Complete GENOMICS™

DNBSEQ-G400RS System Guide

For Research Use Only.
Not for use in diagnostic procedures.

Complete Genomics, Inc.

About this guide

CG intends to provide this product solely for research use.

This guide is applicable to Genetic Sequencer (DNBSEQ-G400RS) and DNBSEQ-G400RS High-throughput Sequencing Set. The guide version is 4.0 and the software version is V1.

CG makes no commitment to this guide, including (but not limited to) any special commercial purpose and any reasonable implied warranties. CG has taken measures to ensure the correctness of this guide. However, CG is not responsible for any missing parts of the guide and reserves the right to revise the guide and modify the device, so as to improve the reliability, performance or design.

Figures in this guide are for illustrative purpose only. The content may be slightly different from the device. For the most up-to-date details, refer to the device purchased.

Revision history

	Date	Version
Revision	September 19, 2025	4.0
	July 3, 2024	3.0
	June 1, 2023	2.0
Initial release	February 14, 2023	1.0

Contents

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01

Safety

This chapter describes basic safety information about the device. Carefully read and understand the information before use to ensure correct operations, best performance, and personnel safety. Keep this guide at hand for reference at any time.

Conventions used in this guide

The following table describes conventions that are used in this guide:

Item	Description
shall	Means compliance with a requirement or it is mandatory for compliance with this document
should	Means compliance with a requirement but it is not mandatory for compliance with this document
may	Used to describe possibility or probability
can	Used to describe permission and capability
must	Used to express a constraint
Boldface	Indicates the printings and on-screen characters on the device
Reagent name	Indicates the name of a reagent

General safety

DANGER

- Ensure that the device is operated under the conditions specified in this guide. Not doing so may cause altered experiment results, device malfunction, or even personal injury.
- Ensure that the components of the device are completely installed before operation. Not doing so may result in personal injury.
- A laser is installed in the device. Laser radiation can cause eye injury and skin burns. Before performing a sequencing run, ensure that the flow cell compartment door of the device is closed. Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.
- Maintain the device by following the instructions described in this guide to ensure best performance. Not doing so may result in device malfunction or even personal injury.
- Do not operate the device in the presence of flammable or explosive liquids, vapors, or gases. Doing so may result in device damage or even personal injury.
- Do not operate the device during maintenance or transportation.

WARNING

- Only CG Technical Support or qualified and trained personnel may unpack, install, move, debug and maintain the device. Incorrect operations may cause altered experiment results or damage to the device.
- Do not move the device after CG Technical Support has installed and debugged the device. Unauthorized moves of the device may cause altered experiment results. If the device needs to be moved, contact CG Technical Support.
- Only trained personnel can operate the device.
- Do not disconnect the power cord when the device is on. Doing so may result in device malfunction.
- Only the components provided by the manufacturer can be used for device maintenance. Unapproved components may degrade device performance or result in device malfunction.
- Do not reuse disposable items, except where noted in this guide.
- Do not place tubes or reagent kits on the device. Liquids seeping into the device may damage it.

CAUTION

- Only the peripheral devices and consumables specified by the manufacturer can be used.
- If you have maintenance questions that are not mentioned in this guide, contact CG Technical Support.
- The device has been verified before delivery. If serious deviation occurs during use, contact CG Technical Support for calibration.
- Ensure that you are familiar with the operation of all the laboratory apparatus to be used.
- This sequencing reagent kit is for one sequencing run only and cannot be reused.
- The components and packaging are batched separately. Keep the components in the packaging until use and do not remove them. Mixed use of reagent components from different batches of kits is not recommended.

Electrical safety

DANGER

- Ensure that the device is properly grounded, and that the grounding resistance meets the requirements. Failure to do so may result in altered experiment results, electrical leakage, or even electric shock. If you have concerns about proper device grounding, please contact CG Technical Support.
- Do not remove the device cover and expose the inner components. Doing so may result in electrical shock.

WARNING

Do not use the device in close proximity to sources of strong electromagnetic fields, such as unshielded sources of radiated emissions. Radiated signals may reduce the accuracy of the results.

CAUTION

- Before initial use of the device, assess the electromagnetic environment in which the device will be used. The electromagnetic environment should meet Federal Communications Commission-Part 15 Subpart B. For details, contact CG Technical Support.
- Ensure that the input voltage meets the device requirements.
- Ensure that the voltage of the power outlet in your laboratory or the UPS (if any) meets the voltage requirements before using the device. Failure to do so may damage the electrical components.
- Prepare the laboratory and power supply according to the instructions described in this guide.

Mechanical safety

DANGER

To avoid device damage and personal injury, place the device on a level surface that meets the load-bearing requirements, and ensure that the device cannot be easily moved.

Components safety

WARNING

- Only the software that has been provided by the manufacturer can be installed and used on the device. Other software may interfere with normal device functions, or even cause data loss.
- Do not uninstall the control software by yourself. If any problem occurs during software operation, contact CG Technical Support.
- In case of a blown fuse, replace the fuse with the specified type. For details, contact CG Technical Support.

CAUTION

Ensure that the peripheral devices meet the requirements of IEC/EN 62368-1.

Biological safety

- 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 your skin or into your eyes, immediately flush the affected area with large amounts of water and seek medical aid immediately.
- When disposing of expired reagents, waste liquids, waste samples, and consumables, comply with local regulations.

WARNING

- Use and store the reagents according to this guide. Failure to do so may negatively impact performance.
- Check the expiration date of all reagents before use. Using expired reagents may cause inaccurate results.

Symbols

Packaging

The following table describes symbols on the packaging or on the label of the packaging:

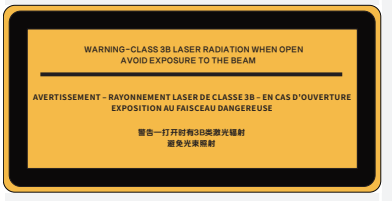
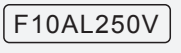
Symbol	Name	Description
	This way up	Indicates the correct upright position of the crated unit for transport and/or storage
	Fragile, handle with care	Indicates a device that can be broken or damaged if not handled carefully
	Keep dry	Indicates a device that needs to be protected from moisture

Symbol	Name	Description
	Do not stack	Indicates that stacking of the crated unit is prohibited and no item shall be placed on top during transport or storage
	Do not roll	Indicates that the crated unit shall not be rolled or turned over. It shall remain in the upright position at all times
	Temperature limit	Indicates the temperature limits to which the device can be safely exposed
	Humidity limitation	Indicates the range of humidity to which the device can be safely exposed
	Atmospheric pressure limitation	Indicates the range of atmospheric pressure to which the device can be safely exposed

Device

The following table describes symbols on the device or reagent kit:

Symbol	Name	Description
	General warning sign	Signifies a general warning
	Warning; biological hazard	Biological hazard warning
	Caution; hot surface	Indicates that the marked item can be hot and should not be touched without taking proper safety precautions
	Warning; dangerous voltage	Indicates hazards arising from dangerous voltages

Symbol	Name	Description
	Protective earth	Indicates the terminal of a protective earth (ground) electrode
	Warning; laser beam	Warns of a hazard from laser beam Class 3B laser
	"ON" (power)	Indicates the main power supply is on
	"OFF" (power)	Indicates the main power supply is off
	Fuse specification	Indicates the fuse specification
	USB 2.0 port	Connects to the USB device
	USB 3.0 port	Connects to the USB device
	Network port	Ethernet connection

Label

The following table describes symbols on the labels of the device or reagent kit:

Symbol	Name	Description
	Model number	Indicates the model number or type number of a product
	Manufacturer	Indicates the name and address of the device manufacturer
	Date of manufacture	Indicates the date when the device was manufactured
	Serial number	Indicates the manufacturer's serial number so that a specific device can be identified

Symbol	Name	Description
	Consult instructions for use	Indicates the need for the user to consult the instructions for use
	Catalog number	Indicates the manufacturer's catalog number (Cat. No.) so that the device can be identified
	Use by date	Indicates the date after which the device is not to be used
	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified
	Keep away from sunlight	Indicates a device that needs protection from light sources
	Do not re-use	Indicates a component or reagent that is intended for a single use only
	Part number	Indicates the part number of an individual box in the reagent set
	Version	Indicates the version of the device or reagent kit
	Caution	Indicates that caution is necessary when operating the device, or that the current situation needs operator awareness or operator action in order to avoid undesirable consequences

System guide

The following table describes symbols that are used in this guide:

Symbol	Description
DANGER	Indicates that the operator should operate the device according to the instructions in this guide. Failure to do so will result in death or serious injury
WARNING	Indicates that the operator should operate the device according to the instructions in this guide. Failure to do so could result in death or serious injury
CAUTION	Indicates that the operator should operate the device according to the instructions in this guide. Failure to do so could result in minor or moderate injury
	Indicates that the operator should pay special attention to the noted information, and operate the device by following the instructions
	Indicates biological risk. The operator should operate the device by following the instructions

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02

Device overview

This chapter describes the intended use, working principle, and structural composition of the device.

Intended use

WARNING **This device is intended only for research use and should not be used for clinical diagnosis.**

This device is a sequencing instrument that measures optical and electronic signals of the reporting molecules, which decode the sequence information of a DNA or RNA fragment. This is accomplished through instrument-specific reagents, flow cells, imaging hardware, and data analysis software. The sequencing input is intended to be prepared as DNA Nanoball (DNB) libraries, which can be used for whole genome, whole exosome, and de novo sequencing.

Working principle

The device adopts the advanced DNA Nanoball (DNB) and the core technology of combinatorial probe-anchor synthesis (cPAS). It uses a regular, arrayed flow cell with special surface sites. Each site contains a single DNB, which is evenly arrayed across the flow cell, ensuring that the optical signals of nearby Nanoballs cannot be interrupted by one another. This improves the accuracy of signal processing.

The following figure demonstrates how to make DNBs:

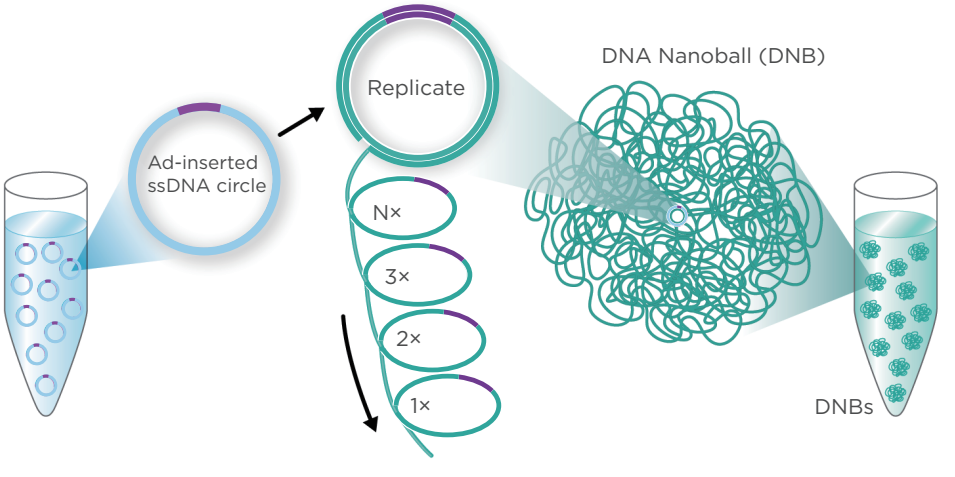

Figure 1 Making DNBs

The following figure demonstrates how to load DNBs:

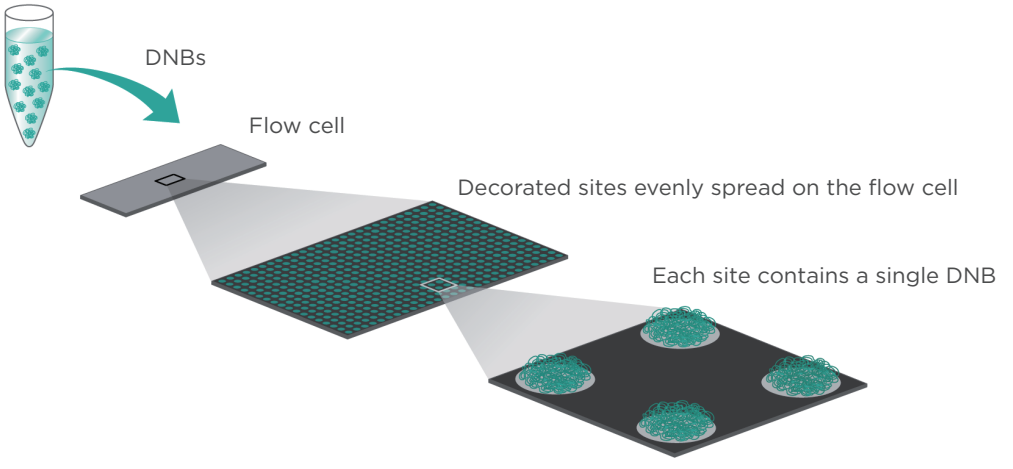

Figure 2 Loading DNBs

The DNBs and sequencing reagents are pumped into the sequencing flow cell through the device's liquid delivery system, where each DNB is combined with the respective fluorescence group. The laser excites the fluorescence group to emit light, and the optical signals are acquired by the camera. The optical signals are converted to digital intensities and processed by the computer to determine the nucleotide sequence of the DNB.

Sequencer overview

Structural composition

The device consists of the main unit and pre-installed control software. The main unit includes the shell, host, optical system, XYZ-stage, flow cell stage, gas-liquid system, electric control system, reagent storage system, power supply system, and display system.

The following table describes the function of each component:

Component	Description
Shell	Provides stable support for the main unit.
Host	Controls the device, and collects, analyzes, and stores data.
Optical system	Images the fluorescence signal on the flow cell.
XYZ-stage	Moves the flow cell and focuses automatically.

Component	Description
Flow cell stage	Connects the flow cell to fluidics lines and controls the temperature of the flow cell.
Gas-liquid system	Provides the gas-liquid support that is required for the biochemical reaction.
Electric control system	Controls the electric system.
Reagent storage system	Provides the reagent storage environment.
Power supply system	Provides power to the device.
Display system	Provides the human-computer interaction interface.

Basic components

Front view

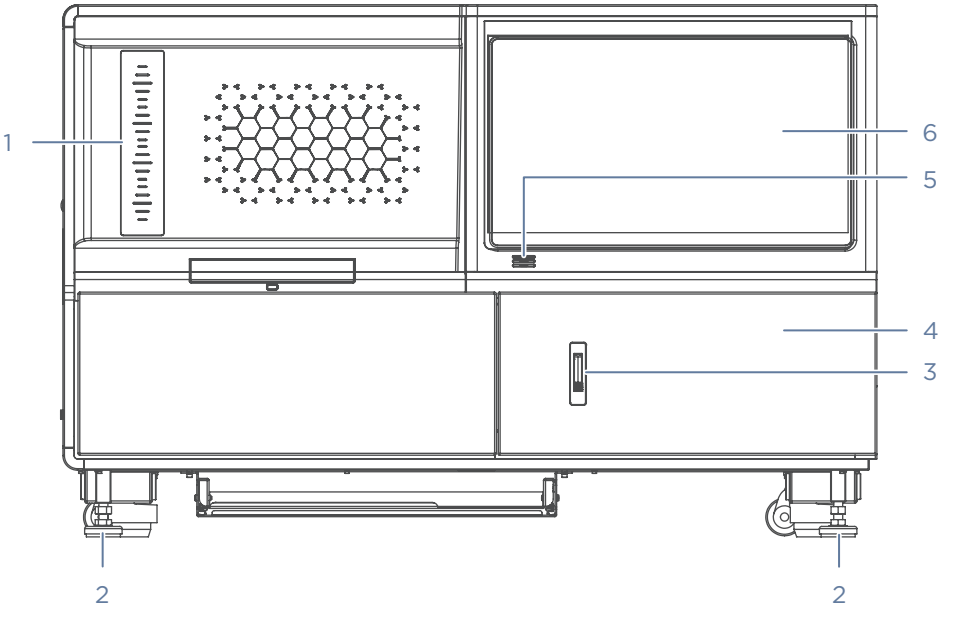

Figure 3 Front view of DNBSEQ-G400RS

No.	Name	Description
1	Status indicator	Displays the current status of the device: • Green: the device is running. • Blue: the device is in standby status. • Red: an error occurred. • Yellow: a warning notification appears.
2	Supporting feet	Supports the main unit to ensure stability.
3	Latch on the reagent compartment	Press the button on the latch, and pull the pop-up ring to open the reagent compartment door.
4	Reagent compartment	Holds the reagent cartridge and tube at appropriate temperatures.
5	Speaker	Alerts when warnings appear, or errors occur.
6	Touch screen monitor	Facilitates on-screen operation and displays information.

Back view

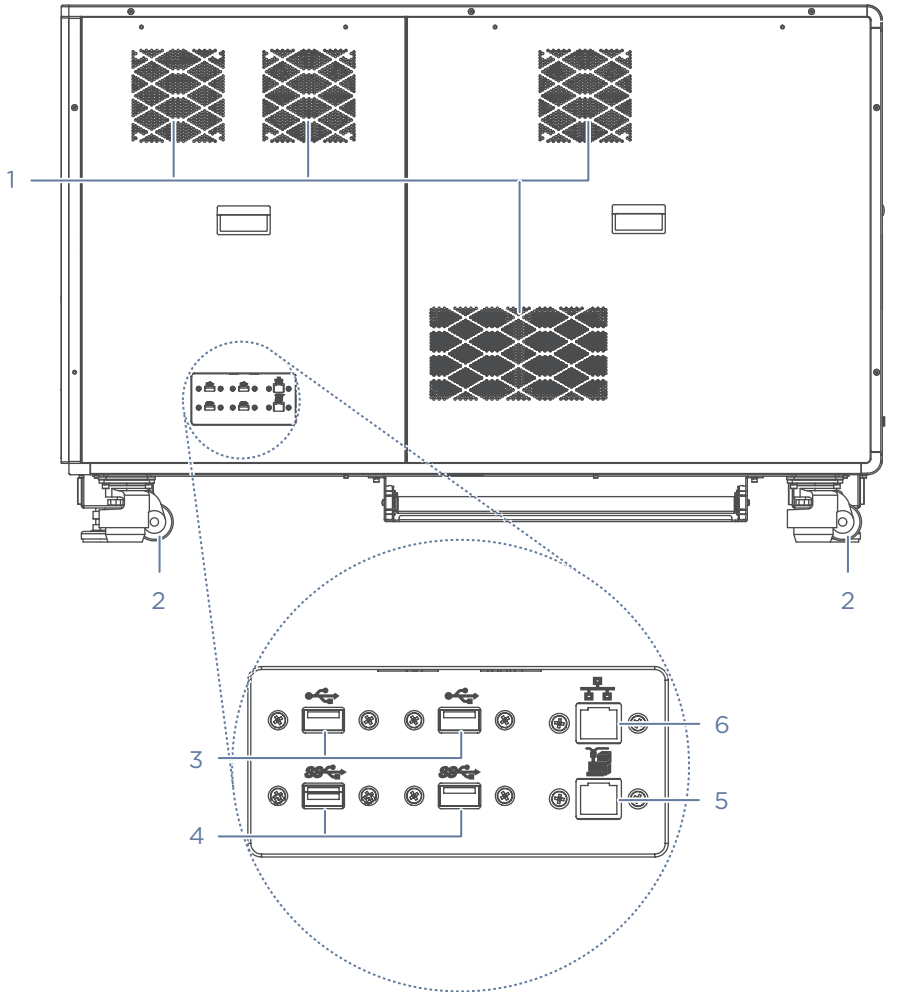

Figure 4 Back view of DNBSEQ-G400RS

It is recommended that the length of the cables that are connected to the following ports (excluding network ports and port unavailable to user) should be less than 3 m (118 inches), and the length of the Ethernet cable should be from 3 m to 30 m (118 inches to 1181 inches).

No.	Name	Description
1	Ventilation outlet	Ventilates the device.
2	Wheel	Used for moving the device.
3	USB 2.0 port	Connects to USB devices such as the keyboard, mouse, and scanner.

No.	Name	Description
4	USB 3.0 port	Connects to USB devices such as the keyboard, mouse, and scanner.
5	Network port	Reserved for future use.
6	Network port	Connects to the network.

Left view

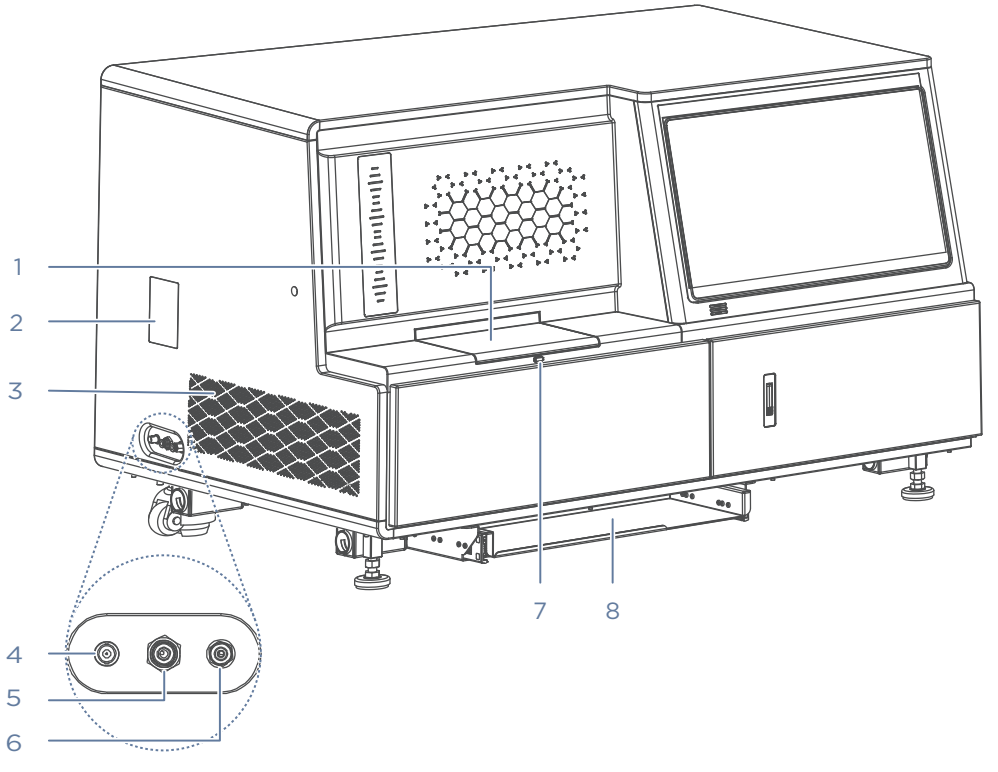

Figure 5 Left view of DNBSEQ-G400RS

No.	Name	Description
1	Flow cell compartment	Holds the flow cells and controls the temperature for biochemical reactions.
2	Window	Allows you to observe the status of the fluidics system and negative pressure gauge.
3	Ventilation inlet	Ventilates the device.
4	Level sensor port	Connects the waste level sensor in the waste container.

No.	Name	Description
5	Condensed water port	Dispenses the condensed water from the cooling system to the waste container through the condenser tube.
6	Waste port	Connects the waste tube to dispense the waste to the waste container.
7	Button on the flow cell compartment door	Press to open the flow cell compartment door.
8	Keyboard drawer	Holds the keyboard and mouse.

Right view

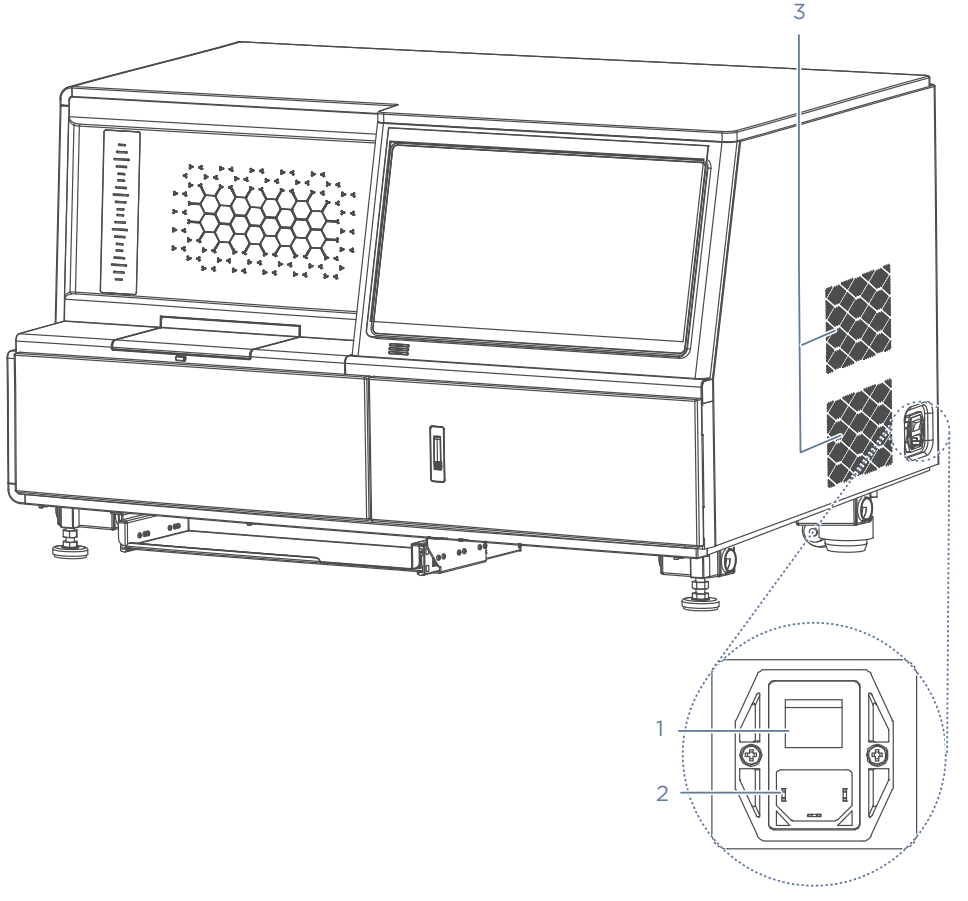

Figure 6 Right view of DNBSEQ-G400RS

It is recommended that the length of the cables that are connected to the following ports (excluding network ports and port unavailable to user) should be less than 3 m (118 inches), and the length of the Ethernet cable should be from 3 m to 30 m (118 inches to 1181 inches).

No.	Name	Description
1	Power switch	Powers the device on or off. • Switch to the | position to power the device on. • Switch to the ◯ position to power the device off.
2	Power port	Connects the power cord. Fuses are installed in the port.
3	Ventilation inlet	Ventilates the device.

Flow cell compartment

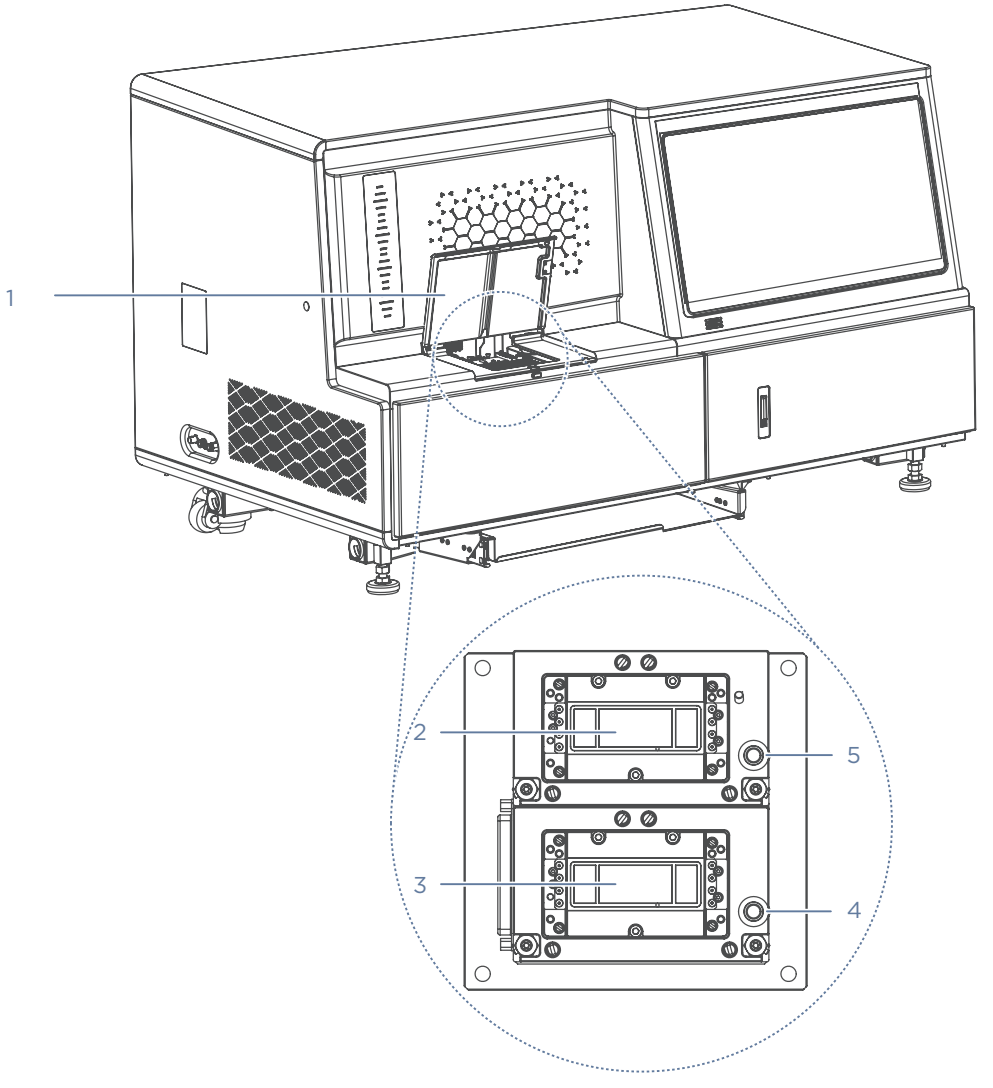

Figure 7 Flow cell compartment of DNBSEQ-G400RS

No.	Name	Description
1	Flow cell compartment door	Press the button of the flow cell compartment door to open the door.
2	Flow cell stage B	Holds the flow cell B and controls the temperature that is required for biochemical reaction.
3	Flow cell stage A	Holds the flow cell A and controls the temperature that is required for biochemical reaction.

No.	Name	Description
4	Flow cell attachment button A	Press to activate the vacuum for attachment or release of the flow cell A.
5	Flow cell attachment button B	Press to activate the vacuum for attachment or release of the flow cell B.

Reagent compartment

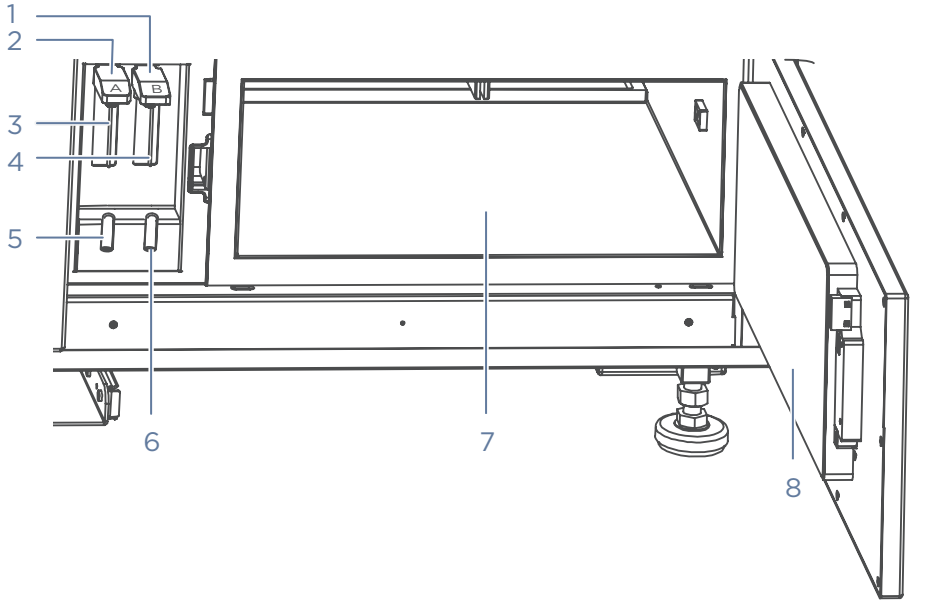

Figure 8 Reagent compartment of DNBSEQ-G400RS

No.	Name	Description
1	Base B	Controls needle B.
2	Base A	Controls needle A.
3	DNB loading needle A	Aspirates DNBs from tube A.
4	DNB loading needle B	Aspirates DNBs from tube B.
5	DNB tube rack A	Holds DNB tube A.
6	DNB tube rack B	Holds DNB tube B.

No.	Name	Description
7	Reagent compartment	Holds the reagent cartridge at appropriate temperatures: • Reagent cartridge A is placed on the left. It provides the required reaction mixture for flow cell A. • Reagent cartridge B is placed on the right. It provides the required reaction mixture for flow cell B.
8	Reagent compartment door	Press the button on the latch of the door, and pull the pop-up ring to open the door.

Control software

Overview

The system control software initiates the communication protocol through physical ports to coordinate with the hardware, control gas lines, fluidics lines, temperature control, mechanical components, and optical components. The software detects the signal on the sequencing flow cell, transfers the photographic information to the base sequence files in standard format, and guides users to perform different processes on the device, such as maintenance and experimental protocols.

The following table describes the function of each functional module:

Item	Description
Self-test	Checks whether the components of the system are functional.
Sequence	Performs different types of sequencing processes.
Wash	Performs wash and maintenance for fluidics lines in the system.
Software running	Monitors the status of the system components.

Main interface

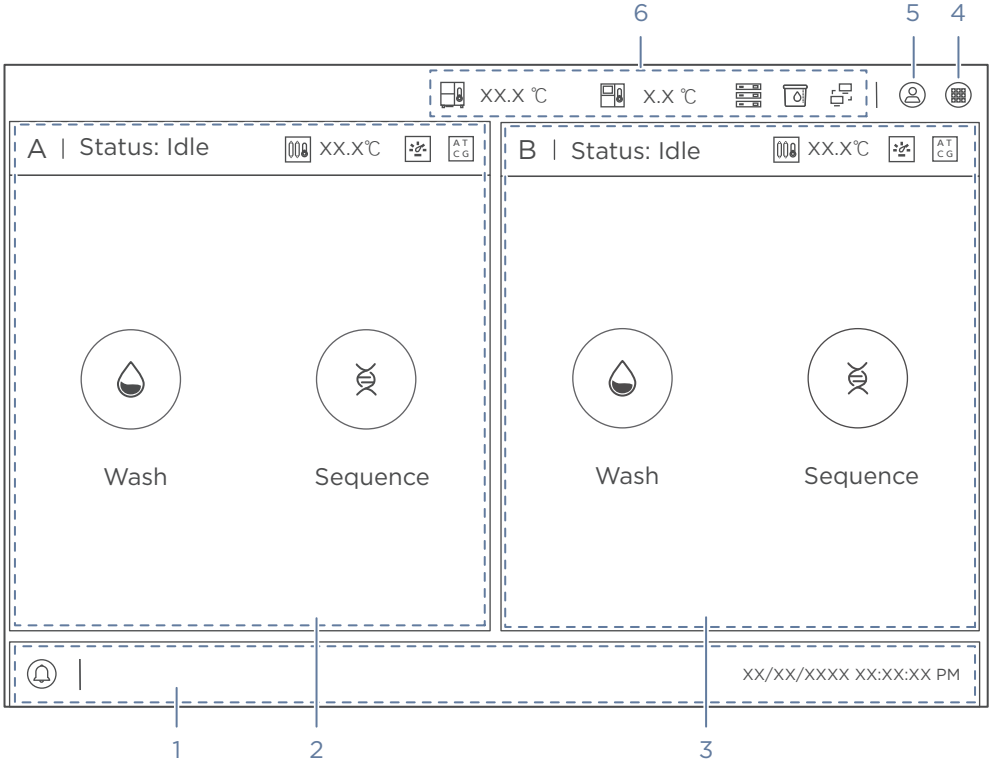

Figure 9 Main interface

The following table describes the function of each area or icon in the main interface:

No.	Name	Description
1	Notification area	Indicates warnings, errors, date, and time.
2	Flow cell A operation area	Indicates the status of flow cell A and provides wash and sequence options.
3	Flow cell B operation area	Indicates the status of flow cell B and provides wash and sequence options.
4		Select to view the function menu. The function menu provides the following functions:
		Select to view the logs.
		Select to change settings.
		Select to perform maintenance.

No.	Name	Description
4		Select to lock the screen.
		Select to shut down the system.
		Select to restart the system.
		Select to check the system information.
5		Select to log in to the system.
6	Status area	Indicates the status of critical components of the device.

Notification area

The following table describes the function of the controls in the notification area:

Item	Description
	The notification icon indicates: • Blue: the device is operating normally. • Flashing yellow: a warning notification appears. • Flashing red: an error occurred. General information, warnings, or error messages are displayed to the right of the icon.

Operation area

The following table describes the function of the controls in the operation area:

Item	Description
A/B	Indicates either flow cell A operation area or flow cell B operation area.
Status	Displays the status of the selected operation area.
	Temperature of the flow cell stage is normal.
	Temperature of the flow cell stage is outside the normal range.
	Negative pressure is normal.
	Negative pressure is outside the normal range.

Item	Description
	Basecalling is connected.
	Errors occurred in the basecalling connection.
	The Basecall software is processing image data. This icon is dynamic.
Wash	Select to set the wash type, and perform the relevant operations by following the on-screen instructions. For details, refer to *Wash on Page 128*.
Sequence	Select to set sequencing parameters, and perform a sequencing run by following the on-screen instructions. For details, refer to *Sequencing on Page 47*.

Status area

The following table describes the function of the controls in the status area:

Item	Description
	Inner temperature of the device is normal.
	Inner temperature of the device is outside the normal range.
	Temperature of the reagent compartment is normal.
	Temperature of the reagent compartment is outside the normal range.
	Sufficient storage drive space.
	Insufficient storage drive space.
	Sufficient space remaining in the waste container.
	Insufficient space remaining in the waste container.
	The system is running independently, and the ZLIMS software server is not connected.
	The system is connected normally to the ZLIMS software server.
	An error has occurred in the connection to the ZLIMS software server.

Log interface

You can view log information in this interface.

Select > to open the log interface.

The following table describes the function of the controls in this interface:

Item	Description
✕	Select to exit the log interface and return to the previous interface.
All	Select to view all logs.
Info	Select to view information logs.
Warning	Select to view warning logs.
Error	Select to view error logs.
⌄	Select the date in the pop-up calendar.
Flow Cell	Select the check box to view the logs for flow cell A or B, or both.
<	Select to return to the previous page of logs.
x/x	Displays the current page and the total number of pages of logs.
>	Select to open the next page of logs.

System settings interface

You can change system settings in this interface.

To open the system settings interface, perform the following steps:

1. Log in to your account. For details, refer to *Logging in to the control software on Page 45.*
2. Select > .

The following table describes the function of the controls in this interface:

Item	Description
✕	Select to exit the system settings interface and return to the previous interface.
Language	Select to change the language of the software. Changes take effect after you restart the system.
Network	Select to enter the IP address and port number of the ZLIMS server.
Upload	Select **Upload enabled** to upload the data to the specified server. ⓘ Data can be uploaded to up to 5 servers simultaneously.
Customize	Select to change the wait time before the screen locks automatically. Move the slider to change the speaker volume.

Upload interface

- When uploading the data to regular servers, fill in the fields accordingly.
- When uploading the data to Amazon Cloud, fill in the fields according to the following table:

Item	Description
Server addr.	Must be aws.
Group	Must be a combination of only lowercase English letters and digits with a length of 3-60 characters.
User name	Access Key ID and Secret access Key provided by CG.
Password	Access Key ID and Secret access Key provided by CG.

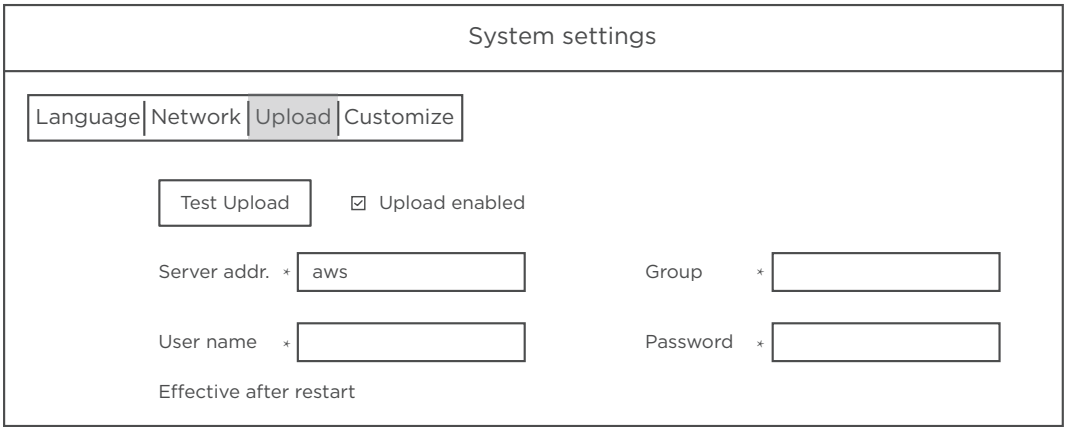

Figure 10 Upload interface

Maintenance interface

You can maintain the device, set scripts and barcodes, and check lifetime statistics of key hardware components in this interface.

To open the system maintenance interface, perform the following steps:

1. Log in to your account.
2. Select > .

The following table describes the functions of the controls in this interface:

Item		Description
✕		Select to exit the system maintenance interface and return to the previous interface.
Device maintenance	**Empty fluidics line**	Select to discharge the residual liquid in all fluidics lines to the waste container. The fluidics line that is being emptied is highlighted.
	Self-test	Select to perform a hardware self-test. When the test is finished, a notification and the results will be displayed on the screen.
	Clear history data	Select to clear all sequencing run history data, except for the data from the most recent run.
	Export data	• Select a data type and export the data to the specified directory of the external storage device. • Select **Uploading data** and upload the data to the specified server.
Script settings	**Import scripts**	Select to import scripts from the external storage server to the device.
	Export scripts	Select to export scripts from the device to the external storage server.
Barcode settings	**Import barcode**	Import the barcode file that is saved in the external storage server or hard drive to the device.
	Export template	Export the template that is saved in the device to the external storage server or hard drive.
User management		Select to add or delete users, modify information, or reset passwords.
DeviceLifecycle		Select to view total used times and total working time of key hardware components.

User account or administrator account can be used to log in to the software and access corresponding permissions.

The user management interface in Maintenance is as follows:

Account type	User name	Password	Permission
User account	user	Password123	Modify user's password
Administrator account	research	Admin123	Add users, delete users, modify user's password, and reset user's password

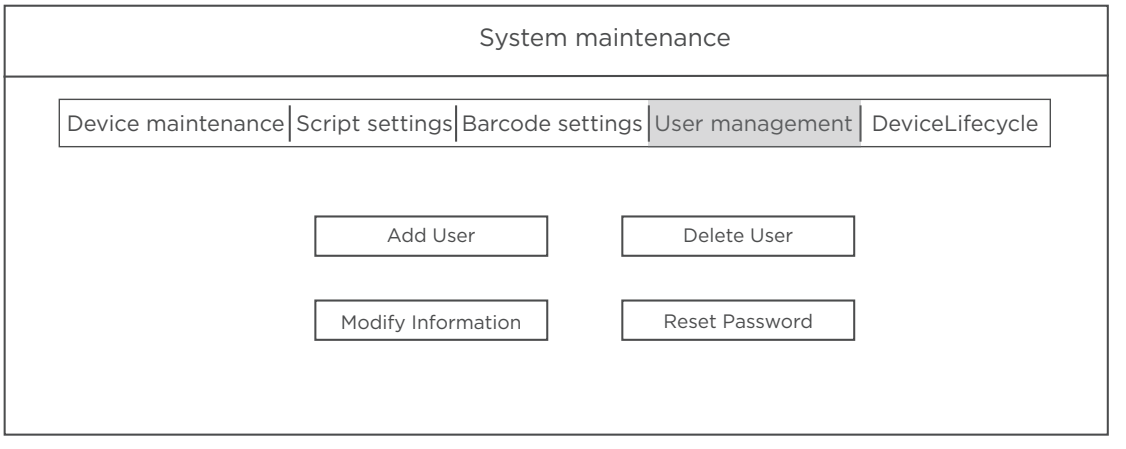

Figure 11 User management interface

- The account will be locked for 5 min after 5 consecutive incorrect login attempts.
- To protect information, it is recommended that you change the password every 90 days. Password expiration (over 90 days) enables account lockout, which requires the administrator account to unlock/reset the password.

Shutdown or restart interface

You can shut down or restart the computer in the interface.

To open the Shutdown or Restart interface, perform one of the following steps:

- Select > , and select **Yes** when you are prompted.
- Select > , and select **Yes** when you are prompted.

About interface

You can view basic device information in this interface, such as the release version, full version of the control software, and serial number.

To open the About interface, select > .

DNB loader overview

Overview

The Portable DNB Loader (DL-200H) is used with the sequencer. Use it to load the prepared DNBs into the sequencing flow cells.

The service life of DL-200H is one year.

Basic component

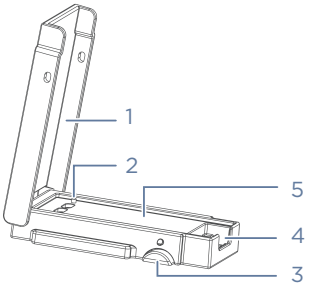

Figure 12 Side view of DL-200H

No.	Name	Description
1	Upper cover	Fastens the flow cell.
2	Alignment pin	Helps align the flow cell with the loader.
3	Latch	Locks the upper cover.
4	Sealing gasket groove	Holds the sealing gasket.
5	Loader body	Loads the flow cell.

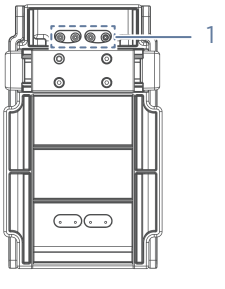

Figure 13 Back view of DL-200H

No.	Name	Description
1	Fluidics inlet	Loads DNBs into the flow cell.

03

Sequencing sets overview

This chapter describes the sequencing sets, data output, sequencing read length, and sequencing run times.

Introduction

This section describes the sequencing sets, data output, sequencing read length, and sequencing run times.

