Complete GENOMICS™

DNBSEQ-T7RS System Guide

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

Complete Genomics, Inc.

CSS-00165 DNBSEQ-T7RS System Guide SW V5.1 | Released by Complete Genomics on 09/10/2025 07:40:38 AM

Part No.: CSS-00165

About this guide

CG intends to provide this product solely for research use.

This guide is applicable to Genetic Sequencer (DNBSEQ-T7RS) and DNBSEQ-T7RS High-throughput Sequencing Set. The guide is Rev A and the software version is V1.

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

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

Revision history

	Date	Version
Initial release	September 04, 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

General safety

DANGER

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

CSS-00165 DNBSEQ-T7RS System Guide SW V5.1 | Released by Complete Genomics on 09/10/2025 07:40:38 AM

WARNING

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

CAUTION

- Only the peripheral devices and consumables specified by the manufacturer can be used.
- If you have maintenance questions that are not mentioned in this guide, contact CG Technical Support.
- The device has been verified before delivery. If serious deviation occurs during use, contact CG Technical Support for calibration.
- After the device is powered off, a key is needed to open the fluidics maintenance door. The key is only accessible to CG Technical Support, or other trained/qualified individuals. Please do not open the fluidics maintenance door by force.
- After the device is powered off, a screwdriver is needed to open the optics maintenance door. The door should only be opened by CG Technical Support, or other trained/qualified individuals. Please do not open the optics maintenance door by force.

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, electric leakage, or even electric shock.
- Do not remove the device cover and expose the inner components. Otherwise, electric shock may be caused.

WARNING

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

CAUTION

- Before initial use of the device, assess the electromagnetic environment in which the device will be used.
- Ensure that the input voltage meets the device requirements.
- Ensure that the voltage of the power outlet in your laboratory or the UPS (if any) meets the voltage requirements before using the device. Failure to do so may damage the electrical components.
- Prepare the laboratory and power supply according to the instructions described in this guide.

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.

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

Biological safety

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

WARNING

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

Symbols

Packaging

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

Symbol	Name	Description
	This way up	Indicates the correct upright position of the crated unit for transport and/or storage
	Fragile, handle with care	Indicates a device that can be broken or damaged if not handled carefully
	Keep dry	Indicates a device that needs to be protected from moisture
	Do not stack	Indicates that stacking of the crated unit is prohibited and no item shall be placed on top during transport or storage
	Do not roll	Indicates that the crated unit shall not be rolled or turned over. It shall remain in the upright position at all times
	Temperature limit	Indicates the temperature limits to which the device can be safely exposed
	Humidity limitation	Indicates the range of humidity to which the device can be safely exposed
	Atmospheric pressure limitation	Indicates the range of atmospheric pressure to which the device can be safely exposed

Device

The following table describes symbols on the device:

Symbol	Name	Description
	General warning sign	Signifies a general warning
	Warning; biological hazard	Biological hazard warning
	Caution; hot surface	Indicates that the marked item can be hot and should not be touched without taking proper safety precautions
	Warning; dangerous voltage	Indicates hazards arising from dangerous voltages
	Protective earth	Indicates the terminal of a protective earth (ground) electrode
	Warning; laser beam	Warns of a hazard from laser beam
	"ON" (power)	Indicates the main power supply is on
	"OFF" (power)	Indicates the main power supply is off
SBC-LAN	Network port	Connects to the network
BCS-LAN	Network port	Connects the server to the network
SBC-USB 3.0	USB 3.0 port	Connects USB devices, such as the keyboard and mouse, to the computer
BCS-USB 3.0	USB 3.0 port	Connects USB devices, such as the keyboard and mouse, to the server
VGA	VGA port	Used for display adjustment
BCS-Fiber	Optical fiber port	10 Gigabit network port

Symbol	Name	Description
RFID	RFID (Radio frequency identification) reader indication	Scans the ID of the flow cell or cartridge placed near the area
SWITCH	Power switch	Powers the device on or off
SOCKET	Power port	Connects to the main power supply
LAN	RJ45 network port	Connects the network of the computer and server
	USB 2.0 port	Connects to the USB device
SS	USB 3.0 port	Connects to the USB device

Label

The following table describes symbols on the label:

Symbol	Name	Description
	Manufacturer	Indicates the name and address of the device manufacturer
	Date of manufacture	Indicates the date when the device was manufactured
SN	Serial number	Indicates the manufacturer's serial number so that a specific device can be identified
	Consult instructions for use	Indicates the need for the user to consult the instructions for use
REF	Catalog number	Indicates the manufacturer's catalog number so that the device can be identified
	Use by date	Indicates the date after which the device is not to be used
LOT	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

Symbol	Name	Description
	Do not re-use	Indicates a component or reagent that is intended for a single use only
	Part number	Indicates the part number of an individual box in the reagent set
	Version	Indicates the version of the device or reagent kit
	Caution	Indicates that caution is necessary when operating the device, or that the current situation needs operator awareness or operator action in order to avoid undesirable consequences

System guide

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

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

02

Devices overview

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

Intended use

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

Working principle

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

The following figure demonstrates how to make DNBs:

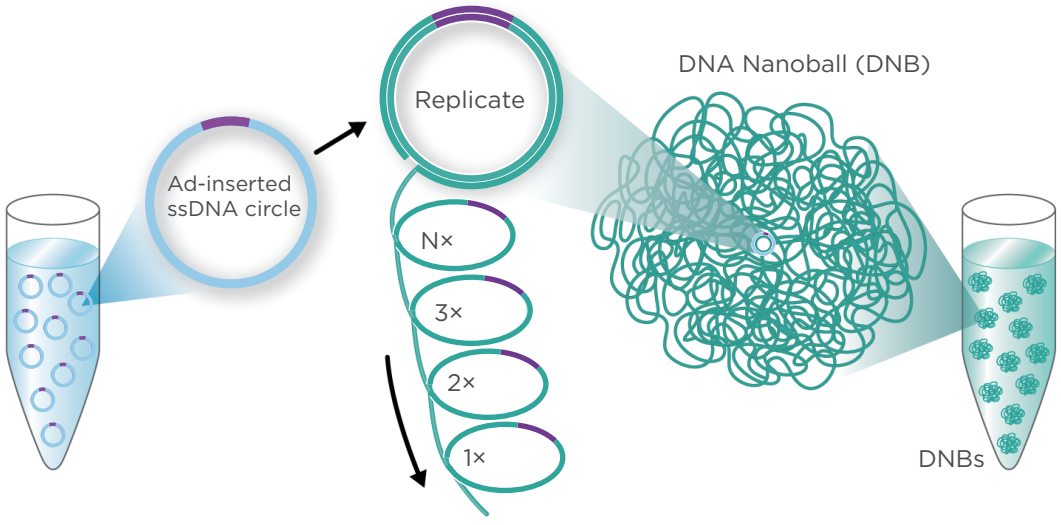

Figure 1 Making DNBs

The following figure demonstrates how to load DNBs:

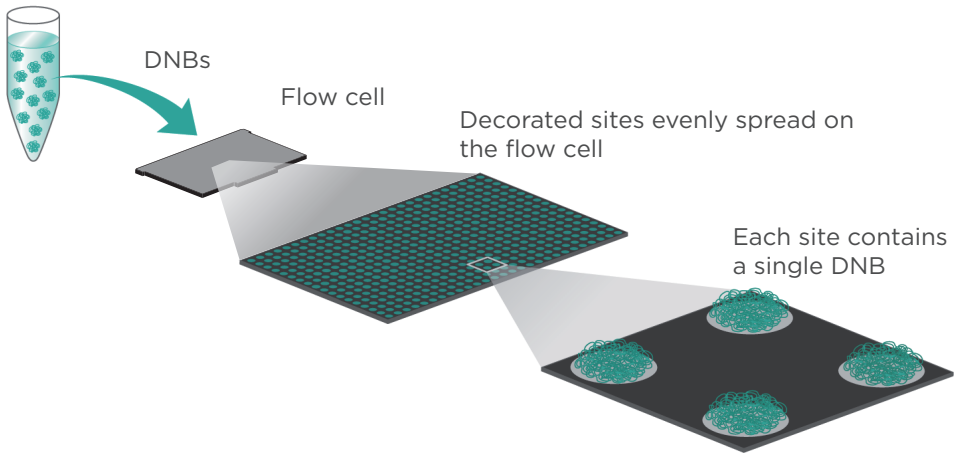

Figure 2 Loading DNBs

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

Sequencer overview

Structural composition

The device consists of the main unit and pre-installed control software (software version: V1). The main unit includes the shell, host, optical system, XYZT-stage, flow cell stage, gas-liquid system, electric control system, reagent storage system, power supply system, display system, robotic arm, flow cell drive, and flow cell retrieval compartment.

The following table describes the function of each component:

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

Component	Description
XYZT-stage	Moves the flow cell and focuses automatically.
Flow cell stage	Connects the flow cell to fluidics lines and controls the temperature of the flow cell.
Gas-liquid system	Provides the gas-liquid support that is required for the biochemical reaction.
Electric control system	Controls the electric system.
Reagent storage system	Provides the reagent storage environment.
Power supply system	Provides the power supply for the device.
Display system	Provides the human-computer interaction interface.
Robotic arm	Transfers and loads flow cells.
Flow cell drive	Loads a flow cell for sequencing or washing.
Flow cell retrieval compartment	Holds used flow cells.

Basic components

Front view

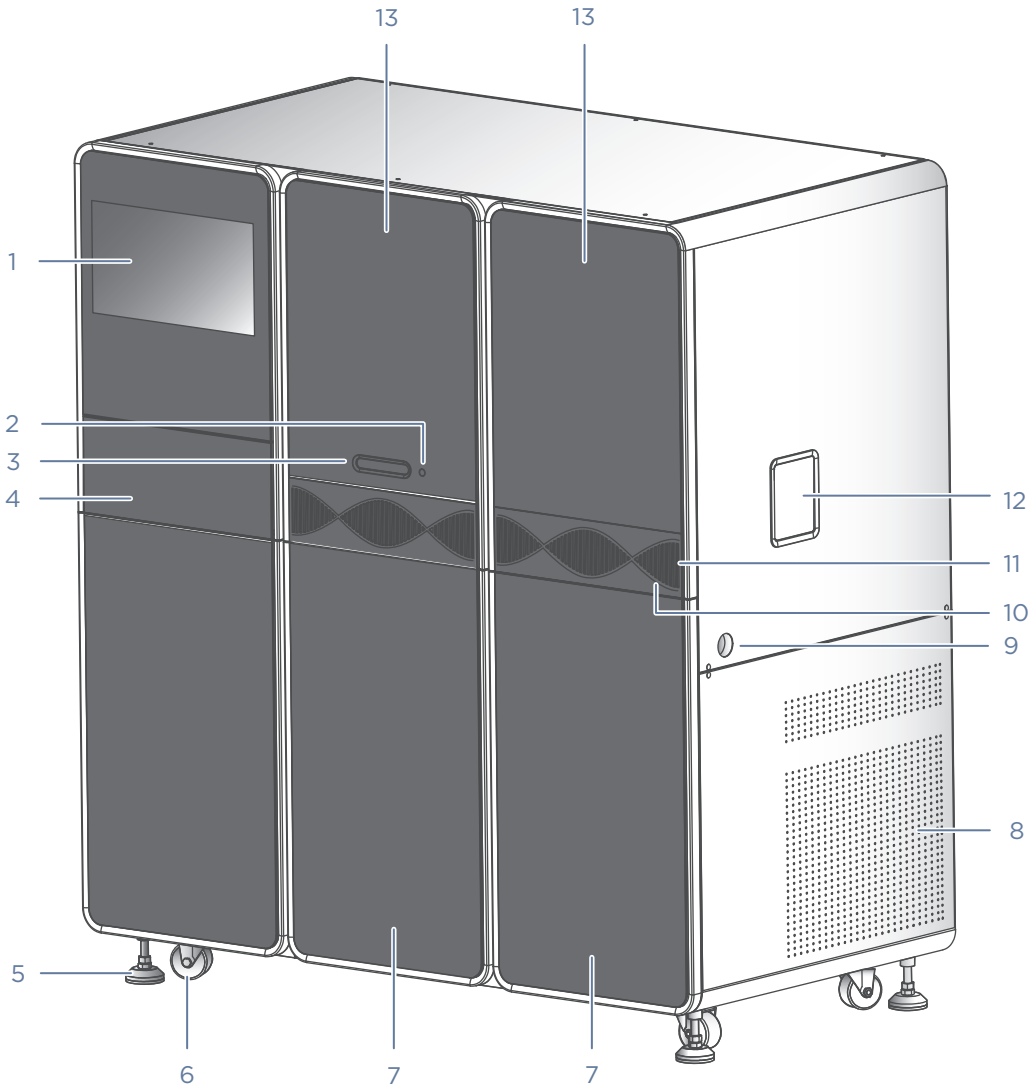

Figure 3 **Front view of the sequencer**

No.	Name	Description
1	Touch screen monitor	Facilitates on-screen operations and displays information.
2	Flow cell drive button	Touch to eject the flow cell drive.

No.	Name	Description
3	Flow cell drive	Touch the flow cell drive button to eject the flow cell drive.
4	Keyboard tray	When unfolded, you can power the computer on or off and connect USB devices, such as the keyboard and mouse, to the computer.
5	Supporting feet	Supports the main unit to ensure stability.
6	Caster	Used for moving the device.
7	Reagent compartment	Holds reagent kits and samples at appropriate temperatures. You can press to open the door.
8	Ventilation inlet	Ventilates the device.
9	Waste container port	Used for connecting the waste container.
10	Status indicator	Displays the current status of the device: • Green: the device is running. • Blue: the device is in standby status. • Red: an error occurs. • Yellow: a warning notification appears.
11	Ventilation inlet	Air enters the air filter in the device through this inlet.
12	Flow cell retrieval compartment	Retrieves used flow cells.
13	Fluidics maintenance door	Used by CG Technical Support or trained personnel to maintain the fluidics system.

Keyboard and ports

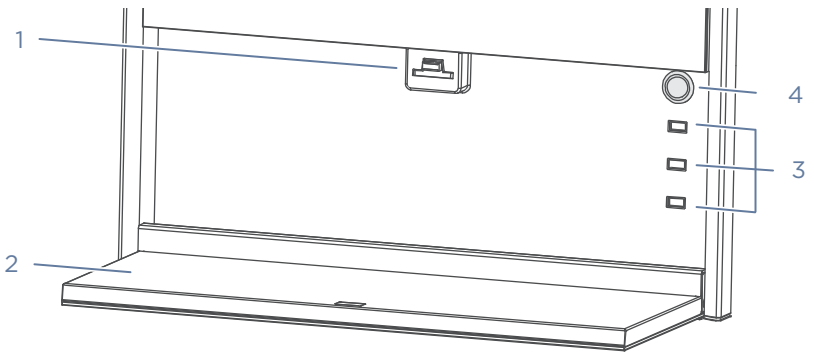

Figure 4 Keyboard and ports

No.	Name	Description
1	Keyboard tray latch	Used to secure the keyboard tray in the closed position.
2	Keyboard tray	Used to support the keyboard and mouse. When not in use, fold the keyboard tray up and press it towards the latch until you hear a click.
3	USB 3.0 port	Connects to USB devices, such as the keyboard and mouse.
4	Host power button	Press to power the computer on or off.

Reagent compartment

The reagent compartments include the sequencing cartridge compartments and washing cartridge compartments. The system automatically identifies the QR code of the cartridges by built-in RFID readers.

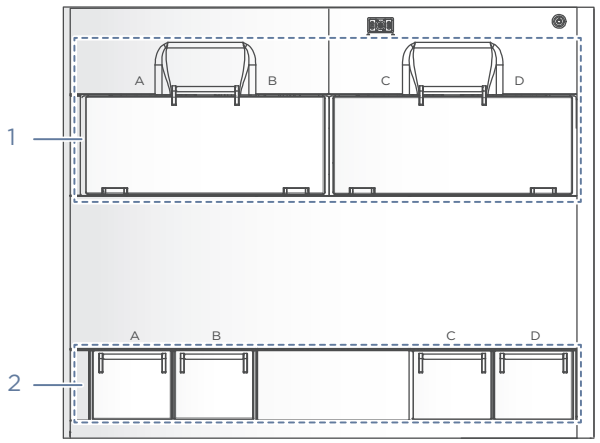

Figure 5 Reagent compartment

No.	Name	Description
1	Sequencing cartridge compartment	Holds the sequencing cartridges at appropriate temperatures.
2	Washing cartridge compartment	Holds the washing cartridges.

Flow cell

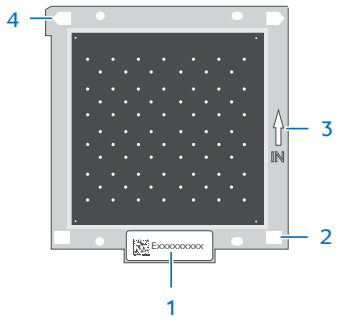

Figure 6 Top view (front side) of the flow cell

No.	Name	Description
1	Label location	Shows the QR (quick response) code of the flow cell.
2	Fluidic inlet hole	/
3	Flow cell orientation marker	Shows correct flow cell orientation.
4	Fluidic outlet hole	/

Back view

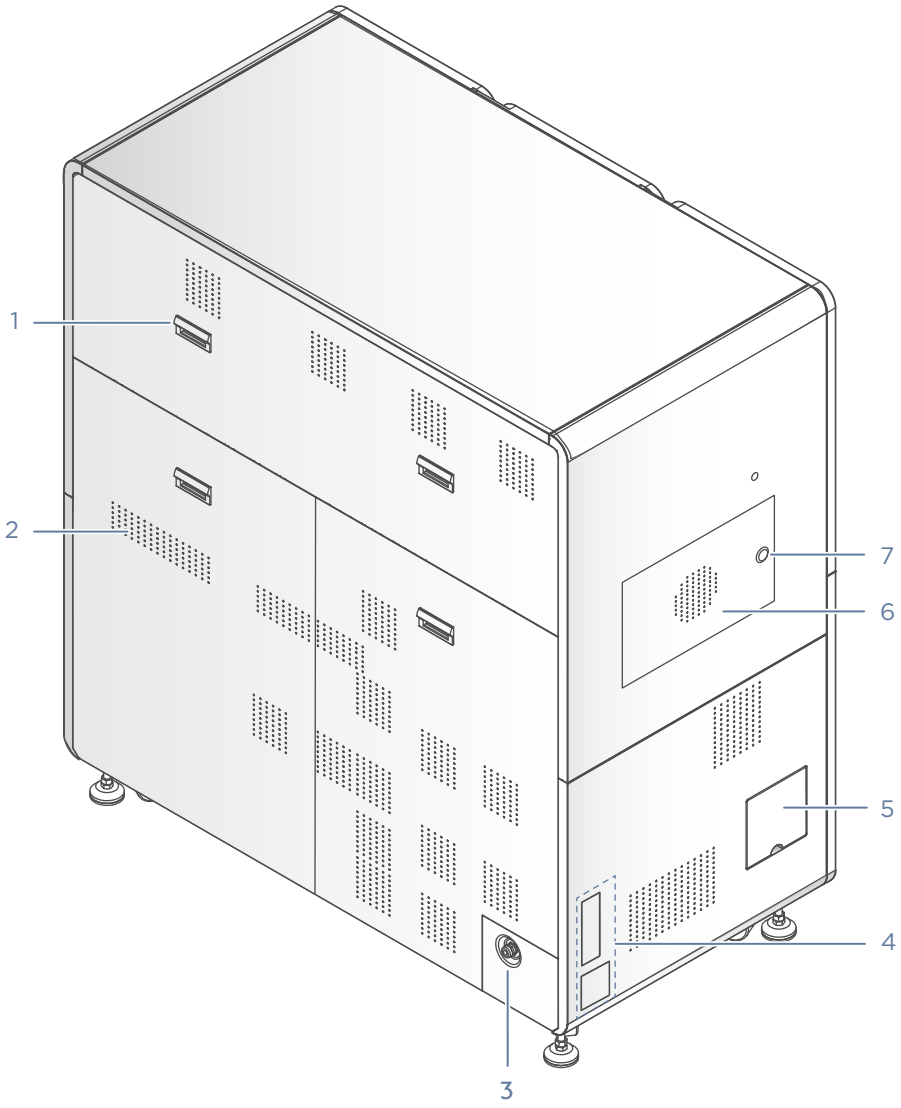

Figure 7 Back view of the sequencer

No.	Name	Description
1	Handle	Used to easily remove the rear panel during maintenance.
2	Ventilation outlet	Ventilates the device.
3	Power inlet	Connects to the power cord.
4	Ports	Used for cable connections.

No.	Name	Description
5	Pure water container port	Connects to the pure water container.
6	Optics maintenance door	Used by CG Technical Support to maintain the optical system.
7	Maintenance door button	Used for opening the door after unlocking.

Ports

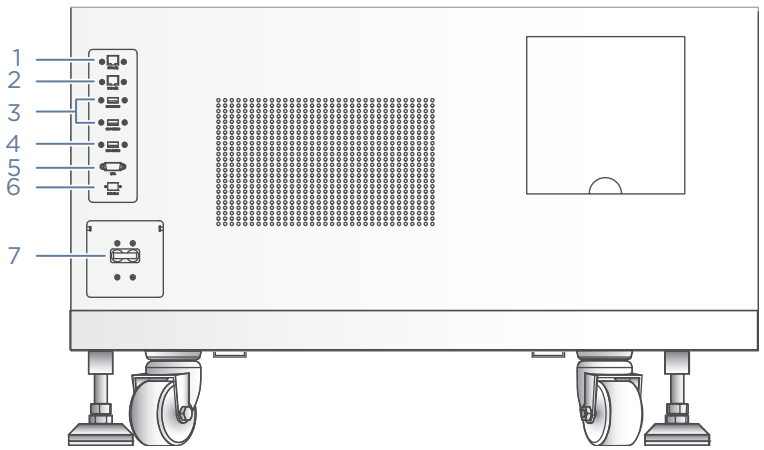

Figure 8 Ports

No.	Name	Description
1	Network port 1	Connects the computer to the network.
2	Network port 2	Connects the server to the network.
3	USB 3.0 port 1	Connects USB devices, such as the keyboard and mouse, to the computer.
4	USB 3.0 port 2	Connects USB devices such as the keyboard and mouse to the server.
5	VGA port	Connects to the LCD screen for adjustment.
6	Optical fiber port	10 Gigabit network port.
7	Power switch	Powers the device on or off: • Switch to the ON position to power the device on. • Switch to the OFF position to power the device off.

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, mechanical components, and optical components. The software detects the signal on the sequencing flow cell, transfers the photographic information to the base sequence files in standard format, and guides users to perform different processes, such as maintenance and experimental protocols.

The following table describes the function of each functional module:

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

- Because the flow cell stages have the same functions, flow cell stage A is used as an example in the subsequent sections.
- For interface control, you can use either the touch screen monitor or keyboard and mouse.

Self-test interface

After you power the device on, self-test starts. If the self-test succeeds, the main interface appears.

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

1. In the main interface, select , and select **Log** to check the detailed self-test results that are recorded in the log.
2. Follow the on-screen instructions or the solutions that are mentioned in *FAQs on Page 131*.
3. Perform a self-test again:
   - Select > **Maintenance** > **Self-test**.
   - Select > **Restart**.

If the problem persists, contact CG Technical Support.

Main interface

The main interface appears after a successful self-test, as shown in the figure below:

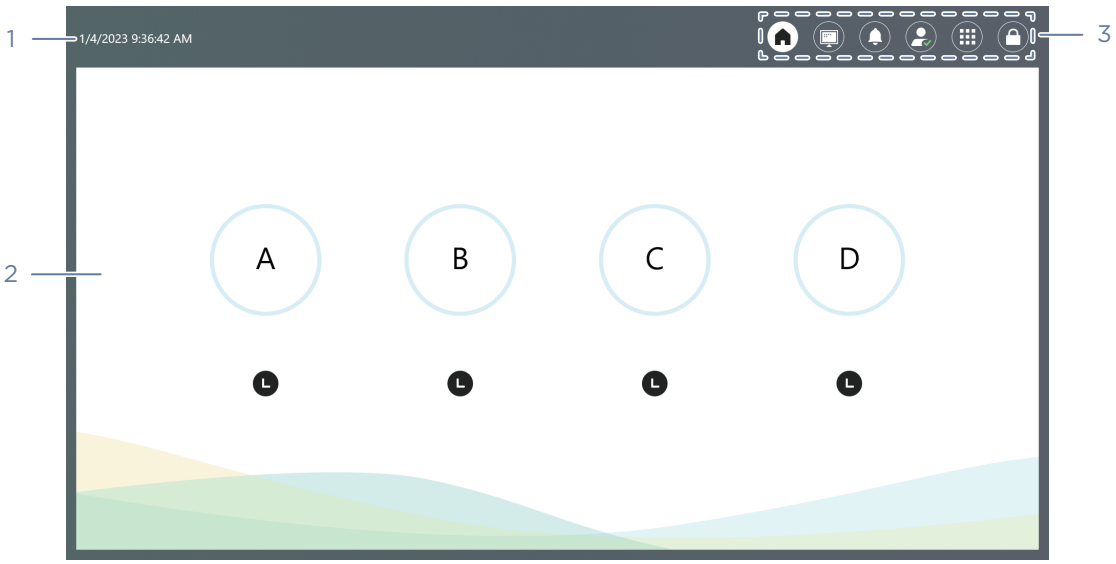

Figure 9 Main interface

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	Operation area	Indicates the status of flow cells and provides wash and sequence options when you enter the main interface of the selected flow cell stage.
3	Menu area	Select the buttons to perform relative operations.

Operation area

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

Item	Description
A	Flow cell stage name If an error occurs in a flow cell stage, an error indicator appears on the progress bar.
xx%	Task progress

Item	Description
	The flow cell stage is in sequencing.
	The fluidics lines of the flow cell stage are being washed.
L	The flow cell stage is in idle status.
	The flow cell stage is preparing for sequencing or washing.
	Sequencing or washing is in the process of being paused.
	Sequencing or washing is paused.
	Sequencing or washing is in the process of stopping.

Main interface A

Select a flow cell stage name to enter the main interface for that flow cell stage.

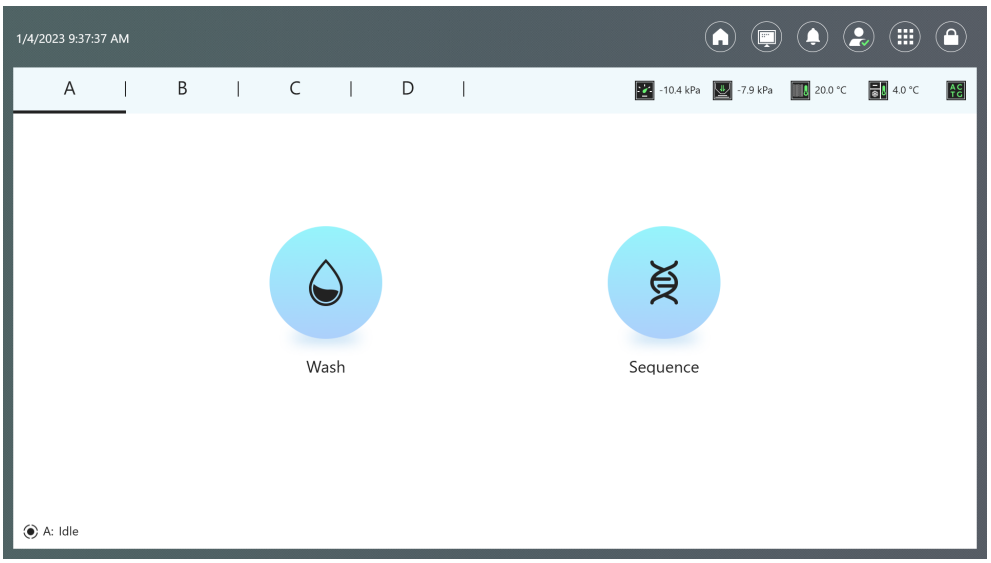

Figure 10 Main interface A

The following table describes the function of the sensor status indicators on the main interface of the selected flow cell stage.

Item	Description
ACTG	Basecalling is connected.

Item	Description
	Errors occur in the Basecalling connection.
	The Basecall software is processing image data. This icon is dynamic.
	The fluidics chuck vacuum is normal. The real-time value is displayed to the side.
	The fluidics chuck vacuum is outside the normal range. The real-time value is displayed to the side.
	The fluidics chuck vacuum is almost outside the normal range. The real-time value is displayed to the side.
	The imager vacuum is normal. The real-time value is displayed to the side.
	The imager vacuum is outside the normal range. The real-time value is displayed to the side.
	The fluidics chuck temperature is normal. The real-time value is displayed to the side.
	The fluidics chuck temperature is outside the normal range. The real-time value is displayed to the side.
	The sequencing cartridge compartment temperature is normal. The real-time value is displayed to the side.
	The sequencing cartridge compartment temperature is outside the normal range. The real-time value is displayed to the side.

Menu area

The following table describes control functions in the menu area:

Item	Description
	Select to return to the main interface when the flow cell stage is not preparing for sequencing or washing.
	Sensor status indicator Select to check the status of sensors for all flow cell stages. A red dot appears on the icon when an error occurs.

Item	Description
	Select to view notification details. The notification icon indicates: • Yellow: a warning notification appears. • Red: an error occurs.
	Select to log in to the system.
	Menu button When the device is idle or paused, the system information and logs can be viewed by selecting the menu button.
	After logging into the system, you can select this button to lock the screen.

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

Item	Description
	Error occurs in connection with the server on which the ZLIMS software is installed.
	The device is not connected to the server on which the ZLIMS software is installed and is running independently.
	The device is connected normally to the server on which the ZLIMS software is installed.
	Sufficient storage drive space
	Insufficient storage drive space
	Inner temperature of the device is normal. The real-time value is displayed to the side.
	Inner temperature of the device is outside the normal range. The real-time value is displayed to the side.
	The device humidity is normal. The real-time value is displayed to the side.
	The device humidity is outside the normal range. The real-time value is displayed to the side.
	Sufficient space remaining in the built-in waste container.

Item	Description
	Insufficient space remaining in the built-in waste container. If this occurs, contact CG Technical Support.

Log interface

You can view log information in this interface.

To open the log interface, select  in the main interface, and select **Log**.

The following table describes control functions in the log interface:

Item	Description
	Select to exit the log interface and return to the previous interface.
All	Select to view all logs.
Info	Select to view information logs.
Warning	Select to view warning logs.
Error	Select to view error logs.
	Select to select the date in the pop-up calendar.
Flow cell	Select the check box to view the logs of that flow cell.
Sort by	Set the display order of the logs.

Settings interface

You can manage recipes and change system settings in this interface.

To open the settings interface, log in to your account, select , and select **Settings**.

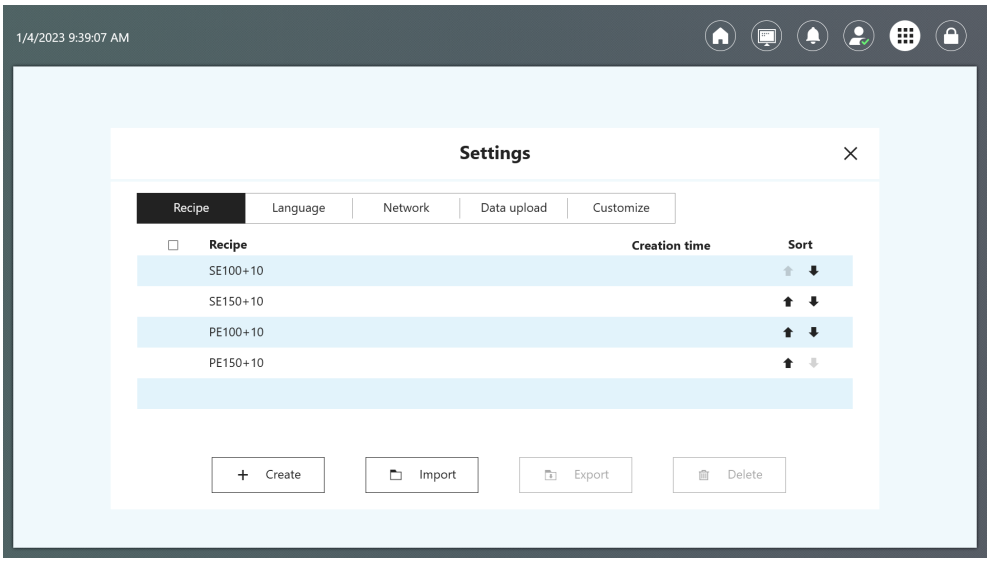

Figure 11 Settings interface

The following table describes control functions in the settings interface:

Item	Description
Recipe	Select to perform the following settings: • Select **Sort** to change the display order of the recipes. • Select **Create** to customize a recipe. • Select **Import** to import recipes. • Select **Export** to export customized recipes. • Select **Delete** to delete the customized or imported recipes.
Language	Select to change the language of the software. Restart the computer to apply the change.
Network	Select to input the IP address and port number of the ZLIMS server. Restart the computer to apply the changes.
Data upload	Select to set data processing methods.
Customize	Select to change the wait time before the screen locks automatically and change the buzzer volume.

