Complete GENOMICS™

DNBSEQ-G99RS & DNBSEQ-G99ARS System Guide

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

Complete Genomics, Inc.

Part No.: CSS-00038

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 High-throughput Sequencing Reagent Set. The guide version is Rev B and the software version is V1.4.0.

Revision history

	Date	Revision
Revision	December 05, 2025	B
Initial release	April 11, 2025	A

Contents

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

Packaging

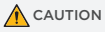
The following table describes symbols on the packaging or on the label of the packaging:

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

Symbol	Title	Description
	Do not stack	Indicates that stacking of the crated unit is prohibited and that 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 limits	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	Title	Description
	General warning sign	Signifies a general warning
	Warning; biological hazard	Biological hazard warning
	Caution; hot surface	Indicates that the marked item can be hot and should not be touched without taking proper safety precautions
	Warning; dangerous voltage	Indicates hazards arising from dangerous voltages

Symbol	Title	Description
	Protective earth	Indicates the terminal of a protective earth (ground) electrode
	Warning; laser beam	Warns of a hazard from laser beam Class 3B laser
	Warning; crushing of hands	Take care to avoid injury to hands when in the vicinity of equipment with closing mechanical parts
	"ON" (power)	Indicates connection to the main 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	Allows device debug

Labels

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

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

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

System guide

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

Symbol	Description
 DANGER	Indicates that the operator should operate the device according to the instructions in this guide. Failure to do so will result in death or serious injury

Symbol	Description
 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

General safety

 DANGER

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

WARNING

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

CAUTION

- Only the peripheral devices and consumables specified by the manufacturer can be used.
- If you have maintenance questions that are not mentioned in this guide, contact CG Technical Support.
- The device has been 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.
- One sequencing reagent kit is for one sequencing run only and cannot be reused.
- The components and packaging are batched separately. Keep the components in the packaging until use and do not remove them. Mixed use of reagent components from different batches of kits is not recommended.

Electrical safety

DANGER

- Ensure that the device is properly grounded, and the grounding resistance meets the requirements. Failure to do so may result in altered experiment 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. Doing so may cause electrical shock.

WARNING

- This equipment is not intended for use in residential environments and may not provide adequate protection to radio reception in such environments.
- 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.

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. This is accomplished through instrument-specific reagents, flow cells, imaging hardware, and data analysis software. The sequencing input is intended to be prepared as DNA Nanoball (DNB) libraries, which can be used for whole genome, whole exosome, and de novo sequencing.

Working principle

The device adopts the advanced DNA Nanoball (DNB) and the core technology of combinatorial probe-anchor synthesis (cPAS) 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:

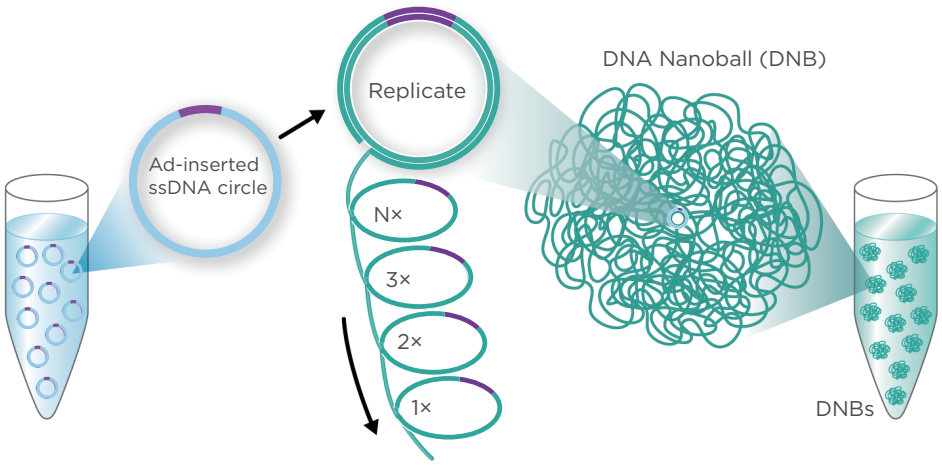


Figure 1 **Making DNBs**

The following figure demonstrates how to load DNBs:

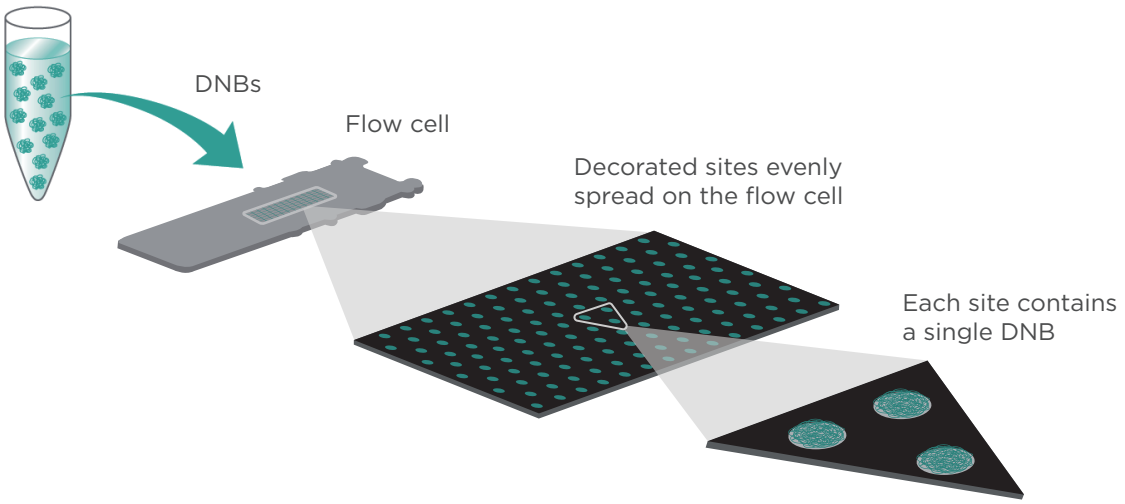


Figure 2 Loading DNBs

The sequencing reagents are pumped into the DNB-loaded 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 storage system, power supply system, display system, and server.

i The server and relevant bioinformation analysis function are available only to DNBSEQ-G99ARS, which is not available in the U.S. or Canada.

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.

Component	Description
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.
Bioinformatic analysis server	Performs bioinformation analysis.

Basic components

Front view

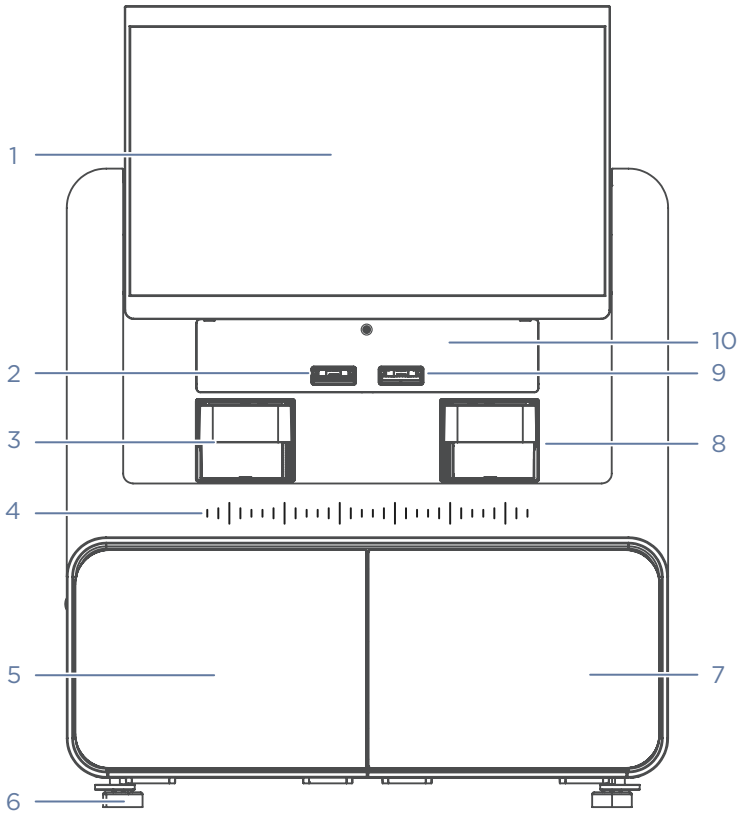


Figure 3 **Front view**

No.	Name	Description
1	Auto-sliding screen	Facilitates on-screen operation and displays information. The screen slides up and down at the touch of the on-screen controls. When the screen is moved up, 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.
3	Reagent compartment A	Holds the cartridge.

No.	Name	Description
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 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 compartment door B	Allows you to remove the waste container after the system automatically opens compartment door B.
8	Reagent compartment B	Holds the cartridge.
9	Flow cell stage B	Holds and moves Flow Cell B and controls the temperature of Flow Cell B.
10	Flow cell maintenance compartment door	Allows you to access the flow cell stage. To open the door, remove the M3 screw by using a hexagon wrench.

Back view

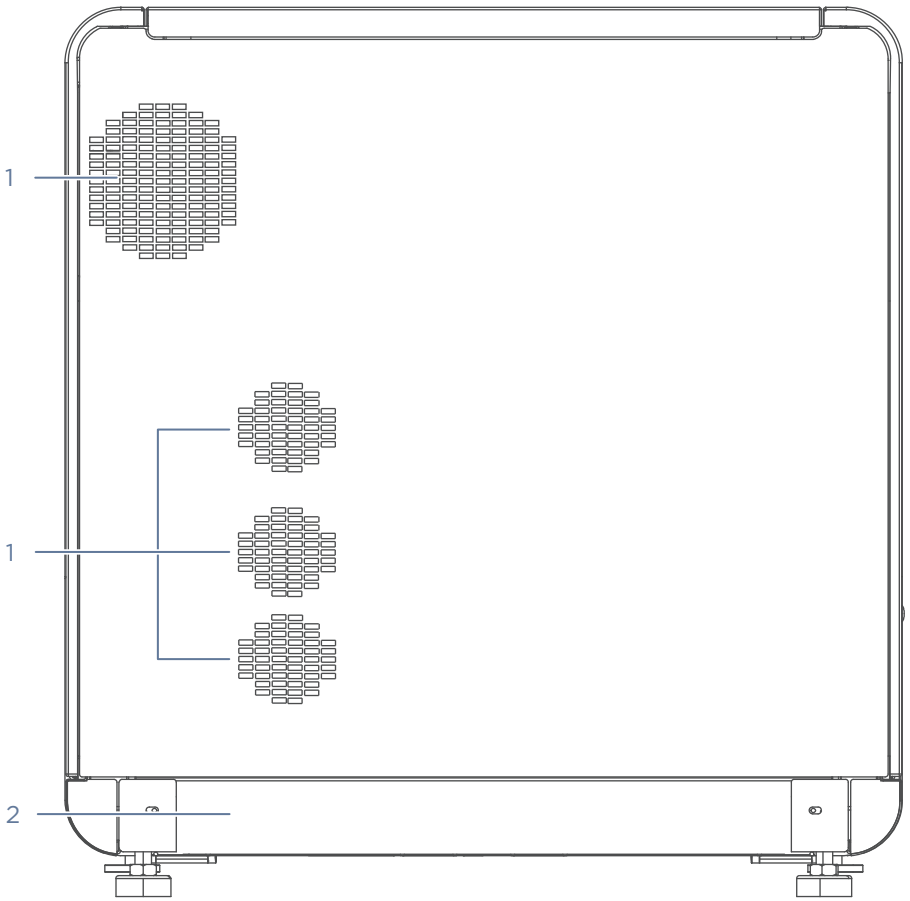


Figure 4 Back view

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

Left view

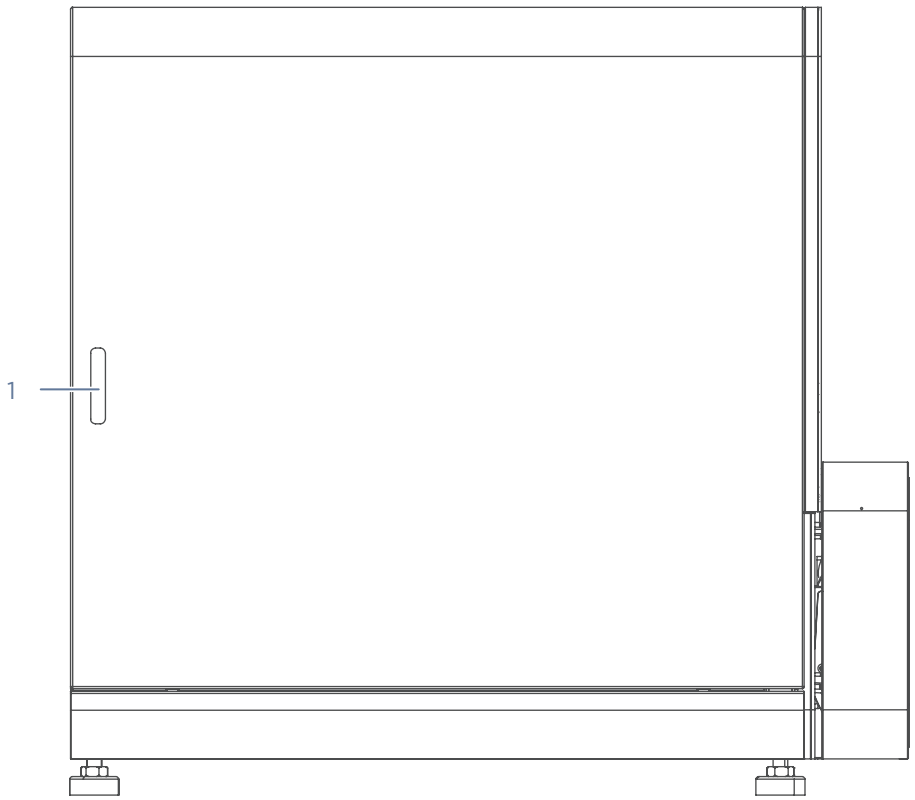


Figure 5 Left view

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

Right view

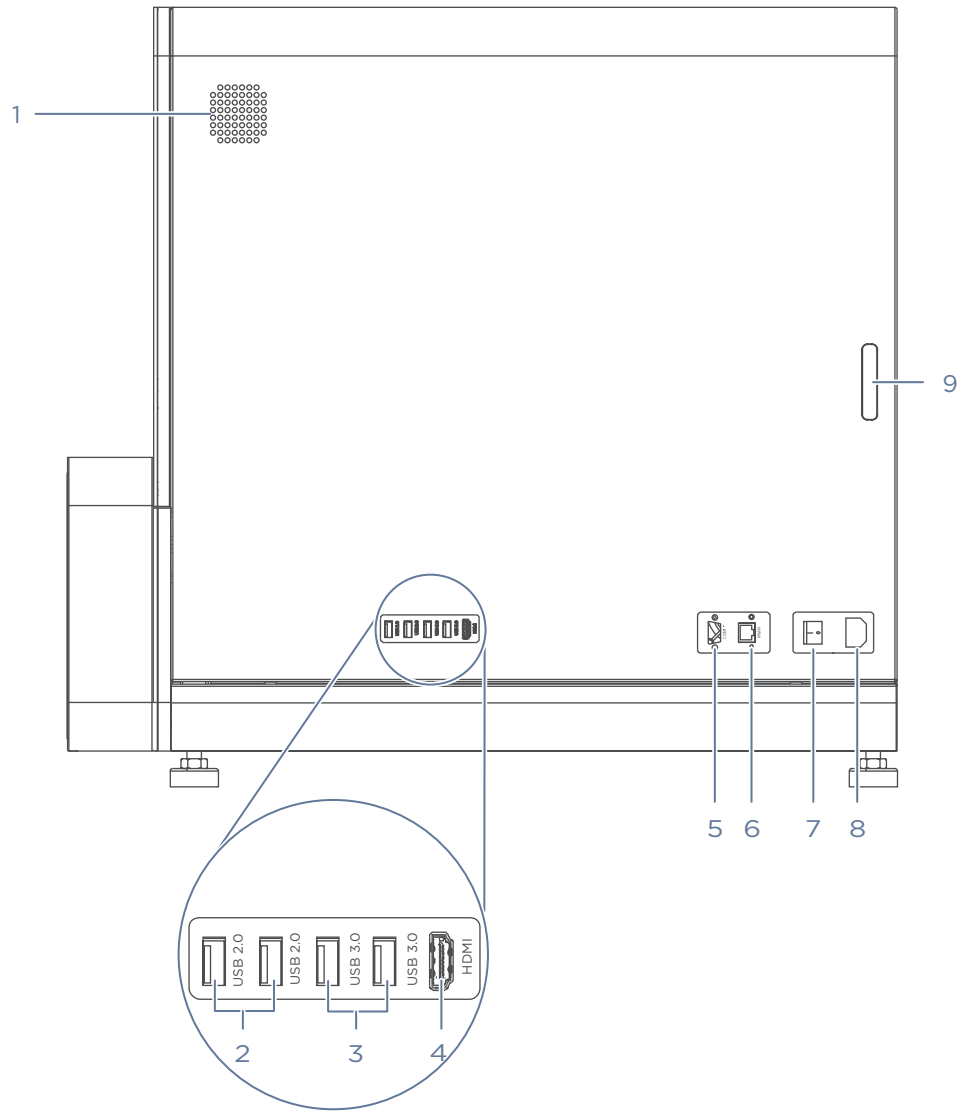


Figure 6 Right view

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

No.	Name	Description
1	Speaker	Provides sound.

No.	Name	Description
2	USB 2.0 port	Connects USB devices to the device.
3	USB 3.0 port	
4	HDMI port	Allows you to debug the device.
5	COM port	Indicates a cluster communication port
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	Checks whether the components of the system are functional.
Sequence	Performs different types of sequencing processes.
Wash	Performs wash and maintenance for fluidics lines in the system.

Main interface

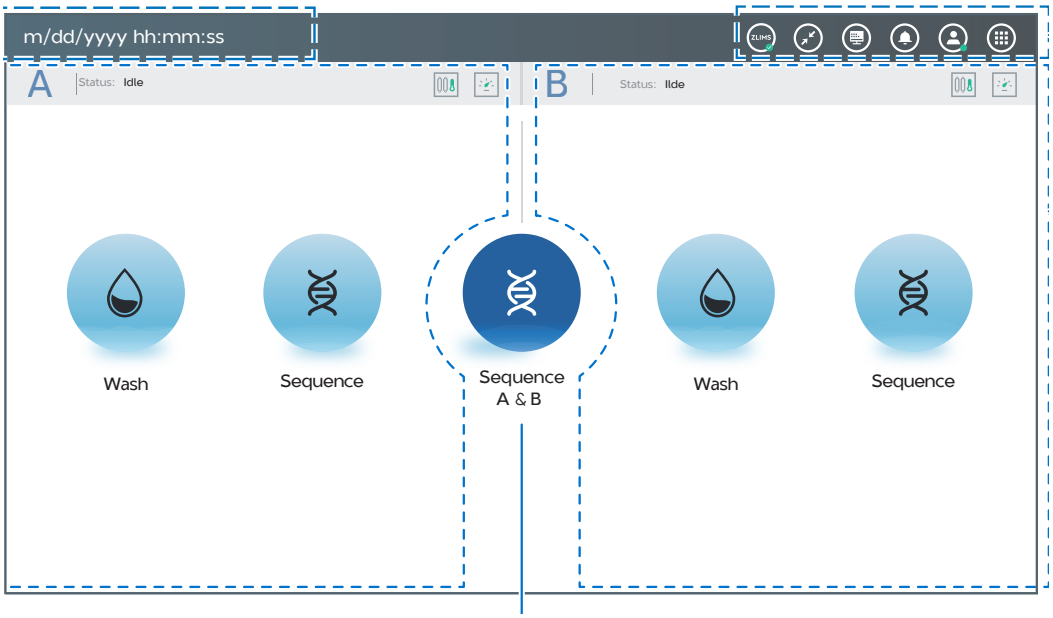


Figure 7 Main interface

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. For details, refer to *Menu area on Page 22*.

Operation area

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 that the negative pressure of the flow cell stage is normal.
	Indicates that the negative pressure of the flow cell stage is beyond the normal range.
	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 to set the sequencing parameters and perform sequencing.
Wash	Select to perform washing and the relevant operations by following the on-screen instructions. For details, refer to *Device maintenance on Page 117*.

Menu area

The following table describes control functions in the menu area:

Item	Description
	The device is running independently without being connected to the server that ZLIMS is installed on.
	The device is connected normally to the server on which ZLIMS is installed.
	An error has occurred with the connection to the server that ZLIMS is installed on.
	Select to minimize the control software.
	Sensor status indicator Select to check the status of sensors for all flow cell stages. The icon includes the following status: • Green: the device is running. • Yellow: an alarm appears in the device. • Orange: the device is abnormal.

Item	Description
	Select to view warnings, errors, or other abnormal information. The prompt icon includes the following status: • No color marking: the device is running. • Flashing yellow: a warning appears. • Flashing orange: 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 is connected normally to the Basecall.
	An error has occurred with the connection to the Basecall
	Indicates the device temperature is normal. The real-time value is displayed next to the icon.
	Indicates that a temperature alarm of the device appears. The real-time value is displayed tnext to the icon.
	Indicates the device humidity is normal. The real-time value is displayed next to the icon.
	Indicates that a humidity alarm of the device appears. The real-time value is displayed next to the icon.

Log interface

Select  > **Log** 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.
	Select to choose a time period and view the logs in the period.
Close	Select to exit the log interface and return to the previous interface.

Item	Description
<	Select to open the previous log page.
X/X	Displays the current page and the total pages of logs.
>	Select to open the next log page.

System settings interface

Select ⋮⋮⋮ > **Setting** 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 perform server settings.
Others	Select to change the wait time before the screen locks automatically. Move the slider to change the volume of the speaker.
Language	Select to change the language of the software. Changes take effect after you restart the system.
Save	Select to save the modifications.
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 sort the recipes in ascending or descending order by creation time.
Order	Select ▲ or ▼ to adjust the order of the recipes.
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 files.
misMatch1	Displays the Barcode mismatch number in the barcode files.
misMatch2	Displays the DualBarcode mismatch number in the barcode files.
Import time	Select to sort the barcode files in ascending or descending order by import time.
Order	Select ▲ or ▼ to adjust the order of the barcode files.
Close	Select to exit the Barcode settings interface and return to the main interface.

Maintenance interface

Select ⠿ > **Maintenance** to maintain the system.

Tools

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

Item	Description
Check	Select **initialize & Check** in this interface to initialize and check the device without restarting the system.
Auto-sliding screen	Select to move the screen up and down.
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 fluidics

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

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

Upload file

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

Item	Description
Server Type	Select to choose the server type.
Flow cell ID	Select to choose the flow cell ID.
File Type	Select to choose the type of the result files to be uploaded.
Upload	Select to upload the file to the specified server.
Close	Select to exit the maintenance interface and return to the main interface.

Service life stat.

You can view the residual service life of the consumables. When the residual service life is less than 5%, and alarm is prompted in . Please contact technical support for maintainance.

The alarm prompt does not affect device operations.

Shut down or restart interface

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

To open the shut down or restart interface, select > **Shut down**.

About interface

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

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

Lock screen

If you need to perform other operations, log in to the software.

To log out of the software, select > **Lock screen**.

Sequencing interface

When sequencing has started, the sequencing interface appears.

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

Table 1 Sequencing interface description

Item	Description
A&B	Indicates flow cell A operation area or flow cell 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.
Load cartridge	Placing the Sequencing Reagent Cartridge phase.
Load flow cell	Placing the Flow Cell phase.
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.

Item	Description
	Select this button and select **Yes** in the pop-up dialog box to stop sequencing. The stop phase and whether resume sequencing is available is displayed in the Sequencing interface. For details about resuming a sequencing run, refer to *Q: What should I do if I want to resume a stopped sequencing run? on Page 141* **CAUTION** Sequencing cannot be resumed from all phases. Please operate with caution.
	After the first base is imaged, select this button to open the first base report.
	After the sequencing starts, select to check sequencing information or change the auto wash settings.
	After the sequencing is completed, select to view the sequencing results.
QC type	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.

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.

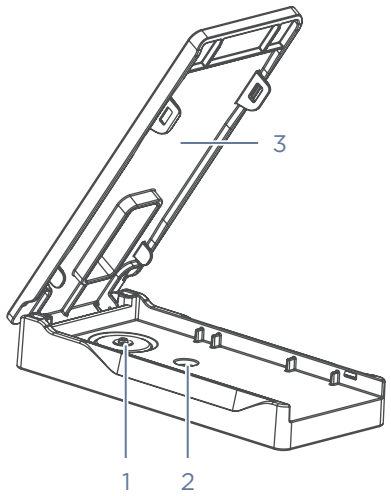


Figure 8 DL-G99 side view

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	Fastens the flow cell.

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03

Sequencing sets overview

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

Available sequencing sets

Table 2 Available sequencing sets

Cat. No.	Model	Name	Version
940-001874-00	App-D FCL SE100	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V2.0
940-002777-00	App-D FCU SE100	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V1.0
940-002648-00	App-D FCS PE150	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V1.0
940-001871-00	App-D FCL PE150	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V2.0
940-002781-00	App-D FCU PE150	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V1.0
940-001717-00	App-D FCL PE300	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V1.0
940-002774-00	App-D FCU PE300	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V1.0

- Sequencing Reagent Cartridges can be stacked for storage. It is recommended that the number of stacked layers do not exceed three.
- App-D FCL/FCU SE100 sequencing set can perform SE35, SE50 and SE100 sequencing.
- App-D FCS/FCL/FCU PE150 sequencing set can perform PE50, PE150 and PE100 sequencing.
- If third-party library preparation kits are used, contact CG Technical Support for conversion options.
- App-D applies to CG and third-party libraries (TruSeq and Nextera libraries).
- PE means Paired-end sequencing; SE means Single-end sequencing.

List of sequencing set components

A sequencing set includes a Sequencing Flow Cell, a Sequencing Reagent Cartridge and reagents for sequencing.

Table 3 DNBSEQ-G99RS High-throughput Sequencing Reagent Set V2.0 (App-D FCL SE100)
Cat. No.: 940-001874-00

Component	Cap color	Spec.&quantity	Storage temperature	Transportation temperature	Expiration date
DNBSEQ-G99 Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C	as stated on label
Low TE Buffer		100 µL/tube×1 tube			
App 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			
Microcentrifuge Tube 0.5 mL (Empty)		1 tube			
Puncher	/	1 EA			
Sequencing Reagent Cartridge V2.0	/	1 EA			

Table 4 DNBSEQ-G99RS High-throughput Sequencing Reagent Set V2.0 (App-D FCU SE100)
Cat. No.: 940-002777-00

Component	Cap color	Spec.&quantity	Storage temperature	Transportation temperature	Expiration date
DNBSEQ-G99 FCU Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C	as stated on label
Low TE Buffer		100 µL/tube×1 tube			
App 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			
Microcentrifuge Tube 0.5 mL (Empty)		1 tube			
Puncher	/	1 EA			
Sequencing Reagent Cartridge V2.0	/	1 EA			

Table 82 DNBSEQ-G99RS High-throughput Sequencing Reagent Set V2.0 (App-D FCU PE150)
Cat. No.: 940-002781-00

Component	Cap color	Spec.&quantity	Storage temperature	Transportation temperature	Expiration date
DNBSEQ-G99 FCUL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C	as stated on label
Low TE Buffer		100 µL/tube×1 tube			
App 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			
Microcentrifuge 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 V2.0	/	1 EA			

Table 5 DNBSEQ-G99RS High-throughput Sequencing Reagent Set V2.0 (App-D FCS PE150)
Cat. No.: 940-002648-00

Component	Cap color	Spec.&quantity	Storage temperature	Transportation temperature	Expiration date
DNBSEQ-G99 FCS Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C	as stated on label
Low TE Buffer		100 µL/tube×1 tube			
App 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			
Microcentrifuge 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 V2.0	/	1 EA			

Table 83 DNBSEQ-G99RS High-throughput Sequencing Reagent Set (App-D FCU PE300)
Cat. No.: 940-002774-00

Component	Cap color	Spec.&quantity	Storage temperature	Transportation temperature	Expiration date
DNBSEQ-G99 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C	as stated on label
Low TE Buffer		100 µL/tube×1 tube			
App Make DNB Buffer		40 µL/tube×1 tube			
Make DNB High-efficiency Enzyme Mix V		80 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		60 µL/tube×1 tube			
Microcentrifuge 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 84 DNBSEQ-G99 Cleaning Reagent Kit
Cat. No.: 940-000903-00

Component	Spec.&quantity	Storage temperature	Transportation temperature	Expiration date
Washing Cartridge	1 EA	0 °C to 30 °C	Below 40 °C	as stated on label

Table 6 DNBSEQ-G99RS High-throughput Sequencing Reagent Set V2.0 (App-D FCL PE150)
Cat. No.: 940-001871-00

Component	Cap color	Spec.&quantity	Storage temperature	Transportation temperature	Expiration date
DNBSEQ-G99 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C	as stated on label
Low TE Buffer		100 µL/tube×1 tube			
App 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			
Microcentrifuge 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 V2.0	/	1 EA			

Table 7 DNBSEQ-G99RS High-throughput Sequencing Reagent Set (App-D FCL PE300)
Cat. No.: 940-001717-00

Component	Cap color	Spec.&quantity	Storage temperature	Transportation temperature	Expiration date
DNBSEQ-G99 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C	as stated on label
Low TE Buffer		100 µL/tube×1 tube			
App Make DNB Buffer		40 µL/tube×1 tube			
Make DNB High-efficiency Enzyme Mix V		80 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		60 µL/tube×1 tube			
Microcentrifuge 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 8 DNBSEQ-G99 Cleaning Reagent Kit
Cat. No.: 940-000903-00

Component	Spec.&quantity	Storage temperature	Transportation temperature	Expiration date
Washing Cartridge	1 EA	0 °C to 30 °C	Below 40 °C	as stated on label

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 9 Sequencing cycle

Sequencing read length	Read1 length	Read2 length	Barcode read length	DualBarcode read length	Maximum cycles
SE100	100	0	10	10	132
PE150	150	150	10	10	332
PE300	300	300	10	10	632

- 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 DualBarcode read length is 10, plus 1 correction cycle for Read1 and 1 correction cycle for Read2 (barcodes do not require correction). The maximum cycle number of this sequencing is 322.
- Among the maximum cycles of each sequencing, the additional 10 cycles are 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 want to resume a stopped sequencing run? on Page 141*.
  - For information on examples of customized run, refer to *Examples of customized runs on Page 182*.

Sequencing time

Table 10 Average sequencing time and analysis time for each read length (h)

	Type		Single flow cell	Dual flow cells	Data Analysis (Single flow cell)	Data analysis (Dual flow cells)
Read length	FCL	SE35	2.2	2.4	0.05	0.1
		SE50	2.6	2.8	0.05	0.1
		SE100	4.2	4.4	0.05	0.1
		PE50	5.2	5.4	0.05	0.1
		PE100	8.2	8.4	0.05	0.1
		PE150	11.8	12.0	0.05	0.1
		PE300	27.5	28	0.1	0.2
	FCS	PE150	10.5	10.8	0.05	0.1
	FCU	SE100	4.9	5.5	0.05	0.1
		PE150	15.0	15.9	0.05	0.1
		PE300	32.5	32.8	0.1	0.2

- DNBSEQ-G99RS FCL Sequencing Flow Cell, also referred to as FCL, only has one lane that can output 80 M raw reads. DNBSEQ-G99RS FCS Sequencing Flow Cell, also referred to as FCS, only has one lane that can output 40 M raw reads. DNBSEQ-G99RS FCU Sequencing Flow Cell, also referred to as FCU, only has one lane that can output 200 M raw reads.
- Any two of the three flow cells (FCS, FCL, FCU) can be loaded simultaneously..
- The sequencing time (Single flow cell/Dual flow cells) in the table above includes the time required from loading through sequencing completion. The data analysis time includes the time required for barcode demultiplexing (if **Split Barcode** is selected) and generation of the FASTQ output files when sequencing is completed.
- The time in the table above is measured for single barcode sequencing.
- The time in the table above is the average value. The actual run time may vary slightly among individual sequencers.

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04

Getting Started

This chapter describes sequencing preparation.

User-supplied equipment and consumables

Before using the device, prepare the following equipment:

Table 11 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 12 Recommended reagents/consumables

Reagent/Consumable	Recommended brand	Purpose
2 M NaOH	General lab supplier	Diluting to 0.1 M for washing reagents
Sterile pipette tips (various types)	General lab supplier	For pipetting during dilutions, loading the wash and loading reagents, and so on
Sterile 200 µL wide-bore, non-filtered pipette tips	AXYGEN, Cat. No.: T-205 WB-C	Mixing DNBs

Reagent/Consumable	Recommended brand	Purpose
Qubit ssDNA Assay Kit	Thermo Fisher	Library and DNB QC
Qubit Assay Tubes	Thermo Fisher	Library and DNB QC
Sterile PCR tubes (0.2 mL)	General lab supplier	Making DNB reaction mixture
Sterile microcentrifuge tubes (1.5 mL)	VWR, Cat. No. 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

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 may 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 rights of the system will be changed and the device may 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 upper- and lower-case letters, numbers, and symbols. The password should be changed 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 powering on, the login interface is displayed.
4. Select a user account and enter the corresponding password. The device will perform a self-check.

Table 13 Windows user accounts

Account type	User name	Password	Permission
User account	user	Password123	Modifying user's password
Administrator account	DNBSEQ-G99	CGftat!138	Adding users, deleting users, modifying user's password, and resetting user's password

- If the check is successful, the main interface is displayed. Proceed to the next step.
- If the check fails, perform the following steps:
  a. Log in to the control software.

     For details, refer to *Logging in to the control software on Page 47*.

  b. Select > **Logs** to check the result in the logs.

  c. If any problems are identified, resolve them according to the on-screen instructions or *Sequencer FAQs on Page 150*.

  d. 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

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 the user name *user* and password *123*, select **Log in**.

