Part No.: SOP-013-B01-033

High-throughput (Rapid)Sequencing Set

MGISEQ-200RS

Instructions for use

Version: 11.0

Leading Life Science Innovation

Address 1: Main Building and Second floor of No. 11 Building, Beishan Industrial Zone, Yantian District, 518083 Shenzhen, Guangdong, P.R.China

Address 2: Building B13, No.818, Gaoxin Avenue, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China

Address 3: 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

Research Use Only

MGI Tech Co., Ltd.

Wuhan MGI Tech Co., Ltd.

About the instructions for use

This instructions for use is applicable to MGISEQ-200RS High-throughput (Rapid) Sequencing Set. The version of the instructions for use is 11.0 and the kit version is 4.0.

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*Unless otherwise informed, StandardMPS sequencing reagents are not available in Germany, UK, Sweden, and Switzerland.

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Revision history

Version	Date	Description
11.0	June 2024	• Updated the use-by-date of sequencing flow cell.
10.0	November 2023	• Updated the components in sequencing sets. • Deleted cPAS Barcode Primer 3 & 4 Reagent Kit. • Updated the configuration of the sequencing cartridge. • Changed the template of the instructions for use. • Updateded some of the figures. • Added two chapterd: 1.3 Sample requirements and 7.8 Data access. • Updated some of the sequencing time. • Updated login password.
9.0	January 2022	• Updated the company logo. • Changed the sale statement. • Updated the transport temperature. • Changed the operation pictures.
8.0	July 2021	• Increased the validity of reagents. • Changed the Storage Temperature of the flow cell.
A6	December 2020	• Updated the company Logo, website address and mailbox. • Added config 2.
A5	June 2020	• Changed the Storage Temperature of the flow cell. • Updated the catalog number and Spec &Quantity of cPAS Barcode Primer 3 Reagent Kit. • Updated the filling volume of wash reagents.

Version	Date	Description
A4	March 2020	• Updated the figures of sequencer interface. • Updated the product name, catalog number and version. • Updated the loading volume of dNTPs Mix III, dNTPs Mix II and Sequencing Enzyme Mix. • Added the High-throughput Rapid Sequencing Set products. • Added the PE150 read length.
A4	March 2020	• Added dual barcode sequencing in PE sequencing. • Added the solution of crystal precipitation in DNB Load Buffer.
A4	March 2020	• Explained the possible dark green crystals in well No.18. • Updated the loading well positions.
A3	Dececmber 2019	• Added a new chapter “Attention”. • Updated the equations used to calculate library input. • Added the “Revision History”.
A2	August 2019	Changed the DNBseq™ to DNBSEQ™.
A1	March 2019	• Updated the storage temperature. • Added the SE100 read length.
A0	November 2018	Initial release.

Sequencing set

Catalog number	Name	Model	Version
940-001629-00	MGISEQ-200RS High-throughput Sequencing Set	FCL SE50	4.0
940-001640-00		FCL SE100	4.0
940-001616-00		FCL PE50	4.0
940-001623-00		FCL PE100	4.0
940-001619-00		FCL PE150	4.0
940-001626-00	MGISEQ-200RS High-throughput Rapid Sequencing Set	FCS SE100	4.0
940-001618-00		FCS PE100	4.0
940-001627-00		FCS PE150	4.0

Contents

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Chapter 1 Introduction

This instructions for use explains how to perform sequencing by using the MGISEQ-200RS High-throughput Sequencing Set and MGISEQ-200RS High-throughput Rapid Sequencing Set. This instructions for use includes instructions regarding sample preparation, flow cell preparation, sequencing kit storage, the sequencing protocol and device maintenance.

1.1 Applications

MGISEQ-200RS High-throughput Sequencing Set and MGISEQ-200RS High-throughput Rapid Sequencing Set are specifically designed for DNA or RNA sequencing on MGISEQ-200RS. This sequencing set is intended to be used for scientific research only and cannot be used for clinical diagnosis.

1.2 Sequencing technology

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

1.3 Sample requirements

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

1.4 Data analysis

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

1.5 Sequencing read length

Sequencing read length determines the number of sequencing cycles for a given sequencing run. For example, a PE150 cycle run performs reads of 150 cycles (2×150) for a total of 300 cycles. At the end of the insert sequencing run, one or two extra 10 cycles of barcode read can be performed, 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	Read1 read length	Read2 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
PE50	50	50	10	10	102+10+10	122
PE100	100	100	10	10	202+10+10	222
PE150	150	150	10	10	302+10+10	322

Tips To perform SE35 sequencing, use the MGISEQ-200RS High-throughput Sequencing Set (FCL SE50).

1.6 Sequencing and analysis time

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

Time (hours)	SE35	SE50	SE100	PE50	PE100	PE150
FCL	7.7	10.1	16.0	22.7	48.0	65.5
FCS	-	-	11.6	-	30.7	42.0
Data analysis (FCL)	0.5	0.6	1.0	1.0	2.0	2.8
Data analysis (FCS)	-	-	0.3	-	0.5	0.7

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

Time (hours)	SE35	SE50	SE100	PE50	PE100	PE150
FCL	5.8	5.8	12.7	14.5	25.0	38.7

Time (hours)	SE35	SE50	SE100	PE50	PE100	PE150
FCS	-	-	9.5	-	18.9	26.7
Data analysis (FCL)	0.2	0.15	0.3	0.5	0.9	1.3
Data analysis (FCS)	-	-	0.1	-	0.1	0.3

Tips

- The sequencing time in the table above includes the time required from DNB loading 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 for single barcode sequencing and the actual run time may vary among various sequencing instruments.

1.7 Attention

- This product is for research use only. Please read the instructions for use 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 or reagents. Don't swallow. Please wash with plenty of water immediately and go to the hospital if this happens.
- All the samples and waste materials should be disposed of according to relevant laws and regulations.
- This product is for one sequencing run only and cannot be reused.
- The components and packages are batched separately. Keep the components in the packages until use and do not remove them. Mixed use of reagent components from different batches is not recommended.
- Do not use expired products.

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

2.1 List of sequencing set components

Tips To perform SE35 sequencing, please use the MGISEQ-200RS High-throughput Sequencing Set (FCL SE50).

