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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 2.0 and the software version is V1.

Revision history

	Date	Version
Revision	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. Otherwise, it may cause altered experimental results, device malfunction, or even personal injury.
- Ensure that the components of the device are completely installed before operation. Otherwise, it 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. Otherwise, it 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. Otherwise, it 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 experimental results or damage to the device.
- Do not move the device after CG Technical Support have installed and debugged the device. Unauthorized moves of the device may cause altered experimental results. If you require to move the device, contact CG Technical Support.
- Only trained personnel can operate the device.
- Do not disconnect the power cord when the device is on. Otherwise, it 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 packages are batched separately. Keep the components in the packages until use and do not remove them. Mixed use of reagent components from different batches of kits is not recommended.

Electrical safety

DANGER

- Ensure that the device is properly grounded, and 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. Otherwise, electric shock may be caused.

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-Part15A. 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.
- If the fuse blew, 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

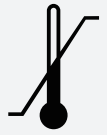
- Use and store the reagents according to the guide. Failure to do so may negatively impact performance.
- Check the expiration date of all reagents before use. Using expired reagents may cause inaccurate results. .

Symbols

Packaging

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

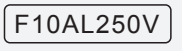
Symbol	Name	Description
	This way up	Indicates the correct upright position of the crated unit for transport and/or storage
	Fragile, handle with care	Indicates a device that can be broken or damaged if not handled carefully
	Keep dry	Indicates a device that needs to be protected from moisture

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
	"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
	/	Indicates a device that is for research use only, and cannot be used for clinical diagnosis
	Model number	Indicates the model number or type number of a product
	NRTL Listing and Certification Mark	Used to designate conformance to nationally recognized product safety standards. The mark bears the name and/or logo of the testing laboratory, product category, safety standard to which conformity is
	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 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

Devices 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 the use of 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 each other. This improves the accuracy of signal processing.

The following figure demonstrates how to make DNBs:

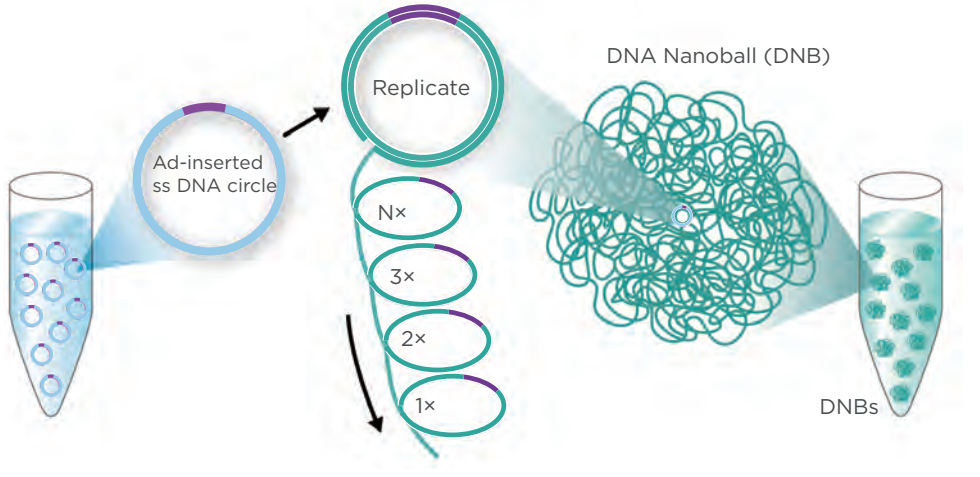

Figure 1 **Making DNBs**

The following figure demonstrates how to load DNBs:

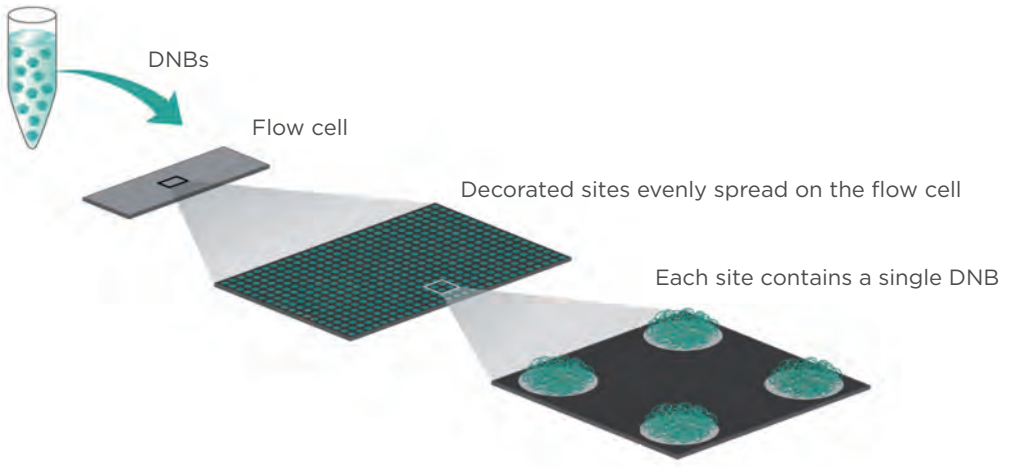

Figure 2 Loading DNBs

The DNBs and sequencing reagents are pumped into the sequencing flow cell through the device's liquid delivery system. Each DNB combines 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 (software version: V1). 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, 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 the power supply for the device.
Display system	Provides the human-computer interaction interface.

Basic components

Front view

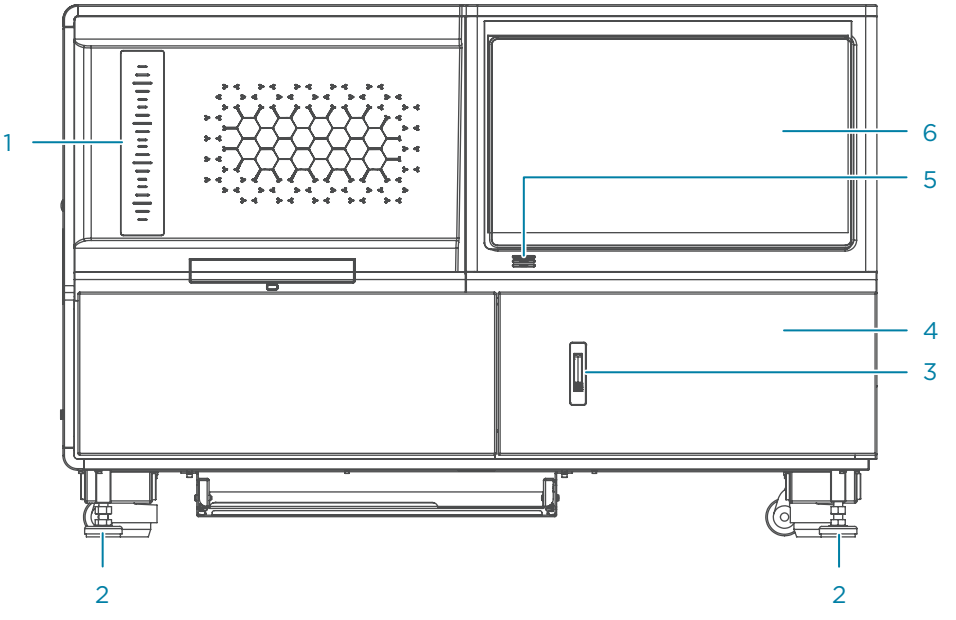

Figure 3 Front view of DNBSEQ-G400RS

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 of 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	Buzzer	Alerts when warnings appear, or errors occur.
6	Touch screen monitor	Facilitates on-screen operation and displays information.

Back view

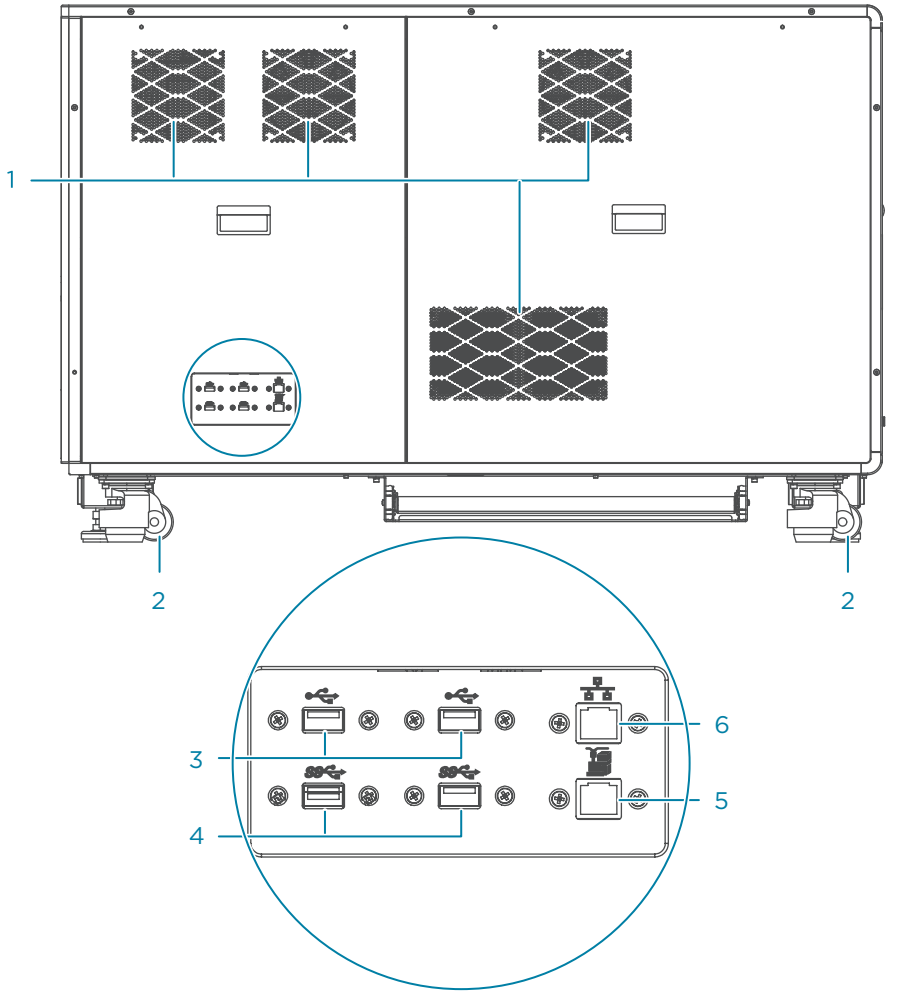

Figure 4 Back view of DNBSEQ-G400RS

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.
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

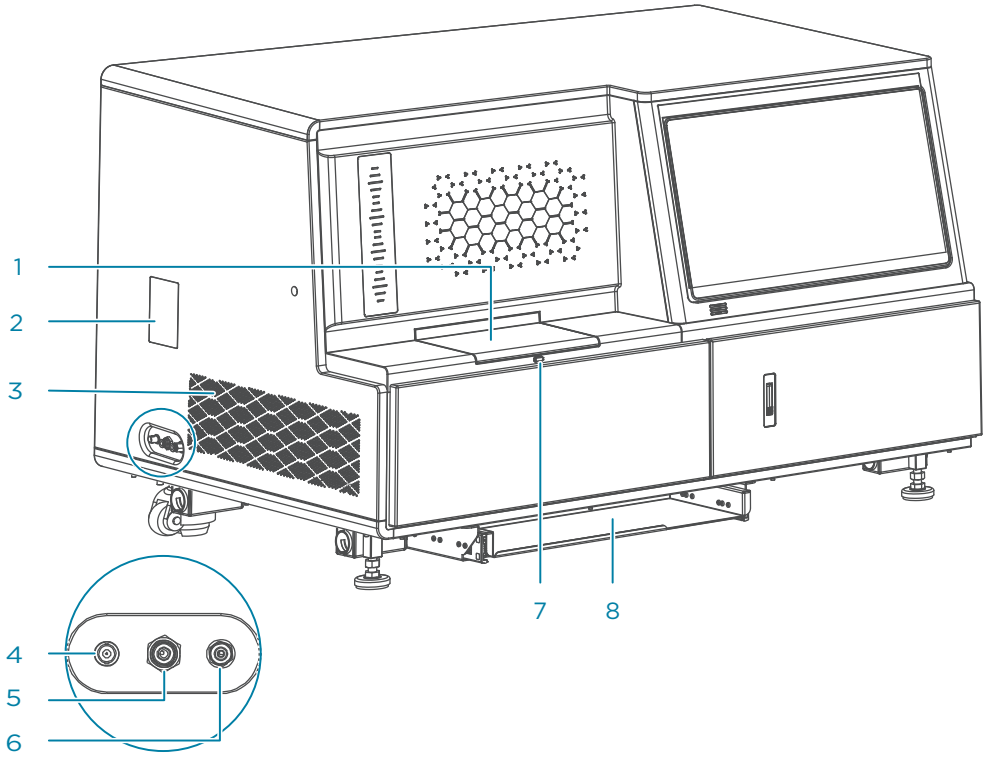

Figure 5 Left view of DNBSEQ-G400RS

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 through the window.
3	Ventilation inlet	Ventilates the device.
4	Level sensor port	Connects the waste level sensor in the waste container.
5	Condensed water port	Connects the condenser tube to dispense the condensed water that is produced by the cooling system to the waste container.
6	Waste port	Connects the waste tube to dispense the waste to the waste container.
7	Button of the flow cell compartment door	Presses to open the flow cell compartment door.
8	Keyboard drawer	Holds the keyboard and mouse.

Right view

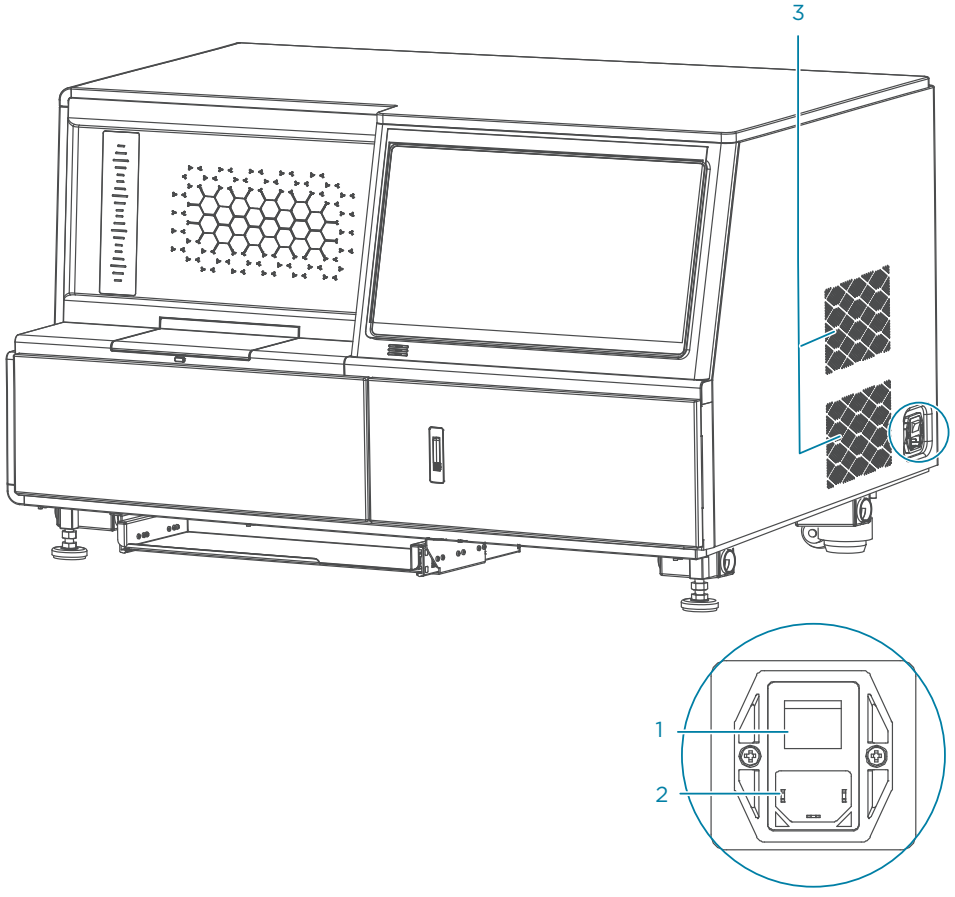

Figure 6 Right view of DNBSEQ-G400RS

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

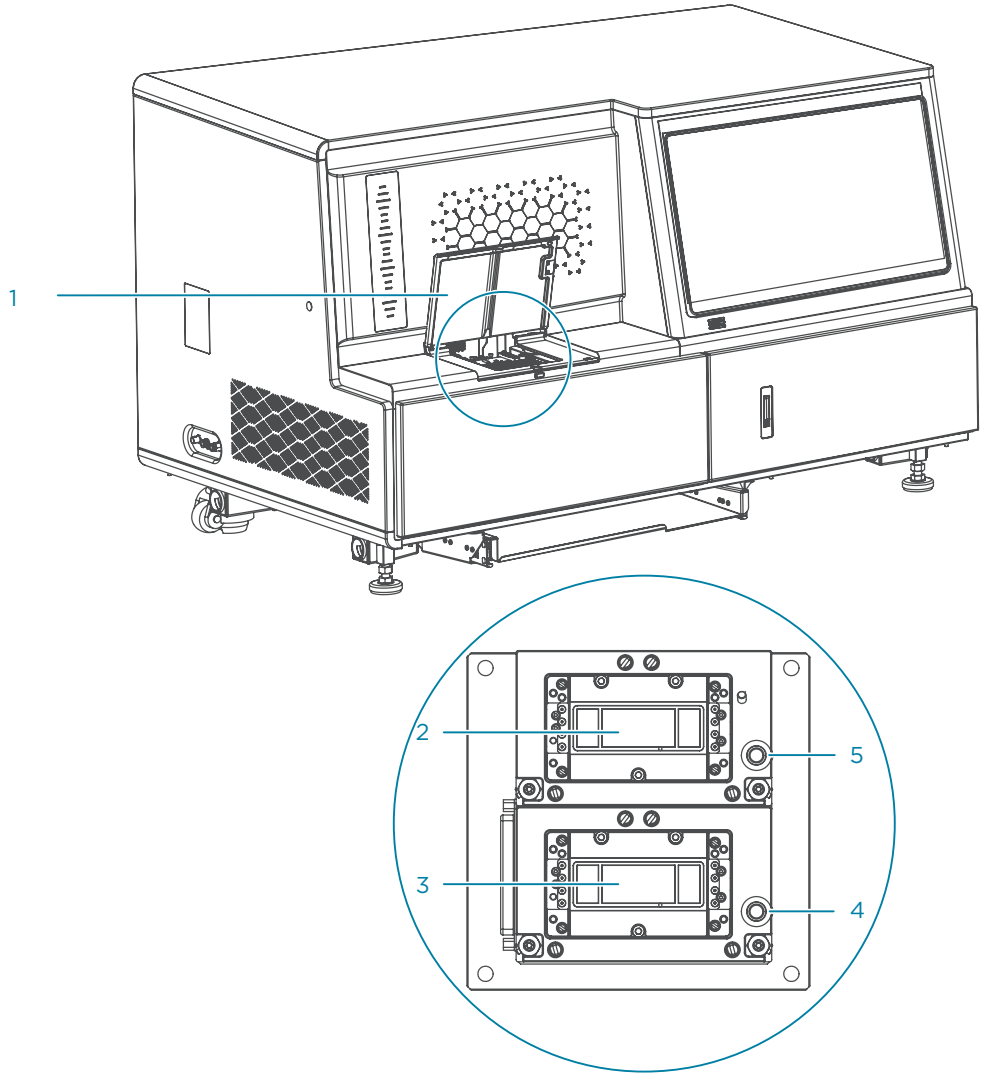

Figure 7 Flow cell compartment of DNBSEQ-G400RS

No.	Name	Description
1	Flow cell compartment door	Presses 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	Presses to activate the vacuum for attachment or release of the flow cell A.
5	Flow cell attachment button B	Presses to activate the vacuum for attachment or release of the flow cell B.

Reagent compartment

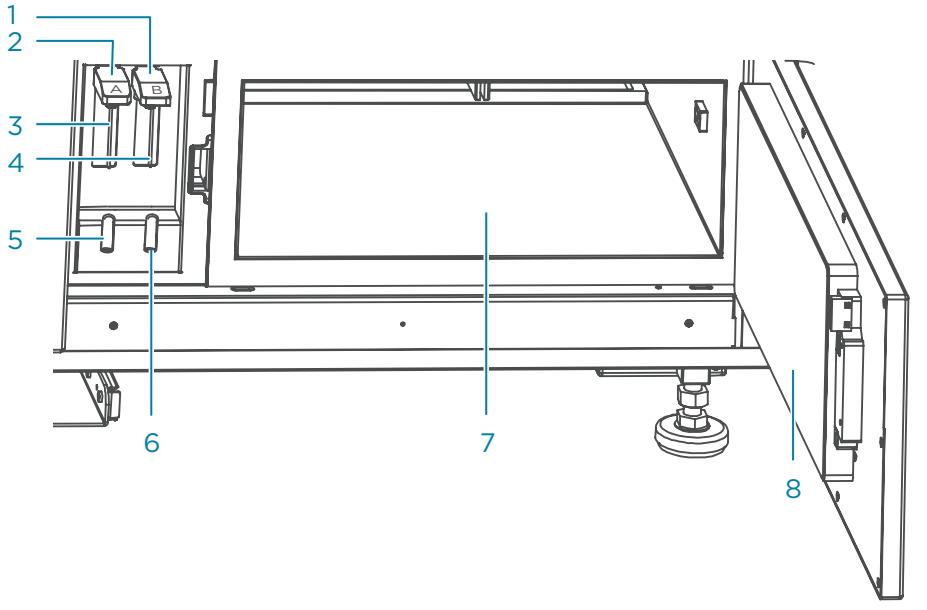

Figure 8 Reagent compartment of DNBSEQ-G400RS

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	Tube rack A	Holds tube A.
6	Tube rack B	Holds 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	Allows you to 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 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 components status of the system.

Self-test

After you power the device on and log in to the computer with the password that is provided by the manufacturer, self-test starts. If the self-test succeeds, the main interface appears.

If the self-test fails, perform the following steps:

1. In the main interface, select , and select **Log** to check the detailed self-test results that are recorded in the log.
2. Follow the on-screen instructions or the solutions that are mentioned in *Sequencer FAQs on Page 128*.
3. Perform a self-test again:
   - Select , select **Maintenance > Self-test**.
   - Select **> Restart**.

   If the problem persists, contact CG Technical Support.

Main interface

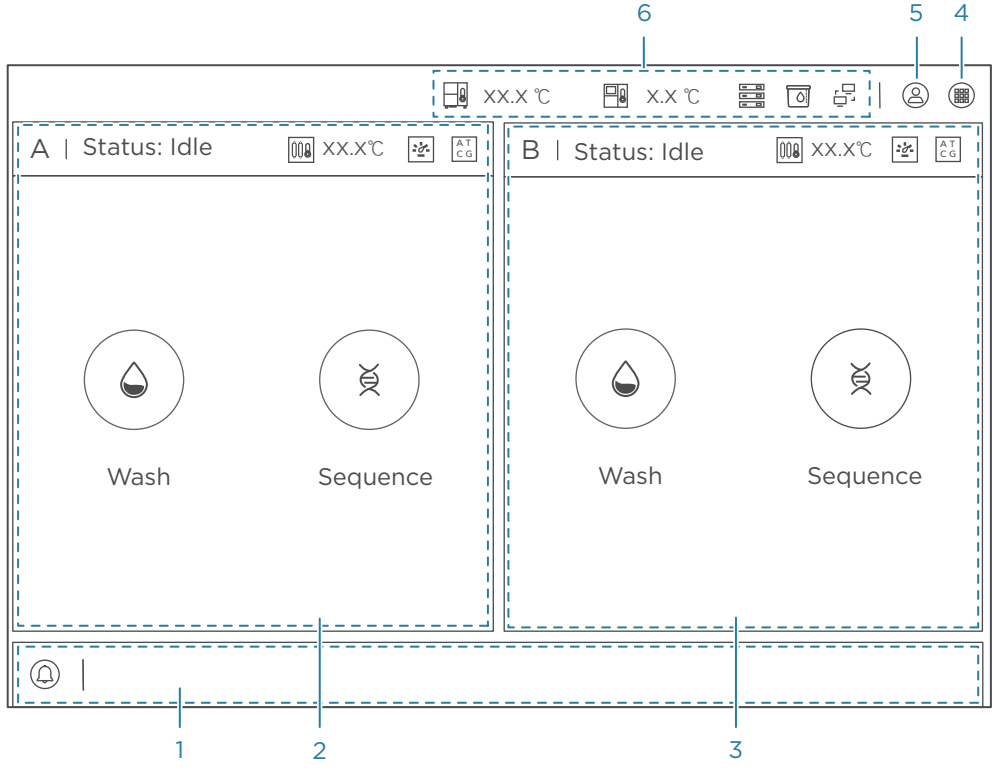

Figure 9 Main interface

The following table describes the function of each area or button 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	Menu button	Select to view the logs, change settings, perform maintenance, lock screen, shut down or restart the system, or check the system information.
5	Login button	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 control functions in the notification area:

Item	Description
	The notification icon indicates: • Blue: the device is operating normally. • Yellow and flash: a warning notification appears. • Red and flash: an error occurs. General information, warnings, or error messages are displayed on the right of the icon.

Operation area

The following table describes control functions in the operation area:

Item	Description
A/B	Operation area of flow cell A or B.
Status	System status.
	Temperature of the flow cell stage is normal.
	Temperature of the flow cell stage is beyond the normal range.
	Negative pressure is normal.

Item	Description
	Negative pressure is beyond the normal range.
	Basecalling is connected.
	Errors occur 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 114*.
Sequence	Select to set sequencing parameters, and perform a sequencing run by following the on-screen instructions. For details, refer to *Sequencing on Page 35*.

Status area

The following table describes control functions in the status area:

Item	Description
	Inner temperature of the device is normal.
	Inner temperature of the device is beyond the normal range.
	Temperature of the reagent compartment is normal.
	Temperature of the reagent compartment is beyond 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.

Item	Description
	The system runs independently, and the server of ZLIMS software is not connected.
	The system is connected normally to the server on which ZLIMS software is installed.
	Errors occur in connection with the server on which ZLIMS software is installed.

Log interface

You can view log information in this interface.

To open the log interface, select  **> Log**.

The following table describes control functions in the log 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 of that 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 page of logs.
>	Select to turn to 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 51*.
2. Select  and select **Settings**.

The following table describes the function of controls in the 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. Restart the device to apply the changes.
Upload	Select **Upload enabled** to upload the data to the specified server.
Customize	Select to change the wait time before the screen locks automatically. Move the slider to change the buzzer volume.

Maintenance interface

You can maintain the device, import or export the data, and import barcode files in this interface.

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

1. Log in to your account.
2. Select  and select **Maintenance**.

The following table describes control functions in the maintenance 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 self-test for the hardware of the device. When the test is finished, you will get a notification and the results will be displayed on the screen.
	Clear history data	Select to clear all history data of sequencing runs, except for the data of 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.
Barcode settings	**Import barcode**	Import the barcode file that is saved in the external storage server or hard drive to the device.

Item	Description
User management	Select to reset the passwords of the current user account.

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

About interface

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

To open the About interface, select > **About**.

DNB loader overview

Overview

The Portable DNB Loader (DL-200H) is used with the sequencer. It is intended for loading the prepared DNBs into the sequencing flow cells.

Basic components

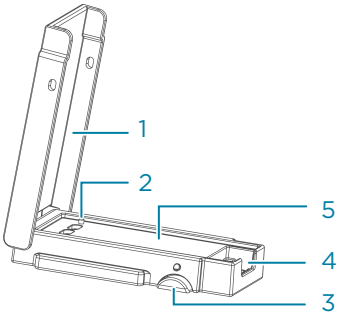

Figure 10 Side view of DL-200H

No.	Name	Description
1	Upper cover	Fastens the flow cell.
2	Alignment pin	Helps alignment of 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.

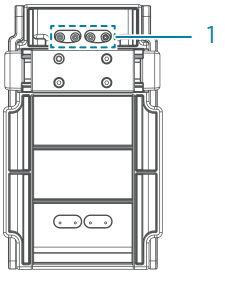

Figure 11 Back view of DL-200H

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

03

Sequencing sets overview

This chapter describes the sequencing sets information.

Introduction

This section describes the sequencing sets, sequencing run times, and data output. Two flow cell types are described: FCS (Flow Cell Small) with two lanes per flow cell and FCL (Flow Cell Large) with four lanes per flow cell.

Available sequencing set list

Table 1 Available sequencing set list

Catalog number	Model	Name	Version	Data output (GB/flow cell)
940-000830-00	FCL SE50	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	18.7 to 22.5
940-000826-00	FCL SE100	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	37.5 to 45.0
940-000828-00	FCL SE400	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	150.0 to 180.0
940-000812-00	FCL PE100	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	75.0 to 90.0
940-000810-00	FCL PE150	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	112.5 to 135.0
940-000814-00	FCL PE200	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	150.0 to 180.0
940-000831-00	Small RNA FCL SE50	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	/
940-000824-00	FCS SE100	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	V1.0	About 27.5
940-000820-00	FCS PE100	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	V1.0	About 55.0
940-000818-00	FCS PE150	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	V1.0	About 82.5
940-000816-00	FCS PE300	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	V1.0	About 90
940-000822-00	stLFR FCL PE100	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	75.0 to 90.0

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. At the end of the sequencing run an extra 10 cycles or 20 cycles of barcode read can be performed to aid in identifying a specific library, if required. For stLFR (Single-tube long fragment read) libraries, at the end of the sequencing run, an extra 42 or 52 cycles of barcode read will be performed.

Table 2 Sequencing cycle

Sequencing read length	Read1 read length	Read2 read length	Barcode read length	Dual barcode read length	Maximum cycles
SE50	50	--	10	10	71
SE100	100	--	10	10	121
SE400	400	--	10	10	421
PE100	100	100	10	10	222
PE150	150	150	10	10	322
PE200	200	200	10	10	422
PE300	300	300	10	10	622
stLFR FCL PE100	100	100	42	10	254

- To ensure sequencing quality, when Read1 and Read2 sequencing is complete, the sequencer will automatically perform one more cycles 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 cycle number of this sequencing is 222.
- PE means Pair-end sequencing; SE means Single-end sequencing.

Sequencing time

Table 3 FCL[a] Sequencing time and analysis time for each read length (hours)

/	SE50	SE100	SE400	stLFR PE100	PE100	PE150	PE200
Single flow cell	12.0	22.0	104.0	52.2	34.0	51.0	100.0
Dual flow cells	13.0	23.0	104.0	53.2	35.0	52.0	102.0
Data analysis (Single flow cell)	0.5	0.8	2.5	1.5	1.0	1.4	2.5
Data analysis (Dual flow cells)	1.0	1.5	5.0	3.3	1.6	2.4	5.0

Table 4 FCS[b] Sequencing time and analysis time for each read length (hours)

/	SE100	PE100	PE150	PE300
Single flow cell	12.5	24.9	35.4	95.5
Dual flow cells	12.7	25.0	35.6	96.3
Data analysis (Single flow cell)	0.4	0.5	0.6	2.0
Data analysis (Dual flow cells)	0.7	1.0	1.2	4.0

- a: DNBSEQ-G400 FCL Sequencing Flow Cell, hereinafter referred to as FCL. Each FCL has four lanes, each lane with 600 M DNB binding sites.
- b: 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.

User-supplied equipment and consumables

Before using the device, prepare the following equipment:

Table 5 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 to use the following reagents/consumables:

WARNING Tips are disposable consumables. Do not reuse them.