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05

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

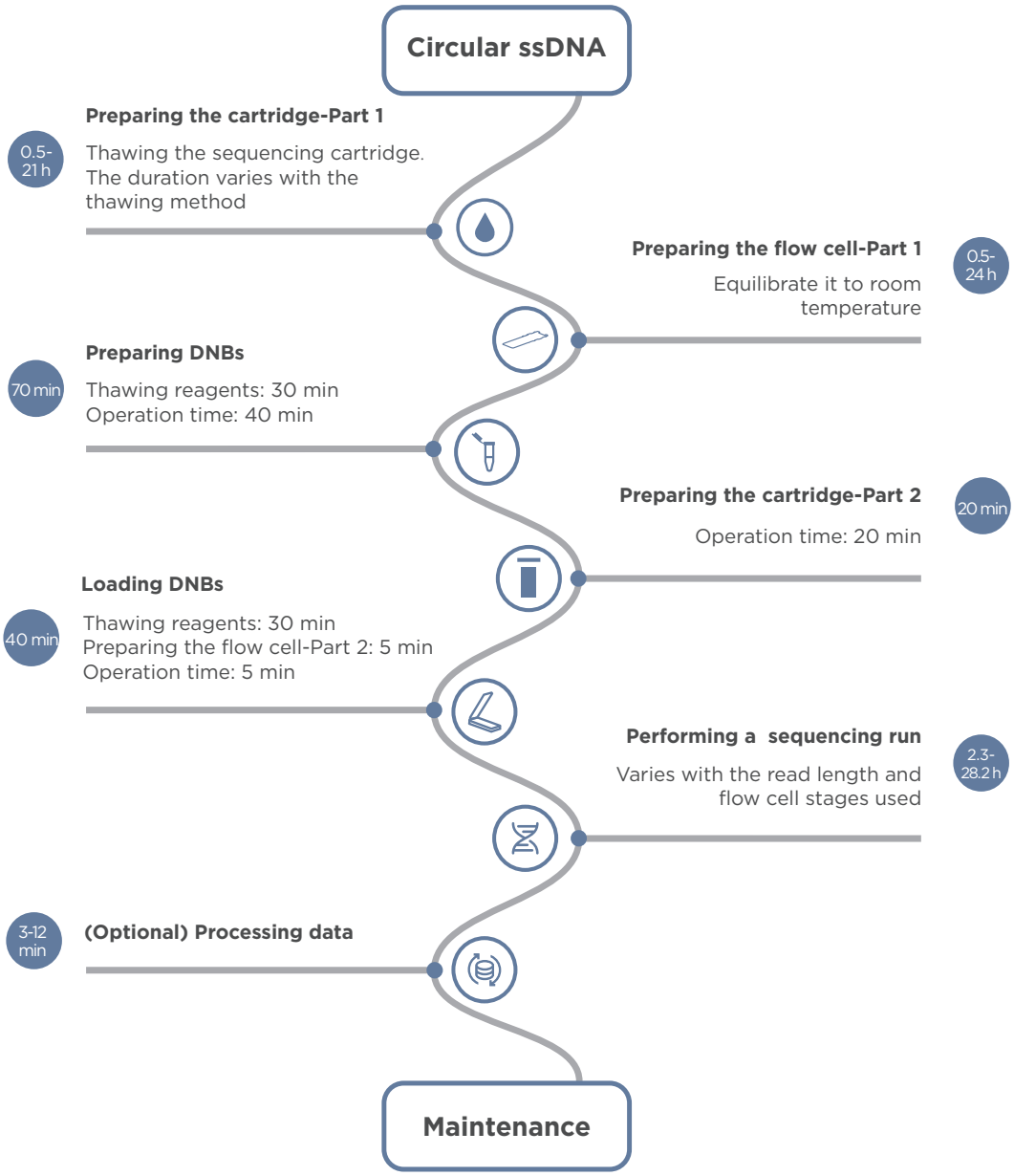

Figure 9 Sequencing workflow

The manual operation duration mentioned above is for reference only. The actual duration may vary with your proficiency level.

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

Preparing the Sequencing Reagent Cartridge-Part 1

Perform the following steps:

1. Remove the Sequencing Reagent Cartridge from the sequencing set.
   Do not open the outer plastic packaging at this moment.
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. For temporary storage, refer to Q: What rules should I follow if I need to store a reagent cartridge temporarily? on Page 147

Table 14 Approximate thaw time for various models

Model	Method		
	Water bath at room temperature (h)	Refrigerator at 2 °C to 8 °C overnight then water bath at room temperature (h)	
		Refrigerator at 2 °C to 8 °C (h)	Water bath at room temperature (h)
App-D FCL SE100 App-D FCU SE100	2.0	8.0	0.5
App-D FCS PE150 App-D FCL PE150 App-D FCU PE150	3.0	14.0	0.5
App-D FCL PE300 App-D FCU PE300	4.5	21.0	0.5

Preparing the flow cell-Part 1

Perform the following steps:

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

   Do not open the outer plastic package at this moment.

2. Place the flow cell at room temperature for 30 min to 24 h.

Preparing DNBs

Recommended library insert size

This sequencing set is compatible with the libraries prepared using CG Library Prep Kits. If third-party library preparation kits are used, it is recommended that you use the following conversion kits:

Table 15 Third-party compatibility

Catalog Number	Name
940-000963-00	DNBSEQ Universal Library Conversion Kit
940-001648-00	DNBSEQ OneStep Library Conversion Kit (Third Party)

- Select sequencing sets according to the insert size and the required data output.
- The recommended size distribution of inserts ranges between 200 bp and 600 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, the requirements of the kit should be followed.

Table 16 Recommended library insert size

Model	Recommended library insert distribution (bp)
App-D FCL SE100 App-D FCU SE100	200 to 400
App-D FCS PE150 App-D FCL PE150 App-D FCU PE150	300 to 500
App-D FCL PE300 App-D FCU PE300	400 to 600

DNA library concentration and amount requirement

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

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

  N represents the number of nucleotides (average library length including the adapter) as determined by fragment size analysis. Typically, fragment size analysis is determined during library preparation.

- If there are any special requirements or specifications for the CG library preparation kit, the requirements of the kit should be followed.

Table 17 Circular ssDNA library concentration requirement

Library type	Library concentration
PCR libraries	≥2 fmol/μL
PCR-free libraries	≥3.75 fmol/μL
Third-party PCR libraries	≥3 fmol/μL
Third-party PCR-free libraries	≥3.75 fmol/μL

Third-party libraries refer to TruSeq or Nextera adapter libraries.

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 you use pipettes from suggested brands and catalog numbers. Using other brands may yield suboptimal results.

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

- *Making DNBs for App-D SE100 and App-D PE150 on Page 54.*
- *Making DNBs for App-D PE300 on Page 57*.

Making DNBs for App-D SE100 and App-D PE150

App Make DNB Buffer can be used to make DNBs for both CG and third-party libraries.

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. Remove the reagents from storage and thaw them according to the table below:

Table 18 Thawing reagents for making DNBs

Component	Cap color	Thawing method
Low TE Buffer		At room temperature for approximately 30 min
App Make DNB Buffer		
Stop DNB Reaction Buffer		
Make DNB Enzyme Mix I		On ice for approximately 30 min

3. After thawing, mix all the reagents for 5 s using a vortex mixer, 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 53*.

  - If there are any special requirements or specifications for the CG library preparation kit, 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 19 Required amount of ssDNA libraries

Library type	Volume (μL)
PCR libraries	V=20 fmol/C
PCR-free libraries	V=37.5 fmol/C
Third-party PCR libraries	V = 30 fmol / C
Third-party PCR-free libraries	V = 37.5 fmol / C

- Calculate the required volume of ssDNA library for each Make DNB reaction and fill it as *V* in *Make DNB reaction mixture 1 on Page 55*.

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 20 Make DNB reaction mixture 1

Component	Cap color	Volume (μL)
Low TE Buffer		10 - V
App 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 by using a vortex mixer. Centrifuge for 5 s and place it on ice until use.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown in the table below:

Table 21 Primer hybridization reaction conditions

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

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

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

Table 22 Make DNB Enzyme Mix II (LC)

Component	Cap color
Make DNB Enzyme Mix II (LC)	

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

Table 23 Make DNB reaction mixture 2

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

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

Table 24 RCR conditions

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

- When a reaction protocol is running, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating 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 by 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 h.

Table 25 Stop DNB Reaction Buffer

Component	Cap color
Stop DNB Reaction Buffer	

10. For the next step, refer to *Quantifying and pooling DNBs on Page 61*.

Making DNBs for App-D PE300

App Make DNB Buffer can be used to make DNBs for both CG and Thrid-party libraries.

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. Remove the reagents from storage thaw them according to the table below:

Table 26 Thawing reagents for making DNBs

Component	Cap color	Thawing method
Low TE Buffer		At room temperature for approximately 30 min
App Make DNB Buffer		
Stop DNB Reaction Buffer		
Make DNB High-efficiency Enzyme Mix V		On ice for approximately 30 min

3. After thawing, mix all the reagents for 5 s using a vortex mixer, 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 53*.

  - If there are any special requirements or specifications for the CG library preparation kit, 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 27 Required amount of ssDNA libraries

Library type	Volume (μL)
PCR libraries	V=20 fmol/C
PCR-free libraries	V=37.5 fmol/C
Third-party PCR libraries	V = 30 fmol / C
Third-party PCR-free libraries	V = 37.5 fmol / C

- Calculate the required volume of ssDNA library for each Make DNB reaction and fill it as *V* in *Make DNB reaction mixture 1 on Page 59*.

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 28 Make DNB reaction mixture 1

Component	Cap color	Volume (µL)
Low TE Buffer		10 - V
App 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 by using a vortex mixer. Centrifuge for 5 s and place it on ice until use.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown in the table below:

Table 29 Primer hybridization reaction conditions

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

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

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

Table 30 Make DNB Enzyme Mix II (LC)

Component	Cap color
Make DNB Enzyme Mix II (LC)	

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

Table 31 Make DNB reaction mixture 2

Component	Cap color	Volume (µL)
Make DNB High-efficiency Enzyme Mix V		20
Make DNB Enzyme Mix II (LC)		0.8

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

Table 32 RCR conditions

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

- When a reaction protocol is running, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating 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 take the tubes out of thermal cycler when the temperature reaches 4 °C. Add Stop DNB Reaction Buffer according to the table below. Mix gently by pipetting 8 times using a wide-bore, non-filtered pipette tip.

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

Table 33 Stop DNB Reaction Buffer

Component	Cap color
Stop DNB Reaction Buffer	

10. For the next step, refer to *Quantifying and pooling DNBs on Page 61*.

Quantifying and pooling DNBs

Quantifying DNBs

Perform the following steps:

1. When the make DNB process 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 in *Instructions for using Qubit to quantify the DNBs on Page 189*.

Table 34 DNB concentration standard

Model	DNB concentration
App-D FCL SE100 App-D FCU SE100 App-D FCS PE150 App-D FCL PE150 App-D FCU PE150	≥8 ng/µL
App-D FCL PE300 App-D FCU PE300	≥25 ng/µL

i If the concentration of libraries is lower than that specified in the table above, refer to *Q:What should I do if DNB concentration is low? on Page 136*.

2. If the concentration exceeds 40 ng/µL, the DNBs should be diluted to 20 ng/µL with Low TE Buffer.

i This requirement does not apply to App-D FCL PE300. or App-D FCU PE300

(Optional) Pooling DNBs

To balance the base proportion and improve sequencing quality, it is optional to pool DNBs when performing App-D PE300 sequencing.

Perform the following steps:

1. Make DNBs for the balanced library by using the following kit.

Table 35 Balanced library reagent

Kit name	Cat. No.
DNBSEQ ATOPlex E450 Dual Barcode Balanced Library Reagent	940-000637-00

2. Calculate the required DNB volume.

a in the following formulas represents the application library and *b* the balanced library.

$$V_a\ (\mu L) = \frac{84 \times C_b\ (ng/\mu L)}{4 \times C_b\ (ng/\mu L) + C_a\ (ng/\mu L)}$$

Figure 10 Calculating the DNB volume of the application library

$$V_b\ (\mu L) = 21 - V_a\ (\mu L)$$

Figure 11 Calculating the DNB volume of the balanced library

3. Pool the DNBs of the application library and balanced library.

   Use normal pipette tips to aspirate the required DNB volume of each library, and use wide-bore tips to mix.

Preparing the Sequencing Reagent Cartridge-Part 2

- Follow steps 1 through 5 to prepare the Sequencing Reagent Cartridge for SE sequencing.
- Follow steps 1 through 6 to prepare the Sequencing Reagent Cartridge for PE sequencing.
- The MDA mixture (MDA: Multiple Displacement Amplification) must be added into the MDA well if you perform PE sequencing. If the prepared reagent cartridges cannot used immediately, refer to *Q: What rules should I follow if I need to store a reagent cartridge temporarily? on Page 147*.
- A Sequencing Reagent Cartridge can be primed a maximum of 2 times.

Perform the following steps:

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

Firmly punch the wells to pierce the seal.

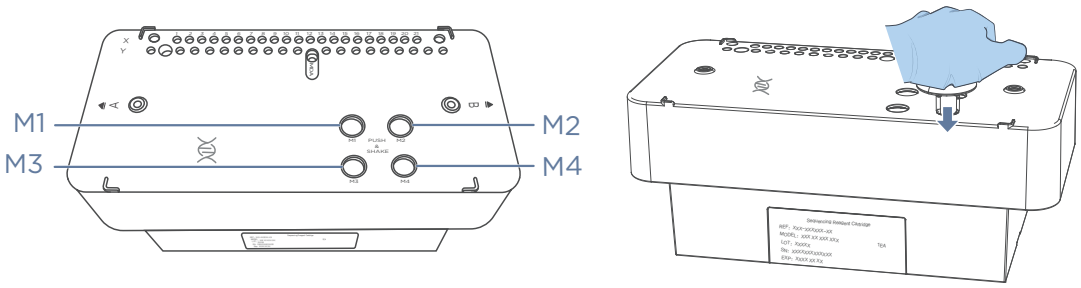

Figure 12 Piercing the M1, M2, M3, and M4 wells of the Sequencing Reagent Cartridge

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

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

5. Pierce the seal of the MDA well by using a clean 1 mL sterile pipette tip. The position of the MDA well is shown in *Figure 13 on Page 64* .

   The App-D FCL/FCU SE100 Sequencing Reagent Cartridge is now ready for use.

6. For PE sequencing, prepare and add reagent into the MDA well:

   1) Take out MDA Reagent and MDA Enzyme Mix from storage.
   2) Add 125 µL of MDA Enzyme Mix to the MDA Reagent tube using a 200 µL pipette.

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

   3) Invert the MDA Reagent tube 6 times to mix the reagents.
   4) Add the entire volume of the mixture into the MDA well.

- When adding the MDA mixture, keep the tip close to the concave side of the MDA well to avoid generating bubbles.
- Transfer the mixture carefully to prevent the mixture from spilling out of the reagent tube.
- The App-D FCS/FCL/FCU PE150 or App-D FCL/FCU PE300 Sequencing Reagent Cartridge is now ready for use.

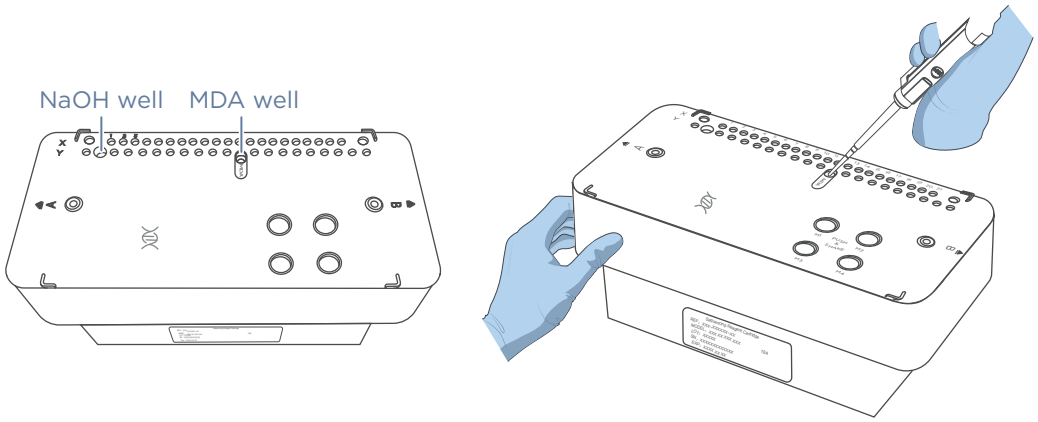

Figure 13 Adding MDA mixture

Performing a sequencing run

Checking before sequencing

Perform the following steps:

1. Select **Sequence** in operation area A or B according to your requirement. If both A and B are required, select **Sequence A&B**.

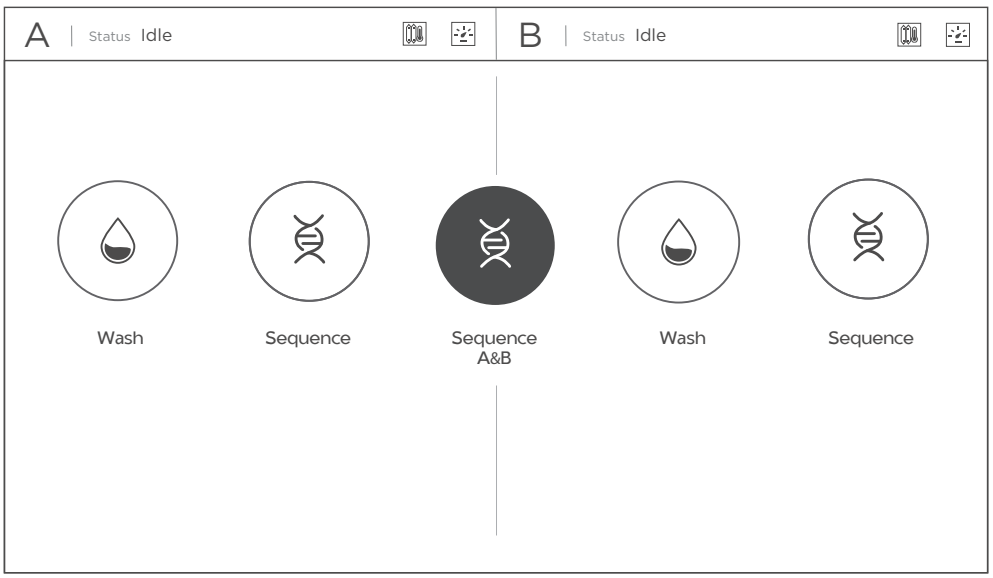

Figure 14 New sequence selection interface

2. The system automatically checks the disk, sensor, optical system, and incubation system one by one.

   - Ensure that the waste container is empty.
   - If any part of this check fails, refer to *Q: What should I do if error messages appear when the system is performing a check before sequencing? on Page 151.*

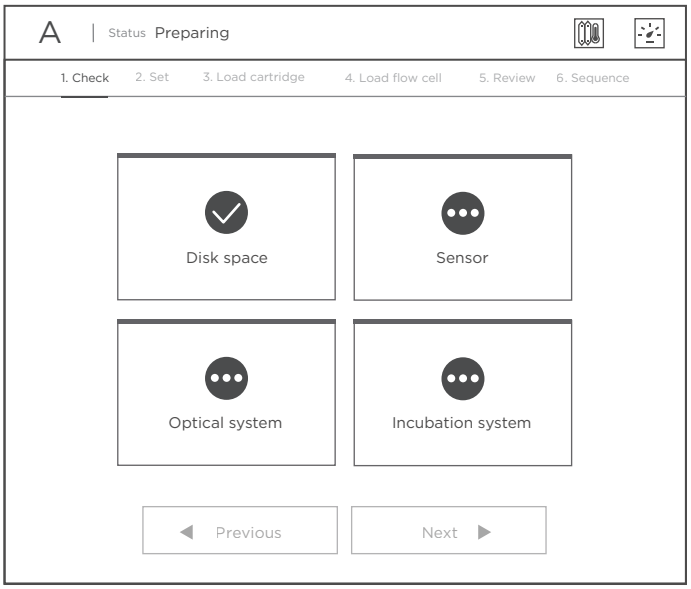

Figure 15 Check interface

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

(Optional) Inputting the DNB sample information

CAUTION If you need to perform bioinformatic analysis, ensure that ZLIMS is successfully connected, the sample information is input in ZLIMS, and the **Workflow type** is set to **Sequence** & **Transmission** when setting the sequencing parameters.

Perform the following steps:

1. Select ZLIMS in the menu area of the control software to open the login page of ZLIMS.
2. Enter the user name *lite* and password *lite123456* and select **Login**. The home page of ZLIMS lite is displayed.
3. Select **Sequencing + Analysis** to go to the corresponding page.
4. (Optional) Enter the task name.
5. Select the analysis product.
6. Input DNB sample information.

   You can input DNB sample information using one of the following methods:

   - Generating the sample ID

     Perform the following steps:

     a. Select **Generate the Sample ID** and select **New**. A pop-up window appears.

b. On the left of the pop-up window, input the DNB ID, and input or select the barcode. By default, the **Sample ID** is **DNB ID_Barcode**.

c. On the right of the pop-up window, fill in the sample attribute information, for example, select the sample type.

d. Select **Save and Close** to complete the current input step, or select **Save and Next** to start a new input step.

After completion, the New Sequencing + Analysis page returns to view.

- Importing the Sample ID

  Perform the following steps:

  a. Select **Import the Sample ID** and select **New**. A pop-up window appears.

  b. Select **Excel template** or **CSV template** to download the sample template in *.xlsx* or *.csv* format.

  c. Open the template and fill in the worksheet according to the template instructions.

  d. Return to the interface from which you downloaded the template. Select **Choose File** to upload the completed worksheet and select **Upload**.

  After completion, the New Sequencing + Analysis page returns to view.

7. Select **Save**, and then select **Ok** in the pop-up dialog box.

Setting the sequencing parameters

Choose one of the following workflow types:

Choose one of the following workflow types:

- **Sequence Only**: Refers to sequencing with the general script.
- **Sequence** & **Transmission**: After sequencing, the data is uploaded to the server for bioinformatic analysis.
- **BBS** (Bioanalysis by Sequencing): First the barcode sequencing is performed, and then the data in the specified node is uploaded for bioinformatic analysis

- The types of **Sequence** & **Transmission** and **BBS** can be performed on DNBSEQ-G99RS and DNBSEQ-G99ARS
- Ensure that the sequencing parameters are correct in this step.

For information on setting parameters, refer to:

- *Setting Sequence Only parameters on Page 68.*
- *Setting Sequence & Transmission parameters on Page 71.*
- *Setting BBS parameters on Page 73.*

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 by using the on-screen keyboard.

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

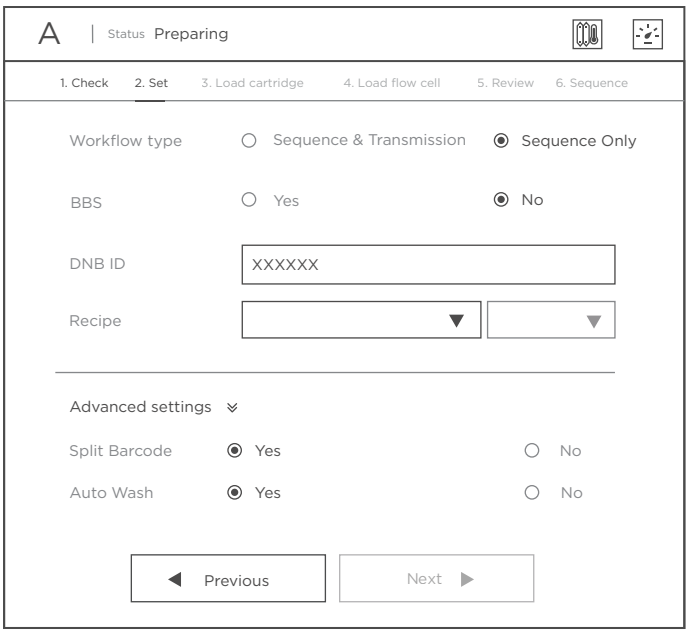

Figure 16 Selecting a workflow type

2. Select an appropriate sequencing recipe from the **Recipe** list. There are preset sequencing recipes as well as an option (**Customize**) to create a customized sequencing recipe.

   WARNING For PE300 sequencing, ensure that the insert size range is set correctly. Otherwise, the sequencing results will be adversely affected.

   - To view detailed information about a recipe, hover the cursor over the recipe in the **Recipe** list
   - For dual barcode sequencing and other recipes not in the recipe list (such as SE35, SE50, PE50, PE100, and so on), select **Customize** from the **Recipe** list. For information on customizing a recipe, refer to *Instructions for customizing a run on Page 179*.

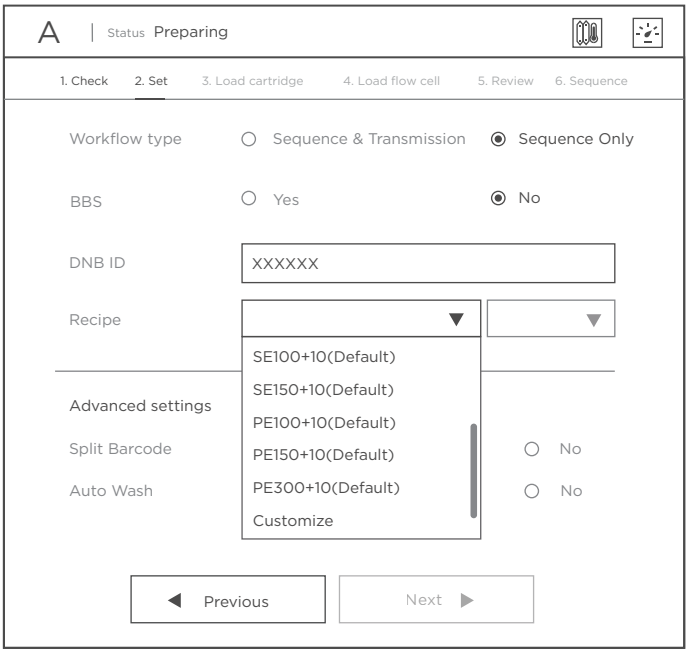

Figure 17 Selecting a sequencing recipe

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

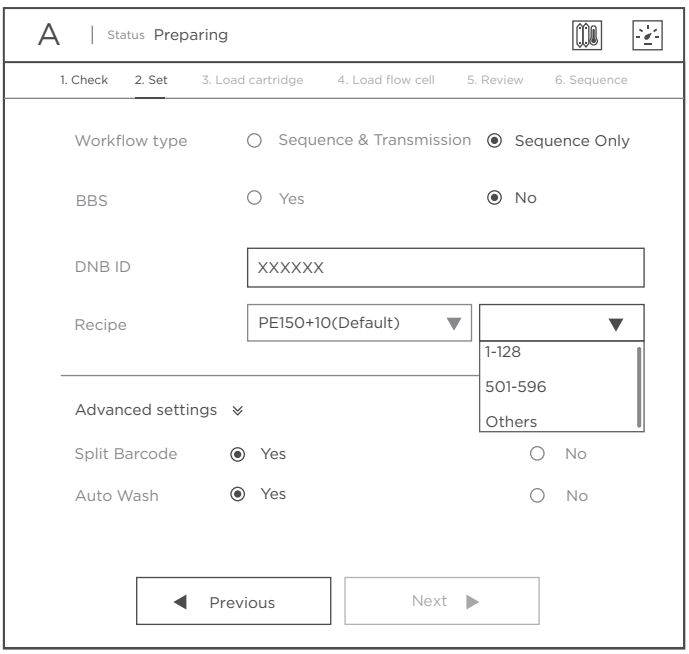

Figure 18 Selecting a barcode range

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.

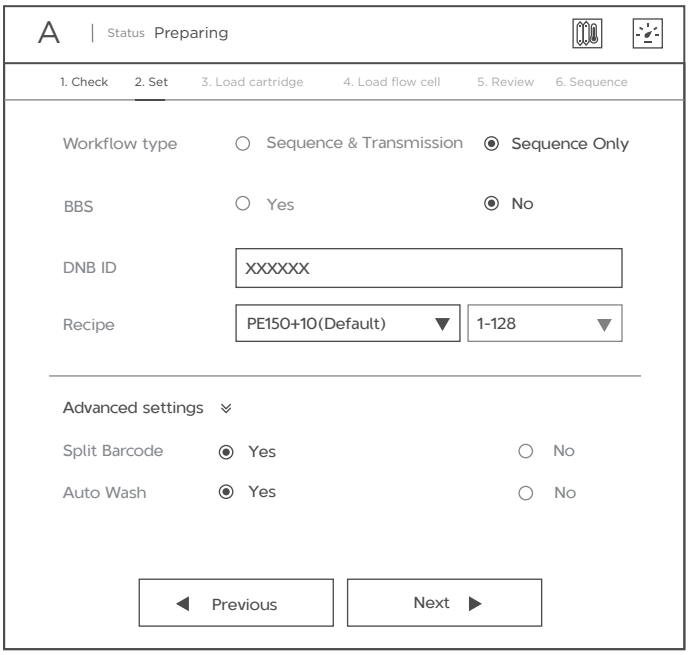

Figure 19 Advanced settings

5. Select **Next** and proceed to *Loading the sequencing cartridge on Page 75.*

Setting Sequence & Transmission parameters

Perform the following steps:

1. Select **Sequence & Transmission** workflow type. BBS will default to **No**. You can also set BBS to **Yes** and the BBS sequencing recipe will be displayed in the **Recipe** list.

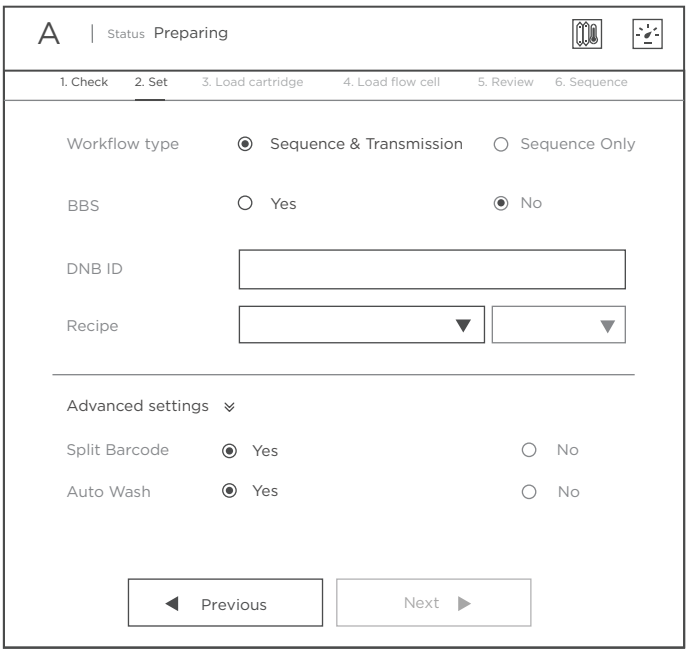

Figure 20 Sequence & Transmission workflow type

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

WARNING For PE300 sequencing, ensure that the insert size range is set correctly. Otherwise, the sequencing results will be adversely affected.

- To view detailed information about a recipe, hover the cursor over the recipe in the **Recipe** list
- For dual barcode sequencing and other recipes not in the recipe list (such as SE35, SE50, PE50, PE100, and so on), select **Customize** from the **Recipe** list. For information on customizing a recipe, refer to *Instructions for customizing a run on Page 179*.

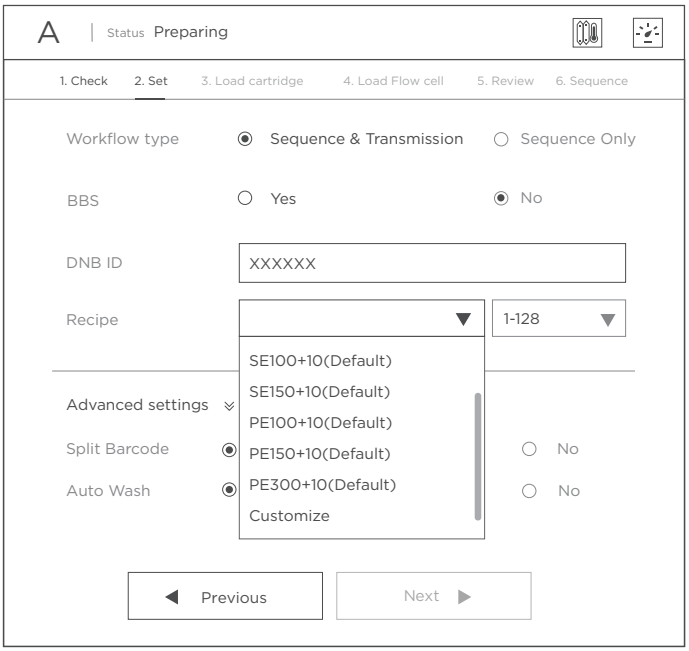

Figure 21 Entering DNB ID and selecting the sequencing recipe

For information on barcode sequence and advanced settings, refer to *Figure 18 on Page 69* and *Figure 19 on Page 70* .

3. Select **Next** and proceed to *Loading the sequencing cartridge on Page 75.*

Setting BBS parameters

Perform the following steps:

1. Select **Sequence & Transmission** for workflow type. Select **Yes** for BBS and fill in the BBS box next to **Yes** for data analysis. or example, for BBS PE100 sequencing, input **10,110**, the data analysis will be performed at the 10th cycle of Read1 and the 10th cycle of Read2.