Table 4 MGISEQ-200RS High-throughput Sequencing Set (FCL SE50)Catalog number:940-001629-00

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
MGISEQ-200RS Sequencing Flow Cell Catalog number: 1000020206					
Sequencing Flow Cell	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	12 months
MGISEQ-200RS High-throughput Sequencing Kit (FCL SE50/FCS SE100) Catalog number: 940-001632-00					
Low TE Buffer		100 µL/ tube×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Make DNB Buffer		50 µL/ tube×1 tube			
Make DNB Enzyme Mix I		100 µL/ tube×1 tube			
Make DNB Enzyme Mix II (LC)		13 µL/ tube×1 tube			
Stop DNB Reaction Buffer		50 µL/ tube×1 tube			
DNB Load Buffer I		300 µL/ tube×1 tube			
DNB Load Buffer II		120 µL/ tube×1 tube			

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
Micro Tube 0.5 mL (Empty)		1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
dNTPs Mix III		0.32 mL/ tube×1 tube			
dNTPs Mix II		0.56 mL/ tube×1 tube			
Sequencing Enzyme Mix		0.60 mL/ tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			

Table 5 MGISEQ-200RS High-throughput Sequencing Set (FCL SE100)Catalog number:940-001640-00

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
MGISEQ-200RS Sequencing Flow Cell Catalog number: 1000020206					
Sequencing Flow Cell	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	12 months
MGISEQ-200RS High-throughput Sequencing Kit (FCL SE100) Catalog number: 940-001622-00					
Low TE Buffer		100 μL/ tube×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Make DNB Buffer		50 μL/ tube×1 tube			
Make DNB Enzyme Mix I		100 μL/ tube×1 tube			
Make DNB Enzyme Mix II (LC)		13 μL/ tube×1 tube			

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
Stop DNB Reaction Buffer		50 μL/ tube×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
DNB Load Buffer I		300 μL/ tube×1 tube			
DNB Load Buffer II		120 μL/ tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
dNTPs Mix III		0.44 mL/ tube×1 tube			
dNTPs Mix II		0.76 mL/ tube×1 tube			
Sequencing Enzyme Mix		0.82 mL/ tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			

Table 6 MGISEQ-200RS High-throughput Sequencing Set (FCL PE50) Catalog number:940-001616-00

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
MGISEQ-200RS Sequencing Flow Cell Catalog number: 1000020206					
Sequencing Flow Cell	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	12 months
MGISEQ-200RS High-throughput Sequencing Kit (FCL PE50/FCS PE100) Catalog number: 940-001635-00					
Low TE Buffer		100 μL/ tube×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Make DNB Buffer		50 μL/ tube×1 tube			

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
Make DNB Enzyme Mix I		100 μL/ tube×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Make DNB Enzyme Mix II (LC)		13 μL/ tube×1 tube			
Stop DNB Reaction Buffer		50 μL/ tube×1 tube			
DNB Load Buffer I		300 μL/ tube×1 tube			
DNB Load Buffer II		120 μL/ tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
dNTPs Mix III		0.56 mL/ tube×1 tube			
dNTPs Mix II		0.92 mL/ tube×1 tube			
Sequencing Enzyme Mix		1.02 mL/ tube×1 tube			
MDA Reagent		1.40 mL/ tube×1 tube			
MDA Enzyme Mix II		0.20 mL/ tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			

Table 7 MGISEQ-200RS High-throughput Sequencing Set (FCL PE100)Catalog number:940-001623-00

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
MGISEQ-200RS Sequencing Flow Cell Catalog number: 1000020206					
Sequencing Flow Cell	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	12 months
MGISEQ-200RS High-throughput Sequencing Kit (FCL PE100/FCS PE150) Catalog number: 940-001636-00					
Low TE Buffer		100 μL/ tube×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Make DNB Buffer		50 μL/ tube×1 tube			
Make DNB Enzyme Mix I		100 μL/ tube×1 tube			
Make DNB Enzyme Mix II (LC)		13 μL/ tube×1 tube			
Stop DNB Reaction Buffer		50 μL/ tube×1 tube			
DNB Load Buffer I		300 μL/ tube×1 tube			
DNB Load Buffer II		120 μL/ tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
dNTPs Mix III		0.74 mL/ tube×1 tube			
dNTPs Mix II		1.48 mL/ tube×1 tube			
Sequencing Enzyme Mix		1.48 mL/ tube×1 tube			
MDA Reagent		1.40 mL/ tube×1 tube			

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
MDA Enzyme Mix II		0.20 mL/ tube×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			

Table 8 MGISEQ-200RS High-throughput Sequencing Set (FCL PE150)Catalog number:940-001619-00

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
MGISEQ-200RS Sequencing Flow Cell Catalog number: 1000020206					
Sequencing Flow Cell	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	12 months
MGISEQ-200RS High-throughput Sequencing Kit (FCL PE150) Catalog number: 940-001615-00					
Low TE Buffer		100 μL/ tube×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Make DNB Buffer		50 μL/ tube×1 tube			
Make DNB Enzyme Mix I		100 μL/ tube×1 tube			
Make DNB Enzyme Mix II (LC)		13 μL/ tube×1 tube			
Stop DNB Reaction Buffer		50 μL/ tube×1 tube			
DNB Load Buffer I		300 μL/ tube×1 tube			
DNB Load Buffer II		120 μL/ tube×1 tube			

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
Micro Tube 0.5 mL (Empty)		1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
dNTPs Mix III		0.96 mL/ tube×1 tube			
dNTPs Mix II		1.28 mL/ tube×2 tubes			
Sequencing Enzyme Mix		0.99 mL/ tube×2 tubes			
MDA Reagent		1.40 mL/ tube×1 tube			
MDA Enzyme Mix II		0.20 mL/ tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			

Table 9 MGISEQ-200RS High-throughput Rapid Sequencing Set (FCS SE100)
Catalog number:940-001626-00

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
MGISEQ-200RS Rapid Sequencing Flow Cell Catalog number: 1000020207					
Sequencing Flow Cell	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	12 months
MGISEQ-200RS High-throughput Sequencing Kit (FCL SE50/FCS SE100) Catalog number: 940-001632-00					
Low TE Buffer		100 μL/ tube×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Make DNB Buffer		50 μL/ tube×1 tube			

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
Make DNB Enzyme Mix I		100 μL/ tube×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Make DNB Enzyme Mix II (LC)		13 μL/ tube×1 tube			
Stop DNB Reaction Buffer		50 μL/ tube×1 tube			
DNB Load Buffer I		300 μL/ tube×1 tube			
DNB Load Buffer II		120 μL/ tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
dNTPs Mix III		0.32 mL/ tube×1 tube			
dNTPs Mix II		0.56 mL/ tube×1 tube			
Sequencing Enzyme Mix		0.60 mL/ tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			

Table 10 MGISEQ-200RS High-throughput Rapid Sequencing Set (FCS PE100)
Catalog number:940-001618-00

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
MGISEQ-200RS Rapid Sequencing Flow Cell Catalog number: 1000020207					
Sequencing Flow Cell	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	12 months

