 4. When adding the reagent, make sure there are no bubbles at the bottom of the tube.

Chapter 8 Sequencing

8.1 Entering the main interface

Perform the steps below:

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

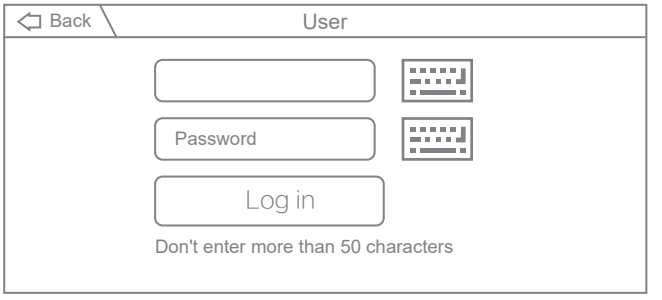


Figure 23 Log-in interface

2. The main interface is as below:

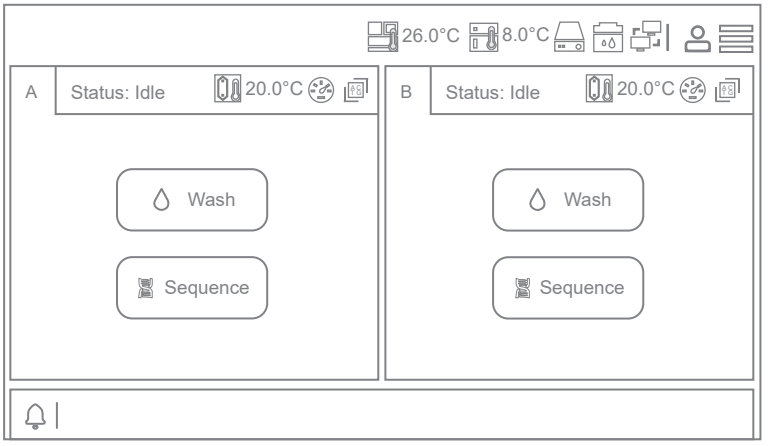


Figure 24 Main interface

8.2 Loading the DNBs

Perform the steps below:

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

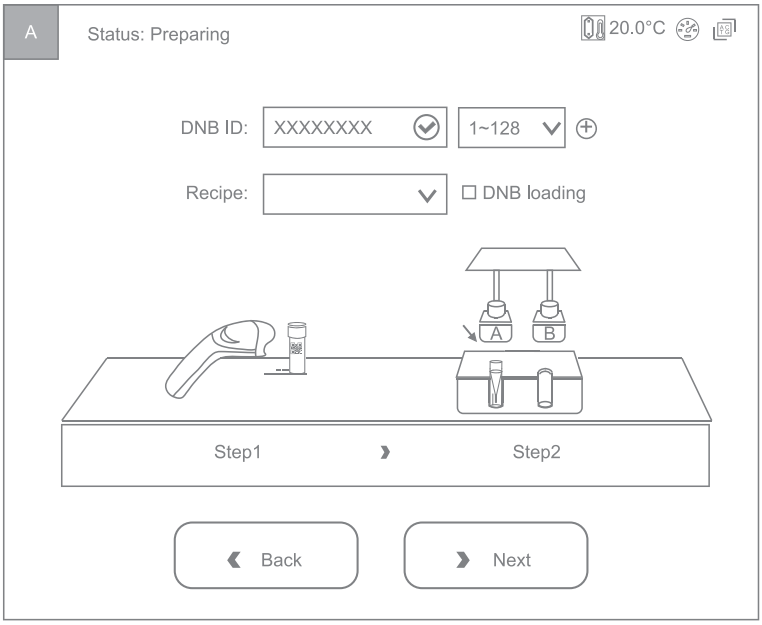


Figure 25 DNB loading interface

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

   NOTE Select 4 lanes for FCL and 2 lanes for FCS.