Maintenance interface

You can empty the fluidics lines and perform self-tests in the maintenance interface.

To open the maintenance interface, log in to your account, select , and select **Maintenance**.

The following table describes control functions in the maintenance interface:

Item		Description
Device maintenance	**Cleaning tool replacement**	Select to replace the cleaning tool.
	Empty fluidics line	Select this button and select a flow cell stage to discharge the residual liquid in its fluidics line to the waste container.
	Self-test	Select to perform a self-test for the hardware of the device. When the test is finished, you will get a notification and the results will be displayed on the screen.
Door control	**Unlock optics maintenance door**	Select to unlock the optics maintenance door. Only CG Technical Support or trained personnel can maintain the optical system.
	Unlock fluidics maintenance door	Select to unlock the fluidics maintenance door. Only CG Technical Support or trained personnel can maintain the fluidics system.
Service life stat.	**Lifetime alarm notification**	If enabled, the system automatically displays notifications when the consumables listed are nearing the end of their service life.
	Residual service life	Displays the residual service life of the consumables.
User management		Select to reset the password of the current user account.

Shutdown or restart interface

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

To open the shutdown or restart interface, select and select **Shut down** or **Restart**.

About interface

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

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

Sequencing interface

The sequencing interface displays real-time sequencing progress.

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

Item	Description
Estimated completion time	Shows the sequencing completion time.
QC type	You can select a QC value graph from the QC type list to assess the sequencing quality.
	Select to pause sequencing. Select **Yes** when prompted.
	Select to resume sequencing.
	Select to stop sequencing. Select **Yes** when prompted.
	Select to view the First Base Report.
	Select to view the summary after a sequencing run completes.
	Select to view sequencing information or change auto wash settings after a sequencing run starts.

DNB loader overview

Working principle

The device loads the sample libraries and/or reagent to a sequencing flow cell through defined and optimized vacuum procedures.

Structural composition

The device consists of the touch screen monitor, PCR board assembly, Y-Z motion stage, flow cell stage, syringe pump, vacuum pump, and RFID reader.

The following table describes functions of each component:

Component	Description
Touch screen monitor	Displays information and performs on-screen operations.
PCR board assembly	Controls the system, drives the components, collects status, and sends feed back to the system.
Y-Z motion stage	Switches among different reagents.
Flow cell stage	Connects the fluidics line to the flow cells, and controls the flow cell stage temperature.
Syringe pump	Aspirates flow cell reagents and discharges the waste to the DNB loading plate.
Vacuum pump	Firmly attaches the flow cell to the inlet and outlet of the sealing rings to avoid liquid leakage.
RFID reader	Identifies the ID of an item.

Front view

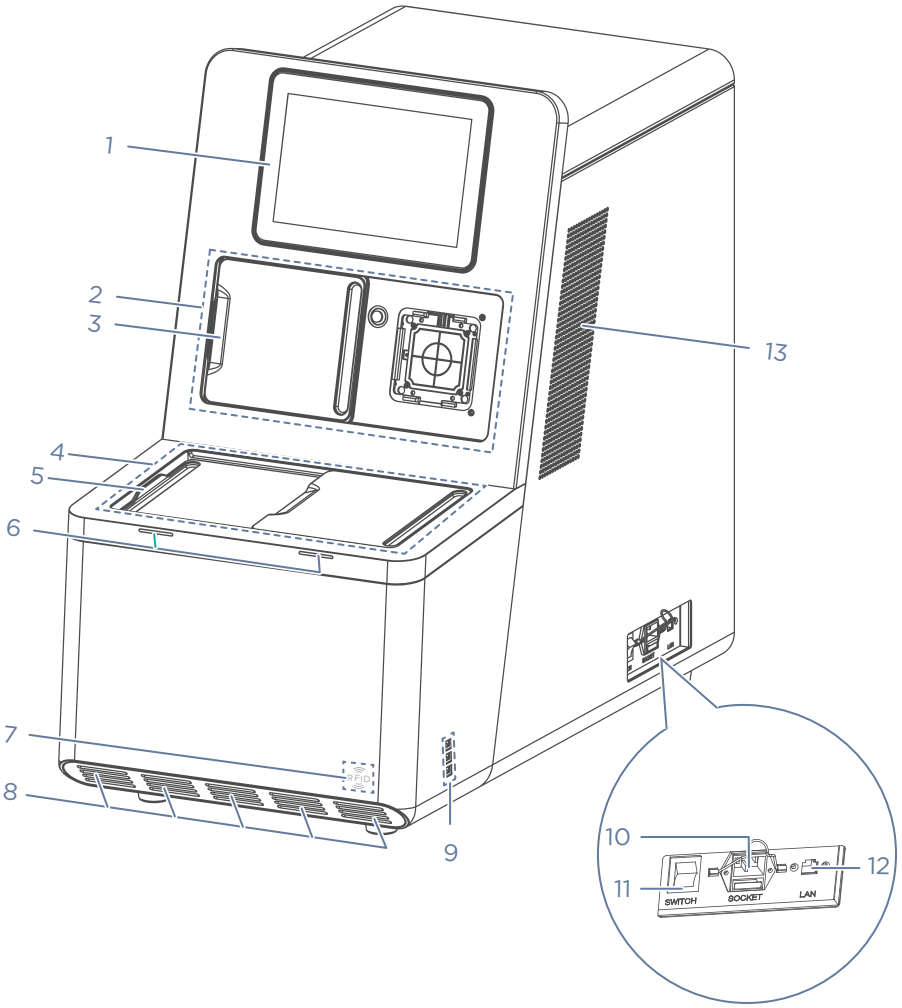

Figure 12 **Front view of the DNB loader**

No.	Name	Description
1	Touch screen monitor	Facilitates on-screen operations and displays information.
2	Flow cell compartment	Holds the flow cell.
3	Flow cell compartment door	Slide to open or close the flow cell compartment door.
4	Loading compartment	Area to place the post-load plate and to load liquids.

No.	Name	Description
5	Loading compartment door	Slide to open or close the loading compartment door. When opening or closing the left compartment door, handle with care to avoid injury to hands.
6	Status indicator	Displays the current status of the device. • Blue: the device is in standby status. • Green: the device is running. • Yellow: a warning notification appears. • Red: an error occurs.
7	RFID scanning area	Identifies the ID of the item placed near the area.
8	Ventilation outlet	Ventilates the device.
9	USB port panel	Connects to USB devices such as the keyboard and mouse.
10	Power port	Connects to the main power supply. Fuses are installed in the port.
11	Power switch	Powers the device on or off. • Switch to the | position to power the device on. • Switch to the ◯ position to power the device off.
12	RJ45 Network port	Connects to the network of the computer and server.
13	Ventilation outlet	Ventilates the device.

Back view

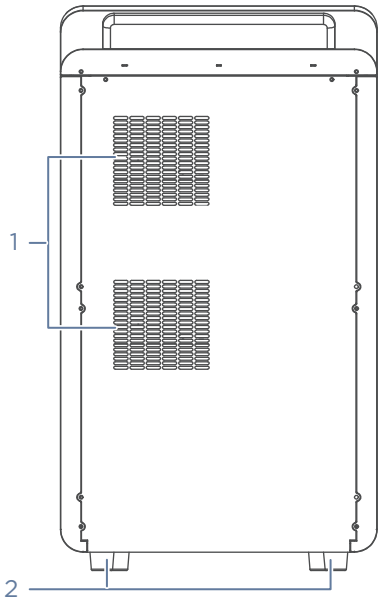

Figure 13 Back view of the DNB loader

No.	Name	Description
1	Ventilation outlet	Ventilates the device.
2	Supporting feet	Supports the main unit to ensure stability.

Flow cell stage A

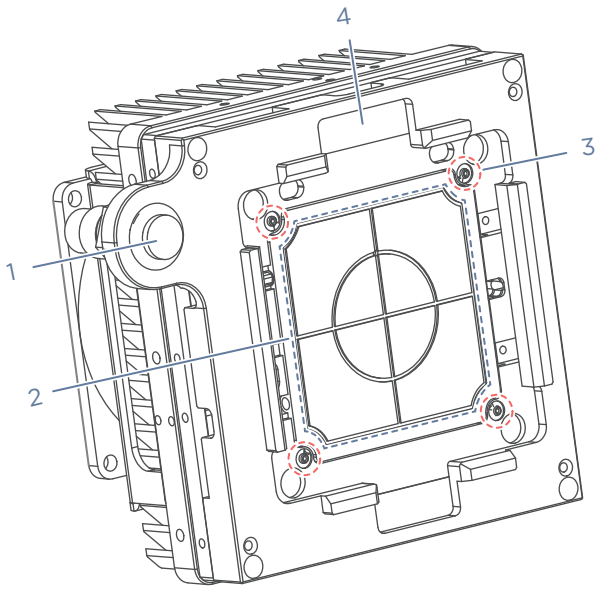

Figure 14 Flow cell stage A

The following description uses flow cell stage A as an example.

No.	Name	Description
1	Flow cell attachment button A	Press to activate the vacuum for attachment or release of the flow cell.
2	Aluminum chuck	Loads and attaches the flow cell.
3	Sealing rings	Connects and seals the flow cell to the flow cell stage. Allows reagents to be pumped into the flow cell through the sealing rings.
4	Alignment groove	Used for aligning the flow cell.

Plate tray unit

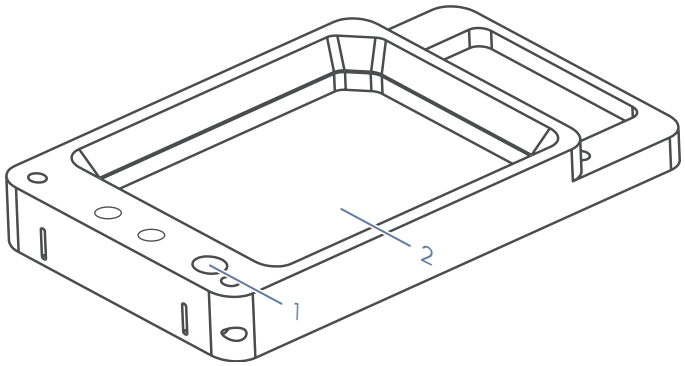

Figure 15 Plate tray unit

No.	Name	Description
1	DNB tube hole	Holds the DNB tube.
2	Plate tray	Holds the DNB loading plate and brings it to the specified position while Y-Z motion stage moves.

Control software

The software of the device can guide the user to load different sample libraries and/or reagents to a sequencing flow cell according to experimental requirements.

The following table describes the function of each functional module:

Item	Description
Self-test	Checks whether the components of the system are functional.
Loading	Loads the required sample library and/or reagent to the flow cell.
Wash	Performs wash and maintenance for the fluidics lines in the system.

Self-test interface

After you power the device on, self-test starts. If the self-test succeeds, the main interface appears.

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

1. In the main interface, select > **Log** to check the detailed self-test results that are recorded in the log.

2. Follow the on-screen instructions or the solutions that are mentioned in *FAQs on Page 131*.
3. Perform a self-test again:
   - Select > **Maintenance** > **Self-test**.
   - Select > **Restart**.

If the problems persist, contact CG Technical Support.

Main interface

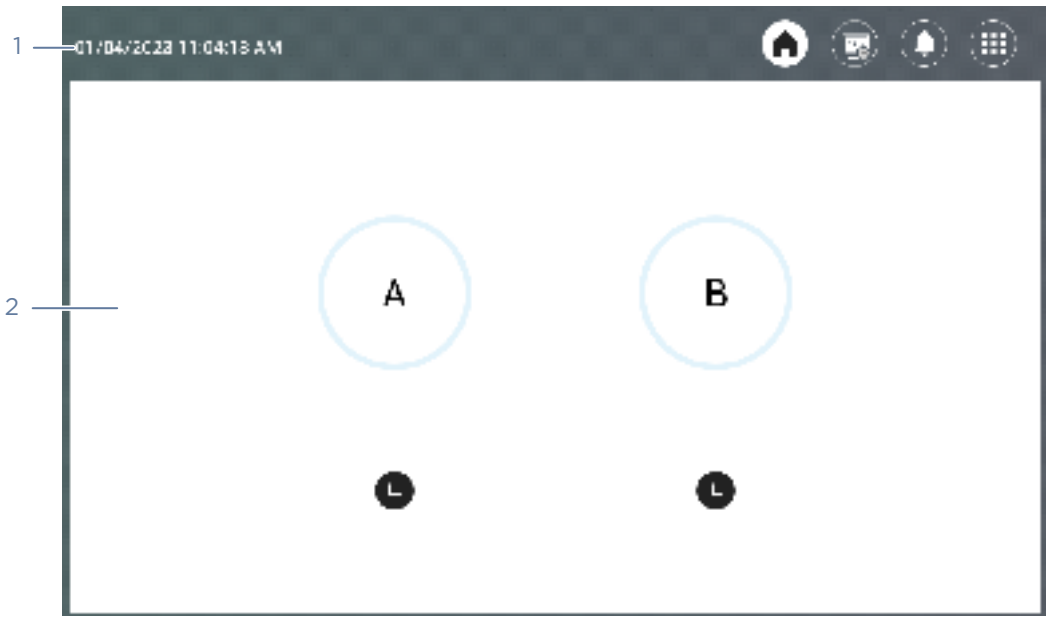

Figure 16 **Main interface**

The main interface includes the following areas.

No.	Name	Description
1	Icon and button area	Displays the local time, home, status, notification and menu buttons.
2	Operation area	Select a flow cell stage and perform the relevant operations.

Operation area

Item	Description
A	Flow cell stage name.

Item	Description
xx%	Task progress.
	The flow cell stage is in a loading process.
	The fluidics lines of the flow cell stage are being washed.
	The flow cell stage is in idle status.
	Loading or washing is in the process of being paused.

Main interface A

If you select flow cell stage A, main interface A appears.

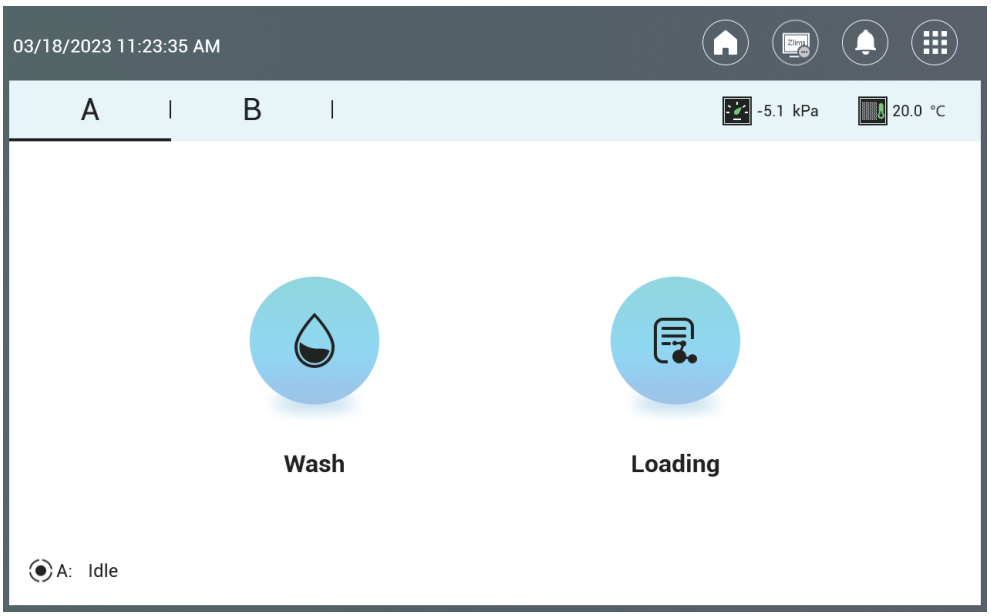

Figure 17 Main interface A

The following table describes statuses of the flow cell stage in this interface:

Item	Description
	The temperature of the flow cell stage is normal.
	The temperature of the flow cell stage is outside the normal range.
	Negative pressure is normal.

Item	Description
	Negative pressure is outside the normal range.

Icon and button area

The following table describes control functions in the icon the button area:

Item	Description
	Select to return to the main interface.
	Displays the connection status of the device and the server on which ZLIMS is installed.
	Select to view notification details. The notification icon indicates: • Yellow: a warning notification appears. • Red: an error occurs.
	Menu button Select to view logs, change system settings, perform system maintenance, shut down or restart the system, or view system information.

Log interface

You can view log information in this interface.

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

The following table describes control functions in the log interface:

Item	Description
	Select to exit the log interface and return to the previous interface.
All	Select to view all logs.
Info	Select to view information logs.
Warning	Select to view warning logs.
Error	Select to view error logs.
	Select to select the date in the pop-up calendar.
Flow cell	Select the check box to view the logs for that flow cell stage.
Sort by	Set the display order of the logs.

Settings interface

You can change system settings in this interface.

To open the settings interface, select > **Settings**.

The following table describes control functions in the settings interface:

Item	Description
Language	Select to change the language of the software. Restart the device to apply the change.
Network	Input the IP address and port number of the ZLIMS server. Restart the device to apply the changes.
Customize	Move the slider to change the speaker volume.

Maintenance interface

You can empty the fluidics lines and perform self-tests in this interface.

To open the maintenance interface, select > **Maintenance**.

The following table describes the maintenance menu:

Item	Description
Self-test	Select to perform a self-test for the hardware of the device. When the test is finished, you will get a notification and the results will be displayed on the screen.
Empty fluidics line A/B	Select to discharge the residual liquid in its fluidics line to the DNB loading plate.

Shutdown or restart interface

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

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

About interface

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

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

Loading interface

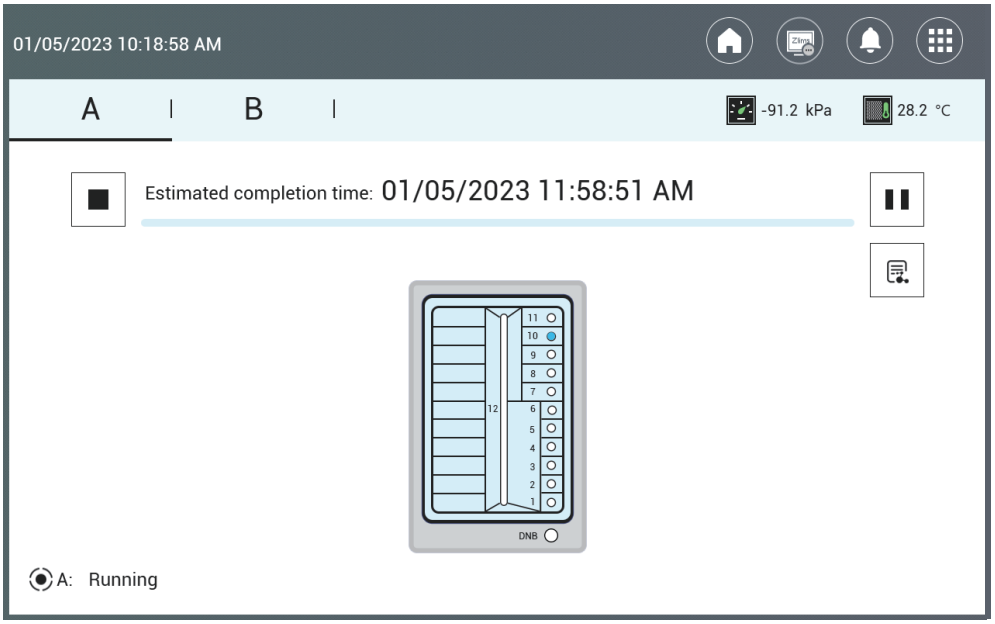

Figure 18 DL-T7RS flow cell loading interface

The estimated completion time for loading is shown on the screen. The following table describes control functions in the loading interface:

Item	Description
	Select to view the loading information, such as DNB ID, and select **Back** to return to the loading interface.
	The status of the device is displayed next to this icon.
	Select to pause loading. Select **Yes** when prompted.
	Select to resume loading.
	Select to stop loading. Select **Yes** when prompted.

CG-ZTRON-LITE overview

Introduction

Data Analysis Appliance (CG-ZTRON-LITE) is designed to support DNBSEQ-T7 sequencers. It receives sequencing data (cal files) from a sequencer and runs the built-in write_fastq pipeline to convert the cal files into FASTQ files.

CG-ZTRON-LITE functions include data delivery, data storage and data governance. “Delivery” means the transfer of data to the user’s cloud data center. “Storage” means the transfer of data to the user’s local data center. “Governance” means the handling of data stored on CG-ZTRON-LITE, such as deletion. The user can configure relevant rules on the visualized system interface as needed, and CG-ZTRON-LITE will automatically execute these rules to complete data delivery/storage/governance.

CG-ZTRON-LITE integrates hardware with software. CG-ZTRON-LITE is an independent tower server that houses computing, storage and network resources. CG-ZTRON-LITE provides lab information management, automated analysis, and data governance.

Figure 19 CG-ZTRON-LITE

WARNING For data security, do not use the USB ports on the case of CG-ZTRON-LITE.

Technical specifications

Name	Description
CPU	Intel Xeon Gold 5218R CPU×2, or other CPU with equivalent or better performance
Memory	192GB DDR4
GPU	Nvidia Quadro RTX4000 or other GPU with equivalent or better performance
OS Disk	480GB non-hot-swappable SSD×2
Data Disk	2.5-inch 3.84TB hot-swappable SSD×10
RAID Card	Support RAID 0, RAID 1, RAID 5 and RAID 6 with super-capacitors
Network	10/25GbE SFP28 dual-port PCIe×2
Fiber Optic Module	10G multi-mode module×3 and 10G single-mode module×1
Power Supply	1100W 100~240VAC hot-swappable module×2

Bandwidth requirement

To ensure the best performance of CG-ZTRON-LITE, it is recommended that the bandwidth at the customer site should reach at least 10 GB/s.

03

Sequencing sets overview

This chapter describes the sequencing sets information.

Introduction

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

Available sequencing set list

Table 1 Sequencing set

Catalog number	Model	Name	Version	Theoretical Data output (GB)
940-000838-00	FCL PE100	DNBSEQ-T7RS High-throughput Sequencing Set	V1.0	1160
940-000836-00	FCL PE150	DNBSEQ-T7RS High-throughput Sequencing Set	V1.0	1740

List of sequencing set components

Table 2 DNBSEQ-T7RS High-throughput Sequencing Set (FCL PE100)
Catalog number: 940-000838-00

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiration date
DNBSEQ-T7RS Sequencing Flow Cell (T7-2 FCL PE100) **Catalog number: 930-000076-00**					
Sequencing Flow Cell (T7-2 FCL)	/	1 EA	2 °C to 8 °C	2 °C to 8 °C	as stated on label
DNBSEQ-T7RS DNB Make Reagent Kit (FCL PE100) **Catalog number: 940-000848-00**					
Low TE Buffer		960 µL/tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	as stated on label
Make DNB Buffer		400 µL/tube×1 tube			
Make DNB Enzyme Mix I		800 µL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		80 µL/tube×1 tube			
Stop DNB Reaction Buffer		400 µL/tube×1 tube			
DNBSEQ-T7RS DNB Load Reagent Kit (FCL PE100) **Catalog number: 940-000844-00**					
DNB Load Buffer I		300 µL/tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	as stated on label
DNB Load Buffer II		150 µL/tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
Post Load Plate (T7 FCL PE100)	/	1 EA			

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiration date
DNBSEQ-T7RS High-throughput Sequencing Kit (FCL PE100) **Catalog number:940-000837-00**					
dNTPs Mix II		8.28 mL / tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	as stated on label
dNTPs Mix V		2.76 mL / tube×1 tube			
Sequencing Enzyme Mix		5.52 mL / tube×1 tube			
MDA Reagent		4.20 mL / tube×1 tube			
MDA Enzyme Mix		0.60 mL / tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent Sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (FCL PE100) **Catalog number: 940-000842-00**					
Washing Cartridge	/	1 EA	below 40 °C	0 °C to 30 °C	as stated on label

Table 3 DNBSEQ-T7RS High-throughput Sequencing Set (FCL PE150)
Catalog number: 940-000836-00

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiration date
DNBSEQ-T7RS Sequencing Flow Cell (T7-2 FCL PE150) Catalog number: 930-000869-00					
Sequencing Flow Cell (T7-2 FCL)	/	1 EA	2 °C to 8 °C	2 °C to 8 °C	as stated on label
DNBSEQ-T7RS DNB Make Reagent Kit (FCL PE150) Catalog number: 940-000847-00					
Low TE Buffer		960 µL/tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	as stated on label
Make DNB Buffer		400 µL/tube×1 tube			
Make DNB Rapid Enzyme Mix II		800 µL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		80 µL/tube×1 tube			
Stop DNB Reaction Buffer		400 µL/tube×1 tube			
DNBSEQ-T7RS DNB Load Reagent Kit (FCL PE150) Catalog number: 940-000845-00					
DNB Load Buffer IV		200 µL/tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	as stated on label
Micro Tube 0.5 mL (Empty)		1 tube			
Post Load Plate (T7 FCL PE150)	/	1 EA			
DNBSEQ-T7RS High-throughput Sequencing Kit (FCL PE150) Catalog number: 940-000835-00					

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiration date
dNTPs Mix II		5.61 mL/tube×2 tube	-80 °C to -15 °C	-25 °C to -15 °C	as stated on label
dNTPs Mix V		3.74 mL/tube×1 tube			
Sequencing Enzyme Mix		7.48 mL/tube×1 tube			
MDA Reagent		4.20 mL/tube×1 tube			
MDA Enzyme Mix		0.60 mL/tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent Sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (FCL PE150) **Catalog number: 940-000841-00**					
Washing Cartridge	/	1 EA	below 40 °C	0 °C to 30 °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 PE100 cycle run performs reads of 100 cycles (2×100) for a total of 200 cycles or 200 bases sequenced. At the end of the sequencing run an extra 10 cycles or 20 cycles of barcode read can be performed to aid in identifying a specific library, if required.

Table 4 Sequencing cycle

Sequencing read length	Read1 length	Read2 length	Barcode read length	DualBarcode read length	Maximum cycles
PE100	100	100	10	10	240
PE150	150	150	10	10	340

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

Sequencing time

Table 5 Theoretical sequencing time (hours)

Model	Single flow cell	Four flow cells	DNB preparation	DNB loading
FCL PE100	15.0 to 16.0	16.0 to 20.0	1	2
FCL PE150	21.0 to 23.0	23.0 to 28.0	1	2

- Sequencing run time for a single flow cell and four flow cells only refer to the time elapsing from the “start” to the “finish” of the sequencing run. The time used for DNB preparation, DNB loading and Write FQ is not included. Write FQ for a single flow cell will take approximately 1.5 hours.
- Two flow cells can be loaded with DNBs concurrently by using one DNB loader. The total time is approximately two hours.
- The time in the table above is the average value. The actual run time may vary slightly among individual sequencers.
- Sequencing run time includes the time for the single barcode (10 cycles) sequencing.

---This page is intentionally left blank.---

04

Getting Started

This chapter describes sequencing preparations.

Preparing user-supplied equipment and consumables

Before using the device, prepare the following equipment:

Table 6 User-supplied equipment list

Equipment	Recommended brand
Ultra-pure water machine	General lab supplier
Freezer, -25 °C to -15 °C	General lab supplier
Refrigerator, 2 °C to 8 °C	General lab supplier
Graduated cylinder, 500 mL	General lab supplier
Ice bucket	General lab supplier
Pipette, 20 µL	Eppendorf or equivalent
Pipette, 200 µL	Eppendorf or equivalent
Pipette, 1000 µL	Eppendorf or equivalent
Electronic pipette	Integra or equivalent
Vortex mixer	General lab supplier
Qubit Fluorometer	Thermo Fisher
Thermal cycler	Bio-Rad or equivalent
Mini spinner	General lab supplier
96 well plate centrifuge	General lab supplier

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

Table 7 Recommended reagent/consumable list

Reagent/Consumable	Recommended brand	Purpose
2 M NaOH	General lab supplier	Diluting to 0.1 M for denaturing
5 M NaCl	General lab supplier	Diluting to 1 M for washing reagents
Tween-20	Sigma-Aldrich, catalog number: P7949	Performing a maintenance wash, diluting to 0.05% for washing reagents
Sterile pipette tip (various types)	General lab supplier	Pipetting for diluting and loading wash and loading reagents
Sterile 200 µL wide-bore, non-filtered pipette tip	AXYGEN, catalog number: T-205-WB-C	Mixing DNBs

Reagent/Consumable	Recommended brand	Purpose
Qubit ssDNA Assay Kit	General lab supplier	Library and DNB QC
Qubit Assay Tubes	Thermo Fisher	Library and DNB QC
Sterile PCR 8-strip tube, 0.2 mL	Thermo Fisher	Making DNB reaction mixture
Sterile microcentrifuge tube, 1.5 mL	VWR, catalog number: 20170-038, or equivalent	Combining volumes when diluting NaOH and library
Canned air duster	General lab supplier	Cleaning
Disposable gloves, powder-free	General lab supplier	General purpose
Kimwipes	VWR	Cleaning
Low-lint cloth	General lab supplier	Cleaning
Laboratory-grade water	General lab supplier	Sequencing and cleaning

WARNING Tips are disposable consumables, do not reuse them.

Recommended laboratory-grade water types include:

- 18 Megohm (MΩ) water
- Milli-Q water
- Super-Q water
- similar molecular biology-grade water

Preparing the pure water container

Perform the following steps:

1. Ensure that the pure water volume is sufficient.

   WARNING Insufficient pure water volume will result in sequencing failure.

   For information on pure water consumption, refer to *Filling the pure water container on Page 87.* For information on maintaining the pure water container, refer to *Maintaining the pure water container on Page 126.*

2. Connect the pure water container to the device.

   1) Place the fixing plate on the lid and align the holes. Insert the pure water tube into the pure water container through the aligned holes until the tube reaches the bottom of the container.

Ensure that the tube goes through the handle, as shown in the figure below:

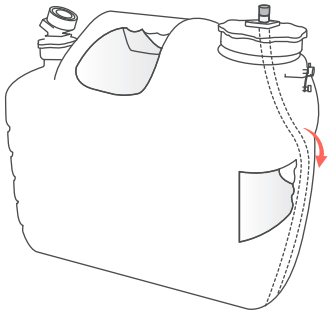

Figure 20 Inserting the pure water tube

2) Secure the fixing plate and lid.

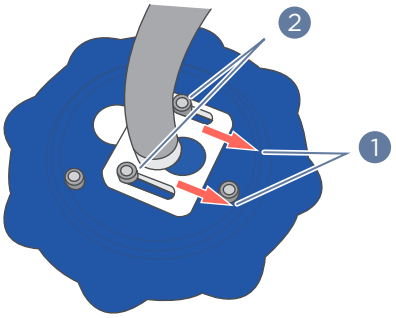

Figure 21 Securing the fixing plate and lid

3) Open the airway.

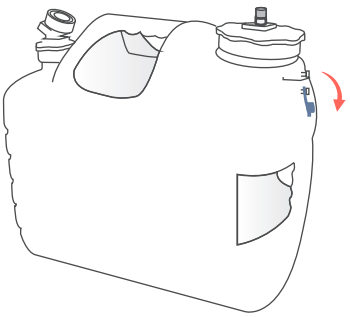

Figure 22 Opening the airway

3. If you need to add pure water into the pure water container during sequencing, perform the following steps:

   1) Open the lid in position 1 according to the direction indicator on the lid.

2) Insert the water output tube of the pure water machine into the pure water container through the water inlet in position 2.

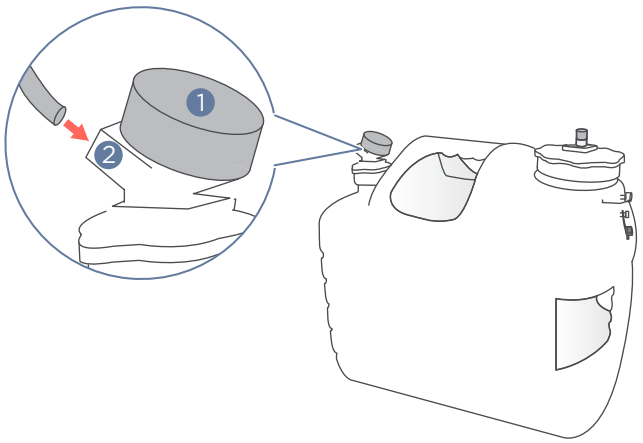

Figure 23 Adding pure water during sequencing

3) Fill the pure water container with fresh pure water.
4) Remove the water output tube of the pure water machine from position 2 and secure the lid in position 1.