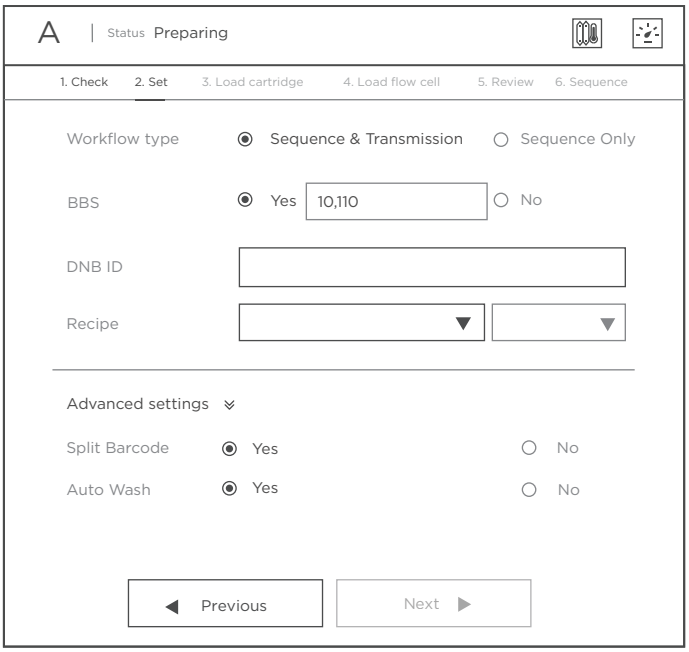

Figure 22 Selecting BBS sequencing type

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

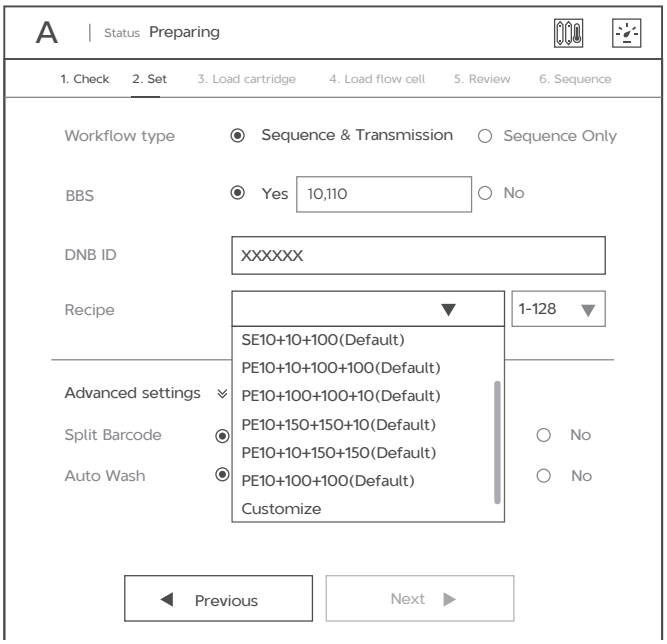

Figure 23 Entering DNB ID and selecting BBS recipe

For information on barcode range and advanced settings, refer to *Figure 18 on Page 69* and *Figure 19 on Page 70* .

3. Select **Next** and proceed to *Loading the sequencing cartridge on Page 75*.

Loading the sequencing cartridge

Perform the following steps:

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

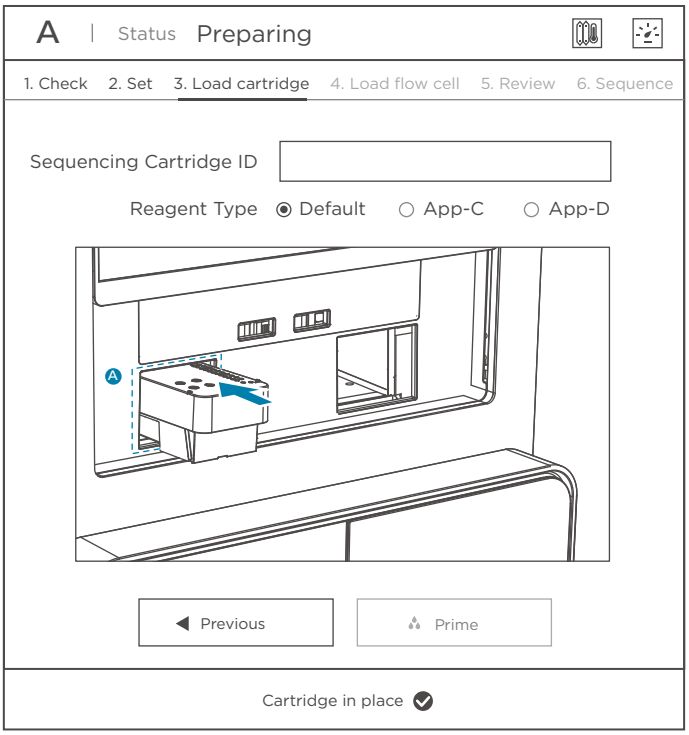

Figure 24 Loading the Sequencing Reagent Cartridge

The RFID (Radio Frequency Identification) scanner will automatically identify the cartridge ID and reagent type.

Enter the cartridge ID manually if the RFID scanner fails to identify the ID. The format of the sequencing cartridge ID is “REF-SN”.

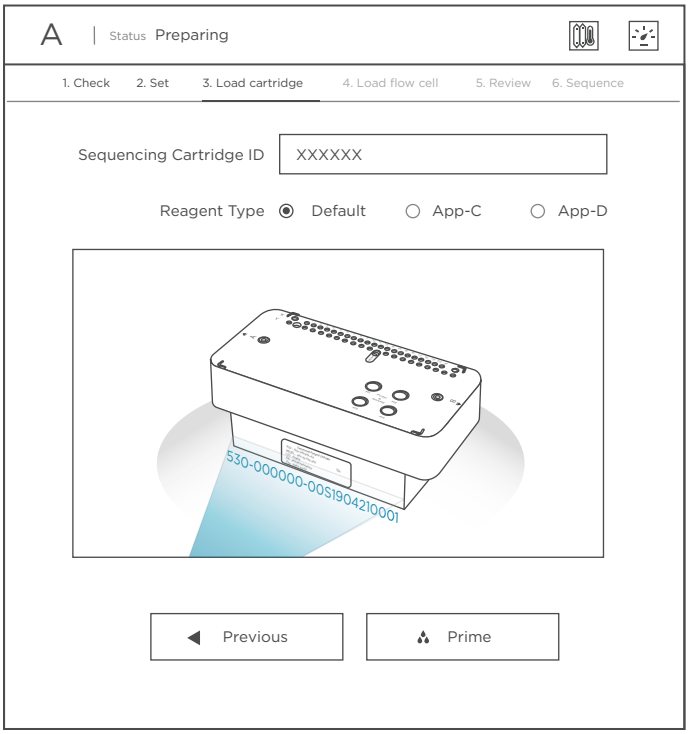

Figure 25 **Scanning Sequencing Reagent Cartridge ID**

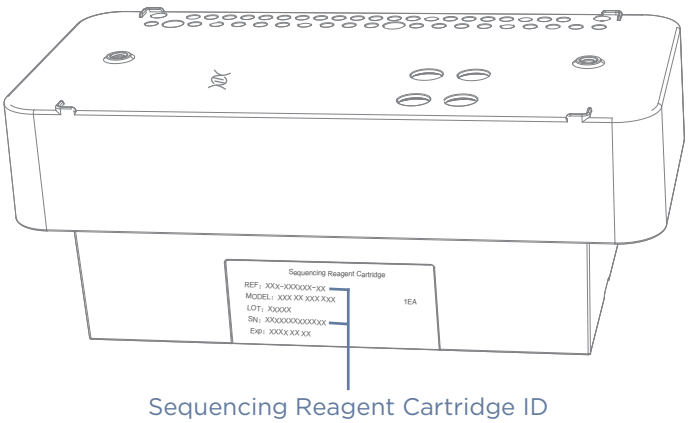

Figure 26 **Location of Sequencing Reagent Cartridge ID**

2. Select **Prime** > **Yes** to start priming. The priming process takes at least 3 min.

CAUTION If a returning to the main interface option is provided after priming but before sequencing starts, follow the on-screen instructions to resolve the issue before operation. Direct returning to the main interface may result in sequencing failure.

Figure 27 Confirming prime interface

Loading DNBs using DL-G99

Preparing reagents

Perform the following steps:

1. Remove DNB Load Buffer II from storage and thaw it on ice for approximately 30 min.

Table 36 DNB Load Buffer II

Component	Cap color
DNB Load Buffer II	

2. After thawing, mix the reagent for 5 s using a vortex mixer, centrifuge briefly, and place on ice until use.

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

3. Add the following reagents to the 0.5 mL microcentrifuge tube provided in the sequencing set:

Table 37 DNB loading mixture

Component	Cap color	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. Immediately load the mixture on the flow cell.

- Do not centrifuge, vortex, or shake the tube.
- Prepare a fresh DNB loading mixture immediately before the sequencing run.
- Load the appropriate volume of the DNB loading mixture according to the flow cell type.

Table 38 Loading volume by flow cell type

Flow cell type	Loading volume (µL)
FCL	10.0
FCS	10.0
FCU	13.0

Preparing the flow cell-Part 2

Perform the following steps:

1. Unwrap the outer plastic packaging before use.

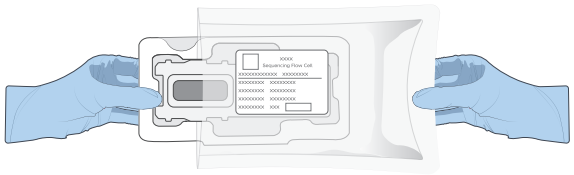

Figure 28 Unwrapping the outer plastic packaging

- If the flow cell is not used within 24 h after being placed at room temperature and the outer plastic packaging is intact, the flow cell can be returned to -25 °C to -15 °C for storage. But the switch between room temperature and -25 °C to -15 °C must not exceed 3 times.
- If the outer plastic packaging has been opened but the flow cell will not be used immediately, store the flow cell at room temperature and use within 24 h. It is not recommended that you use the flow cell after 24 h.

2. Take out the flow cell from the inner packaging and ensure that the flow cell is free from dirt, scratches, or debonding.
3. (Optional) Clean the back of the flow cell by using a canned air duster.

Loading DNBs

Perform the following steps:

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

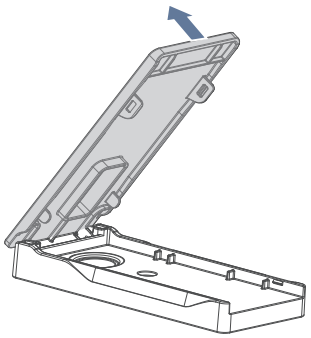

Figure 29 Opening the cover

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

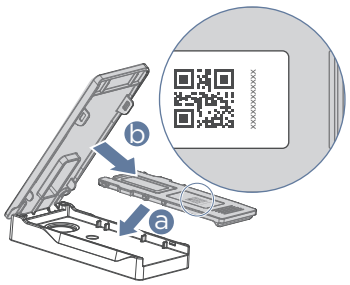

Figure 30 Placing the flow cell

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

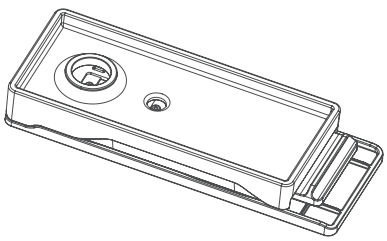

Figure 31 Placing the loader with back facing up

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

- Use a 200 µL non-filtered, sharp pipette tip instead of a wide-bore tip for this loading method.
- Do not tilt or rotate the tip.

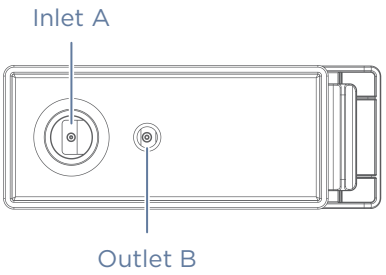

Figure 32 Inlet and outlet of loader

5. While holding the pipette tip in inlet A with one hand, press the tip ejector to unload the tip with the other hand. Observe the liquid level in the tip:
   - If the liquid level drops after ejecting the tip and the DNB loading mixture automatically flows into the flow cell, proceed to step 7.
   - If the liquid level does not drop and the DNB loading mixture does not flow into the flow cell, proceed to step 6.

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

   Do not press the plunger button during the loading process.

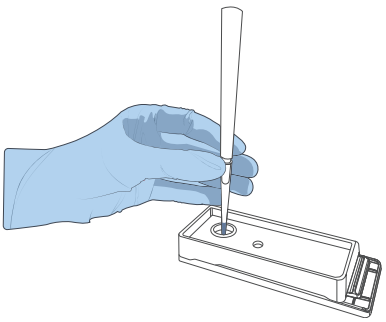

Figure 33 Loading DNBs using DL-G99

6. (Optional) If the liquid level in the pipette tip does not drop, perform the following steps:
   1) Leave the tip with the DNB loading mixture in inlet A.
   2) Adjust the aspirate volume to 2 µL and load a new 200 µL non-filtered pipette tip.
   3) Press and hold the plunger button, and gently insert the new tip into outlet B.

4) Hold the new tip in outlet B with the other hand, gently release the hand from the plunger button, and remove the tip from outlet B after the liquid level of the tip in inlet A drops..

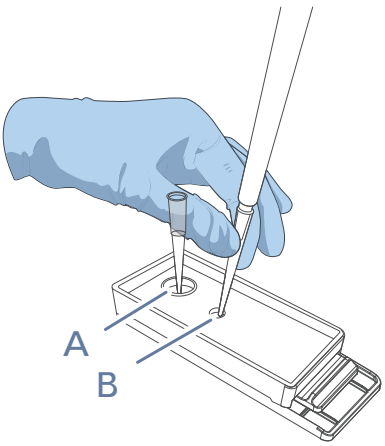

Figure 34 Loading DNBs using DL-G99

7. After the liquid level in the pipette tip stops dropping, remove the pipette tip from inlet A.
8. Turn the loader upside down, open the cover, remove the flow cell, and transfer it to the sequencer immediately.

Loading the flow cell

Perform the following steps:

1. Insert the flow cell into the flow cell compartment after priming is finished. The built-in RFID scanner will automatically identify the flow cell ID.

   Enter the flow cell ID manually if the RFID scanner fails to identify the ID. Ensure that the ID is correct.

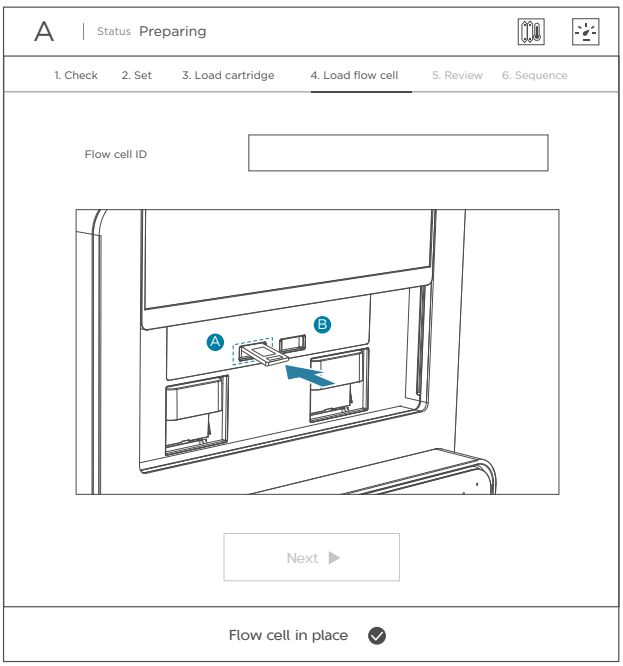

Figure 35 Loading the flow cell

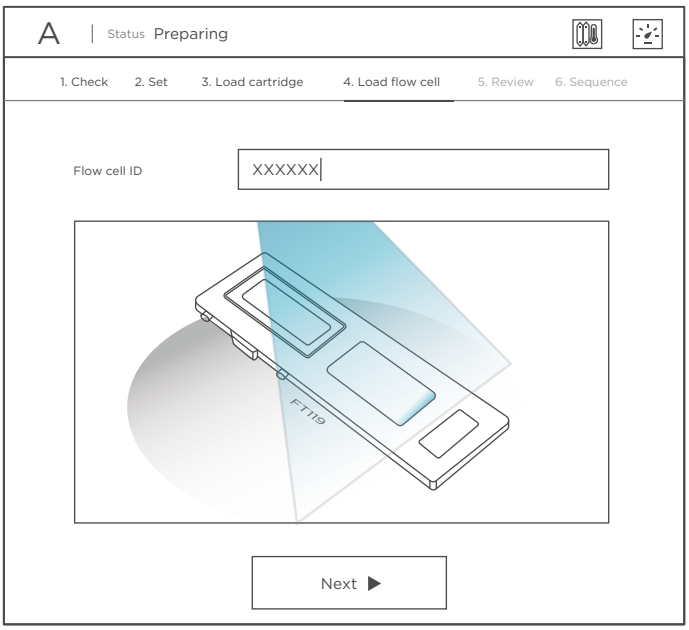

Figure 36 Scanning flow cell ID

CAUTION

- If the flow cell is not loaded 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 KimWipes tissue moistened with 75% ethanol and then let it air-dry to ensure that the flow cell can be properly loaded to the stage.
- Do not move the flow cell after it has been loaded. Doing so may cause misalignment between the flow cell inlet and outlet and the gasket.

2. Select **Next**. A stage flatness verification starts. Proceed to next step when passed.

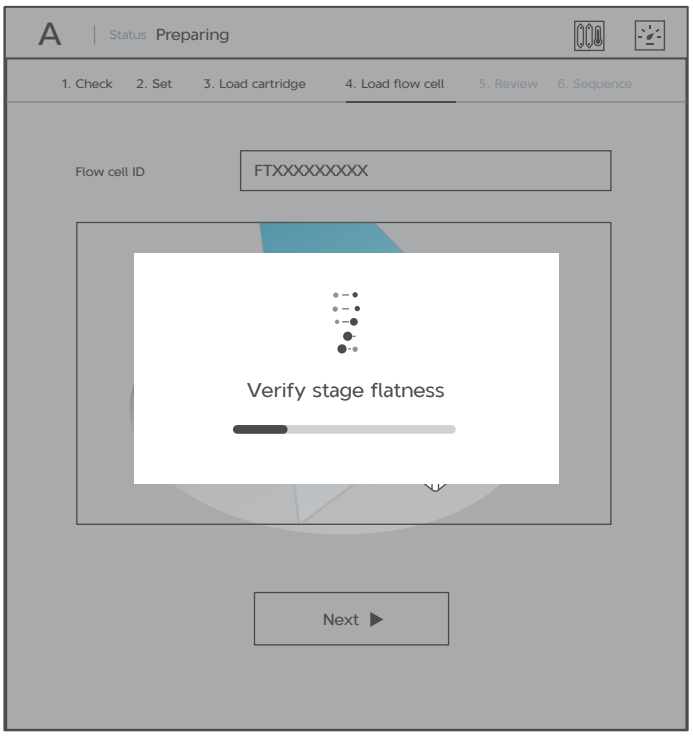

Figure 37 Verifying the stage flatness

Reviewing parameters

Review the parameters and ensure that all information is correct. If the parameters are not correct, select  to modify the information.

- The modification button is available only for new sequencing runs.
- Modification of the workflow type is not supported.

A typical PE150 Barcode sequencing run interface is shown below.

For information on the extra cycle of Read1 and Read2, refer to *Sequencing read length on Page 40.*

Figure 38 Reviewing information

Starting sequencing

Perform the following steps:

1. After confirming that the information is correct on *Reviewing parameters on Page 85*, select **Sequence**, and select **Yes** in the pop-up dialog box to start sequencing.

Figure 39 Confirming sequencing interface

CAUTION If you select **No**, you will be prompted whether to return to the main interface:

- Select **No** to continue reviewing sequencing information.
- Select **Yes** to return to the main interface, which may result in sequencing failure.

2. If the insufficient disk space is detected, a pop-up window is displayed. Perform the following steps to clear the disk space:
   1) Select **Confirm** to close the window.
   2) Select to return to the desktop.
   3) Open the folder in the relevant path to clear the data:
      - **Data** folder stores the images and operating data of the device.
      - **Result** folder stores the sequencing run data. The default folder paths are *D:\DATA* and *D:\result*, and the actual folder paths for storing the images and files shall prevail.

CAUTION The data cannot be restored once be deleted. Please operate it with caution. If you need to keep the data, copy it to the external storage device.

3. The real-time sequencing progress appears in the Sequencing interface. Perform any one of the following operations as needed:

- Select to view the sequencing information or change the auto wash settings.
- Select to pause sequencing. Select to resume sequencing that has been paused.
- Select and select **Yes** in the pop-up dialog box to stop sequencing.

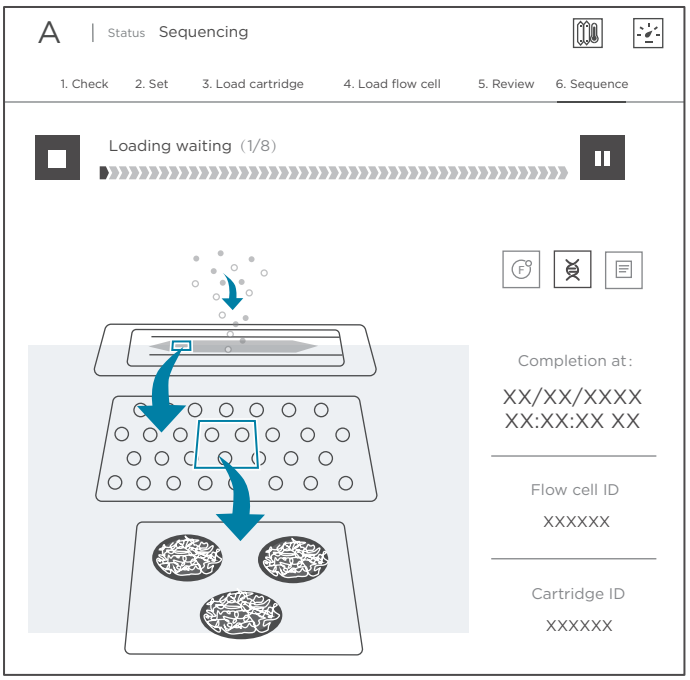

Figure 40 Sequencing interface

(Optional) Viewing the analysis report

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

Perform the following steps:

1. Select ZLIMS in the menu area of the control software to open the ZLIMS login page.
2. Input the username *lite* and password *lite123456*, and select **Login** to open the home page of ZLIMS.
3. Select **Completed** or the corresponding number in the **Task Status** area to open the task list.

   The task list displays all tasks that have been completed in the last month by default.
4. Select any position of the target task to open the **Analysis Info** page.

To locate a history report, perform the following steps:

1) Select a time range on the upper right of the **Task List** area.
2) Select on the upper left to open the **Advanced Query** window.
3) Enter query terms and select **Search** to search for the target task.
4) Tap any position of the target task.

5. Select in the **Report** column to view the analysis report.
6. (Optional) Select in the **Result File** column to access the analysis result directory:
   - Select **Result**, and then select the **.html* file to view the analysis report,
   - Select **Result**, and then select the **.tar.gz* file to download the result compression package to the default directory.
7. (Optional) Select **Back** to return to the to the analysis result directory, and tap **logs** to view the analysis logs.

Performing the post-sequencing operations

If **Auto Wash** is selected when you set sequence parameters, the sequencer will perform an auto wash after the sequencing is completed.

If **Auto Wash** is unselected, perform a manual wash within 12 h. For details, refer to *Performing a manual wash on Page 120*.

After the auto wash is completed, perform the following steps:

1. Select **Finish**. The auto-sliding screen will move up and the waste compartment door will open automatically.
2. Remove the Sequencing Flow Cell, Sequencing Reagent Cartridge, and waste container.

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

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

   Wipe the bottom and two sides of the reagent compartment with a KimWipes tissue or a low-lint cloth moistened with laboratory-grade water and keep the compartment clean and dry.
4. Wash the waste container.

   CAUTION The waste container can be reused for up to one month. Replace the waste container promptly when it expires.

1) Remove the waste container from the waste 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.

   It is recommended to use laboratory-grade water such as 18 MΩ·cm water, Milli-Qwater, Super-Q water, or similar molecular biology-grade water.

4) Pour the waste into an appropriate container.
5) 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 compartment and close the waste compartment door.
6. Select **Return home** to return to the main interface after all items are completed.
7. Dispose of the waste in accordance with local regulations and your laboratory safety standards.
8. Dispose of the the Sequencing Flow Cell and Sequencing Reagent Cartridge in accordance with the disposal standards of medical waste.

(Optional) Powering the device off

CAUTION

- If the sequencer is to be powered off for more than 7 days, perform an auto wash and deep wash before powering off and after powering on.
- 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 > **Shut down**, and select **Shut down** > **Yes** in the pop-up dialog box.
2. Turn the power switch to the position.
3. Disconnect the power cord from the main power supply or UPS.

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06

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 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 parameters

The following table describes parameters for Tab1 of summary report:

Table 39 Parameters for Tab1 in the summary report

Parameter	Description
SoftwareVersion	Version of BasecallLite. Ensure that the version of BasecallLite is the officially release version
TemplateVersion	Version of summary report template
Reference	Mark the species category of the sample. When the species category is known, this parameter displays the species category of the sample. When the species category is unknown, this parameter is not displayed
CycleNumber	The total cycles 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: $$\textbf{ChipProductivity} = \frac{\textbf{ValidFovNumber} \times \textbf{ESR}}{\textbf{ImageArea}} \times \textbf{100\%}$$

Parameter	Description
ImageArea	The total number of FOVs (fields of view) in a lane. The system reads the total number of FOVs from the *QC.csv* file under the metrics directory generated by the basecall software
TotalReads(M)	Reads included in the FASTQ file (Reads after filtering)
Q30(%)	The percentage of bases with a quality score ≥30. A base with a quality score of 30 implies that the chances of this base being called incorrectly are 1 in 1000
Q40(%)	The percentage of bases with a quality score ≥40. A base with a quality score of 40 implies that the chances of this base being called incorrectly are 1 in 10000
SplitRate(%)	The proportion of FASTQ data that can be split according to the barcode list. 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 first strand sequencing • Lag2(%) is the slope of the Lag curve for the second strand sequencing • Runon1(%) is the slope of the runon curve for the first strand sequencing • Runon2(%) is the slope of the runon curve for the second strand sequencing
ESR(%)	Effective spot rate. Percentage of effective spots after filtering in the flow cell
RecoverValue(AVG)	The ratio of the second strand signal to the first strand signal. This indicator is only for PE sequencing

The following table describes parameters for Tab2 of summary report:

Table 40 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 selected 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 unselected
Sequencing Cartridge ID	Serial number of the Sequencing Reagent Cartridge
Flow Cell ID	Serial number of the Sequencing Flow Cell
DNB ID	DNB ID that you entered
Flow Cell Pos	Position of the flow cell (stage A or stage B)
Barcode Type	The barcode file that you selected during sequencing
Read1 Cycles	First-strand read length
Read2 Cycles	Second-strand read length
Barcode	Read length of Barcode
Dual Barcode	Read length of DualBarcode
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	The cycle number for 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.

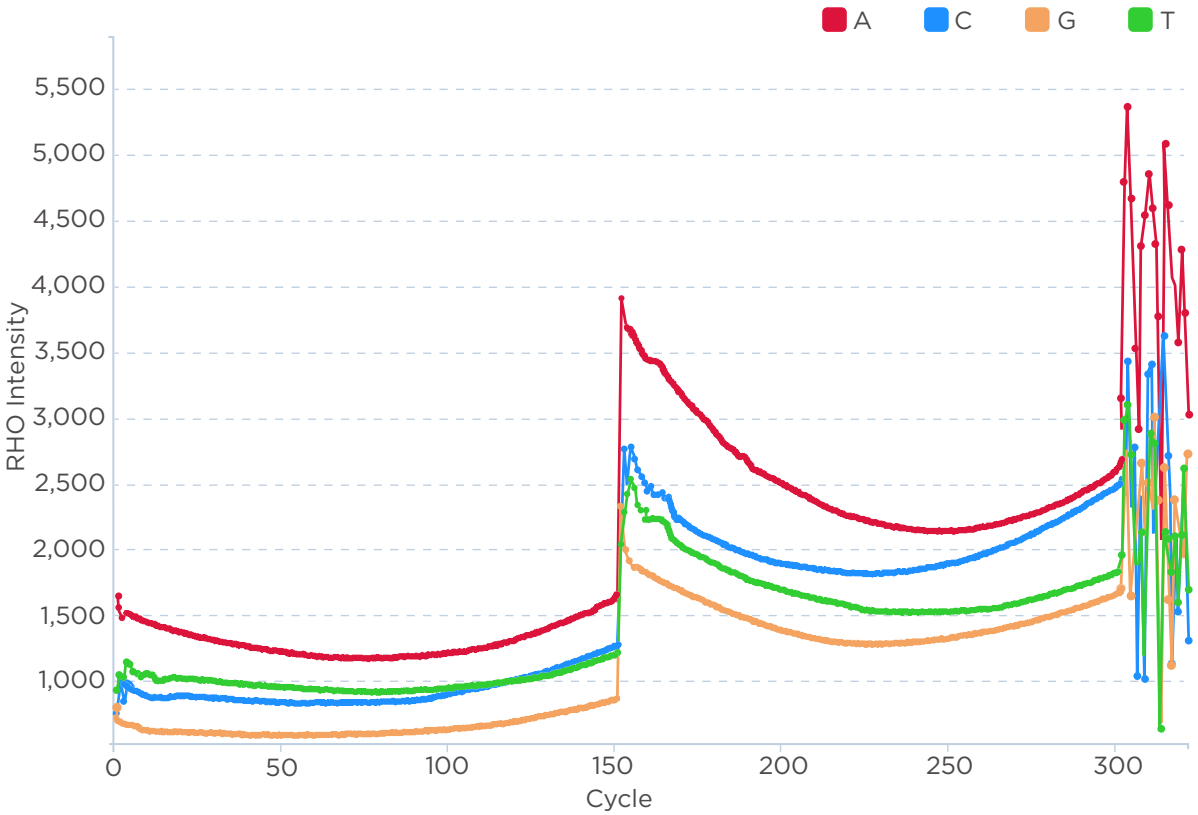


Figure 41 RHO Intensity

Table 41 Diagram description for RHO Intensity

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

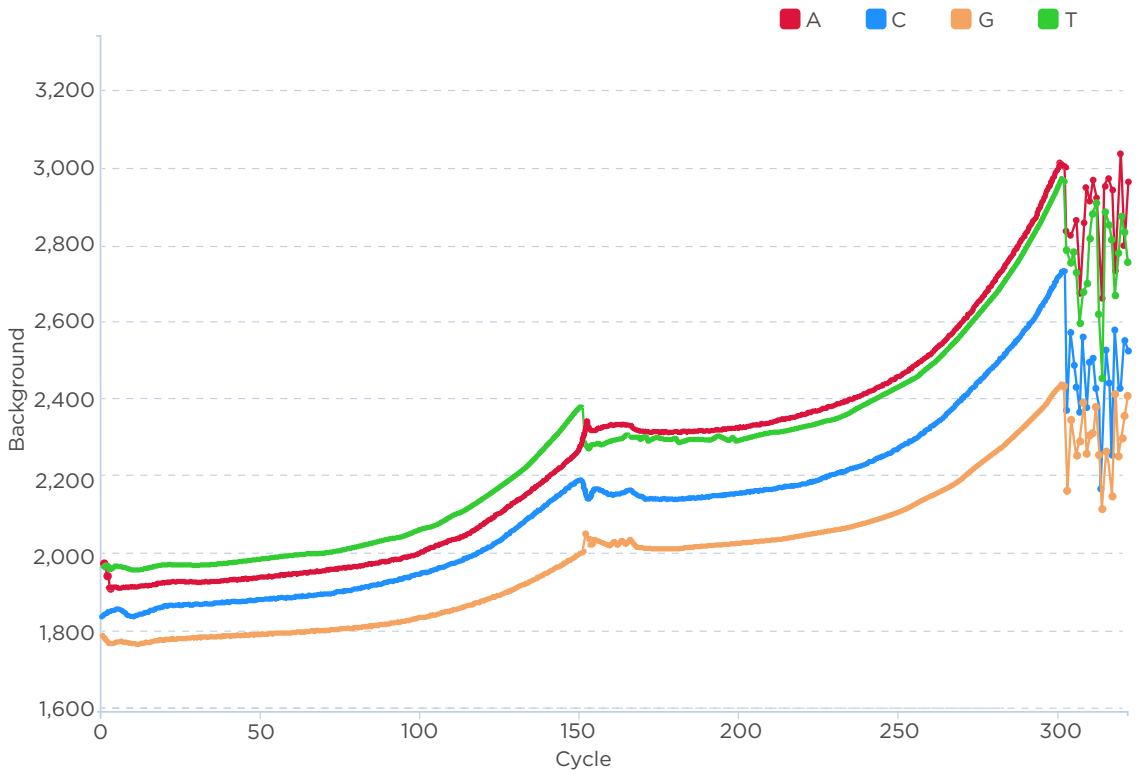


Figure 42 Background

Table 42 Diagram description for Background

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

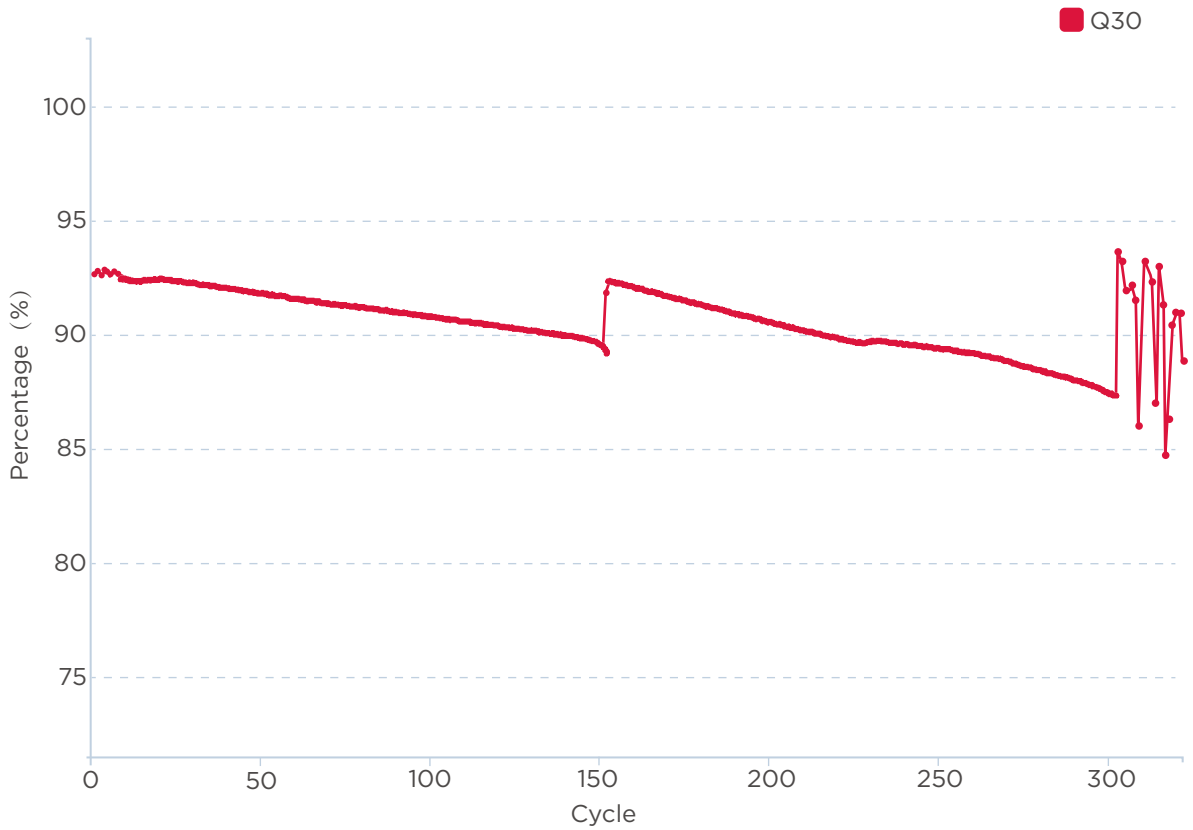


Figure 43 **Unfiltered Q30**

Table 43 **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.