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
MGISEQ-200RS High-throughput Sequencing Kit (FCL PE50/FCS PE100) Catalog number: 940-001635-00					
Low TE Buffer		100 μL/ tube×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Make DNB Buffer		50 μL/ tube×1 tube			
Make DNB Enzyme Mix I		100 μL/ tube×1 tube			
Make DNB Enzyme Mix II (LC)		13 μL/ tube×1 tube			
Stop DNB Reaction Buffer		50 μL/ tube×1 tube			
DNB Load Buffer I		300 μL/ tube×1 tube			
DNB Load Buffer II		120 μL/ tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
dNTPs Mix III		0.56 mL/ tube×1 tube			
dNTPs Mix II		0.92 mL/ tube×1 tube			
Sequencing Enzyme Mix		1.02 mL/ tube×1 tube			
MDA Reagent		1.40 mL/ tube×1 tube			
MDA Enzyme Mix II		0.20 mL/ tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			

Table 11 MGISEQ-200RS High-throughput Rapid Sequencing Set (FCS PE150)
Catalog number:940-001627-00

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
MGISEQ-200RS Rapid Sequencing Flow Cell Catalog number: 1000020207					
Sequencing Flow Cell	/	1 EA	2 ℃ to 8 ℃	2 ℃ to 8 ℃	12 months
MGISEQ-200RS High-throughput Sequencing Kit (FCL PE100/FCS PE150) Catalog number: 940-001636-00					
Low TE Buffer		100 μL/ tube×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Make DNB Buffer		50 μL/ tube×1 tube			
Make DNB Enzyme Mix I		100 μL/ tube×1 tube			
Make DNB Enzyme Mix II (LC)		13 μL/ tube×1 tube			
Stop DNB Reaction Buffer		50 μL/ tube×1 tube			
DNB Load Buffer I		300 μL/ tube×1 tube			
DNB Load Buffer II		120 μL/ tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
dNTPs Mix III		0.74 mL/ tube×1 tube			
dNTPs Mix II		1.48 mL/ tube×1 tube			
Sequencing Enzyme Mix		1.48 mL/ tube×1 tube			
MDA Reagent		1.40 mL/ tube×1 tube			

Component	Cap color	Spec & Quantity	Storage temperature	Transportation temperature	Use-by date
MDA Enzyme Mix II		0.20 mL/ tube×1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			

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 12 User-supplied equipment and consumables

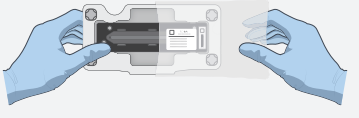
Name	Recommended brand	Catalog number
Ultra-pure water machine	Major Laboratory Supplier (MLS)	/
Freezer, -25 ℃ to -15 ℃	MLS	/
Refrigerator, 2 ℃ to 8 ℃	MLS	/
Graduated cylinder, 500 mL	MLS	
Ice bucket	MLS	/
Pipette, 20 μL	Eppendorf or equivalent	/
Pipette, 200 μL	Eppendorf or equivalent	/
Pipette, 1000 μL	Eppendorf or equivalent	/
Electronic pipette	Intergra or equivalent	/
Vortex mixer	MLS	/
Qubit 4 fluorometer	Thermo Fisher	Q33226
Thermal cycler	Bio-Rad or equivalent	/
Mini spinner	MLS	/
2 M NaOH solution	MLS	/
5 M NaCl solution	MLS	/
Tween-20	MLS	/
Sterile pipette tip(box)	AXYGEN	/
Sterile 200 μL wide-bore, non-filtered pipette tips	AXYGEN	T-205-WB-C
Sterile 200 μL wide-bore, non-filtered pipette tips	MGI	091-000355-00
Qubit ssDNA Assay Kit	Thermo Fisher	Q10212

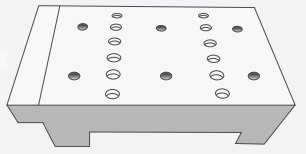
Name	Recommended brand	Catalog number
Qubit assay tube	Thermo Fisher	Q32856
Sterile PCR 8-strip tube, 0.2 mL	AXYGEN	/
Sterile PCR tube, 0.2 mL	AXYGEN	/
Sterile microcentrifuge tube, 1.5 mL	AXYGEN	MCT-150-C
Canned air duster	MATIN	M-6318
Disposable gloves, powder-free	MLS	/
Kimwipes	VWR	/
Low-lint cloth	MLS	/
Laboratory-grade water	MLS	/

Chapter 3 Sequencing workflow

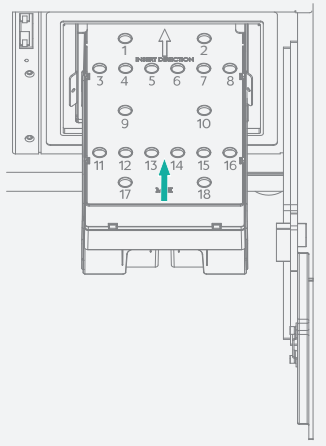
Tips

- Reagents and waste chemicals may cause personal injury through skin, eye, or mucosal contact. Follow the safety standards of your laboratory and wear protective equipment (such as a laboratory coat, protective glasses, mask, gloves, and shoe covers) when using the device.
- If you accidentally splash reagents or waste liquids on the skin or into eyes, immediately flush the affected area with large amounts of water, and then seek medical aid.
- When disposing of expired reagents, waste liquids, waste DNBs, and consumables, comply with local regulations.

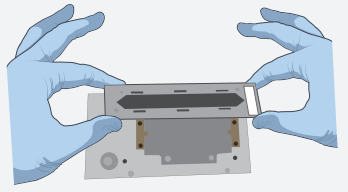
	Preparing DNBs: use DNB preparation kit for making DNBs
	Preparing the new Flow Cell: take out the Flow Cell from package and inspect to ensure that the Flow Cell is intact



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



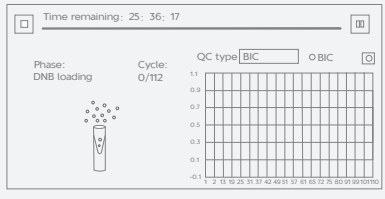
Load the reagent cartridge into the sequencer



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

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

Performing a sequencing run: 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 Preparing DNBs

4.1 Recommended library insert size

The size distribution of inserts ranges between 50 and 500 bp, with the main insert size fragment 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 instructions for use. For general libraries, the ssDNA library concentration should be not less than 2 fmol/μL and each Make DNB reaction requires 40 fmol library.
- If the library concentration is unknown, it is recommended that you perform ssDNA library quantitation (ng/μL) by using Qubit ssDNA Assay Kit and the Qubit Fluorometer. Use the equation below to convert the concentration of the ssDNA library from ng/μL to fmol/μL:

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

  N represents the number of nucleotides (average library length including the adapter) as determined by fragment size analysis. Typically, fragment size analysis is determined during library preparation.
- If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.

4.3 Making DNBs

4.3.1 Preparing reagents for DNB making

Tips
- Mixed use of reagent components from different batches is not recommended.
- Avoid making and loading DNBs by the filtered pipette tips.
- Use the wide-bore, non-filtered pipette tips to make, mix and load DNBs.

