Part No.: SOP-013-B01-080

High-throughput Sequencing Set

DNBSEQ-T7RS

Instructions for use

Version: 8.0

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 8.0.

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Revision history

Version	Date	Description
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 .
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.

Version	Date	Description
A0	December 2019	Initial release

Sequencing set

Catalog number	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
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

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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 primer kit is applicable to MGI libraries and App libraries. 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
SE35	35	/	10	10	36+10+10	56
SE50	50	/	10	10	51+10+10	71
SE100	100	/	10	10	101+10+10	121
PE100	100	100	10	10	202+10+10	222
PE150	150	150	10	10	302+10+10	322
App-A PE100	100	100	10	10	202+10+10	222
App-A PE150	150	150	10	10	302+10+10	322
App-D SE50	50	/	10	10	51+10+10	71
App-D SE100	100	/	10	10	101+10+10	121
App-D PE100	100	100	10	10	202+10+10	222
App-D 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)
SE35	4.5	5.0	1	2
SE50	5.5	6.0	1	2
SE100	9.0	10.5	1	2
PE100	15.0 to 16.0	16.0 to 20.0	1	2
PE150	21.0 to 23.0	23.0 to 28.0	1	2
App-A PE100	15.0 to 16.0	16.0 to 20.0	1	2
App-A PE150	21.0 to 23.0	23.0 to 28.0	1	2
App-D SE50	5.5	6.0	1	2
App-D SE100	9.0	10.5	1	2
App-D PE100	15.0 to 16.0	16.0 to 20.0	1	2
App-D 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 manual carefully before use.

- Ensure that you are familiar with the SOP&Attention of all the laboratory apparatus to be used.
- Avoid direct skin and eye contact with any samples and reagents. 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.
- This product is for one sequencing run only and cannot be reused.
- The components and packages are batched separately. Keep the components in the packages until use and do not remove them. Mixed use of reagent components from different batches is not recommended.
- Do not use expired products.

Chapter 2 List of sequencing set components and User-supplied equipment and consumables

2.1 List of sequencing set components

Tips It is worth reminding that:

- A dual barcode FCL SE35/SE50/SE100 sequencing run requires the cPAS Barcode Primer 4 Reagent Kit (Cat. 1000014048), in addition to its corresponding sequencing set.
- A dual barcode FCL PE100/PE150 sequencing run requires the cPAS Barcode Primer 3 Reagent Kit (Cat. 1000020834), in addition to its corresponding sequencing set.
- A dual barcode App-A FCL PE100/PE150 sequencing run requires the High-throughput Barcode Primer 3 Reagent Kit (App-A) (Cat. 1000014047), in addition to its corresponding sequencing set.
- An App-D FCL SE50/SE100 sequencing run requires the High-throughput Single-End Sequencing Primer Kit (App-D) (Cat. 940-000857-00), in addition to its correspongding sequencing set.
- An App-D FCL PE100/PE150 sequencing run requires the High-throughput Pair-End Sequencing Primer Kit (App-D) (Cat. 1000028550), in addition to its correspongding sequencing set.

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

Component	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 tubee			
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			
DNBSEQ-T7RS High-throughput Sequencing Kit (FCL SE35) **Catalog number: 1000019813**				
dNTPs Mix II	4.50 mL/tube×1 tube	-80 ℃ to -15 ℃	-25 ℃ to -15 ℃	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 ℃	0 ℃ to 30 ℃	12 months

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

Component	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			
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	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			
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	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			
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	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 **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			
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	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			
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	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			
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

Component	Spec&quantity	Transportation temperature	Storage temperature	Expiry date
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	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			
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			

Component	Spec&quantity	Transportation temperature	Storage temperature	Expiry date
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	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	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	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	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	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			

Tips App-D primer kits support the sequencing of mixed libraries with Truseq, Nextera and AD153 adapters, and it should be used together with DNBSEQ-T7RS High-throughput Sequencing Set.

2.2 User-supplied equipment and consumables

Tips
- Avoid making and loading DNBs by the filtered pipette tips.
- It is highly recommended that pipettes and tips of the suggested brands and catalog numbers be used. Using other brands may yield negative results.

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	/
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	/
Power dust remover	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	BI-200K-H
Qubit assay tubes	Thermo Fisher	Q32856

Equipment and consumables	Recommended brand	Catalog number
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 Transport tubes	AXYGEN	/
Lint-free paper	MLS	/
Ziplock bag	MLS	/

Chapter 3 Sequencing workflow

1	Making DNB: use reagents from DNB Making Kit and DNA library from user to make DNB.
2	Loading DNB: load DNB onto the flow cell using reagents from DNB Load Reagent Kit at MGIDL-T7RS loader.
3	Preparing sequencing reagent kit: inspect, thaw the reagent kit (for 4 to 24 hours) and then add the required reagents, as well as check the pure water container and waste liquid container.
4	Click **Start,** and the machine starts self-check.
5	Sequencing
6	Data analysis

Chapter 4 Making DNB

4.1 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 17 Recommended insert size and theoretical throughput for each flow cell

Sequencing kit	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	200 to 400	WGS, WES, RNAseq, Single Cell	5800	1160
FCL PE150	300 to 500	WGS, WES, RNAseq	5800	1740
App-A FCL PE100	200 to 400		5500	1100
App-A FCL PE150	300 to 500		5500	1650
App-D FCL SE50	50 to 230		5500	275
App-D FCL SE100	200 to 400		5500	550
App-D FCL PE100	200 to 400		5500	1100
App-D FCL PE150	300 to 500		5500	1650
stLFR FCL PE100	200 to 1500	stLFR	4000	800

4.2 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 18 Library requirement

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

4.3 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 82*

4.3.1 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 17 on Page 19*, 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 19 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

4.3.2 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.

4.4 Making DNB

Tips
- Mixed use of reagent components from different batches is not recommended.
- Avoid making and loading DNBs by the filtered pipette tips.
- It is highly recommended that pipettes and tips of the suggested brands and catalog numbers be used. Using other brands may yield negative results.

Seven DNB making protocols are listed below, please select the appropriate one according to the sequencing kit used.

- Section *4.4.1 Preparing DNB for the FCL SE35/SE50/SE100/PE100 kits on Page 23*.
- Section *4.4.2 Preparing DNB for the FCL PE150 kit on Page 26*.
- Section *4.4.3 Preparing DNB for the App-A FCL PE100 kit on Page 29*.
- Section *4.4.4 Preparing DNBs for the App-A FCL PE150 kit on Page 32*.
- Section *4.4.5 Preparing DNB for the App-D FCL SE50/SE100/PE100 kit on Page 35*.
- Section *4.4.6 Preparing DNB for the App-D FCL PE150 kit on Page 38*.
- Section *4.4.7 Preparing DNB for the stLFR FCL PE100 kit on Page 41*.

4.4.1 Preparing DNB for the FCL SE35/SE50/SE100/PE100 kits

4.4.1.1 Calculating the required amount of ssDNA library

- 270 μL of DNB is required to load one flow cell for FCL SE35/SE50/SE100/PE100.
- One DNB making 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 either 100 μL or 50 μL of DNBs are shown in the table below.

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

Table 20 FCL SE35/SE50/SE100/PE100 required ssDNA volume

Sample type	Required ssDNA volume: V (μL)	
	100 μL DNB reaction	50 μL DNB reaction
Regular library	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.

4.4.1.2 Preparing reagents for DNB making

Perform the steps below:

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

4.4.1.3 Making DNB

Perform the steps below:

1. Take out a 0.2 mL 8-strip tube or PCR tubes, and prepare the reaction mix on ice according to the table below. The following 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 *4.4.1.1 Calculating the required amount of ssDNA library on Page 23*.

Table 21 FCL SE35/SE50/SE100/PE100 Make DNB Reaction Mixture 1

Component	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

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 1 into a thermal cycler, and start the primer hybridization reaction according to the following table:

Tips As some thermal cyclers are slow in temperature adjustment, when the lid is being heated or cooled, the sample block may remain at room temperature and the procedure is not performed. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at working temperature during the DNB reaction.

Table 22 FCL SE35/SE50/SE100/PE100 primer hybridization reaction condition

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

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. Mix the Make DNB Enzyme Mix II (LC) with Make DNB Enzyme Mix I by gently pipetting 6 to 8 times before use as described in the table below.

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

Table 23 FCL SE35/SE50/SE100/PE100 Make DNB Reaction Mixture 2

Component	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

5. Take the PCR tube out of the thermal cycler after the reaction becomes **Hold** at 4 °C . Centrifuge the tube briefly for 5 seconds, then place the tube on ice and add all the Make DNB Reaction Mixture 2 to the tube.
6. Mix the mixture thoroughly by using a vortex mixer for 5 seconds. Centrifuge it briefly and place it on ice until use
7. Place the tubes into a thermal cycler for the next reaction. The condition is shown in the table below:

Tips It is recommended to set the temperature of the heated lid to 35 °C or as close as possible to 35 °C .

Table 24 FCL SE35/SE50/SE100/PE100 rolling circle replication condition

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

8. Add 20 μL (for 100 μL of DNB reaction) or 10 μL (for 50 μL of DNB reaction) Stop DNB Reaction Buffer to the tube immediately when the reaction becomes **Hold** at 4 ℃ . Mix the tube gently by pipetting 5 to 8 times by using a wide-bore, non-filtered pipette tip.

   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 4 ℃ and perform sequencing within 48 hours.