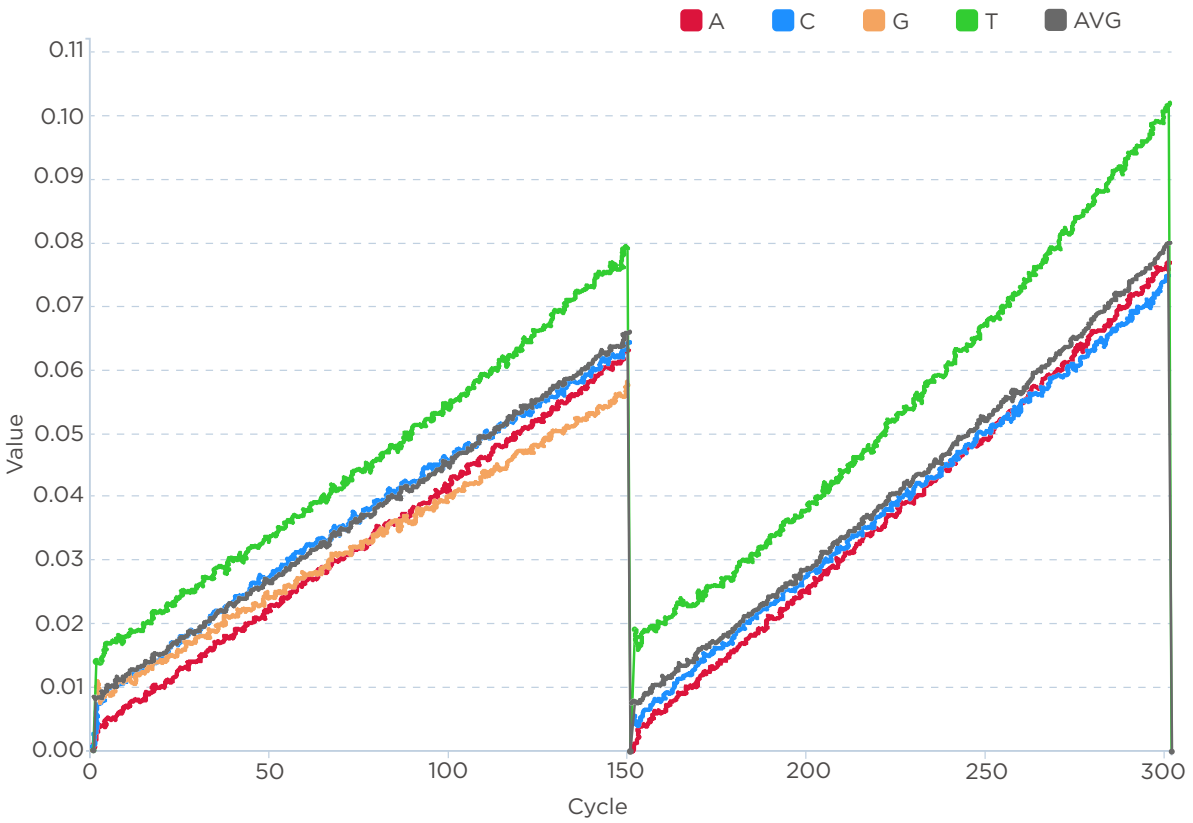


Figure 44 Runon

Table 44 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.

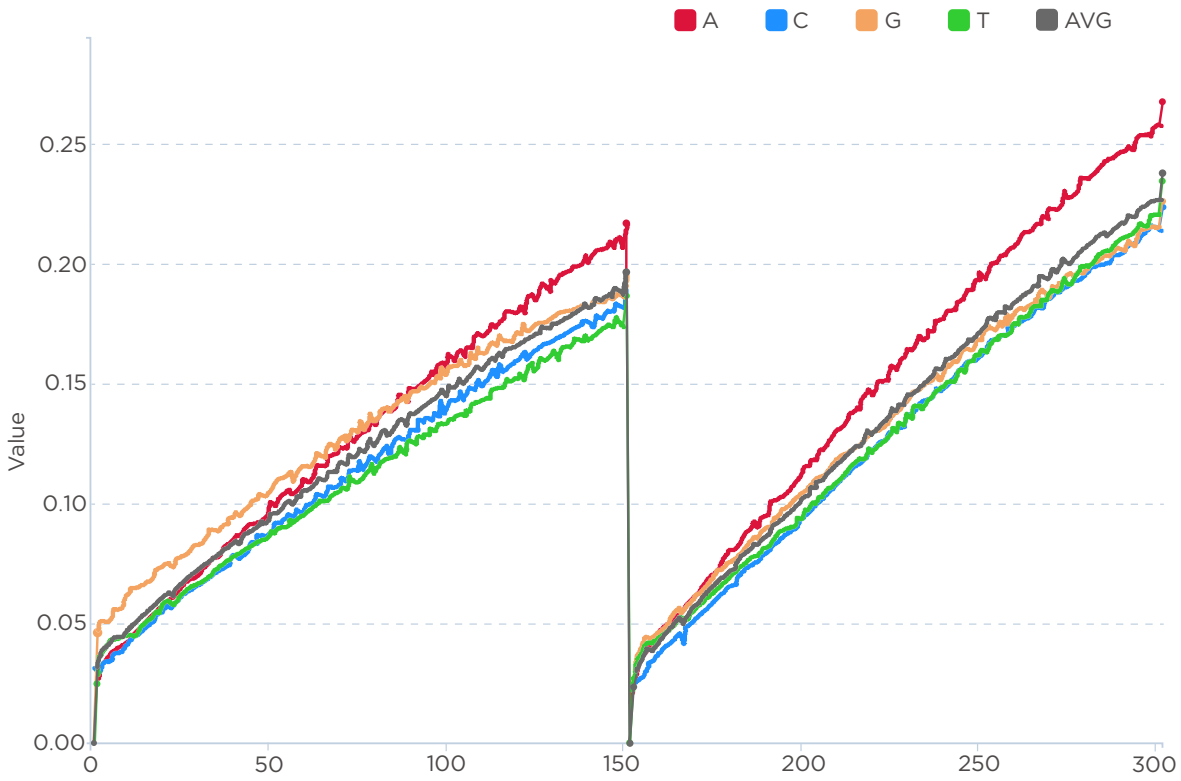


Figure 45 Lag

Table 45 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.

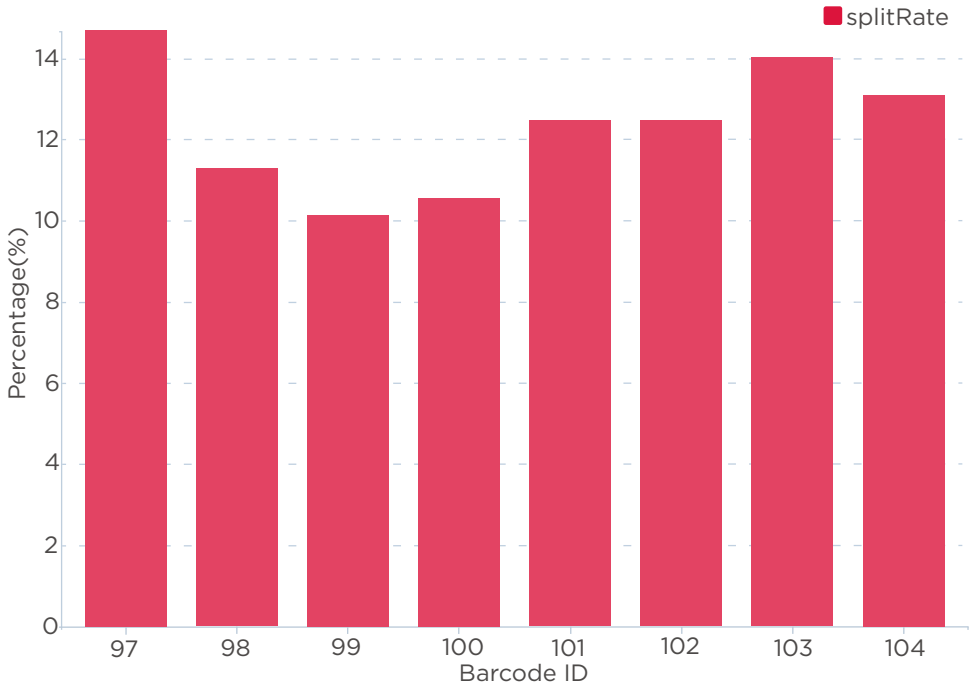


Figure 46 Barcode Split Rate

Table 46 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%.

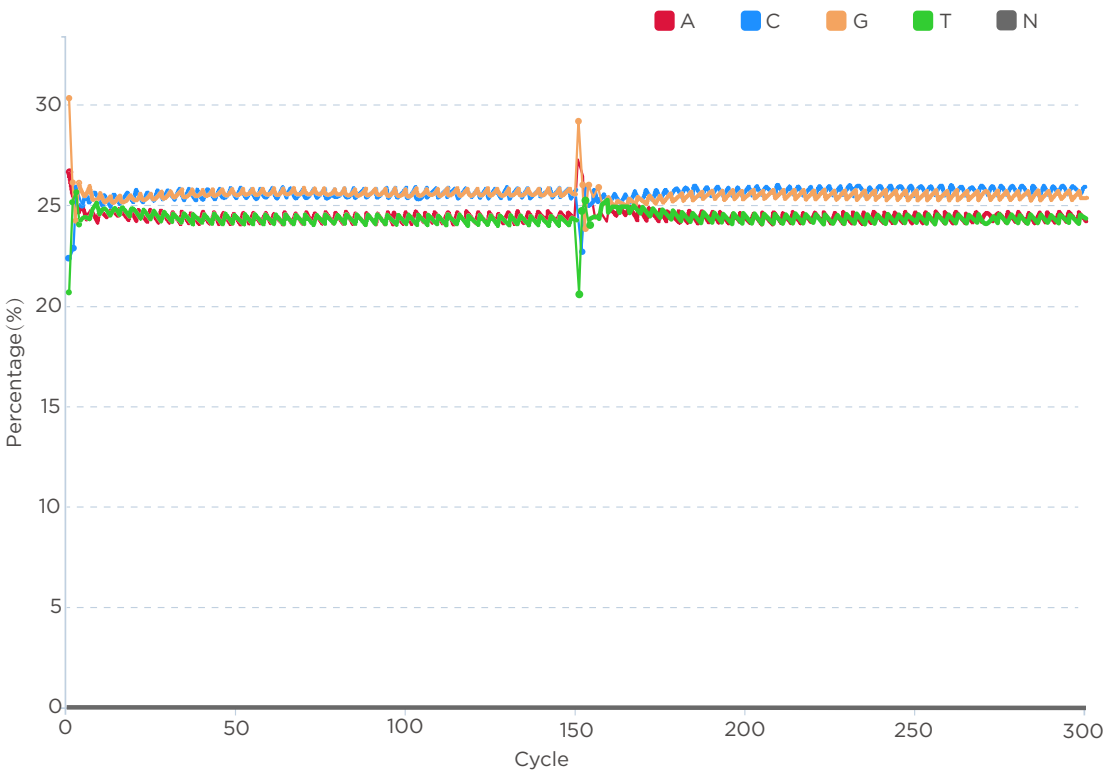


Figure 47 **Bases Distribution**

Table 47 **Diagram description for Bases Distribution**

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

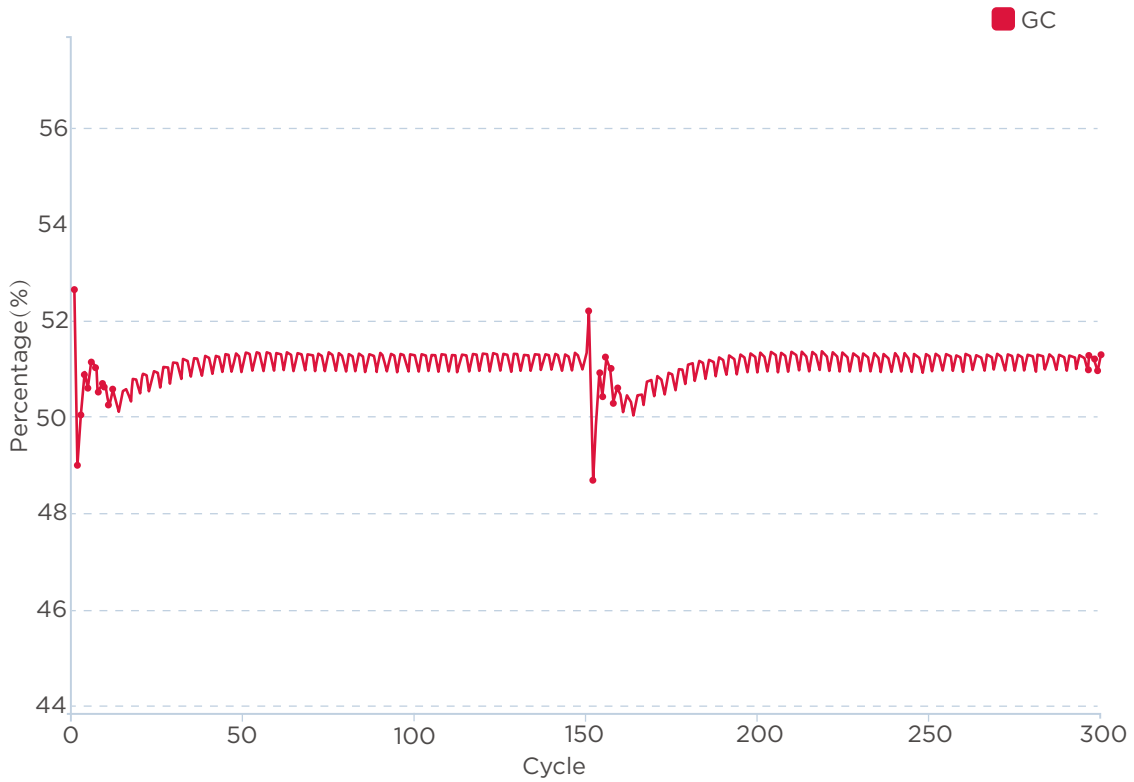


Figure 48 GC Distribution

Table 48 Diagram description for GC Distribution

X axis	Cycle
Y axis	Percentage (%): GC percentage calculated from FASTQ.

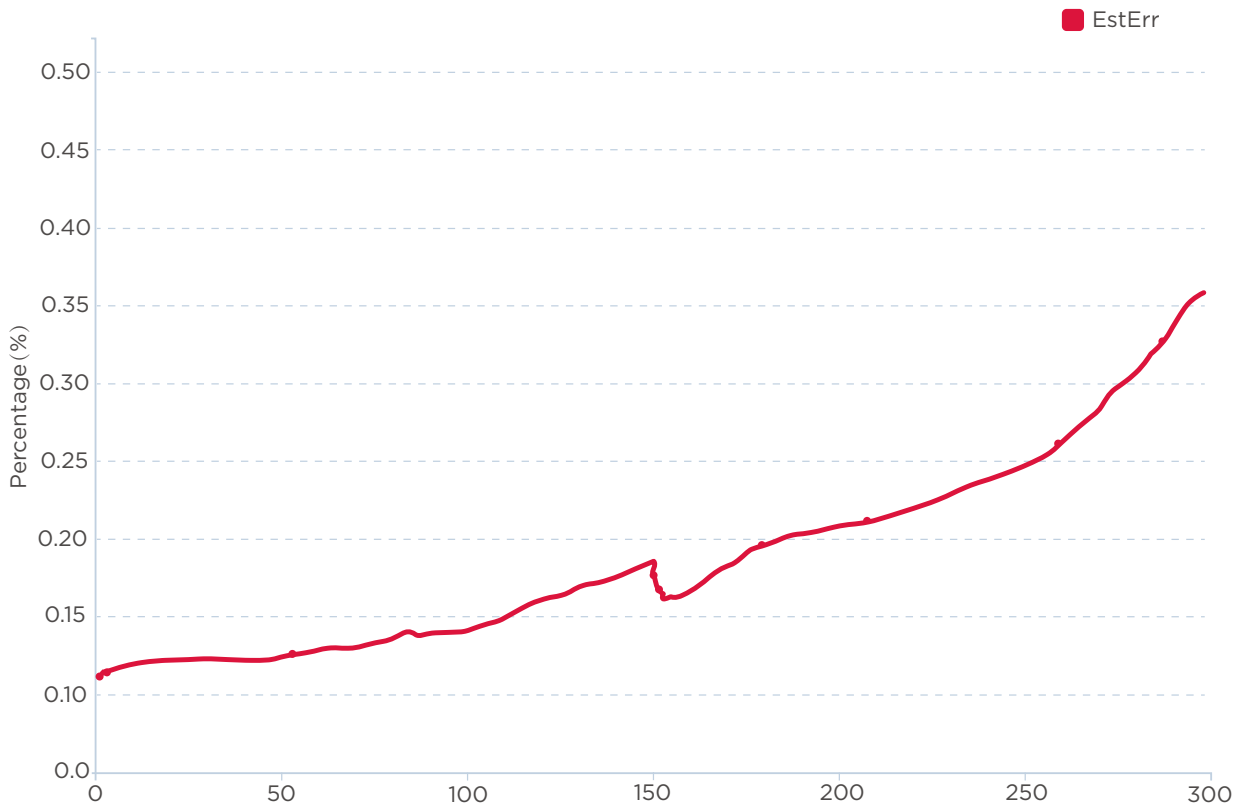


Figure 49 **Estimated Error Rate**

Table 49 **Diagram description for Estimated Error Rate**

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

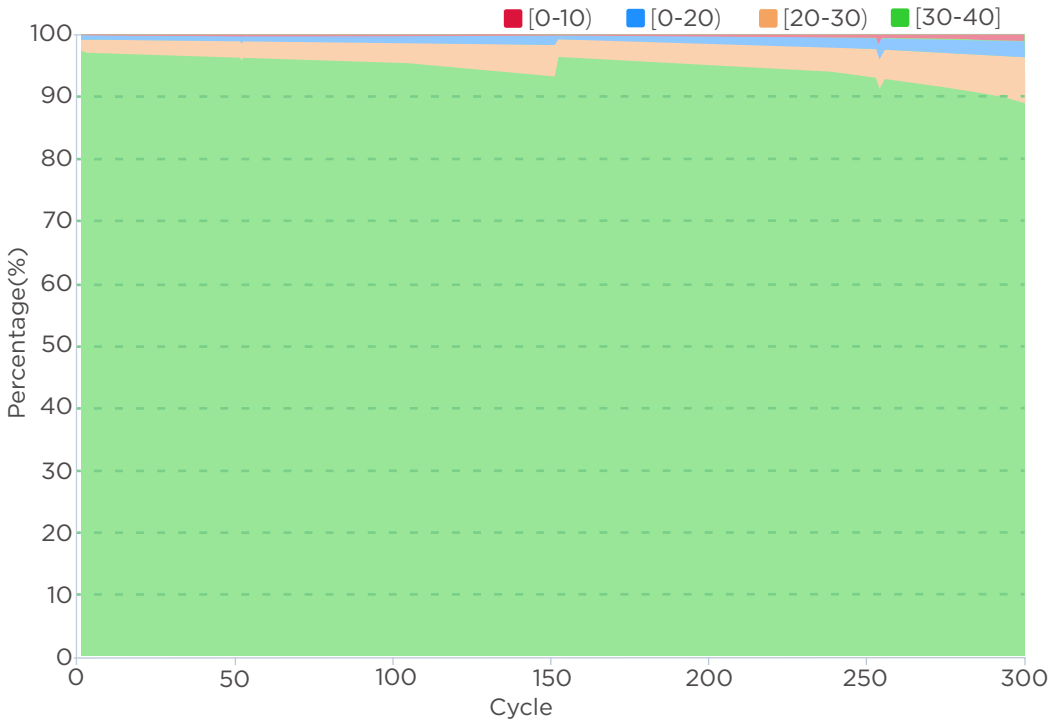


Figure 50 Quality Proportion Distribution

Table 50 Diagram description for Quality Proportion Distribution

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

Other reports

Table 51 Other report description

Name	Description
XXXXXXXX_L01.heatmapReport.html	Contains information on each FOV in the lane generated during sequencing, including AvgQ30, offset_x, offset_y, lag1, lag2, runon1, and runon2. "1" and "2" stand for the first strand and second strand, 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 Writing FASTQ on sequencer automatically) or manually (termed Writing FASTQ on sequencer manually).

The two Write FASTQ methods are described below.

Writing 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.

Writing FASTQ on sequencer manually

FASTQ files may need to be written manually in the following situations:

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

Preparation before writing FASTQ manually

Perform the following steps:

1. Ensure that the sequencer is in idle status, and not in the sequencing or base calling phase.
2. Select  to return to the desktop.
3. 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+1+150+1+10= 312

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

   D:/Result/workspace/FTXXXXXXXXX/L01/Metrics

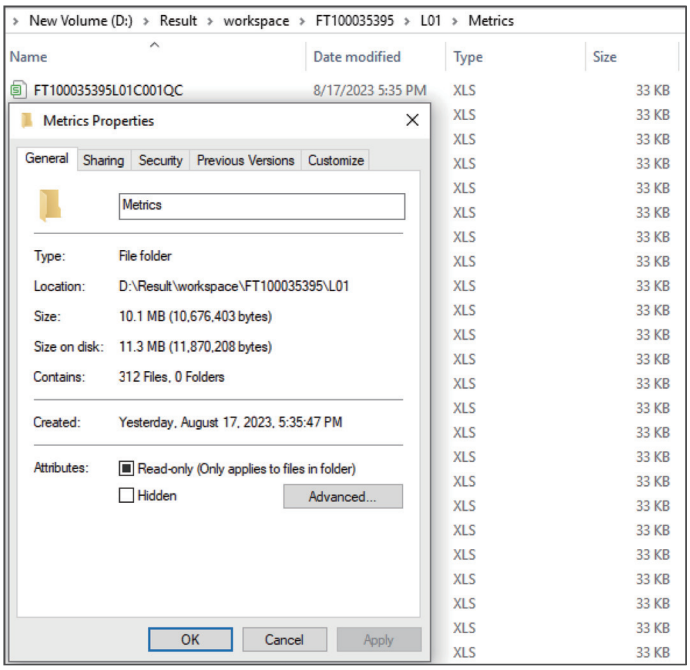


Figure 51 **Metrics file number**

4. Rename the original FASTQ folder. For example, rename *FTXXXXXXXXX* to *FTXXXXXXXXX_old*, or rename *L01* to *L01_old*.

   If the folder is not renamed, it will be overwritten during the manual writing FASTQ process.

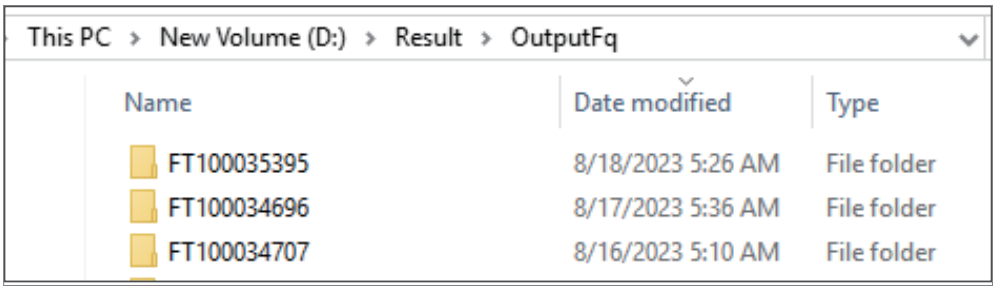


Figure 52 **Renaming the FASTQ folder**

5. Prepare the barcode file path.

   For details, refer to *Instructions for importing barcodes on Page 153*.

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

Using BasecallLite to write FASTQ manually

Perform the following steps:

1. Copy the *BasecallLite* folder in drive C and rename it, for example, *BasecallLite-Copy.* Place the *BasecallLite-Copy* folder in drive C.

   All the following modifications take place in the folder *BasecallLite-Copy*.

2. Edit the *Client.ini* file.

   1) Select the *Client.ini* file and open it with a text editor.

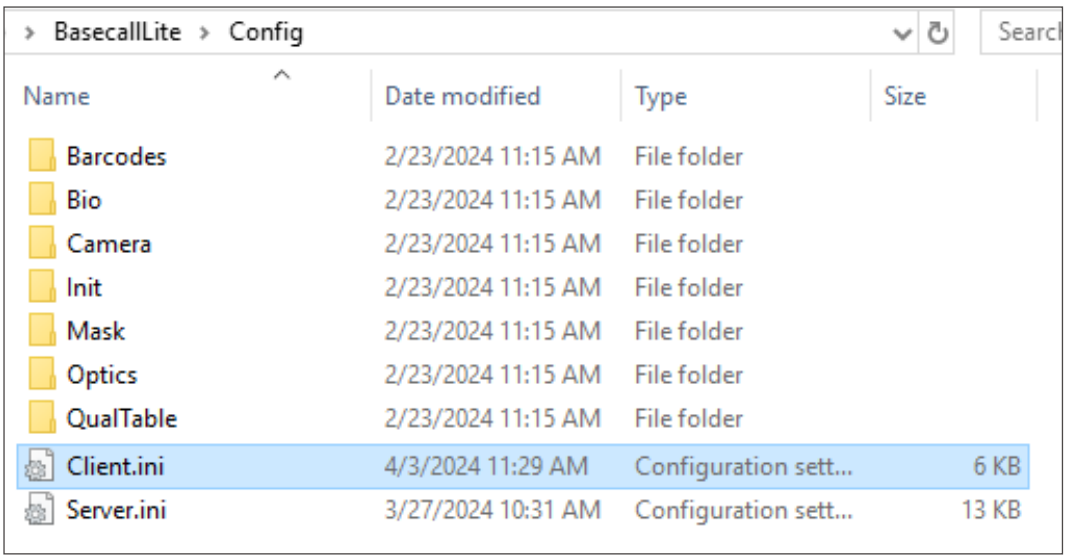


Figure 53 Location of Client.ini file

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

```
# This file predefined all the parameters which basecall client needed

[Run]

# Input path: the path of raw image
SourcePath = F:\FT100049910C\L01

# Cycle information: including read1&2 length, barcode1&2 length and position, and whether do one more cycle for lag correction for strand
# eg: r50e1r50e1b10b10, a PE50 run with postfix cycle in each strand for lag correction, and dual indexes at the end
# eg: r100, a SE100 run
# eg: r50e50, a PE50 run
Cycle = r100e1r100e1b10b10

# whether upload cal and metrics to remote storage
UploadCal = false

# The upload path of cal and metrics of remote storage
UploadPath = E:\data\result


[Communication]

# Client connection string of ice
ConnectionStr = tcp -t 10000 -p 5065 -h 127.0.0.1


[Workflow]

# Whether submit images to basecall server. If set to false, will skip images and directly writefastq from cal.
SubmitImages = false

# Cal file path, only apply when SubmitImages = false
# eg: D:\Result\workspace\FT100003261L01\Cal\  cal path should follow basecall directory rule and set to cal folder
CalFilePath = D:\Result\workspace\FT100042538\L01\calFile

# Whether write fastq nor not.
WriteFastQ = true

# Whether duplicate the images of first fov of each batch, to accelerate the speed of submit. For speed testing only.
DuplicateImage = false

# Only enabled when DuplicateImage is true. It decides how many fovs to duplicate. { ColMax, RowMax }
DuplicateColRow = { 6, 72 }

# Thread number of Submit Image
SubmitImageThreadNum = 4

# Interval Time of Submit Image (ms)
SubmitImageIntervalMs = 0

# If true, image will be loaded by server instead of client for efficiency. Only apply when SubmitImages = true
LoadImageFromServer = true

# Postfix name of slide, default is null string. The length of it should not be longger than 10.
# eg: Postfix = _Test1, the output data path will be D:\Result\OutputFq\xxxxxx_Test1, xxxxxx is slide name.
# $TIME$ is macro, it will be replaced with current time string for convenience.
Postfix =
```

Change the number of cycles

Change CAL file path

Figure 54 Editing Client.ini file

Table 52 Parameter settings descriptions

Parameter settings	Description
Change the number of cycles	Cycle=r[Read1 cycle number]e1r[Read2 cycle 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 • PE300+10(301+301+10), Cycle=r300e1r300e1b10 • PE300+10+10+300(301+10+10+301), Cycle=r300e1Mb10b10r300e1 • PE300+10+300(301+10+301), Cycle=r300e1Mb10r300e1 • PE300+10+10(301+301+10+10), Cycle=r300e1r300e1b10b10 • PE300+10+10(Read1 dark cycle:11-20, Read2 dark cycle:21-40), Cycle=r290e1r280e1b10b10
Change SubmitImages value from true to false	This parameter setting means writing FASTQ from CAL file.
Change CalFilePath	This parameter describes the CAL file storage path. For details, refer to *Figure 55 on Page 111* .
Change BarcodeFile	You need to input the barcode file path here if you used a user defined barcode.
Change FilterLength	This parameter setting determines the insert size to be filtered: • For PE300 sequencing, set it to 300 • For sequencing of other read lengths, set it to 100. If the actual read length is < 100 bp, the system performs filtering by the actual read length. If the actual read length is ≥ 100 bp, the system performs filtering by 100 bp.

- The text displayed in green in the file represents comments. Refer to the comments to modify the relevant parameters.