Perform the following steps:

1. Place the libraries on ice until use.

2. Remove Low TE Buffer, Make DNB Buffer and Stop DNB Reaction Buffer from storage and thaw reagents at room temperature.
3. Take out Make DNB Enzyme Mix I and thaw the reagent for approximately 30 minutes on ice.
4. After thawing, mix all the reagents thoroughly by using a vortex mixer for 5 seconds. Centrifuge them briefly and place them on ice until use.

4.3.2 Calculating the required amount of ssDNA library

- The required volume of ssDNA libraries is determined by the required library amount (fmol) and library concentration quantified in *4.2 Library concentration and amount requirement on Page 18*.
- The volume of each DNB making reaction is 100 µL and the required library input for each DNB making reaction is calculated as follows:

  Tips
  - If there are any special requirements or specifications for 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.
  - *C* mentioned in the following formula represents the concentration of libraries (fmol/µL).

$$\mathbf{V\ (\mu L) = 40\ fmol / C\ (fmol/\mu L)}$$

- Calculate the required ssDNA library for each DNB making reaction and fill it in *Table 13 on Page 20* as V.

4.3.3 Making DNBs

Perform the following steps:

1. Take out a 0.2 mL PCR 8-strip tube or PCR tubes. Prepare Make DNB reaction mixture 1 according to the table below:

Table 13 Make DNB reaction mixture 1

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

2. Mix Make DNB reaction mixture 1 thoroughly by using a vortex mixer for 5 seconds. Centrifuge it briefly and place it on ice until use.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown in the table below:

Table 14 Primer hybridization reaction conditions

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

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

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

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

Table 15 Make DNB reaction mixture 2

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

7. Add all Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer for 5 seconds. Centrifuge it briefly and place it on ice until use.
8. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

Tips
- When a reaction protocol is ran, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
- It is recommended to set the temperature of the heated lid to 35 ℃ or as close as possible to 35 ℃ .

Table 16 RCR (Rolling Circle Replication) conditions

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

9. 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, 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, or shake the tube.
- Store DNBs at 4 ℃ and perform sequencing within 48 hours.

4.4 Quantifying DNBs

When DNB making is completed, take out 2 μL DNBs, and use Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify the DNBs. For details, refer to *Appendix 1 Instructions for using Qubit to quantify the DNBs on Page 48*

Tips
- Sequencing requires a minimum DNB concentration of 8 ng/μL. If the concentration is lower than 8 ng/μL, refer to *9.1 Low DNB concentration on Page 46*.
- If the concentration exceeds 40 ng/μL, the DNBs should to be diluted to 20 ng/μL with DNB Load Buffer I for loading.
- 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 DNBs at 4 ℃ and perform sequencing within 48 hours.

4.5 Loading DNBs

4.5.1 Preparing reagents

Tips Prepare a fresh DNB loading mix immediately before the sequencing run. It is recommended to prepare the DNB loading mix mentioned above after finishing *4.5 Loading DNBs on Page 22*.

Perform the following steps:

1. Remove DNB Load Buffer I and DNB Load Buffer II from storage and thaw the reagents on ice for approximately 30 minutes.
2. After thawing, mix these reagents thoroughly 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 by using a vortex mixer to re-dissolve the precipitation before use..
3. Take out a 0.5 mL Micro Tube from the sequencing kit and add the following reagents according to the table below:

Table 17 DNB loading mix

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

4. Combine components and mix by gently pipetting 5 to 8 times by using a wide- bore, non-filtered tip. Place the mixture at 4 ℃ until use.
   Tips Do not centrifuge, vortex, or shake the tube.

4.5.2 Loading DNBs

After finishing sequencing cartridge preparing according to *Chapter 6 Preparing the sequencing cartridge on Page 25*, load DNBs referring to *7.2 Loading DNBs on Page 31*.

Chapter 5 Preparing the flow cell

Perform the following steps:

1. Take the flow cell box out of storage and remove the flow cell plastic package from the box.
   Tips Do not open the outer plastic package yet.
2. Place the plastic package at room temperature for 1 hour to 24 hours.
3. Unwrap the outer plastic package before use.

Tips

- If the flow cell is not used within 24 hours after being placed at room temperature and the outer plastic package is intact, the flow cell can be returned to 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 is open but the flow cell cannot be used immediately, store the flow cell at room temperature and use it within 24 hours. If 24 hours is exceeded, it is not recommended to use the flow cell.

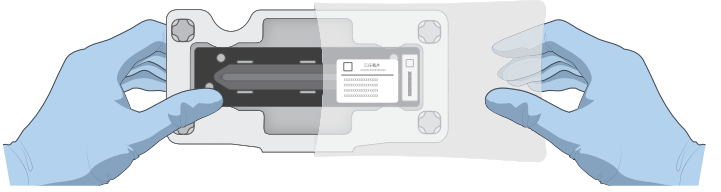


Figure 1 Unwrapping the outer package

4. Take out the flow cell from the inner package and inspect to ensure the flow cell is intact.

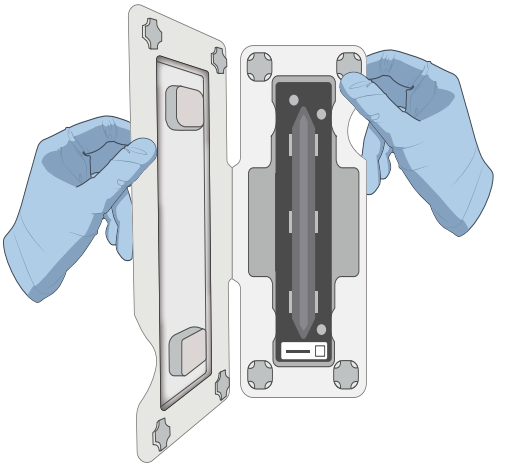


Figure 2 Inspecting the flow cell

Chapter 6 Preparing the sequencing cartridge

Sequencing enzyme and dNTP mixes are provided in different tubes and packaged together with the sequencing reagent cartridge. Before the sequencing run starts, an appropriate amount of sequencing enzyme and dNTP mix needs to be added to well No. 1 and well No. 2 of the sequencing reagent cartridge. Furthermore, MDA Enzyme Mix (MDA, Multiple displacement amplification) needs to be added to well No. 15 if you perform PE sequencing. If prepared reagent cartridges are not used immediately, refer to *9.6 Reagent kit storage on Page 47*

Perform the following steps:

1. Remove the sequencing reagent cartridge from storage and thaw it in a water bath at room temperature until completely thawed (or thaw in a 2 ℃ to 8 ℃ refrigerator 1 day in advance). Store it in a 2 ℃ to 8 ℃ refrigerator until use.
2. Shake the cartridge vigorously in all directions for 10 to 20 times. Ensure that the reagents are fully mixed, especially for reagents in well No.17 and No.18.