4.4.2 Preparing DNB for the FCL PE150 kit

4.4.2.1 Calculating the required amount of ssDNA library

- 300 μL of DNB is required to load one flow cell for the FCL PE150 kit. One DNB making reaction can make 90 μL of DNB.
- The required ssDNA library volume needed to make 90 μL of DNB (one DNB reaction) is shown in the table below.

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

Table 25 FCL PE150 required ssDNA volume

Sample type	Required ssDNA volume: V (μL)
Regular Library	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.

4.4.2.2 Preparing reagents for DNB making

Perform the steps below:

1. Place the library on ice until use.
2. Take the Low TE Buffer, Make DNB Buffer and Stop DNB Reaction Buffer out of the DNB Rapid Make Reagent Kit. Thaw reagents at room temperature.
3. Thaw the Make DNB Rapid Enzyme Mix II on ice for approximately 0.5 hours.
4. After thawing, mix the reagents by using a vortex mixer for 5 seconds. Centrifuge them briefly, and place them on ice until use.

4.4.2.3 Making DNB

Perform the steps below:

1. Take out a 0.2 mL 8-strip tube or PCR tubes and prepare the reaction mix on ice following the table below. The following table only illustrates one make DNB reaction. The amount of input ssDNA and required number of make DNB reactions are determined by the actual application as described in *4.4.2.1 Calculating the required amount of ssDNA library on Page 26*.

   Tips Do not throw away the Low TE Buffer after this step. The remaining Low TE buffer will be used in DNB loading.

Table 26 FCL PE150 Make DNB Reaction Mixture 1

Component	Volume/90 µL DNB reaction (µL)
Low TE Buffer	20-V
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 mix 1 into a thermal cycler and start the primer hybridization reaction accoring to the following table:

   Tips As some thermal cyclers are slow in temperature adjustment, when the lid is being heated or cooled, the sample block may remain at room temperature and the procedure is not performed. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at working temperature during the DNB reaction.

Table 27 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.

5. Mix the Make DNB Enzyme Mix II (LC) with Make DNB Rapid Enzyme Mix II by gently pipetting 6 to 8 times before use as described in the table below.

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

Table 28 FCL PE150 Make DNB Reaction Mixture 2

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

6. Take the PCR tube out of the thermal cycler after the reaction becomes **Hold** at 4 ℃ . Centrifuge it briefly for 5 seconds.
7. Place the tube on ice, and add all the Make DNB Reaction Mixture 2 to the tube.
8. Mix the tube thoroughly by using a vortex mixer for 5 seconds.Centrifuge it briefly and place the tube into a thermal cycler for the next reaction. The condition is shown in the table below:

Tips It is recommended to set the temperature of the heated lid to 35 ℃ or as close as possible to 35 ℃ .

Table 29 FCL PE150 rolling circle replication condition

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

9. Add 10 µL of Stop DNB Reaction Buffer to the tube immediately when the reaction becomes **Hold** at 4 ℃ . Mix it gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

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 4 ℃ and perform sequencing within 8 hours.

4.4.3 Preparing DNB for the App-A FCL PE100 kit

4.4.3.1 Calculating the required amount of ssDNA library

- 270 μL of DNB is required to load one flow cell for the App-A FCL PE100 kit. One DNB making reaction can make either 100 μL or 50 μL of DNB.
- The required ssDNA library volume to make either 100 μL or 50 μL of DNB is shown in the table below.

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

Table 30 App-A FCL PE100 required ssDNA volume

Sample type	Required ssDNA volume: V (μL)	
	100 μL DNB reaction	50 μL DNB reaction
Regular library	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 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.

4.4.3.2 Preparing reagents for DNB making

Perform the steps below:

1. Place the library on ice until use.
2. Take the App-A Make DNB Buffer out of High-throughput Pair-End Sequencing Primer Kit (App-A) and thaw reagents at room temperature.
3. Take the Low TE Buffer and Stop DNB Reaction Buffer ot of the DNB Make Reagent Kit and thaw reagents at room temperature.
4. Thaw the Make DNB Enzyme Mix I on ice for approximately 0.5 hours.

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

4.4.3.3 Making DNB

Perform the steps below:

1. Take a 0.2 mL 8-strip tube or PCR tubes and prepare the reaction mix on ice following the table below. The following table only illustrates one make DNB reaction. The amount of input ssDNA and required number of make DNB reactions are determined by the actual application as described in *4.4.3.1 Calculating the required amount of ssDNA library on Page 29*.

Table 31 App-A FCL PE100 Make DNB Reaction Mixture 1

Component	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

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 1 into a thermal cycler and start the primer hybridization reaction accoring to the following table:

Tips As some thermal cyclers are slow in temperature adjustment, when the lid is being heated or cooled, the sample block may remain at room temperature and the procedure is not performed. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at working temperature during the DNB reaction.

Table 32 App-A FCL PE100 primer hybridization reaction condition

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

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.
5. Mix the Make DNB Enzyme Mix II (LC) with Make DNB Enzyme Mix I by gently pipetting 6 to 8 times before use as described in the table below.

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

Table 33 App-A FCL PE100 Make DNB Reaction Mixture 2

Component	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

6. Take the PCR tube out of the thermal cycler when the temperature reaches 4 ℃ . Centrifuge it briefly for 5 seconds.
7. Place the tube on ice and add all the Make DNB Reaction Mixture 2 to the tube.
8. Mix the mixture thoroughly by using a vortex mixer for 5 seconds. Centrifuge it briefly and place it on ice until use.
9. Place the tubes into a thermal cycler for the next reaction. The condition is shown in the table below:

Tips It is recommended to set the temperature of the heated lid to 35 ℃ or as close as possible to 35 ℃ .

Table 34 App-A FCL PE100 rolling circle replication condition

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

10. Add 20 µL (for a 100 µL of DNB reaction) or 10 µL (for a 50 µL of DNB reaction) of Stop DNB Reaction Buffer to the tube immediately once the temperature reaches to 4 ℃. Mix the tube gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

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 4 ℃ and perform sequencing within 48 hours.

4.4.4 Preparing DNBs for the App-A FCL PE150 kit

4.4.4.1 Calculating the required amount of ssDNA library

- 300 µL of DNB is required to load one flow cell for the FCL PE150 kit. One DNB making reaction can make 90 µL of DNBs.
- The required ssDNA library volume needed to make 90 µL of DNBs (one DNB reaction) is shown in the table below.

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

Table 35 App-A FCL PE150 required ssDNA volume

Sample type	Required ssDNA volume: V (µL)
Regular Library	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.

4.4.4.2 Preparing reagents for DNB making

Perform the steps below:

1. Place the library on ice until use.
2. Take the App-A Make DNB Buffer out of the High Throughput Pair-End Sequencing Primer Kit (App-A).
3. Take the Low TE Buffer and Stop DNB Reaction Buffer out of the DNB Rapid Make Reagent Kit and thaw reagents at room temperature.
4. Thaw the Make DNB Rapid Enzyme Mix II on ice for approximately 0.5 hours.
5. After thawing, mix the reagents thoroughly by using a vortex mixer for 5 seconds. Centrifuge them briefly and place them on ice until use.

4.4.4.3 Making DNB

Perform the steps below:

1. Take a 0.2 mL 8-strip tube or PCR tubes and prepare the reaction mix on ice following the table below. The following table only illustrates one make DNB reaction. The amount of input ssDNA and required number of make DNB reactions are determined by the actual application as described in *4.4.4.1 Calculating the required amount of ssDNA library on Page 32.*

   Tips Do not throw away the Low TE Buffer after this step. The remaining Low TE buffer will be used in DNB loading.

Table 36 App-A FCL PE150 Make DNB Reaction Mixture 1

Component	Volume / 90 µL DNB reaction (µL)
App-A Make DNB Buffer	20
Low TE Buffer	20 - V
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 the tube into a thermal cycler and start the primer hybridization reaction accoring to the following table:

   Tips As some thermal cyclers are slow in temperature adjustment, when the lid is being heated or cooled, the sample block may remain at room temperature and the procedure is not performed. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at working temperature during the DNB reaction.

Table 37 App-A FCL PE150 primer hybridization reaction condition

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

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

4. Mix the Make DNB Enzyme Mix II (LC) with Make DNB Rapid Enzyme Mix II by gently pipetting 6 to 8 times before use as described in the table below.

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

Table 38 App-A FCL PE150 Make DNB Reaction Mixture 2

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

5. Take the PCR tube out of the thermal cycler when the temperature reaches 4 ℃ . Centrifuge it briefly for 5 seconds.
6. Place the tube on ice and add all the Make DNB Reaction Mixture 2 to the tube.
7. Mix it thoroughly by using a vortex mixer for 5 seconds, Centrifuge it briefly and place the tubes into a thermal cycler for the next reaction. The condition is shown in the table below:

Tips It is recommended to set the temperature of the heated lid to 35 ℃ or as close as possible to 35 ℃ .

Table 39 App-A FCL PE150 rolling circle replication condition

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

8. Add 10 μL of Stop DNB Reaction Buffer to the tube immediately once the temperature reaches to 4 ℃. Mix gently by pipetting 5 to 8 times using a wide bore tip.

Tips
- It is very important to mix DNB gently by using a wide bore pipette tip. Do not centrifuge, vortex, pipette vigorously or shake the tube.
- Store the DNB at 4 ℃ and perform sequencing within 8 hours.

4.4.5 Preparing DNB for the App-D FCL SE50/SE100/PE100 kit

4.4.5.1 Calculating the required amount of ssDNA library

- 270 µL of DNB is required to load one flow cell for the FCL SE50, SE100 or PE100 kit. One DNB making reaction can make 100 µL or 50 µL of DNB.
- The required ssDNA library volume needed to make one DNB reaction is as follows.