Available sequencing sets

Table 1 Available sequencing sets

Cat. No.	Model	Name	Version	Specification	Data output (Gb/flow cell)
940-000812-00	FCL PE100	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	220 Cycles/ Set	300 to 360
940-000810-00	FCL PE150	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	320 Cycles/ Set	450 to 540
940-000818-00	FCS PE150	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	V1.0	320 Cycles/ Set	Approx 165
940-000917-00	/	DNBSEQ High-throughput Sequencing Primer Kit (App-D) (Paired-End)	V1.0	1 Rxn/Kit	/
940-001750-00	/	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode)	V4.0	4 Rxn/Kit	/
940-001648-00	/	DNBSEQ OneStep Library Conversion Kit (Third party)	V2.0	4 Rxn/Kit	/

- Specifications are based on Complete Genomics *E. coli* control library using Complete Genomics standard library preparation method. Actual performance may vary based on library type, library quality, insert size, and other experimental factors.
- DNBSEQ High-throughput Sequencing Primer Kit (App-D) (Paired-End) is suitable for PE single barcode and PE dual barcode sequencing of CG libraries and third-party libraries with TruSeq and Nextera adapters.
- DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) requires the dual barcode dsDNA libraries with CG adapters.
- DNBSEQ OneStep Library Conversion Kit (Third party) requires dsDNA libraries that include either TruSeq or Nextera adapters.
- PE means Pair-end sequencing.

List of sequencing set components

Table 2 DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE100)
Cat. No.: 940-000812-00

Component	Cap color	Spec. & quantity	Storage temperature	Transportation temperature	Expiration date
DNBSEQ-G400RS Sequencing Flow Cell **Cat. No.: 940-000862-00**					
DNBSEQ-G400 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C	12 months
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL PE100) **Cat. No.: 940-000811-00**					
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
Make DNB Buffer		100 µL/tube×1 tube			
Make DNB Enzyme Mix I		200 µL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		25 µL/tube×1 tube			
Stop DNB Reaction Buffer		100 µL/tube×1 tube			
DNB Load Buffer I		200 µL/tube×1 tube			
DNB Load Buffer II		200 µL/tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
dNTPs Mix		1.80 mL/tube×1 tube			
dNTPs Mix II		1.50 mL/tube×1 tube			
Sequencing Enzyme Mix		3.30 mL/tube×1 tube			
MDA Reagent		3.50 mL/tube×1 tube			
MDA Enzyme Mix II		0.50 mL/tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			

Table 3 DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150)
Cat. No.: 940-000810-00

Component	Cap color	Spec. & quantity	Storage temperature	Transportation temperature	Expiration date
DNBSEQ-G400RS Sequencing Flow Cell **Cat. No.: 940-000861-00**					
DNBSEQ-G400 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C	12 months
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL PE150) **Cat. No.: 940-000809-00**					
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
Make DNB Buffer		100 µL/tube×1 tube			
Make DNB Enzyme Mix I		200 µL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		25 µL/tube×1 tube			
Stop DNB Reaction Buffer		100 µL/tube×1 tube			
DNB Load Buffer I		200 µL/tube×1 tube			
DNB Load Buffer II		200 µL/tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
dNTPs Mix		1.20 mL/tube×2 tubes			
dNTPs Mix II		1.05 mL/tube×2 tubes			
Sequencing Enzyme Mix		4.50 mL/tube×1 tube			
MDA Reagent		3.50 mL/tube×1 tube			
MDA Enzyme Mix II		0.50 mL/tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			

Table 4 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE150)
Cat. No.: 940-000818-00

Component	Cap color	Spec. & quantity	Storage temperature	Transportation temperature	Expiration date
DNBSEQ-G400RS Rapid Sequencing Flow Cell **Cat. No.: 940-000867-00**					
DNBSEQ-G400 FCS Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C	12 months
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE150) **Cat. No.: 940-000817-00**					
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
Make DNB Buffer		100 µL/tube×1 tube			
Make DNB Enzyme Mix I		200 µL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		25 µL/tube×1 tube			
Stop DNB Reaction Buffer		100 µL/tube×1 tube			
DNB Load Buffer I		200 µL/tube×1 tube			
DNB Load Buffer II		200 µL/tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
dNTPs Mix		1.90 mL/tube×1 tube			
dNTPs Mix II		1.90 mL/tube×2 tubes			
Sequencing Enzyme Mix		3.80 mL/tube×1 tube			
MDA Reagent		3.50 mL/tube×1 tube			
MDA Enzyme Mix II		0.50 mL/tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			

Table 5 DNBSEQ-G400RS High-throughput Sequencing Primer Kit (App-D) (Paired-End)
Cat. No.: 940-000917-00

Component	Cap color	Spec. & quantity	Storage temperature	Transportation temperature	Expiration date
App-D Insert Primer 1 Cat. No.: 530-002536-00		2.2 mL/tube ×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
App-D MDA Primer Cat. No.: 530-002540-00		4.2 mL/tube ×1 tube			
App-D Insert Primer 2 Cat. No.: 530-002538-00		4.2 mL/tube ×1 tube			
App-D Barcode Primer 2 Cat. No.: 530-002541-00		3.5 mL/tube ×1 tube			
App-D Barcode Primer 3 Cat. No.: 530-002535-00		3.5 mL/tube ×1 tube			
App Make DNB Buffer Cat. No.: 530-002539-00		400 µL/tube×1 tube			

Table 6 DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode)
Cat. No.: 940-001750-00 Specification: 4 Rxn/Kit

Component	Cap color	Spec. & quantity	Storage temperature	Transportation temperature	Expiration date
Low TE Buffer Cat. No.: 530-003851-00		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
Make DNB Buffer (OS-DB) Cat. No.: 530-003893-00		80 µL/tube×1 tube			
Make DNB Enzyme Mix I (OS) Cat. No.: 530-003852-00		160 µL/tube×1 tube			
Make DNB Enzyme Mix II (OS) Cat. No.: 530-003854-00		8 µL/tube×1 tube			
Stop DNB Reaction Buffer Cat. No.: 530-003849-00		100 µL/tube×1 tube			

Table 7 DNBSEQ OneStep Library Conversion Kit (Third party)
Cat. No.: 940-001648-00 Specification: 4 Rxn/Kit

Component	Cap color	Spec. & quantity	Storage temperature	Transportation temperature	Expiration date
Low TE Buffer Cat. No.:530-003851-00		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
Make DNB Buffer (OS-App) Cat. No.: 530-003853-00		80 µL/tube×1 tube			
Conversion Enzyme Cat. No.: 530-003848-00		5 µL/tube×1 tube			
Make DNB Enzyme Mix I (OS) Cat. No.: 530-003852-00		160 µL/tube×1 tube			
Make DNB Enzyme Mix II (OS) Cat. No.: 530-003854-00		8 µL/tube×1 tube			
Stop DNB Reaction Buffer Cat. No.: 530-003849-00		100 µL/tube×1 tube			

Sequencing read length

Sequencing read length determines the number of sequencing cycles for a given sequencing run. One sequencing cycle equates to one base pair of sequence data. For example, a PE150 cycle run performs reads of 150 cycles (2×150) for a total of 300 cycles or 300 bases sequenced.

Table 8 Sequencing cycle

Sequencing read length	Read1 read length	Read2 read length	Barcode read length	Dual barcode read length	Maximum cycles
PE100	100	100	10	10	222
PE150	150	150	10	10	322

i To ensure sequencing quality, when Read1 and Read2 sequencing is complete, the sequencer will automatically perform one more cycle for correction. For example, for PE100 dual barcode sequencing, Read1 length is 100, Read2 length is 100, barcode read length is 10 and dual barcode read length is 10, plus 1 correction cycle for Read1 and 1 correction cycle for Read2 (barcode does not require correction). The total number of cycles this sequencing is 222.

Sequencing time

Table 9 FCL[a] Sequencing time and analysis time for each read length (h) (ECR 6.0)

Type	Read length	
	PE100	PE150
Single flow cell	35.0	51.0
Dual flow cells	36.0	52.0
Data analysis (Single flow cell)	1.4	1.9
Data analysis (Dual flow cells)	2.7	3.7

Table 10 FCS[b] Sequencing time and analysis time for each read length (h) (ECR 6.0)

Type	Read length
	PE150
Single flow cell	36.4
Dual flow cells	36.6
Data analysis (Single flow cell)	0.7
Data analysis (Dual flow cells)	1.4

Table 11 FCL[a] Sequencing time and analysis time for each read length (h) (ECR 7.0)

Type	Read length	
	PE100	PE150
Single flow cell	33.6	47.0
Dual flow cells	34.6	48.0
Data analysis (Single flow cell)	0.75	1.0
Data analysis (Dual flow cells)	1.35	1.9

Table 12 FCS[b] Sequencing time and analysis time for each read length (h) (ECR 7.0)

Type	Read length
	PE150
Single flow cell	30.4
Dual flow cells	30.5
Data analysis (Single flow cell)	0.4
Data analysis (Dual flow cells)	0.7

- a. "FCL" in the titles of *Table 9 on Page 38* and *Table 11 on Page 38* indicates DNBSEQ-G400 Sequencing Flow Cell, hereinafter referred to as FCL. Each FCL has four lanes, each lane with 600 M DNB binding sites.
- b. "FCS" in the titles of *Table 10 on Page 38* and *Table 12 on Page 39* indicates DNBSEQ-G400 FCS Sequencing Flow Cell, hereinafter referred to as FCS. Each FCS has two lanes, each lane with 400 M DNB binding sites.
- The sequencing time (Single flow cell/Dual flow cells) in the table above includes the time required from Post loading prime to sequencing completion. The data analysis time includes the time required for barcode demultiplexing (if **Split barcode** is selected) and FASTQ files output when sequencing is completed.
- The time in the table above is measured for single barcode.
- The time in the table above is an average value. The actual run time may vary slightly among individual sequencers.

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04

Getting Started

This chapter describes sequencing preparations.

User-supplied equipment and consumables

Before using the device, prepare the following equipment:

Table 13 User-supplied equipment list

Equipment	Recommended brand
Ultra-pure water machine	General lab supplier
Freezer, -25 °C to -15 °C	General lab supplier
Refrigerator, 2 °C to 8 °C	General lab supplier
Graduated cylinder, 500 mL	General lab supplier
Ice bucket	General lab supplier
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	General lab supplier
Qubit Fluorometer	Thermo Fisher
Thermal cycler	Bio-Rad or equivalent
Mini spinner	General lab supplier
Ultrasonic cleaner	General lab supplier

It is recommended that you use the following reagents/consumables:

WARNING Tips are disposable consumables. Do not reuse them.

Table 14 Recommended reagent/consumable list

Reagent/Consumable	Recommended brand	Purpose
2 M NaOH	General lab supplier	Diluting to 0.1 M for washing reagents
5 M NaCl	General lab supplier	Diluting to 1 M for washing reagents
Tween-20	Sigma-Aldrich, Cat. No.: P7949	Performing a maintenance wash, diluting to 0.05% for washing reagents
Sterile pipette tip (various types)	General lab supplier	Pipetting for diluting and loading wash and loading reagents

Reagent/Consumable	Recommended brand	Purpose
Sterile 200 µL wide-bore, non-filtered pipette tip	MGI, Cat. No.: 091-000355-00	Mixing DNBs
Qubit ssDNA Assay Kit	General lab supplier	Library and DNB QC
Qubit Assay Tubes	Thermo Fisher	Library and DNB QC
Sterile PCR 8-strip tube, 0.2 mL	Thermo Fisher	Making DNB reaction mixture
Sterile microcentrifuge tube, 0.5 mL	General lab supplier	Making DNB loading mixture 2
Sterile microcentrifuge tube, 1.5 mL	VWR, Cat. No.: 20170-038, or equivalent	Combining volumes when diluting NaOH and library
Sterile microcentrifuge tube, 2.0 mL	General lab supplier	DNB Loading needle washing tube
Sterile centrifuge tube, 5 mL	General lab supplier	For reagent mix
Sterile centrifuge tube, 10 mL	General lab supplier	For reagent mix
Sterile centrifuge tube, 15 mL	General lab supplier	For reagent mix
Sterile centrifuge tube, 25 mL	General lab supplier	For reagent mix
Canned air duster	General lab supplier	Cleaning
Disposable gloves, powder-free	General lab supplier	General purpose
KimWipes tissues	VWR	Cleaning
Low-lint cloth	General lab supplier	Cleaning
Laboratory-grade water	General lab supplier	/

Preparing the device

Powering the device on

WARNING

- Only the power cord provided by the manufacturer can be used to connect to the power supply, and the power cord can be only used with this device. Failure to do so may damage the power cord or device.
- Ensure that the power switch is in the OFF position before connecting to the power supply.
- Do not switch the account after you log in to the computer. Otherwise, the access rights of the system will be changed, and the device may stop running.

CAUTION

- It is recommended that you change the password after you log in to the computer for the first time.
- For security purpose, it is recommended that you set a long and complex password which should include the uppercase and lowercase letters, numbers, and symbols, and that you change the password every three months.

Perform the following steps:

1. Connect the device to the main power supply.
2. (Optional) If a UPS is prepared, connect the device to the main power supply using the UPS power cord.
3. Turn the power switch to the | position.
4. Log in to the computer with the user name *Zebra* and password *123*. The device starts a self-test.
   - If the self-test succeeds, proceed to the next step.
   - If the self-test fails, perform the following steps:
     a. Select > to check the detailed self-test results.
     b. Follow the on-screen instructions or the solutions in *Sequencer FAQs on Page 144*.
     c. Select > > **Self-test** to initialize and check the device again.
     Or select > .
     If the problem persists, contact CG Technical Support.

Logging in to the control software

You can perform the sequencing and wash procedures only after you log in to the control software.

Perform the following steps:

1. Power the device on.
2. Log in to the computer with the user name *Zebra* and password *123*.
3. Select in the main interface.
4. Log in to the control software with the account information provided below:

Account type	User name	Password
User account	user	Password123
Administrator account	research	Admin123

Please note that the usernames are case sensitive.

Performing pre-run checks

Before each sequencing run, perform the following checks:

- Check whether the remaining storage drive space is greater than 4.6 TB. If the remaining space is insufficient, clear history data according to *Reviewing parameters on Page 85*.
- Check the waste container, and fix any problems prior to sequencing:
  1) If the waste level approaches 80% of the maximum volume of the waste container, empty the waste container.
     For details, refer to *Maintaining the waste container on Page 139*.
  2) If the waste container icon turns to , empty the waste container.
     For details, refer to *Maintaining the waste container on Page 139*.
  3) If the float of the waste level sensor is not properly placed at the lower position of the waste container, clean and move the sensor to the lower position.
  4) If any problem occurs other than those listed above, restart the sequencer control software.
  5) If the problem persists, contact CG Technical Support.
- Check whether the environmental temperature and humidity meet the requirements in *Device specifications on Page 199*. Ensure that the temperature fluctuates within the specified range throughout the sequencing and that the humidity is constant.

Performing a pre-run wash

Before each sequencing run, perform a pre-run wash to flush impurities out and to empty the fluidics line.

For details, refer to *Performing a pre-run wash (~54 min) on Page 133.*

05

Sequencing

This chapter describes the sequencing workflow, sequencing and analysis, and post-sequencing procedures by using the flow cell A operation area as an example. Read and follow the instructions to ensure correct operations.

Workflow

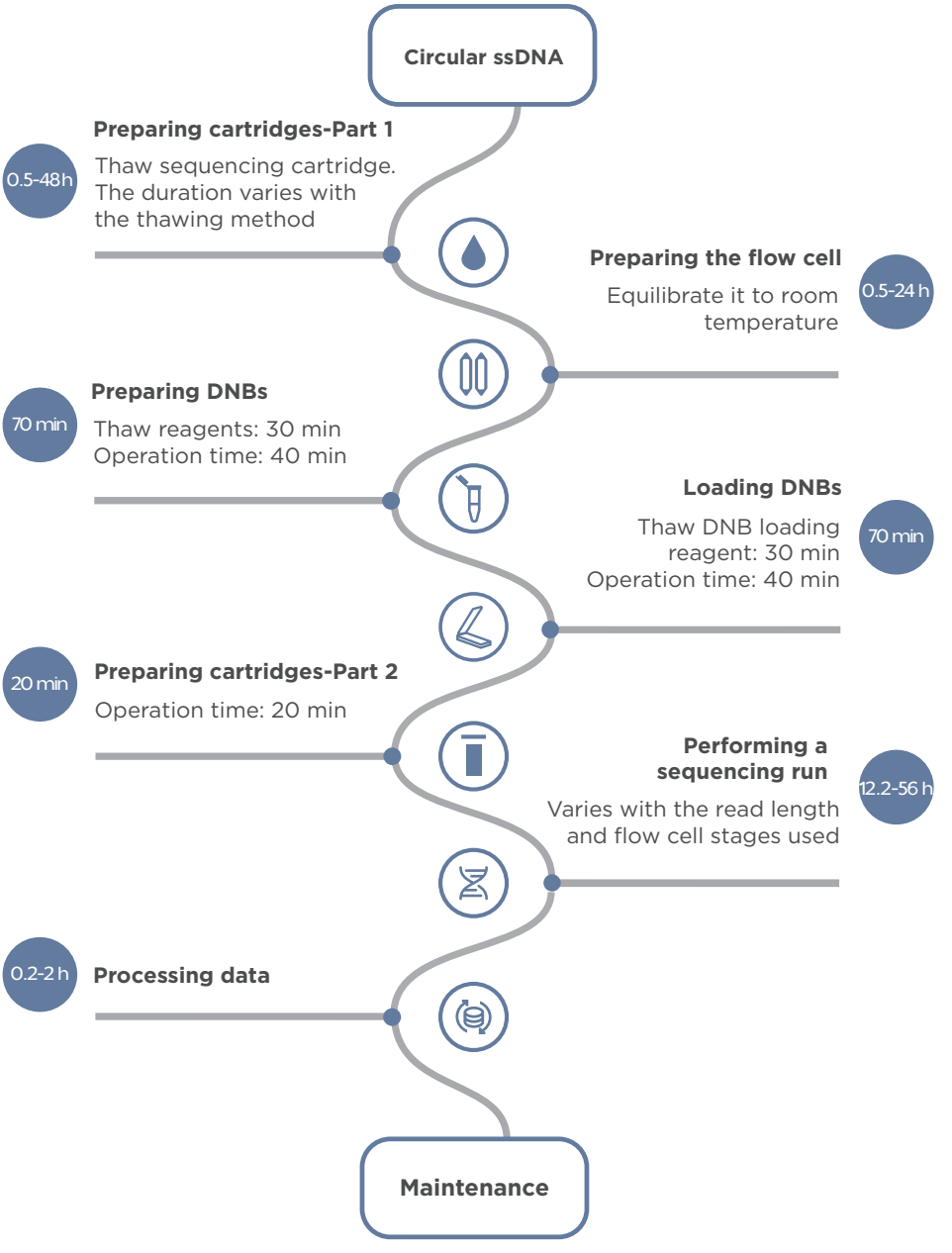


Figure 14 Sequencing workflow

- The manual operation duration shown above is for reference only. The actual duration may vary with your proficiency level.
- If you load DNBs by the sequencer, prepare the sequencing cartridge first according to *Preparing the Sequencing Reagent Cartridge-Part 2 on Page 70,* then proceed to the loading process.

- 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 seek medical aid immediately.
- When disposing of expired reagents, waste liquids, waste DNBs, and consumables, comply with local regulations.

Preparing the Sequencing Reagent Cartridge-Part 1

Perform the following steps:

1. Remove the Sequencing Reagent Cartridge from storage.
2. Thaw in a water bath at room temperature until completely thawed (or thaw in a 2 °C to 8 °C refrigerator 1 to 2 days in advance). The approximate time to thaw is listed in the following table. Store in a 2 °C to 8 °C refrigerator until use.

Table 15 Approximate thaw times for various sequencing kits

Model	Method		
	Water bath at room temperature (h)	Refrigerate at 2 °C to 8 °C overnight, then water bath at room temperature (h)	Refrigerate at 2 °C to 8 °C (h)
FCL PE100	3.0	1.5	36.0
FCL PE150	5.0	2.0	48.0
FCS PE150	3.0	1.5	36.0

Preparing the flow cell

Perform the following steps:

1. Remove the box containing the flow cell from storage and take out the flow cell.

   Do not open the outer plastic packaging yet.

2. Place the flow cell at room temperature for 30 min to 24 h.
3. Unwrap the outer plastic packaging before use.

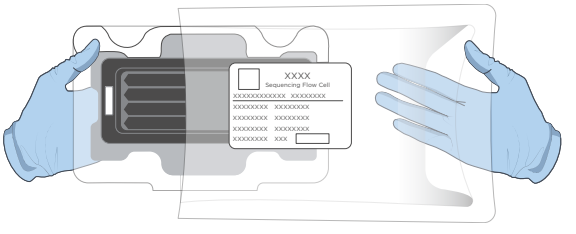


Figure 15 Unwrapping the outer plastic packaging

- If the flow cell is not used within 24 h after being placed at room temperature and the outer plastic packaging is intact, the flow cell can be returned to -25 °C to -15 °C for storage. The number of freeze-thaw events must not exceed three cycles.
- If the outer plastic packaging has been opened but the flow cell cannot be used immediately, store the flow cell at room temperature and use it within 24 h. It is not recommended that you use the flow cell after 24 h.

4. Take the flow cell out of the inner packaging and inspect it to ensure that it is intact and clean, without scratches.

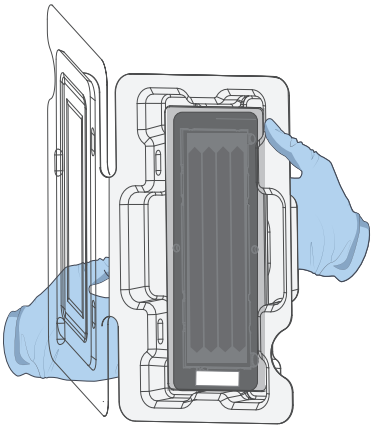


Figure 16 Inspecting the flow cell

Preparing DNBs

Recommended library insert size

The high-throughput sequencing set is compatible with the libraries prepared by CG Library Prep Kits. If third-party library preparation kits are used, contact CG Technical Support for conversion options. The recommended size distribution of inserts ranges between 200 bp and 500 bp, with the main insert size fragment centered within ±100 bp.

- If there are any special requirements or specifications for the CG library preparation kit, then the requirements of the kit should be followed.
- Select sequencing kits according to the insert size and the required data output.
- Average data output will vary with library types and applications.

Table 16 Recommended library insert sizes

Model	Recommended library insert distribution (bp)
FCL PE100	200 to 400
FCL PE150	300 to 500
FCS PE150	300 to 500

DNA library concentration and amount requirements

ssDNA library

- If the library concentration is unknown, it is recommended that you perform library quantitation (ng/μL) by using the 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:

$$C\ (\text{fmol}/\mu\text{L}) = \frac{3030 \times C\ (\text{ng}/\mu\text{L})}{N}$$

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 any special requirements or specifications for the CG library preparation kit, then the requirements of the kit should be followed.

Table 17 Circular ssDNA library concentration requirements

Library type	Library adapter	Library concentration
PCR libraries	CG	≥ 2 fmol / μL
PCR-free libraries		≥ 3.75 fmol / μL
Third-party PCR libraries	TruSeq, Nextera	≥ 3 fmol / μL
Third-party PCR-free libraries		≥ 3.75 fmol / μL

dsDNA library

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

$$C\ (\text{fmol}/\mu\text{L}) = \frac{1515 \times C\ (\text{ng}/\mu\text{L})}{N}$$

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 any special requirements or specifications for the CG library preparation kit, then the requirements of the kit should be followed.
- DNBSEQ OneStep DNB Make Reagent Kit is available.

Table 18 dsDNA library concentration requirements

Library type	Library adapter	Library concentration
PCR libraries	CG	≥ 6.0 fmol / μL
PCR-free libraries		≥ 3.5 fmol / μL
Third-party PCR libraries without 5′-Phosphorylation	TruSeq, Nextera	≥ 10.0 fmol / μL
Third-party PCR-free libraries without 5′-Phosphorylation		≥ 7.5 fmol / μL
Third-party PCR libraries with 5′-Phosphorylation		≥ 7.5 fmol / μL
Third-party PCR-free libraries with 5′-Phosphorylation		≥ 6.0 fmol / μL

Making DNBs for ssDNA libraries

- App Make DNB Buffer can be used to make DNBs for both CG and third-party libraries.
- Mixed use of reagent components from different batches is not recommended.
- For transferring or mixing DNBs, use the wide-bore pipette tips.
- For other reagents, use a proper pipette tip according to the actual situation. It is recommended that you use the pipette tips from recommended brands and catalog numbers.

Select the appropriate protocol for the sequencing kit you choose to use, and follow the protocol carefully.

Making DNBs

Preparing reagents for making 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 the reagents at room temperature.

   For sequencing of third-party libraries, remove App Make DNB Buffer from the DNBSEQ High-throughput Sequencing Primer Kit (App-D) packaging.
3. Remove Make DNB Enzyme Mix I from storage, and thaw the reagent for approximately 30 min on ice.
4. Mix the reagents by using a vortex mixer for 5 s. Centrifuge briefly and place on ice until use.

Calculating the number of DNB reactions

- Using the sequencer to load DNBs

  All lanes in the flow cell must be loaded with the same DNBs.
- Using DL-200H to load DNBs

  Different DNBs can be loaded into different lanes.

Table 19 Minimum number of required Make DNB reactions for each flow cell

Flow Cell type	Loading system	Minimum number of required Make DNB reactions / flow cell	Make DNB reaction (µL)	DNB volume (µL) / lane
FCL	Sequencer	2	100	50
	DL-200H	2	50	25

Flow Cell type	Loading system	Minimum number of required Make DNB reactions / flow cell	Make DNB reaction (µL)	DNB volume (µL) / lane
FCS	Sequencer	1	100	50
	DL-200H	1	50	25

The specific number of Make DNB reactions depends on the number of samples required by the customer.

Calculating the required amount of ssDNA libraries

The required volume of ssDNA libraries is determined by the required library amount (fmol) and library concentration quantified in *DNA library concentration and amount requirements on Page 51*.

- If there are any special requirements or specifications for the CG 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 and local regulations.
- *C* in the following table represents the concentration of libraries (fmol/µL).

Table 20 Required amount of ssDNA libraries

Library type	Library adapter	Library concentration	
		100 µL DNB reaction	50 µL DNB reaction
PCR libraries	CG	V = 40 fmol / C	V = 20 fmol / C
PCR-free libraries		V = 75 fmol / C	V = 37.5 fmol / C
Third-party PCR libraries	TruSeq, Nextera	V = 60 fmol / C	V = 30 fmol / C
Third-party PCR-free libraries		V = 75 fmol / C	V = 37.5 fmol / C

Calculate the required volume of ssDNA libraries for each Make DNB reaction and fill it in the following tables as *V*.

Making DNBs

Perform the following steps:

1. Take out a 0.2 mL 8-strip tube or PCR tubes. Prepare Make DNB reaction mixture 1 according to different ssDNA libraries.

Table 21 Make DNB reaction mixture 1 for CG libraries

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

Table 22 Make DNB reaction mixture 1 for third-party libraries

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

2. Mix the reaction mixture thoroughly by using a vortex mixer. Centrifuge for 5 s by using a mini spinner, and place it on ice until use.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. The thermal cycler settings are shown in the table below:

Table 23 Primer hybridization reaction conditions

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

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

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

5. Remove the PCR tube from the thermal cycler when the temperature has reached 4 °C.
6. Centrifuge briefly for 5 s, place the tube on ice, and prepare Make DNB reaction mixture 2 according to the table below:

Table 24 Make DNB reaction mixture 2

Component	Cap color	Volume of 100 µL DNB reaction (µL)	Volume of 50 µL DNB reaction (µL)
Make DNB Enzyme Mix I		40	20
Make DNB Enzyme Mix II (LC)		4	2
Total volume		44	22

7. Add all of Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer, and centrifuge for 5 s by using a mini spinner.
8. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below.

- When a reaction protocol is running, 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 that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 25 RCA (Rolling Circle Amplification) conditions

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

9. Immediately add Stop DNB Reaction Buffer when the temperature reaches 4 °C. The volume of Stop DNB Reaction Buffer is shown in the table below. Mix gently by pipetting 8 times by using a wide-bore pipette tip.

Table 26 Volume of Stop DNB Reaction Buffer

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

- It is very important to mix DNBs gently by using a wide-bore pipette tip. Do not centrifuge, vortex, or shake the tube.
- Store DNBs at 2 °C to 8 °C, and perform sequencing within 48 h.

10. Quantify the DNBs. For details, refer to *Quantifying DNBs on Page 63.*

Making DNBs for dsDNA libraries

- App Make DNB Buffer can be used to make DNBs for both CG and third-party libraries.
- Mixed use of reagent components from different batches is not recommended.
- For transferring or mixing DNBs, use the wide-bore pipette tips.
- For other reagents, use a proper pipette tip according to the actual situation. It is recommended that you use the pipette tips from recommended brands and catalog numbers.

Select the appropriate protocol for the sequencing kit you choose to use, and follow the protocol carefully.

Making DNBs

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. Remove the reagents according to the following conditions:
   - For CG dual barcode libraries, remove Low TE Buffer, Make DNB Buffer (OS-DB), and Stop DNB Reaction Buffer from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) packaging, and thaw the reagents at room temperature.
   - For third-party libraries, remove Low TE Buffer, App Make DNB Buffer, and Stop DNB Reaction Buffer from the DNBSEQ OneStep Library Conversion Kit (Third party) packaging, and thaw the reagents at room temperature.

3. Remove Make DNB Enzyme Mix I (OS) from storage and thaw the reagents for approximately 30 min on ice.
4. Mix the reagents by using a vortex mixer for 5 s. Centrifuge briefly and place on ice until use.

Calculating the number of DNB reactions

- Using the sequencer to load DNBs

  All lanes in the flow cell must be loaded with the same DNBs.

- Using DL-200H to load DNBs

  Different DNBs can be loaded into different lanes.

Table 27 Minimum number of required Make DNB reactions for each flow cell

Flow cell type	Loading system	Minimum number of required Make DNB reactions/flow cell	Make DNB reaction (µL)	DNB volume (µL)/lane
FCL	Sequencer	2	100	50
	DL-200H	1	100	25
FCS	Sequencer	1	100	50
	DL-200H	1	100	25

The specific number of Make DNB reactions depends on the number of samples required by the customer.

Calculating the required amount of dsDNA libraries

The required volume of dsDNA libraries is determined by the required library amount (fmol) and library concentration quantified in *DNA library concentration and amount requirements on Page 51*.

- If there are any special requirements or specifications for the CG 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 and local regulations.
- *C* in the following table represents the concentration of libraries (fmol/µL).

Table 28 Required amount of dsDNA libraries

Library type	Library adapter	Library concentration
		100 µL DNB reaction
PCR libraries	CG	V = 120 fmol / C
PCR-free libraries		V = 70 fmol / C
Third-party PCR libraries without 5′-Phosphorylation	TruSeq, Nextera	V = 200 fmol / C
Third-party PCR-free libraries without 5′-Phosphorylation		V = 150 fmol / C
Third-party PCR libraries with 5′-Phosphorylation		V = 150 fmol / C
Third-party PCR-free libraries with 5′-Phosphorylation		V = 120 fmol / C

Calculate the required volume of dsDNA libraries for each Make DNB reaction and fill it in the following tables as V.

Making DNBs (CG libraries)

1. Take out a 0.2 mL 8-strip tube or PCR tubes. Prepare Make DNB reaction mixture 1 according to different dsDNA libraries.

Table 29 Make DNB reaction mixture 1 for CG dual barcode libraries

Component	Cap color	Volume of 100 µL DNB reaction (µL)
Low TE Buffer		20 – V
Make DNB Buffer (OS-DB)		20
dsDNA libraries	/	V
Total volume		40

2. Mix the reaction mixture thoroughly by using a vortex mixer. Centrifuge for 5 s by using a mini spinner, and place it on ice until use.

3. Place the mixture into a thermal cycler and start the primer hybridization reaction. The thermal cycler settings are shown in the table below:

Table 30 Primer hybridization reaction conditions

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

4. Remove Make DNB Enzyme Mix II (OS) from storage and place it on ice. Centrifuge briefly for 5 s by using a mini spinner, and hold on ice.

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

5. Remove the PCR tube from the thermal cycler when the temperature has reached 4 °C.
6. Centrifuge briefly for 5 s by using a mini spinner, place the tube on ice, and prepare the Make DNB reaction mixture 2 according to the table below:

Table 31 Make DNB reaction mixture 2

Component	Cap color	Volume of 100 µL DNB reaction (µL)
Make DNB Enzyme Mix I (OS)		40
Make DNB Enzyme Mix II (OS)		2
Total volume		42

7. Add all of Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer, centrifuge for 5 s by using a mini spinner, 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.

- When a reaction protocol is running, 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 that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 32 RCA conditions

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

9. Immediately add 20 µL of Stop DNB Reaction Buffer when the temperature reaches 4 °C. Mix gently by pipetting 8 times by using a wide-bore pipette tip.

   - It is very important to mix DNBs gently by using a wide-bore pipette tip. Do not centrifuge, vortex, or shake the tube.
   - Store DNBs at 2 °C to 8 °C, and perform sequencing within 48 h.

Table 33 Volume of Stop DNB Reaction Buffer

Component	Cap color	Volume of 100 µL DNB reaction (µL)
Stop DNB Reaction Buffer		20

10. Quantify the DNBs. For details, refer to *Quantifying DNBs on Page 63.*

Making DNBs (third-party libraries)

Perform the following steps:

1. Remove the Conversion Enzyme from DNBSEQ OneStep Library Conversion Kit (Third party). Centrifuge briefly for 5 sec, and place it on ice until use.
2. Take out a 0.2 mL 8-strip tube or PCR tubes. Prepare Make DNB reaction mixture 1 according to the table below:

Table 34 Make DNB reaction mixture 1 for third-party libraries

Component	Cap color	Volume of 100 µL DNB reaction (µL)
Low TE Buffer		20 - V
Make DNB Buffer (OS-App)		20
dsDNA libraries	/	V
Conversion Enzyme		0.5
Total volume		40.5

3. Mix the reaction mixture thoroughly by using a vortex mixer. Centrifuge for 5 s by using a mini spinner, and place it on ice until use.

4. Place the mixture into a thermal cycler and start the primer hybridization reaction. The thermal cycler settings are shown in the table below:

Table 35 Primer hybridization reaction conditions

Temperature	Time
Heated lid (105 °C)	On
37 °C	5 min
95 °C	3 min
40 °C	3 min
4 °C	Hold

5. Remove Make DNB Enzyme Mix II (OS) from storage and place it on ice. Centrifuge briefly for 5 s by using a mini spinner, and hold on ice.

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

6. Remove the PCR tube from the thermal cycler when the temperature has reached 4 °C.
7. Centrifuge briefly for 5 s by using a mini spinner, place the tube on ice, and prepare the Make DNB reaction mixture 2 according to the table below:

Table 36 Make DNB reaction mixture 2

Component	Cap color	Volume of 100 µL DNB reaction (µL)
Make DNB Enzyme Mix I (OS)		40
Make DNB Enzyme Mix II (OS)		2
Total volume		42

8. Add all of Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer, and centrifuge for 5 s by using a mini spinner.
9. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below.

   - When a reaction protocol is running, 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 that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 37 RCA conditions

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

10. Immediately add 20 µL of Stop DNB Reaction Buffer when the temperature reaches 4 °C. Mix gently by pipetting 8 times by using a wide-bore pipette tip.

    - It is very important to mix DNBs gently by using a wide-bore pipette tip. Do not centrifuge, vortex, or shake the tube.
    - Store DNBs at 2 °C to 8 °C, and perform sequencing within 48 h.

Table 38 Volume of Stop DNB Reaction Buffer

Component	Cap color	Volume of 100 µL DNB reaction (µL)
Stop DNB Reaction Buffer		20

11. Quantify the DNBs. For details, refer to *Quantifying DNBs on Page 63*.

Quantifying DNBs

Perform the following steps:

1. When DNB making is completed, take out 2 µL of DNBs, and use Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify the DNBs. For details, refer to *Instructions for using Qubit to quantify the DNBs on Page 191*.

   - The concentration of DNBs should be no less than 8 ng/µL. If the concentration is below 8 ng/µL, it is not qualified. Refer to *Q: What should I do if DNB concentration is low? on Page 149*.
   - If there are too many samples in a single test, it is recommended that you quantify in batches to avoid inaccurate DNB quantification as a result of fluorescence quenching.

2. If the concentration exceeds 40 ng/µL, the DNBs should be diluted to 20 ng/µL with DNB Load Buffer I.

   For DNBSEQ OneStep DNB Make Reagent Kit V4.0/DNBSEQ OneStep Library Conversion Kit (Third party), DNB dilution is not mandatory.

Loading DNBs

If you load DNBs by the sequencer, prepare the sequencing cartridge first according to *Preparing the Sequencing Reagent Cartridge-Part 2 on Page 70,* then proceed to the loading process.

Loading DNBs by the sequencer

Preparing reagents

Perform the following steps:

1. Remove DNB Load Buffer II from storage and thaw it on ice for approximately 30 min.
2. Mix it by using a vortex mixer for 5 s, centrifuge briefly by using a mini spinner, and place on ice until use.

   If crystal precipitation is visible in DNB Load Buffer II, vigorously mix the reagent for 2 min by using a vortex mixer to re-dissolve the precipitation before use.
3. Take out a 0.5 mL microcentrifuge tube from the sequencing kit and add the following reagents according to different sequencing read lengths:

Table 39 DNB loading mixture 1

Model	Component	Volume (µL)	
		FCL	FCS
FCL PE100, FCL PE150, FCS PE150	DNB Load Buffer II	64	32
	Make DNB Enzyme Mix II (LC)	2	1
	DNBs	200	100
	Total volume	266	133

If the DNBSEQ OneStep DNB Make Reagent Kit is used for making DNB, prepare DNB loading mixture 1 according to the table below:

Table 40 DNB loading mixture 1

Model	Component	Volume (µL)	
		FCL	FCS
FCL PE100, FCL PE150, FCS PE150	DNB Load Buffer II	64	32
	Make DNB Enzyme Mix II (OS)	2	1
	DNBs	200	100
	Total volume	266	133

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

   - Do not centrifuge, vortex, or shake the tube.
   - Prepare a fresh DNB loading mixture 1 immediately before the sequencing run.
   - Each FCL requires 266 µL of DNB loading mixture 1, and each FCS requires 133 µL of DNB loading mixture 1.

Loading DNBs

Perform the following steps:

1. Open the reagent compartment door.
2. Gently lift the DNB loading needle with one hand, remove the cleaning reagent tube with the other hand, load the sample tube prepared in *Preparing reagents on Page 64*, and slowly lower the DNB loading needle until the tip reaches the bottom of the tube.

   Perform this step if you load DNBs by the sequencer; otherwise, use an empty tube.

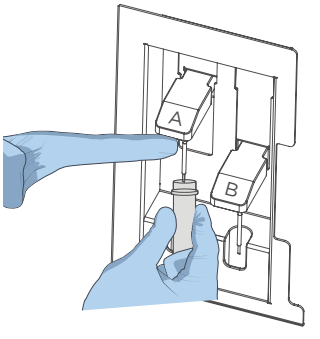


Figure 17 Loading the DNB tube

3. Close the reagent compartment door.
4. Select the **DNB loading** box in the DNB ID entry interface. After you prepare the Sequencing Reagent Cartridge, perform the sequencing run according to *Performing a sequencing run on Page 78*.

Loading DNBs by DL-200H

Preparing reagents

Perform the following steps:

1. Take out a new 1.5 mL sterile microcentrifuge tube or 0.5 mL sterile microcentrifuge tube and add the reagents by model as shown in the tables below:

Table 41 DNB loading mixture 2

Model	Component	Volume (µL)	
		FCL	FCS
FCL PE100, FCL PE150, FCS PE150	DNB Load Buffer II	8	8
	Make DNB Enzyme Mix II (LC)	0.25	0.25
	DNBs	25	25
	Total volume	33.25	33.25

If the DNBSEQ OneStep DNB Make Reagent Kit is used for making DNBs, prepare DNB loading mixture 2 according to the table below:

Table 42 DNB loading mixture 2

Model	Component	Volume (µL)	
		FCL	FCS
FCL PE100, FCL PE150, FCS PE150	DNB Load Buffer II	8	8
	Make DNB Enzyme Mix II (OS)	0.25	0.25
	DNBs	25	25
	Total volume	33.25	33.25

2. Combine the components and mix by gently pipetting 8 times by using a wide-bore pipette tip. Place the mixture at 2 °C to 8 °C until use.

- Do not centrifuge, vortex, or shake the tube.
- Each lane requires at least 30 µL of DNB loading mixture 2.
- Prepare a fresh DNB loading mixture 2 immediately before the sequencing run.

Loading DNBs

- Ensure that DL-200H is properly maintained. If necessary, perform maintenance according to *DL-200H and sealing gasket maintenance on Page 141*.
- Ensure that the sealing gasket of DL-200H is clean and properly maintained. If necessary, perform maintenance according to *DL-200H and sealing gasket maintenance on Page 141*.

Perform the following steps:

1. Install the sealing gasket and the flow cell.

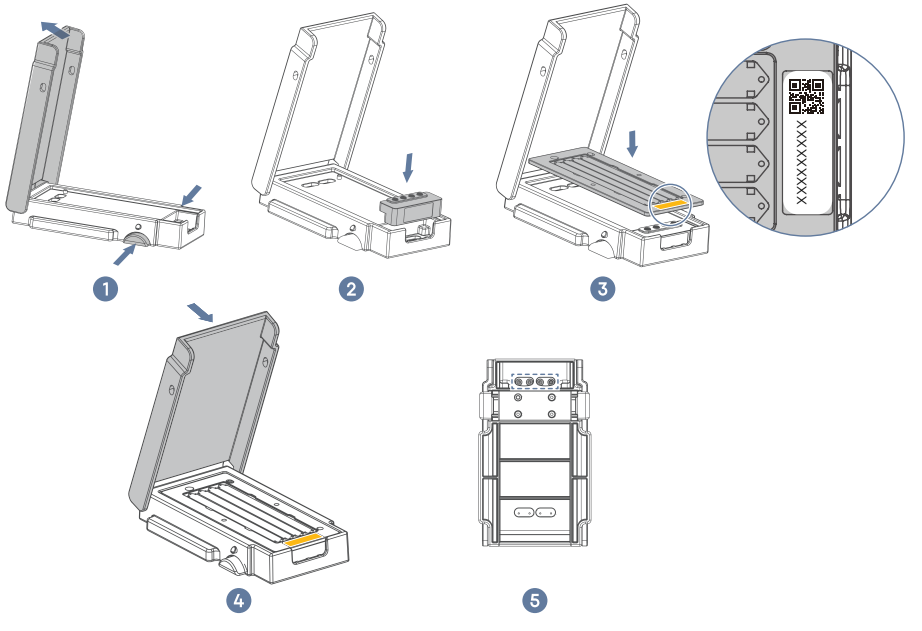


Figure 18 Installing sealing gasket and flow cell

1) Squeeze the latches and open the cover as shown above.
2) Place a clean sealing gasket into the groove and ensure that the gasket surface is even.
3) Align the holes of the flow cell with the alignment pins of the device and place the flow cell on it.
   - Ensure that the label of the flow cell is facing up and in the same position as the sealing gasket.
   - For the label location on the flow cell, refer to the area marked in yellow in *Figure 18 on Page 68* .
4) Close the cover and ensure that the cover is securely closed.
5) With the back of the DL-200H facing up, verify that the fluidic inlets align with the holes of the sealing gasket, and ensure that all four fluidic inlets are clearly visible and free from contamination.

2. Load DNBs by using DL-200H.

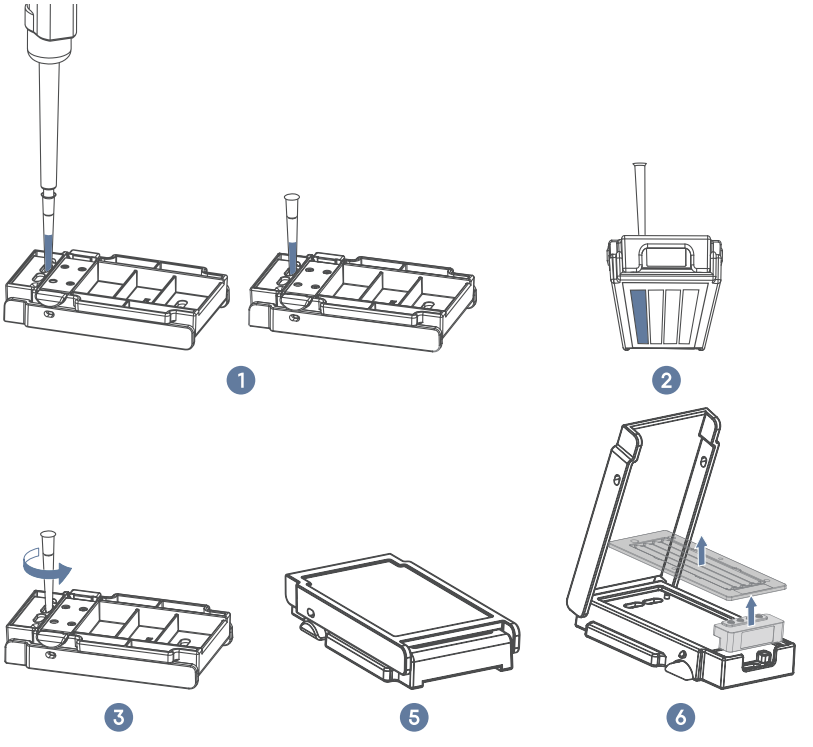


Figure 19 Loading DNBs by using DL-200H

1) Place DL-200H on the laboratory bench with the back facing up. Aspirate 30 µL of DNB loading mixture 2 with a wide-bore pipette tip, and insert the tip into the fluidics inlet. Eject the tip from the pipette. DNBs automatically flow into the flow cell.

   i Do not touch or move the tip when ejecting the tip. Doing so may introduce bubbles into the flow cell.

2) Lift up the DL-200H, but do not tilt it (keep it parallel to the bench), and check whether the DNBs flow through the flow cell.

   ⚠ **WARNING** During observation, do not tilt the DL-200H. Doing so may cause liquid leakage or biological contamination.

   i If DNBs do not flow into the flow cell, slightly press the top of the pipette tip until DNBs start to flow into the flow cell.

3) Ensure that all DNBs flow into the flow cell, and then hold the device and rotate the tip counterclockwise to remove it.

