Part No.: SOP-013-B01-081

DNBSEQ-G50RS

High-throughput (Rapid) Sequencing Set

User Manual | Version: 7.0

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Wuhan MGI Tech Co., Ltd.

About the user manual

This user manual is applicable to DNBSEQ-G50RS High-throughput (Rapid) Sequencing Set. The manual version is 7.0.

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Revision history

Version	Date	Description
7.0	January 2022	• Update the logo. • Update the Transport temperature to -80 ℃ to -15 ℃. • Added the disclaimer. • Change the operation pictures.
6.0	July 2021	• Increase the validity of reagents. • Change the Flow Cell temperature from "0 ℃ to 30 ℃" to "2 ℃ to 8 ℃"
A4	Dec 2020	• Update the logo, website address and mailbox. • Add configuration 2.
A3	Jun 2020	• Change the Storage Temperature of Flow Cell from "Room Temperature" to "0 ℃ to 30 ℃". • Update the catalog number and Spec&Quantity of cPAS Barcode Primer 3 Reagent Kit. • Update the filling volume of wash reagents.
A2	March 2020	• Update the figures of sequencer interface. • Update the product name, catalog number and version. • Update the loading volume of dNTPs Mix III, dNTPs Mix II and Sequencing Enzyme Mix. • Add the High-throughput Rapid Sequencing Set products. • Add the PE150 read length. • Add dual barcode sequencing in PE sequencing. • Add the solution of crystal precipitation in DNB Load Buffer II in "4.5 DNB Loading". • Explain the possible dark green crystals in well No.18 in "5 Prepare the sequencing cartridge". • Update the loading well positions in Figure 3, Figure 4 and Figure 5.

Version	Date	Description
A1	December 2019	• Add a new chapter “Attention”. • Update the equations used to calculate library input. • Add the “Revision History”.
A0	August 2019	Initial release.

Sequencing set

Catalog number	Name	Version
1000019855	DNBSEQ-G50RS High-throughput Sequencing Set (FCL SE50)	V3.1
1000019856	DNBSEQ-G50RS High-throughput Sequencing Set (FCL SE100)	V3.1
1000019857	DNBSEQ-G50RS High-throughput Sequencing Set (FCL PE50)	V3.1
1000019859	DNBSEQ-G50RS High-throughput Sequencing Set (FCL PE100)	V3.1
1000019858	DNBSEQ-G50RS High-throughput Sequencing Set (FCL PE150)	V3.1
1000019860	DNBSEQ-G50RS High-throughput Rapid Sequencing Set (FCS SE100)	V3.1
1000019861	DNBSEQ-G50RS High-throughput Rapid Sequencing Set (FCS PE100)	V3.1
1000019862	DNBSEQ-G50RS High-throughput Rapid Sequencing Set (FCS PE150)	V3.1
1000020834	CPAS Barcode Primer 3 Reagent Kit	V2.0
1000014048	CPAS Barcode Primer 4 Reagent Kit	V1.0

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Contents

Chapter 1 Introduction

This manual describes how to perform sequencing using DNBSEQ-G50RS High-throughput Sequencing Set and DNBSEQ-G50RS High-throughput Rapid Sequencing Set. Includes instructions regarding sample preparation, Flow Cell preparation, sequencing kit storage, sequencing protocol, and device maintenance.

1.1 Applications

DNBSEQ-G50RS High-throughput Sequencing Set and DNBSEQ-G50RS High-throughput Rapid Sequencing Set are specifically designed for DNA or RNA sequencing on DNBSEQ-G50RS. This sequencing set is intended to be used for scientific research only, Which cannot be used for clinical diagnosis.

1.2 Sequencing technology

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

1.3 Data analysis

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

1.4 Sequencing read length

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

Table 1 Sequencing cycle

Sequencing read length	Read 1 read length	Read 2 read length	Barcode read length	Total read length	Maximum cycles
SE35	35	--	10	35+10	55
SE50	50	--	10	50+10	70
SE100	100	--	10	100+10	120
PE50	50	50	10	50+50+10	120
PE100	100	100	10	100+100+10	220
PE150	150	150	10	150+150+10	320

NOTE To perform SE35 sequencing, please use the DNBSEQ-G50RS High-throughput Sequencing Set (FCL SE50).

1.5 Sequencing time and analysis time

Table 2 Sequencing time for each read length (hours) of config 1

Type	Read length	Sequencing time	Analysis time
FCL	SE35	7.7	0.5
	SE50	9.7	0.6
	SE100	16.0	1.0
	PE50	22.7	1.0
	PE100	45.0	2.0
	PE150	63.5	2.8
FCS	SE100	9.5	0.3
	PE100	30.7	0.5
	PE150	42.0	0.7

Table 3 Sequencing time for each read length (hours) of config 2

Type	Read length	Sequencing time	Analysis time
FCL	SE35	5.8	0.2
	SE50	7.7	0.3
	SE100	12.7	0.3
	PE50	14.5	0.5
	PE100	25.0	0.9
	PE150	38.7	1.3
FCS	SE100	8.9	0.1
	PE100	18.9	0.1
	PE150	26.7	0.3

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

1.6 Attention

- This product is for research use only, please read the manual carefully before use.
- Make sure that you are familiar with the SOP&Attention of all the laboratory apparatus to be used.
- Avoid direct skin and eye contact with any samples and reagents. Don't swallow. Please wash with plenty of water immediately and go to the hospital if this happened.
- All the samples and waste materials should be disposed of according to relevant laws and regulations.
- This product is for one sequencing run only and cannot be reused.
- Do not use expired products.

Chapter 2 Sequencing sets and self-prepared consumables

2.1 List of sequencing set components

Table 4 DNBSEQ-G50RS High-throughput Sequencing Set (FCL SE50)
Catalog number: 1000019855

Component	Spec & quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G50RS Sequencing Flow Cell Catalog number: 1000020208				
Sequencing Flow Cell	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	8 months
DNBSEQ-G50RS High-throughput Sequencing Kit (FCL SE50/FCS SE100) Catalog number: 1000019849				
Low TE Buffer	100 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	10 months
Make DNB Buffer	50 μL×1 tube			
Make DNB Enzyme Mix I	100 μL×1 tube			
Make DNB Enzyme Mix II (LC)	13 μL×1 tube			
Stop DNB Reaction Buffer	50 μL×1 tube			
DNB Load Buffer I	300 μL×1 tube			
DNB Load Buffer II	120 μL×1 tube			
Micro Tube 0.5 mL (Empty)	1 tube			
dNTPs Mix III	0.32 mL×1 tube			
dNTPs Mix II	0.56 mL×1 tube			
Sequencing Enzyme Mix	0.60 mL×1 tube			
Sequencing Reagent Cartridge	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	10 months
Transparent sealing film	2 sheets			

NOTE ● To perform FCL SE35 sequencing, please use the DNBSEQ-G50RS High-throughput Sequencing Set (FCL SE50).