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

Table 40 App-D FCL SE50/SE100/PE100 required ssDNA volume

Sample type	Required ssDNA volume of: V (µL)	
	100 µL DNB reaction	50 µL DNB reaction
Regular Library	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).
  - The number of the 100 µL DNB making reactions is equal to round (V/100)+1, the number of the 50 µL DNB making reactions is equal to round (V/50)+1.

4.4.5.2 Preparing reagents for DNB making

Perform the steps below:

1. Place the library on ice until use.
2. Take the App Make DNB Buffer out of the High-throughput Pair-End/Single-End Sequencing Primer Kit (App-D).
3. Take the Low TE Buffer and Stop DNB Reaction Buffer out of the DNB Make Reagent Kit and thaw reagents at room temperature.
4. Thaw the Make DNB Enzyme Mix I on ice for approximately 0.5 hours.
5. After thawing, mix the reagents thoroughly by using a vortex mixer for 5 seconds. Centrifuge them briefly and place them on ice until use.

4.4.5.3 Making DNB

Perform the steps below:

1. Take a 0.2 mL 8-strip tube or PCR tubes and prepare the reaction mix on ice following the table below. The following table only illustrates one make DNB reaction. The amount of input ssDNA and required number of make DNB reactions are determined by the actual application as described in *4.4.5.1 Calculating the required amount of ssDNA library on Page 35*.

   Tips Do not throw away the Low TE Buffer after this step. The remaining Low TE buffer will be used in DNB loading.

Table 41 App-D FCL SE50/SE100/PE100 Make DNB Reaction Mixture 1

Component	Volume (μL) for 100 μL DNB reaction	Volume (μL) for 50 μL DNB reaction
App Make DNB Buffer	20	10
Low TE Buffer	20-V	10-V
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 the mixture into a thermal cycler, and start the primer hybridization reaction accoring to the following table:

   Tips As some thermal cyclers are slow in temperature adjustment, when the lid is being heated or cooled, the sample block may remain at room temperature and the procedure is not performed. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at working temperature during the DNB reaction.

Table 42 App-D FCL PE150 primer hybridization reaction condition

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

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

4. Mix the Make DNB Enzyme Mix II (LC) with Make DNB Enzyme Mix I by gently pipetting 6 to 8 times before use as described in the table below.

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

Table 43 App-D FCL SE50/SE100/PE100 Make DNB Reaction Mixture 2

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

5. Take the PCR tube out of the thermal cycler when the temperature reaches 4 ℃ . Centrifuge the tube briefly for 5 seconds.
6. Place the tube on ice and add all the Make DNB Reaction Mixture 2 to the tube.
7. Mix it thoroughly by using a vortex mixer for 5 seconds. Centrifuge it briefly and place the tubes into a thermal cycler for the next reaction. The condition is shown in the table below:

   Tips It is recommended to set the temperature of the heated lid to 35 ℃ or as close as possible to 35 ℃ .

Table 44 App-D FCL SE50/SE100/PE100 rolling circle replication condition

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

8. Add Stop DNB Reaction Buffer to the tube immediately once the temperature reaches to 4 ℃. Mix it 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 tip. Do not centrifuge, vortex, pipette vigorously or shake the tube.
   - Store the DNB at 4 ℃ and use within 48 hours.

Table 45 Volume of Stop DNB Reaction Buffer

Component	Volume (µL) for 100 µL DNB reaction	Volume (µL) for 50 µL DNB reaction
Stop DNB Reaction Buffer	20	10

4.4.6 Preparing DNB for the App-D FCL PE150 kit

4.4.6.1 Calculating the required amount of ssDNA library

- 300 μL of DNB is required to load one flow cell for the FCL PE150 kit. One DNB making reaction can make 90 μL of DNB.
- The required ssDNA library volume needed to make 90 μL of DNB (one DNB reaction) is shown in the table below.

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

Table 46 App-D FCL PE150 required ssDNA volume

Sample type	Required ssDNA volume: V (μL)
Regular Library	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.

4.4.6.2 Preparing reagents for DNB making

Perform the steps below:

1. Place the library on ice until use.
2. Take the App Make DNB Buffer out of the High-throughput Pair-End Sequencing Primer Kit (App-D).
3. Take the Low TE Buffer and Stop DNB Reaction Buffer out of the DNB Rapid Make Reagent Kit and thaw reagents at room temperature.
4. Thaw the Make DNB Rapid Enzyme Mix II on ice for approximately 0.5 hours.
5. After thawing, mix the reagents thoroughly by using a vortex mixer for 5 seconds. Centrifuge them briefly and place them on ice until use.

4.4.6.3 Making DNB

Perform the steps below:

1. Take a 0.2 mL 8-strip tube or PCR tubes and prepare the reaction mix on ice following the table below. The following table only illustrates one make DNB reaction. The amount of input ssDNA and required number of make DNB reactions are determined by the actual application as described in *4.4.6.1 Calculating the required amount of ssDNA library on Page 38*.

   Tips Do not throw away the Low TE Buffer after this step. The remaining Low TE buffer will be used in DNB loading.

Table 47 App-D FCL PE150 make DNB Reaction Mixture 1

Component	Volume (µL) for 90 µL DNB reaction
App Make DNB Buffer	20
Low TE Buffer	20-V
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 the mixture into a thermal cycler, and start the primer hybridization reaction accoring to the following table:

   Tips As some thermal cyclers are slow in temperature adjustment, when the lid is being heated or cooled, the sample block may remain at room temperature and the procedure is not performed. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at working temperature during the DNB reaction.

Table 48 App-D FCL PE150 primer hybridization reaction condition

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

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

4. Mix the Make DNB Enzyme Mix II (LC) with Make DNB Rapid Enzyme Mix II by gently pipetting 6 to 8 times before use as described in the table below.

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

Table 49 App-D FCL PE150 Make DNB Reaction Mixture 2

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

5. Take the PCR tube out of the thermal cycler when the temperature reaches 4 ℃ . Centrifuge the tube briefly for 5 seconds.
6. Place the tube on ice and add all the Make DNB Reaction Mixture 2 to the tube.
7. Mix it thoroughly by using a vortex mixer for 5 seconds. Centrifuge it briefly and place the tubes into a thermal cycler for the next reaction. The condition is shown in the table below:

Tips It is recommended to set the temperature of the heated lid to 35 ℃ or as close as possible to 35 ℃ .

Table 50 App-D FCL PE150 rolling circle replication condition

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

8. Add 10 µL of Stop DNB Reaction Buffer to the tube immediately once the temperature reaches to 4 ℃. Mix it gently by pipetting 5 to 8 times using a wide bore tip.

Tips
- It is very important to mix DNB gently by using a wide bore pipette tip. Do not centrifuge, vortex, pipette vigorously or shake the tube.
- Store the DNB at 4 ℃ and perform sequencing within 8 hours.

4.4.7 Preparing DNB for the stLFR FCL PE100 kit

4.4.7.1 Calculating the required amount of dsDNA library

- 270 μL of DNB is required to load one flow cell for the stLFR FCL PE100. 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.

4.4.7.2 Preparing reagents for DNB making

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 0.5 hours.
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.

   Tips Mixed use of reagent components from different batches is not recommended.

4.4.7.3 Making DNB

Perform the steps below:

1. Take a 0.2 mL 8-strip tube or PCR tubes and prepare the reaction mix on ice following the table below. The following table only illustrates one make DNB reaction. The amount of input ssDNA and required number of make DNB reactions are determined by the actual application as described in *4.4.7.1 Calculating the required amount of dsDNA library on Page 41.*

Table 51 stLFR FCL PE100 Make DNB Reaction Mixture 1

Component	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 mix 1 into a thermal cycler, and start the primer hybridization reaction accoring to the following table:

Tips As some thermal cyclers are slow in temperature adjustment, when the lid is being heated or cooled, the sample block may remain at room temperature and the procedure is not performed. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at working temperature during the DNB reaction.

Table 52 stLFR FCL PE100 primer hybridization reaction condition

Temperature	Time
Heated lid (105 °C)	On
95 °C	3 min
40 °C	3 min
4 °C	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.
5. Mix the Make DNB Enzyme Mix IV with Make DNB Enzyme Mix III by gently pipetting 6 to 8 times before use as described in the table below.

Tips Do not place Make DNB Enzyme Mix IV at room temperature and avoid holding the tube for a prolonged time.

Table 53 stLFR FCL PE100 Make DNB Reaction Mixture 2

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

6. Take the PCR tube out of the thermal cycler when the temperature reaches 4 ℃ . Centrifuge it briefly for 5 seconds.
7. Place the tube on ice, and add all the Make DNB Reaction Mixture 2 to the tube.
8. Mix the tube thoroughly by using a vortex mixer. Centrifuge it for 5 seconds by using mini centrifuge, and place the tubes into a thermal cycler for the next reaction. The condition is shown in the table below:

   Tips It is recommended to set the temperature of the heated lid to 35 ℃ or as close as possible to 35 ℃ .

Table 54 stLFR FCL PE100 rolling circle replication condition

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

9. Add 16 μL of Stop DNB Reaction Buffer to the tube immediately once the temperature reaches to 4 ℃. Mix gently by pipetting 5 to 8 times using a wide bore tip.

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

4.5 Quantifying DNB and pooling

4.5.1 Quantifying DNB

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 make a new DNB.

