Complete GENOMICS™
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DNBSEQ-G99RS & DNBSEQ-G99ARS 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-G99RS & DNBSEQ-G99ARS) and DNBSEQ-G99RS & DNBSEQ-G99ARS High-throughput Sequencing Set. The guide version is 1.0 and the software version is V1.

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

Revision history

	Date	Version
Initial release	April 19, 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

Symbols

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

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
	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:

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
	Protective earth	Indicates the terminal of a protective earth (ground) electrode
	Warning; laser beam	Warns of a hazard from laser beam
	Warning; crushing of hands	Taking care to avoid injury to hands when in the vicinity of equipment with closing mechanical parts
	"ON" (power)	Indicates connection to the mains power supply
	"OFF" (power)	Indicates disconnection from the main power supply
T10AH250V	Fuse specification	Indicates the fuse specification
USB 2.0 **USB 3.0**	USB port	Connects USB devices to the device
WLAN	Network port	Connects the device to the network
COM	COM port	Indicates the cluster communication port
HDMI	HDMI port	Debugs the device

Labels

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

Symbol	Name	Description
FOR RESEARCH USE ONLY Not for use in diagnostic procedures	/	Indicates a device that is for research use only, and cannot be used for clinical diagnosis
	Manufacturer	Indicates the name and address of the device manufacturer
	Date of manufacture	Indicates the date when the device was manufactured
SN	Serial number	Indicates the manufacturer's serial number so that a specific device can be identified
	Consult instructions for use	Indicates the need for the user to consult the instructions for use

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 cause in personal injury.
- A laser is installed in the device. Laser radiation may 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 can 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 inspected and validated before delivery. If serious deviation occurs during use, contact CG Technical Support for troubleshooting and 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 experimental results, electrical leakage, or even electrical 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, electrical 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 (uninterruptible power supply) (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.

FCC statement

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions:

1. This device may not cause harmful interference, and
2. This device must accept any interference received, including interference that may cause undesired operation.

Any changes or modifications not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

This equipment should be installed and operated with a minimum distance of 25 mm between the radiator and your body.

This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.

- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

IC statement

This device complies with Industry Canada's licence-exempt RSSs. Operation is subject to the following two conditions:

1. This device may not cause interference; and
2. This device must accept any interference, including interference that may cause undesired operation of the device.

The distance between user and products should be no less than 20 cm.

Le présent appareil est conforme aux CNR d'Industrie Canada applicables aux appareils radio exempts de licence. L'exploitation est autorisée aux deux conditions suivantes:

1. l'appareil ne doit pas produire de brouillage, et
2. l'utilisateur de l'appareil doit accepter tout brouillage radioélectrique subi, même si le brouillage est susceptible d'en compromettre le fonctionnement.

La distance entre l'utilisateur et de produits ne devrait pas être inférieure à 20 cm.

Industry Canada ICES-003 Compliance:CAN ICES-3(B)/NMB-3(B)

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 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.

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02

Device overview

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

Intended use

WARNING **This device is intended only for scientific research 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 through the use of instrument specific reagents and flow cells imaging hardware, and data analysis software. The device is intended for decoding the sequence of DNAs or RNAs prepared into specific sequencing libraries such as DNA nanoball libraries. It can be used for whole genome sequencing and de novo sequencing.

Working principle

The device adopts the advanced DNA Nanoball (DNB) and the core technology of combinatorial probe-anchor synthesis (cPAS) and uses a regular arrayed flow cell with the special decorated surface. Each decorated site of the flow cell contains a single DNB, and the decorated site is evenly arranged on the flow cell, ensuring that the optical signals of different Nanoballs cannot be interrupted by one another. Therefore, the accuracy of signal process is improved.

The following figure demonstrates how to make DNBs:

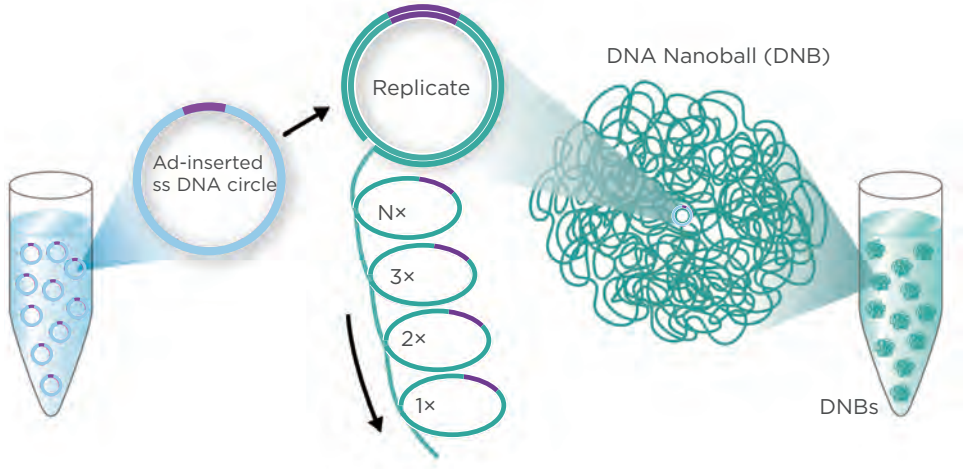

Figure 1 **Making DNBs**

The following figure demonstrates how to load DNBs:

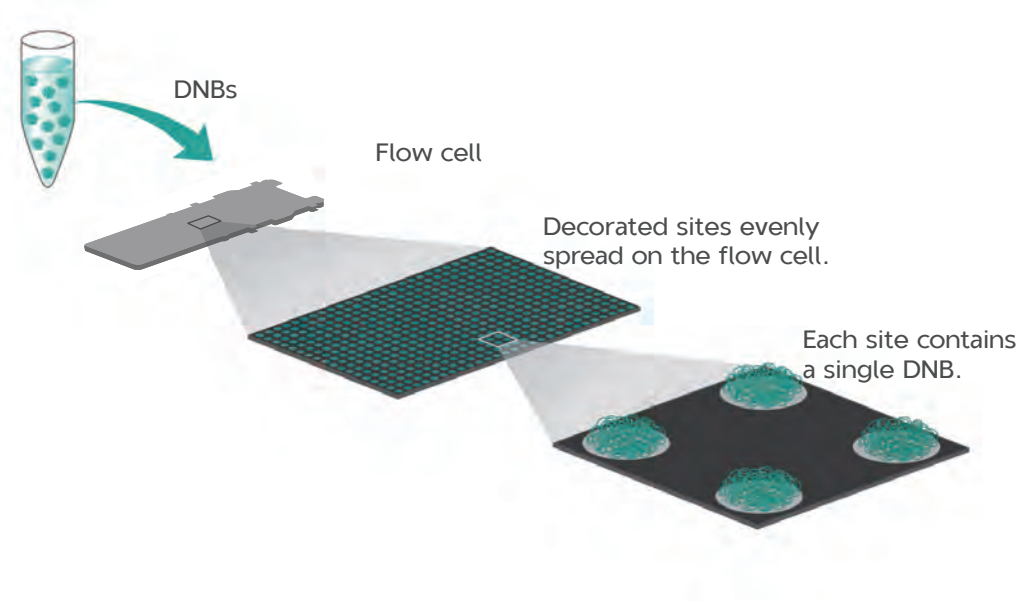

Figure 2 Loading DNBs

The DNBs and sequencing reagents are pumped into the sequencing flow cell through the liquid delivery system. Each DNB combines 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 acquire the nucleotide sequence of the DNB.

Sequencer overview

Structural composition

The sequencer consists of the main unit and pre-installed control software. The main unit includes the main structure, host, optical system, XYZ-stage, flow cell stage, gas-liquid system, electric control system, reagent compartment, power supply system, display system, and bioinformatic analysis server.

The bioinformatic analysis server and its relevant functions are intended only for DNBSEQ-G99ARS.

The following table describes the function of each component:

Component	Description
Main structure	Provides the stable support for the device.
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.
Flow cell stage	Connects the flow cell to the 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

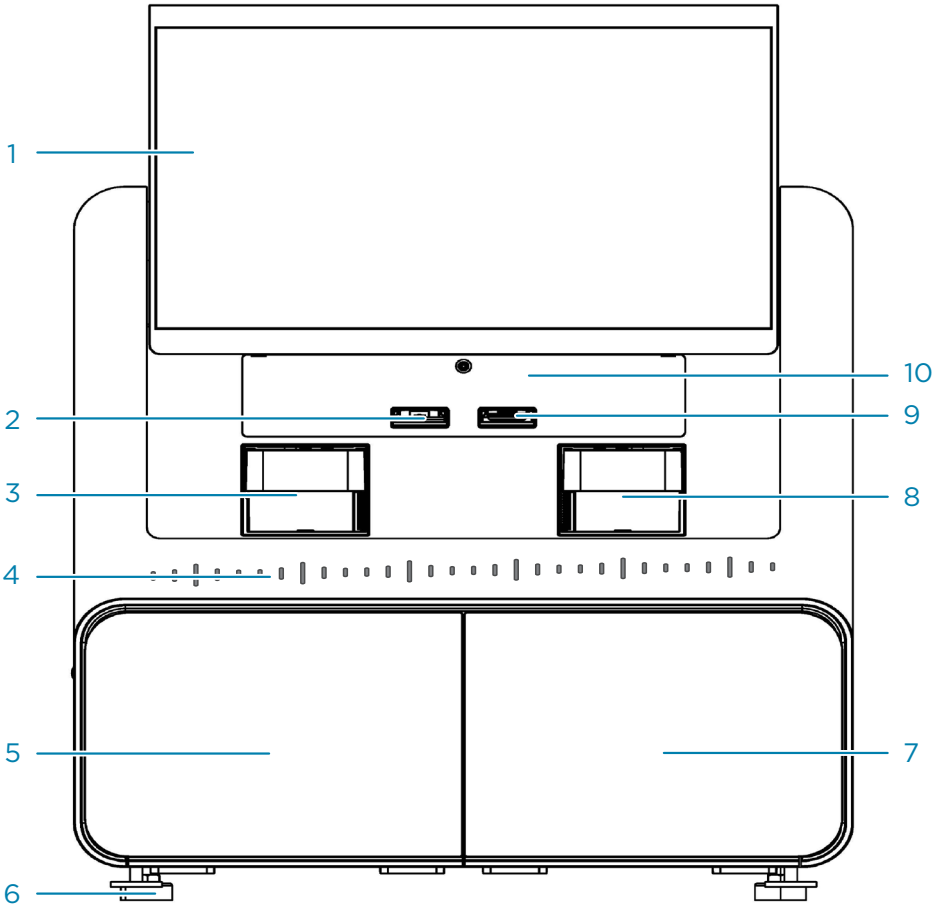

Figure 3 Front view

No.	Name	Description
1	Touch screen monitor	Facilitates on-screen operation and displays information. The monitor slides up and down at the touch of on-screen controls. When the monitor is open, the flow cell compartment door, the flow cell stage, and the reagent compartment are accessible.
2	Flow cell stage A	Holds and moves Flow Cell A and controls the temperature of Flow Cell A.

No.	Name	Description
3	Reagent compartment A	Holds the reagent cartridge.
4	Status indicator	Displays the current status of the device: • Green: the device is running. • Blue: the device is in standby status. • Yellow: a warning notification appears, but the device keeps running. • Red: an error occurred.
5	Waste container compartment door A	Allows you to remove the waste container after the system automatically opens compartment door A.
6	Supporting feet	Supports the device to ensure stability.
7	Waste container compartment door B	Allows you to remove the waste container after the system automatically opens compartment door B.
8	Reagent compartment B	Holds the reagent cartridge.
9	Flow cell stage B	Holds and moves Flow Cell B and controls the temperature of Flow Cell B.
10	Flow cell compartment door	Allows you to maintain the flow cell stage. To open the door, remove the M3 screw using a hexagon wrench.

Back view

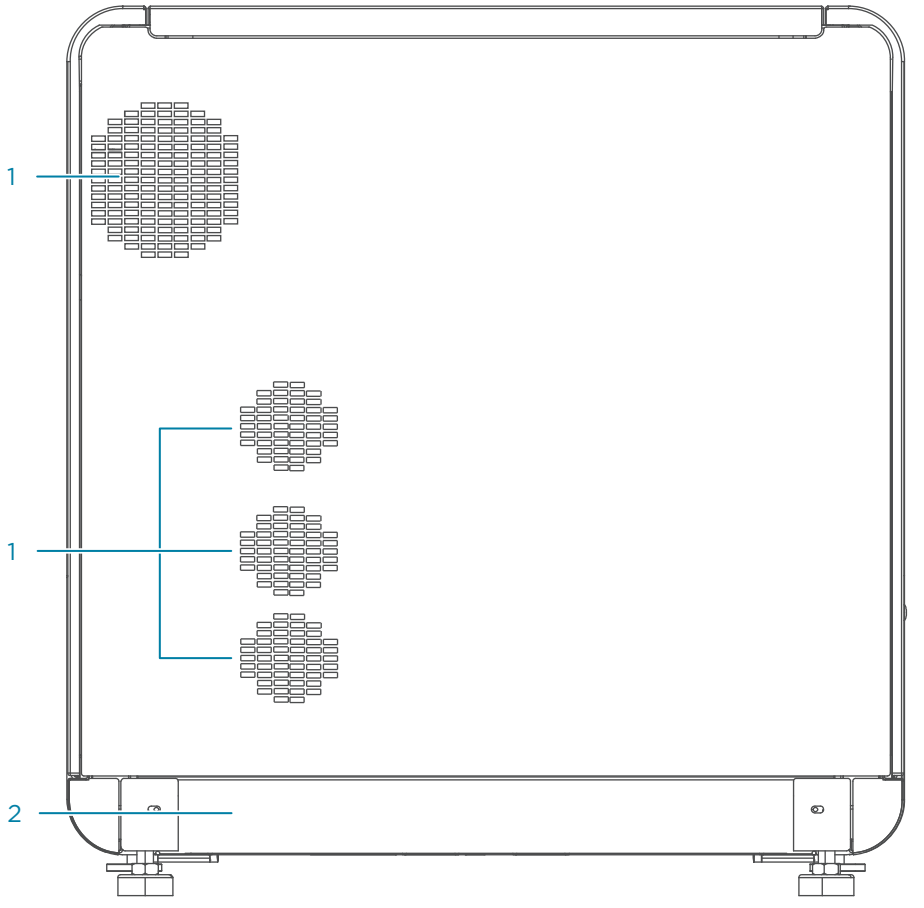

Figure 4 Back view

No.	Name	Description
1	Ventilation outlet	Ventilates the device.
2	Bioinformatic analysis module	Perform bioinformation analyzes. This component is only available on DNBSEQ-G99ARS.

Left view

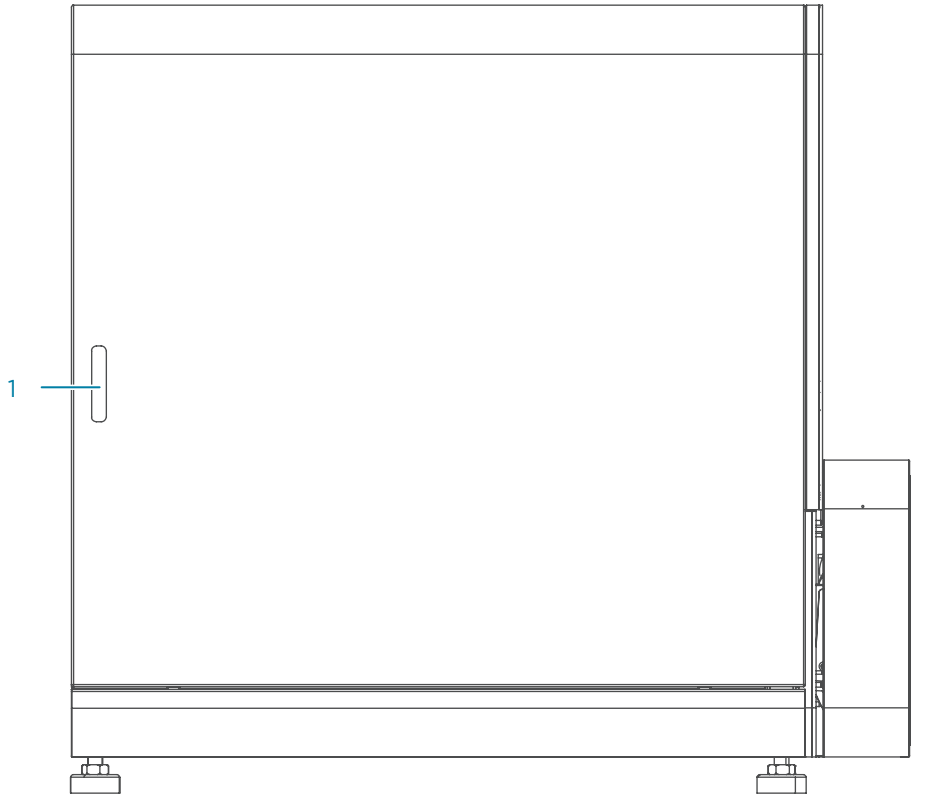

Figure 5 Left view

No.	Name	Description
1	Window	Allows you to observe the status of the fluidics system.

Right view

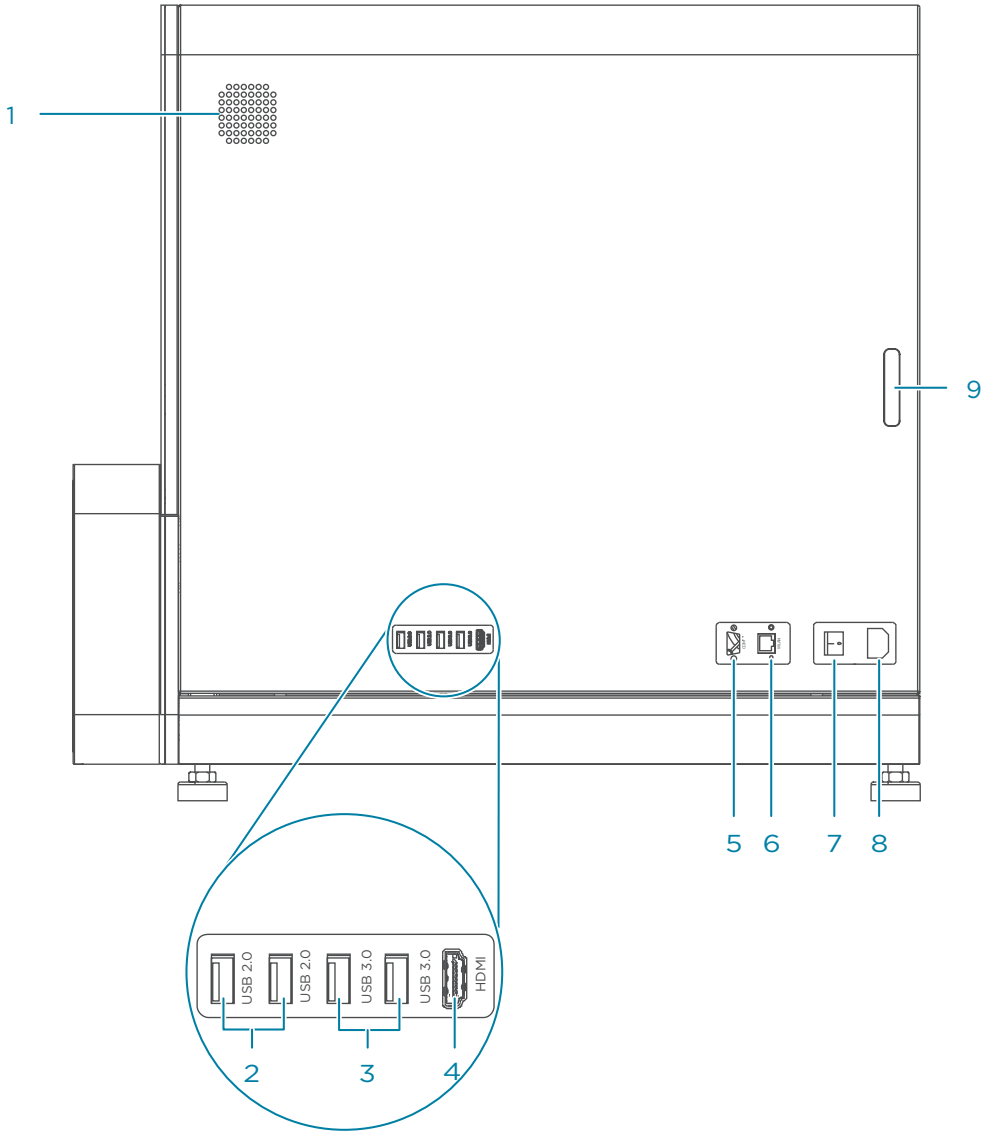

Figure 6 Right view

No.	Name	Description
1	Speaker	Provides sound.
2	USB 2.0 port	Connects USB devices to the device.
3	USB 3.0 port	
4	HDMI port	Debugs the device.
5	COM port	Connects a UPS device to the device.

No.	Name	Description
6	Network port	Connects the device to the network.
7	Power switch	Powers the device on and off. • Switch to the | position to power the device on. • Switch to the ◯ position to power the device off.
8	Power port	Connects to the power cord.
9	Window	Allows you to observe the status of the fluidics system.

Control software

Overview

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

The following table describes the function of each functional module:

Item	Description
Check	Performs a check to determine if the system components are functional.
Sequence	Performs different types of sequencing processes.
Wash	Performs wash and maintenance on the fluidics lines.

Main interface

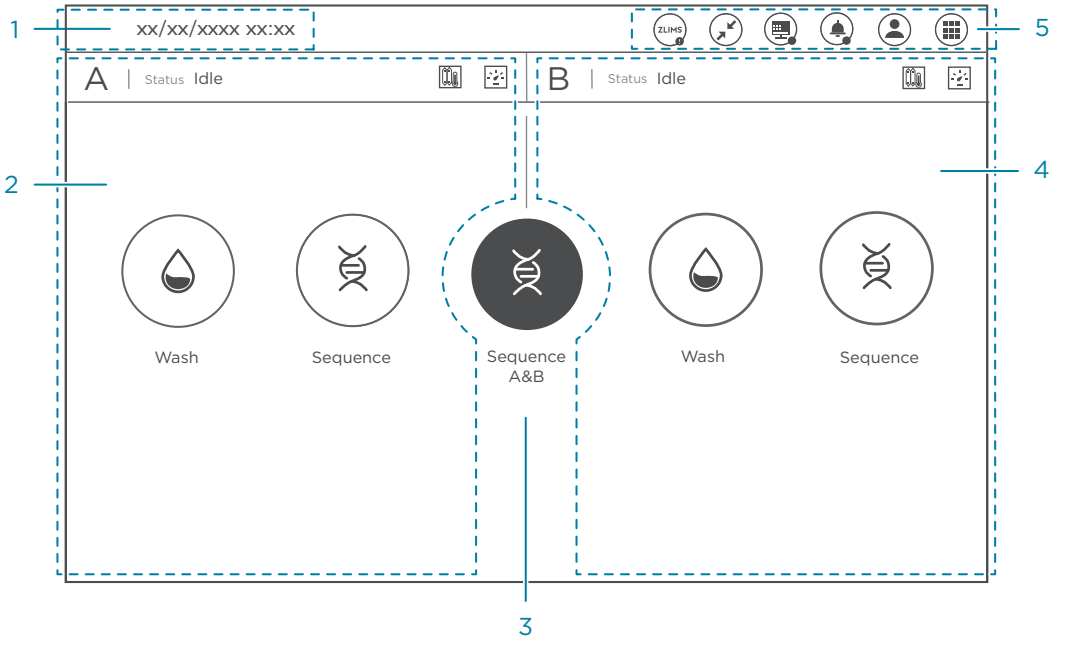

Figure 7 Main interface

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

No.	Name	Description
1	Date and time area	Displays the local date and time.
2	Flow cell A operation area	Also referred to as Operation area A. Indicates the status of Flow Cell A and provides **Wash** and **Sequence** options.
3	**Sequence A&B**	Select to simultaneously perform sequencing on the flow cell stages A and B.
4	Flow cell B operation area	Also referred to as Operation area B. Indicates the status of Flow Cell B and provides **Wash** and **Sequence** options.
5	Menu area	Select the buttons to perform relative operations.

Operation area

The icons include the following statuses:

- Green: the device is running.
- Salmon pink: the device is abnormal.

The following table describes the function of icons and buttons in the area:

Item	Description
A&B	Indicates either flow cell A operation area or flow cell B operation area.
Status	Displays the status of the selected operation area.
	Indicates the negative pressure of the flow cell stage. The real-time value is displayed to the side.
	Indicates that the temperature of the flow cell stage is normal.
	Indicates that the temperature of the flow cell stage is beyond the normal range.
Sequence	Select the sequencing recipe, and perform sequencing by following the on-screen instructions. For details, refer to *Sequencing on Page 35*.
Wash	Select to perform washing and follow the on-screen instructions for the relevant operation. For details, refer to *Device maintenance on Page 95*.

Menu area

The following table describes control functions in the menu area:

The icons change to reflect the following statuses:

- Green: the device is running.
- Salmon pink: the device is abnormal.

Item	Description
	Displays the connection status of the device and the server that ZLIMS is installed on.
	Select to minimize the control software.

Item	Description
	Sensor status indicator Select to check the status of sensors for all flow cell stages. A red dot appears on the icon when an error occurs.
	Select to view warnings, errors or other abnormal information. The prompt icon includes the following status: • No color marking: the device is running. • Yellow and flash: a warning appears. • Salmon pink and flash: the device is abnormal.
	Select to log in to the system.
	Select to view logs, change settings, perform maintenance, lock screen, shut down or restart the system, or view the system information.

The following table describes the function of the sensor status indicators in the menu area.

Item	Description
	The device runs independently, without being connected to the server that ZLIMS is installed on.
	Errors occurs in connection with the server that ZLIMS is installed on.
	The device is connected normally to the server on which the ZLIMS software is installed.
	Basecall connection status.
	Images are being uploaded to the Basecall. This icon is dynamic to reflect real-time status.
	Indicates the device temperature. The real-time value is displayed to the left.
	Indicates the device humidity. The real-time value is displayed to the left.

Log interface

Select  > **Logs** to view the logs in this interface.

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

Item	Description
Time	Select to sort the logs in ascending or descending order of time.
Close	Select to exit the log interface and return to the previous interface.
<	Select to open the previous log page.
X/X	Displays the current page and the total page of logs.
>	Select to open the next log page.

System settings interface

Select ⋮⋮⋮ and select **Settings** to change system settings in this interface.

General settings

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

Item	Description
Data upload	Select to upload the sequencing status or the data to the specified server. Changes take effect after you restart the system.
Language	Select to change the language of the software. Changes take effect after you restart the system.
Customize	Select to change the wait time before the screen locks automatically. Move the slider to change the volume of the speaker.
Close	Select to exit the settings interface and return to the main interface.

Sequencing recipe settings

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

Item	Description
Create	Select to customize a recipe.
Delete	Select to delete the selected recipes.
Creation time	Select to display the recipes according to the creation time.
Order	Select ▲ or ▼ to adjust the recipe display order.
Close	Select to exit the settings interface and return to the main interface.

Barcode settings

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

Item	Description
Template	Select to download the customized Barcode template.
Import	Select to import the Barcode files from external devices to the device.
Export	Select to export the customized Barcode files.
Delete	Select to delete the selected customized Barcode recipes.
misMatch1	Displays the Barcode mismatch number in the Barcode recipes.
misMatch2	Displays the DualBarcode mismatch number in the Barcode recipes.
Import time	Select to sort the Barcode files in ascending or descending order by import time.
Order	Select to adjust the order of the selected recipes.
Close	Select to exit the barcode settings interface and return to the main interface.

Maintenance interface

Select  and select **Maintenance** to maintain the system.

