Part No.: SOP-013-B01-080

High-throughput Sequencing Set

DNBSEQ-T7RS

Instructions for use

Version: 9.0

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-T7RS High-throughput Sequencing Set. The instructions for use version is 9.0.

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For StandardMPS and CoolMPS: Unless otherwise informed, StandardMPS and CoolMPS sequencing reagents, and sequencers for use with such reagents are not available in Germany, Spain, UK, Sweden, Belgium, Italy, Finland, Czech Republic, Switzerland, Portugal, Austria and Romania. Unless otherwise informed, StandardMPS sequencing reagents, and sequencers for use with such reagents are not available in Hong Kong.

Revision history

Version	Date	Description
9.0	September 2024	• Updated the Data output. • Revised the Preparation before sequencing, the operations order of Loading DNBs, and added the descriptions for dual barcode primers replacement. • Deleted the related information about Shenzhen MGI in the appendix 3 and added the appendix 4.
8.0	August 2023	• Updated the user name and password of MGIDL-T7RS and DNBSEQ-T7RS. • Updated App-D Primer Kit. • Revised the enzyme in DNB loading mixture for stLFR FCL PE100. • Updated disclaimer. • Added sample requirements and data access.
7.0	October 2022	• Updated the catalog number of PE100 and PE150 sequencing kits. Revised the loading volume in the "Preparing the sequencing reagent cartridge" section. • Updatded the catalog number of Load Reagent Kit for SE read length. • The input amount of general libraries was updated from 40 fmol to 60 fmol.
6.0	February 2022	• Updated logo of MGI. • Updated transport temperature. • Deleted the description of the small RNA adapter in App-D.
5.0	July 2021	• Updated disclaimer. • Added the validity of reagents.
4.0	July 2021	• Added App-D PE150. • Updated the DNB preparation and load DNB for PE150. • Updated the sequencing cartridge well No.9 and No.10 reagent adding.
A2	December 2020	Updated the logo, website address and mailbox, and template of the instructions for use .

Version	Date	Description
A1	November 2020	• Added the SE35, SE50, SE100 and PE150 read length. • Added Dual barcode PE sequencing. • Added the stLFR PE100. • Added the App-A PE100 and App-A PE150. • Updated part of PUI figures. • Revised DNB pooling. • Added an attachment for quantify DNB.
A0	December 2019	Initial release

Sequencing set

Cat. No.	Name	Model	Version
940-000270-00	DNBSEQ-T7RS High-throughput Sequencing Set	FCL SE35	V2.0
940-000271-00	DNBSEQ-T7RS High-throughput Sequencing Set	FCL SE50	V2.0
940-000272-00	DNBSEQ-T7RS High-throughput Sequencing Set	FCL SE100	V2.0
940-000269-00	DNBSEQ-T7RS High-throughput Sequencing Set	FCL PE100	V3.0
940-000268-00	DNBSEQ-T7RS High-throughput Sequencing Set	FCL PE150	V3.0
1000019251	DNBSEQ-T7RS High-throughput Sequencing Set	stLFR FCL PE100	V1.0
940-000298-00	DNBSEQ-T7RS High-throughput Sequencing Set	App-A FCL PE100	V3.0
940-000300-00	DNBSEQ-T7RS High-throughput Sequencing Set	App-A FCL PE150	V3.0
1000020834	CPAS Barcode Primer 3 Reagent Kit	/	V2.0
1000014048	CPAS Barcode Primer 4 Reagent Kit	/	V1.0

Cat. No.	Name	Model	Version
1000014047	High-throughput Barcode Primer 3 Reagent Kit (App-A)	/	V1.0
1000028550	High-throughput Pair-End Sequencing Primer Kit (App-D)	/	V2.0
940-000857-00	High-throughput Single-End Sequencing Primer Kit (App-D)	/	V2.0

Tips

- The cPAS Barcode Primer 4 Reagent Kit (Cat. 1000014048) is suitable for SE dual barcode sequencing of MGI libraries. It needs to be used in conjunction with High-throughput Sequencing Set (Models: FCL SE35/FCL SE50/FCL SE100) for sequencing.
- cPAS Barcode Primer 3 Reagent Kit (Cat. 1000020834) is suitable for PE dual barcode sequencing of MGI libraries. It needs to be used in conjunction with High-throughput Sequencing Set (Models: FCL PE100/FCL PE150) for sequencing.
- High-throughput Barcode Primer 3 Reagent Kit (App-A) (Cat. 1000014047) is suitable for PE dual barcode sequencing of App-A libraries with TruSeq and Nextera adapters. It needs to be used in conjunction with High-throughput Sequencing Set (Models: App-A FCL PE100/App-A FCL PE150) for sequencing.
- High-throughput Single-End Sequencing Primer Kit (App-D) (Cat. 940-000857-00) is suitable for SE single barcode and SE dual barcode sequencing of MGI libraries and App-D libraries with TruSeq and Nextera adapters. It needs to be used in conjunction with High-throughput Sequencing Set (Models: FCL SE50/FCL SE100) for sequencing.
- High-throughput Paired-End Sequencing Primer Kit (App-D) (Cat. 1000028550) is suitable for PE single barcode and PE dual barcode sequencing of MGI libraries and App-D libraries with TruSeq and Nextera adapters. It needs to be used in conjunction with High-throughput Sequencing Set (Models: FCL FCL PE100/FCL PE150) for sequencing.

Contents

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Chapter 1 Introduction

This instructions for use explains how to perform sequencing by using the DNBSEQ-T7RS High-throughput Sequencing Set and includes instructions on sample preparation, Flow Cell preparation, sequencing kit storage, the sequencing protocol and device maintenance.

1.1 Applications

DNBSEQ-T7RS High-throughput Sequencing Set is specifically designed for DNA or RNA sequencing on DNBSEQ-T7RS. This sequencing set is intended to be used for scientific research only and cannot be used for clinical diagnosis.

1.2 Sequencing principle

This sequencing set utilizes DNBSEQ technology. A sequencing run starts with the hybridization of a DNA anchor, then a fluorescent probe is attached to the DNA Nanoball (DNB) using combinatorial probe anchor sequencing (cPAS) chemistry. Finally, the high-resolution imaging system captures the fluorescent signal. After digital processing of the optical signal, the sequencer generates high quality and highly accurate sequencing information.

1.3 Sample requirements

This High-throughput Sequencing Set is in conjunction with the Primer Kit for MGI libraries and App libraries sequencing. The MGI library is the library prepared by MGI Library Prep Kits. After being converted from the third-party library by the MGIEasy Universal Library Conversion Kit (Cat. No.: 1000004155) or other MGI Library Conversion Kits, the App library (including TruSeq and Nextera adapter) is applicable to the MGI sequencing platforms.

1.4 Data analysis

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

1.5 Sequencing read length

In the sequencing run, the number of sequencing cycles depends on the sequencing read length. For example, a PE100 cycle run performs reads of 100 cycles from each end, for a total of 200 (2×100) cycles. At the end of the insert sequencing run, an extra 10 cycles of barcode read can be carried out, if required.

Tips Both read 1 and read 2 need an extra calibration cycle. Barcode does not need calibration. The calibration cycle is generated automatically in the system based on the sequencing read length without the need for specific settings.

Table 1 Sequencing cycle

Sequencing read length	Read 1 read length	Read 2 read length	Barcode read length	Dual barcode read length	Total read length	Maximum cycles
FCL SE35	35	/	10	10	36+10+10	56
FCL SE50	50	/	10	10	51+10+10	71
FCL SE100	100	/	10	10	101+10+10	121
FCL PE100	100	100	10	10	202+10+10	222
FCL PE150	150	150	10	10	302+10+10	322
stLFR PE100	100	100	42	10	202+42+10	254

1.6 Sequencing time

Tips

- Sequencing run time for both single flow cell and four flow cells only refer to the time elapsing from the “start” to the “finish” of the sequencing run. The time used for DNB preparation, DNB loading and Write FQ is not included. Write FQ for a single flow cell will take about 1.5 hours.
- Two flow cells can be loaded with DNB concurrently using one MGIDL-T7RS instrument. Total time is about 2 hours.
- Sequencing run time is based on the DNBSEQ-T7RS instrument with standard model, actual sequencing run time could vary among different instruments.
- Sequencing run time includes the time for the single barcode (10 cycles) sequencing, except for the stLFR PE100 where the time for 42+10 barcode cycles run is included.

Table 2 Theoretical sequencing time

Read length	Single flow cell (hours)	Four flow cells (hours)	DNB preparation (hours)	DNB loading (hours)
FCL SE35	4.5	5.0	1	2
FCL SE50	5.5	6.0	1	2
FCL SE100	9.0	10.5	1	2
FCL PE100	15.0 to 16.0	16.0 to 20.0	1	2
FCL PE150	21.0 to 23.0	23.0 to 28.0	1	2
stLFR PE100	21.0	24.5	1	2

1.7 Precautions and warnings

- This product is for research use only. Please read the instructions 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. Do not 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.
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- 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 set components and User-supplied equipment and consumables

2.1 List of sequencing set components

Tips
- App Make DNB Buffer can be used to make DNBs for both MGI and App libraries.
- Mixed use of reagent components from different batches is not recommended.

Table 3 DNBSEQ-T7RS High-throughput Sequencing Set (FCL SE35) V2.0
Catalog number: 940-000270-00

Component	Cap color	Spec&quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS Sequencing Flow Cell (T7-2 FCL) Catalog number: 930-000054-00					
Sequencing Flow Cell (T7-2 FCL)	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	10 months
DNBSEQ DNB Make Reagent Kit Catalog number 1000016115					
Low TE Buffer		960 μL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
Make DNB Buffer		400 μL/tube×1 tube			
Make DNB Enzyme Mix I		800 μL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		80 μL/tube×1 tube			
Stop DNB Reaction Buffer		400 μL/tube×1 tube			

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS DNB Load Reagent Kit V2.0 Catalog number: 1000028452					
DNB Load Buffer I		300 µL / tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	12 months
DNB Load Buffer II		150 µL / tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
Post Load Plate (T7 FCL) V2.0	/	1 EA			
DNBSEQ-T7RS High-throughput Sequencing Kit (FCL SE35) Catalog number: 1000019813					
dNTPs Mix II		4.50 mL / tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	12 months
dNTPs Mix IV		1.70 mL / tube×1 tube			
Sequencing Enzyme Mix		3.20 mL / tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent Sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (FCL SE35) Catalog number: 1000019812					
Washing Cartridge	/	1 EA	below 40 °C	0 °C to 30 °C	12 months

Table 4 DNBSEQ-T7RS High-throughput Sequencing Set (FCL SE50) V2.0
Catalog number: 940-000271-00

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS Sequencing Flow Cell (T7-2 FCL) **Catalog number: 930-000054-00**					
Sequencing Flow Cell (T7-2 FCL)	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	10 months
DNBSEQ DNB Make Reagent Kit **Catalog number: 1000016115**					
Low TE Buffer		960 μL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
Make DNB Buffer		400 μL/tube×1 tube			
Make DNB Enzyme Mix I		800 μL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		80 μL/tube×1 tube			
Stop DNB Reaction Buffer		400 μL/tube×1 tube			
DNBSEQ-T7RS DNB Load Reagent Kit V2.0 **Catalog number: 1000028452**					
DNB Load Buffer I		300 μL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
DNB Load Buffer II		150 μL/tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
Post Load Plate (T7 FCL) V2.0	/	1 EA			

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS High-throughput Sequencing Kit (FCL SE50) Catalog number: 1000016108					
dNTPs Mix II		2.70 mL/tube×2 tubes	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
dNTPs Mix IV		2.00 mL/tube×1 tube			
Sequencing Enzyme Mix		3.80 mL/tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent Sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (FCL SE50) Catalog number: 1000016117					
Washing Cartridge	/	1 EA	below 40 ℃	0 ℃ to 30 ℃	12 months

Table 5 DNBSEQ-T7RS High-throughput Sequencing Set (FCL SE100) V2.0
Catalog number: 940-000272-00

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS Sequencing Flow Cell (T7-2 FCL) **Catalog number: 930-000054-00**					
Sequencing Flow Cell (T7-2 FCL)	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	10 months
DNBSEQ DNB Make Reagent Kit **Catalog number: 1000016115**					
Low TE Buffer		960 μL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
Make DNB Buffer		400 μL/tube×1 tube			
Make DNB Enzyme Mix I		800 μL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		80 μL/tube×1 tube			
Stop DNB Reaction Buffer		400 μL/tube×1 tube			
DNBSEQ-T7RS DNB Load Reagent Kit V2.0 **Catalog number: 1000028452**					
DNB Load Buffer I		300 μL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
DNB Load Buffer II		150 μL/tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
Post Load Plate (T7 FCL) V2.0	/	1 EA			

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS High-throughput Sequencing Kit (FCL SE100) Catalog number: 1000016109					
dNTPs Mix II		4.05 mL/tube×2 tubes	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
dNTPs Mix IV		3.00 mL/tube×1 tube			
Sequencing Enzyme Mix		2.85 mL/tube×2 tubes			
Sequencing Reagent Cartridge	/	1 EA			
Transparent Sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (FCL SE100) Catalog number: 1000016118					
Washing Cartridge	/	1 EA	below 40 ℃	0 ℃ to 30 ℃	12 months

Table 6 DNBSEQ-T7RS High-throughput Sequencing Set (FCL PE100) V3.0
Catalog number: 940-000269-00

Component	Cap color	Spec&quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS Sequencing Flow Cell (T7-2 FCL) **Catalog number: 930-000054-00**					
Sequencing Flow Cell (T7-2 FCL)	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	10 months
DNBSEQ DNB Make Reagent Kit **Catalog number: 1000016115**					
Low TE Buffer		960 μL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
Make DNB Buffer		400 μL/tube×1 tube			
Make DNB Enzyme Mix I		800 μL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		80 μL/tube×1 tube			
Stop DNB Reaction Buffer		400 μL/tube×1 tube			
DNBSEQ-T7RS DNB Load Reagent Kit V2.0 **Catalog number: 1000028452**					
DNB Load Buffer I		300 μL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
DNB Load Buffer II		150 μL/tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
Post Load Plate (T7 FCL) V2.0	/	1 EA			

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS High-throughput Sequencing Kit (FCL PE100) V3.0 Catalog number: 940-000267-00					
dNTPs Mix II		8.28 mL / tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
dNTPs Mix V		2.76 mL / tube×1 tube			
Sequencing Enzyme Mix		5.52 mL / tube×1 tube			
MDA Reagent		4.20 mL / tube×1 tube			
MDA Enzyme Mix		0.60 mL / tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent Sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (FCL PE100) V3.0 Catalog number: 940-000299-00					
Washing Cartridge	/	1 EA	below 40 ℃	0 ℃ to 30 ℃	12 months

Table 7 DNBSEQ-T7RS High-throughput Sequencing Set (FCL PE150) V3.0
Catalog number: 940-000268-00