Table 55 Requires the DNB concentration

Read length	Regular library	Other library
FCL PE100 and below	≥15 ng/μL	≥8 ng/μL
FCL PE150	≥8 ng/μL	≥5 ng/μL

- If the concentration exceeds 40 ng/μL, the DNB need to be diluted to 20 ng/μL before loading, please refer to the following table for DNB dilution.

Tips Only Low TE Buffer can be used as DNB dilution reagent for FCL PE150, App-A FCL PE150 and App-D FCL PE150 kits.

Table 56 DNB dilution buffer

Sequencing kit	Dilution reagent	DNB storage temperature	Maximum DNB storage time (hour)
FCL SE35	DNB Load Buffer I	4 ℃	≤48
FCL SE50	DNB Load Buffer I	4 ℃	≤48
FCL SE100	DNB Load Buffer I	4 ℃	≤48
FCL PE100	DNB Load Buffer I	4 ℃	≤48
App-A FCL PE100	DNB Load Buffer I	4 ℃	≤48
stLFR FCL PE100	DNB Load Buffer I	4 ℃	≤48
App-D FCL SE50	DNB Load Buffer I	4 ℃	≤48
App-D FCL SE100	DNB Load Buffer I	4 ℃	≤48
App-D FCL PE100	DNB Load Buffer I	4 ℃	≤48
FCL PE150	Low TE Buffer	4 ℃	≤8
App-A FCL PE150	Low TE Buffer	4 ℃	≤8
App-D FCL PE150	Low TE Buffer	4 ℃	≤8

4.5.2 DNB pooling

Tips Use normal pipette tips to aspirate the required volume of each DNB and use wide bore 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.

4.5.2.1 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.

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

V = A1 + B1 + … + H1

4.5.2.3 Calculating the DNB volume needed for each sample

For each FCL flow cell used for SE35 / SE50 / SE100 / PE100, App-A PE100, stLFR PE100 and App-D SE50/SE100/PE100 requiring 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 each FCL flow cell used for PE150, App-A PE150 and App-D PE150 requiring 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 5 Loading DNB

5.1 Preparing the post load plate and buffers

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

- Section *5.1.1 Preparing the post load plate and buffer for FCL SE35/SE50/SE100/PE100, App-A FCL PE100, stLFR FCL PE100, App-D FCL SE50, App-D FCL SE100 and App-D FCL PE100 sequencing on Page 46*.
- Section *5.1.2 Post load plate and buffers preparation for FCL PE150, App-A FCL PE150 and App-D FCL PE150 sequencing on Page 48*.

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

5.1.1.1 Thawing the post load plate

Perform the steps below:

1. Take out Post Load Plate. Perform the following steps according to different situations:
   - For the libraries except stLFR: take Post Load Plate out of the DNB Load Reagent Kit
   - For stLFR libraries: 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.
4. Gently invert the Post Load Plate to mix it 5 times and then centrifuge for 1 minute before use.

5.1.1.2 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 the libraries except App-A and App-D: Please skip to the next step.
   - For App-A libraries: take the App-A Insert Primer 1 out of the High-throughput Pair-End Sequencing Primer Kit (App-A).
   - For App-D libraries: 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 0.5 hours.
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.

5.1.1.3 Preparing the 0.1 M NaOH reagent

Prepare 0.1 M NaOH according to the procedure described in *Preparing wash reagents on Page 77*. Each Post Load plate requires at least 4 mL of 0.1 M NaOH.

5.1.1.4 Preparing DNB loading mixture

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

Perform the steps below:

1. Take out a new 0.5 mL microfuge tube, and add the following components.

Tips DNB in the above table refers to the pooled DNB in *4.5.2 DNB pooling on Page 44*.

Table 57 DNB loading mixture for FCL SE35/SE50/SE100/PE100, App-A FCL PE100, App-D FCL SE50, App-D FCL SE100 and App-D FCL PE100

Adding order	Component	volume (μL)
1	DNB	270
2	DNB Load Buffer II	90
3	Make DNB Enzyme Mix II (LC)	1

Table 58 DNB loading mixture for stLFR FCL PE100

Adding order	Component	volume (μL)
1	DNB	270
2	DNB Load Buffer II	90
3	Make DNB Enzyme Mix IV	1

2. Gently pipette the DNB loading mix 5 to 8 times by using a wide bore tip.

Tips Do not centrifuge, vortex, vigorously pipette or shake the tube.

5.1.2 Post load plate and buffers preparation for FCL PE150, App-A FCL PE150 and App-D FCL PE150 sequencing

5.1.2.1 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 ℃ to 8 ℃ refrigerator one day in advance.
3. Once the Rapid Post Load Plate is thoroughly thawed, place it in a 2 ℃ to 8 ℃ refrigerator until use.
4. Gently invert the Rapid Post Load Plate to mix it 5 times, and then centrifuge it for 1 minute before use.

5.1.2.2 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 the libraries except App-A and App-D: Please skip to the next step.
   - For App-A library: take the App-A Insert Primer 1 out of the High-throughput Pair-End Sequencing Primer Kit (App-A).
   - For App-D library: 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. After thawing, mix the reagents by using a vortex mixer for 5 seconds. Centrifuge them briefly and place them on ice until use.

5.1.2.3 Preparing the 0.1 M NaOH reagent

Prepare 0.1 M NaOH according to the procedure described in *Preparing wash reagents on Page 77*. Each Post Load plate requires at least 4 mL of 0.1 M NaOH.

5.1.2.4 Preparing DNB loading mixture

Perform the steps below:

1. Take a new 0.5 mL microfuge tube and add reagents following the table below.

   Tips DNB in the above table refers to the pooled DNB in *4.5.2 DNB pooling on Page 44*.

Table 59 DNB loading mixture for FCL PE150, App-A FCL PE150 and App-D FCL PE150

Adding order	Component	volume (µL)
1	DNB	300
2	DNB Load Buffer IV	150

2. Gently pipette the DNB loading mix 5 to 8 times by using a wide bore tip.

   Tips
   - Do not centrifuge, vortex, vigorously pipette or shake the tube.
   - DNB loading mixture must be prepared fresh and used it within 30 minutes.

5.2 Preparing the sequencing flow cell

Perform the steps below:

1. Take out the flow cell from the DNBSEQ-T7RS sequencing flow cell box.

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

2. Balance the flow cell at room temperature for at least 30 minutes, but no longer than 24 hours.
3. Unwrap the outer package before use and start DNB loading.

   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 out the flow cell from the inner package and inspect if the flow cell is intact.
5. Clean the back of the flow cell using dust remover.

5.3 DNB loading

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.

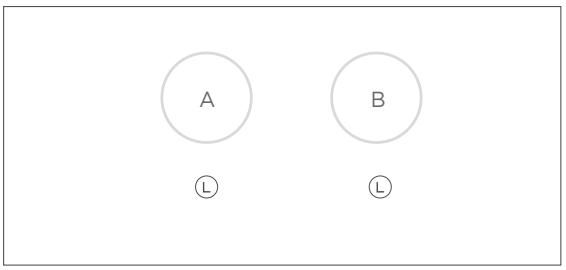

Figure 1 MGIDL-T7RS main interface

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

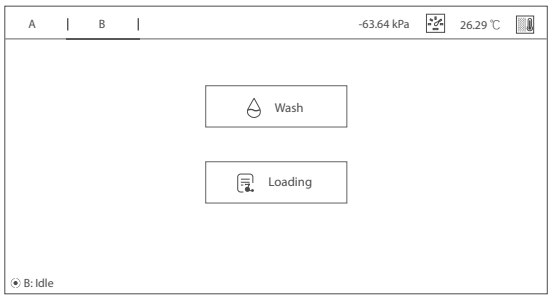

Figure 2 MGIDL-T7RS selection interface

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

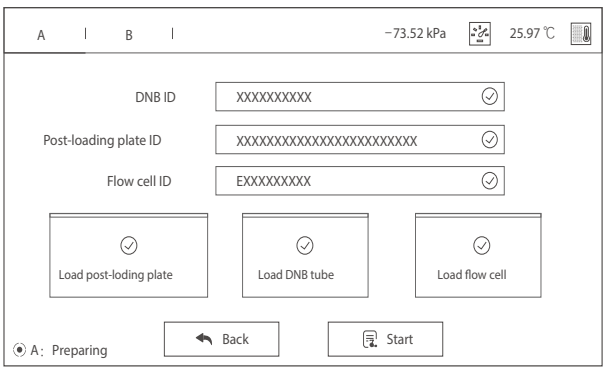

Figure 3 MGIDL-T7RS information input interface

5. Open the loading compartment door.
6. 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.

7. Place the 0.5 mL micro tube containing DNB loading mix into the DNB tube hole (see *Figure 6 on Page 53*), the screen will prompt that the DNB tube is loaded.