Table 6 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
ProClin 300	Sigma, catalog number: 48912-U	Performing a maintenance wash, diluting to 0.03% for washing reagents

Reagent/Consumable	Recommended brand	Purpose
Tween-20	Sigma-Aldrich, catalog number: 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
Sterile 200 µL wide-bore, non-filtered pipette tip	AXYGEN, catalog number: T-205-WB-C	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, 1.5 mL	VWR, catalog number: 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	VWR	Cleaning
Low-lint cloth	General lab supplier	Cleaning
Laboratory-grade water	General lab supplier	/

04

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

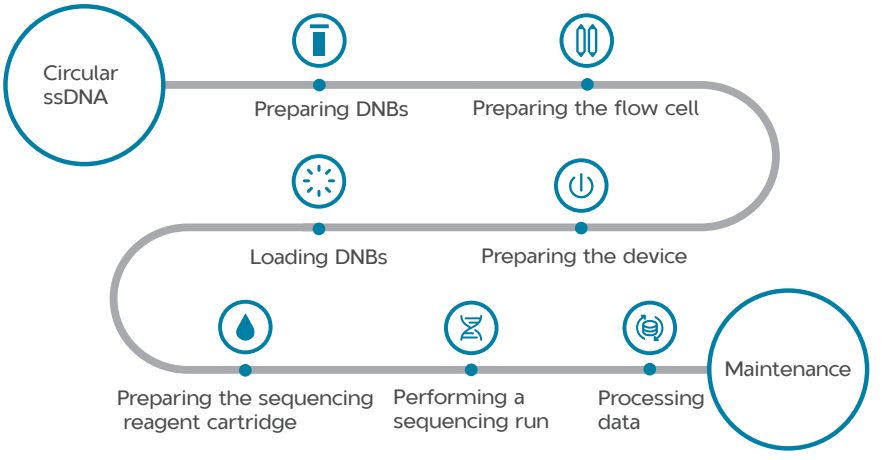

Figure 12 DNDSEQ-G400RS Sequencing workflow

- 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 DNBs

Recommended library insert size

The sequencing set is compatible with the libraries prepared by CG Library Prep Kits and the stLFR libraries prepared by CG stLFR Library Prep Kits. If third party library preparation kits are used, please contact CG Technical Support for conversion options.

- For stLFR libraries, the recommended size distribution of inserts ranges between 200 bp and 1500 bp.
- For other libraries, the recommended size distribution of inserts ranges between 20 bp and 800 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.

Table 7 Recommended library insert size

Model	Recommended library insert distribution (bp)
FCL SE50	50 to 230
FCL SE100	200 to 400
FCL SE400	400 to 600
FCL PE100	200 to 400
FCL PE150	300 to 500
FCL PE200	400 to 600
Small RNA FCL SE50	20 to 60
FCS SE100	200 to 400
FCS PE100	200 to 400
FCS PE150	300 to 500
FCS PE300	400 to 700

- Select sequencing kits according to the insert size and the required data output.
- Average data output will vary with library types and applications.

DNA library concentration and amount requirement

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

  C (fmol/μL)=3030×C (ng/μL)/N

  N represents the number of nucleotides (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 8 Circular ssDNA library concentration requirement

Library type	Library concentration
General libraries	≥ 2 fmol / μL
Small RNA libraries	≥ 3 fmol / μL
stLFR libraries	≥ 1.5 ng / μL
PCR free libraries	≥ 3.75 fmol / μL

Making DNBs

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

DNB making protocols for different sequencing kits are listed below. Please select the appropriate protocol and follow it carefully, depending on the sequencing kit you choose to use.

- *Making DNBs for FCL SE50, FCL SE100, FCL SE400, FCL PE100, FCL PE150, FCL PE200, Small RNA FCL SE50, FCS SE100, FCS PE100, and FCS PE150 on Page 38.*
- *Making DNBs for FCS PE300 on Page 42.*
- *Making DNBs for stLFR FCL PE100 on Page 45.*

Making DNBs for FCL SE50, FCL SE100, FCL SE400, FCL PE100, FCL PE150, FCL PE200, Small RNA FCL SE50, FCS SE100, FCS PE100, and FCS PE150

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.
3. Remove Make DNB Enzyme Mix I from storage and thaw the reagent for approximately 0.5 hours on ice.
4. Mix the reagents by using a vortex mixer for 5 seconds. Centrifuge briefly and place on ice until use.

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

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 9 Required number of make DNB reactions for each flow cell

Flow Cell type	Loading system	DNB volume (μL) / lane	Make DNB reaction (μL)	Required number of make DNB reactions / flow cell
FCL	Sequencer	50	100	2
	DL-200H	25	50	1 to 4
FCS	Sequencer	50	100	1
	DL-200H	25	50	1 to 2

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 Section *DNA library concentration and amount requirement on Page 37*.

- 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 10 Required amount of ssDNA libraries

Library type	Volume of 100 μL DNB reaction (μL)	Volume of 50 μL DNB reaction (μL)
General libraries	V = 40 fmol / C	V = 20 fmol / C
Small RNA libraries	V = 60 fmol / C	V = 30 fmol / C
PCR free libraries	V = 75 fmol / C	V = 37.5 fmol / C

Calculate the required ssDNA libraries for each Make DNB reaction. The value of *V* obtained from the above equation will be used in *Table 11 on Page 40* .

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:

Table 11 Make DNB reaction mixture 1

Component	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

2. Mix the reaction mixture thoroughly by using a vortex mixer, centrifuge for 5 seconds 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. Thermal cycler settings are shown in the table below:

Table 12 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 on ice. Centrifuge briefly for 5 seconds 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. Take the PCR tube out of the thermal cycler when the temperature reaches 4 °C.

6. Centrifuge briefly for 5 seconds, place the tube on ice, and prepare Make DNB reaction mixture 2 according to the table below:

Table 13 Make DNB reaction mixture 2

Component	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

7. Add all Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer and centrifuge it for 5 seconds 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 run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
- It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 14 RCR (Rolling circle replication) 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, non-filtered pipette tip.

Table 15 Volume of Stop DNB Reaction Buffer

Component	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, non-filtered pipette tip. Do not centrifuge, vortex, or shake the tube.
- Store DNBs at 2 °C to 8 °C and perform sequencing within 48 hours.

Making DNBs for FCS PE300

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 reagents at room temperature.
3. Remove Make DNB rapid Enzyme Mix II from storage and thaw the reagent for approximately 0.5 hours on ice.
4. Mix the reagents by using a vortex mixer for 5 seconds. Centrifuge briefly by using a mini spinner and place on ice until use.

Calculating the number of DNB reactions

Each FCS contains 2 lanes. DNBs can be loaded into the flow cell by using the sequencer or DL-200H.

- 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 16 Required number of make DNB reactions for each flow cell

Loading system	Model	DNB volume (μL)/lane	Make DNB reaction (μL)	Required number of make DNB reactions / flow cell
Sequencer	FCS PE300	45	90	1
DL-200H		22.5	90	1 to 2

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 Section *DNA library concentration and amount requirement on Page 37*.

- 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 regulations.
- *C* in the following table represents the concentration of libraries（fmol/μL).

Table 17 Volume of ssDNA libraries for FCS PE300

Library type	Volume of 90 µL DNB reaction (µL)	Volume of 45 µL DNB reaction (µL)
General libraries	V = 40 fmol / C	V = 20 fmol / C
PCR free libraries	V = 75 fmol / C	V = 37.5 fmol / C

Calculate the required ssDNA libraries for each Make DNB reaction. The value of *V* obtained from the above equation will be used in *Table 18 on Page 43* .

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:

Table 18 Make DNB reaction mixture 1 for FCS PE300

Component	Volume of 90 µL DNB reaction (µL)	Volume of 45 µL DNB reaction (µL)
Low TE Buffer	20 - V	10 - V
Make DNB Buffer	20	10
ssDNA libraries	V	V
Total Volume	40	20

i Do not discard the Low TE Buffer after you finish this step, it will be used in DNB dilution operations.

2. Mix the reaction mixture thoroughly by using a vortex mixer, centrifuge for 5 seconds 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. Thermal cycler settings are shown in the table below:

Table 19 Primer hybridization reaction conditions for FCS PE300

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

Table 20 Make DNB reaction mixture 2 for FCS PE300

Component	Volume of 90 µL DNB reaction (µL)	Volume of 45 µL DNB reaction (µL)
Make DNB rapid Enzyme Mix II	40	20
Make DNB Enzyme Mix II (LC)	1.6	0.8

7. Add all Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer, centrifuge for 5 seconds 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 run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
   - It is recommended to set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 21 RCR conditions for FCS PE300

Temperature	Time
Heated lid (35 °C)	On
30 °C	15 min
4 °C	Hold

9. Immediately add Stop DNB Reaction Buffer once the temperature reaches 2 °C to 8 °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, non-filtered pipette tip.

   - Keep DNBs on ice during the entire operation to prevent DNBs from performing secondary replication.
   - It is very important to mix DNBs gently by using a wide-bore, non-filtered pipette tip. Do not centrifuge, vortex, or shake the tube.
   - This is not a STOP point, immediately go to the next step: *Quantifying DNBs on Page 48*.

Table 22 Volume of Stop DNB Reaction Buffer for FCS PE300

Component	Volume of 90 µL DNB reaction (µL)	Volume of 45 µL DNB reaction (µL)
Stop DNB Reaction Buffer	10	5

Making DNBs for stLFR FCL PE100

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. Remove Low TE Buffer, stLFR Make DNB Buffer, and Stop DNB Reaction Buffer from storage and thaw the reagents at room temperature.
3. Remove Make DNB Enzyme Mix III from storage nd thaw it on ice for approximately 0.5 hours.
4. Mix all the reagents by using a vortex mixer for 5 seconds. Centrifuge briefly by using a mini spinner and place on ice until use.

Calculating the number of DNB reactions

The FCL contains 4 lanes. DNBs can be loaded into the flow cell by using the sequencer or DL-200H.

- Using the sequencer to load DNBs

  All lanes must be loaded with the same DNBs.

- Using DL-200H to load DNBs

  Different DNBs can be loaded into 4 different lanes.

Table 23 Required number of make DNB reactions for each flow cell

Loading system	Model	DNB volume (µL) / lane	Make DNB reaction (µL)	Required number of make DNB reactions / flow cell
Sequencer	stLFR FCL PE100	50	80	3
DL-200H		25	80	2 to 4

Calculating the required amount of ssDNA libraries

The required volume of stLFR libraries is determined by the required library amount (ng) and library concentration quantified in Section *DNA library concentration and amount requirement on Page 37*.

Tips

- 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 regulations.
- *C* mentioned in the following table represents the concentration of libraries (ng/µL).

Table 24 Volume of ssDNA libraries for stLFR FCL PE100

Library type	The volume of 80 µL DNB reaction (µL)
stLFR libraries	V = 20 ng / C

Calculate the required ssDNA libraries for each Make DNB reaction. The value of *V* obtained from the above equation will be used in *Table 25 on Page 46* .

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:

Table 25 Make DNB reaction mixture 1 for stLFR FCL PE100

Component	Volume (µL)
Low TE Buffer	16 - V
stLFR Make DNB Buffer	16
dsDNA libraries	V
Total Volume	32

2. Mix the reaction mixture thoroughly by using a vortex mixer, centrifuge for 5 seconds 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. Thermal cycler settings are shown in the table below:

Table 26 Primer hybridization reaction conditions for stLFR FCL PE100

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

4. Remove Make DNB Enzyme Mix IV from storage and place on ice. Centrifuge briefly for 5 seconds and hold on ice.

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

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

Table 27 Make DNB reaction mixture 2 for stLFR FCL PE100

Component	Volume (µL)
Make DNB Enzyme Mix III	32.0
Make DNB Enzyme Mix IV	3.2

7. Add all Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer, centrifuge for 5 seconds 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 run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
- It is recommended to set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 28 RCR conditions for stLFR FCL PE100

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

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

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

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 171*.

Table 29 DNB concentration standard

Model	DNB concentration
FCL SE50, FCL SE100, FCL SE400, FCL PE100, FCL PE150, FCL PE200, Small RNA FCL SE50, FCS SE100, FCS PE100, FCS PE150	≥12 ng/µL
FCS PE300	≥8 ng/µL
stLFR FCL PE100	≥6 ng/µL

   - If the concentration of libraries prepared by customers is lower than that specified in the table above, refer to *Q: What should I do if DNB concentration is low? on Page 132* for details.
   - If there are too many samples in a single test, it is recommended to quantify in batches to avoid inaccurate DNB quantification due to fluorescence quenching.

2. If the concentration exceeds 40 ng/µL, the DNBs should be diluted to 20 ng/µL according to the table below:

Table 30 DNB dilution scheme

Model	Dilution reagent	Storage conditions	Storage time
FCL SE50, FCL SE100, FCL SE400, FCL PE100, FCL PE150, FCL PE200, Small RNA FCL SE50, FCS SE100, FCS PE100, FCS PE150	DNB Load Buffer I	4 °C	≤48 h
FCS PE300	Low TE Buffer	4 °C	≤4 h
stLFR FCL PE100	DNB Load Buffer I	4 °C	≤48 h

For FCS PE300, do not use DNB Loading Buffer I to dilute the DNBs, use Low TE Buffer instead.

Preparing the flow cell

Perform the following steps:

1. Take the flow cell box out of storage and remove the flow cell plastic package from the box.

   Do not open the outer plastic package yet.

2. Place the plastic package at room temperature for 1 hour to 24 hours.
3. Unwrap the outer plastic package before use.

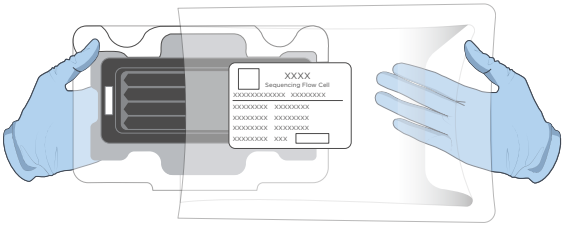

Figure 13 Unwrapping the outer plastic package

- If the flow cell is not used within 24 hours after being placed at room temperature and the outer plastics package is intact, the flow cell can be returned to -25 °C to -15 °C for storage. But the switch between room temperature and -25 °C to -15 °C must not exceed 3 times.
- If the outer plastic package has been opened but the flow cell cannot be used immediately, store the flow cell at room temperature and use it within 24 hours. If 24 hours is exceeded, it is not recommended to use the flow cell.

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

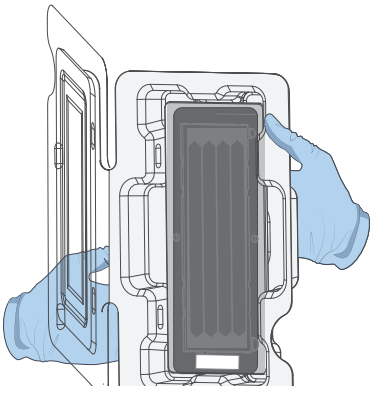

Figure 14 Inspecting the flow cell

Preparing the device

Powering the device on

CAUTION

- Ensure that the power switch is in the OFF position before connecting to the power supply.
- Ensure that the grounding cable is connected in accordance with the relevant standard or under the guidance of an experienced electrician.
- Only the power cord of the manufacturer can be used, and the power cord can be only used with this device. Failure to do so may damage the power cord or device.

Perform the following steps:

1. Connect one end of the power cord to the power port of the device, and the other end to the main power supply.
2. (Optional) If a UPS is prepared, connect the one end of the UPS power cord to the device, and the other end to the main power supply.
3. Turn the power switch of the device to the | position. After you power the device on, self-test begins.

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 password that is provided by the manufacturer.
3. Select in the main interface.
4. Log in to the control software with the user name and password.

Performing pre-run checks

Before each sequencing run, perform the following checks:

- Check whether the remaining space of storage drive is greater than 4.6 TB. If the remaining space is insufficient, clear history data according to *Reviewing parameters on Page 72*.
- Check the waste container, and fix the problem before 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 122*.
  2) If the waste container icon turns to , empty the waste container.
     For details, refer to *Maintaining the waste container on Page 122*.
  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 mentioned 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 mentioned in *Device specifications on Page 191*, and ensure that the temperature fluctuates within the specified range throughout the sequencing, and 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 (~48 min) on Page 118.*

Loading DNBs

Loading DNBs by the sequencer

Preparing reagents

Perform the following steps:

1. Depending upon the sequencing read length, remove the following reagents from storage and thaw the reagents on ice for approximately 0.5 hours.

Table 31 DNB loading buffer

Model	Component
FCL SE50, FCL SE100, FCL SE400, FCL PE100, FCL PE150, FCL PE200, Small RNA FCL SE50, FCS SE100, FCS PE100, FCS PE150	DNB Load Buffer II
FCS PE300	DNB Load Buffer IV
stLFR FCL PE100	DNB Load Buffer II

2. Mix the reagents by using a vortex mixer for 5 seconds, centrifuge briefly by using a mini spinner, and place on ice until use.

   If crystal precipitation is found in DNB Load Buffer II, vigorously mix the reagent for 2 minutes by using a vortex mixer to re-dissolve the precipitation before use.

3. Take out a Micro Tube 0.5 mL from the sequencing kit and add the following reagents according to different sequencing read length:

Table 32 DNB loading mixture 1

Model	Component	Volume (μL)	
		FCL	FCS
FCL SE50, FCL SE100, FCL SE400, FCL PE100, FCL PE150, FCL PE200, Small RNA FCL SE50, FCS SE100, FCS PE100, FCS PE150	DNB Load Buffer II	64	32
	Make DNB Enzyme Mix II (LC)	2	1
	DNBs	200	100
	Total Volume	266	133
FCS PE300	DNB Load Buffer IV	/	45
	DNBs	/	90
	Total Volume	/	135

Model	Component	Volume (µL)	
		FCL	FCS
stLFR FCL PE100	DNB Load Buffer II	64.0	/
	Make DNB Enzyme Mix IV	2.5	/
	DNBs	200.0	/
	Total Volume	266.5	/

4. Combine components and mix by gently pipetting 8 times by using a wide-bore, non-filtered 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 52*, 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, if not, place an empty tube.

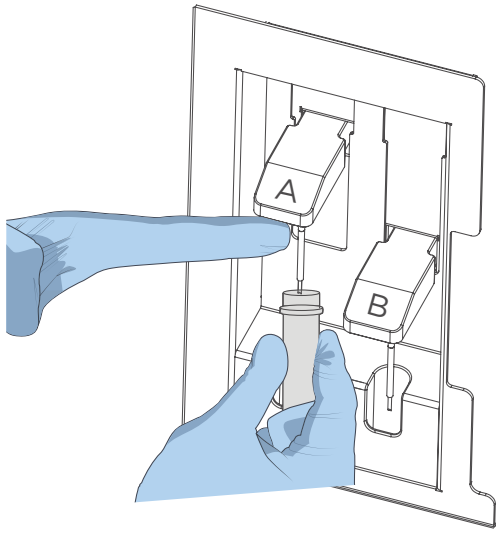

Figure 15 Loading the DNB tube

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 66.*

Loading DNBs by DL-200H

Preparing reagents

Perform the following steps:

1. Take out a new PCR 8-strip tube and add the reagents shown in the table below according to different models:

Table 33 DNB loading mixture 2

Model	Component	Volume (μL)	
		FCL	FCS
FCL SE50, FCL SE100, FCL SE400, FCL PE100, FCL PE150, FCL PE200, Small RNA FCL SE50, FCS SE100, FCS PE100, 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
FCS PE300	DNB loading buffer IV	/	11.5
	DNBs	/	22.5
	Total Volume	/	34
stLFR FCL PE100	DNB Load Buffer II	8.0	/
	Make DNB Enzyme Mix IV	0.31	/
	DNBs	25.00	/
	Total Volume	33.31	/

2. Combine components and mix by gently pipetting 8 times by using a wide-bore, non-filtered 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 not, perform maintenance according to *DL-200H and sealing gasket maintenance on Page 124*.
- Ensure that the sealing gasket of DL-200H is clean and properly maintained. If not, perform maintenance according to *DL-200H and sealing gasket maintenance on Page 124*.

Perform the following steps:

1. Install the sealing gasket and the flow cell.

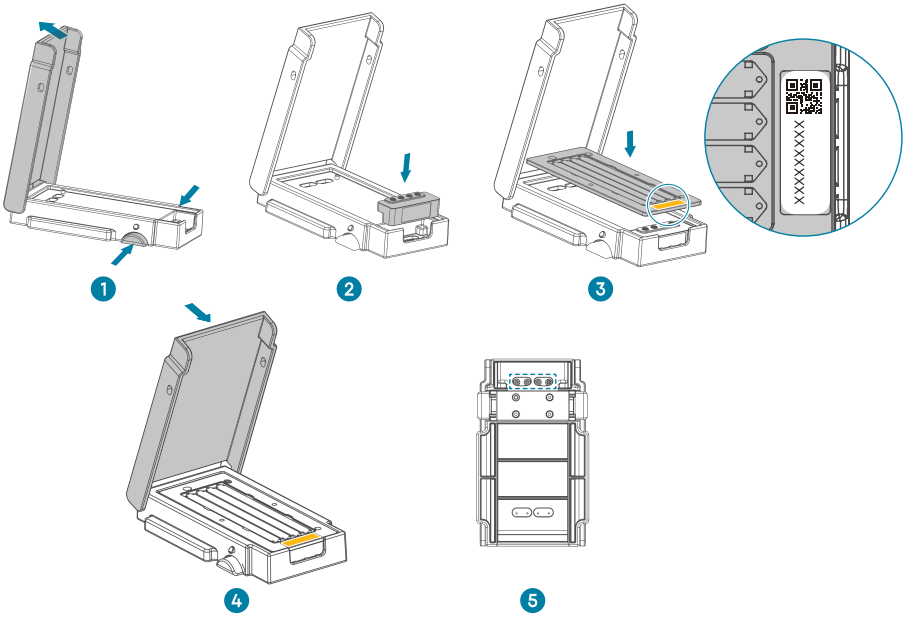

Figure 16 Installing sealing gasket and flow cell

1) Press the latches and open the cover.
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 upward and in the same position of sealing gasket.
   - For the label location on the flow cell, refer to the area marked in yellow in *Figure 16 on Page 55*.
4) Close the cover and ensure that the cover is securely closed.
5) Place the back of DL-200H upward, and check whether the fluidics inlets align with the holes of the sealing gasket and ensure that the holes are clean.

2. Load DNBs by using DL-200H.

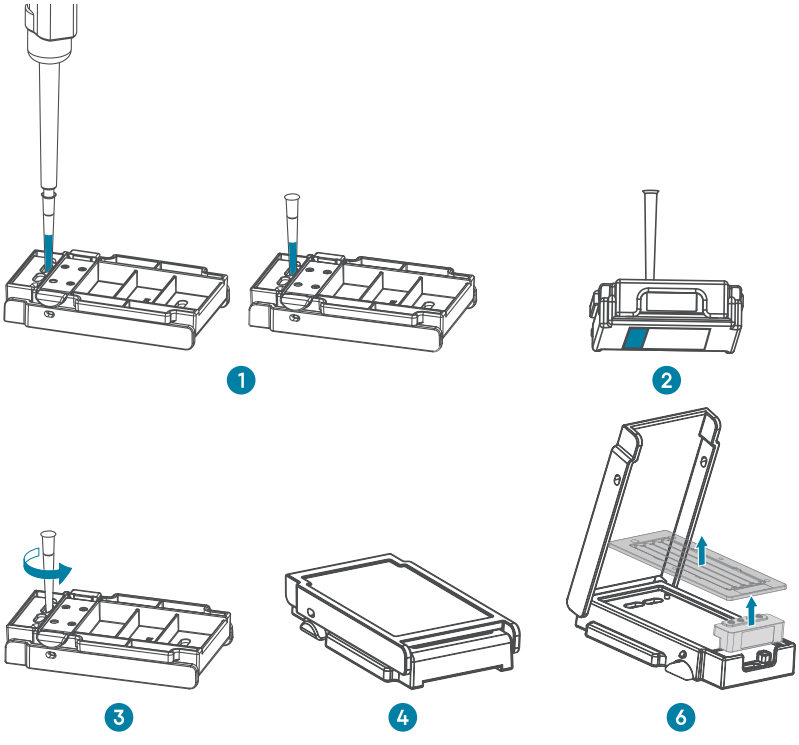

Figure 17 Loading DNBs by using DL-200H

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

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

2) Keep DL-200H parallel to the bench and keep the back upward. Hold up the device vertically to check whether the DNBs flow into the flow cell.

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

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

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

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

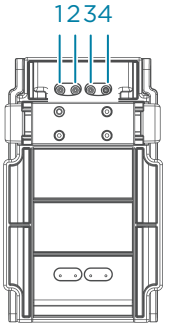

Figure 18 Lane order of DNB loading

5) Place DL-200H on the bench with the front upward.

   For FCS PE300 sequencing, wait 75 minutes for the DNB loading process.

   For other sequencing models, wait 30 minutes 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 take the flow cell out and transfer it 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 66*.

Preparing the sequencing reagent cartridge

Sequencing enzyme mix and dNTP mixes are provided in different tubes and packaged together with the Sequencing Reagent Cartridge. Before the sequencing run starts, an appropriate amount of sequencing enzyme mix and dNTP mixes needs to be added to well No. 1 and well No. 2 of Sequencing Reagent Cartridge. Furthermore, MDA Enzyme Mix (MDA, Multiple displacement amplification) 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 135*.

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 34 Approximate thaw times for various sequencing kits

Model	Method		
	Water bath at room temperature (hours)	Refrigerator at 2°C to 8°C overnight then water bath at room temperature (hours)	Refrigerator at 2°C to 8°C (hours)
FCL SE50	2.0	0.5	24.0
FCL SE100	2.0	0.5	24.0
FCL SE400	8.0	3.0	48.0
FCL PE100	3.0	1.5	36.0
FCL PE150	5.0	2.0	48.0
FCL PE200	6.0	3.5	48.0
FCS SE100	1.0	0.5	24.0
FCS PE100	2.0	0.5	36.0
FCS PE150	3.0	1.5	36.0
FCS PE300	6.0	3.5	48.0
stLFR FCL PE100	3.0	1.5	36.0

After removal from -25 °C to -15 °C, the flow cell must be placed at room temperature for at least 1 hour and no more than 24 hours before DNB loading.

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

   Presence of dark green crystals in well No. 10 is normal due to crystallization of reagent materials in this well. When the cartridge is thawed, mix the reagents in the cartridge thoroughly and the crystals will dissolve. Sequencing quality will not be affected. Refer to *Q: What should I do if dark green crystals appear in well No. 10? on Page 133* in this guide for details.

5. Wipe any water condensation on the cartridge cover and well surround with a Kimwipes tissue.

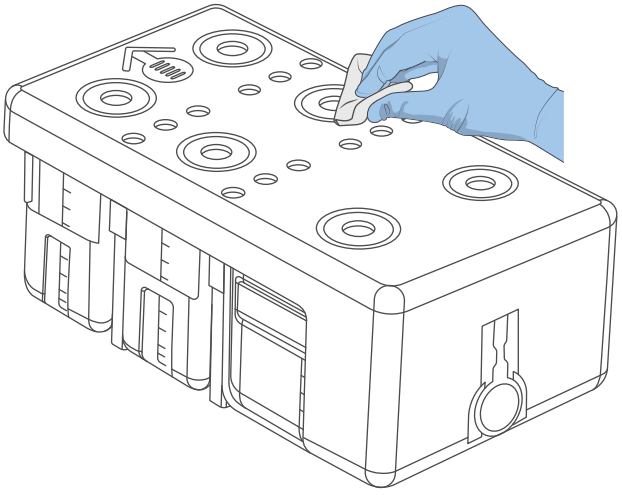

Figure 19 Wiping cartridge cover

6. Remove dNTPs Mix and dNTPs Mix II from -25 °C to -15 °C storage 1 hour in advance and thaw at room temperature. Store at 2 °C to 8 °C until use.
7. Remove the Sequencing Enzyme Mix from -25 °C to -15 °C storage and place on ice until use.
8. Remove reagents from storage according to your model:
   - For PE sequencing, remove MDA Reagent from storage and place on ice until use.
   - For Small RNA FCL SE50 Sequencing, remove Wash Buffer For Small RNA Sequencing from storage and thaw at room temperature. Store at 2 °C to 8 °C until use.
   - For FCL SE400 sequencing, remove Wash Buffer For Sequencing from storage and thaw at room temperature. Store at 2 °C to 8 °C until use.

Well positions are shown in the figure below:

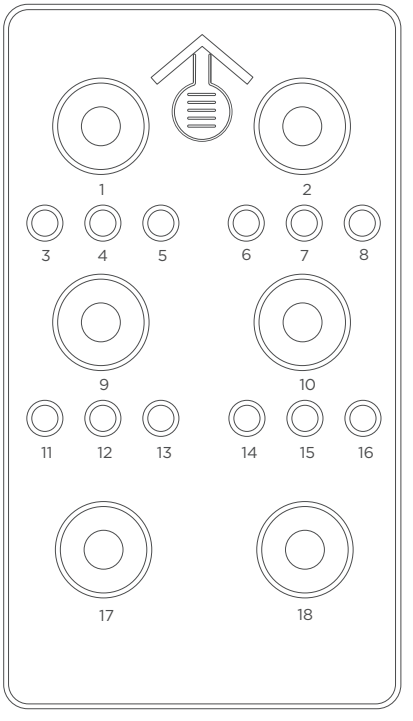

Figure 20 Well positions

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

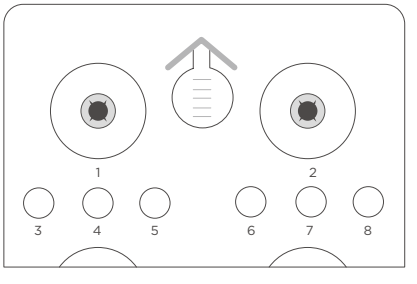

Figure 21 Piercing the seal of cartridge

10. 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 *Table 35 on Page 61* .

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

Table 35 Reagent preparation for well No. 1

Model	dNTPs Mix loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
FCL SE50	0.700	0.700
FCL SE100	1.100	1.100
FCL SE400	4.000	4.000
FCL PE100	1.800	1.800
FCL PE150	2.400	2.400
FCL PE200	3.800	3.800
Small RNA FCL SE50	0.700	0.700
FCS SE100	0.800	0.800
FCS PE100	1.400	1.400
FCS PE150	1.900	1.900
FCS PE300	3.800	3.800
stLFR FCL PE100	2.000	2.000

11. 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.

12. Take out a pipette with the appropriate volume range and add reagents according to *Table 36 on Page 62* . 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 seconds and centrifuge briefly before use.
    - Invert Sequencing Enzyme Mix 6 times before use.

Table 36 Reagent preparation for well No. 2

Model	dNTPs Mix II loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
FCL SE50	0.600	0.600
FCL SE100	0.900	0.900
FCL SE400	12.000	4.000
FCL PE100	1.500	1.500
FCL PE150	2.100	2.100
FCL PE200	5.700	3.800
Small RNA FCL SE50	0.600	0.600
FCS SE100	1.600	0.800
FCS PE100	2.800	1.400
FCS PE150	3.800	1.900
FCS PE300	5.700	3.800
stLFR FCL PE100	1.700	1.700

13. 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.

14. Seal loading well No. 1 and No. 2 with transparent sealing films.

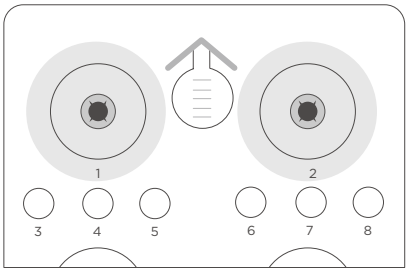

Figure 22 Sealing the loading wells of the cartridge

15. Press the film with your finger around the well. Ensure that the well is tightly sealed and that no air bubbles exist between the film and cartridge surface, so that the reagents would not flow over the cartridge.

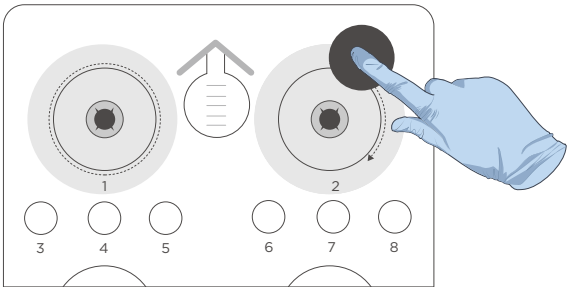

Figure 23 Sealing the loading wells of the cartridge tightly

16. Lift the cartridge horizontally, 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.

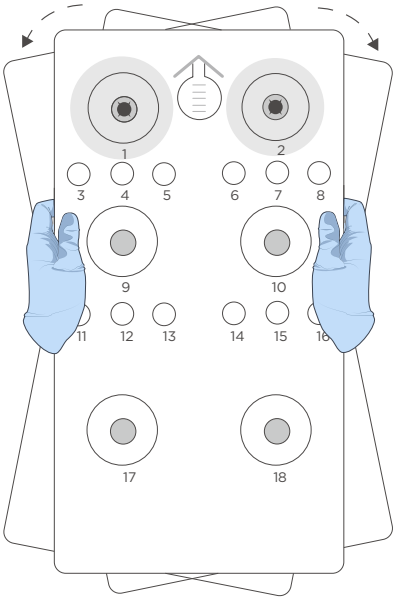

Figure 24 Mixing reagents after loading

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

- Do not reuse the used sealing film.
- Ensure that the surface around wells No. 1 and No. 2 is clean to avoid cross contamination.