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS Sequencing Flow Cell (T7-2 FCL) **Catalog number: 930-000054-00**					
Sequencing Flow Cell (T7-2 FCL)	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	10 months
DNBSEQ DNB Rapid Make Reagent Kit V2.0 **Catalog number: 1000028453**					
Low TE Buffer		960 µL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
Make DNB Buffer		400 µL/tube×1 tube			
Make DNB Rapid Enzyme Mix II		800 µL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		80 µL/tube×1 tube			
Stop DNB Reaction Buffer		400 µL/tube×1 tube			
DNBSEQ-T7RS DNB Rapid Load Reagent Kit V2.0 **Catalog number: 1000028451**					
DNB Load Buffer IV		200 µL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
Micro Tube 0.5 mL (Empty)		1 tube			
Rapid Post Load Plate (T7 FCL) V2.0	/	1 EA			

Component	Cap color	Spec&quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS High-throughput Sequencing Kit (FCL PE150) V3.0 Catalog number : 940-000266-00					
dNTPs Mix II		5.61 mL/tube×2 tubes	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
dNTPs Mix V		3.74 mL/tube×1 tube			
Sequencing Enzyme Mix		7.48 mL/tube×1 tube			
MDA Reagent		4.20 mL/tube×1 tube			
MDA Enzyme Mix		0.60 mL/tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent Sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (FCL PE150) V3.0 Catalog number: 940-000297-00					
Washing Cartridge	/	1 EA	below 40 ℃	0 ℃ to 30 ℃	12 months

Table 8 DNBSEQ-T7RS High-throughput Sequencing Set (stLFR FCL PE100)
Catalog number: 1000019251

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS Sequencing Flow Cell Catalog number: 1000016269					
Sequencing Flow Cell (T7 FCL)	/	1 EA	0 ℃ to 30 ℃	0 ℃ to 30 ℃	10 months
DNBSEQ DNB Make Reagent Kit (stLFR) Catalog number: 1000019257					
Low TE Buffer		480 μL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
stLFR Make DNB Buffer		160 μL/tube×1 tube			
Make DNB Enzyme Mix III		320 μL/tube×1 tube			
Make DNB Enzyme Mix IV		42 μL/tube×1 tube			
Stop DNB Reaction Buffer		200 μL/tube×1 tube			
DNBSEQ-T7RS DNB Load Reagent Kit (stLFR) Catalog number: 1000019256					
DNB Load Buffer I		500 μL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
DNB Load Buffer II		500 μL/tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
Post Load Plate (stLFR)	/	1 EA			

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS High-throughput Sequencing Kit (stLFR FCL PE100) Catalog number: 1000019252					
dNTPs Mix II		4.90 mL/tube×3 tubes	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
dNTPs Mix IV		5.40 mL/tube×1 tube			
Sequencing Enzyme Mix		5.15 mL/tube×2 tubes			
MDA Reagent		4.20 mL/tube×1 tube			
MDA Enzyme Mix		0.60 mL/tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent Sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (stLFR FCL PE100) Catalog number: 1000019254					
Washing Cartridge	/	1 EA	below 40 ℃	0 ℃ to 30 ℃	12 months

Table 9 DNBSEQ-T7RS High-throughput Sequencing Set (App-A FCL PE100) V3.0
Catalog number: 940-000298-00

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS Sequencing Flow Cell (T7-2 FCL) **Catalog number: 930-000054-00**					
Sequencing Flow Cell (T7-2 FCL)	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	10 months
DNBSEQ DNB Make Reagent Kit **Catalog number: 1000016115**					
Low TE Buffer		960 µL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
Make DNB Buffer		400 µL/tube×1 tube			
Make DNB Enzyme Mix I		800 µL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		80 µL/tube×1 tube			
Stop DNB Reaction Buffer		400 µL/tube×1 tube			
DNBSEQ-T7RS DNB Load Reagent Kit V2.0 **Catalog number: 1000028452**					
DNB Load Buffer I		300 µL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
DNB Load Buffer II		150 µL/tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
Post Load Plate (T7 FCL) V2.0	/	1 EA			

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS High-throughput Sequencing Kit (FCL PE100) V3.0 Catalog number: 940-000267-00					
dNTPs Mix II		8.28 mL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
dNTPs Mix V		2.76 mL/tube×1 tube			
Sequencing Enzyme Mix		5.52 mL/tube×1 tube			
MDA Reagent		4.20 mL/tube×1 tube			
MDA Enzyme Mix		0.60 mL/tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent Sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (FCL PE100) V3.0 Catalog number: 940-000299-00					
Washing Cartridge	/	1 EA	below 40 ℃	0 ℃ to 30 ℃	12 months
High-throughput Pair-End Sequencing Primer Kit (App-A) Catalog number: 1000020832					
App-A Make DNB Buffer		400 µL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
1 µM App-A Insert Primer 1		2.20 mL/tube×1 tube			
1 µM App-A Insert Primer 2		4.20 mL/tube×1 tube			
1 µM App-A MDA Primer		4.20 mL/tube×1 tube			
1 µM App-A Barcode Primer 2		3.50 mL/tube×1 tube			

Table 10 DNBSEQ-T7RS High-throughput Sequencing Set (App-A FCL PE150) V3.0
Catalog number: 940-000300-00

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS Sequencing Flow Cell (T7-2 FCL) Catalog number: 930-000054-00					
Sequencing Flow Cell (T7-2 FCL)	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	10 months
DNBSEQ DNB Rapid Make Reagent Kit V2.0 Catalog number: 1000028453					
Low TE Buffer		960 µL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
Make DNB Buffer		400 µL/tube×1 tube			
Make DNB Rapid Enzyme Mix II		800 µL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		80 µL/tube×1 tube			
Stop DNB Reaction Buffer		400 µL/tube×1 tube			
DNBSEQ-T7RS DNB Rapid Load Reagent Kit V2.0 Catalog number: 1000028451					
DNB Load Buffer IV		200 µL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
Micro Tube 0.5 mL (Empty)		1 tube			
Rapid Post Load Plate (T7 FCL) V2.0	/	1 EA			

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS High-throughput Sequencing Kit (FCL PE150) V3.0 Catalog number: 940-000266-00					
dNTPs Mix II		5.61 mL/tube×2 tubes	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
dNTPs Mix V		3.74 mL/tube×1 tube			
Sequencing Enzyme Mix		7.48 mL/tube×1 tube			
MDA Reagent		4.20 mL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
MDA Enzyme Mix		0.60 mL/tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent Sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (FCL PE150) V3.0 Catalog number: 940-000297-00					
Washing Cartridge	/	1 EA	below 40 ℃	0 ℃ to 30 ℃	12 months
High-throughput Pair-End Sequencing Primer Kit (App-A) Catalog number: 1000020832					
App-A Make DNB Buffer		400 μL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
1 μM App-A Insert Primer 1		2.20 mL/tube×1 tube			
1 μM App-A Insert Primer 2		4.20 mL/tube×1 tube			
1 μM App-A MDA Primer		4.20 mL/tube×1 tube			
1 μM App-A Barcode Primer 2		3.50 mL/tube×1 tube			

Table 11 CPAS Barcode Primer 3 Reagent Kit Catalog number: 1000020834

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
Primer for dual barcode sequencing (Pair End Sequencing use only)					
1 μM AD153 Barcode Primer 3		3.50 mL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months

Table 12 CPAS Barcode Primer 4 Reagent Kit Catalog number: 1000014048

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
Primer for dual barcode sequencing (Single End Sequencing use only)					
1 μM AD153 Barcode Primer 4		3.50 mL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months

Table 13 High-throughput Barcode Primer 3 Reagent Kit (App-A) Catalog number: 1000014047

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
Primer for dual barcode sequencing (Pair End Sequencing use only)					
1 μM App-A Barcode Primer 3		3.50 mL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months

Table 14 High-throughput Pair-End Sequencing Primer Kit (App-D) Catalog number: 1000028550

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
Primer for dual barcode sequencing (FCL PE100/PE150 Sequencing use)					
1 μM App-D Insert Primer 1		2.20 mL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
1 μM App-D MDA Primer		4.20 mL/tube×1 tube			
1 μM App-D Insert Primer 2		4.20 mL/tube×1 tube			
1 μM App-D Barcode Primer 2		3.50 mL/tube×1 tube			
1 μM App-D Barcode Primer 3		3.50 mL/tube×1 tube			
App Make DNB Buffer		400 μL/tube×1 tube			

Table 15 High-throughput Single-End Sequencing Primer Kit (App-D)
Catalog number: 940-000857-00

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
Primer for dual barcode sequencing (FCL SE50/SE100 Sequencing use)					
1 μM App-D Insert Primer 1		2.20 mL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	12 months
1 μM App-D Barcode Primer 1		3.50 mL/tube×1 tube			
1 μM App-D Barcode Primer 4		3.50 mL/tube×1 tube			
App Make DNB Buffer		400 μL/tube×1 tube			

2.2 User-supplied equipment and consumables

Table 16 Self-prepared equipment and consumables

Equipment and consumables	Recommended brand	Catalog number
Qubit 4.0 Fluorometer	Thermo Fisher	Q33226
Thermal cycler	Bio-Rad	/
MPC2000 96-well plate centrifuge	Major Laboratory Supplier (MLS)	/
Pipette	Eppendorf	/
Electronic pipette	Labnet	FASTPETTEV-2
Mini centrifuge	MLS	/
Vortex mixer	MLS	/
2 ℃ to 8 ℃ Refrigerator	MLS	/
-25 ℃ to -15 ℃ Freezer	MLS	/

Equipment and consumables	Recommended brand	Catalog number
Qubit ssDNA assay kit	Thermo Fisher	Q10212
2 M NaOH solution	Aladdin	S128511-1L
100% Tween-20	BBI	A600560-0500
5 M NaCl solution	SIGMA	S5150-4L
75% Ethanol	MLS	/
Canned air dust	MATIN	M-6318
Sterile pipette tip (box)	AXYGEN	/
5 mL Sterile pipette tip (box)	AXYGEN	/
200 µL wide-bore, non-filtered pipette tips	AXYGEN	T-205-WB-C
200 µL wide-bore, non-filtered pipette tips	MGI	091-000355-00
Qubit assay tubes	Thermo Fisher	Q32856
0.2 mL PCR 8-strip tube	AXYGEN	/
1.5 mL microcentrifuge tube	AXYGEN	MCT-150-C
Ice box	MLS	/
Ice machine	MLS	/
100 mL Serological pipet	CORNING	4491
25 mL Serological pipet	CORNING	4489
10 mL Serological pipet	CORNING	4488
15 mL Sterile tube	SARSTEDT	60.732.001
Microfiber clean wiper	DUSTFREE TECHNOLOGY CO.,LTD	LJ618180B1
5 mL Sterile tube	AXYGEN	/
Kimwipes	MLS	/
Lint-free cloth	MLS	/
Ziplock bag	MLS	/

Chapter 3 Sequencing workflow

1	Preparation before sequencing: check the integrity of the reagent cartridge and thaw the Sequencing Reagent Cartridge (4-24 hours), prepare the wash reagents and fill the pure water container, and check the available capacity of waste liquid container and storage space.
2	Making DNB: make DNB using reagents from DNB Make Reagent Kit and DNA library.
3	Loading DNB: load DNB onto the flow cell using reagents from DNB Load Reagent Kit at MGIDL-T7RS loader.
4	Preparing Sequencing Reagent Cartridge: Add the reagents in tubes from the Sequencing Kit into the Sequencing Reagent Cartridge and mix well.
5	Click **Start,** and the machine starts self-check.
6	Sequencing
6	Data analysis

Chapter 4 Preparation before sequencing

4.1 Thawing the Sequencing Reagent Cartridge

Perform the steps below:

1. Take the Sequencing Reagent Cartridge out of the DNBSEQ-T7RS High-throughput Sequencing Kit.

2. Thaw in a water bath at room temperature until completely thawed (or thaw in a 2 ℃ to 8 ℃ refrigerator 1 days in advance). The approximate time to thaw is listed in the following table. Store in a 2 ℃ to 8 ℃ refrigerator until use.

Table 17 Approximate thaw times for various sequencing kits

Model	Method		
	Water bath at room temperature (h)	Refrigerate at 2 ℃ to 8 ℃ overnight, then water bath at room temperature (h)	Refrigerate at 2 ℃ to 8 ℃ (h)
FCL SE35	1.0	0.5	24.0
FCL SE50	1.5	0.5	24.0
FCL SE100	2.0	1.0	24.0
FCL PE100/ App-A FCL PE100	2.5	1.5	24.0
FCL PE150/ App-A FCL PE150	3.0	2.0	24.0
stLFR FCL PE100	2.5	1.5	24.0

4.2 Preparing wash reagents

Tips The following Wash Reagents are stored at 2 ℃ to 8 ℃ and are valid for 28 days.

- Prepare the Wash Reagent I (1 M NaCl+0.05% Tween-20) following the table below:

Table 18 Wash reagent I: 1 M NaCl+0.05% Tween

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

- Prepare the Wash Reagent II (0.1 M NaOH) following the table below:

Table 19 Wash reagent II: 0.1 M NaOH

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

4.3 Filling the pure water container

Fill the pure water container with laboratory-grade water according to the table below:

Tips

- Check whether the water in the pure water container is sufficient. If it is insufficient, the sequencing will fail. Replenish pure water in time, and pay attention to opening the air vent of the pure water container.
- Prepare pure water before sequencing. If you need to add water during the sequencing process, gently pour it along the wall of the water container to avoid bubbles entering the pump water pipeline, which could affect sequencing quality.
- The pure water will be used in sequencing so it must be kept clean. Renew the pure water in the pure water container on a weekly basis.
- Before refilling the pure water container, empty the container and spray 75% ethanol on the inner surface of the container lid and the surface of the pure water tube. Wipe and clean the surfaces with new microfiber clean wipers. Rinse the container with fresh pure water 3 times.
- Refer to *H-020-000157-00 DNBSEQ-T7RS Genetic Sequencer User Manual* for the preparation of the water container.

Table 20 Pure water consumption (L)

Model	1 flow cell	2 flow cell	3 flow cell	4 flow cell
FCL SE35	1.0	2.0	3.0	4.0
FCL SE50	1.0	2.0	3.0	4.0
FCL SE100	1.5	3.0	4.5	6.0
FCL PE100/ App-A FCL PE100	3.0	6.0	9.0	12.0
FCL PE150/ App-A FCL PE150	4.5	9.0	13.5	18.0
stLFR FCL PE100	3.0	6.0	9.0	12.0

4.4 Performing pre-run checks

Before each sequencing run, perform the following checks:

Check the storage drive space, for 1 flowcell (PE150), including FASTQ files, the remaining storage drive space must be at least 4.7 TB. If the remaining space is insufficient, clear history data.

Check the waste container, for 1 flowcell (PE150), the available capacity of waste container needs to be ≥7L. If it is insufficient, please empty the waste liquid before sequencing.