Preparing the waste container

The waste container is connected to the device through the tubes. Ensure that the space is sufficient before connecting the waste container to the device. When the space is insufficient, replace the waste container.

For information on estimating the space and replacing the waste container, refer to *Replacing the waste container on Page 127.*

Preparing the devices

Powering the device on

CAUTION

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

Powering the sequencer on

Perform the following steps:

1. If a UPS is prepared, connect the UPS to the device.
2. Turn the power switch of the device to the ON position.

   After you power the device on, self-test begins.

Powering the DNB loader on

Perform the following steps:

1. Connect the power port of the device and the main power supply socket by using the power cord.
2. Turn the power switch to the | position.

   After you power the device on, self-test begins.

Logging in to the control software

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

Perform the following steps:

1. Power the device on.
2. Log in to the computer with the password that is provided by the manufacturer.
3. Select in the main interface.
4. Log in to the control software with the user name and password.

05

Sequencing

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

Workflow

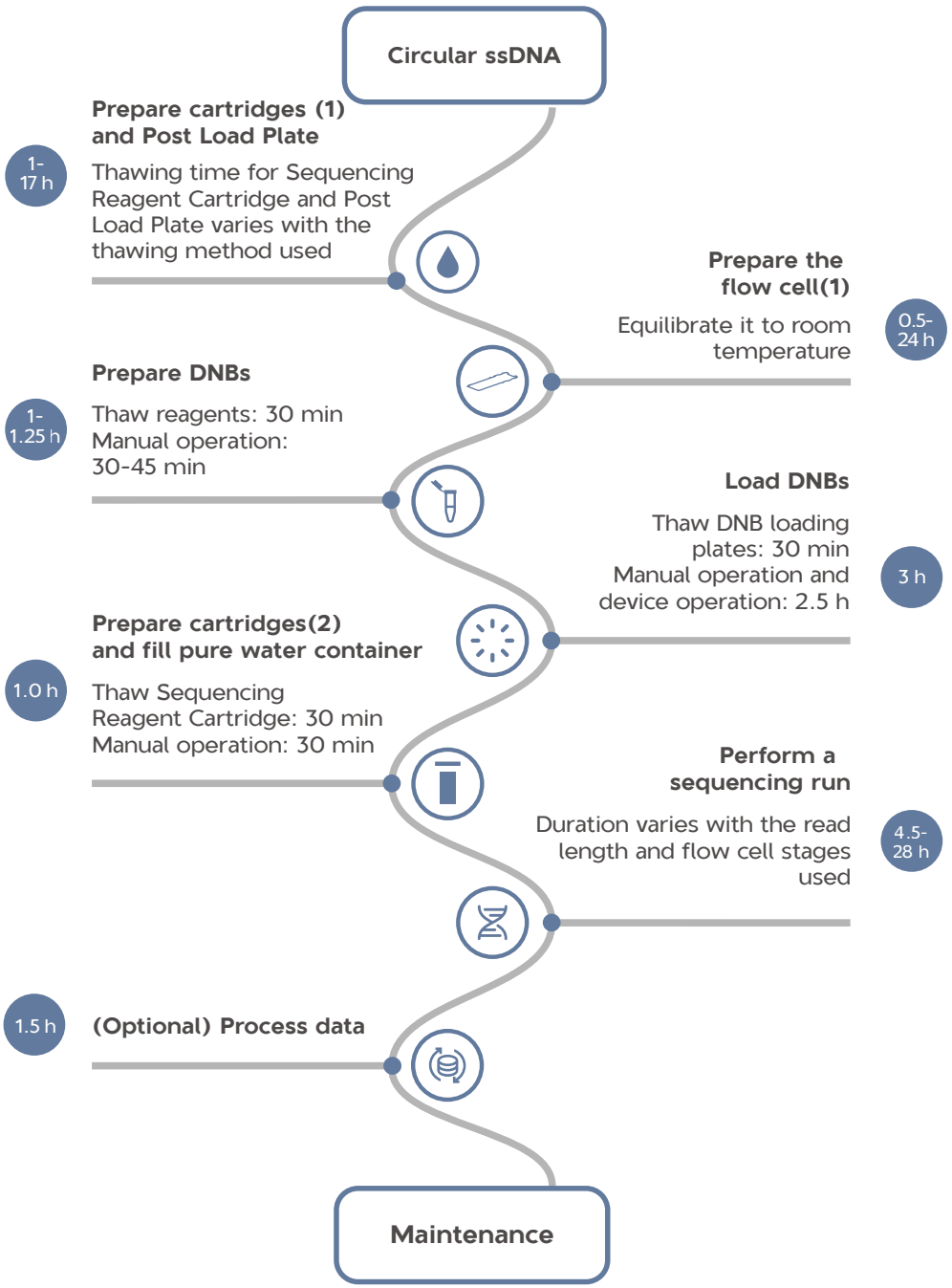

Figure 24 Sequencing workflow

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

Preparing the Sequencing Reagent Cartrige - part 1 and Preparing the Post Load Plate

Preparing the Sequencing Reagent Cartridge - part 1

Perform the following steps:

1. Remove the Sequencing Reagent Cartridge from the DNBSEQ-T7RS High-throughput Sequencing Kit.
2. Thaw the Sequencing Reagent Cartridge using one of the following methods:
   - Thaw it in a water bath at room temperature.
   - Thaw it in 2 °C to 8 °C refrigerator at least 16 hours in advance, followed by water bath thawing.

Table 8 Approximate thaw times for various sequencing kits

Model	Method	
	Water bath at room temperature (hours)	Overnight storage at 2 °C to 8 °C then water bath at room temperature (hours)
FCL PE100	2.5	2.0
FCL PE150	4.0	2.0

Overnight refers to 16 hours.

3. Ensure that the reagents are completely thawed. Store the thawed reagents at 2 °C to 8 °C until use.

Preparing the Post Load Plate

Perform the following steps:

1. Remove Post Load Plate from the DNBSEQ-T7RS DNB Load Reagent Kit.
2. Thaw Post Load Plate using one of the following methods:
   - Thaw it in a water bath at room temperature for 2 hours.
   - Thaw it in 2 °C to 8 °C refrigerator at least 16 hours in advance, followed by water bath thawing.

Table 9 Approximate thaw times for Post Load Plate in water bath

Method	
Water bath at room temperature (hours)	**Overnight at 2 °C to 8 °C then water bath at room temperature (hours)**
2.0	0.5

3. Once Post Load Plate is thoroughly thawed, place it in a 2 °C to 8 °C refrigerator until use.

Preparing the flow cell - part 1

Perform the following steps:

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

   i Do not open the outer plastic package yet.

2. Place the flow cell at room temperature for 0.5 hours to 24 hours.

Preparing DNBs

Recommended library insert size

The sequencing set is compatible with the libraries prepared by CG Library Prep Kits. If third-party library preparation kits are used, please select one of the options below for compatibility with DNBSEQ sequencers. For more information, please contact CG Technical Support.

.

Table 10 Library Conversion Kit

Catalog Number	Name
940-000917-00	DNBSEQ High-throughput Sequencing Primer Kit (App-D) (Paired-End)
940-001648-00	DNBSEQ OneStep Library Conversion Kit (Third Party)

- The recommended size distribution of inserts ranges between 200 bp and 500 bp, with the main insert size fragment centered within ±100 bp.

- The selection of specific reagent kits needs to consider the fragment size and the required data volume.
- If there are any special requirements or specifications for the CG library preparation kit, then the requirements of the kit should be followed.

Table 11 Recommended library insert size and applications

Model	Recommended library insert distribution (bp)	Applications
FCL PE100	200 to 400	WGS, WES, RNAseq, Single Cell
FCL PE150	300 to 500	WGS, WES, RNAseq

DNA library concentration and amount requirement

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

  C (fmol/μL)=3030×C (ng/μL)/N

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

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

Table 12 Circular ssDNA library concentration requirement

Library type	Library concentration
PCR libraries	≥3 fmol/μL
PCR free libraries	≥3.75 fmol/μL

Library pooling

Number of samples that can be pooled together

The sequencer can simultaneously perform sequencing of 4 flow cells. For PE100 sequencing, one flow cell can produce 1160 Gb of data in theory. The number of samples that can be pooled together for each flow cell depends on the required data output, read length, and specific application.

Do not pool more samples if their total data output is larger than 90% of the theoretical data output. This is due to variation in pooling and the fact that not all barcodes will generate the same amount of the data output from the same amount of DNBs.

$$\textbf{Maximum number of samples pooled} = \frac{\textbf{Theoretical data output of one flow cell} \times \textbf{90\%}}{\textbf{Required data per sample}}$$

- Example 1: Human Whole-genome Sequencing (WGS)

  When using the PE100 sequencing kit, 10 samples on each flow cell are recommended.

- Example 2: 50Gb data output is required for each sample

  When using the PE100 sequencing kit, if 50Gb is required for each sample, then a maximum of 20 samples is recommended to be pooled for each flow cell.

- Example 3: Pooling samples with various applications

  When using the PE150 sequencing kit, if samples to be sequenced include WGS (100Gb / sample) and RNASeq (50Gb / sample), it is recommended to pool 4 WGS samples and 23 RNASeq samples for each flow cell.

  Expected pooling variation are within ±10%.

Table 13 Examples of various sample pooling

Model	Theoretical data output range for each flow cell (Gb/flow cell)	Minimum data for each sample (Gb)	Pooling sample number	Theoretical data output range for each sample (Gb)
FCL PE100	1160	100	10	104 to 127
FCL PE100	1160	50	20	52 to 63
FCL PE150	1740	50 - RNAseq	23 RNAseq + 4 WGS	51 to 62 - RNAseq
	1740	100 - WGS		102 to 122 - WGS

Verifying the base balance for barcode

- A balanced base composition in each sequencing cycle is very important for high sequencing quality. It is strongly recommended that the minimum base composition of A, C, G, T for each position in the barcode is not lower than 12.5%. For a given pooling of samples, if the minimum base composition of A, C, G, T within the barcode is between 5% and 12.5%, the barcode split rate may be compromised. If the minimum base composition of A, C, G, T in any position of the barcode is less than 5%, re-design the pooling strategy to have a more balanced base composition in the barcode.
- It is also important to note that two or more samples with an identical barcode should not be pooled together, otherwise, it is impossible to assign the read correctly.

Making DNBs

- Mixed use of reagent components from different batches is not recommended.
- For transferring or mixing DNBs, use the wide-bore, non-filtered pipette tips.
- For operating other reagents, it is recommended to use the specified pipette tips according to the actual situation.

DNB making protocols are listed below. Please select the appropriate one according to the sequencing kit used.

- *Making DNBs for FCL PE100 on Page 62*

- *Making DNBs for FCL PE150 on Page 65*.

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. Remove the following reagents from the DNBSEQ-T7 DNB Make Reagent Kit and thaw according to the following table:

Table 14 Approximate thaw times for DNB Make reagents

Component	Cap color	Thawing method
Low TE Buffer		Thaw at room temperature for 30 mins
Make DNB Buffer		
Stop DNB Reaction Buffer		
Make DNB Enzyme Mix I (PE100) Make DNB Rapid Enzyme Mix II (PE150)		Thaw on ice for 30 mins

3. Mix all the reagents by using a vortex mixer for five seconds. Centrifuge briefly and place on ice until use.

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

Making DNBs for FCL PE100

Calculating the required amount of ssDNA libraries

270 µL of DNBs is required to load one flow cell for FCL PE100.

One DNB making reaction can make either 100 µL or 50 µL of DNBs. The volume of the DNB making reaction system depends on the amount of data required for sequencing per sample and the types of DNA libraries.

The required ssDNA library volume to make either 100 µL or 50 µL of DNBs are shown in the table below.

- If there are any special requirements or specifications for the CG library preparation kit, then the requirements of the kit should be followed.
- *C* in the following table represents the concentration of libraries (fmol/µL).

Table 15 Volume of ssDNA libraries for FCL PE100

Library type	Required ssDNA volume: V (µL)	
	100 µL DNB reaction	50 µL DNB reaction

PCR libraries	V = 60 to 90 fmol / C	V = 30 to 45 fmol / C
PCR free libraries	V = 75 to 150 fmol / C	V = 37.5 to 75 fmol / C

Calculate the required ssDNA libraries for each Make DNB reaction. The value of *V* obtained from the above table will be used in *Table 16 on Page 63*.

For a given sample A, if it requires "a" million base data output and the total theoretical expected data output for this flow cell is "b" million bases, then the required DNB volume (*V'*) in the pooling for sample A is as follows:

$$V' = a / b \times 270\ (\mu L)$$

- If the total sample number pooled is <6, it is recommended that you select the volume of 100 µL for each DNB reaction. The number of 100 µL DNB making reactions is equal to (V'/100)+1 rounded down to the nearest whole number.

  For example:

  - If V' = 80, it requires one 100 µL DNB making reaction.
  - If V' = 120, it requires two 100 µL DNB making reactions.

- If the total sample number pooled is ≥ 6, it is recommended that you select the volume of 50 µL for each DNB reaction, and the number of 50 µL DNB making reactions is equal to (V'/50)+1 rounded down to the nearest whole number.

Making DNBs

Perform the following steps:

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

   - Do not discard the Low TE buffer after finishing this step. It might be used in DNB dilution operations.

Table 16 Make DNB reaction mixture 1 for FCL PE100

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

2. Mix Make DNB reaction mixture 1 thoroughly by using a vortex mixer, centrifuge it for five seconds, and place 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 17 Primer hybridization reaction conditions for FCL PE100

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

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

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

5. Immediately take the tubes out of thermal cycler when the temperature reaches 4 °C. Centrifuge briefly for five seconds and place the tube on ice.
6. Prepare Make DNB reaction mixture 2 according to the table below.

Table 18 Make DNB reaction mixture 2 for FCL PE100

Component	Cap color	Volume of 100 µL DNB reaction (µL)	Volume of 50 µL DNB reaction (µL)
Make DNB Enzyme Mix I		40	20
Make DNB Enzyme Mix II (LC)		4	2
Total volume		84	42

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

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

Table 19 RCR (Rolling Circle Replication) conditions for FCL PE100

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

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 by using a wide-bore, non-filtered pipette tip.

Table 20 Volume of Stop DNB Reaction Buffer for FCL PE100

Component	Cap color	Volume of 100 µL DNB reaction (µL)	Volume of 50 µL DNB reaction (µL)
Stop DNB Reaction Buffer		20	10
Total volume		104	52

- Keep DNBs on ice during the entire operation to prevent DNBs from performing secondary replication.
- It is very important to mix DNBs gently by using a wide-bore, non-filtered pipette tip. Do not centrifuge, vortex, or shake the tube.
- DNBs can be stored at 2 °C to 8 °C and sequenced within 48 hours.
- This is not a STOP point, immediately go to the next step: *Quantifying DNBs and pooling on Page 69*.

Making DNBs for FCL PE150

Calculating the required amount of ssDNA libraries

300 µL of DNBs is required to load one flow cell for the FCL PE150 kit. One DNB making reaction can make either 90 µL or 45 µL of DNB. The volume of the DNB making reaction system depends on the amount of data required for sequencing per sample and the types of DNA libraries.

The required ssDNA library volume needed to make 90 µL or 45 µL of DNBs (one DNB reaction) is shown in the table below.

- If there are any special requirements or specifications for the CG library preparation kit, then the requirements of the kit should be followed.
- *C* in the following table represents the concentration of libraries (fmol / µL).

Table 21 Volume of ssDNA libraries for FCL PE150

Library type	Required ssDNA volume: V (µL)	
	90 µL DNB reaction	45 µL DNB reaction
PCR libraries	V = 60 to 90 fmol / C	V = 30 to 45 fmol / C
PCR free libraries	V = 75 to 150 fmol / C	V = 37.5 to 75 fmol / C

Calculate the required ssDNA libraries for each Make DNB reaction. The value of *V* obtained from the above equation will be used in *Table 22 on Page 67*.

For a given sample A, if it requires "a" million base data output and the total theoretical expected data output for this flow cell is "b" million bases, then the required DNB volume (*V'*) in the pooling for sample A is as follows:

$$V' = a / b \times 300 \text{ (µL)}$$

The number of the 90 µL DNB making reactions is equal to (V'/90)+1 rounded down to the nearest whole number.

The number of the 45 µL DNB making reactions is equal to (V'/45)+1 rounded down to the nearest whole number.

Making DNBs

Perform the following steps:

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

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

Table 22 Make DNB reaction mixture 1 for FCL PE150

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

2. Mix Make DNB reaction mixture 1 thoroughly by using a vortex mixer. Centrifuge it for five seconds and place 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 23 Primer hybridization reaction conditions for FCL PE150

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

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

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

5. Immediately take the tubes out of thermal cycler when the temperature reaches 4 °C. Centrifuge briefly for five seconds and place the tube on ice.
6. Prepare Make DNB reaction mixture 2 according to the table below:

Table 24 Make DNB reaction mixture 2 for FCL PE150

Component	Cap color	Volume of 90 µL DNB reaction (µL)	Volume of 45 µL DNB reaction (µL)
Make DNB Rapid Enzyme Mix II		40	20
Make DNB Enzyme Mix II (LC)		1.6	0.8
Total volume		81.6	40.8

7. Add all the Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer. Centrifuge for five seconds.
8. Place the tubes into a thermal cycler for the next reaction. The condition is shown in the table below.

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

Table 25 RCR conditions for FCL PE150

Temperature	Time
Heated lid (35 °C)	On
30 °C	10 min
4 °C	Hold

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 by using a wide-bore, non-filtered pipette tip.

Table 26 Volume of Stop DNB Reaction Buffer for FCL PE100

Component	Cap color	Volume of 90 µL DNB reaction (µL)	Volume of 45 µL DNB reaction (µL)
Stop DNB Reaction Buffer		10	5
Total volume		91.6	45.8

- Keep DNBs on ice during the entire operation to prevent DNBs from performing secondary replication.
- It is very important to mix DNBs gently by using a wide-bore, non-filtered pipette tip. Do not centrifuge, vortex, or shake the tube.
- This is not a STOP point, immediately go to the next step: *Quantifying DNBs and pooling on Page 69*.

Quantifying DNBs and pooling

Quantifying DNBs

Perform the following steps:

1. When DNB making is completed, take out 2 µL of DNBs, and use Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify the DNBs. For details, refer to *Instructions for using Qubit to quantify the DNBs on Page 167.*

   - If the concentration of libraries prepared by customers is lower than that specified in the table below, refer to *Q: What should I do if DNB concentration is low? on Page 135* for details.
   - If there are too many samples in a single test, it is recommended to quantify in batches to avoid inaccurate DNB quantification due to fluorescence quenching.

Table 27 DNB concentration standard

Model	DNB concentration
FCL PE100	≥8 ng/µL
FCL PE150	≥5 ng/µL

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

Table 28 DNB dilution buffer and storage conditions

Model	Dilution reagent	Storage conditions	Storage time (hours)
FCL PE100	DNB Load Buffer I	2 °C to 8 °C	≤48
FCL PE150	Low TE Buffer	2 °C to 8 °C	≤8

To ensure sequencing quality, it is recommended that you pool and load DNBs for FCL PE150 as soon as possible. If sequencing for four flow cells is performed simultaneously, you can make the DNBs together. Load the remaining flow cells immediately after loading the first two flow cells.

DNB pooling

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

Amount of DNBs (μL) needed for each sample in the pool depends on the relative amount for this sample and the total amount of DNBs needed for loading one flow cell which is defined by the specific type of sequencing kit.

Calculating the relative amount for each sample

Assuming there are 8 samples (A to H) in the pool, the relative amount for each sample is defined as:

The relative amount of A sample (A1) = data output required for sample A / the concentration of DNBs for sample A.

The relative amount of B sample (B1) = data output required for sample B / the concentration of DNBs for sample B.

.....

The relative amount of H sample (H1) = data output required for sample H / the concentration of DNBs for sample H.

Calculating the total relative amount (V) for all sample

V = A1 + B1 + ... + H1

Calculating the DNB volume needed for each sample

For each FCL flow cell used for PE100 requiring 270 μL of DNB, the DNB volume for pooling is calculated as follows:

DNB volume for sample A: A2 = 270×A1 / V

DNB volume for sample B: B2 = 270×B1 / V

...

DNB volume for sample H: H2 = 270×H1 / V

For each FCL flow cell used for PE150 requiring 300 μL of DNB, the DNB volume for pooling is calculated as follows:

DNB volume for sample A: A2 = 300×A1 / V

DNB volume for sample B: B2 = 300×B1 / V

...

DNB volume for sample H: H2 = 300×H1 / V

Preparing the flow cell - part 2

Perform the following steps:

1. Unwrap the outer package before use.

   - If the flow cell cannot be used within 24 hours after being placed at room temperate and the outer plastics package is intact, the flow cell can be placed back in 2 °C to 8 °C for storage. But the switch between room temperature and 2 °C to 8 °C must not exceed three times.
   - If the outer plastic package has been opened, but the flow cell cannot be used immediately, store the flow cell at room temperature and use it within 24 hours. If 24 hours is exceeded, it is not recommended that you use the flow cell.

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

Loading DNBs

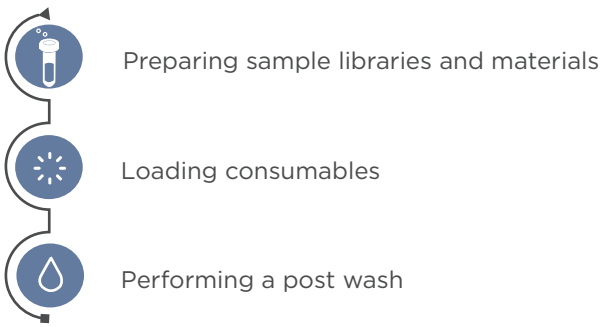

Figure 25 DNB loading workflow

DNB loading protocols are listed below, please select the appropriate one depending on the sequencing kit used:

- *Preparing load buffers for FCL PE100 sequencing on Page 72.*
- *Preparing load buffers for FCL PE150 sequencing on Page 73.*

Preparing load buffers for FCL PE100 sequencing

Preparing DNB Load Buffer II

Perform the following steps:

1. Remove DNB Load Buffer II from the DNBSEQ-T7RS DNB Load Reagent Kit (FCL PE100).
2. Thaw the reagent in a water bath at room temperature for approximately 0.5 hours.
3. Mix the reagent by using a vortex mixer for 5 seconds. Centrifuge briefly and place on ice until use.

   If crystallized precipitation is found in DNB Load Buffer II, vigorously mix the reagent for 1 to 2 minutes by using a vortex mixer to re-dissolve the precipitation before use.

Preparing the 0.1 M NaOH reagent

Prepare 0.1 M NaOH according to the procedure described in *Preparing washing reagents on Page 120*. Each Post Load Plate (T7 FCL PE100) requires at least 4 mL of 0.1 M NaOH.

Preparing DNB loading mixture

Perform the following steps:

1. Remove Micro Tube 0.5 mL (Empty) from the DNBSEQ-T7RS DNB Load Reagent Kit (FCL PE100) and add the following components in order.

   DNB in the table below refers to the pooled DNBs in *DNB pooling on Page 70*.

Table 29 DNB loading mixture for FCL PE100

No.	Component	Volume (µL)
1	DNB	270
2	DNB Load Buffer II	90
3	Make DNB Enzyme Mix II (LC)	1

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

   - Do not centrifuge, vortex, or shake the tube.
   - DNB loading mixture must be prepared fresh and used within 30 minutes.

Preparing load buffers for FCL PE150 sequencing

Preparing DNB Load Buffer IV

Perform the following steps:

1. Remove DNB Load Buffer IV from the DNBSEQ-T7RS DNB Load Reagent Kit (FCL PE150).
2. Thaw the reagent in a water bath at room temperature for approximately 0.5 hours.
3. Mix the reagent by using a vortex mixer for 5 seconds. Centrifuge briefly and place on ice until use.

Preparing the 0.1 M NaOH reagent

Prepare 0.1 M NaOH according to the procedure described in *Preparing washing reagents on Page 120*. Each Post Load Plate (T7 FCL PE150) requires at least 4 mL of 0.1 M NaOH.

Preparing DNB loading mixture

Perform the following steps:

1. Remove Micro Tube 0.5 mL (Empty) from the DNBSEQ-T7RS DNB Load Reagent Kit (FCL PE150) and add the following components in order.

   DNB in the table below refers to the pooled DNBs in *DNB pooling on Page 70*.

Table 30 DNB loading mixture for FCL PE150

No.	Component	volume (µL)
1	DNB	300
2	DNB Load Buffer IV	150

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

   - Do not centrifuge, vortex, or shake the tube.
   - DNB loading mixture must be prepared fresh and used within 30 minutes.

Perform DNB Loading

Perform the following steps:

1. Ensure that the compartment doors of DL-T7RS are closed and start the device. After a successful initialization, the main interface of DL-T7RS appears.

2. Start the DL-T7RS program, enter the user name and password, and then select **Log in** to enter the main interface.
3. Select **A** or **B** to continue the operation, see the figure below:

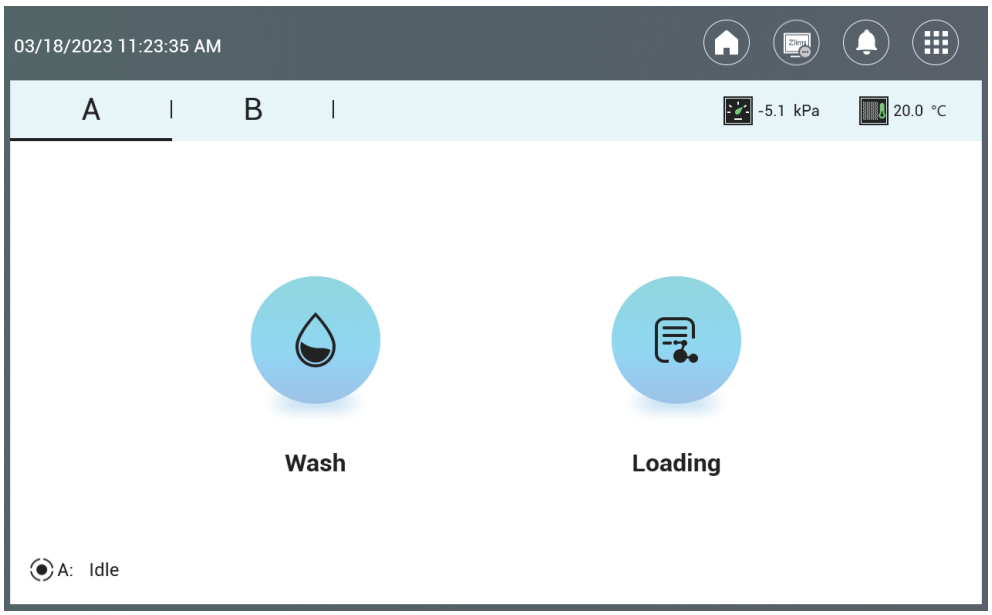

Figure 26 DL-T7RS selection interface

4. Select **Loading** to enter the information input interface, see the figure below:

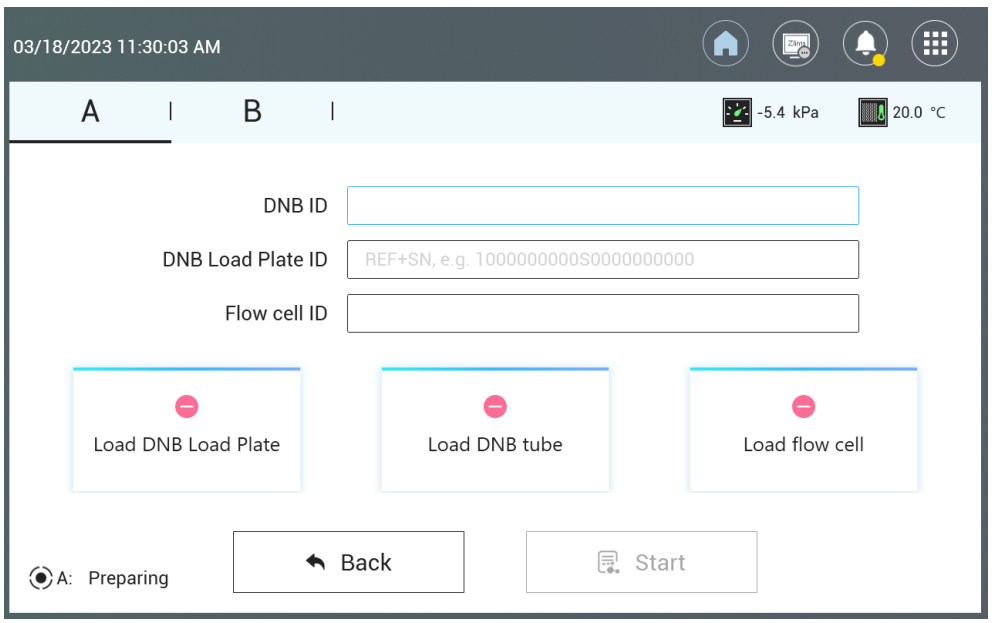

Figure 27 DL-T7RS information input interface

5. Open the loading compartment door.

6. Select the text box next to **DNB ID**, enter the DNB information into the text box.

   Use only numbers or letters or a combination of numbers and letters for DNB ID.

7. Place the Micro Tube 0.5 mL containing DNB loading mixture into the DNB tube hole, the screen will prompt that the DNB tube is loaded.

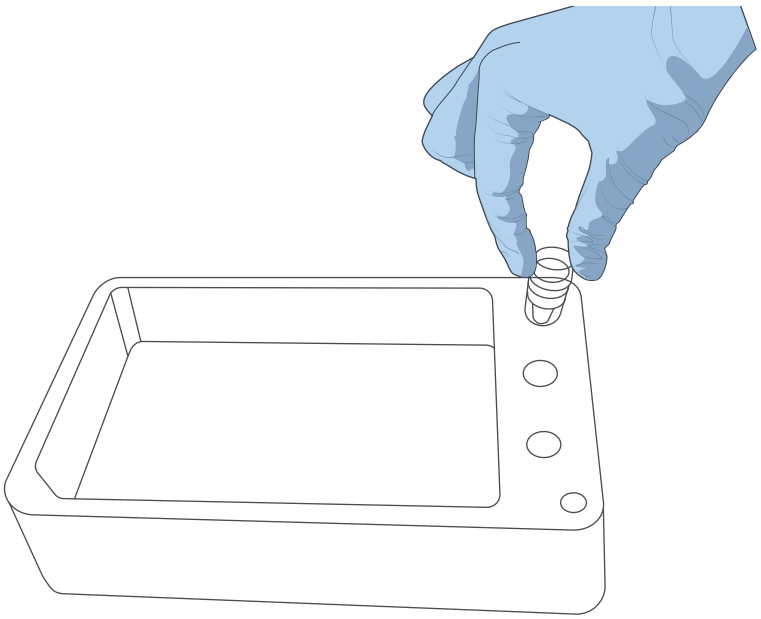

Figure 28 Placing DNB tube

8. Gently invert Post Load Plate to mix it 5 times and then centrifuge for 1 minute or gently tap the seal of the Post Load Plate and let it stand for 2~3 minutes. Remove the seal of the Post Load Plate and add 4 mL of 0.1 M NaOH into well No. 11.

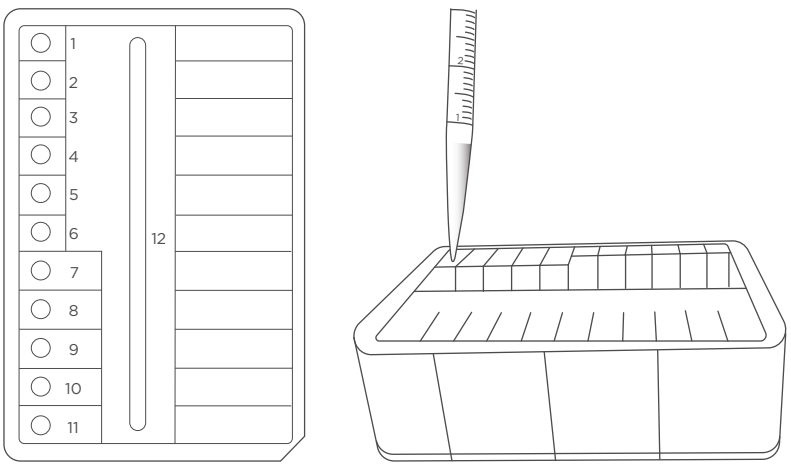

Figure 29 Adding 4 mL of 0.1 M NaOH into well No. 11

9. Align the Post Load Plate to the RFID scanning area and the ID information will appear in the text box.

   - If scanning fails, input the plate ID with the on-screen keyboard.
   - Ensure that the ID format is correct when you input ID manually. Otherwise, you will be prompted that the ID is incorrect and the procedure cannot continue.
   - The plate ID consists of 10-digit catalog numbers and 11-character serial numbers.