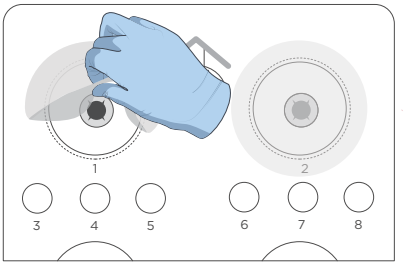

Figure 25 Removing the seal from cartridge

18. Gently tap the cartridge on the bench to reduce air bubbles in the reagents.
    - The FCL SE50/FCL SE100/FCS SE100 sequencing reagent cartridge is now ready for use.
    - For the next step, refer to *Performing a sequencing run on Page 66.*
19. Perform the following steps for Small RNA Sequencing or SE400:

    Wash Buffer For Small RNA Sequencing and Wash Buffer For Sequencing for SE400 contains highly concentrated formamide which may have potential reproductive toxicity. Avoid breathing vapor and wear protective gloves/protective clothing/protective eye mask/protective mask when using these reagents.

    - For Small RNA FCL SE50
        a. Mix Wash Buffer For Small RNA Sequencing by using a vortex mixer for 5 seconds and centrifuge briefly before use.
        b. Pierce the seal of well No. 7 and add 4.50 mL of Wash Buffer For Small RNA Sequencing. When adding the reagent, ensure that no bubbles exist at the bottom of the tube.
            - The Small RNA FCL SE50 sequencing reagent cartridge is now ready for use.
            - For the next step, refer to *Performing a sequencing run on Page 66.*
    - For FCL SE400
        a. Mix Wash Buffer For Sequencing by using a vortex mixer for 5 seconds and centrifuge briefly before use.
        b. Pierce the seal of well No. 7 and add 2.70 mL of Wash Buffer For Sequencing. When adding the reagent, ensure that no bubbles exist at the bottom of the tube.
            - The FCL SE400 sequencing reagent cartridge is now ready for use.
            - For the next step, refer to *Performing a sequencing run on Page 66.*

20. Perform the following steps for FCL PE100/FCL PE150/FCL PE200/FCS PE100/FCS PE150/FCS PE300 sequencing:
    1) Pierce the seal of well No. 15 by using a 1 mL sterile pipette tip.
    2) Add 500 µL of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette.

       When using MDA Enzyme Mix, do not touch the wall of the tube. The heat from your hands may affect the enzyme activity.

    3) Invert the tube 6 times to mix the reagents.
    4) Add the mixture to well No. 15. When adding the mixture, ensure that no bubbles appear at the bottom of the tube.

       - When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.
       - The FCL PE100/FCL PE150/FCL PE200/FCS PE100/FCS PE150/FCS PE300 sequencing reagent cartridge is now ready for use.
       - For the next step, refer to *Performing a sequencing run on Page 66.*

Performing a sequencing run

Entering DNB ID

Perform the following steps:

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

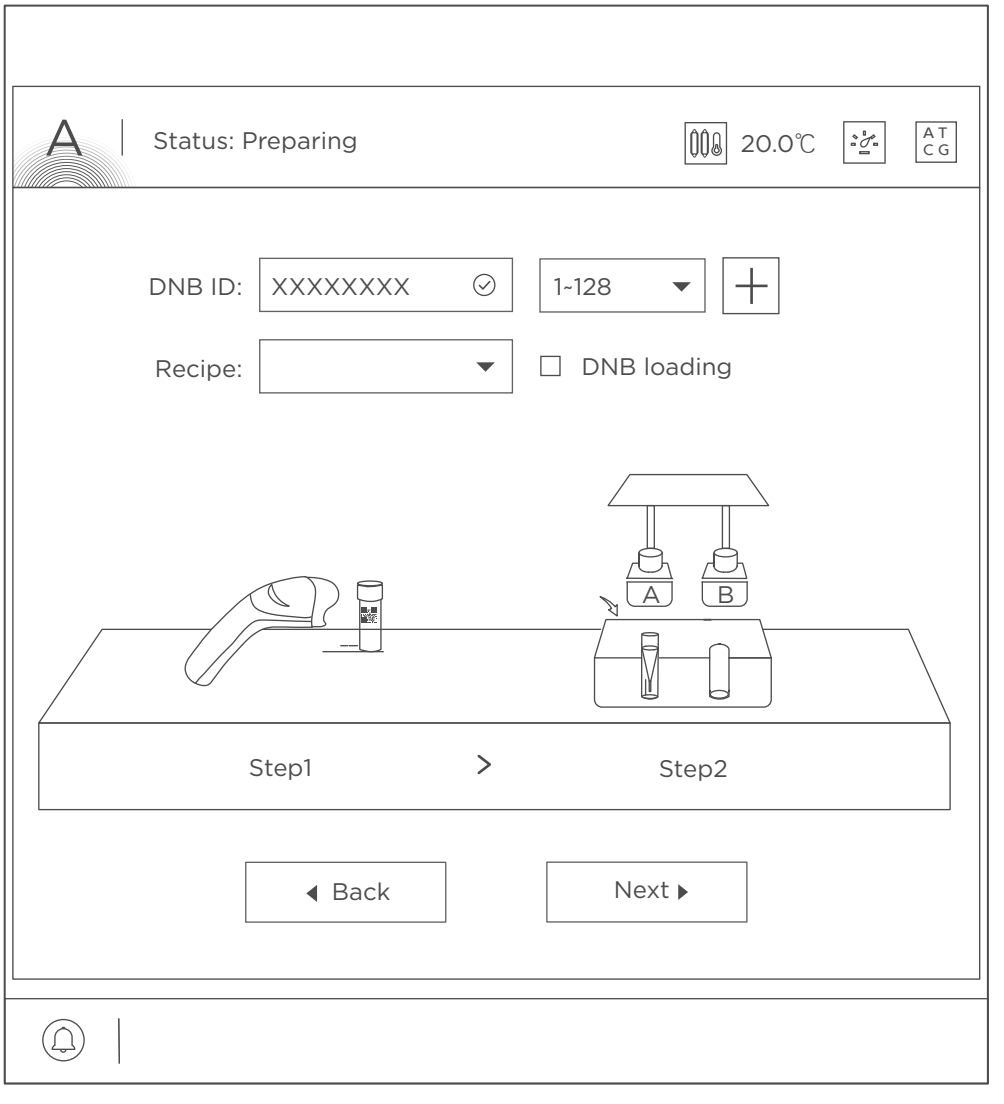

Figure 26 DNB ID entry interface

2. Select the **DNB ID** box, scan the QR code on the tube or 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.

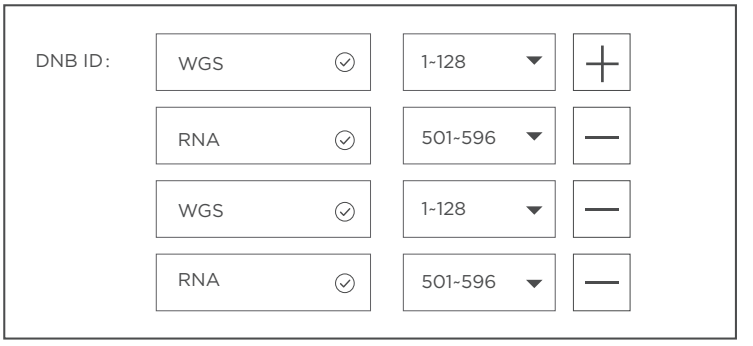

Figure 27 DNBs and information selection interface

Selecting sequencing parameters

Perform the following steps:

1. Select an appropriate recipe from the **Recipe** list. One-click sequencing runs (for example, SE50, and so on) and a user-customized run (Customize) are available.

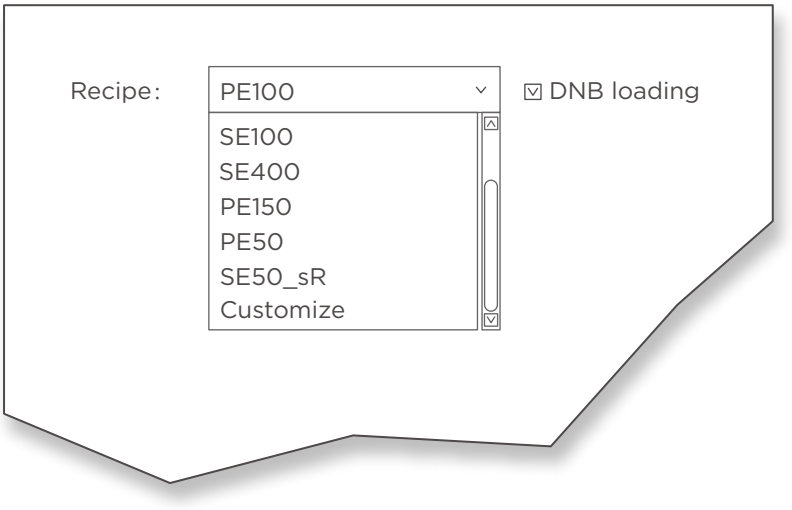

Figure 28 Selecting sequencing recipe

Sequencing recipe **SE50_sR** is for Small RNA FCL SE50 sequencing. For Dual Barcode sequencing, select **Customize** from the **Recipe** list. For details about customizing a recipe, refer to *Instructions for customizing a run on Page 145.*

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, enter the cartridge ID manually or use the barcode scanner to scan the cartridge barcode at the lower right corner of the sequencing reagent cartridge label.

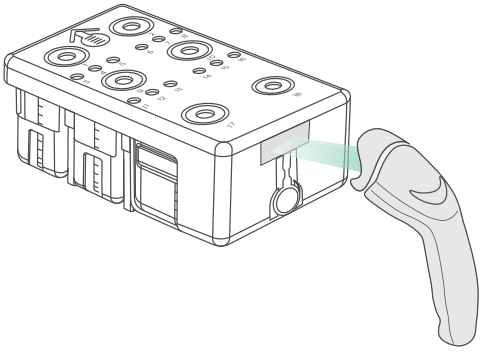

Figure 29 Scanning sequencing reagent cartridge ID

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

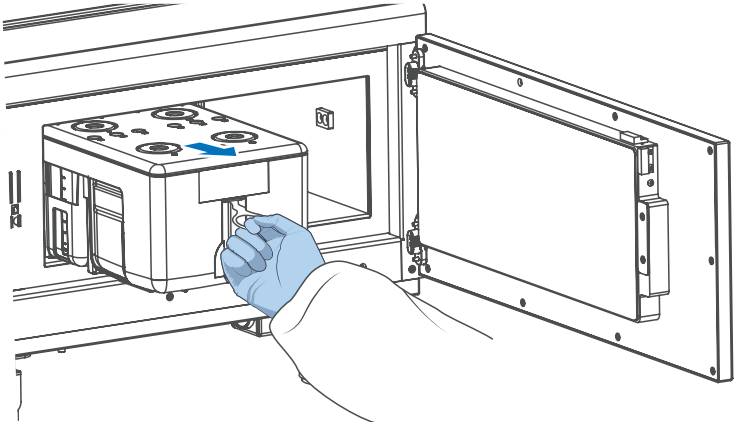

Figure 30 Removing cleaning cartridge

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.

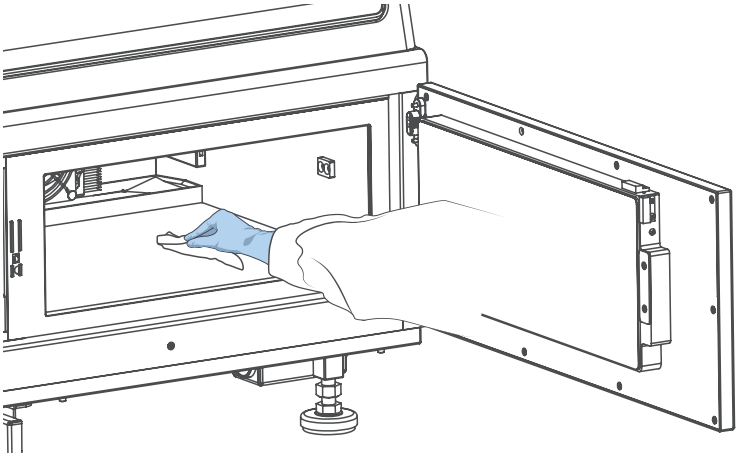

Figure 31 Maintaining the reagent compartment

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.

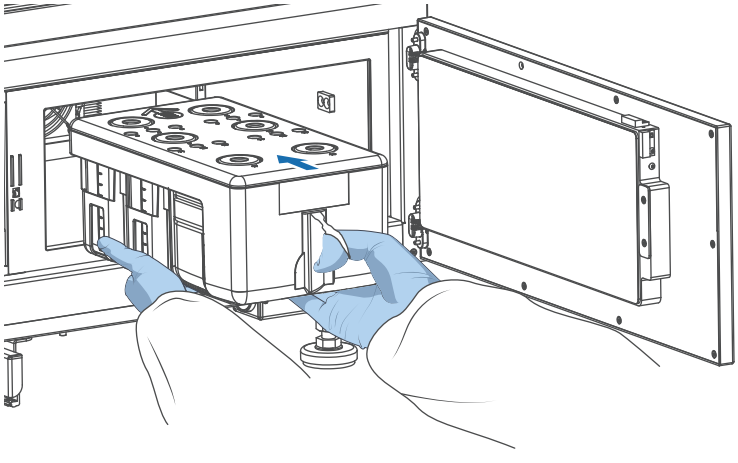

Figure 32 Sliding the new sequencing reagent cartridge into the reagent compartment

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 washing flow cell, 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 on the flow cell stage and the back of the flow cell.

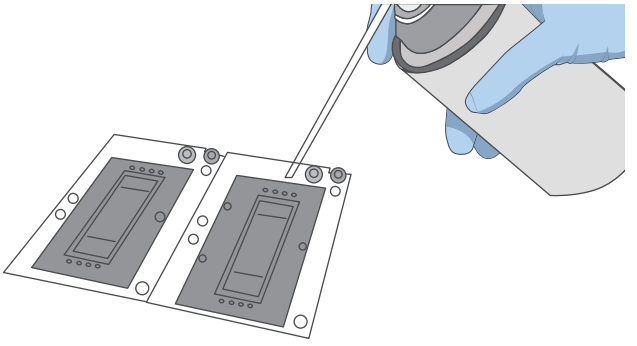

Figure 33 Cleaning the flow cell stage

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 hole on the right side. The label is on the right. Hold the edge of the flow cell with both hands.

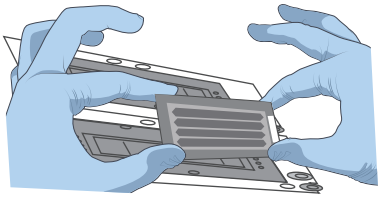

Figure 34 Loading the flow cell

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, please handle it with caution.

9. Ensure that the negative pressure is within the range of -80 kPa to -99 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 136* in this guide for troubleshooting.

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

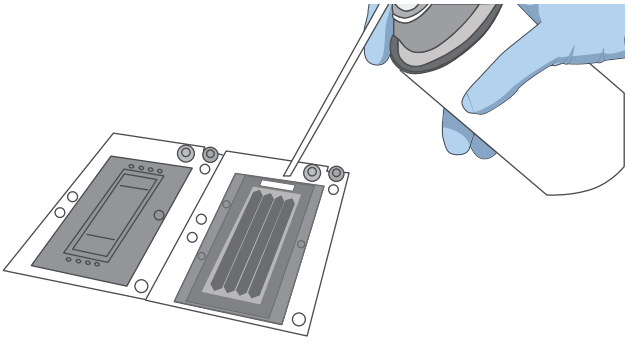

Figure 35 Cleaning the flow cell

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

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

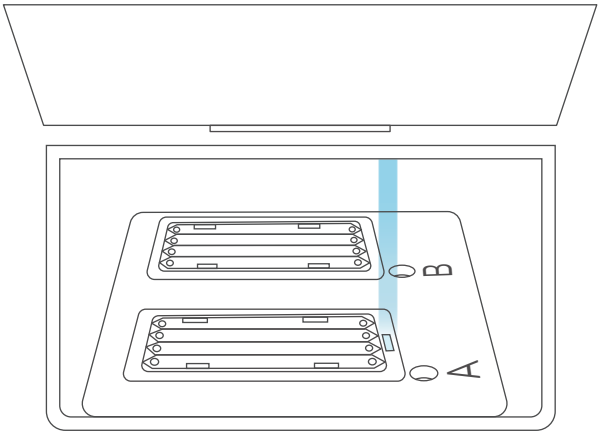

Figure 36 Scanning flow cell ID

12. Select **Next**.

WARNING If the flow cell accidentally drops 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, to ensure that the flow cell can be firmly attached to the stage.
- Do not move the flow cell once it is loaded. Otherwise, it may cause misalignment between the flow cell inlet, outlet, and the gasket.

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 37 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, perform the following steps:
  a. In the prompted dialog box, select the data that you want to delete and 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** when prompted to begin sequencing.

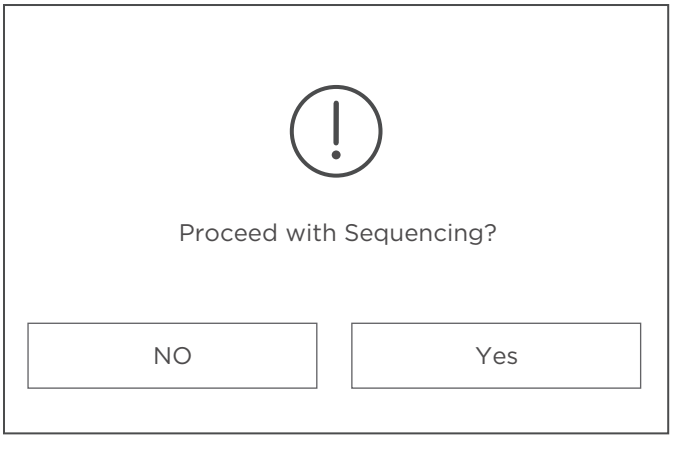

Figure 38 Confirming sequencing interface

2. Once sequencing has started, immediately open the flow cell compartment door to inspect the flow cells, and ensure that DNBs or reagents are flowing through the flow cell, if not, fix the problems before you restart sequencing. For details, refer to *Instructions for customizing a run on Page 145.*

CAUTION

- Do not bump, move, vibrate, or impact the device during sequencing, as 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 128.*
- 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 37 Item description for the sequencing interface

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	You can 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
	Select and a confirmation dialog box appears. Select **Yes** to stop sequencing
	After imaging of the first cycle, when the sequencing is paused, you can select this button to move up the needles. Open the reagent compartment and take out the sequencing reagent cartridge
	After imaging of the first base, select this button to open the first base report
	Select this button to open the Review interface, and you can check sequencing information

3. When the sequencer interface shows the information of processing data or idle, it means that the sequencing reaction has finished. Perform the wash process according to *Wash on Page 114*.

Performing a regular wash

After the sequencing run, perform a regular wash within 24 hours.

For details, refer to *Performing a maintenance wash (~76 min) on Page 119*.

Disposing of the sequencing reagent cartridge and flow cell

WARNING If the flow cell accidentally drops 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 avoid 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 control software is shut down, and the flow cell compartment door is closed. Failure to do so may damage the control software.

Perform the following steps:

1. Select and select **Shut down**. 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 socket or UPS.

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05

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, mainly contains pictures and data (such as metrics) generated during the device operation.
- The result folder, named after the flow cell ID, mainly 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 use, 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 data range for the logs that you want to export.

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

     You can only exports logs of up to 15 days before the very day. Logs of the very day cannot be exported.
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 where the ZLIMS software is installed, select **Uploading data**.

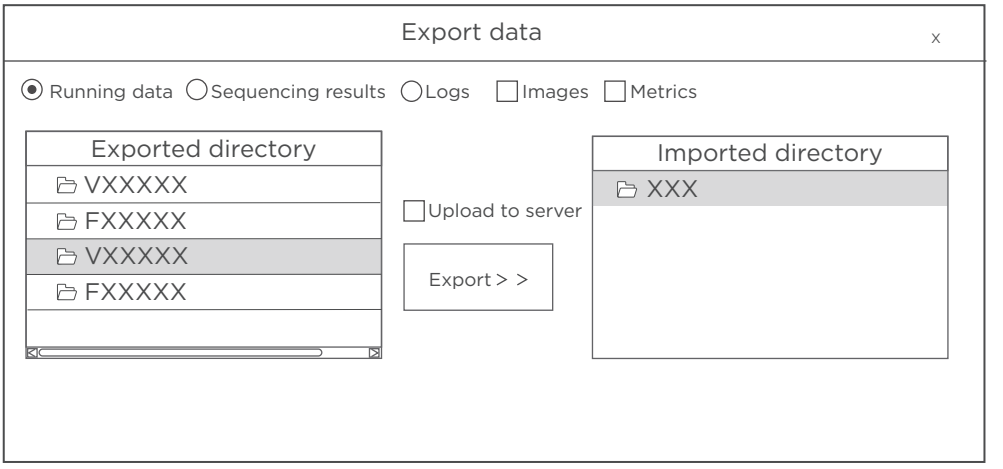

Figure 39 Export data interface

Summary report

Report parameter overview

The following table describes parameters for Tab1 of summary report:

Table 38 Parameter description for Tab1 of 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 Ecoli, 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: $\textbf{ChipProductivity} = \frac{\textbf{ValidFovNumber} \times \textbf{ESR}}{\textbf{ImageArea}} \times \textbf{100\%}$
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)
MappedReads(M)	Number of reads mapped to the reference genome. For PE sequencing, a mapped read implies that both Read1 and Read2 are mapped to the reference genome
Q30(%)	The percentage of bases with quality score ≥30. A base with a quality score of 30 implies that the chances that this base 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
MappingRate (%)	The ratio of mapped reads to total reads. The indicator is defined as the following: $\mathbf{MappingRate} = \frac{\mathbf{MappedReads}}{\mathbf{TotalReads}} \times \mathbf{100\%}$
AvgErrorRate(%)	After the mapping analysis of TotalReads, the error rate of the number of reads mapped to the reference genome. AvgErroRate(%) is defined as the following: $\mathbf{AvgErrorRate(\%)} = \frac{\mathbf{TotalMismatchBaseNumber}}{\mathbf{MappedReadsNumber} \times \mathbf{ReadLength}} \times \mathbf{100\%}$
AvgErrorRate!N(%)	The average error rate after removing the mismatches caused by call N
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(AVG)	The average ratio of second strand signal to first strand signal of 4 (ACGT) base. This indicator is only for PE sequencing

The following table describes parameters for Tab2 of summary report:

Table 39 Parameter description for Tab2 of the summary report

Parameter	Description
ISW Version	Version of 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.

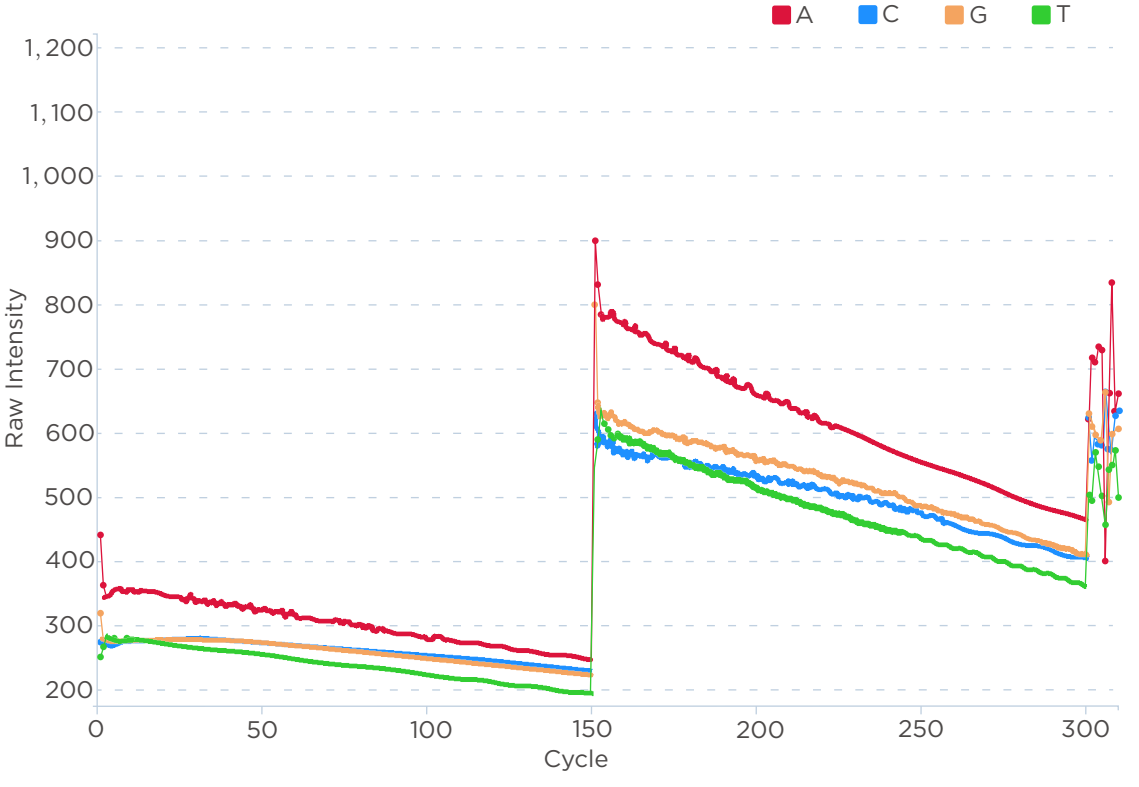

Figure 40 Raw Intensity

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

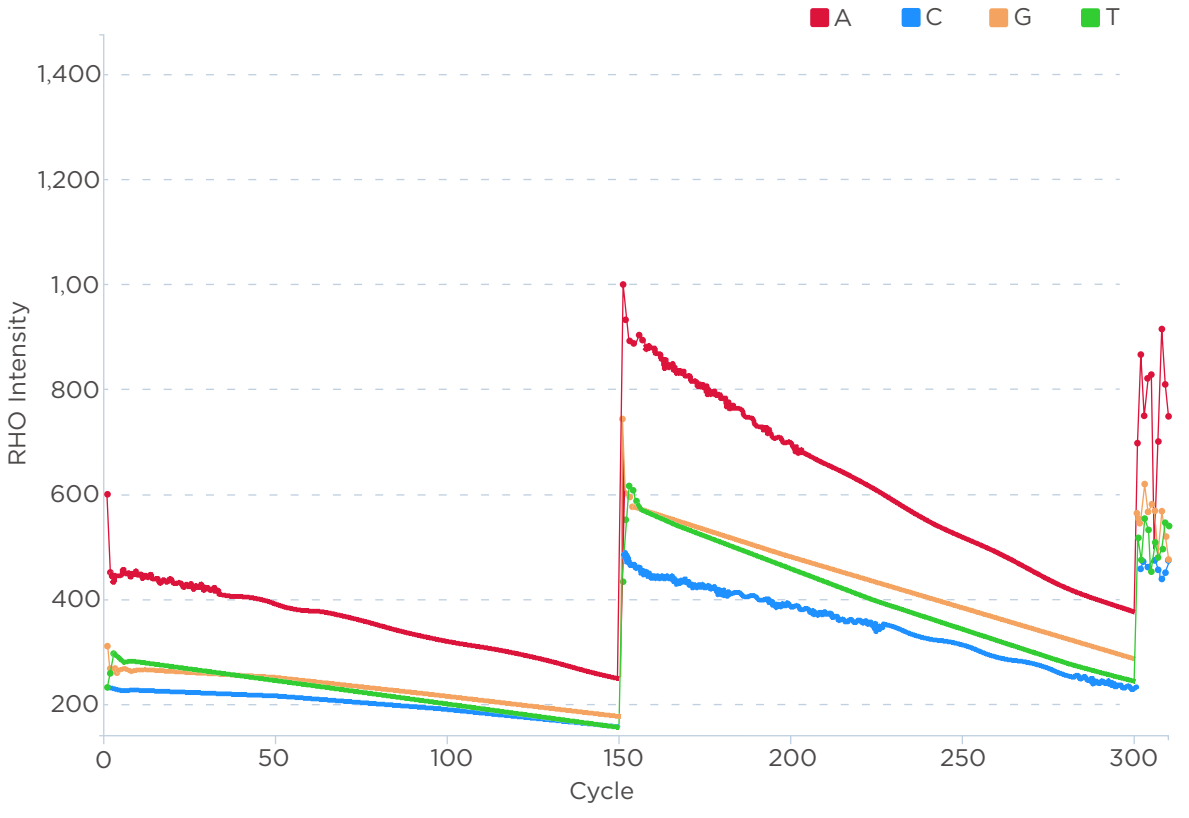

Figure 41 RHO Intensity

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.

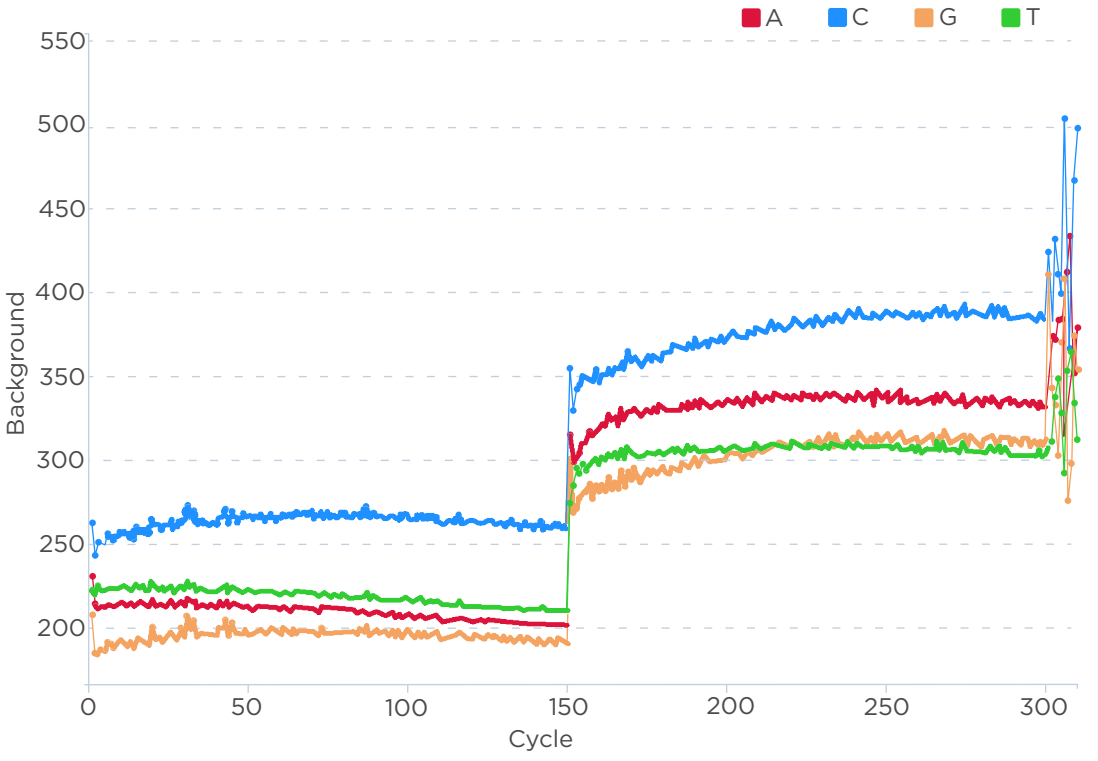

Figure 42 **Background**

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

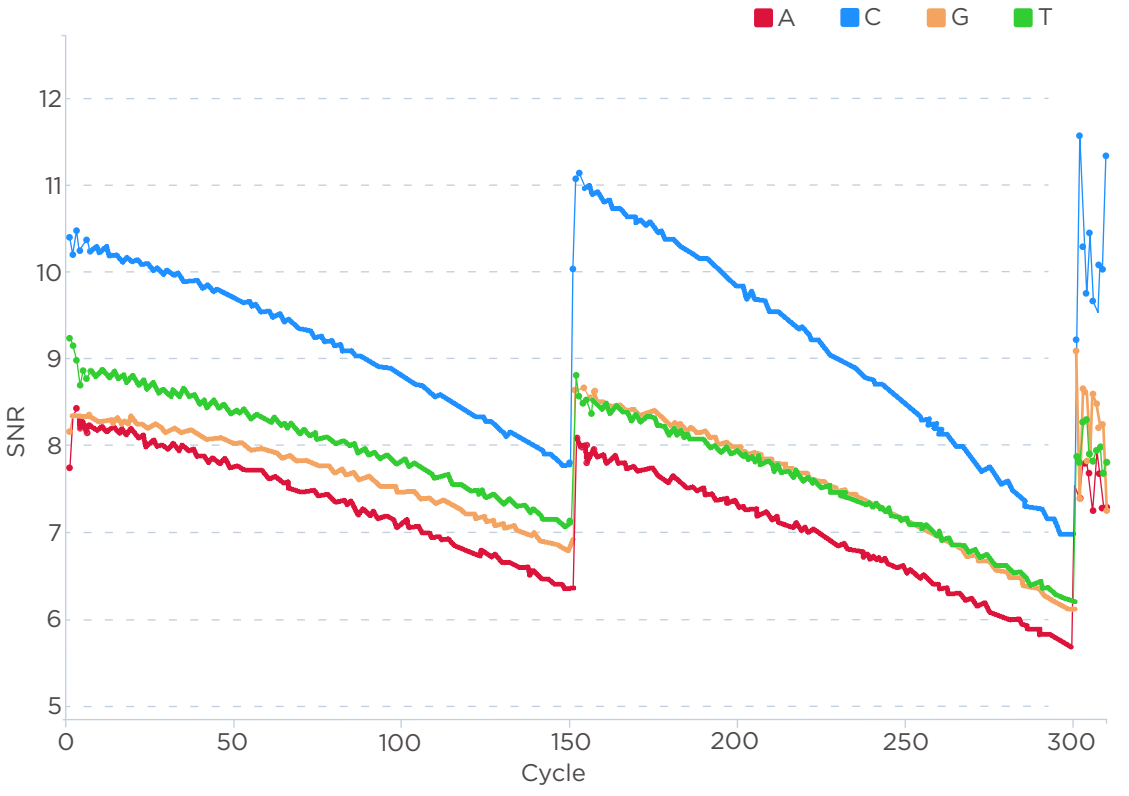

Figure 43 SNR

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.