4) Repeat steps 1) through 3) to load the DNBs to the rest of the lanes of the flow cell. Ensure that you load DNBs from Lane No. 1 to Lane No. 4 in ascending order, as shown in the figure below:

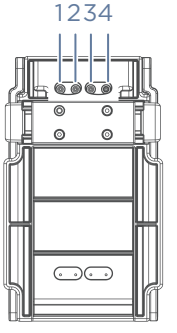


Figure 20 Lane order of DNB loading

5) Place the DL-200H on the bench with the front facing up, wait 30 min for the DNB loading process.

6) Open the cover and take out the flow cell and the sealing gasket.

3. After the DNB loading process has completed, immediately transfer the flow cell to the sequencer for sequencing. After you have prepared the Sequencing Reagent Cartridge, perform the sequencing run according to *Performing a sequencing run on Page 78*.

Preparing the Sequencing Reagent Cartridge-Part 2

Sequencing enzyme mix and dNTP mixes are provided in different tubes and are packaged together with the Sequencing Reagent Cartridge. Before the sequencing run starts, an appropriate amount of sequencing enzyme mix and dNTP mixes needs to be added to well No. 1 and well No. 2 of the Sequencing Reagent Cartridge. Furthermore, MDA Enzyme Mix II needs to be added to well No. 15 if you perform PE (Pair-End) sequencing. If prepared reagent cartridges are not used immediately, refer to *Q: What rules should I follow if I need to store a reagent kit temporarily? on Page 151*.

Perform the following steps:

1. Invert the cartridge 3 times to mix before use.
2. Shake the cartridge vigorously clockwise 20 times, and then shake it counterclockwise 20 times. Ensure that the reagents are fully mixed.

Presence of dark green crystals in well No. 10 is normal as the result of the 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. Refer to *Q: What should I do if dark green crystals appear in well No. 10? on Page 149*.

3. Wipe any water condensation from the cartridge cover and well surround with a KimWipes tissue.

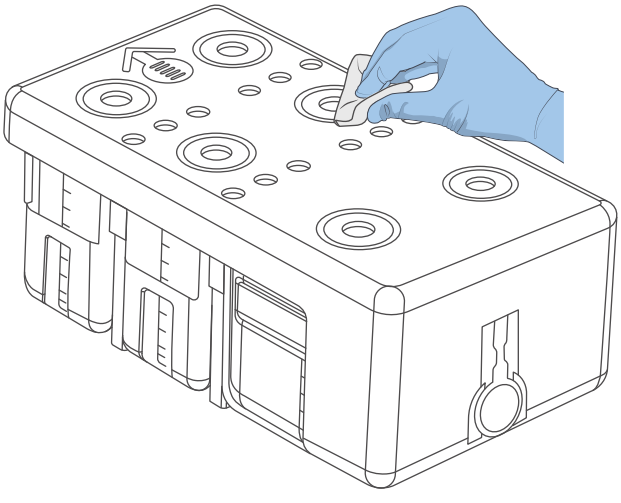


Figure 21 Wiping cartridge cover

4. Remove dNTPs Mix and dNTPs Mix II from -25 °C to -15 °C storage 1 h in advance, and thaw them at room temperature. After they have thawed, store at 2 °C to 8 °C until use.
5. Remove the Sequencing Enzyme Mix from -25 °C to -15 °C storage and place it on ice until use.
6. Remove MDA Reagent from storage and place it on ice until use.

   For third-party libraries, remove primers reagents from DNBSEQ High-throughput Sequencing Primer Kit (App-D) (Pair-End) and thaw them at room temperature. Store at 2 °C to 8 °C until use.

   - App-D Insert Primer 1, App-D Insert Primer 2, App-D MDA Primer, and App-D Barcode Primer 2 are for single barcode libraries.
   - App-D Insert Primer 1, App-D Insert Primer 2, App-D MDA Primer, App-D Barcode Primer 2, and App-D Barcode Primer 3 are for dual barcode libraries.

Well positions are shown in the figure below:

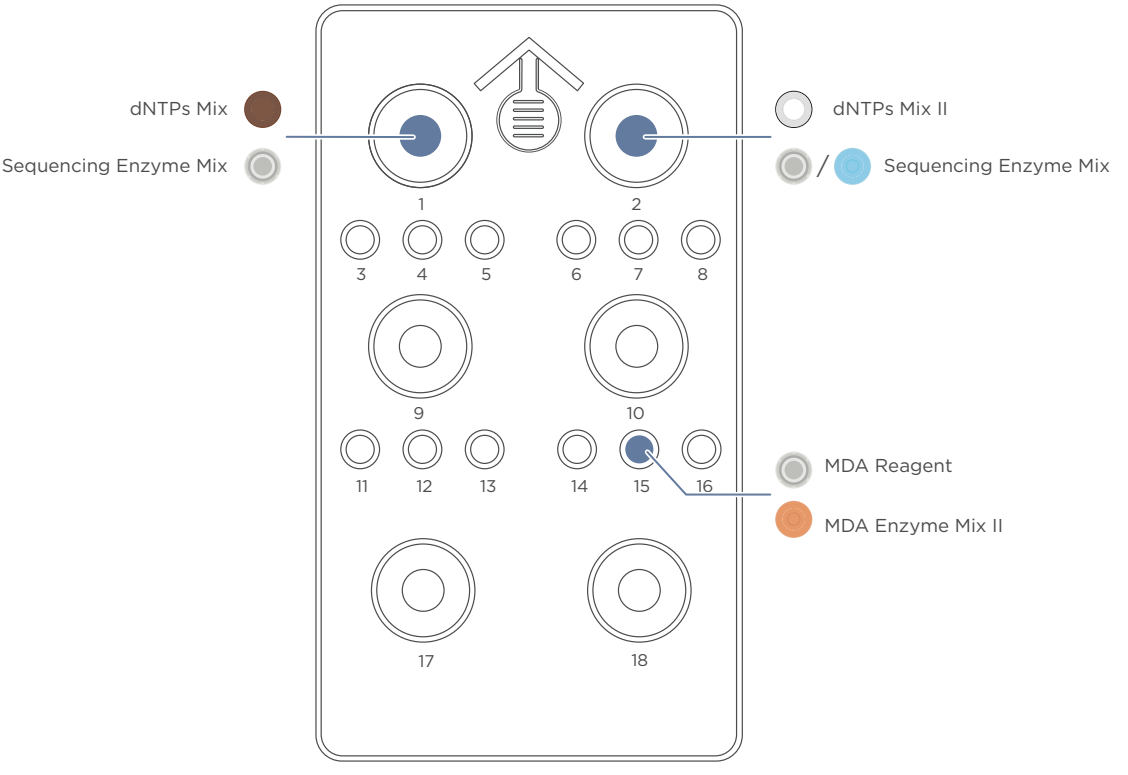


Figure 22 Well positions

7. Pierce the seals in the center of wells No. 1 and No. 2 to make a hole approximately 2 cm in diameter by using a 1 mL sterile pipette tip.

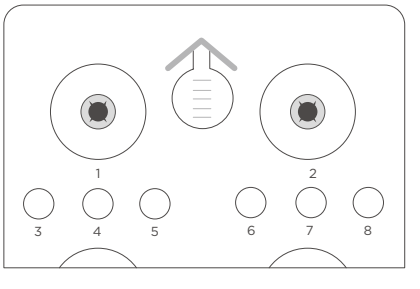


Figure 23 Piercing the seal of the cartridge

8. Take out a pipette with the appropriate volume range. Add dNTPs Mix into a new 5 mL/10 mL sterile tube, and then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube according to the table below.

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

Table 43 Reagent preparation for well No. 1

Model	dNTPs Mix loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
FCL PE100	1.800	1.800
FCL PE150	2.400	2.400
FCS PE150	1.900	1.900

9. Invert the tube 6 times to mix the reagents in the tube before adding all of them into well No. 1.

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

10. Take out a pipette with the appropriate volume range and add reagents according to the table below. Add dNTPs Mix II into a new 5 mL/10 mL/15 mL/25 mL sterile tube, and then add Sequencing Enzyme Mix into the dNTPs Mix II in the same tube.

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

Table 44 Reagent preparation for well No. 2

Model	dNTPs Mix II loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
FCL PE100	1.500	1.500
FCL PE150	2.100	2.100
FCS PE150	3.800	1.900

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

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

12. Seal loading wells No. 1 and No. 2 with transparent sealing films.

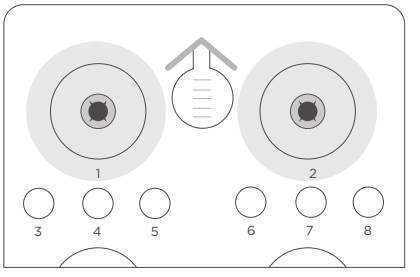


Figure 24 Sealing the loading wells of the cartridge

13. Press the film around the well with your finger. Ensure that the well is tightly sealed and that there are no air bubbles between the film and cartridge surface. This ensures that the reagents will not flow over the cartridge.

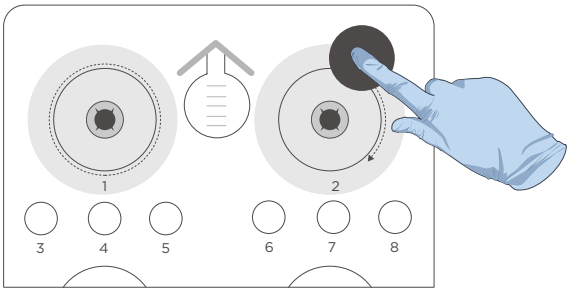


Figure 25 Sealing the loading wells of the cartridge tightly

14. Lift the cartridge horizontally and hold both sides of the cartridge with both hands. Shake the cartridge 20 times in a clockwise and counterclockwise direction. Ensure that the reagents are fully mixed.

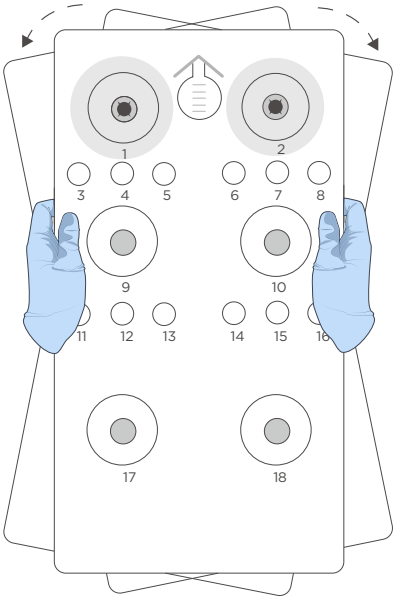


Figure 26 Mixing reagents after loading

15. Carefully remove the seals from the loading wells after fully mixing.

- Do not reuse the used sealing film.
- To prevent cross contamination, ensure that the surface around wells No. 1 and No. 2 is clean.

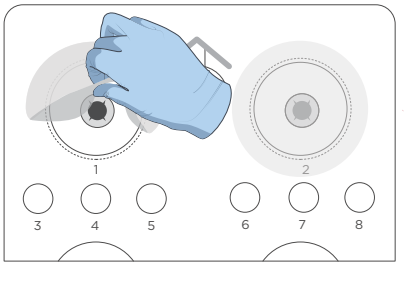


Figure 27 Removing the seals from the cartridge

16. It is recommended that you handle reagent in well No. 10 according to the following steps:
    1) Open the reagent cartridge cover and remove the reagent bottle from well No.10.
    2) Fill the ultrasonic cleaner with ultra-pure water. The recommended power for the ultrasonic cleaner is 80 W to 600 W, with a capacity of 2 L to 10 L.

    It is recommended that you change the ultra-pure water every 7 days.

3) Pierce the sealing film of the reagent bottle with a pipette tip. Place the reagent bottle into the ultrasonic cleaner, ensure that the water level of the cleaner is above the level of reagent inside the bottle, and avoid water from entering the reagent bottle.

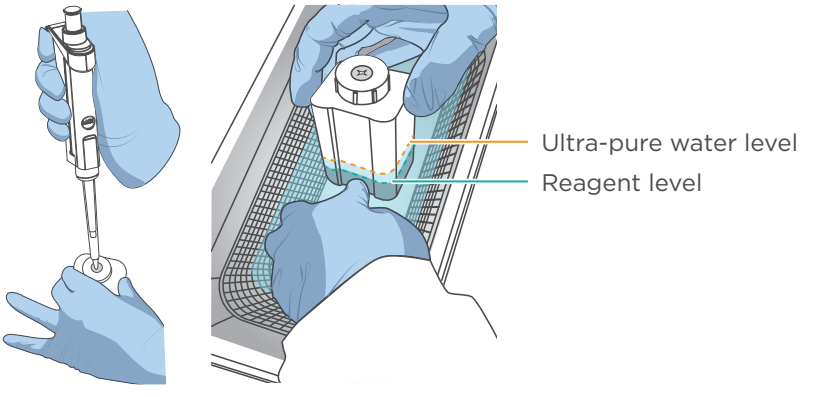


Figure 28 Piercing and placing No. 10 bottle

4) Start the ultrasonic cleaner and vibrate for 5 min to 10 min.
5) After completion, remove the reagent bottle and avoid shaking it.
6) Use a KimWipes tissue to wipe off any moisture on the surface of the reagent bottle.
7) Place the reagent bottle back into the reagent cartridge and close the cartridge cover.

17. Gently tap the cartridge on the bench to reduce air bubbles in the reagents, and the Sequencing Reagent Cartridge is ready for sequencing CG libraries.

    For the next step, refer to *Performing a sequencing run on Page 78*. Otherwise, proceed to *Step 18* to sequence third-party libraries.

18. For sequencing of third-party libraries, perform the following steps:
    1) Pierce the foil seals with clean pipettes (Well No. 3, No. 6, No. 7, and No. 8 for single barcode sequencing; No. 3, No. 4, No. 6, No. 7, and No. 8 for dual barcode sequencing).
    2) Discard the reagents in each tube by using a pipette.
    3) Add the primers using appropriate pipettes according to the table below.

       - App-D barcode primer 3 is only for dual barcode App-D PE sequencing.
       - When adding the mixture, ensure that there are no bubbles at the bottom of the tube.

Table 45 Primer loading correspondence for App-D PE sequencing

Reagent name	Well	Volume (mL)
App-D Insert Primer 1	No. 3	2.20
App-D Barcode Primer 3	No. 4	2.90
App-D Barcode Primer 2	No. 6	2.90
App-D MDA Primer	No. 7	3.10
App-D Insert Primer 2	No. 8	3.30

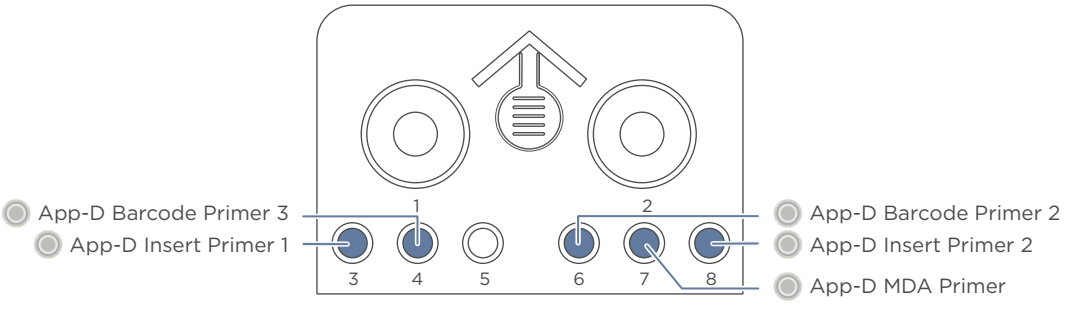


Figure 29 DNBSEQ-G400RS PE sequencing primer adding diagram

19. Pierce the seal of well No. 15 by using a 1 mL sterile pipette tip.
20. Add 500 µL of MDA Enzyme Mix II to the MDA Reagent tube with a 1 mL pipette.

    When using MDA Enzyme Mix II, do not touch the wall of the tube. The heat from your hands may affect the enzyme activity.
21. Invert the tube 6 times to mix the reagents.
22. Add the mixture to well No. 15. When adding the mixture, ensure that there are no bubbles at the bottom of the tube.

    - When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.
    - The Sequencing Reagent Cartridge is now ready for use.
    - For the next step, refer to *Performing a sequencing run on Page 78*.

Performing a sequencing run

Entering DNB ID

Perform the following steps:

1. In the main interface, select **Sequence** to open the DNB ID entry interface:

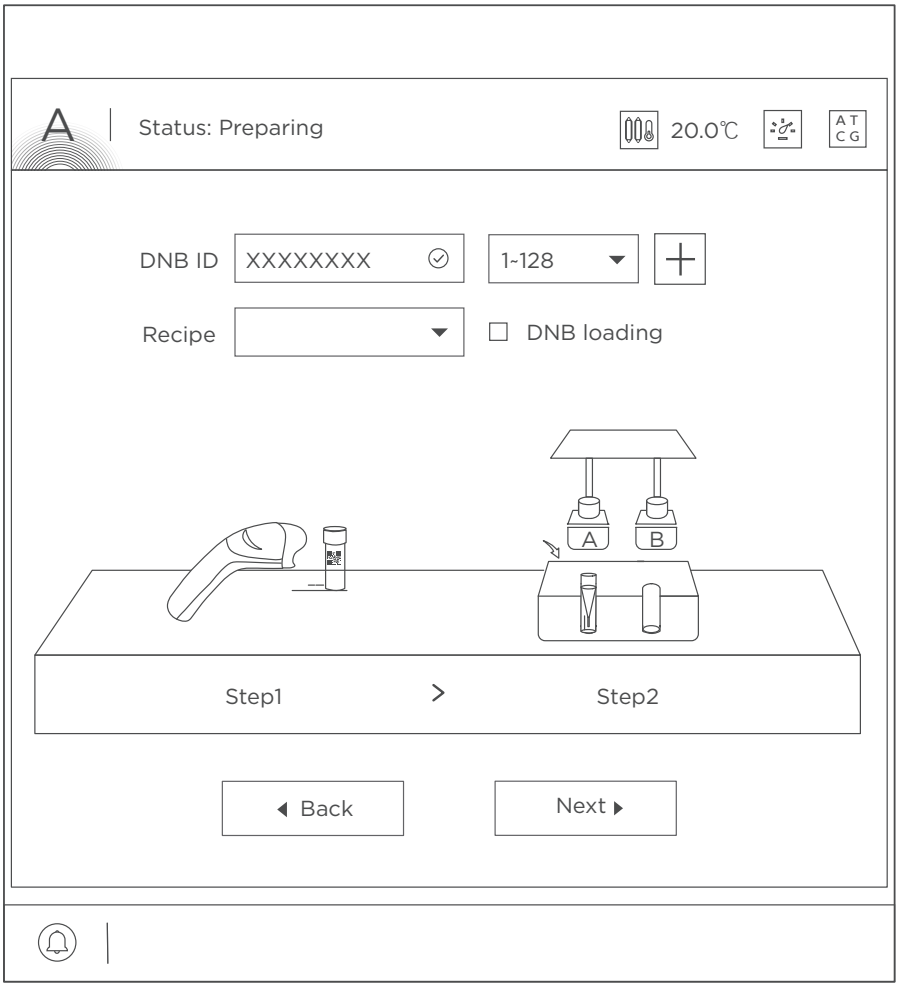


Figure 30 DNB ID entry interface

2. Select the **DNB ID** box, and enter the DNB ID manually by using the on-screen keyboard.
3. Select a barcode range of different lanes from the list next to the **DNB ID** box.

   Select [+] or [—] to add or remove a line of DNB ID if needed.

Select 4 lanes for FCL and 2 lanes for FCS.

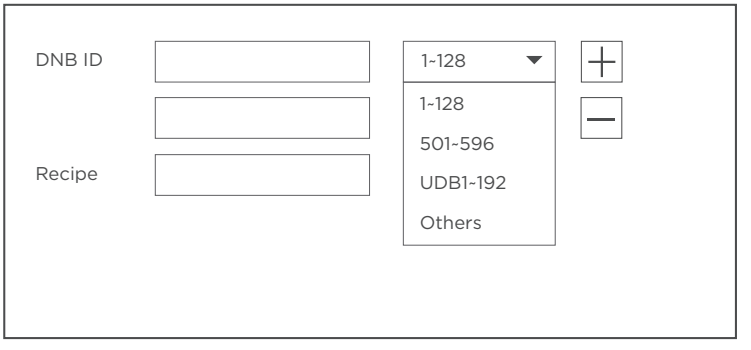


Figure 31 **DNBs and information selection interface**

UDB1~192 in the barcode range list is for dual barcode sequencing, and the barcode file in the Customize interface will be split by UDB1~192 by default. The barcode list is currently limited to customized scripts.

Selecting sequencing parameters

Perform the following steps:

1. Select an appropriate recipe from the **Recipe** list. One-click sequencing runs (for example, **PE150**, and so on) and a user-customized run (**Customize**) are available.

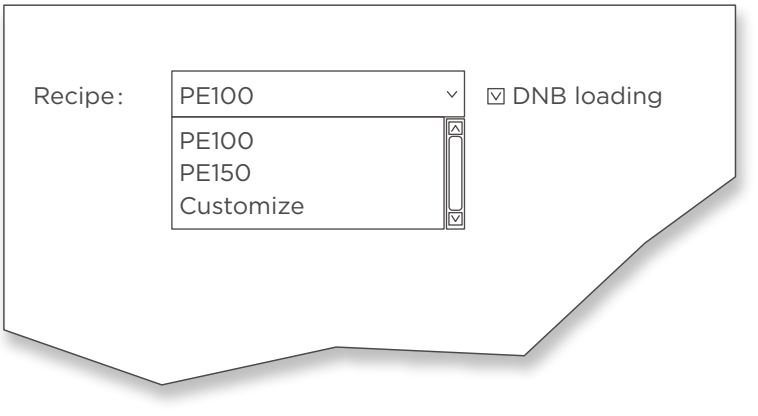


Figure 32 **Selecting sequencing recipe**

2. If you choose one-click sequencing and the DNBs are loaded by the sequencer, select **DNB loading** on the right of the **Recipe** list.

Loading the Sequencing Reagent Cartridge

Perform the following steps:

1. Select the **Sequencing cartridge ID** field, manually enter the cartridge ID according to the serial number (SN) printed on the cartridge label, or use the barcode scanner to scan the cartridge barcode at the lower-right corner of the Sequencing Reagent Cartridge label.

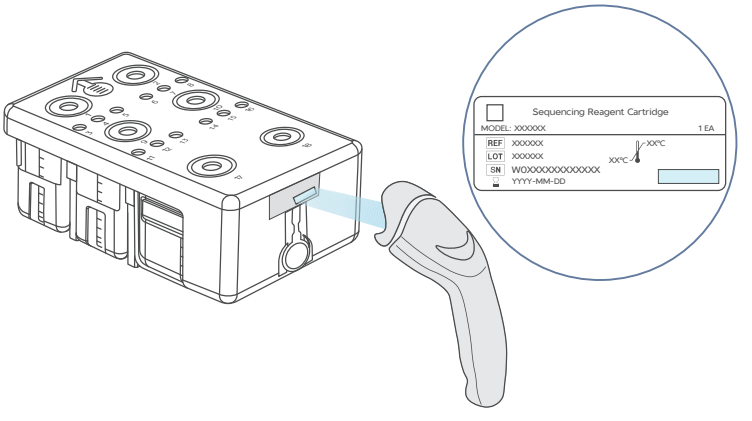


Figure 33 Scanning Sequencing Reagent Cartridge ID

- Ensure that you select the applicable Sequencing Reagent Cartridge. If necessary, replace the appropriate cartridge according to the notes in the software.
- Reagents of the same type must be used when performing sequencing on both sides simultaneously.

CAUTION
- Do not scan the QR code in the upper-left corner by mistake.
- Ensure that you enter the correct serial number. Entering an incorrect serial number will result in sequencing errors or sequencing failures.

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

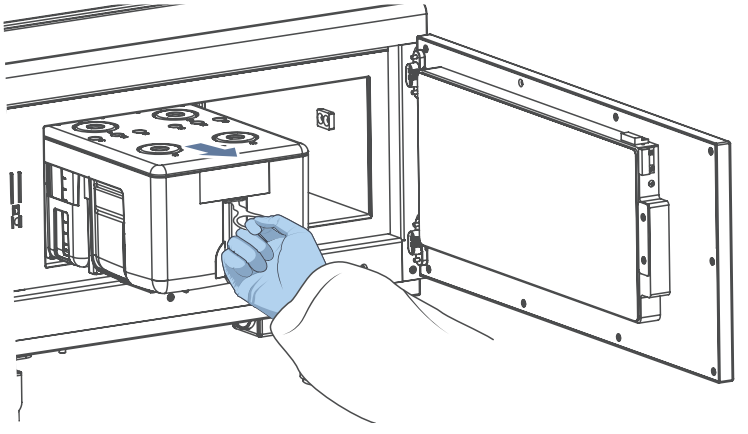


Figure 34 Removing cleaning cartridge

3. Moisten a KimWipes tissue with laboratory-grade water and use it to wipe the bottom and sides of the compartment to keep it clean and dry.

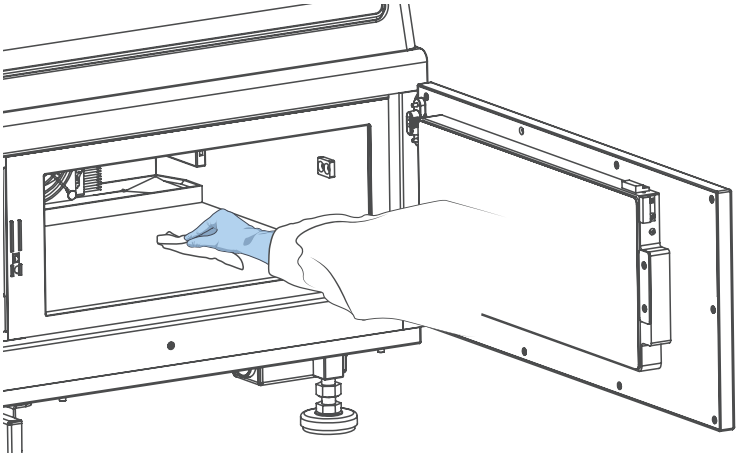


Figure 35 Maintaining the reagent compartment

4. Hold the handle of a new Sequencing Reagent Cartridge with one hand and place the other hand underneath for support.

5. Slide the cartridge into the compartment by following the direction printed on the cover until it stops.

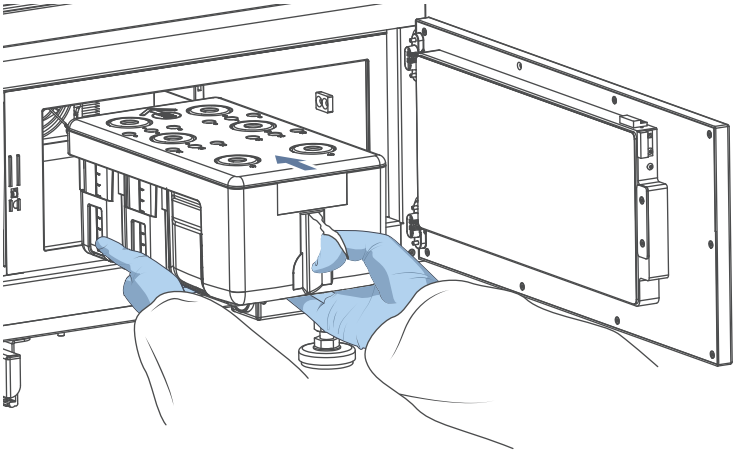


Figure 36 Sliding the new Sequencing Reagent Cartridge into the reagent compartment

6. Ensure that the cartridge is in the correct position and close the reagent compartment door.

Loading the flow cell

Perform the following steps:

1. Open the flow cell compartment door.
2. Press both sides of the flow cell with one hand, and press the flow cell attachment button with the other hand.
3. After the vacuum is released, remove the washing flow cell from the stage.
4. Use a canned air duster to remove the dust from the flow cell stage and the back of the flow cell.

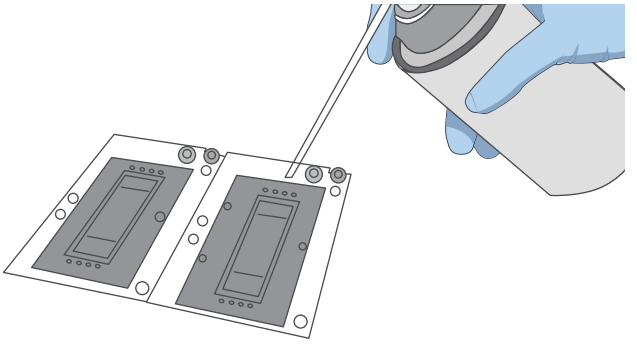


Figure 37 Cleaning the flow cell stage

If there are impurities on the stage surface, gently wipe the surface with a wet KimWipes tissue to ensure that the flow cell can be held properly.

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

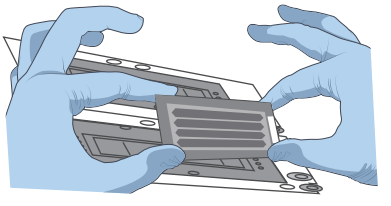


Figure 38 Loading the flow cell

7. Align the holes on the flow cell with the locating pins on the flow cell stage. Gently slide the flow cell at an angle of 45 degrees to the upper-left corner to keep the flow cell aligned with the pin.
8. Press the flow cell attachment button. Press the left and right sides of the flow cell on the stage at the same time to ensure that the flow cell is properly seated on the stage.

   The flow cell is fragile. Handle it with caution.

9. Ensure that the negative pressure is within the range of -99 kPa to -80 kPa before continuing.

   If the negative pressure is abnormal, refer to *Q: What should I do if abnormal negative pressure appears during flow cell attachment? on Page 152*.

10. Use a canned air duster to remove the dust from the flow cell surface, and then close the flow cell compartment door.

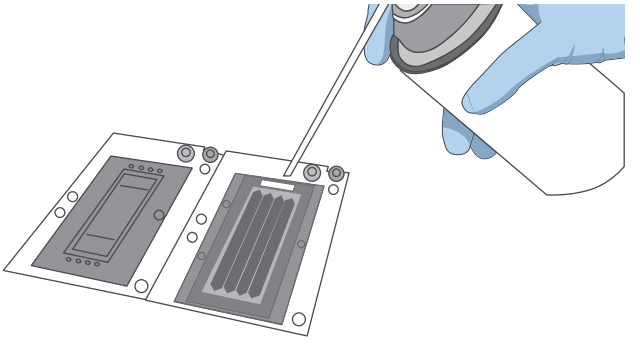


Figure 39 Cleaning the flow cell

11. Select **Next**. The flow cell ID can be entered using the barcode scanner.

If automated entry does not work, move the cursor to the **Flow cell ID** box and enter the ID manually.

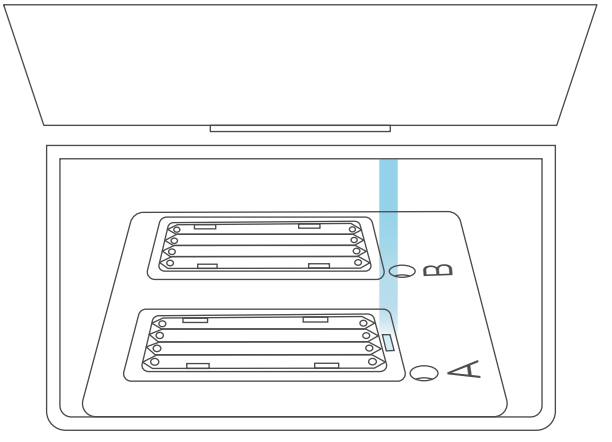


Figure 40 Scanning flow cell ID

12. Select **Next**.

WARNING If the flow cell accidentally falls to the floor and breaks, handle with care to prevent personal injury.

CAUTION

- If the flow cell is not attached properly, use a canned air duster to blow off the dust on the flow cell stage and the back of the flow cell. If there are crystals on the surface of the stage, wipe it gently with a damp KimWipes tissue and then let it air-dry.
- Do not move the flow cell after it is loaded. Otherwise, the flow cell inlet, outlet, and the gasket may become misaligned.

Reviewing parameters

To ensure sequencing quality, when sequencing of Read1 and Read2 is completed, the sequencer will automatically perform another cycle for calibration. For example, for PE100 sequencing, the length of Read1 is 100, the length of Read2 is 100, the length of barcode is 10. Adding 1 correction cycle for Read1 and 1 correction cycle for Read2 (barcode does not need to be corrected), the total number of sequencing cycles is 212.

Review	
Item	Content
User name	user
DNB ID Lane 1	WGS|1 ~ 128
DNB ID Lane 2	RNA| 501 ~ 596
DNB ID Lane 3	WGS|1 ~ 128
DNB ID Lane 4	RNA| 501~ 596
Sequencing cartridge ID	AA000012
Flow cell ID	V300001234
Recipe	PE100
Start phase	DNB Loading
Cycles	212
Read 1	100
Read 2	100
Barcode	10
Split barcode	Yes | Yes | Yes | Yes

Figure 41 Reviewing information

Carefully check each item in the review interface, and do one of the following:

- If you find errors, select **Previous** to return to the previous interface and reset.
- If all parameters are correct, select **Start**. The software automatically checks the available storage drive space:

- If the storage space is sufficient, a confirmation dialog box appears. Select **Yes** to start a sequencing run.
- If the storage space is insufficient, a pop-up dialog box appears. Perform the following steps:

  a. In the pop-up dialog box, select the data that you want to delete and then select **Clear history data**.

  b. When the storage drive space icon turns green, select **Back** to return to the parameter review interface, and then select **Start**.

Starting sequencing

Perform the following steps:

1. After confirming that the information is correct, select **Start**, and select **Yes** in the pop-up dialog box to start sequencing.

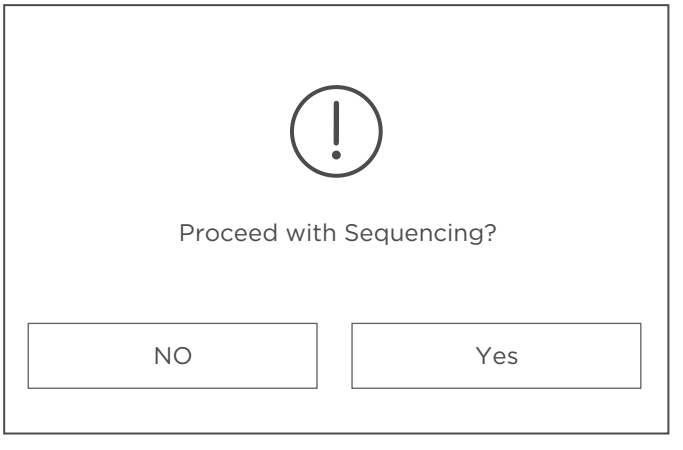


Figure 42 Confirming sequencing interface

2. When sequencing has started, immediately open the flow cell compartment door to inspect the flow cells, ensure that the DNBs or reagents are flowing through the flow cell, and close the compartment door.

   If the DNBs and reagents are not flowing through the flow cell, fix the problems before you restart sequencing. For details, refer to *Instructions for customizing a run on Page 163.*

CAUTION

- Do not bump, move, vibrate, or impact the device during sequencing because it may cause inaccurate sequencing results.
- If malfunctions related to fluidics lines (for example, bubbles) occur during sequencing, fix the problems before you restart sequencing. For details, refer to *Sequencer FAQs on Page 144*.
- Pay special attention to the LED status indicator or the on-screen instructions. If errors occur, troubleshoot the problem by following the instructions and this guide. If errors persist, contact CG Technical Support.

The Sequencing interface displays real-time sequencing progress, and you can check sequencing parameters or operate the device as described in the following table during the run.

The following table describes the function of each item in the interface:

Table 46 Sequencing interface description

Item	Description
Time remaining	Shows the remaining time for sequencing
Phase	Shows the current phase of sequencing
Step	Shows the current step and total sequencing steps
Cycle	Shows the current read length and total read length of sequencing
QC type	Select a QC value graph from the QC type list to assess the sequencing quality
Lane	Shows the serial number of the lane that is being imaged, and the total number of flow cell lanes
Row	Shows the serial number of the row that is being imaged, and the total number of flow cell rows
Column	Shows the column of the flow cell lane that is being imaged, and the total columns
⏸	Select to pause sequencing
▷	Select to resume sequencing that has been paused
■	Select this button and a confirmation dialog box appears. Select **Yes** in the dialog box to stop sequencing

Item	Description
	After imaging of the first cycle, when the sequencing is paused, select this button to move up the needles. Open the reagent compartment door and take out the Sequencing Reagent Cartridge
	After imaging of the first base is completed, select this button to open the first base report
	Select this button to open the Review interface, and then check the sequencing information

3. When the interface shows that the device is processing data or is in idle status, it means that the sequencing reaction has finished. Perform the wash process according to *Wash on Page 128*.

(Optional) Resuming a sequencing run

- Resuming a run is only applicable to the flow cell on the sequencer whose run is not completed.
- The run can be resumed if it is stopped in Sequencing prime, Incorporation of Read1, Read2 and barcode sequencing, Imaging of Read1, Read2 and barcode sequencing, and Cleavage of Read1, Read2 and barcode sequencing.

Resume the sequencing run according to the following situations:

- Errors that do not need to be handled, and the sequencing run can be resumed directly. For example, the customer manually stops sequencing.

  Perform the following steps:

  1) Select **Finish** on the sequencing stopped interface. When you are prompted **Are you sure you want to wash?**, select **No** to return to the main interface.
  2) Select **Sequence.** When you are prompted **Last run stopped abnormally. Do you want to continue the run?**, select **Yes** to open the Review interface.
  3) Carefully check each item in the Review interface and ensure that each item is correct.
  4) Select **Start** and select **Yes** when prompted to resume sequencing.
- Errors that can be solved only after you restart the system; for example, software and hardware errors.

  Perform the following steps:

  1) Select **Finish** on the sequencing stopped interface. When you are prompted **Are you sure you want to wash?**, select **No** to return to the main interface.
  2) Select > . In the pop-up dialog box, select **Yes** to restart the device.

3) Input the user name and password to log in to the computer. The control software starts automatically.
4) Input the user name and password to log in to the control software.
5) Select **Sequence.** When you are prompted **Last run stopped abnormally. Do you want to continue the run?**, select **Yes** to open the Review interface.
6) Carefully check each item in the Review interface and ensure that each item is correct.
7) Select **Start** and select **Yes** when prompted to resume sequencing.

- Errors that can be solved only after you power off and start the system again; for example, flow cell stage communication error and IO board communication error.

1) Select **Finish** on the sequencing stopped interface. When you are prompted **Are you sure you want to wash?**, select **No** to return to the main interface.
2) Select > . In the pop-up dialog box, select **Yes** to restart the device.
3) Input the user name and password to log in to the computer. The control software starts automatically.
4) Input the user name and password to log in to the control software.
5) Select **Sequence.** When you are prompted **Last run stopped abnormally. Do you want to continue the run?**, select **Yes** to open the Review interface.
6) Carefully check each item in the Review interface and ensure that each item is correct.
7) Select **Start** and select **Yes** when prompted to resume sequencing.

Performing a maintenance wash

After the sequencing run, perform a maintenance wash within 24 h.

For details, refer to *Performing a maintenance wash (~94 min) on Page 134*.

Disposing of the Sequencing Reagent Cartridge and flow cell

WARNING If the flow cell accidentally falls to the floor and breaks, handle with care to prevent personal injury.

Perform the following steps:

1. Wear protective equipment.
2. Open the flow cell compartment and remove the flow cell:
   1) Hold the flow cell by the edges with one hand to prevent the flow cell from falling into the device, and to prevent damage to the flow cell.

2) Press the flow cell attachment button with the other hand to release the flow cell. Remove the flow cell.

3. Open the reagent compartment door, pull out the Sequencing Reagent Cartridge by using the pull ring and remove the cartridge. Move up the base, and remove the tube.
4. Empty the remaining solution in the Sequencing Reagent Cartridge and tube into an appropriate waste container.
5. Dispose of the tube, flow cell, and Sequencing Reagent Cartridge in accordance with local regulations and safety standards of your laboratory.

(Optional) Powering the device off

CAUTION

- Power the device off and disconnect the power cord if you do not plan to use the device for an extended period of time.
- Before you power the device off, ensure that the sequencing run and wash are completed, the flow cell compartment door is closed, and then exit the control software. Failure to do so may damage the device.

Perform the following steps:

1. Select > . In the pop-up dialog box, select **Shut down**.
2. Turn the power switch to the position.
3. Disconnect the power cord from the main power supply or UPS.

06

Sequencing data

This chapter describes the sequencing output data.

Sequencing output files

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

After sequencing starts, the sequencing results generated by the control software will appear in the D drive of the computer.

- The data folder, named after the flow cell ID, primarily contains pictures and data (such as metrics) generated during the device operation.
- The result folder, named after the flow cell ID, primarily contains Bioinfo files and FASTQ files, reports and, *.cal* files.

Exporting data

You can export running data, sequencing results, and logs from sequencer to external storage device according to your needs.

- When the device is exporting data, you can go back to the main interface to perform a wash, but sequencing is not allowed.
- Before using an external storage device, we recommend that you format the external storage device (for example, a USB storage drive).

Perform the following steps:

1. In the system maintenance interface, select **Device maintenance** > **Export data**.
2. Select a data type:
   - If you select **Running data** or **Sequencing results**, select the file types that you need next to **Logs**:
     - Running data file types: images, metrics of each cycle.
     - Sequencing result file types: FASTQ, metrics, report, and others of each flow cell.
   - If you select **Logs**, select a date range for the logs that you want to export.

     Log files are exported according to the date range that you select.

     Only logs of up to 15 days prior to the current day can be exported. not including logs from runs performed on the current day.
3. Select the desired folders from the **Exported directory** and the **Imported directory** boxes.
4. Select **Export**. The data will be exported to the target folder in the external storage device.

5. (Optional) If you want to upload the data to a storage server or to the server on which the ZLIMS software is installed, select **Uploading data**.

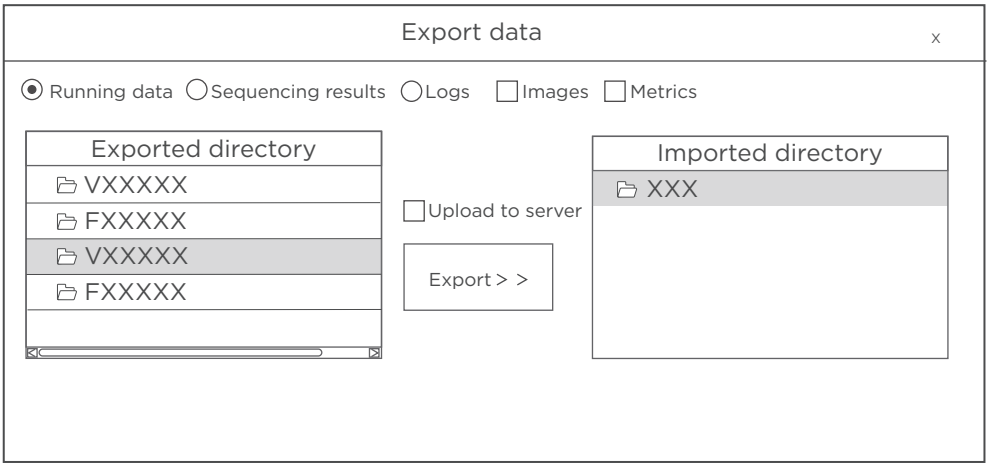


Figure 43 Export data interface

Summary report

Report parameters

The following table describes parameters for Tab1 in the summary report:

Table 47 Parameters for Tab1 in the summary report

Parameter	Description
SoftwareVersion	Version of BasecallLite. Ensure that the BasecallLite is in the official release version
TemplateVersion	Version of summary report template
Reference	The species category of the sample. When the species category is unknown or when the category is not *E. coli*, the reference will be indicated as NULL
CycleNumber	The total cycle of the sequencing run (not including the extra cycles, but including barcode, regardless of whether the barcode is split or not)
ChipProductivity(%)	Flow cell productivity. The yield of the flow cell is estimated by the following formula: $\mathbf{ChipProductivity} = \frac{\mathbf{ValidFovNumber} \times \mathbf{ESR}}{\mathbf{ImageArea}} \times \mathbf{100\%}$
ImageArea	The total number of FOVs (field of view) in a lane The system reads the total number of FOVs from the *QC.csv* file under the metrics directory generated by the basecall software
TotalReads(M)	Reads included in the FASTQ file (Reads after filtering)
Q30(%)	The percentage of bases with quality score of ≥30. A base with a quality score of 30 implies that the chances that this base being called incorrectly are 1 in 1000
SplitRate(%)	The proportion of FASTQ data that can be split according to barcodelist. This indicator is obtained from the *BarcodeStat.txt* file, and the split results are included in *Sequencestat.txt*. The Split Rate is counted from the filtered reads only

Parameter	Description
Lag/Runon	• Lag1 (%) is the slope of the Lag curve for the first-strand sequencing • Lag2 (%) is the slope of the Lag curve for the second-strand sequencing • Runon1 (%) is the slope of the runon curve for the first-strand sequencing • Runon2 (%) is the slope of the runon curve for the second-strand sequencing
ESR(%)	Effective spot rate. Percentage of effective spots after filtering in the flow cell
MaxOffsetX/ MaxOffsetY	During the sequencing process, the basecall program will output the Offset value of each FOV in channel A compared with the standard template in cycle 1. The two values are based on the results of channel A base fluorescent imaging
InitialOffsetX/ InitialOffsetY	The offset value of A base fluorescent channel in cycle 1
RecoverValue	RecoverValue(A) is the ratio of second-strand signal to first-strand signal of A RecoverValue(C) is the ratio of second-strand signal to first-strand signal of C RecoverValue(G) is the ratio of second-strand signal to first-strand signal of G RecoverValue(T) is the ratio of second-strand signal to first-strand signal of T RecoverValue(AVG) is the average ratio of second-strand signal to first-strand signal of 4 (ACGT) bases This indicator is only for PE sequencing

The following table describes parameters for Tab2 in the summary report:

Table 48 Parameters for Tab2 in the summary report

Parameter	Description
ISW Version	Version of the control software for the sequencer
Machine ID	Serial number of the sequencer
Sequence Type	The sequencing recipe that you select when sequencing
Recipe Version	Version of the sequencing recipe script
Sequence Date	The date on which the sequencing started
Sequence Time	The time at which the sequencing started
Reagent ID	Serial number of the reagent cartridge
Flowcell Pos	Position of the flow cell (stage A or stage B)
DNB ID	DNB ID that you enter
Barcode Type	The barcode file that you select during sequencing
Barcode File	The name of the barcode file used for barcode split
Read1 Cycles	First-strand read length
Read2 Cycles	Second-strand read length
Barcode	Read length of barcode
Dual Barcode	Read length of dual barcode
Read1 Dark Cycles	The number of cycles for the first strand to perform a dark reaction
Read2 Dark Cycles	The number of cycles for the second strand to perform a dark reaction

Diagrams in summary report

Diagrams in this section are for illustrative purposes only. The actual diagrams may vary.