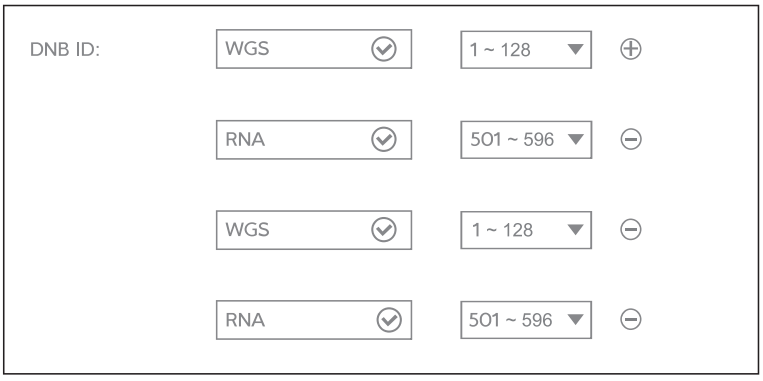


Figure 26 DNBs and information selection interface

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.
5. 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, Place an empty tube.

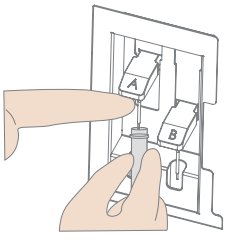


Figure 27 Loading the DNBs tube

6. Close the reagent compartment door.

8.3 Selecting the sequencing parameters

Perform the steps below:

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

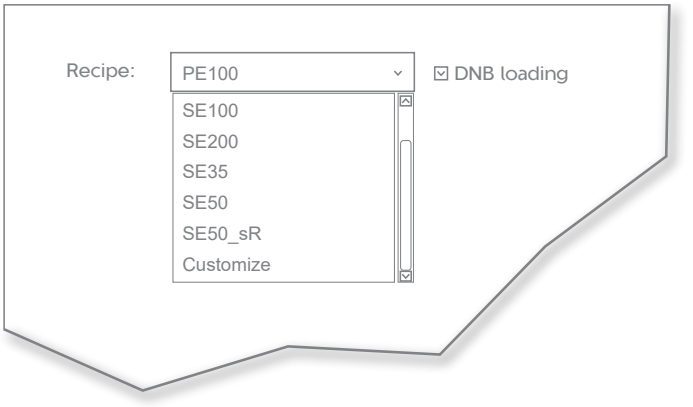


Figure 28 Selecting sequencing solutions

NOTE 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 54*. If you choose **Customize**, continue performing the steps below.
3. In the beginning, please select a step to start the sequencing run.

Figure 29 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.

Figure 30 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.

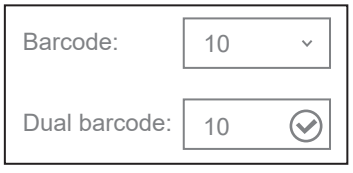


Figure 31 Selecting the barcode length

6. Select the lane for barcode demultiplexing.

Figure 32 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.

Figure 33 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.