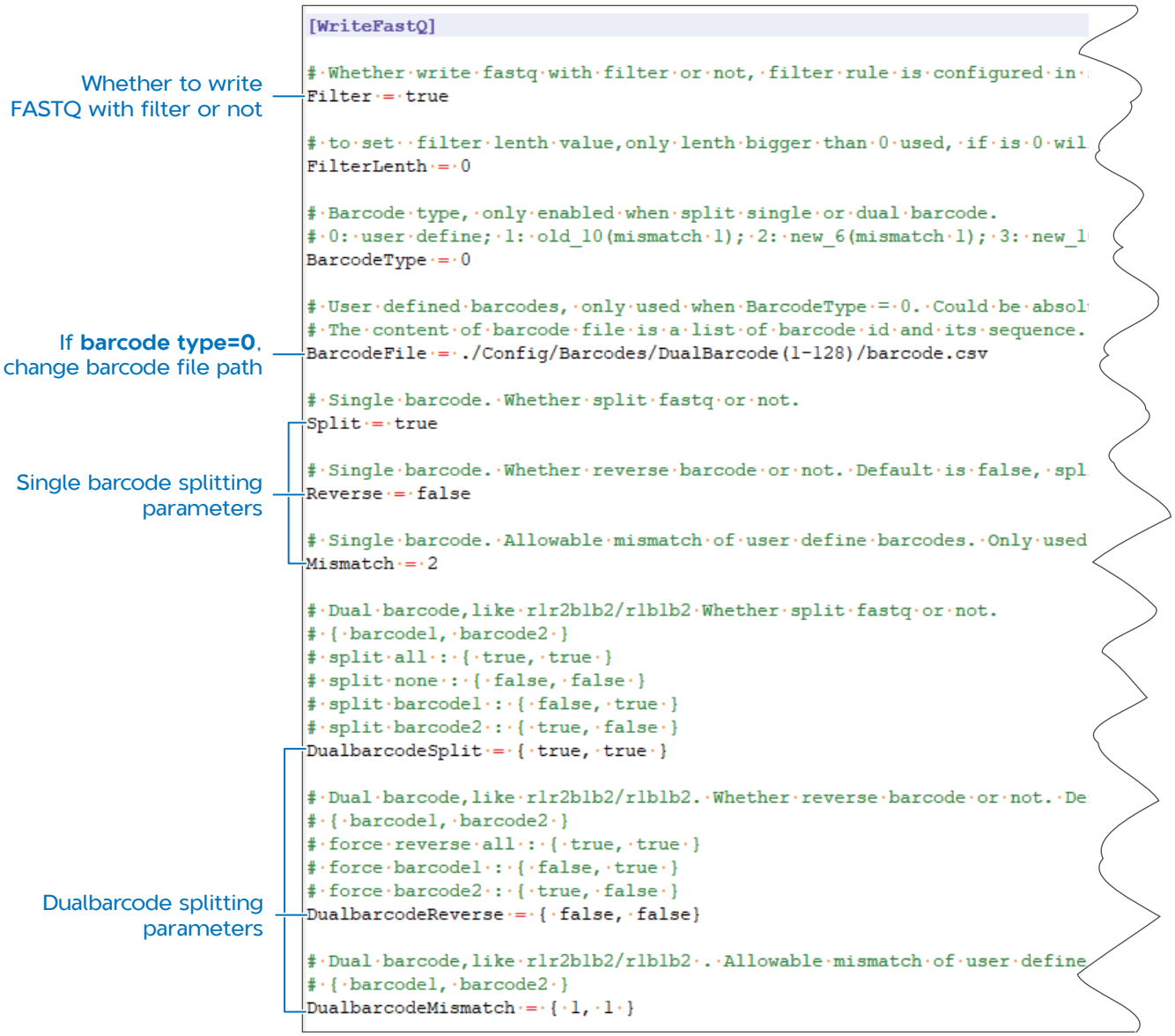


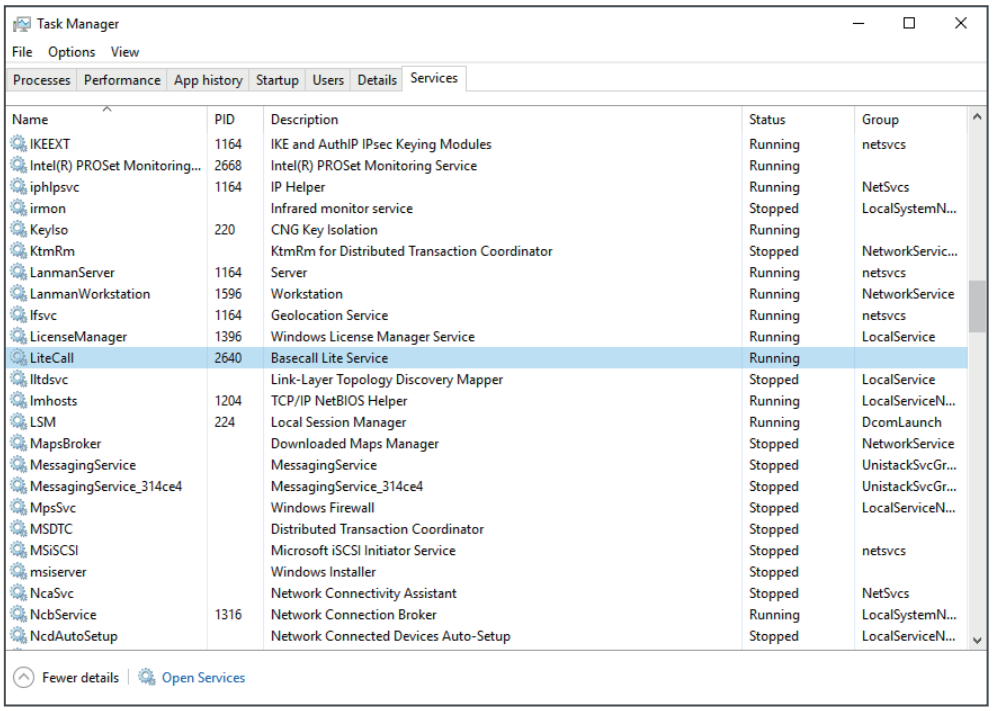
Figure 55 Changing CAL file path and barcode splitting parameters

3) Save the modification and close the *Client.ini* file.

3. (Optional) Edit the *Server.ini* file.

The step is required only when you want to save all raw reads.

1) Open Task Manager, select the Services tab, and check the LiteCall status. If LiteCall is running, right-click to stop it.



2) Select the *Server.ini* file and open it with a text editor.

3) Change the SaveDiscardedReads setting and save the file.

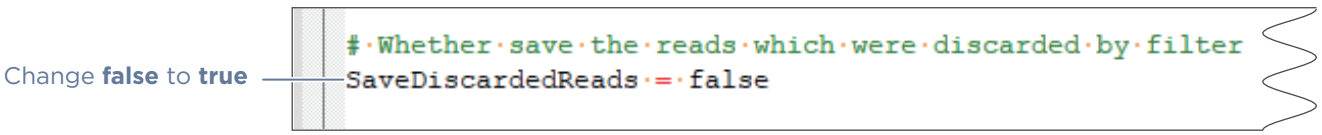


Figure 56 Changing the SaveDiscardedReads setting

4) Double-click to launch *Basecall.Server.exe*.

4. In the folder *BasecallLite-Copy*, double-click to launch *Basecall.Server.exe*, which will run the write FASTQ program automatically.
5. Close the application after writing FASTQ finishes.
6. Upload the FASTQ file:

   1) Restore the display of the control software from the task bar.

   2) In the main interface, select > **Maintenance** > **Upload file**.

   3) Select **Server** and **Storage server** for Server Type.

   4) Select the appropriate flow cell ID according to the written FASTQ file in the **Flow cell ID** list.

      You can select no more than 3 flow cell IDs.

5) Select **Fq** for File Type and select **Upload** to upload the manual FASTQ file.

Example of parameter setting (PE100+10+10)

Perform the following steps:

1. Set sequencing parameters with the following assumptions:
   - Sequencing run: PE100+10+10
   - Length of Read1: 100
   - Length of Read2: 100
   - Length of DualBarcode: 10
   - Length of barcode1: 10
   - CAL file path: *D:\Result\workspace\FTXXXXXXXXX\L01\calFile*.

```
# Cycle information: including read1&2 length, barcode1&2 length and position, and whether do one more cycle for lag co
# eg: r50e1r50e1b10b10, a PE50 run with postfix cycle in each strand for lag correction, and dual indexes at the end
# eg: r100, a SE100 run
# eg: r50r50, a PE50 run
Cycle = r100e1r100e1b10b10

# whether upload cal and metrics to remote storage
UploadCal = false

# The upload path of cal and metrics of remote storage
UploadPath = E:\data\result


[Communication]

# Client connection string of ice
ConnectionStr = tcp -t 10000 -p 5065 -h 127.0.0.1


[Workflow]

# Whether submit images to basecall server. If set to false, will skip images and directly writefastq from cal.
SubmitImages = false

# Cal file path, only apply when SubmitImages = false
# eg: D:\Result\workspace\V300008361\L01\Cal\, cal path should follow basecall directory rule and set to cal folder
CalFilePath = D:\Result\workspace\FT100036396\L01\calFile

# Whether write fastq nor not.
WriteFastQ = true
```

Figure 57 Parameter settings

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

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

     BarcodeFile path:

     C:\ISW\barcode\CustomizeDualBarcode\DualBarcode-10_10\barcode.csv

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

# Dual barcode. Whether reverse barcode or not. Defa
# {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 ba
# {barcode2, barcode1}
DualbarcodeMismatch = {1, 1}
```

Figure 58 Splitting both barcode1 and barcode2

   - Splitting only barcode2:

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

# Dual barcode. Whether reverse barcode or not. D
# {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
# {barcode2, barcode1}
DualbarcodeMismatch = {1, 1}
```

Figure 59 Spitting barcode2 only

- Splitting only barcode1:

```
#·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·no
#·{·barcode2,·barcode1·}
#·force·reverse·all·:·{·true,·true·}
#·force·barcode1·:·{·false,·true·}
#·force·barcode2·:·{·true,·false·}
DualbarcodeReverse·=·{·false,·false}

#·Dual·barcode.·Allowable·mismatch·of·user·de
#·{·barcode2,·barcode1·}
DualbarcodeMismatch·=·{·1,·1·}
```

Figure 60 **Spitting only barcode1**

3. If you want to save all raw reads, change the **SaveDiscardedReads** setting in the *Server.ini* file.

   Restart the server application after modification.

Change **false** to **true**

```
#·Whether·save·the·reads·which·were·discarded·by·filter
SaveDiscardedReads·=·false
```

Figure 61 **Changing the SaveDiscardedReads setting**

FASTQ file introduction

FASTQ file name format

The name format of the FASTQ file is as follows:

Figure 62 FASTQ file name format

FASTQ file format

Here is an example of a single entry in a Read1 FASTQ file for PE sequencing:

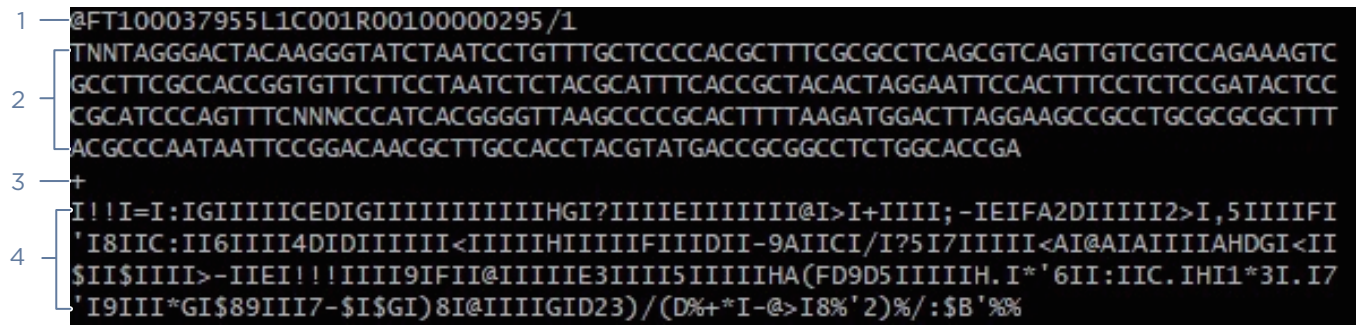


Figure 63 An example of Read1 FASTQ file for PE sequencing

Each entry in a FASTQ file consists of 4 lines:

No.	Description
1	A sequence identifier with information about the sequencing run and the read @FTxxxxxxxxxL1CxxxRxxxxxxxxxx/x Read identifier for PE sequencing DNB serial number FOV position Flow cell ID Lane
2	Nucleotide sequences
3	A separator, which is simply a plus (+) sign
4	Quality scores (Phred +33 encoded)

07

Device maintenance

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

DANGER

- Ensure that the device is powered off before cleaning 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 that you use other 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

To prevent cross contamination, perform a wash to remove the remaining reagents from the fluidics lines and flow cell stage. Select the appropriate wash type depending on sequencer conditions.

CAUTION If the sequencer is to be idle or powered off for more than 7 days, perform both an auto wash and a deep wash before powering off and after powering on.

A used Sequencing Reagent Cartridge can be used in a manual wash if it has not been used in an auto wash.

Table 53 Wash types

Wash type	Cartridge type	Approximate process time (min)	Description
Auto wash	Sequencing Reagent Cartridge	26	If **Auto Wash** is selected in advanced settings before sequencing starts, the system will automatically perform the wash after each sequencing.
Manual wash	Sequencing Reagent Cartridge (Not used in an auto wash after sequencing)	20	If **Auto Wash** is unselected in advanced settings before sequencing starts or if sequencing fails, perform a manual wash within 12 h after sequencing.
Deep wash	Washing Cartridge	30	• If the sequencer is to be idle or powered off for more than 7 days, perform an auto wash and deep wash before powering off and after powering on. • Under normal use, perform a deep wash every month. *i* Normal use means that the sequencing interval of each flow cell stage is less than 7 days, and sequencing and auto wash are performed smoothly each time.

Performing an auto wash

If **Auto Wash** is selected when setting sequence parameters, the sequencer will perform an auto wash after sequencing is completed.

For details, refer to *Performing the post-sequencing operations on Page 88.*

Performing a manual wash

If **Auto Wash** is unselected in advanced settings before sequencing, the Sequencing Reagent Cartridge can be used to perform a manual wash.

Perform the following steps:

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

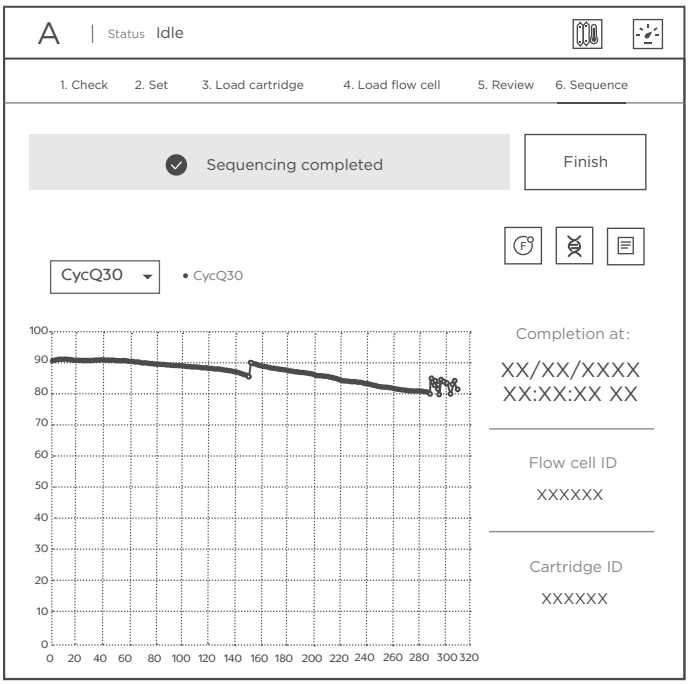


Figure 64 Sequencing completed interface

2. Remove the Sequencing Fow Cell, Sequencing Reagent Cartridge, and waste container.

Press the flow cell down or lift it up before removing it.

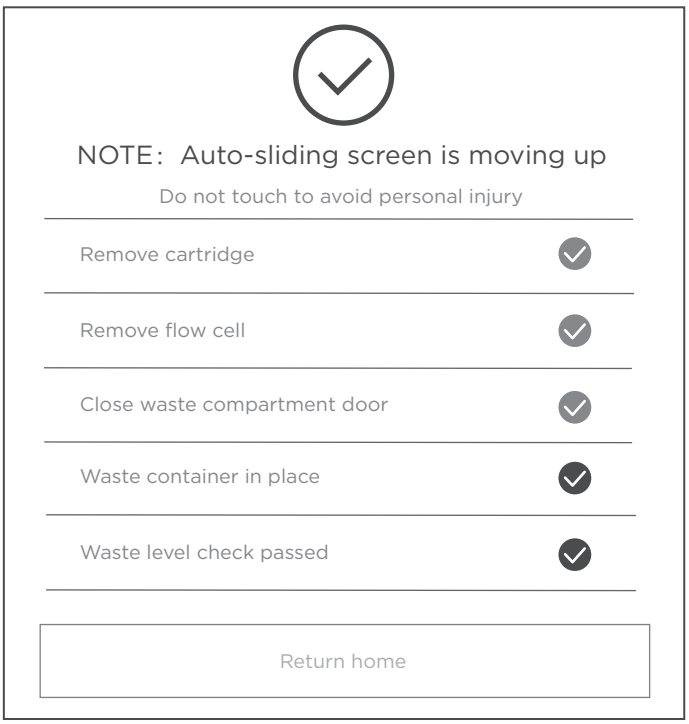


Figure 65 Operations after sequencing finished

3. Clean the reagent compartment.

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

   Wipe the bottom and two sides of the reagent compartment with a KimWipes tissue or a low-lint cloth moistened with laboratory-grade water and keep the compartment clean and dry.

4. Clean the waste container.

   CAUTION The waste container can be reused for up to one month. Replace the waste container promptly when it expires.

   1) Remove the waste container from the waste 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.

      It is recommended to use laboratory-grade water such as 18 MΩ·cm water, Milli-Qwater, Super-Q water, or similar molecular biology-grade water.

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 compartment and close the waste compartment door.
6. Select **Return home** after all items are completed.
7. Select **Wash**. The auto-sliding screen will move up and the waste compartment door will open automatically.

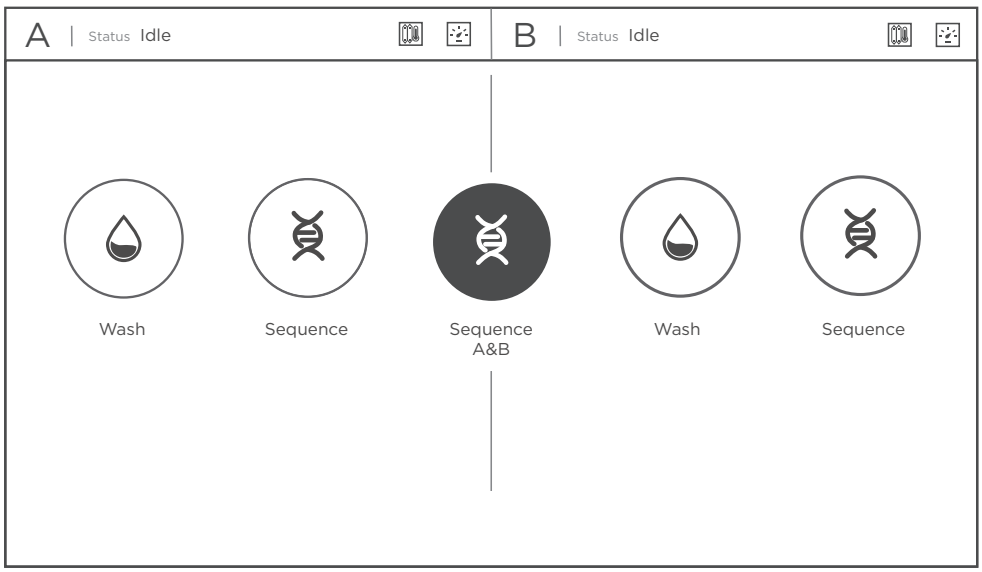


Figure 66 Main interface

8. Place the Sequencing Reagent Cartridge which is not used to perform an auto wash into the reagent compartment until it stops, select the cartridge type, and close the waste compartment door.

The RFID scanner automatically identifies the cartridge type. Ensure that the cartridge type is consistent with the actual one. If the RFID scanner fails to identify the type, select the cartridge type manually.

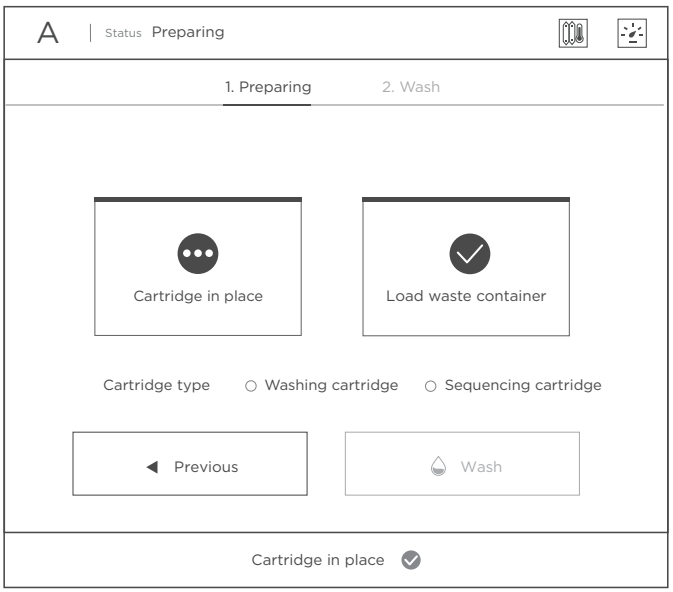


Figure 67 Placing the Sequencing Reagent Cartridge

9. Select **Wash** and select **Yes** in the pop-up dialog box to start washing.

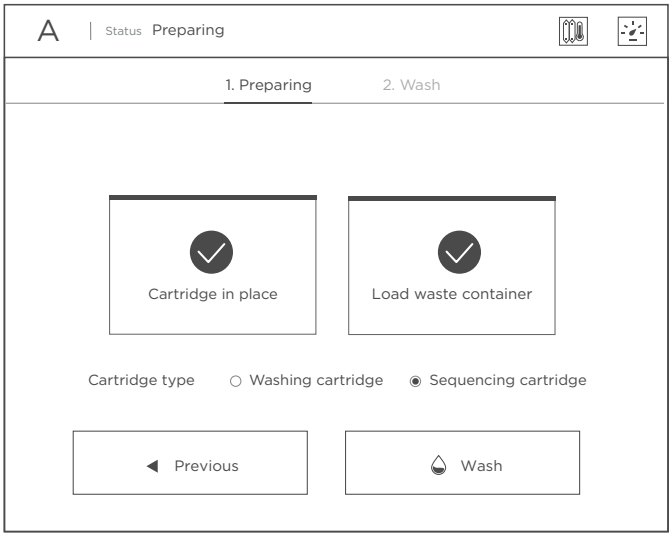


Figure 68 Check completed

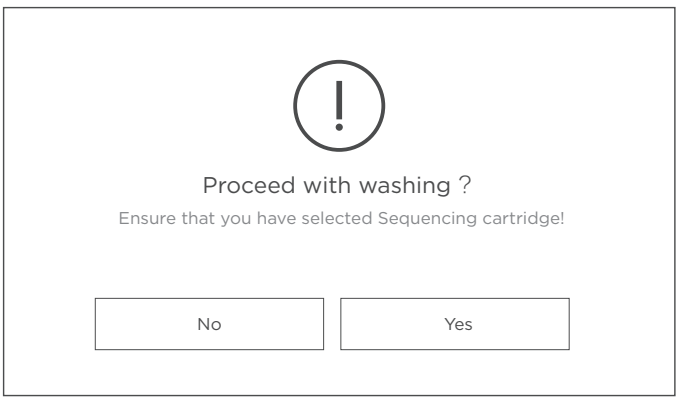


Figure 69 Confirming washing interface

10. Select **Finish** after washing is completed. Remove the Sequencing Reagent Cartridge and waste container.

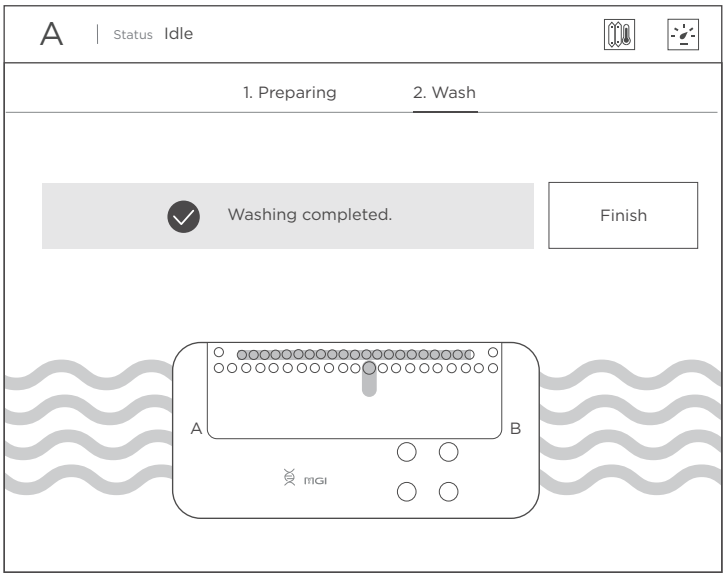


Figure 70 Washing completed interface

11. Clean the waste container according to step 4, put the waste container into the waste compartment, and then close the waste compartment door.

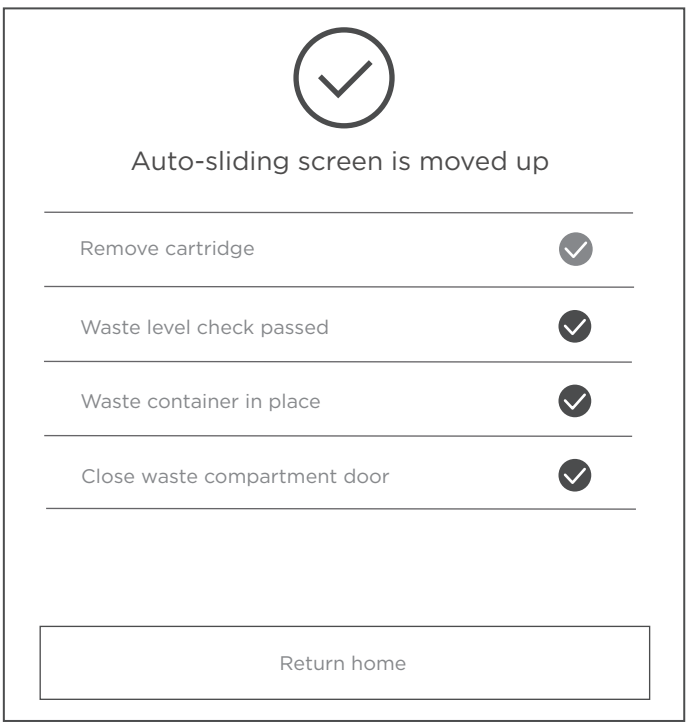


Figure 71 Operations after washing completed

12. Select **Return home** to return to the main interface after all items are completed.
13. Dispose of the waste in accordance with local regulations and your laboratory safety standards.
14. Dispose of the Sequencing Flow Cell and Sequencing Reagent Cartridge in accordance with the disposal standards of medical waste.

Performing a deep wash

Perform the following steps:

1. Prepare the washing reagent according to the table below:

Table 54 Washing reagent: 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	
Validity period	1 month at 4 °C	

2. Prepare the Washing Cartridge.

Washing Cartridge can be ordered as needed (DNBSEQ-G99 Cleaning Reagent Kit, Cat. No.: 940-000903-00).

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

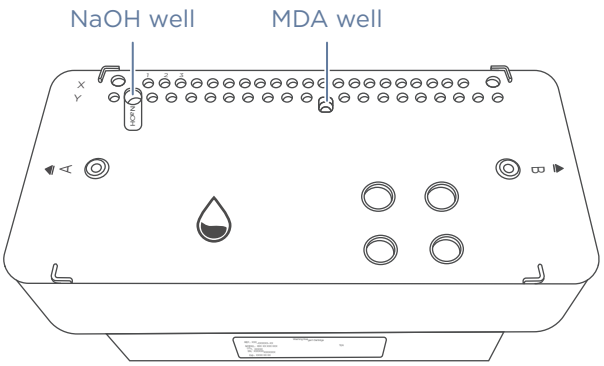


Figure 72 Position of MDA and NaOH wells

2) Add 7.5 mL 0.1 M NaOH into the NaOH well of the Washing Cartridge

3. Check the waste container:

- If an empty waste container is already placed in the waste compartment, proceed to the next step.
- If the waste container is not in place or is not empty, the waste compartment door will pop to open simultaneously. Empty and clean the waste container, place it back into the waste compartment, and close the waste compartment door. After you are prompted that the operation is completed, select **Return home**.

4. Select **Wash** in the main interface. The auto-sliding screen will move up and the waste compartment door will open automatically.

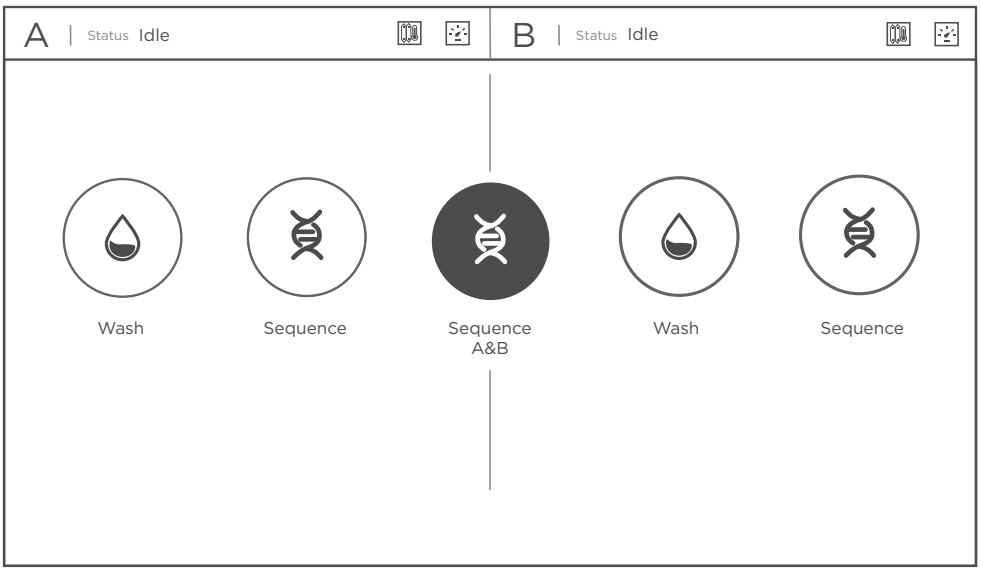


Figure 73 Main interface

5. Confirm that NaOH is added into the NaOH well of the Washing Cartridge, place the Washing Cartridge into the reagent compartment until it stops, select the cartridge type, and close the waste compartment door.

   The RFID scanner automatically identifies the cartridge type. Ensure that the selected cartridge type is consistent with the actual one. If the RFID scanner fails to identify the type, select the cartridge type manually.

6. Select **Wash** and select **Yes** in the pop-up dialog box to start washing.

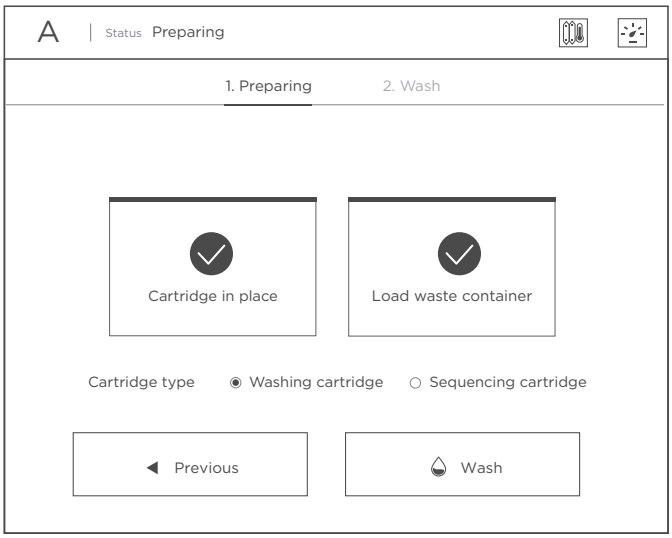


Figure 74 Check completed

Figure 75 Confirming washing interface

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

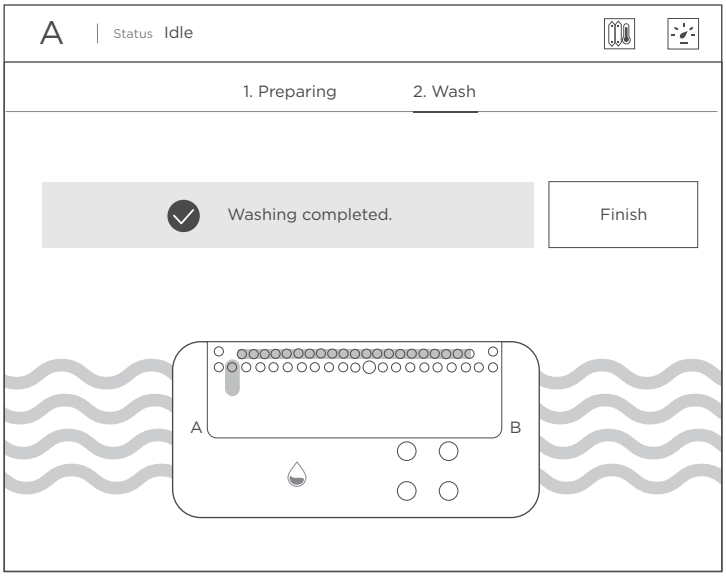


Figure 76 Washing completed interface

8. After the auto-sliding screen moves up, perform the following steps:
   1) Remove the washing cartridge and waste container.
   2) Clean the waste container.

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

      a. Remove the waste container from the waste compartment and empty the waste into an appropriate container according to local regulations and your laboratory safety standards.
      b. 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.
      c. Pour the waste into an appropriate waste container.
      d. Clean the surface and opening of the waste container with a 75% ethanol wipe. Ensure that no waste remains in the container.

3) Place the waste container back into the waste compartment and close the waste compartment door.

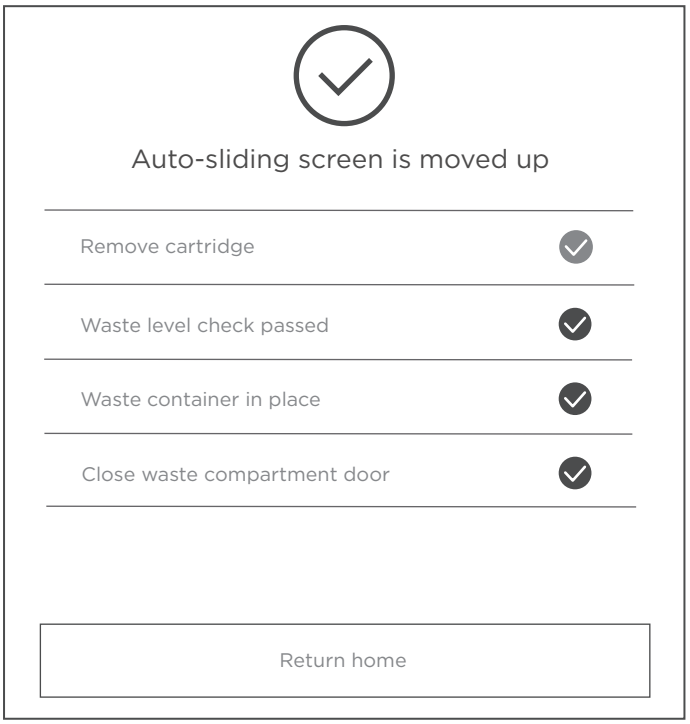


Figure 77 Removing the washing cartridge

9. Select **Return home** to return to the main interface after all items are completed.
10. Dispose of the waste in accordance with local regulations and your laboratory safety standards.
11. Dispose of the washing cartridge in accordance with the disposal standards of medical waste.