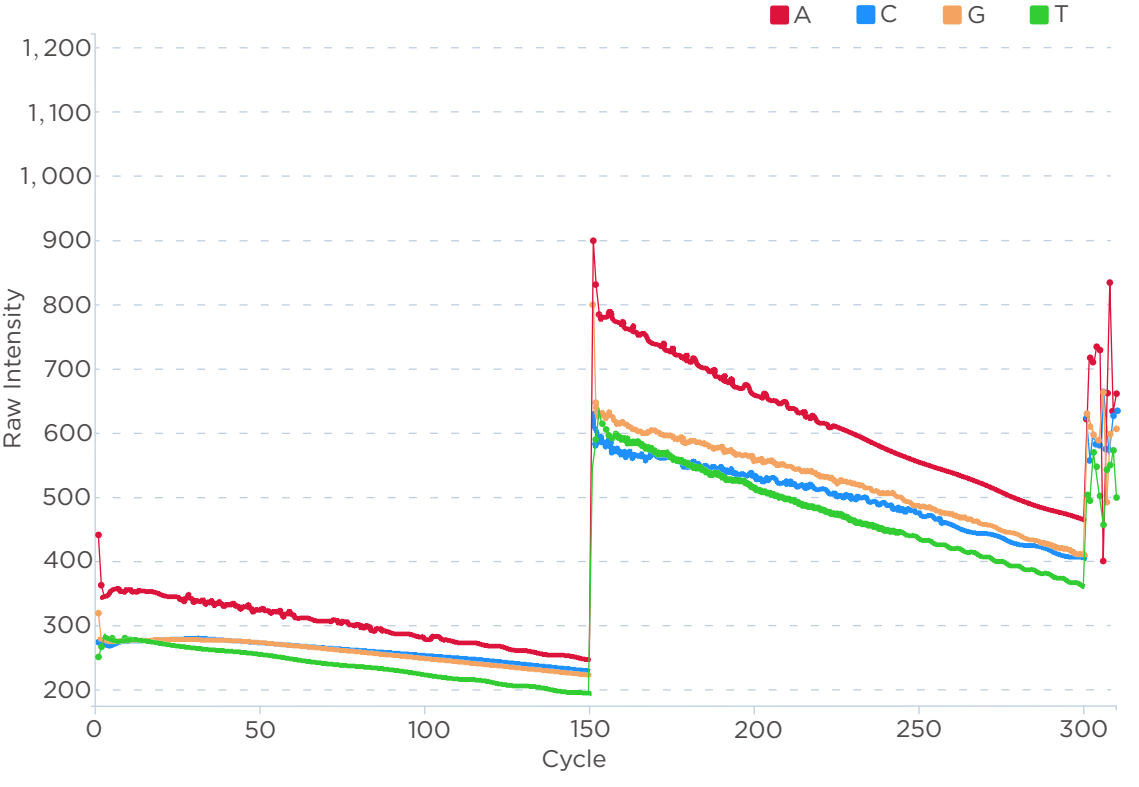


Figure 44 **Raw Intensity**

X axis	Cycle
Y axis	Raw Intensity: Signal intensity of images after preliminary treatment.

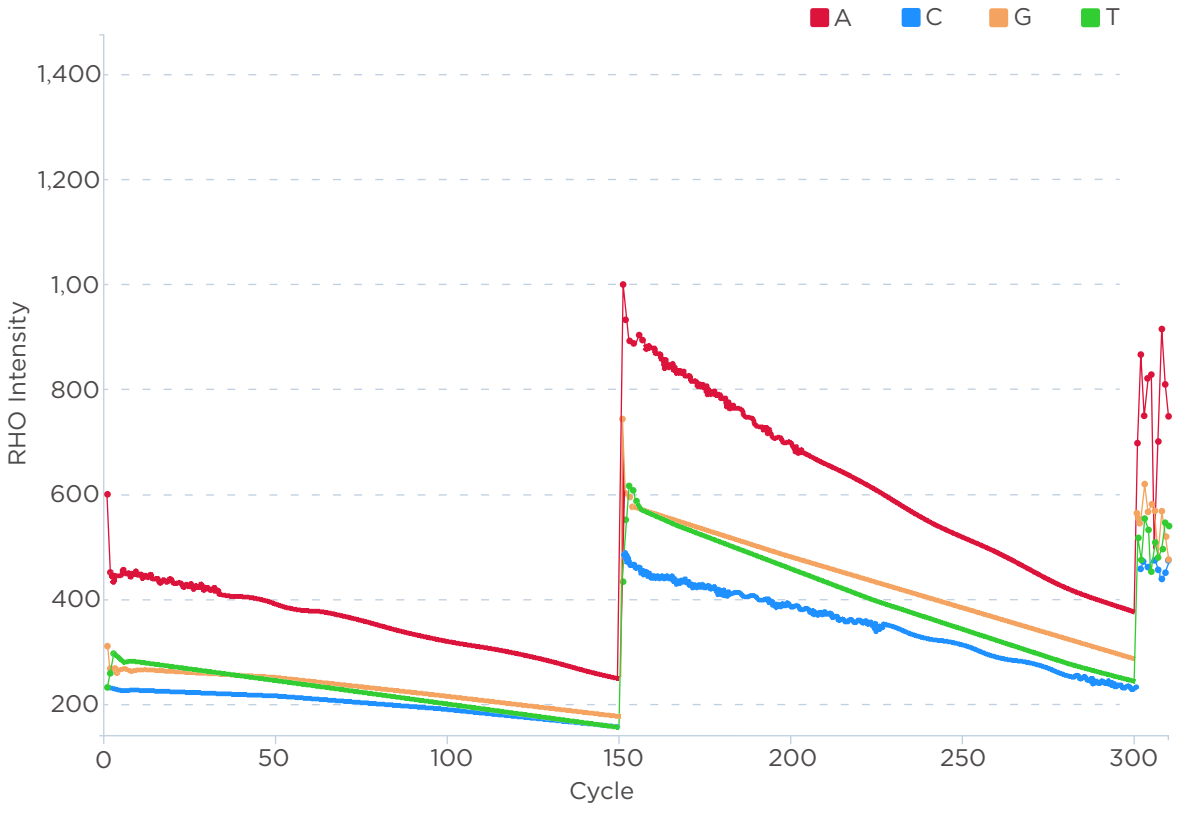


Figure 45 RHO Intensity

X axis	Cycle
Y axis	RHO(ρ) Intensity: Intensity of raw signals. RHO is the orthogonalized, background subtracted, spot intensity in 4 (ACGT)-space. RHO A is the average RHO A of all DNBs with basecall A.

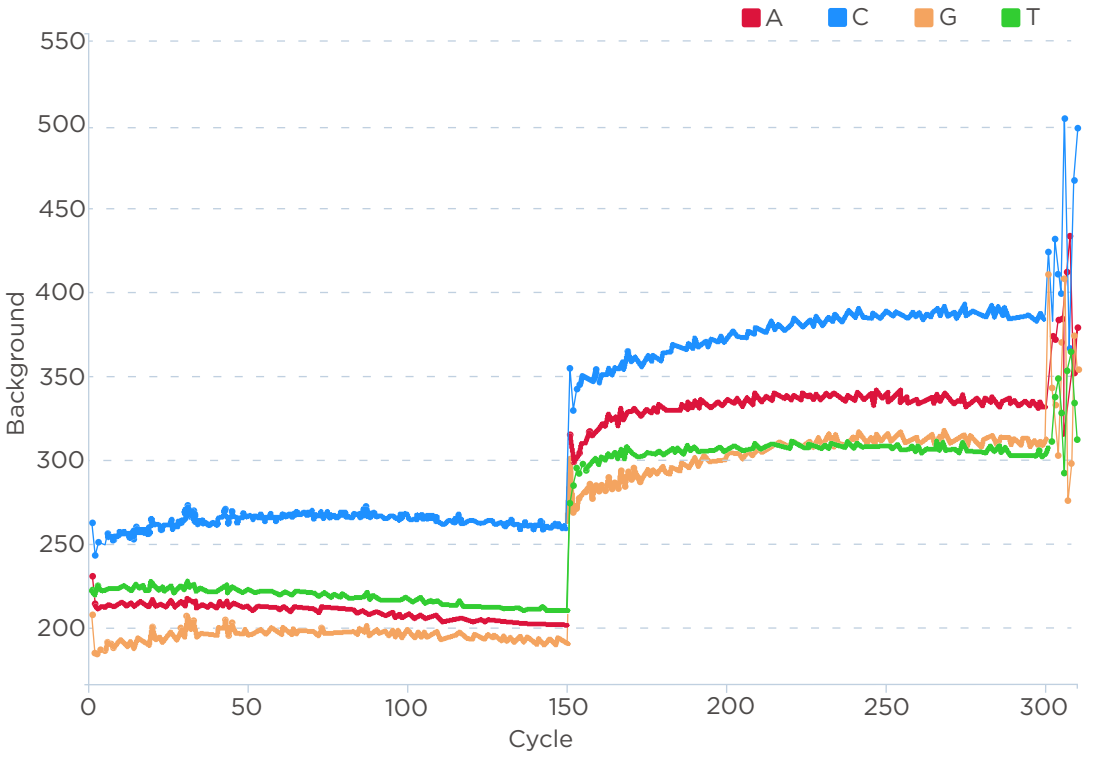


Figure 46 Background

X axis	Cycle
Y axis	Background: Signal intensity in the area where no DNBs are loaded.

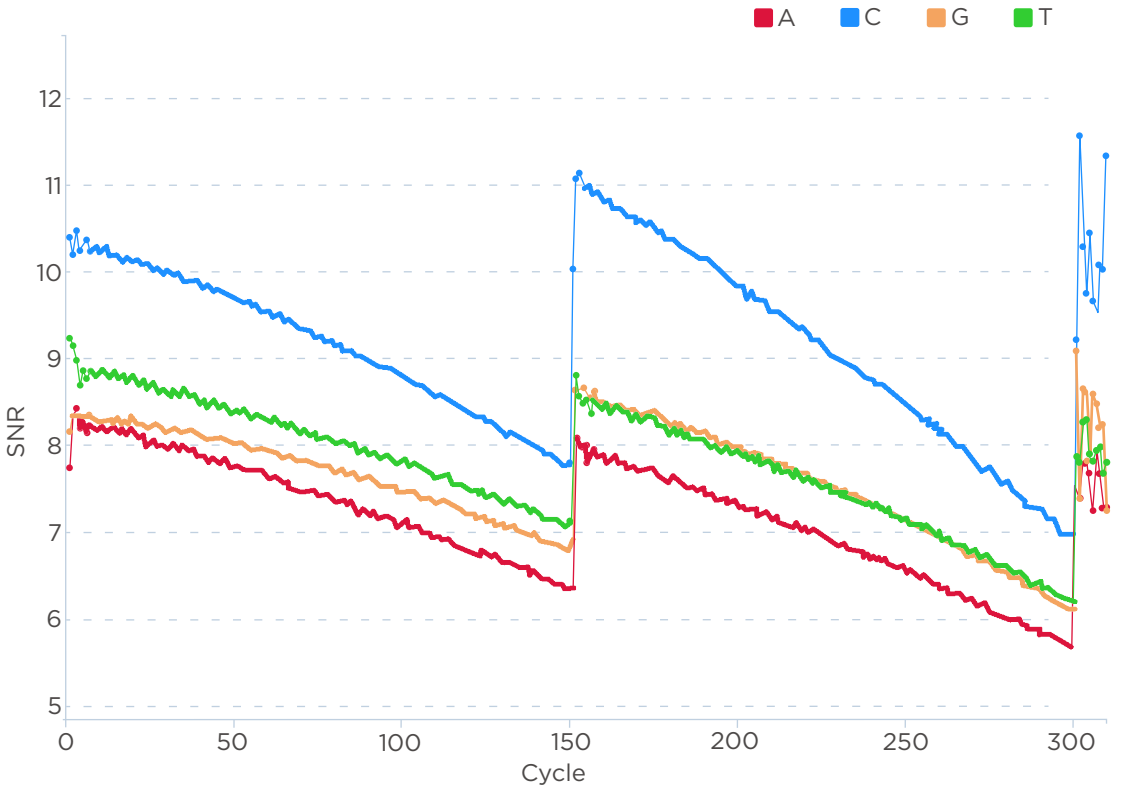


Figure 47 SNR

X axis	Cycle
Y axis	SNR: Signal to Noise Ratio.

A, T, C, and G represent the 4 base types and correspond to 4 channels of images.

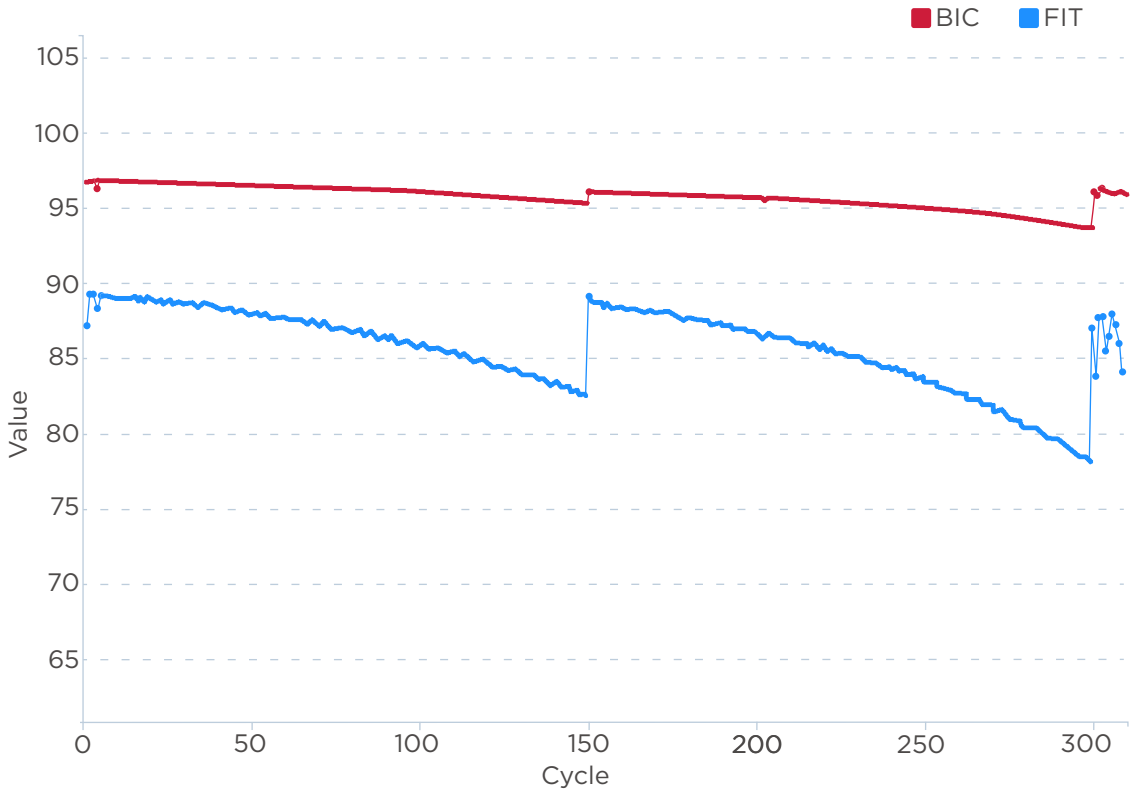


Figure 48 BIC And FIT

X axis	Cycle
Y axis	BIC and FIT Value: • BIC (Basecall Information Content): Percentage of spots that can be used for basecalling. • FIT (Least square fit to the DNB intensities in 4 color space to represent the overall quality of the clusters): Discrete degree of the signals of A/T/C/G bases.

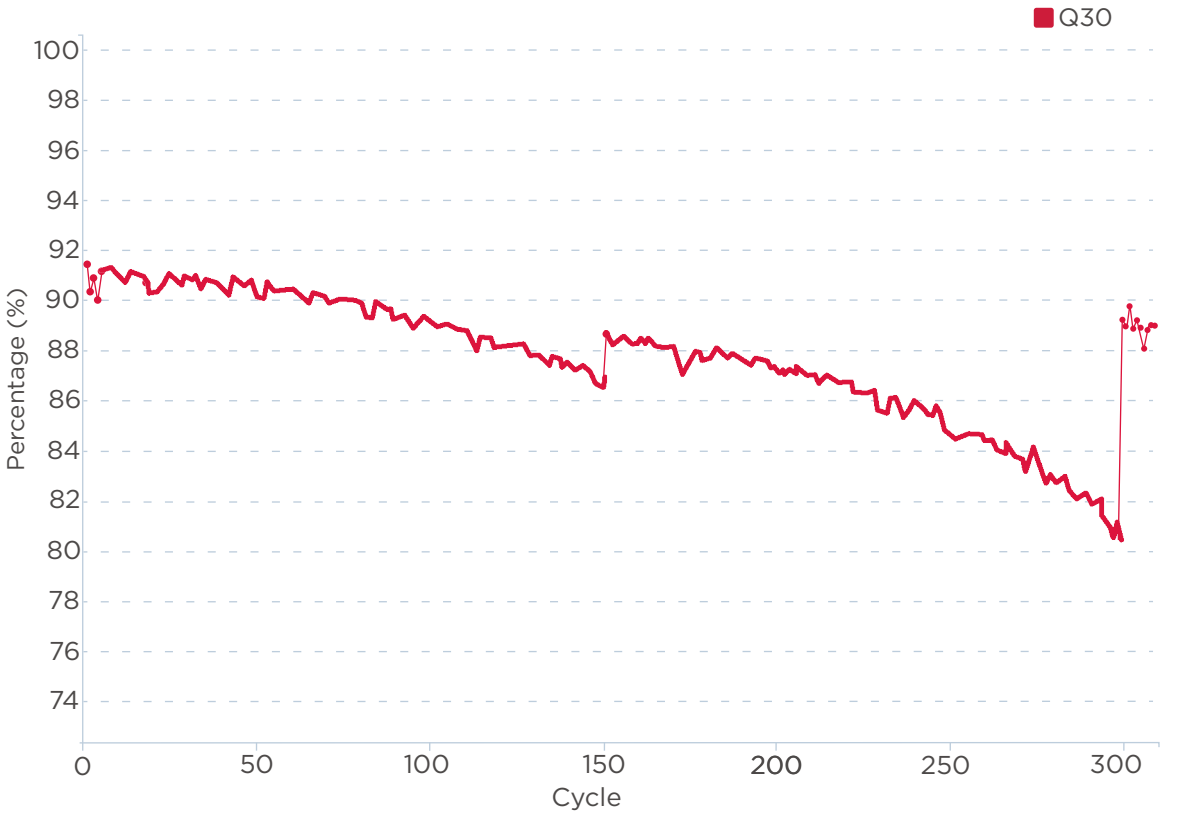


Figure 49 Unfiltered Q30

X axis	Cycle
Y axis	Percentage (%): The percentage of bases with a quality score of no less than 30 in each cycle before filtering.

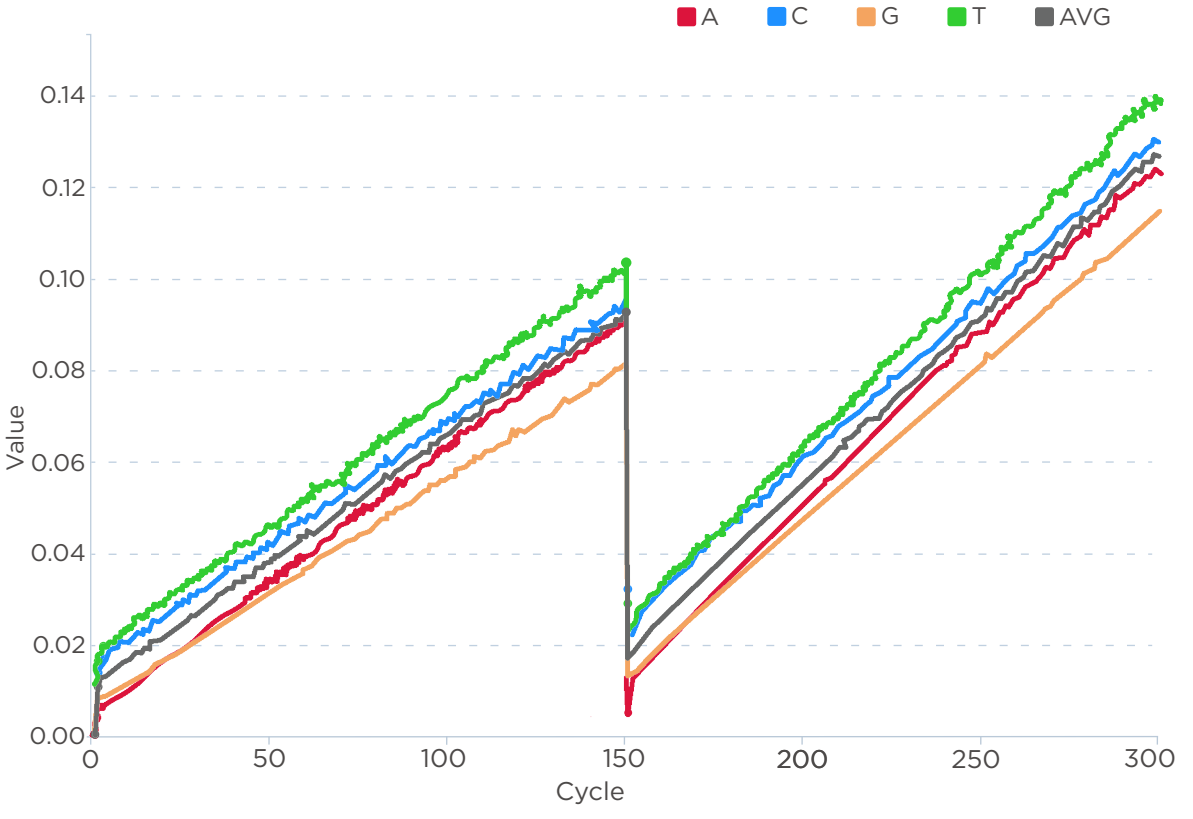


Figure 50 **Runon**

X axis	Cycle
Y axis	Runon: For a DNB with m copies of DNA fragments, while sequencing at cycle i, n copies of DNA fragments react at i+1 cycle, the runon is defined as n/m.

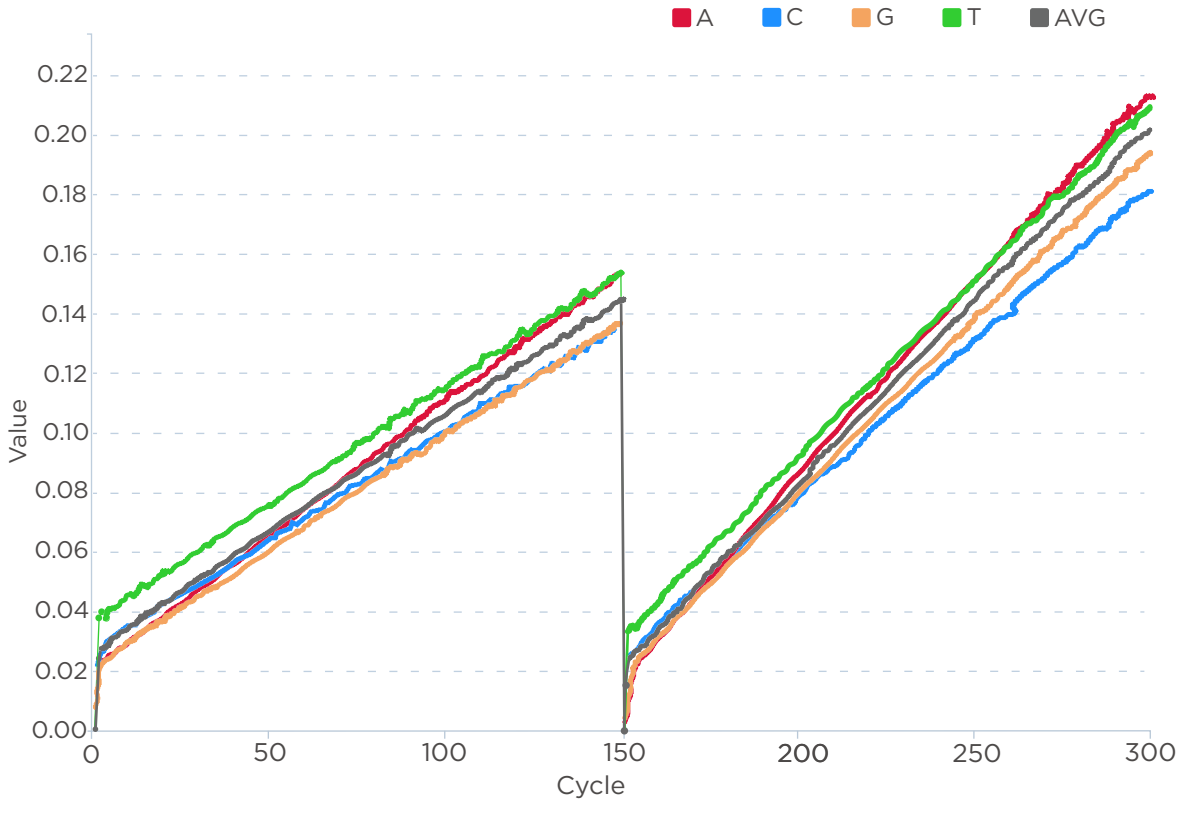


Figure 51 Lag

X axis	Cycle
Y axis	Value: Lag value for each cycle. Lag: For a given DNB with m copies of DNA fragments, while sequencing at cycle i, n copies of DNA fragments react at i-1 cycle, the Lag is defined as n/m.

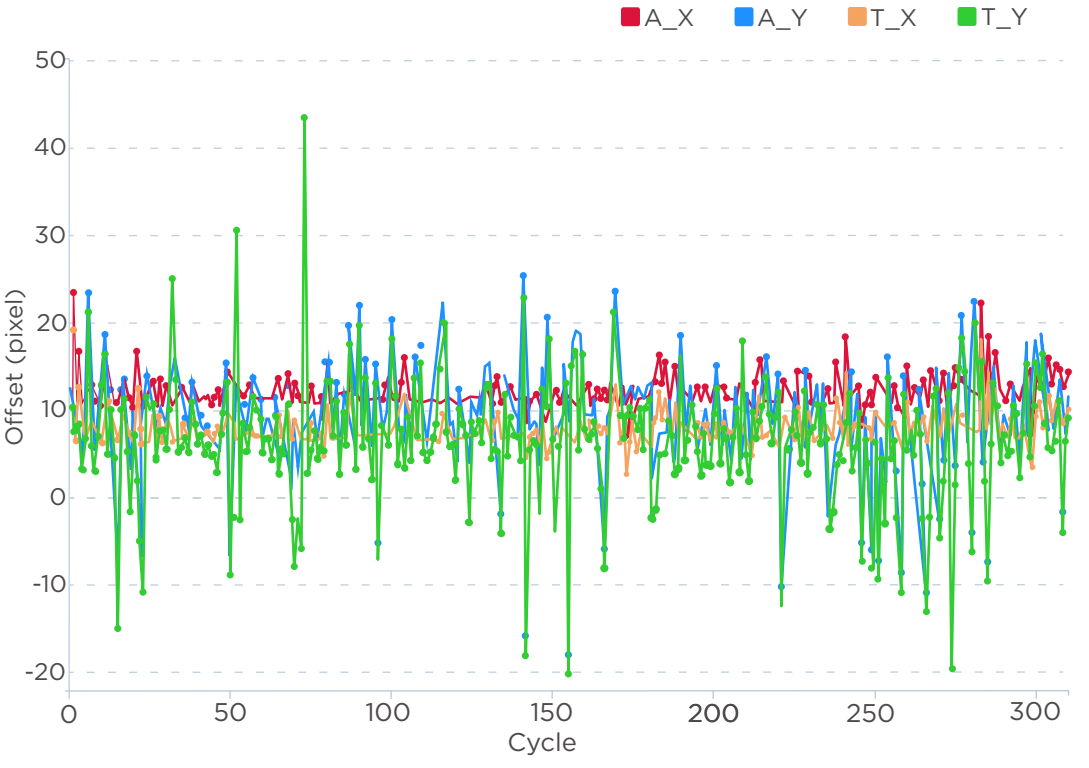


Figure 52 Offset

X axis	Cycle
Y axis	Offset: Offset value of each cycle, coordinate offset of the image center in the standard FOV, compared with the reference standard.

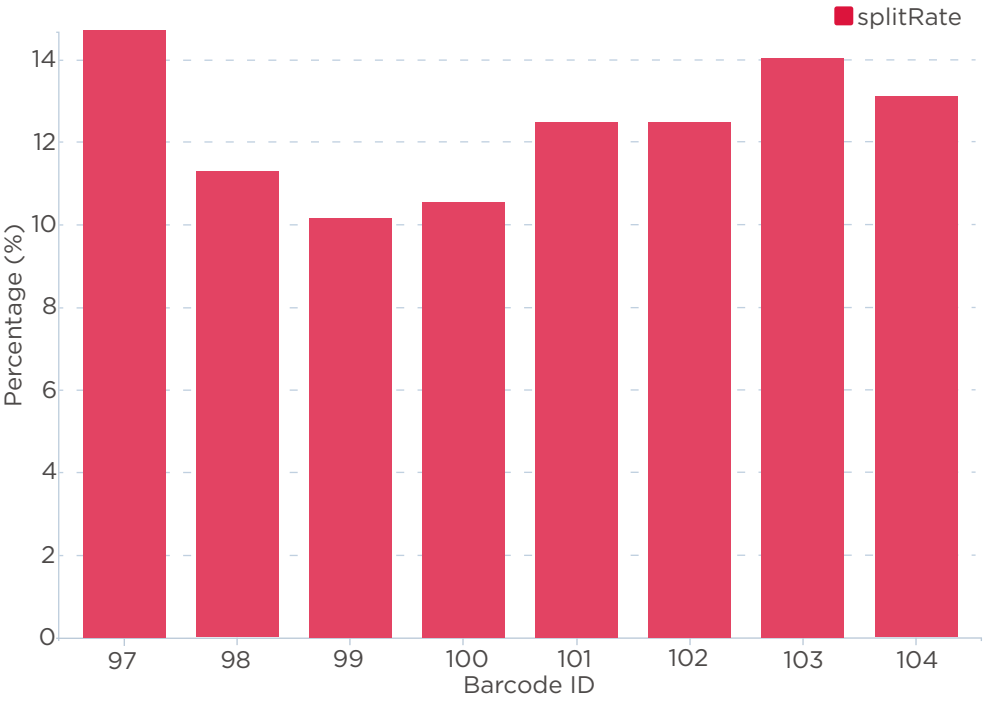


Figure 53 **Barcode Split Rate**

X axis	Barcode ID
Y axis	Percentage (%): A histogram that shows the percentage of the barcode when the split rate is greater than 0.5%.

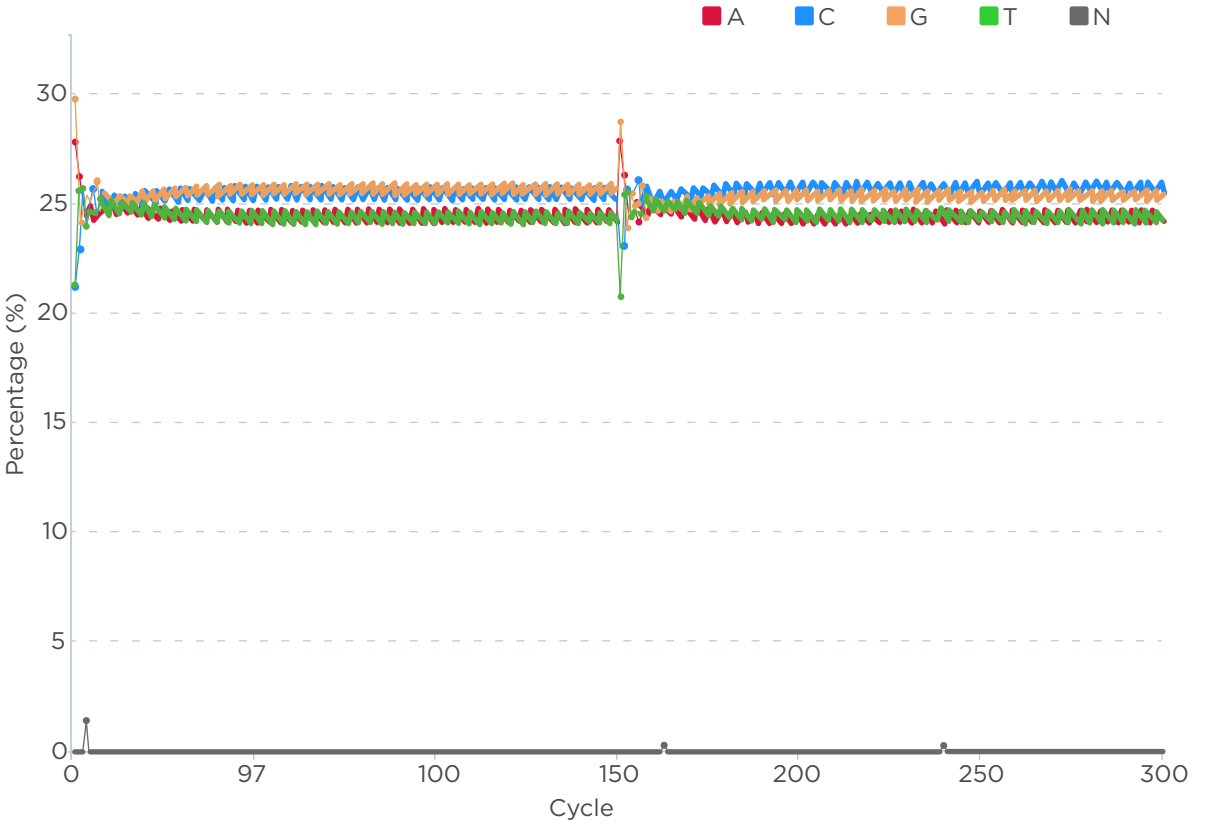


Figure 54 **Bases Distribution**

X axis	Cycle
Y axis	Percentage (%): Base distribution calculated from FASTQ.

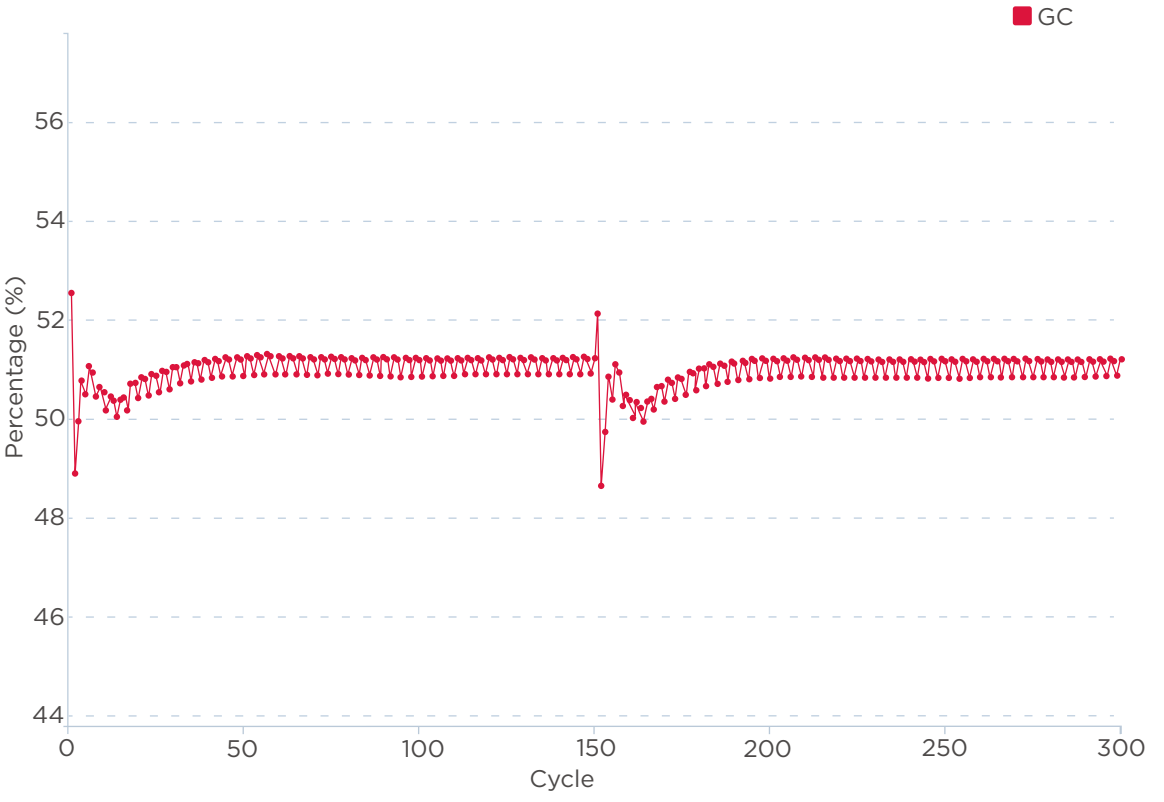


Figure 55 GC Distribution

X axis	Cycle
Y axis	Percentage (%): G+C percentage calculated from FASTQ.

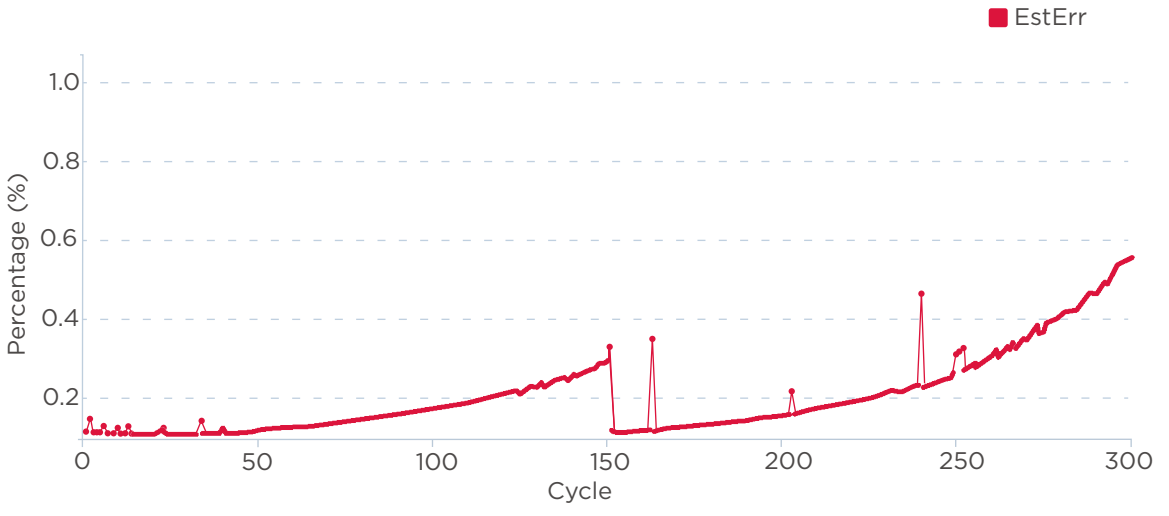


Figure 56 **Estimated Error Rate**

X axis	Cycle
Y axis	Percentage (%): The error rate that is estimated according to the Q value.

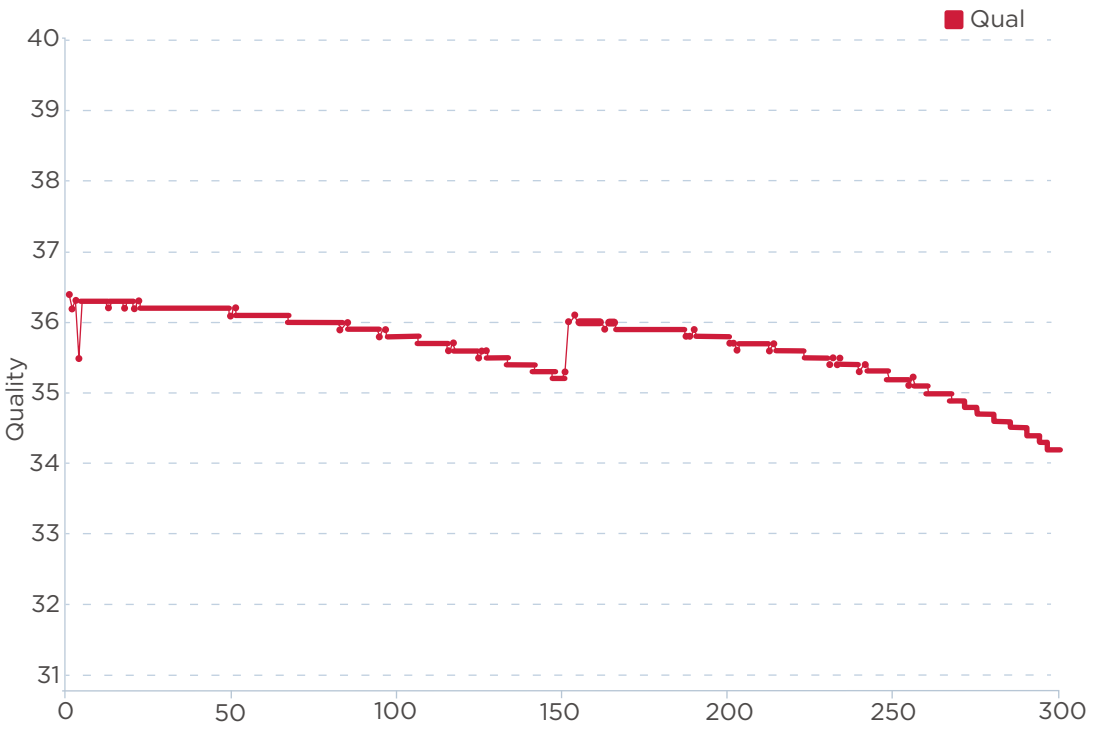


Figure 57 Average Quality Distribution

X axis	Cycle
Y axis	Quality: Average quality score distribution for each cycle.

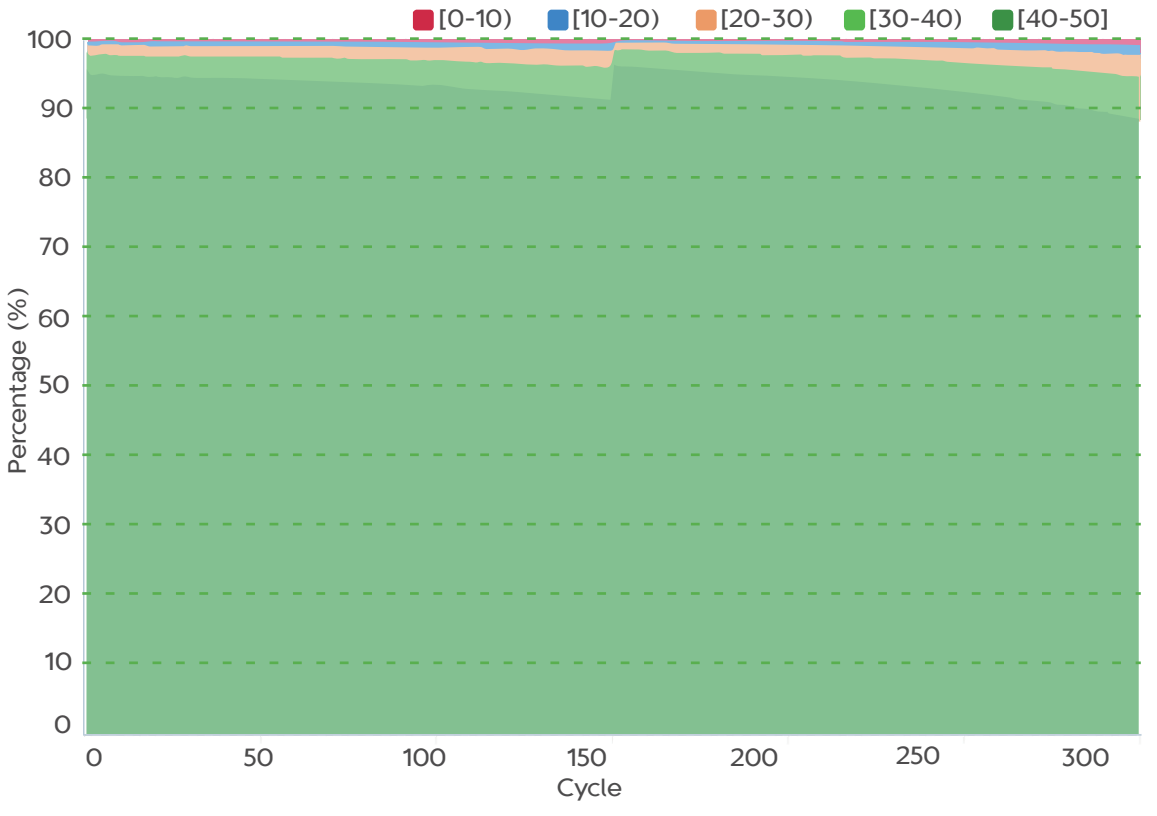


Figure 58 **Quality Proportion Distribution**

X axis	Cycle
Y axis	Percentage (%): Quality distribution for each quality score range.

Other reports

Table 49 Other report descriptions

Name	Description
XXXXXXXX_L0X.heatmapReport.html	Contains information on each FOV in the lane generated during sequencing, including AvgQ30, offset_x, offset_y, lag1, lag2, runon1, and runon2. *XXXXXXXX_L0X* represents: flow cell ID_Lane No.
XXXXXXXX_L0X.bestFovReport.html	The summary of the best FOV and basecall information during the entire sequencing run.
XXXXXXXX_L0X.allCycleHeatmap.html	Information in each FOV of every cycle, including LoadedDNB, Offset, Signal, Background, RHO, SNR, Q30, BIC, Fit, A-T, G-C, Lag, and Runon.

XXXXXXXX_L0X represents: flow cell ID_Lane No.

Data processing

Introduction

The sequencer processes the image files to generate a base call at each position of the read, and the base sequence information is saved in the FASTQ format. The FASTQ file and report file are both output using the split rate obtained by barcode analysis.

During a sequencing run, the control software will automatically generate *.cal* files in real time by the BasecallLite application. After the sequencing run has finished, the BasecallLite application will generate FASTQ files based on *.cal* files from all FOVs, either automatically (termed "Write FASTQ" on sequencer automatically), or manually (termed "Write FASTQ" on the sequencer manually).