Figure 30 RFID scanning area of Post Load Plate

10. Place the prepared Post Load Plate on the plate tray of DL-T7RS. The screen will prompt that the Post Load Plate is loaded.

Figure 31 Placing Post Load Plate

11. Align the flow cell to the RFID scanning area and ID information will appear in the text box.

If ID information does not appear after scanning, please enter it manually according to the prompt.

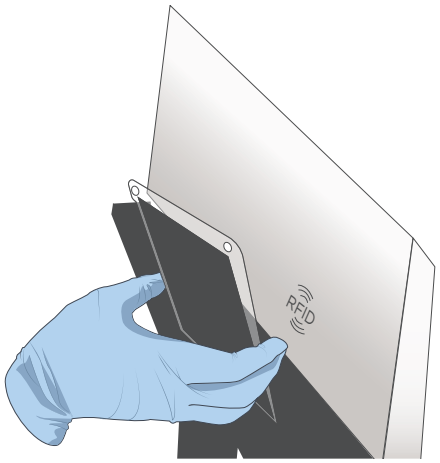

Figure 32 **Scanning the Flow cell ID**

12. Orient the flow cell upwards by holding the sides of the flow cell. Align the locating bulge on the flow cell to the locating groove on the flow cell stage. Gently press down the edges of the flow cell, see the figure below:

    Ensure that all the four rubber sealing rings are on the four corners of the flow cell.

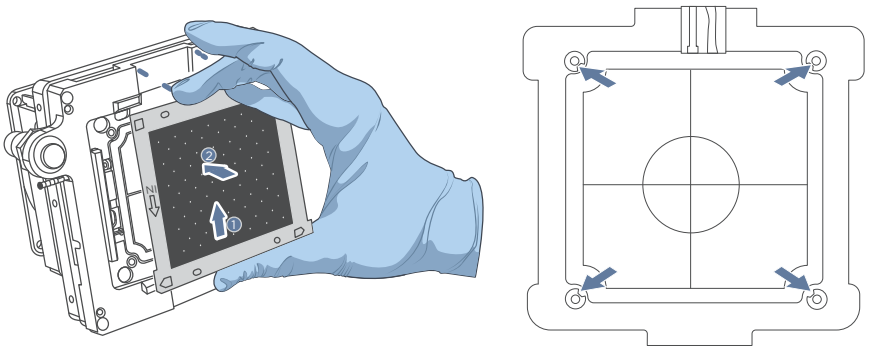

Figure 33 **Flow cell locating**

13. Press the flow cell attachment button on the flow cell stage to ensure that the flow cell is securely seated and held on the stage. The screen will prompt that the flow cell is loaded.

- Remove the dust on both sides of the flow cell with a canned air duster.
- Do not press or touch the glass cover of the flow cell to avoid leaving finger prints or impurities on the glass surface, and possibly damaging the flow cell.
- Do not move the flow cell after installing the flow cell onto the stage, or it may cause the sealing gaskets to misalign with holes of the fluidics line.
- If flow cell attachment fails, gently wipe the back of the flow cell and flow cell stage with a clean low-lint cloth moistened with 75% ethanol. Dust the flow cell with a canned air duster.

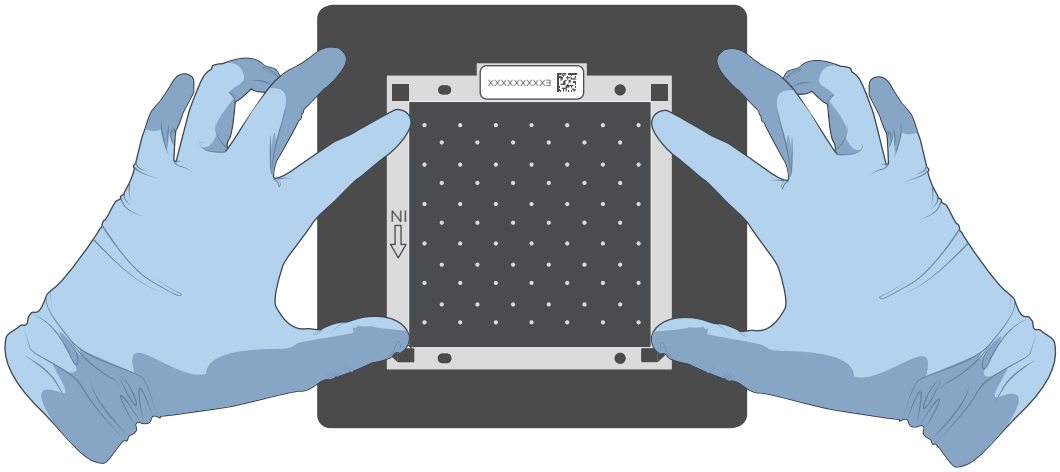

Figure 34 Flow cell loaded

14. Close the loading compartment door.

15. Select **Start** and select **Yes** when prompted to start loading. Flow cell loading starts as shown in the table below.

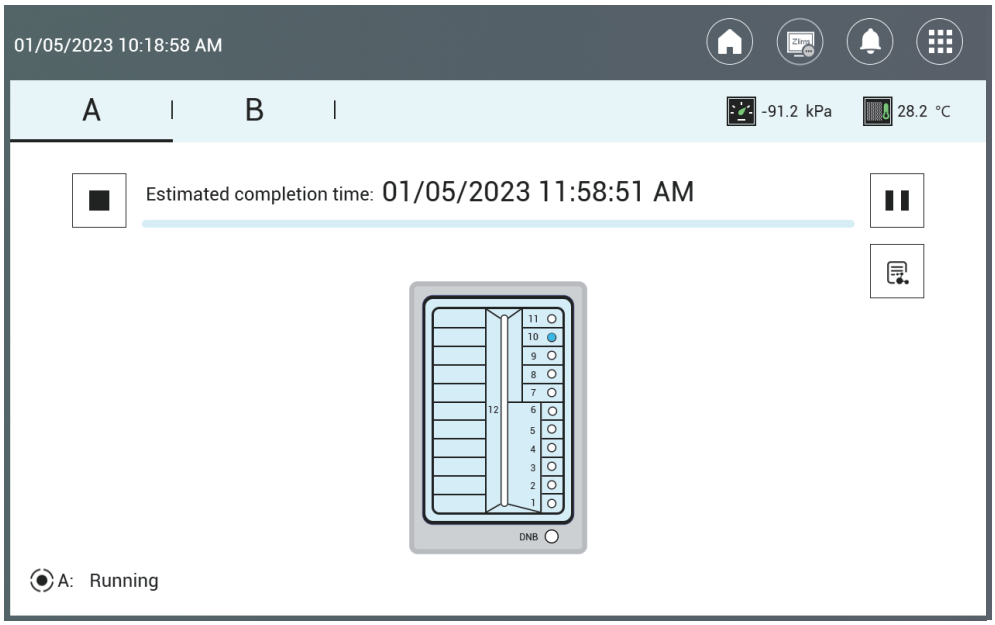

Figure 35 DL-T7RS flow cell loading interface

16. The process takes around two hours. When the screen is shown as *Figure 36 on Page 80*, the flow cell loading is complete.

- Do not open the loading compartment door during loading as it will stop the loading process.
- Do not bump, move, vibrate or impact the device during loading as it may cause inaccurate results.
- Do not place other instruments such as a centrifuge or vortex on the same bench where the loader is placed. Other instruments may cause vibrational interference to the loader.
- Pay special attention to the LED status indicator, icons, and prompts. If errors occur, a message appears on the screen. Follow the prompt to troubleshoot and fix the problem. For information about the troubleshooting, refer to *FAQs on Page 131*. If the problem persists, contact CG Technical Support.

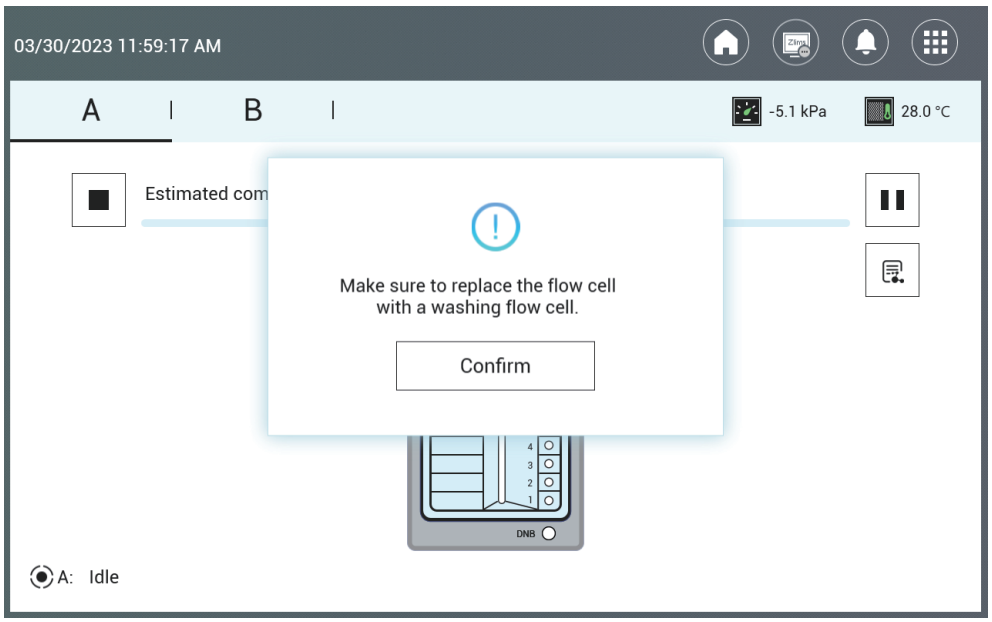

Figure 36 DL-T7RS flow cell loading complete status interface

17. Press the flow cell attachment button and remove the loaded flow cell from the stage. The flow cell is now ready for sequencing.

    - If sequencing cannot be performed immediately, put the loaded flow cell in a clean zip bag and store it at 2 °C to 8 °C until use.
    - The maximum storage time for loaded flow cell is 48 hours.

18. When the loading is complete, install the washing flow cell onto the flow cell stage and press the flow cell attachment button. Close the flow cell compartment door. Select **Confirm** as shown in *Figure 36 on Page 80*.

19. Select **Post-wash** and select **Yes** when prompted to start DL-T7RS wash, which will take approximately 20 minutes.

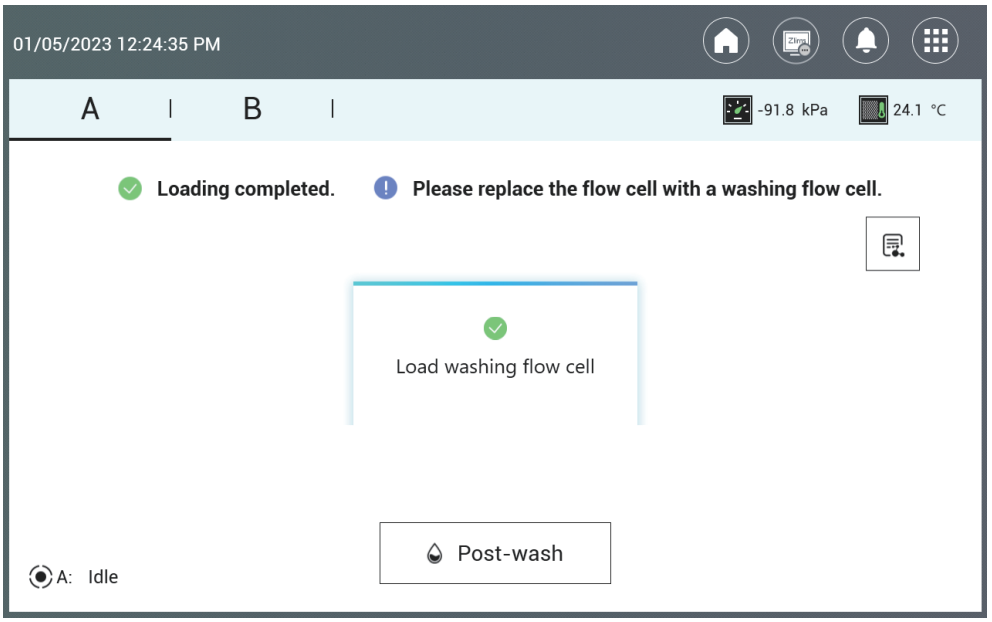

Figure 37 DL-T7RS post-wash interface

20. The DL-T7RS wash starts, and the estimated time to completion is displayed:

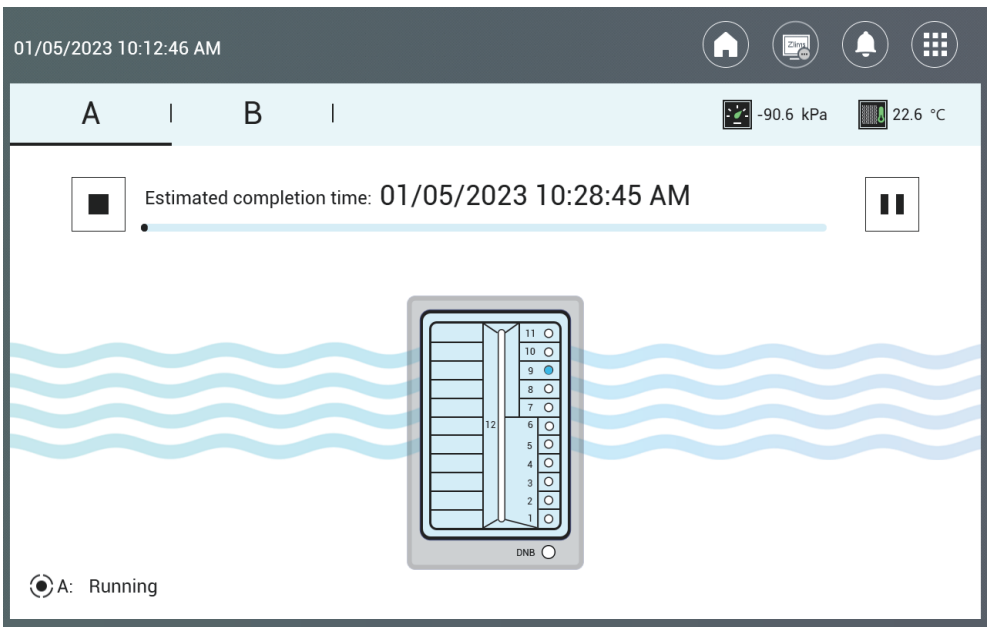

Figure 38 DL-T7RS wash interface

21. When the screen is shown as the figure below, the wash is complete. Complete the loading process by selecting **Finish** to return to the main interface.

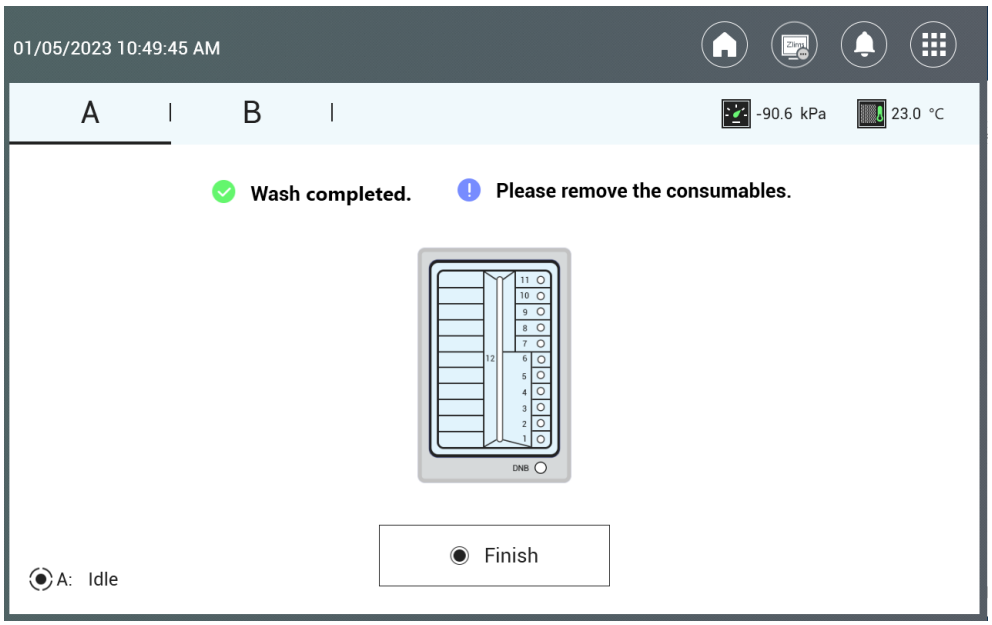

Figure 39 DL-T7RS wash complete status interface

22. Remove the washing flow cell and store it at room temperature.
23. Empty any remaining washing solution in the Post Load Plate into an appropriate waste container.
24. Dispose of the waste and DNB tube.

Preparation before sequencing

CAUTION If prepared cartridges are not used immediately, refer to *Q: What rules should I follow if I need to store a reagent kit temporarily? on Page 139* for details.

Preparing the Sequencing Reagent Cartridge - part 2

Sequencing Enzyme Mix and dNTP Mix are provided in different tubes and packaged together with the Sequencing Reagent Cartridge. Before the sequencing run starts, an appropriate amount of sequencing enzyme and dNTP mix needs to be added to well No. 9 and well No. 10 of the Sequencing Reagent Cartridge. Furthermore, the MDA mixture (MDA, Multiple displacement amplification) needs to be added to well No. 8 if performing PE (Pair-end) sequencing. If the prepared Sequencing Reagent Cartridge is not used immediately, please refer to *Q: What rules should I follow if I need to store a reagent kit temporarily?*

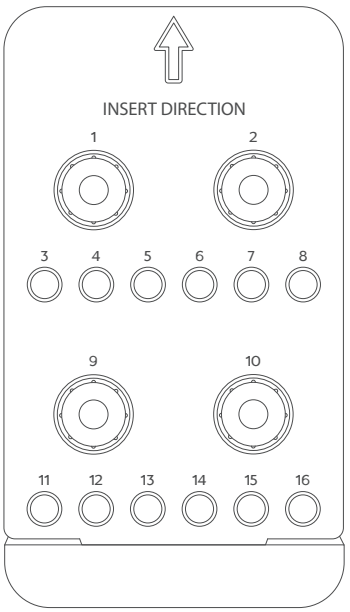

Figure 40 Sequencing cartridge wells

Perform the following steps:

1. Remove dNTPs Mix V and dNTPs Mix II from the DNBSEQ-T7RS High-throughput Sequencing Kit one hour before sequencing preparation and thaw them at room temperature.
2. After thawing the cartridge, invert the cartridge 10 times to mix before use.
3. Shake the cartridge vigorously clockwise 20 times, and then counterclockwise 20 times. Ensure that reagents are fully mixed.

> *i* Presence of dark green crystals in well No. 1 is normal due to crystallization of reagent materials in this well. When the cartridge is thawed, mix the reagents in the cartridge well and the crystals will dissolve. Sequencing quality will not be affected.

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

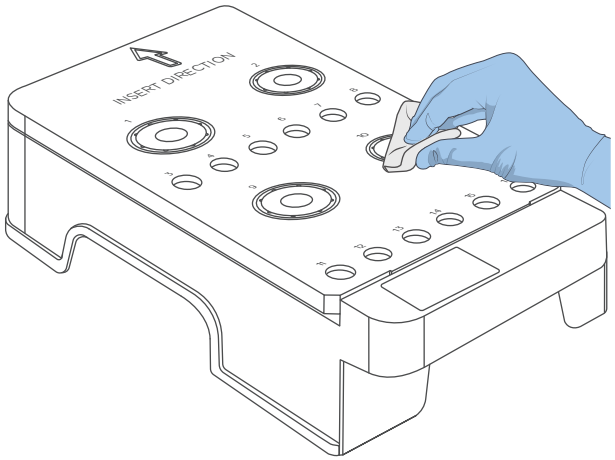

Figure 41 Wiping cartridge cover

5. Remove Sequencing Enzyme Mix from the DNBSEQ-T7RS High-throughput Sequencing Kit and place on ice until use.
6. For PE sequencing, remove the MDA Reagent and place on ice until use.
7. Invert the dNTPs Mix V and dNTPs Mix II 6 times. Gently tap the tube on the bench to bring the liquid to the bottom.
8. Invert the Sequencing Enzyme Mix 6 times.
9. Pierce the seal in the center of well No. 9 and No. 10 to make a hole around 2 cm in diameter by using a 1 mL sterile tip.
10. Take out a pipette with the appropriate volume range. Add the dNTPs Mix V and Sequencing Enzyme Mix into well No. 9 according to the table below:

Table 31 Reagent preparation for well No. 9

Model	Volume (mL)	
	dNTPs mix V	Sequencing enzyme mix
FCL PE100	2.760	2.760
FCL PE150	3.740	3.740

11. Take out a pipette with the appropriate volume range. Add the dNTPs Mix II and Sequencing Enzyme Mix into well No. 10 following the table below:

Table 32 Reagent preparation for well No. 10

Model	Volume (mL)	
	dNTPs mix II	Sequencing enzyme mix
FCL PE100	8.280	2.760
FCL PE150	11.220	3.740

12. Seal the loading wells of well No. 9 and No. 10 with the transparent sealing film.
13. Press the film with your finger around the well. Ensure that the well is tightly sealed and that no air bubbles exist between the film and cartridge surface, so that the reagents will not flow over the cartridge.
14. Lift the cartridge horizontally, hold both sides of the cartridge with both hands. Shake it clockwise 20 times, and then counterclockwise 20 times. Ensure that reagents are fully mixed.
15. Carefully remove the seals from the loading wells after full mixing.

    - Do not reuse the used sealing film.
    - Ensure that the surface around wells No.9 and No.10 is clean to avoid cross-contamination.

16. Prepare well No. 8:

    1) Pierce the seal of well No. 8 by using a 1 mL sterile tip.
    2) Add 600 µL of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette and invert the tube 6 times to mix the reagents.

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

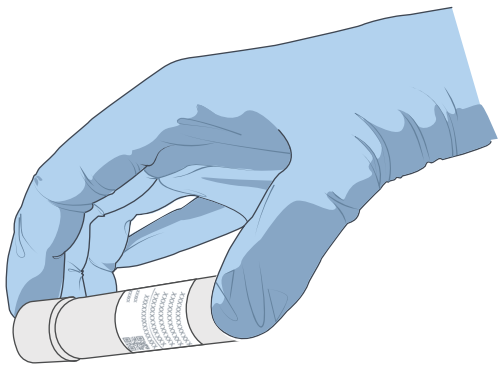

Figure 42 MDA mixture

    3) Add all of the MDA mixture to well No. 8. When adding the MDA mixture, ensure that there is no bubble at the bottom of the tube.

When transferring the mixture, handle it carefully to prevent spillage from the test tube.

4) Gently tap the Sequencing Reagent Cartridge on the bench to reduce air bubbles in the reagents. The sequencing cartridge is ready.

Preparing the Washing Cartridge

Perform the following steps:

1. Shake the cartridge clockwise 10 times, and then counterclockwise 10 times to ensure the reagents are fully mixed.
2. Clean the foil seal on the wells with a Kimwipes tissue. Pierce either side of well No. 2 by using a 1 mL sterile tip.

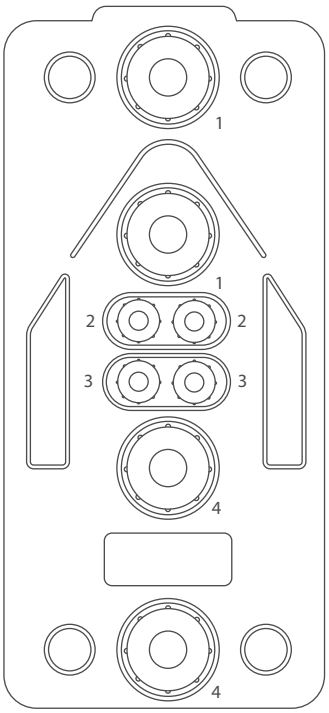

Figure 43 Washing Cartridge

3. Add 45 mL of 0.1 M NaOH into well No. 2 through the pierce by using an electronic pipette. Refer to *Preparing washing reagents on Page 120* for the preparation of 0.1 M NaOH.

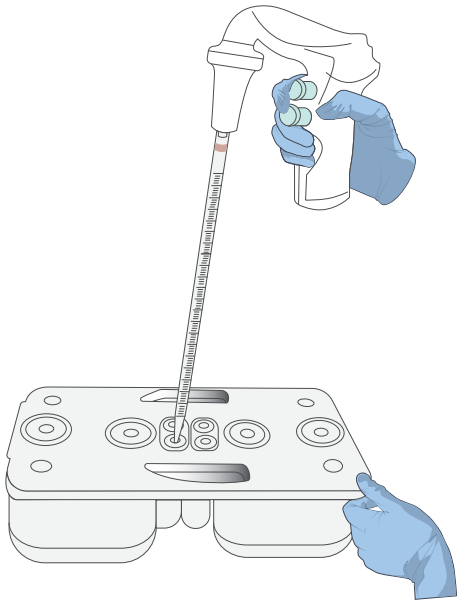

Figure 44 Washing Cartridge added 0.1 M NaOH

Filling the pure water container

Fill the pure water container with laboratory-grade water according to *Table 33 on Page 87*.

- Check to make sure the volume level of the water in the pure water container is sufficient. If the volume is insufficient, sequencing will fail. Replenish the pure water before starting the run. Make sure the air vent opening is unobstructed.
- The pure water will be used in sequencing so it must be kept clean. Change the pure water in the pure water container on a weekly basis.
- Before refilling the pure water container, empty the container and spray 75% ethanol on the inner surface of the container lid and the surface of the pure water tube. Wipe and clean the surfaces with new Kimwipes tissues. Rinse the container with fresh pure water three times.

Table 33 Pure water consumption (L)

Model	1 flow cell	2 flow cells	3 flow cells	4 flow cells
FCL PE100	3.0	6.0	9.0	12.0
FCL PE150	4.5	9.0	13.5	18.0

Performing a sequencing run

Loading the cartridges

Perform the following steps:

1. Open the reagent compartment door and clean the inner walls with a Kimwipes tissue moistened with laboratory-grade water. Keep the compartment clean and dry.

   Be cautious of sharp objects, such as the sampling needles, inside the reagent compartment when cleaning.

2. Place the Sequencing Reagent Cartridge into the sequencing cartridge compartment and place the Washing Cartridge into the washing cartridge compartment.

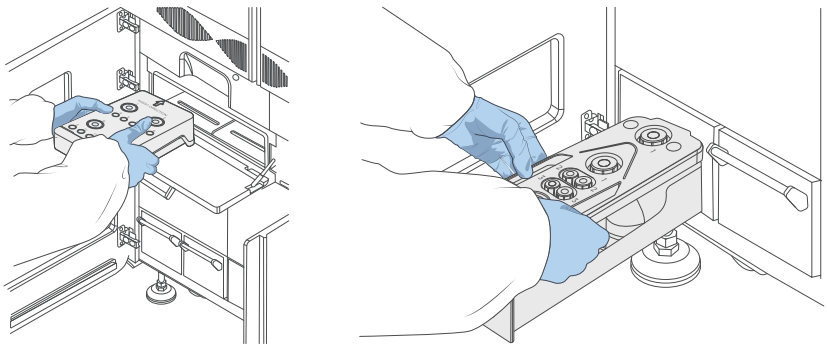

Figure 45 Loading the cartridges

3. Close the doors of both the sequencing cartridge compartment and washing cartridge compartment, and then close the door of the reagent compartment.

Loading the flow cell

Perform the following steps:

1. Select A/B/C/D respectively according to sequencing demand. Select **Sequence** and select **New run**.

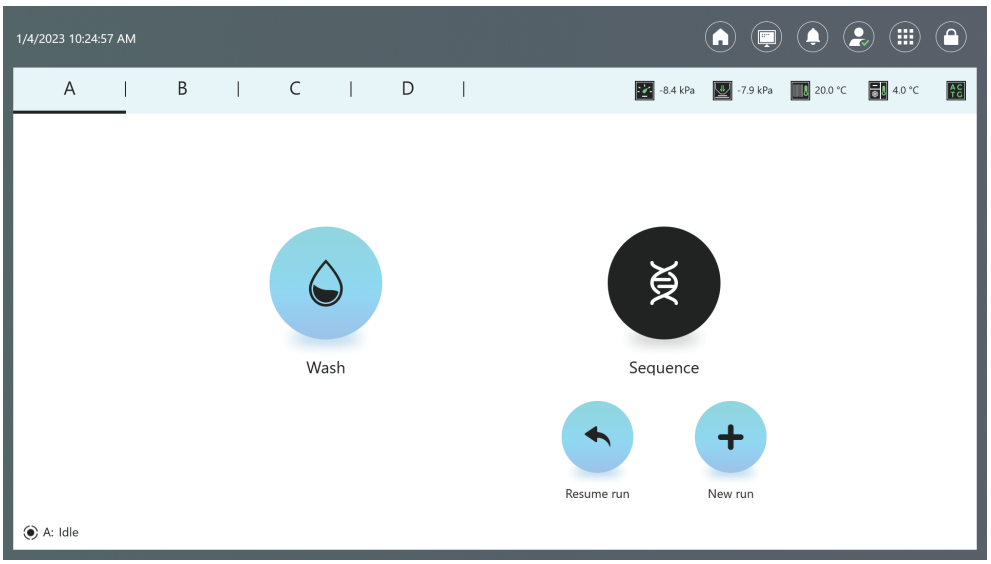

Figure 46 DNBSEQ-T7RS selection interface

2. Clean the loaded flow cell with a canned air duster to ensure that there is no visible dust on the surface and back of the flow cell. Put the flow cell on the flow cell drive, and touch the flow cell drive button to load the flow cell into the device.

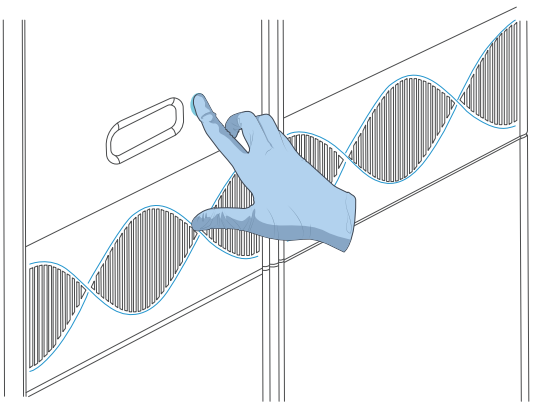

Figure 47 Flow cell drive

- If contaminants are present on the backside of the flow cell, clean it using lint-free wipes or paper. Avoid contact with the fluid inlet/outlet of the flow cell during wiping.
- When using the canned air duster to remove the dust on the back of the flow cell, avoid blowing air into the inlet holes.
- If the flow cell accidentally drops to the floor and breaks, handle with care to prevent personal injury.

Sequencing parameters

Perform the following steps:

1. Align the Sequencing Reagent Cartridge, Washing Cartridge and flow cell respectively to the RFID scanning area, the ID information will automatically display in the corresponding text box.

   - If scanning fails, input the cartridge ID with the on-screen keyboard.
   - The cartridge ID consists of the catalog number (REF on the label), serial number (SN on the label), and special characters. When inputting the ID manually, ensure that both the ID format and content are accurate. Otherwise, you will be prompted that the ID is invalid and the procedure cannot continue.