   Tips Presence of dark green crystals in well No. 18 is normal due to crystallization of reagent materials in this well. When the cartridge is thawed, mix the reagents in the cartridge thoroughly and the crystals will dissolve. Sequencing quality will not be affected.

3. Remove dNTPs Mix III and dNTPs Mix II from -25 ℃ to -15 ℃ storage 1 hour in advance and thaw at room temperature. Store it at 4 ℃ until use. Mix these reagents thoroughly by using a vortex mixer for 5 seconds and centrifuge them briefly. Place them on ice until use.

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

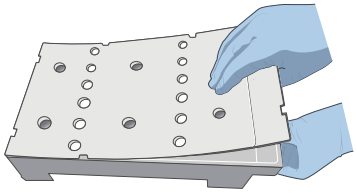


Figure 3 Opening and cleaning the cartridge

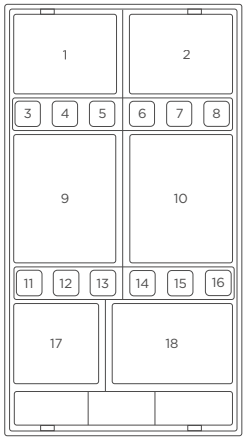


Figure 4 Well position

5. Remove Sequencing Enzyme Mix from -20 °C storage and place it on ice until use. Invert Sequencing Enzyme Mix 4 to 6 times before use.
6. Pierce the seal at the edge of well No.1 and No.2 to make a hole around 1 cm in diameter using 1 mL sterile tip (see the Figure below):

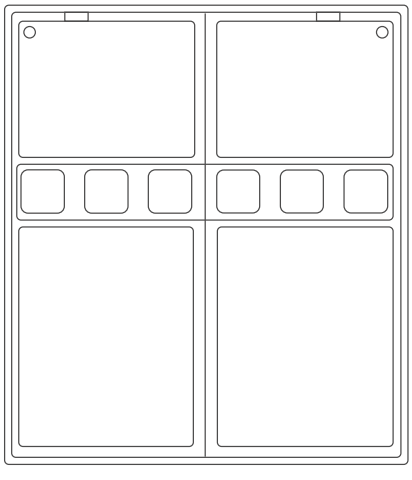


Figure 5 Piercing the seal on the cartridge

7. Take a pipette with the appropriate volume range and add reagents to well No.1 following the table below:

Table 18 dNTPs Mix III loading

Sequencing Kit	Reagent name	Cap color	Loading volume (mL)
FCL SE50/FCS SE100	dNTPs Mix III		0.320
FCL SE100	dNTPs Mix III		0.440
FCL PE50/FCS PE100	dNTPs Mix III		0.560
FCL PE100/FCS PE150	dNTPs Mix III		0.740
FCL PE150	dNTPs Mix III		0.960

8. Take a pipette with the appropriate volume range and add reagents to the well No.2 following the table below:

Table 19 dNTPs Mix II loading

Sequencing Kit	Reagent name	Cap color	Loading volume (mL)
FCL SE50/FCS SE100	dNTPs Mix II		0.560
FCL SE100	dNTPs Mix II		0.760
FCL PE50/FCS PE100	dNTPs Mix II		0.920
FCL PE100/FCS PE150	dNTPs Mix II		1.480
FCL PE150	dNTPs Mix II		2.560

9. Take a pipette with the appropriate volume range and add reagents to well No.1 and No.2 following the table below:

Table 20 Sequencing Enzyme Mix loading

Sequencing Kit	Reagent name	Cap color	Well No.1 loading volume (mL)	Well No.2 loading volume (mL)
FCL SE50/FCS SE100	Sequencing Enzyme Mix		0.320	0.280
FCL SE100	Sequencing Enzyme Mix		0.440	0.380
FCL PE50/FCS PE100	Sequencing Enzyme Mix		0.560	0.460
FCL PE100/FCS PE150	Sequencing Enzyme Mix		0.740	0.740
FCL PE150	Sequencing Enzyme Mix		0.960	1.020

10. Seal the loading wells with the transparent sealing film. Do not cover the center of the well to avoid blocking the sampling needles.

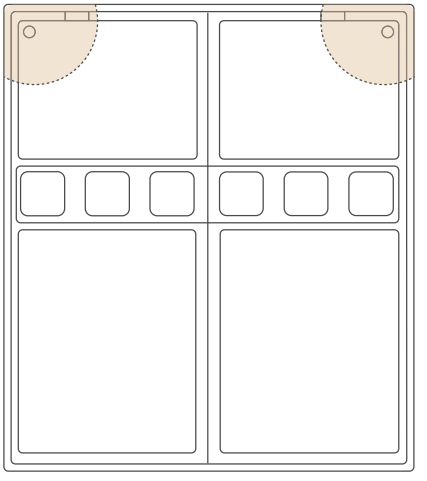


Figure 6 Sealing the loading wells

11. 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.

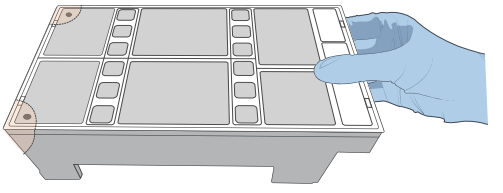


Figure 7 Mixing reagents after loading

12. Gently tap the cartridge on the bench to reduce air bubbles in the reagents.
13. Perform the following steps according to different situations:

    - For PE cartridges

      a. Pierce the seal of well No.15 by using a 1 mL sterile pipette tip.

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

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

      d. Add all the mixture to well No.15. When adding the mixture, ensure that there are no bubbles exist at the bottom of the tube.

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

    - For SE cartridges

      Please skip to the next step.

Chapter 7 Sequencing

7.1 Entering the main interface

Perform the following steps:

1. Enter the user name **user** and password **Password123**, click **Log in** to enter the main interface.

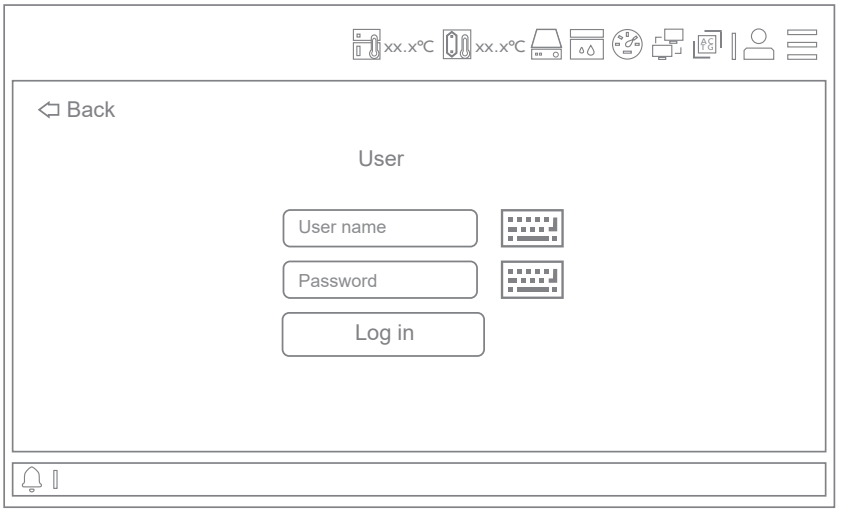


Figure 8 Log-in interface

2. The main interface is as below:

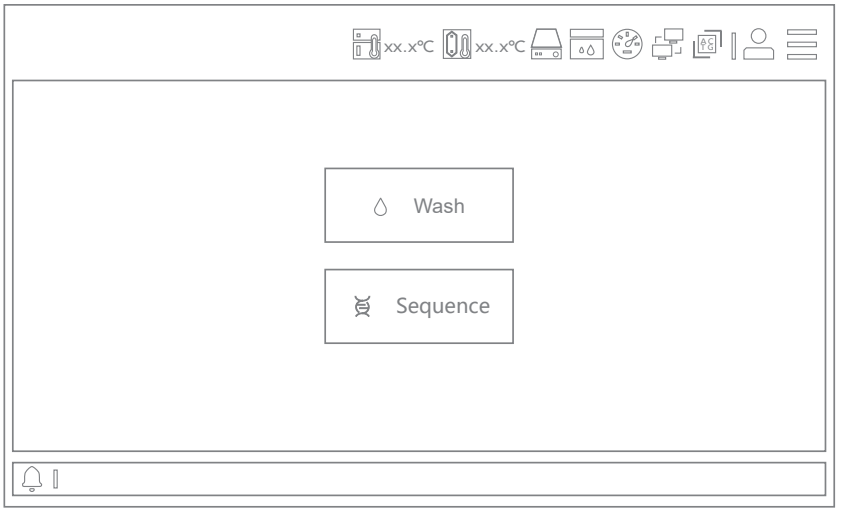


Figure 9 Main interface

7.2 Loading DNBs

Perform the following steps:

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

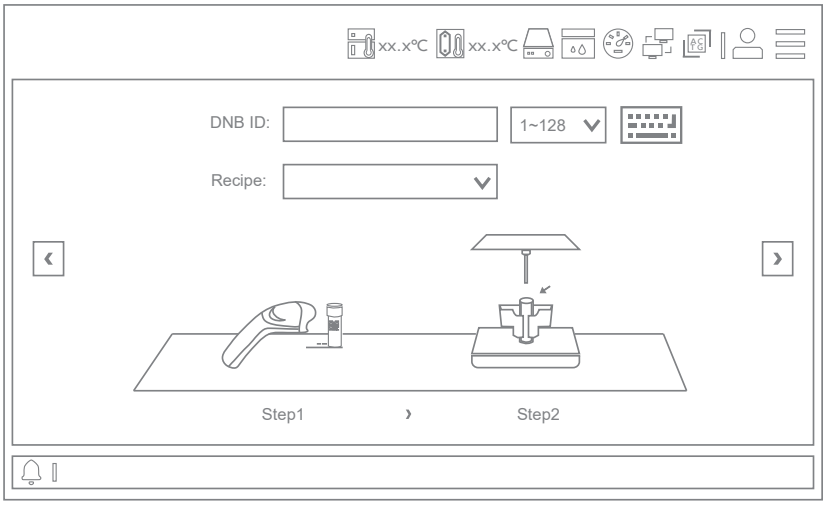


Figure 10 DNB loading interface

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.

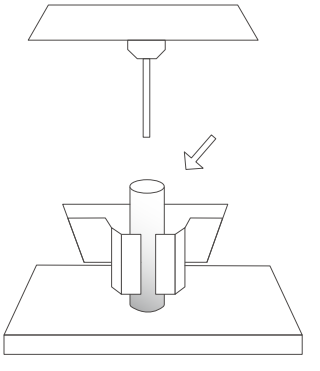


Figure 11 Loading the DNB tube

4. Close the reagent compartment door.

7.3 Selecting the sequencing parameters

Perform the following steps:

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

   Tips
   - Sequencing recipes with **FCL** are for the MGISEQ-200RS High-throughput Sequencing Set (FCL). Sequencing recipes with **FCS** are for the MGISEQ-200RS High-throughput Rapid Sequencing Set (FCS).
   - To perform SE35 sequencing, use the MGISEQ-200RS High-throughput Sequencing Set (FCL SE50), and select recipe **SE35_FCL** or **Customize**.
   - For dual barcode sequencing, select recipe **Customize**.

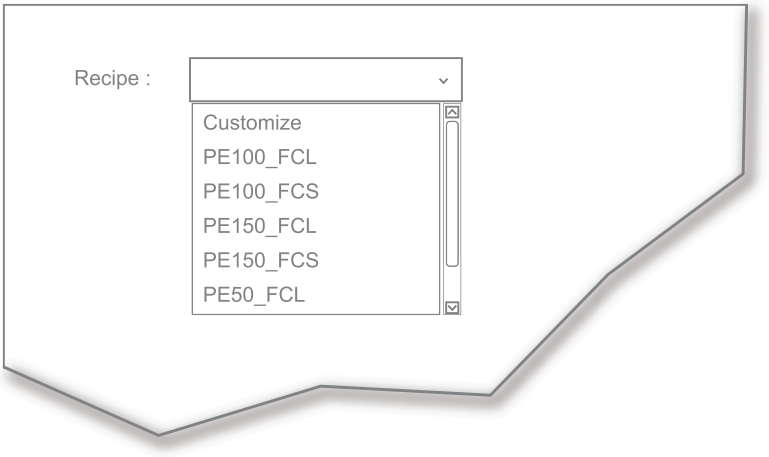


Figure 12 Selecting sequencing solutions

2. If you choose one-click sequencing, go to step *7.4 Loading the reagent cartridge on Page 34*. If you choose **Customize**, continue performing the following steps.
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 the 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.

Tips Dark reaction: only chemical reaction without optical information capture.