Table 5 DNBSEQ-G50RS High-throughput Sequencing Set (FCL SE100)
Catalog number: 1000019856

Component	Spec&quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G50RS Sequencing Flow Cell Catalog number: 1000020208				
Sequencing Flow Cell	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	8 months
DNBSEQ-G50RS High-throughput Sequencing Kit (FCL SE100) Catalog number: 1000019850				
Low TE Buffer	100 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	10 months
Make DNB Buffer	50 μL×1 tube			
Make DNB Enzyme Mix I	100 μL×1 tube			
Make DNB Enzyme Mix II (LC)	13 μL×1 tube			
Stop DNB Reaction Buffer	50 μL×1 tube			
DNB Load Buffer I	300 μL×1 tube			
DNB Load Buffer II	120 μL×1 tube			
Micro Tube 0.5 mL (Empty)	1 tube			
dNTPs Mix III	0.44 mL×1 tube			
dNTPs Mix II	0.76 mL×1 tube			
Sequencing Enzyme Mix	0.82 mL×1 tube			
Sequencing Reagent Cartridge	1 EA			
Transparent sealing film	2 sheets			

Table 6 DNBSEQ-G50RS High-throughput Sequencing Set (FCL PE50)
Catalog number: 1000019857

Component	Spec & quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G50RS Sequencing Flow Cell Catalog number: 1000020208				
Sequencing Flow Cell	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	8 months
DNBSEQ-G50RS High-throughput Sequencing Kit (FCL PE50/FCS PE100) Catalog number: 1000019851				
Low TE Buffer	100 µL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	10 months
Make DNB Buffer	50 µL×1 tube			
Make DNB Enzyme Mix I	100 µL×1 tube			
Make DNB Enzyme Mix II (LC)	13 µL×1 tube			
Stop DNB Reaction Buffer	50 µL×1 tube			
DNB Load Buffer I	300 µL×1 tube			
DNB Load Buffer II	120 µL×1 tube			
Micro Tube 0.5 mL (Empty)	1 tube			
dNTPs Mix III	0.56 mL×1 tube			
dNTPs Mix II	0.92 mL×1 tube			
Sequencing Enzyme Mix	1.02 mL×1 tube			
MDA Reagent	1.40 mL×1 tube			
MDA Enzyme Mix	0.30 mL×1 tube			
Sequencing Reagent Cartridge	1 EA			
Transparent sealing film	2 sheets			

Table 7 DNBSEQ-G50RS High-throughput Sequencing Set (FCL PE100)
Catalog number: 1000019859

Component	Spec & quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G50RS Sequencing Flow Cell Catalog number: 1000020208				
Sequencing Flow Cell	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	8 months
DNBSEQ-G50RS High-throughput Sequencing Kit (FCL PE100/FCS PE150) Catalog number: 1000019853				
Low TE Buffer	100 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	10 months
Make DNB Buffer	50 μL×1 tube			
Make DNB Enzyme Mix I	100 μL×1 tube			
Make DNB Enzyme Mix II (LC)	13 μL×1 tube			
Stop DNB Reaction Buffer	50 μL×1 tube			
DNB Load Buffer I	300 μL×1 tube			
DNB Load Buffer II	120 μL×1 tube			
Micro Tube 0.5 mL (Empty)	1 tube			
dNTPs Mix III	0.74 mL×1 tube			
dNTPs Mix II	1.48 mL×1 tube			
Sequencing Enzyme Mix	1.48 mL×1 tube			
MDA Reagent	1.40 mL×1 tube			
MDA Enzyme Mix	0.30 mL×1 tube			
Sequencing Reagent Cartridge	1 EA			
Transparent sealing film	2 sheets			

Table 8 DNBSEQ-G50RS High-throughput Sequencing Set (FCL PE150)
Catalog number: 1000019858

Component	Spec & quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G50RS Sequencing Flow Cell Catalog number: 1000020208				
Sequencing Flow Cell	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	8 months
DNBSEQ-G50RS High-throughput Sequencing Kit (FCL PE150) Catalog number: 1000019852				
Low TE Buffer	100 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	10 months
Make DNB Buffer	50 μL×1 tube			
Make DNB Enzyme Mix I	100 μL×1 tube			
Make DNB Enzyme Mix II (LC)	13 μL×1 tube			
Stop DNB Reaction Buffer	50 μL×1 tube			
DNB Load Buffer I	300 μL×1 tube			
DNB Load Buffer II	120 μL×1 tube			
Micro Tube 0.5 mL (Empty)	1 tube			
dNTPs Mix III	0.96 mL×1 tube			
dNTPs Mix II	1.02 mL×2 tube			
Sequencing Enzyme Mix	0.99 mL×2 tube			
MDA Reagent	1.40 mL×1 tube			
MDA Enzyme Mix	0.30 mL×1 tube			
Sequencing Reagent Cartridge	1 EA			
Transparent sealing film	2 sheets			

Table 9 DNBSEQ-G50RS High-throughput Rapid Sequencing Set (FCS SE100) Catalog number: 1000019860

Component	Spec&quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G50RS Rapid Sequencing Flow Cell Catalog number: 1000020209				
Sequencing Flow Cell	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	8 months
DNBSEQ-G50RS High-throughput Sequencing Kit (FCL SE50/FCS SE100) Catalog number: 1000019849				
Low TE Buffer	100 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	10 months
Make DNB Buffer	50 μL×1 tube			
Make DNB Enzyme Mix I	100 μL×1 tube			
Make DNB Enzyme Mix II (LC)	13 μL×1 tube			
Stop DNB Reaction Buffer	50 μL×1 tube			
DNB Load Buffer I	300 μL×1 tube			
DNB Load Buffer II	120 μL×1 tube			
Micro Tube 0.5 mL (Empty)	1 tube			
dNTPs Mix III	0.32 mL×1 tube			
dNTPs Mix II	0.56 mL×1 tube			
Sequencing Enzyme Mix	0.60 mL×1 tube			
Sequencing Reagent Cartridge	1 EA			
Transparent sealing film	2 sheets			

Table 10 DNBSEQ-G50RS High-throughput Rapid Sequencing Set (FCS PE100) Catalog number: 1000019861

Component	Spec & quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G50RS Rapid Sequencing Flow Cell Catalog number: 1000020209				
Sequencing Flow Cell	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	8 months
DNBSEQ-G50RS High-throughput Sequencing Kit (FCL PE50/FCS PE100) Catalog number: 1000019851				
Low TE Buffer	100 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	10 months
Make DNB Buffer	50 μL×1 tube			
Make DNB Enzyme Mix I	100 μL×1 tube			
Make DNB Enzyme Mix II (LC)	13 μL×1 tube			
Stop DNB Reaction Buffer	50 μL×1 tube			
DNB Load Buffer I	300 μL×1 tube			
DNB Load Buffer II	120 μL×1 tube			
Micro Tube 0.5 mL (Empty)	1 tube			
dNTPs Mix III	0.56 mL×1 tube			
dNTPs Mix II	0.92 mL×1 tube			
Sequencing Enzyme Mix	1.02 mL×1 tube			
MDA Reagent	1.40 mL×1 tube			
MDA Enzyme Mix	0.30 mL×1 tube			
Sequencing Reagent Cartridge	1 EA			
Transparent sealing film	2 sheets			