Tools

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

Item	Description
Check	Select to initialize and check the device without restarting the system.
Auto-sliding screen	Select to move up and down the screen and hold it.
Waste compartment door	Select to open the selected waste compartment door. Manually close it when you finish the operation.
Verify stage flatness	Select to verify that the flow cell stage is flat, and remove the flow cell after verifying.
Close	Select to exit the maintenance interface and return to the main interface.

Empty

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

Item	Description
Empty	Select to empty the waste liquid in A/B fluidics line into the waste container only when the waste container is in place.
Close	Select to exit the maintenance interface and return to the main interface.

File Upload

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

Item	Description
Server Type	Select to choose a server type.
Flow cell ID	Select to enter the flow cell ID.
File Type	Select to choose the file type of Fastq and/or Cal.
Upload	Select to upload the file to the specified server.
Close	Select to exit the maintenance interface and return to the main interface.

Shutdown or restart interface

You can shut down or restart the system in this interface.

To open the shutdown or restart interface, select > **Shutdown** or **Restart**.

About interface

You can view the software version, serial number, other information of the device, and the manufacturer information in this interface.

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

DNB loader overview

The Portable DNB Loader (DL-G99) is used with the sequencer. It is intended for loading the prepared DNBs into sequencing flow cells.

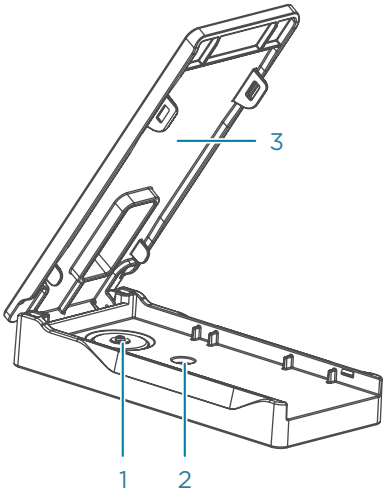

Figure 8 DL-G99 side view

No.	Name	Description
1	O-ring	Seals the reagent to prevent leakage. Remove the gasket before use.
2	Observation hole	Allows you to observe the flow cell loading.
3	Cover	Fasten the flow cell.

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03

Sequencing sets overview

This chapter describes the sequencing sets information.

Introduction

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

Available sequencing set

Table 1 Available sequencing set

Catalog number	Model	Name	Version	Data output (GB/flow cell)
940-000904-00	FCL SE100/PE50	DNBSEQ-G99RS High-throughput Sequencing Set	V1.0	8.0
940-000905-00	FCL PE150	DNBSEQ-G99RS High-throughput Sequencing Set	V1.0	24.0
940-000906-00	App-C FCL SE100	DNBSEQ-G99RS High-throughput Sequencing Set	V1.0	8.0
940-000907-00	App-C FCL PE150	DNBSEQ-G99RS High-throughput Sequencing Set	V1.0	24.0

- FCL SE100/PE50 sequencing set can perform SE100, SE50, SE35 or PE50 sequencing.
- FCL PE150 sequencing set can perform PE150 and PE100 sequencing.
- If third party library preparation kits are used for App-C sequencing, contact CG Technical Support for conversion options.

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.

Table 2 Sequencing cycle

Sequencing read length	Read1 length	Read2 length	Barcode read length	Dual barcode read length	Maximum cycles
SE100	100	--	10	10	132
PE50	50	50	10	10	132
PE150	150	150	10	10	332

- To ensure sequencing quality, when Read1 and Read2 sequencing is completed, the sequencer will automatically perform 1 more cycle for correction. For example, for PE150 dual barcode sequencing, read1 length is 150, read2 length is 150, 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 maximum cycle number of this sequencing is 322.
- Among the maximum cycles of each sequencing, the additional 10 cycles is reserved for resuming a stopped sequencing run, or for a customized run.
  - For information on resuming a stopped run, refer to *Q: What should I do if I forgot to add reagent into MDA well for PE sequencing run or I want to resume a stopped sequencing run? on Page 110*.
  - For information on examples of customized run, refer to *Examples of customized run on Page 128*.
- PE means Pair-end sequencing; SE means Single-end sequencing.

Sequencing time

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

/	SE35	SE50	SE100	PE50	PE100	PE150
Single flow cell	2.15	2.63	4.16	5.16	8.17	11.83
Dual flow cells	2.29	2.79	4.30	5.30	8.31	11.96
Data analysis (Single flow cell)	0.17	0.17	0.17	0.17	0.17	0.17
Data analysis (Dual flow cells)	0.34	0.34	0.34	0.34	0.34	0.34

- DNBSEQ-G99RS FCL Sequencing Flow Cell, also referred to as FCL, has one lane, and each lane will output 80 M raw reads.
- The sequencing time (Single flow cell/Dual flow cells) in the *Table 3 on Page 31* includes the time required from loading to sequencing completion. The data analysis time includes the time required for barcode demultiplexing (if **Split barcode** is selected) and FASTQ files output when sequencing is completed.
- The time in the table above is measured for single barcode.
- The time in the table above is the average value. The actual run time may vary with sequencers in different batches.

User-supplied equipment and consumables

Before using the device, prepare the following equipment:

Table 4 User-supplied equipment

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

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

Table 5 Recommended reagents/consumables

Reagent/Consumable	Recommended brand	Purpose
2 M NaOH	General lab supplier	Diluting to 0.1 M 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 tube, 0.2 mL	Thermo Fisher	Making DNB reaction mixture
Sterile microcentrifuge tube, 1.5 mL	VWR, catalog number 20170-038, or equivalent	For reagent mix
Canned air duster	General lab supplier	Cleaning the flow cell stage
Disposable gloves, powder-free	General lab supplier	General purpose
Kimwipes tissue	VWR	Cleaning
Low-lint cloth	General lab supplier	Cleaning
Laboratory-grade water	General lab supplier	Sequencing and cleaning

WARNING Tips are disposable consumables. Do not reuse them.

Recommended laboratory-grade water types include:

- Deionized water
- 18 Megohms (MΩ) water
- Milli-Q water
- Super-Q water
- Molecular biology-grade water

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04

Sequencing

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

Workflow

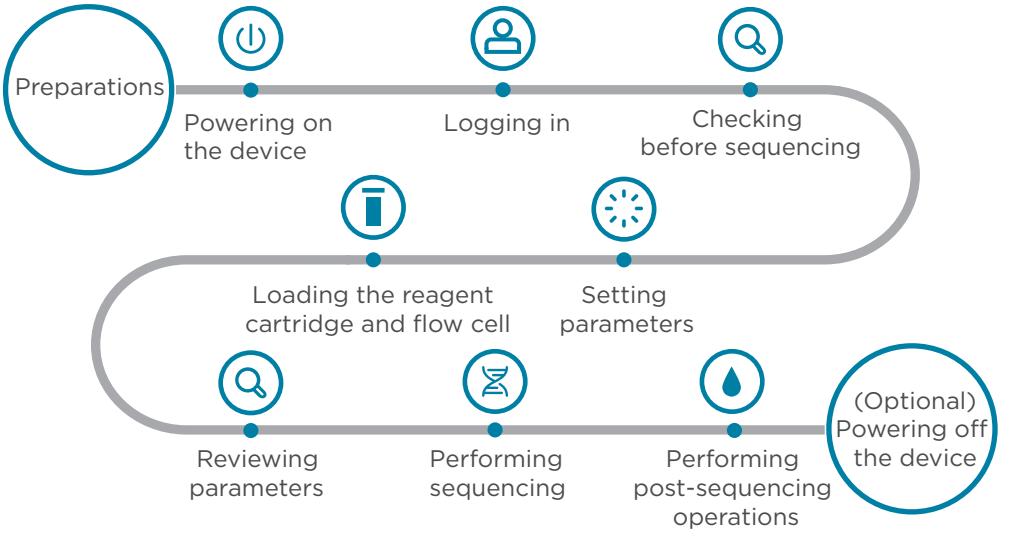


Figure 9 Sequencing workflow

- 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 then 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

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

The recommended size distribution of inserts ranges between 200 bp and 500 bp, with the main insert size fragment centered within ±100 bp. If there are any special requirements or specifications for the CG library preparation kit, then the requirements of the kit should be followed.

Table 6 Recommended library insert size

Model	Recommended library insert distribution (bp)
FCL SE100/PE50	200 to 400
FCL PE150	300 to 500
App-C FCL SE100	200 to 400
App-C FCL PE150	300 to 500

- Average data output will vary with different 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) 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 7 Circular ssDNA library concentration requirement

Library type	Library concentration
General libraries	≥2 fmol/μL
App-C PCR libraries	≥3 fmol/μL
PCR free libraries	≥3.75 fmol/μL

Making DNBs

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

DNB making protocols are listed in the sections listed below. Select the appropriate one according to the sequencing sets used.

- *Making DNBs for FCL SE100/PE50 and FCL PE150 on Page 38.*
- *Making DNBs App-C FCL SE100 and App-C FCL PE150 on Page 40.*

Making DNBs for FCL SE100/PE50 and FCL 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 reagents at room temperature.
3. Remove Make DNB Enzyme Mix I from storage and thaw the reagent for approximately 30 minutes on ice.
4. After thawing, mix all the reagents above using a vortex mixer for 5 seconds. Centrifuge briefly and place on ice until use.

Calculating the required amount of ssDNA libraries

- The required volume of ssDNA libraries is determined by the required library amount (fmol) and library concentration quantified in *DNA library concentration and amount 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 8 Required amount of ssDNA libraries

Library type	Volume (μL)
General libraries	V = 20 fmol / C
PCR free libraries	V = 37.5 fmol / C

- Calculate the required ssDNA libraries for each Make DNB reaction and fill it in *Table 9 on Page 39* as *V*.

Making DNBs

Perform the following steps:

1. Take out 0.2 mL PCR tubes. Prepare Make DNB reaction mixture 1 according to *Make DNB reaction mixture 1 on Page 39*:

Table 9 Make DNB reaction mixture 1

Component	Volume (µL)
Low TE Buffer	10 – V
Make DNB Buffer	10
ssDNA libraries	V
Total Volume	20

Keep Low TE Buffer on ice after use. It may be used for DNB dilution.

2. Mix the reaction mixture thoroughly using a vortex mixer. Centrifuge it 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 10 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. Remove the PCR tube from the thermal cycler when the temperature reaches 4 °C.
6. Centrifuge for 5 seconds, place the tube on ice, and prepare Make DNB reaction mixture 2 according to the table below:

Table 11 Make DNB reaction mixture 2

Component	Volume (µL)
Make DNB Enzyme Mix I	20
Make DNB Enzyme Mix II (LC)	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.
8. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

Table 12 RCR (Rolling circle replication) conditions

Temperature	Time
Heated lid (35 °C)	On
30 °C	20 min
4 °C	Hold

- 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 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.

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

- It is very important to mix DNBs gently using a wide-bore, non-filtered pipette tip. Do not centrifuge, vortex, or shake the tube.
- Store DNBs at 4 °C and perform sequencing within 48 hours.

Making DNBs App-C FCL SE100 and App-C FCL PE150

Preparing reagents for making DNBs

Perform the following steps:

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

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 13 Required amount of ssDNA libraries

Library type	Volume (μL)
App-C libraries	V = 30 fmol / C
PCR free libraries	V = 37.5 fmol / C

- Calculate the required ssDNA libraries for each Make DNB reaction and fill it in *Make DNB reaction mixture 1 on Page 41* as *V*.

Making DNBs

Perform the following steps:

1. Take out 0.2 mL PCR tubes. Prepare Make DNB reaction mixture 1 according to the table below:

Table 14 Make DNB reaction mixture 1

Component	Volume (μL)
Low TE Buffer	10 – V
App-C Make DNB Buffer	10
ssDNA libraries	V
Total Volume	20

Keep Low TE Buffer on ice after use. It can be used for DNB dilution.

2. Mix the reaction mixture thoroughly 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 15 Primer hybridization reaction conditions

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

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

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

5. Remove the PCR tube from the thermal cycler when the temperature 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 16 Make DNB reaction mixture 2

Component	Volume (µL)
Make DNB Enzyme Mix I	20
Make DNB Enzyme Mix II (LC)	2

7. Add all Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly using a vortex mixer. Centrifuge for 5 seconds.
8. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table *RCR conditions on Page 43*:

- When a reaction protocol is executed, 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 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 17 RCR conditions

Temperature	Time
Heated lid (35 °C)	On
30 °C	20 min
4 °C	Hold

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

   - It is very important to mix DNBs gently using a wide-bore, non-filtered pipette tip. Do not centrifuge, vortex, or shake the tube.
   - Store DNBs at 4 °C and perform sequencing within 48 hours.

Quantifying DNBs

Perform the following steps:

1. When DNB making is completed, take 2 µL of DNBs, and use the Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify the DNBs. For details, refer to the instructions for using Qubit to quantify the DNBs on *Instructions for using Qubit to quantify the DNBs on Page 133*.

Table 18 DNB concentration standard

Model	DNB concentration
FCL SE100, FCL PE150, App-C FCL SE100, App-C FCL SE150	≥10 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 110* for details.
   - If there are too many samples in a single test, it is recommended that you 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 with Low TE Buffer.

Preparing the flow cell

Perform the following steps:

1. Remove the flow cell box from the sequencing set.

   Do not open the outer plastic package at this moment.

2. Place the flow cell at room temperature for 30 minutes to 24 hours.
3. Unwrap the outer plastic package before use.

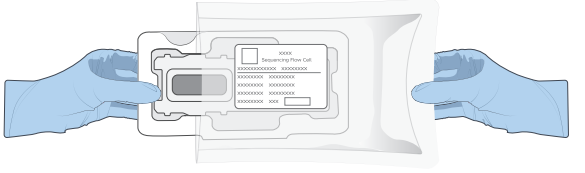


Figure 10 Unwrapping the outer plastic package

- If the flow cell is not used within 24 hours after being placed in 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 is not used immediately, store the flow cell at room temperature and use within 24 hours. If 24 hours is exceeded, it is not recommended that you use the flow cell.

4. Take the flow cell out of the inner package and inspect it to ensure that the flow cell is intact.
5. Clean the back of the flow cell by using a canned air duster.

Preparing the device

Powering the device on

WARNING

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

CAUTION

- For the ports of the computer and how to use them, refer to the computer user manual.
- It is recommended that you change the password after you log in to the computer for the first time.
- To protect the information, it is recommended that you set a long and complex password which should include the upper and lower case letters, numbers, and symbols, and that you change the password every three months.

Perform the following steps:

1. Ensure that the device is powered off.
2. Connect the device to the power supply.
3. Power the device on. After power-on, the login interface is displayed. After you power on the device, the login interface is displayed.
4. Select your user name and enter your password. The device performs a self-check.
   - If the check succeeds, the main interface is displayed.
   - If the check fails, perform the following steps:
     a. Select > **Logs** to check the result in the logs.
     b. Solve the problem according to the on-screen instructions or *Sequencer FAQs on Page 118*.
     c. Select > **Maintenance** > **Tools** > **Check** > **Initialize & Check** to initialize and check the device again.
     If the problem persists, contact CG Technical Support.

Logging in to the control software

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

Perform the following steps:

1. Select in the main interface.
2. Enter your user name and password, select **Log in**.

Preparing the Sequencing Reagent Cartridge

- Follow steps 1 through 7 to prepare the Sequencing Reagent Cartridge for SE sequencing.

- Follow steps 1 through 8 to prepare the Sequencing Reagent Cartridge for PE sequencing.
- The MDA mixture (MDA, Multiple displacement amplification) needs to be added into MDA well if you perform PE sequencing. If prepared reagent cartridges are not used immediately, refer to *Q: What rules should I follow if I need to store a reagent cartridge temporarily? on Page 115*.

Perform the following steps:

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

Table 19 Approximate thaw time for various models

Model	Method		
	Water bath at room temperature (hours)	Refrigerator at 2°C to 8°C overnight then water bath at room temperature (minutes)	Refrigerator at 2°C to 8°C (hours)
FCL SE100/PE50	2.0	30	24.0
FCL PE150	3.0	30	24.0
App-C FCL SE100	2.0	30	24.0
App-C FCL PE150	3.0	30	24.0

3. Invert the cartridge 5 times to mix before use.
4. Wipe any water condensation on the cartridge cover and wells with a Kimwipes tissue.
5. Use the Puncher to pierce the M1, M2, M3, M4 wells of the cartridge with the pre-mixed reagents.

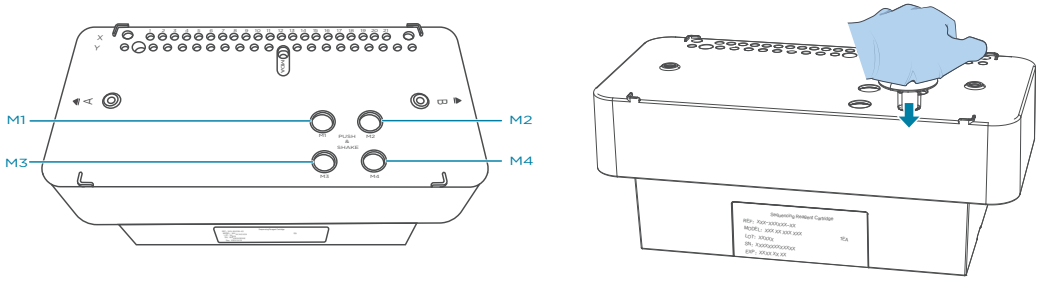


Figure 11 Piercing the M1, M2, M3, M4 wells of the Sequencing Reagent Cartridge

6. Shake the cartridge vigorously 20 times in an up-and-down motion and 20 times in a clockwise and counterclockwise direction. Ensure that reagents are fully mixed.

   Failure to mix adequately will affect the results of the experiment.

7. Pierce the seal of MDA well by using a clean 1 mL sterile pipette tip. The position of MDA well is on *Figure 12 on Page 47* .

   The FCL SE100/App-C FCL SE100 Sequencing Reagent Cartridge is now ready for use. For the next step, refer to *Performing a sequencing run on Page 48.*

8. For PE sequencing, take out MDA Reagent from storage.
9. Add 125 µL of MDA Enzyme Mix to the MDA Reagent tube with a 200 µL pipette and invert the tube 6 times to mix the reagents.

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

11. Add the mixture to MDA well.

    - Transfer the mixture carefully to prevent the mixture from spilling out of the reagent tube.
    - The FCL PE50/FCL PE150/App-C FCL PE150 Sequencing Reagent Cartridge is now ready for use. For the next step, refer to *Performing a sequencing run on Page 48.*

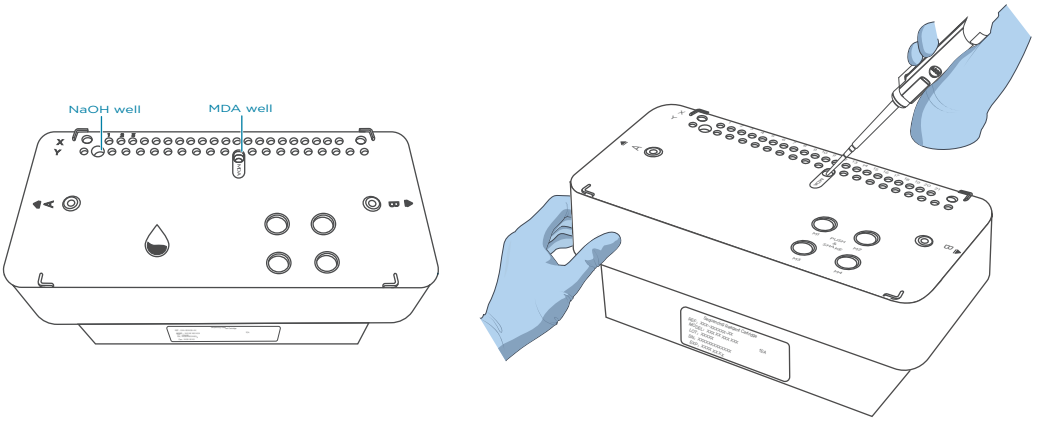


Figure 12 Adding MDA mixture

Performing a sequencing run

Checking before sequencing

Perform the following steps:

1. Select (DNA) in operation A according to your requirement. If both A and B are required, select (DNA).

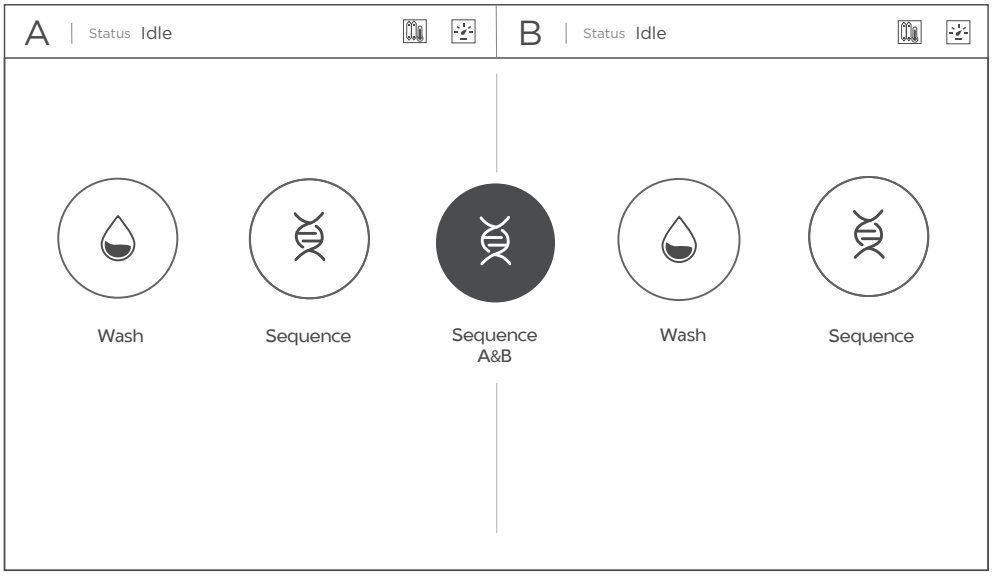


Figure 13 New sequence selection interface

2. The system automatically checks the Disk space, Sensor, Optical system and Incubation system one by one.

- Ensure that the liquid level in the waste container is lower than the upper limit line. If not, empty the waste container.
- If any part of this check fails, refer to *Troubleshooting on Page 119*.

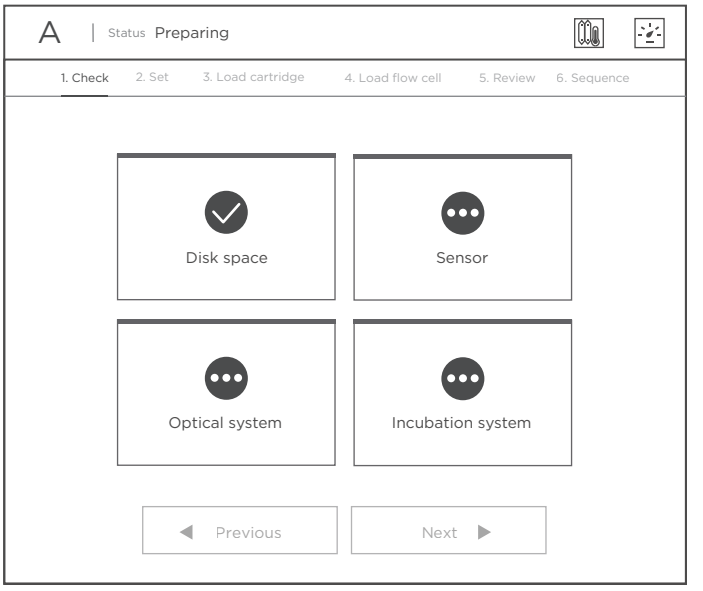


Figure 14 Check interface

3. After the check is completed, select **Next**.

Setting the sequencing parameters

Choose one of the following workflow types:

- **Sequence Only**: Testing general script.
- **Sequence & Analysis**: After general sequencing, upload data to the server for bioinformatic analysis.
- **BBS** (Bioanalysis By Sequencing): Test Barcode first, and then upload data to the specified node for bioinformatic analysis.

- The settings of **Sequence & Analysis** and **BBS** can only be performed on DNBSEQ-G99ARS.
- Ensure that the sequencing parameters are correct in this step. When the sequencing parameters are confirmed, they cannot be modified in subsequent steps.

For information on setting parameters, refer to:

- *Setting sequence only parameters on Page 50.*
- *Setting sequence & analysis parameters on Page 52.*
- *Setting BBS parameters on Page 54.*

Setting sequence only parameters

Perform the following steps:

1. Select **Sequence Only** workflow type, and **BBS** will default to No. Select the **DNB ID** box and enter the DNB ID using the on-screen keyboard.

   When naming a DNB ID, use only letters, numbers, "+", "-" and "_".

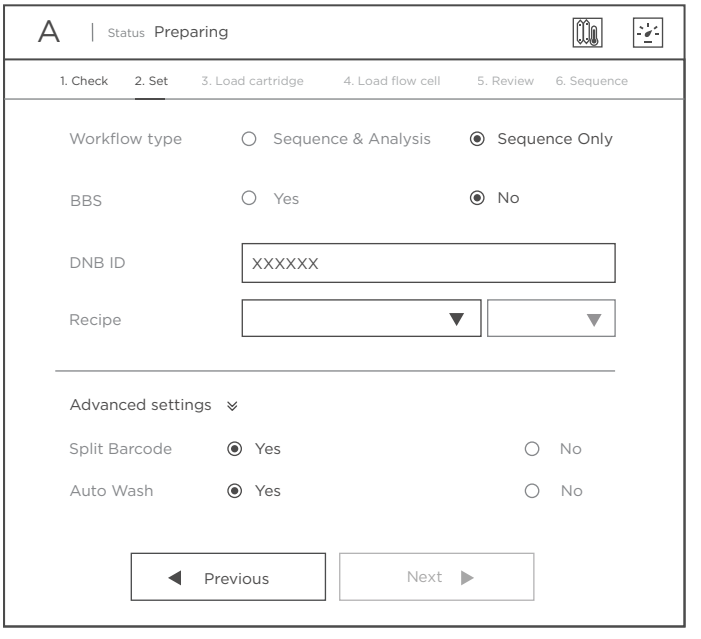


Figure 15 Selecting a workflow type

2. Select the sequencing recipe from the **Recipe** list. There are one-click sequencing runs (for example, SE100+10) and a user-customized run (Customize).

   For Dual Barcode sequencing and other recipes not in the recipe list, select **Customize** from the **Recipe** list. For information on customizing a recipe, refer to *Instructions for customizing a run on Page 125.*

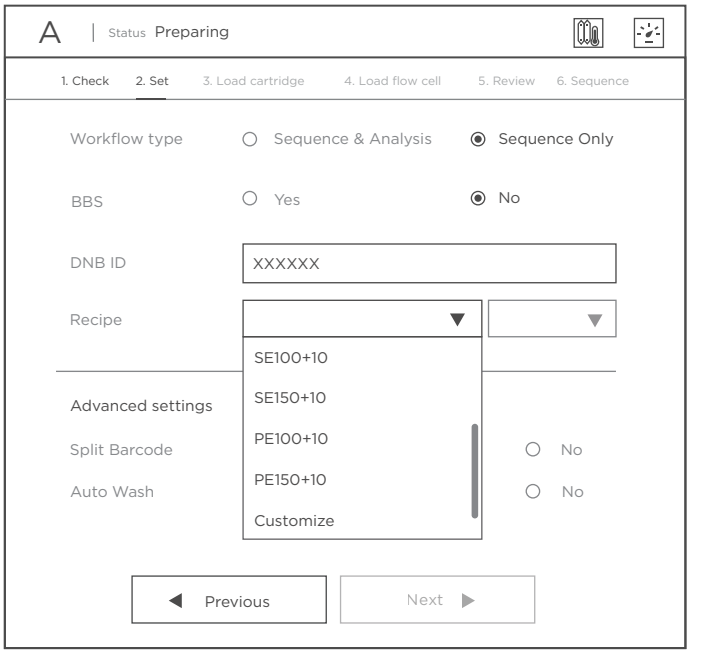


Figure 16 Selecting a sequencing recipe

3. Select a barcode range sequence from the list of barcode ranges next to the **Recipe** list.

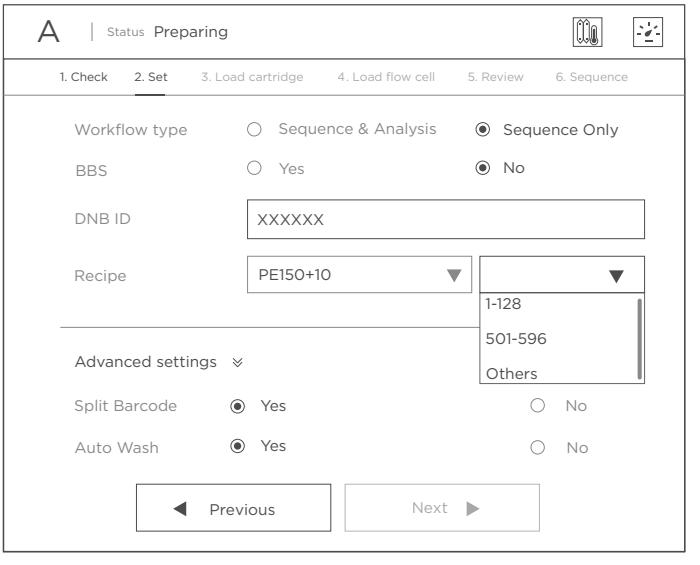


Figure 17 Selecting a barcode range

4. In Advanced settings, select either **Yes** or **No** for **Split Barcode** and **Auto Wash**, according to your needs. Yes is the default for both settings.