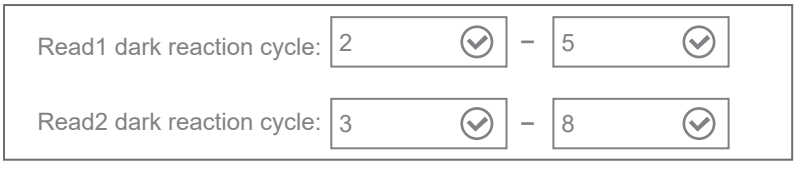


Figure 17 Selecting the dark reaction

8. Click Confirm.

7.4 Loading the reagent cartridge

Perform the following steps:

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

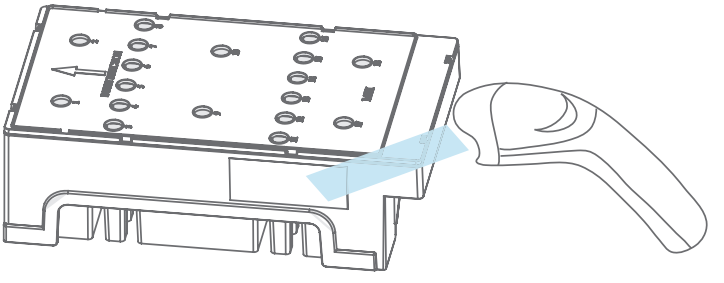


Figure 18 Reagent cartridge information entry interface

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.

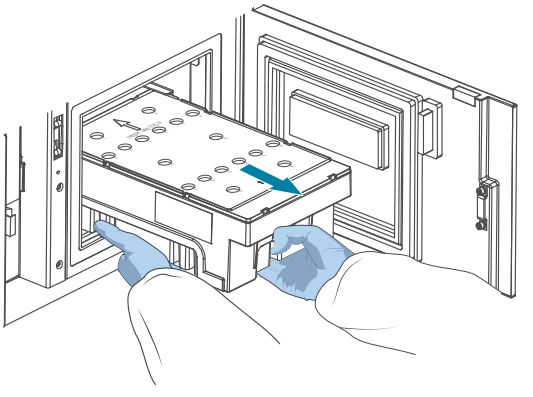


Figure 19 Removing cleaning cartridge

3. Moisten a Kimwipes tissue or a low-lint cloth with laboratory-grade water and use it to wipe the bottom and sides of the compartment to keep it clean and dry.

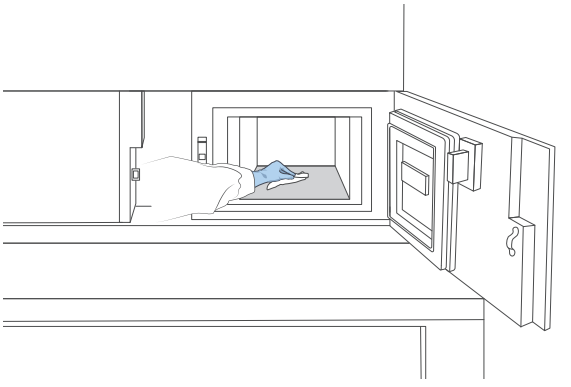


Figure 20 Maintaining the reagent compartment

4. Hold the handle of the reagent cartridge with one hand and place the other hand underneath for support. Slide the new cartridge into the compartment following the direction printed on the cover until it stops.

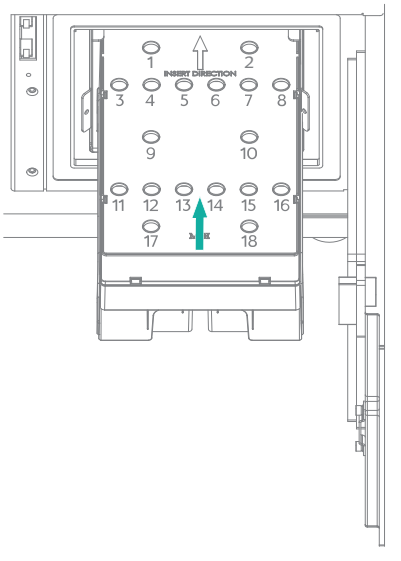


Figure 21 Sliding the new reagent cartridge into the reagent compartment

5. Check that the reagent cartridge is in the correct position and close the reagent compartment door.

7.5 Loading the flow cell

Perform the following steps:

1. Open the flow cell compartment door, press one side of 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.

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

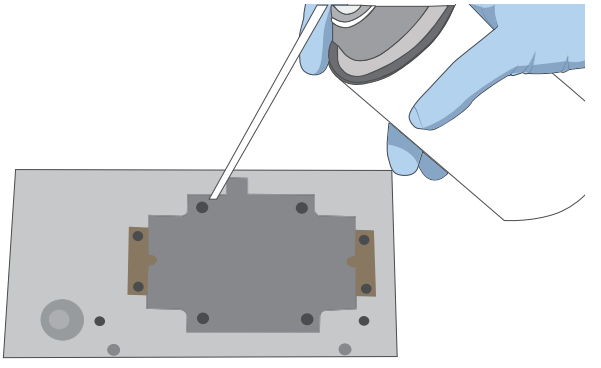


Figure 22 Cleaning the flow cell stage

3. Press the flow cell attachment button.
4. Take out a new 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.

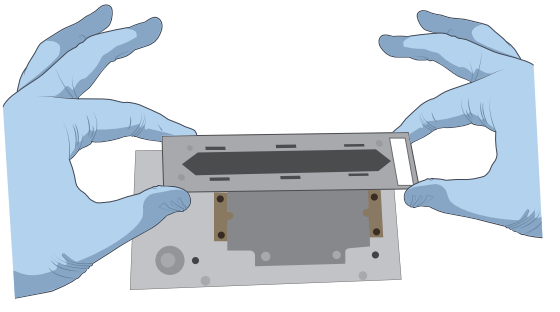


Figure 23 Loading the flow cell

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 that the flow cell is properly seated on the stage.

   Tips 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.

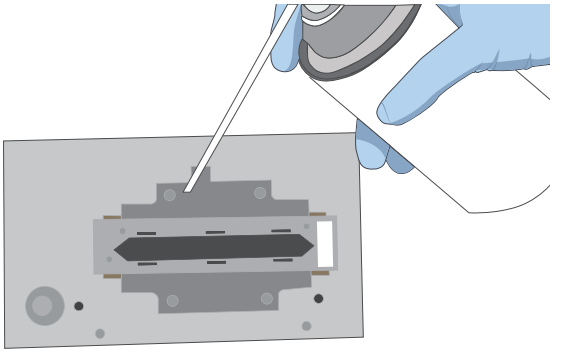


Figure 24 Cleaning the flow cell

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.

   Tips 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.

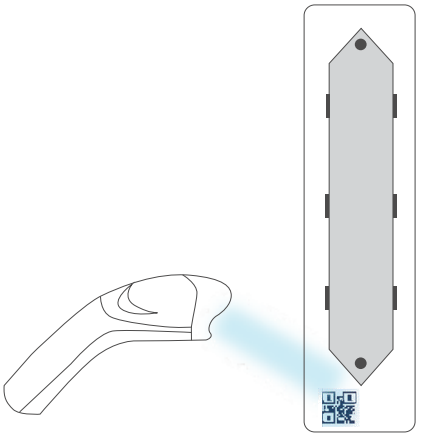


Figure 25 Flow cell information entry interface

7.6 Reviewing parameters

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

Tips 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.