Table 11 DNBSEQ-G50RS High-throughput Rapid Sequencing Set (FCS PE150) Catalog number: 1000019862

Component	Spec & quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G50RS Rapid Sequencing Flow Cell Catalog number: 1000020209				
Sequencing Flow Cell	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	8 months
DNBSEQ-G50RS High-throughput Sequencing Kit (FCL PE100/FCS PE150) Catalog number: 1000019853				
Low TE Buffer	100 μL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	10 months
Make DNB Buffer	50 μL×1 tube			
Make DNB Enzyme Mix I	100 μL×1 tube			
Make DNB Enzyme Mix II (LC)	13 μL×1 tube			
Stop DNB Reaction Buffer	50 μL×1 tube			
DNB Load Buffer I	300 μL×1 tube			
DNB Load Buffer II	120 μL×1 tube			
Micro Tube 0.5 mL (Empty)	1 tube			
dNTPs Mix III	0.74 mL×1 tube			
dNTPs Mix II	1.48 mL×1 tube			
Sequencing Enzyme Mix	1.48 mL×1 tube			
MDA Reagent	1.40 mL×1 tube			
MDA Enzyme Mix	0.30 mL×1 tube			
Sequencing Reagent Cartridge	1 EA			
Transparent sealing film	2 sheets			

Table 12 CPAS Barcode Primer 3 Reagent Kit
Catalog number: 1000020834

Product	Component	Spec & quantity	Storage temperature	Transportation temperature	Expiry date
Primer for dual barcode sequencing (Pair End Sequencing use only)	1 μM AD153 Barcode Primer 3	3.5 mL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months

Table 13 CPAS Barcode Primer 4 Reagent Kit
Catalog number: 1000014048

Product	Component	Spec & quantity	Storage temperature	Transportation temperature	Expiry date
Primer for dual barcode sequencing (Single End Sequencing use only)	1 μM AD153 Barcode Primer 4	3.5 mL×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months

2.2 Self-prepared equipment and consumables

Table 14 Self-prepared equipment and consumables

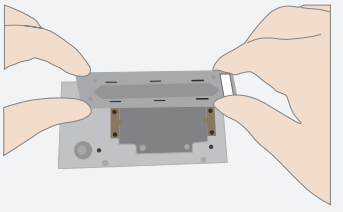
Equipment and consumables	Recommended brand	Catalog number
Qubit 3.0 fluorometer	Thermo Fisher	Q33216
Mini centrifuge	Major Laboratory Supplier (MLS)	/
Vortex mixer	MLS	/
Thermal cycler	Bio-Rad	/
Pipette	Eppendorf	/
2 ℃ to 8 ℃ refrigerator	MLS	/
-25 ℃ to -15 ℃ refrigerator	MLS	/
Qubit ssDNA assay kit	Thermo Fisher	Q10212
Power dust remover	MATIN	M-6318
Sterile pipette tip(box)	AXYGEN	/
200 µL Wide-bore pipette tips	AXYGEN	T-205-WB-C
Qubit assay tubes	Thermo Fisher	Q32856
100%Tween-20	MLS	/
5 M NaCl solution	MLS	/
2 M NaOH solution	MLS	/
0.2 mL PCR 8-tube strip	AXYGEN	/
1.5 mL Microcentrifuge tube	AXYGEN	MCT-150-C
2.0 mL Cryotube	SARSTEDT	72.609.003
Ice box	MLS	/

Chapter 3 Sequencing workflow

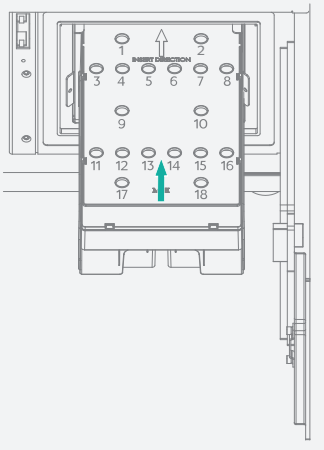
Making DNB: use DNB preparation kit for making DNB

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

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



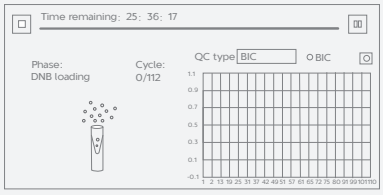
Loading the Flow Cell: place the Flow Cell on the stage of the sequencer



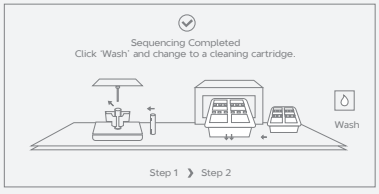
Load the reagent cartridge into the sequencer

Review	Content
User name	user
DNB ID	XXX
Sequencing Kit ID	XXXXX
Flow cell ID	XXXXXXXXXX
Recipe	Customize
Cycles	XXX

Follow the instructions to enter sequencing information and start the run



Monitor the sequencing run from the control software interface



Perform device maintenance when sequencing is completed

Chapter 4 Making DNB

4.1 Insert size recommendation

- This sequencing set is compatible with the libraries prepared by MGI Library Prep Kits.
- Recommended library insert size: The size distribution of inserts should be between 50 to 500 bp, with the main band centered within ±100 bp. If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.

4.2 Library concentration and amount requirement

- Library requirement is subject to the corresponding library preparation kit user manual. For general libraries, the ssDNA library concentration should be ≥2 fmol/μL and each Make DNB reaction requires 40 fmol library.
- If the library concentration is unknown, it is recommended to perform ssDNA library quantitation (ng/μL) using Qubit ssDNA Assay Kit and Qubit Fluorometer. Use the equation below to convert the concentration of the ssDNA library from ng/μL to fmol/μL:

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

4.3 Making DNB

4.3.1 Preparing reagents for DNB making

Perform the steps below:

1. Place the library on ice until use.
2. Take out Make DNB Buffer, Low TE Buffer and Stop DNB Reaction Buffer from storage and thaw reagents at room temperature.

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

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

4.3.2 Calculating the required amount of ssDNA library

- The required volume of ssDNA library is determined by the required library amount (fmol) and library concentration quantified in *4.2 Library concentration and amount requirement on Page 16*.
- The volume of each Make DNB reaction is 100 μL and the required library input for each Make DNB reaction is calculated as followed:

  ssDNA library input (μL) = 40 fmol / library concentration (fmol/μL)

  NOTE
  - If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.
  - All samples should be considered potentially infectious and should be handled in accordance with relevant national regulations.
- Calculate the required ssDNA library for each Make DNB reaction and fill it in the table below as V.