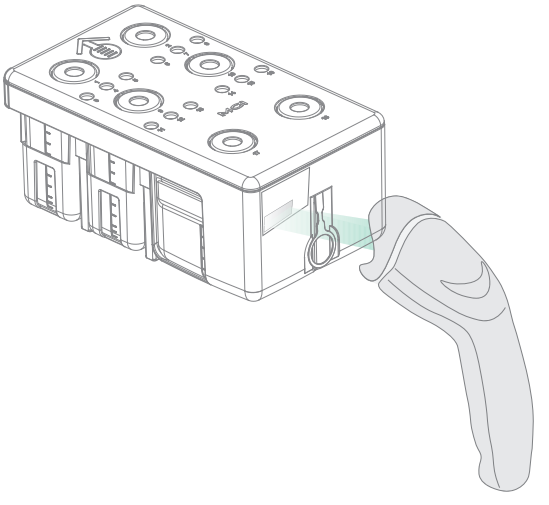


Figure 34 Reagent cartridge information entry interface

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

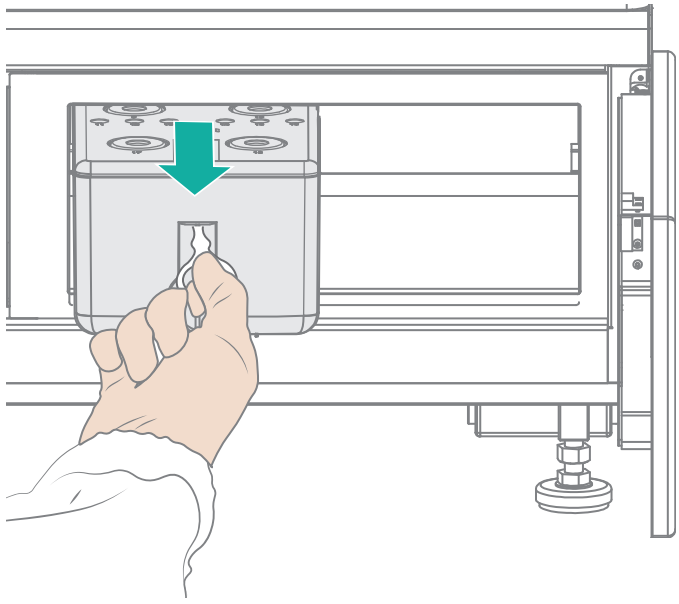


Figure 35 Removing cleaning cartridge

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.

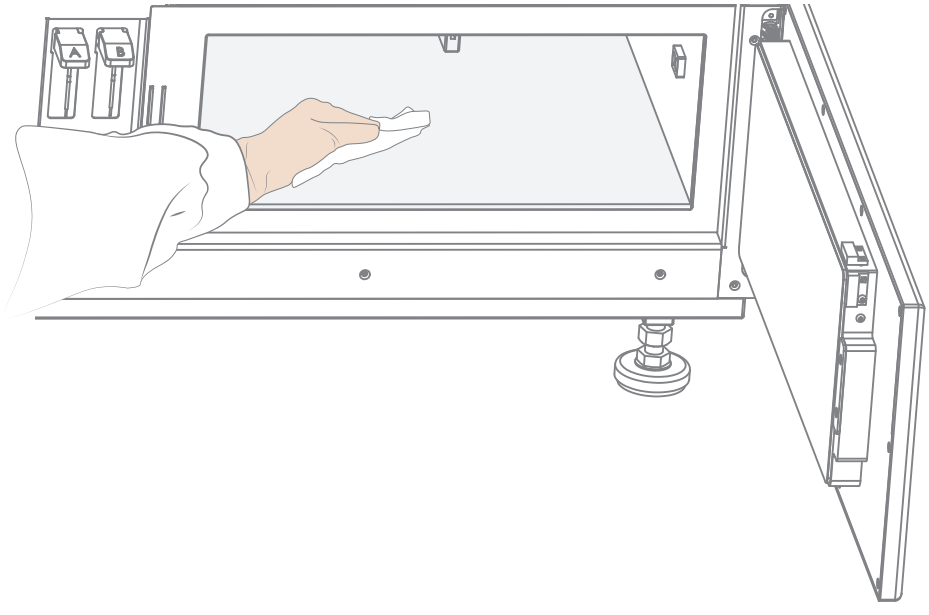


Figure 36 Maintaining the reagent compartment

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.

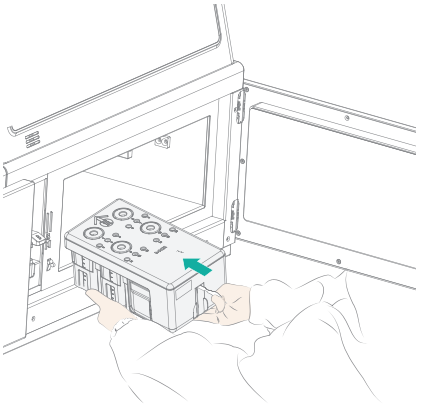


Figure 37 Sliding the new reagent cartridge into the reagent compartment

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.

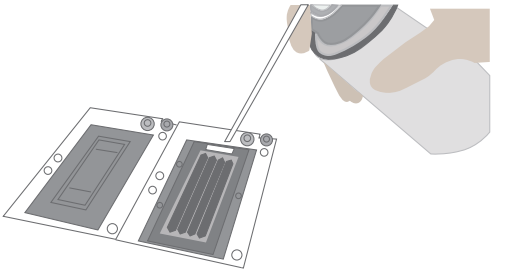


Figure 38 Cleaning the Flow Cell stage

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

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

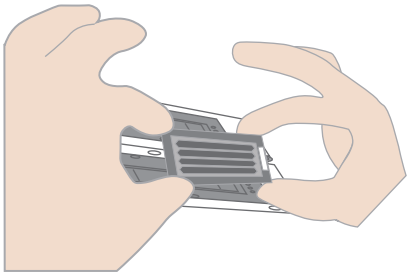


Figure 39 Loading the Flow Cell

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.