If any problem occurs other than those listed above, restart the sequencer control software.

Chapter 5 Making DNBs

5.1 Insert size recommendation

For general purpose, library refers to single stranded circular DNA (ssDNA). For the best sequencing quality, it is recommended that the insert size of the library should be between 50 and 500 nucleotides (nt), and the main band is centered within±100 nt. For the stLFR library prepared with MGIEasy stLFR Library Prep Kit, the library is circularized dsDNA. It is recommended that the insert size of the library be between 200 and 1500 nt.

Tips
- The insert size and required data output should be considered when selecting sequencing kits.
- Average data output will vary with different library type and applications.
- If there is any special requirement or specification from the library preparation kit, then the requirement of the kit should be followed.

Table 21 Recommended insert size and theoretical throughput for each flow cell

Model	Suggested insert distribution (bp)	Applications	Data output (M)	Data output (Gb)
FCL SE35	50 to 230	NIPT	5800	203
FCL SE50	50 to 230	NIPT, PMSEQ	5800	290
FCL SE100	200 to 400	PMSEQ	5800	580
FCL PE100/ App-A FCL PE100	200 to 400	WGS, WES, RNAseq	5800	1160
FCL PE150/ App-A FCL PE150	300 to 500	WGS, WES, RNAseq	5800	1740
stLFR FCL PE100	200 to 1500	stLFR	4500	800

5.2 Library concentration and amount requirement

Tips

- If the library concentration is unknown, it is recommended to perform ssDNA library quantitation (ng/μL) using the Qubit ssDNA Assay Kit and the Qubit 4.0 Fluorometer. Use the following equation to convert the concentration of the ssDNA library from ng/μL to fmol/μL:

  Concentration (fmol/μL) = 3030 × Concentration (ng/μL) / N. N represents the number of nucleotides (total library length including the adapters).

- If there is any special requirement or specification of the library preparation kit, then the requirement of the kit should be followed.

Table 22 Library requirement

Libraries	Library concentration
PCR	≥3 fmol/μL
PCR-free	≥3.75 fmol/μL
stLFR	≥1.9 ng/μL

5.3 Library pooling

Tips When the App libraries need to be pooled with MGI libraries, some libraries with similar barcode sequence in the adapter should avoid being pooled together for sequencing. For details, refer to *Appendix 2 Conflicting adapter list on Page 78*

5.3.1 Number of samples that can be pooled together

The DNBSEQ-T7RS sequencer can simultaneously perform sequencing of 4 flow cells and each flow cell can theoretically produce 5000M reads. For PE100 sequencing, one flow cell can produce 1 Tb of data in theory. The number of samples that can be pooled together for each flow cell depends on the required data output, read length, and specific application.

As a guide, do not pool more samples with their total data output larger than 90% of the theoretical data output as described in *Table 21 on Page 27*, due to variation in pooling and the fact that not all barcodes will generate the same amount of the data output from the same amount of DNB.

$$\text{Maximum number of samples pooled} = \frac{\text{Total data output of one flow cell} \times 90}{\text{required data per sample}}$$

- Example 1: Human Whole-genome Sequencing (WGS)

  When using the PE100 sequencing kit, 10 samples on each flow cell are recommended.

- Example 2: stLFR sample

  When using the PE100 sequencing kit, if the required sequencing depth is 40X, then 6 samples are recommended to be pooled for each flow cell.

- Example 3: 50G are required for each sample

  When using the PE100 sequencing kit, if 50G are required for each sample, then 20 samples are recommended to be pooled for each flow cell.

- Example 4: Pooling samples with various applications

  When using the PE150 sequencing kit, if samples to be sequenced include WGS (100G/sample) and RNASeq (50G/sample), it is recommended to pool 4 WGS samples and 23 RNASeq samples for each flow cell.

Tips Expected pooling variation are within ±10%.

Table 23 Examples of various sample pooling

Index	Read length	Minimum data for each sample	Pooling sample number	Theoretical data output range for each sample
1	PE100	100 Gb	10	104 to 127 Gb
2	stLFR PE100	120 Gb	6	120 to 146 Gb
3	PE100	50 Gb	20	52 to 63 Gb
4	PE150	50 Gb	23 RNAseq	51 to 62 Gb
		100 Gb	4 WGS	102 to 122 Gb

5.3.2 Verifying the base balance for barcode

- A balanced base composition in each sequencing cycle is very important for high sequencing quality. It is strongly recommended that the minimum base composition of A, C, G, T for each position in the barcode is not lower than

12.5%. For a given pooling of samples, if the minimum base composition of A, C, G, T within the barcode is between 5% and 12.5%, the barcode split rate may be compromised. If the minimum base composition of A, C, G, T in any position of the barcode is less than 5%, it is strongly suggested to re-design the pooling strategy to have a more balanced base composition in the barcode.

- It is also important to note that two or more samples with an identical barcode should not be pooled together, otherwise, it is impossible to assign the read correctly.

5.4 Making DNBs

Tips
- Mixed use of reagent components from different batches is not recommended.
- For mixing DNBs, use the wide-bore, non-filtered pipette tips.
- For other reagents, use a proper pipette tip according to the actual situation.
- It is recommended that you use the pipette tips from recommended brands and catalog numbers

DNB making protocols are listed below, please select the appropriate one according to the Sequencing Set used.

- Section *5.4.1 Preparing DNBs for the FCL SE35, FCL SE50, FCL SE100, FCL PE100/App-A FCL PE100 on Page 30*.
- Section *5.4.2 Preparing DNBs for the FCL PE150/App-A FCL PE150 on Page 35*.
- Section *5.4.3 Preparing DNBs for the stLFR FCL PE100 on Page 39*.

5.4.1 Preparing DNBs for the FCL SE35, FCL SE50, FCL SE100, FCL PE100/App-A FCL PE100

5.4.1.1 Calculating the required amount of ssDNA library

- For FCL SE35, FCL SE50, FCL SE100, FCL PE100/App-A FCL PE100 Sequence Set, 270 µL of DNB is required to load one flow cell.
- One Make DNB Reaction can make either 100 µL or 50 µL of DNBs. The volume of the DNB making reaction system depends on the amount of data required for sequencing per sample and the types of DNA libraries.

- The required ssDNA library volume to make one DNB reaction are shown in the table below.

  Tips *C* refers to the concentration of the ssDNA library (fmol/μL).

Table 24 FCL SE35/SE50/SE100/PE100 required ssDNA volume

Sample type	Required ssDNA volume: V (μL)	
	100 μL DNB reaction	50 μL DNB reaction
PCR	V=60 fmol/C	V=30 fmol/C
PCR free	V=75 fmol/C	V=37.5 fmol/C

- For a given sample A, if it requires "a" million base data output and the total theoretical expected data output for this flow cell is "b" million bases, then the required DNB volume (V) in the pooling for sample A is as follows: V=a/b×270 (μL).
  - If the total sample number pooled is<6, it is suggested to select the volume of 100 μL DNB reaction, and the number of 100 μL DNB making reactions is equal to round (V/100)+1.(for example: If V=80, it requires one 100 μL of DNB making reaction; If V=120, it requires two 100 μL DNB making reactions)
  - If the total sample number pooled is≥6, it is suggested to select the volume of 50 μL DNB reaction, and the number of 50 μL DNB making reactions is equal to round (V/50)+1.

5.4.1.2 Preparing reagents for making DNBs

Perform the steps below:

1. Place the library on ice until use.
2. Remove the Low TE Buffer, Make DNB Buffer and Stop DNB Reaction Buffer from the DNB Make Reagent Kit packaging and thaw reagents at room temperature.

- For App-A libraries sequencing, remove App-A Make DNB Buffer from the High-throughput Pair-End Sequencing Primer Kit (App-A) packaging and thaw reagents at room temperature.
- For App-D libraries sequencing, remove App Make DNB Buffer from the High-throughput Pair-End Sequencing Primer Kit (App-D)/High-throughput Single-End Sequencing Primer Kit (App-D) packaging and thaw reagents at room temperature.

3. Thaw the Make DNB Enzyme Mix I on ice for approximately 30 minutes.
4. After thawing, mix the reagents by using a vortex mixer for 5 seconds. Centrifuge them briefly, and place them on ice until use.

5.4.1.3 Making DNBs

Perform the steps below:

1. Take out a 0.2 mL 8-strip tube or PCR tubes, and prepare the Make DNB Reaction Mixture 1 on ice according to the different library types:

Tips
- The above table only illustrates one Make DNB Reaction.
- The amount of input ssDNA and the required number of Make DNB Reactions are determined by the actual application as described in *5.4.1.1 Calculating the required amount of ssDNA library on Page 30*.

Table 25 Make DNB Reaction Mixture 1 for MGI libraries (FCL SE35/SE50/SE100/PE100)

Component	Cap color	Volume/100 μL DNB reaction (μL)	Volume/50 μL DNB reaction (μL)
Low TE Buffer		20-V	10-V
Make DNB Buffer		20	10
ssDNA libraries	/	V	V
Total volume		40	20

Table 26 Make DNB Reaction Mixture 1 for App-A libraries (FCL SE35/SE50/SE100/PE100)

Component	Cap color	Volume/100 μL DNB reaction (μL)	Volume/50 μL DNB reaction (μL)
Low TE Buffer		20-V	10-V
App-A Make DNB Buffer		20	10
ssDNA libraries	/	V	V
Total volume		40	20

Table 27 Make DNB Reaction Mixture 1 for App-D libraries (FCL SE35/SE50/SE100/PE100)

Component	Cap color	Volume/100 µL DNB reaction (µL)	Volume/50 µL DNB reaction (µL)
Low TE Buffer		20-V	10-V
App Make DNB Buffer		20	10
ssDNA libraries	/	V	V
Total volume		40	20

2. Mix the Make DNB Reaction Mixture 1 thoroughly by using a vortex mixer for 5 seconds. Centrifuge it briefly and place it on ice until use.
3. Place the mixture into a thermal cycler, and start the primer hybridization reaction according to the following table:

Tips When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.

Table 28 FCL SE35/SE50/SE100/PE100 primer hybridization reaction condition

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

4. Take the Make DNB Enzyme Mix II (LC) out of freezer. Centrifuge it briefly for 5 seconds, and place it on ice until use.

Tips
- Do not place the 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 ℃ . Centrifuge briefly for five seconds, place the tube on ice.

6. Prepare Make DNB Reaction Mixture 2 according to the table below:

Table 29 Make DNB Reaction Mixture 2 (FCL SE35/SE50/SE100/PE100)

Component	Cap color	Volume/100 µL DNB reaction (µL)	Volume/50 µL DNB reaction (µL)
Make DNB Enzyme Mix I		40	20
Make DNB Enzyme Mix II (LC)		4	2

7. Add all the Make DNB Reaction mixture 2 into Make DNB Reaction Mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer. Centrifuge it for five seconds and place it on ice until use.
8. Place the tubes into a thermal cycler for the next reaction. The condition is shown in the table below:

Tips
- For thermal cyclers, pre-heating or pre-cooling of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
- It is recommended to set the temperature of the heated lid to 35 ℃ or as close as possible to 35 ℃ .

Table 30 FCL SE35/SE50/SE100/PE100 rolling circle replication condition

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

9. Immediately add Stop DNB Reaction Buffer to the tube when the temperature reaches 4 ℃ . Mix the tube gently by pipetting 5 to 8 times by using a wide-bore, non-filtered pipette tip.

Table 31 Volume of Stop DNB Reaction Buffer

Component	Cap color	Volume/100 µL DNB reaction (µL)	Volume/50 µL DNB reaction (µL)
Stop DNB Reaction Buffer		20	10

Tips
- It is very important to mix DNBs gently by using a wide-bore, non-filtered pipette tip. Do not centrifuge, vortex, pipette vigorously or shake the tube.
- Store the DNBs at 2 ℃ to 8 ℃ and perform sequencing within 48 hours.

10. Quantify the DNBs. For details, refer to *5.5.1 Quantifying DNBs on Page 42.*

5.4.2 Preparing DNBs for the FCL PE150/App-A FCL PE150

5.4.2.1 Calculating the required amount of ssDNA library

- For FCL PE150/App-A FCL PE150 Sequence Set, 300 µL of DNB is required to load one flow cell. One DNB making reaction can make 90 µL of DNB.
- The required ssDNA library volume (1 DNB reaction) is shown in the table below.

  Tips C refers to the concentration of the ssDNA library (fmol/µL).

Table 32 FCL PE150 required ssDNA volume

Sample type	Required ssDNA volume: V (µL)
PCR	V=60 fmol/C
PCR free	V=75 fmol/C

- For a given sample A, if it requires "a" million base data output and the total theoretical expected data output for this flow cell is "b" million bases, then the required DNB volume (V) in the pooling for sample A is as follows: V=a/b×300 (µL).
  - The number of the 90 µL DNB making reactions is equal to round (V/90)+1.

5.4.2.2 Preparing reagents for making DNBs

Perform the steps below:

1. Place the library on ice until use.
2. Remove the Low TE Buffer, Make DNB Buffer and Stop DNB Reaction Buffer from the DNB Rapid Make Reagent Kit packaging and thaw reagents at room temperature.

- For App-A libraries sequencing, remove App-A Make DNB Buffer from the High-throughput Pair-End Sequencing Primer Kit (App-A) packaging and thaw reagents at room temperature.
- For App-D libraries sequencing, remove App Make DNB Buffer from the High-throughput Pair-End Sequencing Primer Kit (App-D)/High-throughput Single-End Sequencing Primer Kit (App-D) packaging and thaw reagents at room temperature.

3. Thaw the Make DNB Rapid Enzyme Mix II on ice for approximately 30 minutes.
4. After thawing, mix the reagents by using a vortex mixer for 5 seconds. Centrifuge them briefly, and place them on ice until use.

5.4.2.3 Making DNBs

Perform the steps below:

1. Take out a 0.2 mL 8-strip tube or PCR tubes, and prepare the Make DNB Reaction Mixture 1 on ice according to the different library types:

Tips
- The above table only illustrates one Make DNB Reaction.
- The amount of input ssDNA and the required number of Make DNB Reactions are determined by the actual application as described in *5.4.2.1 Calculating the required amount of ssDNA library on Page 35*.

Table 33 Make DNB Reaction Mixture 1 for MGI libraries (FCL PE150)

Component	Cap color	Volume/90 μL DNB reaction (μL)
Low TE Buffer		20-V
Make DNB Buffer		20
ssDNA libraries	/	V
Total volume		40

Table 34 Make DNB Reaction Mixture 1 for App-A libraries (FCL PE150)

Component	Cap color	Volume/90 μL DNB reaction (μL)
Low TE Buffer		20-V
App-A Make DNB Buffer		20
ssDNA libraries	/	V
Total volume		40

Table 35 Make DNB Reaction Mixture 1 for App-D libraries (FCL PE150)

Component	Cap color	Volume/90 μL DNB reaction (μL)
Low TE Buffer		20-V
App Make DNB Buffer		20
ssDNA libraries	/	V
Total volume		40

2. Mix the Make DNB Reaction Mixture 1 thoroughly by using a vortex mixer for 5 seconds. Centrifuge it briefly and place it on ice until use.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction according to the following table:

   Tips When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.