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

7.7 Starting sequencing

Perform the following steps:

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.

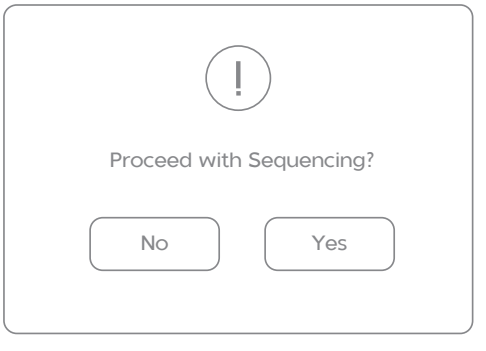


Figure 27 Confirming sequencing interface

3. Once sequencing has started, immediately open the flow cell compartment door to ensure that DNBs (or reagents) are flowing through the flow cell. Then close the flow cell compartment door.

7.8 Data access

Tips For detailed information, please refer to *MGISEQ-200&MGISEQ-200RS Gene Sequencer Software Operation Guide*.

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 Maintaining Device

8.1 Terminology and definition

Table 21 Wash solution

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: 3. Cleaning cartridge 4 4. Cleaning cartridge 3 5. Cleaning cartridge 2	About 45 min
Regular Wash	To remove residual reagents, reducing the risk of cross-contamination. Procedure: 6. Cleaning cartridge 1 7. Air Prime.	About 30 min

8.2 Wash instruction

When the interface below appears, please perform a wash.

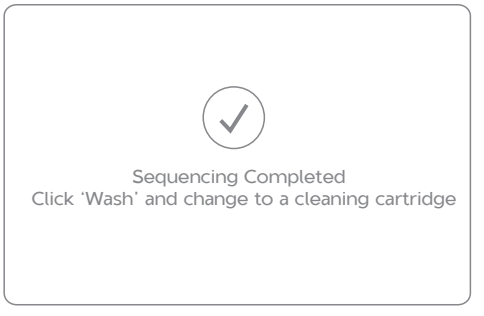


Figure 28 Wash interface

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 o r 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

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

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

Table 22 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 23 Wash reagents 2 preparation

Reagent	Weight/Volume
5 M NaCl solution	200.0 mL
100% Tween-20	0.5 mL
Laboratory-grade water	799.5 mL

- Prepare 0.1 M NaOH following the table below.

Table 24 Wash reagents 3 preparation

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

8.4 Washing cartridge

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

Table 25 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 following steps:

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.

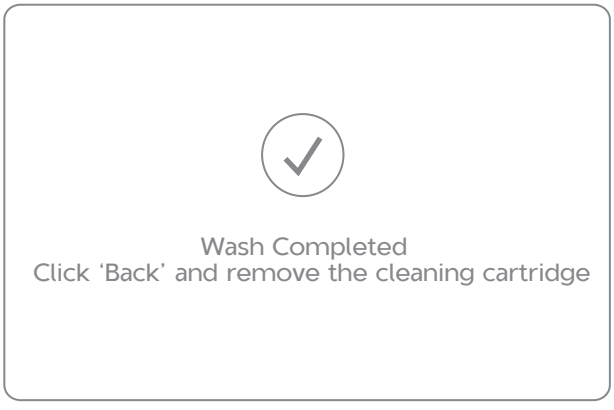


Figure 30 Regular Wash end interface

8.5.2 Maintenance Wash

Perform the following steps:

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 interface appears as figure below, 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 interface appears as figure below, 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 interface appears as Figure 9-6, 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, perform the steps below:

- Check if the DNB preperation kit has expired.
- Check if the libraries meet the requirements.
- Make a new DNB preperation. If the DNB concentration still does not meet the requirements after a new sample preparation, please contact a technical support.

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 that no dust is left.
- Blow the back of the flow cell with a dust remover to ensure that no dust is left.
- If the problem persists, please contact a technical support.

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 technical support.

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 technical support.

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 technical support.

9.6 Reagent kit storage

- If a kit has been thawed (not including dNTPs) and cannot be used within 24 hours, it can be frozen and thawed at most one time.
- If a kit has been thawed (including dNTPs) but cannot be used immediately, store it at 4 ℃ . It is strongly recommended to use it within 24 hours. Mix the reagents in the cartridge following instruction in *Chapter 6 Preparing the sequencing cartridge on Page 25* before use.
- If dNTPs and Sequencing Enzyme Mix have been added into the cartridge, i.e. the cartridge has been prepared but cannot be used immediately, store it at 4 ℃ and use it within 24 hours. Mix the reagents in the cartridge following instruction in *Chapter 6 Preparing the sequencing cartridge on Page 25* before use.
- If dNTPs and Sequencing Enzyme Mix have been added into the cartridge, i.e. the cartridge has been prepared and the sampling needles have started aspiration, but the cartridge cannot be used immediately, 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 Instructions for using Qubit to quantify the DNBs

Tips
- Working solution should be used within 0.5 hours after preparation.
- Avoid touching the wall of tapered detection tubes.
- Avoid introducing bubbles in detection tubes.

Perform the steps below:

1. Prepare the Qubit working solution by diluting the Qubit ssDNA Reagent 1:199 in Qubit ssDNA Buffer. Use a clean Qubit assay tube each time you prepare Qubit working solution. Do not mix the solution in a glass container.

   Tips The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit working solution, and each sample tube requires anywhere from 180 to 199 µL of Qubit working solution.

   Prepare sufficient Qubit working solution to accommodate all standards and samples.

   For example: for 8 samples, prepare enough working solution for the samples and 2 standards. ~200 µL per tube in 10 tubes yields a total of 2 mL of working solution (10 µL of Qubit reagent plus 1990 µL of Qubit Buffer).

2. Add 190 µL of Qubit working solution to each of the tubes used for standards.
3. Add 10 µL of each Qubit standard to the appropriate tube and mix by vortexing 3 to 5 seconds. Be careful not to create bubbles.
4. Set up the required number of 0.5-mL tubes for standards and samples. The Qubit ssDNA Assay requires 2 standards.

   Tips
   - Use only thin-wall, clear, 0.5 mL PCR tubes. Acceptable tubes include Qubit assay tubes (Cat. No. Q32856) or Axygen PCR-05-C tubes (Part No. 10011-830).
   - Number of Qubit test tubes needed are the number of samples plus 2 standards tubes. For example, if you have 3 samples, you will need 5 tubes.

5. Label the tube lids. Do not label the side of tube.
6. Prepare the solutions used for standards and sample tests 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

7. Mix tubes by using a vortex mixer and centrifuge briefly for 5 seconds. Incubate at room temperature for 2 minutes.
8. Proceed instructions in section "Reading standards and samples" of relevant Qubit user guide; follow the procedure appropriate for your instrument.

Appendix 2 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
E-mail	MGI-service@mgi-tech.com
Website	www.mgi-tech.com

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