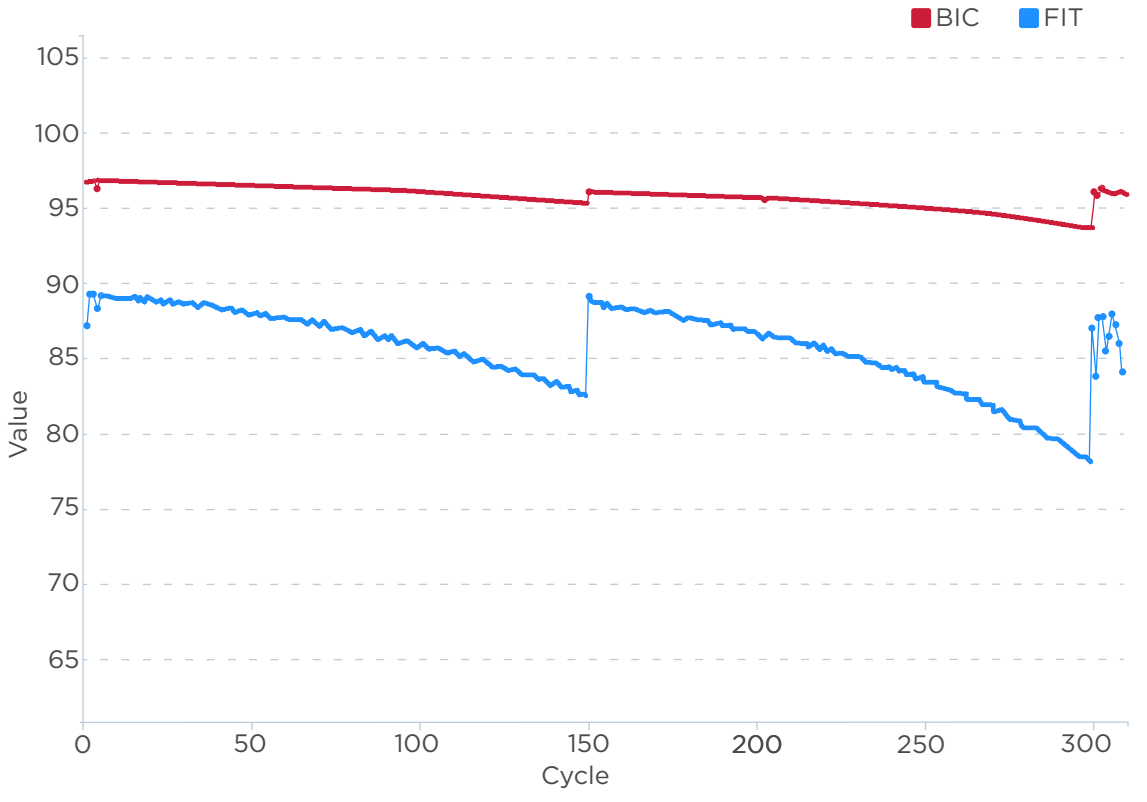

Figure 44 BIC And FIT

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.

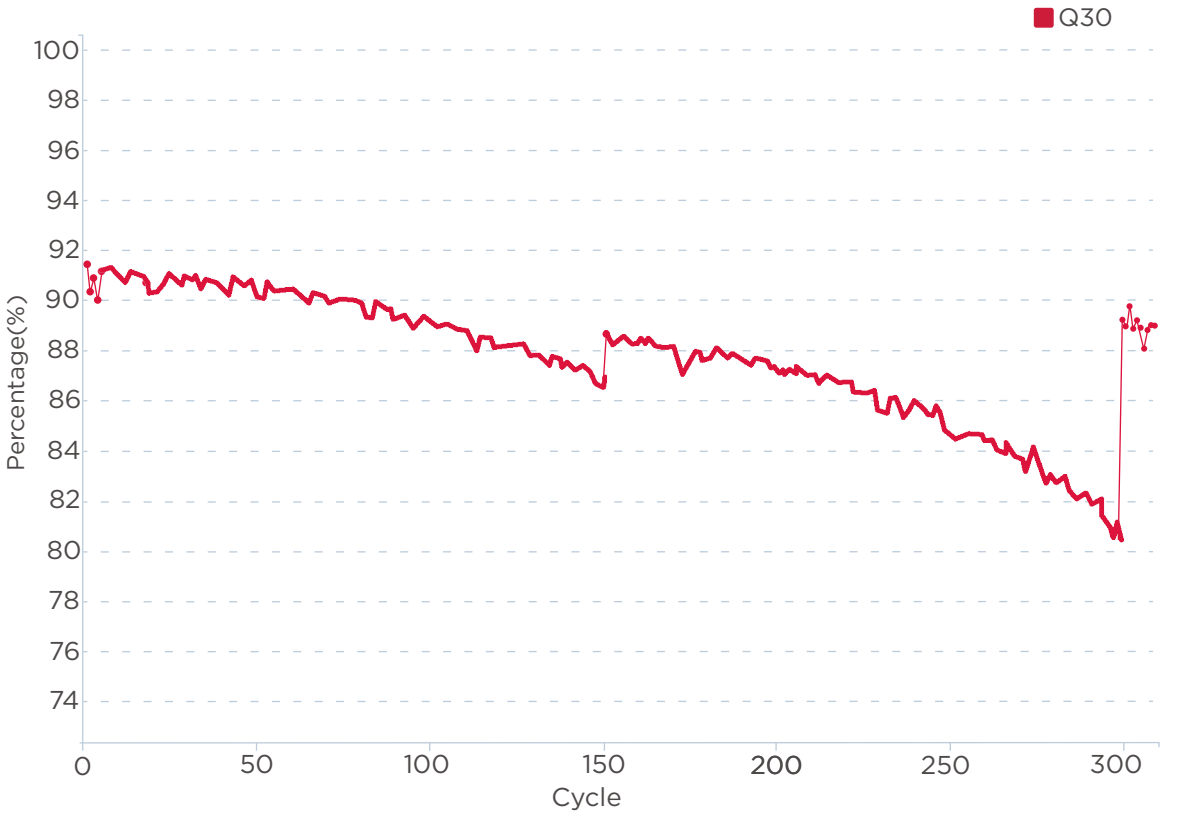

Figure 45 **Unfiltered Q30**

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

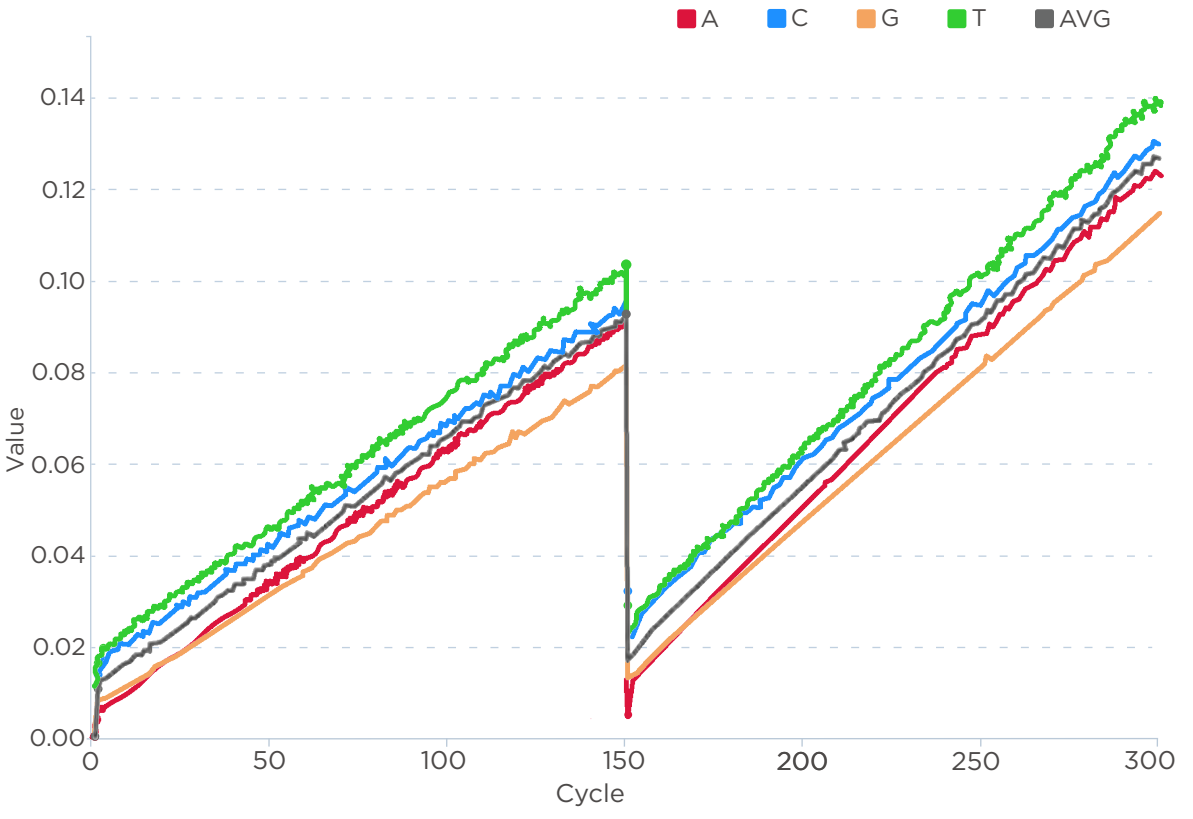

Figure 46 Runon

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.

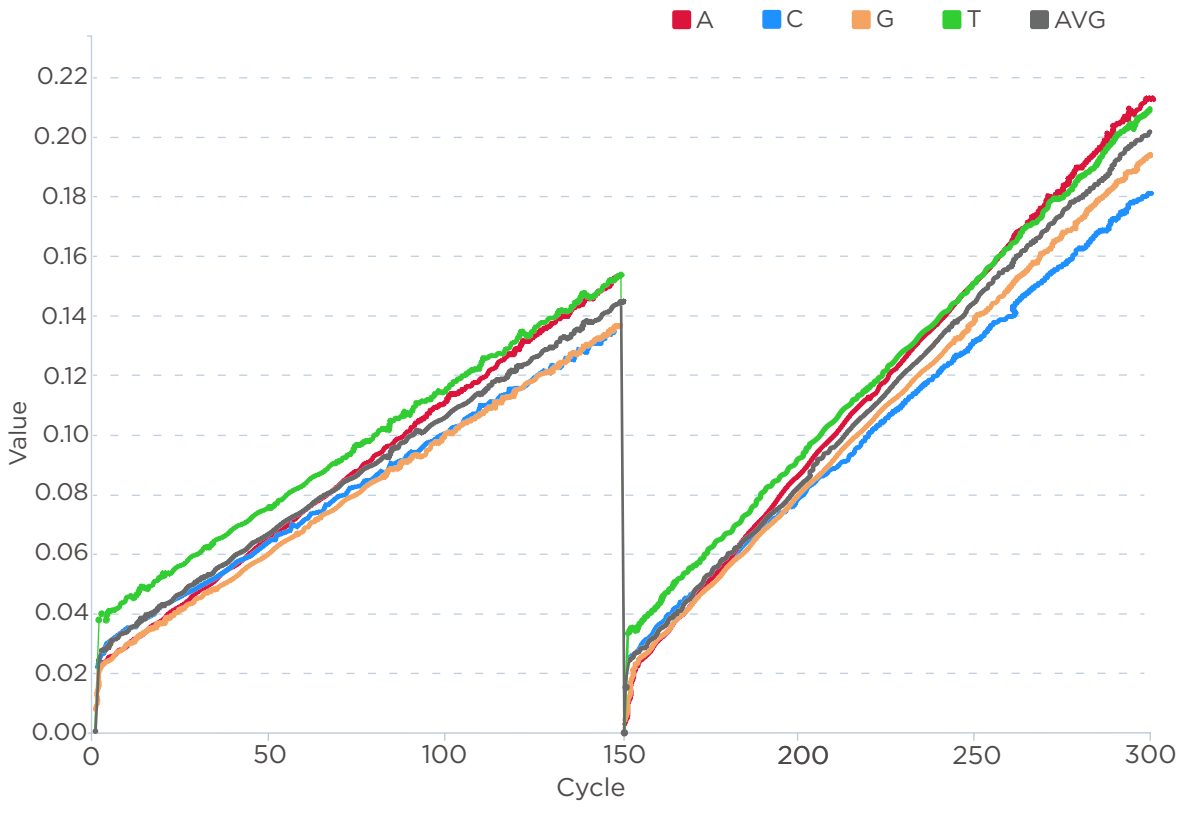

Figure 47 Lag

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.

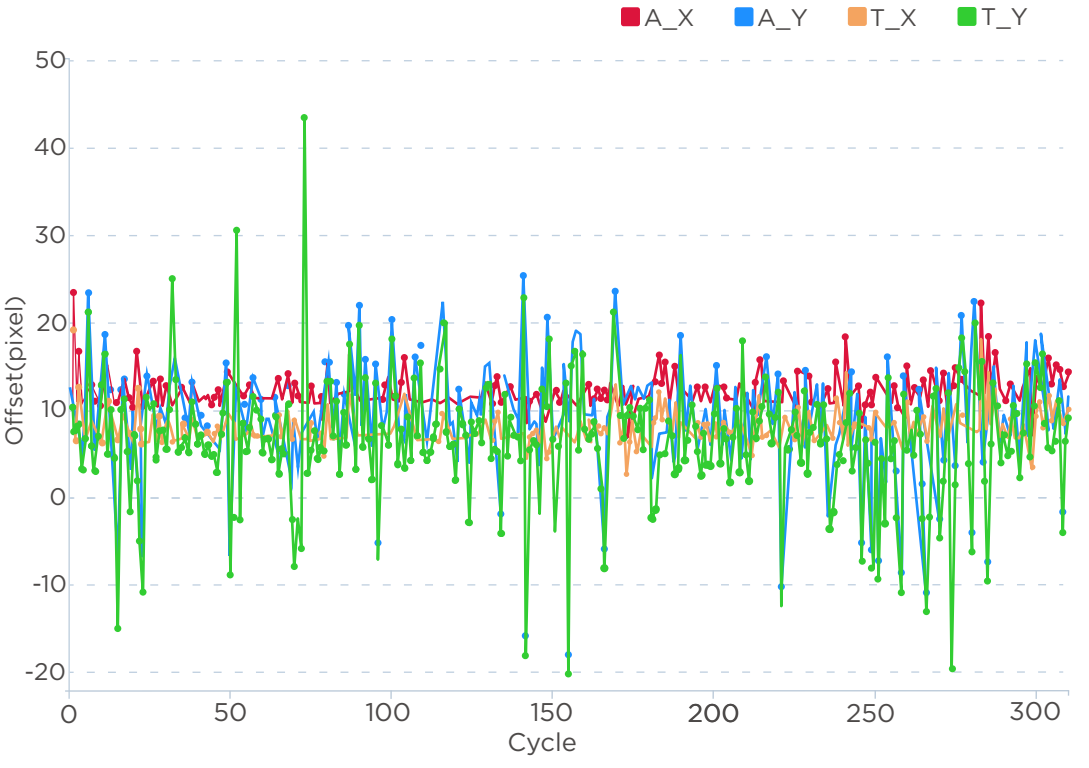

Figure 48 Offset

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.

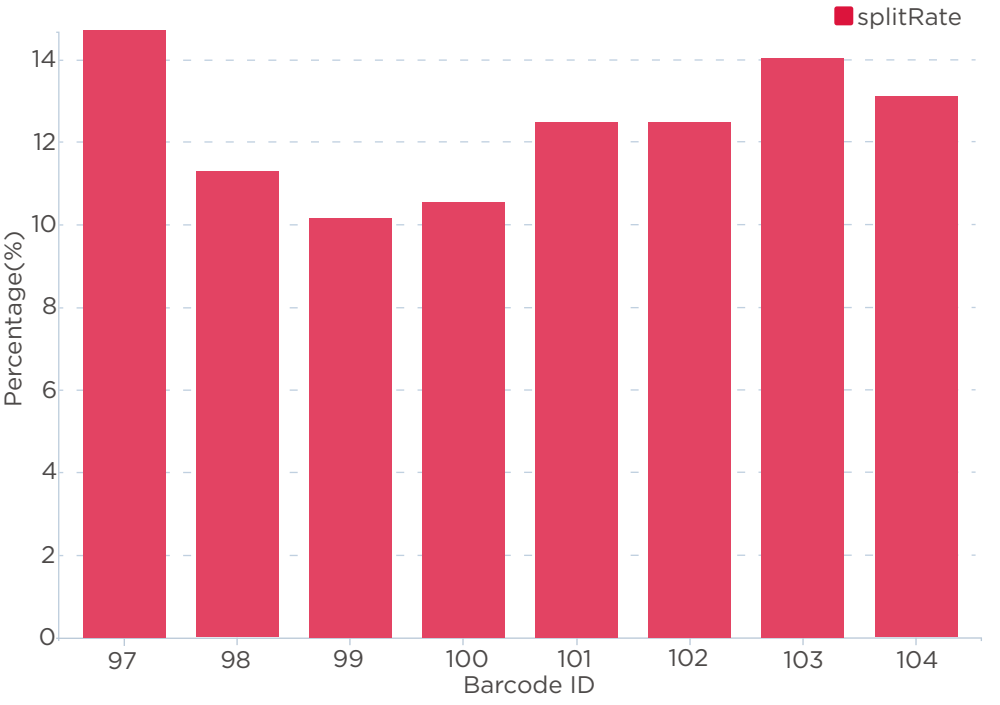

Figure 49 **Barcode Split Rate**

X axis	Barcode ID
Y axis	Percentage (%): a histogram that shows the percentage of the barcode when the splitting rate is over 0.5%.

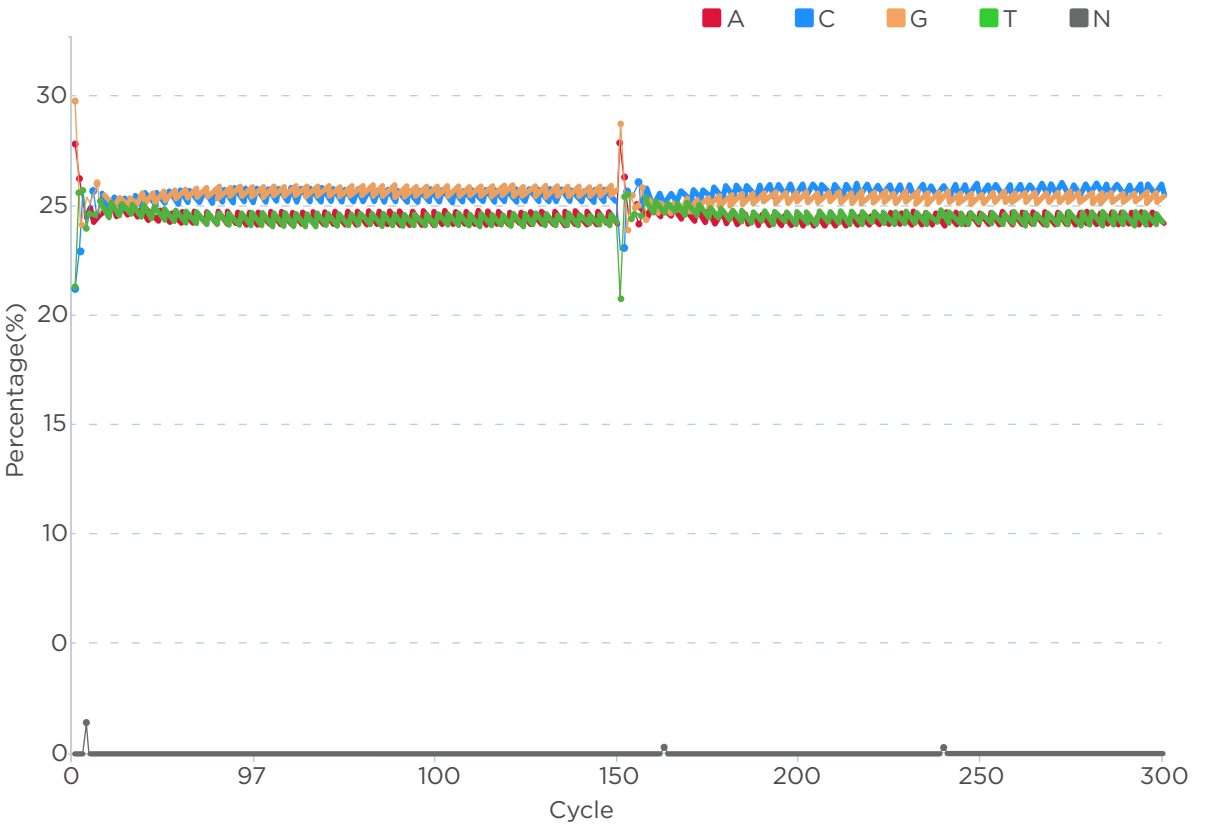

Figure 50 **Bases Distribution**

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

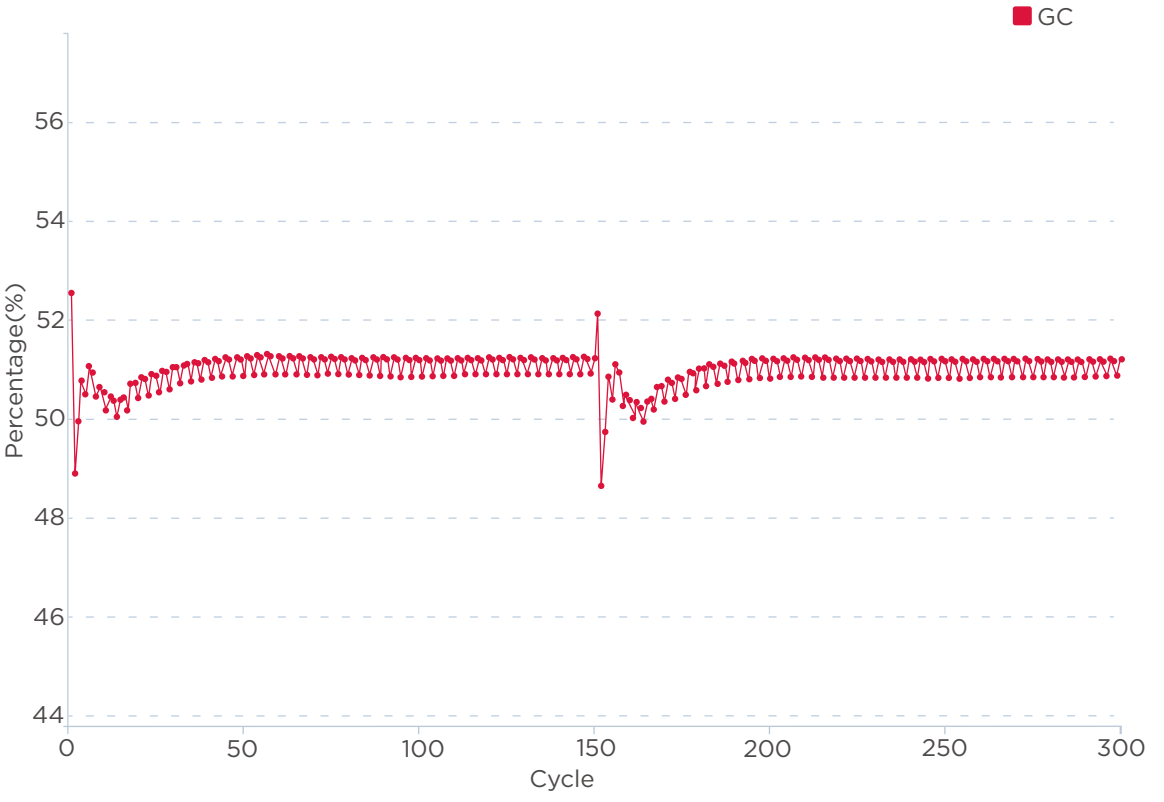

Figure 51 GC Distribution

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

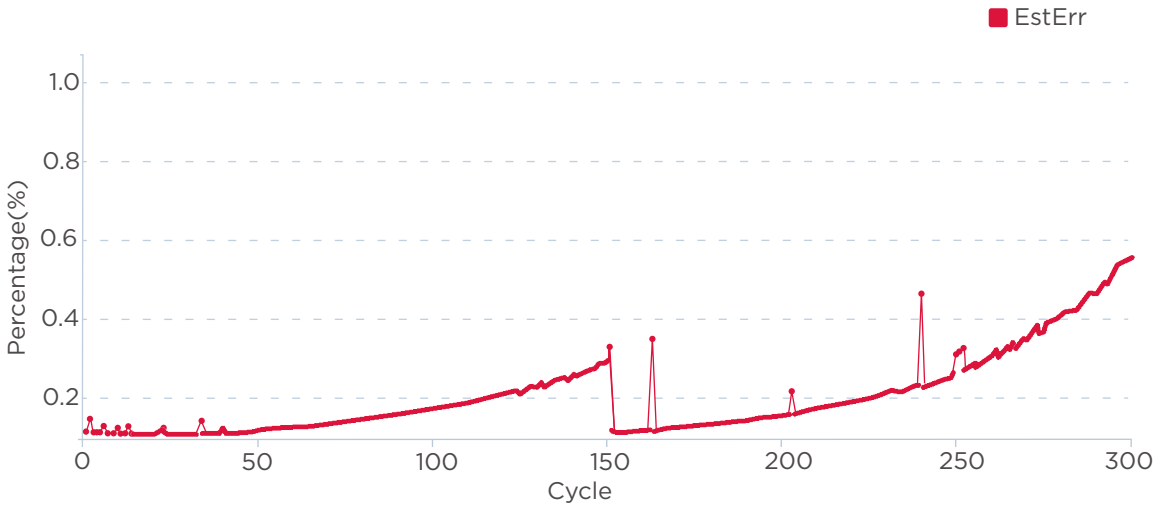

Figure 52 **Estimated Error Rate**

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

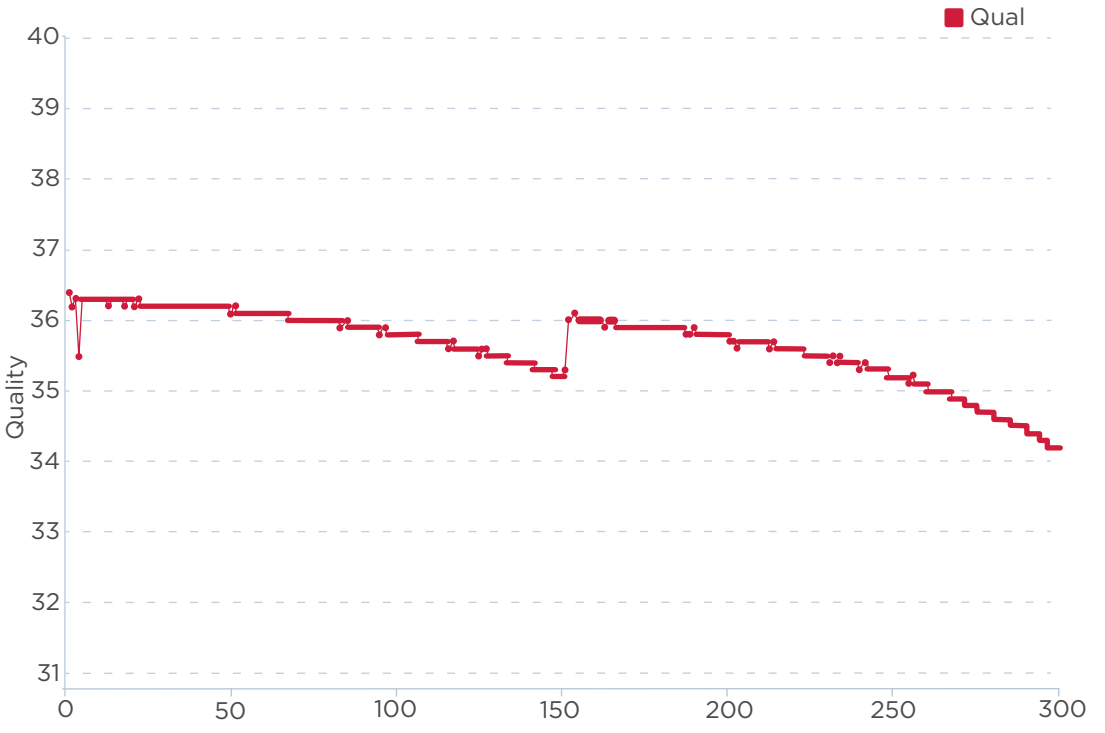

Figure 53 **Average Quality Distribution**

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

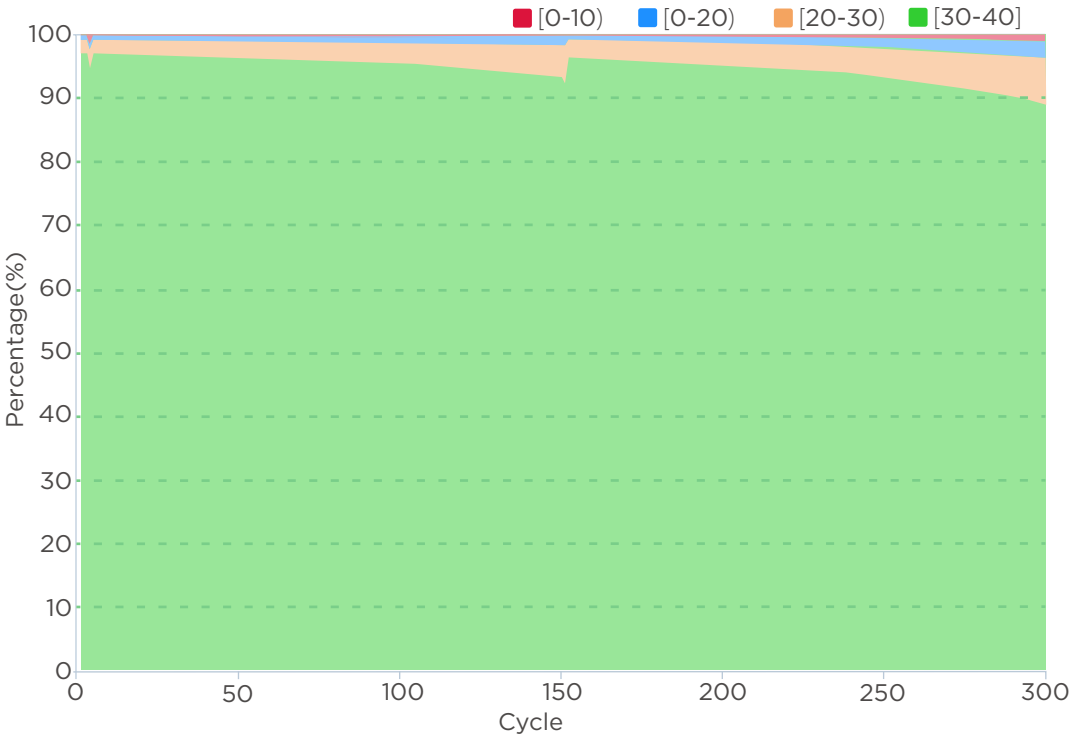

Figure 54 Quality Proportion Distribution

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

Other reports

Table 40 Other report description

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.