Table 36 FCL PE150 primer hybridization reaction condition

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

4. Take the Make DNB Enzyme Mix II (LC) out of freezer. Centrifuge it briefly for 5 seconds, and place it on ice until use.

   Tips
   - 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 ℃ . Centrifuge briefly for five seconds, place the tube on ice.
6. Prepare Make DNB Reaction Mixture 2 according to the table below:

Table 37 Make DNB Reaction Mixture 2 (FCL PE150)

Component	Cap color	Volume / 90 µL DNB reaction (µL)
Make DNB Rapid Enzyme Mix II		40
Make DNB Enzyme Mix II (LC)		1.6

7. Add all the Make DNB Reaction mixture 2 into Make DNB Reaction Mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer. Centrifuge it for five seconds and place it on ice until use.

8. Place the tubes into a thermal cycler for the next reaction. The condition is shown in the table below:

   Tips
   - For thermal cyclers, pre-heating or pre-cooling of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
   - It is recommended to set the temperature of the heated lid to 35 ℃ or as close as possible to 35 ℃ .

   Table 38 FCL PE150 rolling circle replication condition

Temperature	Time
Heated lid (35 ℃)	On
30 ℃	10 min
4 ℃	Hold

9. Immediately add 10 µL of Stop DNB Reaction Buffer to the tube when the temperature reaches to 4 ℃. Mix it gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

   Tips
   - Keep DNBs on ice during the entire operation to prevent DNBs from performing secondary replication.
   - It is very important to mix DNBs gently by using a wide-bore, non-filtered pipette tip. Do not centrifuge, vortex, or shake the tube.
   - Store the DNBs at 2 ℃ to 8 ℃ and perform sequencing within 8 hours. To ensure sequencing quality, it is recommended that you pool and load DNBs for FCL PE150 as soon as possible.
   - This is not a STOP point, immediately go to the next step :*5.5 Quantifying DNBs and pooling on Page 42*.

5.4.3 Preparing DNBs for the stLFR FCL PE100

5.4.3.1 Calculating the required amount of dsDNA library

- For the stLFR FCL PE100, 270 μL of DNB is required to load one flow cell. One DNB making reaction can make 80 μL of DNB. 30 ng dsDNA libraries are needed to make 80 μL of DNB; Therefore, the volume of stLFR library needed for each 80 μL of DNB preparation reaction is defined as follows:V (μL) = 30 ng / C

  Tips C refers to the concentration of stLFR dsDNA library (ng / μL).

- For a given sample A, if it requires "a" million base data output and the total theoretical expected data output for this flow cell is "b" million bases, then the required DNB volume (V) in the pooling for sample A is as follows: V = a / b × 270 (μL).
  - The number of the 80 μL DNB making reactions is equal to round (V / 80) + 1.

5.4.3.2 Preparing reagents for making DNBs

Perform the steps below:

1. Place the library on ice until use.
2. Take the Low TE Buffer, stLFR Make DNB Buffer and Stop DNB Reaction Buffer out of the DNB Make Reagent Kit (stLFR) and thaw reagents at room temperature.
3. Thaw the Make DNB Enzyme Mix III on ice for approximately 30 minutes.
4. After thawing, mix the reagents thoroughly by using a vortex mixer for 5 seconds. Centrifuge them briefly and place them on ice until use.

5.4.3.3 Making DNBs

Perform the steps below:

1. Take a 0.2 mL 8-strip tube or PCR tubes and prepare the Make DNB Reaction Mixture 1 on ice following the table below:

   Tips
   - The above table only illustrates one Make DNB reaction.
   - The amount of input ssDNA and the required number of Make DNB Reactions are determined by the actual application as described in *5.4.3.1 Calculating the required amount of dsDNA library on Page 39*.

Table 39 Make DNB Reaction Mixture 1 (stLFR FCL PE100)

Component	Cap color	Volume / 80 μL DNB reaction (μL)
stLFR Make DNB Buffer		16
Low TE Buffer		16 - V
dsDNA libraries	/	V
Total volume		32

2. Mix the Make DNB Reaction Mixture 1 thoroughly by using a vortex mixer for 5 seconds. Centrifuge it briefly and place it on ice until use.
3. Place the mixture into a thermal cycler, and start the primer hybridization reaction according to the following table:

   Tips When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.

Table 40 stLFR FCL PE100 primer hybridization reaction condition

Temperature	Time
Heated lid (105 ℃)	On
95 ℃	3 min
40 ℃	3 min
4 ℃	Hold

4. Take out the Make DNB Enzyme Mix IV from freezer and place on ice. Centrifuge it briefly for 5 seconds and place on ice until use.

Tips
- Do not place Make DNB Enzyme Mix IV 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 ℃ . Centrifuge briefly for five seconds, place the tube on ice.
6. Prepare Make DNB Reaction Mixture 2 according to the table below:

Table 41 Make DNB Reaction Mixture 2 (stLFR FCL PE100)

Component	Cap color	Volume/80 μL DNB reaction (μL)
Make DNB Enzyme Mix III		32
Make DNB Enzyme Mix IV		3.2

7. Add all the Make DNB Reaction mixture 2 into Make DNB Reaction Mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer. Centrifuge it for five seconds and place it on ice until use.
8. Place the tubes into a thermal cycler for the next reaction. The condition is shown in the table below:

Tips
- For thermal cyclers, pre-heating or pre-cooling of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
- It is recommended to set the temperature of the heated lid to 35 ℃ or as close as possible to 35 ℃ .

Table 42 stLFR FCL PE100 rolling circle replication condition

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

9. Immediately add 16 μL of Stop DNB Reaction Buffer to the tube once the temperature reaches to 4 ℃. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered tip.

Tips
- It is very important to mix DNB gently by using a wide-bore, non-filtered pipette tip. Do not centrifuge, vortex, pipette vigorously or shake the tube.
- Store the DNB at 2 ℃ to 8 ℃ and perform sequencing within 48 hours.

5.5 Quantifying DNBs and pooling

5.5.1 Quantifying DNBs

When the DNB making is complete, use the Qubit ssDNA Assay Kit and Qubit 4.0 Fluorometer to quantify the DNBs.

Tips 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.

- If the concentration is not qualified, please remake DNB.

Table 43 DNB concentration standard

Model	DNB concentration
FCL SE35, FCL SE50, FCL SE100, FCL PE100/App-A FCL PE100	≥8 ng/μL
stLFR FCL PE100	≥6 ng/μL
FCL PE150/App-A FCL PE150	≥5 ng/μL

- If the concentration exceeds 40 ng/μL, the DNBs need to be diluted to 20 ng/μL according to the table below before loading:.

Tips If dilution is required, dilute it right before use.

Table 44 DNB dilution buffer

Model	DNB storage temperature	Maximum DNB storage time (hour)	Dilution reagent	Cap color
FCL SE35, FCL SE50, FCL SE100, FCL PE100/ App-A FCL PE100, stLFR FCL PE100	2 ℃ to 8 ℃	≤48	DNB Load Buffer I	
FCL PE150/ App-A FCL PE150	2 ℃ to 8 ℃	≤8	Low TE Buffer	

5.5.2 DNBs pooling

Tips Use normal pipette tips to gently aspirate the required volume of each DNB and use wide-bore, non-filtered tips to mix.

Amount of DNB (μL) needed for each sample in the pool depends on the relative amount for this sample and the total amount of DNB needed for loading one flow cell which is defined by the specific type of sequencing kit.

5.5.2.1 Calculating the relative amount for each sample

Assuming there are 8 samples (A to H) in the pool, the relative amount for each sample is defined as:

The relative amount of A sample (A1) = data output required for sample A / the concentration of DNB for sample A.

The relative amount of B sample (B1) = data output required for sample B / the concentration of DNB for sample B.

……

The relative amount of H sample (H1) = data output required for sample H / the concentration of DNB for sample H.

5.5.2.2 Calculating the total relative amount (V) for all sample

V = A1 + B1 + … + H1

5.5.2.3 Calculating the DNB volume needed for each sample

For FCL SE35, FCL SE50, FCL SE100, FCL PE100/App-A FCL PE100 Sequence Set, each FCL flow cell requires 270 μL of DNB, the DNB volume for pooling is calculated as follows:

DNB volume for sample A: A2 = 270 × A1 / V

DNB volume for sample B: B2 = 270 × B1 / V

…

DNB volume for sample H: H2 = 270 × H1 / V

For FCL PE150/App-A FCL PE150 Sequence Set, each flow cell requires 300 μL of DNB, the DNB volume for pooling is calculated as follows:

DNB volume for sample A: A2 = 300 × A1 / V

DNB volume for sample B: B2 = 300 × B1 / V

…

DNB volume for sample H: H2 = 300 × H1 / V

Chapter 6 Loading DNBs

6.1 Preparing the post load plate and load buffer

Two DNB loading protocols are listed below, please select the appropriate one depending on the Sequencing Set used:

- Section *6.1.1 Preparing the post load plate and load buffer for FCL SE35, FCL SE50, FCL SE100, FCL PE100/App-A FCL PE100, stLFR FCL PE100 on Page 44*.
- Section *6.1.2 Preparing the post load plate and load buffer for FCL PE150/App-A FCL PE150 on Page 45*.

6.1.1 Preparing the post load plate and load buffer for FCL SE35, FCL SE50, FCL SE100, FCL PE100/App-A FCL PE100, stLFR FCL PE100

6.1.1.1 Thawing the post load plate

Perform the steps below:

1. Perform the following steps according to different situations:
   - For FCL SE35, FCL SE50, FCL SE100, FCL PE100/App-A FCL PE100,: take Post Load Plate out of the DNB Load Reagent Kit
   - For stLFR FCL PE100: take the Post Load Plate (stLFR) out of the DNB Load Reagent Kit (stLFR)).
2. Thawing Post Load Plate. Perform the following steps according to different situations:
   - Thaw it in a water bath at room temperature for 2 hours.
   - Thaw it in 2 ℃ to 8 ℃ refrigerator one day in advance.
3. Once the Post Load Plate is thoroughly thawed, place it in a 2 ℃ to 8 ℃ refrigerator until use.

6.1.1.2 Preparing the DNB loading reagents

Perform the steps below:

1. Take the DNB Load Buffer II out of the DNBSEQ-T7RS DNB Load Reagent Kit.
2. Perform the following steps according to different situations:
   - For App-A libraries sequencing: take the App-A Insert Primer 1 out of the High-throughput Pair-End Sequencing Primer Kit (App-A).
   - For App-D libraries sequencing: take the App-D Insert Primer 1 out of the High-throughput Single-End/Pair-End Sequencing Primer Kit (App-D).
3. Thaw reagents at room temperature for approximately 30 minutes.
4. After thawing, mix the reagents by using a vortex mixer for 5 seconds. Centrifuge them briefly and place them on ice until use.

   Tips If crystal precipitation is found in DNB Load Buffer II, vigorously mix the reagent with 1 to 2 minutes of continuous vortex to re-dissolve the precipitate before use.

6.1.1.3 Preparing the 0.1 M NaOH reagent

Prepare 0.1 M NaOH according to the procedure described in *4.2 Preparing wash reagents on Page 24*. Each Post Load plate requires at least 4 mL of 0.1 M NaOH.

6.1.2 Preparing the post load plate and load buffer for FCL PE150/App-A FCL PE150

6.1.2.1 Thawing the post load plate

Perform the steps below:

1. Take the Rapid Post Load Plate out of the DNB Rapid Load Reagent Kit.
2. Thawing the Rapid Post Load Plate. Perform the following steps according to different situations:
   - Thaw it in a water bath at room temperature for 2 hours.
   - Thaw it in 2 °C to 8 °C refrigerator one day in advance.
3. Once the Rapid Post Load Plate is thoroughly thawed, place it in a 2 °C to 8 °C refrigerator until use.

6.1.2.2 Preparing the DNB loading reagents

Perform the steps below:

1. Take the DNB Load Buffer IV out of the DNB Rapid Load Reagent Kit.
2. Perform the following steps according to different situations:
   - For App-A libraries sequencing: take the App-A Insert Primer 1 out of the High-throughput Pair-End Sequencing Primer Kit (App-A).
   - For App-D libraries sequencing: take the App-D Insert Primer 1 out of the High-throughput Pair-End Sequencing Primer Kit (App-D).
3. Thaw reagents in a water bath at room temperature for approximately 30 minutes.
4. After thawing, mix the reagents by using a vortex mixer for 5 seconds. Centrifuge them briefly and place them on ice until use.

6.1.2.3 Preparing the 0.1 M NaOH reagent

Prepare 0.1 M NaOH according to the procedure described in *4.2 Preparing wash reagents on Page 24*. Each Post Load plate requires at least 4 mL of 0.1 M NaOH.

6.2 Preparing the sequencing flow cell

Perform the steps below:

1. Take the flow cell out of the DNBSEQ-T7RS Sequencing Flow Cell packing.

   Tips Do not open the outer plastic package yet..

2. Place the flow cell at room temperature for 0.5 hours to 24 hours.
3. Unwrap the outer package before use.

   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 2 ℃ to 8 ℃ for storage. But the switch between room temperature and 2 ℃ to 8 ℃ 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 it within 24 hours. If exceed 24 hours, it is not recommended to use the flow cell.

4. Take the flow cell out of the inner package and inspect it to ensure that the flow cell is intact.
5. Clean the back of the flow cell by using a canned air duster.

6.3 Loading DNBs

Perform the steps below:

1. When starting the MGIDL-T7RS, the compartment doors need to be closed.
2. Start the MGIDL-T7RS program. Enter the user name **research** and password **Admin123**, or the user name **user** and password **Password123**, then tap **Log in** to enter the main interface.