4.3.3 Making DNB

Perform the steps below:

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

Table 15 Make DNB reaction mix 1

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

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

Table 16 Primer hybridization reaction condition

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

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

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

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

Table 17 Make DNB reaction mix 2

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

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

Table 18 Rolling circle amplification conditions

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

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

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

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

4.4 Quantifying DNB

Perform the steps below:

1. When DNB making is completed, take 2 µL DNB, use Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify the DNB.
2. Sequencing requires a minimum DNB concentration of 8 ng/µL. If the concentration is lower than 8 ng/µL, see *9.1 Low DNB concentration on Page 42*.

NOTE
- Sequencing requires a minimum DNB concentration of 8 ng/µL. If the concentration is lower than 8 ng/µL, make a new DNB preparation.
- Because DNB is viscous, it is recommended to take 2 µL for quantification.
- 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.
- Store DNB at 4 ℃ and perform sequencing within 48 hours.

3. If the concentration exceeds 40 ng/μL, the DNB need to be diluted to 20 ng/μL with DNB Load Buffer I for loading.

4.5 Loading DNB

Perform the steps below:

1. Take out DNB Load Buffer I and DNB Load Buffer II from storage and thaw reagents on ice for approximately 0.5 hours.
2. After being thawed, mix reagents by using a vortex mixer for 5 seconds, centrifuge briefly and place on ice until use.

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

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

Table 19 Making DNB reaction mix 1 (For sequencer loading)

Component	Volume (μL)
DNB Load Buffer I	50
DNB Load Buffer II	50
Make DNB Enzyme Mix II (LC)	1
DNB	100

NOTE Prepare a fresh DNB loading mix before the sequencing run. It is recommended to prepare the DNB loading mix mentioned above after finishing *Chapter 5 Preparing the sequencing reagent cartridge on Page 21*.

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

   NOTE ● Do not centrifuge, vortex, or shake the tube.

Chapter 5 Preparing the sequencing reagent cartridge

Perform the steps below:

1. Take out the Sequencing Reagent Cartridge from storage.
2. Thaw them in water bath with room temperature until completely thawed. Store at 2 ℃ to 8 ℃ storage until use(or thaw in 2 ℃ to 8 ℃ fridge one day in advance).
3. Shake the cartridge violently in all directions for 10 to 20 times and making sure reagents are fully mixed.

   NOTE This is the normal phenomenon that dark green crystals appear in well No.18, which is precipitation of raw materials of the reagent. When the cartridge is thawed, mix the reagents in the cartridge well and the crystals will dissolve. Sequencing quality will not be affected.

4. Open the cartridge cover and wipe any water condensation with lint-free paper. Well positions are shown in the following figure.

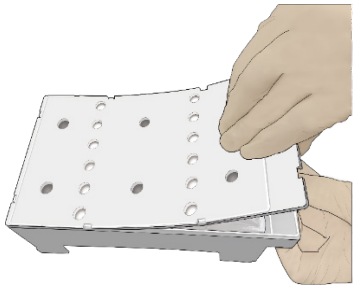


Figure 1 Opening and clean the cartridge

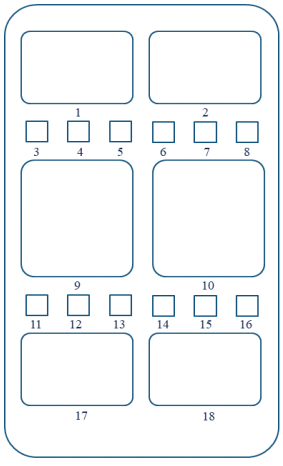


Figure 2 Well position

5. Take out dNTPs Mix III and dNTPs Mix II from -20 ℃ storage one hour in advance, thaw at room temperature.Store at 4 ℃ until use. Mix the reagents by using a vortex mixer for 5 seconds and centrifuge briefly before use.
6. Take out Sequencing Enzyme Mix from -20 ℃ storage and place, invert Sequencing Enzyme Mix 4 to 6 times before use.
7. Pierce the seal in the center of well No.1 and No.2 to make a hole around 1 cm in diameter by using a 1 mL sterile tip.

Figure 3 Piercing the seal on the cartridge

8. Take a pipette with the appropriate volume range and add them into well No.1 following the table below:

Table 20 Sequencing cartridge well No.1 reagent loading

Product model	dNTPs Mix III loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
FCL SE50/FCS SE100	0.320	0.320
FCL SE100	0.440	0.440
FCL PE50/FCS PE100	0.560	0.560
FCL PE100/FCS PE150	0.740	0.740
FCL PE150	0.960	0.960

9. Take a pipette with the appropriate volume range and add the reagents into well No.2 following the table below.

Table 21 Sequencing cartridge well No.2 reagent loading

Product model	dNTPs Mix II loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
FCL SE50/FCS SE100	0.560	0.280
FCL SE100	0.760	0.380
FCL PE50/FCS PE100	0.920	0.460
FCL PE100/FCS PE150	1.480	0.740
FCL PE150	2.040	1.020

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

    NOTE Do not cover the center of the well to avoid blocking the sampling needles.

Figure 4 Sealing the loading wells

12. Place the cartridge horizontally on the table, hold both sides of the cartridge with both hands. Shake it clockwise 10 to 20 times, and then counterclockwise 10 to 20 times, until the reagent color in well No,1 is uniform. Make sure that you see the vortex to ensure reagents are fully mixed.

Figure 5 Mixing reagents after loading

13. Perform the following steps according to different situations:

- For PE cartridges

  a. Add 200 μL of MDA Enzyme Mix to the MDA Reagent tube with a 200 μL pipette.

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

  b. Invert the tube for 4 to 6 times to mix the reagents.

  c. Add the mixture to well No.15. When adding the mixture, make sure there are no bubbles at the bottom of the tube.

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

- Dual barcode sequencing for the PE cartridge

  NOTE If dual barcode sequencing is required, perform the following steps after preparing the PE cartridge.

  a. Take out the 1 μM AD153 Barcode Primer 3 from the cPAS Barcode Primer 3 Reagent Kit and thaw at room temperature.

  b. Mix the 1 μM AD153 Barcode Primer 3 by using a vortex mixer for 5 seconds and centrifuge briefly before use.

  c. Pierce the seal of well No.12 by using a sterile tip.

  d. Add 1.30 mL of the 1 μM AD153 Barcode Primer 3. When adding the reagent, make sure there are no bubbles at the bottom of the tube.

- Dual barcode sequencing for the SE cartridge

  a. Take out the 1 μM AD153 Barcode Primer 4 from the cPAS Barcode Primer 4 Reagent Kit and thaw at room temperature.

  b. Mix the 1 μM AD153 Barcode Primer 4 by using a vortex mixer for 5 seconds and centrifuge briefly before use.

  c. Pierce the seal of well No.12 by using a sterile tip.

  d. Add 1.30 mL of the 1 μM AD153 Barcode Primer 4. When adding the reagent, make sure there are no bubbles at the bottom of the tube.