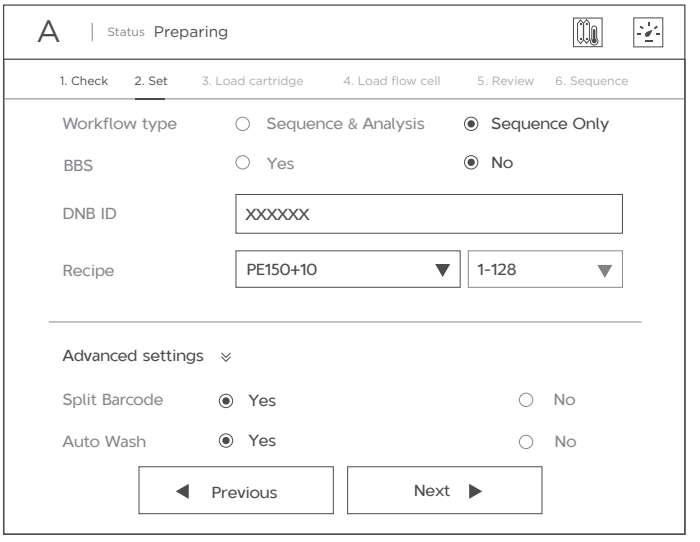


Figure 18 Advanced settings

5. Select **Next**, and for the next step, refer to *Loading the sequencing cartridge on Page 55.*

Setting sequence & analysis parameters

Perform the following steps:

1. Select **Sequence & Analysis** workflow type. Select **No** for BBS.

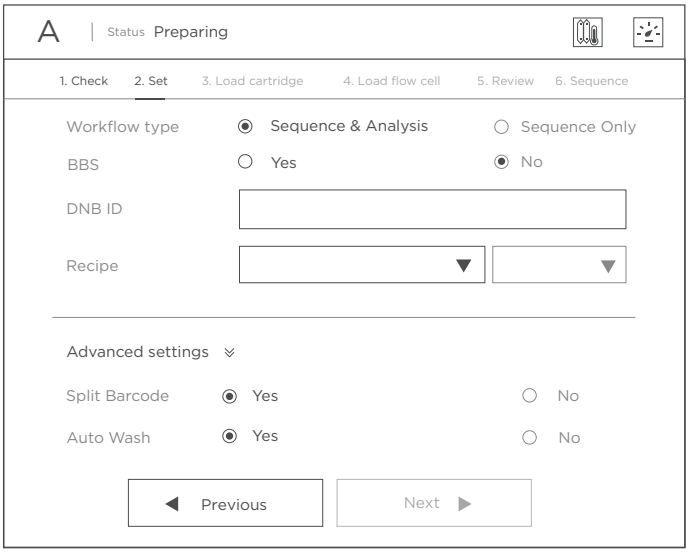


Figure 19 Sequence & Analysis workflow type

2. Select the **DNB ID** box and enter the DNB ID using the on-screen keyboard. Select the sequencing recipe from the **Recipe** list.

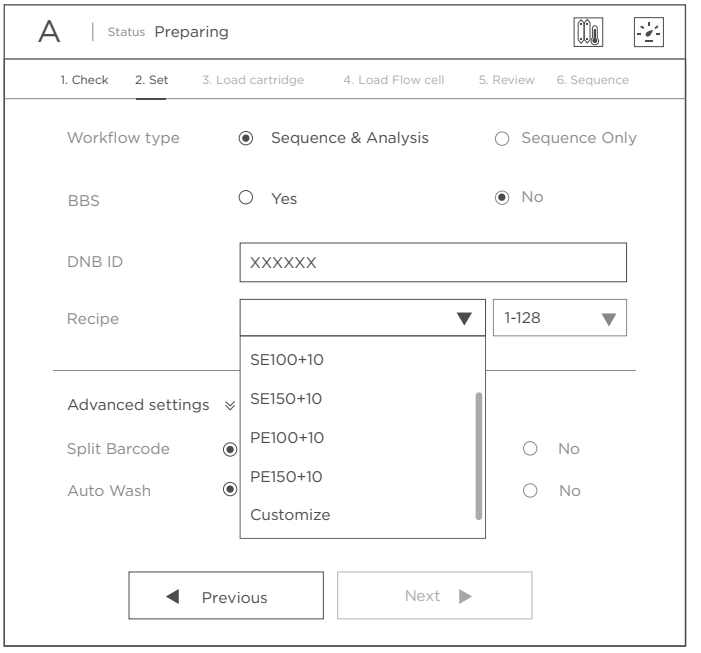


Figure 20 Entering DNB ID and selecting the sequencing recipe

For information on barcode sequence and advanced settings, refer to *Figure 17 on Page 51* and *Figure 18 on Page 52*

3. Select **Next**, and for the next step, refer to *Loading the sequencing cartridge on Page 55.*

Setting BBS parameters

Perform the following steps:

1. Select **Sequence & Analysis** workflow type. Select **Yes** for BBS and fill in the BBS box next to **Yes** for data analysis. For example, *10,110*, means that the data analysis will be performed at the 10th cycle of read1 and the 10th cycle of read2 for a BBS PE100 sequencing.

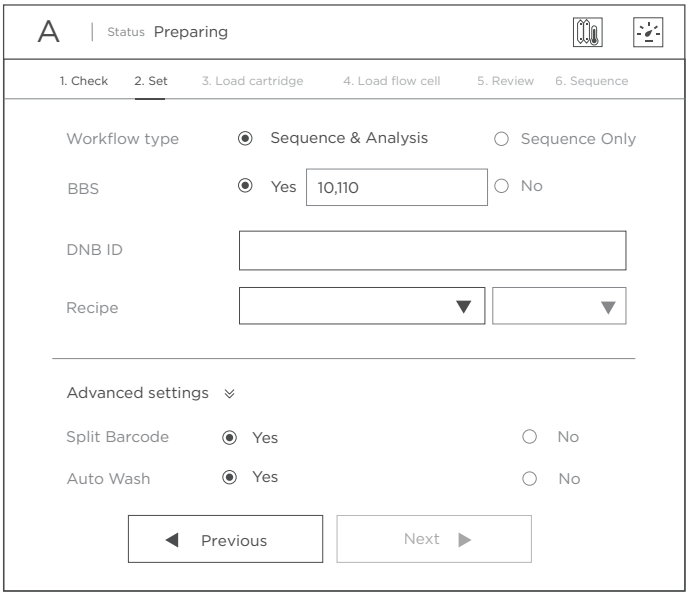


Figure 21 Selecting BBS sequencing type

2. Select the **DNB ID** box and enter the DNB ID using the on-screen keyboard. Select a sequencing recipe from the **Recipe** list.

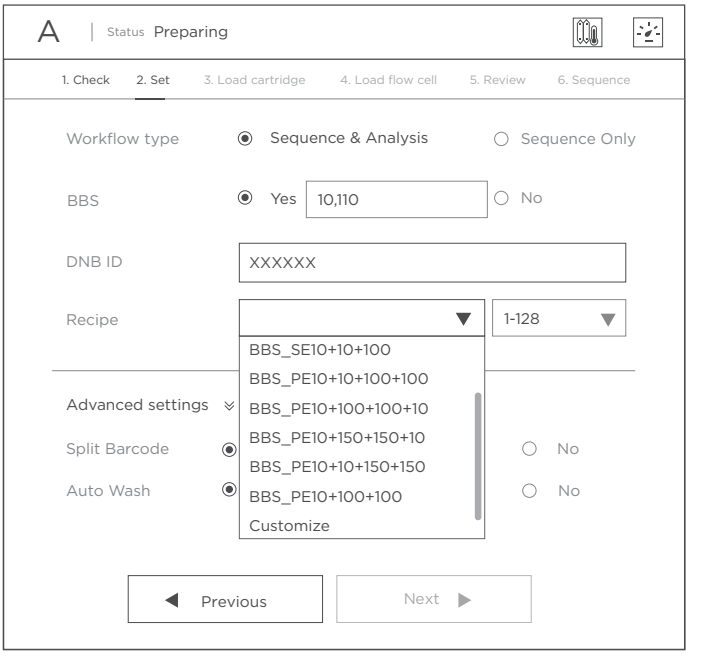


Figure 22 Entering DNB ID and selecting BBS recipe

For information on barcode range and advanced settings, refer to *Figure 17 on Page 51* and *Figure 18 on Page 52* .

3. Select **Next**, and for the next step, refer to *Loading the sequencing cartridge on Page 55*.

Loading the sequencing cartridge

Perform the following steps:

1. Slide the Sequencing Reagent Cartridge into the reagent compartment until it stops.

   If you perform App-C sequencing, select **Yes** for App-C. Otherwise, select **No**.

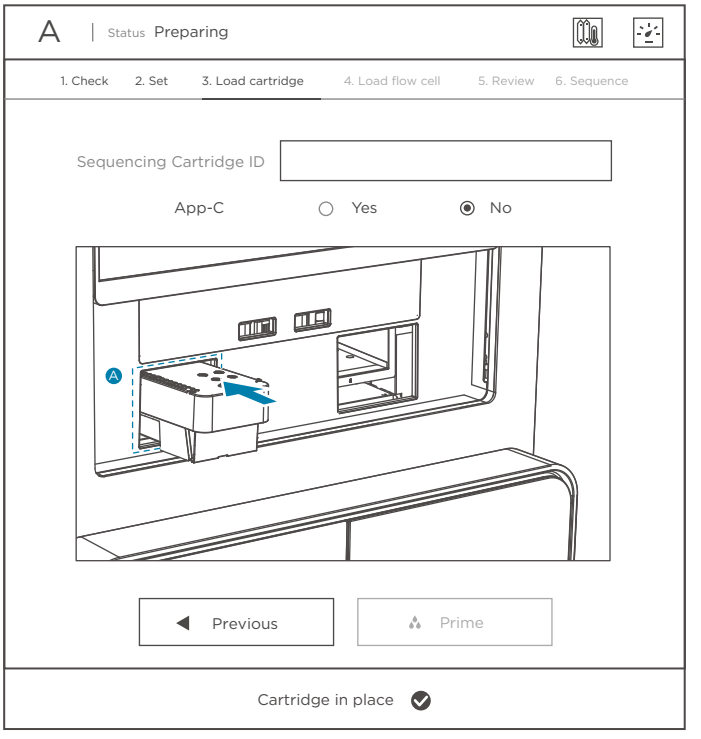


Figure 23 Loading the sequencing cartridge

The RFID (Radio Frequency Identification) scanner will automatically identify the sequencing cartridge ID.

Enter the cartridge ID manually if the RFID scanner fails to recognize the ID.

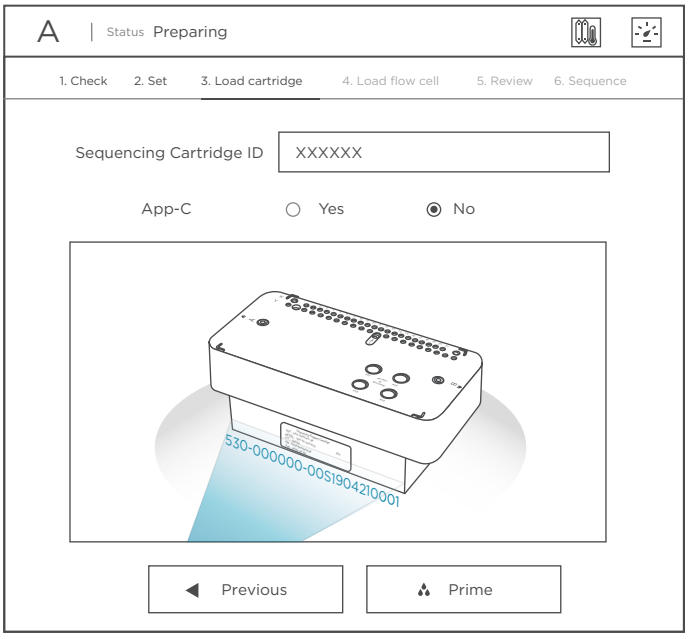


Figure 24 Scanning Sequencing Reagent Cartridge ID

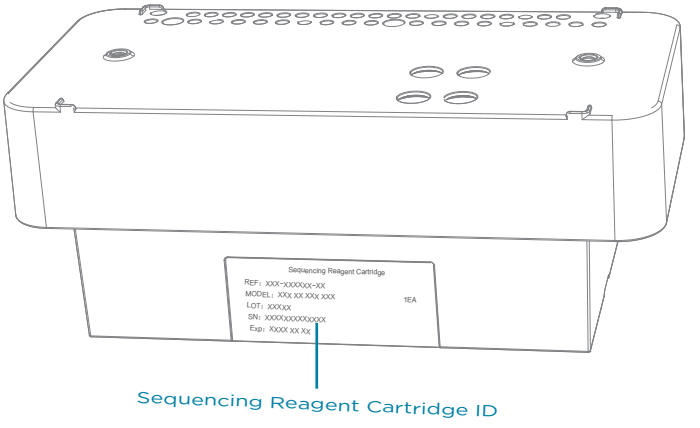


Figure 25 Location of Sequencing Reagent Cartridge ID

2. Select **Prime**.
3. Select **Yes** to start priming. The priming process takes about 2 minutes. If pumping failure occurs during priming, contact CG Technical Support.

Figure 26 **Confirming prime interface**

Loading DNBs by DL-G99

Preparing reagents

Perform the following steps:

1. Remove DNB Load Buffer II from storage and thaw the reagents on ice for approximately 30 minutes.
2. After thawing, mix the reagents using a vortex mixer for 5 seconds. Centrifuge briefly and place on ice until use.

   If crystal precipitation is found in DNB Load Buffer II, vigorously mix the reagent for 1 to 2 minutes using a vortex mixer to re-dissolve the precipitation before use.
3. Remove the Micro Tube 0.5 mL (Empty) from the sequencing set and add the following reagents:

Table 20 **DNB loading mixture**

Component	Volume (µL)
DNB Load Buffer II	7.0
Make DNB Enzyme Mix II (LC)	1.0
DNBs	21.0
Total Volume	29.0

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

   - Do not centrifuge, vortex, or shake the tube.
   - Prepare a fresh DNB loading mixture immediately before the sequencing run.
   - Each FCL requires 10 µL of DNB loading mixture.

Loading DNBs

Perform the following steps:

1. Hold the loader with one hand, and open the cover with the other hand.

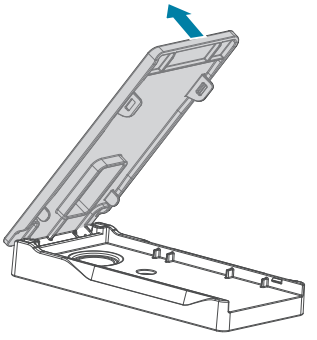


Figure 27 Opening the cover

2. Place the flow cell into the loader, and ensure that the QR code is facing up. Close the cover.

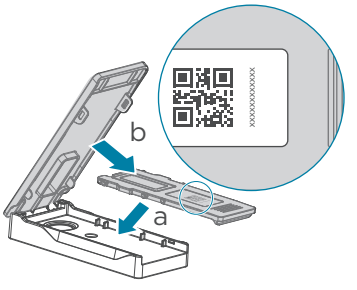


Figure 28 Placing the flow cell

3. Place the loader on the laboratory bench with the back facing up.

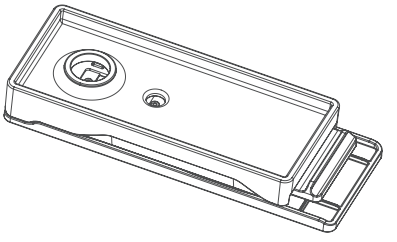


Figure 29 Placing the loader with back facing up

4. Aspirate 10 µL of DNB loading mixture using a 200 µL non-filtered sharp tip, and vertically insert the tip into inlet A as shown in the following figure:

Use a 200 µL non-filtered sharp tip but not a wide-bore tip in this loading method.

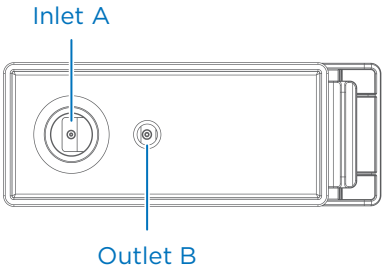


Figure 30 Inlet and outlet of loader

5. Fix the tip with one hand, press the tip ejector on the pipette to unload the tip with the other hand, and observe the liquid level in the tip:
   - If the liquid level drops automatically, the DNB loading mixture will automatically flow into the flow cell, skip step 6.
   - If the liquid level does not drop, continue to step 6.

   During DNB library loading, do not press the button of the pipette.

   WARNING Do not rotate the tip or move the flow cell during the loading process.

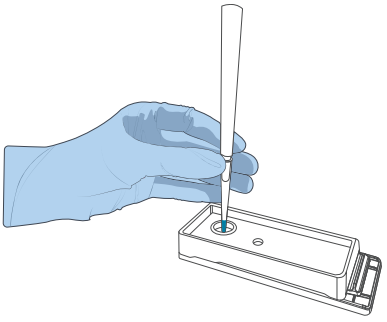


Figure 31 Loading DNBs by using DL-G99

6. (Optional) If the liquid level does not drop, perform the following steps:
   1) Leave the tip with DNB loading mixture in inlet A.
   2) Pipette a new tip and adjust the aspirate volume to 1 µL.
   3) Hold the new empty tip with one hand and gently insert it into outlet B while pressing the button down with the other hand.

4) Gently release the button and remove the tip after the liquid level of the tip at outlet A drops.

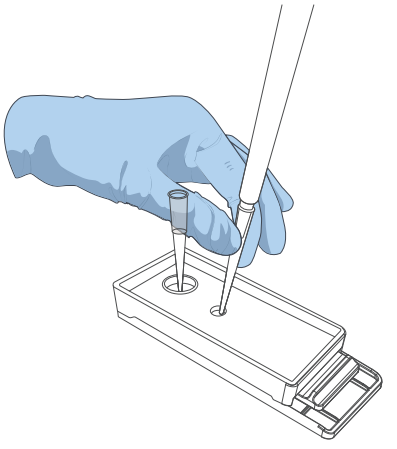


Figure 32 Loading DNBs by using DL-G99

7. After ensuring that the tip at inlet A is empty, remove the tip, and pipette 1.5 µL of DNB loading mixture into inlet A. Ensure that inlet A is full of liquid.
8. Turn the loader upside down, open the cover, remove the flow cell, and transfer it to the sequencer immediately.

Loading flow cell

Perform the following steps:

1. Insert the flow cell into the flow cell compartment after priming is finished. The RFID scanner will automatically recognize the flow cell ID.

   Enter the flow cell ID manually if the RFID scanner fails to recognize the ID.

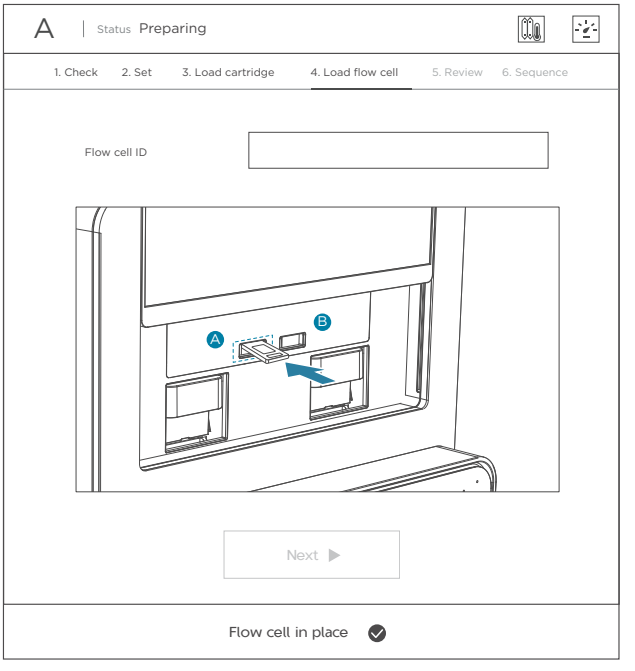


Figure 33 Loading flow cell

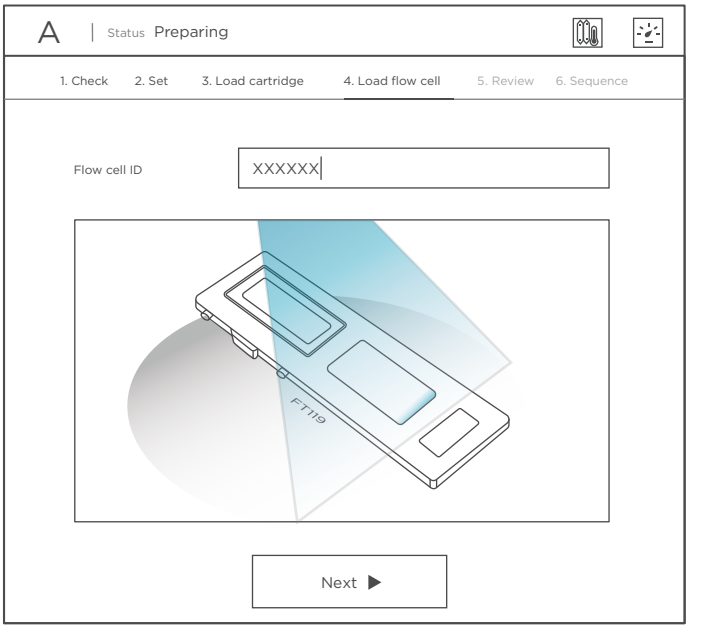


Figure 34 Scanning flow cell ID

2. Select **Next**.

CAUTION

- If the flow cell is not attached properly, use a canned air duster to blow the dust off 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 after it has been loaded. Otherwise, it may cause misalignment between the flow cell inlet and 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 PE150 sequencing, the length of read1 is 150, the length of read2 is 150, and 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 312.

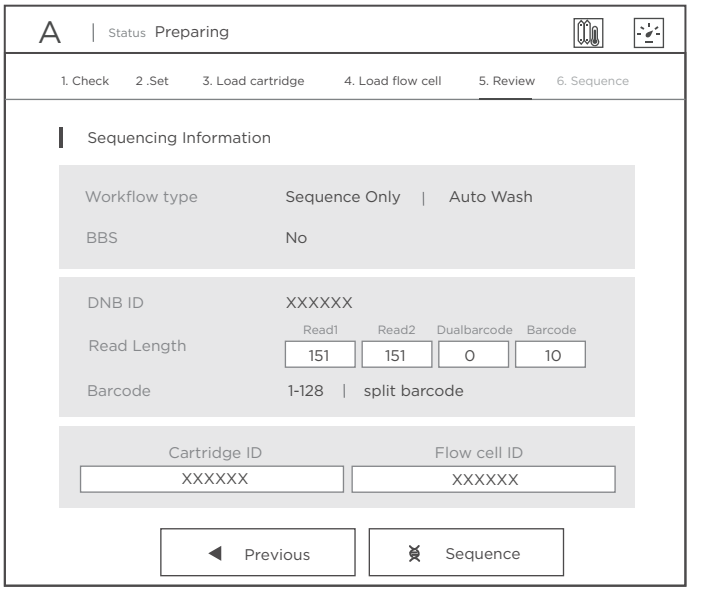


Figure 35 Reviewing information

Starting sequencing

Perform the following steps:

1. After confirming that the information is correct on *Reviewing parameters on Page 63*, select **Sequence**, and select **Yes** in the pop-up dialog box to start sequencing.

Figure 36 Confirming sequencing interface

2. When sequencing has started, the following interface will appear.

CAUTION

- Do not bump, move, vibrate, or impact the device during sequencing, as it may cause inaccurate sequencing results.
- Pay special attention to the LED status bar 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.

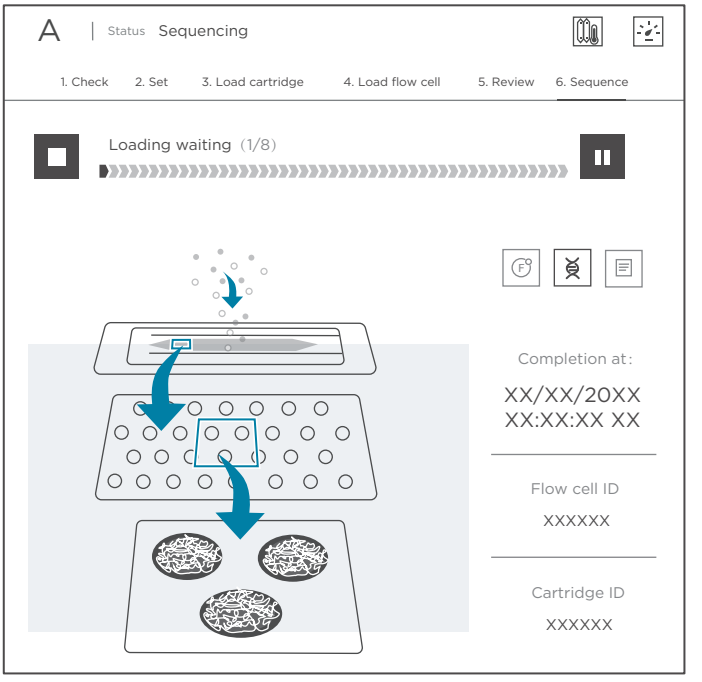


Figure 37 Sequencing interface

While real-time sequencing progress appears in the Sequencing interface, you can still operate the device if needed.

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

Table 21 Sequencing interface description

Item	Description
A&B	Indicates flow cell A operation area or B operation area.
Status	Indicates the current sequencing run phase.
	Indicates the temperature status of the flow cell stage.
	Indicates the negative pressure of the flow cell stage. The real-time value is displayed to the side.
Check	Checking phase.
Set	Setting sequencing parameters phase.
Cartridge in place	Placing the Sequencing Reagent Cartridge phase.
Flow cell in place	Placing the flow cell phase.

Item	Description
Review	Reviewing parameters phase.
Sequence	Sequencing phase.
	Shows the current phase of sequencing.
	Select to pause sequencing.
	Select to resume sequencing that has been paused.
	Select to stop sequencing, and then select Yes in the pop-up dialog box.
	After the first base is imaged, select this button to open the first base report.
	Select to open the Review interface, and you can check sequencing information.
	Select to view the sequencing results.
QC type	You can select a QC value graph from the QC type list to assess the sequencing quality.
Completion at	Shows the completion time for sequencing.
Flow cell ID	Shows the flow cell ID.
Cartridge ID	Shows the Sequencing Reagent Cartridge ID.