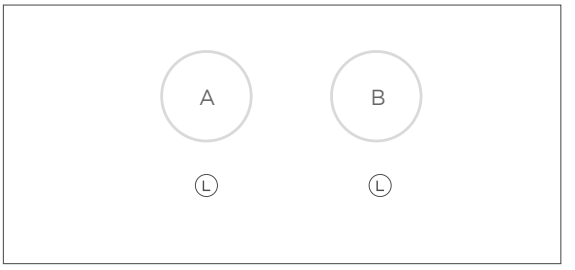

Figure 1 MGIDL-T7RS main interface

3. Tap on **A** or **B** to continue the operation, see the figure below:

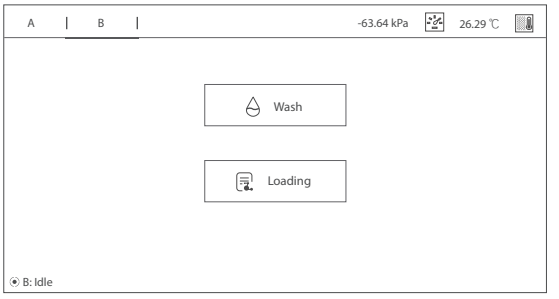

Figure 2 MGIDL-T7RS selection interface

4. Tap on **Loading** and enter the information input interface, see the figure below:

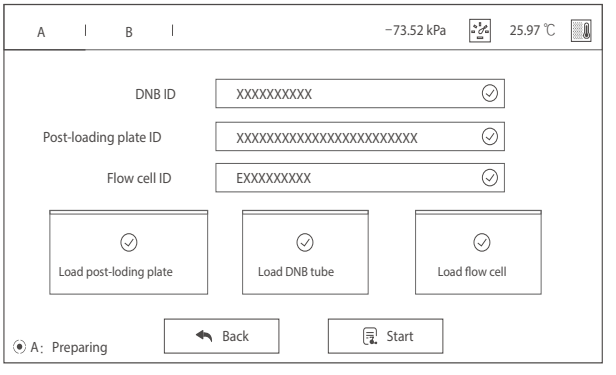

Figure 3 MGIDL-T7RS information input interface

5. Open the loading compartment door.
6. Take out the thawed Post load plate/Rapid post load plate, align the post load plate to the RFID scanning area and the ID information will appear in the text box.

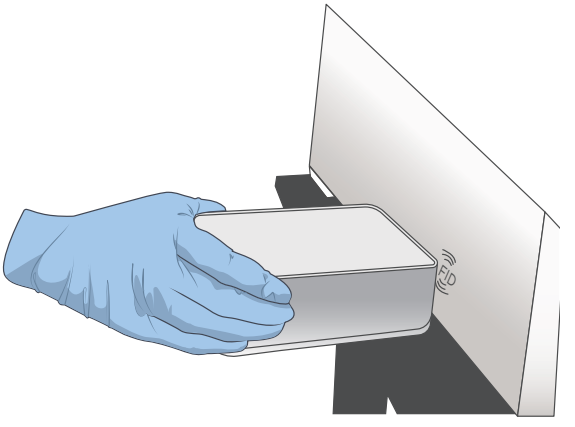

Figure 4 The RFID scanning area of post load plate

7. Gently invert the load plate 5 times, then centrifuge for 1 minute or gently tap the sealing film and let it sit for 2-3 minutes. Remove the seal of the load plate and add 4 mL of 0.1 M NaOH into well No.11.

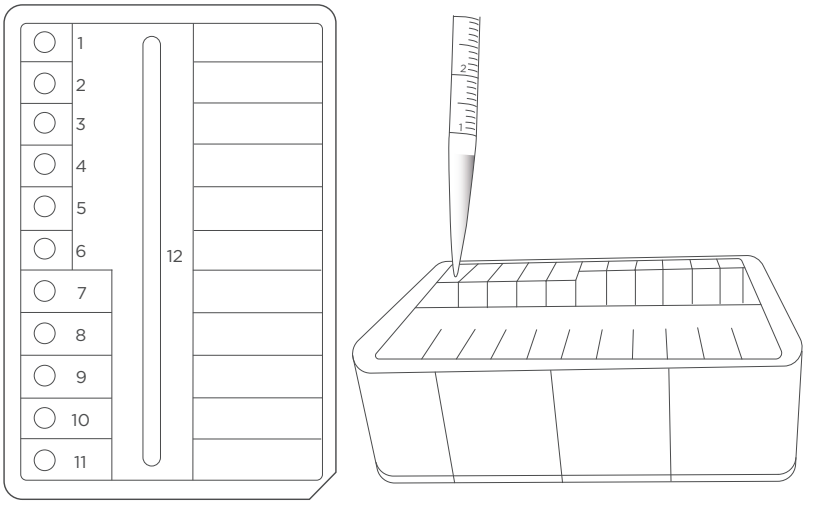

Figure 5 Adding 4 mL of 0.1 M NaOH into well No.11

8. Perform the following steps according to different situations:
   - For App-A libraries sequencing: Use a pipette to completely remove all the reagent in well No.1 of Post Load Plate/Rapid Post Load Plate, then add 2 mL of App-A Insert primer 1 from High-throughput Pair-End Sequencing Primer Kit (App-A).
   - For App-D libraries sequencing: Use a pipette to completely remove all the reagent in well No.1 of Post Load Plate/Rapid Post Load Plate, then add 2 mL of App-D Insert primer 1 from High-throughput Single-End/Pair-End Sequencing Primer Kit (App-D).
9. Place the prepared load plate on the plate tray of MGIDL-T7RS. The screen will prompt that the post load plate is loaded.

Figure 6 Post-loading plate placement diagram

10. Align the flow cell to the RFID scanning area and ID information will appear in the text box.

Tips If ID information does not appears, please enter it manually according to the prompts.

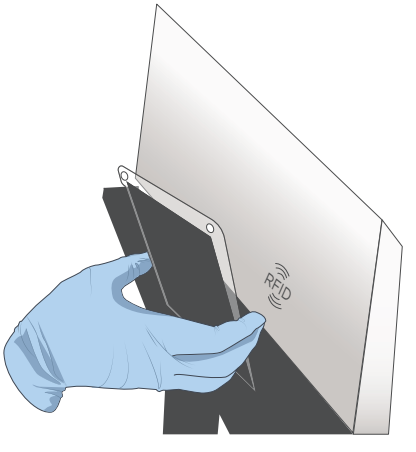

Figure 7 Scaning the Flow cell ID

11. Hold both sides of the flow cell, upwardly align the locating bulge on the flow cell to the locating groove on the flow cell stage. See the figure below:

Tips Ensure that all the four rubber sealing rings are on the four corners of the flow cell.

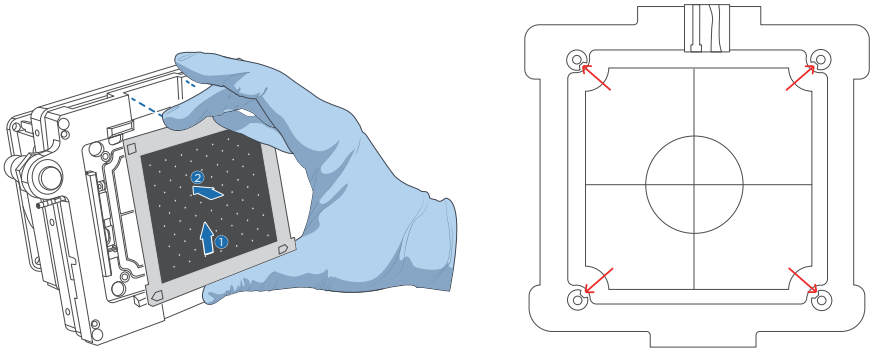

Figure 8 Flow cell locating

12. Press the flow cell attachment button on the flow cell stage and gently press down the edges of the flow cell to ensure that the flow cell is securely seated and held on the stage. The green light of the flow cell attachment button will be lit and the screen will prompt that the flow cell is loaded.

Tips
- Remove the dust on both sides of the flow cell with a canned air dust.
- Do not press or touch the glass cover of the flow cell to avoid flow cell damage or fingerprints and impurities left on the glass surface.
- Do not move the flow cell after installing the flow cell onto the stage, or it may cause the sealing gaskets to misalign with holes of the fluidics line.
- If flow cell attachment fails, gently wipe the back of the flow cell and flow cell stage with microfiber clean wiper moistened with 75% ethanol, then clean with a gas canned air dust.

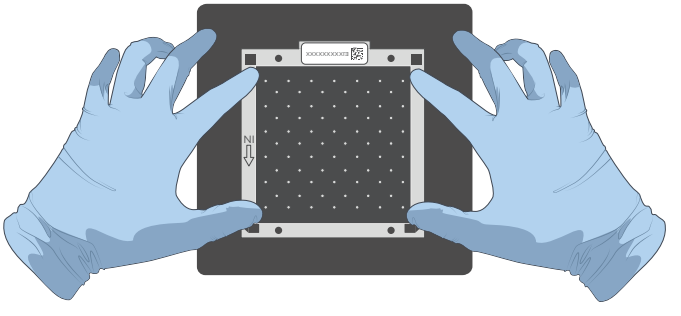

Figure 9 Flow cell loaded

13. Take out a new 0.5 mL microfuge tube from the Load Reagent Kit and add reagents following the table below, then gently pipette the DNB loading mixture 5 to 8 times by using a wide-bore, non-filtered tip.

Table 45 DNB loading mixture for FCL SE35/SE50/SE100/PE100

Adding order	Component	Cap color	volume (μL)
1	DNB	/	270
2	DNB Load Buffer II		90
3	Make DNB Enzyme Mix II (LC)		1
/	Total Volume		361

Table 46 DNB loading mixture for stLFR FCL PE100

Adding order	Component	Cap color	volume (μL)
1	DNB	/	270
2	DNB Load Buffer II		90
3	Make DNB Enzyme Mix IV		1
/	Total Volume		361

Table 47 DNB loading mixture for FCL PE150

Adding order	Component	Cap color	volume (μL)
1	DNB	/	300
2	DNB Load Buffer IV		150
/	Total Volume		450

Tips

- DNB in the above table refers to the pooled DNB in *5.5.2 DNBs pooling on Page 43*.
- Do not centrifuge, vortex, vigorously pipette or shake the tube.
- Prepare a fresh DNB loading mixture immediately on ice before the loading run, and use it as soon as possible once prepared.
- For PE150, used the DNB loading mixture within 30 minutes.

14. Tap on the text box next to **DNB ID**, enter the DNB information into the text box.

    Tips Use only numbers or letters or a combination of numbers and letters for DNB ID.

15. Place the 0.5 mL micro tube containing DNB loading mixture into the DNB tube hole, the screen will prompt that the DNB tube is loaded.

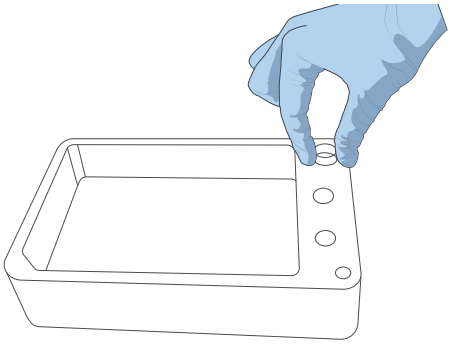

Figure 10 Placing DNB tube

16. Close the loading compartment door.
17. Tap the **Start** button and select **Yes** as shown in the in the figure below.

    Tips For PE150 sequencing, **Are you sure to loading Rapid Post Load Plate?** shows here.

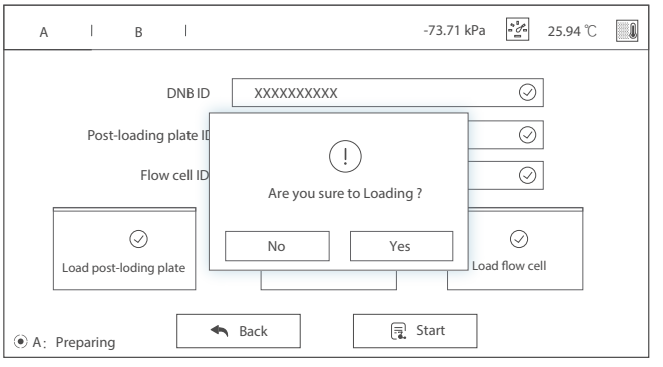

Figure 11 MGIDL-T7RS loading confirmation dialog box

18. Flow cell loading starts as shown in the figure below.

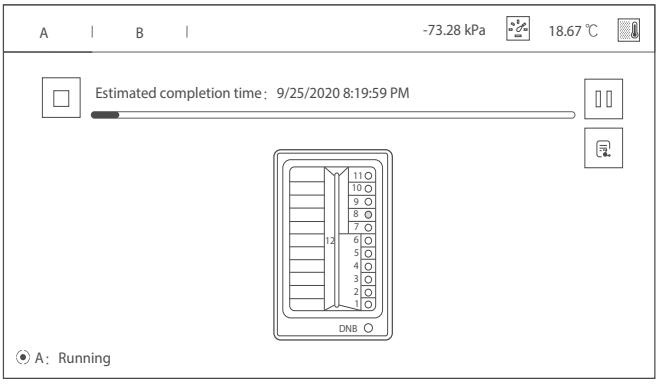

Figure 12 MGIDL-T7RS flow cell loading interface

19. The process take around 2 hours. When the screen is shown as in the figure below, the flow cell loading is complete.

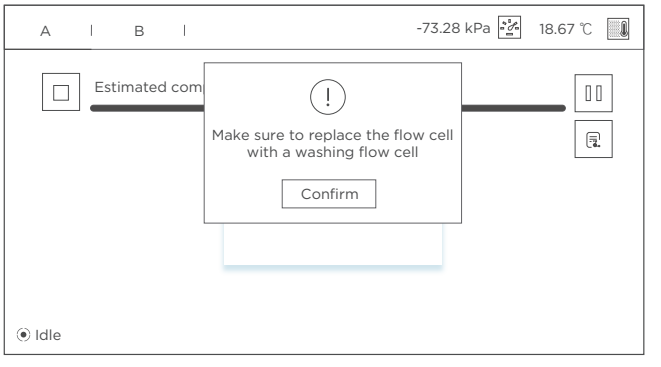

Figure 13 MGIDL-T7RS flow cell loading complete status window

20. Press the flow cell attachment button and remove the loaded flow cell from the stage. The flow cell is now ready for sequencing.

Tips

- If sequencing cannot be performed immediately, put the loaded flow cell in a clean zip bag and store it at 2 ℃ to 8 ℃ until use.
- The maximum storage time for loaded flow cell is 48 hours.

21. Tap **Confirm** button as shown in *Figure 13 on Page 53*, install the washing flow cell onto the flow cell stage and press the flow cell attachment button.

22. Tap **Post-wash.**

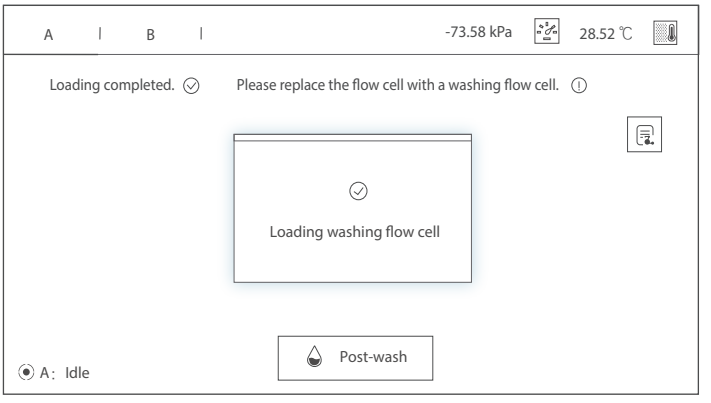

Figure 14 MGIDL-T7RS post-wash interface

23. and select **Yes** to start MGIDL-T7RS wash (see the figures below), which will take around 20 minutes.