   NOTE 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 69* 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.

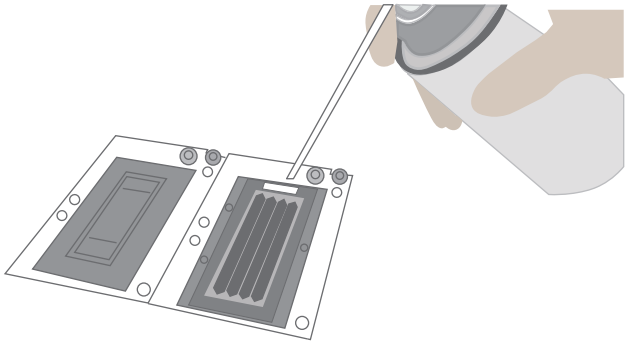


Figure 40 Cleaning the Flow Cell

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.

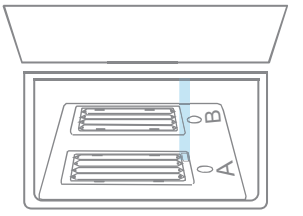


Figure 41 Flow Cell information entry interface

12. Click **Next**.

8.6 Reviewing parameters

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

Item	Content
User name	user
DNB ID Lane 1	WGS | 1 ~ 128
DNB ID Lane 2	RNA | 501 ~ 596
DNB ID Lane 3	WGS | 1 ~128
DNB ID Lane 4	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 42 Reviewing 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 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.

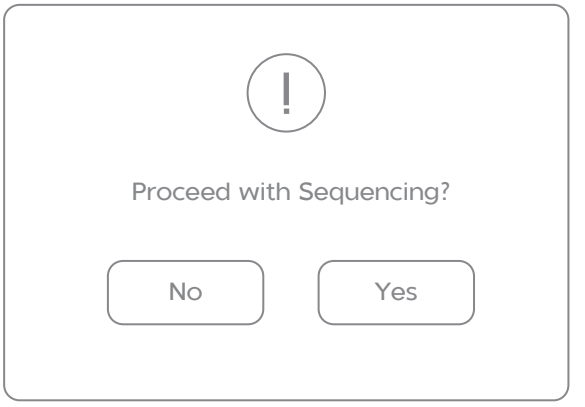


Figure 43 Confirming sequencing interface

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

Chapter 9 Device Maintenance

9.1 Wash type

Table 48 Wash requirments

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

9.2 Wash instruction

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

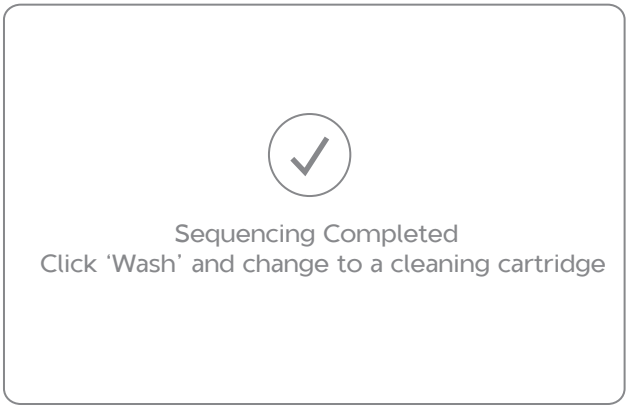


Figure 44 Wash interface

- When the sequencing is completed, the device needs to be washed within 24 hours.
- A Full Wash is required if the sequencer is used for A) a PE run or B) a DNB loading/post-load. 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.
- After the replacement of pipelines, sample needles and other accessories exposed to reagents, please perform a full wash.
- If the sequencer is to be powered off for more than 7 days, perform a maintenance wash before being powered off and after being powered on.
- If the sequencer was left unused for more than 7 days after a full wash, please perform a full wash before use.
- If impurities are found on the Flow Cell, perform a full wash.