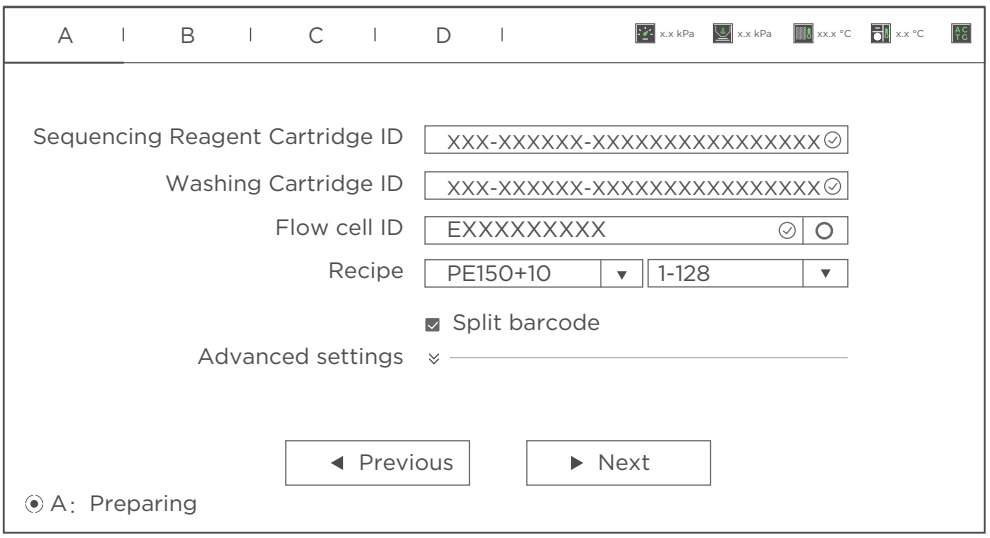

Figure 48 DNBSEQ-T7RS sequencing parameters

2. Select the first ▼ next to **Recipe**. Select an appropriate sequencing recipe from the list.

If a customized recipe is required, select **Customize** from the **Recipe** list. For information on recipe customization, refer to *Instructions for customizing a run on Page 149*.

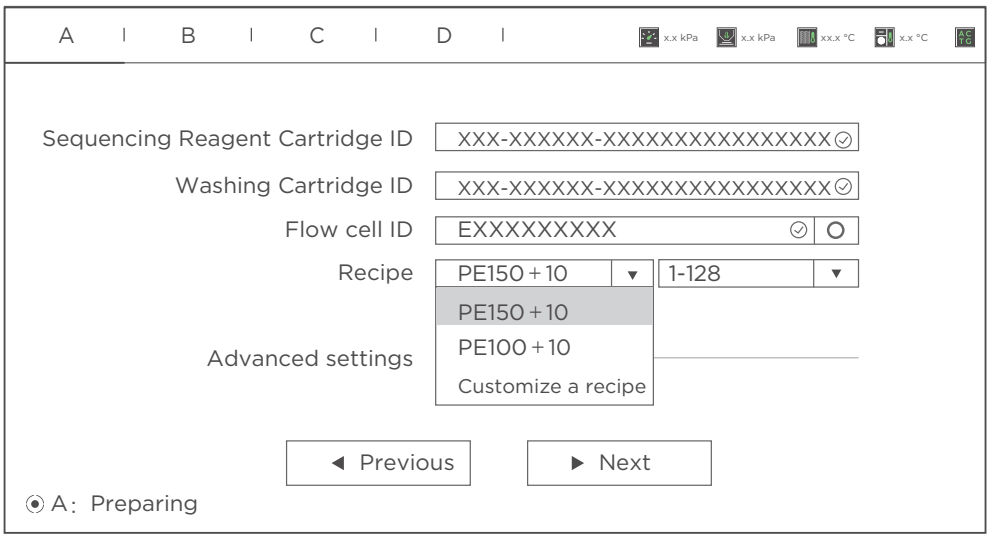

Figure 49 Set the sequencing recipe

3. Select the second ▼ next to **Recipe** and select the corresponding barcode sequence.

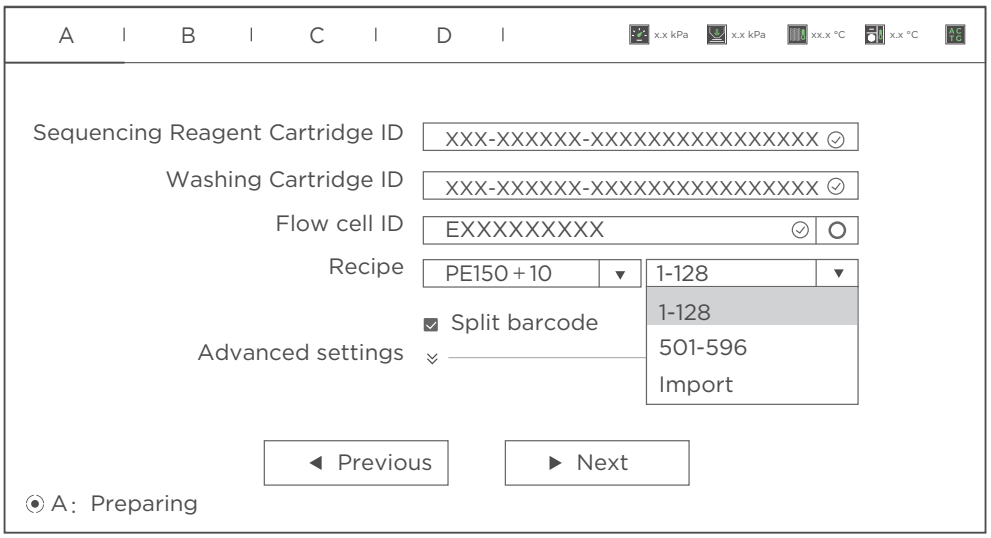

Figure 50 Set the barcode sequence

4. Select the **Split barcode** check box.

5. Select next to **Advanced settings** to enter the interface as shown in the figure below. You can indicate whether primers are custom and whether an auto wash is to be performed.

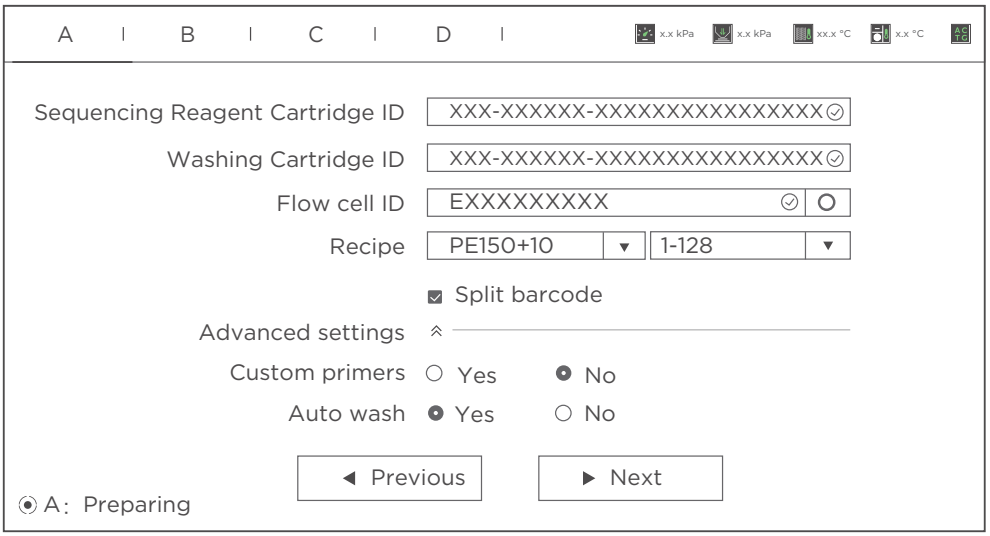

Figure 51 DNBSEQ-T7RS advanced settings

6. Select **Next**.

Reviewing parameters

Review the parameters and ensure that all information is correct. An example for PE150 is shown in the figure below:

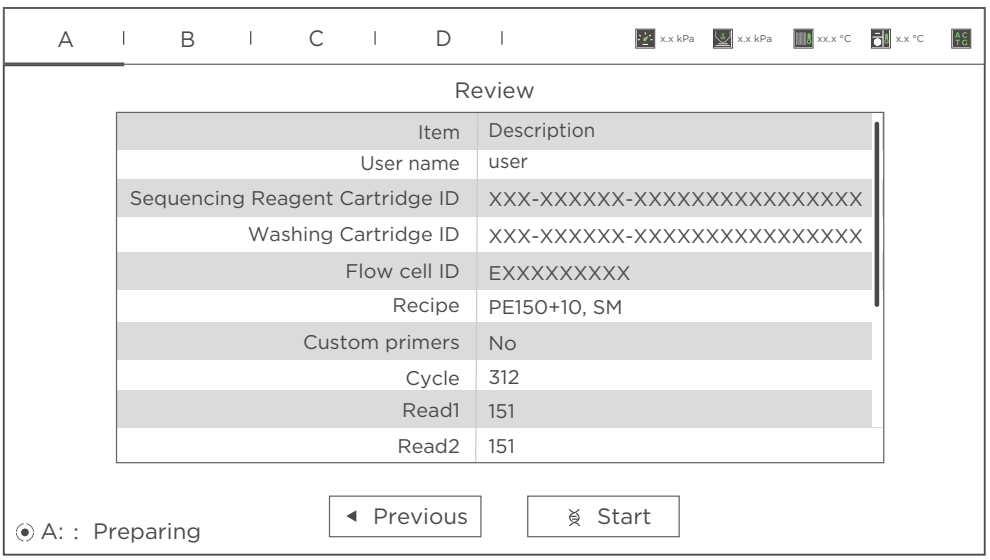

Figure 52 Reviewing information

Starting sequencing

After confirming that all the information is correct, select **Start** and select **Yes** when prompted to begin sequencing.

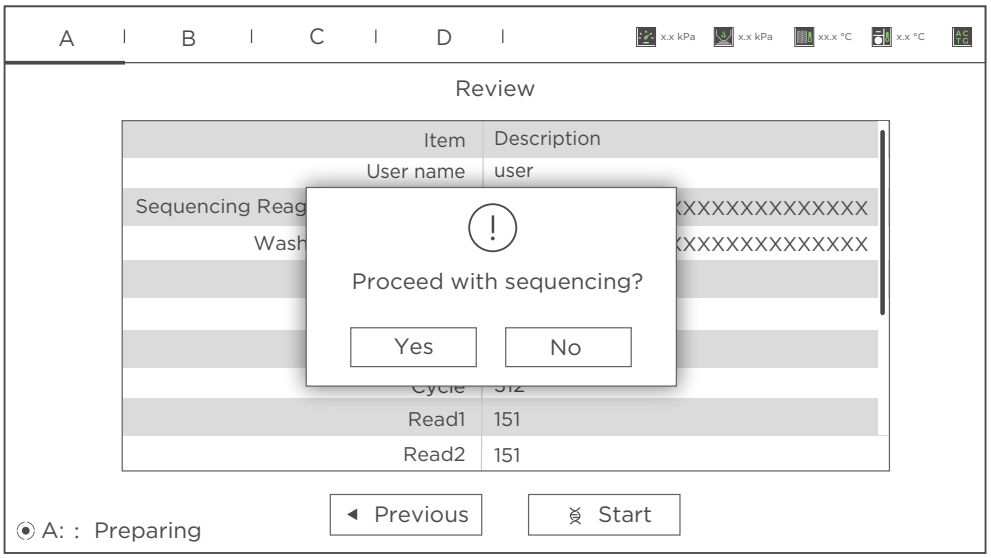

Figure 53 Confirm sequencing interface

When the following screen appears, the sequencing has begun.

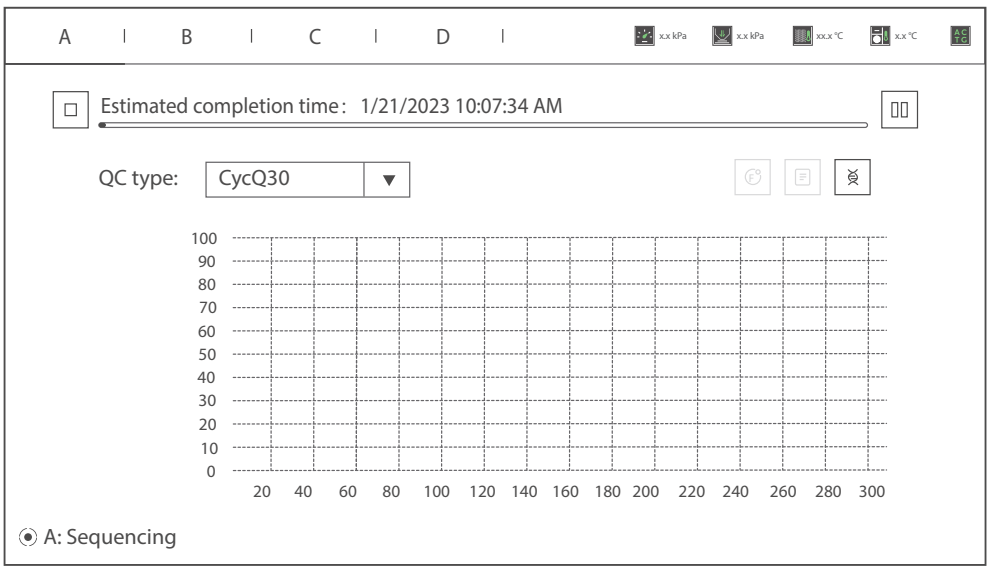

Figure 54 Sequencing start interface

When the sequencing and wash process for this run are complete, the following screen appears:

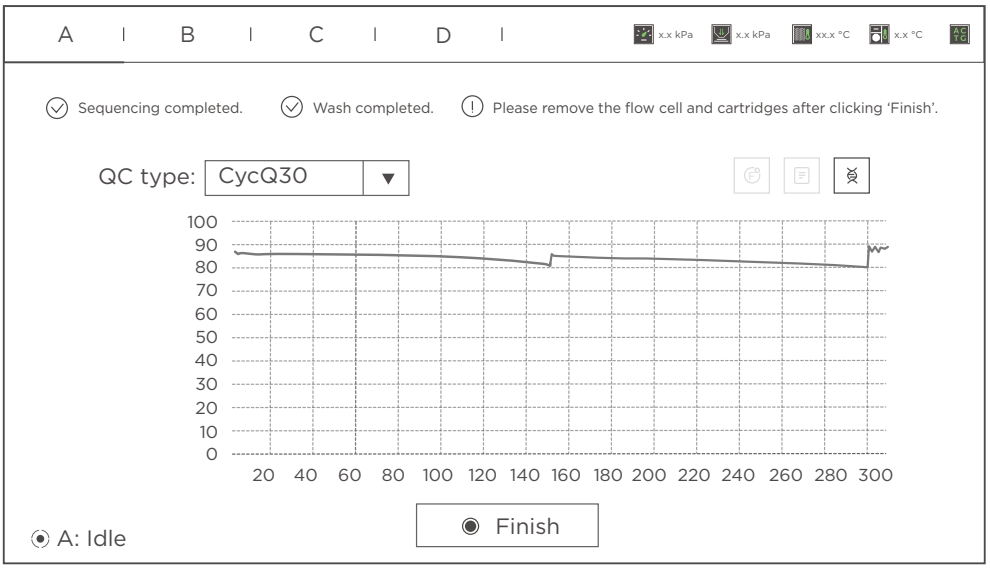

Figure 55 Sequencing and wash complete interface

CAUTION

- Ensure that all compartment doors are closed. The sequencing run cannot be started when the reagent compartment door is open.
- Only open the reagent compartment door when necessary to avoid adverse effects on sequencing results or even damage to the device.
- Do not bump, move, vibrate, or impact the device during sequencing as it may cause inaccurate results.
- If malfunctions related to fluidics lines (for example, bubbles) occur during sequencing, fix the problems before you restart sequencing.
- Pay special attention to the LED status bar or the on-screen instructions. If errors occur, troubleshoot the problem by following the instructions and this guide. If errors persist, contact CG Technical Support.

Automatic post-wash

Auto wash is enabled by default. The system automatically performs a post-wash after each sequencing run.

During troubleshooting, you can set **Auto wash** to **No** when necessary and perform a wash manually right after troubleshooting. For information on how to perform a wash manually, refer to *Wash procedures on Page 123.*

Disposing of cartridges and flow cells

After sequencing and post-wash, or before powering the device off, perform the following steps:

1. Wear protective equipment.
2. Open the flow cell retrieval compartment and remove the flow cells.
3. Open the reagent compartment door and remove the cartridges.
4. Empty the remaining solution in the cartridges into an appropriate waste container.
5. Dispose of the flow cell and cartridges.

For information on reusing cartridges and flow cells, refer to *Reusing the washing flow cell, washing cartridge, and washing plate on Page 124.*

(Optional) Powering the devices off

CAUTION

- Before you power the sequencer off, ensure that the sequencing run and wash are completed, the control software is shut down, and the flow cell drive is withdrawn. Failure to do so may damage the device.
- Power the loader off and disconnect the power cord if you do not plan to use the device for an extended period of time.

Powering the sequencer off

Perform the following steps:

1. Ensure that the sequencing run and wash are completed, and the flow cell drive is withdrawn.
2. Tap  > **Shut down** to open the shutdown/restart interface.
3. Select **SBC and Basecall server** and tap **Shut down** . Tap **Yes** in the pop-up dialog box.
4. After the screen turns off, wait for one more minute, and then turn the power switch to the OFF position.

Powering the DNB loader off

Perform the following steps:

1. Select  > **Shut down**. Select **Yes** when prompted to shut down the control software. Wait until the screen turns off.
2. Turn the power switch to the  position.

---This page is intentionally left blank.---

06

Sequencing data

This chapter describes the sequencing output data.

Sequencing output files

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

Folder structure

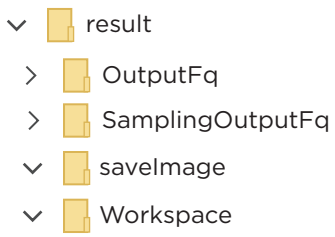

Figure 56 *result* folder of BCS or BIS

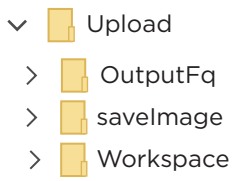

Figure 57 *Upload* folder of CG-ZTRON-LITE

- When configured on BCS (GPU) or BIS (FPGA), you need to visit the *result* and *Upload* folder by using the vnc viewer on the desktop (GPU) or Remote Desktop Connection of Windows (FPGA). In this case:
  - The *result* folder is located in the *data* folder of BCS or drive D of BIS.
  - The *upload* folder is located in the *storeData* folder of BCS or drive Z of BIS.
- When configured on SBC (GPU/FPGA):
  - The *result* folder is located in drive Z of SBC.
  - The *upload* folder is located in the designated drive.

When CG-ZTRON-LITE is connected:

- For BCS configuration, data in the *saveImage* and *Workspace* folders is stored on CG-ZTRON-LITE only. If network problems occur, data will continue to be stored on BCS.
- For FPGA version, data in the *saveImage* folder is stored on BIS only, but data in the *Workspace* folder is stored on both BIS and CG-ZTRON-LITE. If network problems occur, data will continue to be stored on BIS.

Item	Description
result	Sequencing result folder for algorithm software
OutputFq	FASTQ and report folder. In this folder, a folder named with the flow cell ID is produced, and Bioinfo file is generated in it promptly after the sequencing run starts. Writing FASTQ is finished when the summary report is generated.

Item	Description
SamplingOutputFq	Summary report folder
saveimage	Images folder
Workspace	Intermediate files. Only cal and metrics files are saved by default.

File type description

Folder structure		Description
Result	**OutputFq**	• *"XX.fq.gz"* is the FASTQ file generated by the sequencer. • *"XX.Report.html"* is the report file that includes results of the entire sequencing run.
	Workspace	Intermediate files, including cal and metrics files.
	saveImage	Raw images received from Basecall. 9 FOVs (field of view) are saved by default.

Summary report

In the device sequencing interface, select F5 to view the first base report.

You can view the detailed sequencing report in the default server directory.

Report parameter review

The following table describes critical report parameters:

Parameter	Description
SoftwareVersion	Version of BasecallLite. Ensure that the BasecallLite is in the official release version.
TemplateVersion	Version of summary report template
Reference	The species category of the sample. When the species category is unknown or when the category is not Ecoli, the reference will be indicated as NULL.
CycleNumber	The total cycle of the sequencing run (not including the extra cycles, but including barcode regardless of whether the barcode is split or not)
ChipProductivity(%)	Flow cell productivity. The yield of the flow cell is estimated by the following formula: $$\textbf{ChipProductivity} = \frac{\textbf{ValidFovNumber} \times \textbf{ESR}}{\textbf{ImageArea}} \times \textbf{100\%}$$
ImageArea	The total number of FOVs 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 that this base 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 that this base called incorrectly are 1 in 1000.
SplitRate(%)	The proportion of FASTQ data that can be split according to barcode sequences. This indicator is obtained from the *BarcodeStat.txt* file, and the split results are included in *Sequencestat.txt*. The split rate is counted from the filtered reads only.

Parameter	Description
Lag/Runon	• Lag1 (%) is the slope of the Lag curve for the first-strand sequencing. • Lag2 (%) is the slope of the Lag curve for the second-strand sequencing. • Runon1 (%) is the slope of the runon curve for the first-strand sequencing. • Runon2 (%) is the slope of the runon curve for the second-strand sequencing.
ESR(%)	Effective spot rate. Percentage of effective spots after filtering in the flow cell.
RecoverValue(AVG)	The ratio of second-strand signal to first-strand signal. This indicator is only for PE sequencing.

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

Table 34 Parameter description for Tab2 of the summary report

Parameter	Description
ISW Version	Version of control software for the sequencer
Machine ID	Serial number of the sequencer
Sequence Type	The sequencing recipe that you select when sequencing
Recipe Version	Version of the sequencing recipe script
Sequence Start Date	The date on which the sequencing started
Sequence Start Time	The time at which the sequencing started
Sequencing Cartridge ID	Serial number of the Sequencing Reagent Cartridge
Washing Cartridge ID	Serial number of the Washing Cartridge
Flow Cell ID	Serial number of the flow cell
Flow Cell Pos	Position of the flow cell (stage A, B, C, or D)
Barcode Type	The barcode file that you select during sequencing
Barcode File	The name of the barcode file used for barcode split
Read1	First-strand read length
Read2	Second-strand read length
Barcode	Read length of Barcode
DualBarcode	Read length of DualBarcode
Read1 Dark Reaction	The number of cycles for the first-strand to perform a dark reaction
Read2 Dark Reaction	The number of cycles for the second-strand to perform a dark reaction
Resume Cycles	Cycles in which sequencing resume started

Diagram description

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

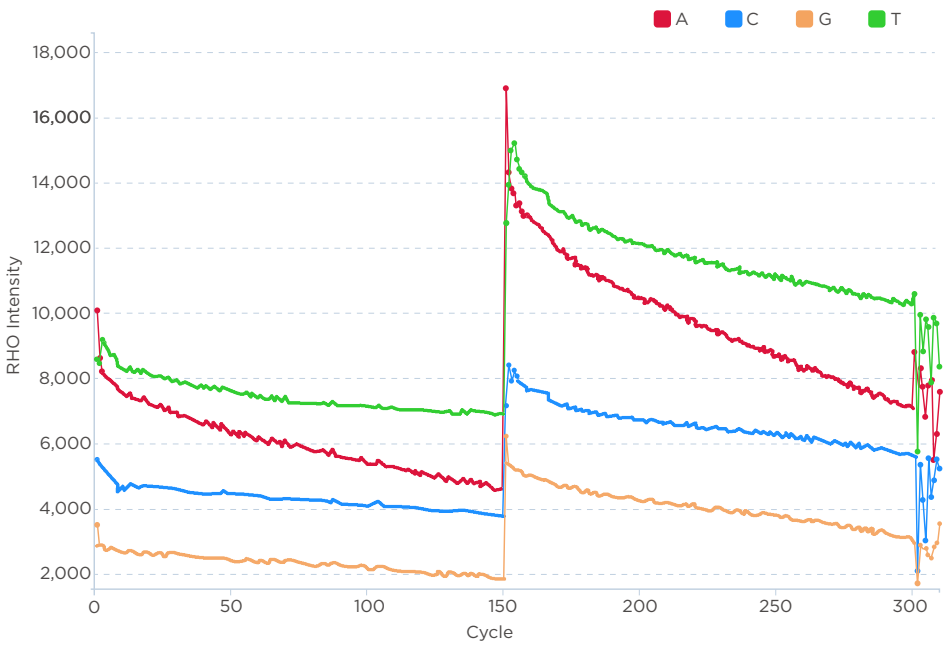

Figure 58 RHO Intensity

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

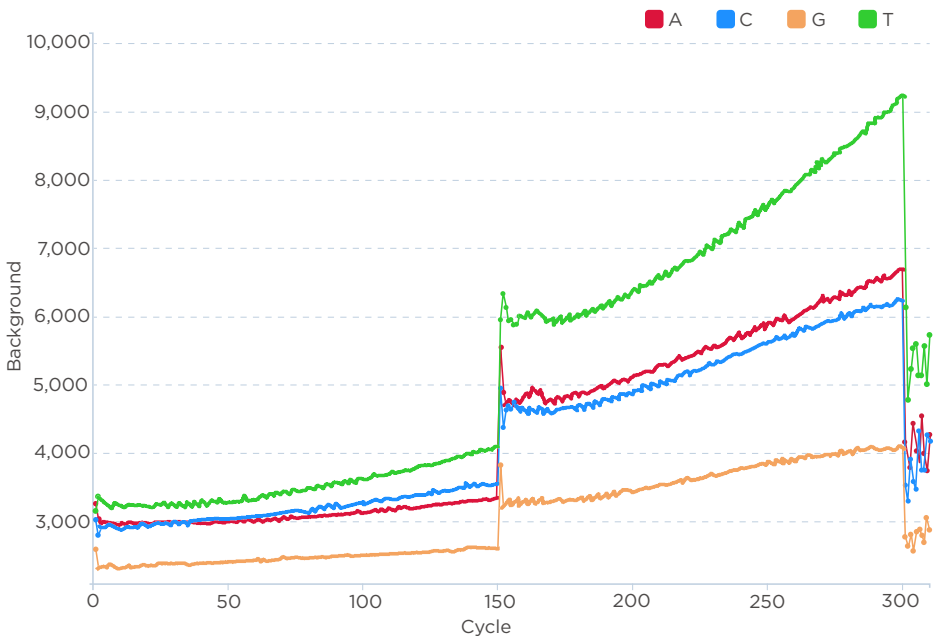

Figure 59 Background

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

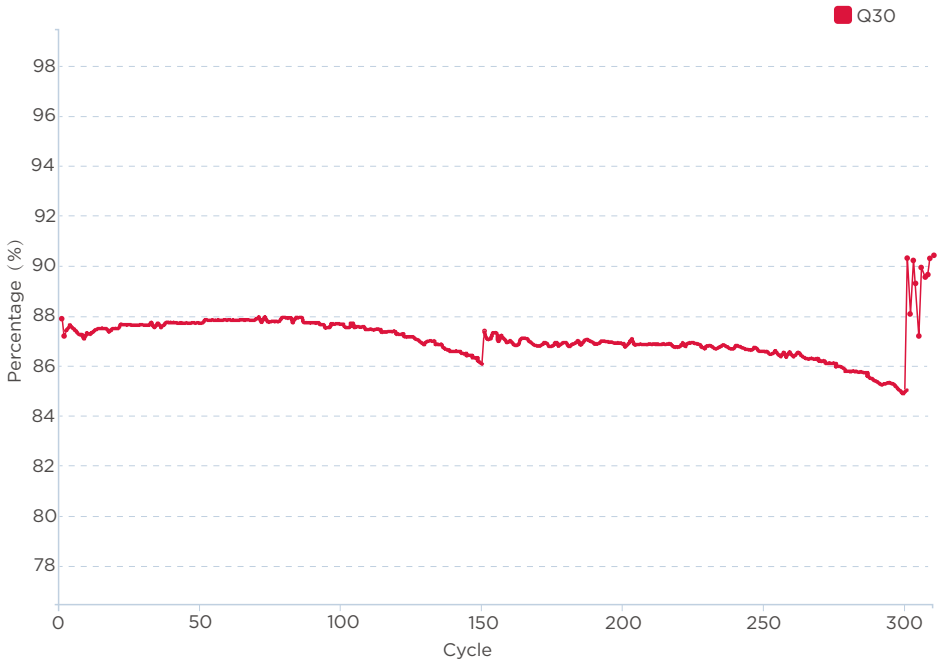

Figure 60 Unfiltered Q30

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

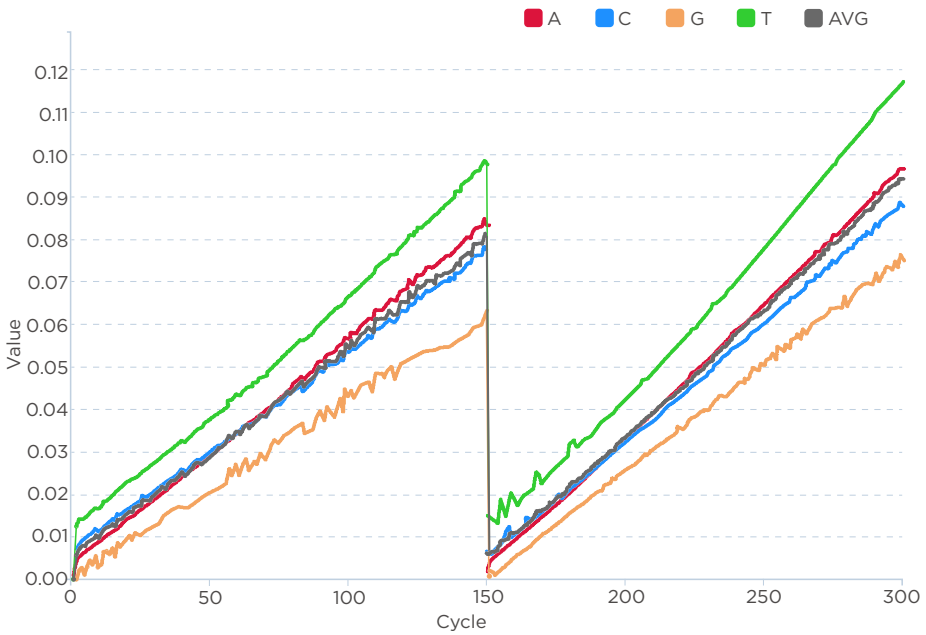

Figure 61 **Runon**

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*.

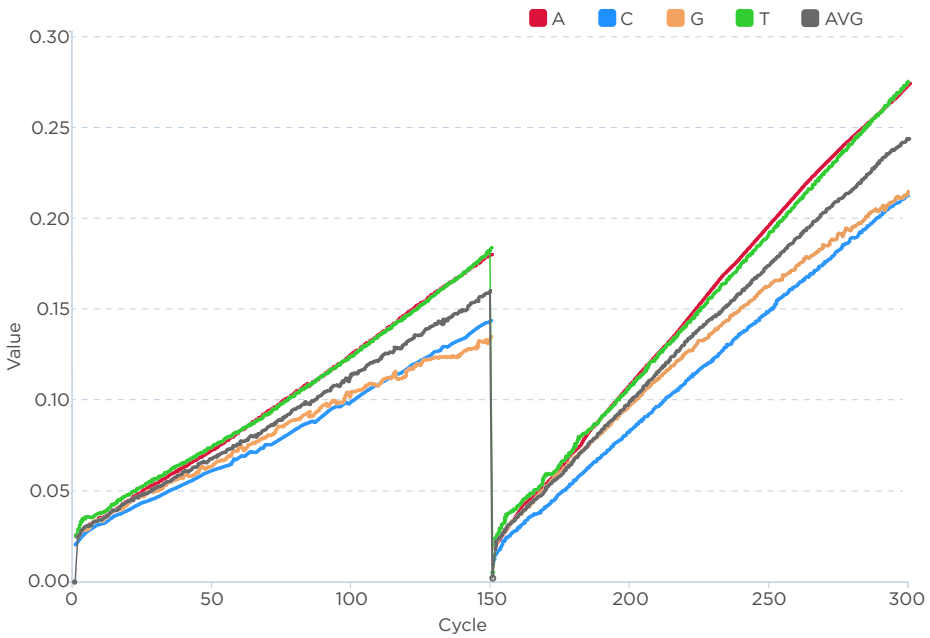

Figure 62 Lag

X axis	Cycle
Y axis	Value: Lag value for each cycle. 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*.