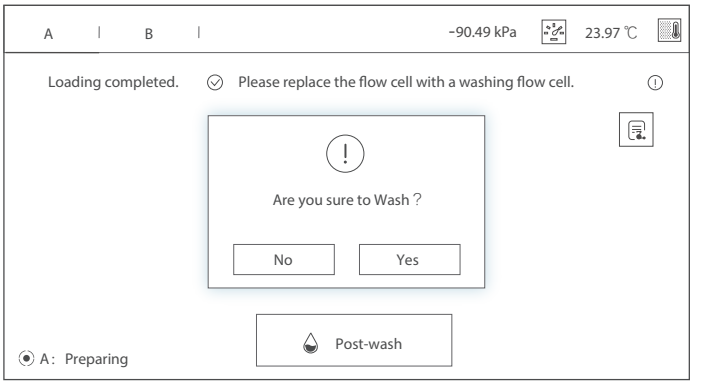

Figure 15 MGIDL-T7RS post-wash confirmation interface

24. MGIDL-T7RS wash starts, see the figure below:

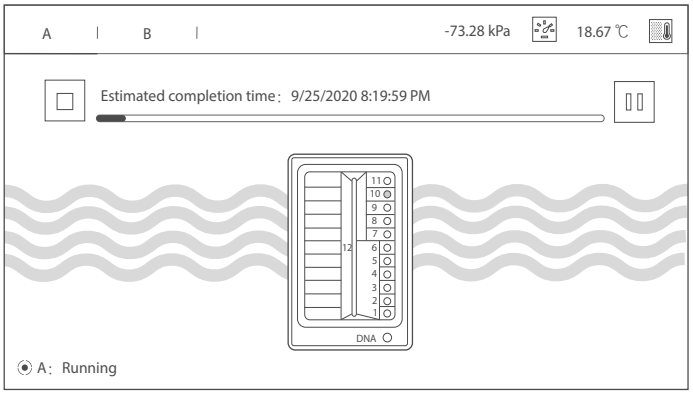

Figure 16 MGIDL-T7RS wash interface

25. When the screen is shown as the figure below, the wash is complete. Tap **Finish** and another flow cell loading can be performed.

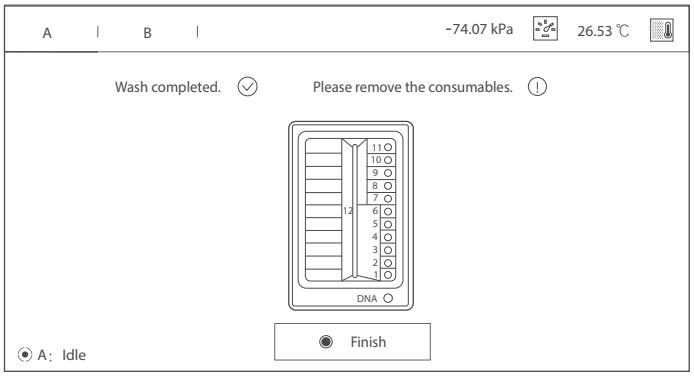

Figure 17 MGIDL-T7RS wash complete status window

Chapter 7 Preparing the Sequencing Reagent Cartridge and Washing Cartridge

7.1 Preparing the Sequencing Reagent Cartridge

Perform the steps below:

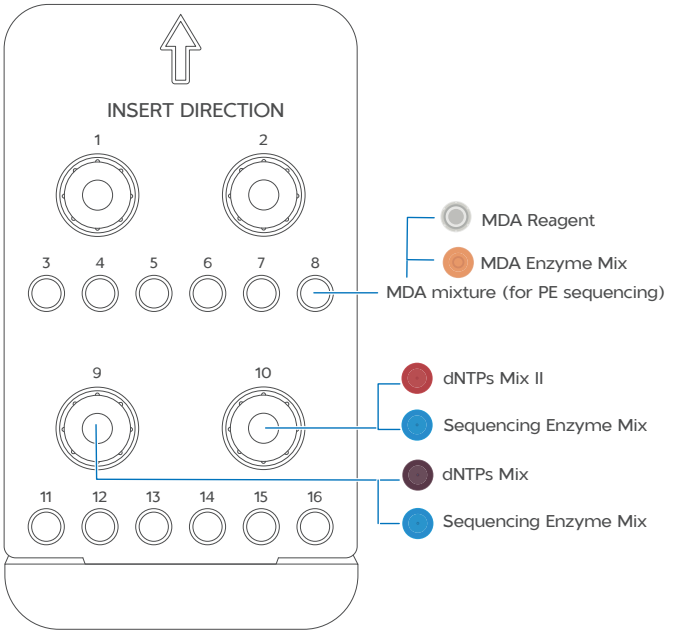

Figure 18 Sequencing cartridge wells

1. Invert the cartridge 3 times to mix before use.
2. Shake the cartridge vigorously in all directions 10 to 20 times to mix well.

   Tips It is the normal phenomenon that dark green crystal appears in well No.1, which is crystallization of raw materials of the reagent in this well. When the cartridge is thawed, mix the reagents in the cartridge well and the crystals will dissolve. Sequencing quality will not be affected.

3. Remove the dNTPs Mix IV (or dNTPs Mix V) and dNTPs Mix II from -25 ℃ to -15 ℃ storage 1 hour in advance and thaw at room temperature. Store at 2 ℃ to 8 ℃ until use.

4. Process the primer according to different situations:
   - For SE Dual barcode sequencing
     a. Take the 1 μM AD153 Barcode Primer 4 out of the CPAS Barcode Primer 4 Reagent Kit.
     b. After thawing at room temperature, mix the reagent thoroughly by using a vortex mixer for 5 seconds. Centrifuge it briefly and place it on ice until use.
   - For PE Dual barcode sequencing
     a. Take the 1 μM AD153 Barcode Primer 3 out of the CPAS Barcode Primer 3 Reagent Kit.
     b. After thawing at room temperature, mix the reagent thoroughly by using a vortex mixer for 5 seconds. Centrifuge it briefly and place it on ice until use.
   - For PE sequencing of App-A libraries
     a. Take the 1 μM App-A Insert Primer 2, 1 μM App-A MDA primer and 1 μM App-A Barcode Primer 2 out of the High-throughput Pair-End Sequencing Primer Kit (App-A).
     b. Take the 1 μM App-A Barcode Primer 3 (just for Dual barcode App-A PE sequencing) out of the High-throughput Barcode Primer 3 Reagent Kit (App-A).
     c. After thawing at room temperature, mix the reagents thoroughly by using a vortex mixer for 5 seconds. Centrifuge them briefly and place them on ice until use.
   - For PE sequencing of App-D libraries
     a. Take the 1 μM App-D Insert Primer 2, 1 μM App-D MDA primer and 1 μM App-D Barcode Primer 2 out of the High-throughput Pair-End Sequencing Primer Kit (App-D).
     b. Take the 1 μM App-D Barcode Primer 3 (just for Dual barcode App-D PE sequencing) out of the High-throughput Pair-End Sequencing Primer Kit (App-D).
     c. After thawing at room temperature, mix the reagents thoroughly by using a vortex mixer for 5 seconds. Centrifuge them briefly and place them on ice until use.

- For SE sequencing of App-D libraries

  a. Take the 1 μM App-D Barcode Primer 1 out of the High-throughput Single-End Sequencing Primer Kit (App-D).

  b. Take the 1 μM App-D Barcode Primer 4 (just for Dual barcode App-D SE sequencing) out of the High-throughput Single-End Sequencing Primer Kit (App-D).

  c. After thawing at room temperature, mix the reagents thoroughly by using a vortex mixer for 5 seconds. Centrifuge them briefly and place them on ice until use.

5. Open the cartridge cover and wipe any water condensation with lint-free paper. Spray 75% ethanol on the surface of the cartridge seal and clean the seal with lint-free paper.
6. Pierce the seal in the center of well No.9 and No.10 to make a hole around 2 cm in diameter by using a 1 mL sterile tip.
7. Remove the Sequencing Enzyme Mix from -25 ℃ to -15 ℃ storage and place on ice until use.
8. Use a pipette with the appropriate volume range and add the dNTPs Mix IV (or dNTPs Mix V) and Sequencing Enzyme Mix into well No.9 according to the table below.

Tips
- Mix dNTPs Mix IV (or dNTPs Mix V) for 5 s by using a vortex mixer and centrifuge briefly before use.
- Invert Sequencing Enzyme Mix 6 times before use..

Table 48 Sequencing cartridge well No.9 reagent adding

Model	dNTPs mix IV volume (mL)	dNTPs mix V volume (mL)	Sequencing enzyme mix volume (mL)
FCL SE35	1.7	/	1.7
FCL SE50	2.0	/	2.0
FCL SE100	3.0	/	3.0
FCL PE100/ App-A FCL PE100	/	2.76	2.76
FCL PE150/ App-A FCL PE150	/	3.74	3.74
stLFR FCL PE100	5.4	/	5.4

9. Use a pipette with the appropriate volume range and add the dNTPs Mix II and Sequencing Enzyme Mix into well No.10 following the table below:

   Tips
   - Mix dNTPs Mix II for 5 s by using a vortex mixer and centrifuge briefly before use.
   - Invert Sequencing Enzyme Mix 6 times before use..

   Table 49 Sequencing cartridge well No.10 reagent adding

Model	dNTPs mix II volume (mL)	Sequencing enzyme mix volume (mL)
		
FCL SE35	4.5	1.5
FCL SE50	5.4	1.8
FCL SE100	8.1	2.7
FCL PE100/ App-A FCL PE100	8.28	2.76
FCL PE150/ App-A FCL PE150	11.22	3.74
stLFR FCL PE100	14.7	4.9

10. Seal the loading wells of well No.9 and No.10 with the transparent sealing film.
11. When applying the sealing film, rotate your fingers to press the sealing film at the lid. Ensure that the sticker is firm and free of air bubbles, and the reagent will not overflow from the sample hole.
12. Place the cartridge horizontally on the table, and hold both sides of the cartridge with both hands. Shake it vigorously clockwise 10 to 20 times, and then counterclockwise 10 to 20 times, ensure that reagents are fully mixed.

    Tips Avoid shaking the cartridge too hard, vertically or holding the cartridge too slanted in case the reagent overflows from the sample hole.
13. Take the seal of loading wells out of the cartridge carefully after fully mixing.

    Tips
    - Avoid reusing the used sealing film.
    - Avoid cross-contamination of the reagents in wells 9 and 10.
14. Gently tap the cartridge on the bench to reduce air bubbles in the reagents.

    Tips The FCL SE35/FCL SE50/FCL SE100 cartridge for single barcode sequecing is ready, go to *17 Close the sequencing cartridge cover. on Page 62* for the next step.

15. Perform the following steps according to different situations:

- For SE Dual barcode sequencing

  a. Pierce the seal of well No.3 by using a 1 mL sterile tip.

  b. Add 3.5 mL of 1 μM AD153 Barcode Primer 4 into well No.3 with a 1 mL pipette.

  Tips The FCL SE35/FCL SE50/FCL SE100 cartridge for dual barcodes sequecing is ready, go to *17 Close the sequencing cartridge cover. on Page 62* for the next step.

- For PE Dual barcode sequencing:

  a. Pierce the seal of well No.3 by using a 1 mL sterile tip.

  b. Add 3.5 mL of 1 μM AD153 Barcode Primer 3 into well No.3 with a 1 mL pipette.

- For PE sequencing of App-A libraries:

  a. Pierce the seals of well No.3, No.4, No.6 and No.13.

  b. Add the reagents by using the appropriate pipette according to the table below:

  Tips App-A barcode primer 3 is just for App-A PE dual barcode sequencing.

Table 50 App-A Reagents for PE sequencing

Primer working solution	Well	Volume (mL)
1 μM App-A Barcode Primer 2	No.4	3.5
1 μM App-A MDA Primer	No.6	4.2
1 μM App-A Insert Primer 2	No.13	4.2
1 μM App-A Barcode Primer 3	No.3	3.5

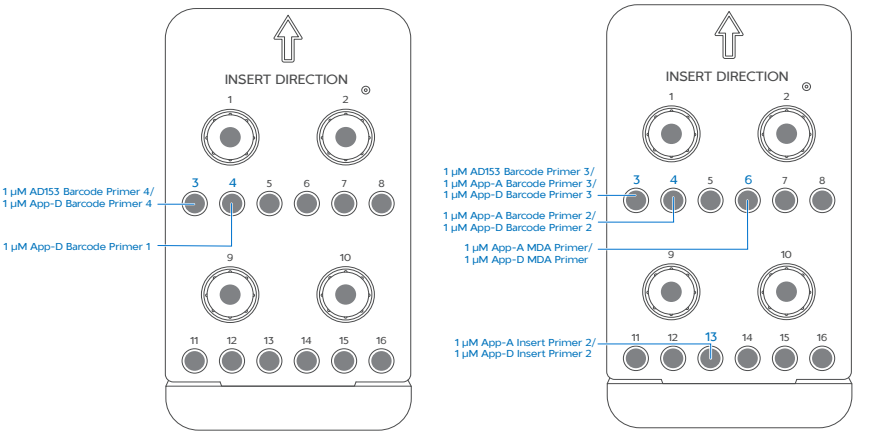

Figure 19 Primers added wells (Left: SE; Right: PE)

- For SE sequencing of App-D libraries:

  a. Pierce the seals of well No.3 and No.4.

  b. Add the reagents by using the appropriate pipette according to the table below:

  Tips App-D barcode primer 4 is just for App-D SE dual barcode sequencing.

Table 51 App-D Reagents for SE sequencing

Primer working solution	Well	Volume (mL)
1 μM App-D Barcode Primer 1	No.4	3.5
1 μM App-D Barcode Primer 4	No.3	3.5

Tips The FCL SE50/FCL SE100 sequencing cartridge for App-D libraries sequencing is ready, go to *17 Close the sequencing cartridge cover. on Page 62* for the next step.

- For PE sequencing of App-D libraries:

  a. Pierce the seals of well No.3, No.4, No.6 and No.13.

  b. Add the reagents by using the appropriate pipette according to the table below:

  Tips App-D barcode primer 3 is just for App-D PE dual barcode sequencing.

Table 52 App-D Reagents for PE sequencing

Primer working solution	Well	Volume (mL)
1 μM App-D Barcode Primer 2	No.4	3.5
1 μM App-D MDA Primer	No.6	4.2
1 μM App-D Insert Primer 2	No.13	4.2
1 μM App-D Barcode Primer 3	No.3	3.5

16. Perform the following steps for PE sequencing:
    1) Pierce the seal of well No.8 by using a 1 mL sterile tip.
    2) Add 600 μL of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette.
    3) Invert the tube 4 to 6 times to mix the reagents.
    4) Add all the mixture to well No.8. When adding the mixture, ensure that there is no bubble at the bottom of the tube.