The two Write FASTQ methods are described below.

Write FASTQ on sequencer automatically

After sequencing has started, the sequencing results generated by the control software will be saved in the D drive.

Bioinfo files and *.cal* files are contained within the Result folder named after the flow cell ID.

After the sequencing process has finished, the BasecallLite application will automatically write FASTQ files based on *.cal* files, and generate a summary report.

Write FASTQ on sequencer manually

This section describes how to write FASTQ manually in the following situations:

- The FASTQ generation fails after sequencing.
- The barcode file is selected incorrectly.
- There is a need to change some FASTQ parameters, including but not limited to, filtering of FASTQ file, barcode splitting, and selection of SaveDiscardedReads.

Preparation before writing FASTQ manually

Perform the following steps:

1. Ensure that the sequencer is in idle status and not in the sequencing or base calling phase.
2. Check whether the *.cal* file generation is complete or not.

   Also check whether the number of Metrics files for one lane is consistent with the total cycle number.

   For example, set sequencing parameters with the following assumptions:

   - Sequencing run: PE150+10
   - Length of Read1: 150
   - Length of Read2: 150
   - Length of Barcode: 10
   - Total cycles = 150 + 1 + 150 + 1 + 10 = 312

   When checking the Metrics file path, as shown below, ensure that the expected Metrics file number is 312:

   D:\Result\workspace\V3XXXXXX\L0X\Metrics

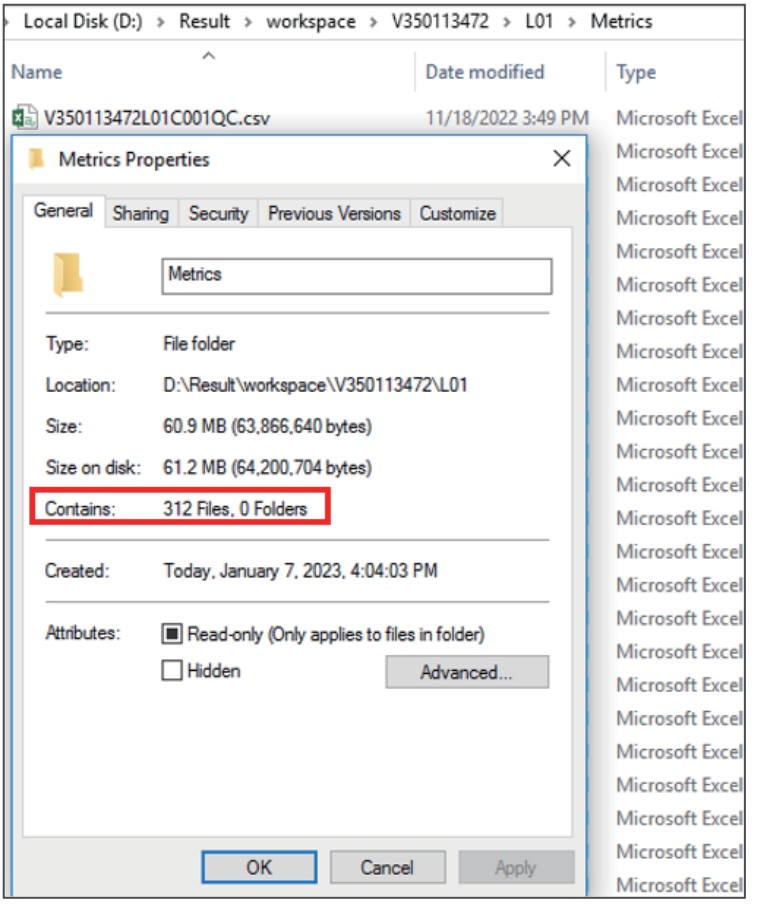


Figure 59 Metrics file number

3. Rename the original FASTQ folder. For example, rename *V3000XXXX* to *V3000XXXX_old*, or to *L01 rename to L01_old*.

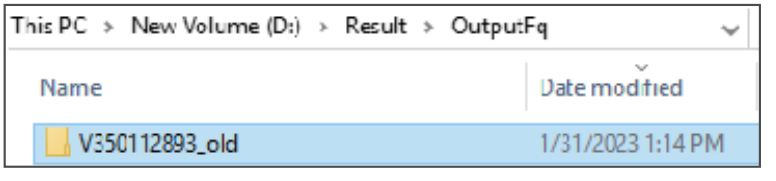


Figure 60 Renaming the FASTQ folder

4. Prepare the barcode file that you need to write FASTQ manually.
   For details, refer to *Instructions for importing barcode on Page 153.*

   Using the wrong barcode file to write FASTQ manually may cause a failure to split barcode correctly or may report an error as the result of incorrect formatting.

BasecallLite (Litecall) write FASTQ manually

Perform the following steps:

1. Open the *C:\BasecallLite\1.5.0.323\Config* folder, select the *Client.ini* file, and right-click **Edit with Notepad++** to open.

   - Select the corresponding software version folder according to the current version of the sequencer. The current basecall software version may vary among sequencers.
   - It is recommended that you back up the *Client.ini* file before editing it.

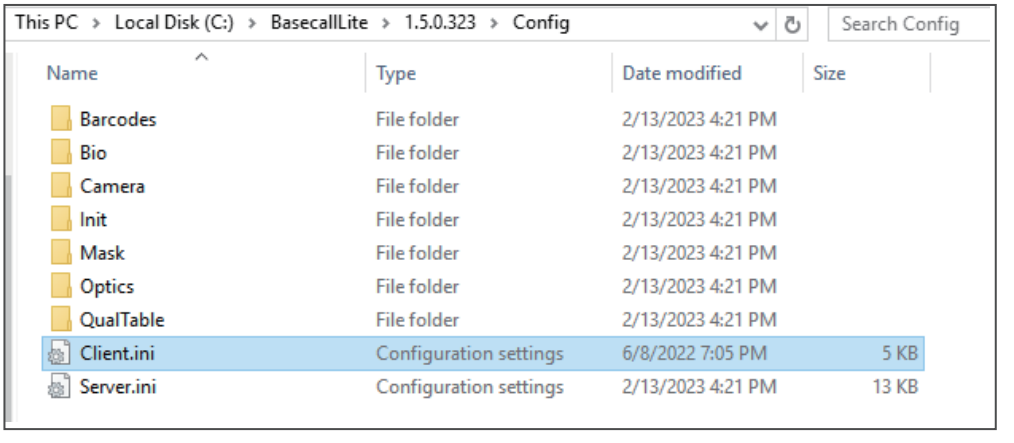


Figure 61 Location of Client.ini file

2. Edit the *Client.ini* file as follows.

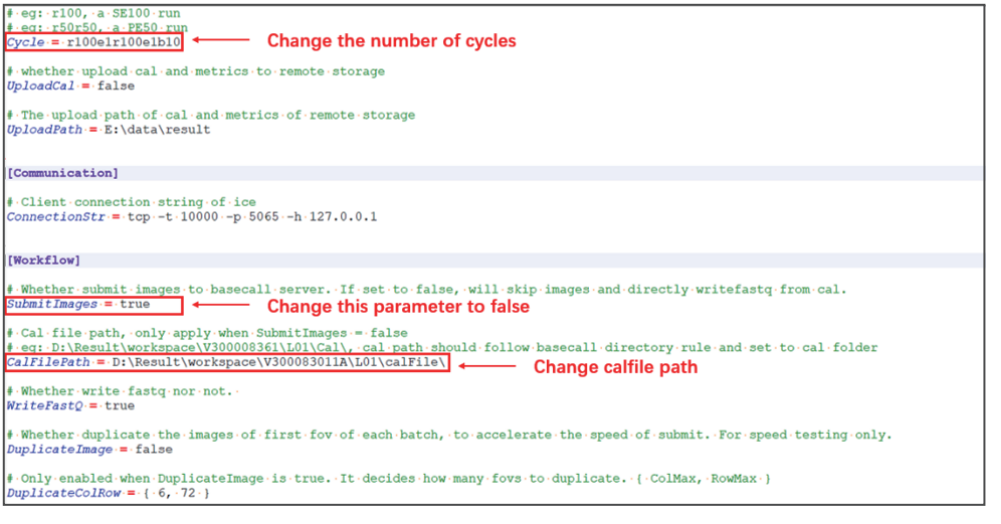


Figure 62 Editing Client.ini file

Table 50 Parameters

Parameter settings	Description
Change the number of cycles	Cycle=r[Read1cycle number]e1r[Read2cycle number] e1b[dualbarcode cycle number]b[barcode cycle number]. e1 means end cycle process mode. Assumptions: • PE100+10(101+101+10), Cycle=r100e1r100e1b10 • PE100+10(100+100+10), Read1 dark reaction cycle: 1-10 Read2 dark reaction cycle: 161-180 Cycle=r90e1r80e1b10 • PE100+10+10(101+101+10+10), Cycle=r100e1r100e1b10b10 • PE100+10+42(101+101+10+42), Cycle=r100e1r100e1b10b42
Change SubmitImages value from true to false	This parameter setting indicates writing FASTQ from *.cal* file.
Change *.cal* file path	This parameter describes the *.cal* file storage path. For details, refer to *Figure 63 on Page 117*
Change BarcodeType	BarcodeType=0, User define barcode, if BarcodeType=0, you need to change the barcode file path. BarcodeType=1, 501-596, 10 bp, mismatch is 1. BarcodeType=2, 1-128, 6 bp, mismatch is 1. BarcodeType=3, 1-128, 10 bp, mismatch is 2.
Change BarcodeFile path	You need to input the barcode file path here if you use a user defined barcode. *i* Comment text is displayed in green in the file. Refer to the comments to modify the relevant parameters.

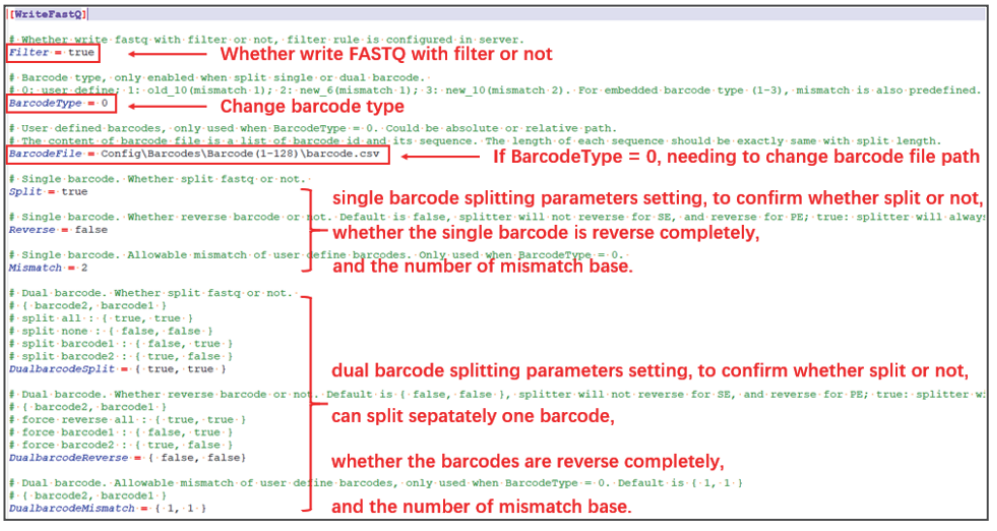


Figure 63 Changing .cal file path and barcode splitting parameters

3. Select **Save** and close the *Client.ini* file.
4. Execute the manual write FASTQ command.
   Use either method below to run the write FASTQ program.
   - Method 1
     a. Open the Task Manager, select the **Services** tab, and start LiteCall.

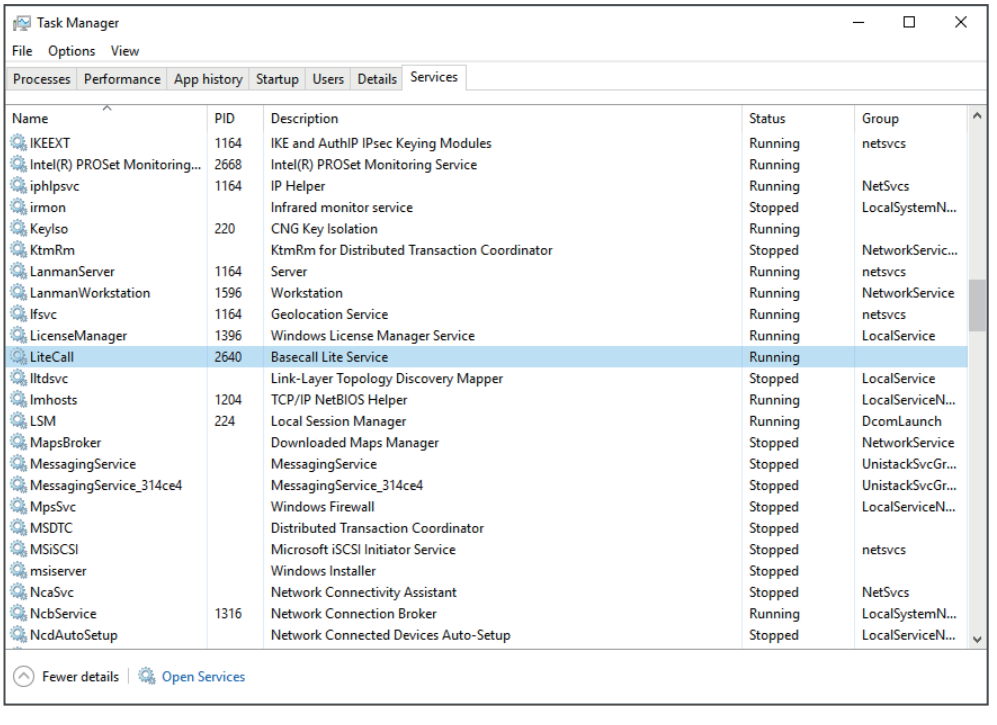


Figure 64 Starting LIteCall service

b. Open the *C:\BasecallLite\1.5.0.323* folder, and double-click to open *MGI.Basecall.Client.exe* program, and run the write FASTQ program.

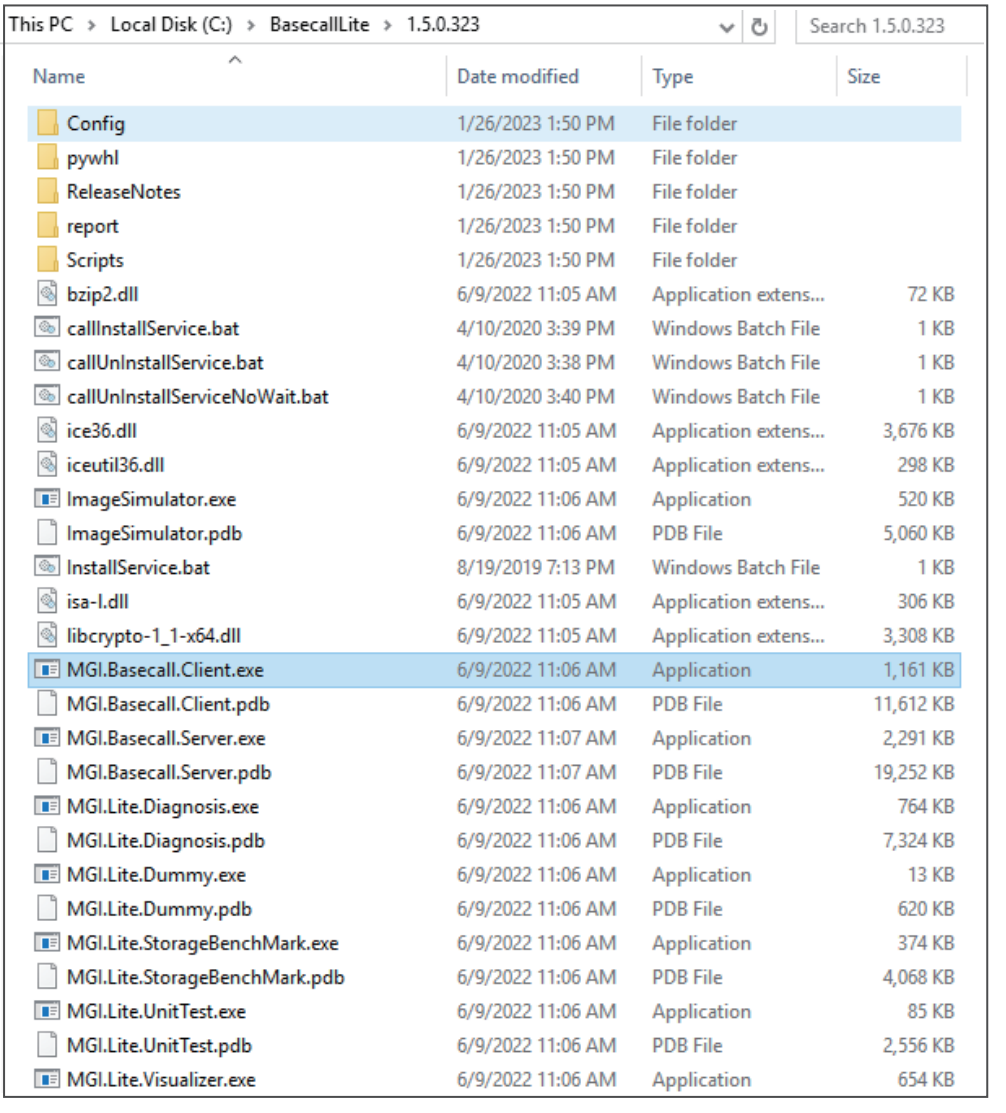


Figure 65 Opening the MGI.Basecall.Client.exe program

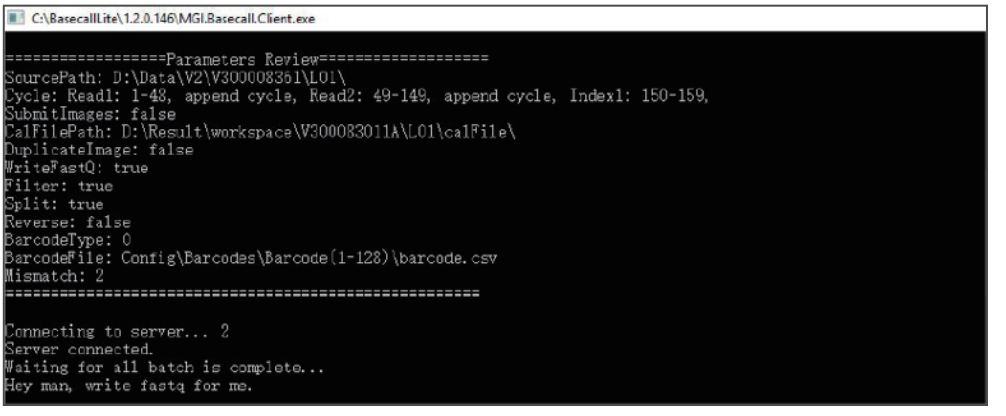


Figure 66 Starting the MGI.Basecall.Client.exe program

- Method 2

  a. Open the Task Manager, select the **Services** tab, and stop Litecall.

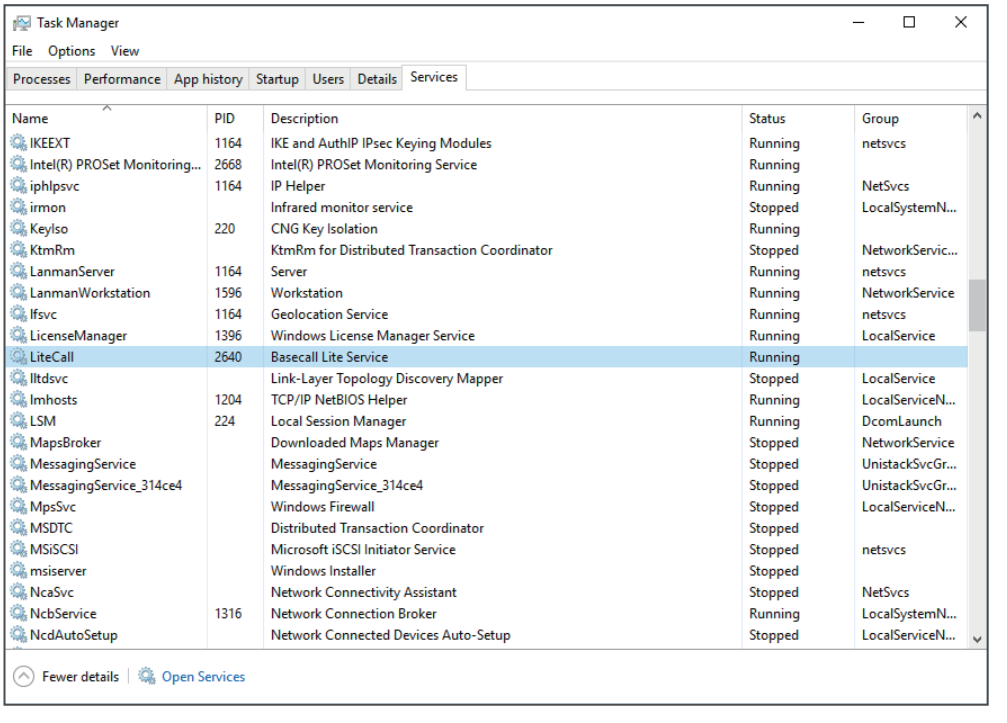


Figure 67 Stopping the LiteCall service

b. Open the *C:\BasecallLite\1.5.0.323* folder, and double-click to open the *MGI.Basecall.Server.exe*.

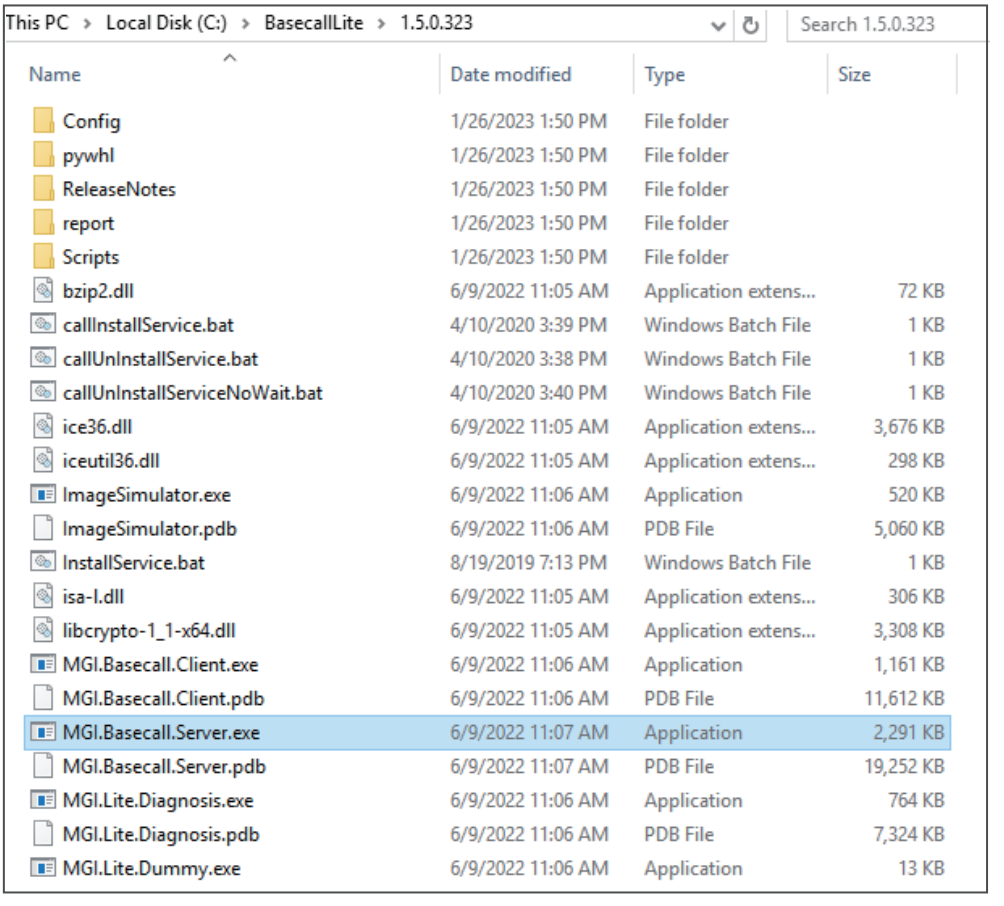


Figure 68 Opening the MGI.Basecall.Server.exe

c. Double-click to open *MGI.Basecall.Client.exe*, and then run the write FASTQ program.

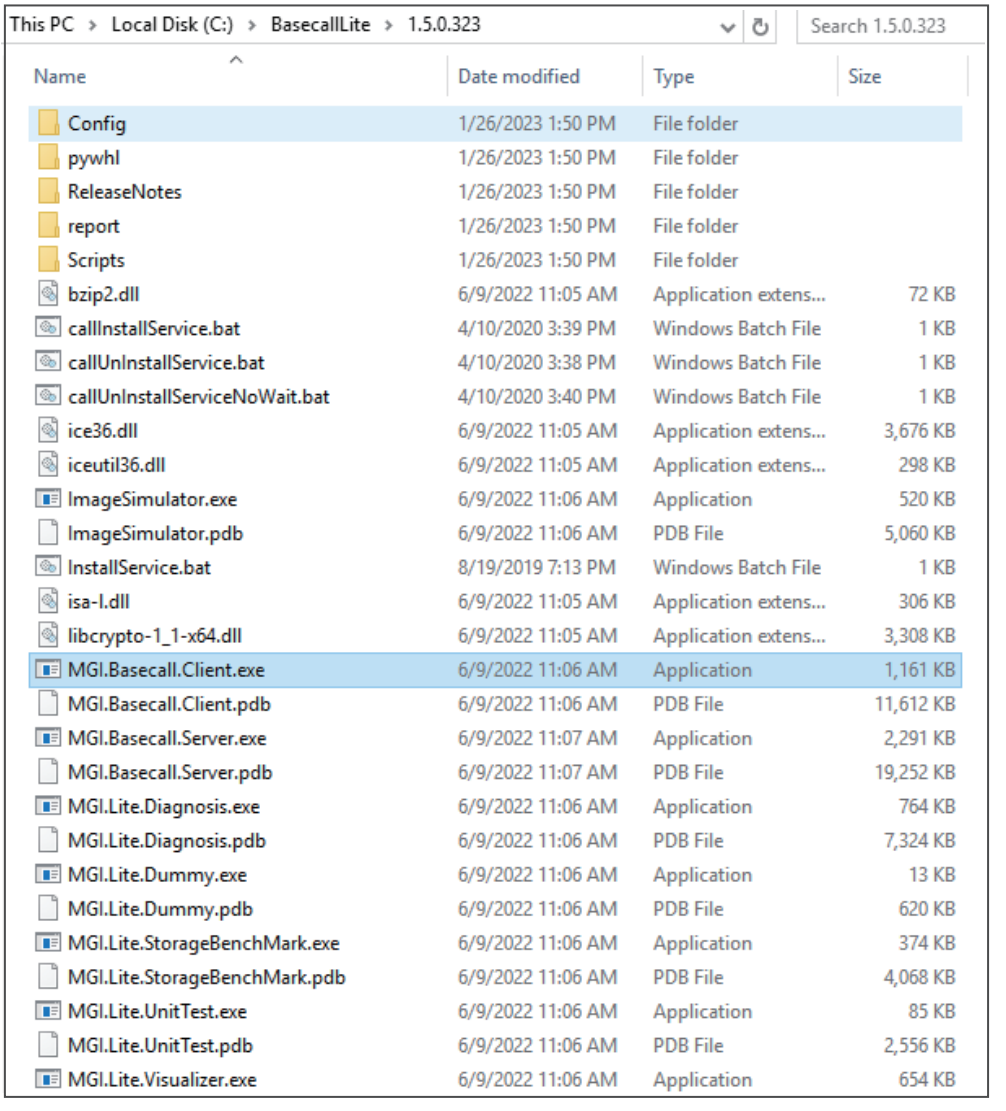


Figure 69 Opening the MGI.Basecall.Client.exe

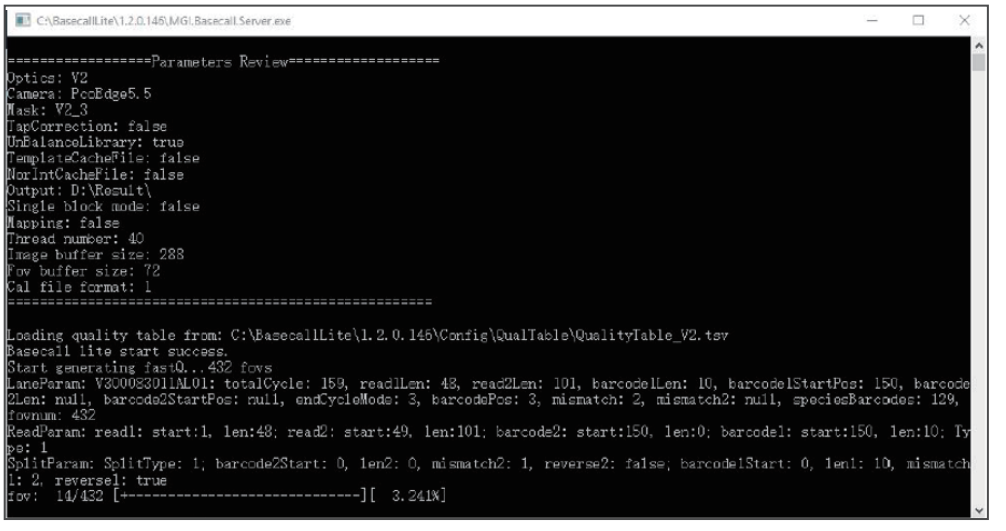


Figure 70 **Starting the MGI.Basecall.Server.exe**

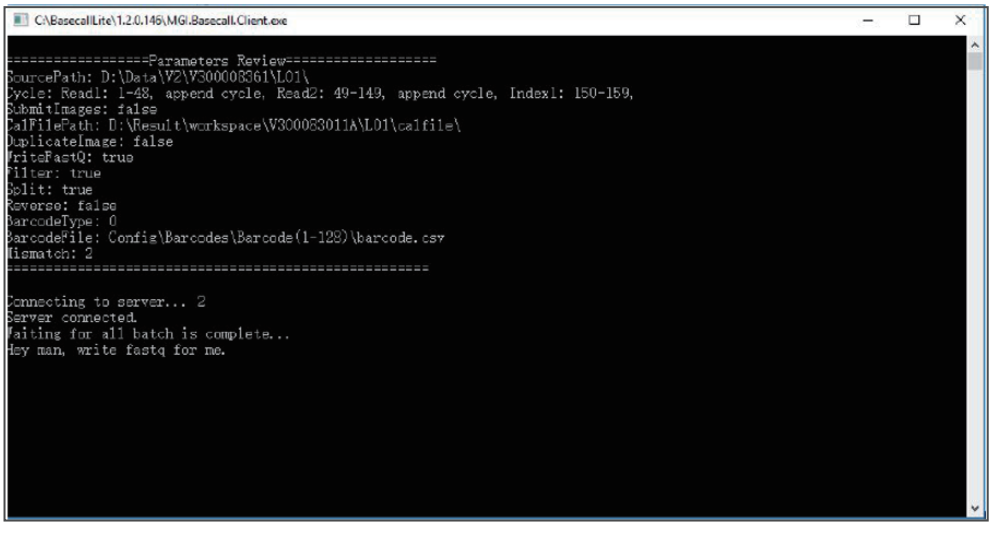


Figure 71 **Starting the MGI.Basecall.Client.exe**

The advantage of this method is that you can view the progress of the write FASTQ process in the *Server.exe* program.

Example of parameter setting (PE100+10+8)

Perform the following steps:

1. Set sequencing parameters with the following assumptions:
   - Sequencing run: PE100+10+8
   - Length of Read1: 100
   - Length of Read2: 100
   - Length of Dual barcode: 10
   - Length of barcode1: 8
   - *.cal* file path: *D:\Result\workspace\V350060903\L02\calFile*

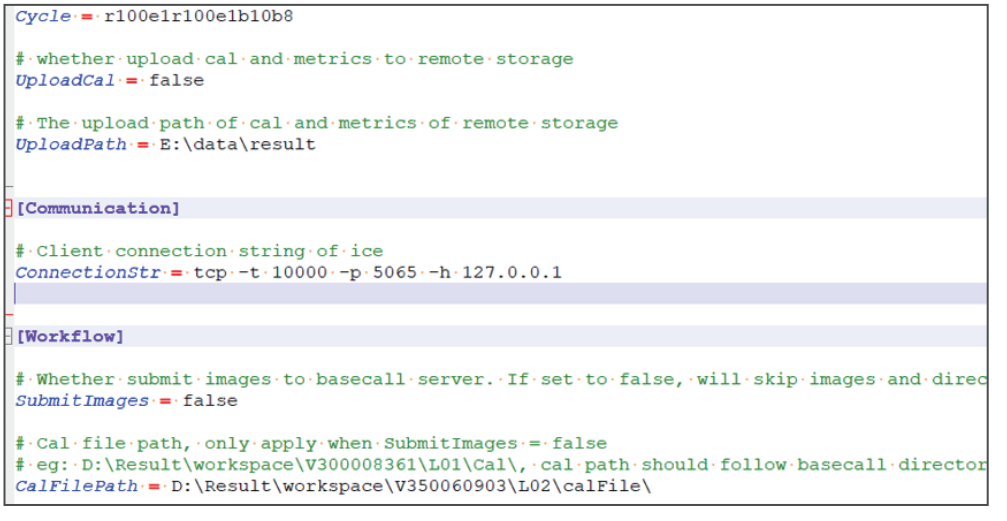


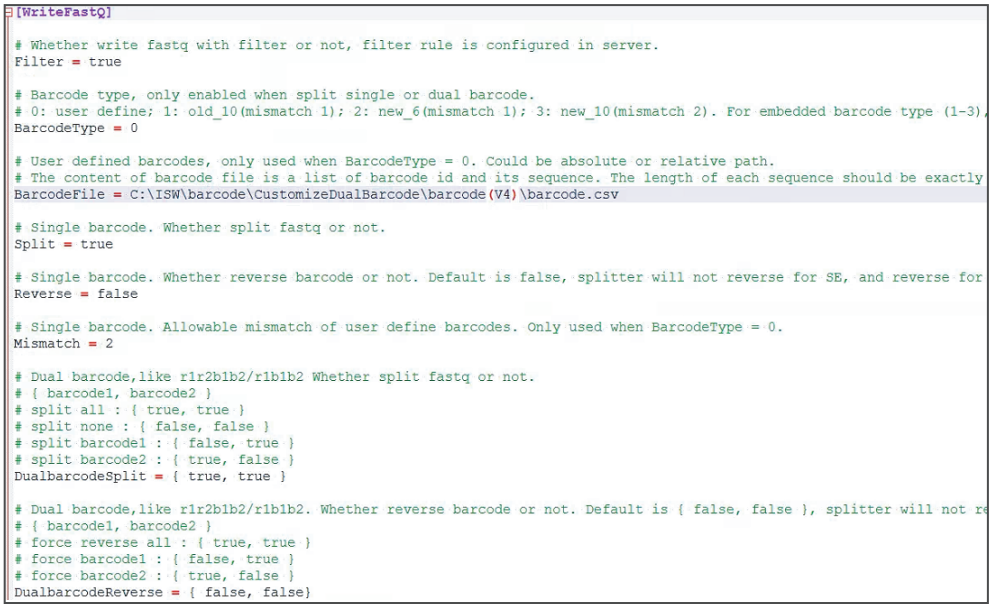
Figure 72 Parameter settings

These parameter settings are consistent, regardless of whether or not the barcode is split.

2. Set Barcode file for barcode splitting.
   - Splitting both barcode1 and barcode2:

     BarcodeFile path:

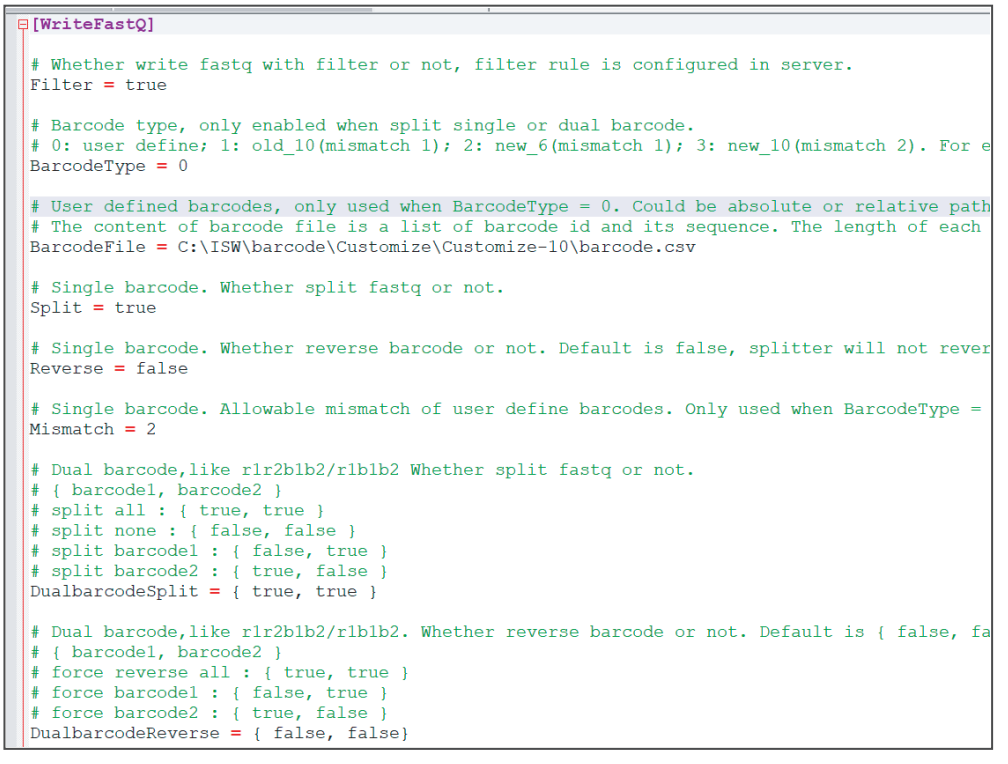
     C:\ISW\barcode\CustomizeDualBarcode\barcode(V4)\barcode.csv



```
[WriteFastQ]

# Whether write fastq with filter or not, filter rule is configured in server.
Filter = true

# Barcode type, only enabled when split single or dual barcode.
# 0: user define; 1: old_10(mismatch 1); 2: new_6(mismatch 1); 3: new_10(mismatch 2). For embedded barcode type (1-3),
BarcodeType = 0

# User defined barcodes, only used when BarcodeType = 0. Could be absolute or relative path.
# The content of barcode file is a list of barcode id and its sequence. The length of each sequence should be exactly
BarcodeFile = C:\ISW\barcode\CustomizeDualBarcode\barcode(V4)\barcode.csv

# Single barcode. Whether split fastq or not.
Split = true

# Single barcode. Whether reverse barcode or not. Default is false, splitter will not reverse for SE, and reverse for
Reverse = false

# Single barcode. Allowable mismatch of user define barcodes. Only used when BarcodeType = 0.
Mismatch = 2

# Dual barcode,like r1r2b1b2/r1b1b2 Whether split fastq or not.
# { barcode1, barcode2 }
# split all : { true, true }
# split none : { false, false }
# split barcode1 : { false, true }
# split barcode2 : { true, false }
DualbarcodeSplit = { true, true }

# Dual barcode,like r1r2b1b2/r1b1b2. Whether reverse barcode or not. Default is { false, false }, splitter will not re
# { barcode1, barcode2 }
# force reverse all : { true, true }
# force barcode1 : { false, true }
# force barcode2 : { true, false }
DualbarcodeReverse = { false, false}
```

Figure 73 Splitting both barcode1 and barcode2

- Splitting barcode2 only:



```
[WriteFastQ]

# Whether write fastq with filter or not, filter rule is configured in server.
Filter = true

# Barcode type, only enabled when split single or dual barcode.
# 0: user define; 1: old_10(mismatch 1); 2: new_6(mismatch 1); 3: new_10(mismatch 2). For e
BarcodeType = 0

# User defined barcodes, only used when BarcodeType = 0. Could be absolute or relative path
# The content of barcode file is a list of barcode id and its sequence. The length of each
BarcodeFile = C:\ISW\barcode\Customize\Customize-10\barcode.csv

# Single barcode. Whether split fastq or not.
Split = true

# Single barcode. Whether reverse barcode or not. Default is false, splitter will not rever
Reverse = false

# Single barcode. Allowable mismatch of user define barcodes. Only used when BarcodeType =
Mismatch = 2

# Dual barcode,like r1r2b1b2/r1b1b2 Whether split fastq or not.
# { barcode1, barcode2 }
# split all : { true, true }
# split none : { false, false }
# split barcode1 : { false, true }
# split barcode2 : { true, false }
DualbarcodeSplit = { true, true }

# Dual barcode,like r1r2b1b2/r1b1b2. Whether reverse barcode or not. Default is { false, fa
# { barcode1, barcode2 }
# force reverse all : { true, true }
# force barcode1 : { false, true }
# force barcode2 : { true, false }
DualbarcodeReverse = { false, false}
```

Figure 74 Splitting barcode2 only

- Splitting barcode1 only:

```
[WriteFastQ]

# Whether write fastq with filter or not, filter rule is configured in server.
Filter = true

# Barcode type, only enabled when split single or dual barcode.
# 0: user define; 1: old_10(mismatch 1); 2: new_6(mismatch 1); 3: new_10(mismatch 2). For embe
BarcodeType = 0

# User defined barcodes, only used when BarcodeType = 0. Could be absolute or relative path.
# The content of barcode file is a list of barcode id and its sequence. The length of each seq
BarcodeFile = C:\ISW\barcode\Customize\Customize-8\barcode.csv

# Single barcode. Whether split fastq or not.
Split = true

# Single barcode. Whether reverse barcode or not. Default is false, splitter will not reverse
Reverse = false

# Single barcode. Allowable mismatch of user define barcodes. Only used when BarcodeType = 0.
Mismatch = 2

# Dual barcode,like r1r2b1b2/r1b1b2 Whether split fastq or not.
# { barcode1, barcode2 }
# split all : { true, true }
# split none : { false, false }
# split barcode1 : { false, true }
# split barcode2 : { true, false }
DualbarcodeSplit = { true, true }

# Dual barcode,like r1r2b1b2/r1b1b2. Whether reverse barcode or not. Default is { false, false
# { barcode1, barcode2 }
# force reverse all : { true, true }
# force barcode1 : { false, true }
# force barcode2 : { true, false }
DualbarcodeReverse = { false, false}
```

Figure 75 Splitting barcode1 only

3. Change the SaveDiscardedReads setting.

```
[WriteFastQ]  File: Server.ini

# true: partial filter, false: global filter
# true: partial filter, false: global filter
PartialFilter = false

# Use in global filter
FilterReadLen = 100

# Whether save the reads which were discarded by filter
SaveDiscardedReads = false   <-- Change false to true

# Whether generate md5 for each fq file
GenMD5 = true

# Base order, {0, 1, 2, 3} means ACGT
BaseOrder = { 0, 1, 2, 3 }
```

Figure 76 Changing the SaveDiscardedReads setting

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07

Device maintenance

This chapter describes maintenance procedures for the device and its components. Perform maintenance regularly to ensure that the device runs smoothly.

DANGER

- To prevent personal injury, ensure that the device is powered off before cleaning or disinfecting.
- To prevent device damage, do not spray the wash solutions or disinfectants into the device during cleaning or disinfecting.

WARNING

- It is not recommended that you use other disinfectants or wash solutions, except for those that are mentioned in this guide. Other solutions are not verified for use and their effects on the device are unknown.
- If you have questions about the compatibility of wash solutions, contact CG Technical Support.

Service plan

A free preventive maintenance service is provided in the first year during the warranty period. To purchase additional services, contact CG Technical Support.

Sequencer maintenance

Wash

Wash type introduction

There are four different wash types based on sequencer conditions:

Table 51 Wash types

Wash type	Wash selection	Cartridge type	Description
Pre-run wash	Regular (54 min)	Cleaning cartridge 1 (Laboratory-grade water)	• Before and after each sequencing run. • It has been more than 24 h since the last maintenance wash.

Wash type	Wash selection	Cartridge type	Description
Maintenance wash	Maintenance (20 min)	Cleaning cartridge 3 (Tween-20)	• After a sequencing run. • During initial installation or upgrade installation. • Weekly if the sequencer has been used. • Biweekly if the sequencer is idle or powered off. • When impurities are visible in the image. • After the sequencer maintenance is performed by an engineer. This includes, but is not limited to the replacement of pipelines, sample needles, and other accessories exposed to reagents.
	Maintenance (20 min)	Cleaning cartridge 2 (NaOH)	
	Regular (54 min)	Cleaning cartridge 1 (Laboratory-grade water)	
Ultra deep wash	Ultra deep (11 h)	Cleaning cartridge 1 (Laboratory-grade water)	• During initial installation or upgrade installation. • The sequencer remains unwashed for more than 14 days. • After the sequencer maintenance is performed by an engineer. This includes, but is not limited to the replacement of pipe lines, sample needles and other accessories exposed to reagents.
DNBTube wash (Optional)	DNBTube (5 min)	Cleaning cartridge	If you need an extra wash for the DNB loading tubes after pre-wash and maintenance wash.
	DNBTube (5 min)		
	DNBTube (5 min)		

Preparing for wash

Preparing washing reagents

Prepare the washing reagents according to the tables below:

Table 52 Washing reagent 1: 0.05% Tween-20

Reagent name	Volume (mL)	Final concentration
100% Tween-20	0.5	0.05%
Laboratory-grade water	999.5	/
Total volume	1000	
Validity period	1 month at 2 °C to 8 °C	

Table 53 Washing reagent 2: 0.05% Tween-20+1 M NaCl

Reagent name	Volume (mL)	Final concentration
100% Tween-20	0.5	0.05%
5 M NaCl solution	200	1 M
Laboratory-grade water	799.5	/
Total volume	1000	
Validity period	1 month at 2 °C to 8 °C	

Table 54 Washing reagent 3: 0.1 M NaOH

Reagent name	Volume (mL)	Final concentration
2 M NaOH	50	0.1 M
Laboratory-grade water	950	/
Total volume	1000	
Validity period	1 month at 2 °C to 8 °C	

Preparing cleaning cartridges, DNB loading needle washing tubes, and washing flow cell

Preparing cleaning cartridges

Fill the cleaning cartridges with washing reagents according to the table below:

Table 55 Reagents and volume of the cleaning cartridges

Cleaning cartridges	Well position	Washing reagent	Volume (mL)
Cleaning cartridge 1	1, 9, 10	Laboratory-grade water	300
	2		380
	17, 18		700
	3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16 (all small wells)		9
Cleaning cartridge 2	1, 2, 9, 10, 17, 18	Washing reagent 3: 0.1 M NaOH	50
	3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16 (all small wells)		8.5
Cleaning cartridge 3	1, 2, 9, 10, 17, 18	Washing reagent 1: 0.05% Tween-20	50
	3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 16 (all small wells, except 15)		8.5
	15 (small well)	Washing reagent 2: 0.05% Tween-20+1 M NaCl	8.5

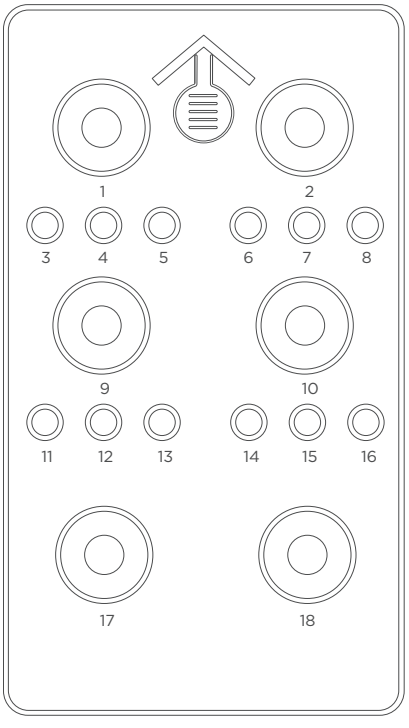

Figure 77 **Top view of cleaning cartridge**

- Large wells are No. 1, 2, 9, 10, 17, 18.
- Small wells are No. 3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16.