Chapter 6 Preparing a Flow Cell

Perform the steps below:

1. Take the Flow Cell out of storage and take out the Flow Cell form the box.
2. Unwrap the outer package.

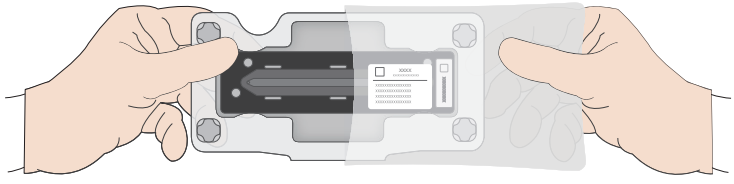


Figure 6 Unwrapping the outer package

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

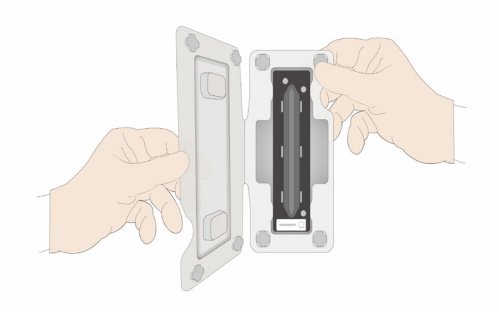


Figure 7 Inspecting the Flow Cell

Chapter 7 Sequencing

7.1 Entering the main interface

Perform the steps below:

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

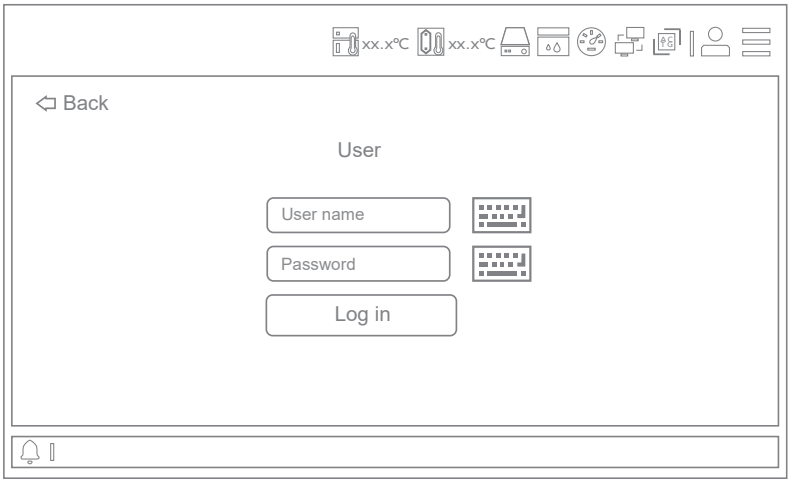


Figure 8 Log-in interface

2. The main interface is as below:

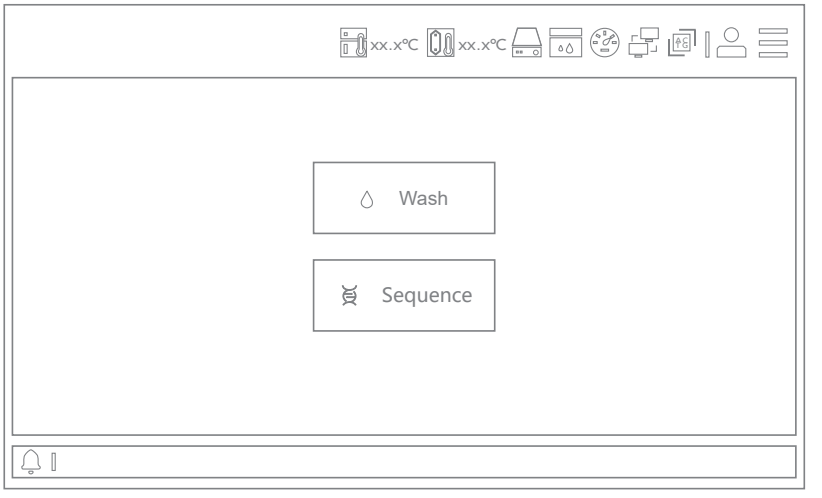


Figure 9 Main interface

7.2 Loading DNB

Perform the steps below:

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

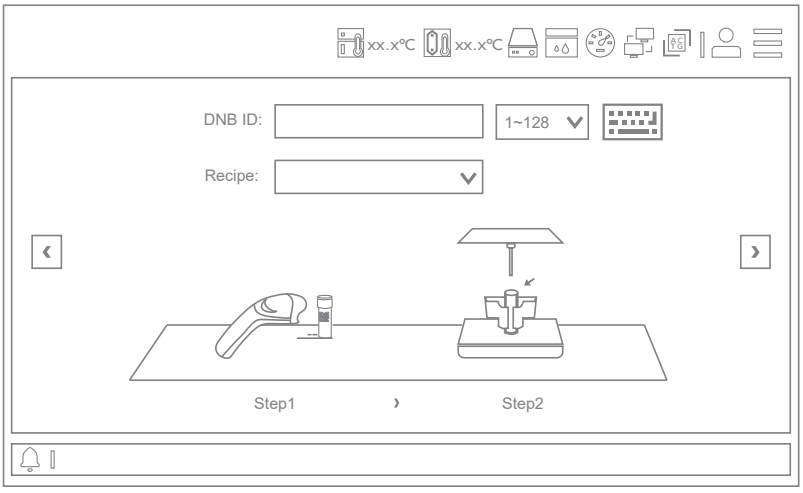


Figure 10 DNB loading interface

2. Move the cursor to the blank area next to the **DNB ID** and enter the library name or number.
3. Open the reagent compartment door, gently lift the sampling needle with one hand, remove the cleaning reagent tube with the other hand, load the sample tube, then slowly lower the sampling needle until the tip reaches the bottom of the tube.

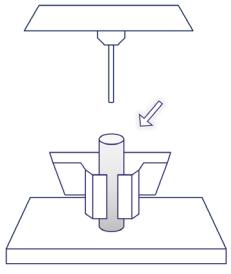


Figure 11 Loading the DNBs tube

4. Close the reagent compartment door.

7.3 Selecting the sequencing parameters

Perform the steps below:

1. Select the sequencing recipe in the **Recipe** drop-down menu. There are one-click sequencing run (PE100_FCL,PE100_FCS etc.) and user-customized run (Customize).