8. Align the post load plate to the RFID scanning area and the ID information will appear in the text box.

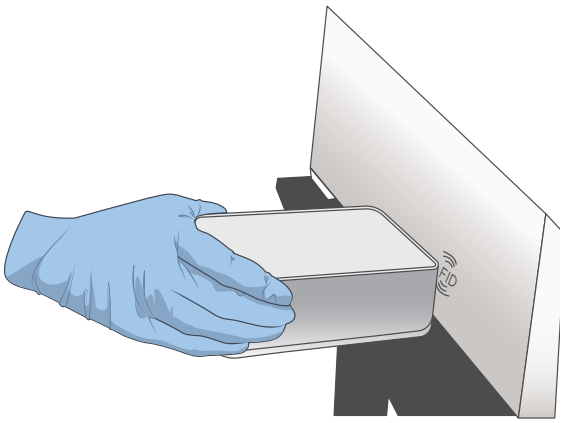

Figure 4 The RFID scanning area of post load plate

9. Remove the seal of the post load plate and add 4 mL of 0.1 M NaOH into well No.11.

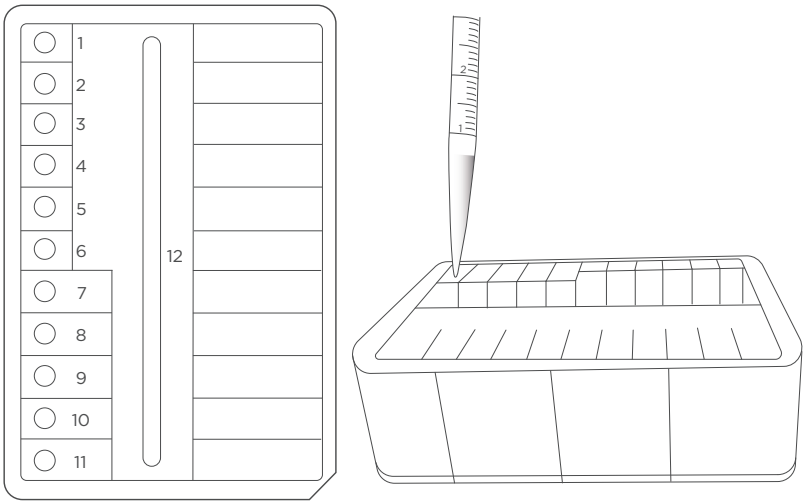

Figure 5 Adding 4 mL of 0.1 M NaOH into well No.11

10. Perform the following steps according to different situations:
    - For the libraries except App-A and App-D: Please skip to the next step.
    - For App-A libraries: Use a pipette to completely remove all the reagent in well No.1 of 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: Use a pipette to completely remove all the reagent in well No.1 of 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).

11. Place the prepared post load plate on the plate tray of MGIDL-T7RS. The screen will prompt that the post load plate is loaded.

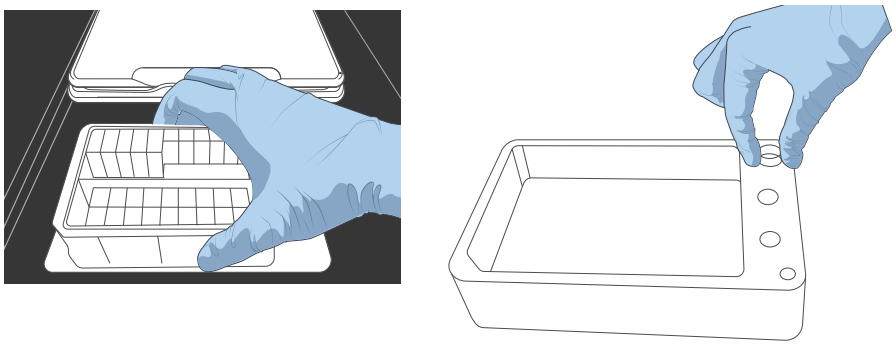

Figure 6 Post-loading plate placement diagram

12. 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.

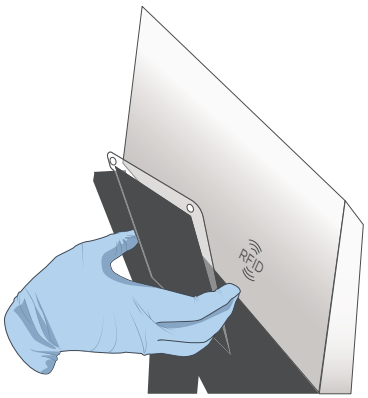

Figure 7 Scaning the Flow cell ID

13. 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.

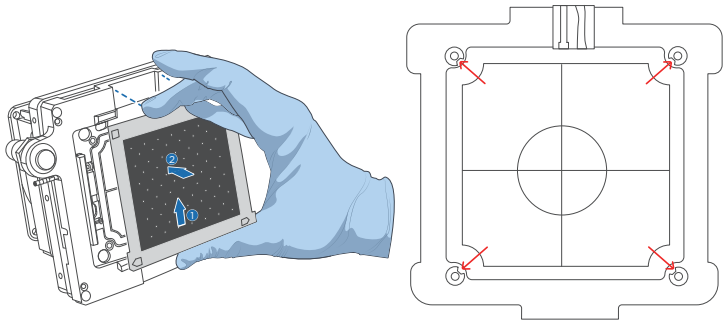

Figure 8 Flow cell locating

14. 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 gas dust remover.
    - 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 dust remover.

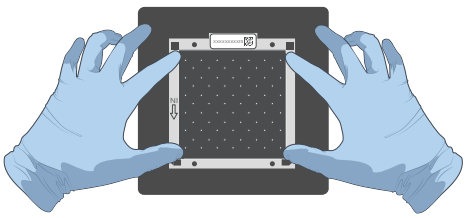

Figure 9 Flow cell loaded

15. Close the loading compartment door.
16. Tap the **Start** button and select **Yes** as shown in the *Figure 10 on Page 55*. Flow cell loading starts as shown in the *Figure 11 on Page 55*.

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

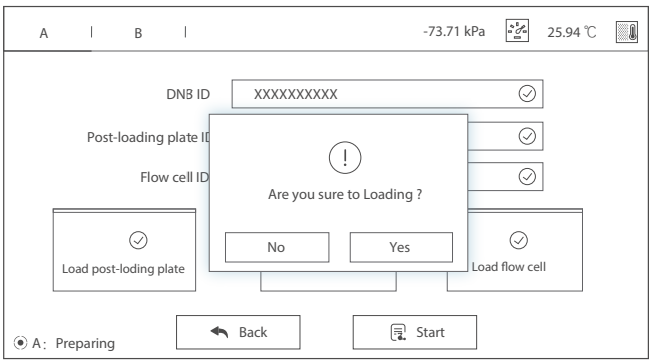

Figure 10 MGIDL-T7RS loading confirmation dialog box

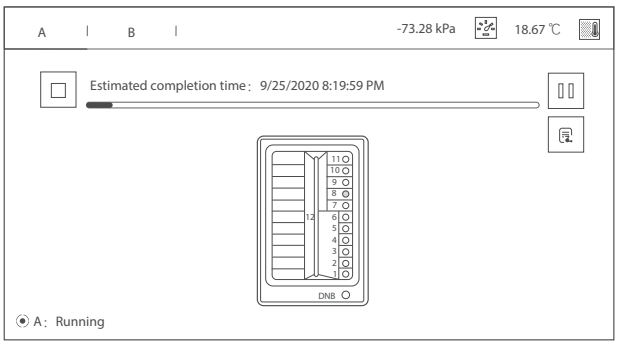

Figure 11 MGIDL-T7RS flow cell loading interface

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

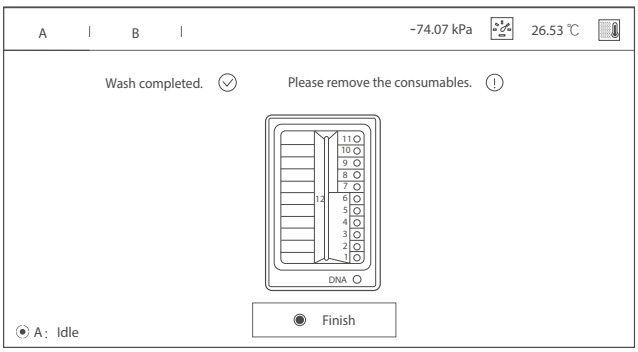

Figure 12 MGIDL-T7RS flow cell loading complete status window

18. 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 4 ℃ until use.
    - The maximum storage time for loaded flow cell is 48 hours.

19. When the loading is complete, install the washing flow cell onto the flow cell stage and press the flow cell attachment button. Tap **Confirm** button as shown in *Figure 12 on Page 55*.
20. Tap **Post-wash** and select **Yes** to start MGIDL-T7RS wash (see the figures below), which will take around 20 minutes.