If **Auto wash** is selected, the system will automatically perform a post-wash after each sequencing.

(Optional) Viewing the analysis report

 CAUTION Perform the following steps only when you choose the **Sequence & Analysis** workflow type.

1. In the main interface of ZLIMS, select the number under **Report Today** to enter the Analysis Report interface.
2. Select  in the **Report** column to view the analysis report.

Performing a regular wash

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

For details, refer to *Performing a regular wash on Page 66*.

Disposing of the Sequencing Reagent Cartridge and Flow Cell

Perform the following steps:

1. Select **Finish**. The auto-sliding screen will move up and the waste compartment door will be open automatically.
2. Wash the waste container.

   CAUTION The waste container cannot be reused for more than one month, timely replace the waste container.

   1) Remove the waste container from the waste container compartment and empty the waste into an appropriate container according to local regulations and your laboratory safety standards.
   2) Add sufficient laboratory-grade water into the waste container, and gently shake the container until all inner walls are cleaned. If necessary, attach the lid back onto the waste container.
   3) Pour the waste into an appropriate waste container.
   4) Clean the surface and opening of the waste container with a 75% ethanol wipe. Ensure that no waste remains in the container.
   5) Place the waste container back into the waste container compartment and close the waste compartment door.
3. Remove the reagent cartridge and the flow cell.

   Press the flow cell down or lift it up before removing it.
4. Clean the reagent compartment.

   CAUTION Mind the reagent needles in the upper part of the reagent compartment during cleaning.

   Wipe the reagent compartment with a dust-free paper or a dust-free cloth moistened with laboratory-grade water and keep it clean and dry.
6. Dispose of the waste in accordance with local regulations and your laboratory safety standards.
7. Dispose of the flow cell and the reagent cartridge in accordance with the disposal standards of medical waste.
8. If **Auto wash** is not selected in advance, perform a maintenance wash within 24 hours.

   For details, refer to *Cleaning the fluidics lines on Page 105*.
9. (Optional) Select **Return home** to return to the login interface of the control software.

(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.

Perform the following steps:

1. Select and select **Shut down**. In the pop-up dialog box, select **Shut down**.
2. Power the device off.
3. Disconnect the power cord from the main power supply or UPS.

05

Sequencing data

This chapter describes the sequencing output data.

Sequencing output files

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

After sequencing starts, the sequencing results generated by the control software will be saved to the D drive of the computer.

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

Summary report

Report parameter overview

The following table describes parameters for Tab1 of summary report:

Table 22 Parameters for Tab1 in the summary report

Parameter	Description
SoftwareVersion	Version of BasecallLite. Ensure that the BasecallLite is in the official release version.
TemplateVersion	Version of summary report template.
Reference	The species category of the sample. When the species category is unknown or when the category is not 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).
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.

Parameter	Description
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 a 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.
Lag/Runon	• Lag1 (%) is the slope of the Lag curve for the 1st strand sequencing. • Lag2 (%) is the slope of the Lag curve for the 2nd strand sequencing. • Runon1 (%) is the slope of the runon curve for the 1st strand sequencing. • Runon2 (%) is the slope of the runon curve for the 2nd 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 follows: $\mathbf{MappingRate} = \frac{\mathbf{MappedReads}}{\mathbf{TotalReads}} \times \mathbf{100\%}$
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.
RecoverValue(AVG)	The ratio of second strand signal to first strand signal. This indicator is only for PE sequencing.

The following table describes parameters for Tab2 of summary report:

Table 23 Parameters for Tab2 in 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 Start Time	The time at which the sequencing started
Workflow Type	Type of sequencing
BBS	BBS is selected or not selected
Sequencing Cartridge ID	Serial number of the sequencing reagent cartridge
Flow Cell ID	Serial number of the flow cell
DNB ID	DNB ID that you enter
Flow Cell Pos	Position of the flow cell (stage A or stage B)
Barcode Type	The barcode file that you select during sequencing
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
Resume Cycles	Cycles of resume sequencing
Full Flow Cell ID	Full information of flow cell ID

Diagrams in summary report

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

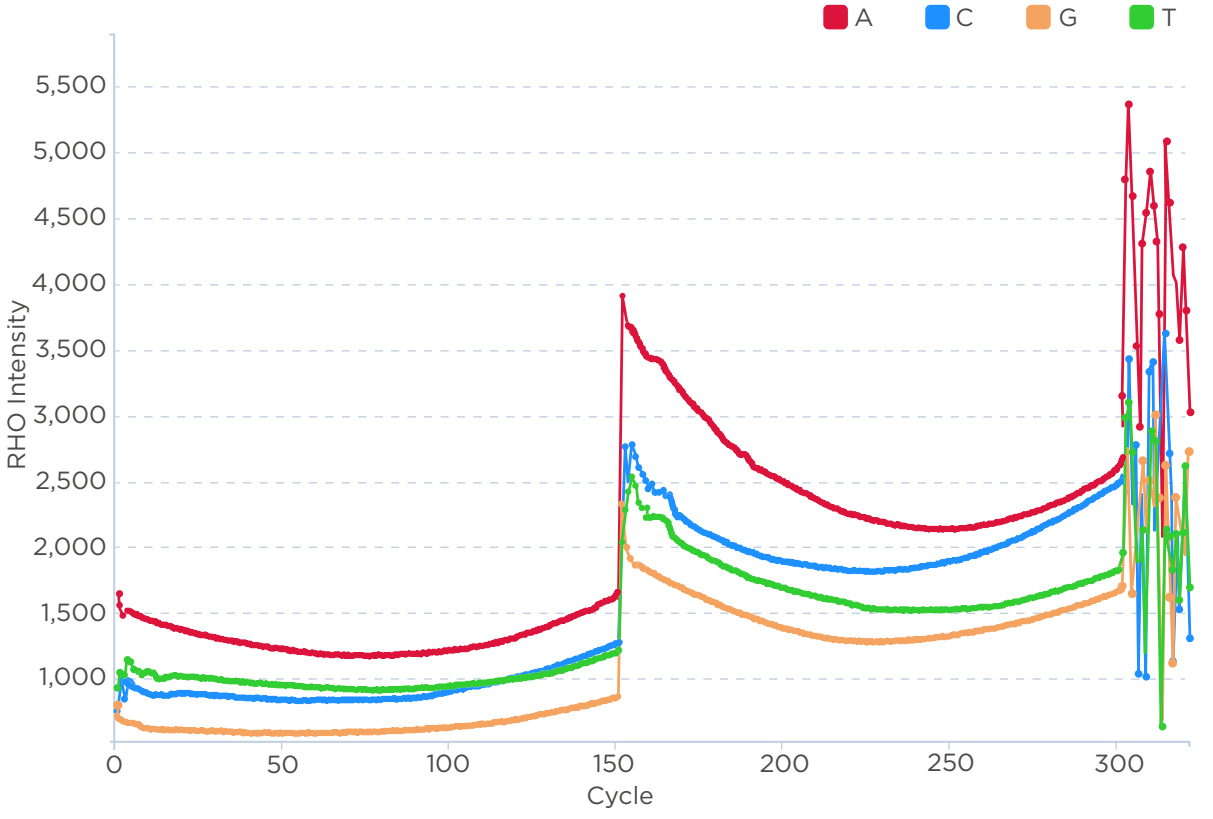


Figure 38 RHO Intensity

Table 24 Diagram description for RHO Intensity

X axis	Cycle
Y axis	RHO 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.

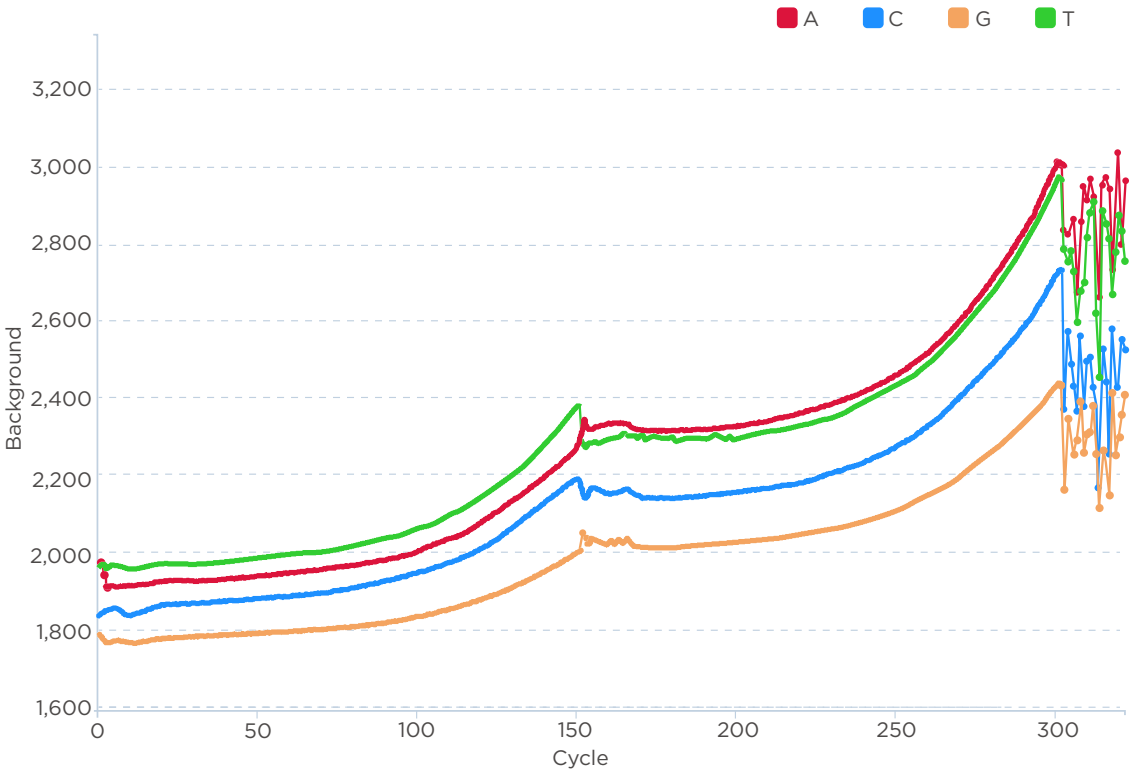


Figure 39 Background

Table 25 Diagram description for Background

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

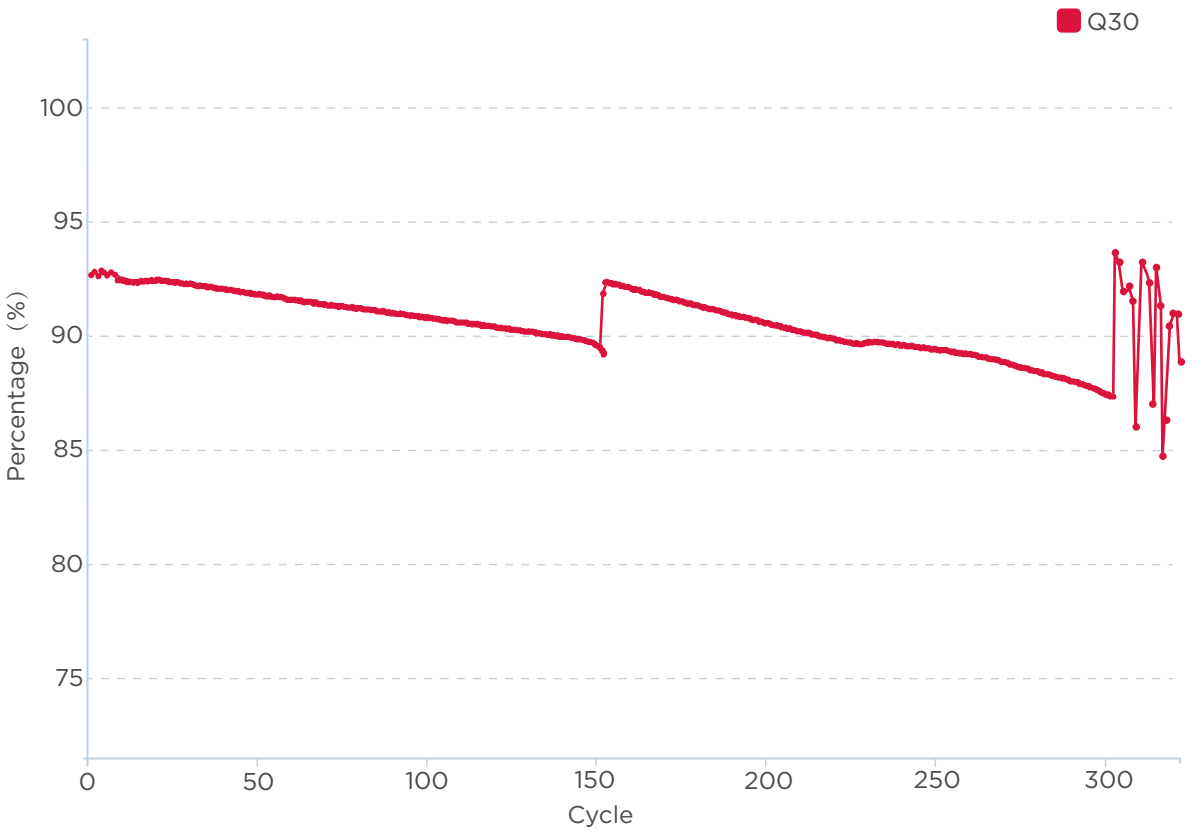


Figure 40 **Unfiltered Q30**

Table 26 **Diagram description for Unfiltered Q30**

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

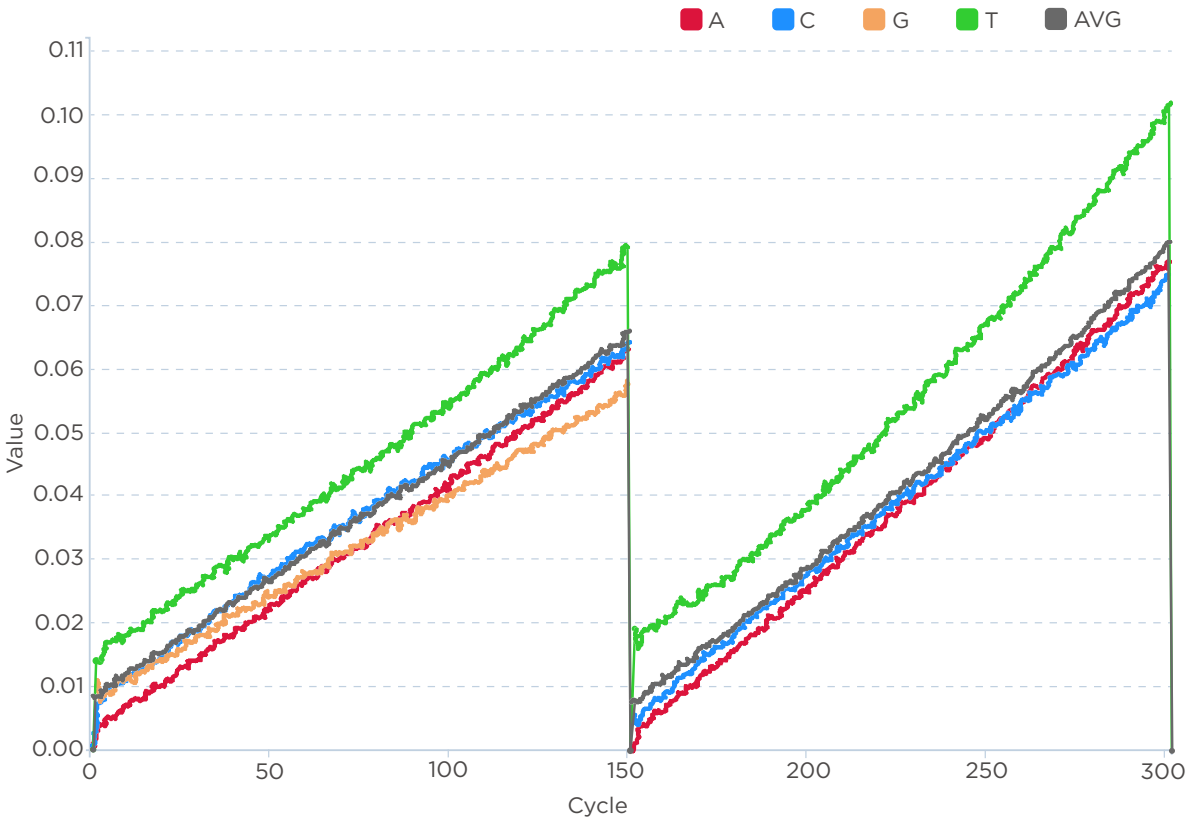


Figure 41 Runon

Table 27 Diagram description for Runon

X axis	Cycle
Y axis	Runon: Runon value for each cycle. 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.

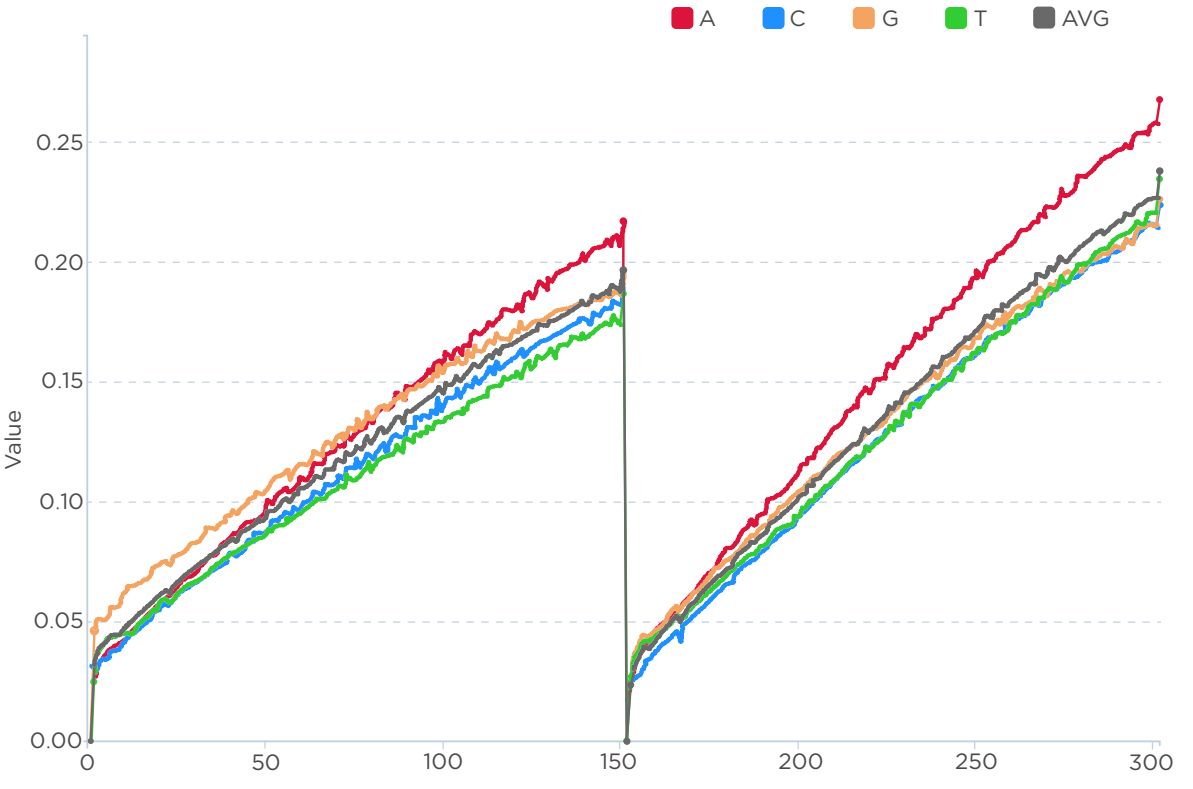


Figure 42 Lag

Table 28 Diagram description for 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.

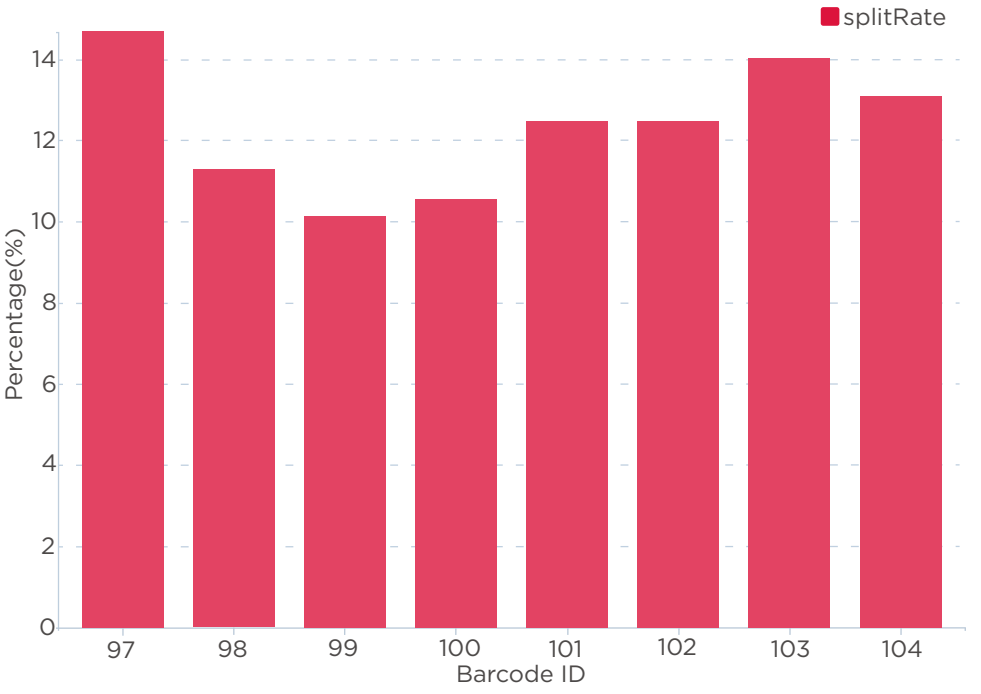


Figure 43 **Barcode Split Rate**

Table 29 **Diagram description for 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%.