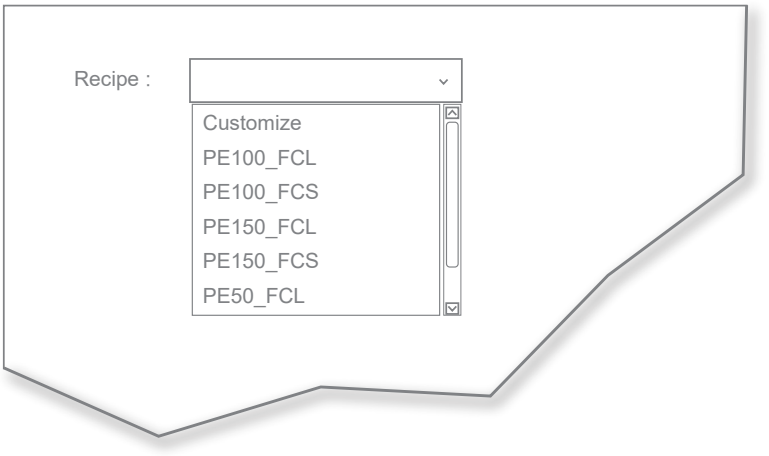


Figure 12 Selecting sequencing solutions

NOTE
- Sequencing recipes with **FCL** are for the DNBSEQ-G50RS High-throughput Sequencing Set (FCL). Sequencing recipes with **FCS** are for the DNBSEQ-G50RS High-throughput Rapid Sequencing Set (FCS).
- To perform SE35 sequencing, use the DNBSEQ-G50RS High-throughput Sequencing Set (FCL_SE50), and select recipe **SE35_FCL** or **Customize**.
- For dual barcode sequencing, select recipe **Customize**.

2. If you choose one-click sequencing, go to *7.4 Loading the reagent cartridge on Page 30*. If you choose **Customize**, continue performing the steps below.
3. In the beginning, please select a step to start the sequencing run.

Figure 13 Selecting the step to start sequencing

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

Figure 14 Selecting the read length

5. Select the barcode length. For Dual Barcode sequencing, fill in the length of Barcode and Dual barcode. Leave the Dual barcode blank if it is a Single Barcode sequencing run.

Figure 15 Selecting the barcode length

6. Check the barcode for demultiplexing and select the barcode sequence.

Figure 16 Barcode demultiplexing

7. Select the dark reaction for any position of read length in read 1 or 2. If dark reaction is not required, leave the table below blank. Dark reaction: only chemical reaction without optical information capture.

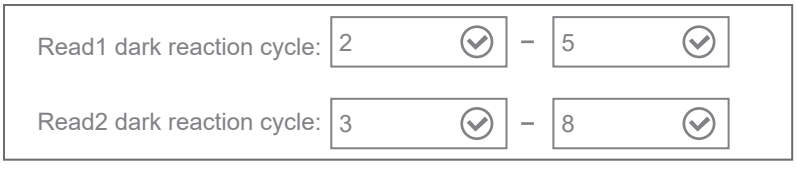


Figure 17 Selecting the dark reaction

8. Click **Confirm**.

7.4 Loading the reagent cartridge

Perform the steps below:

1. Move the cursor to the **Reagent cartridge ID** blank, enter the cartridge information manually or use the barcode scanner to scan the cartridge barcode.

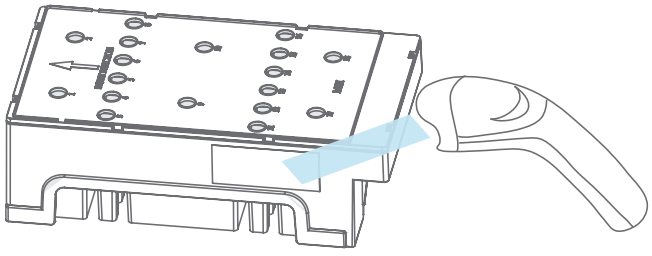


Figure 18 Reagent cartridge information entry interface

2. Open the reagent compartment door.Hold the handle of the cleaning cartridge 1 with one hand, place the other hand underneath the cartridge for support, and slowly remove it from the compartment.

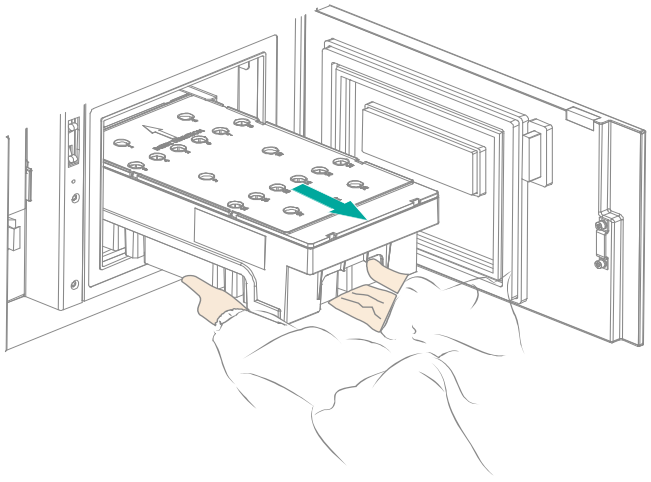


Figure 19 Removing cleaning cartridge

3. Moisten dust-free paper or a dust-free cloth with laboratory-grade water and use it to wipe the bottom and sides of the compartment to keep it clean and dry.

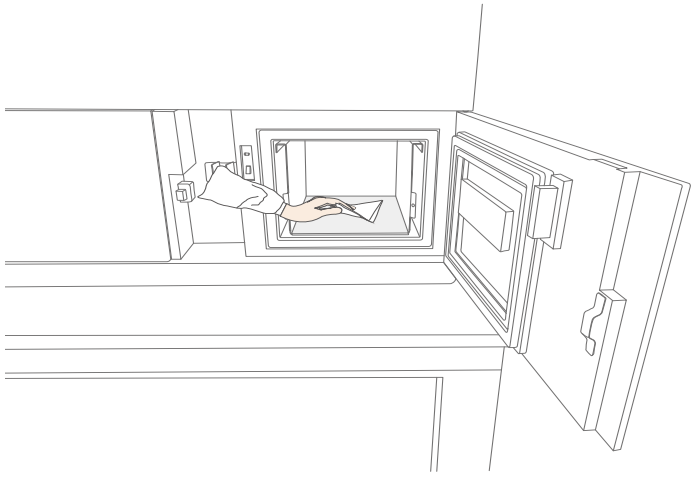


Figure 20 Maintaining the reagent compartment

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

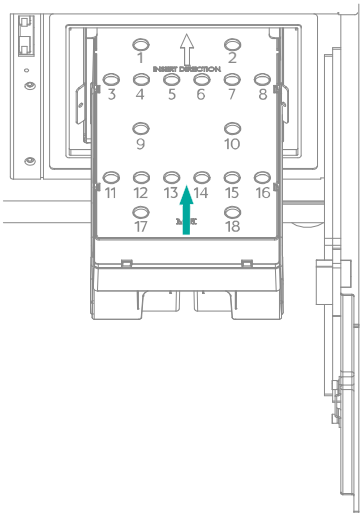


Figure 21 Sliding the new reagent cartridge into the reagent compartment

7.5 Loading the Flow Cell

Perform the steps below:

1. Open the Flow Cell compartment door, press one side of the Flow Cell used for washing, and press the Flow Cell attachment button with the other hand. After the vacuum is released, remove the Flow Cell for washing from the stage.
2. Use dust remover to remove the dust on the Flow Cell stage and the back of the Flow Cell. If there are impurities on the stage surface, please gently wipe it with wet dust-free paper to ensure that the Flow Cell can be held properly.