Name	Description
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 utilizing 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 sequencer manually).

The specific details of 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 provides a guide for writing FASTQ manually for situations including:

- If the FASTQ generation fails after sequencing.
- If the barcode file is selected incorrectly.
- If there is a need to change some FASTQ parameters, including but not limited to, whether filtering of FASTQ is used or not, whether splitting of barcodes is used or not, and whether save SaveDiscardedReads is used or not.

Preparation before writing FASTQ manually

Perform the following steps:

1. Ensure that the sequencer is in idle status, and not in 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+150+10+2= 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

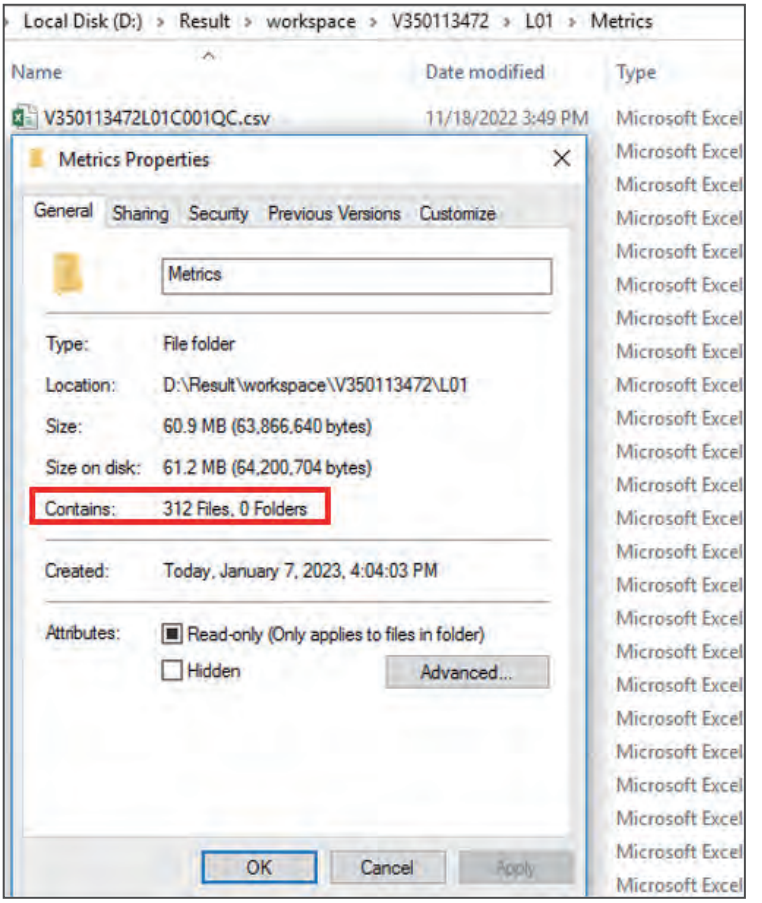

Figure 55 Metrics file number

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

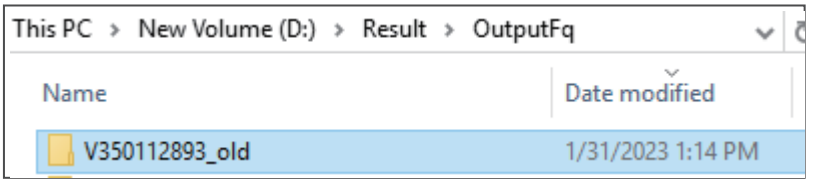

Figure 56 Renaming the FASTQ folder

4. Prepare the barcode file that you need to write FASTQ manually.
   For details, refer to *Instructions for importing barcode on Page 137.*

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

BasecallLite (Litecall) write FASTQ manually

Perform the following steps:

1. Enter 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 to backup the *Client.ini* file before editing it.

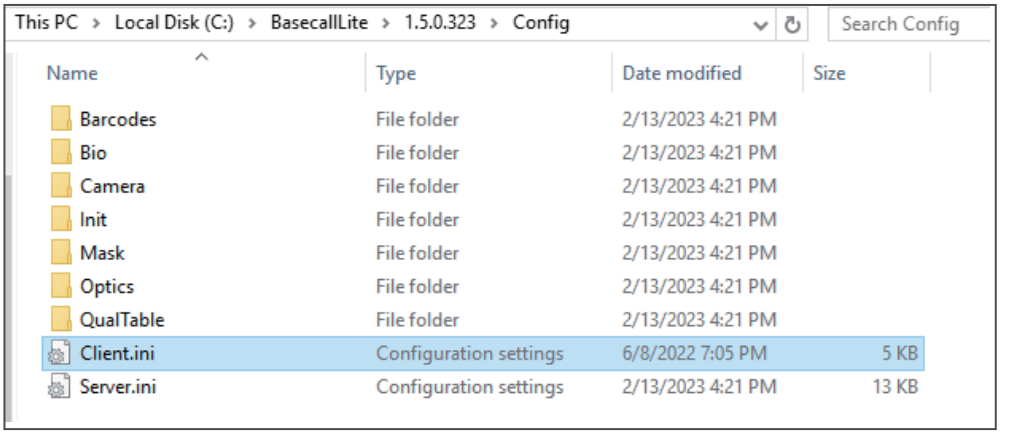

Figure 57 Location of Client.ini file

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

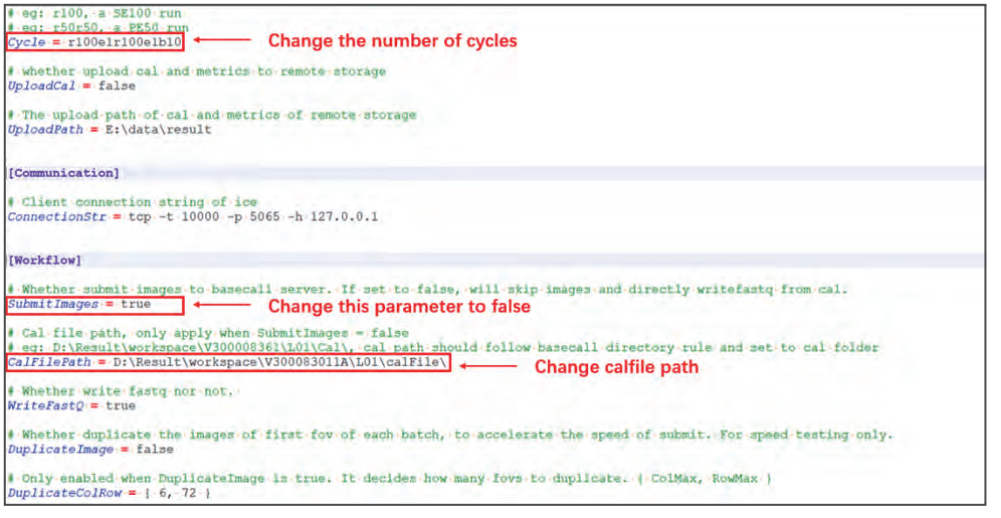

Figure 58 Editing Client.ini file

Table 41 Parameter description

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), Cycle=r100r100b10 • PE100+10+10(101+101+10+10), Cycle=r100e1r100e1b10b10 • PE100+10+42(101+101+10+42), Cycle=r100e1r100e1b10b42 • SE50+10(51+10), Cycle=r50e1b10
Change SubmitImages value from true to false	This parameter setting means writing FASTQ from *cal.* file.
Change *cal.* file path	This parameter describes the *cal.* file storage path, for details, refer to *Figure 59 on Page 103* .
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* The text displayed in green in the file are comments. You can refer to the comments to modify the relevant parameters.

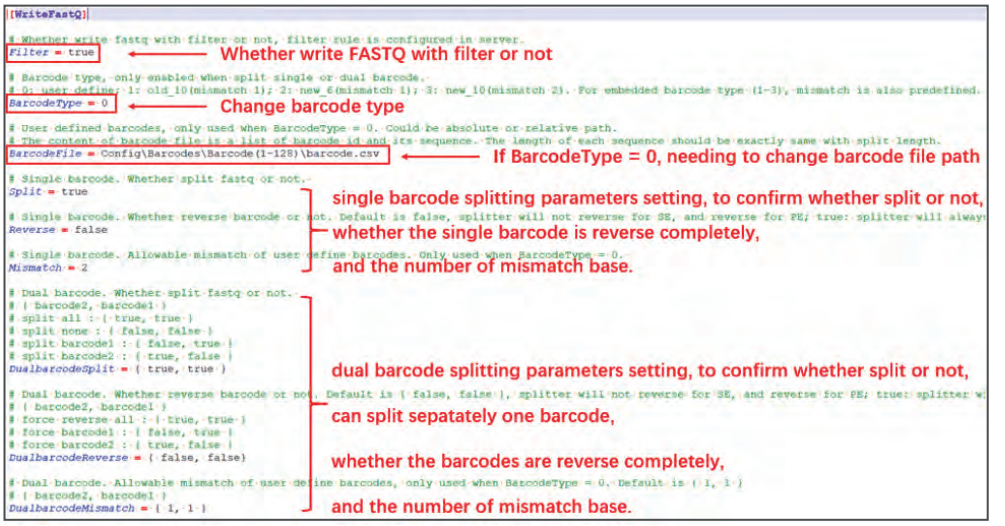

Figure 59 Changing cal. file path and barcode splitting parameters

3. Click **Save** and close the *Clint.ini* file.
4. Execute the manual write FASTQ command.

   There are two ways to execute the manual write FASTQ command, choose one of the following:

   - Open the Task Manager, select the **Services** tab, and start LiteCall.

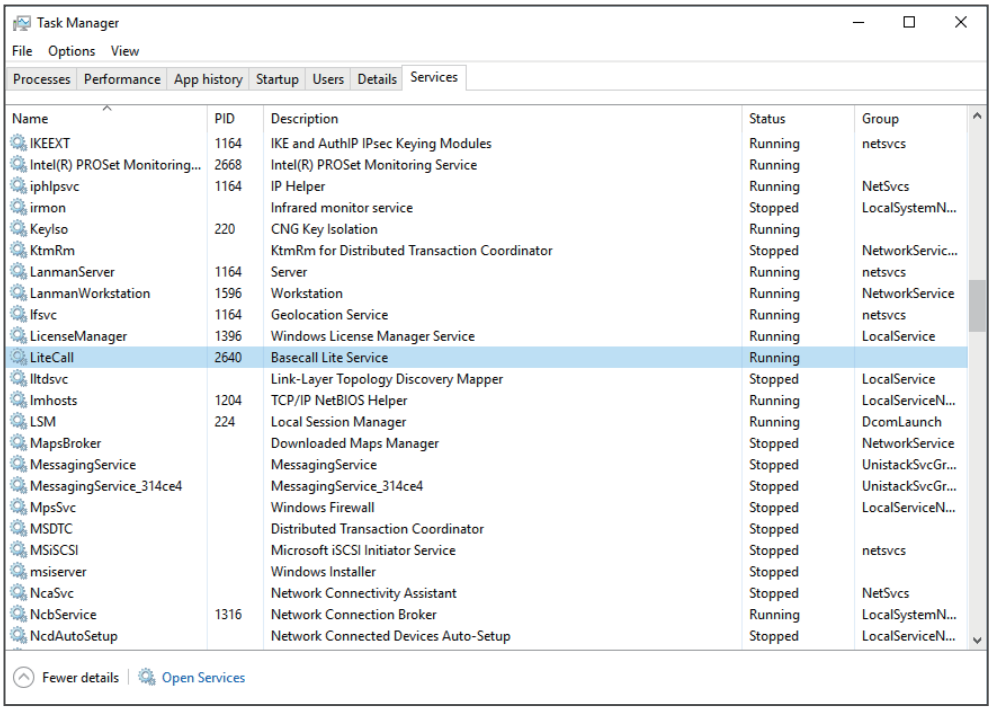

Figure 60 Starting LIteCall service

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

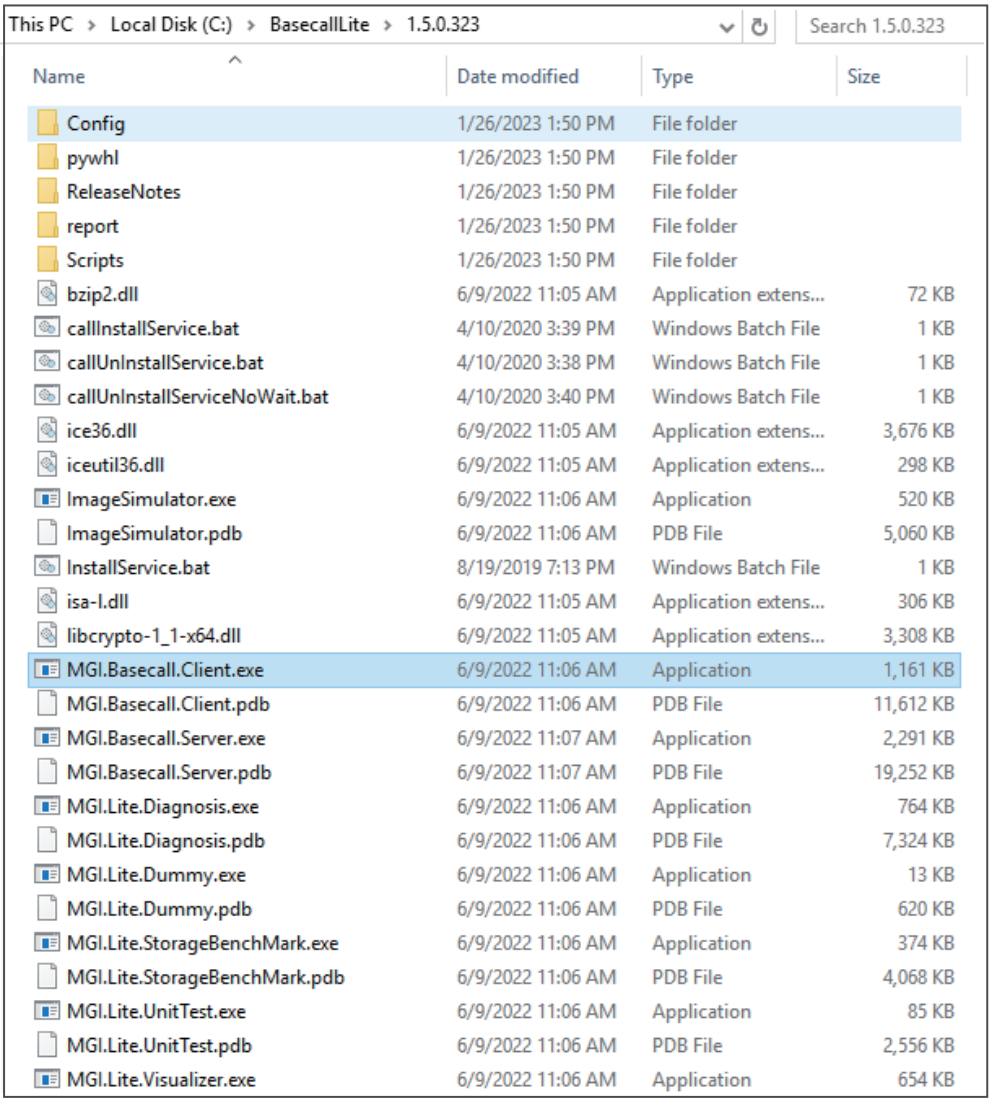

Figure 61 Opening the MGI.Basecall.Client.exe program

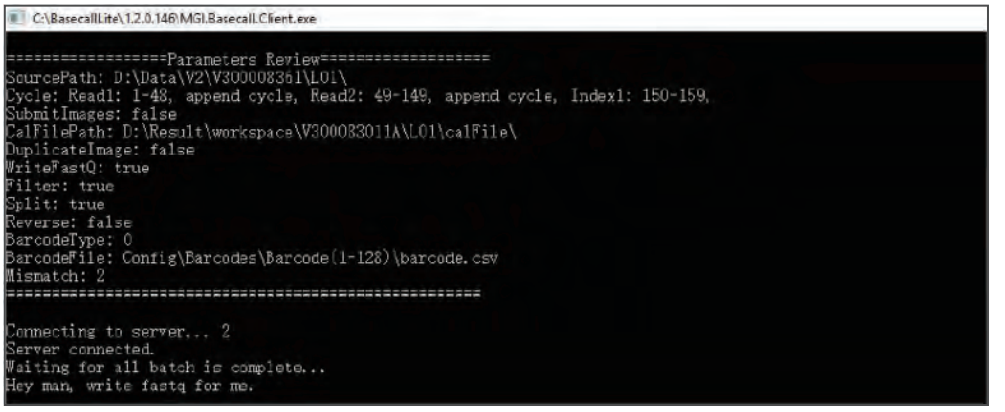

Figure 62 Starting the MGI.Basecall.Client.exe program

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

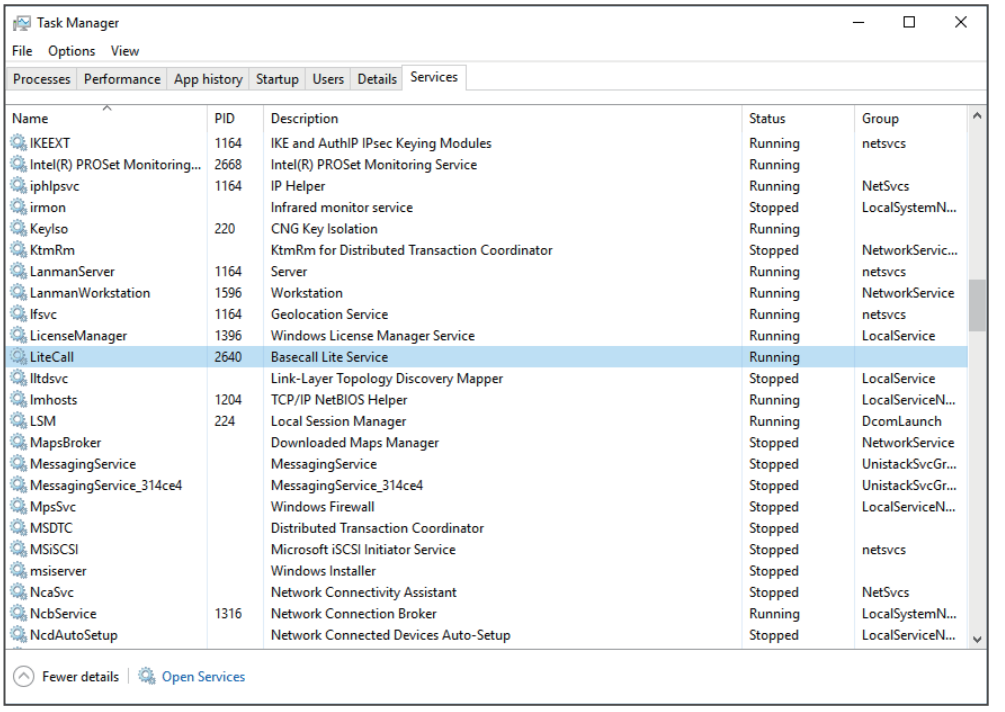

Figure 63 Stopping the LiteCall service

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

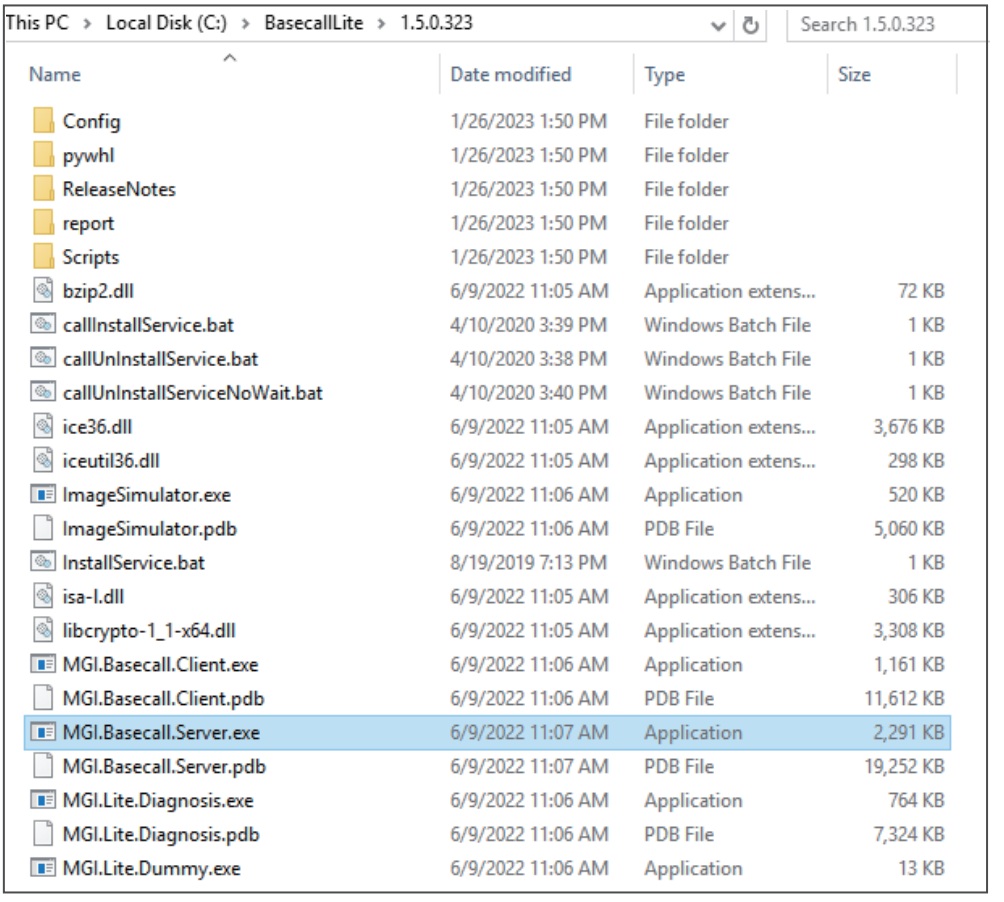

Figure 64 Opening the MGI.Basecall.Servicer.exe

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

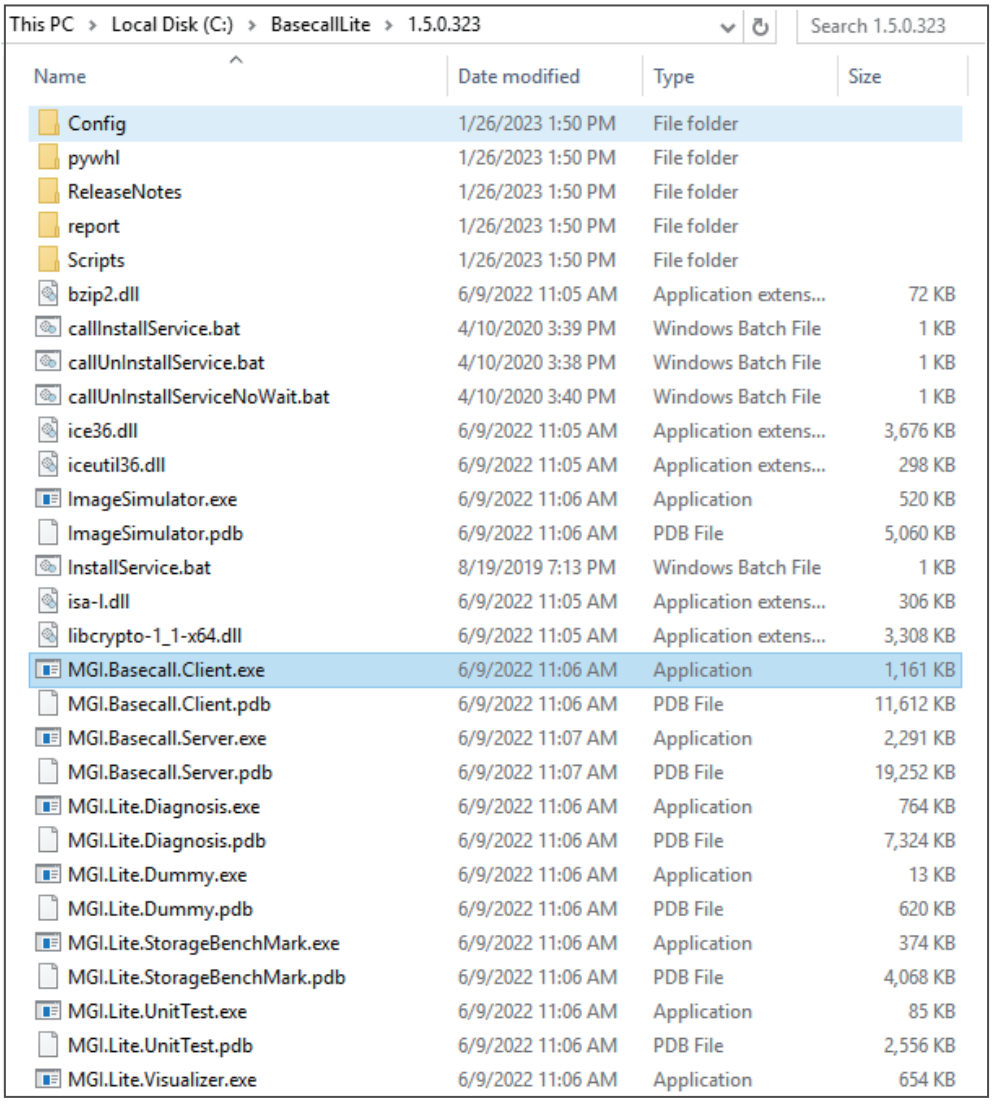

Figure 65 Opening the MGI.Basecall.Client.exe

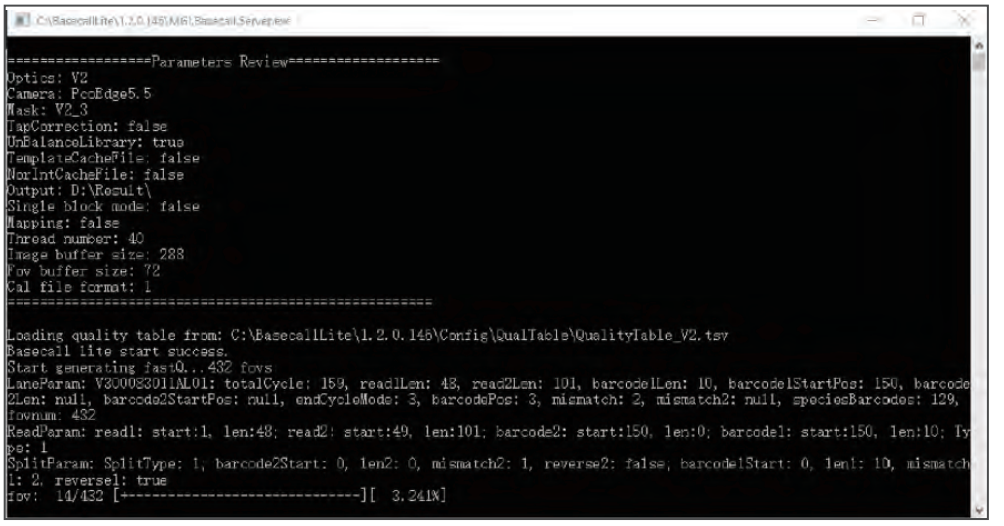

Figure 66 Starting the MGI.Basecall.Servicer.exe

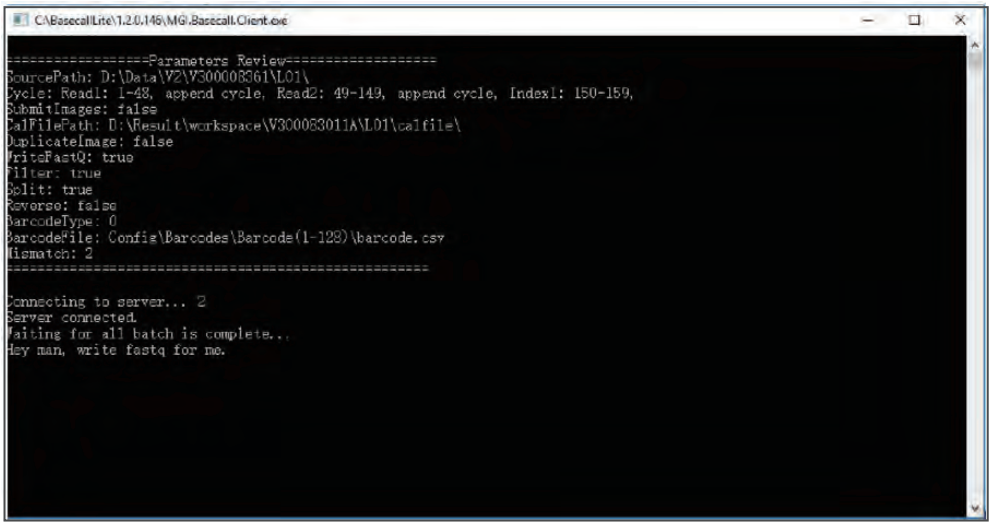

Figure 67 Starting the MGI.Basecall.Client.exe

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 assumptions as below:
   - 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*.

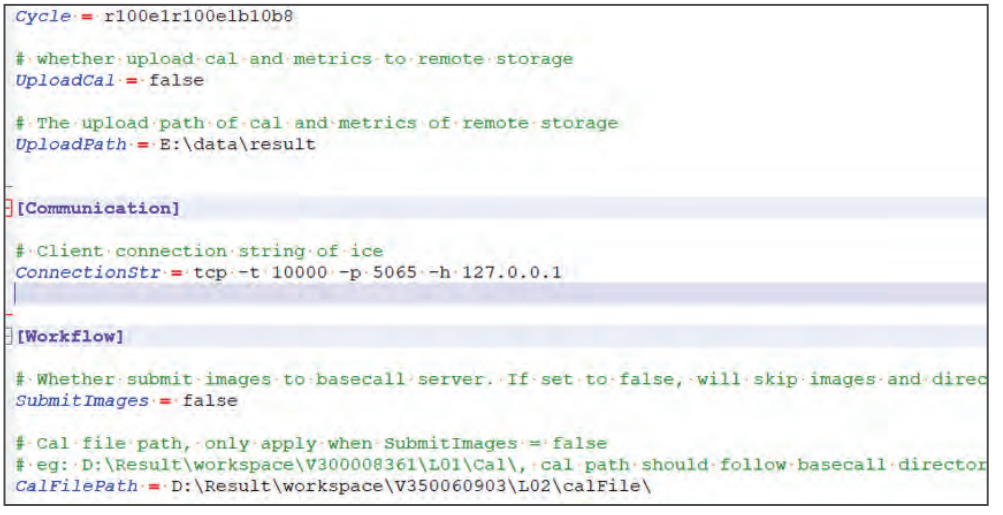

Figure 68 Parameter settings

These parameter settings are consistent no matter whether the barcode is split or not.