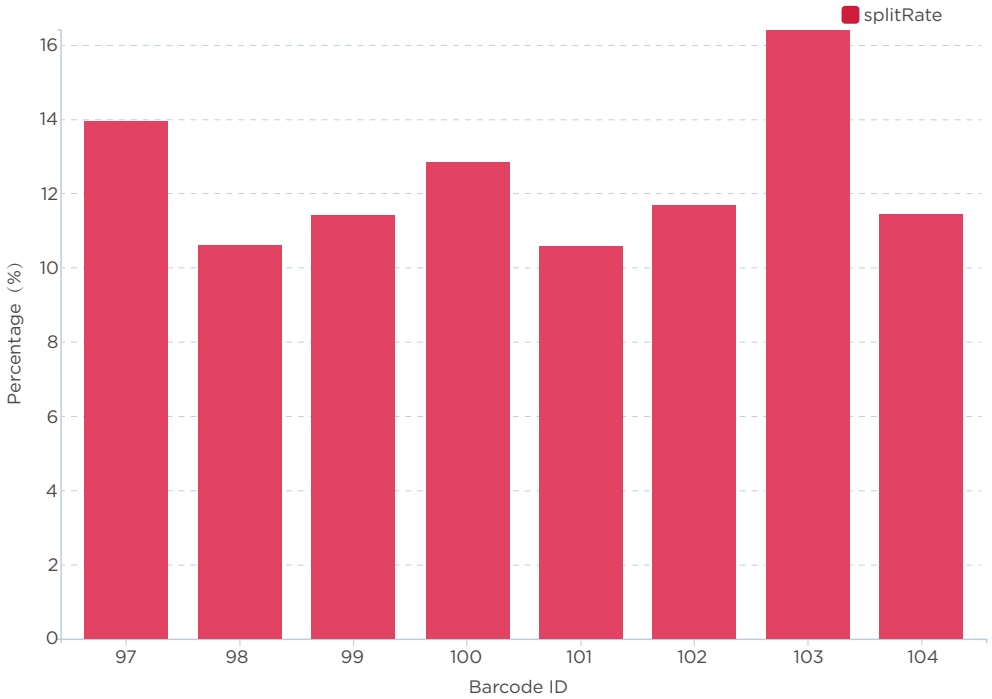

Figure 63 Barcode Split Rate

X axis	Barcode ID
Y axis	Percentage (%): a histogram that shows the percentage of the barcode when the splitting rate is over 1%.

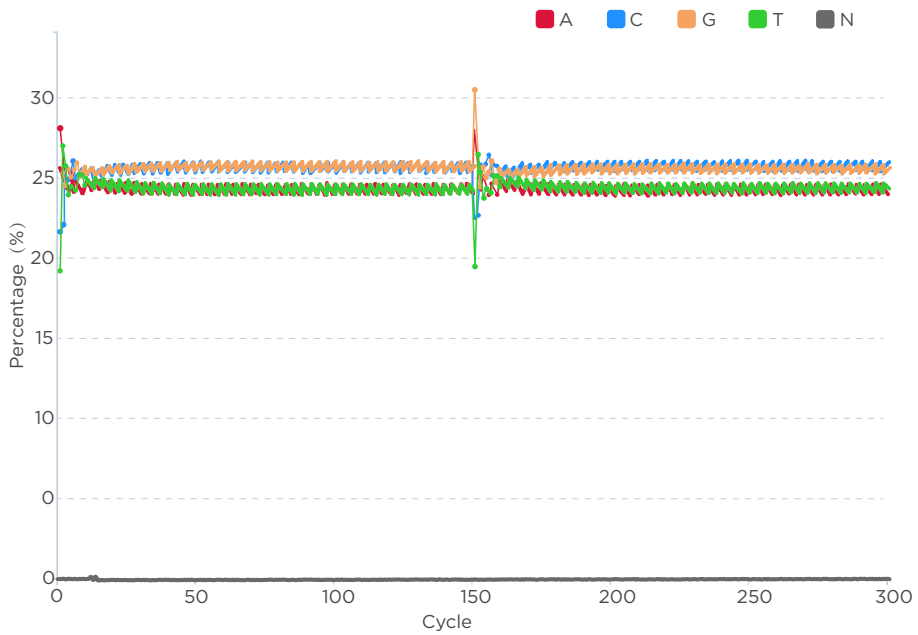

Figure 64 Bases Distribution

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

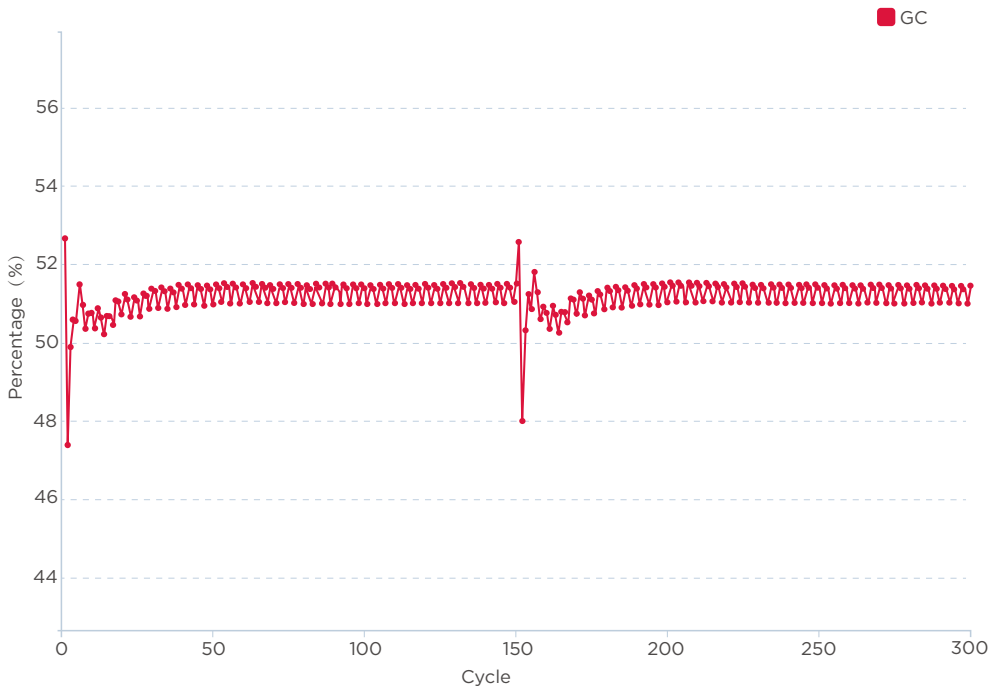

Figure 65 GC Distribution

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

Figure 66 Estimated Error Rate

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

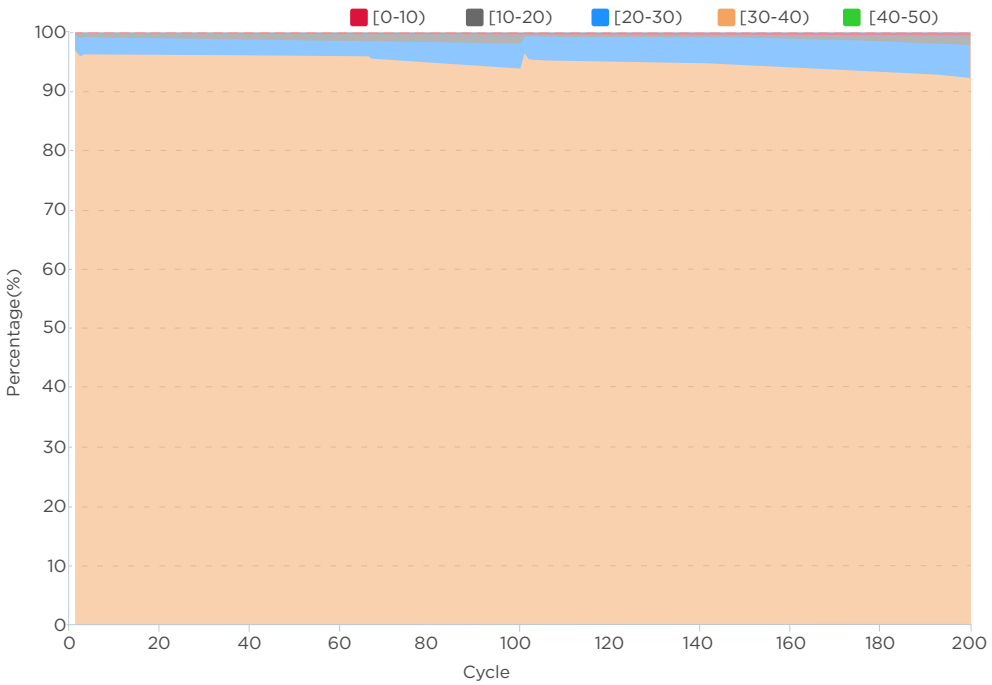

Figure 67 Quality Proportion Distribution

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

Other reports

Table 35 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. *XXXXXXXX* represents flow cell ID.
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 (Signal to Noise Ratio), Q30, BIC (Basecall Information Content), Fit, A-T, G-C, Lag, and Runon.

---This page is intentionally left blank.---

07

Data Processing

This chapter describes data processing.

CAUTION

- To protect your data, please change the password when you log into the device for the first time, and change the password regularly.
- To protect your data, it is recommended that you enable synchronous data uploading from the device to the server after connecting the device to the server.
- The logs system does not record data deletion or revision through Windows. Please ensure that you have backed up the data before deletion or revision.

Figure 68 Data processing workflow

If CG-ZTRON-LITE server is deployed and connected to the sequencer, ZLIMS will monitor the status of the sequencer.

For deployment of CG-ZTRON-LITE, contact CG Technical Support.

After a sequencing run is complete. the sequencing data will be uploaded to the CG-ZTRON-LITE server automatically, and ZLIMS can automatically trigger bioinformatics analysis.

For the operation of CG-ZTRON-LITE, refer to the relevant user manual.

08

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 into the device during cleaning to avoid device damage.

WARNING

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

Service plan

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

Wash

Wash introduction

Table 36 Wash type introduction

Equipment	Wash type	Cartridge type	Process time (minutes)	Description
DNB Loader	Post-wash	Post Load Plate	15	When DNB loading is complete, the loader can perform the wash after you replace the washing flow cell and click **Post-wash** with no need to change the Post Load Plate.
	Manual wash	Washing plate	20	• The device is used for the first time. • The device has not been used for 7 days or longer. • Impurities are found in the device or flow cell. • Tubing, sampling needles or other accessories exposed to the reagents were replaced.
Sequencer	Auto wash	Sequencing Reagent Cartridge and Washing Cartridge	40	If **Auto wash** is enabled, the system will automatically perform a wash after each sequencing run.
	Manual wash	Sequencer Cleaning Cartridge and Cleaning Cartridge	40	• The device is used for the first time. • The device has not been used for 7 days or longer. • **Auto wash** is disabled when setting sequencing parameters. • Any maintenance by technical support, or when impurities are found.

Wash preparation

Preparing washing reagents

Prepare the washing reagents according to the table below.

You can use laboratory-grade water such as 18 Megohm (MΩ) water, Milli-Q water, Super-Q water, or similar molecular biology-grade water.

Table 37 Washing reagent 2: 0.05% Tween-20+1 M NaCl

Reagent name	Volume (mL)	Final concentration
100% Tween-20	0.5	0.05%
5 M NaCl solution	200	1 M
Laboratory-grade water	799.5	/
Total volume	1000	
Shelf life	1 month at 4 °C	

Table 38 Washing reagent 3: 0.1 M NaOH

Reagent name	Volume (mL)	Final concentration
2 M NaOH	50	0.1 M
Laboratory-grade water	950	/
Total volume	1000	
Shelf life	1 month at 4 °C	

Preparing the loader washing plate

Top view of the loader washing plate is shown as follows:

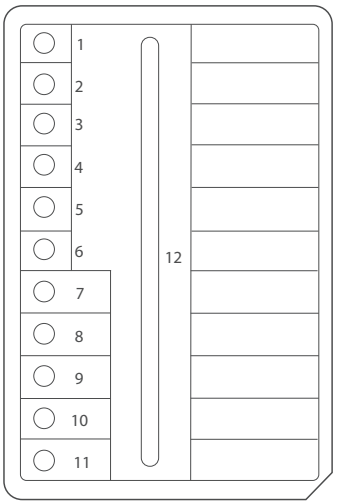

Figure 69 Post Load Plate (no Reagent)

- Before being refilled with fresh washing reagents, used washing plates must be cleaned 3 to 5 times with laboratory-grade water.
- After being cleaned 3 to 5 times with laboratory-grade water, used DNB loading plates may be used as washing plates.

Prepare the loader washing plate by using Post Load Plate according to the table below:

Well position	Washing reagent	Volume (mL)
9	Laboratory-grade water	4
12		20
10	Washing reagent 2: 0.05% Tween-20+1 M NaCl	4
11	Washing reagent 3: 0.1 M NaOH	4

Preparing washing cartridges

Top views of the washing cartridges are shown in the figures below:

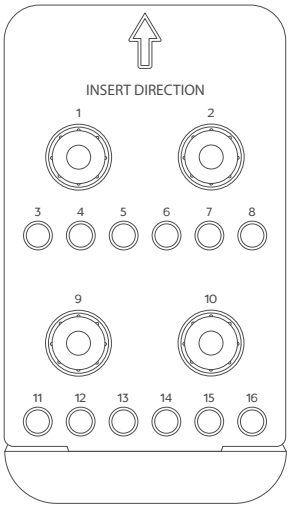

Figure 70 Sequencer Cleaning Cartridge

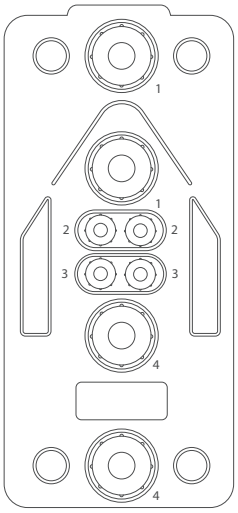

Figure 71 Cleaning Cartridge

Prepare washing cartridges for the sequencer according to the table below:

Cartridge type	Well position	Washing reagent	Volume (mL)
Sequencer Cleaning Cartridge	All	NA	NA
Cleaning Cartridge	2	Washing reagent 3: 0.1 M NaOH	45
	3	Washing reagent 2: 0.05% Tween-20+1 M NaCl	45

We recommend that you dispose of the following consumables every month or after they have been used for 10 times:

- Post Load Plate (no Reagent)
- Sequencer Cleaning Cartridge
- Cleaning catridge

Preparing the washing flow cell

Flow cells from previous runs can be used as washing flow cells. Replace the washing flow cell every month or after it has been used 10 times.

Wash procedures

Automatic wash and manual wash need to be performed on each flow cell stage independently.

Performing a manual wash on the loader (~20 minutes)

Perform the following steps:

1. Start the loader, enter the password, and then select **Log in** to enter the main interface.
2. Select the flow cell stage that needs to be washed. Open the loading compartment door.
3. Place the prepared washing plate into the flow cell stage that needs to be washed. Close the compartment door.
4. Press the flow cell attachment button and wait until the negative pressure is released. Remove the flow cell from the stage.

   Skip this step if no flow cell is on the stage.

5. Place the washing flow cell on the flow cell stage. Press the flow cell attachment button and gently press down on the flow cell to ensure that the flow cell is securely attached to the stage.
6. Return to the main interface. Select **Start** > **Yes** to begin the wash, which takes approximately 20 minutes.
7. When the wash is complete, take out all the consumables by following the on-screen instructions.
8. Select **Back** to return to the main interface.

Performing a manual wash on the sequencer (~40 minutes)

You can perform a wash to remove the remaining reagents from the fluidics lines and flow cell stages to avoid cross-contamination.

When **Auto wash** is enabled, the system automatically performs a wash after each sequencing run. If **Auto wash** is set to **No**, or if the device has not been used for seven days or longer, perform a wash manually.

Perform the following steps:

1. Ensure that the pure water container is filled with at least 4.5 L (approximately at the 1/3 volume of the pure water container) of laboratory-grade water before performing the wash.

   We recommend that you dispose of Post Load Plate (no Reagent), Sequencer Cleaning Cartridge, and Cleaning Cartridge every month or after they have been used 10 times.

2. Start the sequencer. Enter the user name and password, select **Log in** to enter the main interface
3. Select **Wash**. Touch the flow cell drive button to install a washing flow cell. Touch the flow cell drive button again to load the washing flow cell into the device.
4. Place the prepared Sequencer Cleaning Cartridge into the sequencing cartridge compartment on the flow cell stage that needs to be washed. Close the sequencing cartridge compartment door.
5. Place the prepared Cleaning Cartridge into the washing cartridge compartment on the flow cell stage that needs to be washed. Then close the washing cartridge compartment and reagent compartment doors.
6. Select **Start** and select **Yes** when prompted to begin the manual wash, which takes approximately 40 minutes.
7. When the wash is complete, select **Finish** to return to the main interface.
8. Remove the washing flow cell, Sequencer Cleaning Cartridge, and Cleaning Cartridge.

Reusing the washing flow cell, washing cartridge, and washing plate

Washing flow cell

- Store the washing flow cell at room temperature.
- Replace the washing flow cell every month or after it has been used 10 times.
- Used sequencing flow cells can be used as washing flow cells.

Washing cartridge

- Store the washing cartridge at room temperature.
- Replace the washing cartridge every month or after it has been used 10 times.
- Used sequencing cartridges can be used as washing cartridges.

Washing plate

- Store the washing plate at room temperature.
- Replace the washing plate every month or after it has been used 10 times.
- Before refilled with fresh washing reagents, used washing plates must be cleaned 3 to 5 times with laboratory-grade water.
- After cleaned 3 to 5 times with laboratory-grade water, used DNB loading plates can be used as washing plates.

Sequencer maintenance

Daily maintenance

- Check whether the fan at the rear of the device is operational. If not, contact CG Technical Support.
- During sequencing, pay attention to error messages and check whether the relevant parts are functioning properly. Contact CG Technical Support if needed. For information about how to find error messages, refer to *Menu area on Page 22*, and *Log interface on Page 24*.
- Clean the interior of the reagent compartment before sequencing:

  1) Open the reagent compartment, open the sequencing cartridge compartment door and pull out the washing cartridge drawer.

     For information on powering the device off, refer to *(Optional) Powering the devices off on Page 95.*

  2) Clean the interior of the reagent compartment with a 75% ethanol wipe. Ensure that the surface is free of DNBs, reagents, blood, and saliva.

Weekly cleaning

Perform the following steps:

1. Power the device off and open the reagent compartment.

   For information on powering the device off, refer to *(Optional) Powering the devices off on Page 95.*

2. Clean the touch screen and exterior of the reagent compartment door, sequencing cartridge compartment door, and washing cartridge drawer with a 75% ethanol wipe. Ensure that the surface is free of DNBs, reagents, blood, and saliva.

Monthly maintenance

Maintaining the device

Perform the following steps:

1. Power the device off and open the reagent compartment.

   For information on powering the device off, refer to *(Optional) Powering the devices off on Page 95.*

2. Clean the surface of the device with a 75% ethanol wipe. Ensure that the surface is free of DNBs, reagents, blood, and saliva.

Maintaining the power supply

- When the device is not in use for seven days or longer, perform a wash manually according to *Performing a manual wash on the sequencer (~40 minutes) on Page 123*. Power the device off after the wash.

  For information on powering the device off, refer to *(Optional) Powering the devices off on Page 95*.
- Check whether the power cord and cables are in good condition regularly. Contact CG Technical Support if new cables are required.

Maintaining the software

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

Maintaining the pure water container

Perform the following maintenance every week:

1. Empty the pure water container.
2. Spray 75% ethanol onto the inner surface of the lid and the surface of the pure water tube, and then wipe them with a clean Kimwipes tissue.
3. Add 2 L laboratory-grade water into the pure water container, and reattach the lid.
4. Gently swirl the container until all inner walls are cleaned.
5. Empty the container.
6. Repeat steps 3 through 5 twice.

Replacing the waste container

The waste container is connected to the device through tubes. To avoid liquid leakage and biological hazard exposure, monitor the waste container status frequently. Dispose of the waste and waste container when the waste approaches the maximum volume.

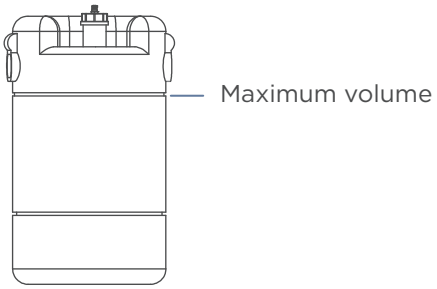

Figure 72 Maximum waste volume

Perform the following steps:

1. Wear protective equipment.
2. Remove the lid with tubes from the waste container, install a new lid with sealing gasket, and secure the lid until you hear a click.
3. Dispose of the waste and waste container.

DNB loader maintenance

Daily maintenance

Perform the following maintenance when the device is powered on:

- Check whether the fan of the device is operational. If not, contact CG Technical Support.
- During loading, pay attention to error messages and check whether the relevant parts are functioning properly. Contact CG Technical Support if needed. For information about how to find error messages, refer to *Log interface on Page 36*.

Weekly cleaning

Perform the following steps:

1. Power the device off and open the loading compartment door and flow cell compartment door.

2. Clean the touch screen, plate tray, and flow cell stage with a 75% ethanol wipe. Ensure that the surface is free of DNBs, reagents, blood, and saliva.

Monthly cleaning

Maintaining the device

Perform the following steps:

1. Power the device off.
2. Clean the surface of the device with a 75% ethanol wipe. Ensure that the surface is free of DNBs, reagents, blood, and saliva.

Maintaining the power supply

- When the device is not in use for seven days or longer, perform a maintenance wash, power the device off, and disconnect the power cord.
- Check whether the power cord and cables are connected correctly and in good condition before each use. Re-connect the cables if needed (ensure that the device is powered off), or contact CG Technical Support if new cables are required.

Annual maintenance

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

Maintaining the flow cell stage

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

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

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

Perform the following steps:

1. Wear protective gloves.

2. Check for dust, debris, damage, or particulate matter on the back of the flow cell and the surface of the aluminum chuck of the flow cell stage.
3. If necessary, wipe the back of the flow cell or the surface of the aluminum chuck with a low-lint cloth moistened with 75% ethanol, and then let it air-dry.

   Do not wipe the inlet holes and vacuum attachment slot to prevent absolute ethanol from entering the holes and damaging the device.

4. Use a canned air duster to carefully blow particulate matter and dust from the surface of the silicon chip and aluminum chuck until they are clean.
5. Place the flow cell on the flow cell stage. Ensure that the flow cell and label are facing upward. Press the edges of the flow cell with your hands to ensure that it is securely seated.
6. Press the flow cell attachment button on the flow cell stage.

Maintaining the software

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.

---This page is intentionally left blank.---

09

FAQs

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

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

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

Sequencer FAQs

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

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

Perform the following steps:

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

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

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

Perform the following steps:

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

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

If the sequencer has been turned off after a long period of time, the sequencing cartridge compartment will be at room temperature. The sensor may detect that the sequencing cartridge compartment is exceeding the preset temperature. Issues may also occur when there is an error with the temperature control board.

Perform the following steps:

1. Let the sequencer run and let the sequencing cartridge compartment cool. The error message should disappear when the sequencing cartridge compartment is at operating temperature.
2. Restart the sequencer.

Q: What should I do if temperature error messages and warnings related to the LT (Laser Temperature) board appear in the sequencing interface?

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

Q: What should I do if the waste level sensor alarm is activated?

The waste level sensor alarm may activate if the waste level exceeds the preset limit, the level sensor is not installed properly, and/or the level sensor is damaged. It is recommended to record the warning and the related logs of the sequencing run and contact CG Technical Support.

DNB loader FAQs

Q: What should I do if a message, indicating that the compartment door is opened, is displayed in the interface?

This message is displayed when the compartment door is open. The resolve this issue, ensure that the compartment door is closed.

Q: What should I do if the sampling needle bumps into the post-loading plate and bends during operation?

The sample needle may make contact with the post-loading plate if the position settings are incorrect. If this occurs, contact CG Technical Support for assistance.

Q: What should I do if bubbles are present in the fluidics lines of the flow cell?

Bubbles may be present in the fluidics lines of the flow cell when aspirating reagents.

To resolve the issue, perform the following steps:

1. After loading, press the flow cell attachment button to release the flow cell.
2. Check whether the sealing rings installed evenly and properly. If not, re-install the sealing ring.

Q: Why is the flow cell not attaching to the flow cell stage?

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

To resolve the issue, perform the following steps:

1. Check whether the flow cell attachment button is pressed.
2. Check the flow cell stage for dust, debris, or damage. Clean the flow cell stage. For further details, refer to *Maintaining the flow cell stage on Page 128*.

Q: Why is liquid not passing through the fluidics lines of the flow cell?

When foreign particles are present on the sealing ring, or the sealing ring is damaged, liquid may not be able to pass through the fluidics lines. Foreign particles on the rear of the flow cell and blockages in fluidics line may also be present if liquid is unable to pass through the lines.

To resolve the issue, perform the following steps:

1. Check whether the sealing ring on the flow cell stage is intact or if any foreign particles are blocking the holes of the sealing ring.
2. Check whether there are any foreign particles on the rear of the flow cell or the surface of the flow cell stage. If particles are present, clean the flow cell stage. For details, refer to *Maintaining the flow cell stage on Page 128*.

Q: What should I do if the flow cell stage is leaking?

The flow cell stage may leak if:

- Sealing rings are not installed.
- Sealing rings are not correctly installed.
- There are foreign particles on the back side of the flow cell.
- The fluidics lines are blocked.

To resolve the issue, perform the following steps:

1. Check whether the sealing rings are installed.
2. Check whether the sealing rings on the flow cell stage are intact or if any foreign particles are blocking the holes of the sealing ring.
3. Check whether there are any foreign particles on the back side of the flow cell or the surface of the flow cell stage. If particles are present, clean the flow cell stage. For detail, refer to *Maintaining the flow cell stage on Page 128*.

Reagent FAQs

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

When DNB concentration is lower than that specified in *Table 27 on Page 69*, perform the following steps:

1. Check whether the DNB preparation kit has expired.
2. Check whether the library meets the requirements.
3. Make DNBs again. If the DNB concentration still does not meet the requirements after a new sample preparation, please contact CG Technical Support.

Q: What should I do if I forgot to add reagent into well No. 8 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 must be added to well No. 8. If MDA mixture was not added into well No. 8 before starting the sequencing run, this can be resolved by performing the following steps, as long as the sequencing run is in the sequencing phase of Read1:

1. Pause the run: Select the pause button ⏸ in the sequencing interface and select **Yes** when prompted.

2. Lift the needle:
   1) Select the stop button ■ and select **Yes** when prompted.
   2) Select **Finish**.
3. Fill well No. 8 of the Sequencing Reagent Cartridge:
   1) Open the reagent compartment door and take out the Sequencing Reagent Cartridge.
   2) Prepare the MDA mixture by adding the appropriate amount of MDA Enzyme Mix into the MDA Reagent tube.
   3) Mix thoroughly and transfer all solution into well No. 8. as described in *Preparing the Sequencing Reagent Cartridge - part 2 on Page 83*.
   4) Insert the filled sequencing cartridge back into the sequencer.
4. Resume the run:
   1) Select **Sequence** > **Resume run** on the main interface.
   2) Clean the loaded flow cell with a canned air duster to ensure that no visible dust exists on the surface and back of the flow cell. Place the flow cell on the flow cell drive, and touch the flow cell drive button to load the flow cell into the device.
   3) Select **Next** to review the parameters and ensure that all parameters are correct.
   4) Select **Start** > **Continue**.

Q: How do I resume a sequencing run?

The sequencing run might be stopped due to some unexpected errors during the run, such as mechanical gripper operation failure, flow cell transfer failure, fluidics failure, and photographing failure. This stopped run may be continued after resolving the issues causing the run to stop.

Perform the following steps:

1. When the sequencing run is prematurely halted due to unexpected errors, the sequencer's interface display may resemble that of the figure below. Select **Finish** to end the stopped run.

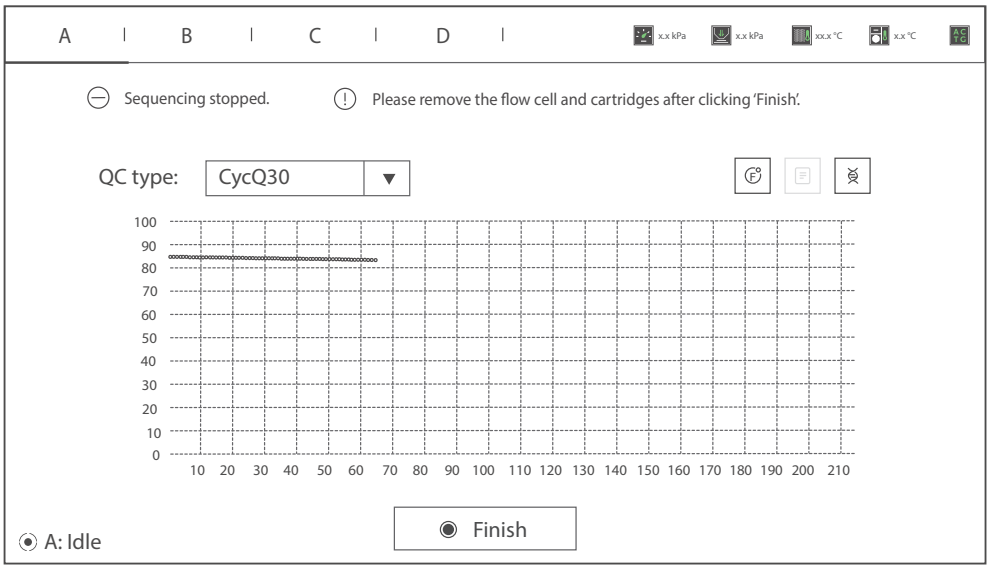

Figure 73 Interface of a stopped run

2. After resolving the issues that caused the run to stop, select **Sequence** > **Resume run** in the main interface.

   If the Sequencing Reagent Cartridge or Washing Cartridge is taken out for processing, ensure that the processed Sequencing Reagent Cartridge or Washing Cartridge is placed back in the corresponding compartment before resuming the sequencing run.

3. Re-load the flow cell:

   1) Dust the loaded flow cell of the interrupted sequencing run with a canned air duster. Ensure that no visible dust is present on the surface and back of the flow cell.

   2) Place the flow cell on the flow cell drive, and touch the flow cell drive button to load the flow cell into the device.

4. Select **Next** to review the parameters and ensure that all information is correct.

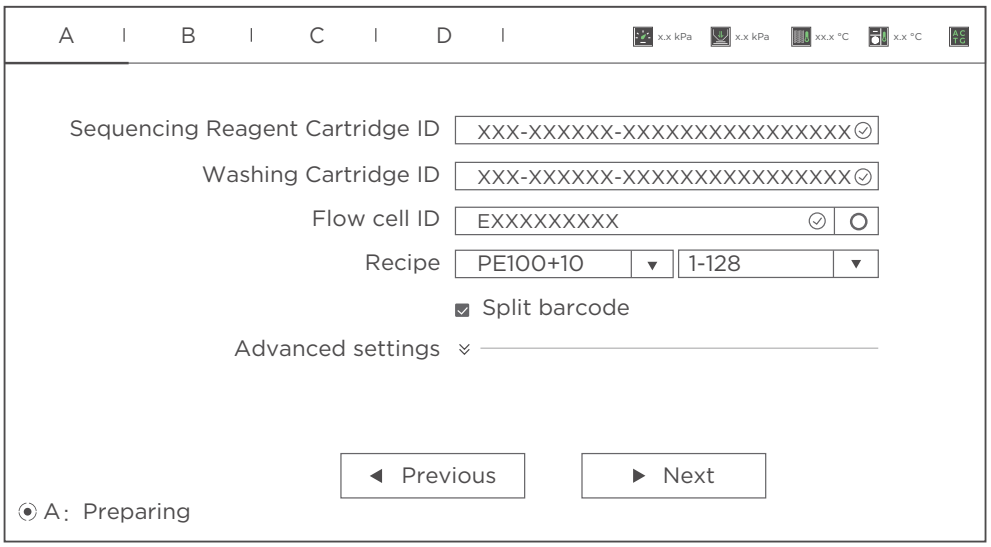

Figure 74 Cartridge ID, flow cell ID interface

5. Select **Start** > **Continue** to resume the sequencing run.

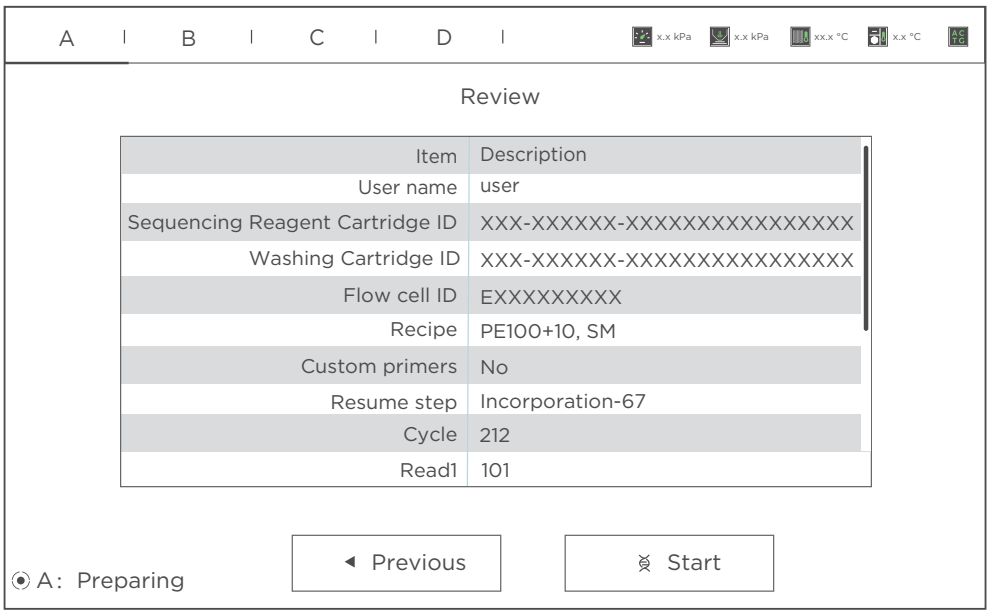

Figure 75 Sequencing parameter interface

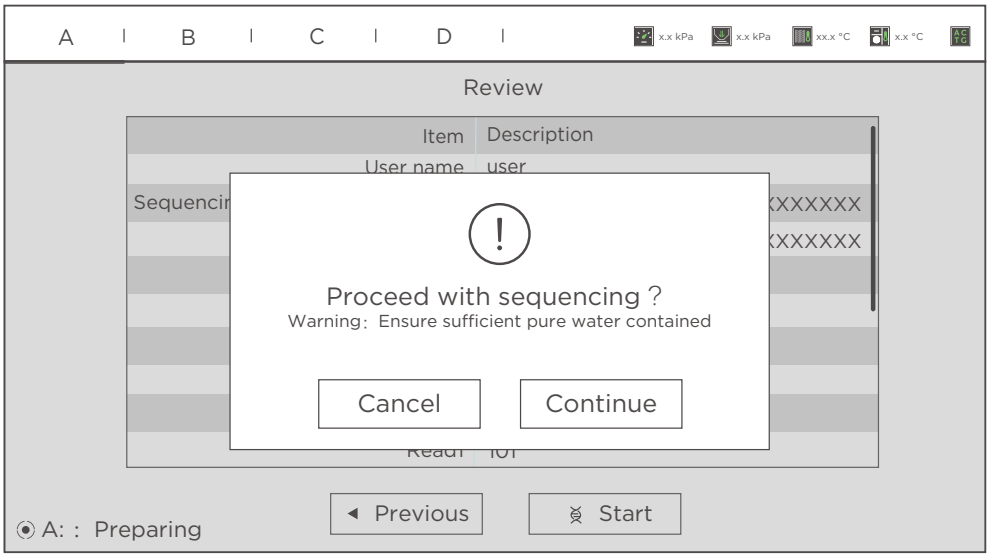

Figure 76 Continue run interface

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

- If a kit has been thawed (including the dNTPs) but cannot be used within 24 hours, it can be frozen and thawed at most one time.