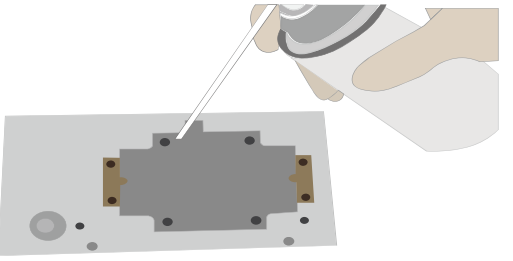


Figure 22 Cleaning the Flow Cell stage

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

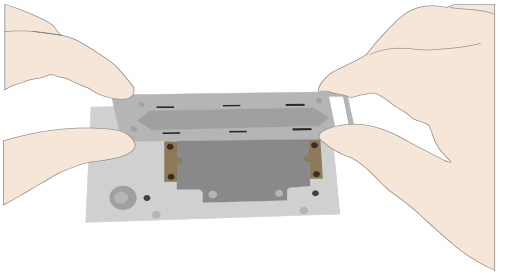


Figure 23 Loading the Flow Cell

5. Align the holes on the Flow Cell with the locating pins on the Flow Cell stage. Gently slide the Flow Cell to keep the Flow Cell aligned with the pin. Press the left and right sides of the Flow Cell on the stage at the same time to ensure the Flow Cell is properly seated on the stage.

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

6. Use a dust remover to remove the dust on the Flow Cell surface and close the Flow Cell compartment door.

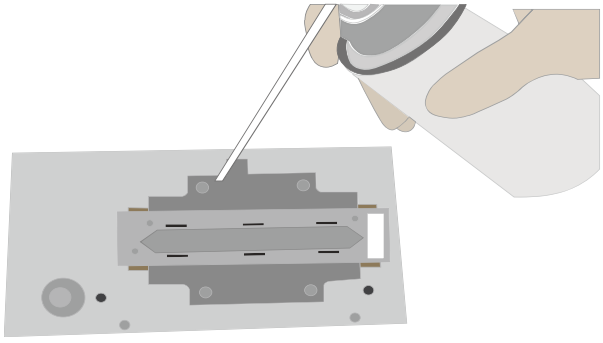


Figure 24 Cleaning the Flow Cell

7. Click **Next**, the device will automatically enter the Flow Cell ID; if automated entry does not work, move the cursor to the **Flow Cell ID** blank and manually enter the ID.

NOTE When entering manually, the Flow Cell ID should be entered strictly according to the Flow Cell number on the label. Different sequencing recipe will be invoked based on the Flow Cell ID entered. Flow Cell ID beginning with S is FCL and Flow Cell ID beginning with K is FCS.

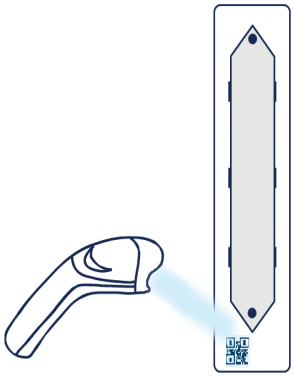


Figure 25 Flow Cell information entry interface

7.6 Reviewing parameters

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

Review	Content
User name	user
DNB ID	123 | 1-128
Sequencing cartridge ID	A0001
Flow cell ID	S200000001
Recipe	PE150_FCL
Cycles	312

Figure 26 Reviewing information

NOTE To ensure sequencing quality, when read 1 and read 2 sequencing is completed, the sequencer will automatically perform one more cycle for correction. For example, for PE150 sequencing, read 1 read length is 150, read 2 read length is 150, barcode read length is 10, plus 1 correction cycle for read 1 and 1 correction cycle for read 2 (barcode does not require correction), the total cycle number of the sequencing is 312.

7.7 Starting sequencing

Perform the steps below:

1. After confirming that the information is correct, click **Start**.
2. The system will display the dialog box **Proceed with Sequencing?**. Click **Yes** to start sequencing.

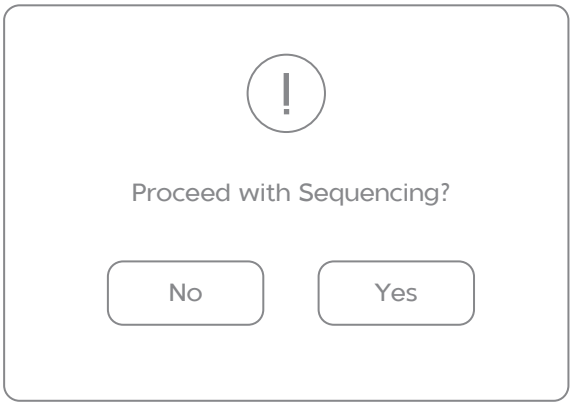


Figure 27 Confirming sequencing interface

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

Chapter 8 Maintaining Device

8.1 Wash type

Table 22 Wash requirments

Wash type	Description	Time
Full wash	1. Maintenance wash 2. Regular wash	About 75 min
Maintenance wash	To remove residual reagents and proteins in the pipeline, reducing the risk of blockage. Procedure: 1. Cleaning cartridge 4 2. Cleaning cartridge 3 3. Cleaning cartridge 2	About 45 min
Regular wash	To remove residual reagents, reducing the risk of cross-contamination. Procedure: 1. Cleaning cartridge 1 2. Air Prime.	About 30 min

8.2 Wash instruction

- When the sequencing is completed and the interface below appears, please perform a wash. The device needs to be washed within 24 hours.

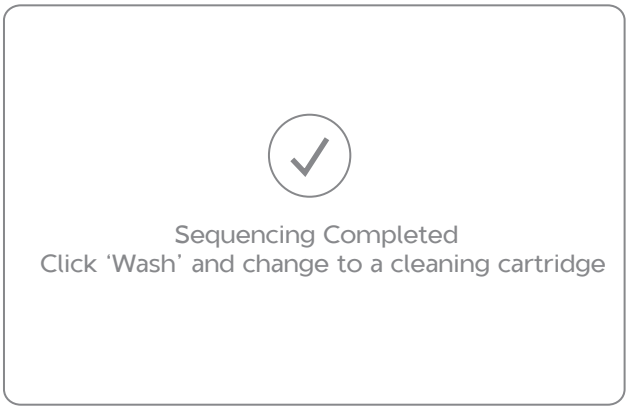


Figure 28 Wash interface

Wash type	Description
Full wash	• The sequencer is used for a PE run. • After the replacement of pipelines, sample needles and other accessories exposed to reagents. • The sequencer was left unused for more than 7 days or longer, perform a wash before use. • Impurities are found on the Flow Cell.
Regular wash	• The device was left unused for more than 12 hours after a full wash, perform a wash again before use. • After the system maintenance performed by an engineer • other situation except for full wash and maintenance wash.
Maintenance wash	The sequencer is to be powered off for more than 7 days, perform a wash before being powered off and after being powered on.