    Tips
    - When using the MDA Enzyme Mix, do not touch the wall of the tube to prevent influencing the enzyme activity.
    - The cartridge for FCL PE100/FCL PE150/stLFR FCL PE100 with single barcodes is ready, refer *Chapter 8 Sequencing on Page 64* for the next step.
    - For PE dual barcode sequencing or App-A/App-D libraries sequencing, please ensure that the primers replaced are correctly added to the reagent wells.

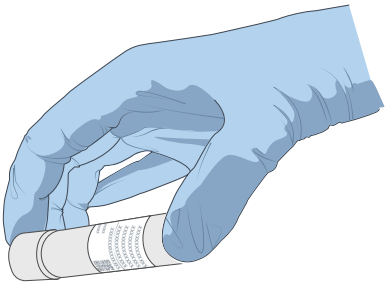

Figure 20 MDA mixing

17. Close the sequencing cartridge cover.

7.2 Preparing the Washing Cartridge

Perform the steps below:

1. Shake the cartridge clockwise 5 to 10 times, and then counterclockwise 5 to 10 times to ensure the reagents are fully mixed.
2. Spray 75% ethanol on the surface of the cartridge seal and clean the seal with lint-free paper. Pierce either of the well No.2 by using a 1 mL sterile tip.

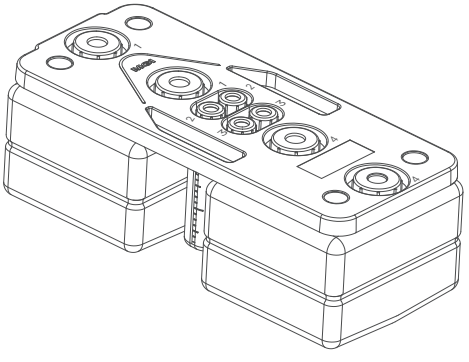

Figure 21 Washing cartridge

3. Add 45 mL of 0.1 M NaOH into well No.2 through the pierce by using an electronic pipette. Refer to *4.2 Preparing wash reagents on Page 24* for the preparation of 0.1 M NaOH.

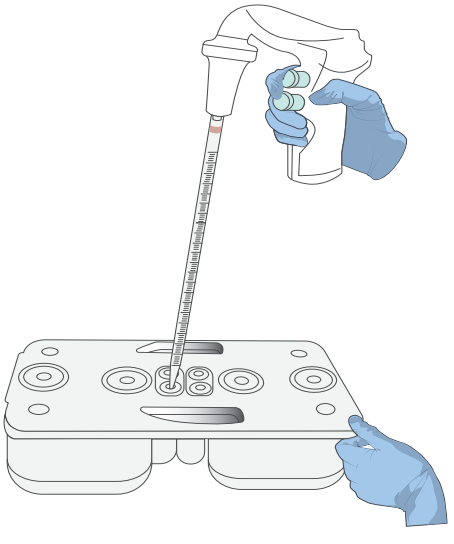

Figure 22 Washing cartridge added 0.1 M NaOH

Chapter 8 Sequencing

8.1 Loading the reagent cartridge

Perform the steps below:

1. Open the reagent compartment door and clean the inner walls with a microfiber clean wiper or lint-free paper moistened with laboratory-grade water. Keep the compartment clean and dry.

   Tips Be careful not to be scratched by the sampling needle above when cleaning the inner walls of the compartment.

2. Place the Sequencing Reagent Cartridge into the sequencing cartridge compartment and place the Washing Cartridge into the washing cartridge compartment.

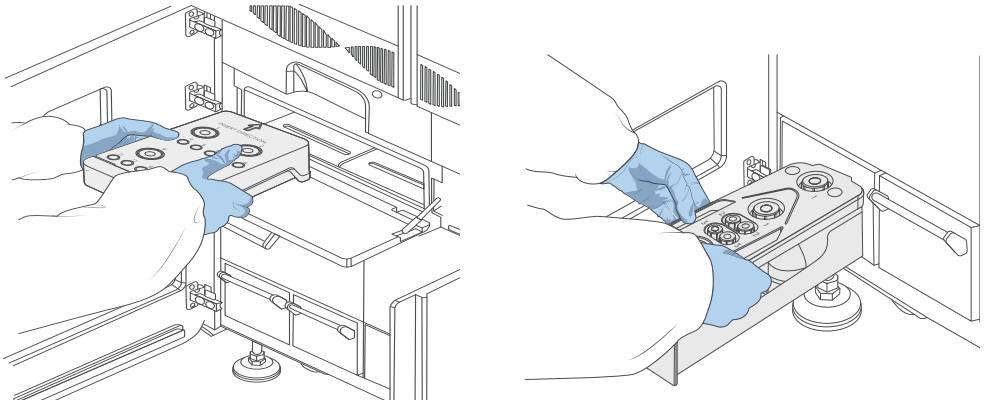

Figure 23 Loading the reagent cartridge

3. Close the doors of both sequencing cartridge compartment and washing cartridge compartment, and then close the door of the reagent compartment.

8.2 Entering sequencing interface

Enter the user name **research** and password **Admin123**, or the user name **user** and password **Password123**, tap **Log in** to enter the main interface.

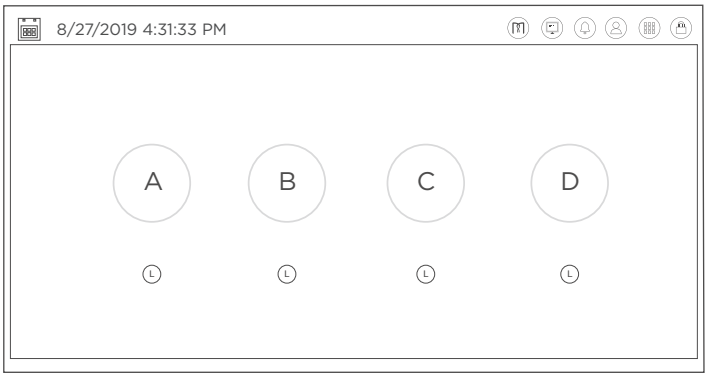

Figure 24 DNBSEQ-T7RS main interface

8.3 Loading the flow cell

Perform the steps below:

1. Select A/B/C/D respectively according to sequencing demand. Tap **Sequencing** and select **New run** (see the figure below).

Figure 25 DNBSEQ-T7RS selection interface

2. Remove the dust on both sides of the flow cell with a canned air dust, If there are any dust on the back, use a lint-free cloth or Kimwipes to wipe it clean. Ensure that there is no visible dust on the flow cell. Put the loaded flow cell on the flow cell drive, and tap the flow cell drive control button to load the flow cell into the device.

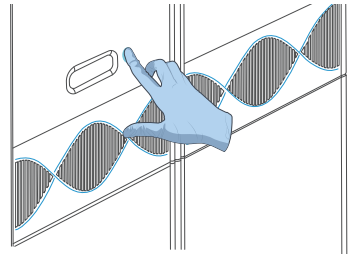

Figure 26 The flow cell drive

Tips Be careful not to touch the flowcell's inlet and outlet during when cleaning.

8.4 Sequencing parameters

Tips
- For App libraries and stLFR sequencing, the barcode file need to be manually imported before sequencing.
- For the App library with a barcode length of 8bp, when mixed sequencing with the MGI library, please customize the Barcode/Dualbarcode read length as 10bp. In addition, please add a 2bp fixed sequence "AC" before the original 8bp Barcode/Dualbarcode sequence in the barcode list of the App library. For example, the original 8bp barcode sequence is "xxxxxxxx", the sequence in the barcode list should be "ACxxxxxxxx".

Perform the steps below:

1. Align the sequencing cartridge, washing cartridge and flow cell respectivly to the RFID scanning area, the ID information will automatically display in the corresponding text box. If the scanning fails, information should be entered manually.

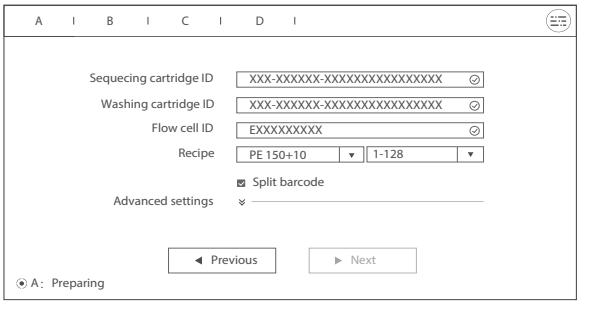

Figure 27 DNBSEQ-T7RS sequencing parameters

2. Tap ▼ next to **Recipe**. Select a appropriate sequencing recipe from the list. If Customize recipe is required, select **Customize** in the drop-down menu to enter the interface as shown in the figure below:

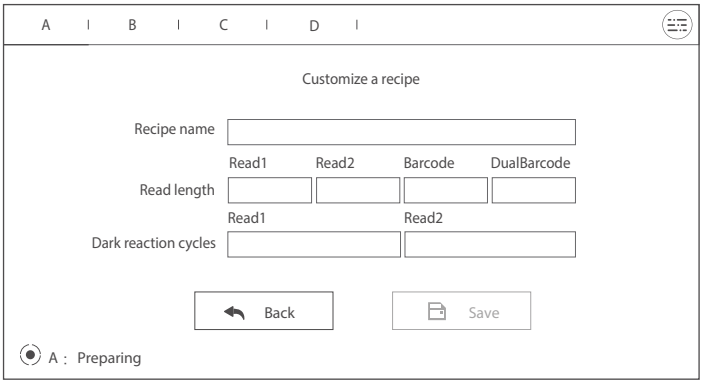

Figure 28 Customize a recipe

- The rules for filling in the **Customize a recipe** interface are as follows:
  - When sequencing recipe is named, use only letters, numbers, "+", "_" and "–".
  - Duplicate name check will be performed to ensure that each sequencing recipe name is unique. for example, a new recipe name must not be the same with an existing recipe.
  - Enter numbers only to the read length of Read1, Read2, Barcode and DualBarcode.
  - Multiple segments of dark reaction cycles can be set in Read1 and Read2. Use "," to separate each segment and the dark reaction cycles of each segment are presented in the format of "number" and "number-number".

- Example:
  - The Read1 read length is 100 cycles and the Read2 read length is 100 cycles.
  - Barcode read length is 10 cycles and Dual Barcode read length is 10 cycles.
  - In the 100 cycles of Read1, the 20th to 30th cycles and the 50th to 60th cycles need to perform dark reactions. In the 100 cycles of Read2, the 20th to 30th cycles need to perform dark reactions.
  - Name this recipe as "PE100+10+10+Dark".
  - Fill the **Customize a recipe** interface as shown in the figure below:

Tips For stLFR FCL PE100, the Read1 read length is 100 cycles and the Read2 read length is 100 cycles, Barcode read length is 42 cycles and DualBarcode read length is 10 cycles.

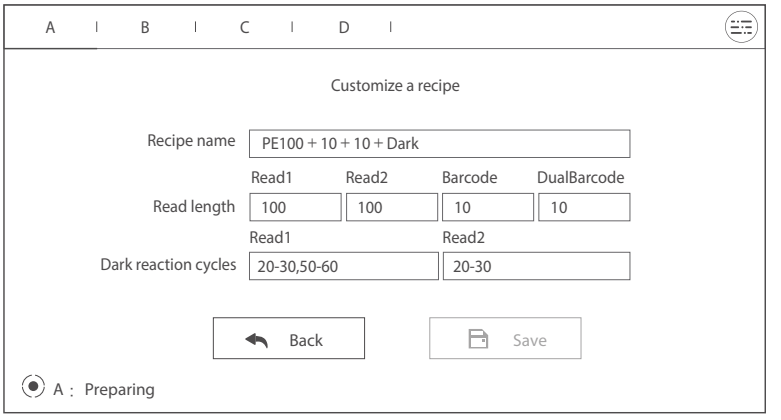

Figure 29 Example

3. Tap ▼ in the red box of the figure below and select the corresponding barcode sequence. If customized barcode sequence is required, selected the inputted barcode sequence. Select whether split barcode and Dual barcode (stLFR FCL PE100 just select split barcode)

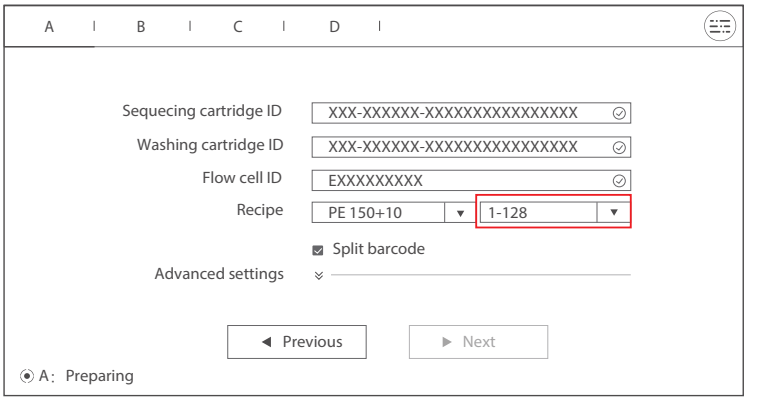

Figure 30 Set the barcode sequence

4. Tap on the **Advanced settings** to enter the interface as shown in the figure below. Users can select whether the primer is **custom primers** and whether to perform **Auto wash**.

   Tips **Custom Primers** refers to primers that need to be replaced before sequencing. The App-A library/App-D library Sequencing uses **Custom Primers**, The stLFR sequencing does not use **Custom Primers**.