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

     BarcodeFile path:

     C:\BGI\barcode\CustomizeDualBarcode\DualBarcode-10_8\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 (
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 exa
BarcodeFile = C:\BGI\barcode\CustomizeDualBarcode\DualBarcode-10_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 for SE, and reverse
Reverse = false

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

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

# Dual barcode. Whether reverse barcode or not. Default is { false, false }, splitter will not reverse for SE, an
# { barcode2, barcode1 }
# force reverse all : { true, true }
# force barcode1 : { false, true }
# force barcode2 : { true, false }
DualbarcodeReverse = { false, false}

# Dual barcode. Allowable mismatch of user define barcodes, only used when BarcodeType = 0. Default is { 1, 1 }
# { barcode2, barcode1 }
DualbarcodeMismatch = { 1, 1 }
```

Figure 69 Spitting both barcode1 and barcode2

- Splitting only barcode2:

```
[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 (
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 exa
BarcodeFile = C:\BGI\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 reverse for SE, and reverse
Reverse = false

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

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

# Dual barcode. Whether reverse barcode or not. Default is { false, false }, splitter will not reverse for SE, an
# { barcode2, barcode1 }
# force reverse all : { true, true }
# force barcode1 : { false, true }
# force barcode2 : { true, false }
DualbarcodeReverse = { false, false}

# Dual barcode. Allowable mismatch of user define barcodes, only used when BarcodeType = 0. Default is { 1, 1 }
# { barcode2, barcode1 }
DualbarcodeMismatch = { 1, 1 }
```

Figure 70 Spitting only barcode2

- Splitting only barcode1:

```
[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 em
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 s
BarcodeFile = C:\BGI\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 revers
Reverse = false

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

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

# Dual barcode. Whether reverse barcode or not. Default is { false, false }, splitter will n
# { barcode2, barcode1 }
# force reverse all : { true, true }
# force barcode1 : { false, true }
# force barcode2 : { true, false }
DualbarcodeReverse = { false, false}