Preparing DNB loading needle washing tubes

Prepare the 2.0 mL sterile microcentrifuge tube for DNB loading needle washing according to the table below:

Table 56 **Reagents and volume of DNB loading needle washing tubes**

DNB loading needle washing tubes	Washing reagent	Volume (mL)
DNB loading needle washing tube 1	Laboratory-grade water	1.8
DNB loading needle washing tube 2	Washing reagent 3: 0.1 M NaOH	1.8
DNB loading needle washing tube 3	Washing reagent 1: 0.05% Tween-20	1.8

Preparing the washing flow cell

A used flow cell without physical damage can be used as a washing flow cell.

Each washing flow cell, stored at room temperature or in a 2 °C to 8 °C refrigerator, can be reused 20 times.

Performing a wash

Selecting wash

When the sequencing run is completed, the device must be washed within 24 h. When the following interface appears, select **Wash** and perform the wash procedures.

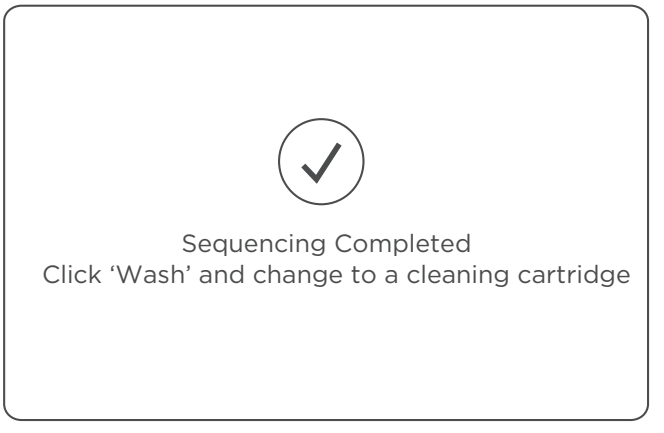

Figure 78 Wash instructions interface

Performing a pre-run wash (~54 min)

Perform the following steps:

1. Slowly insert the Cleaning cartridge 1 into the reagent compartment by following the direction printed on the cartridge cover until it stops.

   Two empty reagent cartridges are provided with the kit. To purchase a new empty reagent cartridge, order it by the catalog number according to *Order information on Page 207*.

2. Place the DNB loading needle washing tube 1 (2.0 mL sterile microcentrifuge tube with 1.8 mL Laboratory-grade water) into the DNB tube rack. Close the reagent compartment door.
3. Load the washing flow cell. Ensure that the washing flow cell is properly loaded. For details, refer to *Loading the flow cell on Page 82*.

4. Select **Wash** in the main interface. Select **Regular** from the **Wash type** list to start pre-run wash, which takes approximately 54 min.

   When you perform the wash, observe the status of the washing flow cell. If bubbles are observed, stop the wash, replace the flow cell, and restart the wash. If no bubbles are observed, continue the wash.

Figure 79 Selecting the wash type

Performing a maintenance wash (~94 min)

Perform the following steps:

1. Slowly insert the Cleaning cartridge 3 into the reagent compartment by following the direction printed on the cartridge cover until it stops.

   Two empty reagent cartridges are provided with the kit. To purchase a new empty reagent cartridge, order it by the catalog number according to *Order information on Page 207*.

2. Place the DNB loading needle washing tube 3 (2.0 mL sterile microcentrifuge tube with 1.8 mL Tween-20) into the DNB tube rack. Close the reagent compartment door.
3. Place the washing flow cell on the stage. Ensure that the washing flow cell is properly seated.

   For details, refer to *Loading the flow cell on Page 82*.

4. Select **Wash** in the main interface. Select **Maintenance** from the **Wash type** list to start the wash. The wash takes approximately 20 min.

Figure 80 Selecting Maintenance

5. When the interface appears as shown below, select **Yes** and the sequencer will automatically lift the sampling needles.

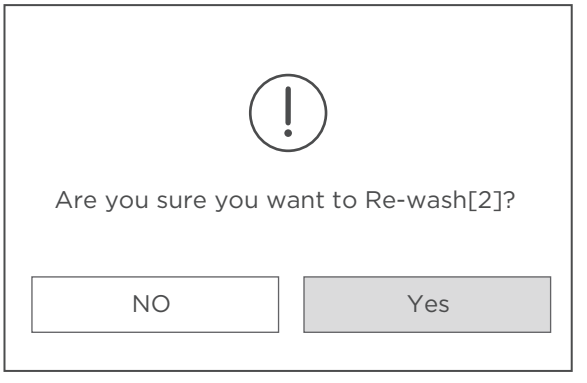

Figure 81 **Maintenance wash [2] prompt**

6. Slowly insert the Cleaning cartridge 2 into the reagent compartment by following the direction printed on the cartridge cover until it stops.
7. Place the DNB loading needle washing tube 2 (2.0 mL sterile microcentrifuge tube with 1.8 mL NaOH) into the DNB tube rack. Close the reagent compartment door.
8. Select **Wash** in the main interface. Select **Maintenance** from the **Wash type** list to start the wash. The wash takes approximately 20 min.
9. When the interface appears as shown below, select **No** and the sequencer will automatically lift the sampling needles.

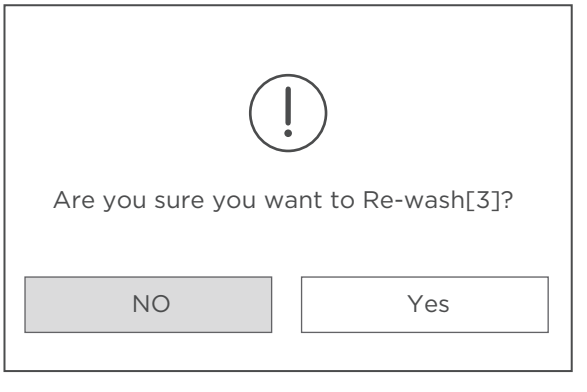

Figure 82 **Maintenance wash [3] prompt**

10. Slowly insert the Cleaning cartridge 1 into the reagent compartment by following the direction printed on the cartridge cover until it stops.
11. Place the DNB loading needle washing tube 1 (2.0 mL sterile microcentrifuge tube with 1.8 mL Laboratory-grade water) into the DNB tube rack.
12. Close the reagent compartment door.

13. Select **Wash** in the wash instructions interface. Select **Regular** from the **Wash type** list to start the wash. The wash takes approximately 54 min.

Performing an ultra deep wash (~11 h)

Perform the following steps:

1. Slowly insert the Cleaning cartridge 1 into the reagent compartment by following the direction printed on the cartridge cover until it stops.
2. Place DNB loading needle washing tube 1 (2.0 mL sterile microcentrifuge tube with 1.8 mL of Laboratory-grade water) into the DNB tube rack. Close the reagent compartment door.
3. Place the washing flow cell on the stage. Ensure that the washing flow cell is properly seated.

   For details, refer to.
4. Select **Wash** in the wash instructions interface. Select **Ultra deep** from the **Wash type** list to start the wash. The wash takes approximately 11 h.

Figure 83 Selecting Ultra deep

(Optional) Performing a DNBTube wash (~15 min)

Once the pre-run wash and maintenance wash are completed, if you need another wash for the DNB loading tubes, perform the following steps:

There is no need to replace the Cleaning cartridge during DNBTube wash.

1. Slowly insert the Cleaning cartridge into the reagent compartment by following the direction printed on the cartridge cover until it stops.
2. Place the DNB loading needle washing tube 3 (2.0 mL sterile microcentrifuge tube with 1.8 mL Tween-20) into the DNB tube rack. Close the reagent compartment door.
3. Place the washing flow cell on the stage. Ensure that the washing flow cell is properly seated.

   For details, refer to *Loading the flow cell on Page 82*.

4. Select **Wash** in the main interface. Select **DNBTube** from the **Wash type** list to start the wash. The wash takes approximately 5 min.

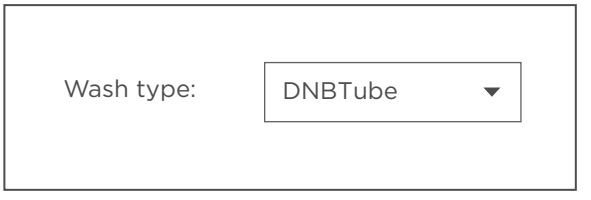

Figure 84 Selecting DNBTube

5. Select **Back** to return to the main interface**.**
6. Place the DNB loading needle washing tube 2 (2.0 mL sterile microcentrifuge tube with 1.8 mL NaOH) into the DNB tube rack. Close the reagent compartment door.
7. Select **Wash** in the main interface. Select **DNBTube** from the **Wash type** list to start the wash. The wash takes approximately 5 min.
8. Select **Back** to return to the main interface**.**
9. Place the DNB loading needle washing tube 1 (2.0 mL sterile microcentrifuge tube with 1.8 mL Laboratory-grade water) into the DNB tube rack.
10. Select **Wash** in the main interface. Select **DNBTube** from the **Wash type** list to start the wash. The wash takes approximately 5 min.

Reusing the cleaning cartridge

A sequencer cleaning cartridge and washing flow cell are provided together with the device.

Rinse the sequencer cleaning cartridge before refilling it with washing reagents. Replace a sequencer cleaning cartridge after it has been used for 20 times or every 6 months.

CAUTION

- While reusing the cleaning cartridges, it is not recommended that you mix various types of sequencer cleaning cartridges with different reagents. For example, the cartridge used for filling NaOH in the previous cleaning procedure should be filled only with NaOH again in the current cleaning procedure.
- It is not recommended that you use the Sequencing Reagent Cartridge as a sequencer cleaning cartridge.

Weekly maintenance

WARNING Wear a laboratory coat, a mask, and gloves before performing the following steps.

Clearing the historical data in the storage drive

Check the storage drive space and timely back up the historical data to the peripheral storage devices. Ensure that the storage drive has enough space for your sequencing run.

For details on clearing history data, refer to *Reviewing parameters on Page 85.*

Powering cycle the device

Perform the following steps:

1. Turn off the computer. Turn off and unplug the device.
2. Wait at least 30 s.
3. Plug in and turn the device on. Turn on the computer again.

Maintaining the power supply

Perform the following steps:

1. Periodically check whether the power cord and cables are connected correctly and in good condition. Contact CG Technical Support if new cables are required.
2. Check whether the area around the power supply is dry and free of moisture.

Cleaning the flow cell stage

Perform cleaning and maintenance for the flow cell stage before each use. Failure to do so may affect the attachment of the flow cell to the chuck.

- Wear protective gloves when cleaning the flow cell stage. Dust, lint, or other particulates can affect flow cell attachment and imaging.
- Ensure that the flow cell does not fall off when cleaning it with a canned air duster.

Prepare the following tools and solutions to clean the flow cell stage:

- Washing flow cell
- Low-lint cloth
- 75% ethanol
- Canned air duster

Perform the following steps:

1. Check for dust, debris, damage, or particulates on the surface of the aluminum chuck of the flow cell stage.
2. Wipe the aluminum chuck of the flow cell stage with a low-lint cloth moistened with 75% ethanol, and then let it air-dry.

   To prevent 75% ethanol from entering the holes and damaging the device, do not wipe the vacuum inlet and the vacuum attachment slot.
3. Check for dust, damage, or debris on each surface of the washing flow cell. Wipe the silicon chip on the back of the flow cell with a low-lint cloth moistened with 75% ethanol, and then let it air-dry.
4. Use a canned air duster to carefully blow particulate matter and dust from the surface of the silicon chip and aluminum chuck until cleaned.
5. Press the flow cell attachment button on the flow cell stage.
6. Place the flow cell on the flow cell stage. Ensure that the front side of the flow cell is facing up with the QR code on the right. Press the edges of the flow cell with your hand to ensure that it is securely seated.

Maintaining the waste container

The waste container is connected to the device through tubes. To prevent liquid leakage and biological hazard exposure, monitor the waste container status frequently and empty it in time. Clean and disinfect the waste container after it is emptied according to the following instructions.

Empty the waste container when either of the following conditions is met:

- The waste level approaches 80% of the maximum volume before sequencing.
- The waste container icon turns to  during sequencing.

Perform the following steps:

1. Wear protective equipment.
2. Remove the lid without tubes from the waste container.
3. Pour the waste into an appropriate waste container, and dispose of the waste according to local regulations and safety standards of your laboratory.
4. Add sufficient laboratory-grade water into the waste container, reattach the lid if necessary, and gently swirl the container until all inner walls are cleaned.
5. Pour the laboratory-grade water into an appropriate waste container.

   If necessary, repeat steps 4 and 5.
6. Clean the surface and opening of the waste container with a 75% ethanol wipe. Ensure that no waste remains in the container.
7. Reattach the lid onto the waste container.

Monthly maintenance

Maintaining the device

The low-lint cloth should be kept moist without droplets.

Perform the following steps:

1. Power the device off.
2. Wipe the surface and the screen of the device with a low-lint cloth moistened with 75% ethanol. Ensure that the surface is free of samples, reagents, blood, and potential biological contaminants.

Checking and cleaning the cooling fan

Perform the following steps:

1. Remove the dust from the ventilation holes with a small brush. Ensure that the device can ventilate normally.
2. Check whether the cooling fan operates normally. If it does not, contact CG Technical Support to replace the fan.

Annual maintenance

It is recommended that you calibrate and maintain critical components, such as the power of the laser, annually. For information on the service plan and preventative maintenance (PM), contact CG Technical Support.

Software maintenance

If necessary, contact CG Technical Support to update and maintain the software.

Storage and transportation

- Store the device according to the environment requirements in this guide.
- If you want to move or transport the device, contact CG Technical Support.

Disposal of the device

The service life of this device is seven years, which is determined by the simulated service life evaluation method. For the date of manufacture, refer to the label on the device. Perform the maintenance according to the requirements in this guide. Dispose of the end-of-life device according to local regulations. However, if it is confirmed that the device is still functioning safely and effectively after maintenance, continue to use the device.

DL-200H and sealing gasket maintenance

WARNING

- Do not immerse the DL-200H into the liquid for cleaning. Doing so may damage the device.
- Do not use other disinfectants such as dichloroethane ($C_2H_4Cl_2$), trichloroethylene (C_2HCl_3), chloroform ($CHCl_3$), and toluene (C_7H_8) to clean the DL-200H. Doing so may damage the device.
- It is recommended that you replace the DL-200H (Cat. No.: 900-000218-00) with a new one after using it for one year.
- If you have questions about the compatibility of disinfectants, contact CG Technical Support.

After each DNB loading, perform the following steps to maintain the DL-200H and sealing gasket:

1. Wipe all sides of the device with a low-lint cloth moistened with 75% ethanol and a low-lint cloth moistened with ultra-pure water.
2. Wipe the device with a low-lint cloth and let it air-dry.
3. Collect the used sealing gasket into a 200 mL beaker.
4. Fill the beaker with ultra-pure water and wash the sealing gasket in the beaker, and then empty the beaker. Repeat the wash twice, for a total of 3 times.
5. Fill the ultrasonic cleaner tank with ultra-pure water, and wash the sealing gasket in the ultrasonic cleaner tank for about 15 min.
6. Repeat step 4, place the cleaned sealing gasket into a clean container, and let it air-dry.
7. (Optional) Replace with a new sealing gasket (Cat. No.: 510-003139-00) if any of the following occurs:
   - The sealing gasket has been cleaned 20 times.
   - The sealing gasket has been used for 3 months.
   - The pipette tip loosens during loading DNBs.

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08

FAQs

This chapter describes frequently asked questions about the reagents and sequencer.

If malfunctions occur during operation, the device alarms, or when a message is displayed on the screen, follow the prompts to troubleshoot and solve the issue.

If the problem persists after you try the recommended actions, contact CG Technical Support.

Sequencer FAQs

Q: What should I do if the device does not power on after I turn the power switch to the ON position?

Power-on issues arise when the main power supply is in an abnormal condition, not connected to the main power supply/UPS, or if the UPS has run out of power. Perform the following steps:

1. Ensure that the power cord is plugged in.
2. Check whether fuses have blown.

Q: What should I do if error messages appear when the control software is running?

Error messages may appear when parameters are not set properly or if an error occurs in software-hardware communication.

Perform the following steps:

1. Perform a self-test in the system maintenance interface. Check the record of the hardware that fails the self-test.
2. Check error messages in the log, and fix the problem according to the on-screen instructions.
3. Restart the device.

Q: Why does the flow cell not attach to the flow cell stage?

If the flow cell does not attach to the flow cell stage, it may be because the flow cell attachment button was not pressed. Any dust, debris, or damage that may be present on the flow cell stage and/or the flow cell can prevent the flow cell from attaching.

To resolve the issue, perform the following steps:

1. Check whether the flow cell attachment button is pressed.

2. Check the flow cell stage for dust, debris, or damage. Clean the flow cell stage. For details, refer to *Cleaning the flow cell stage on Page 138*.

Q: What should I do if a message indicates that the initialization of the reagent needle fails or the operation times out?

This message is displayed when the reagent compartment door is open and/or the interlock or controller is damaged.

Ensure that the reagent compartment door is closed, and perform a self-test in the system maintenance interface.

Q: What should I do if a temperature error message and warning appear in the sequencing interface?

Errors messages may appear when the flow cell temperature exceeds the default limits and/or if there is an error with the temperature sensor error.

It is recommended that you record the warnings and the related logs of the sequencing run and contact CG Technical Support.

Q: What should I do if many bubbles appear in the flow cell during sequencing?

A large number of bubbles may be released from the reagent in well No. 10 during the liquid pumping process, and the degassing chamber cannot completely remove them. To solve the problem, refer to step 16 in *Preparing the Sequencing Reagent Cartridge-Part 2 on Page 70*.

Q: What should I do if many bubbles appear in the flow cell after sequencing?

Bubbles may be present when air leaks between the flow cell and the sealing gasket, the flow cell is damaged, when the pipeline is bent or becomes loose, or the reagent cartridge contains air bubbles.

To resolve the issue, perform the following steps:

1. Remove the flow cell, check the back of the flow cell and the stage for dust, batting, or crystals, and so on.
2. Clean the flow cell stage, and place the flow cell back onto the stage.

3. Check whether the reagent is pumped out or not. If not, add or replace the reagent.
4. If air bubbles appear in the reagent cartridge, tap the surface of the cartridge until the air bubbles disappear.

Q: What should I do if the waste level sensor alarms?

The waste level sensor alarms when the waste container is full, the level sensor is not installed properly, or when a software error occurs. For details, refer to *Performing pre-run checks on Page 45*.

Q: What should I do if a large amount of liquid remains in the flow cell after regular wash?

A large amount of liquid may remain in the flow cell after regular wash when the flow cell leaks or the reagent needle becomes loose.

To resolve the issue, perform the following steps:

1. Check for damage on the glass surface of the flow cell. If damaged, replace the flow cell with a new one, and clean the surface before you place it on the flow cell stage.
2. Perform another regular wash and check whether solution remains in the flow cell.

Reagent FAQs

Q: What should I do if library amount (less than 40 fmol) is inadequate?

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

i The above solution is only applicable for general libraries with amounts greater than 24 fmol.

Calculate the required amount of ssDNA libraries

- The required volume of ssDNA libraries is determined by the required library

amount (fmol) and library concentration quantified in *DNA library concentration and amount requirements on Page 51*. The volume of each Make DNB reaction is 60 µL and the required library input for each Make DNB reaction is calculated as follows:

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

- Calculate the required volume of ssDNA libraries for each Make DNB reaction and fill it in the following table as *V*.

Making DNBs

Perform the following steps:

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

   For sequencing of third-party libraries, use App Make DNB Buffer.

Table 57 Make DNB reaction mixture 1

Component	Cap color	Volume (µL)
Low TE Buffer		12 - V
Make DNB Buffer		12
ssDNA libraries	/	V
Total volume		24

2. Mix the reaction mixture thoroughly by using a vortex mixer, centrifuge for 5 s 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 58 Primer hybridization reaction conditions

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

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

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

5. Remove the PCR tube from the thermal cycler when the temperature has reached 4 °C.
6. Centrifuge briefly for 5 s, place the tube on ice, and prepare the Make DNB reaction mixture 2 according to the table below:

Table 59 Make DNB reaction mixture 2

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

7. Add all of Make DNB reaction mixture 2 into Make DNB reaction mixture 1.
8. Mix the reaction mixture thoroughly by using a vortex mixer, centrifuge for 5 s.
9. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

Table 60 RCA conditions

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

10. Add 12 µL of Stop DNB Reaction Buffer when the temperature reaches 4 °C immediately. Mix gently by pipetting 8 times by using a wide-bore pipette tip.

Table 61 Volume of Stop DNB Reaction Buffer

Component	Cap color	Volume (µL)
Stop DNB Reaction Buffer		12

- It is very important to mix DNBs gently by using a wide-bore pipette tip. Do not centrifuge, vortex, or shake the tube.
- Store DNBs at 2 °C to 8 °C, and perform sequencing within 48 h.

11. Proceed to *Quantifying DNBs on Page 63.*

Q: What should I do if DNB concentration is low?

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

1. Ensure that the DNB preparation reagents have not expired.
2. Ensure that the libraries meet the requirements.
3. Make a new DNB preparation. If the DNB concentration still does not meet the requirements after a new sample preparation, contact CG Technical Support.

Q: What should I do if dark green crystals appear in well No. 10?

- Presence of dark green crystals in well No. 10 is normal as a result of crystallization of reagent materials in this well.
- When the cartridge is thawed, mix the reagents in the cartridge well and the crystals will dissolve. Sequencing quality will not be affected.

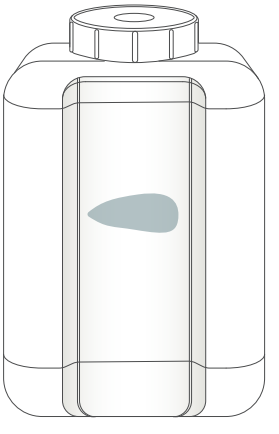

Figure 85 Dark green crystals in well No. 10

Q: What should I do if I forgot to add reagent into well No. 15 for PE sequencing run?

MDA Enzyme is required to make the second-strand template for PE sequencing. When preparing the Sequencing Reagent Cartridge, the appropriate amounts of MDA Enzyme Mix II must be added to well No. 15.

If you forget to add the reagent into well No. 15 before starting the sequencing run, this can be resolved by performing the following steps, as long as the sequencing run is in the sequencing phase of Read1.

1. Pause the run: At any sequencing cycle within Read1, while sequencing is at Step 3/3, and the indicator is at well No. 17 or well No. 18, select , and select **Yes** when you are prompted, as shown in the following two figures.

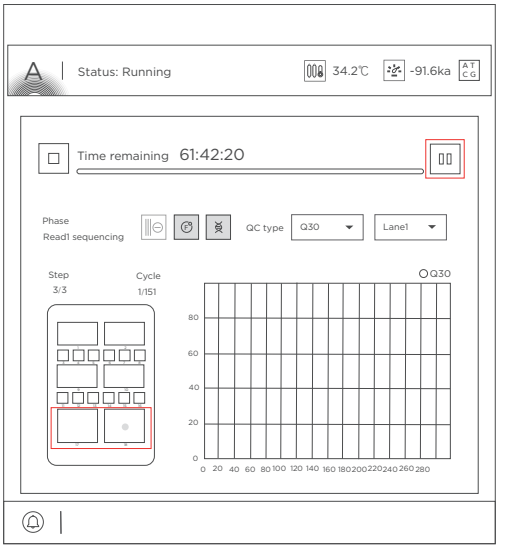

Figure 86 Selecting the sequencing stage to pause

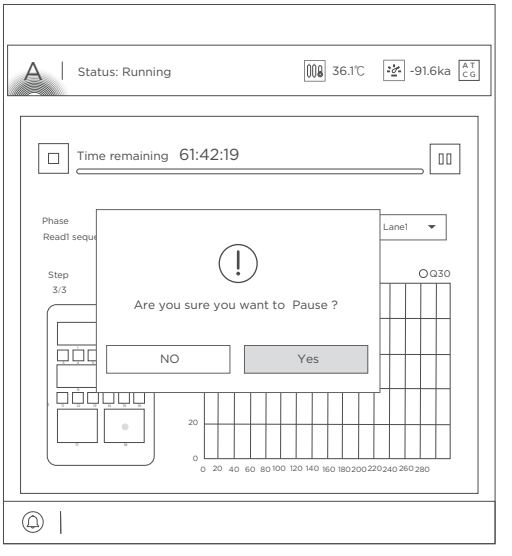

Figure 87 Confirming to pause the run

2. Lift the needle: Select to lift the needle, and select **Yes** when you are prompted, as shown in the following two figures.

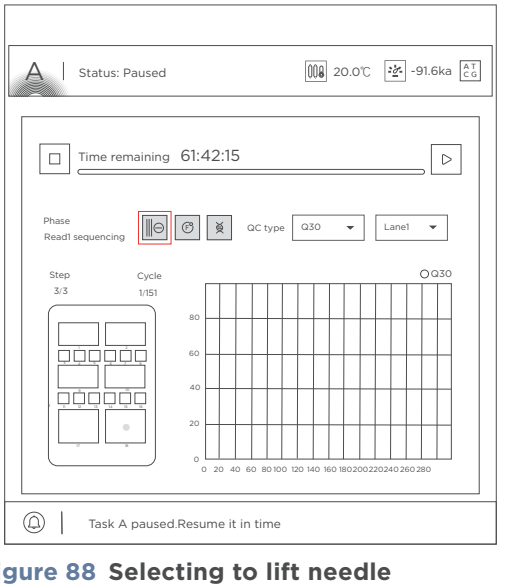

Figure 88 Selecting to lift needle

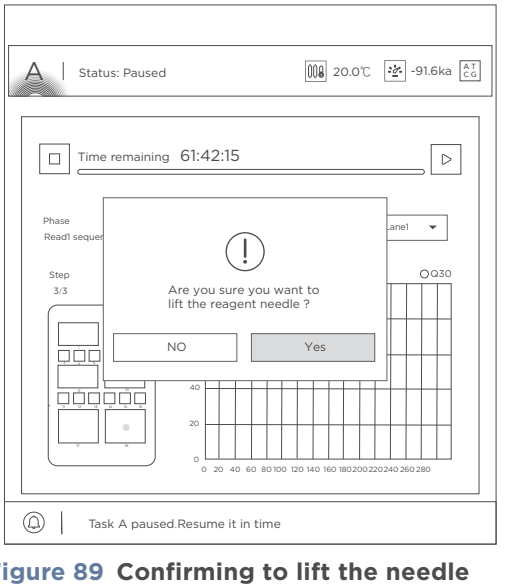

Figure 89 Confirming to lift the needle

3. Prepare the Sequencing Reagent Cartridge: Open the reagent compartment door and take out the Sequencing Reagent Cartridge. Add the appropriate amount of MDA Enzyme Mix II into MDA reagent tube, and mix well. For details, refer to *Preparing the Sequencing Reagent Cartridge-Part 2 on Page 70.*

4. Resume the run: Put the cartridge back to the sequencer and close the reagent compartment door. Select ▷ to resume the run, and select **Yes** when you are prompted, as shown in the following two figures.

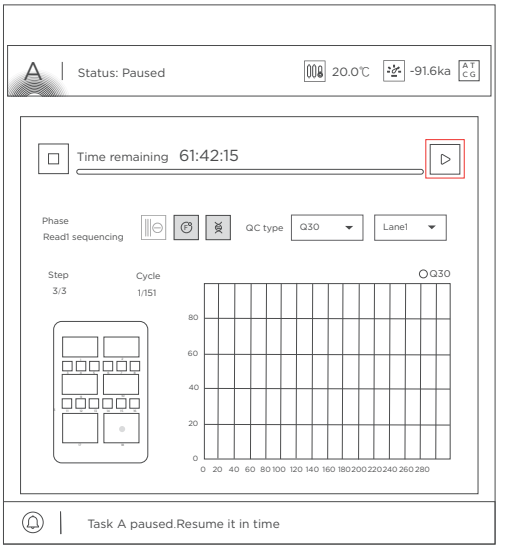

Figure 90 Selecting to resume the run

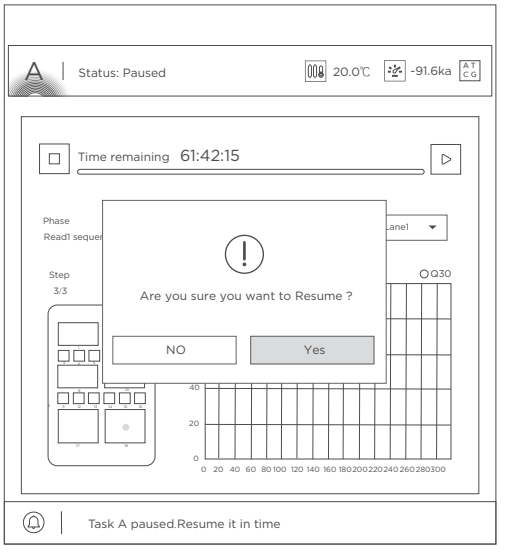

Figure 91 Confirming to resume the run

After the sequencing run is resumed, the sampling needles automatically move down. The sequencer continues to pump reagents into the flow cell. The Read1 sequencing phase continues.

Q: What rules should I follow if I need to store a reagent kit temporarily?

- If a kit has been thawed (not including dNTPs) but cannot be used within 24 h, 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 2 °C to 8 °C. It is strongly recommended that you use it within 24 h. Mix the reagents in the cartridge before use by following the instructions in *Preparing the Sequencing Reagent Cartridge-Part 2 on Page 70*.
- If dNTPs and Sequencing Enzyme Mix have been added into the cartridge, but the cartridge cannot be used immediately, store it at 2 °C to 8 °C and use it within 24 h. Mix the reagents in the cartridge before use by following the instructions in *Preparing the Sequencing Reagent Cartridge-Part 2 on Page 70*.
- If dNTPs and Sequencing Enzyme Mix have been added into the cartridge and the needles have punctured the seal, but the cartridge cannot be used immediately, the cartridge must be sealed with foil or plastic wrap. Store the

cartridge at 2 °C to 8 °C, and use it within 24 h. Gently mix the reagents in the cartridge before use. To prevent reagent contamination when mixing, be careful not to spill any reagent from the needle holes.

Q: What should I do if abnormal negative pressure appears during flow cell attachment?

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

1. Gently wipe the stage surface of flow cell stage with a damp KimWipes tissue and remove the dust from the stage with a canned air duster. Ensure that no dust is present on the flow cell stage.
2. Remove the dust from the back of the flow cell with a canned air duster to ensure that no dust is present.
3. If the problem persists, contact CG Technical Support.

Q: What should I do if pumping failure occurs during DNB loading and sequencing?

If liquids cannot be pumped onto the flow cell, or if large bubbles appear in the flow cell, perform the steps below:

1. The sequencer: remove the flow cell, check for impurities in the sealing gasket, and remove any dust with a canned air duster. Inspect the pump. Place a new flow cell by following the instructions in *Loading the flow cell on Page 82,* and start the pump again.
2. Check the sampling needles to determine if they are moving properly. If the sampling needles are not moving properly, restart the sequencer control software.
3. If the problem persists, contact CG Technical Support.

Q: What should I do if impurities appear in the original sequencing image?

If impurities appear, perform the following steps:

1. Moisten a KimWipes tissue with 75% ethanol and use it to wipe sealing gaskets on the flow cell stage, and perform a maintenance wash on the sequencer according to *Performing a maintenance wash (~94 min) on Page 134*.
2. If the problem persists after a full wash, contact CG Technical Support.

Instructions for importing barcode

Preparing a barcode file

Ensure that the barcode file meets the following requirements:

- The barcode file to be imported should be named “barcode.csv”. In the imported directory, only one “barcode.csv” file is available.
- It is recommended that you use the “Notepad++” program to open the barcode file. Barcode ID and barcode data in the file should be separated by a comma.
- The barcode file should not contain blank lines or full-width characters. The barcode data should include no fewer than two bases.
- Both Barcode sequence and Barcode ID should be unique, and neither should be empty.
- Barcode data of a dual barcode file should not contain any characters other than “A”, “T”, “C”, “G”, and “N”.
- Barcode data of a single barcode file should not contain any characters other than “A”, “T”, “C”, and “G”.

Single barcode file

An example for single barcode file is shown in the figure below:

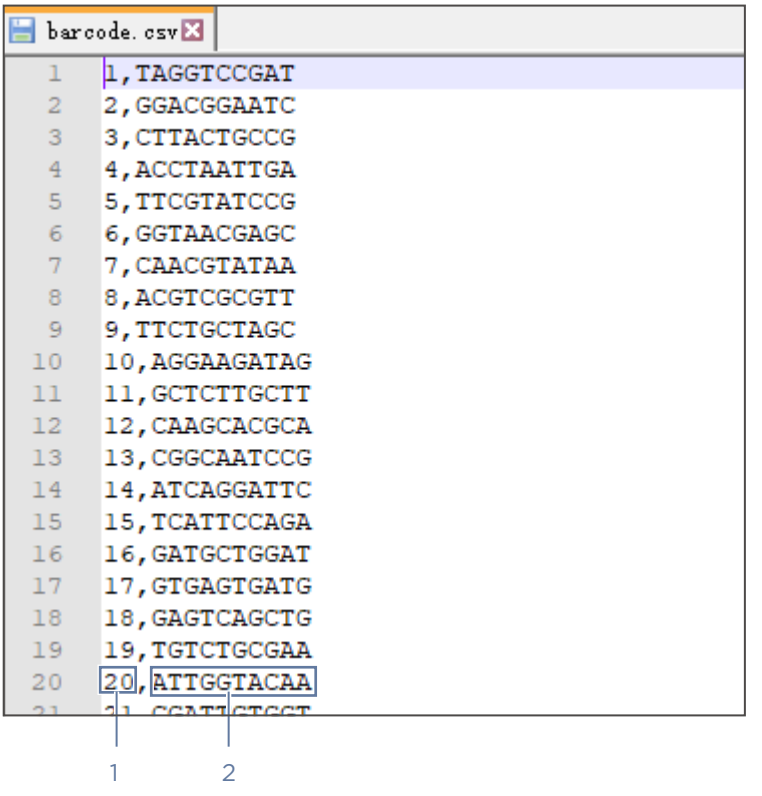

Figure 92 **Single barcode file**

No.	Name	No.	Name
1	Barcode ID	2	Barcode data

Dual barcode file

Examples for dual barcode file is shown in the figure below:

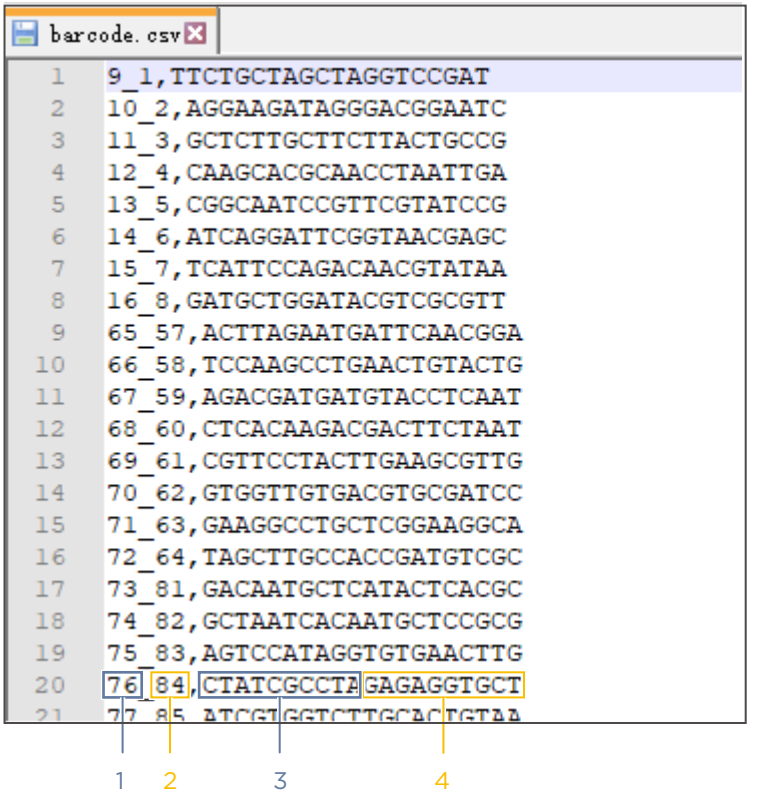

Figure 93 Dual barcode file

No.	Description
1	Corresponds to ID of **Dual barcode** in the Customized parameter interface
2	Corresponds to ID of **Barcode** in the Customized parameter interface
3	Corresponds to data of **Dual barcode** in the Customized parameter interface
4	Corresponds to data of **Barcode** in the Customized parameter interface

Single and dual barcode file

Mixed barcode splitting (both single barcode and dual barcode splitting) is supported in the following two cases:

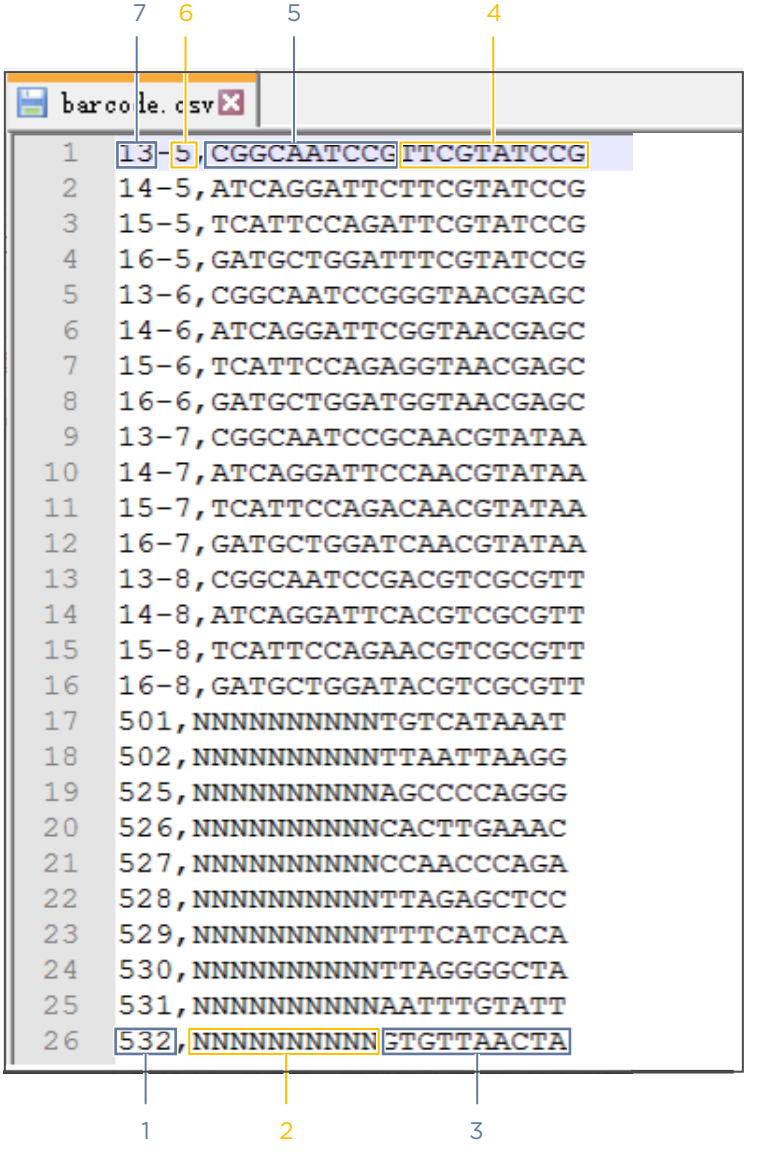

Figure 94 Single and dual barcode file1

No.	Description
1	Corresponds to ID of **Barcode** in the Customized parameter interface
2	Placeholder
3	Corresponds to data of **Barcode** in the Customized parameter interface

No.	Description
4	Corresponds to data of **Barcode** in the Customized parameter interface
5	Corresponds to data of **Dual barcode** in the Customized parameter interface
6	Corresponds to ID of **Barcode** in the Customized parameter interface
7	Corresponds to ID of **Dual barcode** in the Customized parameter interface

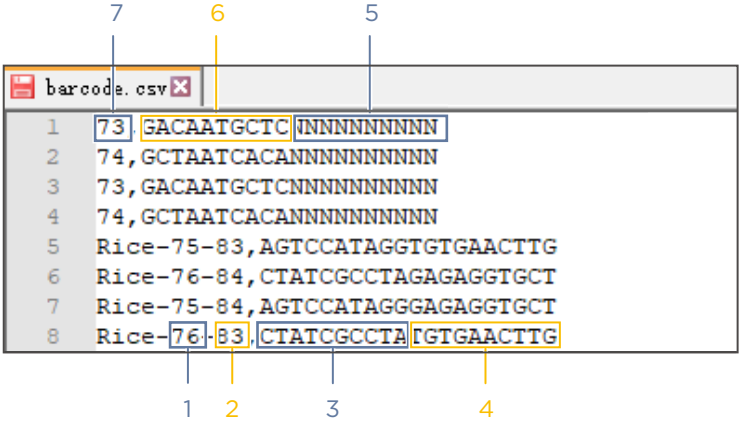

Figure 95 Single and dual barcode file2

No.	Description
1	Corresponds to ID of **Dual barcode** in the Customized parameter interface
2	Corresponds to ID of **Barcode** in the Customized parameter interface
3	Corresponds to data of **Dual barcode** in the Customized parameter interface
4	Corresponds to data of **Barcode** in the Customized parameter interface
5	Placeholder
6	Corresponds to data of **Barcode** in the Customized parameter interface
7	Corresponds to ID of **Barcode** in the Customized parameter interface

Importing a barcode file

Before using an external storage device, it is recommended that you format the external storage device (for example, a USB storage drive).

Perform the following steps:

1. Obtain an external storage device (for example, a USB storage drive), and create a folder in the root directory. Ensure that the folder name is in English. Copy the prepared "*barcode.csv*" file to the folder.
2. In the system maintenance interface, select **Barcode settings**.
3. Select a barcode type:
   - If you want to import a single barcode file, do not select the **Dual barcode** check box.
   - If you want to import a dual barcode file, select the **Dual barcode** check box. Ensure that barcode 1 and barcode 2 have been combined in the dual barcode file.

- Ensure that the barcode file type is consistent with the barcode type.
- Only alphabetic letters, numbers, dashes, and underscores are allowed in Barcode ID.
- Control software automatically recognizes and removes white spaces in barcode file.