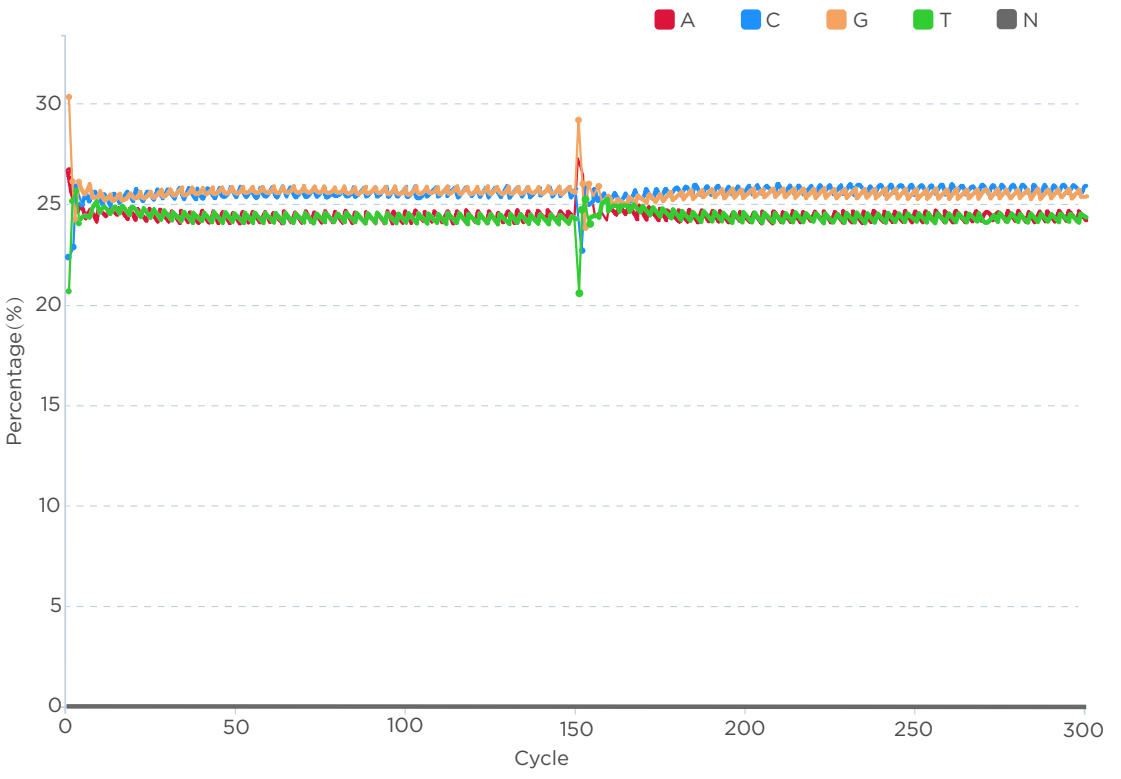


Figure 44 Bases Distribution

Table 30 Diagram description for Bases Distribution

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

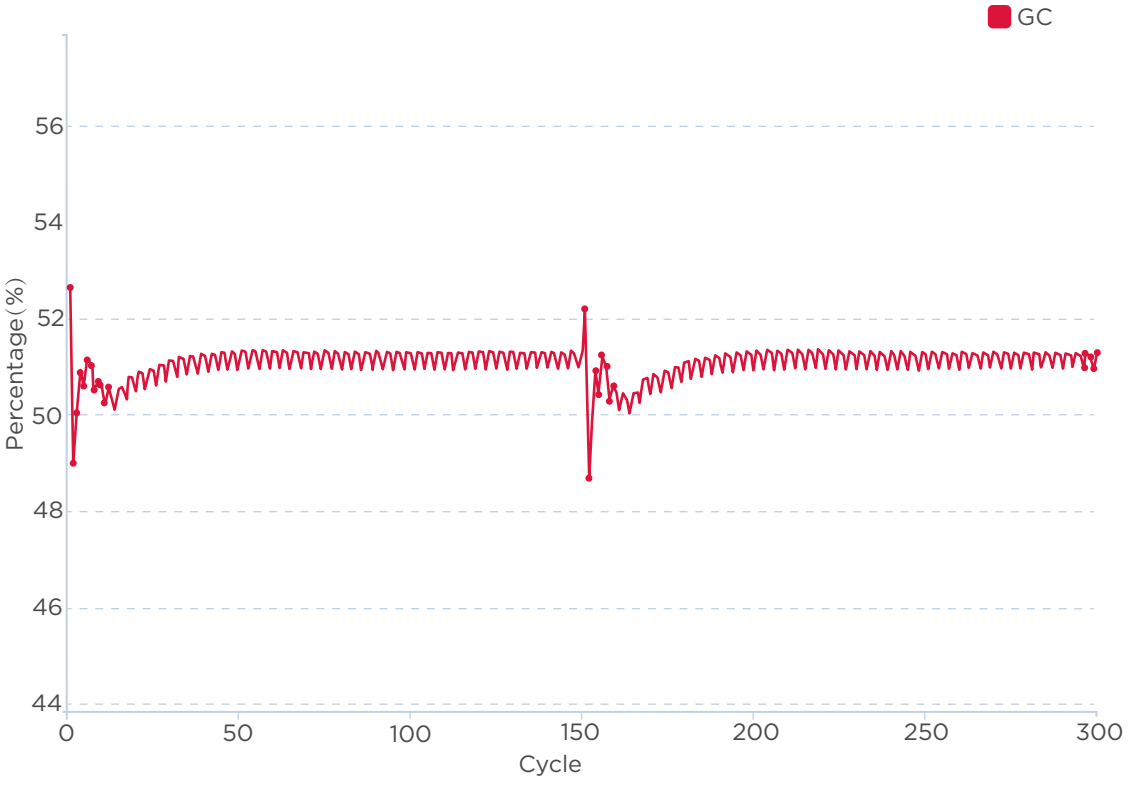


Figure 45 GC Distribution

Table 31 Diagram description for GC Distribution

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

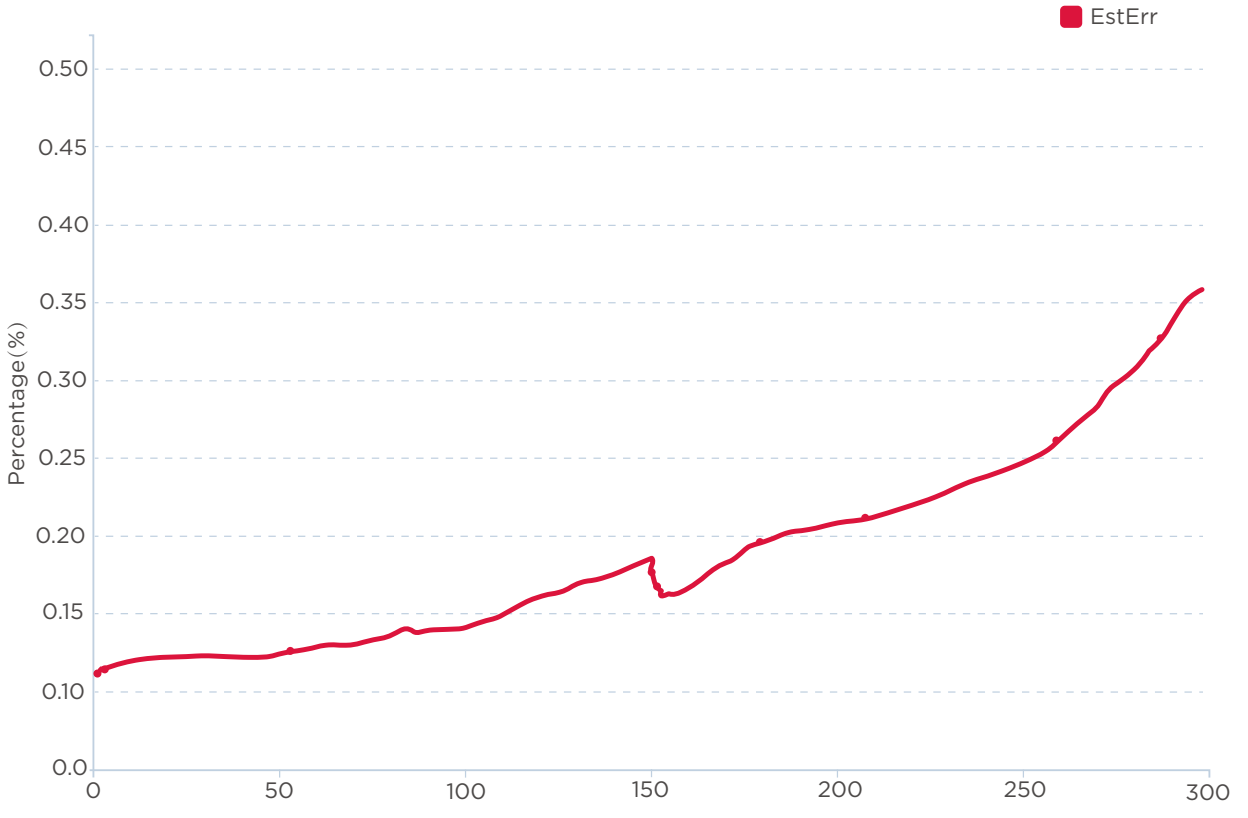


Figure 46 Estimated Error Rate

Table 32 Diagram description for Estimated Error Rate

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

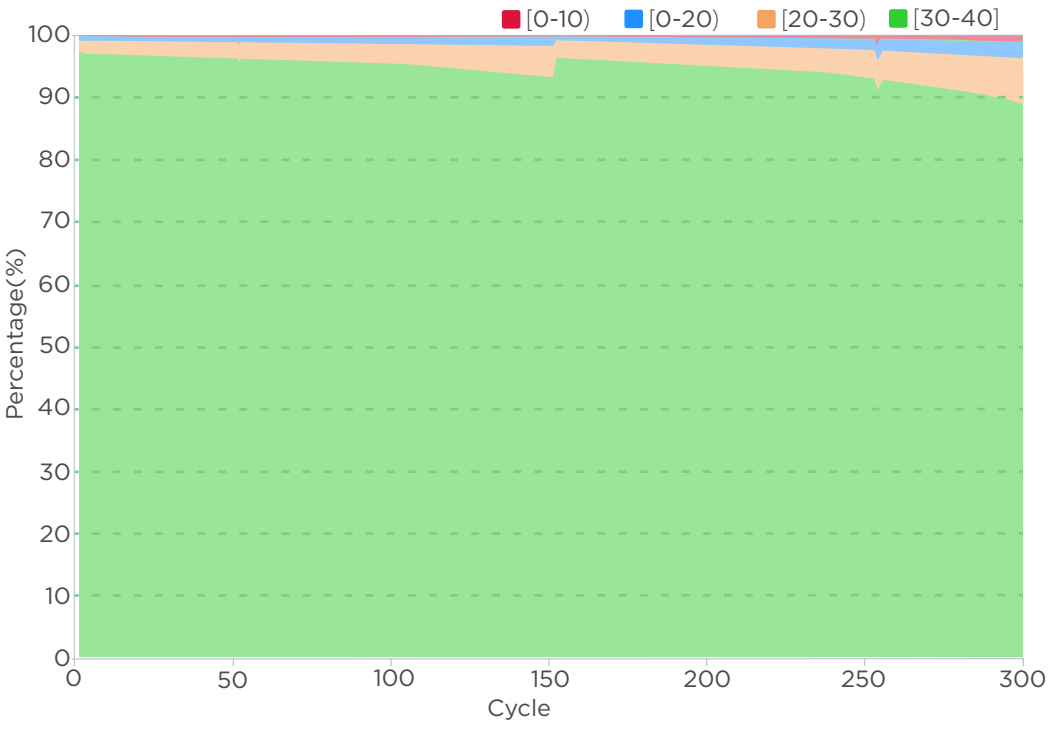


Figure 47 Quality Proportion Distribution

Table 33 Diagram description for Quality Proportion Distribution

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

Other reports

Table 34 Other report description

Name	Description
XXXXXXXX_L01.heatmapReport.html	Contains information of each FOV in the lane generated during sequencing, including AvgQ30, offset_x, offset_y, lag1, lag2, runon1, and runon2. "1" and "2" stands for strand 1 and strand 2 respectively.

Name	Description
XXXXXXXX_L01.bestFovReport.html	The summary of the best FOV and basecall information during the entire sequencing run.
XXXXXXXX_L01.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_L01 represents: flow cell ID_Lane No.

Data processing

Introduction

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

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

The two Write FASTQ methods are described below.

Write FASTQ on sequencer automatically

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

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

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

Write FASTQ on sequencer manually

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

- The FASTQ generation fails after sequencing.
- The barcode file is selected incorrectly.
- You modify some FASTQ parameter settings, including but not limited to, whether or not filtering of FASTQ is used, whether or not splitting of barcodes is used, and whether or not SaveDiscardedReads is used.

Preparation before writing FASTQ manually

Perform the following steps:

1. Ensure that the sequencer is in idle status, and not in the sequencing or base calling phase.
2. Check the *cal.* file generation and determine if it is completed.

   Check the number of Metrics files for one lane and determine if it 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/FT10000XXXX/L01/Metrics

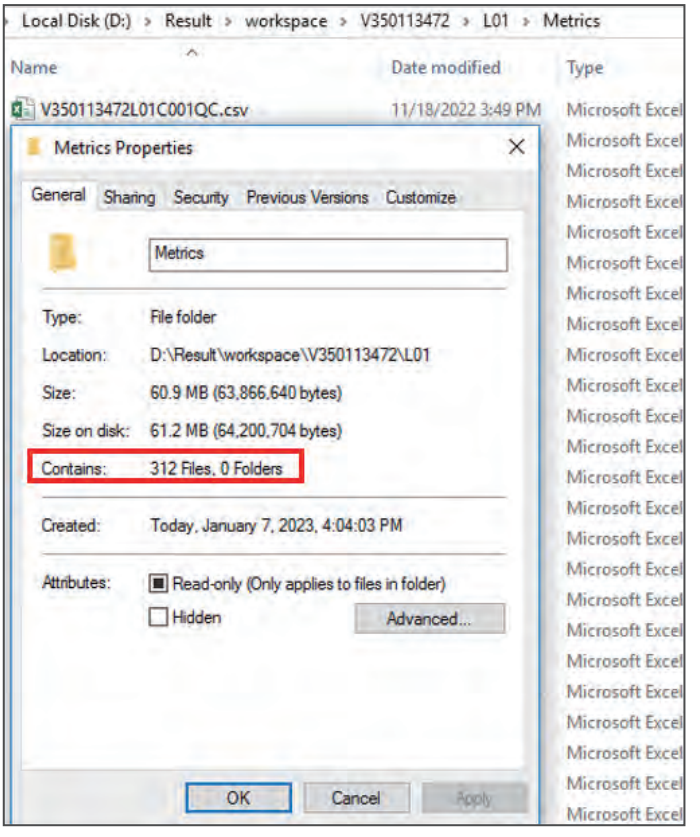


Figure 48 Metrics file number

3. Rename the original FASTQ folder. For example, rename *FT10000XXXX* to *FT10000XXXX_old*, or to *L01 rename to L01_old*.

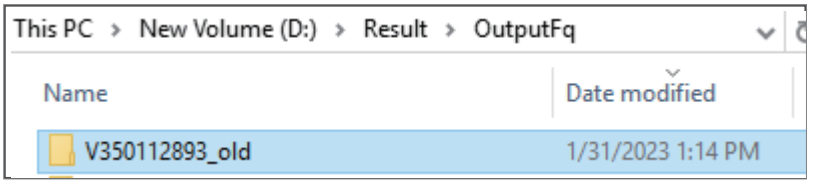


Figure 49 Renaming the FASTQ folder

4. Prepare the barcode file that you need to write FASTQ manually.
   For details, refer to *Instructions for importing barcode on Page 121.*

   Using an invalid 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) writes FASTQ manually

Perform the following steps:

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

   Select the corresponding software version folder according to the current version of the sequencer. The current basecall software version may vary among sequencers.

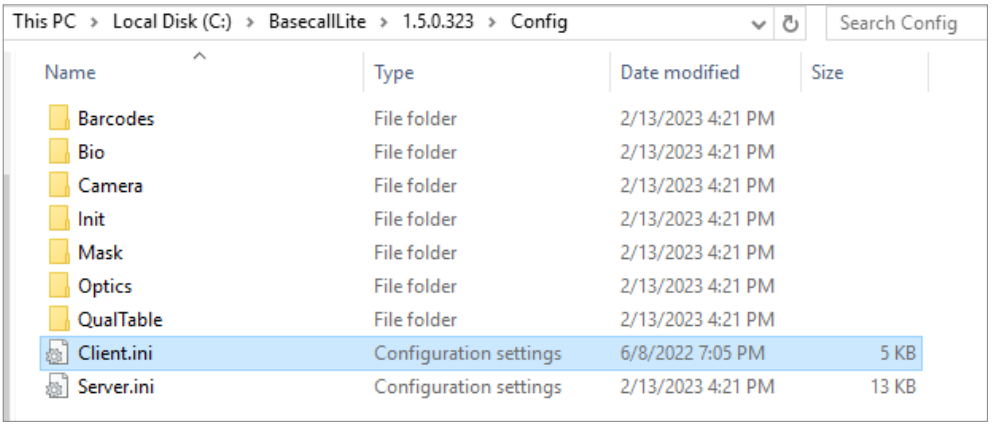


Figure 50 Location of Client.ini file

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

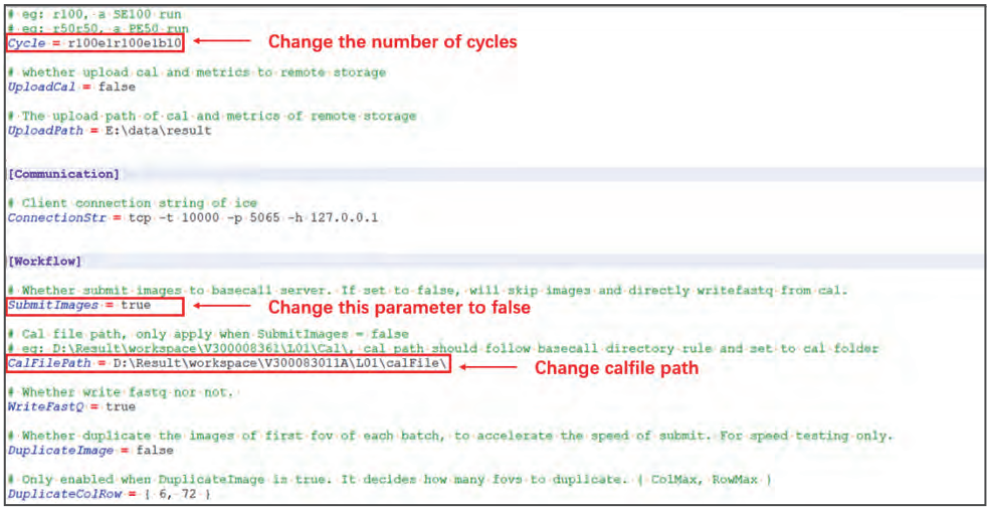


Figure 51 Editing Client.ini file

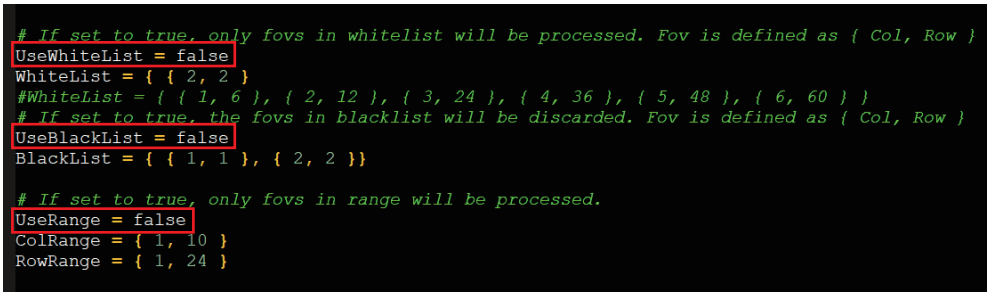


Figure 52 Setting parameters

Changing the three parameters above to true may cause a failure to offline WFQ.

Table 35 Parameter settings descriptions

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 53 on Page 89* .
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 represents comments. You can refer to the comments to modify the relevant parameters.

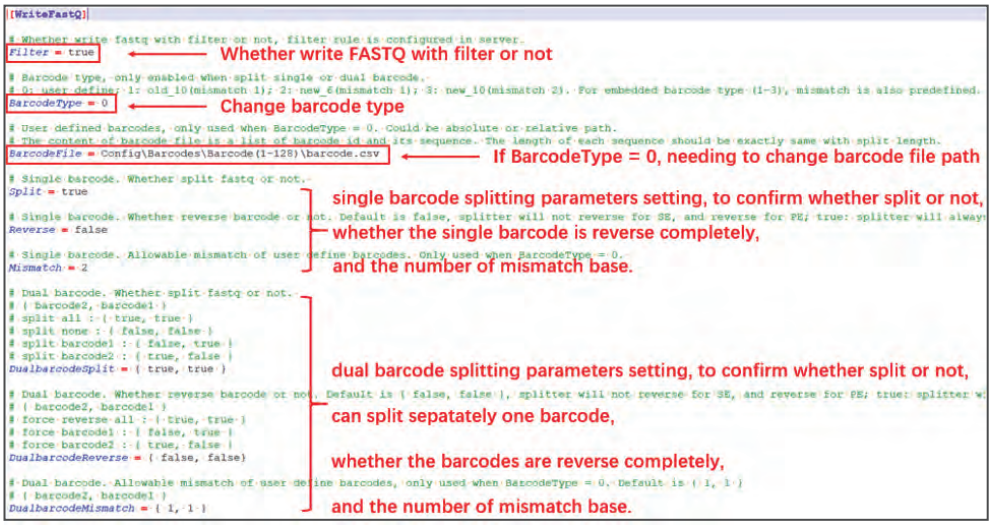


Figure 53 Changing cal. file path and barcode splitting parameters

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

   To execute the manual write FASTQ command, choose one of the following:

   - Option1: Open Task Manager, select the **Services** tab, and start LiteCall.

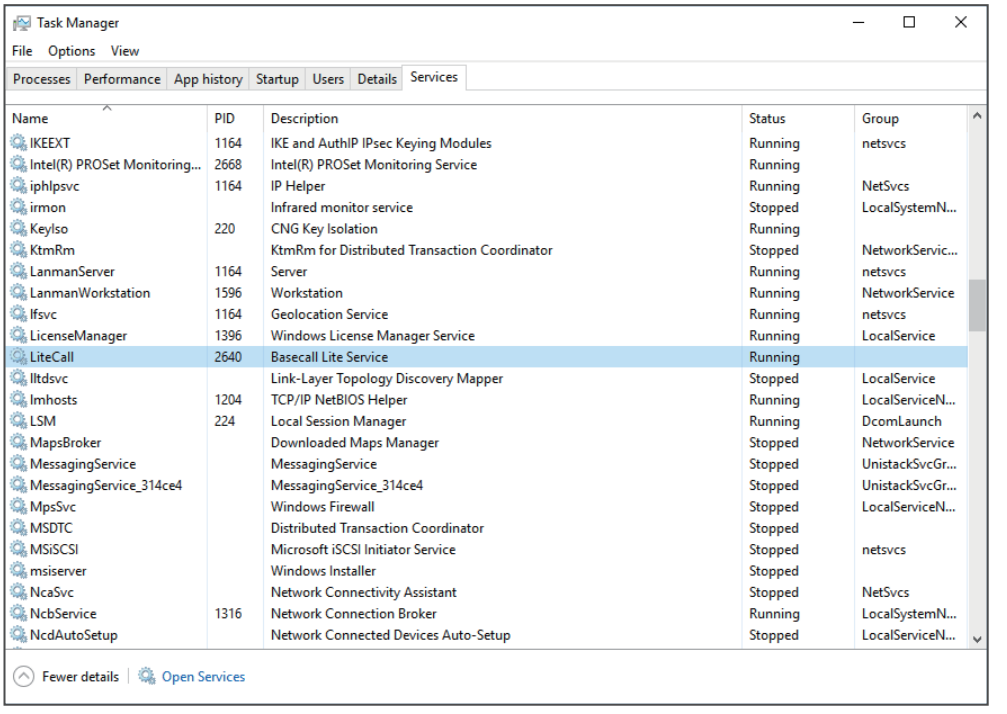


Figure 54 Starting LiteCall service

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.

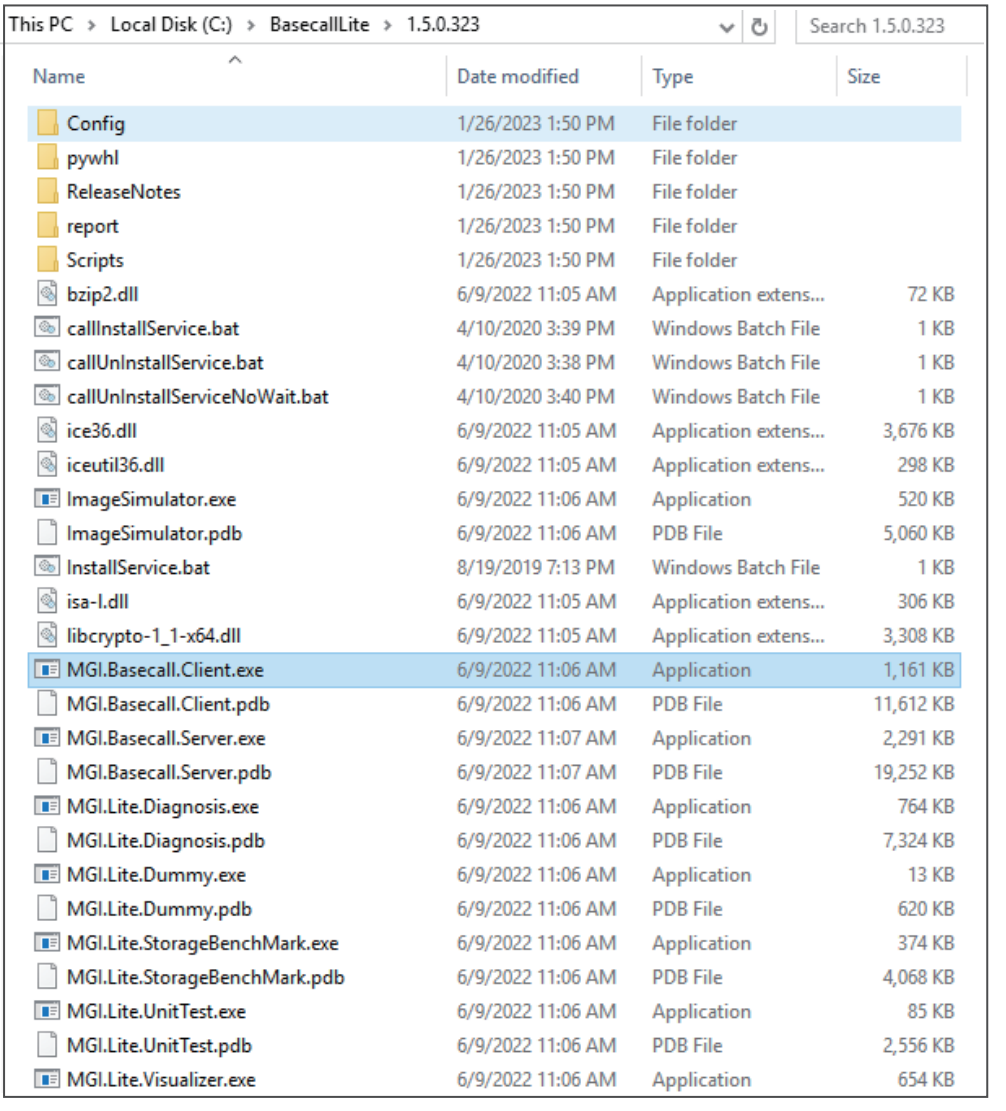


Figure 55 Opening the MGI.Basecall.Client.exe program

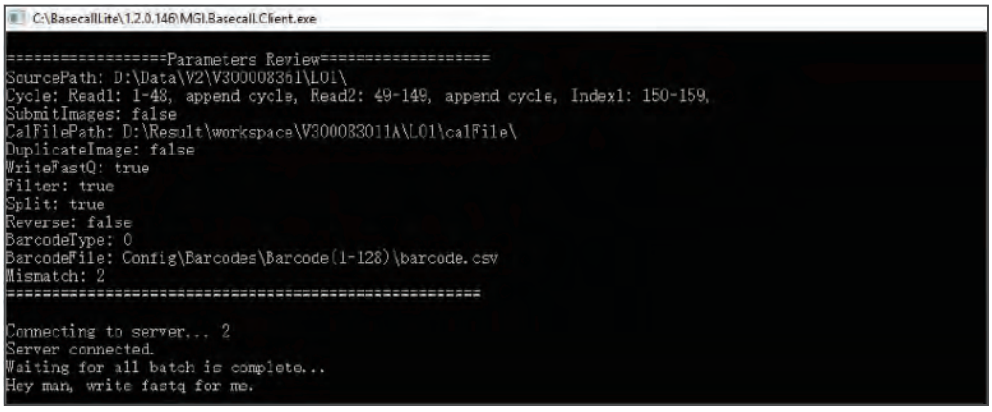


Figure 56 Starting the MGI.Basecall.Client.exe program

- Option 2: Open Task Manager, select the **Services** tab, and stop Litecall.

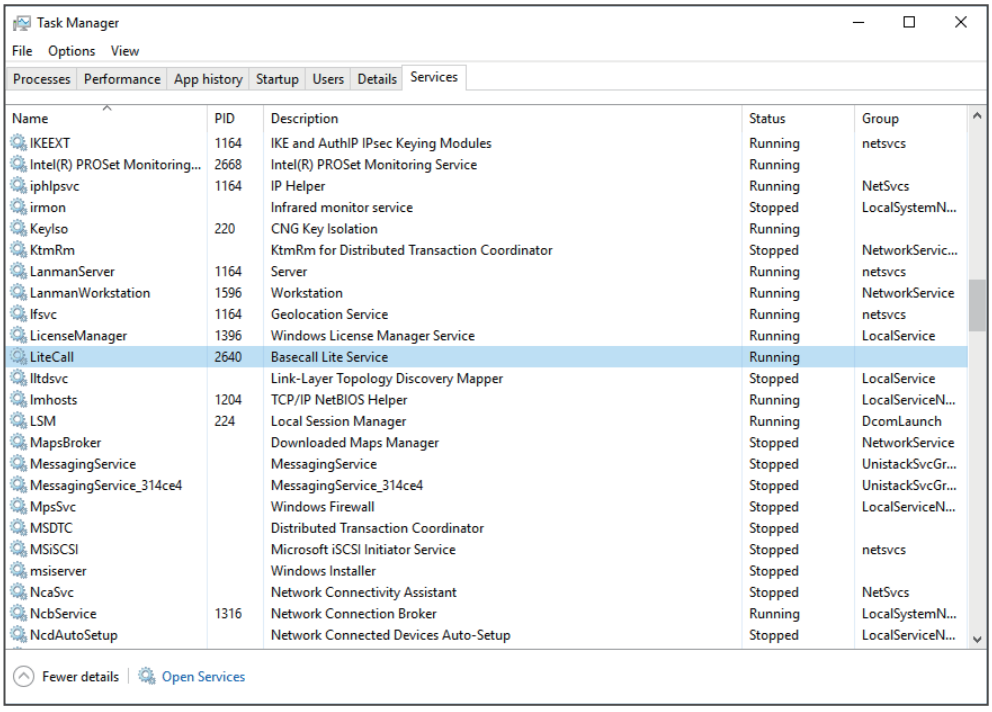


Figure 57 Stopping the LiteCall service

Example of parameter setting (PE100+10+8)

Perform the following steps:

1. Set sequencing parameters with the following assumptions:
   - Sequencing run: PE100+10+8
   - Length of Read1: 100
   - Length of Read2: 100

- Length of Dual barcode: 10
- Length of barcode1: 8
- *Cal.* file path: *D:\Result\workspace\FT10000XXXX\L02\calFile*.