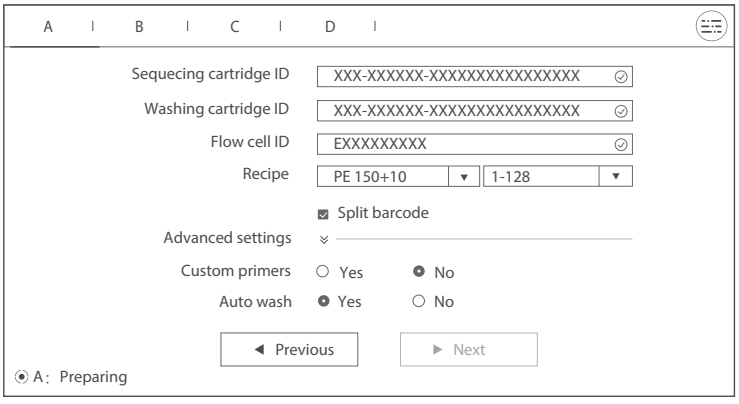

Figure 31 DNBSEQ-T7RS advanced settings

8.5 Reviewing parameters

Tap **Next** to review the parameters and ensure that all information is correct, see the figure below for the example of PE150:

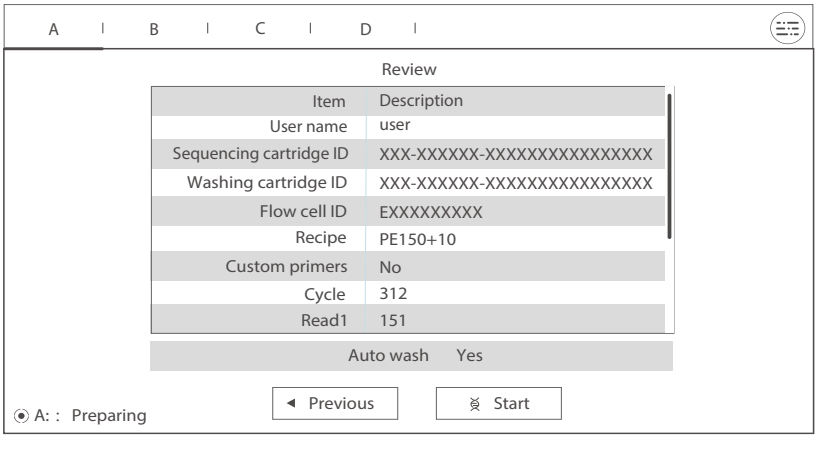

Figure 32 Reviewing information

8.6 Starting sequencing

Perform the steps below:

1. After confirming that all the information is correct, tap **Start** and select **Yes**.

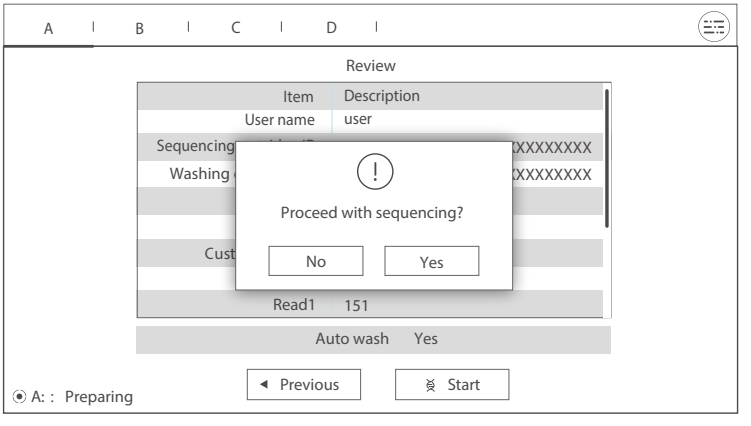

Figure 33 Confirming sequencing interface

2. When the following screen appears, the sequencing has begun.

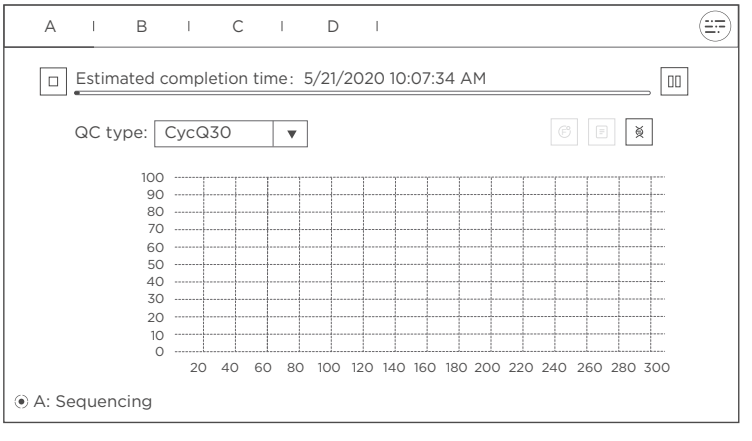

Figure 34 Sequencing Starts Interface

3. During sequencing, tap and the selection of **Auto wash** can be changed as shown in the figure below:

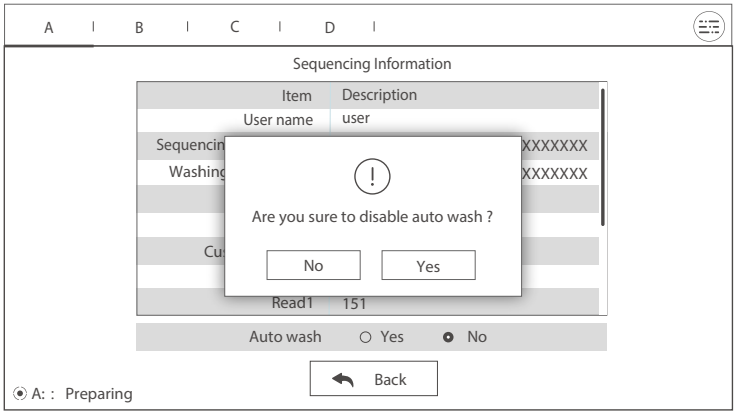

Figure 35 Disable auto wash

4. When the screen appears as shown in the figure below, the sequencing and wash process for this run are complete.

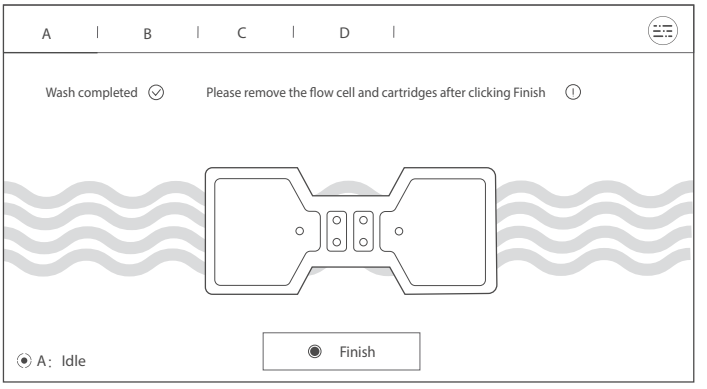

Figure 36 DNBSEQ-T7RS sequencing complete interface

8.7 Data access

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

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

Chapter 9 Device maintenance

9.1 Terminology and definition

Table 53 Wash methods

Wash type	Description
MGIDL-T7RS automatic wash	When the loading is complete, replace the flow cell with a used flow cell and tap **Wash**. The loader will automatically perform the wash without the need to change the post load plate.
DNBSEQ-T7RS automatic wash	Select **Yes** for Auto wash, the system will automatically perform a wash after each sequencing run.
MGIDL-T7RS manual wash	Perform a wash manually under the following conditions: • The device is used for the first time. • The device has not been used for 7 days or longer. • Impurities are found in the device or flow cell. • Replace the tubing, sampling needles, or other accessories. exposed to the reagents.
DNBSEQ-T7RS manual wash	Perform a wash manually under the following conditions: • The device is used for the first time. • The device has not been used for 7 days or longer. • Impurities are found in the device or flow cell. • Replace the tubing, sampling needles, or other accessories exposed to the reagents.

9.2 Washing cartridge

- An empty washing cartridge and washing flow cell for a full wash are provided together with the device.
- The washing plate and washing cartridge must be cleaned before refilled with fresh washing reagents. Replace the washing plate and washing cartridge after three months of continuous use.
- Flow cells from previous runs can be used as washing flow cells. Each flow cell can be used for 3 times.
- Prepare the MGIDL-T7RS washing plate: take a clean and empty post-load plate, add 4 mL of 0.1 M NaOH into well No.11, 4 mL of 1 M Wash Reagent I (1 M NaCl+0.05% Tween-20) into well No.10, 4 mL of laboratory-grade water into well No.9 and 20 mL of laboratory-grade water into well No.12.
- Prepare DNBSEQ-T7RS washing cartridge 1: A clean and empty sequencing cartridge.
- Prepare DNBSEQ-T7RS washing cartridge 2: Take a clean and empty washing cartridge, add 45 mL of Wash Reagent II (0.1 M NaOH) into either of the well No.2, and 45 mL of Washing Reagent I (1 M NaCl+0.05% Tween-20) into either of the well No.3.

9.3 Wash procedures

9.3.1 MGIDL-T7RS manual wash

Perform the steps below:

1. Enter the program. Enter the password **123**, tap **Log in** to enter the main interface.
2. Select the side that needs to be washed, and open the loading compartment door.
3. Place the washing plate filled with wash reagents into the side that needs to be washed. Close the compartment door.
4. Press the flow cell attachment button and wait until the negative pressure is released. Remove the flow cell from the stage. Skip this step if no flow cell is on the stage.
5. Take out the washing flow cell and place it on the flow cell stage. Press the flow cell attachment button and press down the flow cell to ensure the flow cell is securely attached to the stage.
6. Tap **Wash** and select **Yes** to begin the MGIDL-T7RS wash, which will take around 20 minutes.

9.3.2 DNBSEQ-T7RS manual wash

Perform the steps below:

1. Ensure that the pure water container is filled with at least 4.5 L of laboratory-grade water before performing the wash.
2. Enter the program. Enter the user name **research** and password **Admin123**, or the user name **user** and password **Password123**, tap **Log in** to enter the main interface
3. Tap **Wash**. and install a used flow cell from a previous run. Press the flow cell drive control button again to withdraw the flow cell drive.
4. Place the clean and empty DNBSEQ-T7RS washing cartridge 1 into the low-temperature compartment on the side that needs to perform a wash, and then close the low-temperature compartment door.
5. Place the DNBSEQ-T7RS washing cartridge 2 filled with wash reagents into the room-temperature compartment on the side that needs to perform the wash, and then close the room-temperature compartment door and the reagent compartment door.
6. Tap **Start** and select **Yes** to begin the DNBSEQ-T7RS manual wash, which will take around 40 minutes.

Chapter 10 Troubleshooting

10.1 Low DNB concentration

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

- Check whether the kit has expired.
- Check whether the library meets the requirements.
- If DNB concentration still does not meet the requirements after a new sample preparation, please contact the engineer.

10.2 Abnormal negative pressure

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

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

10.3 Bubbles

10.3.1 Bubbles in MGIDL-T7RS

- Check the rubber sealing ring to ensure that it is in the right position.
- Check the DNB loading plate to ensure that enough reagent is in each well
- Replace the used Flow Cell and inspect the pump.
- If the problem persists, please contact the field service engineer.

10.3.2 Bubbles in DNBSEQ-T7RS

- Check the water container to ensure that water is enough.
- Check the water tube in the water container to ensure that it inserts to almost the bottom of the container.
- Check the reagent needles to ensure that they can immerse fully into the reagent cartridge. Otherwise, restart the sequencing software.
- If the problem persists, please contact the field service engineer.

10.4 Impurities

- Perform a manual wash on MGIDL-T7RS and DNBSEQ-T7RS.
- If there is still no improvement after manual wash, follow *4.2 Preparing wash reagents on Page 24* to reconstitute wash reagents, and perform manual wash again on MGIDL-T7RS and DNBSEQ-T7RS.
- If there is still no improvement, please contact the field service engineer.

10.5 Pump fails

- Check if the pure water volume is sufficient.
- When it happens in the MGIDL-T7RS and DNBSEQ-T7RS:
  - Remove the flow cell, check if there are impurities on the sealing gasket and remove any dust with a canned air dust.
  - Place the flow cell following the instruction and start the pump again.
- Check if the sampling needles can move properly.
- If the sampling needles cannot move properly, restart the sequencing software.
- If the problem persists, please contact the field service engineer.

10.6 Reagent kit storage

- If the kit has been thawed (including the dNTPs) but cannot be used within 24 hours, it can be frozen and thawed at most one time.
- If the kit has been thawed (including the dNTPs) but cannot be used immediately, store it at 2 ℃ to 8 ℃ . It is strongly recommended to use it within 24 hours. A thawed kit that is stored at 2 ℃ to 8 ℃ may still be used within seven days, although it may compromise sequencing quality. It is not recommended that you use a kit that has been thawed and stored at 2 ℃ to 8 ℃ for more than seven days.
- If the dNTPs and Sequencing Enzyme Mix have been added into the cartridge, i.e. the cartridge has been prepared and the needles have punctured the seal but the cartridge cannot be used immediately, the cartridge must be covered with foil or plastic wrap. Store the kit at 2 ℃ to 8 ℃ 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.

Tips For MDA reagent, it is recommended to transfer to -20 ℃ to -15 ℃ storage. Invert to mix before use.

Appendix 1 Qubit ssDNA assay kit

Tips Be careful not to create bubbles.

Perform the steps below:

1. Prepare the Qubit working solution.
   1) Diluting the Qubit ssDNA Reagent 1:199 in Qubit ssDNA Buffer. Each sample requires 190 µL of Qubit working solution.
   2) Mix by vortexing 2 to 3 seconds.

      Tips
      - Each sample DNB quantification requires the preparation of a 200 µL of Qubit Working solution.
      - Two additional 200 µL of Qubit Working solutions are required to build the standard curve.
2. Prepare the required number (N+2) of 0.5 mL tubes for standards and samples (N).
3. Prepare the standard tubes and sample tubes to be tested according to the table below.

Component	S1 (µL)	S2 (µL)	D1 (µL)	D2 (µL)	D3 (µL)
working solution	190	190	198	198	198
S1 (0 ng/µL)	10	/	/	/	/
S2 (20 ng/µL)	/	10	/	/	/
Sample	/	/	2	2	2
Tatal	200	200	200	200	200

4. Allow all tubes to incubate at room temperature for 2 minutes.
5. On the Home screen of the Qubit 4.0 Fluorometer, press DNA, then select ssDNA as the assay type. The “Read standards” screen is displayed. Press Read Standards to proceed.
6. Insert the tube containing S1 into the sample chamber, close the lid, then press Read standard.
7. When the reading is complete, put S2 into the sample chamber, close the lid, then press Read standard.
8. When the reading is complete, remove S2. The instrument displays the results on the Read standard screen.
9. Press Run samples. On the assay screen, select the sample volume and units, Insert a sample tube into the sample chamber, close the lid, then press Read tube. When the reading is complete, remove the sample tube. Repeat until all samples have been read.

Appendix 2 Conflicting adapter list

Tips When the App libraries need to be pooled with MGI libraries, the adapters in same row of the following table should avoid being pooled together for sequencing.

Table 1 Conflicting adapter list 1

MGI barcode	Index 1 (i7)
26	[H/N]716
93	[H/N]704
106	[H/N]710
106	UDI0018
126	UDI0071
506	UDI0067
547	[H/N]726

Table 2 Conflicting adapter list 2

MGI barcode	Index 1 (i7)
22	UDI0037
22	UDI0055
86	UDI0087
92	UDI0021
101	UDI0024
101	UDI0092
533	[E/H/N/S]517

Appendix 3 Manufacturer

Manufacturer	Wuhan MGI Tech Co., Ltd.
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
Technical support	Wuhan MGI Tech Co., Ltd.
Technical support E-mail	MGI-service@mgi-tech.com
Website	www.mgi-tech.com

Appendix 4 European representative information

Manufacturer	Latvia MGI Tech, SIA.
Address	“Lidostas parks”, Marupes pag., Marupes nov., LV-2167, Latvia