- If a kit has been thawed (including the dNTPs) but cannot be used immediately, store it at 2 °C to 8 °C. It is strongly recommended that you use it within 24 hours. A thawed kit that is stored at 2 °C to 8 °C may still be used within seven days, although it may compromise sequencing quality. It is not recommended that you use a kit that has been thawed and stored at 2 °C to 8 °C for more than seven days.
- If the dNTPs and Sequencing Enzyme Mix have been added into the cartridge, i.e. the cartridge has been prepared and the needles have punctured the seal but the cartridge cannot be used immediately, the cartridge must be covered with foil or plastic wrap. Store the kit at 2 °C to 8 °C and use it within 24 hours. Gently mix the reagents in the cartridge before use. When mixing, be careful not to spill any reagent from the needle holes to avoid reagent contamination.

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

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

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

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

DL-T7RS

- Check the rubber sealing ring to ensure that it is in the right position.
- Check the DNB loading plate to ensure that enough reagent is in each well.
- Replace the used flow cell and inspect the pump.
- If the problem persists, please contact CG Technical Support.

DNBSEQ-T7RS

- Check the water container to ensure that the water volume is sufficient.
- Ensure that the pure water tube goes through the handle.

  For information on placing the water tube, refer to *Preparing the pure water container on Page 51.*

- Check the reagent needles to ensure that they can immerse fully into the cartridges. Otherwise, restart the sequencing software.
- If the problem persists after a restart, please contact CG Technical Support.

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

- Check if the pure water volume is sufficient.
- When error occurs on DL-T7RS and DNBSEQ-T7RS:
  - Remove the flow cell and check for is dust on the sealing gasket. Remove any dust with a canned air duster.
  - Place the flow cell by following the instructions and start the pump again.
- Check if the sampling needles are moving properly. If the sampling needles are not moving properly, restart the control software of the sequencer.
- If the problem persists, please contact CG Technical Support.

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

If impurities appear, perform the following steps:

1. Perform a manual wash on DL-T7RS and DNBSEQ-T7RS.
2. If there is still no improvement after manual wash, prepare washing reagents again according to *Preparing washing reagents on Page 120*, and perform a manual wash again on DL-T7RS and DNBSEQ-T7RS.
3. If the problem persists, please contact CG Technical Support.

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

DL-T7RS

- Remove the flow cell and check for is dust on the sealing gasket. Remove any dust with a canned air duster.
- Place the flow cell and start the loading again.
- If the problem persists, please contact CG Technical Support.

DNBSEQ-T7RS

- Check if the pure water volume is sufficient.
- Remove the flow cell and check for is dust on the sealing gasket. Remove any dust with a canned air duster.
- Check the reagent needles to ensure that they can immerse fully into the cartridges. Otherwise, restart the control software of sequencer.
- If the problem persists, please contact CG Technical Support.

Instructions for importing barcode

Preparing a barcode file

Ensure that the barcode file meets the following requirements:

-
- The barcode file can only be imported through the control software.
- The barcode file to be imported should be named “barcode.csv”. In the imported directory, only one “barcode.csv” file is available.
- The barcode file should not contain blank lines or full-width characters. The barcode sequence should include no fewer than two bases.
- Barcode ID and barcode sequences in the file should be separated by a comma.
- Barcode sequence should be unique, and barcode ID and barcode sequence should not be empty.
- Barcode sequences of a DualBarcode file should not contain any characters other than “A”, “T”, “C”, “G”, and “N”.
- Barcode sequences of a single barcode file should not contain any characters other than “A”, “T”, “C”, and “G”.
- Barcode name and mismatch number are mandatory for each barcode file.
- The barcodeName can only contain letters, numbers, hyphens (-), plus signs (+), and underscores (_). Its length should be between 1 to 50 characters.

Single barcode file

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

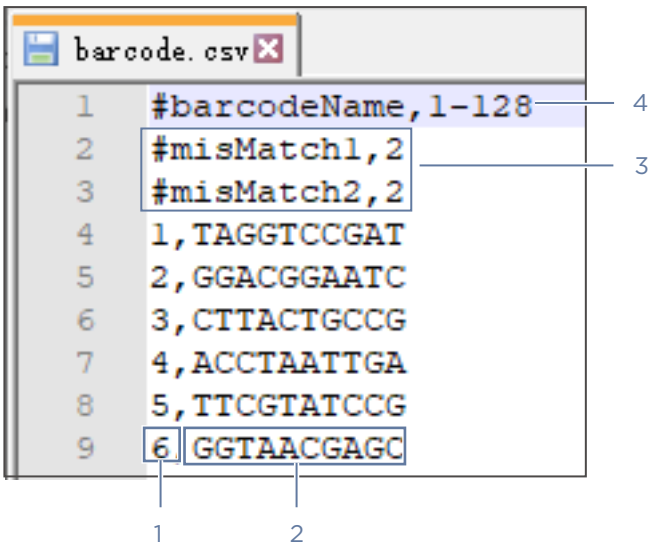

Figure 77 Exemplary single barcode file

Table 39 Description for single barcode file

No.	Name	Description
1	Barcode ID	Correspond to ID of **Barcode** in the Customize a recipe interface
2	Barcode sequence	Correspond to sequence of **Barcode** in the Customize a recipe interface
3	mismatch number	/
4	Barcode name	Barcode list name

Single and DualBarcode file

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

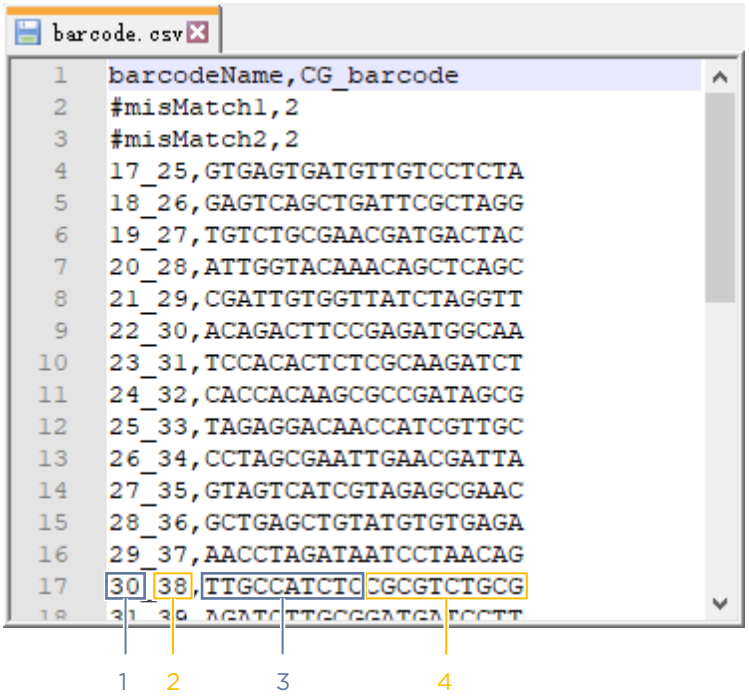

Figure 78 **Single and DualBarcode file1**

Table 40 **Description for mixed barcode file**

No.	Description
1	Corresponds to ID of **DualBarcode** in the **Customize a recipe** interface
2	Corresponds to ID of **Barcode** in the **Customize a recipe** interface
3	Corresponds to sequence of **DualBarcode** in the **Customize a recipe** interface
4	Corresponds to sequence of **Barcode** in the **Customize a recipe** interface

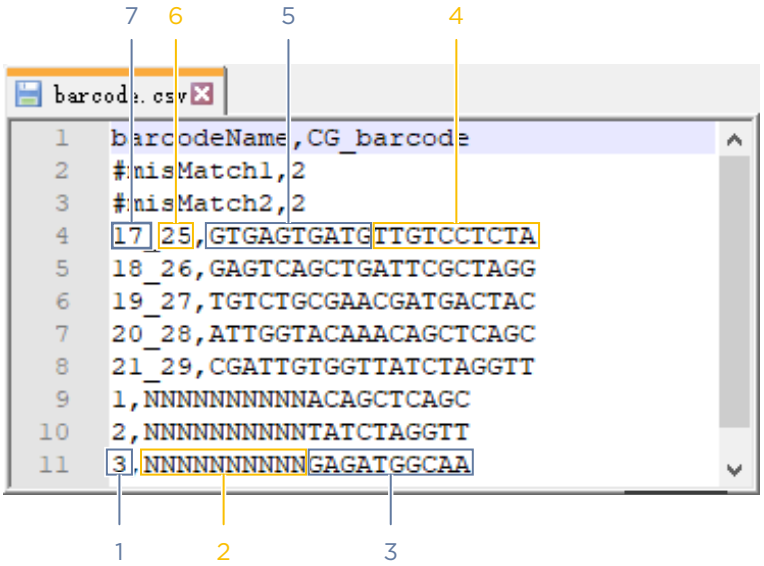

Figure 79 **Single and DualBarcode file 2**

No.	Description
1	Corresponds to ID of **Barcode** in the **Customize a recipe** interface
2	Placeholder
3	Corresponds to sequence of **Barcode** in the **Customize a recipe** interface
4	Corresponds to sequence of **Barcode** in the **Customize a recipe** interface
5	Corresponds to sequence of **DualBarcode** in the **Customize a recipe** interface
6	Corresponds to ID of **Barcode** in the **Customize a recipe** interface
7	Corresponds to ID of **DualBarcode** in the **Customize a recipe** interface

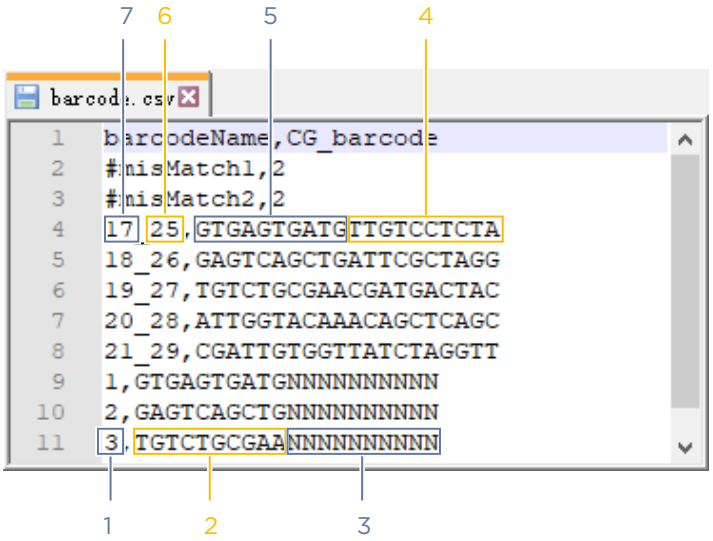

Figure 80 **Single and DualBarcode file 3**

No.	Description
1	Corresponds to ID of **Barcode** in the **Customize a recipe** interface
2	Corresponds to sequence of Barcode in the **Customize a recipe** interface
3	Placeholder
4	Corresponds to sequence of Barcode in the **Customize a recipe** interface
5	Corresponds to sequence of **DualBarcode** in the **Customize a recipe** interface
6	Corresponds to ID of **Barcode** in the **Customize a recipe** interface
7	Corresponds to ID of **DualBarcode** in the **Customize a recipe** interface

Importing a barcode file

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

Perform the following steps:

1. Obtain an external storage device (for example, a USB storage drive), and create a folder in the root directory of the storage device. Ensure that the folder name is in English. Copy the prepared "*barcode.csv*" file to the folder.
2. On the main interface, select **Sequence** > **New run**.

3. Select the second ▼ next to **Recipe**, and select **Import** to import barcodes to the device from the external storage device.

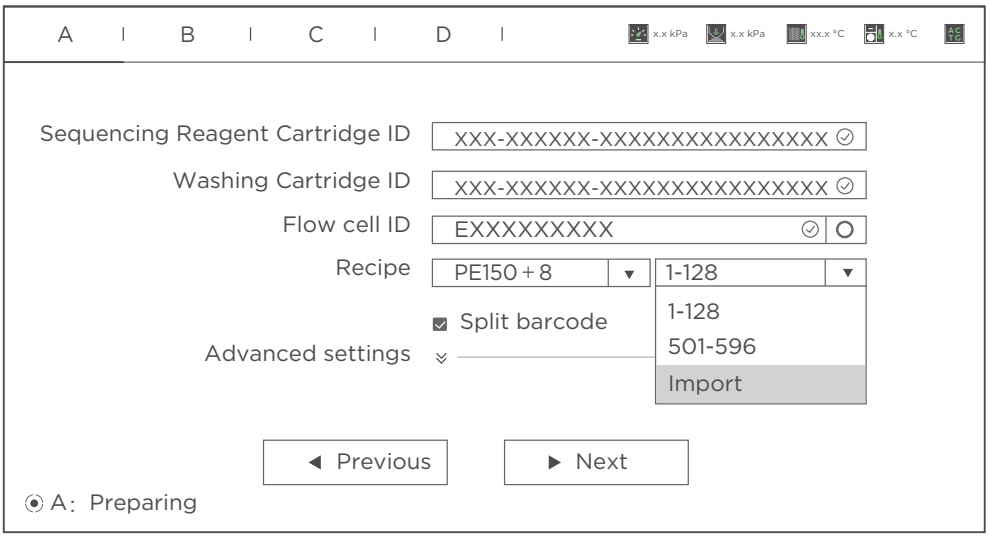

Figure 81 Barcode settings interface

4. Select **Split barcode** if needed.
5. Select **Next** and then **Previous** again to check whether the imported barcodes are displayed in the barcode list.

The same barcode only needs to be imported once.

Instructions for customizing a run

Introductions

This section provides instructions for customizing a sequencing run, which might be needed in the following situations:

- When read length(s) in Read2 and/or Read1 are not the same as those predefined in the Recipe list.
- For a single barcode sequencing run, the barcode sequences are customized.
- All DualBarcode sequencing runs:
  - There are four types of barcode splitting scenarios for DualBarcode sequencing: splitting only the barcode, splitting only the DualBarcode, splitting both the barcode and DualBarcode, and not splitting the barcode and DualBarcode.
  - In the case of DualBarcode sequencing but only one barcode splitting needed, the barcode file needs to be the same as that of splitting both the barcode and DualBarcode, do not delete the contents of the non-split barcode. That is, the base number of the barcode file needs to be consistent with that of the barcode and DualBarcode sequencing, otherwise it cannot be split.
- Dark reaction cycles are required in Read1 and/or Read2 sequencing.
- UMI (Unique Molecular Identifier) +UDI (Unique Dual Index)

Important interfaces for customizing a run

To enter the **Customize a recipe** interface, select the first ▼ next to **Recipe** and select **Customize a recipe** in the sequencing parameters interface.

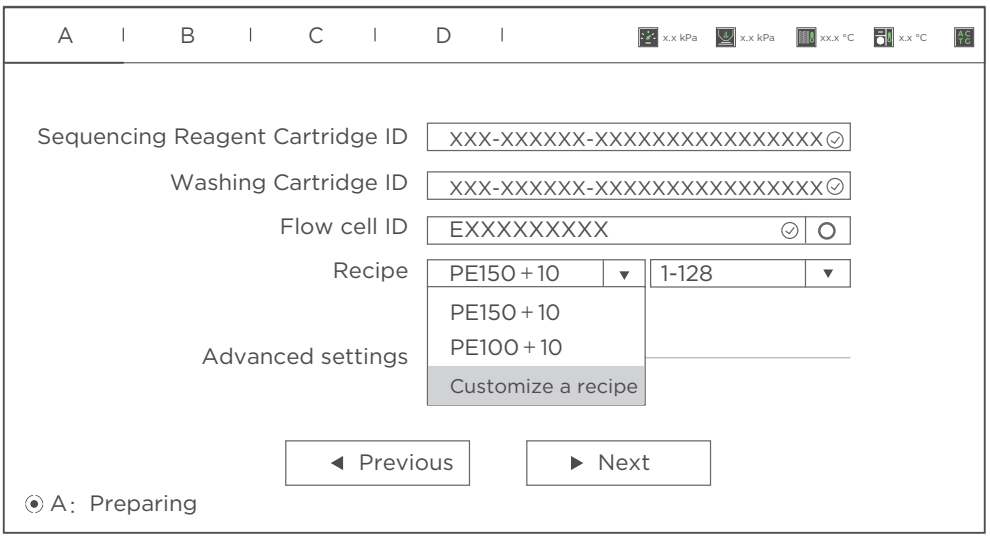

Figure 82 **Selecting Customize a recipe**

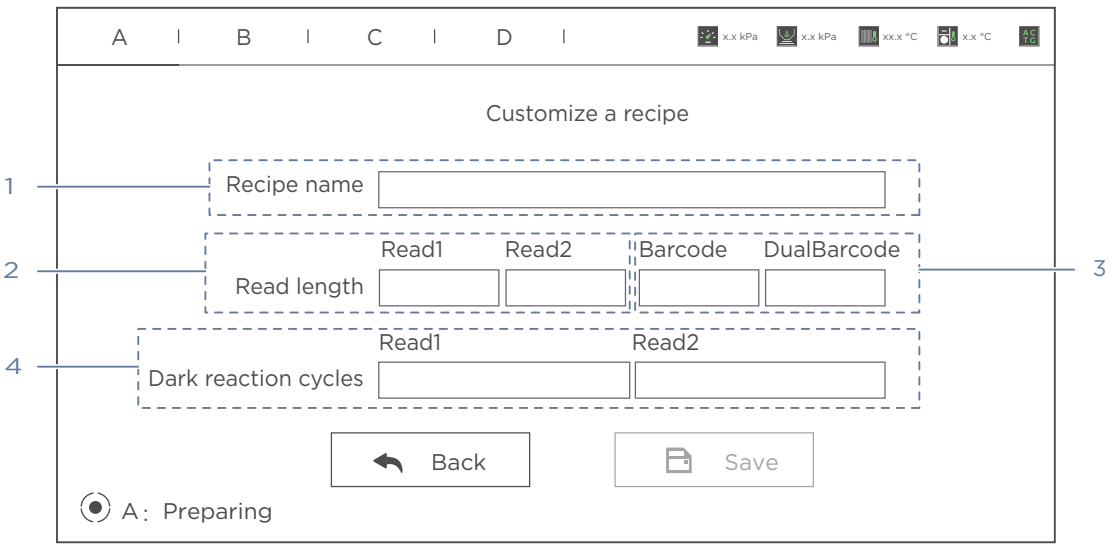

Figure 83 **Customize a recipe interface**

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

No.	Item	Description
1	**Recipe name**	Name the customized recipe.
2	**Read1/Read2**	Customize Read1 and/or Read2 length for a sequencing run.
3	**Barcode/DualBarcode**	Customize Barcode length for a sequencing run. Customize DualBarcode length for a sequencing run.
4	**Dark reaction cycles**	Customize dark reaction range in Read1 and/or Read2.

The rules for filling in the **Customize a recipe** interface are as follows:

- When naming a sequencing recipe, use only letters, numbers, "+", "_" and "–".
- Because a previously named recipe will be saved in the recipe drop-down menu, duplicate name checking will be performed to ensure that each sequencing recipe name is unique (i.e., a new recipe name must not be the same as an existing recipe name).
- Enter numbers in the read length boxes of Read1, Read2, Barcode and DualBarcode.
- Multiple ranges of dark reaction cycles can be set in the Read1 and Read2 entry for "Dark reaction cycles". Use "," to separate the ranges. The dark reaction cycles of the ranges are presented in the format of "number" and "number-number".

Examples of customized run

- Before starting the customized run, confirm that the customized barcode files are already imported into the sequencer. If not, refer to *Instructions for importing barcode on Page 143* 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 kit as defined in *Table 4 Sequencing cycle on Page 46*.
- The maximum read length for both Read1 and Read2 should not exceed that specified in the sequencing kit. For example: If PE150 is used, the maximum customized Read1 length and Read2 length should not exceed 150 bp.
- 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.

You can refer to the following setting examples for your customized run.

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

Assumptions are as below:

- Sequencing run: PE150+10.
- Length of Read1: 120.
- Length of Read2: 140.
- Length of barcode: 10.
- Length of DualBarcode: 0.
- Split barcode: Yes.
- Total cycles = 120+140+10+2 = 272.
- Select a PE150 kit.

The Customize interface is set as follows:

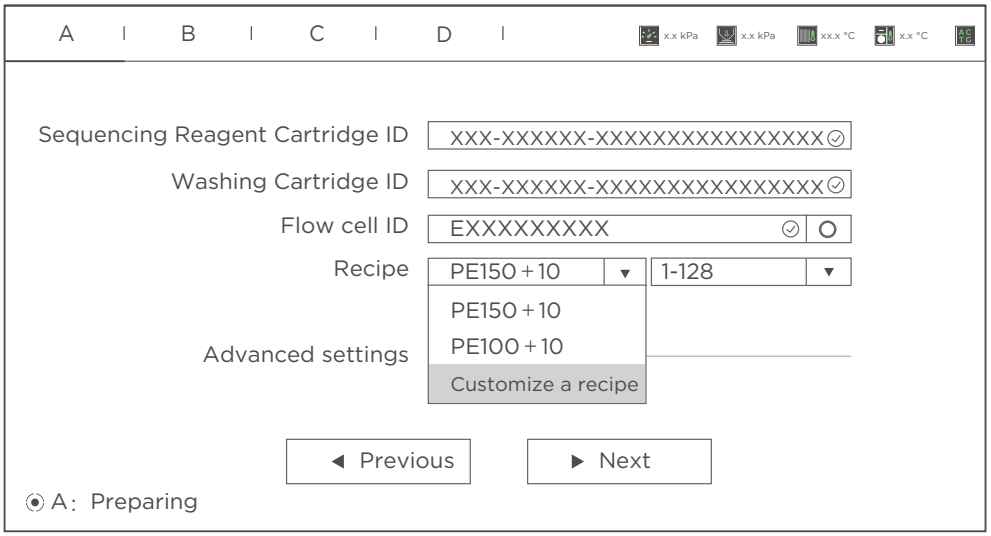

Figure 84 Selecting *Customize a recipe*

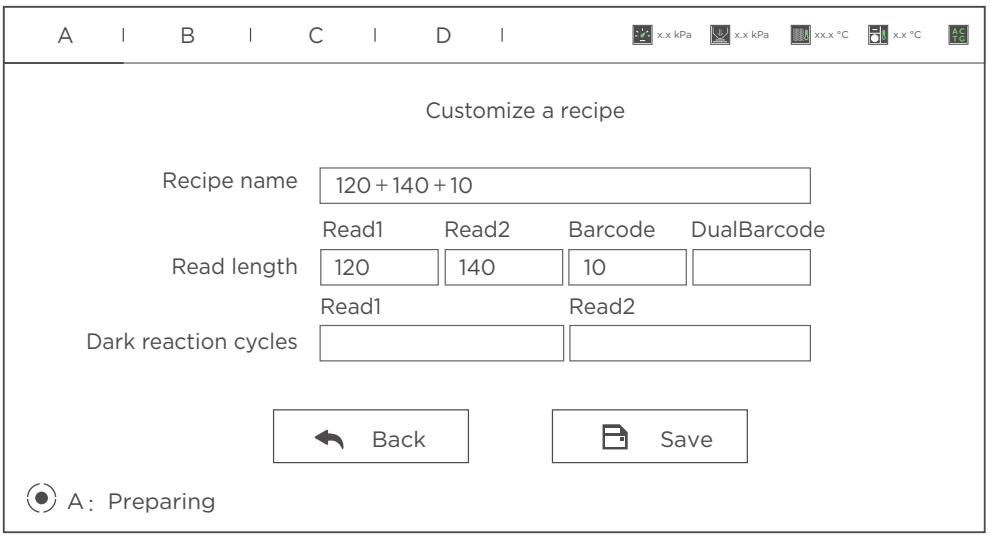

Figure 85 Configuring customized settings

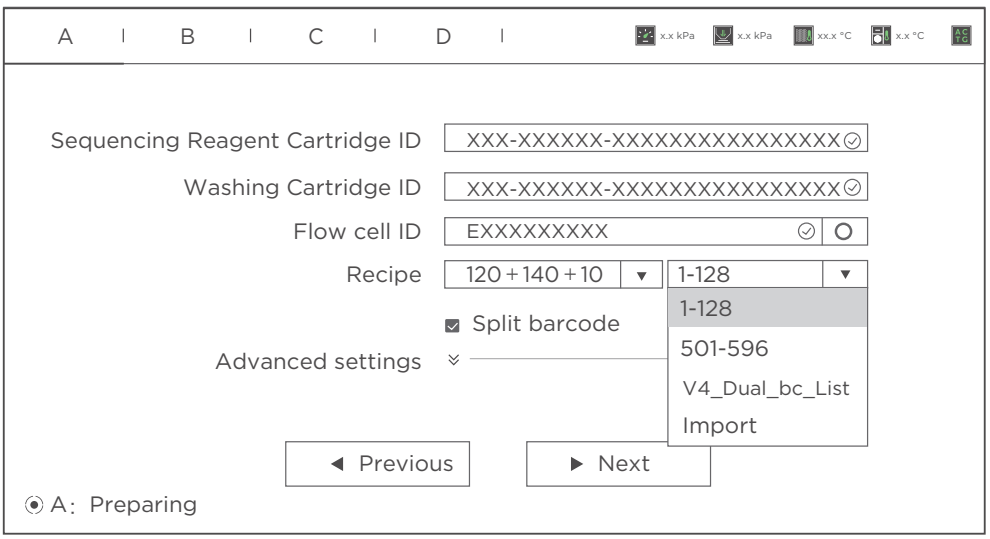

Figure 86 Selecting barcode file

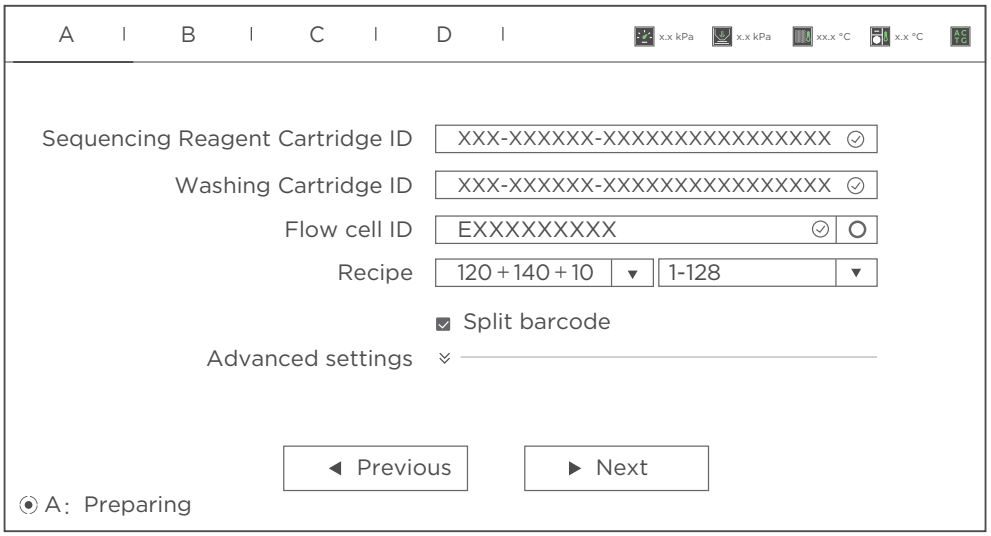

Figure 87 **Checking barcode splitting**

2. Length of the single barcode is not 10

Assumptions are as below:

- Sequencing run: PE150+8.
- Length of Read1: 150.
- Length of Read2: 150.
- Length of barcode: 8.
- Length of DualBarcode: 0.
- Split barcode: Yes.
- Total cycles = 150+150+8+2 = 310.
- Select a PE150 kit.