8.3 Preparing wash reagents

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

Perform the steps below:

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

Table 23 Wash reagents 1 preparation

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

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

Table 24 Wash reagents 2 preparation

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

- Prepare 0.1 M NaOH following the table below.

Table 25 Wash reagents 3 preparation

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

8.4 Wash cartridge

- An empty cleaning cartridge and washing Flow Cell for a full wash are provided together with the device.
- Wash the cleaning cartridge every time before refilling it with cleaning reagents. Replace cleaning cartridge after 20 uses or every half year.
- Used Flow Cells from previous runs can be used as washing Flow Cells. Each Flow Cell can be used for up to 20 full washes.

Table 26 Wash cleaning cartridge preparation

Cartridge name	0.5 mL cryotube	Large wells	No.15 well	Small wells
Wash cleaning cartridge 1	More than 90% volume of laboratory grade water			
Wash cleaning cartridge 2	More than 90% volume of laboratory grade water			
Wash cleaning cartridge 3	80% volume (do not exceed 90%) of Wash reagents 3			
Wash cleaning cartridge 4	80% volume (do not exceed 90%) of Wash reagent 2	80% volume (do not exceed 90%) of Wash reagent 1	80% volume (do not exceed 90%) of Wash reagent 2	80% volume (do not exceed 90%) of Wash reagent 1

8.5 Wash procedures

8.5.1 Regular wash

Perform the steps below:

1. Use cleaning cartridge 1. Open the reagent compartment door. Hold the handle of the cleaning cartridge 1 with one hand and place the other hand underneath the cartridge 1 for support. Slide it into the reagent compartment slowly following the direction printed on the cartridge cover until it stops. Close the reagent compartment door.
2. Click the wash button on the interface.

3. Place the Flow Cell for washing.
4. Select regular wash from the drop-down menu to start the regular wash which takes about 30 minutes.
5. If you perform the regular wash only, observe the status of the washing Flow Cell in this step. If you see many bubbles, continue the wash. If not, stop the wash, replace the Flow Cell and start the wash. If you perform the regular wash after the maintenance wash, skip this step.

Figure 29 Selecting the wash type

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

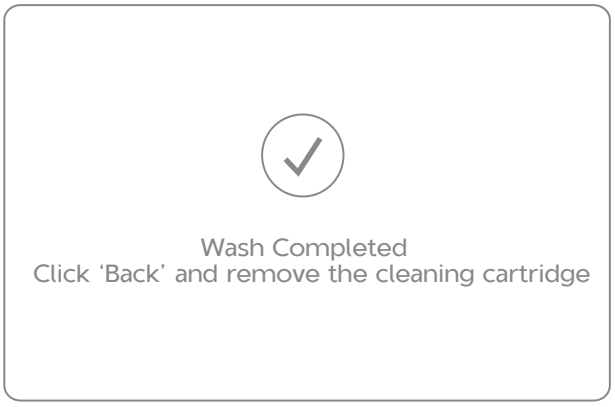


Figure 30 Regular wash end interface

8.5.2 Maintenance wash

Perform the steps below:

1. Use cleaning cartridge 4. Open the reagent compartment door. Hold the handle of the cleaning cartridge 4 with one hand and place the other hand underneath for support. Slide it to the reagent compartment slowly following the direction printed on the cartridge cover until it stops. Close the reagent compartment door.
2. Click the wash button on the interface.
3. Place the Flow Cell for washing.
4. Select the maintenance wash from the drop-down menu to start the maintenance wash which takes about 15 minutes.
5. Observe the status of Flow Cell for wash in this step. If you see many bubbles, continue the wash. If not, stop the wash, replace the Flow Cell and start the wash.
6. When the figure below appears on the interface, click **Yes** and the sequencer will automatically lift the sampling needles. Then open the compartment door and replace the cleaning cartridge.

Figure 31 Maintenance wash [1] end interface

7. Use cleaning cartridge 3 and continue the maintenance wash which takes around 15 minutes.

8. When the figure below appears on the interface, click **Yes** and the sequencer will automatically lift the sampling needles. Then open the compartment door and replace the cleaning cartridge.

Figure 32 Maintenance wash [2] end interface

9. Use cleaning cartridge 2 and continue the maintenance wash which takes around 15 minutes.
10. When the figure below appears on the interface, click **No** to end the maintenance wash.

Figure 33 Maintenance wash end interface

8.5.3 Full wash procedures

Perfrom Maintenance wash, followed by Regular wash, with a total time of around 75 minutes.

Chapter 9 Troubleshooting

9.1 Low DNB concentration

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

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

9.2 Abnormal negative pressure

When the negative pressure is shown in red, the negative pressure is abnormal, try the steps below:

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

9.3 Bubbles

If bubbles appear, try the steps below:

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

9.4 Impurities

If impurities appear, try the steps below:

- Perform a full wash on the sequencer.
- If the problem persists after a full wash, please contact a field service engineer.

9.5 Pump fails

If liquids cannot be pumped into the Flow Cell, or large bubbles appear in the Flow Cell, try the steps below:

- Sequencer: remove the Flow Cell, check if there are impurities in sealing gasket and remove the dust with the dust remover. Place the Flow Cell following the instruction and start the pump again.
- Check if the sampling needles move properly.
- If the sampling needles cannot move properly, restart sequencing software.
- If the problem persists, please contact a field service engineer.

9.6 Reagent kit storage

- If the kit has been thawed (including dNTPs) and cannot be used within 24 hours, it can be frozen and thawed at most once.
- If the kit has been thawed (including dNTPs) but cannot be used immediately, store it at 4 ℃ and use it within 24 hours. Mix the reagents in the cartridge following instruction in *Chapter 5 Preparing the sequencing reagent cartridge on Page 21* before use.
- If dNTPs and enzyme have been added into the cartridge, i.e. the cartridge has been prepared but cannot be used immediately, store it at 4 ℃ and use it within 24 hours. Mix the reagents in the cartridge following instruction in *Chapter 5 Preparing the sequencing reagent cartridge on Page 21* before use.
- If dNTPs and enzyme have been added into the cartridge, i.e. the cartridge has been prepared and the sampling needles have started aspiration, but the cartridge cannot be used in time, the cartridge must be sealed with foil or plastic wrap. Store the cartridge at 4 ℃ and use it within 24 hours. Gently mix the reagents in the cartridge before use. When mixing, be careful not to spill any reagent from the needle holes to avoid reagent contamination.

Appendix 1 Manufacturer

Manufacturer	Wuhan MGI Tech Co., Ltd.
Address	Building B13, No.818, Gaoxin Avenue, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China
	Building 24, Stage 3.1, BioLake Accelerator, No.388, 2nd Gaoxin Road, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China
Service hotline	(+86)4000-966-988
E-mail	MGI-service@genomocs.cn
Website	en.mgi-tech.com