# Dual barcode. Allowable mismatch of user define barcodes, only used when BarcodeType = 0.
# { barcode2, barcode1 }
DualbarcodeMismatch = { 1, 1 }
```

Figure 71 Spitting only barcode1

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 72 Changing the SaveDiscardedReads setting

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06

Device maintenance

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

DANGER

- Ensure that the device is powered off before cleaning or disinfecting to avoid personal injury.
- Do not spray the wash solutions or disinfectants into the device during cleaning or disinfecting to avoid device damage.

WARNING

- It is not recommended to 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, please contact CG Technical Support.

Sequencer maintenance

Wash

Wash type introduction

There are two different wash types based on sequencer conditions:

Table 42 Wash type introduction

Wash type	Cartridge type	Process time (minutes)	Description
Pre-run wash (48 minutes)	Cleaning cartridge 1 (Laboratory-grade water)	48	• Before a sequencing run. • Maintenance wash has been performed more than 24 hours ago.

Wash type	Cartridge type	Process time (minutes)	Description
Maintenance wash (76 minutes)	Cleaning cartridge 3 (Tween-20)	14	• After a sequencing run. • Weekly if the sequencer has been used. • Biweekly if idle or powered off. • When impurities are found in the image. • After the sequencer maintenance is performed by an engineer. Including (but not limited) to the replacement of pipelines, sample needles and other accessories exposed to reagents.
	Cleaning cartridge 2 (NaOH)	14	
	Cleaning cartridge 1 (Laboratory-grade water)	48	

Preparing for wash

Preparing washing reagents

Prepare the washing reagents according to table below:

Table 43 Washing reagent 1: 0.05% Tween-20

Reagent name	Volume (mL)	Final concentration
100% Tween-20	0.5	0.05%
Laboratory-grade water	999.2	N/A
Total volume	1000	
Shelf life	1 month at 2 °C to 8 °C	

Table 44 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	N/A
Total volume	1000	
Shelf life	1 month at 2 °C to 8 °C	

Table 45 Washing reagent 3: 0.1 M NaOH

Reagent name	Volume (mL)	Final concentration
2 M NaOH	50	0.1 M
Laboratory-grade water	950	N/A
Total volume	1000	
Shelf life	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 46 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

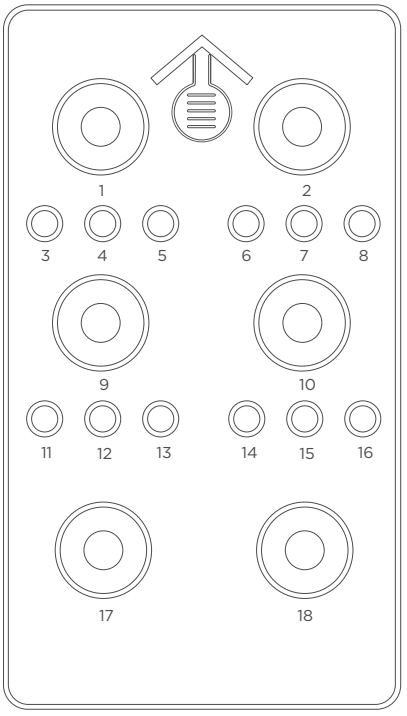

Figure 73 **Top view of cleaning cartridge**

- 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 table below:

Table 47 **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 washing flow cell.

Each washing flow cell, stored at room temperature or at 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 needs to be washed within 24 hours. When the following interface appears, select **Wash** and perform wash procedures.

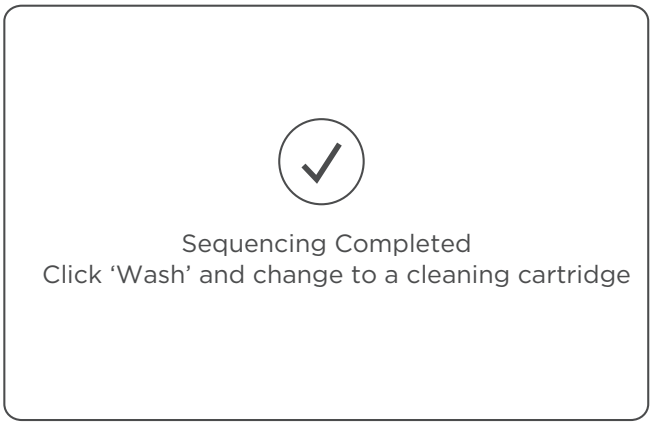

Figure 74 Wash instructions interface

Performing a pre-run wash (~48 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.
2. Put DNB loading needle washing tube 1 (Sterile Microcentrifuge tube, 2.0 mL with 1.8 mL Laboratory-grade water) into the DNB loading position. 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 69.*
4. Select **Wash** in the main interface. Select **Regular** from the **Wash type** list to start pre-run wash, which takes approximately 48 minutes.

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

Figure 75 Selecting the wash type

Performing a maintenance wash (~76 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.
2. Put DNB loading needle washing tube 3 (Sterile Microcentrifuge tube, 2.0 mL with 1.8 mL Tween-20) into the DNB loading position. 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 69*.
4. Select **Wash** in the wash instructions interface. Select **Maintenance** from the **Wash type** list to start the wash. The wash takes approximately 14 minutes.
5. When the interface appears as below, click **Yes** and the sequencer will automatically lift the sampling needles.

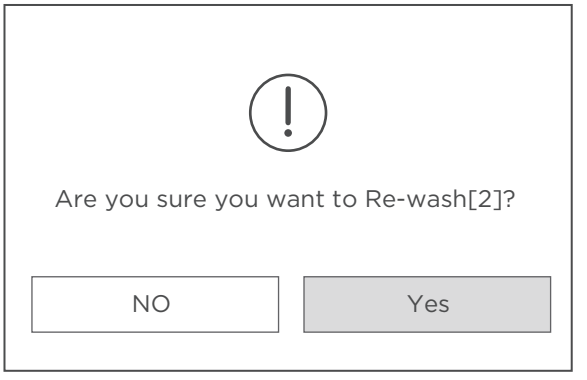

Figure 76 Maintenance wash [2] prompt

6. Slowly insert the Cleaning cartridge 2 into the reagent compartment by following the direction printed on the cartridge cover until it stops.
7. Put DNB loading needle washing tube 2 (Sterile Microcentrifuge tube, 2.0 mL with 1.8 mL NaOH) into the DNB loading position. Close the reagent compartment door.
8. Select **Wash** in the wash instructions interface. Select **Maintenance** from the **Wash type** list to start the wash. The wash takes approximately 14 minutes.

9. When the interface appears as below, click **No** and the sequencer will automatically lift the sampling needles.

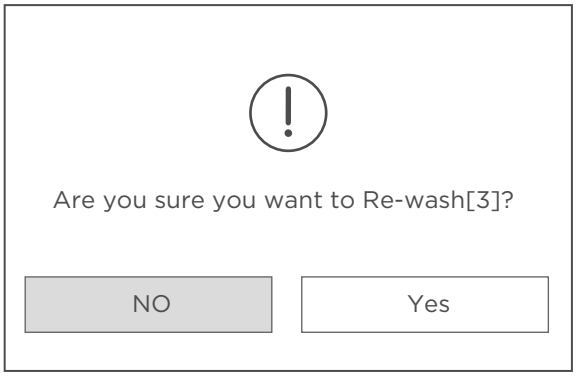

Figure 77 Maintenance wash [3] prompt

10. Slowly insert the Cleaning cartridge 1 into the reagent compartment by following the direction printed on the cartridge cover until it stops.
11. Put DNB loading needle washing tube 1 (Sterile Microcentrifuge tube, 2.0 mL with 1.8 mL Laboratory-grade water) into the DNB loading position.
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 48 minutes.

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 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 recommended that not to 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 to use the sequencing reagent cartridge as 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 with the peripheral storage devices. Ensure that the storage drive has enough space for your sequencing run.

For details about clearing history data, refer to *Reviewing parameters on Page 72.*

Powering cycle the device

Perform the following steps:

1. Turn off the computer. Turn off and unplug the device.
2. Wait at least 30 seconds.
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 flow cell to the chuck.

- Wear protective gloves when cleaning the flow cell stage. Dust, lint, or other particulate matters 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
- Clean cloth
- Absolute ethanol
- Canned air duster

Perform the following steps:

1. Check for dust, debris, damage, or particulate matter on the surface of the aluminum chuck of the flow cell stage.
2. Wipe the aluminum chuck of the flow cell stage with a clean cloth moistened with absolute ethanol, and then let it air-dry.

   Do not wipe the vacuum inlet and vacuum attachment slot to prevent absolute ethanol from entering the holes and damaging the device.
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 clean cloth moistened with absolute 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 flow cell is facing upward, and the QR code is 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 avoid 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, attach the lid back onto the container if necessary, and gently swirl it until all inner walls are cleaned.
5. Pour the laboratory-grade water into an appropriate waste container.

If necessary, repeat step 4 to 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. Attach the lid back onto the waste container.

Monthly maintenance

Maintaining the device

The low-lint cloth should keep 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, and reagents, blood, or potential biological contaminants.

Checking and cleaning the cooling fan

Perform the following steps:

1. Remove the dust on the ventilation holes with a small brush. Ensure that the device can ventilate normally.
2. Check whether the cooling fan operates normally. If 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

DL-200H maintenance

WARNING

- Do not immerse DL-200H into the liquid for cleaning. Doing so may damage the device.
- Do not use disinfectants such as absolute ethanol, dichloroethane ($C_2H_4Cl_2$), trichloroethylene (C_2HCl_3), chloroform ($CHCl_3$), and toluene (C_7H_8) to clean DL-200H. Doing so may damage the device.
- It is recommended to replace DL-200H with a new one after using 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 DL-200H:

1. Wipe all sides of the device with a low-lint cloth moistened with 75% ethanol.
2. Wipe all sides of the device with a low-lint cloth moistened with ultra-pure water.
3. Dry the device with a low-lint cloth or let it air-dry.

Sealing gasket maintenance

After each DNB loading, perform the following steps:

1. Collect the used sealing gasket into a 200 mL beaker. Fill the beaker with ultra-pure water and wash the sealing gasket in the beaker, and empty the beaker after wash. Repeat the wash with ultra-pure water in the beaker for 2 times.
2. Fill the tank of the ultrasonic cleaner with ultra-pure water, and wash the sealing gasket in the tank of the ultrasonic cleaner for approximately 15 minutes.
3. Fill the beaker with ultra-pure water and wash the sealing gasket in the beaker, and empty the ultra-pure water after wash. Repeat the wash with ultra-pure water in the beaker for 2 times.
4. Place the cleaned sealing gasket into a clean container on laboratory bench and let the sealing gasket air-dry.

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07

FAQs

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

If malfunctions occur during operation, the device alarms or 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 turning the power switch to the ON position?

Powering 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. Check whether 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?

Errors 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 is the flow cell not attaching to the flow cell stage?

If the flow cell is not attaching to the flow cell stage, it may be due to the flow cell attachment button not being pressed. Any dust, debris, or damage that may be present on the flow cell stage and/or the flow cell can keep 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 121*.

Q: What should I do if a message, indicating 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 temperature error message and warning appears 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 to 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 after sequencing?

Bubbles may be present when there is air leaks between the flow cell and the sealing gasket, or the flow cell is damaged, or the pipeline is bent or become loose, or 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?

Waste level sensor alarms when the waste container is full, or the level sensor is not installed properly, or software error occurs. For details, refer to *Performing pre-run checks on Page 51.*

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), 60 μL Make DNB reaction can be tried. 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.

The above solution is only applicable for general libraries with amount 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 Section *DNA library concentration and amount requirement on Page 37*. 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 ssDNA libraries for each Make DNB reaction. The value of *V* obtained from the above equation will be used in *Table 48 on Page 131* .

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:

Table 48 Make DNB reaction mixture 1

Component	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 seconds 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 49 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 on ice. Centrifuge briefly for 5 seconds 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. Take the PCR tube out of the thermal cycler when the temperature reaches 4 °C.
6. Centrifuge briefly for 5 seconds, place the tube on ice, and prepare the Make DNB reaction mixture 2 according to the table below:

Table 50 Make DNB reaction mixture 2

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

7. Add all 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 seconds.
9. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

Table 51 RCR conditions

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

10. Immediately add 12 µL of Stop DNB Reaction Buffer once the temperature reaches 4 °C. Mix gently by pipetting 8 times by using a wide-bore, non-filtered pipette tip.

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

11. Proceed to *Quantifying DNBs on Page 48*.

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

When the DNB concentration is lower than that specified in *Table 29 on Page 48* , perform the steps below:

1. Check if the DNB preparation kit has expired.
2. Check if 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, please 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 due to 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.

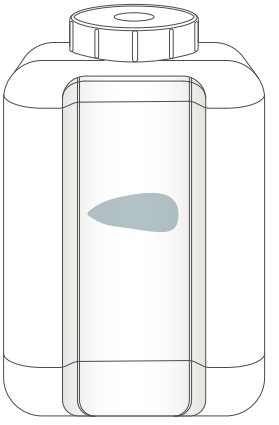

Figure 78 **Dark green crystals in well No. 10**

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 and MDA Reagent need be added to well No. 15. If you mistakenly 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.

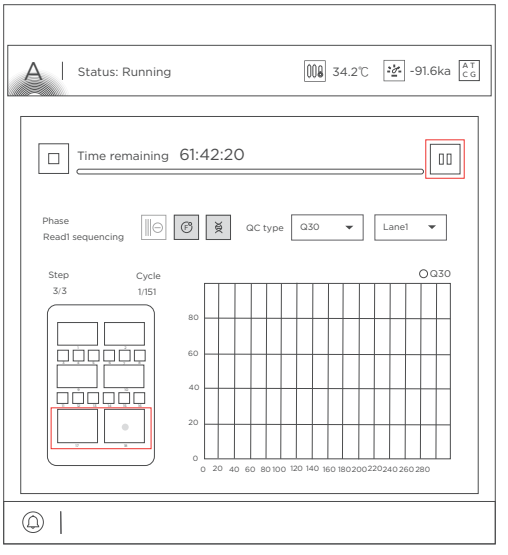

Figure 79 Selecting the sequencing stage to pause

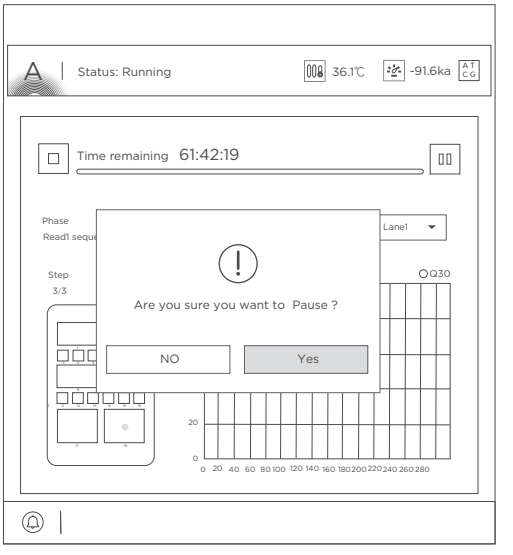

Figure 80 Confirming to pause the run

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

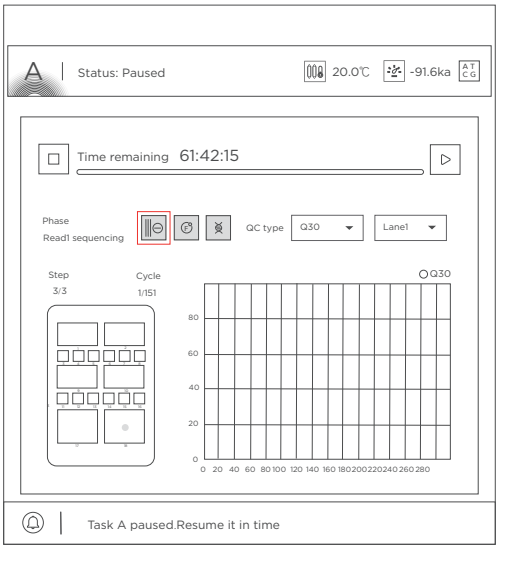

Figure 81 Selecting to lift needle

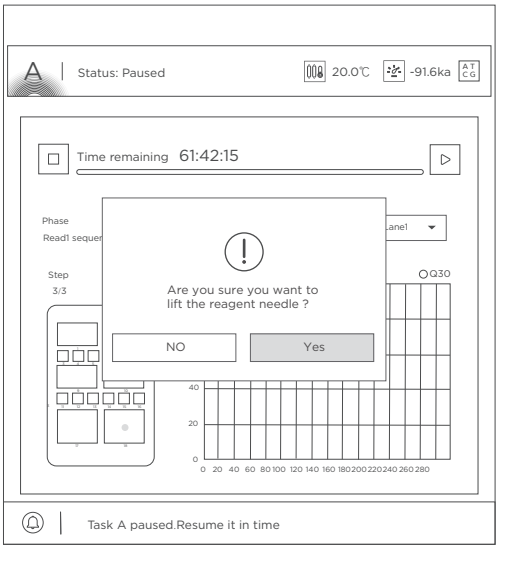

Figure 82 Confirming to lift the needle

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 into MDA reagent tube, mix well. in *"20. Perform the following steps for FCL PE100/FCL PE150/FCL PE200/FCS PE100/FCS PE150/FCS PE300 sequencing:" on Page 129.*

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.

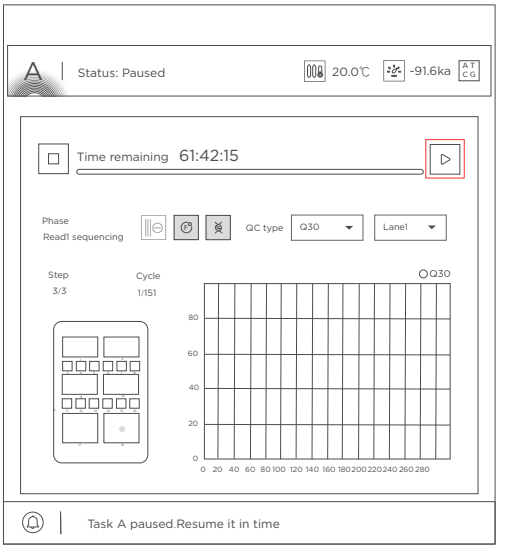

Figure 83 Selecting to resume the run

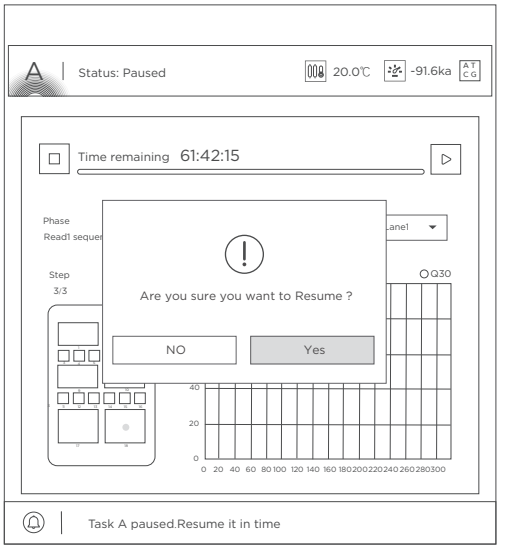

Figure 84 Confirming to resume the run

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 hours, it can be frozen and thawed at most one time.
- If a kit has been thawed (including dNTPs) but cannot be used immediately, store it at 2 °C to 8 °C. It is strongly recommended to use it within 24 hours. Mix the reagents in the cartridge following instruction in *Preparing the sequencing reagent cartridge on Page 57* before use.
- If dNTPs and Sequencing Enzyme Mix have been added into the cartridge, i.e. the cartridge has been prepared but cannot be used immediately, store it at 2 °C to 8 °C and use it within 24 hours. Mix the reagents in the cartridge following instruction in *Preparing the sequencing reagent cartridge on Page 57* before use.
- If dNTPs and Sequencing Enzyme Mix have been added into the cartridge, i.e. the cartridge has been prepared and the needles have punctured the seal, but the cartridge cannot be used immediately, the cartridge must be sealed with foil

or plastic wrap. Store the cartridge at 2 °C to 8 °C and use it within 24 hours. Gently mix the reagents in the cartridge before use. When mixing, be careful not to spill any reagent from the needle holes to avoid reagent contamination.

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 dust the stage with a canned air duster. Ensure that no dust is present on the flow cell stage.
2. Dust the back of the flow cell with a canned air duster to ensure that no dust is present.
3. If the problem persists, please 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 large bubbles appear in the flow cell, perform the steps below:

1. The sequencer: remove the flow cell, check if there are 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 69* and start the pump again.
2. Check if the sampling needles are moving properly. If the sampling needles are not moving properly, restart the control software of the sequencer.
3. If the problem persists, please contact CG Technical Support.

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

If impurities appear, perform the steps below:

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 (~76 min) on Page 119*.
2. If the problem persists after a full wash, please 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 less than two bases.
- Barcode sequence should be unique, and barcode ID and barcode sequence should not 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:

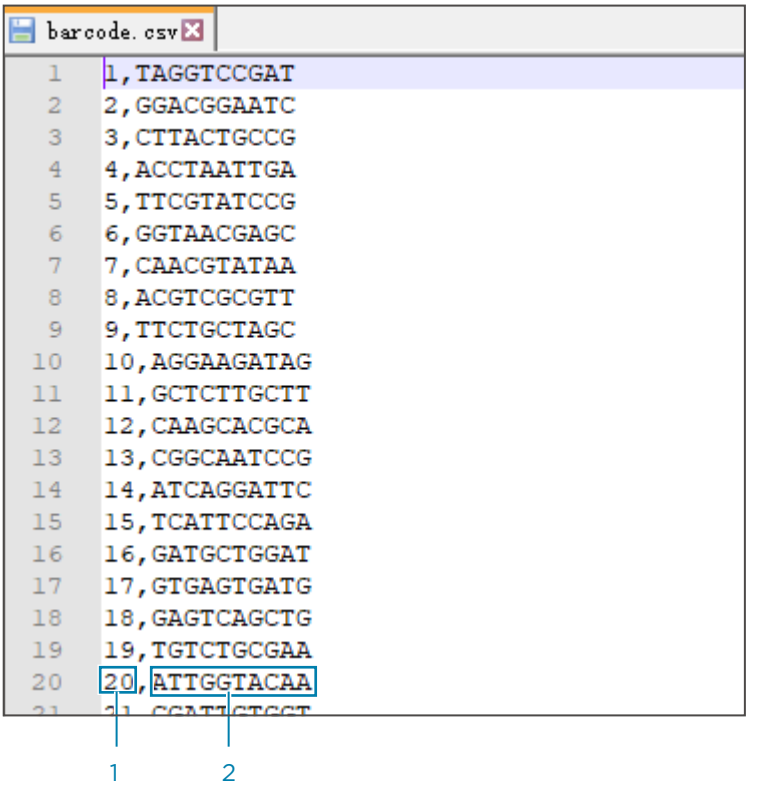

Figure 85 Single barcode file

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

Dual barcode file

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

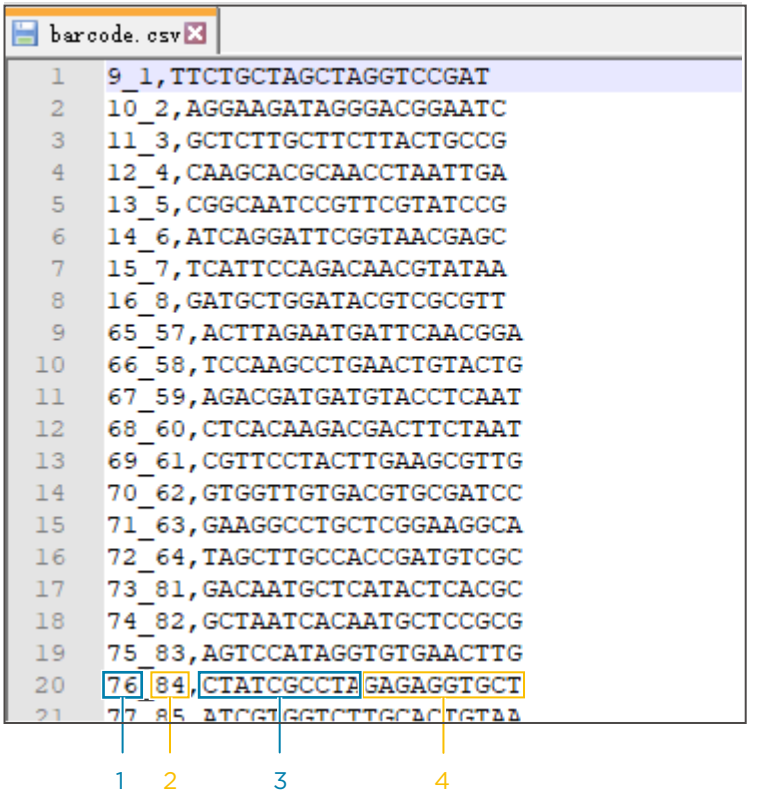

Figure 86 Dual barcode file

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:

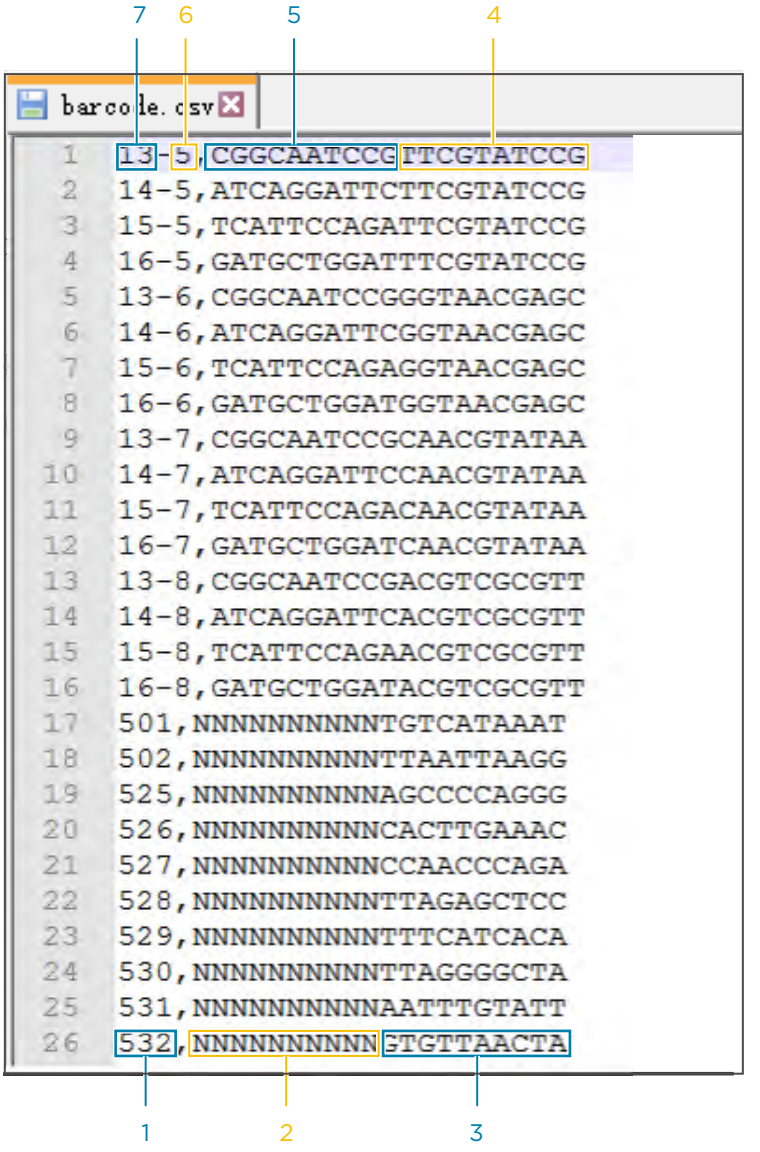

Figure 87 Single and dual barcode file1

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

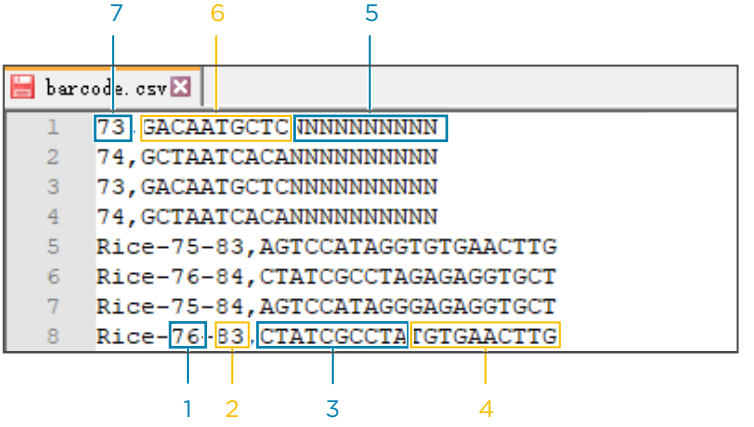

Figure 88 Single and dual barcode file2

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 use, it is recommended to 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 of the storage device. 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 type of barcode file is consistent with the barcode type.

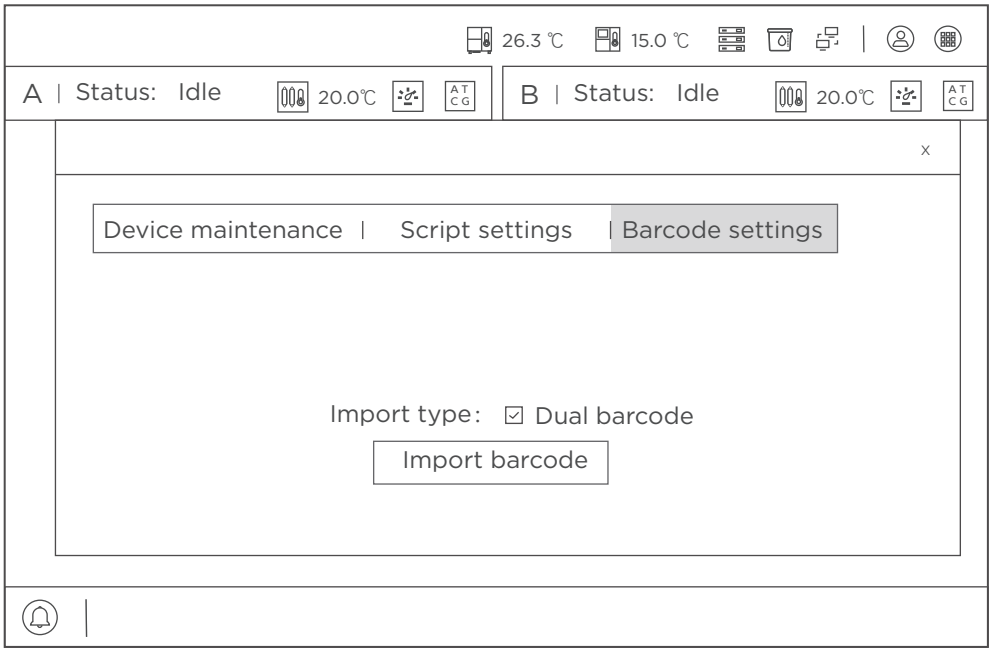

Figure 89 Barcode settings interface

4. Select **Import barcode**.

5. Select the exported and imported directories.

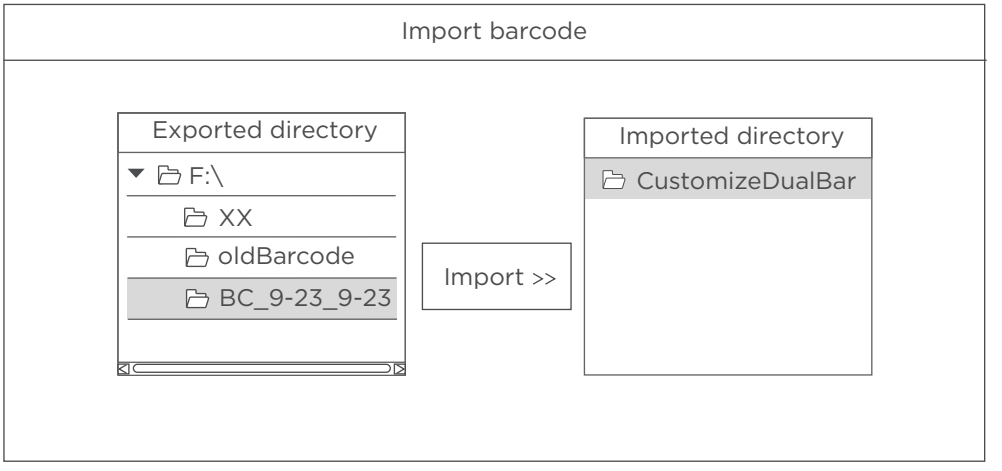

Figure 90 **Importing a dual barcode file**

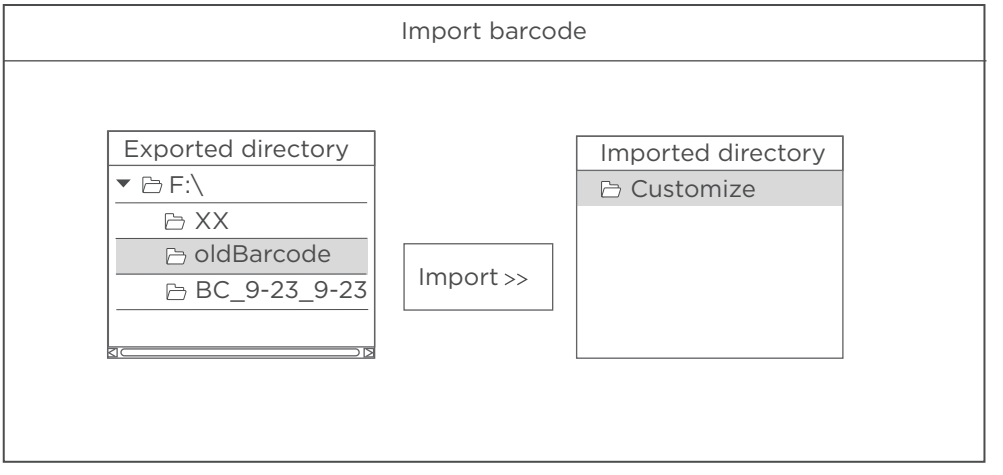

Figure 91 **Importing a single barcode file**

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:\BGI\barcode\CustomizeDualBarcode
   - Single barcode file

     C:\BGI\barcode\Customize

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Instructions for customizing a run

Introductions

This section provides instructions for customizing a sequencing run, which may be needed 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.
- stLFR FCL PE100
- Small RNA
- UMI (Unique Molecular Identifier) +UDI (Unique Dual Index)
- If unexpected problems happen 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

Customize recipe

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

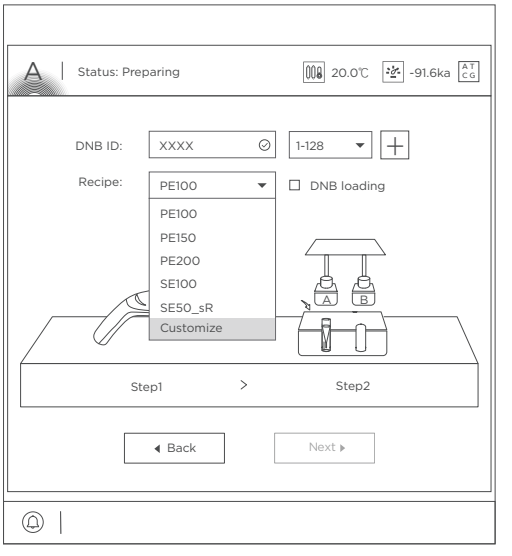

Figure 92 Customize recipe

Customize interface

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

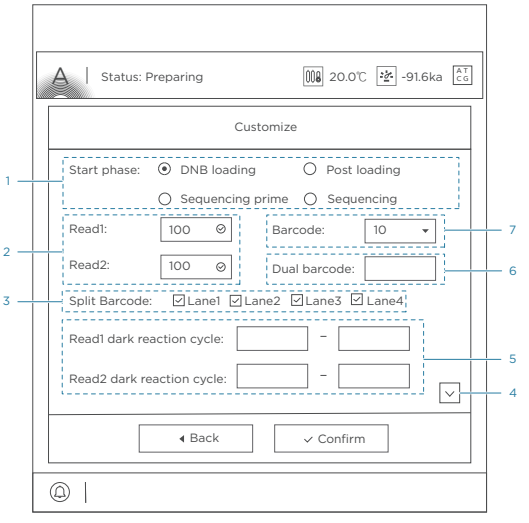

Figure 93 Customize interface

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

No.	Item	Description
1	**Start phase**	Select a start phase for a sequencing run
2	**Read1/Read2**	Customize Read1 and (or) Read2 length for a sequencing run
3	**Split barcode**	Customize whether to split barcode or not for different lanes
4		Select to open barcode type interface, you can select the barcode imported
5	**Read1 dark reaction cycle/ Read2 dark reaction cycle**	Customize dark reaction range in Read1 and (or) Read2
6	**Dual barcode**	Customize Barcode2 (dual barcode) length for a sequencing run
7	**Barcode**	Customize Barcode1 length for a sequencing run

Start phase description

You can select the sequencing start phase according to your needs:

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

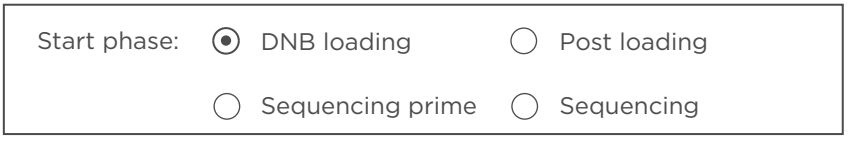

Figure 94 Selecting DNB loading for the start phase

- **Post loading with Prime**: if you have loaded DNBs by DL-200H, select **Post loading** and select **Prime**.

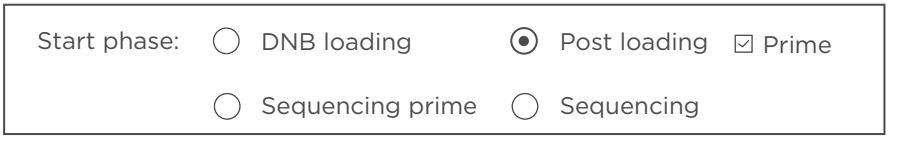

Figure 95 Selecting Post loading (Prime) for the start phase

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

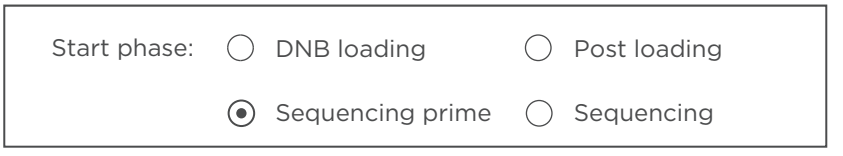

Figure 96 Selecting Sequencing prime for the start phase

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

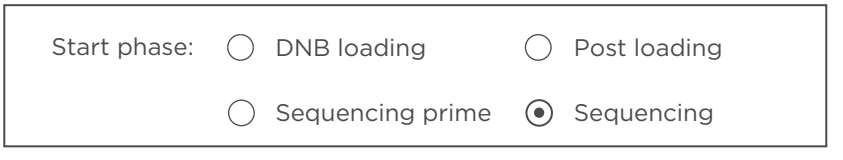

Figure 97 Selecting Sequencing for the start phase

Single barcode (not predefined) interface

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

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

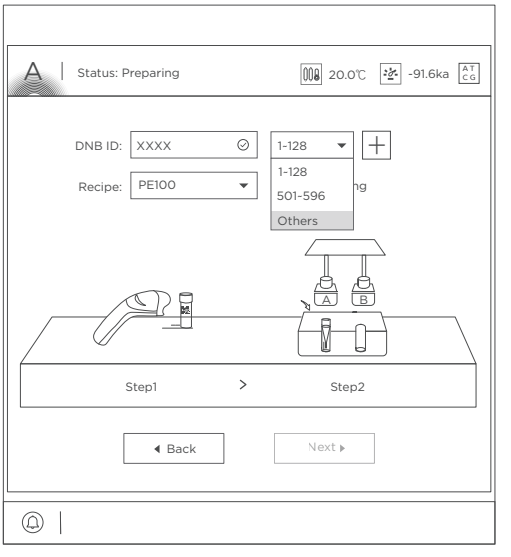

Figure 98 **Selecting Others**

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

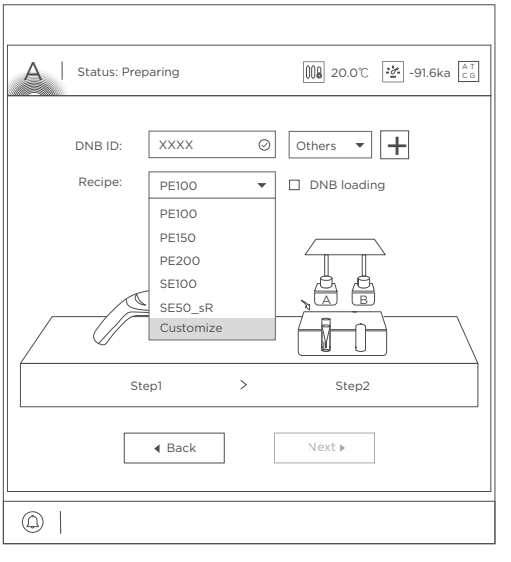

Figure 99 **Selecting Customize**

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

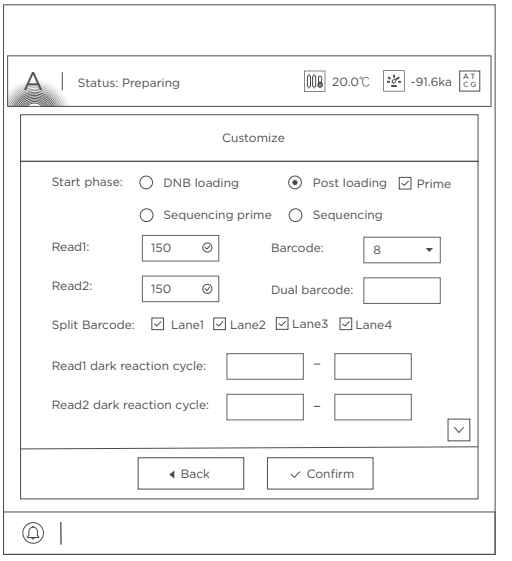

Figure 100 **Configuring customize settings**

4. 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. If not, leave the boxes blank.

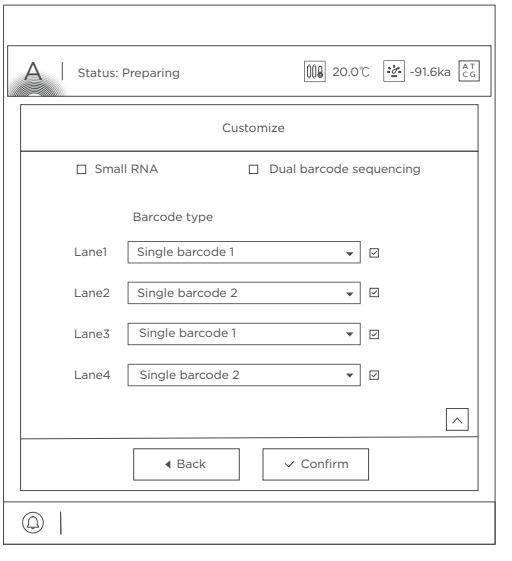

Figure 101 **Selecting barcode type and split strategy**

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 enter 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.

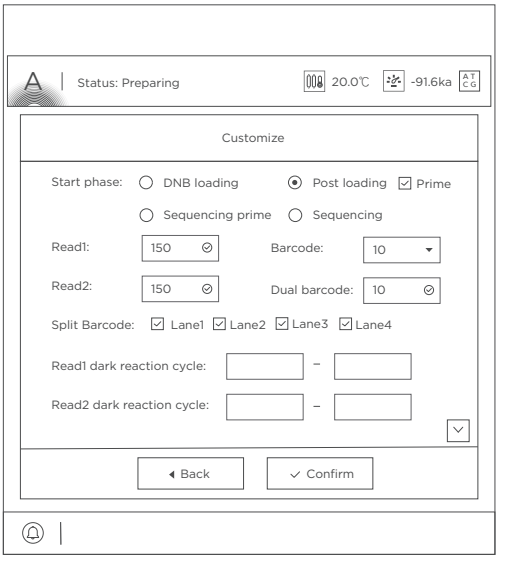

Figure 102 Configuring customize settings

4. 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. If not, leave the boxes blank.

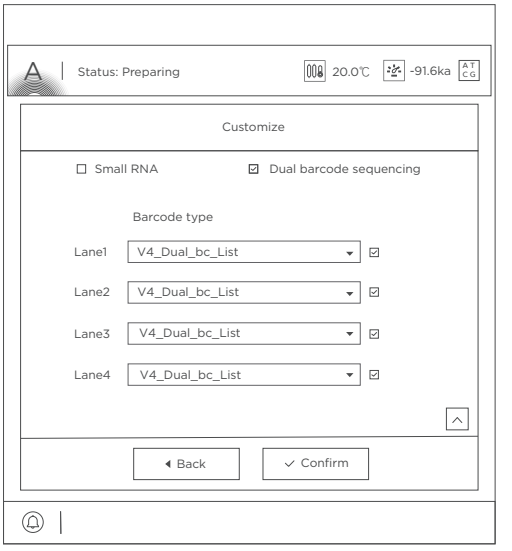

Figure 103 **Selecting barcode type and split strategy**

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 enter 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.

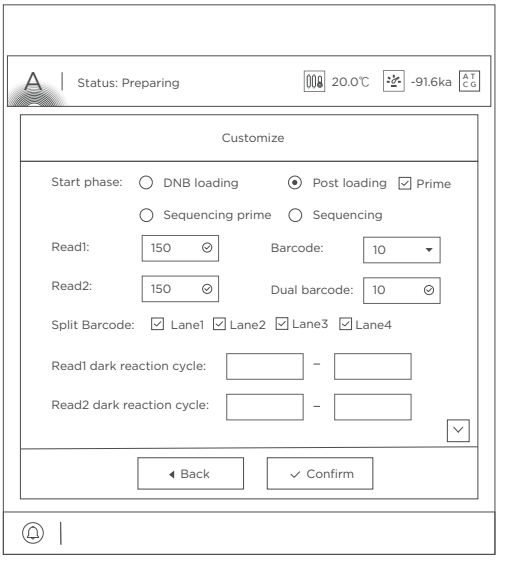

Figure 104 Configuring customize settings

4. Select the **Dual barcode sequencing** box. Select a dual barcode type from the **Barcode type** list and **Dual barcode type** list. If the barcode split is needed, select the boxes next to the **Barcode type** list and **Dual barcode type** list for the split strategy of each lane. If not, leave the boxes blank.