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.

Cleaning the flow cell stage

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

The tools that need to be prepared include 75% ethanol, a low-lint cloth, pipette, and a hexagon wrench.

WARNING To prevent 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 open the tools interface.
2. Select **Auto-sliding screen** > **Maintenance**. 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 parts of the following figure) with a low-lint cloth moistened with 75% ethanol, and then let it air dry.

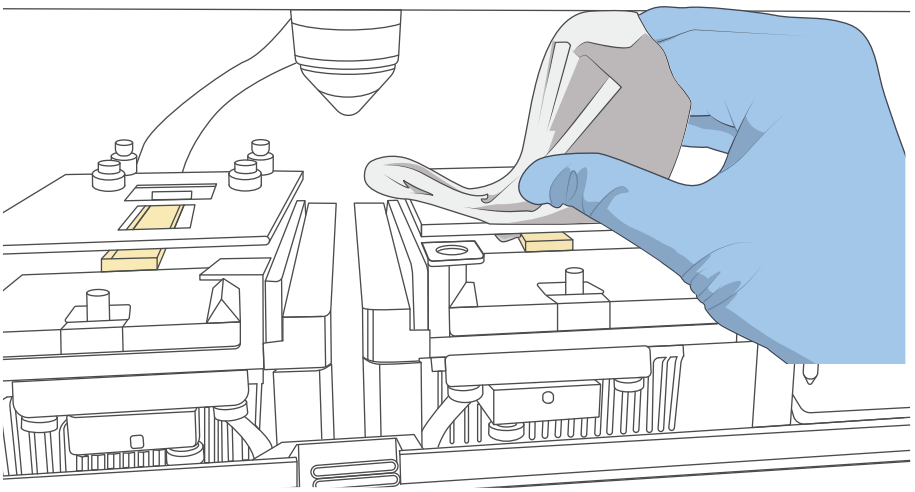


Figure 78 Cleaning the flow cell stage

6. Close the compartment door.
7. Install the M3 screw back.
8. Select **Close**. The auto-sliding door moves down and the flow cell stage maintenance is completed.

Monthly maintenance

Clearing the historical data in the storage drive

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

Maintaining the device

The low-lint cloth should be kept 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 low-lint cloth moistened with 75% ethanol. Ensure that the surface is free of samples and reagents, blood, and saliva.

Annual maintenance

It is recommended 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 end-of-life device according to local regulations. However, if it is confirmed that the device is still functioning safely and effectively after maintenance, continue to use the device.

DL-G99 maintenance

WARNING

- Do not immerse the loader into liquid for cleaning. Doing so may damage the device.
- Do not use any disinfectants to clean the loader. Doing so may damage the device.
- If you have questions about the compatibility of disinfectants, contact CG Technical Support.

After each DNB loading, perform the following steps to maintain the loader:

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

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08

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

Q: What should I do if I forget to add the reagent into MDA well for PE sequencing run?

MDA Enzyme is required to make the second strand template for PE sequencing. When preparing the Sequencing Reagent Cartridge, the appropriate amounts of MDA Enzyme Mix and MDA Reagent need be added to MDA well. If you forget 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.

WARNING A sequencing reagent cartridge can only be reused once.

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:

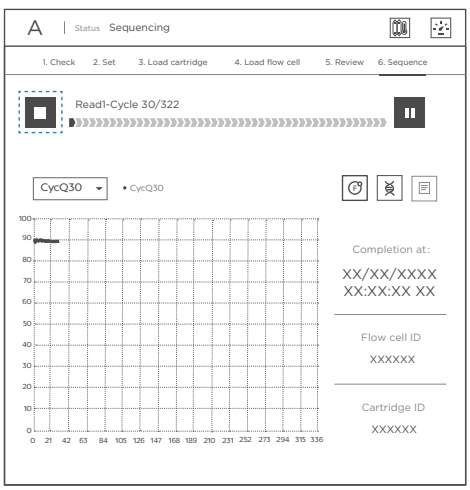

Figure 79 Selecting the sequencing stage to stop

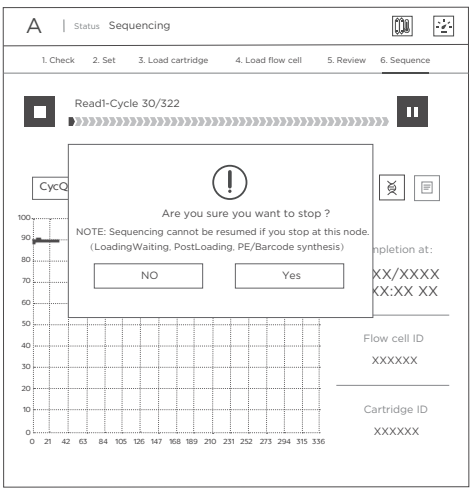

Figure 80 Confirming to stop the run

2. Remove the Sequencing Reagent Cartridge and Flow Cell:

1) Select **Finish**.
2) When the sequencing run is stopped, remove the Sequencing Reagent Cartridge and Flow Cell after the reagent compartment door slides up.
3) Select **Return home**.

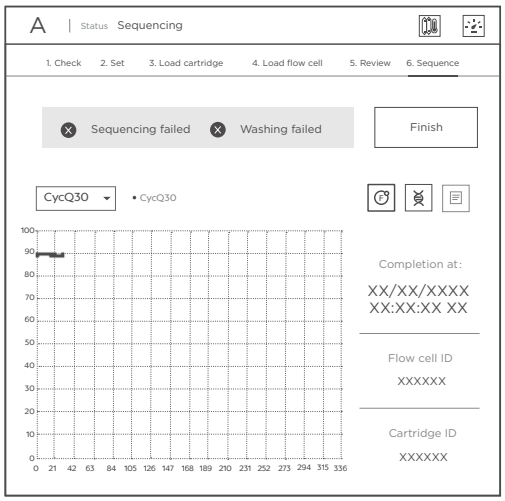

Figure 81 Selecting Finish

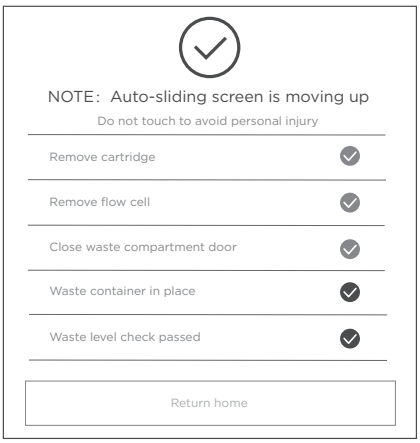

Figure 82 Removing Sequencing Reagent Cartridge and flow cell

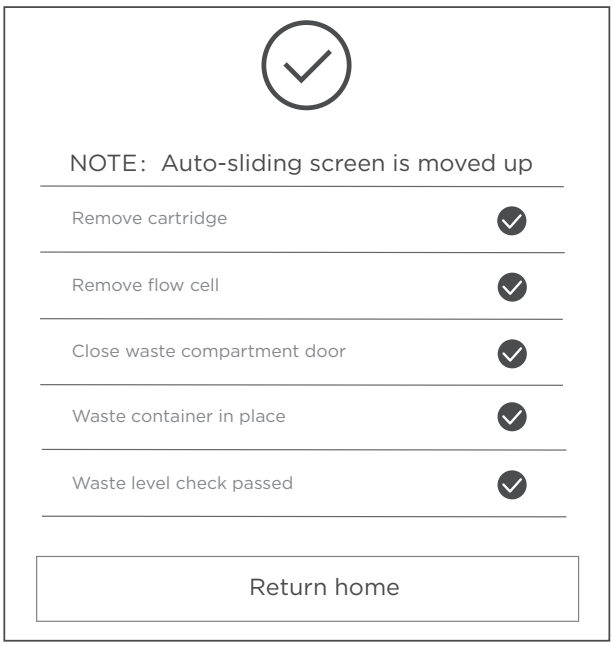

Figure 83 Selecting Return home

3. Add the MDA mixture to the Sequencing Reagent Cartridge: Add 125 µL of MDA Enzyme Mix to the MDA Reagent tube with a 200 µL pipette. Invert the tube 6 times to mix the reagents and transfer the entire volume of the mixture into the MDA well.

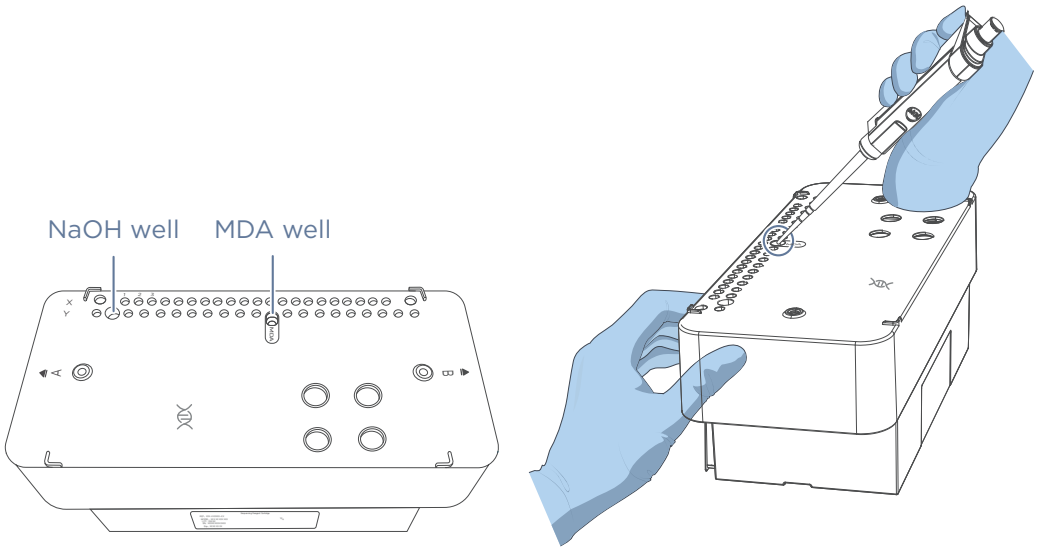

Figure 84 Adding MDA mixture

4. Check before resuming sequencing: Select **Sequence** > **Resume Sequence**. A window displaying the two most recently used flow cells that can be used for resuming sequencing appears. Select one flow cell and select **Confirm**. The system will perform a check before resuming sequencing.

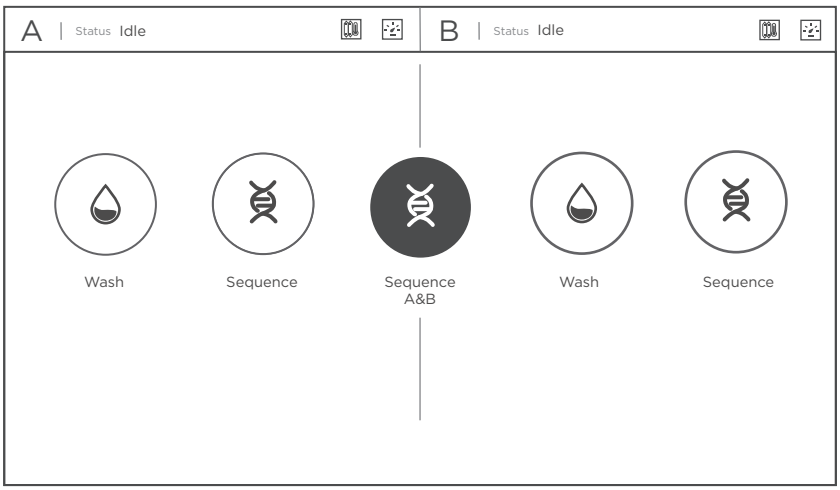

Figure 85 Main interface

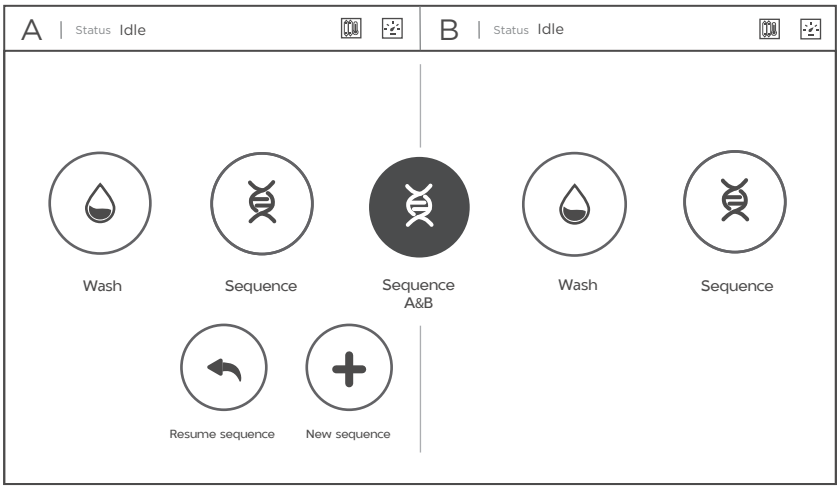

Figure 86 Resume sequence interface

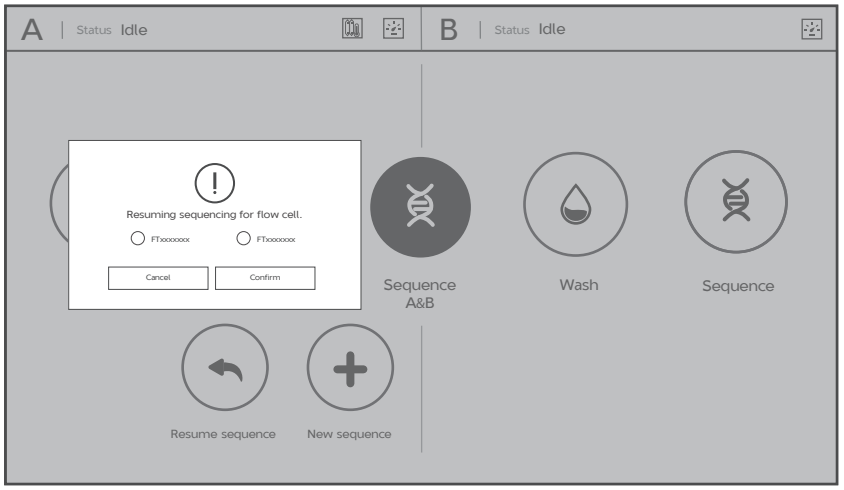

Figure 87 **Select one flow cell for resuming sequencing**

5. Select **Next** after the check is completed.

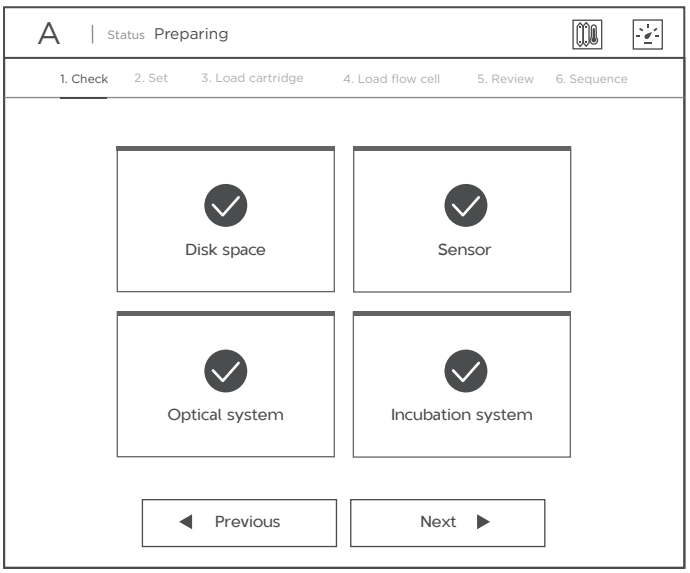

Figure 88 **Checking completed**

6. Resume sequencing:
   1) Put the Sequencing Reagent Cartridge back into the sequencer and select **Prime** to perform priming.
   2) After priming is completed, insert the Flow Cell and select **Next**.
   3) Confirm that all information is correct and select **Sequence** to resume the sequencing run as shown below.

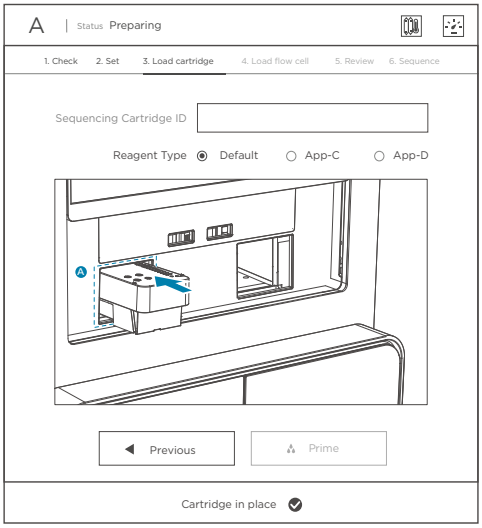

Figure 89 Placing cartridge

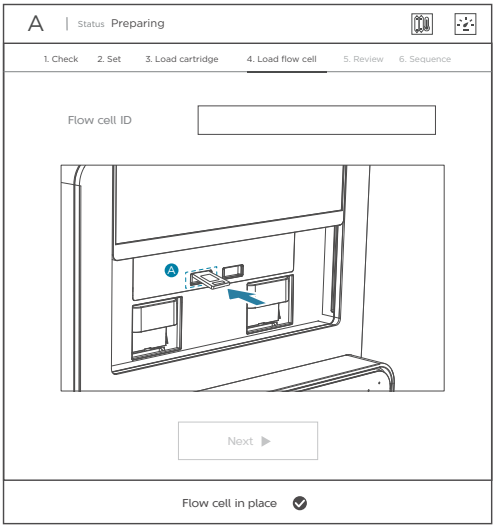

Figure 90 Placing flow cell

Figure 91 Confirming information

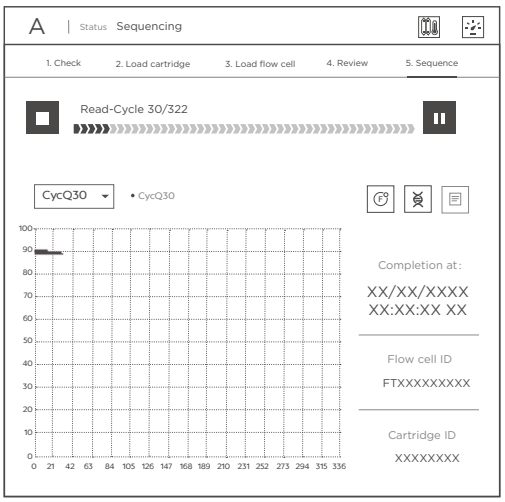

Figure 92 Starting resuming sequencing

Q: What should I do if I want to resume a stopped sequencing run?

WARNING

- Only runs that have been stopped during the Read1, Read2, or barcode sequencing phase can be resumed.
- A sequencing reagent cartridge can only be resumed once.

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:

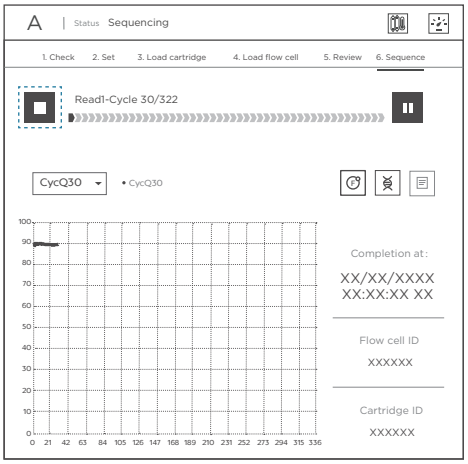

Figure 93 Selecting the sequencing stage to stop

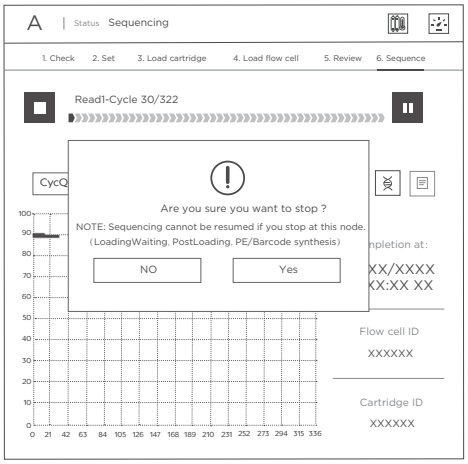

Figure 94 Confirming to stop the run

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

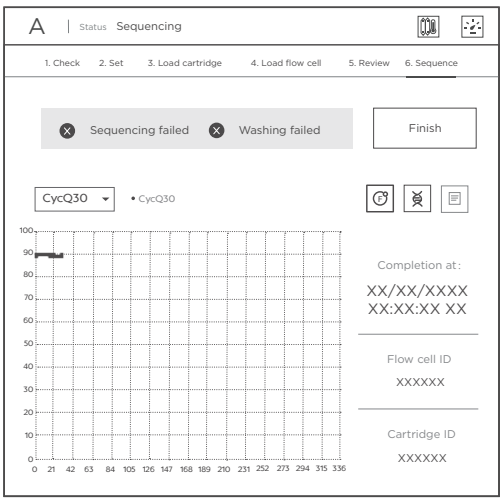

Figure 95 Selecting Finish

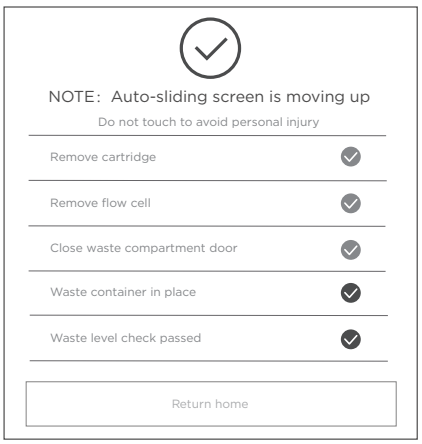

Figure 96 Removing Sequencing Reagent Cartridge and flow cell

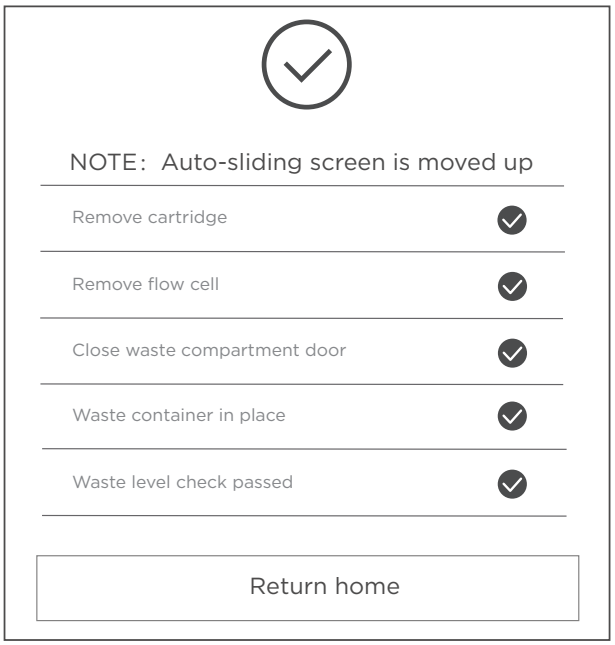

Figure 97 Selecting Return home

3. Check before resuming sequencing: Select **Sequence** > **Resume Sequence**. A window displaying the two most recently used flow cells that can be used for resuming sequencing appears. Select one flow cell and select **Confirm**. The system will perform a check before resuming sequencing.

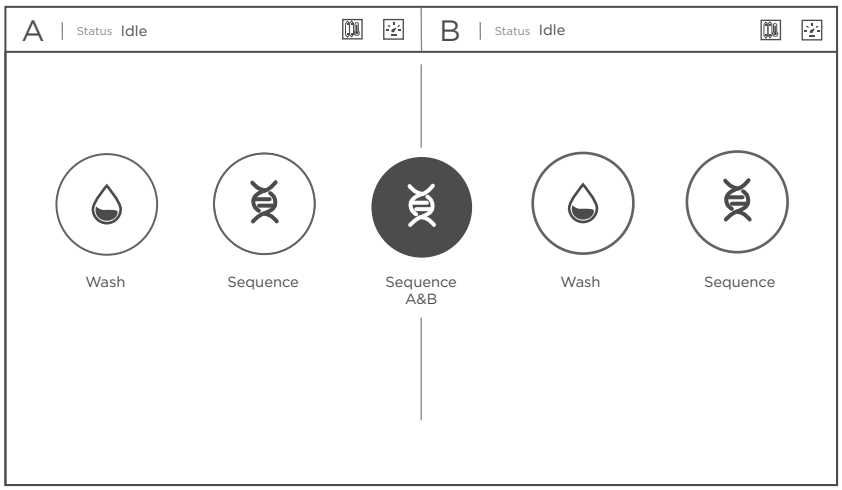

Figure 98 **Main interface**

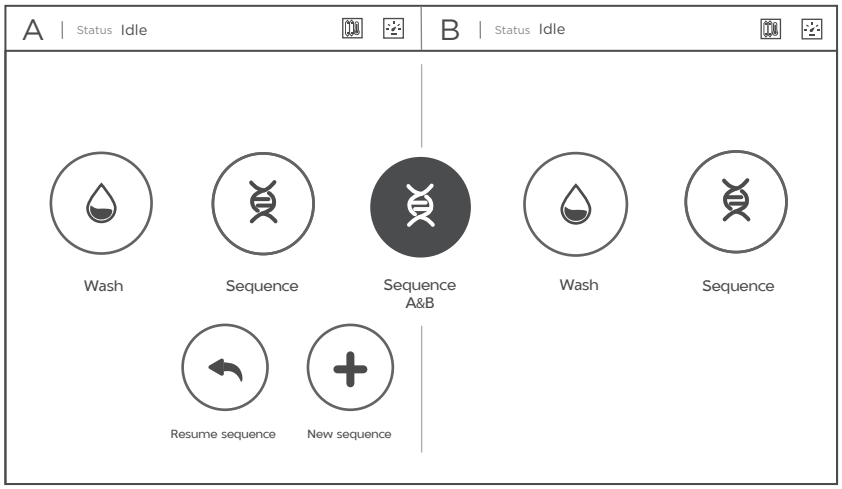

Figure 99 **Resume sequence interface**

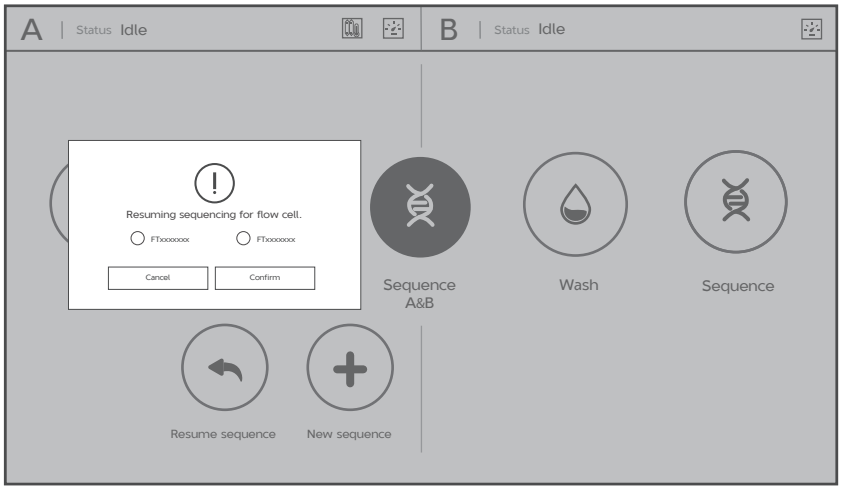

Figure 100 **Select one flow cell for resuming sequencing**

4. Select **Next** after the check is completed.

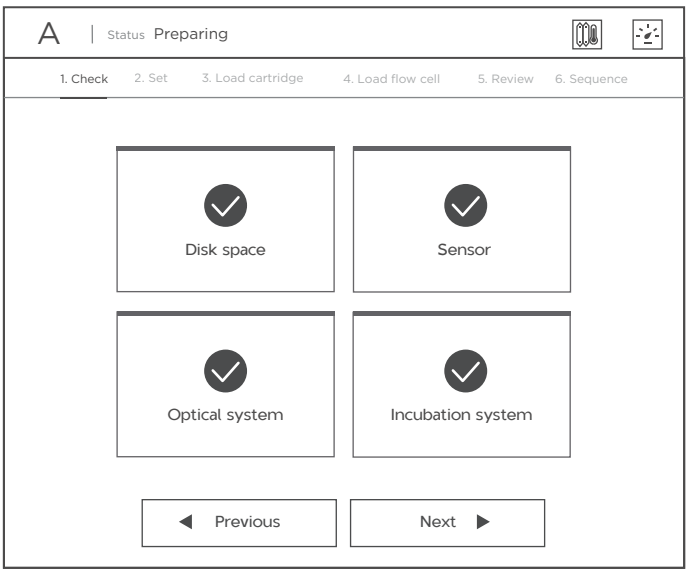

Figure 101 **Checking completed**

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

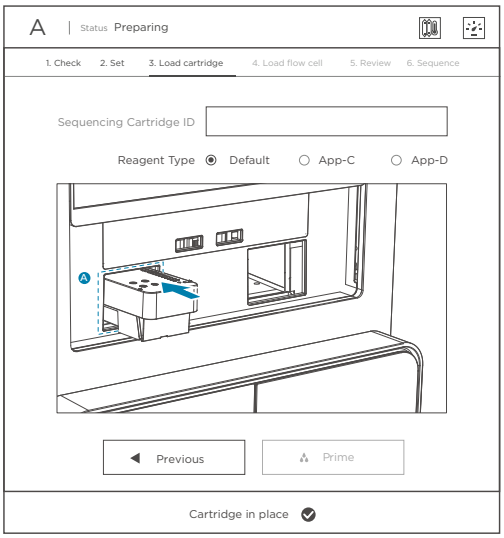

Figure 102 **Placing cartridge**

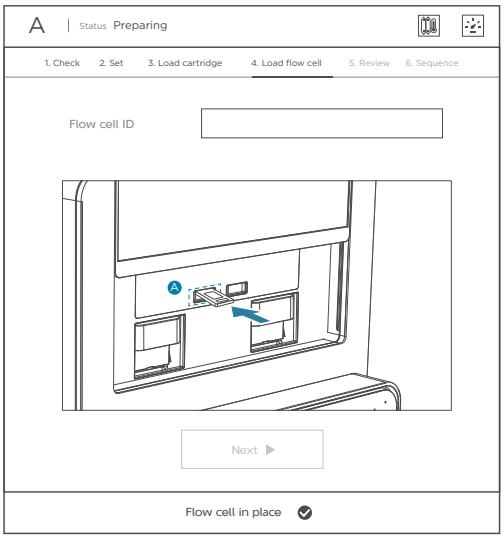

Figure 103 **Placing flow cell**

Figure 104 **Confirming information**

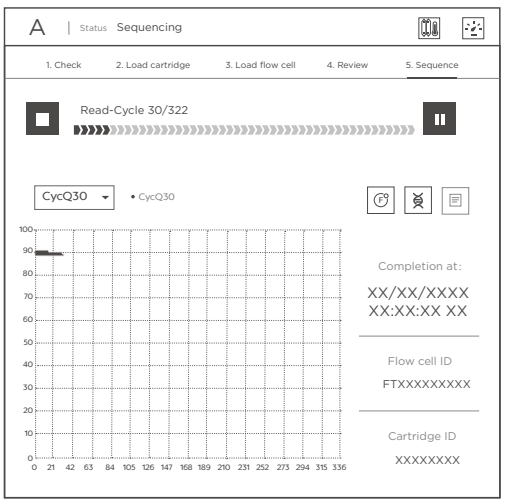

Figure 105 **Starting resuming sequencing**

Q: What should I do if I want to resume a stopped sequencing run after restarting or powering off the device normally?

If you need to restart or power off the device after a sequencing run is stopped, the system will save the data of the stopped run. After restarting or powering off and then powering on the device, you can resume the stopped run.

Tips Resuming a stopped sequencing run is not supported if you manually restart the Basecall server or the device is powerd off abnormally.

Perform the following steps:

1. Stop the run, and remove the Sequencing Reagent Cartridge and Flow Cell.

   For details, refer to steps 1-2 of *Q: What should I do if I want to resume a stopped sequencing run? on Page 141*.

2. Restart or shut down the device:

   1) Select > **Shut down**. The Shut down/Restart interface is displayed. You are prompted that the stopped sequencing run can be resumed.

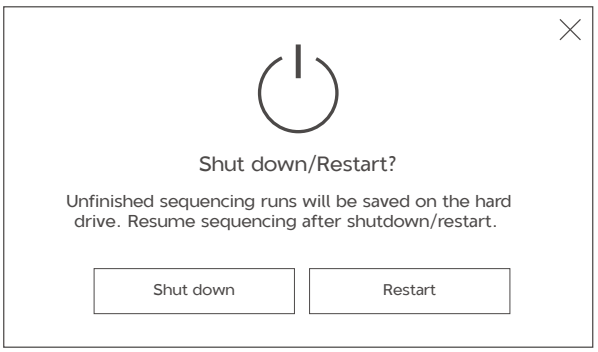

Figure 106 Shut down/Restart interface

   2) Select **Shut down** to shut down the device or select **Restart** to restart the device.

3. After power-on, resume a stopped sequencing run. For details, refer to steps 3-5 of *Q: What should I do if I want to resume a stopped sequencing run? on Page 141*.