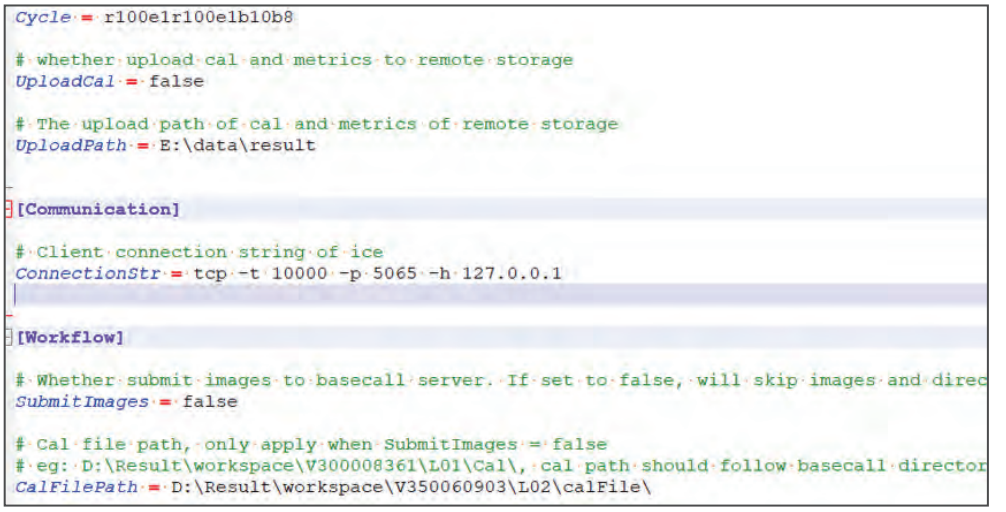


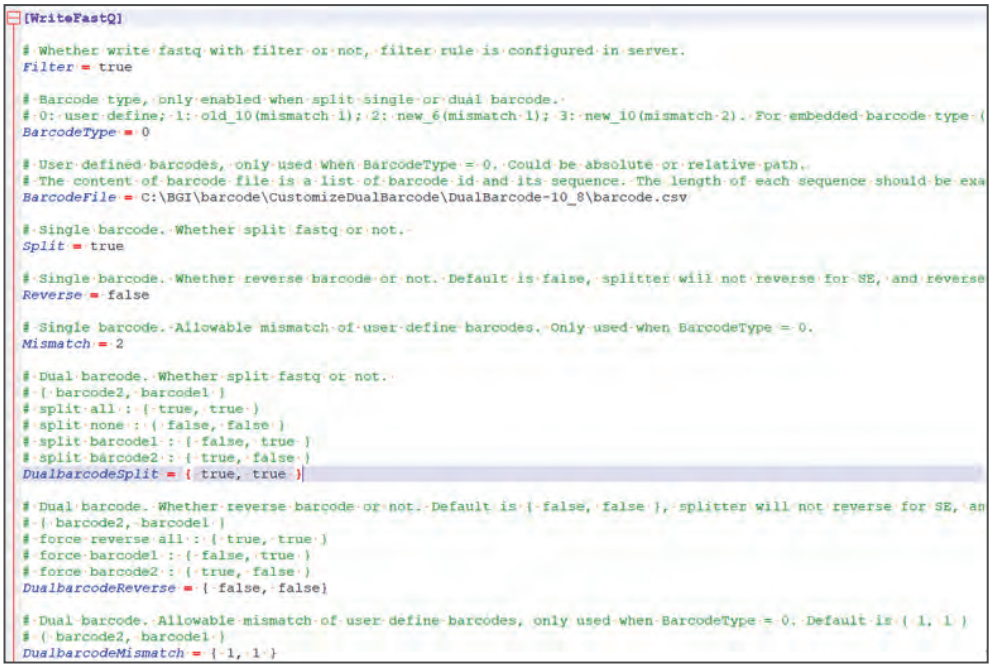
Figure 58 Parameter settings

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

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

     BarcodeFile path:

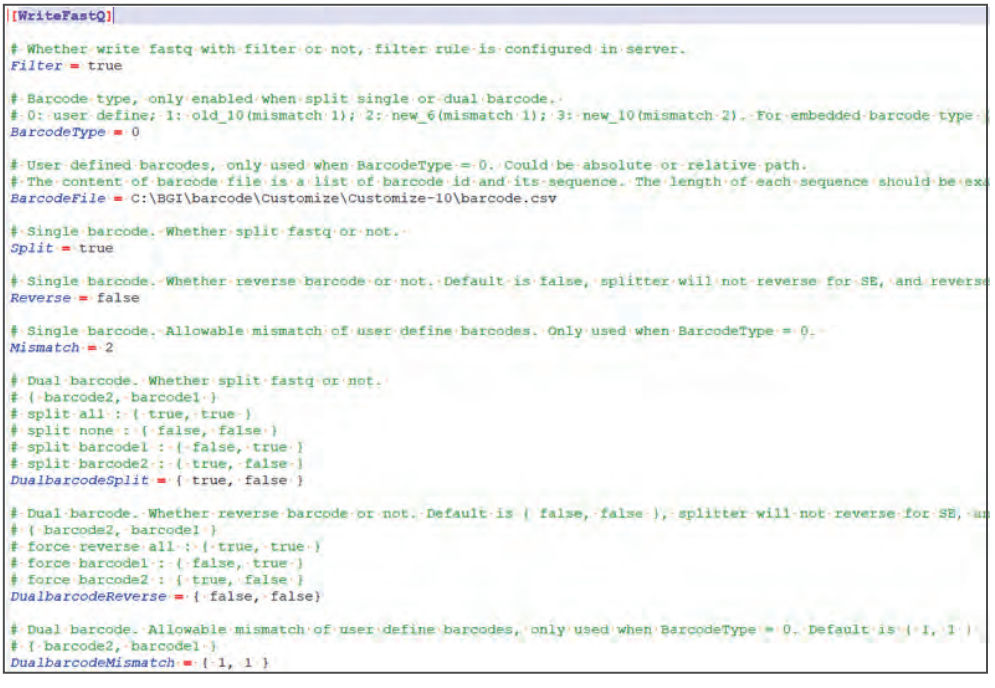
     C:\BGI\barcode\CustomizeDualBarcode\DualBarcode-10_8\barcode.csv



```
[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 59 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 60 Spitting barcode2 only

- 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 61 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 62 Changing the SaveDiscardedReads setting

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 to the device are unknown.
- If you have questions about the compatibility of wash solutions, contact CG Technical Support.

Service plan

Free preventive maintenance service is provided in the first year during the warranty period. For the purchase of additional services, contact CG Technical Support.

Sequencer maintenance

Wash

Wash type introduction

Two wash types are available, depending on sequencer conditions:

Table 36 Wash types

Wash type	Cartridge type	Process time (minutes)	Description
Automatic wash	Sequencing Reagent Cartridge	13	If **Yes** is selected in the advanced settings, the system will automatically perform the wash after each sequencing run.

Wash type	Cartridge type	Process time (minutes)	Description
Manual wash	Washing cartridge	30	• If the Auto Wash is not performed, a manual wash is required after sequencing. • If the sequencer is to be powered off for more than 7 days, perform a manual wash before power off and after power on. • If the sequencer has not been used for 7 days or longer after a manual wash, perform manual wash before use. • Perform a manual wash when impurities are found in the flow cell. • Perform a manual wash after replacing pipelines, sampling needles, or other accessories that have been exposed to the reagents. • Under normal use, perform a manual wash every month.

Preparing for wash

Preparing washing reagents

Prepare the washing reagents according to information in the table below:

Table 37 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 4 °C	

Preparing washing cartridge and washing flow cell

Washing cartridge for manual wash can be ordered as needed (DNBSEQ-G99 Cleaning Reagent Kit. Catalog No. 940-000903-00)

- Pierce the seals of MDA well and NaOH well using a 1 mL sterile tip.

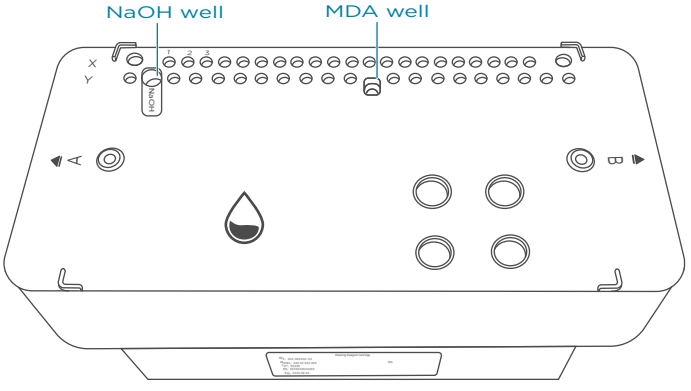


Figure 63 Position of MDA and NaOH wells

- Fill the washing cartridge with washing reagent according to the information in the following table below:

Table 38 Reagents to be added to washing cartridge

Well position	Washing reagent	Volume (mL)
NaOH well	Washing reagent: Washing reagent 3	7.5

Preparing washing flow cell

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

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

Performing a wash

Sequencer automatic wash (~13 min)

If **Auto wash** is selected in setting sequence parameters, the sequencer will perform an automatic wash after sequencing is completed.

Performing a manual wash (~30 min)

- If the **Auto wash** is not selected in the setting sequencing parameters, perform step 1 to step 10.

- Perform step 4 to step 11 in other situations in *Table 36 on Page 96* except when **Auto wash** is not selected in the setting sequencing parameters.

Perform the following steps:

1. Select **Finish** after sequencing is completed.

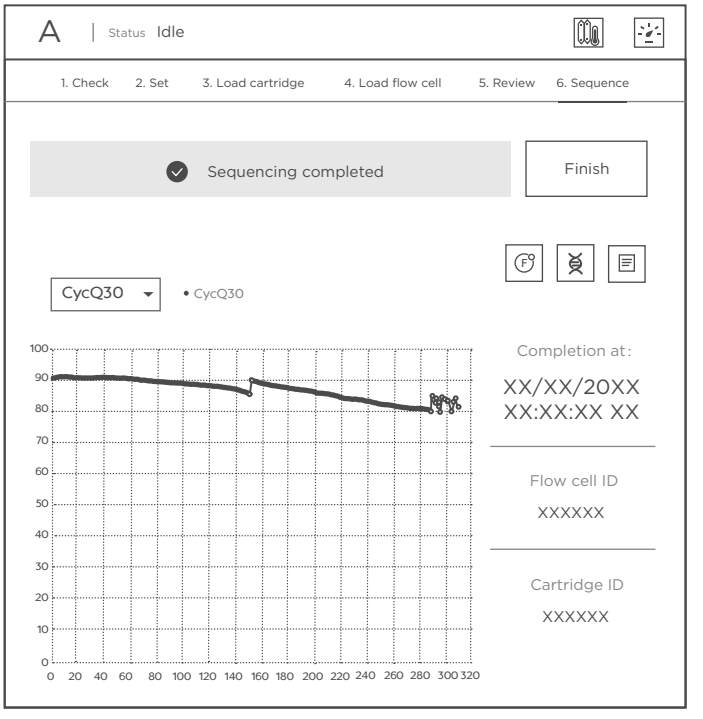


Figure 64 Sequencing completed interface

2. Remove the flow cell and Sequencing Reagent Cartridge after the auto-sliding screen moves up. Pour out the waste and clean the waste container, and then put the waste container into the waste compartment.

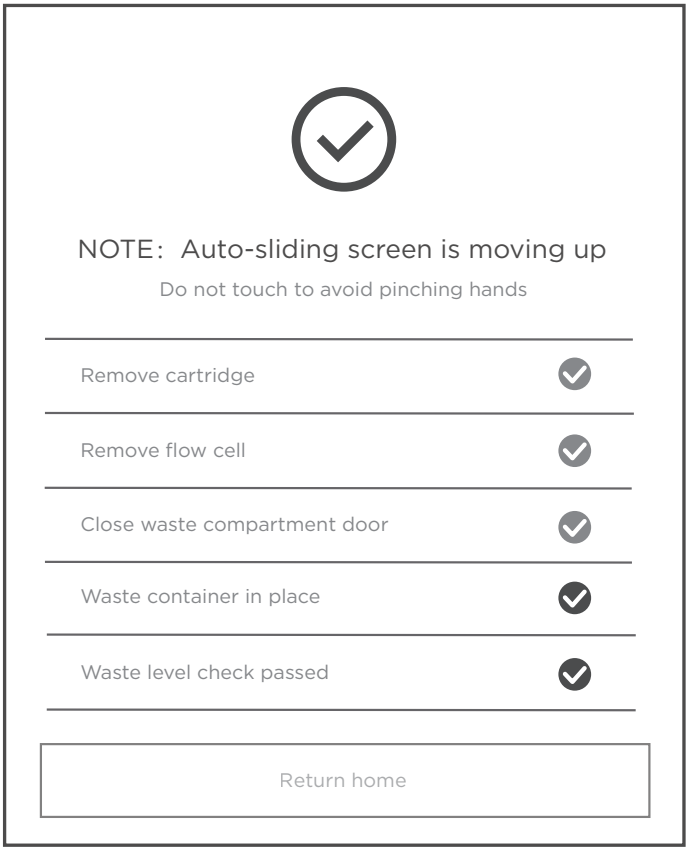


Figure 65 Operations after sequencing finished

3. Select **Return home** after all items are completed.

4. Select .

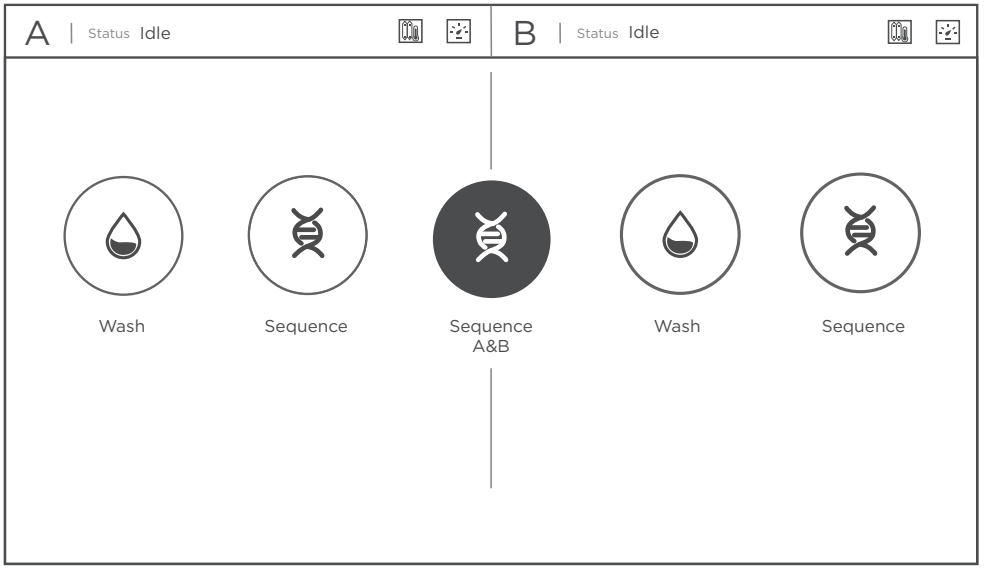


Figure 66 Main interface

5. Placing washing cartridge.

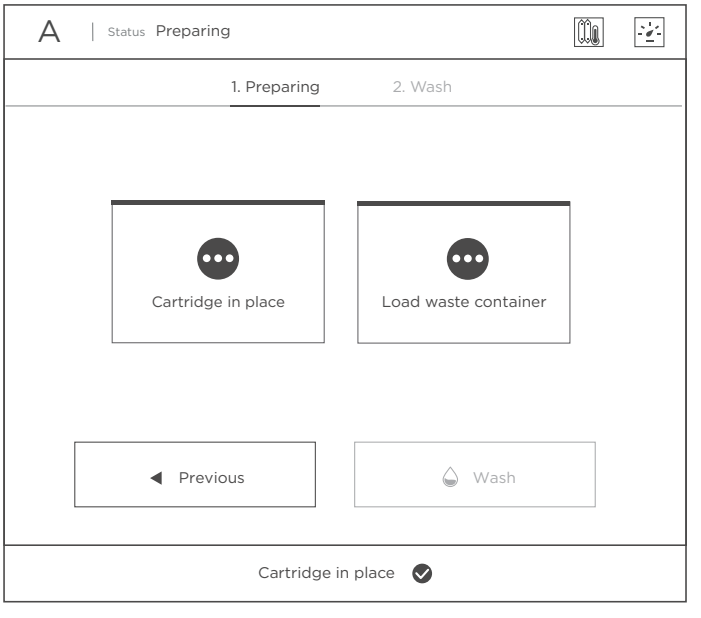


Figure 67 Placing washing cartridge

6. (Optional) Check the waste container.

- If the waste container is in place and the waste level is under the limit, skip this step.
- If the waste container is not in place or the waste level is over the limit, the waste container door will automatically pop open. Pour out the waste and clean the waste container. Then put the waste container into the waste compartment and close the waste compartment door.

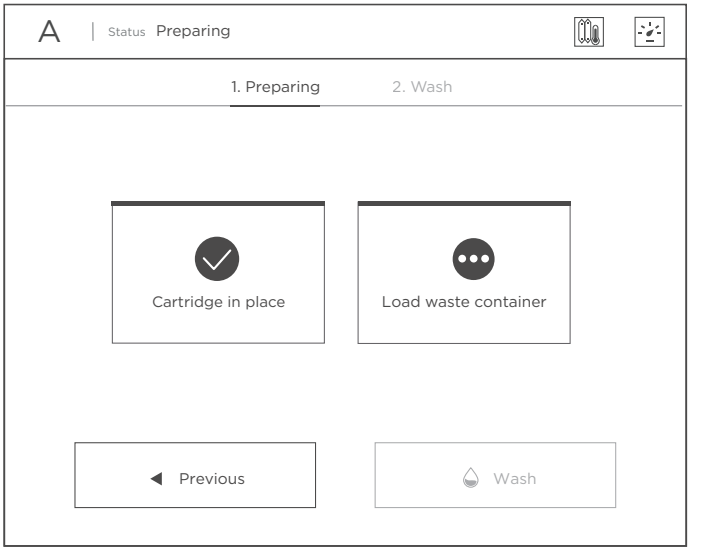


Figure 68 Check waste container

7. Select **Wash**.

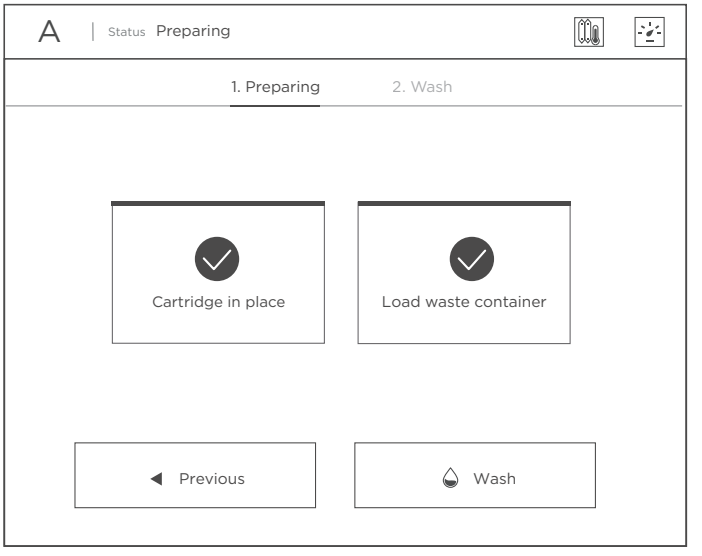


Figure 69 Check completed

8. Select **Yes** to start washing.

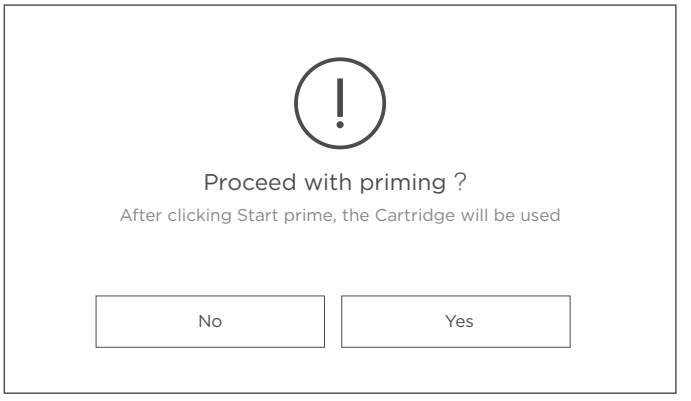


Figure 70 Confirming washing interface

9. Select **Finish** after washing is completed.

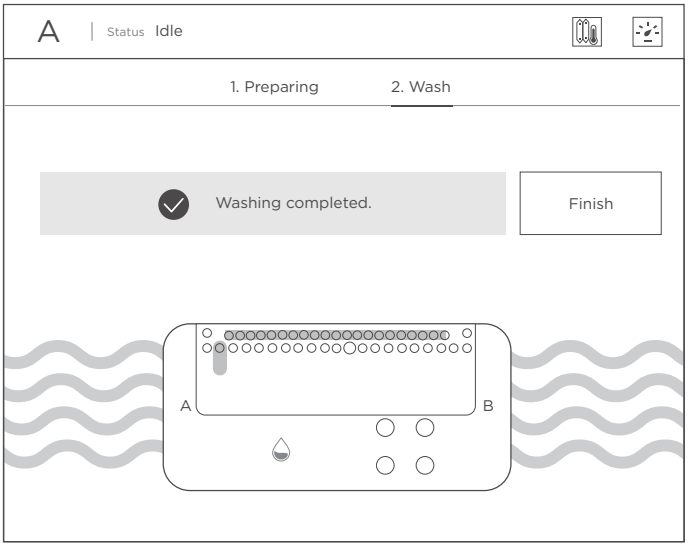


Figure 71 Washing completed interface

10. Remove the washing cartridge. Pour out the waste and clean the waste container, and then put the waste container into the waste compartment.

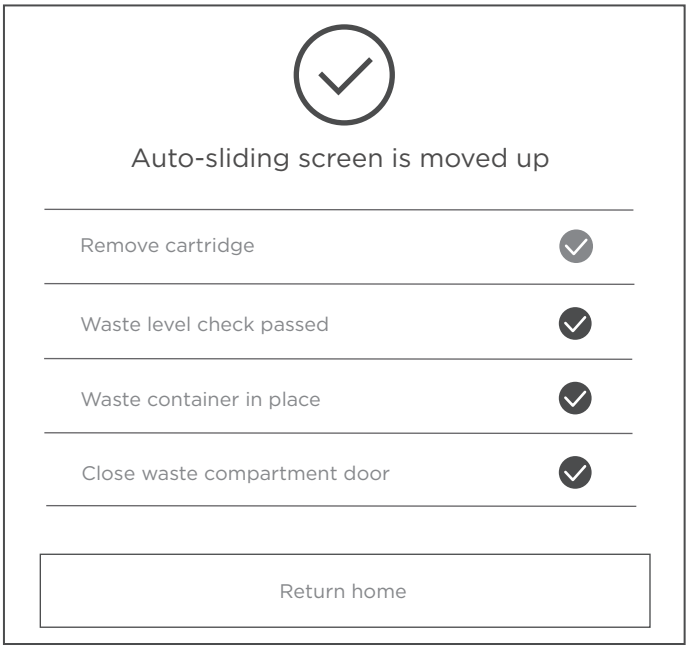


Figure 72 Removing washing cartridge

11. Select **Return home**.

Weekly maintenance

Maintaining the power supply

Perform the following steps:

1. Periodically check the power cord and cables. Ensure that they are connected correctly and are 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.

Checking and cleaning the cooling fan

Perform the following steps:

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

Cleaning the flow cell stage

Perform cleaning and maintenance for the flow cell stage. Failure to do so might affect the attachment of the flow cell to the chuck.

The tools that need to be prepared include absolute ethanol, a clean cloth, pipette, a dust remover, and a hexagon wrench.

WARNING To prevent absolute ethanol from entering the holes and damaging the device, do not wipe the vacuum inlet and vacuum attachment slot.

Perform the following steps:

1. Select > **Maintenance** > **Tools** to enter the tools interface.
2. Select **Auto-sliding screen** > **Maintenance**, and the auto-sliding screen moves up.
3. Use a hexagon wrench to remove the M3 screw.
4. Open the flow cell compartment door by lifting the cover.
5. Wipe the aluminum chuck of the flow cell stage (the highlighted part of the following figure) with a clean cloth moistened with absolute ethanol, and then let it air dry.

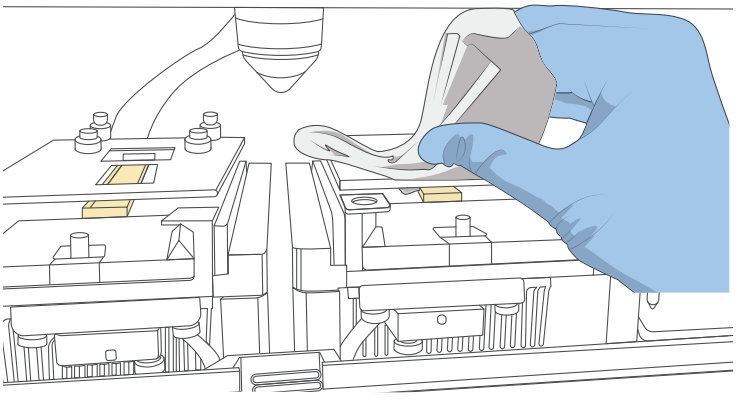


Figure 73 Cleaning the flow cell stage

6. Close the compartment door.
7. Select **Close**, and the auto-sliding door moves down and the flow cell stage maintenance is completed.

Cleaning the fluidics lines

To prevent cross contamination, perform a wash to remove the remaining reagents from the fluidics lines and flow cell stages.

Select the appropriate wash type according to the use frequency of the device and the type of sequence. The following table contains requirements on wash solutions and frequencies.

Table 39 Wash requirements

Wash type	Wash solution	Frequency
Auto wash	Use the sequencing cartridges that contain the wash solutions.	Determine whether perform auto wash for the fluidics lines after sequencing depending on the individual need of experiment.
Maintenance wash	Use the washing cartridge and add 7.5 mL of Washing reagent 3 to the hole highlighted in blue and labeled "NaOH" of the washing cartridge.	• If you do not wash the fluidics lines after previous sequencing, wash them before sequencing. • Wash the fluidics lines monthly. • The device is not in use for 7 days or longer. • Wash the fluidics lines after you perform any device maintenance relevant to the fluidics lines.

- For information on preparing 0.1 M NaOH, refer to *Preparing washing reagents on Page 97*.
- No flow cell needs to be placed during washing.

Perform the following steps:

1. Prepare the washing cartridge.

   For information on preparing the washing cartridge, refer to *Preparing washing cartridge and washing flow cell on Page 98*.

   CAUTION Use only the washing reagent cartridges of the manufacturer for the maintenance. You can purchase them as needed from the authorized sales representatives.

2. Select **Wash** in the main interface.
3. Follow the on-screen instructions to perform the washing.
4. Wash the waste container.
   1) Remove the waste container from the waste container compartment, remove the lid, and empty the waste into an appropriate container according to local regulations and your laboratory safety standards.
   2) Add sufficient laboratory-grade water into the waste container and gently shake the container until all inner walls are cleaned. Attach the lid back onto the waste container according to your need.

3) Pour the waste into an appropriate waste container.
4) Clean the surface and opening of the waste container with a 75% ethanol wipe. Ensure that no waste remains in the container.

5. Place the waste container back into the waste container compartment and close the waste compartment door.
6. Dispose of the waste and waste container according to local regulations and safety standards of your laboratory.

Monthly maintenance

Clearing the historical data in the storage drive

Check the disk space and timely back up the historical data with the peripheral storage devices.

Maintaining the device

The dust-free cloth should keep moist without droplets.

Perform the following steps:

1. Power off the device.
2. Wipe the surface of the device and the auto-sliding screen with a dust-free cloth moistened with 75% ethanol. Ensure that the surface is free of samples and reagents, blood, and saliva.

Annual maintenance

We recommend that you calibrate and maintain critical components, such as the laser power supply, annually. For information on the service plan, 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 device at end of life according to local regulations. Or, if the device is confirmed that it still can work safely and effectively after maintenance, keep using the device.

07

FAQs

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

Reagent FAQs

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

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

1. Determine if the sequencing set has expired.
2. Determine 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, contact CG Technical Support.

Q: What should I do if I forgot to add reagent into MDA well for PE sequencing run or I want to resume a stopped 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 MDA well. If you forgot to add the reagent into MDA well when performing the sequencing run, this can be resolved by performing the following steps, as long as the sequencing run is at the read1 sequencing phase.

If you want to resume a stopped sequencing run, only the run that was stopped during read1, read2 or barcode sequencing phase can be resumed.

WARNING A sequencing reagent cartridge can only be resumed once.

- If you forgot to add reagent into MDA well for PE sequencing run, perform step 1 to step 5.
- If you want to resume a stopped sequencing run, perform step 2, step 4, and step 5.

Perform the following steps:

1. Stop the run: select ■ at any sequencing cycle within read1, and select **Yes** when you are prompted as shown below:

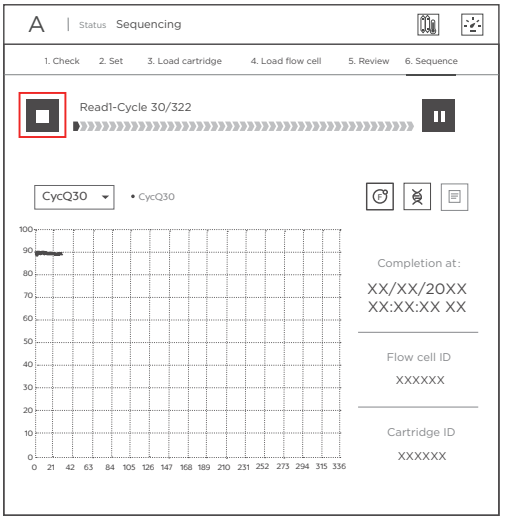


Figure 74 Selecting the sequencing stage to stop

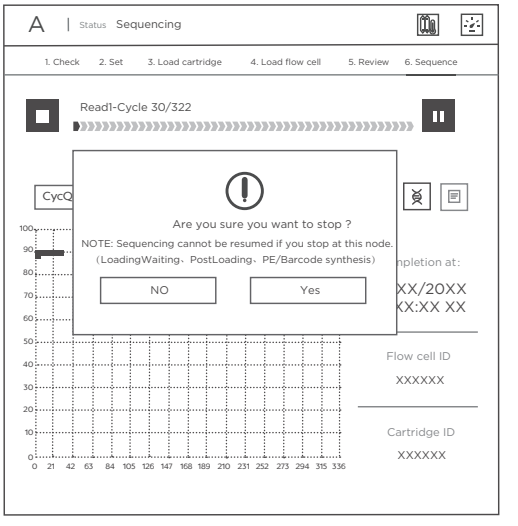


Figure 75 Confirming to stop the run

2. Remove the Sequencing Reagent Cartridge and Flow Cell: Select **Finish**. When the sequencing run is stopped, remove the Sequencing Reagent Cartridge and Flow Cell after the reagent compartment door slides up. Finally, select **Return home** as shown below:

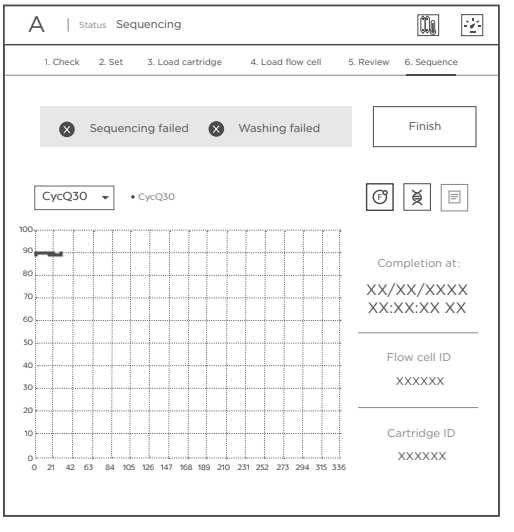


Figure 76 Selecting Finish

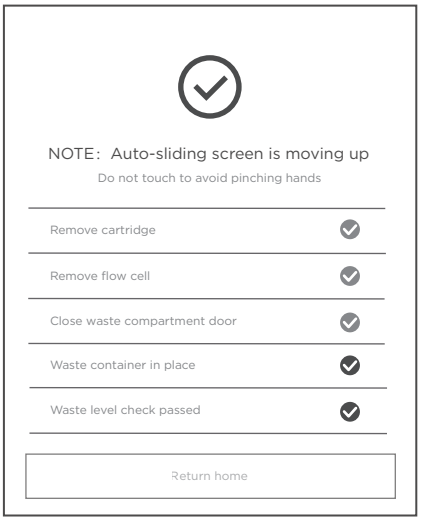


Figure 77 Removing Sequencing Reagent Cartridge and flow cell

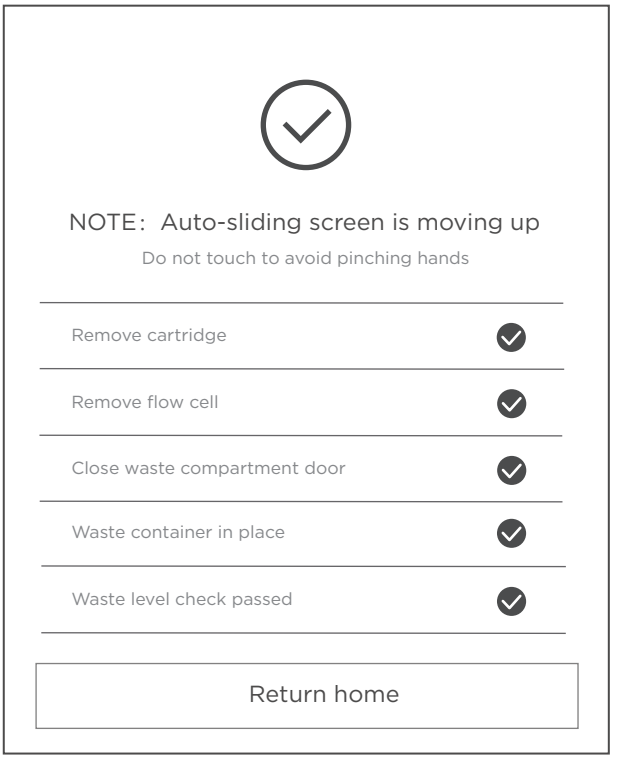


Figure 78 **Selecting Return home**

3. Add MDA mixture to the Sequencing Reagent Cartridge: Add 125 µL of MDA Enzyme Mix to the MDA Reagent tube with a 200 µL pipette. Mix well and transfer all mixture into MDA well.

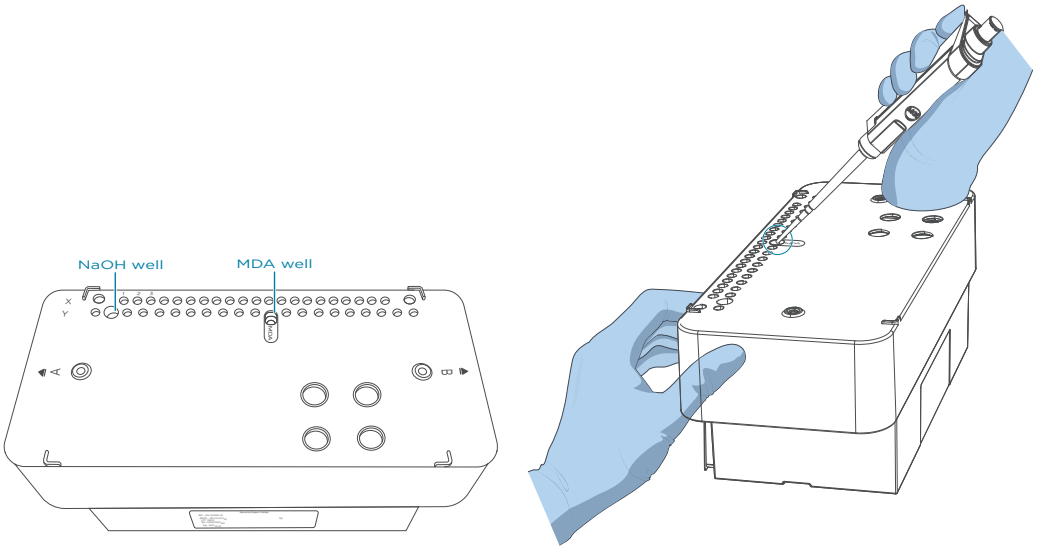


Figure 79 **Adding MDA mixture**

4. Check before resume sequence: select , then select and the system will perform checking before resume sequence. Select **Next** after the check has completed.

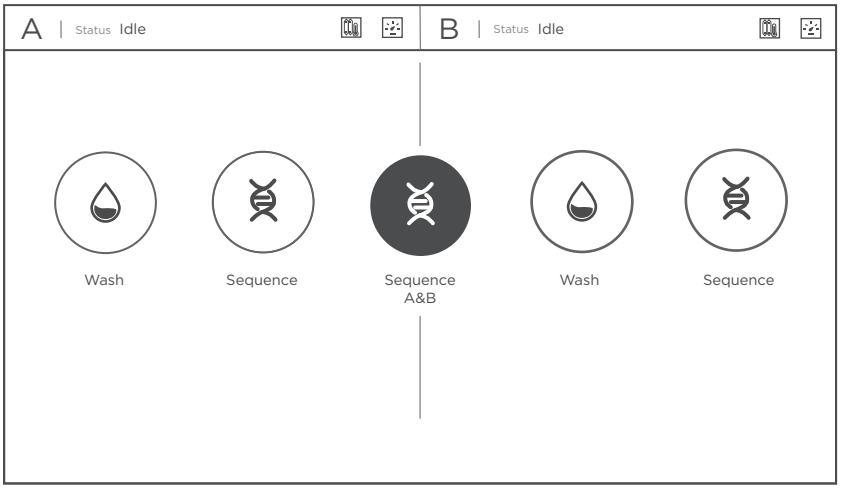


Figure 80 Main interface

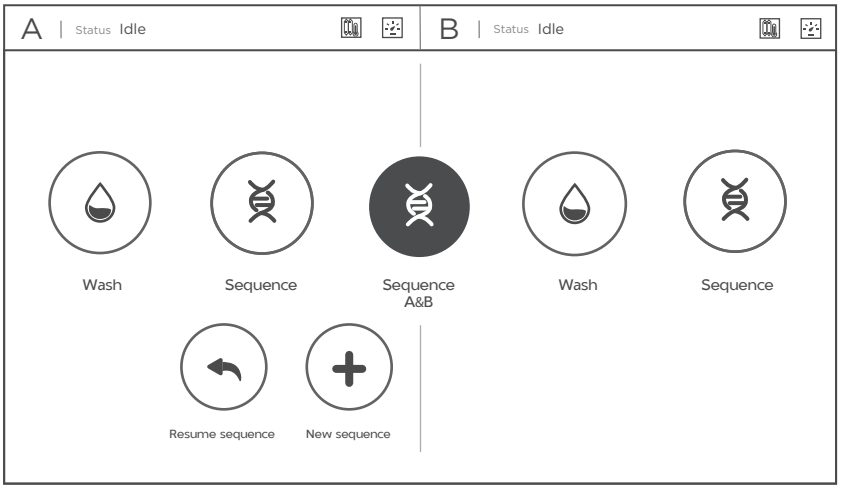


Figure 81 Resume sequence interface

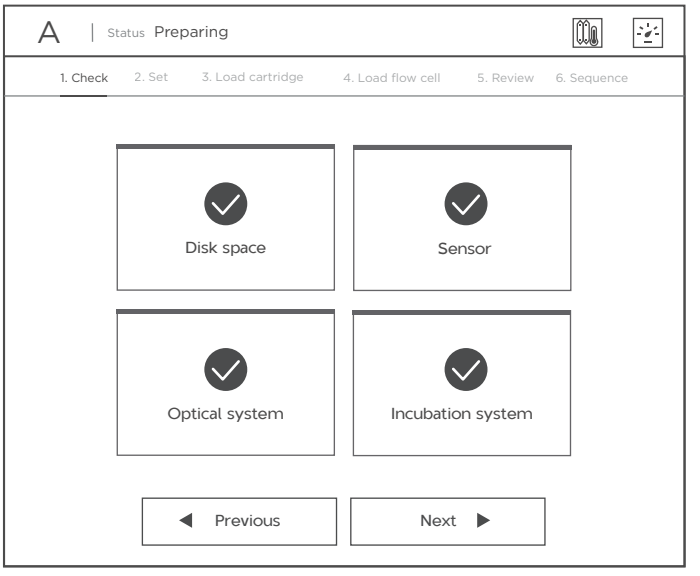


Figure 82 **Checking completed**

5. Resume sequence: Put the Sequencing Reagent Cartridge back into the sequencer and select **Prime** to perform priming. After priming is completed, insert the Flow Cell and select **Next** and confirm that all information is correct. Select **Sequence** to resume the sequencing run as shown below:

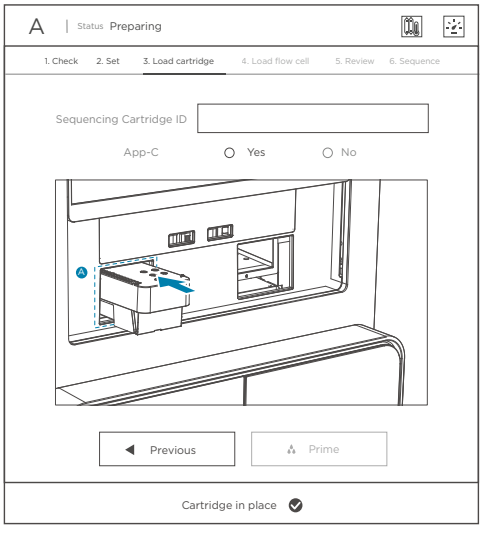


Figure 83 **Placing cartridge**

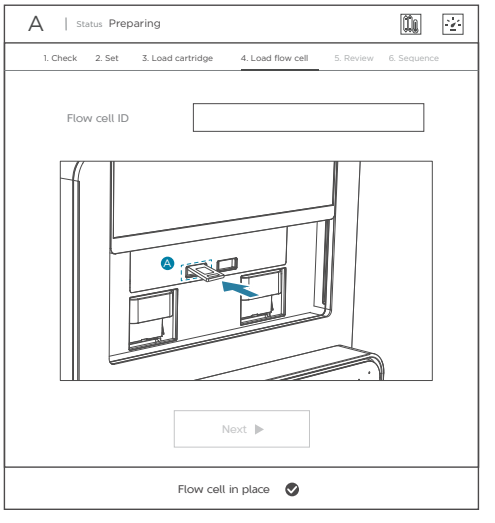


Figure 84 **Placing flow cell**

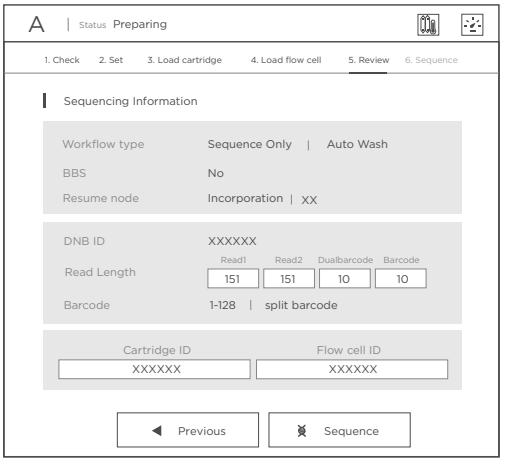


Figure 85 Confirming information

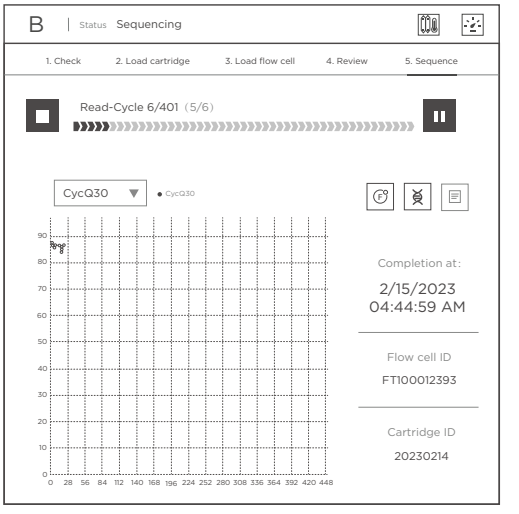


Figure 86 Starting resuming sequencing

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

- If a cartridge has been thawed without pressing M1, M2, M3, M4 wells and cannot be used within 24 hours, the cartridge can be frozen and thawed at most one additional time. Or, store the cartridge at 4 °C, and use it within 24 hours. Mix the reagents in the cartridge following the instructions in *Preparing the Sequencing Reagent Cartridge on Page 45* before use.
- If the reagents of M1, M2 and M3, M4 have been added into the cartridge (the cartridge has been prepared but cannot be used immediately), store it at 4 °C and use it within 24 hours. Mix the reagents in the cartridge following instruction in *Preparing the Sequencing Reagent Cartridge on Page 45* before use.

What should I do if an error occurs before washing?

Perform the following steps:

1. If an error message occurs after selecting wash, select **Confirm**.

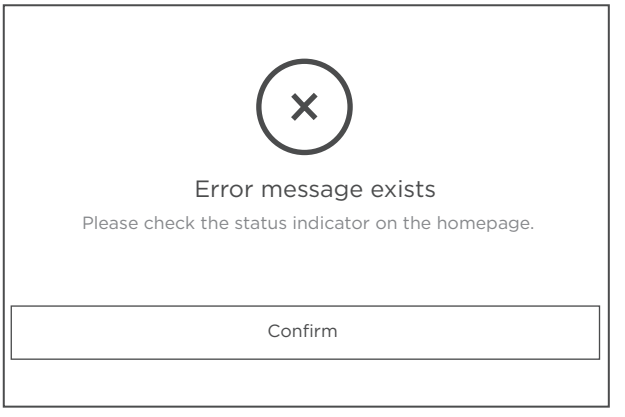


Figure 87 Error message

2. Select , if the following alarm appears, perform the step 3.

Figure 88 Error alarm

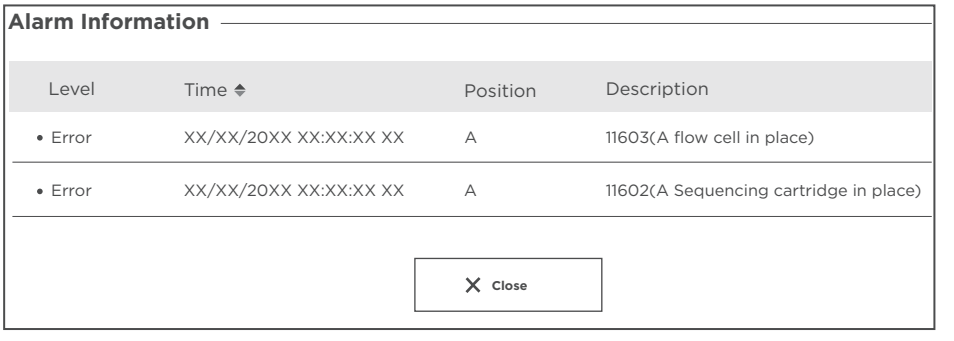


Figure 89 Alarm information

3. Select **Close** to close alarm information. Select > **Maintenance** > **Tools**. Select **Auto-sliding screen** > **Screen Up**, then remove the Sequencing Reagent Cartridge and Flow Cell.

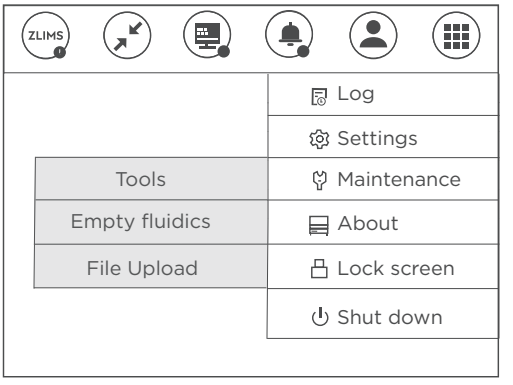


Figure 90 **Maintenance menu**

Figure 91 **Maintenance operation interface**

4. Select **Close**.

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

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

1. Gently wipe the stage surface of flow cell stage with a damp Kimwipes tissue or a low-lint cloth and blow the stage using a canned air duster and ensure that no dust is present.

2. Blow the back of the flow cell using a canned air duster to ensure no dust is present.
3. If the problem persists, contact CG Technical Support.

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

If impurities appear, try the steps below:

1. Moisten a Kimwipes tissue with 75% ethanol and use it to wipe the flow cell stage, and perform a maintenance wash on the sequencer according to *Preparing washing flow cell on Page 98* .
2. If the problem persists after a full wash, contact CG Technical Support.

Sequencer FAQs

Q: What should I do if the device does not turn on after I power the device off?

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 main power supply and UPS is normal.
2. Ensure that the device is connected to the main power supply or UPS.

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 check in the maintenance interface. Check the record of the hardware that fails the check.
2. Check error messages in the log, and solve the problem according to on-screen instructions.
3. Restart the device.

Q: What should I do if temperature error messages and warnings appear in the sequencing interface?

Error messages may appear when the temperature exceeds the preset limits and/or if there is an error with the temperature sensor error. It is recommended that you record the warnings and the related logs of the sequencing run and contact CG Technical Support.

Q: What should I do if error messages appear when the system is performing checking?

- Error messages for the Disk space detection may appear when the Disk space is insufficient.

  Perform the following steps:

  1) Delete the files in the Disk space.
  2) Restart the detection.

- Error messages may appear for the Sensor detection, Optical system detection, and Incubation system detection. It is recommended that you record the warnings and the related logs of the sequencing run and contact CG Technical Support.

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Instructions for importing barcode

Barcode settings

Select > **Settings** > **Barcode** to enter the barcode settings interface. In this interface, you can download the built-in barcode templates, and you can import, export, and delete the customized barcode templates.

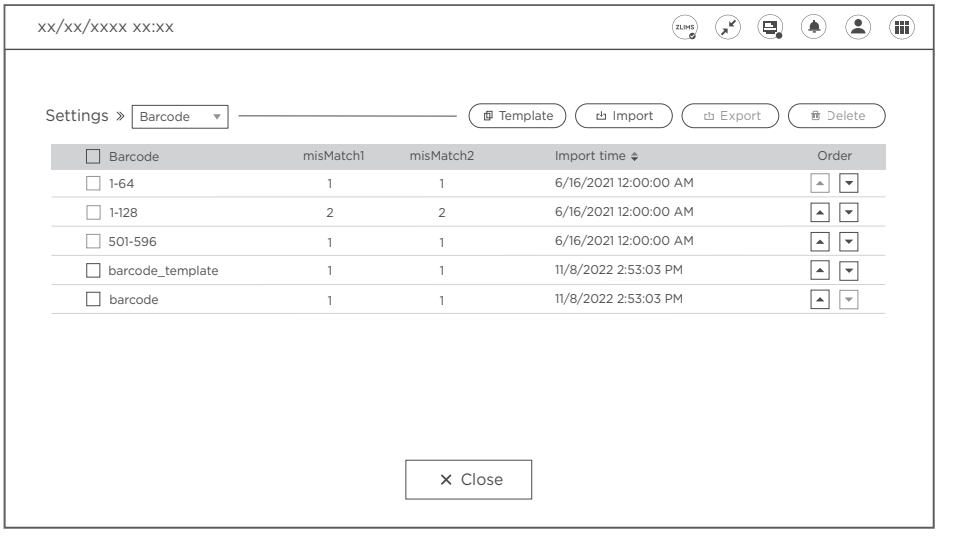

Figure 92 **Barcode settings interface**

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

Table 40 Barcode setting interface description

Item	Description
Template	Select to download the customized barcode template.
Import	Select to import the barcode files from external storage devices to the device.