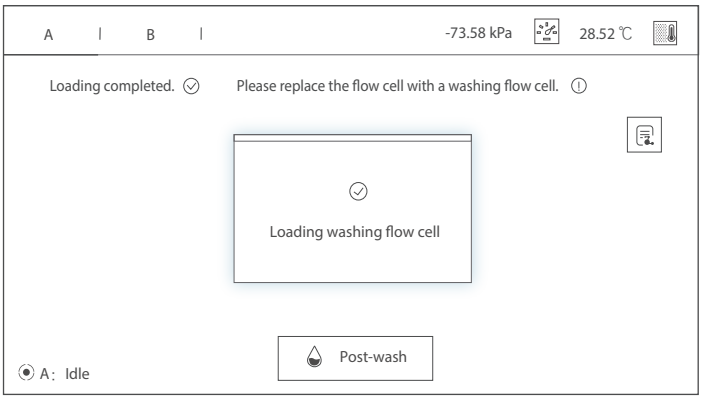

Figure 13 MGIDL-T7RS post-wash interface

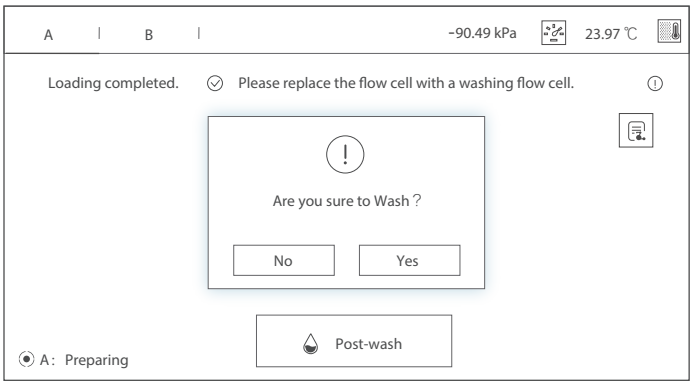

Figure 14 MGIDL-T7RS post-wash confirmation interface

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

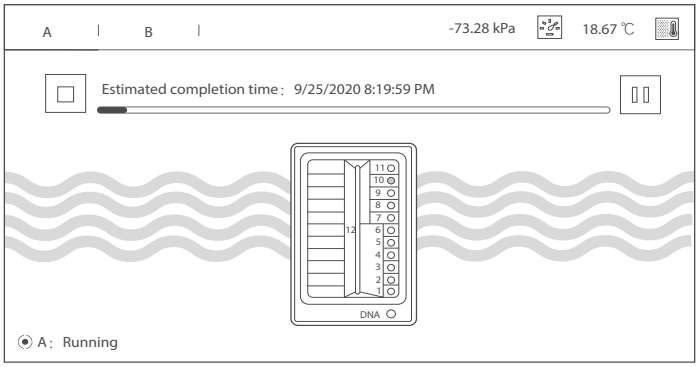

Figure 15 MGIDL-T7RS wash interface

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

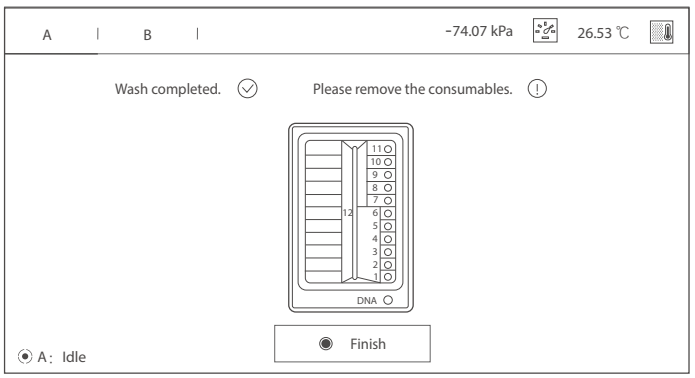

Figure 16 MGIDL-T7RS wash complete status window

Chapter 6 Preparation before sequencing

6.1 Preparing the sequencing reagent cartridge

Perform the steps below:

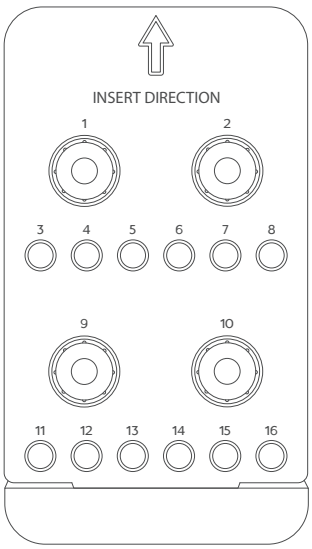

Figure 17 Sequencing cartridge wells

1. Take the Sequencing Reagent Cartridge out of the High-throughput Sequencing Kit.
2. Thawing Cartridge. Perform the following steps according to different situations:
   - Thaw it in a water bath at room temperature for 4 to 5 hours.
   - Thaw it in 2 ℃ to 8 ℃ refrigerator one day in advance.
3. After thoroughly thawing, store it at 2 ℃ to 8 ℃ until use.
4. 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.

5. Take the dNTPs Mix IV (or dNTPs Mix V) and dNTPs Mix II out of the sequencing kit and thaw them at room temperature.
6. After thawing, invert the dNTPs Mix IV (or dNTPs Mix V) and dNTPs Mix II 4 to 6 times. Centrifuge them briefly, and place them on ice until use.

7. Process the primer according to different situations:
   - For Dual barcode SE 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 Dual barcode PE 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 App-A PE sequencing
     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 App-D PE sequencing
     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 App-D SE sequencing
     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.

8. Open the kit 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.
9. 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.
10. Take the Sequencing Enzyme Mix out of the High-throughput Sequencing Kit. Invert the Sequencing Enzyme Mix 4 to 6 times and place it on ice until use.
11. 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.

Table 60 Sequencing cartridge well No.9 reagent adding

Sequencing kit	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	/	2.76	2.76
FCL PE150	/	3.74	3.74
App-A FCL PE100	/	2.76	2.76
App-A FCL PE150	/	3.74	3.74
App-D FCL SE50	2.0	/	2.0
App-D FCL SE100	3.0	/	3.0
App-D FCL PE100	/	2.76	2.76
App-D FCL PE150	/	3.74	3.74
stLFR FCL PE100	5.4	/	5.4

12. 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:

Table 61 Sequencing cartridge well No.10 reagent adding

Sequencing kit	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	8.28	2.76
FCL PE150	11.22	3.74
App-A FCL PE100	8.28	2.76
App-A FCL PE150	11.22	3.74
App-D FCL SE50	5.4	1.8
App-D FCL SE100	8.1	2.7
App-D FCL PE100	8.28	2.76
App-D FCL PE150	11.22	3.74
stLFR FCL PE100	14.7	4.9

13. Seal the loading wells of well No.9 and No.10 with the transparent sealing film.
14. 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.
15. 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.
16. 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.
17. Gently tap the cartridge on the bench to reduce air bubbles in the reagents.

Tips The cartridge for FCL SE35/SE50/SE100 with single barcode is ready, refer *Chapter 7 Sequencing on Page 67* for the next step.

18. Perform the following steps according to different situations:

- For Dual barcode SE 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 cartridge for FCL SE35/SE50/SE100 with dual barcodes is ready, refer *Chapter 7 Sequencing on Page 67* for the next step.

- For single barcode PE sequencing

  a. Pierce the seal of well No.8 by using a 1 mL sterile tip.

  b. Add 600 μL of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette.

  c. Invert the tube 4 to 6 times to mix the reagents.

  d. Add 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/PE150 with single barcodes is ready, refer *Chapter 7 Sequencing on Page 67* for the next step.

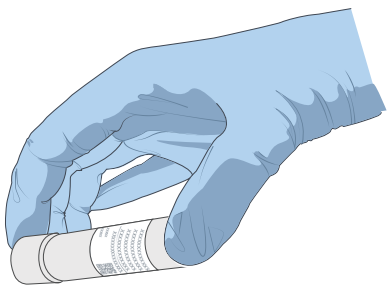

Figure 18 MDA mixing

- For Dual barcode PE sequencing: on the basis of general single barcode PE sequencing operation, the following operations are required.

  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.

  Tips The cartridge for FCL PE100/PE150 with dual barcodes is ready, refer *Chapter 7 Sequencing on Page 67* for the next step.

- For App-A PE sequencing: on the basis of general single barcode PE sequencing operation, the following operations are required.

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

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

  Tips
  - App-A barcode primer 3 is just for dual barcode App-A PE sequencing.
  - The cartridge for App-A FCL PE100/PE150 with dual barcodes is ready, refer *Chapter 7 Sequencing on Page 67* for the next step.

Table 62 Reagents for App-A dual barcode

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

- For App-D SE sequencing: on the basis of general single barcode SE sequencing operation, the following operations are required.

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

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

  Tips
  - App-D barcode primer 4 is just for dual barcode App-D SE sequencing.
  - The cartridge for App-D SE sequencing is ready, refer *Chapter 7 Sequencing on Page 67* for the next step.

Table 63 Reagents for App-D dual barcode

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

- For App-D PE sequencing: on the basis of general single barcode PE sequencing operation, the following operations are required.

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

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

  Tips
  - App-D barcode primer 3 is just for dual barcode App-D PE sequencing.
  - The cartridge for App-D PE sequencing is ready, refer *Chapter 7 Sequencing on Page 67* for the next step.

Table 64 Reagents for App-D dual barcode

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

19. Close the sequencing cartridge cover.

6.2 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.

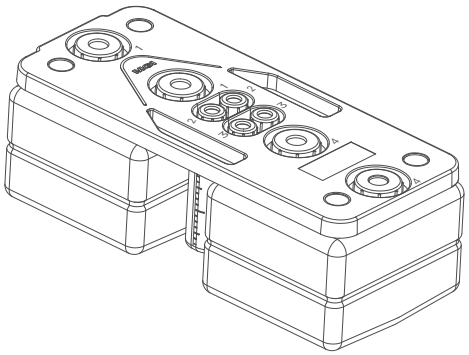

Figure 19 Washing cartridge

3. Add 45 mL of 0.1 M NaOH into well No.2 through the pierce by using an electronic pipette. Refer to *Preparing wash reagents on Page 77* for the preparation of 0.1 M NaOH.

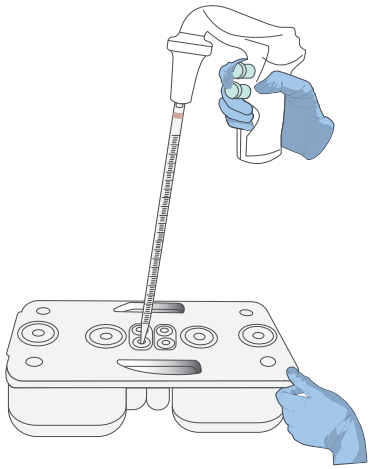

Figure 20 Washing cartridge added 0.1 M NaOH

6.3 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 the pure water is insufficient, the sequencing will fail. Replenish pure water in time, and pay attention to opening the air vent of the pure water container.
- 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 65 Pure water consumption (L)

Sequencing kit	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	3.0	6.0	9.0	12.0
FCL PE150	4.5	9.0	13.5	18.0
App-A FCL PE100	3.0	6.0	9.0	12.0
App-A FCL PE150	4.5	9.0	13.5	18.0
App-D FCL SE50	1.0	2.0	3.0	4.0
App-D FCL SE100	1.5	3.0	4.5	6.0
App-D FCL PE100	3.0	6.0	9.0	12.0
App-D FCL PE150	4.5	9.0	13.5	18.0
stLFR FCL PE100	3.5	7.0	10.5	14.0

Chapter 7 Sequencing

7.1 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 cartridge into the sequencing cartridge compartment and place the washing cartridge into the washing cartridge compartment.