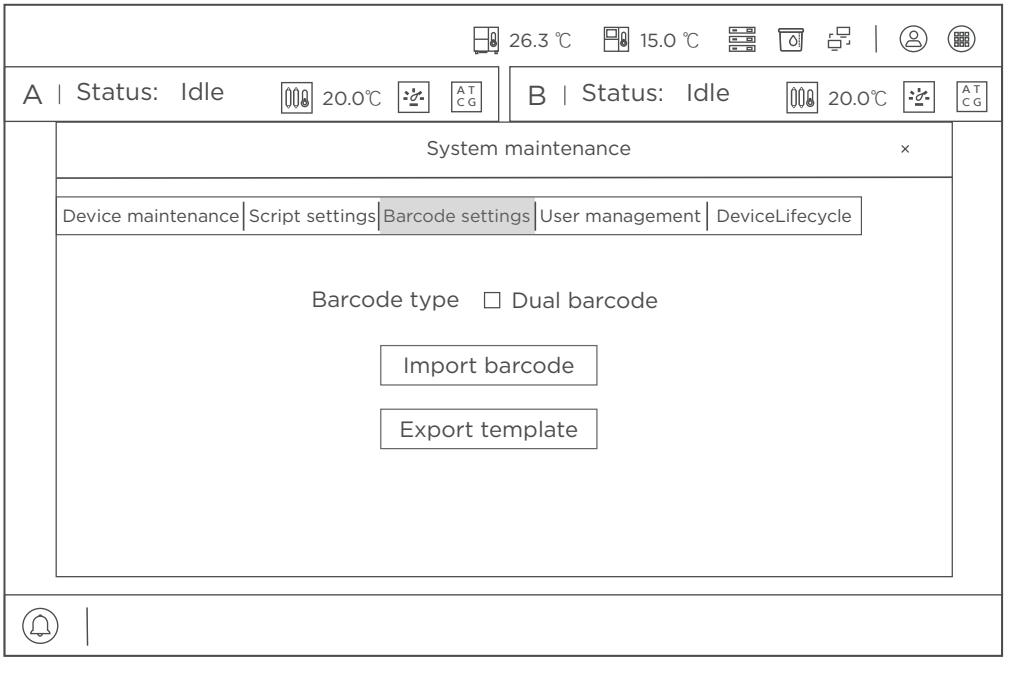

Figure 96 Barcode settings interface (Single barcode file)

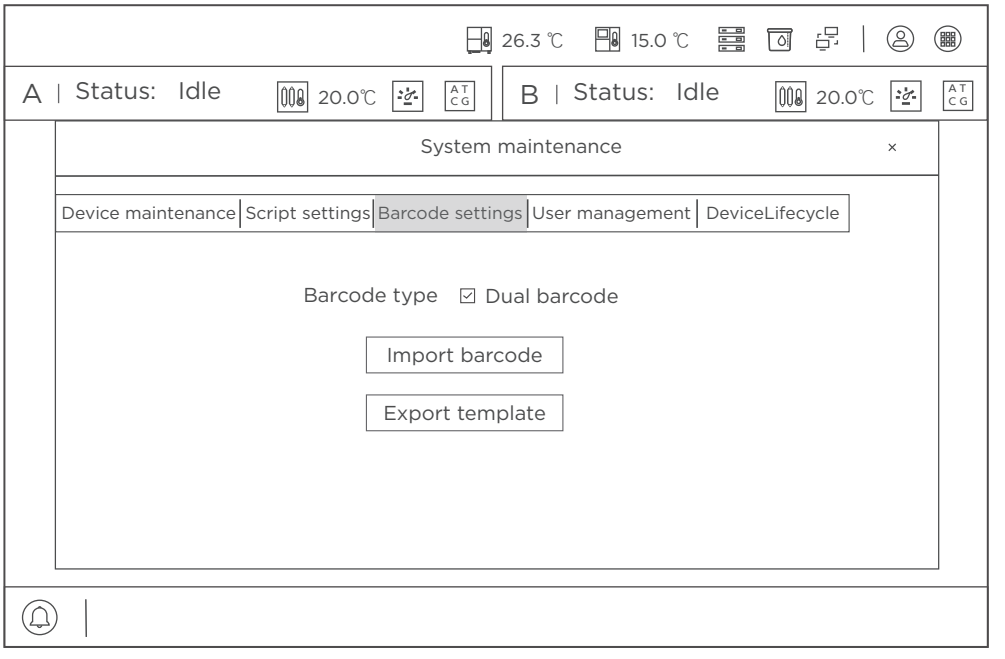

Figure 97 Barcode settings interface (Dual barcode file)

4. Select **Import barcode**.
5. Select the export and import directories.

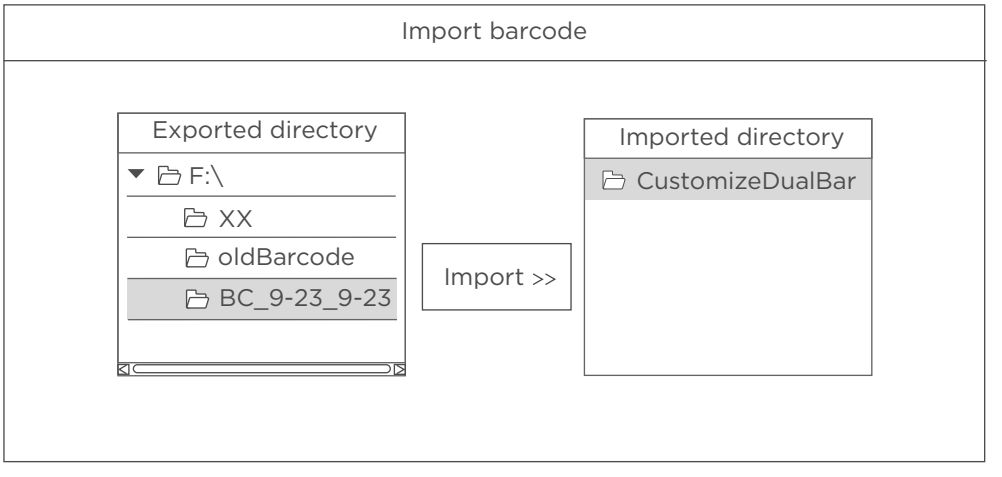

Figure 98 Importing a dual barcode file

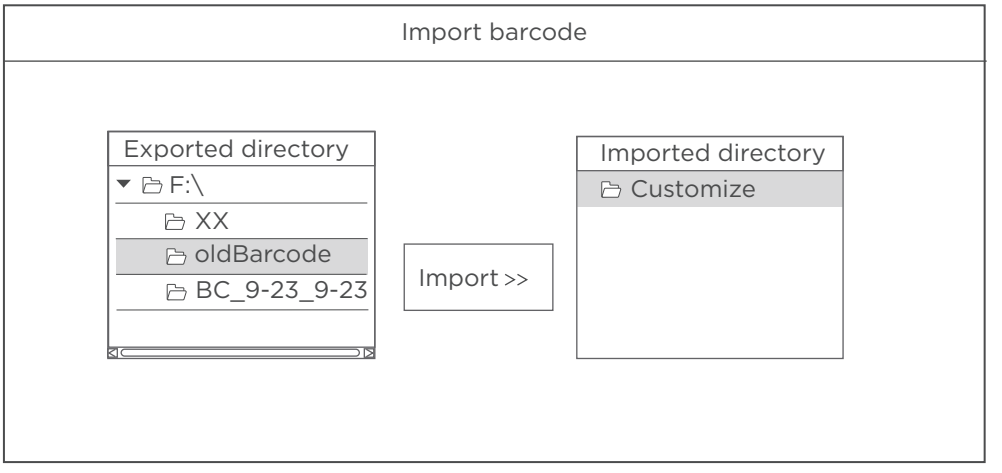

Figure 99 Importing a single barcode file

6. Select **Import** to import barcodes to the device from an external storage device. By default, the barcode file is imported to the following directories:
   - Dual barcode file

     C:\ISW\barcode\CustomizeDualBarcode
   - Single barcode file

     C:\ISW\barcode\Customize

Exporting a barcode template

Perform the following steps:

1. Obtain an external storage device (for example, a USB storage drive).
2. In the system maintenance interface, select **Barcode settings**.
3. Select a barcode type:
   - If you want to export a single barcode template, do not select the **Dual barcode** check box.
   - If you want to export a dual barcode template, select the **Dual barcode** check box.
4. Select **Export template**.
5. Select the exported and imported directories.

   - The storage path for external storage devices can be customized.
   - Exporting a single template (barcode template or dual barcode template) or both templates simultaneously is available.

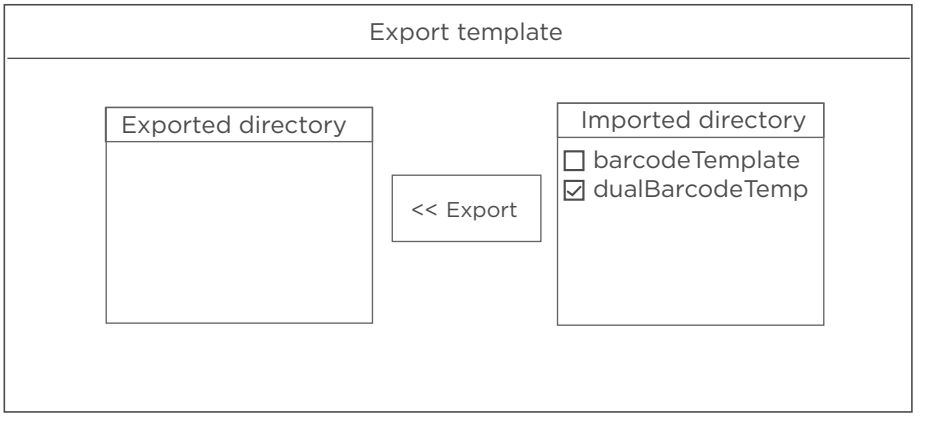

Figure 100 Exporting barcode templates

6. Select **Export** to export a barcode template to an external storage device from the device. By default, the barcode template is exported as *dualbarcodeTemplate.csv* or *barcodeTemplate.csv*.

   If using the template to create a new barcode file, rename the *.csv* file to *barcode.csv* before importing it through the maintenance interface.

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Instructions for customizing a run

Introduction

This section describes how to customize a sequencing run in the following situations:

- When read length(s) in Read2 and/or Read1 are not the same as those predefined in the **Recipe** list.
- For a single barcode sequencing run, the barcode sequences are not within the predefined barcode list.
- All dual barcode sequencing runs.
- Different lanes within a flow cell require different barcodes.
- Different lanes within a flow cell require different barcode split strategies: some lanes need barcode splitting, while other lanes do not.
- Dark reaction cycles are required in Read1 and/or Read2 sequencing.
- UMI (Unique Molecular Identifier) +UDI (Unique Dual Index)
- If unexpected problems occur during DNB loading, Post loading, Sequence priming, or Sequencing, after fixing the problem, you can continue the sequencing run.

Important interfaces for customizing a run

Important interfaces for customizing a run

In the main interface, select **Sequence** to open the DNB ID entry interface. The **Customize** recipe is shown in the following figure:

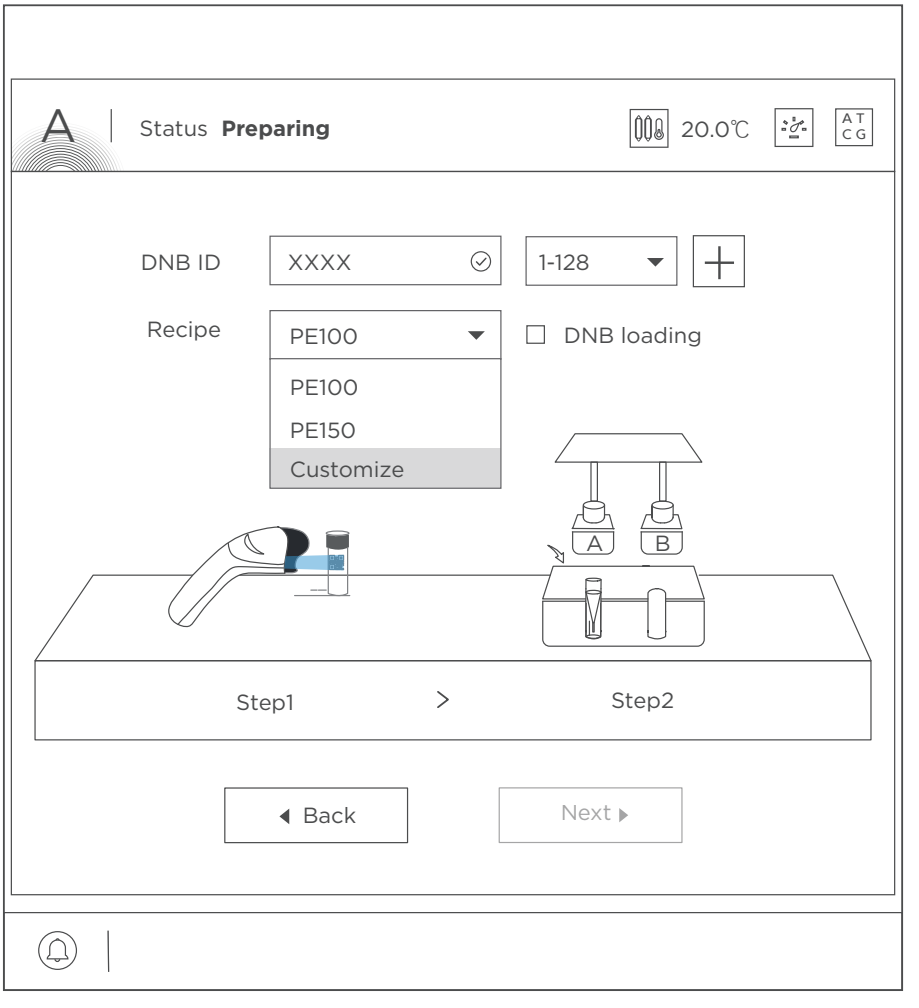


Figure 101 Customize recipe

After you select **Customize** from the **Recipe** list, the Customize interface is displayed as shown below:

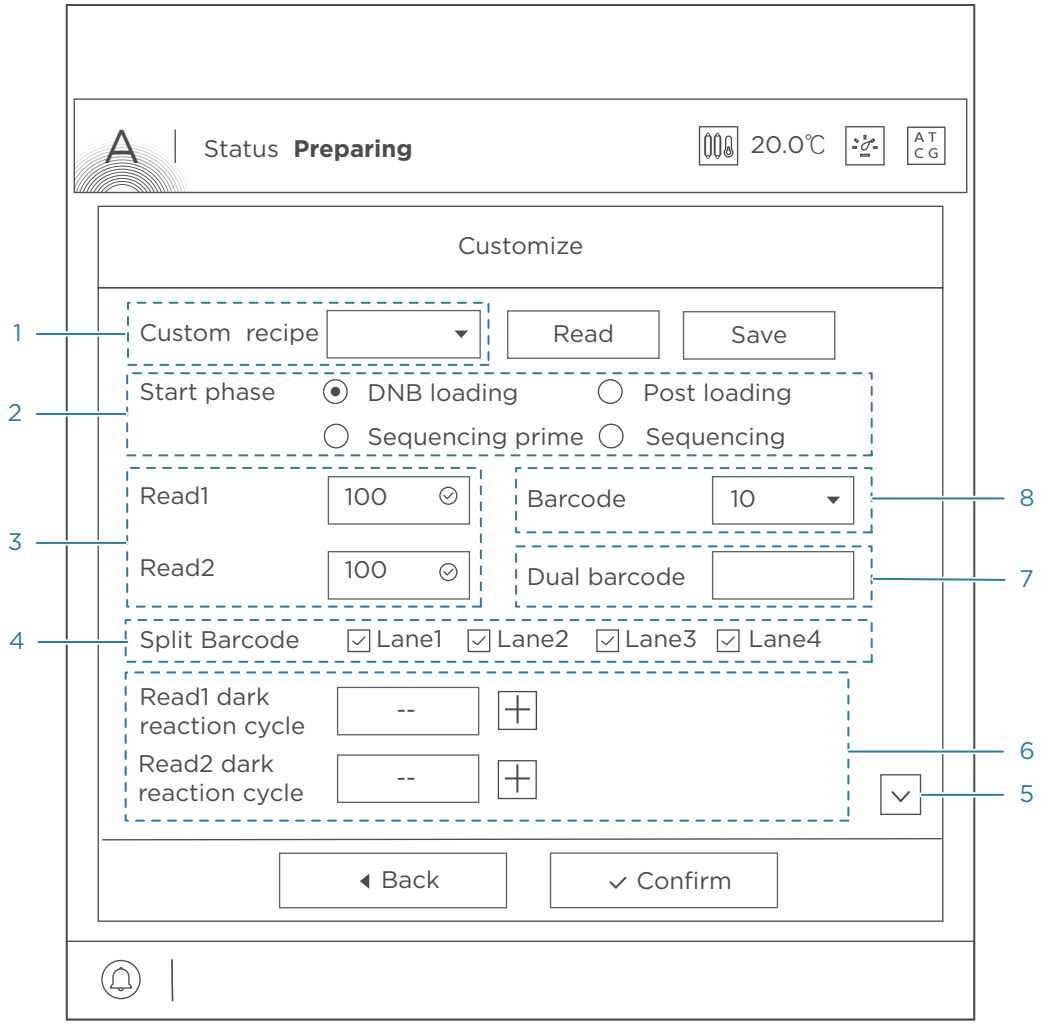


Figure 102 Customize interface

The following table describes the control functions in the Customize interface:

No.	Item	Description
1	**Custom recipe**	Enter a name for the customized recipe; you can create a new one or select a previously saved one • When more than 5 recipes are saved, the following message is displayed: **More than 5 recipes been customized, please select the recipe to be covered**. • Only alphabetic letters, numbers, dashes, and underscores are allowed in recipe names.
2	**Start phase**	Select a start phase for a sequencing run
3	**Read1/Read2**	Customize Read1 and (or) Read2 length for a sequencing run
4	**Split barcode**	Customize whether to split barcode or not for different lanes
5		Select to open barcode type interface; select the imported barcode
6	**Read1 dark reaction cycle/ Read2 dark reaction cycle**	Customize dark reaction range in Read1 and (or) Read2 • Up to 3 cycles can be added to Read1 or Read2 dark reaction cycle. • Ensure that the cycle is within the length of Read1 and Read2. • Each range for a cycle should be unique.
7	**Dual barcode**	Customize Barcode2 (dual barcode) length for a sequencing run
8	**Barcode**	Customize Barcode1 length for a sequencing run

Start phase description

Select the sequencing start phase according to your needs:

To restart the sequencing run at Post loading without Prime, Sequencing Prime, or Sequencing, you should keep the flow cell on the original side of the sequencer where the run was performed.

- **DNB loading**: if you want to load DNBs by the sequencer, select **DNB loading**.

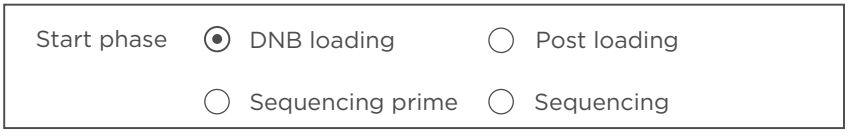


Figure 103 Selecting DNB loading for the start phase

- **Post loading with Prime**: if you have loaded DNBs by DL-200H, select **Post loading** (Prime is selected by default).

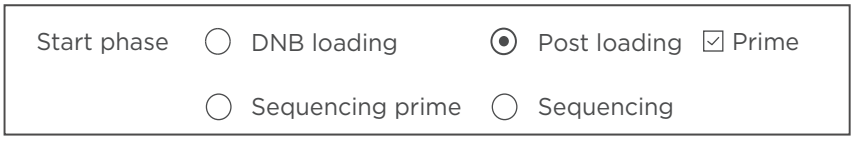


Figure 104 Selecting Post loading (Prime) for the start phase

- If Post loading reagents fail to pump into the flow cell for Post loading after the Post loading prime process has finished, you can restart the Post loading without Prime by selecting **Post loading** and clearing the **Prime** box.
- Restarting Post loading without Prime can be performed one time only.

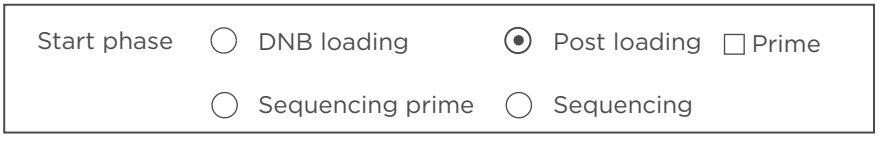


Figure 105 Selecting Post loading for the start phase

- **Sequencing prime**: if the "Post loading" process has been performed on this flow cell already, select **Sequencing prime**, and the sequencing run will start at this step. This is not common in the sequencing procedure.

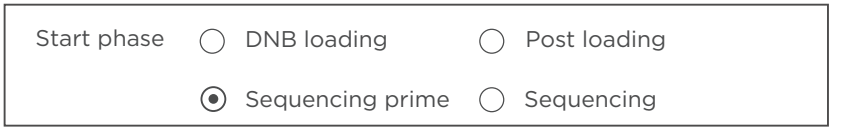


Figure 106 Selecting Sequencing prime for the start phase

- **Sequencing**: if the sequencing run stops before the first cycle imaging step with the error message "Imaging failed", you can restart the sequencing run by selecting **Sequencing**.

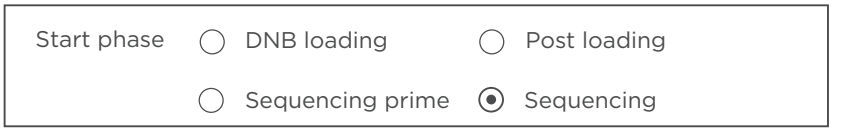


Figure 107 Selecting Sequencing for the start phase

Single barcode (not predefined) interface

If you want to perform single barcode sequencing without using a predefined barcodes list, perform the following steps:

1. Select **Others** from the barcode range list next to the **DNB ID** field.

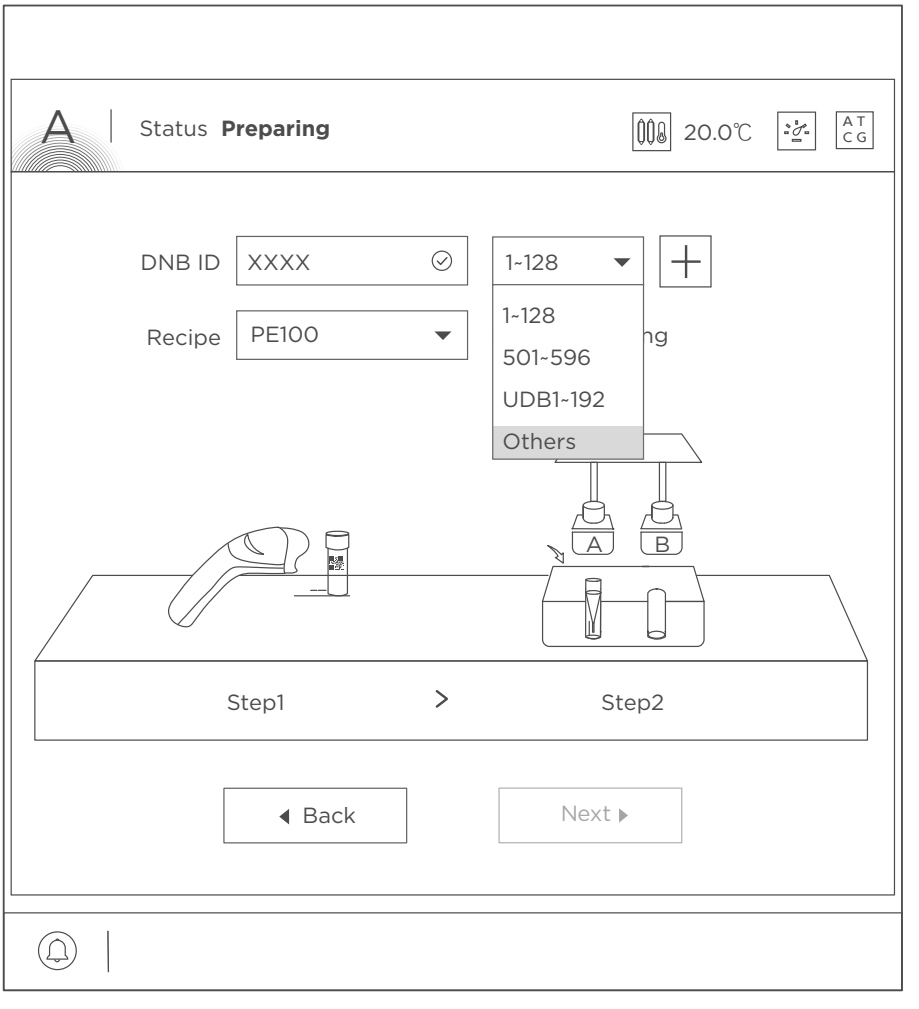


Figure 108 Selecting Others

2. Select **Customize** from the **Recipe** list.

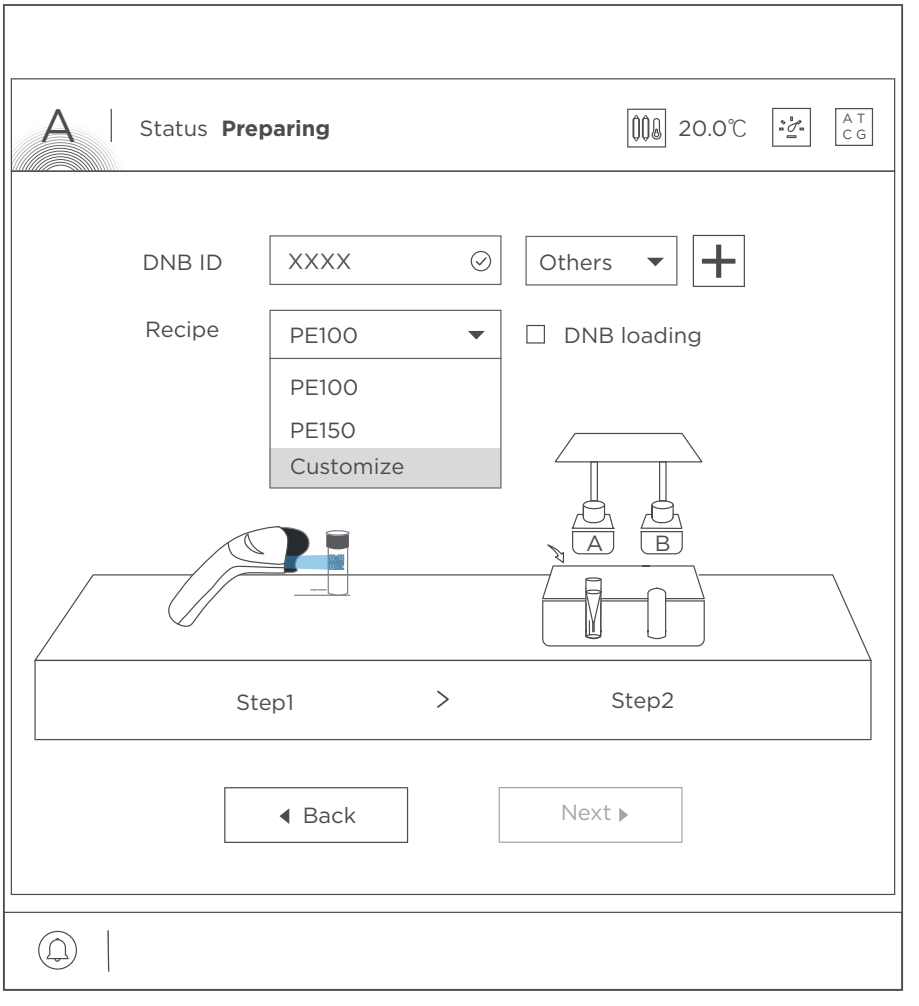


Figure 109 Selecting Customize

3. Configure customize settings according to your needs. Select ⌄ to open the Barcode type interface.

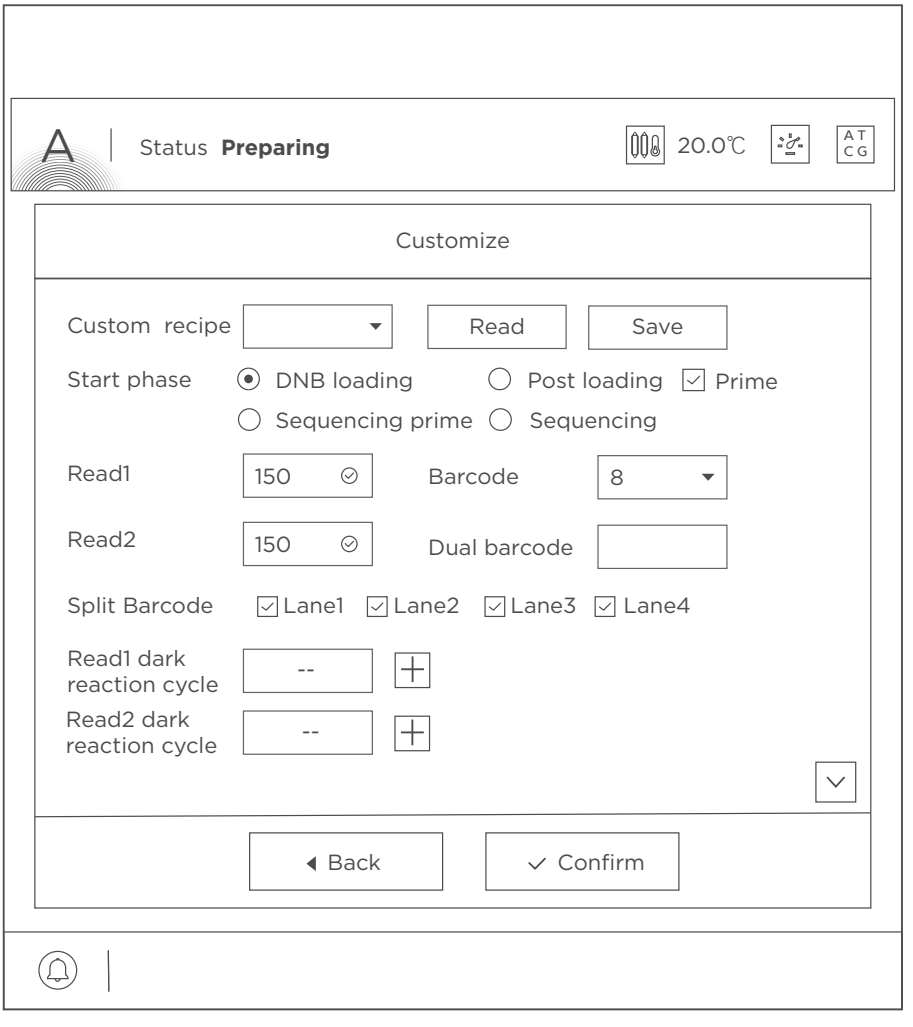
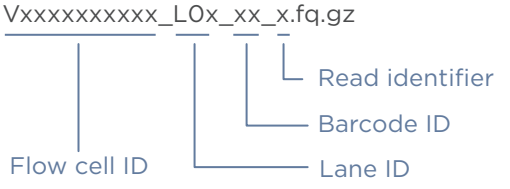


Figure 110 Configuring customize settings

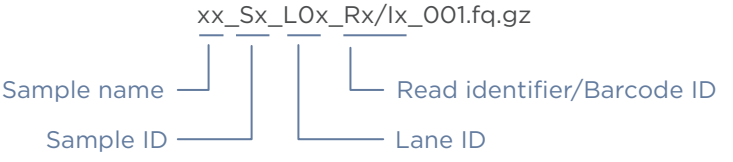
4. Select a desired mode from the **Output fastq mode** list to determine whether to generate the FASTQ file separately.

   Create fastq file for insert only is selected by default.

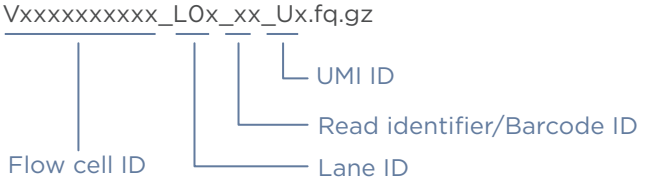
- Select **Create fastq file for insert only**, the format of the Fastq file name will be:



- Select **Create fastq files for insert and barcode**, the format of the Fastq file name will be:



- Select **Create fastq files for insert and UMI**, the format of the Fastq file name will be:



- UMI mode is only available in customized sequencing run and requires specifying the start position and length to take effect.
- **Create fastq files for insert and barcode** and **Create fastq files for insert and UMI** are mutually exclusive.

5. Do not select the **Small RNA** and **Dual barcode sequencing** boxes. Select a type from the **Barcode type** list. If the barcode split is needed, select the boxes next to the **Barcode type** list for the split strategy of each lane. Otherwise, leave the boxes blank.

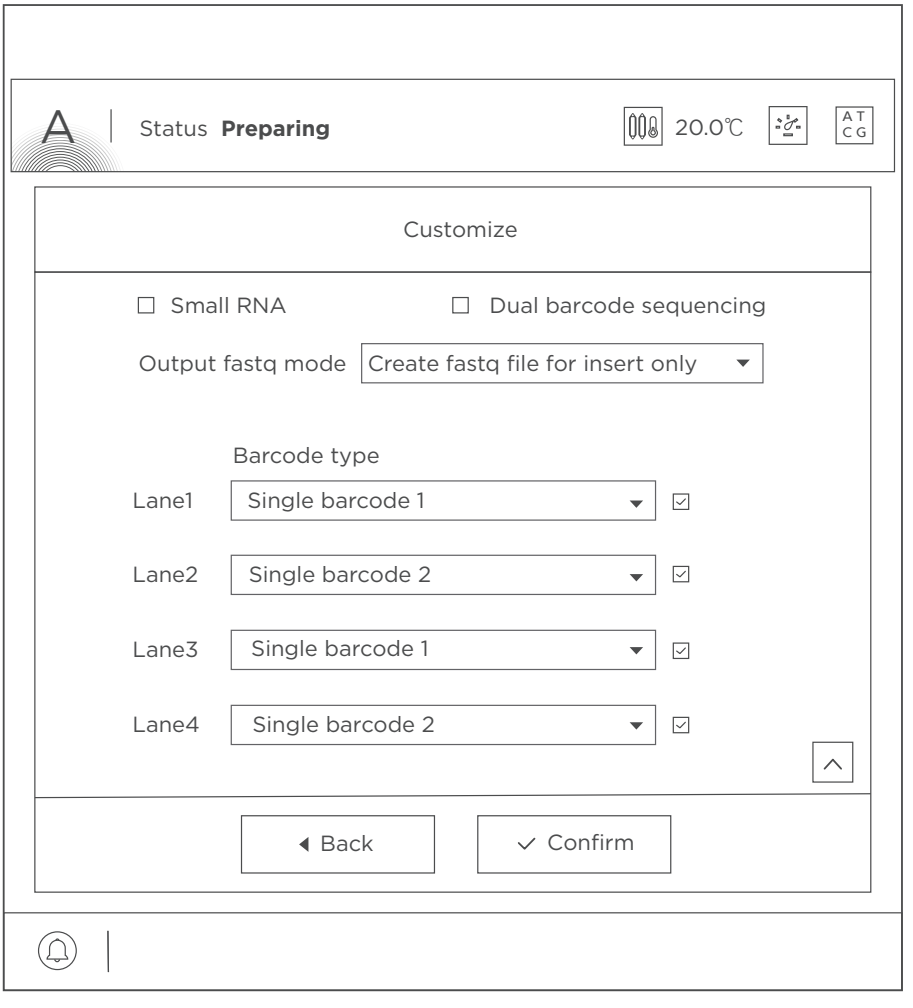


Figure 111 Selecting barcode type and split strategy

Dual barcode (Barcode and Dual barcode combined in the dual barcode file) interface

If you want to perform a dual barcode sequencing, perform the following steps:

1. In the main interface, select **Sequence** to open the DNB ID entry interface.
2. Select **Customize** from the **Recipe** list.
3. Configure customize settings according to your needs. Select ⌄ to open the Barcode type interface.

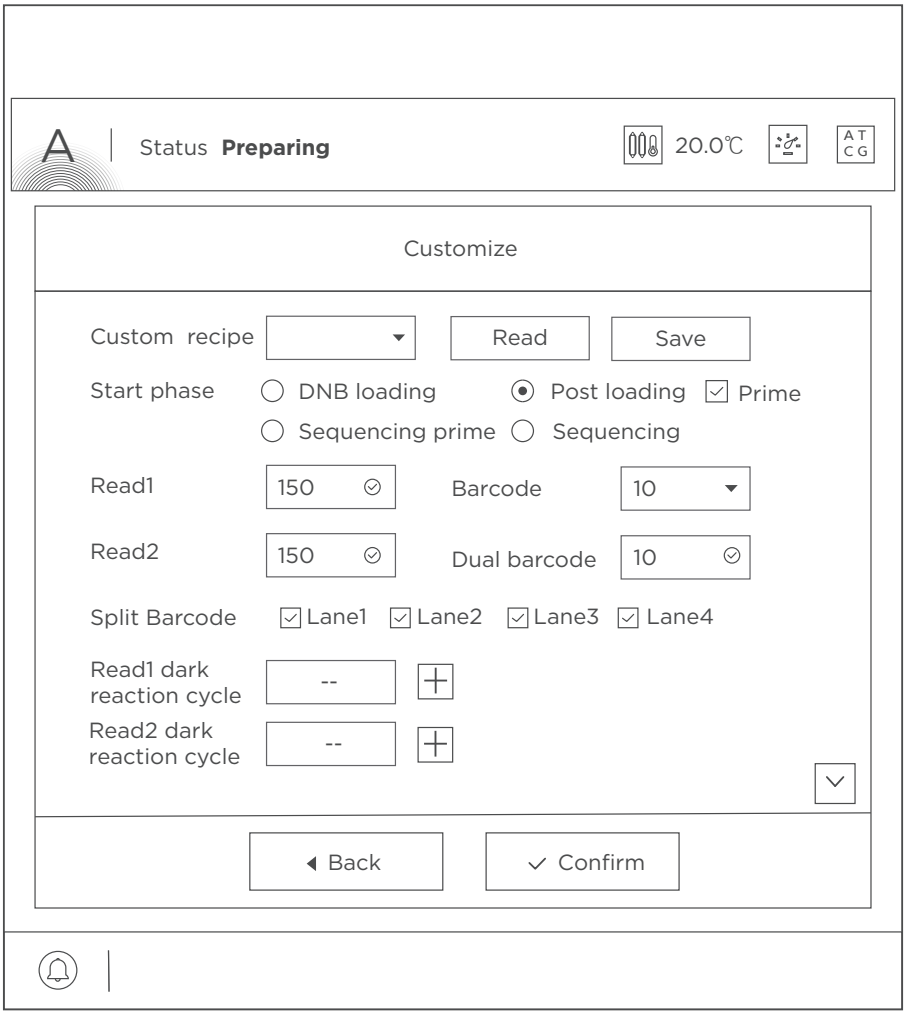


Figure 112 Configuring customize settings

4. Select a desired mode from the **Output fastq mode** list to determine whether to generate the FASTQ file separately.

5. Select the **Dual barcode sequencing** box. Select a dual barcode type from the **Barcode type** list. If the barcode split is needed, select the boxes next to the **Barcode type** list for the split strategy of each lane. Otherwise, leave the boxes blank.

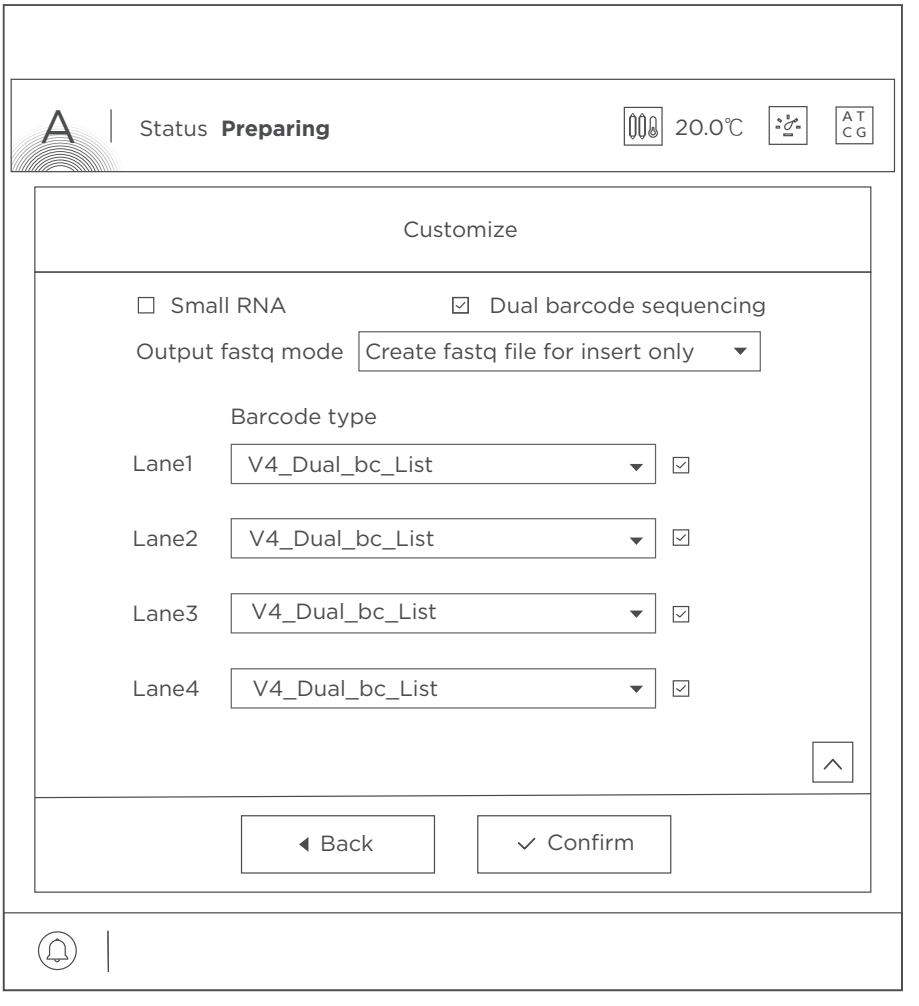


Figure 113 Selecting barcode type and split strategy

The barcode list is contained in the dual barcode file that you have imported. For details on how to import a barcode file, refer to *Importing a barcode file on Page 158*.

Dual barcode (Barcode and Dual barcode used separately in the Customized single barcode file) interface

If you want to perform a dual barcode sequencing, perform the following steps:

1. In the main interface, select **Sequence** to open the DNB ID entry interface.
2. Select **Customize** from the **Recipe** list.
3. Configure customize settings according to your needs. Select ⌄ to open the Barcode type interface.

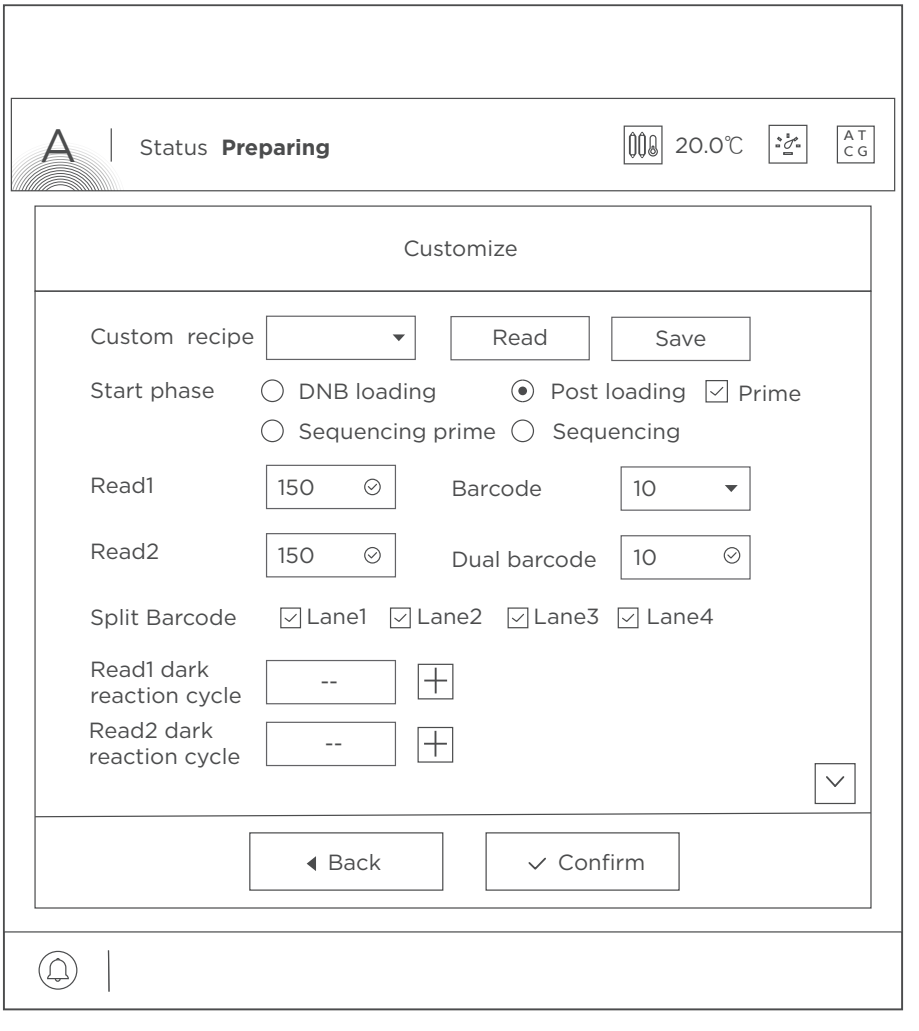


Figure 114 Configuring customize settings

4. Select a desired mode from the **Output fastq mode** list to determine whether to generate the FASTQ file separately.
5. Select the **Dual barcode sequencing** box. Select a dual barcode type from the **Barcode type** list and the **Dual barcode type** list. If the barcode split is needed, select the boxes next to the **Barcode type** list and the **Dual barcode type** list for the split strategy of each lane. Otherwise, leave the boxes blank.

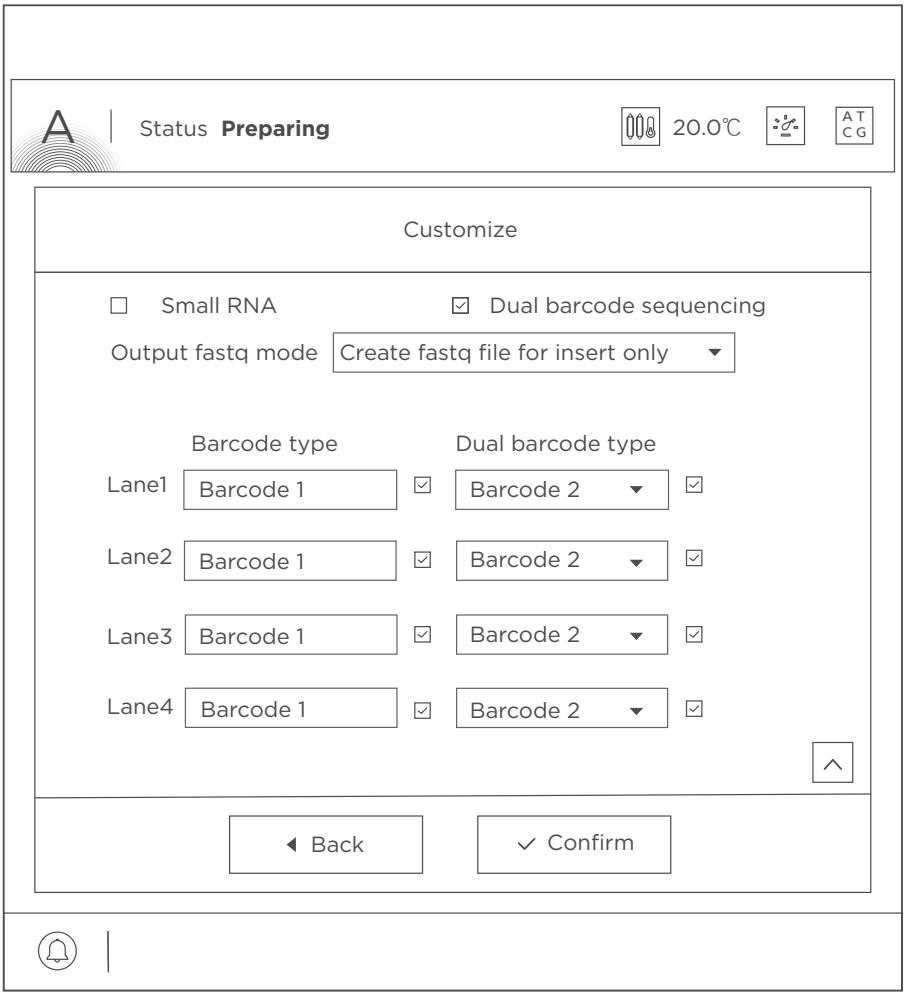


Figure 115 Selecting barcode type and split strategy

The barcode list is contained in the single barcode file that you have imported. For details on how to import a barcode file, refer to *Importing a barcode file on Page 158*.