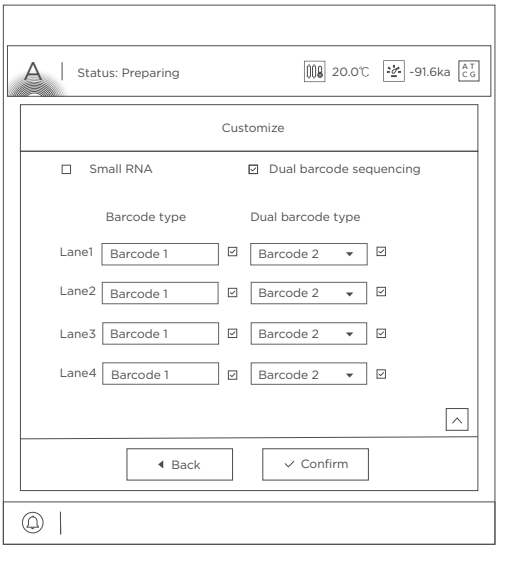

Figure 105 Selecting barcode type and split strategy

Small RNA single barcode (not predefined) interface

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

1. In the main interface, select **Sequence** to enter 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.

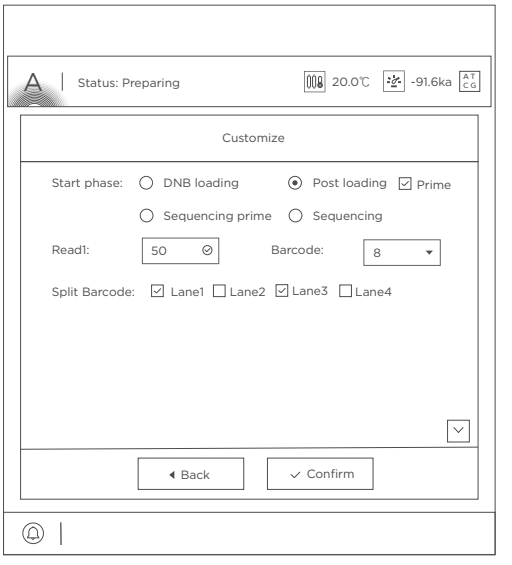

Figure 106 Configuring customize settings

4. Select the **Small RNA** box. Select a single 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. If not, leave the boxes blank.

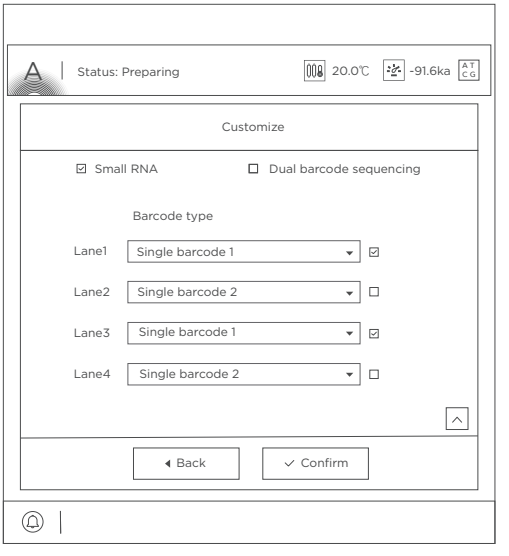

Figure 107 Selecting barcode type and split strategy

Examples of customized run

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 not, refer to *Instructions for importing barcode on Page 137* 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 2 on Page 31* .
- 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 you perform dual barcode sequencing run, it is recommended to use identical settings for the sequencing parameters in both side-A and side-B.
- 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.

You can 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 below:

- 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+140+10+2 = 272
- Select a PE150 kit

The Customize interface is set as follows:

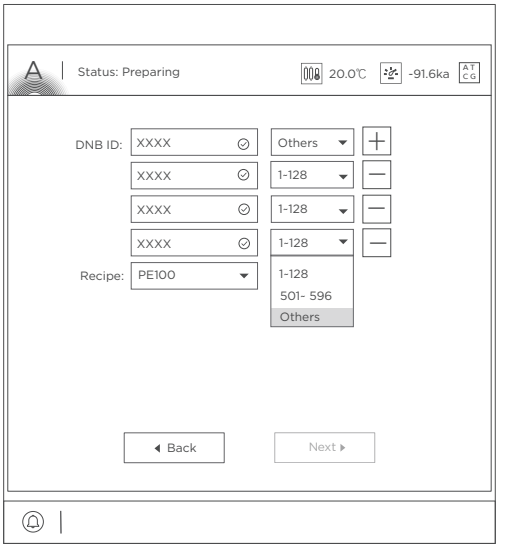

Figure 108 Selecting Others

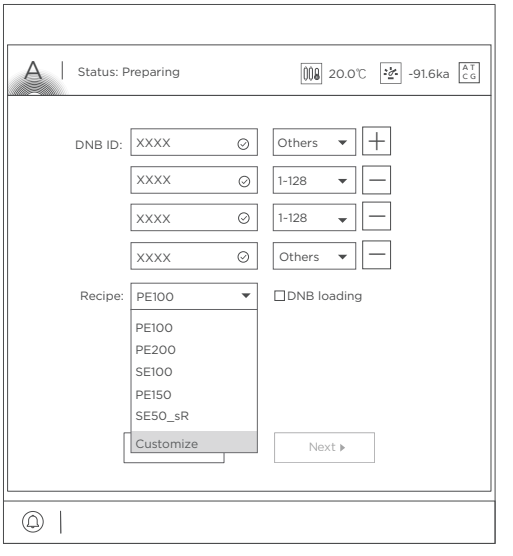

Figure 109 Selecting Customize

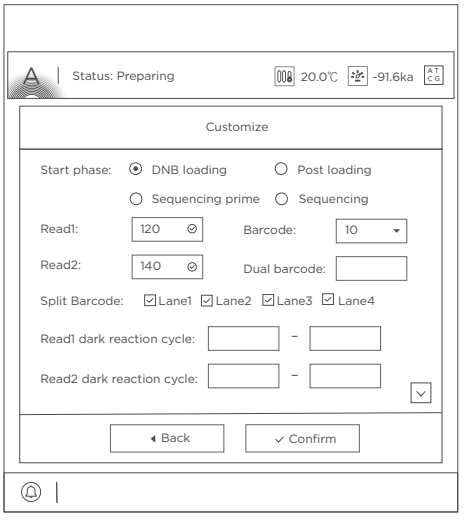

Figure 110 Configuring customize settings for example 1

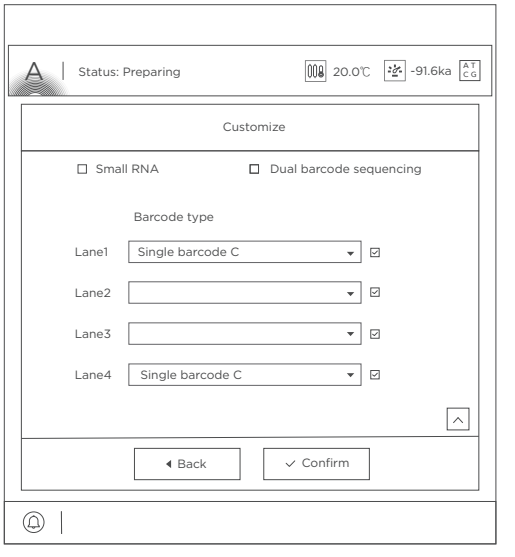

Figure 111 Selecting barcode type and split strategy for example 1

2. Length of the single barcode is not 10

Assumptions are as below:

- 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+150+8+2 = 310
- Select a PE150 kit

The Customize interface is set as follows:

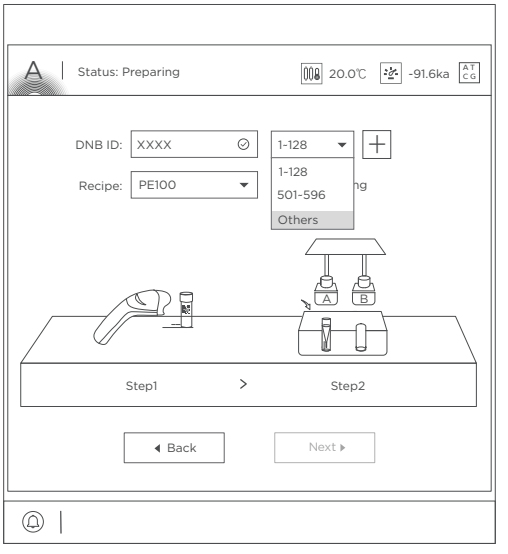

Figure 112 Selecting Others

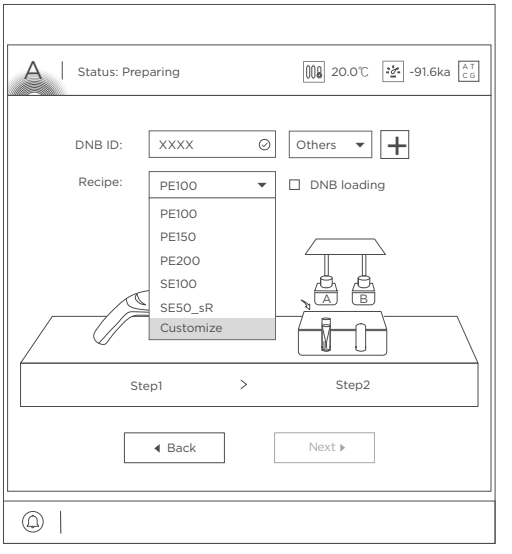

Figure 113 Selecting Customize

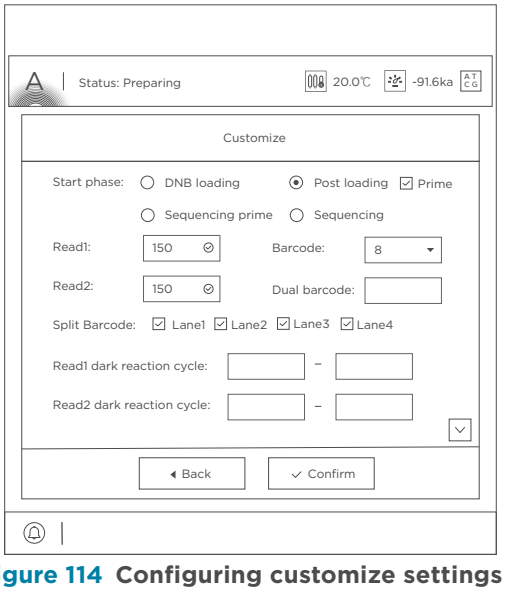

Figure 114 Configuring customize settings for example 2

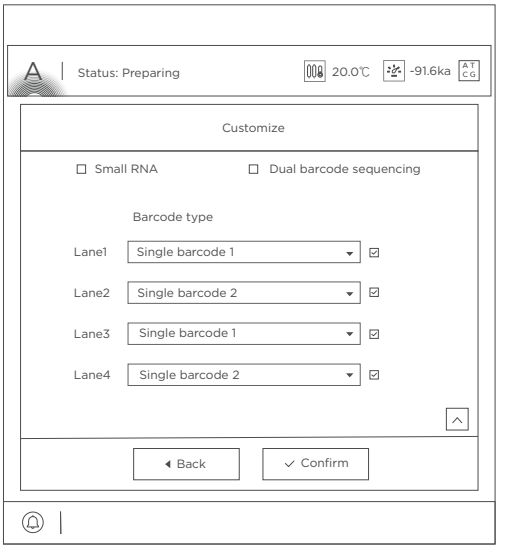

Figure 115 Selecting barcode type and split strategy for example 2

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

Assumptions are as below:

- 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+150+10+10+2 = 322
- Select a PE150 kit

The Customize interface is set as follows:

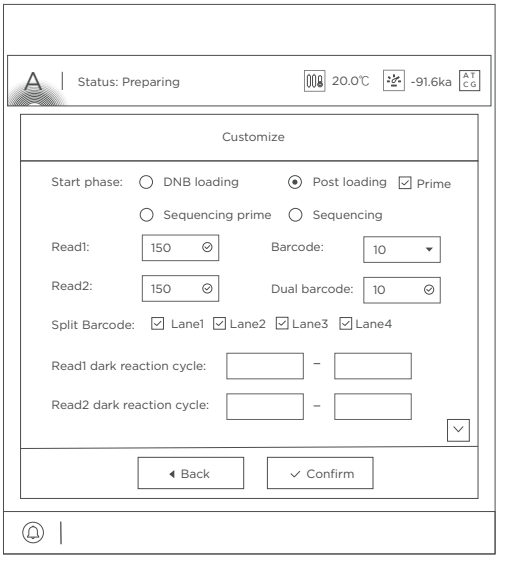

Figure 116 Configuring customize settings for example 3

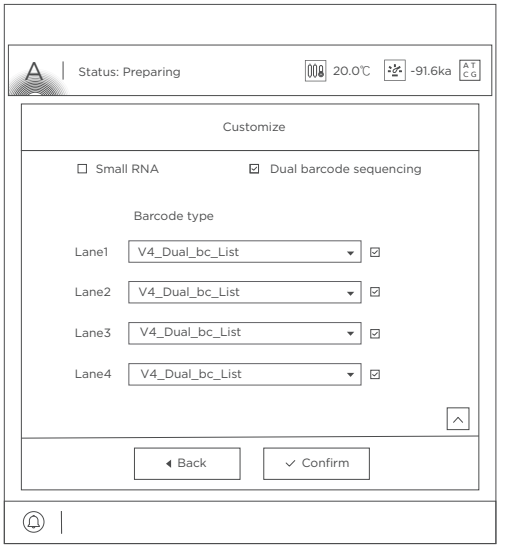

Figure 117 Selecting barcode type and split strategy for example 3

These parameters can be set in both side-A and side-B. it is recommended to 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 below:

- 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+150+8+8+2 = 318
- Select a PE150 kit

The Customize interface is set as follows:

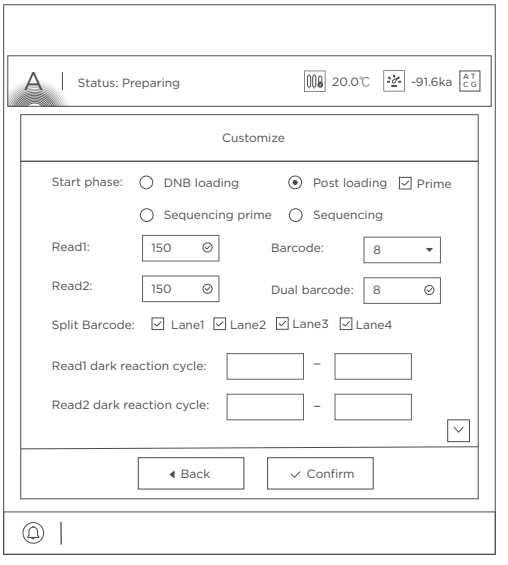

Figure 118 Configuring customize settings for example 4

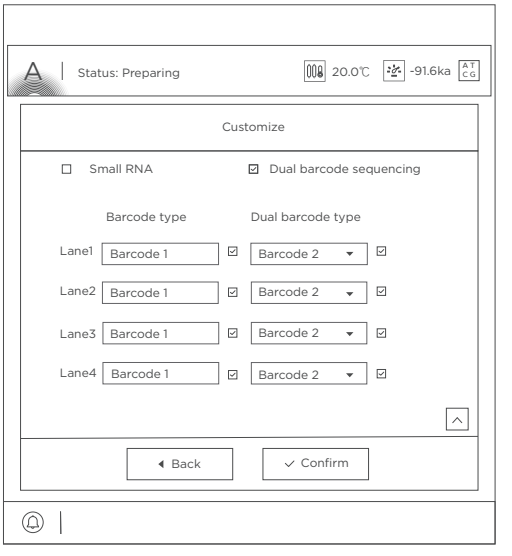

Figure 119 Selecting barcode type and split strategy for example 4

These parameters can be set in both side-A and side-B. it is recommended to 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 below:

- 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+150+8+8+2 = 318
- Select a PE150 kit

The Customize interface is set as follows:

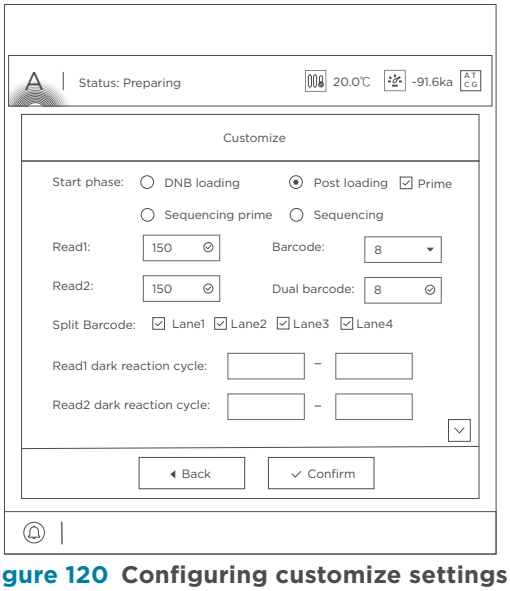

Figure 120 Configuring customize settings for example 5

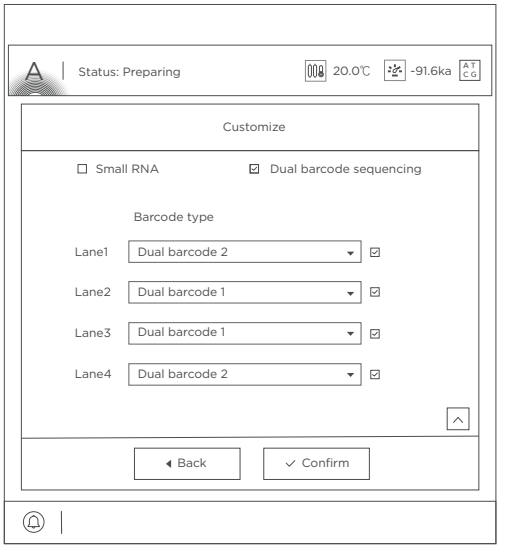

Figure 121 Selecting barcode type and split strategy for example 5

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

Assumptions are as below:

- 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+150+6+10+2 = 318
- Select a PE150 kit

The Customize interface is set as follows:

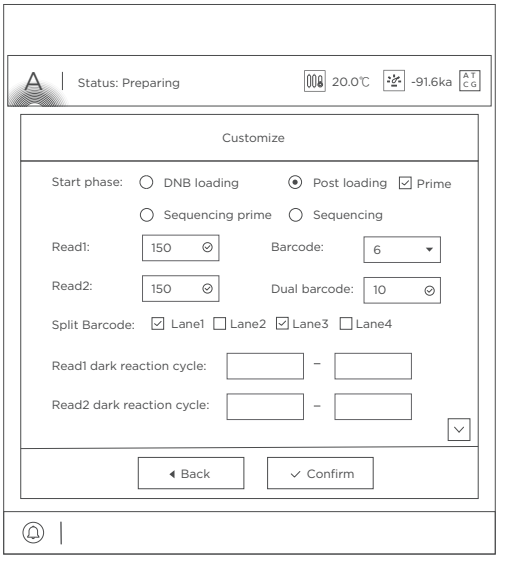

Figure 122 Configuring customize settings for example 6

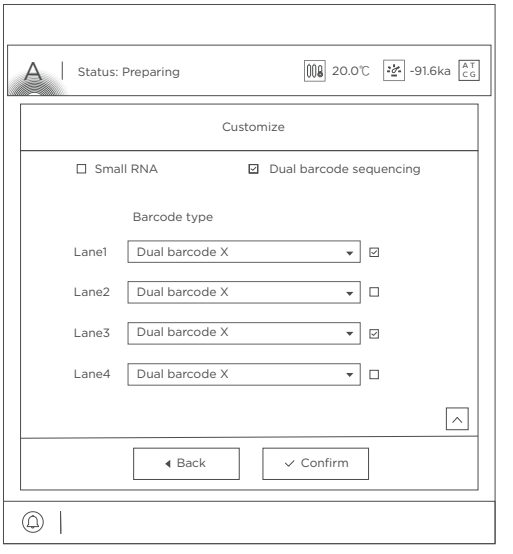

Figure 123 Selecting barcode type and split strategy for example 6

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

Assumptions are as below:

- 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+150+8+8 +2= 318
- Select a PE150 kit

The Customize interface is set as follows:

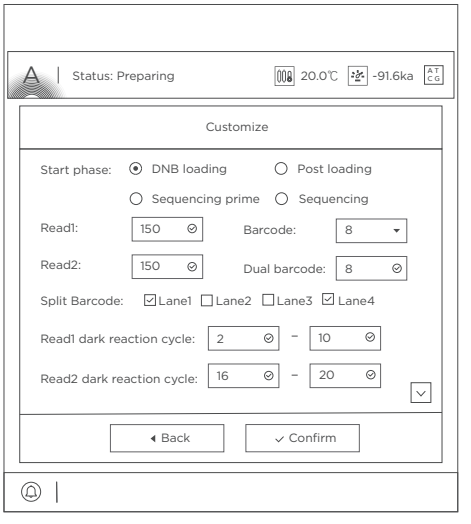

Figure 124 Configuring customize settings for example 7

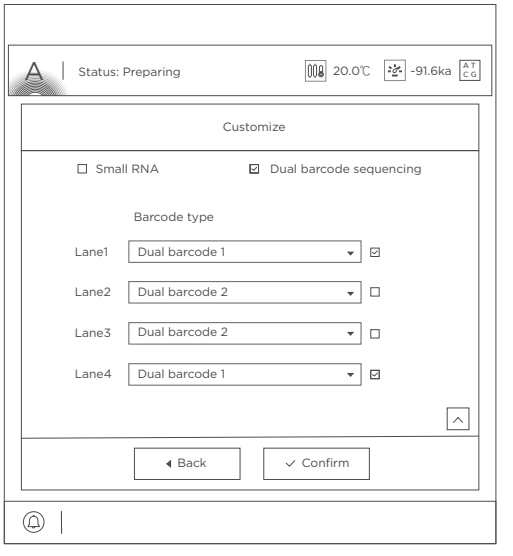

Figure 125 Selecting barcode type and split strategy for example 7

8. stLFR FCL PE100

Assumptions are as below:

- Sequencing run: stLFR FCL PE100
- DNB loading: sequencer
- Length of Read1: 100
- Length of Read2: 100
- Length of Barcode: 42
- Length of Dual barcode: 10
- Split barcode: Yes
- Split dual barcode: Yes
- Total cycles = 100+100+42+10 +2= 254
- Select a stLFR FCL PE100 kit

The Customize interface is set as follows:

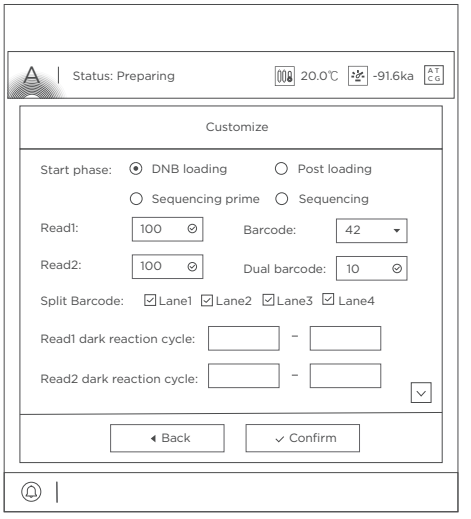

Figure 126 Configuring customize settings for example 8

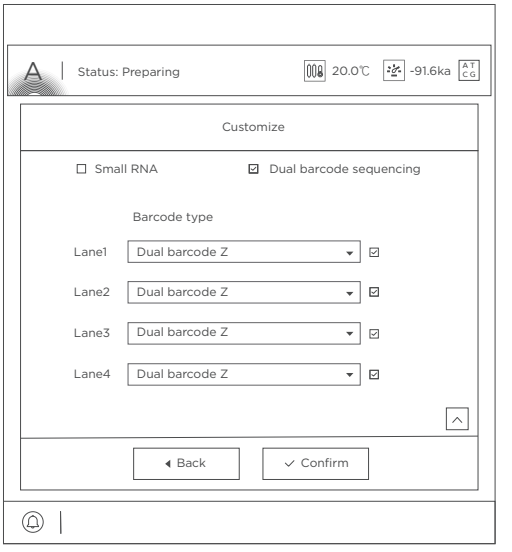

Figure 127 Selecting barcode type and split strategy for example 8

9. Small RNA

Assumptions are as below:

- Sequencing run: SE50+8
- DNB loading: sequencer
- Length of Barcode: 8
- Length of Dual barcode: 0
- Split barcode: Yes
- Split dual barcode: Yes
- Total cycles = 50+8+1 =59
- Select Small RNA SE50 kit

The Customize interface is set as follows:

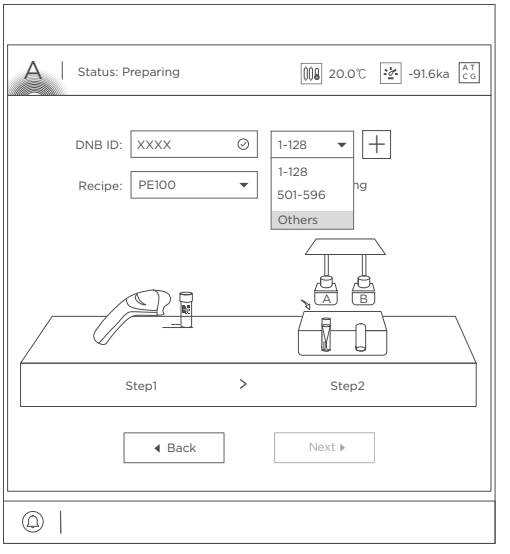

Figure 128 Selecting Others

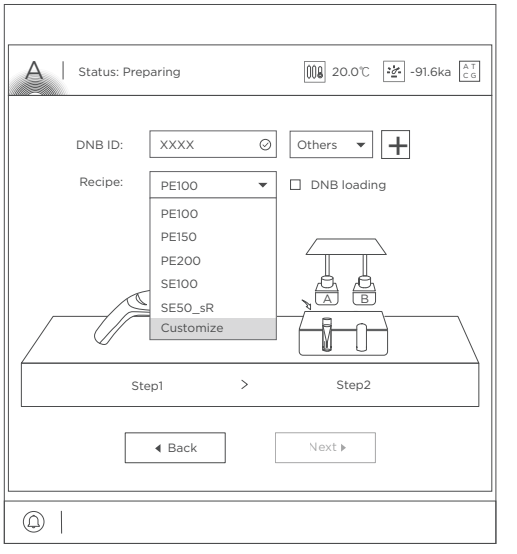

Figure 129 Selecting Customize

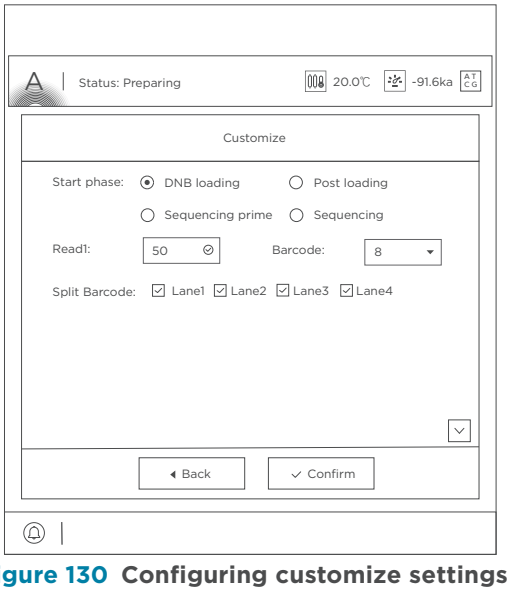

Figure 130 Configuring customize settings for example 9

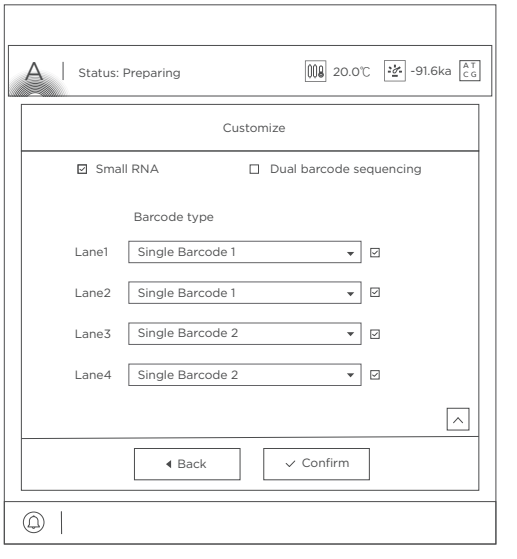

Figure 131 Selecting barcode type and split strategy for example 9

10. UMI+UDI

Assumptions are as below:

- 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+100+8+17+2 = 227
- Select a PE150 kit (a PE100 kit will not have enough reagents for 227 reaction cycles)

The Customize interface is set as follows:

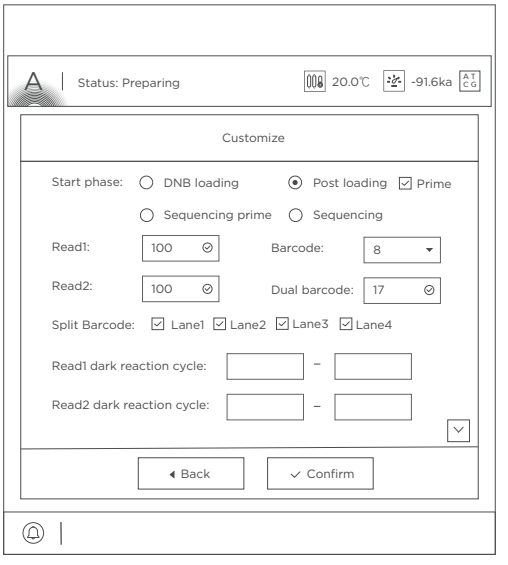

Figure 132 Configuring customize settings for example 10

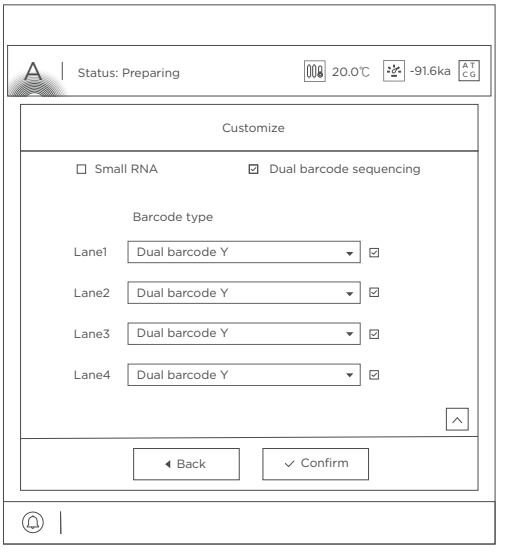

Figure 133 Selecting barcode type and split strategy for example 10

11. 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:

11a. 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 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+150+8+8 +2=318

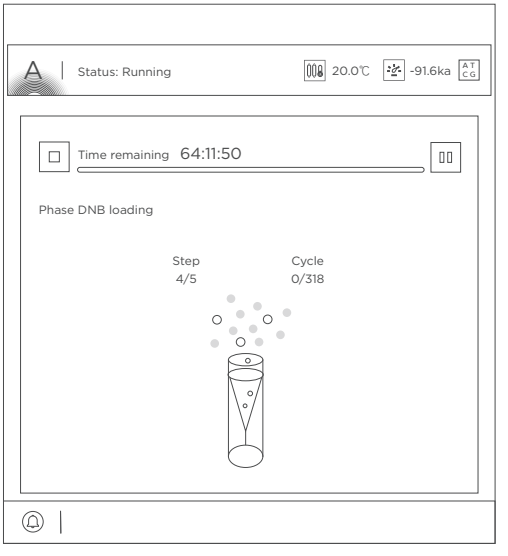

Figure 134 DNB loading interface 4/5

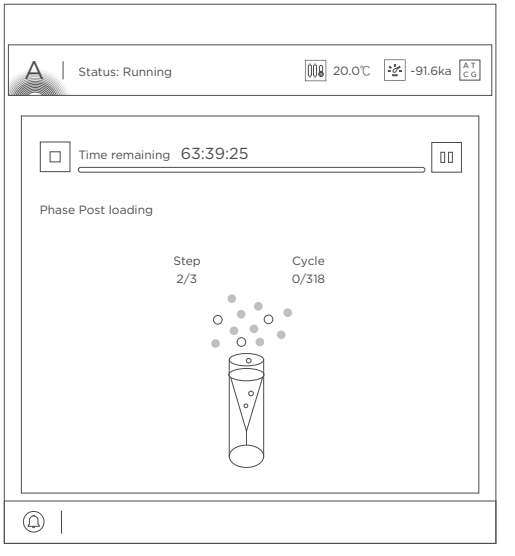

Figure 135 Post loading interface 2/3

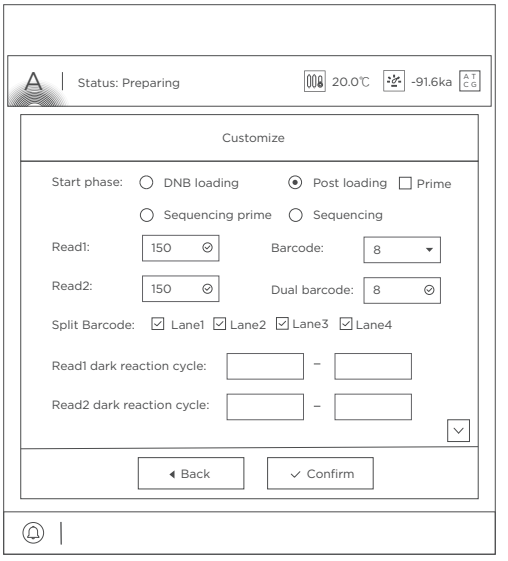

Figure 136 Configuring customize settings for example 11a

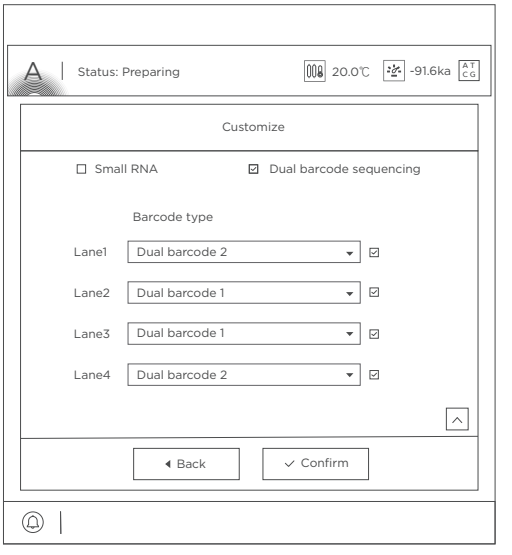

Figure 137 Selecting barcode type and split strategy for example 11a

11b. Sequencing

Assumption:

"Imaging failed" message appeared in the first cycle of sequencing.

You can restart the sequencing run after verifying the reagent cartridge preparation by using 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+150+8+8 +2= 318

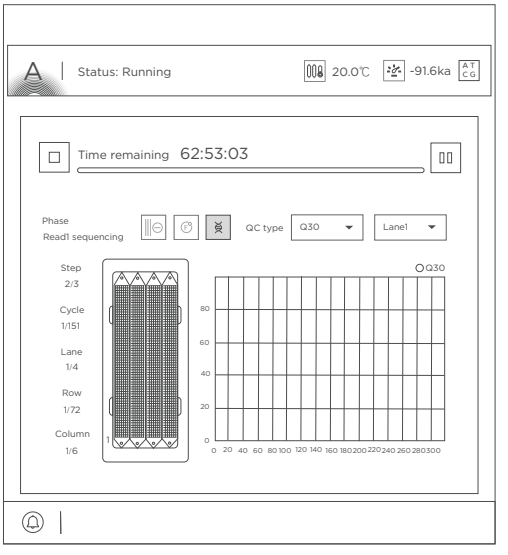

Figure 138 "2/3" Read1 sequencing phase

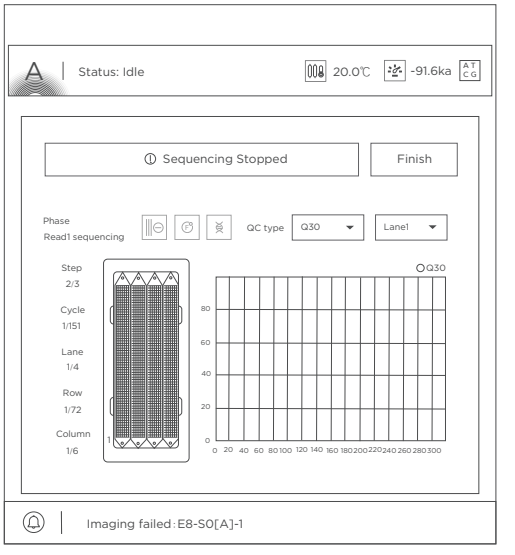

Figure 139 Imaging failed message

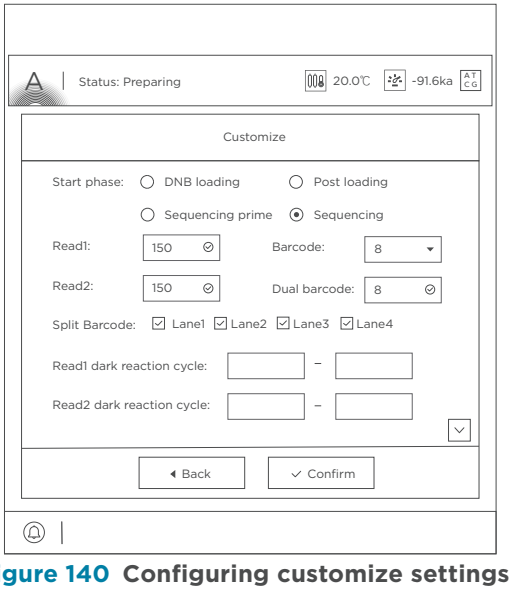

Figure 140 Configuring customize settings for example 11b

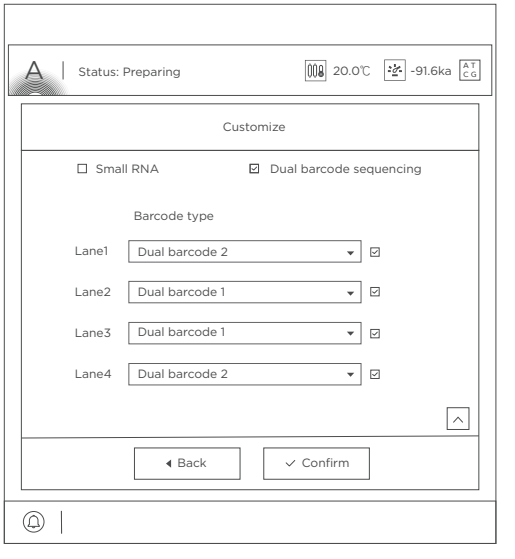

Figure 141 Selecting barcode type and split strategy for example 11b

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Instructions for using Qubit to quantify the DNBs

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

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

   - Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit assay tubes (Cat. No. Q32856) or Axygen PCR-05-C tubes (Part No. 10011-830).
   - Number of Qubit test tubes needed are the number of samples plus 2 standards tubes. For example, if you have 3 samples, you will need 5 tubes.
5. Label the tube lids. Do not label the side of tube.

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

/	S1 (μL)	S2 (μL)	D1 (μL)	D2 (μL)	D3 (μL)
Working solution	190	190	198	198	198
S1 (0 ng/μL)	10	/	/	/	/
S2 (20 ng/μL)	/	10	/	/	/
Sample (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 seconds. Incubate at room temperature for 2 minutes.
8. Refer to the Qubit user manual for instructions on reading standards and samples. Follow the appropriate procedure for your instrument.

Instructions for splitting barcode

Manual barcode splitting

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

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

C:\BasecallLite\Config

This section uses following conditions as examples:

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

Preconditions:

- SubmitImages = false
- CalFilePath is set correctly:

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

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

Table 52 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 53 Splitting BC2 only

Parameter setting	Description
Cycle = r100e1r100e1b10b8	Input the complete sequencing recipe
BarcodeFile =	Input Barcodelist of 10 bp for splitting BC2 only

Parameter setting	Description
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 54 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 through the control software of sequencer, and the control software of sequencer calls the interface of write FASTQ on Basecall to split the specified barcode.

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

1. In the main interface, select **Sequence** to enter the DNB ID entry interface.
2. Select the **DNB ID** box, scan the QR code on the tube or 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 about customizing a run, refer to *Instructions for customizing a run on Page 145.*

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 made in the Customize interface. For details, refer to *Instructions for customizing a run on Page 145*.

This section uses 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 check whether the barcode is split successfully through logs. You can find 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 55 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
barcodePos: 3	The sequencing order is: 1. Insert sequencing 2. Barcode sequencing
mismatch: 1	Fault tolerance of Barcode
mismatch2: 1	Fault tolerance of DualBarcode

Expected parameter passing	Description
speciesBarcodes: 104	The number of barcode in Barcodelist

Splitting BC2 only

You can check whether the barcode is split successfully through logs. You can find 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 56 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
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 check whether the barcode is split successfully through logs. You can find 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 57 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: 8	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
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

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List of sequencing set components

A sequencing set includes a sequencing flow cell and a sequencing kit. A sequencing kit includes a sequencing reagent cartridge and reagents for sequencing.

Table 58 DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE50)
Catalog number: 940-000830-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell **Catalog number: 940-000864-00**				
DNBSEQ-G400 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE50) **Catalog number: 940-000829-00**				
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
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		0.70 mL/tube×1 tube		
dNTPs Mix II		0.60 mL/tube×1 tube		
Sequencing Enzyme Mix		1.30 mL/tube×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 59 DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE100)
Catalog number: 940-000826-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 940-000850-00				
DNBSEQ-G400 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE100) Catalog number: 940-000825-00				
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
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		1.10 mL/tube×1 tube		
dNTPs Mix II		0.90 mL/tube×1 tube		
Sequencing Enzyme Mix		2.00 mL/tube×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 60 DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE400)
Catalog number: 940-000828-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell **Catalog number: 940-000863-00**				
DNBSEQ-G400 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE400) **Catalog number: 940-000827-00**				
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
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		4.00 mL/tube×1 tube		
dNTPs Mix II		12.00 mL/tube×1 tube		
Sequencing Enzyme Mix		8.00 mL/tube×1 tube		
Wash Buffer For Sequencing		2.90 mL/tube×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 61 DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE100)
Catalog number: 940-000812-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 940-000862-00				
DNBSEQ-G400 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit(FCL PE100) Catalog number: 940-000811-00				
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
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		0.50 mL/tube×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 62 DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150)
Catalog number: 940-000810-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 940-000861-00				
DNBSEQ-G400 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit(FCL PE150) Catalog number: 940-000809-00				
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
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		0.50 mL/tube×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 63 DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE200)
Catalog number: 940-000814-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 940-000860-00				
DNBSEQ-G400 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit(FCL PE200) Catalog number: 940-000813-00				
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
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		3.80 mL/tube×1 tube		
dNTPs Mix II		2.85 mL/tube×2 tubes		
Sequencing Enzyme Mix		7.60 mL/tube×1 tube		
MDA Reagent		3.50 mL/tube×1 tube		
MDA Enzyme Mix		0.50 mL/tube×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 64 DNBSEQ-G400RS High-throughput Sequencing Set (Small RNA FCL SE50)
Catalog number: 940-000831-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 940-000864-00				
DNBSEQ-G400 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Sequencing Kit(FCL SE50) Catalog number: 940-000829-00				
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
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		0.70 mL/tube×1 tube		
dNTPs Mix II		0.60 mL/tube×1 tube		
Sequencing Enzyme Mix		1.30 mL/tube×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		
Wash Buffer For Small RNA Sequencing Kit Catalog number: 940-000832-00				
Wash Buffer For Small RNA Sequencing		1.60 mL/tube×3 tubes	2 °C to 8 °C	2 °C to 8 °C

Table 65 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS SE100)
Catalog number: 940-000824-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell **Catalog number: 940-000851-00**				
DNBSEQ-G400 FCS Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS SE100) **Catalog number: 940-000823-00**				
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
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		0.80 mL/tube×1tube		
dNTPs Mix II		1.60 mL/tube×1 tube		
Sequencing Enzyme Mix		1.60 mL/tube×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 66 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE100)
Catalog number: 940-000820-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell **Catalog number: 940-000868-00**				
DNBSEQ-G400 FCS Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE100) **Catalog number: 940-000819-00**				
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
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		1.40 mL/tube×1 tube		
dNTPs Mix II		1.40 mL/tube×2 tubes		
Sequencing Enzyme Mix		2.80 mL/tube×1 tube		
MDA Reagent		3.50 mL/tube×1 tube		
MDA Enzyme Mix		0.50 mL/tube×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 67 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE150)
Catalog number: 940-000818-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell **Catalog number: 940-000867-00**				
DNBSEQ-G400 FCS Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE150) **Catalog number: 940-000817-00**				
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
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		0.50 mL/tube×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 68 DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE300)
Catalog number: 940-000816-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Rapid Sequencing Flow Cell **Catalog number: 940-000866-00**				
DNBSEQ-G400 FCS Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE300) **Catalog number: 940-000815-00**				
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
Make DNB Buffer		100 µL/tube×1 tube		
Make DNB rapid Enzyme Mix II		160 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 IV		200 µL/tube×1 tube		
Micro Tube 0.5 mL (Empty)		1 tube		
dNTPs Mix		3.80 mL/tube×1 tube		
dNTPs Mix II		2.85 mL/tube×2 tubes		
Sequencing Enzyme Mix		7.60 mL/tube×1 tube		
MDA Reagent		3.50 mL/tube×1 tube		
MDA Enzyme Mix		0.50 mL/tube×1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

Table 69 DNBSEQ-G400RS High-throughput Sequencing Set (stLFR FCL PE100)
Catalog number: 090-000822-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing flow cell Catalog number: 940-000865-00				
DNBSEQ-G400 FCL Sequencing Flow Cell	/	1 EA	-25°C to -15°C	-80°C to -15°C
DNBSEQ-G400RS High-throughput Sequencing Kit (stLFR FCL PE100) Catalog number: 940-000821-00				
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
stLFR Make DNB Buffer		100 µL/tube×1 tube		
Make DNB Enzyme Mix III		200 µL/tube×1 tube		
Make DNB Enzyme Mix IV		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		1 tube		
dNTPs Mix		1.00 mL/tube×2 tubes		
dNTPs Mix II		1.70 mL/tube×1 tube		
Sequencing Enzyme Mix		3.70 mL/tube×1 tube		
MDA Reagent		3.50 mL/tube×1 tube		
MDA Enzyme Mix		0.50 mL 1 tube		
Sequencing Reagent Cartridge	/	1 EA		
Transparent sealing film	/	2 sheets		

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 which is one meter from the device and has the maximum sound pressure level.
- Because the temperature and humidity fluctuations influence the accuracy of the experimental 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) indoor used only • Relative humidity: 20% to 80%, non-condensing • Atmospheric pressure: 70 kPa to 106 kPa • Maximum altitude: 3000 m (9843 ft)

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/CAS 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 question, please contact Complete Genomics at +1 (888) 811-9644.

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Manufacturer information

Manufacturer	Complete Genomics, Inc.
Address	2904 Orchard Parkway, San Jose, CA 95134
Technical support	Complete Genomics, Inc.
Technical support E-mail	US-TechSupport@mgi-tech.com
Technical support telephone	+1 (888) 811-9644
Website	www.completegenomics.com

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Order information

Catalog number	Model	Name	Version	Recommended brand
940-000830-00	FCL SE50	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	CG
940-000826-00	FCL SE100	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	CG
940-000828-00	FCL SE400	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	CG
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
940-000814-00	FCL PE200	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	CG
940-000831-00	Small RNA FCL SE50	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	CG
940-000824-00	FCS SE100	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	V1.0	CG
940-000820-00	FCS PE100	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	V1.0	CG
940-000818-00	FCS PE150	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	V1.0	CG
940-000816-00	FCS PE300	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	V1.0	CG
940-000822-00	stLFR FCL PE100	DNBSEQ-G400RS High-throughput Sequencing Set	V1.0	CG
940-000870-00	/	Sequencer Cleaning Cartridge	/	CG
900-000696-00	DL-200H	Portable DNB Loader	/	CG
510-003139-00	/	G400 gasket (sterilization)	/	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
PE	Pair-end sequencing
RCR	Rolling Circle Replication
RHO	Rho (ρ), intensity of raw signals
SE	Single-end sequencing
SNR	Signal to Noise Ratio
stLFR	single-tube Long Fragment Read
UDI	Unique Dual Index
UMI	Unique Molecular Identifier
UPS	Uninterruptible Power Supply
USB	Universal Serial Bus

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