- Refer to the flow chart below for details:

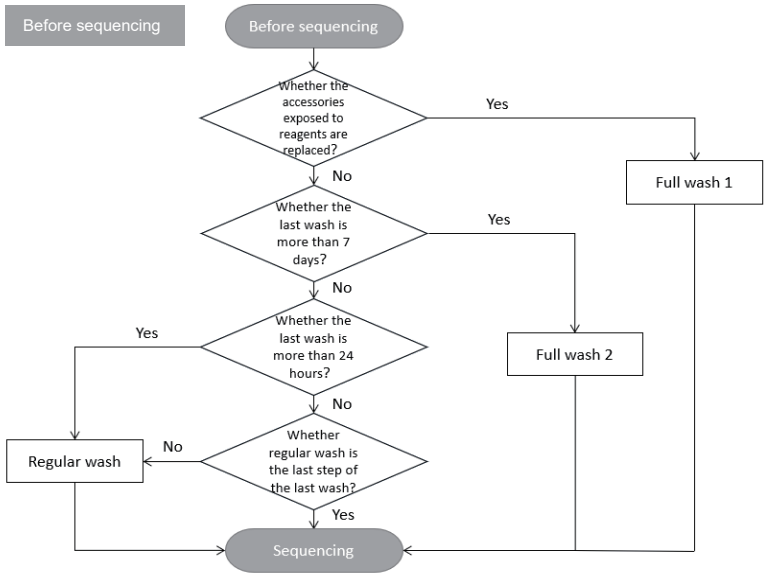


Figure 45 Wash instruction after sequencing

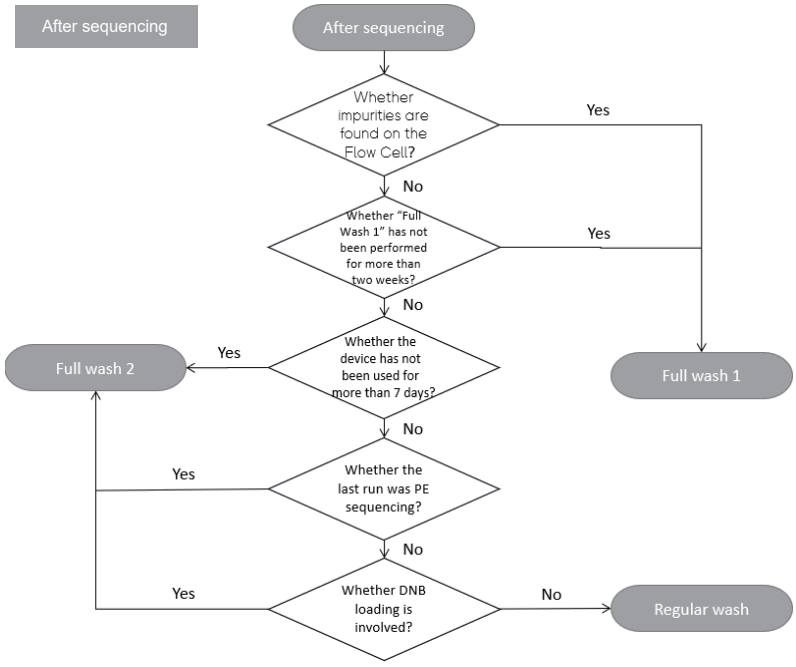


Figure 46 Wash instruction after sequencing

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

9.3 Preparing wash reagents

NOTE Validity period of cleaning reagents for 28 days if stored at 4 ℃ .