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

- If a cartridge has been thawed without piercing M1, M2, M3, M4 wells and cannot be used within 24 h, the cartridge can be frozen and thawed at most one additional time. Alternatively, the cartridge may be stored at 4 °C if it is going to be used within 24 h. Mix the reagents in the cartridge before use by following the instructions in *Performing a sequencing run on Page 65*.
- If the reagents of M1, M2, M3, and 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 h. Mix the reagents in the cartridge before use by following the instructions in *Performing a sequencing run on Page 65*.

Q: 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**.

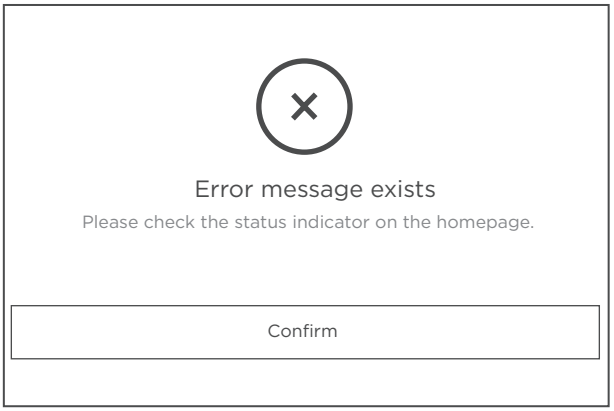

Figure 107 Error message

2. Select . If the following alarm appears, select **Close** to close the alarm information and perform operation according to the alarm description.

Figure 108 Error alarm

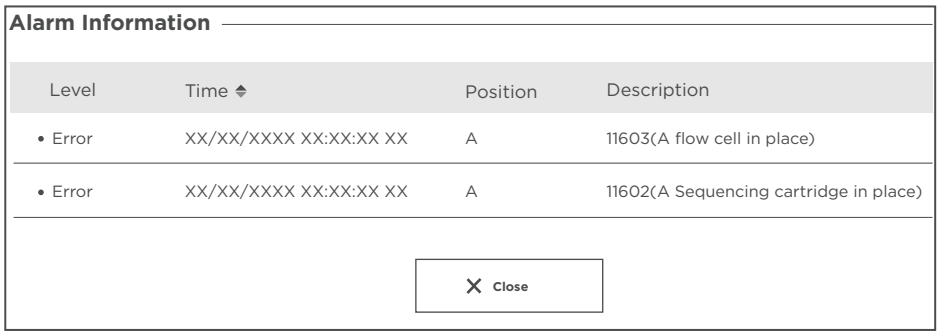

Figure 109 Alarm information

For example, if the alarm description shows that a flow cell or a sequencing cartridge is in place, select > **Maintenance** > **Tools**, select **Auto-sliding screen** > **Screen Up**, and remove the Sequencing Reagent Cartridge and Flow Cell.

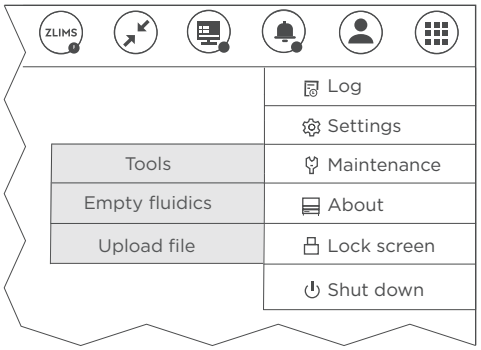

Figure 110 **Maintenance menu**

Figure 111 **Maintenance operation interface**

3. Select **Close**.

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

When the negative pressure icon in the flow cell operation area turns to , it indicates that the negative pressure is abnormal. Try the steps below:

1. Remove the flow cell from the flow cell stage.
2. Wipe the aluminum chuck of the flow cell stage with a low-lint cloth moistened with 75% ethanol.

For details, refer to *Cleaning the flow cell stage on Page 131*.

3. Blow the back of the flow cell by using a canned air duster. Ensure that no dust is present.
4. Re-insert the flow cell into the flow cell compartment.
5. If the problem persists, contact the technical support.

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

Ensure that cleaning and maintenance for the flow cell stage have been performed regularly.

If impurities appear, perform the following steps:

1. After the sequencing is completed, perform a deep wash on the sequencer according to *Performing a deep wash on Page 126 .*
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 power on after I turn the power switch to the ON position?

Powering issues arise when the main power supply is in an abnormal condition, not connected to the main power supply/UPS, or if the UPS has run out of power.

Perform the following steps:

1. Check whether the main power supply and UPS are operating normally.
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 the 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 reported by the temperature sensor. 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 a check before sequencing?

- If the local disk D drive is damaged, an error message may appear during the check. It is recommended that you record the warnings and the related logs of the sequencing run and contact the technical support.
- Error messages may appear during detection of the Sensor, Optical system, and Incubation system. It is recommended that you record the warnings and the related logs of the sequencing run and contact the technical support.

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Instructions for importing barcodes

Barcode settings

Select > **Settings** > **Barcode** to open 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.

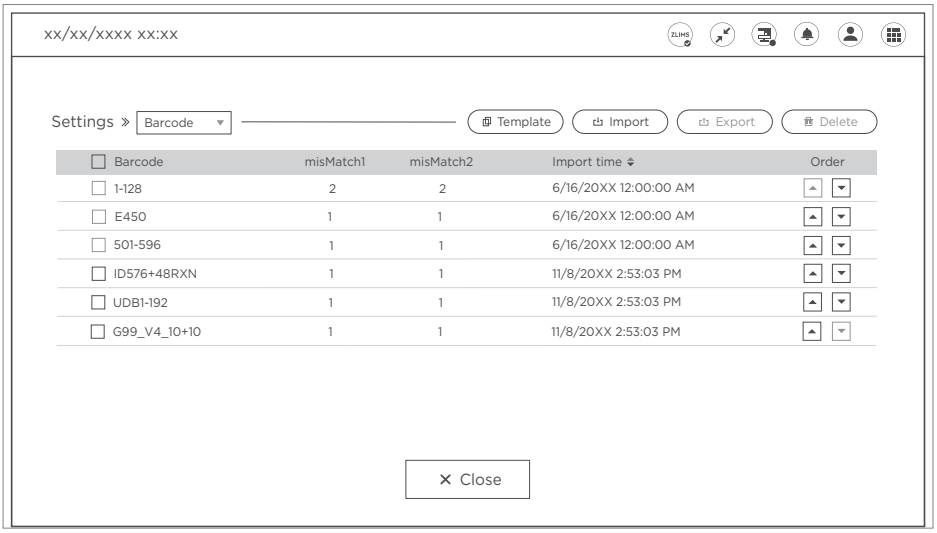

Figure 112 **Barcode settings interface**

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

Table 55 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 files.
misMatch1	Displays the number of Barcode mismatches in the barcode files.
misMatch2	Displays the number of DualBarcode mismatches in the barcode files.
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 barcode files.
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.

The barcode template can be downloaded only when both flow cell stages are in idle status.

Preparing the barcode files

Ensure that the barcode file meets the following requirements:

- The barcode file can be imported only through the control software.
- The barcode file must be in CSV format only.
- If the file name or content of the barcode file to be imported is the same as an existing barcode file, it cannot be imported.
- The barcode ID and mismatch number must be numeric.
- The barcode ID and mismatch number are mandatory for each barcode file.
- The barcode ID and barcode sequence in the file must be separated by a comma.
- The barcode file must not contain blank lines, or full-width characters. The barcode sequence should include no fewer than two bases.
- The barcode sequences of a DualBarcode file must not contain any characters other than “A”, “T”, “C”, “G”, and “N”.
- The barcode sequences of a Barcode file must not contain any characters other than “A”, “T”, “C”, and “G”.
- The barcode ID must not be empty but can be repeated. The barcode sequence must be unique and must not be empty.

Examples for the barcode files are shown in the following sections.

Table 56 Barcode file classification

Type of barcodes	Sequence type	Libraries used	Description
Only Barcode	PE sequencing	CG libraries	Refer to *Figure 113 on Page 158*.
	PE sequencing	Thrid-party libraries	Refer to *Figure 114 on Page 159*.
	PE sequencing	CG and Thrid-party libraries	Refer to *Figure 115 on Page 160*.
	SE sequencing	CG libraries	Refer to *Figure 116 on Page 161*.
	SE sequencing	Thrid-party libraries	Refer to *Figure 117 on Page 162*.
	SE sequencing	CG and Thrid-party libraries	Refer to *Figure 118 on Page 163*.
Only DualBarcode	PE sequencing	CG libraries	Refer to *Figure 119 on Page 164*.
	PE sequencing	Thrid-party libraries	Refer to *Figure 120 on Page 165*.
	SE sequencing	CG and Thrid-party libraries	Refer to *Figure 121 on Page 166*.
	SE sequencing	CG libraries	Refer to *Figure 122 on Page 167*.
	SE sequencing	Thrid-party libraries	Refer to *Figure 123 on Page 168*.
	SE sequencing	CG and Thrid-party libraries	Refer to *Figure 124 on Page 169*.
Barcode and DualBarcode	PE sequencing	CG libraries	Refer to *Figure 125 on Page 170*.
	PE sequencing	Thrid-party libraries	Refer to *Figure 126 on Page 171*.
	PE sequencing	CG and Thrid-party libraries	Refer to *Figure 127 on Page 172*.
	SE sequencing	CG libraries	Refer to *Figure 128 on Page 174*.
	SE sequencing	Thrid-party libraries	Refer to *Figure 129 on Page 175*.
	SE sequencing	CG and Thrid-party libraries	Refer to *Figure 130 on Page 176*.

- *SEi5/PEi7* indicates that *i5 Bases for Sample Sheet* (hereinafter referred to as *i5*) is used for SE sequencing and *i7 Bases for Sample Sheet* (hereinafter referred to as *i7*) is used for PE sequencing. *SEi7/PEi5* indicates that *i7* is used for SE sequencing and *i5* is used for PE sequencing.
- For Barcode sequencing, use *i5* for SE sequencing and *i7* for PE sequencing, both correspond to **Barcode (SEi5/PEi7)** in the **Create Recipe** interface.
- For DualBarcode sequencing, use *i5* for SE sequencing and *i7* for PE sequencing, both correspond to **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface.
- For Barcode and DualBarcode sequencing:
  - SE sequencing order: i7 > i5
    - i7 corresponds to **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface.
    - *i5* correspond to **Barcode (SEi5/PEi7)** in the **Create Recipe** interface.
  - PE sequencing order: i5 > i7
    - *i5* corresponds to **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface.
    - *i7* correspond to **Barcode (SEi5/PEi7)** in the **Create Recipe** interface.

Barcode file

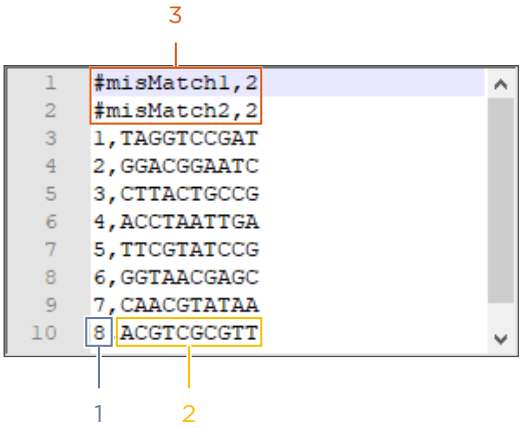

Figure 113 CG Barcode file for PE sequencing

Table 57 Description for CG Barcode file for PE sequencing

No.	Name	Description
1	CG Barcode ID	Corresponds to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
2	CG Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
3	Number of mismatches	/

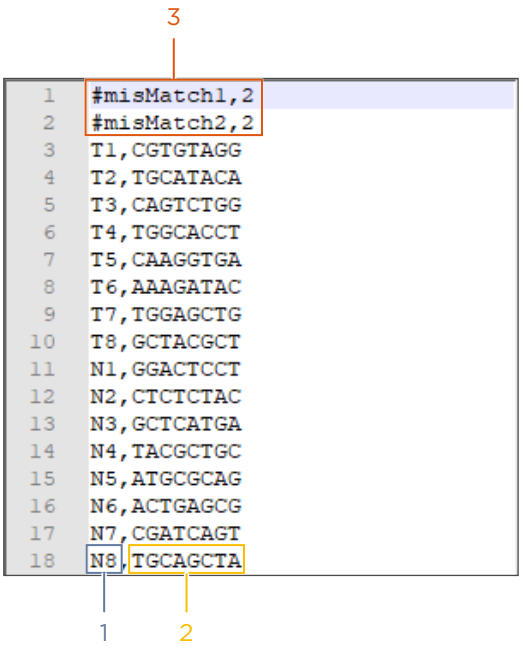

Figure 114 App Barcode file for PE sequencing

Table 58 Description for App Barcode file for PE sequencing

No.	Name	Description
1	App Barcode ID	Corresponds to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
2	App Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
3	Number of mismatches	/

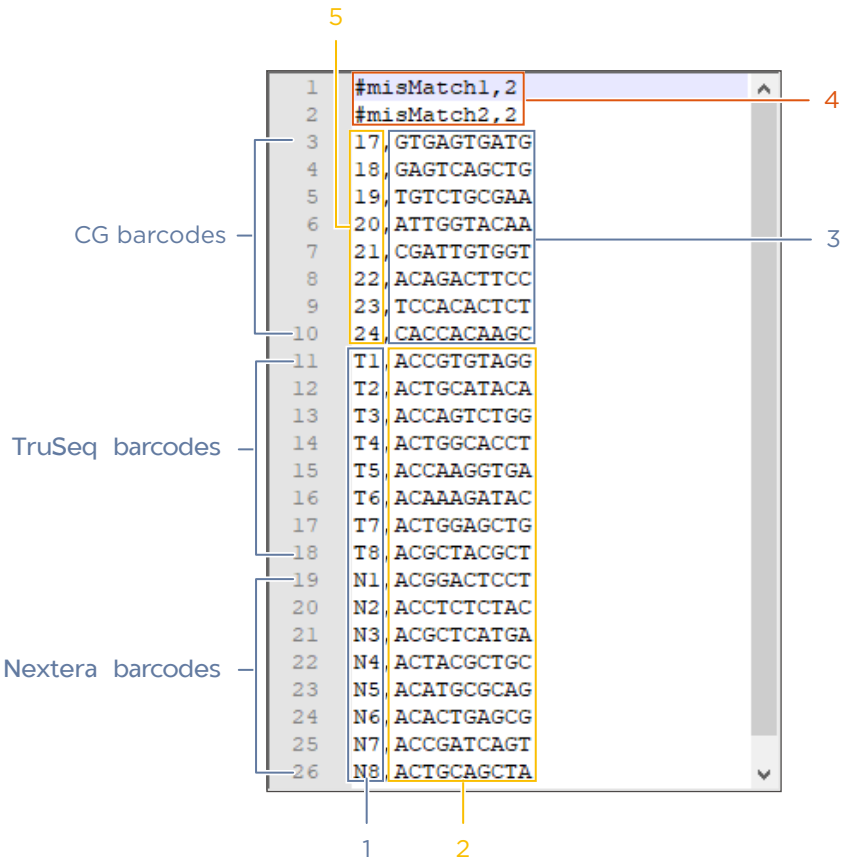

Figure 115 **CG and App Barcode file for PE sequencing**

Table 59 **Description for CG and App Barcode file for PE sequencing**

No.	Name	Description
1	App Barcode IDs	Correspond to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
2	Adapted App Barcode sequence	Correspond to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface The first two bases “AC” of each sequence are added to maintain overall uniformity
3	CG Barcode sequences	Correspond to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
4	Number of mismatches	/
5	CG Barcode IDs	Correspond to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface

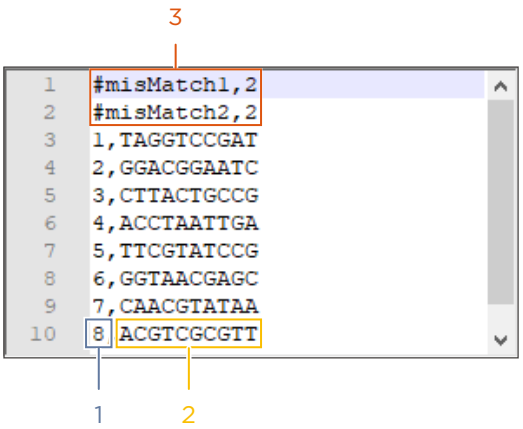

Figure 116 CG Barcode file for SE sequencing

Table 60 Description for CG Barcode file for SE sequencing

No.	Name	Description
1	CG Barcode ID	Corresponds to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
2	CG Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
3	Number of mismatches	/

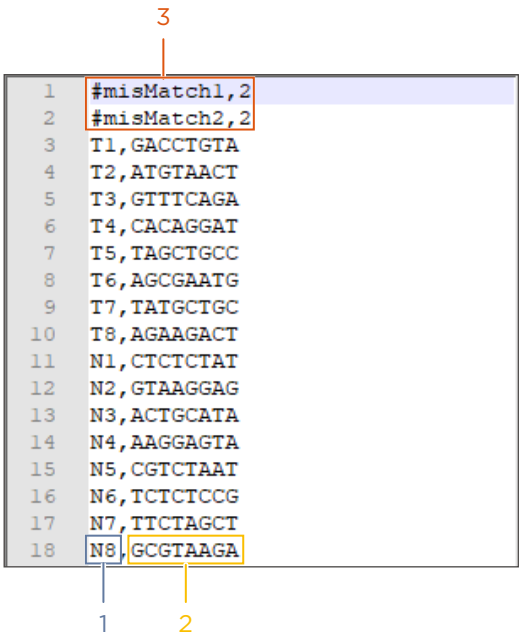

Figure 117 App Barcode file for SE sequencing

Table 61 Description for App Barcode file for SE sequencing

No.	Name	Description
1	App Barcode ID	Corresponds to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
2	App Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
3	Number of mismatches	/

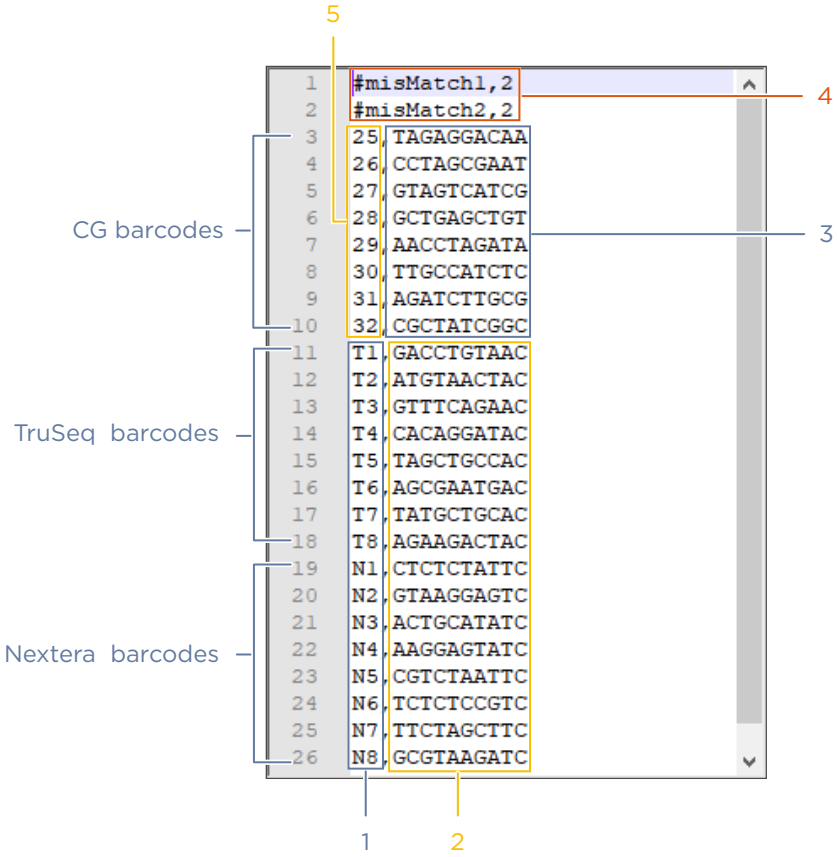

Figure 118 **CG and App Barcode file for SE sequencing**

Table 62 **Description for CG and App Barcode file for SE sequencing**

No.	Name	Description
1	App Barcode IDs	Correspond to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
2	Adapted App Barcode sequence	Correspond to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface The last two bases are added to maintain overall uniformity *i* Use “AC” for TruSeq barcodes and use “TC” for Nextera barcodes.
3	CG Barcode sequences	Correspond to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
4	Number of mismatches	/
5	CG Barcode IDs	Correspond to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface

DualBarcode file

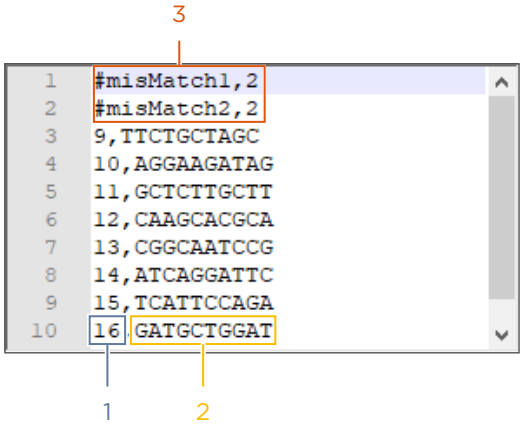

Figure 119 CG DualBarcode file for PE sequencing

Table 63 Description for CG DualBarcode file for PE sequencing

No.	Name	Description
1	CG DualBarcode ID	Corresponds to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
2	CG DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
3	Number of mismatches	/

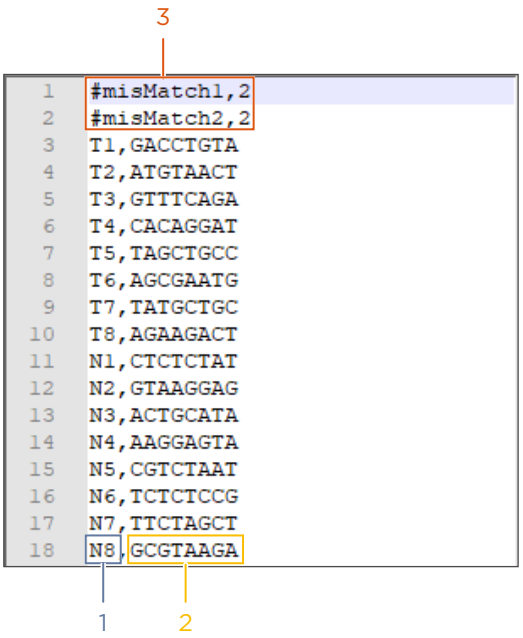

Figure 120 App DualBarcode file for PE sequencing

Table 64 Description for App DualBarcode file for PE sequencing

No.	Name	Description
1	App DualBarcode ID	Corresponds to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
2	App DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
3	Number of mismatches	/

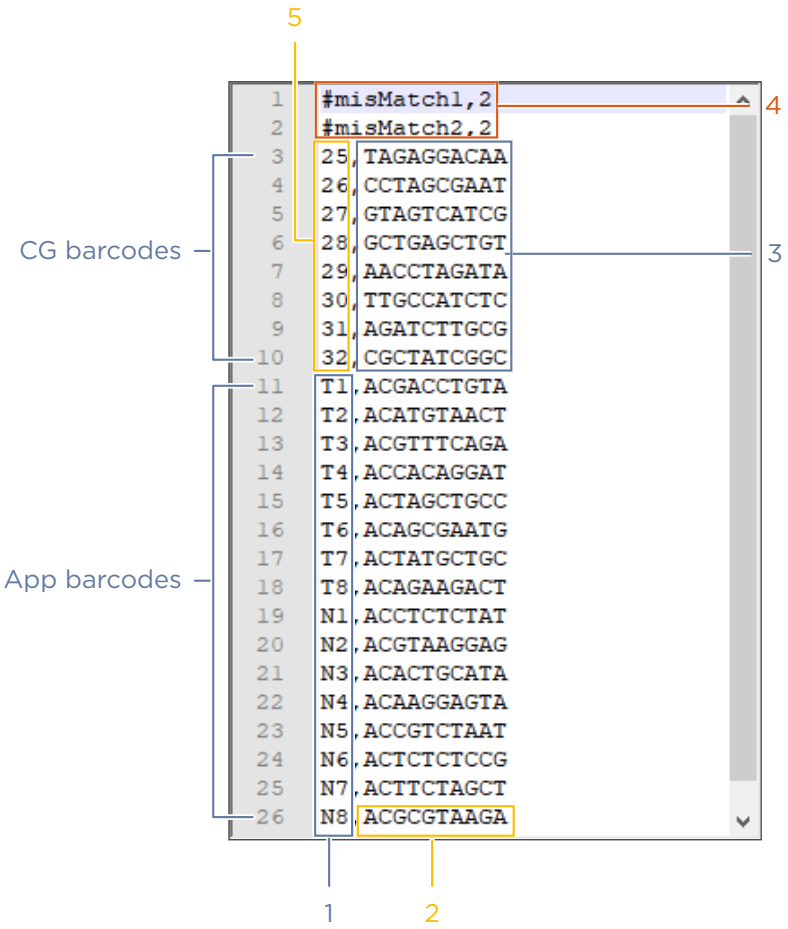

Figure 121 **CG and App DualBarcode file for PE sequencing**

Table 65 **Description for CG and App DualBarcode file for PE sequencing**

No.	Name	Description
1	App DualBarcode IDs	Correspond to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
2	Adapted App DualBarcode sequence	Correspond to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface. The first two bases “AC” of each sequence are added to maintain overall uniformity
3	CG DualBarcode sequences	Correspond to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
4	Number of mismatches	/
5	CG DualBarcode IDs	Correspond to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface

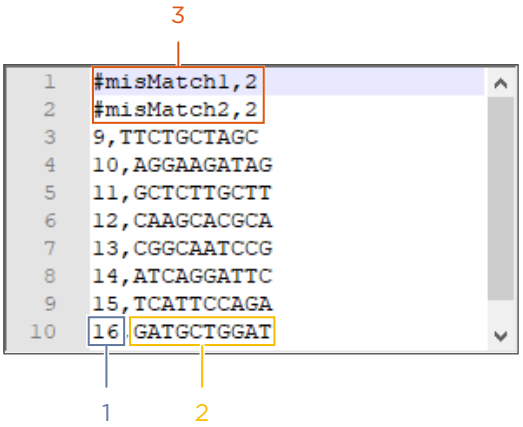

Figure 122 **CG DualBarcode file for SE sequencing**

Table 66 **Description for CG DualBarcode file for SE sequencing**

No.	Name	Description
1	CG DualBarcode ID	Corresponds to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
2	CG DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
3	Number of mismatches	/

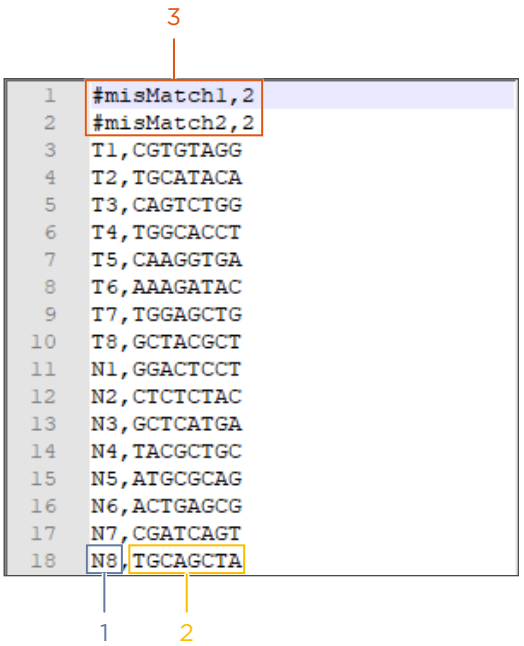

Figure 123 App DualBarcode file for SE sequencing

Table 67 Description for App DualBarcode file for SE sequencing

No.	Name	Description
1	App DualBarcode ID	Corresponds to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
2	App DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
3	Number of mismatches	/

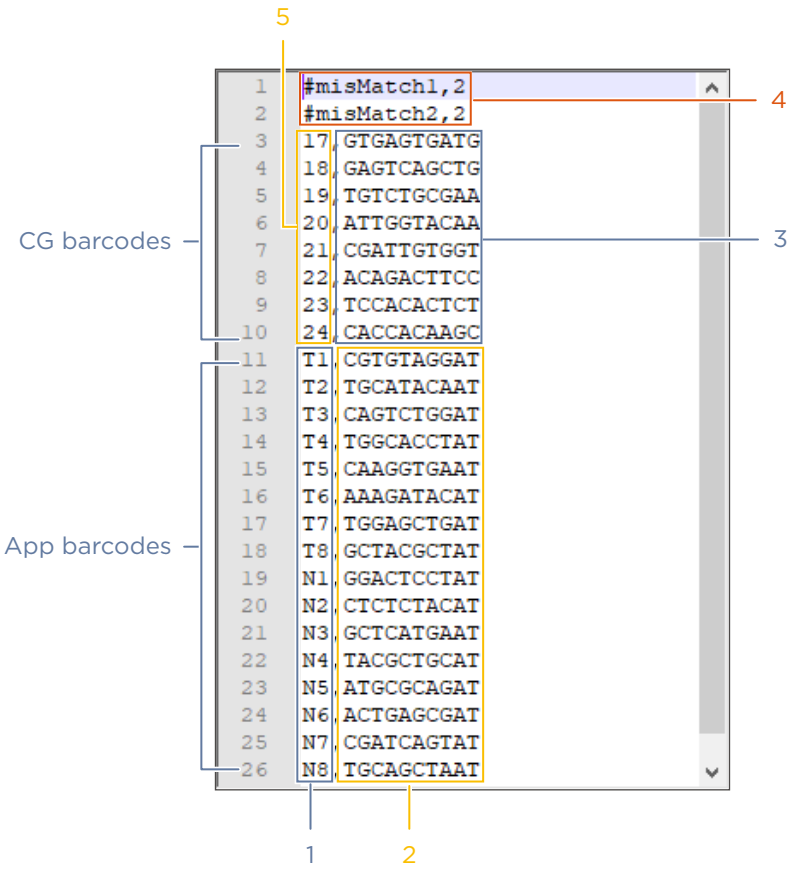

Figure 124 CG and App DualBarcode file for SE sequencing

Table 68 Description for CG and App DualBarcode file for SE sequencing

No.	Name	Description
1	App DualBarcode IDs	Correspond to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
2	Adapted App DualBarcode sequence	Correspond to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface The last two bases "AT" of each sequence are added to maintain overall uniformity
3	CG DualBarcode sequences	Correspond to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
4	Number of mismatches	/
5	CG DualBarcode IDs	Correspond to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface

Barcode and DualBarcode file

Mixed barcode splitting (both Barcode and DualBarcode splitting) is supported in the following cases:

Figure 125 CG Barcode and DualBarcode file for PE sequencing

Table 69 Description for CG Barcode and DualBarcode file for PE sequencing

No.	Name	Description
1	CG Barcode&DualBarcode ID	Corresponds to IDs of **Barcode (SEi5/PEi7)** and **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
2	CG DualBarcode IDs	Correspond to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
3	CG Barcode ID	Corresponds to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
4	Placeholder	/

No.	Name	Description
5	CG Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
6	CG DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
7	CG Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
8	CG DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
9	Number of mismatches	/

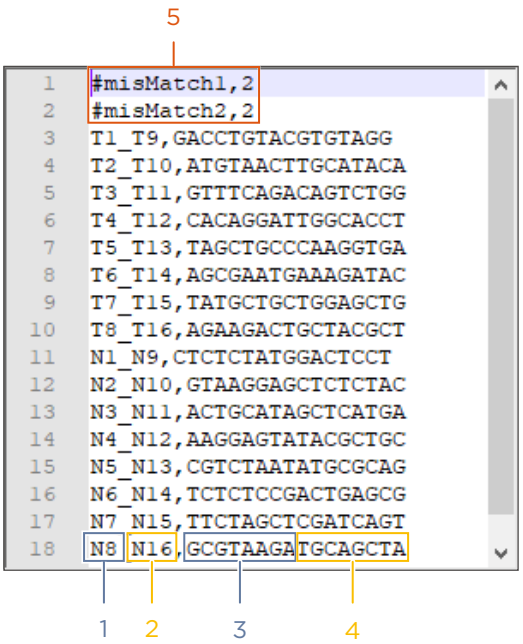

Figure 126 App Barcode and DualBarcode file for PE sequencing

Table 70 Description for App Barcode and DualBarcode file for PE sequencing

No.	Name	Description
1	App DualBarcode ID	Corresponds to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
2	App Barcode ID	Corresponds to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
3	App DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface

No.	Name	Description
4	App Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
5	Number of mismatches	/