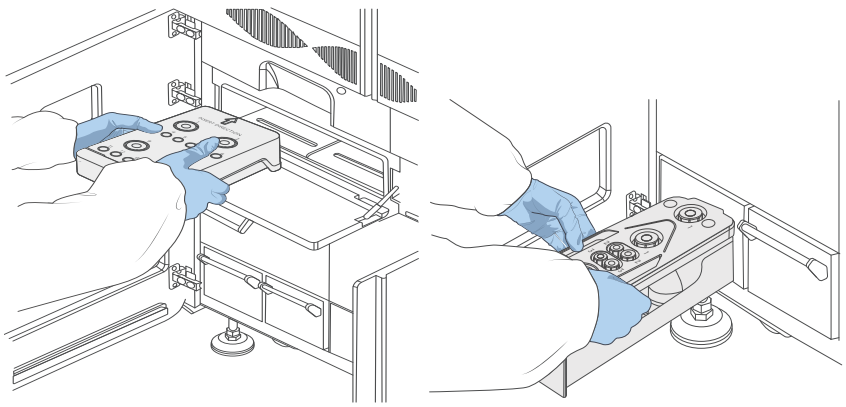

Figure 21 Loading the reagent cartridge

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

7.2 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.

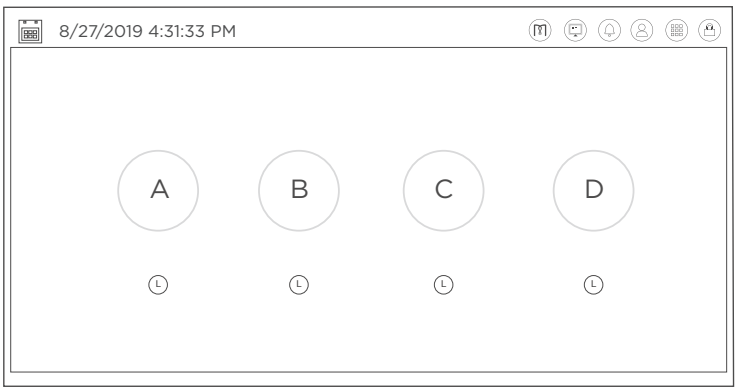

Figure 22 DNBSEQ-T7RS main interface

7.3 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).

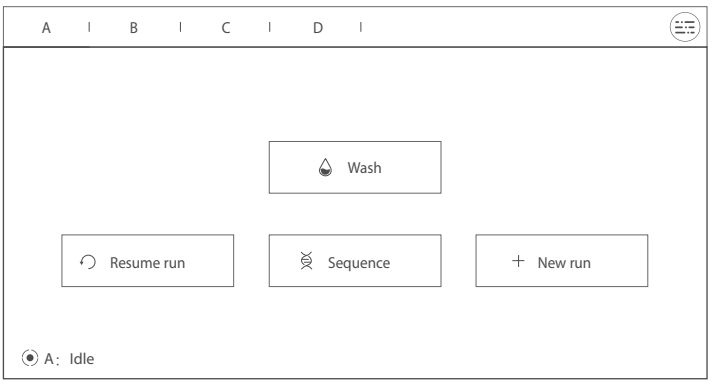

Figure 23 DNBSEQ-T7RS selection interface

2. Clean the loaded flow cell with a dust remover to ensure that there is no visible dust on the surface and the back of the flow cell. Put the flow cell on the flow cell drive, and tap the flow cell drive control button to withdraw the flow cell drive.

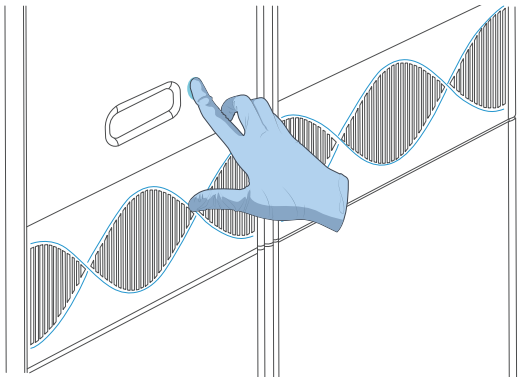

Figure 24 The flow cell drive

7.4 Sequencing parameters

Tips For the App library with a barcode length of 8bp, when sequencing with the MGI library at the same time, 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 scan fails, information should be entered manually.

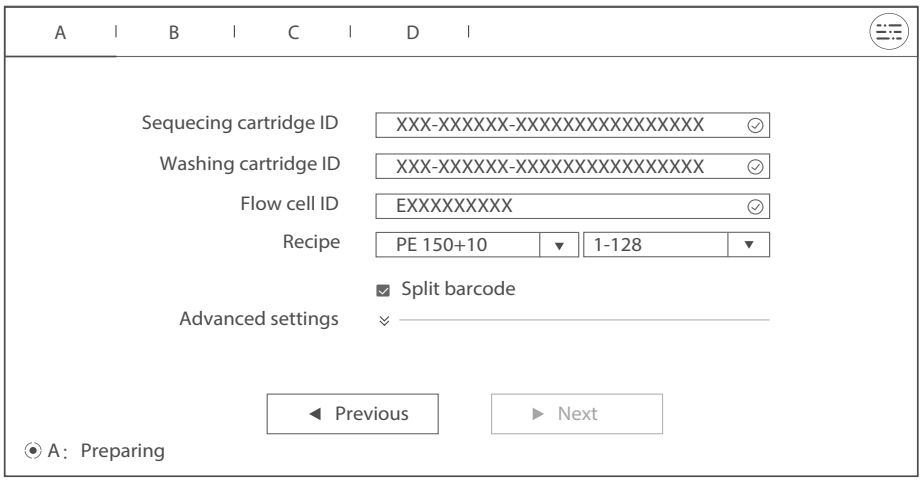

Figure 25 DNBSEQ-T7RS sequencing parameters

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:

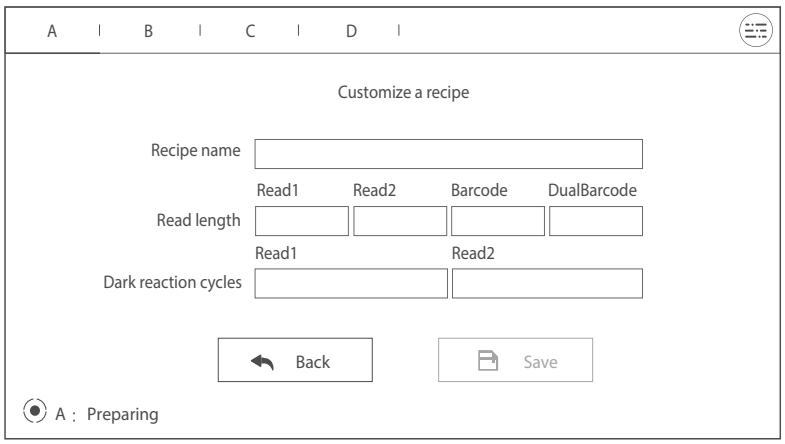

Figure 26 Customize a recipe

- 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.

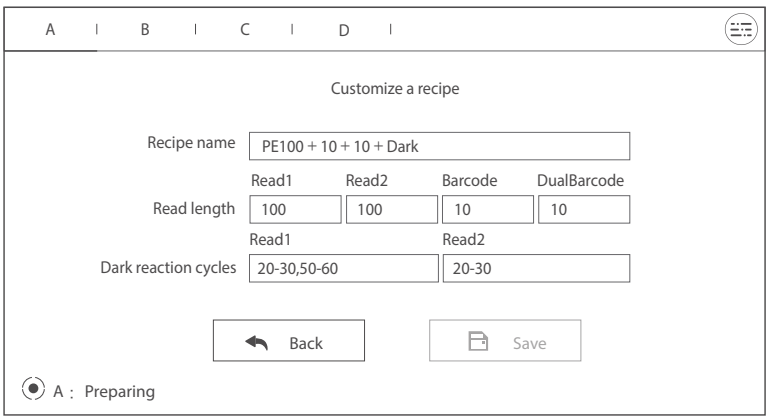

Figure 27 Example

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)

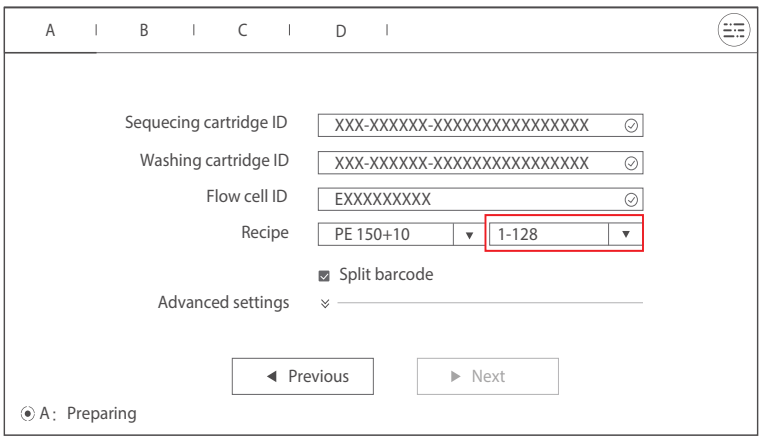

Figure 28 Set the barcode sequence

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 App-A / App-D and others requiring primer replacement. stLFR sequencing does not belong to **Custom primers**.