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

Table 49 Wash reagents preparation 1

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 reagents preparation 2

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 reagents preparation 3

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 reagents preparation 4

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
Wash cleaning cartridge 1	More than 95% volume of laboratory grade water			
Wash cleaning cartridge 2	1800 µL Wash reagent 3	50 mL Wash reagent 3	6 mL Wash reagent 3	6 mL Wash reagent 3
Wash cleaning cartridge 3	1800 µL Wash reagent 2	50 mL Wash reagent 1	6 mL Wash reagent 2	6 mL Wash reagent 1
Wash cleaning cartridge 4	1800 µL Wash reagent 4	50 mL Wash reagent 4	6 mL Wash reagent 4	6 mL Wash reagent 4

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

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.
5. 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 47 Selecting the wash type

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

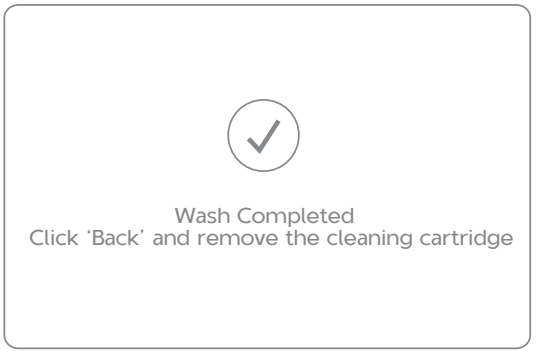


Figure 48 Regular wash end interface

9.5.2 Maintenance 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 to 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 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.
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 49 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 50 Maintenance wash end interface

9.5.3 Full wash procedures

Perfrom Maintenance wash 1, followed by Regular wash, with a total time of around 76 minutes.

9.5.4 Maintenance 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 to 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 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.

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

Figure 51 Maintenance wash end interface

9.5.5 Full wash procedures 2

Perfrom Maintenance wash 2, followed by Regular wash, with a total time of around 62 minutes.

Chapter 10 Troubleshooting

10.1 Low DNB concentration

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

- Check if the kit has expired.
- Check if the library meets the requirements.
- Make a new DNB preparation. You can order DNBSEQ DNB Make Reagent Kit (Catalog No. 1000016115) 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 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 66* 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 steps below:

- 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 Loading the Flow Cell on Page 56* 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 the kit has been thawed (including dNTPs) but cannot be used immediately, store it at 4 ℃ and use it within 24 hours. Mix

the reagents in the cartridge following instruction in *Chapter 7 Preparing the sequencing reagent cartridge on Page 38* 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 4 ℃ and use it within 24 hours. Mix the reagents in the cartridge following instruction in *Chapter 7 Preparing the sequencing reagent cartridge on Page 38* 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 4 ℃ 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 50* and re-load the Flow Cell.
- When selecting *8.3 Selecting the sequencing parameters on Page 52*, 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**.

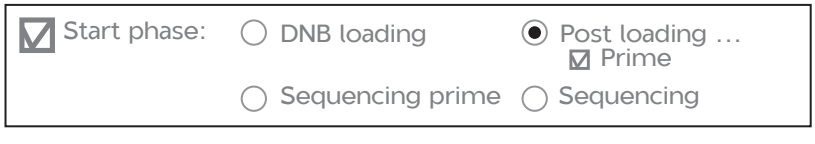


Figure 52 Selecting re-start Post loading

- Other steps please follow *Chapter 8 Sequencing on Page 50* 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 53 Dark green crystals in well No.10

10.9 Library amount less than 40 fmol

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

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 followed:

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

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

2. Making DNB

   1) Take a 0.2 mL 8-tube strip or PCR tubes. Prepare 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

3. Mix gently by vortexing and centrifuge for 5 seconds using a mini centrifuge.
4. 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 ℃)	On
95 ℃	1 min
65 ℃	1 min
40 ℃	1 min
4 ℃	Hold

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

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

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

Table 56 Make DNB reaction mix 2

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

8. 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 ℃)	On
30 ℃	25 min
4 ℃	Hold

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

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

10. Store the DNB at 4 ℃ and perform sequencing within 48 hours. Proceed to *4.4 Quantifying DNB on Page 28*.

Appendix 1 Manufacturer

Manufacturer	MGI Tech Co., Ltd.
	Wuhan MGI Tech Co., Ltd.
	Latvia MGI Tech SIA
Address	Main Building and Second floor of No.11 Building, Beishan Industrial Zone, Yantian District, Shenzhen, 518083, Guangdong, China
	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
Service hotline	4000-966-988
E-mail	MGI-service@genomics.cn
Website	en.mgi-tech.com

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