Examples of customized runs

Ensure that the barcode file meets the following requirements:

- Before starting the customized run, confirm that the customized barcode files are already imported into the sequencer. If they are not, refer to *Instructions for importing barcode on Page 153* to import the customized barcode.
- Ensure that the total number of sequencing cycles including Read1, Read2, Barcode, Dual barcode, and dark cycle is less than the maximum sequencing cycles for a given sequencing kit, as defined in *Table 8 on Page 37* .
- The maximum read length for both Read1 and Read2 should not exceed that specified in the sequencing kit. For example, if PE150 is used, the maximum customized Read1 length and Read2 length should not exceed 150.
- When performing a dual barcode sequencing run, it is recommended that you import single barcode files or dual barcode files that meet sequencing requirements by following the steps in *Importing a barcode file on Page 158*. Both side-A and side-B can only use the same barcode importing strategy if performing dual barcode sequence at the same time.
- Dark reaction cycle: A sequencing cycle in which the chemical reaction is performed, but with no imaging. Therefore, the output FASTQ file will not contain the dark cycle information. For example, for FCL PE150 sequencing, if cycle 2-10 for Read1 are dark cycles, the total cycles in the FASTQ file for Read1 is 141.
- The barcodes for 4 different lanes within a flow cell can be different, but barcode lengths for 4 different lanes within a flow cell have to be the same.

Refer to the following setting examples for your customized run.

1. Read1/Read2 lengths are not the same as those predefined in the Recipe list

Assumptions are as follows:

- Sequencing run: PE150+10
- DNB loading: sequencer
- Length of Read1: 120
- Length of Read2: 140
- Length of Barcode: 10
- Length of Dual barcode: 0
- Split barcode: Yes
- Split dual barcode: Yes
- Two lanes require a non-predefined barcode list

- Total cycles = 120 + 1 + 140 + 1 + 10 = 272
- Select a PE150 kit

The Customize interface is set as follows:

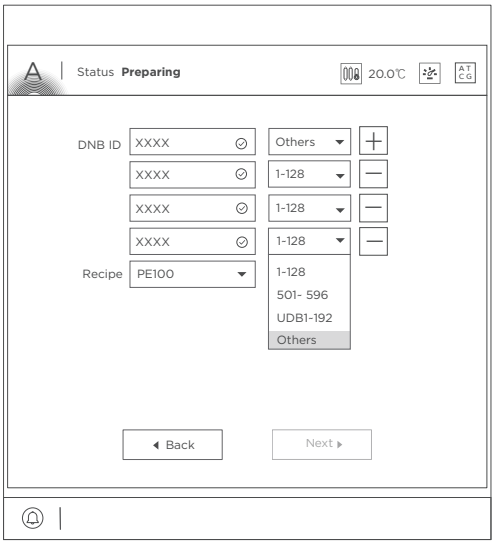


Figure 116 Selecting Others

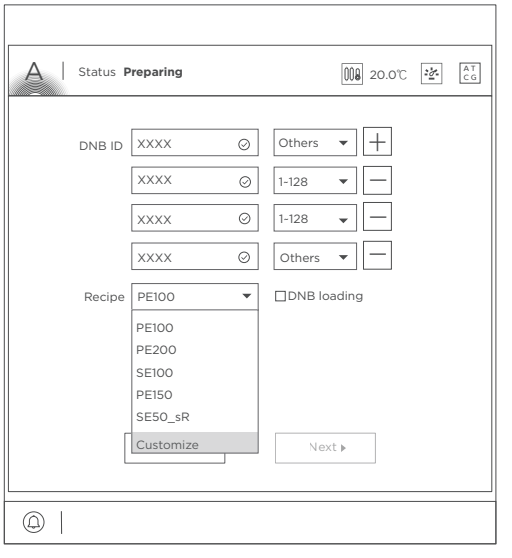


Figure 117 Selecting Customize

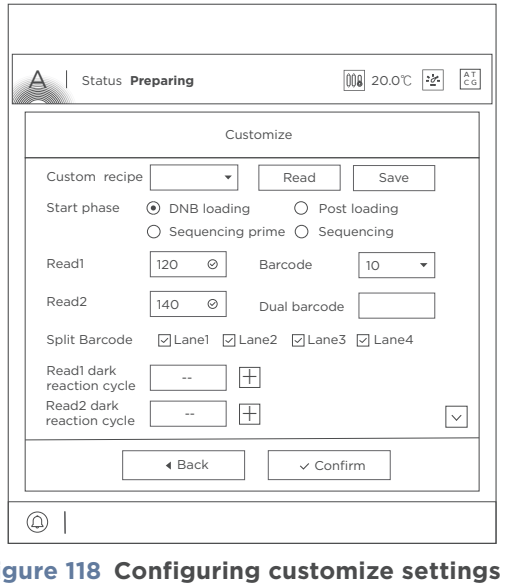


Figure 118 Configuring customize settings for example 1

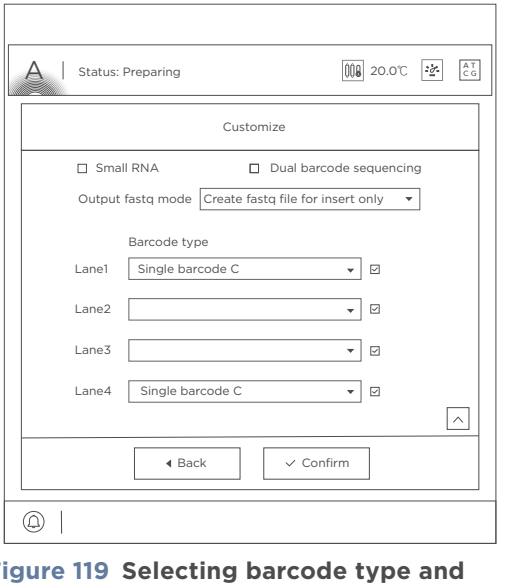


Figure 119 Selecting barcode type and split strategy for example 1

2. Length of the single barcode is not 10

Assumptions are as follows:

- Sequencing run: PE150+8
- DNB loading: DL-200H
- Length of Read1: 150
- Length of Read2: 150
- Length of Barcode: 8
- Length of Dual barcode: 0
- Split barcode: Yes
- Split dual barcode: Yes
- Total cycles = 150 + 1 + 150 + 1 + 8 = 310
- Select a PE150 kit

The Customize interface is set as follows:

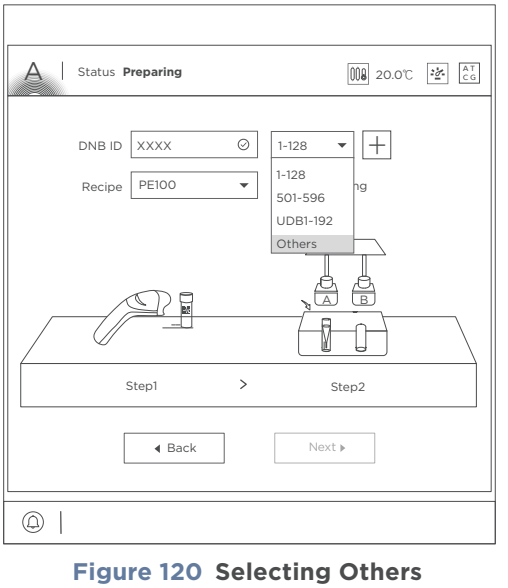


Figure 120 Selecting Others

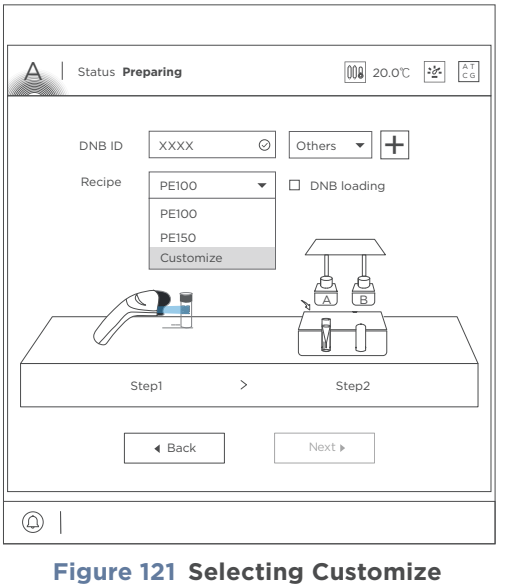


Figure 121 Selecting Customize

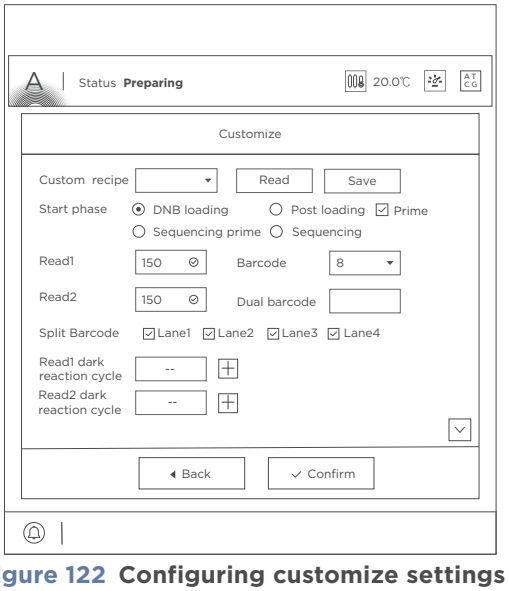


Figure 122 Configuring customize settings for example 2

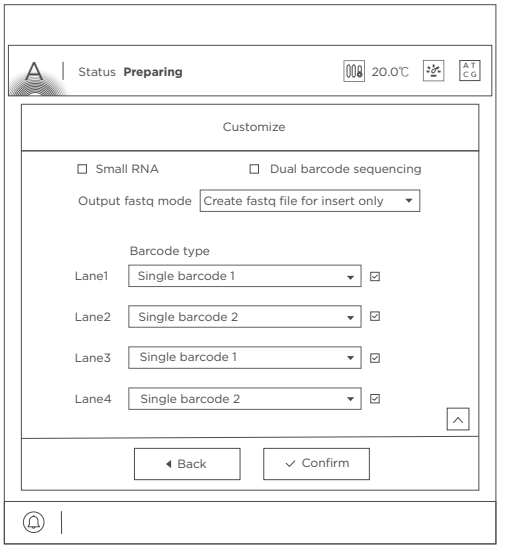


Figure 123 Selecting barcode type and split strategy for example 2

3. A dual barcode sequencing run (Barcode and Dual barcode combined in the dual barcode file)

Assumptions are as follows:

- Sequencing run: PE150+10+10
- DNB loading: DL-200H
- Length of Read1: 150
- Length of Read2: 150
- Length of Barcode: 10
- Length of Dual barcode: 10
- Split barcode: Yes
- Split dual barcode: Yes
- Total cycles = 150 + 1 + 150 + 1 + 10 + 10 = 322
- Select a PE150 kit

The Customize interface is set as follows:

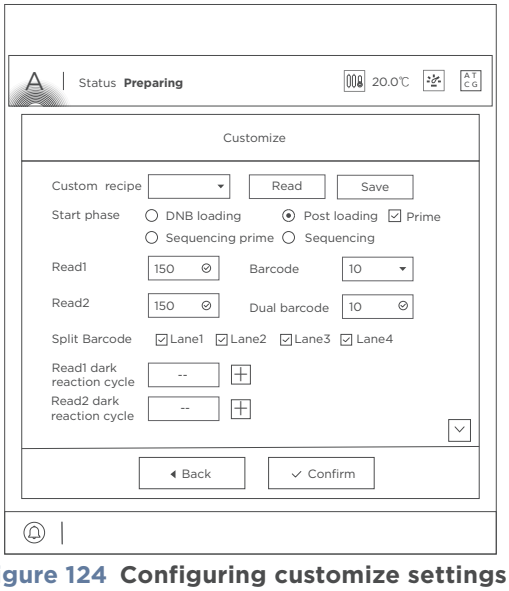


Figure 124 Configuring customize settings for example 3

Figure 125 Selecting barcode type and split strategy for example 3

These parameters can be set in both side-A and side-B. It is recommended that you use identical settings for the sequencing parameters in both side-A and side-B.

4. A dual barcode sequencing run (Barcode and Dual barcode used separately in the dual barcode file)

Assumptions are as follows:

- Sequencing run: PE150+8+8
- DNB loading: DL-200H
- Length of Read1: 150
- Length of Read2: 150
- Length of Barcode: 8
- Length of Dual barcode: 8
- Split barcode: Yes
- Split dual barcode: Yes
- Total cycles = 150 + 1 + 150 + 1 + 8 + 8 = 318
- Select a PE150 kit

The Customize interface is set as follows:

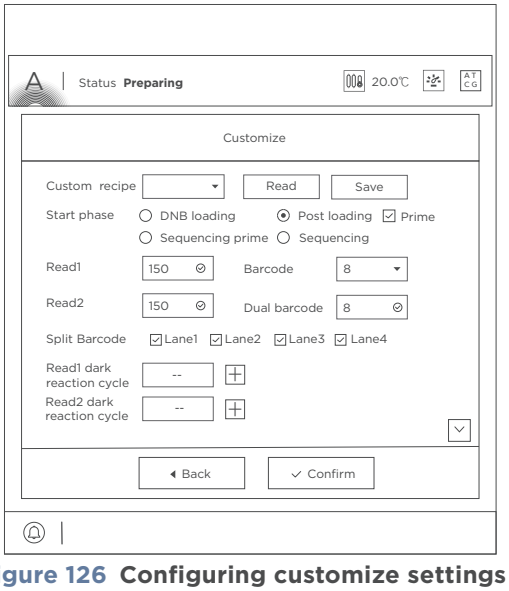


Figure 126 Configuring customize settings for example 4

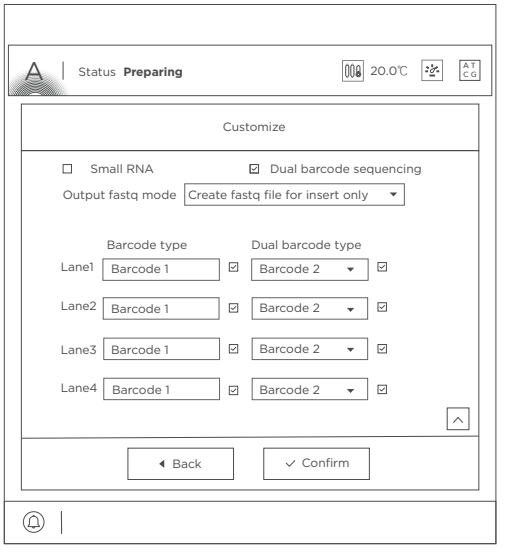


Figure 127 Selecting barcode type and split strategy for example 4

These parameters can be set in both side-A and side-B. It is recommended that you use identical settings for the sequencing parameters in both side-A and side-B.

5. Different lanes within a flow cell require different barcodes

Assumptions are as follows:

- Sequencing run: PE150+8+8
- DNB loading: DL-200H
- Length of Read1: 150
- Length of Read2: 150
- Length of Barcode: 8
- Length of Dual barcode: 8
- Split barcode: Yes
- Split dual barcode: Yes
- Total cycles = 150 + 1 + 150 + 1 + 8 + 8 = 318
- Select a PE150 kit

The Customize interface is set as follows:

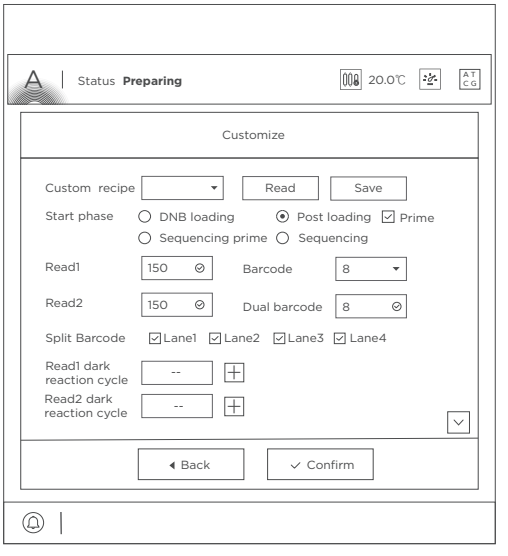


Figure 128 Configuring customize settings for example 5

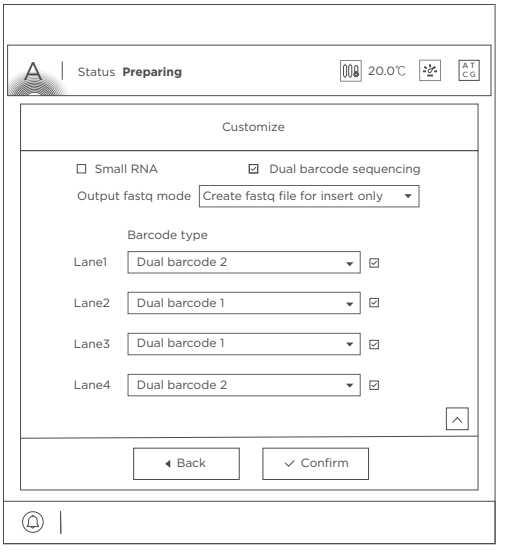


Figure 129 Selecting barcode type and split strategy for example 5

6. Different lanes within a flow cell require different barcode split strategies

Assumptions are as follows:

- Sequencing run: PE150+6+10
- DNB loading: DL-200H
- Length of Read1: 150
- Length of Read2: 150
- Length of Barcode: 6
- Length of Dual barcode: 10
- Lane1 and Lane3 require barcode splitting. Lane2 and Lane4 do not need barcode splitting
- Total cycles = 150 + 1 + 150 + 1 + 6 + 10 = 318
- Select a PE150 kit

The Customize interface is set as follows:

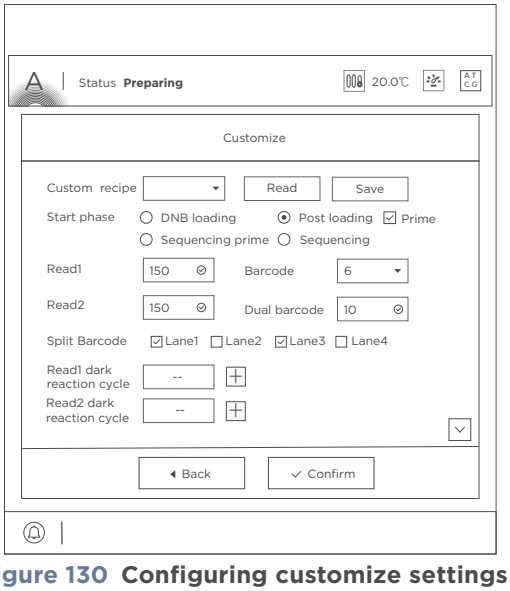


Figure 130 Configuring customize settings for example 6

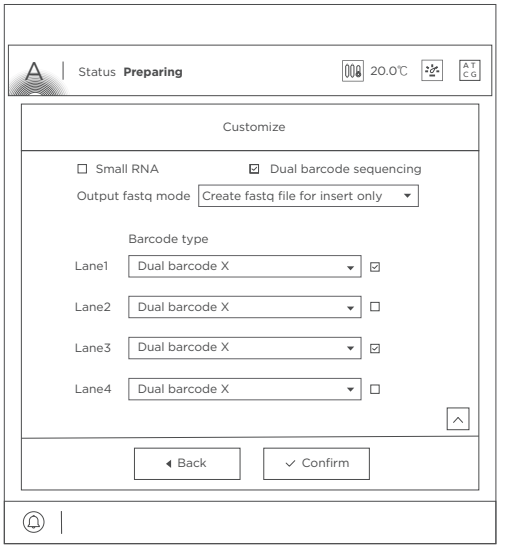


Figure 131 Selecting barcode type and split strategy for example 6

7. Dark reaction cycles are required in Read1 and/or Read2 sequencing

Assumptions are as follows:

- Sequencing run: PE150+8+8
- DNB loading: sequencer
- Length of Read1: 150
- Length of Read2: 150
- Length of Barcode: 8
- Length of Dual barcode: 8
- Lane1 and Lane4 require barcode splitting. Lane2 and Lane3 do not require barcode splitting
- Dark cycles: From cycle-2 to cycle-10 in Read1 and cycle-16 to cycle-20 in Read2.
- Total cycles = 150 + 1 + 150 + 1 + 8 + 8 = 318
- Select a PE150 kit

The Customize interface is set as follows:

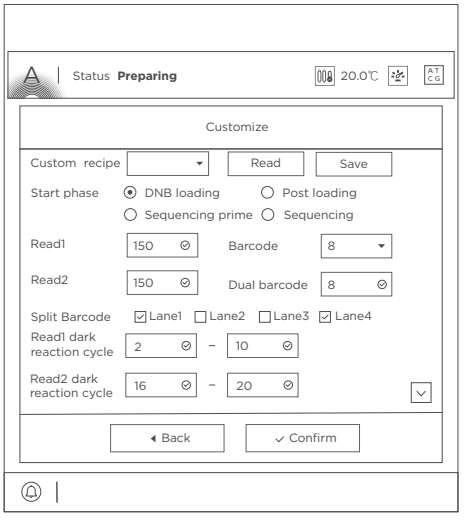


Figure 132 Configuring customize settings for example 7

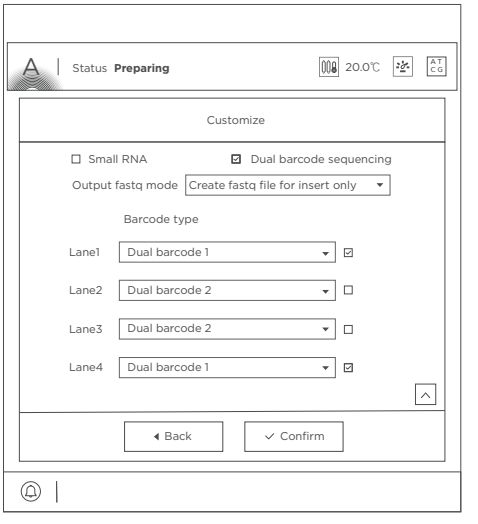


Figure 133 Selecting barcode type and split strategy for example 7

8. UMI+UDI

Assumptions are as follows:

- Sequencing run: PE100+8+(8+9)
- DNB loading: DL-200H
- Length of Read1: 100
- Length of Read2: 100
- Length of Barcode: 8
- Length of Dual barcode: 17
- Split barcode: Yes
- Split dual barcode: Yes
- Total cycles = 100 + 1 + 100 + 1 + 8 + 17 = 227
- Select a PE150 kit (a PE100 kit will not have enough reagents for 227 reaction cycles)

The Customize interface is set as follows:

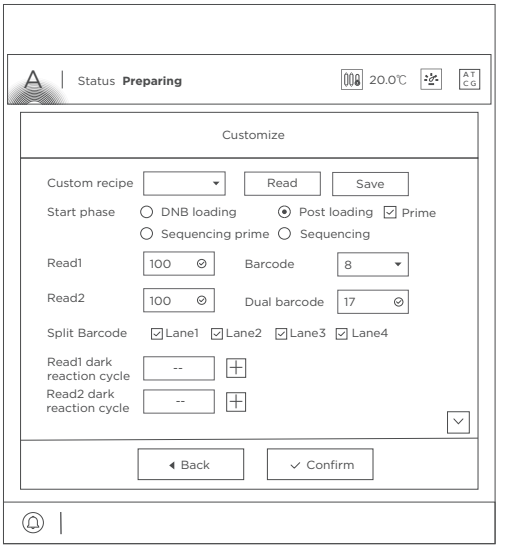


Figure 134 Configuring customize settings for example 8

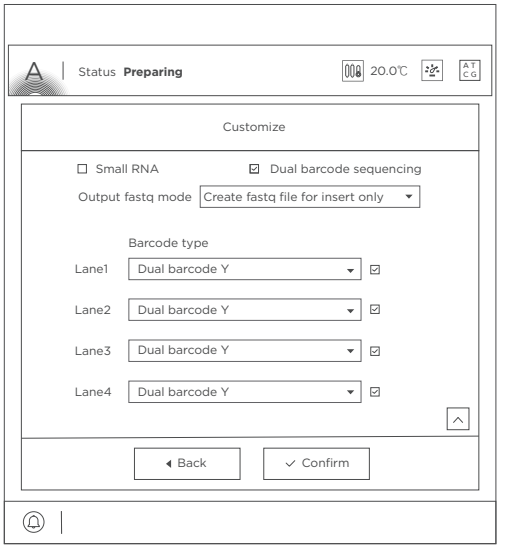


Figure 135 Selecting barcode type and split strategy for example 8

9. Using a customized run when unexpected issues arise

When unexpected problems occur during DNB loading, Post loading, Sequencing prime, or Sequencing, fix the problem first, and continue the sequencing with the following customized settings:

9a. Post loading without Prime

Assumption:

DNBs have been loaded into a flow cell by using either the sequencer or DL-200H, but post loading reagents fail to pump into the flow cell. In addition, the sequencer shows “4/5” for DNB loading or “2/3” for Post loading, which indicates that the post loading prime process has finished. In this case, post loading can be restarted using the Customize interface.

Further assumptions:

- Sequencing run: PE150+8+8
- Start phase: Post loading without Prime
- Length of Read1: 150
- Length of Read2: 150
- Length of Barcode: 8
- Length of Dual barcode: 8
- Split barcode: Yes
- Split dual barcode: Yes
- Total cycles = 150 + 1 + 150 + 1 + 8 + 8 = 318

The Customize interface is set as follows:

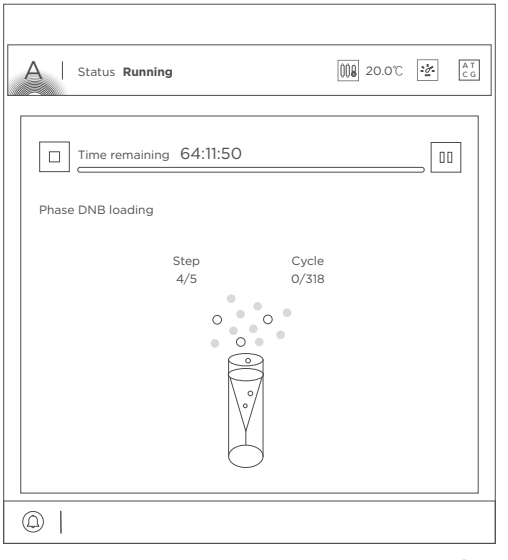


Figure 136 DNB loading interface 4/5

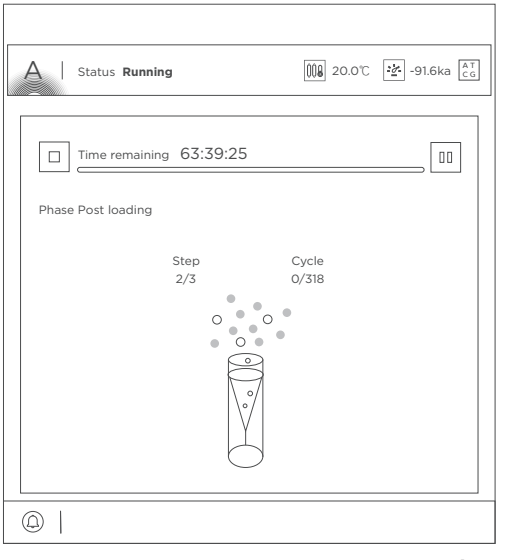


Figure 137 Post loading interface 2/3

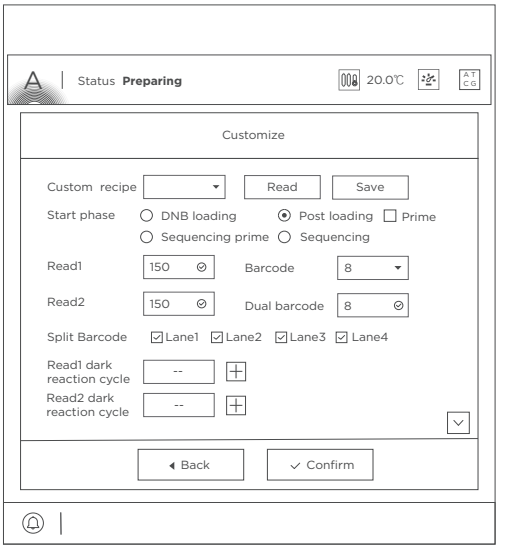


Figure 138 Configuring customize settings for example 9a

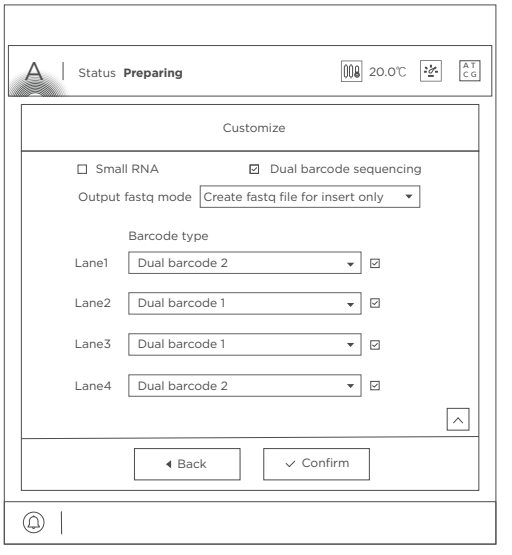


Figure 139 Selecting barcode type and split strategy for example 9a

9b. Sequencing

Assumption:

“Imaging failed” message appeared in the first cycle of sequencing.

Restart the sequencing run after verifying the reagent cartridge preparation by using the following customize settings.

Further assumptions:

- Sequencing run: PE150+8+8
- Start phase: Sequencing
- Length of Read1: 150
- Length of Read2: 150
- Length of Barcode: 8
- Length of Dual barcode: 8
- Split barcode: Yes
- Split dual barcode: Yes
- Total cycles = 150 + 1 + 150 +1 + 8 + 8 = 318

The Customize interface is set as follows:

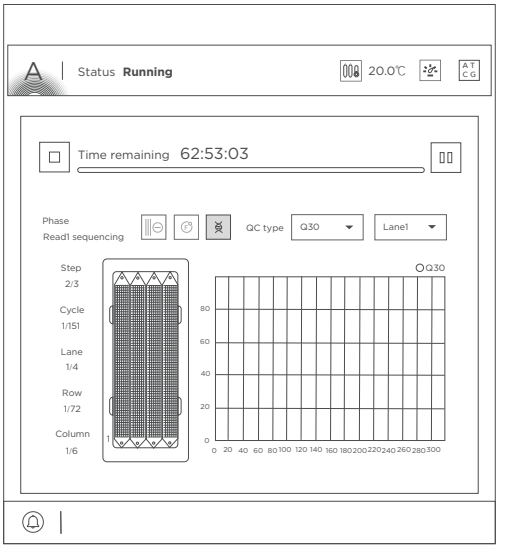


Figure 140 "2/3" Read1 sequencing phase

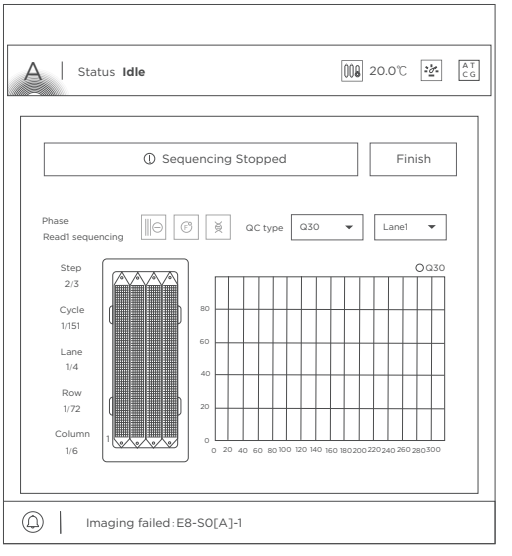


Figure 141 Imaging failed message

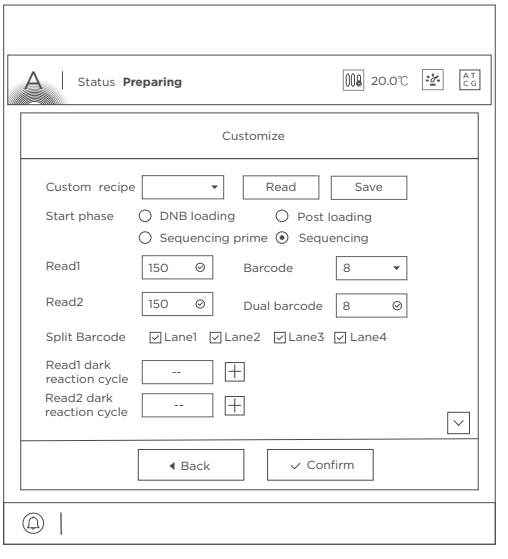


Figure 142 Configuring customize settings for example 9b

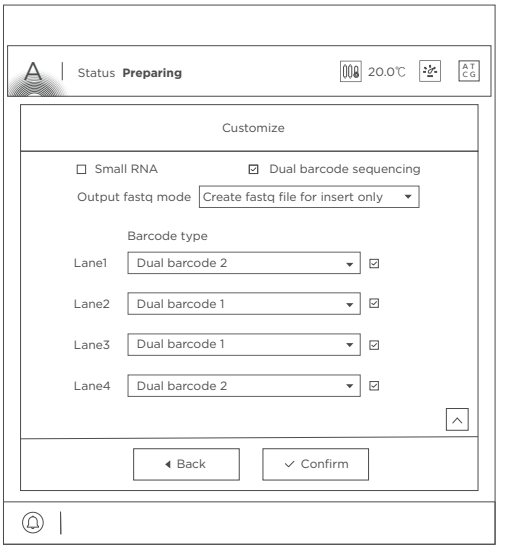


Figure 143 Selecting barcode type and split strategy for example 9b

Instructions for using Qubit to quantify the DNBs

- Working solution should be used within 30 min after preparation.
- It is recommended that the number of quantified samples is no more than 8 per batch to avoid inaccurate DNB quantification as a result of fluorescence quenching.
- Avoid touching the wall of tapered detection tubes.
- Avoid introducing bubbles in detection tubes.

Perform the following steps:

1. Prepare Qubit working solution by diluting Qubit ssDNA Reagent 1:200 in Qubit ssDNA Buffer. Use a clean Qubit assay tube each time you prepare Qubit working solution. Do not mix the working solution in a glass container.

   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 180–199 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 tube used for standards.
3. Add 10 µL of each Qubit standard to the appropriate tube, and mix by vortexing 3 s to 5 s. 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.

   - 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 (Cat. No.: 10011-830).
   - Number of Qubit test tubes needed is 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 the tubes.

6. Prepare the solutions used for standards and sample tests according to the table below:

Table 62 Working solution

Component	Standard volume		Sample volume		
	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（μL）	/	/	2	2	2
Total volume	200	200	200	200	200

7. Mix the tubes by using a vortex mixer and centrifuge briefly for 5 s. Incubate at room temperature for 2 min.
8. Follow the instructions for reading standards and samples in the relevant Qubit user guide. Follow the procedure that is appropriate for your instrument.

Instructions for splitting barcodes

Manual barcode splitting

Offline barcode splitting means that you can split a specified barcode by setting the parameters of *Client.ini*.

You can find *Client.ini* in the following path:

C:\BasecallLite\Config

This section uses the following conditions as examples:

Sequencing recipe is PE100+10+8 (Dualbarcode read length is 10 bp and Barcode read length is 8 bp).

Preconditions:

- SubmitImages = false
- CalFilePath is set correctly:

  D:\Result\workspace\V350060903\L02\calFile

It is recommended that you use the "Notepad++" program to configure the following settings in *Client.ini*.

Table 63 Splitting BC1 and BC2

Parameter setting	Description
Cycle = r100e1r100e1b10b8	Input the complete sequencing recipe
BarcodeFile =	Input Barcodelist of 18 bp with 10 bp at the beginning
DualbarcodeSplit DualbarcodeSplit = { true, true }	Set both DualbarcodeSplit to true
DualbarcodeMismatch = { 1, 1 }	Set both mismatches to 1

Table 64 Splitting BC2 only

Parameter setting	Description
Cycle = r100e1r100e1b10b8	Input the complete sequencing recipe
BarcodeFile =	Input Barcodelist of 10 bp for splitting BC2 only
DualbarcodeSplit DualbarcodeSplit = { true, false}	Set the first DualbarcodeSplit to true, and set the second one to false
DualbarcodeMismatch = { 1, 1 }	Set the first mismatch only

Table 65 Splitting BC1 only

Parameter setting	Description
Cycle = r100e1r100e1b10b8	Input the complete sequencing recipe
BarcodeFile =	Input Barcodelist of 8 bp for splitting BC1 only
DualbarcodeSplit DualbarcodeSplit = { false, true}	Set the first DualbarcodeSplit to false, and set the second one to true
DualbarcodeMismatch = { 1, 1 }	Set the second mismatch only

The input order of Cycle (sequencing read length) is: Read1 length, e1, Read2 length, e1, barcode2 length, barcode1 length; if there is no extra one cycle for calibration, remove e1; barcode2 refers to dual barcode, if no dual barcode is needed, remove the barcode2 length.

Automatic barcode splitting

Automatic barcode splitting means that you can set parameters in the control software of the sequencer, which calls the interface of write FASTQ on Basecall to split the specified barcode.

To set parameters on the sequencer for automatic barcode splitting, perform the following steps:

1. In the main interface, select **Sequence** to open the DNB ID entry interface.
2. Select the **DNB ID** box and enter the DNB ID manually by using the on-screen keyboard.

3. Select a barcode range of different lanes from the list next to the **DNB ID** box; for example, **1~128**, or **501~596**.

   Select [+] or [—] to add or remove a line of DNB ID if needed.

   If you select **Others** from the list, but no barcode file is selected in the **Barcode type** list, the barcode will not be split by the sequencer. For details on customizing a run, refer to *Instructions for customizing a run on Page 163.*

4. Select one-click sequencing recipe from the **Recipe** list; for example, SE50. The sequencer will split barcode automatically.

   If you select **Customize** from the **Recipe** list, more settings need to be set in the Customize interface. For information on customizing a run, refer to *Instructions for customizing a run on Page 163*.

This section uses the following conditions as examples:

Sequencing recipe is PE100+10+8 (Dualbarcode read length is 10 bp, Barcode read length is 8 bp).

Splitting BC1 and BC2

You can determine if the barcode is split successfully in the logs in the following path:

C:\Log

Log example:

|ISW->LITE| generateFastQ: LaneParam: S200032456L01: totalCycle: 220, Read1Len: 101, Read2Len: 101, barcode1Len: 8, barcode1StartPos: 213, barcode2Len: 10, barcode2StartPos: 203, endCycleMode: 3, barcodePos: 3, mismatch: 1, mismatch2: 1, speciesBarcodes: 104

Table 66 Expected parameter passing for splitting BC1 and BC2

Expected parameter passing	Description
totalCycle: 220	Total read length 220
Read1Len: 101	Read1 length 101
Read2Len: 101	Read2 length 101
barcode1Len: 8	The barcode read length
barcode1StartPos: 213	The first cycle of barcode
barcode2Len: 10	The DualBarcode read length
barcode2StartPos: 203	The first cycle of DualBarcode
endCycleMode: 3	Both Read1 and Read2 have an extra cycle for calibration

Expected parameter passing	Description
barcodePos: 3	The sequencing order is: 1. Insert sequencing 2. Barcode sequencing
mismatch: 1	Fault tolerance of Barcode
mismatch2: 1	Fault tolerance of DualBarcode
speciesBarcodes: 104	The number of barcode in Barcodelist

Splitting BC2 only

You can determine if the barcode is split successfully in the logs in the following path:

C:\Log

Log example:

|ISW->LITE| generateFastQ: LaneParam: S200032456L01: totalCycle: 220, Read1Len: 101, Read2Len: 101, barcode1Len: 10, barcode1StartPos: 203, barcode2Len: null, barcode2StartPos: null, endCycleMode: 3, barcodePos: 3, mismatch: 1, mismatch2: 1, speciesBarcodes: 104

Table 67 Expected parameter passing for splitting BC2 only

Expected parameter passing	Description
totalCycle: 220	Total read length 220
Read1Len: 101	Read1 length 101
Read2Len: 101	Read2 length 101
barcode1Len: 10	The barcode read length that needs to be split; that is, read length for DualBarcode
barcode1StartPos: 203	The first cycle of barcode that needs to be split; that is, the first cycle of DualBarcode
barcode2Len: null	If you want to split BC2 only, the value should be null
barcode2StartPos: null	If you want to split BC2 only, the value should be null
endCycleMode: 3	Both Read1 and Read2 have an extra cycle for calibration

Expected parameter passing	Description
barcodePos: 3	The sequencing order is: 1. Insert sequencing 2. Barcode sequencing
mismatch: 1	Fault tolerance of Barcode
mismatch2: 1	Fault tolerance of DualBarcode
speciesBarcodes: 104	The number of barcode in Barcodelist

Splitting BC1 only

You can determine if the barcode is split successfully in the logs in the following path:

C:\Log

Log example:

|ISW->LITE| generateFastQ: LaneParam: S200032456L01: totalCycle: 220, Read1Len: 101, Read2Len: 101, barcode1Len: 8, barcode1StartPos: 213, barcode2Len: null, barcode2StartPos: null, endCycleMode: 3, barcodePos: 3, mismatch: 1, mismatch2: 1, speciesBarcodes: 104

Table 68 Expected parameter passing for splitting BC1 only

Expected parameter passing	Description
totalCycle: 220	Total read length 220
Read1Len: 101	Read1 length 101
Read2Len: 101	Read2 length 101
barcode1Len: 10	The barcode read length that needs to be split; that is, read length for Barcode
barcode1StartPos: 213	The first cycle of barcode that needs to be split; that is, the first cycle of Barcode
barcode2Len: null	If you want to split BC1 only, the value should be null
barcode2StartPos: null	If you want to split BC1 only, the value should be null
endCycleMode: 3	Both Read1 and Read2 have an extra cycle for calibration

Expected parameter passing	Description
barcodePos: 3	The sequencing order is: 1. Insert sequencing 2. Barcode sequencing
mismatch: 1	Fault tolerance of Barcode
mismatch2: 1	Fault tolerance of DualBarcode
speciesBarcodes: 104	The number of barcode in Barcodelist

Device specifications

CAUTION

- The maximum sound pressure level is measured based on the distance between the position where the device operator stands during normal operation and any position that is one meter from the device and has the maximum sound pressure level.
- Because the temperature and humidity fluctuations influence the accuracy of the experiment results, it is recommended that you install an air conditioning system and a humidifier or dehumidifier in the laboratory to maintain the temperature and humidity.

Item	Description
Laser classification of the device	Class 1 laser product
Dimensions	1086 mm × 756 mm × 710 mm (42.8 inches × 29.8 inches × 28 inches)
Net weight	Approximately 193 kg (425.5 lb)
Touch screen	• Type: LCD • Size: 20 inches • Resolution: 1920 × 1080 pixels
Power	• Supply voltage: 100 V to 240 V~ (10% tolerance) • Transient over-voltage category: II • Frequency: 50/60 Hz • Rated power: 1200 VA
Fuse specification	F10AL250V
Maximum sound pressure level	75 dB(A)
Degrees of protection provided by enclosures (IP Code)	IPX0
Operating environment requirements	• Temperature: 19 °C to 25 °C (66 °F to 77 °F) • Relative humidity: 20% to 80%, non-condensing • Atmospheric pressure: 70 kPa to 106 kPa • Maximum altitude: 3000 m (9843 ft) • Site of use: Indoor used only

Item	Description
Transportation/Storage environment requirements	• Temperature: -20 °C to 50 °C (-4 °F to 122 °F) • Relative humidity: 15% to 90%, non-condensing • Atmospheric pressure: 70 kPa to 106 kPa • Maximum altitude: 3000 m (9843 ft)
Accompanying items	Refer to the packing list.

Compliance information

The device complies with the following standards:

Item	Standard
Electromagnetic Compatibility (EMC)	IEC 61326-1 Electrical equipment for measurement, control and laboratory use – EMC requirements – Part 1: General requirements
Safety requirements	• UL 61610-1/CSA C22.2 No.61010-1-12 Safety requirements for electrical equipment for measurement, control, and laboratory use-Part 1: General requirements • UL 61610-2-081/CSA C22.2 No. 61010-2-081 Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes • UL 61010-2-010/CSA C22.2 No. 61010-2-010 Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-010: Particular requirements for laboratory equipment for the heating of materials • IEC 60825-1 Safety of laser product part 1: equipment classification and requirements

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Research use only

Complete Genomics has labeled the product solely for research use only and specified “RS” in the model name which means it should not be used for clinical diagnosis. Please refer to FDA Guidance, *Distribution of In Vitro Diagnostic Products Labeled for Research Use Only or Investigational Use Only* (Nov. 2013) (available at: *https://www.fda.gov/media/87374/download*). If you have any questions, please contact Complete Genomics at +1 (888) 811-9644.

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Contact us

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Order information

Cat. No.	Model	Name	Version	Recommended brand
940-000812-00	FCL PE100	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	CG
940-000810-00	FCL PE150	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	CG
940-000818-00	FCS PE150	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	V1.0	CG
940-000917-00	/	DNBSEQ-G400RS High-throughput Sequencing Primer Kit (App-D) (Paired-End)	V1.0	CG
940-001750-00	/	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode)	V4.0	CG
940-001648-00	/	DNBSEQ OneStep Library Conversion Kit (Third party)	V2.0	CG
940-000870-00	/	Sequencer Cleaning Cartridge	/	CG
900-000218-00	DL-200H	Portable DNB Loader	/	CG
510-003139-00	/	V2L Gasket (Steril)	/	CG

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Acronyms and abbreviations

Item	Description
BIC	Basecall Information Content
cPAS	Combinatorial Probe-anchor Synthesis
DL-200H	Portable DNB Loader
DNB	DNA Nanoball
EMC	Electromagnetic Compatibility
ESR	Effective Spots Rate
FCL	Flow Cell Large, 4 lanes per flow cell in DNBSEQ-G400 Sequencing FCL Flow Cell
FCS	Flow Cell Small, 2 lanes per flow cell in DNBSEQ-G400 Sequencing FCS Flow Cell
FIT	Least square fit to the DNB intensities in 4 color space to represent the overall quality of the clusters
FOV	Field of View
MDA	Multiple Displacement Amplification
PCR	Polymerase Chain Reaction
PE	Pair-end sequencing
PM	Preventive Maintenance
RCA	Rolling Circle Amplification
RHO	Rho (ρ), intensity of raw signals
SE	Single-end sequencing
SNR	Signal to Noise Ratio
UDI	Unique Dual Index
UMI	Unique Molecular Identifier
UPS	Uninterruptible Power Supply
USB	Universal Serial Bus

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