The **Customize a recipe** interface is set as follows:

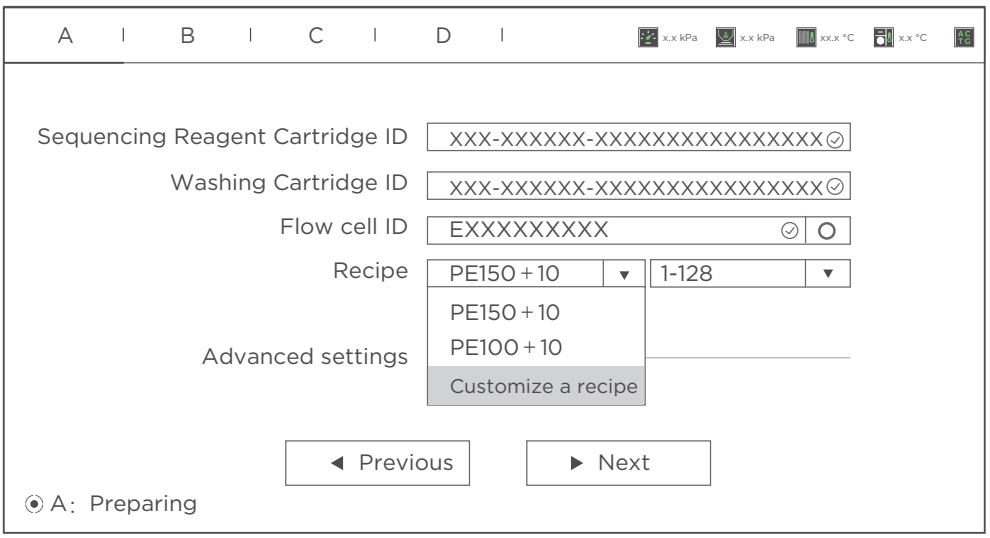

Figure 88 Selecting *Customize a recipe*

Figure 89 Configuring customized settings

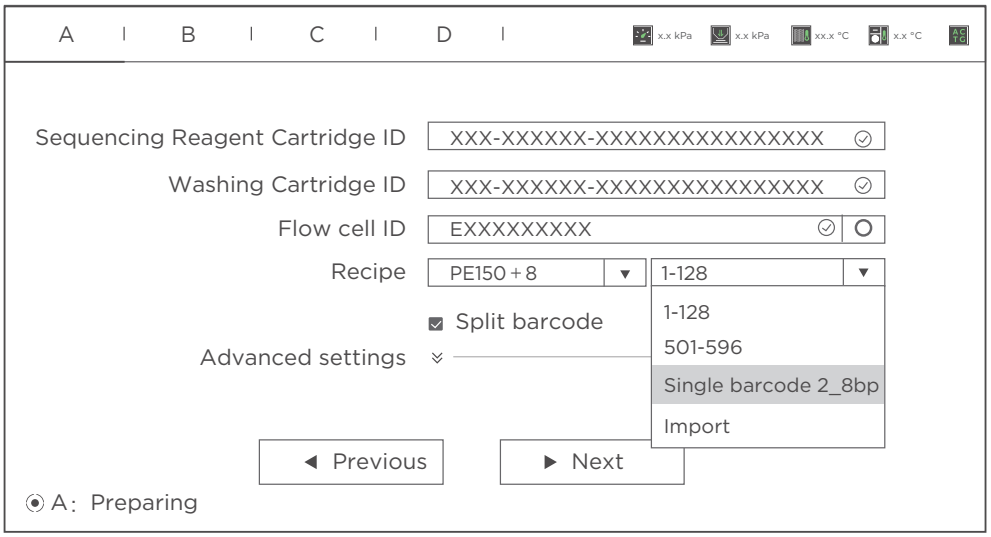

Figure 90 **Selecting barcode file**

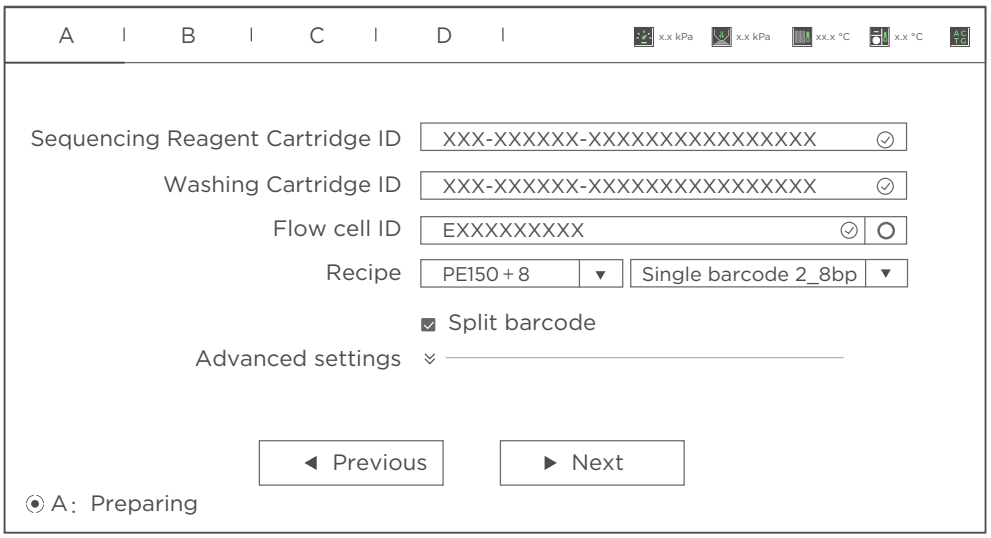

Figure 91 **Checking barcode splitting**

3. Different barcode lengths for DualBarcode sequencing

Assumptions are as below:

- Sequencing run: PE150+6+10.
- Length of Read1: 150.
- Length of Read2: 150.
- Length of barcode: 6.
- Length of DualBarcode: 10.

- Split barcode: Yes.
- Split DualBarcode: Yes.
- Total cycles = 150+150+6+10+2 = 318.
- Select a PE150 kit.

The **Customize a recipe** interface is set as follows:

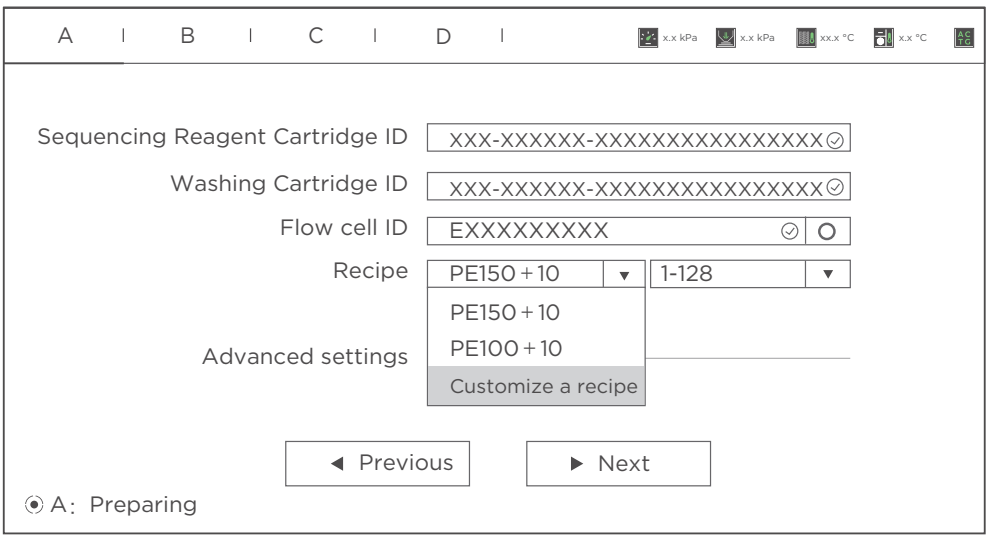

Figure 92 Selecting *Customize a recipe*

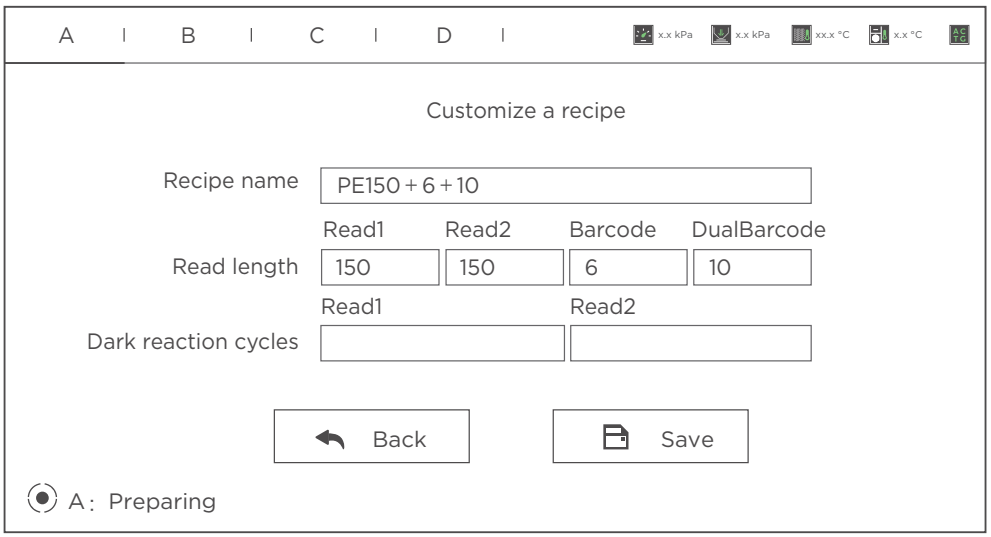

Figure 93 **Configuring customized settings**

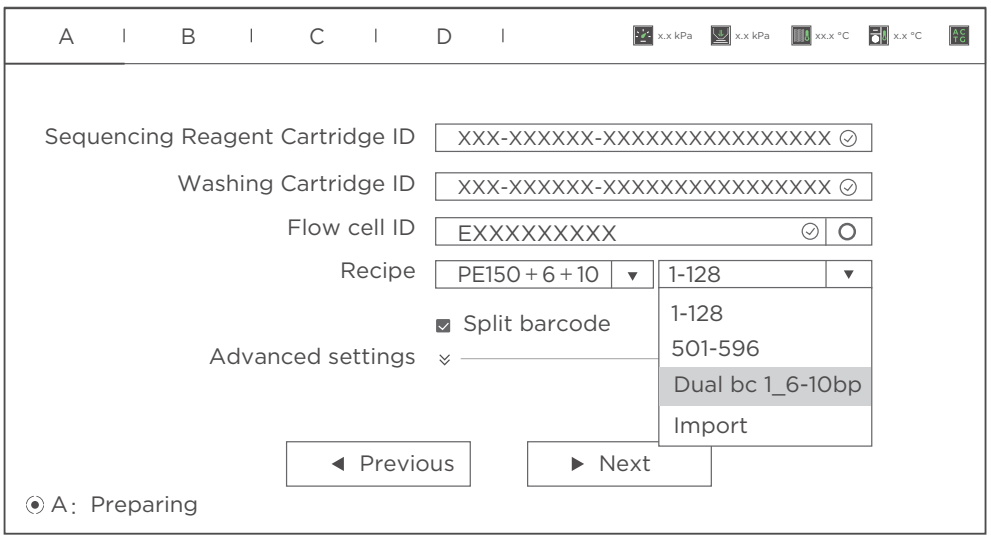

Figure 94 **Selecting barcode file**

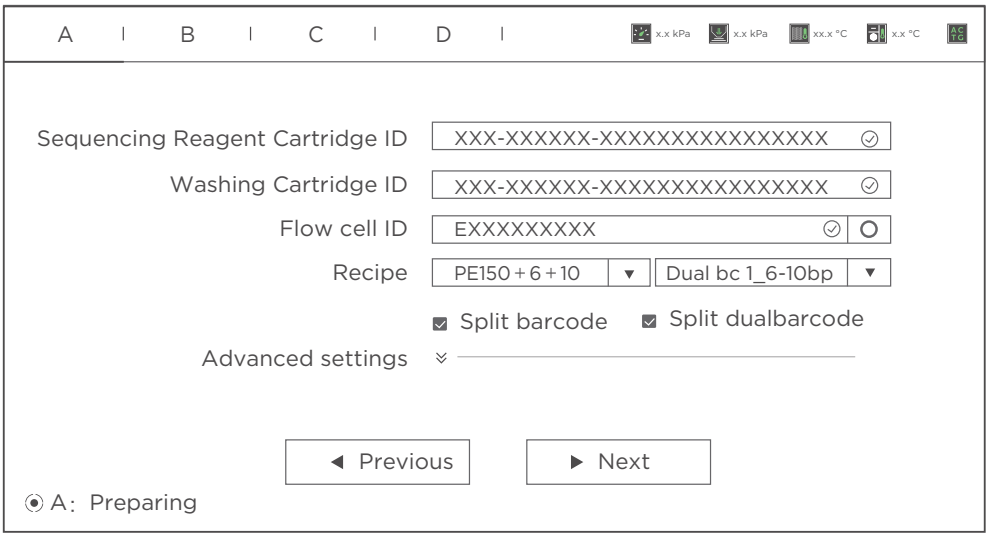

Figure 95 **Checking barcode splitting**

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

Assumptions are as below:

- Sequencing run: PE100+8+8.
- Length of Read1: 100.
- Length of Read2: 100.
- Length of barcode: 8.
- Length of DualBarcode: 8.
- Split barcode: Yes.
- Split DualBarcode: Yes.
- Dark cycles: From cycle-20 to cycle-30 and cycle-50 to cycle-60 in Read1 and cycle-16 to cycle-20 in Read2.
- Total cycles = 100+100+8+8 +2= 218.
- Select a PE100 kit.

The **Customize a recipe** interface is set as follows:

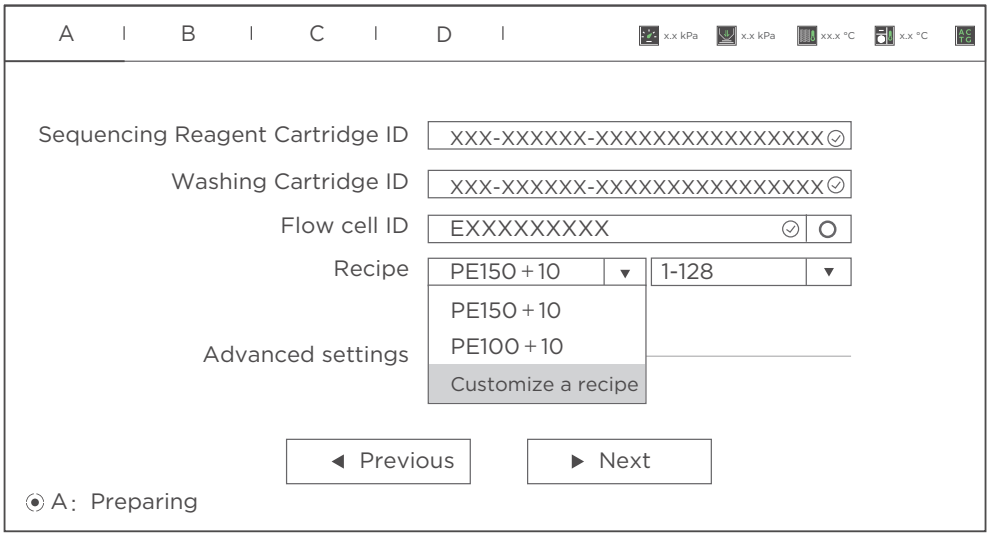

Figure 96 Selecting Customize a recipe

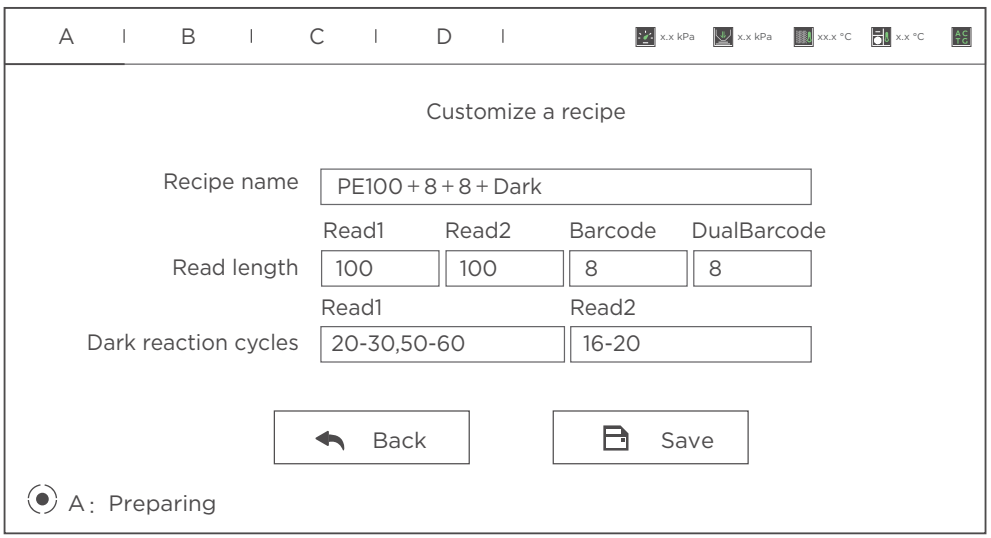

Figure 97 Configuring customized settings

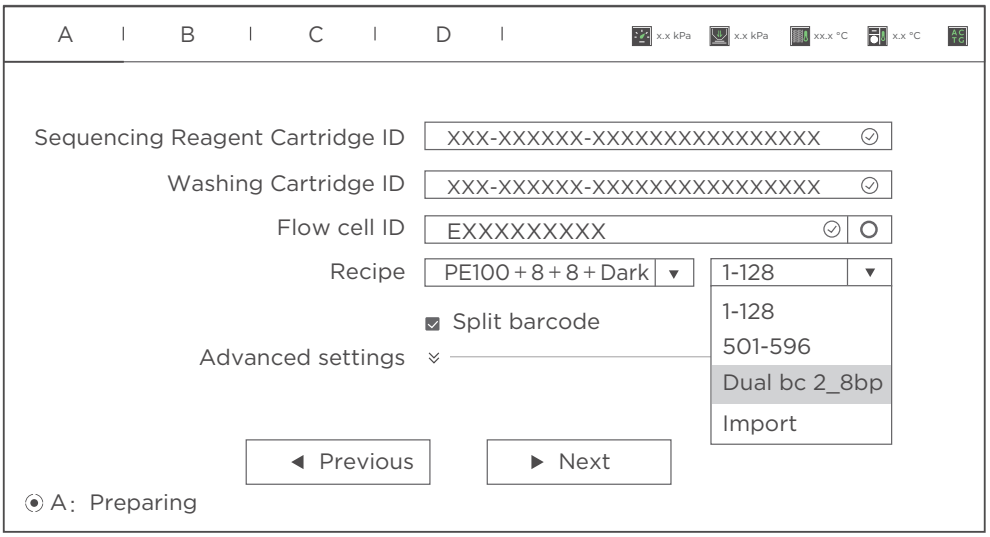

Figure 98 **Selecting barcode file**

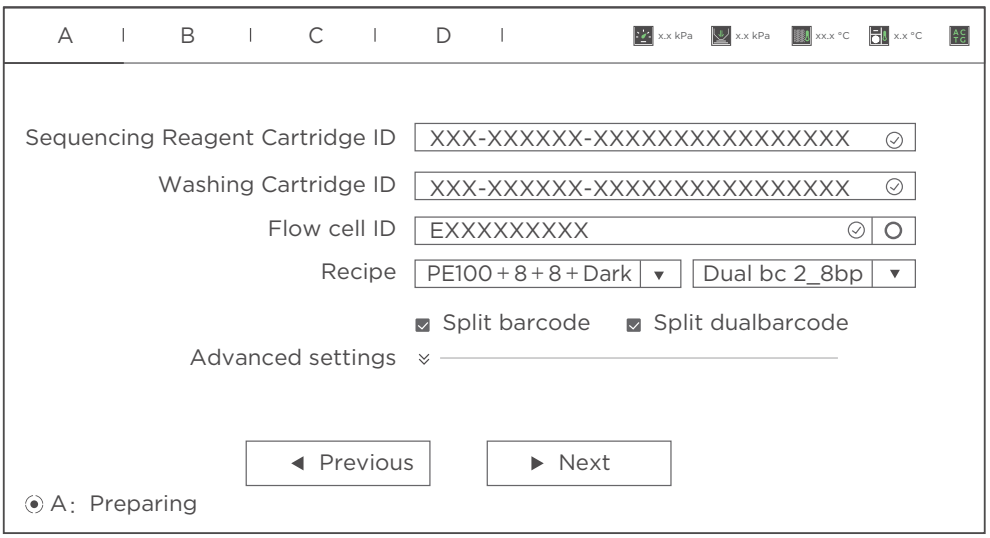

Figure 99 **Checking barcode splitting**

5. UMI+UDI

Assumptions are as below:

- Sequencing run: PE100+8+(8+9).
- Length of Read1: 100.
- Length of Read2: 100.
- Length of barcode: 8.
- Length of DualBarcode: 17.

- Split barcode: Yes.
- Split DualBarcode: Yes.
- Total cycles = 100+100+8+17+2 = 227.
- Select a PE100 kit.

The **Customize a recipe** interface is set as follows:

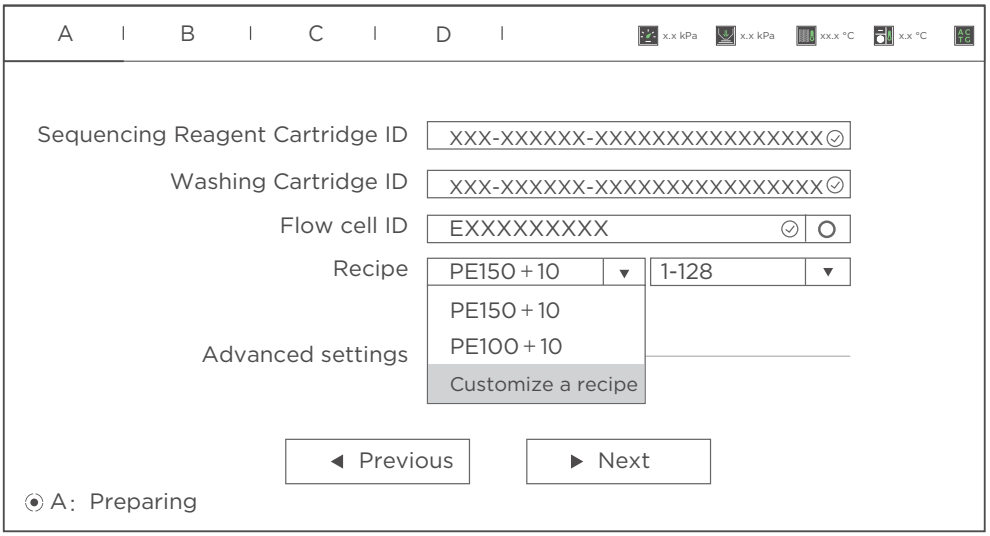

Figure 100 **Selecting *Customize a recipe***

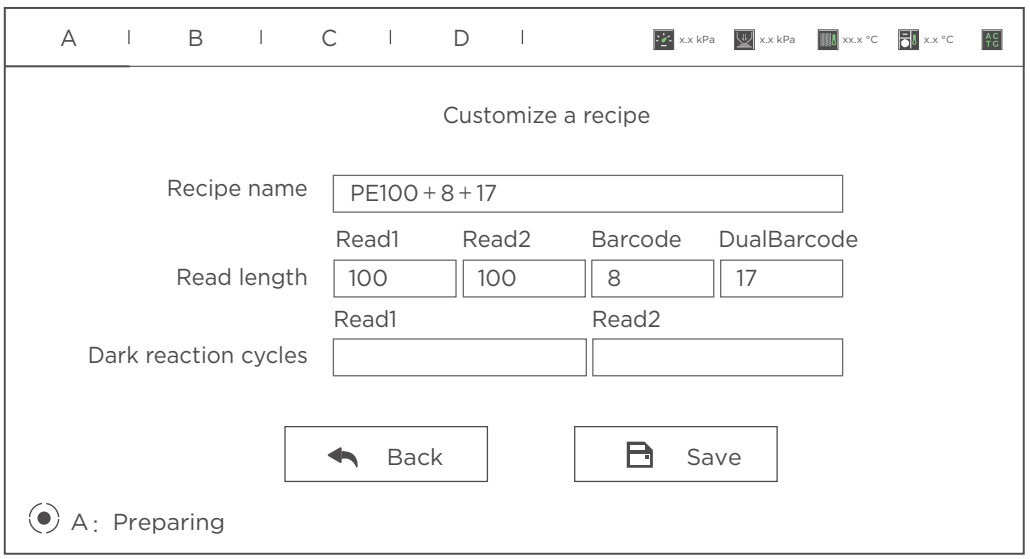

Figure 101 **Configuring customized settings**

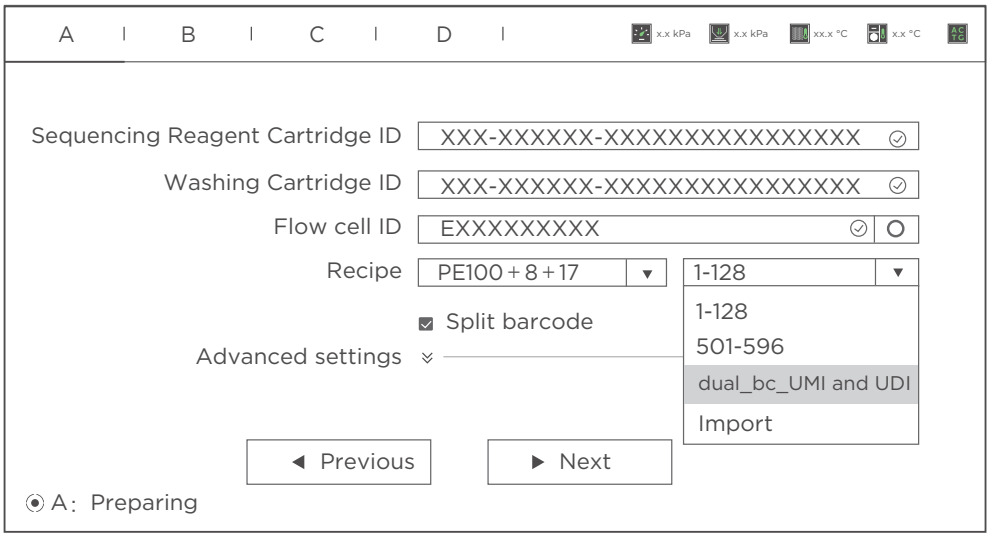

Figure 102 **Selecting barcode file**

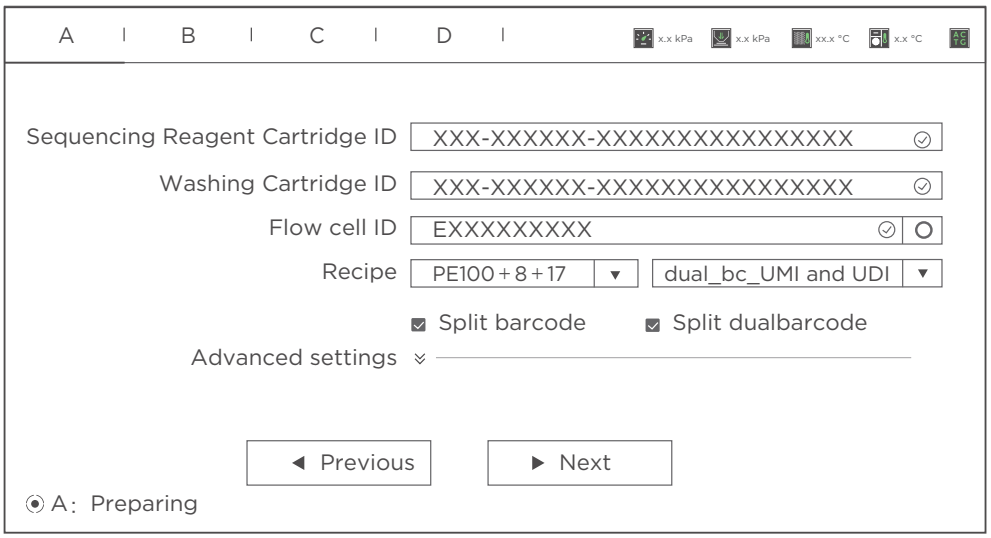

Figure 103 **Checking barcode splitting**

---This page is intentionally left blank.---

Instructions for using Qubit to quantify the DNBs

- Working solution should be used within 30 minutes floowing preparation.
- Avoid touching the wall of tapered detection tubes.
- Avoid introducing bubbles in detection tubes.
- If there are too many samples in a single test, it is recommended to quantify in batches to avoid inaccurate DNB quantification due to fluorescence quenching.
- 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 Qubit assay tube each time you prepare Qubit working solution. Do not mix the working solution in a glass container.

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

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

   For example: for 8 samples, prepare enough working solution for the samples and 2 standards. ~200 µL per tube in 10 tubes yields a total of 2 mL of working solution (10 µL of Qubit reagent plus 1990 µL of Qubit Buffer).
2. Add 190 µL of Qubit working solution to each of the tubes used for standards.
3. Add 10 µL of each Qubit standard to the appropriate tube and mix by vortexing 3 to 5 seconds. Be careful not to create bubbles.
4. Set up the required number of 0.5-mL tubes for standards and samples. The Qubit ssDNA Assay requires 2 standards.

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

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

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

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

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.

Sequencer specifications

Table 41 Sequencer specifications

Item	Description	
Laser classification of the device	Class 1 laser product	
Dimensions	1656 mm (W) × 1815 mm (H) × 903 mm (D) (65.2 inches × 71.5 inches × 35.6 inches)	
Net weight	Approximately 765 kg (1687 lb)	
Touch screen	Type	LCD
	Size	20 inches
	Resolution	1920 × 1080 pixels
Power	Voltage	200 V to 240 V~
	Frequency	50/60 Hz
	Rated power	3000 VA
	Overvoltage category	II
	Cable	min.10AWG
Maximum sound pressure level	75 dBA	

Item	Description	
Degrees of protection provided by enclosures (IP Code)	IPX0	
Accompanying items	Refer to the packing list	
Operating environment requirements	Temperature	19 °C to 25 °C (66 °F to 77 °F)
	Relative humidity	30% RH to 80% RH, non-condensing
	Atmospheric pressure	80 kPa to 106 kPa
	Altitude	≤2000 m
	Pollution degree	2
	Indoor use	
Transportation/ Storage environment requirements	Temperature	-20 °C to 50 °C (-4 °F to 122 °F)
	Relative humidity	15% RH to 85% RH, non-condensing
	Atmospheric pressure	80 kPa to 106 kPa

DNB loader specifications

Table 42 DNB loader specifications

Item	Description
Dimension	430 mm (W) × 750 mm (H) × 780 mm (D) (17 inches × 30 inches × 31 inches)
Net weight	Approximately 81 kg (179 lb)
Touch screen monitor	• Type: LCD touch screen • Size: approximately 13.3 inches • Resolution: 1280 × 600 pixels
Power	• Voltage: 100 V - 240 V~ • Frequency: 50/60 Hz • Rated power: 600 VA • Overvoltage category: II • Cable: min. 16 AWG
Fuse specification	F10AL250V
Maximum sound pressure level	75 dB(A)
Degrees of protection provided by enclosures (IP Code)	IPX0
Operating environment requirements	• Temperature: 19 °C to 25 °C (66 °F to 77 °F) • Relative humidity: 30% RH to 80% RH, non-condensing • Atmospheric pressure: 80 kPa to 106 kPa • Pollution degree: 2 • Indoor use
Storage/transportation environment requirements	• Temperature: -20 °C to 50 °C (-4 °F to 122 °F) • Relative humidity: 15% RH to 85% RH, non-condensing • Atmospheric pressure: 80 kPa to 106 kPa
Accompanying items	Refer to the packing list.

---This page is intentionally left blank.---

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

---This page is intentionally left blank.---

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.

---This page is intentionally left blank.---

Contact us

Manufacturer

Manufacturer	Complete Genomics, Inc.
Technical support	
Address	2904 Orchard Parkway, San Jose, CA 95134
Website	www.completegenomics.com

Customer service (US&Canada)

Telephone	+1 (888) 811-9644 (9:00 AM - 5:00 PM PT, Mon - Fri)
E-mail	US-Customerservice@completegenomics.com

Technical support (US)

Telephone	+1 (888) 811-9644 (6:00 AM - 5:00 PM PT, Mon - Fri)
E-mail	US-TechSupport@completegenomics.com

Technical support (Canada)

Telephone	+1 (888) 811-9644 (6:00 AM - 5:00 PM PT, Mon - Fri)
E-mail	CA-TechSupport@completegenomics.com

---This page is intentionally left blank.---

Order information

Catalog number	Model	Name	Version
940-000838-00	FCL PE100	DNBSEQ-T7RS High-throughput Sequencing Set	V1.0
940-000836-00	FCL PE150	DNBSEQ-T7RS High-throughput Sequencing Set	V1.0
940-000871-00	/	Sequencer Cleaning Cartridge	/
940-000872-00	/	Cleaning Cartridge	/
940-000873-00	/	DNB Load Plate (no Reagent)	/
900-000697-00	DL-T7RS	DNB Loader DL-T7RS	/

---This page is intentionally left blank.---

Acronyms and abbreviations

Item	Description
BCS	Basecall server
BIC	Basecall Information Content
cPAS	Combinatorial Probe-anchor Synthesis
CPU	Central Processing Unit
DL-T7RS	DNB Loader for DNBSEQ-T7
DNA	Deoxyribonucleic Acid
DNB	DNA Nanoball
dsDNA	double-stranded DNA
EMC	Electromagnetic Compatibility
ESR	Effective Spots Rate
FAQ	Frequently Asked Questions
FCC	Federal Communications Commission
FCL	Flow Cell Large
FIT	Least square fit to the DNB intensities in 4 color space to represent the overall quality of the clusters
FOV	Field of View
FPGA	Field-programmable Gate Array
GPU	Graphics Processing Unit
IC	Interference-Causing
ID	Identification
ISW	Instrument Control Software
LAN	Local Area Network
LT	Laser temperature
MDA	Multiple Displacement Amplification
PE	Pair-end sequencing
QC	Quality Control

Item	Description
QR	Quick Response
RCR	Rolling Circle Replication
RFID	Radio Frequency Identification
RHO	Rho (ρ), intensity of raw signals
RNA	Ribonucleic Acid
SBC	Single Board Computer
SNR	Signal to Noise Ratio
ssDNA	single-stranded DNA
TV	Television
UDI	Unique Dual Index
UMI	Unique Molecular Identifier
UPS	Uninterruptible Power Supply
USB	Universal Serial Bus
VGA	Video Graphics Array
WES	Whole Exome Sequencing
WGS	Whole Genome Sequencing
ZLIMS	ZTRON laboratory information management system

Index

P

R

S

Z