Export	Select to export the customized barcode files.
Delete	Select to delete the selected customized barcode recipes.
misMatch1	Displays the number of barcode mismatches in the barcode recipes.
misMatch2	Displays the number of Dualbarcode mismatches in the barcode recipes.
Import time	Select to change the order of the barcode files by import time (ascending order or descending order).
Order	Select to adjust the order of the selected recipes.
Close	Select to exit the barcode settings interface and return to the main interface.

Downloading barcode templates

Select **Template** to download the built-in barcode templates.

Importing barcode templates

Perform the following steps:

1. Prepare a barcode file.

Ensure that the barcode file meets the following requirements:

- The barcode file can be imported only through the control software.
- The barcode file should be named as *.csv* only.
- It is recommended that you use Notepad++ program to open the barcode file. Barcode ID and barcode sequence in the file should be separated by a comma.
- The barcode file should not contain blank lines, or full-width characters. The barcode sequence should include no fewer than two bases.
- Barcode ID and barcode sequence should be unique and should not be empty.

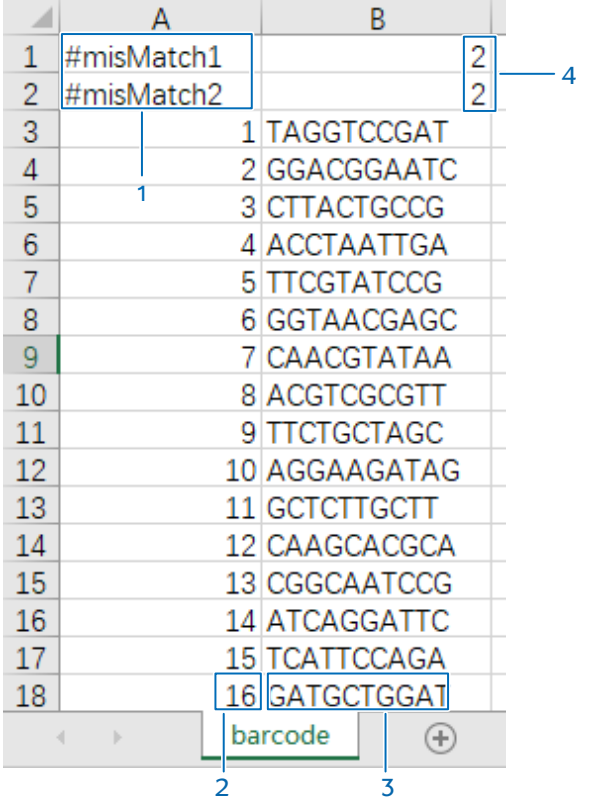

Figure 93 **Barcode template**

No.	Name	No.	Name
1	misMatch	2	Barcode ID
3	Barcode sequence	4	Number of mismatch

2. In the barcode settings interface, select **Import**.
3. Select the prepared barcode file.
4. Select **Open** to import the barcode file to the device from external storage devices.

Exporting barcode templates

Only the barcode templates that are imported from external storage devices can be exported.

Select the barcode templates according to your needs and select **Export**.

Deleting barcode templates

Only the barcode templates that are imported from external storage devices can be deleted.

Select the barcode templates according to your needs and select **Delete**.

Instructions for customizing a run

Introduction

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

- When read length(s) in Read1 and/or Read2 are not the same as those predefined in the **Recipe** list.
- When barcode length(s) in Barcode and/or DualBarcode are not the same as those predefined in the **Recipe** list.
- The recipe you want is not within the predefined recipe list.
- Dark reaction cycles are required in Read1 and/or Read2 sequencing.

Important interfaces for customizing a run

Customize a recipe interface

In the main interface, select **Sequence**. The **Customize** recipe is displayed:

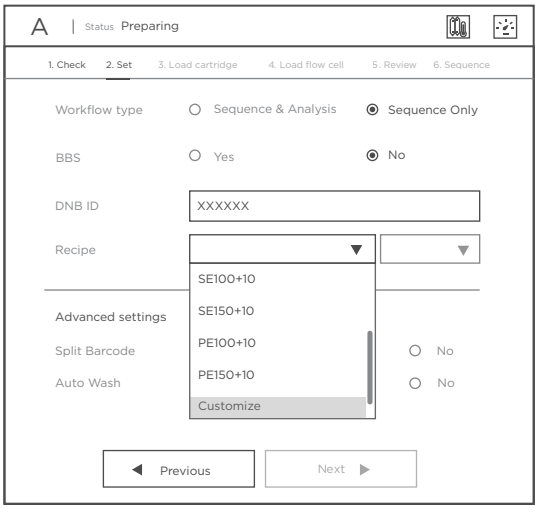

Figure 94 Customize recipe

Customize interface

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

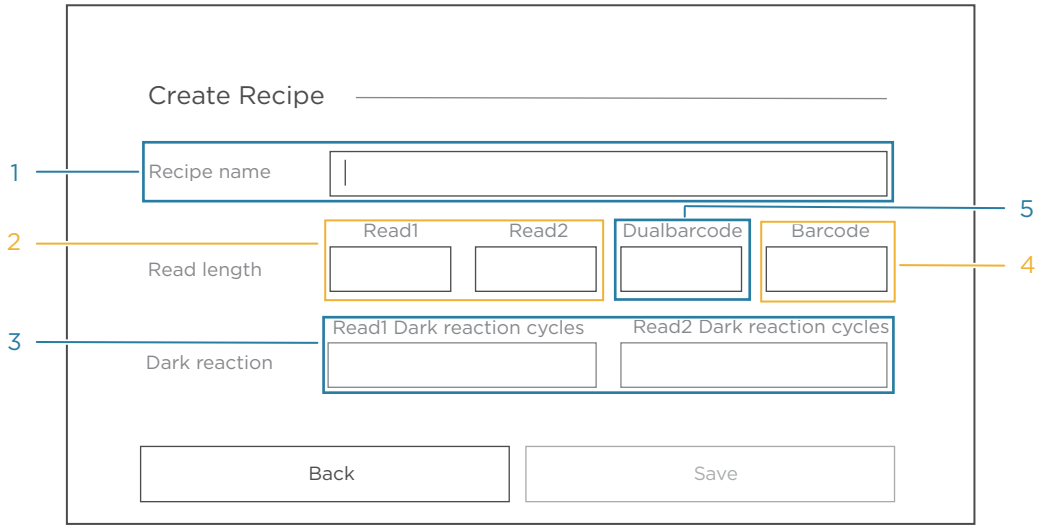

Figure 95 Customize interface

The following table describes the function of buttons and areas in the interface:

No.	Item	Description
1	**Recipe name**	Write a name for a sequencing run
2	**Read1/Read2**	Customize read1 and (or) read2 length for a sequencing run
3	**Read1 dark reaction cycle** / **Read2 dark reaction cycle**	Customize dark reaction range in Read1 and (or) Read2
4	**Barcode**	Customize Barcode length for a sequencing run.
5	**Dualbarcode**	Customize DualBarcode length for a sequencing run.

Barcode (not predefined) interface

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

1. Select **Others** from the barcode range list next to the first **Recipe** box.

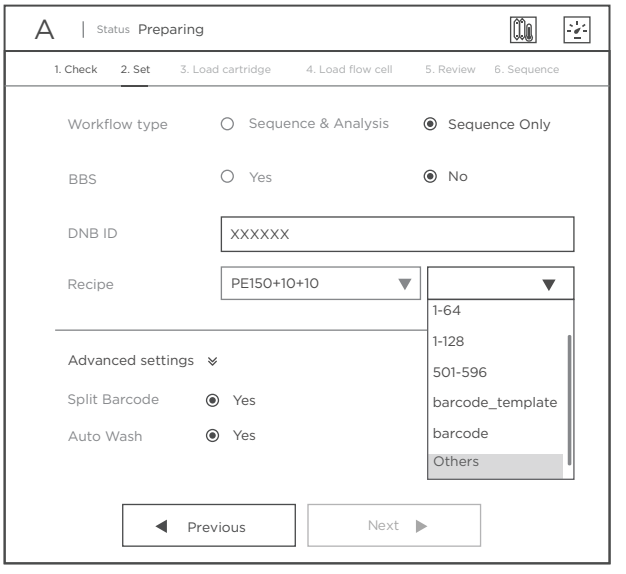

Figure 96 Selecting Others

2. Select next to the **Barcode file**.

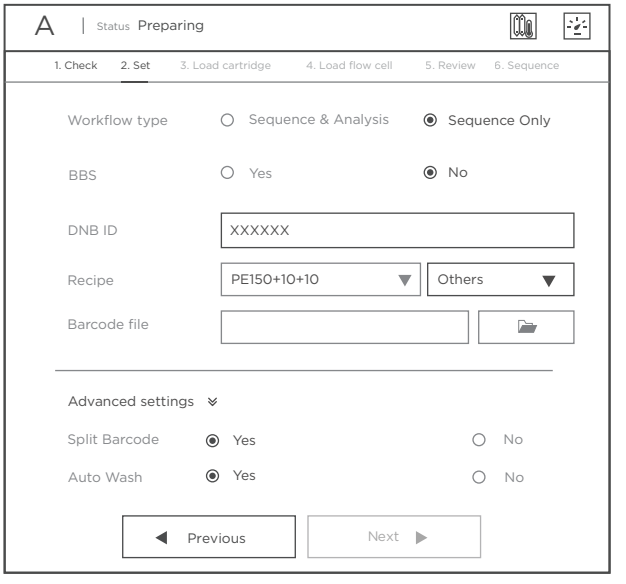

Figure 97 Configuring customize settings

3. Select the barcode file previously imported. For information on barcode importing, refer to Instructions for importing barcode on Page 121.

Examples of customized run

Ensure that you are aware of the following information:

- Before starting the customizing run, confirm that the customized barcode files are already imported into the sequencer. If not, refer to *Instructions for importing barcode on Page 121* to import the customized barcode.
- Ensure that the total number of sequencing cycles including Read1, Read2, Barcode, Dualbarcode, and Dark Cycle is less than the maximum sequencing cycles for a given sequencing set as defined in *Table 2 on Page 31* .
- The maximum read length for both Read1 and Read2 should not be more than that specified in the sequencing set. For example, if PE150 is used, the maximum customized read1 length and read2 length should not be greater than 150.
- When you perform dual barcode sequencing run, it is recommended that you 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 total lengths of barcode and dualbarcode should not be greater than 20.

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 follows:

- Sequencing run: PE120+140+10
- Length of read1: 120
- Length of read2: 140
- Length of barcode: 10
- Length of Dualbarcode: 0
- Split barcode: Yes
- Total cycles = 120+140+10+2 = 272
- Select a PE150 set

The Customize interface is set as follows:

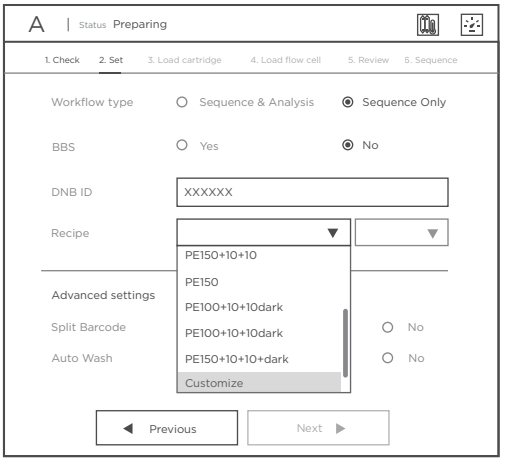

Figure 98 Selecting Customize

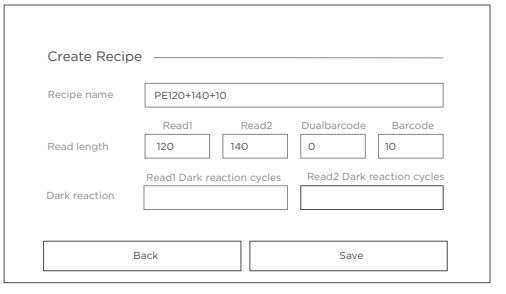

Figure 99 Configuring customize settings for example 1

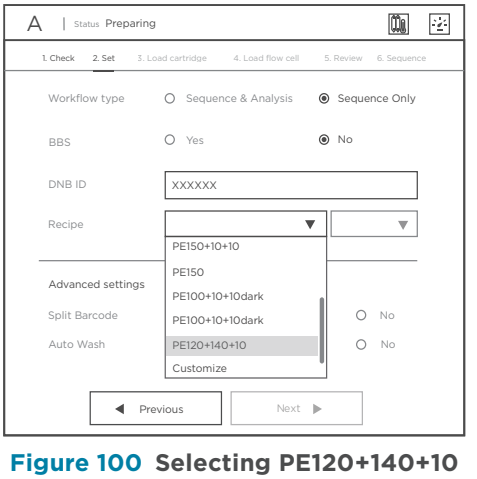

Figure 100 Selecting PE120+140+10

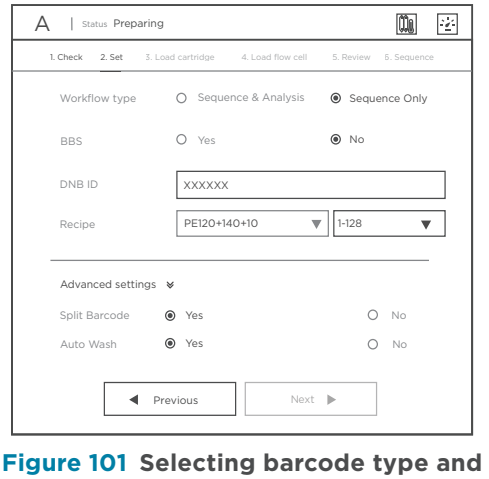

Figure 101 Selecting barcode type and split strategy for example 1

2. Length of the single barcode is not 10

Assumptions are as follows:

- Sequencing run: PE150+8
- Length of read1: 150
- Length of read2: 150
- Length of barcode: 8
- Length of Dualbarcode: 0
- Split barcode: Yes
- Total cycles = 150+150+8+2 = 310

- Select a PE150 set

The Customize interface is set as follows:

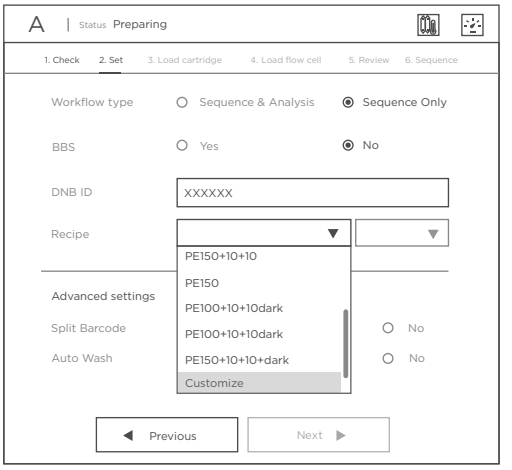

Figure 102 Selecting Customize

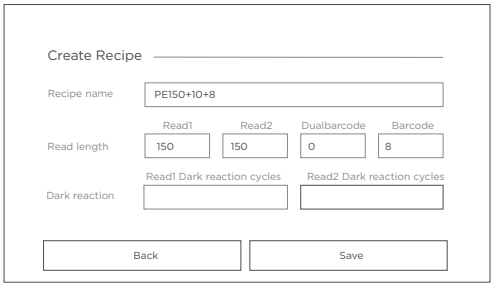

Figure 103 Configuring customize settings for example 2

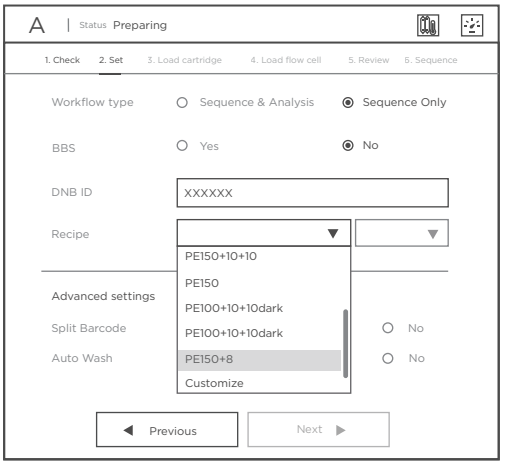

Figure 104 Selecting PE150+8

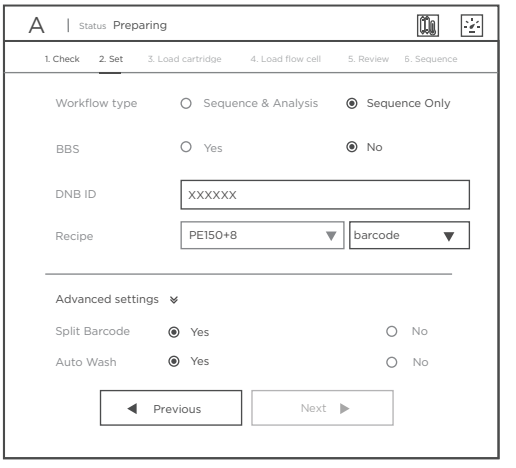

Figure 105 Selecting barcode type and split strategy for example 2

3. A dual barcode sequencing run

Assumptions are as follows:

- Sequencing run: PE150+8+10
- Length of read1: 150
- Length of read2: 150
- Length of barcode: 10
- Length of Dualbarcode: 8
- Split barcode: Yes

- Total cycles = 150+150+8+10+2 = 320
- Select a PE150 set

The Customize interface is set as follows:

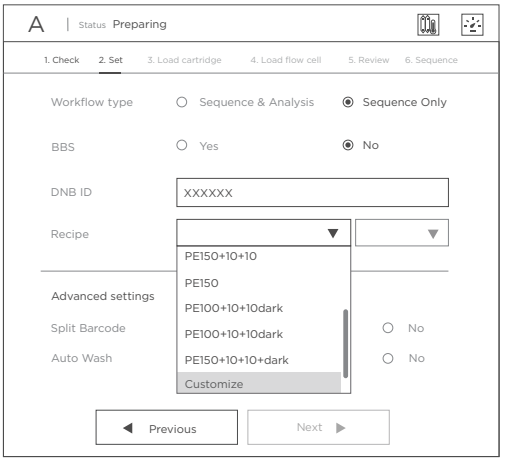

Figure 106 Selecting Customize

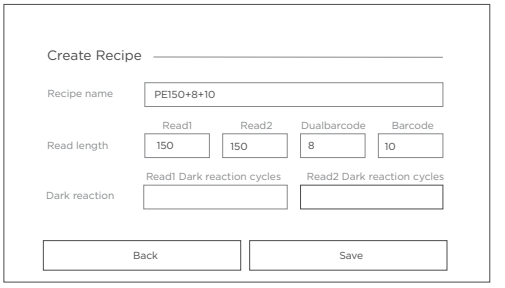

Figure 107 Configuring customize settings for example 3

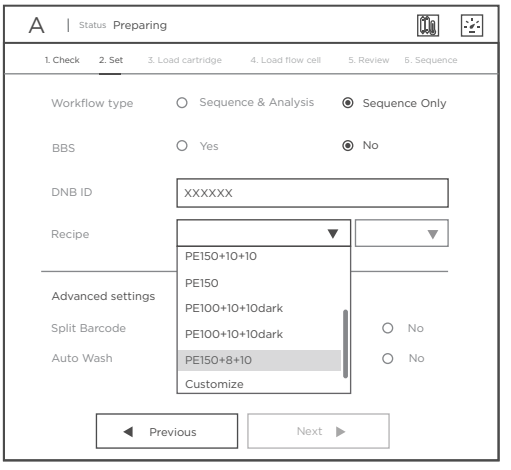

Figure 108 Selecting PE150+8+10

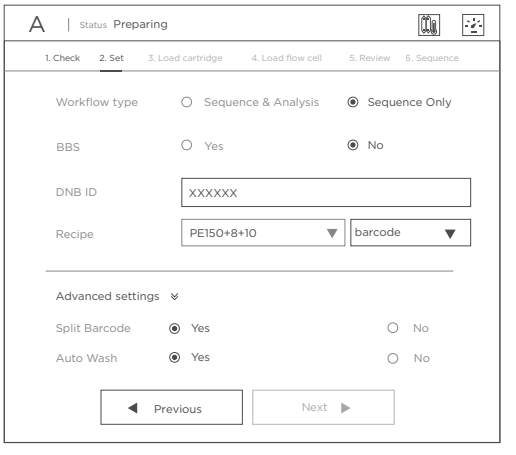

Figure 109 Selecting barcode type and split strategy for example 3

These parameters can be set in both sides. It is recommended that you use identical settings for the sequencing parameters in both sides.

4. Dark reaction cycles are required in read1 and/or read2 sequencing

Assumptions are as follows:

- Sequencing run: PE150+8+8
- Length of read1: 150

- Length of read2: 150
- Length of barcode: 8
- Length of Dualbarcode: 8
- Dark cycles: From cycle-2 to cycle-10, cycle-22 to cycle-30 in read1 and cycle-16 to cycle-20, cycle-30 to cycle-40 in read2.
- Total cycles = 150+150+8+8 +2= 318
- Select a PE150 set

The Customize interface is set as follows:

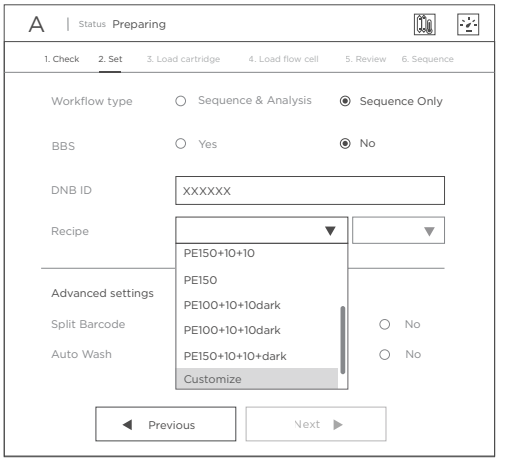

Figure 110 Selecting Customize

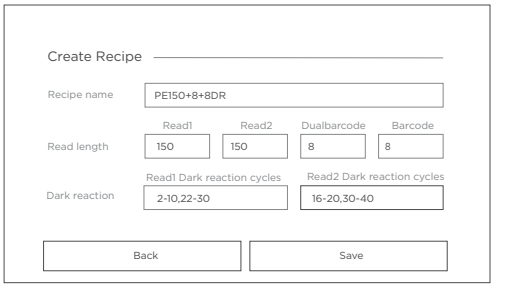

Figure 111 Configuring customize settings for example 4

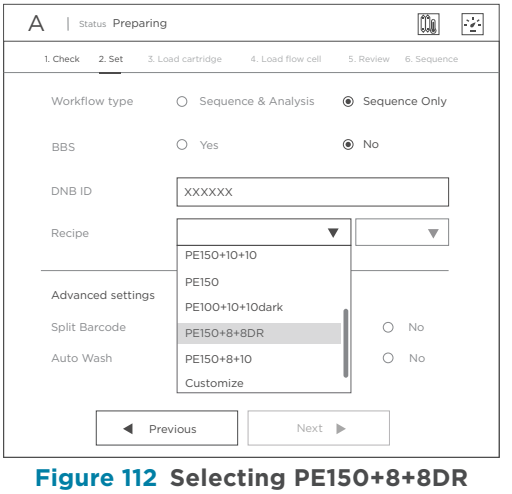

Figure 112 Selecting PE150+8+8DR

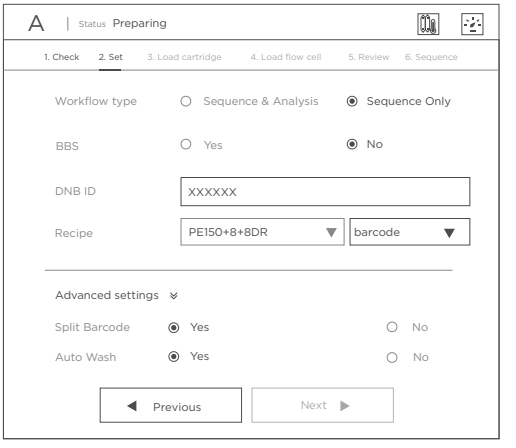

Figure 113 Selecting barcode type and split strategy for example 4

Instructions for using Qubit to quantify the DNBs

- Working solution should be used within 30 minutes after preparation.
- Avoid touching the wall of tapered detection tubes.
- Avoid introducing bubbles in detection tubes.

Perform the following steps:

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

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

   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 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 then 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 (Cat. 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:

Table 41 Working solution

	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	/	/	/

	S1 (µL)	S2 (µL)	D1 (µL)	D2 (µL)	D3 (µL)
Sample (µL)	/	/	2	2	2
Total volume	200	200	200	200	200

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

Instructions for splitting barcode

Manual barcode splitting

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

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

C:\BasecallLite\Config

This section uses the following conditions as examples:

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

Preconditions:

- SubmitImages = false
- CalFilePath is set correctly:

  D:\Result\workspace\FT10000XXXX\L02\calFile

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

Table 42 Splitting Barcode and Dualbarcode

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

Table 43 Splitting Dualbarcode only

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

Parameter setting	Description
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 44 Splitting Barcode only

Parameter setting	Description
Cycle = r100e1r100e1b10b8	Input the complete sequencing recipe
BarcodeFile =	Input Barcodelist of 8 bp for splitting Barcode only
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, dualbarcode length, barcode length; if there is no extra one cycle for calibration, remove e1; barcode2 refer to Dualbarcode, if no Dualbarcode is needed, remove the barcode2 length.

Automatic barcode splitting

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

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

1. In the main interface, select **Sequence** to enter the DNB ID entry interface.
2. Select the **DNB ID** box, scan the QR code on the tube, or enter the DNB ID 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 information on customizing a run, refer to *Instructions for customizing a run on Page 125.*

4. Select one-click sequencing recipe from the **Recipe** list; for example, SE50. The sequencer will split the 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 125*.

This section uses the following conditions as examples:

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

Splitting Barcode and Dualbarcode

You can determine if the barcode is split successfully in the logs located 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 45 Expected parameter passing for splitting Barcode and Dualbarcode

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 Dualbarcode only

You can determine if the barcode is split successfully in the logs located 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 46 Expected parameter passing for splitting Dualbarcode 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, or, read length for DualBarcode
barcode1StartPos: 203	The first cycle of barcode that needs to be split, or, the first cycle of DualBarcode
barcode2Len: null	If you want to split Dualbarcode only, the value should be null
barcode2StartPos: null	If you want to split Dualbarcode 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 Barcode only

You can determine if the barcode is split successfully in the logs located 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 47 Expected parameter passing for splitting Barcode 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, or, read length for Barcode
barcode1StartPos: 213	The first cycle of barcode that needs to be split, or, the first cycle of Barcode
barcode2Len: null	If you want to split Barcode only, the value should be null
barcode2StartPos: null	If you want to split Barcode 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, a Sequencing Reagent Cartridge and reagents for sequencing.

Table 48 DNBSEQ-G99RS High-throughput Sequencing Set (FCL SE100/PE50)
Catalog number: 940-000904-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G99 Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
Low TE Buffer		100 µL/tube×1 tube		
Make DNB Buffer		20 µL/tube×1 tube		
Make DNB Enzyme Mix I		40 µL/tube×1 tube		
Make DNB Enzyme Mix II (LC)		13 µL/tube×1 tube		
Stop DNB Reaction Buffer		50 µL/tube×1 tube		
DNB Load Buffer II		50 µL/tube×1 tube		
Micro Tube 0.5 mL (Empty)		1 tube		
MDA Enzyme Mix		0.125 mL/tube×1 tube		
MDA Reagent		1.0 mL/tube×1 tube		
Puncher	/	1 EA		
Sequencing Reagent Cartridge	/	1 EA		

Table 49 DNBSEQ-G99RS High-throughput Sequencing Set (FCL PE150)
Catalog number: 940-000905-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G99 Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
Low TE Buffer		100 µL/tube×1 tube		
Make DNB Buffer		20 µL/tube×1 tube		
Make DNB Enzyme Mix I		40 µL/tube×1 tube		
Make DNB Enzyme Mix II (LC)		13 µL/tube×1 tube		
Stop DNB Reaction Buffer		50 µL/tube×1 tube		
DNB Load Buffer II		50 µL/tube×1 tube		
Micro Tube 0.5 mL (Empty)		1 tube		
MDA Enzyme Mix		0.125 mL/tube×1 tube		
MDA Reagent		1.0 mL/tube×1 tube		
Puncher	/	1 EA		
Sequencing Reagent Cartridge	/	1 EA		

Table 50 DNBSEQ-G99RS High-throughput Sequencing Set (App-C FCL SE100)
Catalog number: 940-000906-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G99 Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
Low TE Buffer		100 µL/tube×1 tube		
App-C Make DNB Buffer		20 µL/tube×1 tube		
Make DNB Enzyme Mix I		40 µL/tube×1 tube		
Make DNB Enzyme Mix II (LC)		13 µL/tube×1 tube		
Stop DNB Reaction Buffer		50 µL/tube×1 tube		
DNB Load Buffer II		50 µL/tube×1 tube		
Micro Tube 0.5 mL (Empty)		1 tube		
Puncher	/	1 EA		
Sequencing Reagent Cartridge	/	1 EA		

Table 51 DNBSEQ-G99RS High-throughput Sequencing Set (App-C FCL PE150)
Catalog number: 940-000907-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G99 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C
Low TE Buffer		100 µL/tube×1 tube		
Make DNB Buffer		20 µL/tube×1 tube		
Make DNB Enzyme Mix V		40 µL/tube×1 tube		
Make DNB Enzyme Mix II (LC)		13 µL/tube×1 tube		
Stop DNB Reaction Buffer		50 µL/tube×1 tube		
DNB Load Buffer II		50 µL/tube×1 tube		
Micro Tube 0.5 mL (Empty)		1 tube		
MDA Enzyme Mix		0.125 mL/tube×1 tube		
MDA Reagent		1.0 mL/tube×1 tube		
Puncher	/	1 EA		
Sequencing Reagent Cartridge	/	1 EA		

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 experiment results, it is recommended that you install an air conditioning system and a humidifier or dehumidifier in the laboratory to maintain the temperature and humidity.

Item	Description	
Laser classification of the device	Class 1 laser product	
EMC	Class A	
Power	Supply voltage	100-240 V~, 50/60 Hz
	Rated power	1000 VA
Dimensions	607 mm (L) × 680 mm (W) × 640 mm (H) (24 inches × 27 inches × 25 inches)	
Net weight	Approximately 140 kg (308 lb)	
Auto-sliding screen	Type	LCD
	Size	21.5 inches (54.6 cm)
	Resolution	1920 × 1080 pixels
Fuse specification	T10AH250V	
Maximum sound pressure level	75 dBA	
Lab bench bearing capacity	300 kg/m²	

Item	Description	
Operating environment requirements	Altitude	3000 m
	Temperature	15 °C to 30 °C (59 °F to 86 °F)
	Relative humidity	20% to 80%, non-condensing
	Atmospheric pressure	70 kPa to 106 kPa
	Pollution degree	2
Transportation/ Storage environment requirements	Temperature	-20 °C to 50 °C (-4 °F to 122 °F)
	Relative humidity	15% to 85%, non-condensing
	Atmospheric pressure	70 kPa to 106 kPa
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

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Website	www.completegenomics.com

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Order information

Catalog number	Model	Name	Version	Recommended brand
900-000712-00	DNBSEQ-G99ARS	Genetic Sequencer DNBSEQ-G99ARS	V1.0	CG
900-000713-00	DNBSEQ-G99RS	Genetic Sequencer DNBSEQ-G99RS	V1.0	CG
940-000904-00	FCL SE100/PE50	DNBSEQ-G99RS High-throughput Sequencing Set	V1.0	CG
940-000905-00	FCL PE150	DNBSEQ-G99RS High-throughput Sequencing Set	V1.0	CG
940-000906-00	App-C FCL SE100	DNBSEQ-G99RS High-throughput Sequencing Set	V1.0	CG
940-000907-00	App-C FCL PE150	DNBSEQ-G99RS High-throughput Sequencing Set	V1.0	CG
940-000903-00	FCL	DNBSEQ-G99 Cleaning Reagent Kit	/	CG
900-000696-00	DL-G99	Portable DNB Loader	/	CG

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Acronyms and abbreviations

Item	Description
BBS	Bioanalysis By Sequencing
bp	Base-pair
BIC	Basecall Information Content
COM	Component Object Model
cPAS	Combinatorial Probe-anchor Synthesis
DL-G99	Portable DNB Loader
DNB	DNA Nanoball
EMC	Electromagnetic Compatibility
ESR	Effective Spots Rate
FAQ	Frequently Asked Questions
FCC	Federal Communications Commission
FCL	Flow Cell Large, 4 lanes per flow cell in DNBSEQ-G99 Sequencing FCL 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
HDMI	High Definition Multimedia Interface
IC	Interference-Causing
ID	Identification
LCD	Liquid Crystal Display
MDA	Multiple Displacement Amplification
PE	Pair-end sequencing
RCR	Rolling Circle Replication
RFID	Radio Frequency Identification
RHO	Rho (ρ), intensity of raw signals
SE	Single-end sequencing

Item	Description
ssDNA	single-stranded DNA
UPS	Uninterruptible Power Supply
USB	Universal Serial Bus
VGA	Video Graphics Array
WLAN	Wireless Local Area Networks
ZLIMS	ZTRON laboratory information management system

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