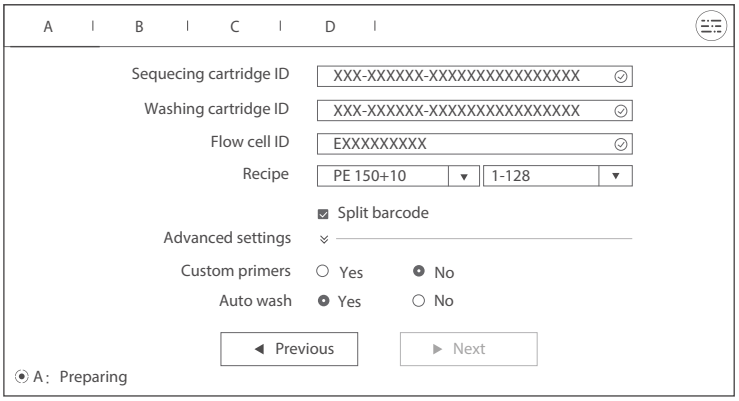

Figure 29 DNBSEQ-T7RS advanced settings

7.5 Reviewing parameters

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

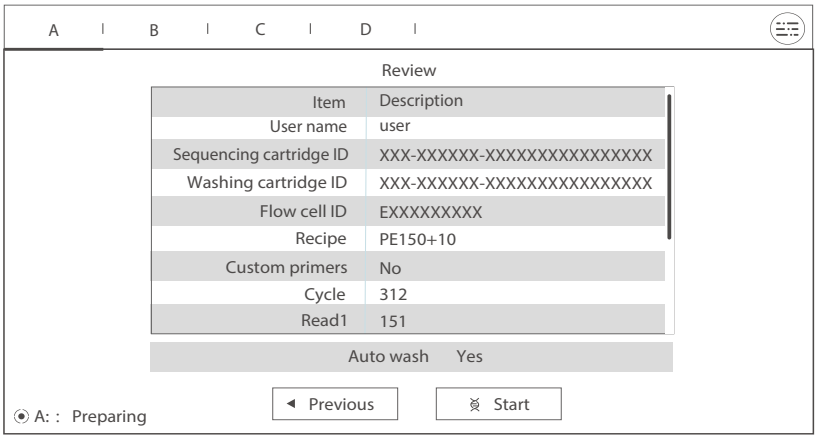

Figure 30 Reviewing information

7.6 Starting sequencing

Perform the steps below:

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

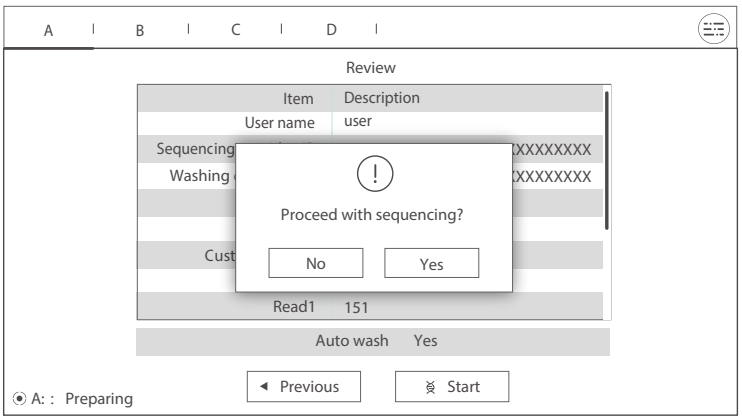

Figure 31 Confirming sequencing interface

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

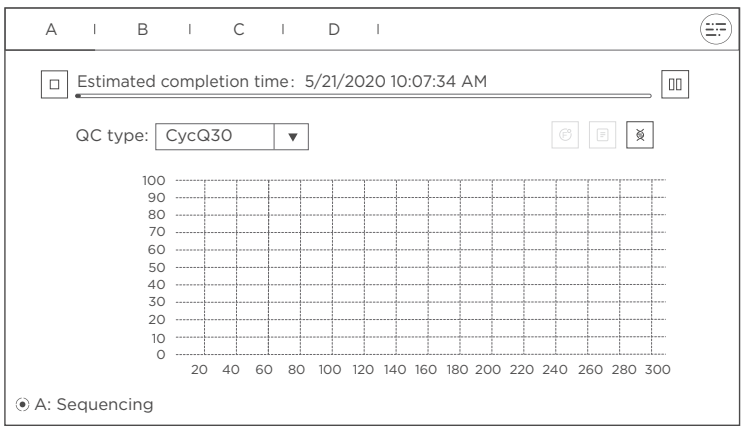

Figure 32 Sequencing Starts Interface

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

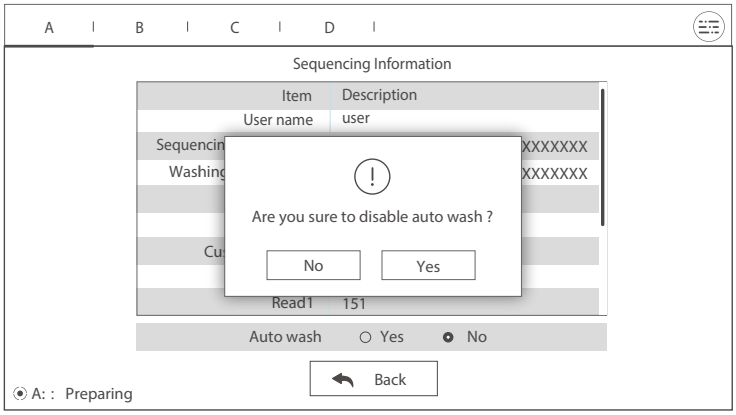

Figure 33 Disable auto wash

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

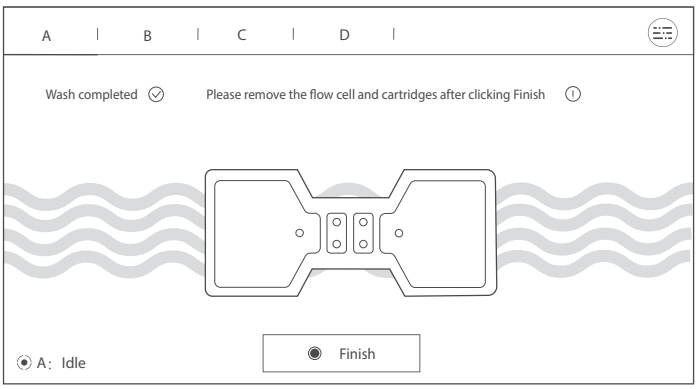

Figure 34 DNBSEQ-T7RS sequencing complete interface

7.7 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 8 Device maintenance

8.1 Terminology and definition

Table 66 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.

Preparing wash reagents

Tips The following Wash Reagents are stored at 4 ℃ and are valid for 28 days.

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

Table 67 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 68 Wash reagent II: 0.1 M NaOH

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

8.2 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.

8.3 Wash procedures

8.3.1 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.

8.3.2 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 9 Troubleshooting

9.1 Low DNB concentration

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

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

9.2 Abnormal negative pressure

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

1. Gently wipe the stage surface with a damp lint-free paper or a lint-free cloth and blow the stage with a dust remover and ensure no dust is left.
2. Blow the back of the Flow Cell with a dust remover to ensure no dust is left.
3. If these solutions cannot solve the problem, please contact the engineer.

9.3 Bubbles

9.3.1 Bubbles in MGIDL-T7RS

- Check the rubber sealing ring to ensure that it is in the right position.
- Check the DNB loading cartridge 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.

9.3.2 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 after restarts, the problem still persists after a restart, please contact the field service engineer.

9.4 Impurities

- Perform a manual wash on MGIDL-T7RS and DNBSEQ-T7RS.
- If there is still no improvement after manual wash, follow *Preparing wash reagents on Page 77* 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.

9.5 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 dust remover.
  - 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.

9.6 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 once only.
- If the kit has been thawed (including the dNTPs) but cannot be used immediately, store it at 4 ℃ and it is strongly recommended to use it within 24 hours. A thawed kit stored at 4 ℃ less than 7 days still can be used, but it may compromise the sequencing quality. It is not recommended to use a thawed kit stored at 4 ℃ more than 7 days.
- If the dNTPs and Sequencing Enzyme Mix II have been added into the cartridge, for example, the cartridge has been prepared and the needles have punctured the seal but the cartridge cannot be used in time, the cartridge must be covered with foil or plastic wrap. Store the kit at 4 ℃ and use it within 24 hours.

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.

/	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	MGI Tech Co., Ltd. / Wuhan MGI Tech Co., Ltd.
Address	Main Building and Second floor of No.11 Building, Beishan Industrial Zone, Yantian District, Shenzhen, 518083, Guangdong, P.R.China
	Building B13, No.818, Gaoxin Avenue, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China
	Building 24, Stage 3.1, BioLake Accelerator, No.388, 2nd Gaoxin Road, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China
E-mail	MGI-service@mgi-tech.com
Website	www.mgi-tech.com