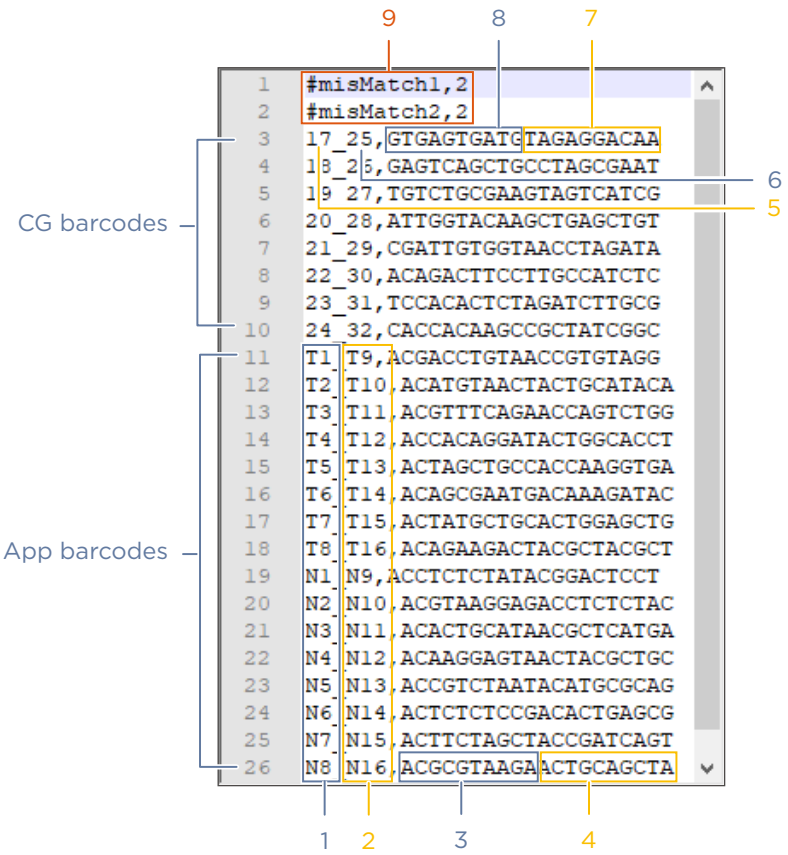

Figure 127 **CG and App Barcode and DualBarcode file for PE sequencing**

Table 71 **Description for CG and App Barcode and DualBarcode file for PE sequencing**

No.	Name	Description
1	App DualBarcode IDs	Correspond to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
2	App Barcode IDs	Corresponds to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface

No.	Name	Description
3	Adapted App DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface. The first two bases “AC” are added to maintain overall uniformity
4	Adapted App Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface. The first two bases “AC” are added to maintain overall uniformity
5	CG DualBarcode ID	Corresponds to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
6	CG Barcode ID	Corresponds to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
7	CG Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
8	CG DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
9	Number of mismatches	/

Figure 128 CG Barcode and DualBarcode file for SE sequencing

Table 72 Description for CG Barcode and DualBarcode file for SE sequencing

No.	Name	Description
1	CG Barcode&DualBarcode ID	Corresponds to IDs of **Barcode (SEi5/PEi7)** and **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
2	CG DualBarcode IDs	Correspond to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
3	CG Barcode ID	Corresponds to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
4	Placeholder	/
5	CG Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
6	CG DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface

No.	Name	Description
7	CG Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
8	CG DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
9	Number of mismatches	/

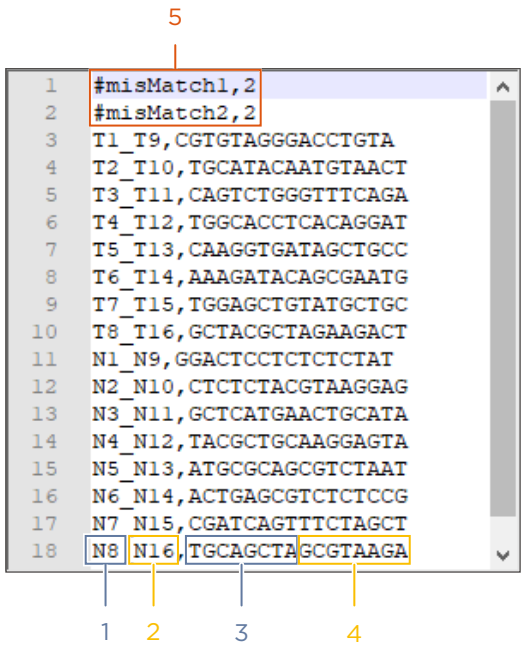

Figure 129 App Barcode and DualBarcode file for SE sequencing

Table 73 Description for App Barcode and DualBarcode file for SE sequencing

No.	Name	Description
1	App DualBarcode ID	Corresponds to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
2	App Barcode ID	Corresponds to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
3	App DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
4	App Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
5	Number of mismatches	/

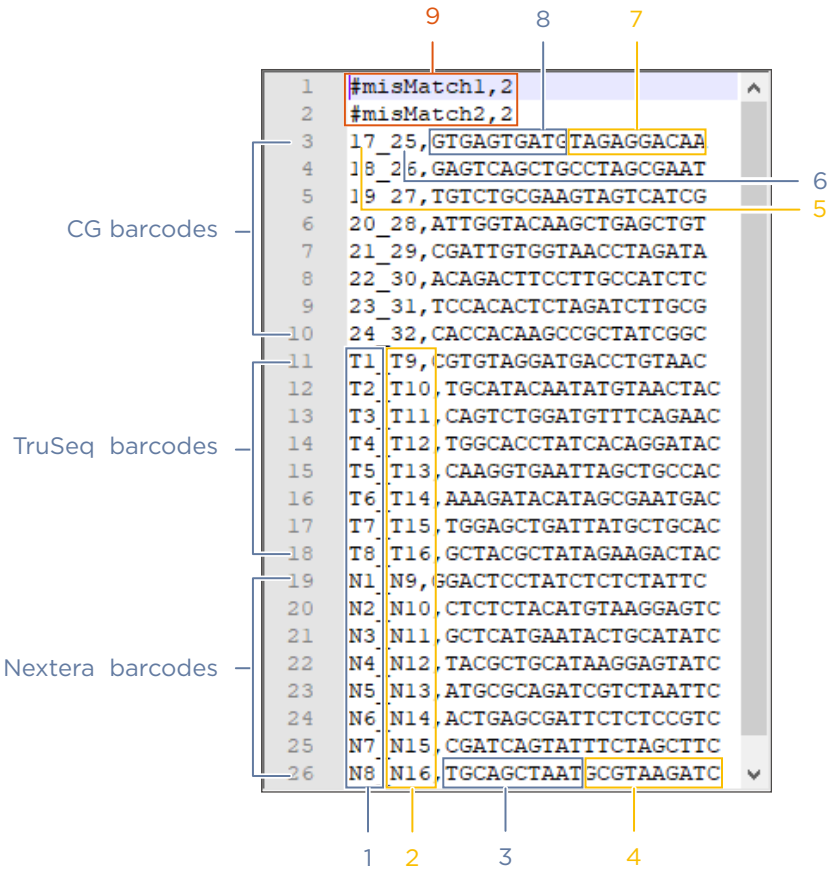

Figure 130 **CG and App Barcode and DualBarcode file for SE sequencing**

Table 74 **Description for CG and App Barcode and DualBarcode file for SE sequencing**

No.	Name	Description
1	App DualBarcode IDs	Correspond to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
2	App Barcode IDs	Correspond to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
3	Adapted App DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface The last two bases “AT” are added to maintain overall uniformity

No.	Name	Description
4	Adapted App Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface. The last two bases "TC" are added to maintain overall uniformity *i* Use "AC" for TruSeq barcodes and use "TC" for Nextera barcodes.
5	CG DualBarcode ID	Corresponds to ID of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
6	CG Barcode ID	Corresponds to ID of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
7	CG Barcode sequence	Corresponds to sequence of **Barcode (SEi5/PEi7)** in the **Create Recipe** interface
8	CG DualBarcode sequence	Corresponds to sequence of **DualBarcode (SEi7/PEi5)** in the **Create Recipe** interface
9	Number of mismatches	/

Importing barcode files

Perform the following steps:

1. Prepare a barcode file.
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 files

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

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

Deleting barcode files

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

Select the barcode files 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:

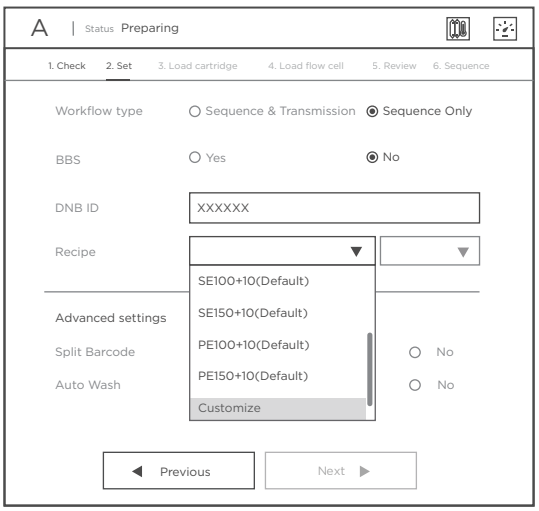

Figure 131 **Customize recipe**

Customize interface

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

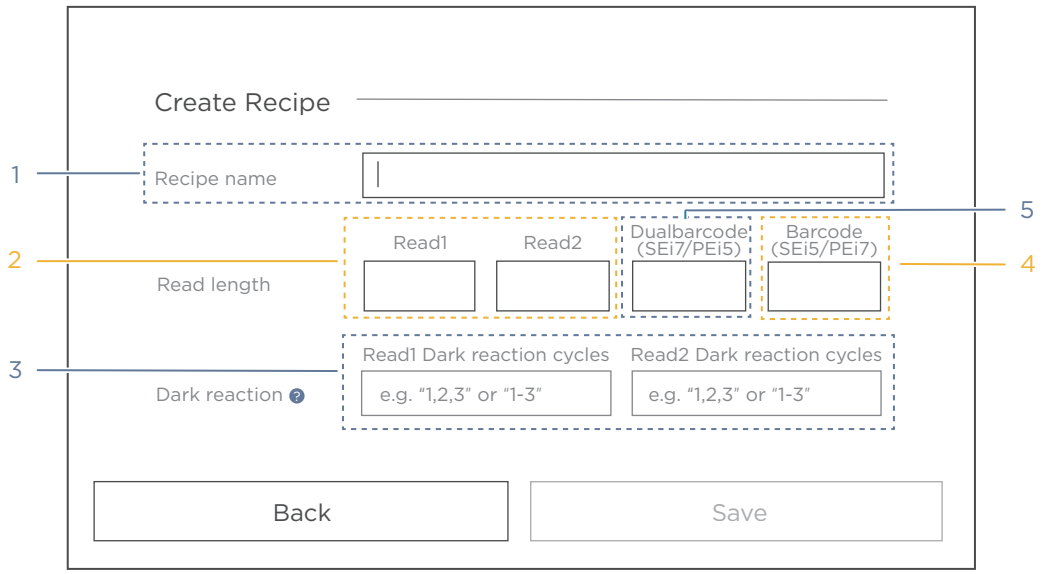

Figure 132 Customize interface

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 cycles** / **Read2 dark reaction cycles**	Customize dark reaction range in Read1 and (or) Read2
4	**Barcode (SEi5/PEi7)**	Customize Barcode length for a sequencing run.
5	**Dualbarcode (SEi7/PEi5)**	Customize DualBarcode length for a sequencing run.

Barcode (not predefined) interface

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

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

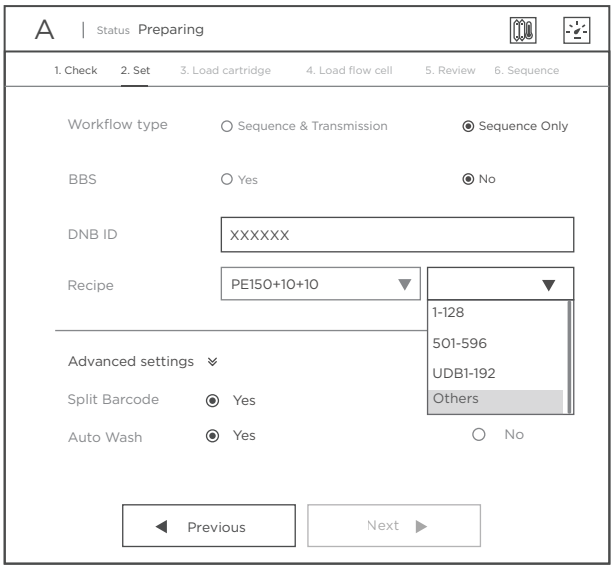

Figure 133 Selecting Others

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

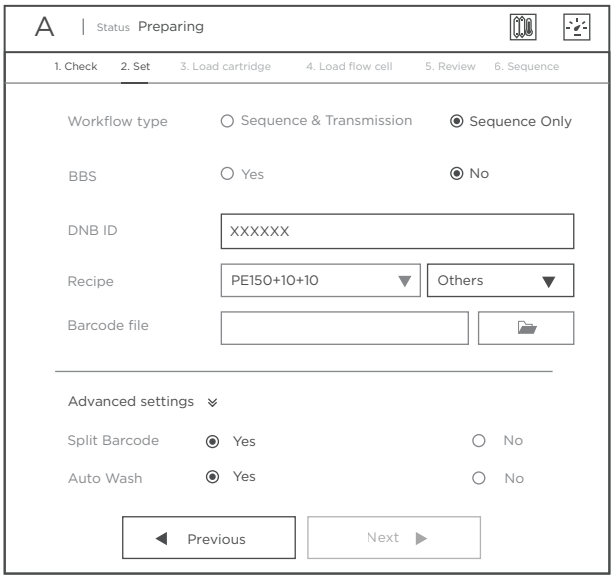

Figure 134 Configuring Customize settings

3. Select the barcode file previously imported. For information on barcode importing, refer to *Instructions for importing barcodes on Page 153*.

Examples of customized runs

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 barcodes on Page 153* 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 9 on Page 40* .
- 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.

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 for customized PE sequencing

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+1+140+1+10 = 272
- Select a PE150 set

The Customize interface is set as follows:

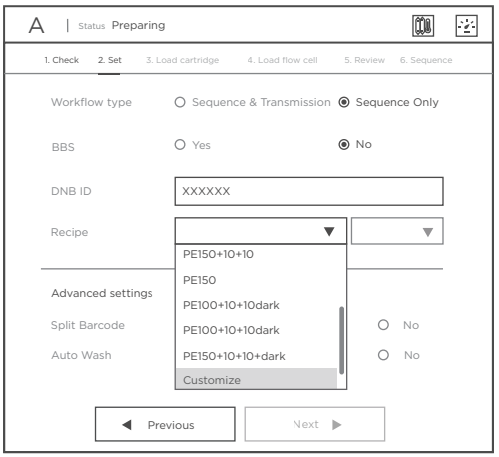

Figure 135 Selecting Customize

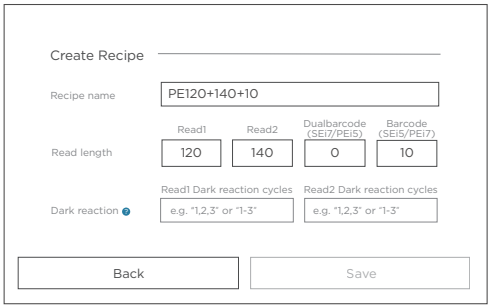

Figure 136 Configuring Customize settings

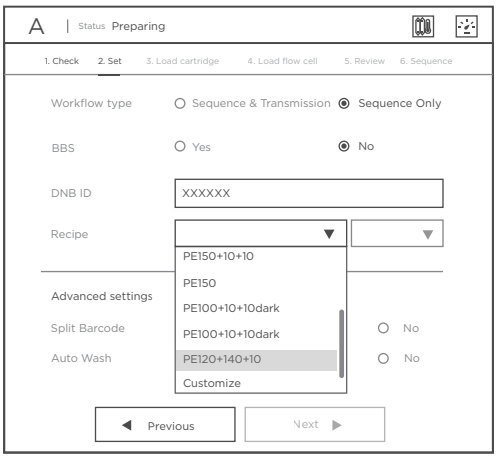

Figure 137 Selecting PE120+140+10

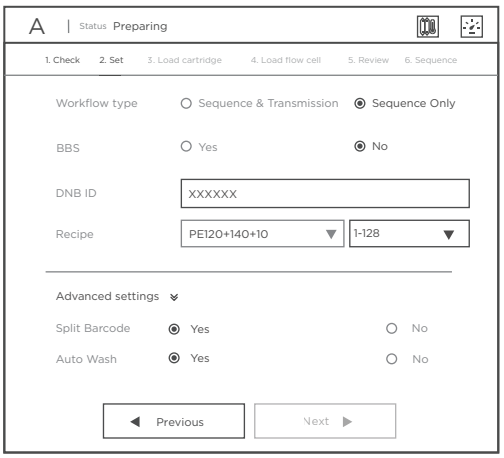

Figure 138 Selecting barcode type and split strategy

2. Single-barcode settings for customized SE sequencing

Assumptions are as follows:

- Sequencing run: SE75+10
- Length of Read1: 75
- Length of Read2: 0
- Length of Barcode: 0
- Length of DualBarcode: 10
- Total cycles = 75+1+10 = 86
- Select an SE100 set

The Customize interface is set as follows:

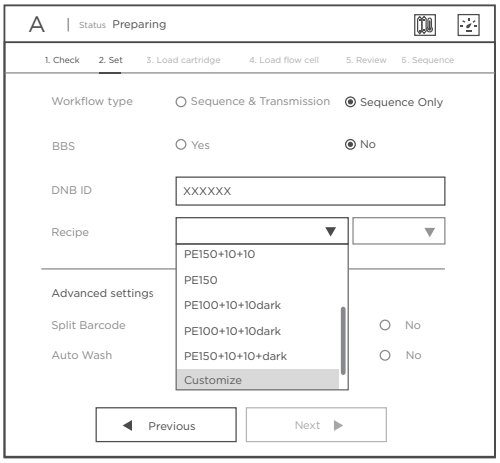

Figure 139 Selecting Customize

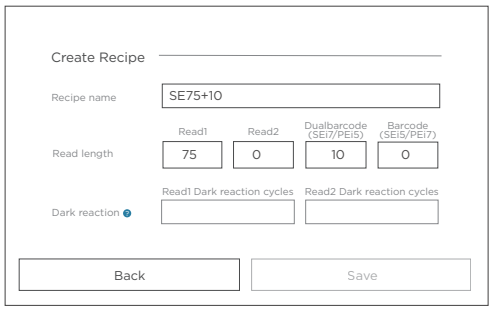

Figure 140 Configuring Customize settings

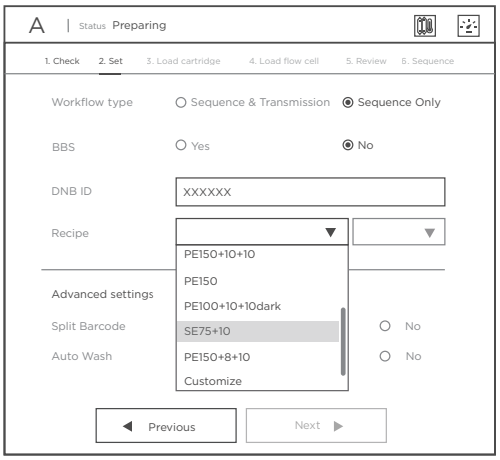

Figure 141 Selecting PE150+8+8DR

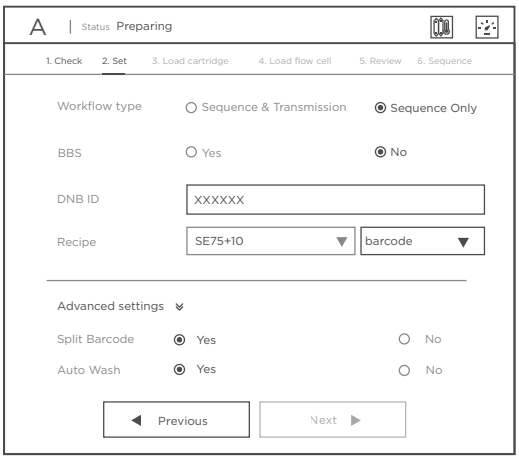

Figure 142 Selecting barcode type and split strategy

3. Length of Barcode is not 10 for customized PE sequencing

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+1+150+1+8 = 310
- Select a PE150 set

The Customize interface is set as follows:

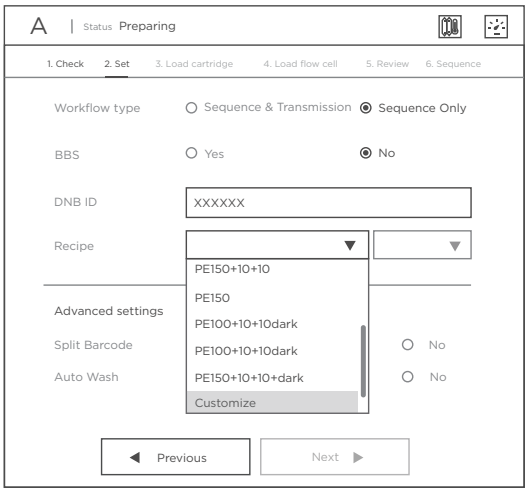

Figure 143 Selecting Customize

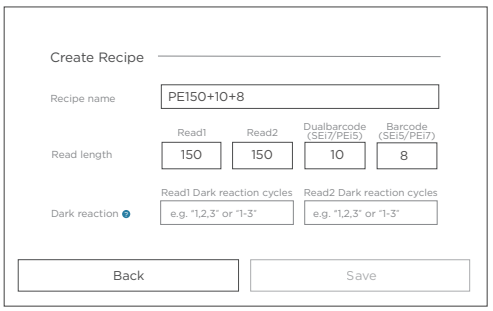

Figure 144 Configuring Customize settings

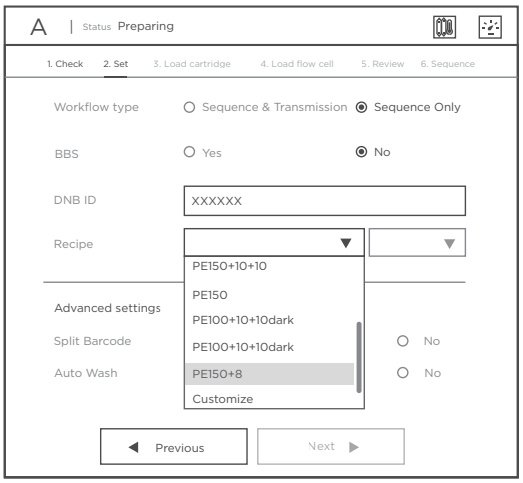

Figure 145 Selecting PE150+8

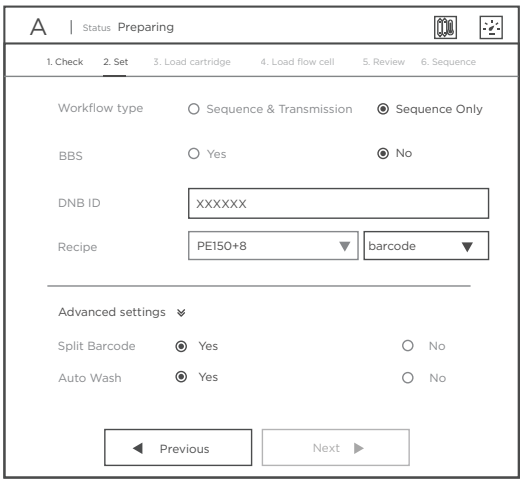

Figure 146 Selecting barcode type and split strategy

4. A dual barcode sequencing run for customized PE sequencing

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+1+150+1+8+10 = 320
- Select a PE150 set

The Customize interface is set as follows:

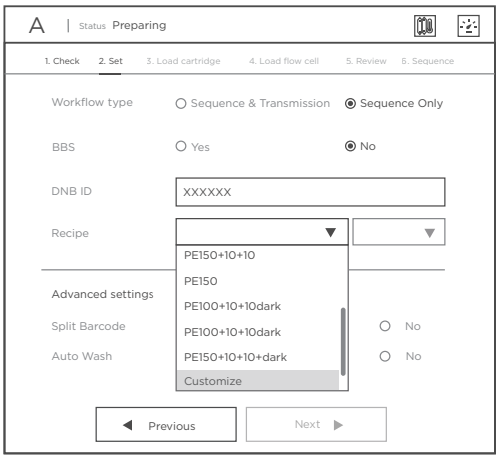

Figure 147 Selecting Customize

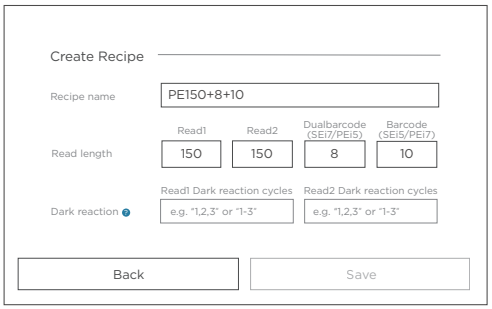

Figure 148 Configuring Customize settings

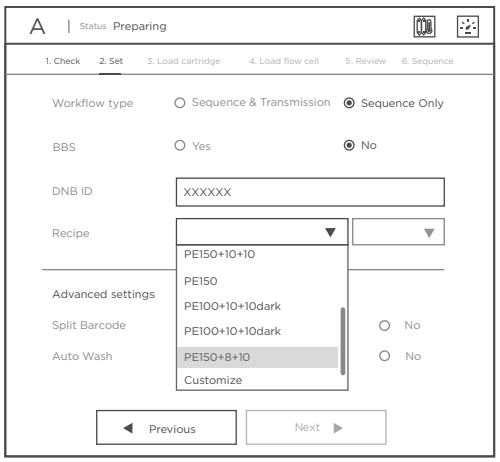

Figure 149 Selecting PE150+8+10

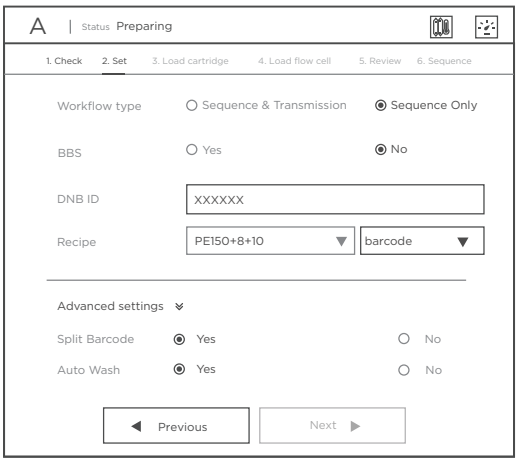

Figure 150 Selecting barcode type and split strategy

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

5. Dark reaction cycles are required in Read1 and/or Read2 sequencing for customized PE 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+1+150+1+8+8 = 318
- Select a PE150 set

The Customize interface is set as follows:

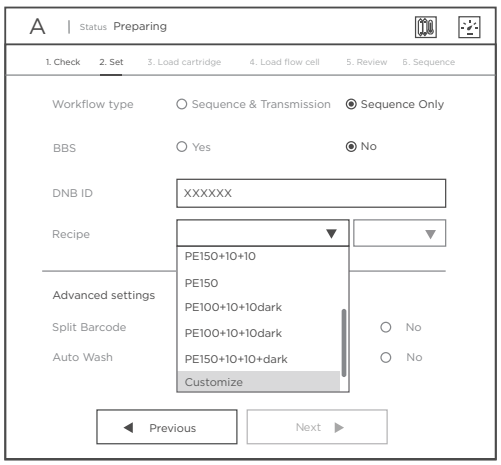

Figure 151 Selecting Customize

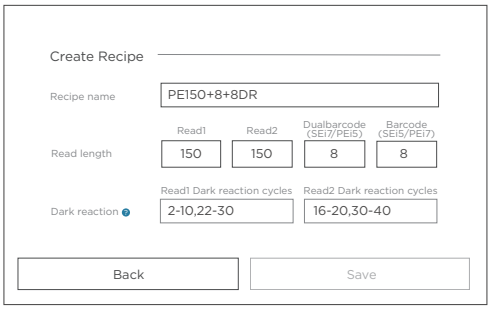

Figure 152 Configuring Customize settings

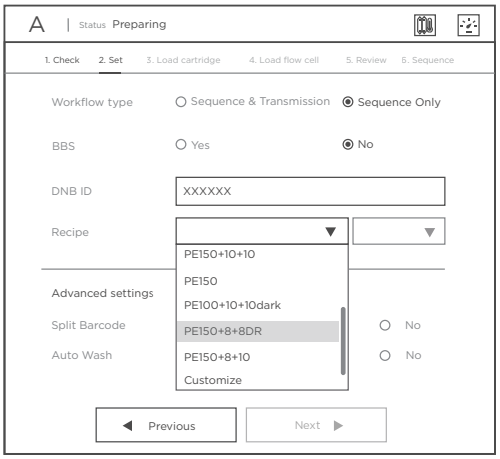

Figure 153 Selecting PE150+8+8DR

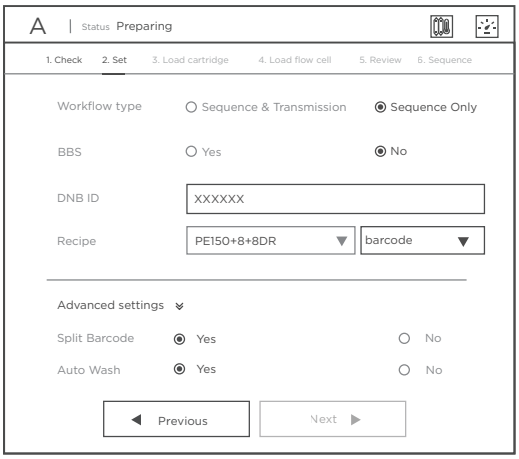

Figure 154 Selecting barcode type and split strategy

Instructions for using Qubit to quantify the DNBs

- Working solution should be used within 30 min following preparation.
- Avoid touching the wall of tapered detection tubes.
- Avoid introducing bubbles in detection tubes.
- After the working solution is added to the DNBs, the mixture should be quantified as soon as possible. Leaving it for a prolonged time may lead to inaccurate results as the result of fluorescence quenching.

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

   - Use only thin-wall, clear, 0.5 mL PCR tubes. Acceptable tubes include Qubit assay tubes (Cat. No. Q32856) or Axygen PCR-05-C tubes (Cat. No. 10011-830).
   - The 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 the tube.
6. Prepare the solutions used for standards and sample tests according to the table below:

Table 75 Working solution

Component	Standard volume		Sample volume		
	S1 (µL)	S2 (µL)	D1 (µL)	D2 (µL)	D3 (µL)
Working solution	190	190	198	198	198
S1 (0 ng/µL)	10	/	/	/	/
S2 (20 ng/µL)	/	10	/	/	/
Sample (µL)	/	/	2	2	2
Total volume	200	200	200	200	200

7. Mix the tubes by using a vortex mixer, centrifuge briefly for 5 s, and then incubate at room temperature for 2 min.
8. Refer to the Qubit user manual for instructions on reading standards and samples. Follow the appropriate procedure for your instrument.

Instructions for splitting barcodes

Manual barcode splitting

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

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

C:\BasecallLite\Config

This section uses the following conditions as examples:

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

Preconditions:

- SubmitImages = false
- CalFilePath is set correctly:

  D:\Result\workspace\FTXXXXXXXXX\L01\calFile

It is recommended that you use a text editor to configure following settings in *Client.ini*.

Table 76 Splitting Barcode and DualBarcode

Parameter setting	Description
Cycle = r100e1r100e1b10b8	Input the complete sequencing recipe
BarcodeFile =	Barcode file path
DualbarcodeSplit = { true, true }	Set both DualbarcodeSplit to true
DualbarcodeMismatch = { 1, 1 }	Set both mismatches to 1

Table 77 Splitting DualBarcode only

Parameter setting	Description
Cycle = r100e1r100e1b10b8	Input the complete sequencing recipe
BarcodeFile =	Barcode file path
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 78 Splitting Barcode only

Parameter setting	Description
Cycle = r100e1r100e1b10b8	Input the complete sequencing recipe
BarcodeFile =	Barcode file path
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 open the DNB ID entry interface.
2. Select the **DNB ID** box, scan the QR code on the tube, or enter the DNB ID by using the on-screen keyboard.
3. Select a barcode range 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 179*.
4. Select a 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 179*.

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: FT1000001566L01: 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 79 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
speciesBarcodes: 104	The number of barcode entries in the barcode file

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: FT1000001566L01: 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 80 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 entries in the barcode file

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: FT1000001566L01: 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 81 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 entries in the barcode file

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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 (W) × 689 mm (D) × 657 mm (H) (24 inches × 27 inches × 26 inches)	
Net weight	Approximately 140 kg (309 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/m^2	

Item	Description	
Operating environment requirements	Maximum altitude	3000 m (9842 ft)
	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/CSA C22.2 No.61010-1-12 Safety requirements for electrical equipment for measurement, control, and laboratory use-Part 1: General requirements • UL 61610-2-081/CSA C22.2 No. 61010-2-081 Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes • UL 61010-2-010/CSA C22.2 No. 61010-2-010 Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-010: Particular requirements for laboratory equipment for the heating of materials • IEC 60825-1 Safety of laser product part 1: equipment classification and requirements

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Research use only

Complete Genomics has labeled the product solely for research use only and specified “RS” in the model name which means that 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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Order information

DNBSEQ-G99ARS is not available in the U.S. or Canada.

Cat. No.	Model	Name	Version
900-000712-00	DNBSEQ-G99ARS	Genetic Sequencer DNBSEQ-G99ARS	V1.0
900-000889-00	DNBSEQ-G99RS	Genetic Sequencer DNBSEQ-G99RS	V1.0
940-001874-00	App-D FCL SE100	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V2.0
940-002777-00	App-D FCU SE100	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V1.0
940-002648-00	App-D FCS PE150	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V1.0
940-001871-00	App-D FCL PE150	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V2.0
940-002781-00	App-D FCU PE150	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V1.0
940-001717-00	App-D FCL PE300	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V1.0
940-002774-00	App-D FCU PE300	DNBSEQ-G99RS High-throughput Sequencing Reagent Set	V1.0
940-000903-00	FCL	DNBSEQ-G99 Cleaning Reagent Kit	/
510-003290-00	DL-G99	Portable DNB Loader	/

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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 Spot Rate
FAQ	Frequently Asked Questions
FCC	Federal Communications Commission
FCL	Flow Cell Large, 1 lane per flow cell in DNBSEQ-G99 FCL Sequencing 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
PCR	Polymerase Chain Reaction
PE	Paired-end sequencing
QC	Quality Control
RCR	Rolling Circle Replication
RFID	Radio Frequency Identification

Item	Description
RHO	Rho (ρ), intensity of raw signals
SE	Single-end sequencing
ssDNA	single-stranded DNA
STR	Short Tandem Repeat
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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