Complete GENOMICS™

DNBSEQ-G800RS System Guide

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

Complete Genomics, Inc.

Part No.: CSS-00018

About this guide

CG intends to provide this product solely for research use.

This guide is applicable to Genetic Sequencer (DNBSEQ-G800RS) and DNBSEQ-G800RS High-throughput Sequencing Set. The guide revision is B and the software version is V1.

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

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

Revision history

	Date	Revision
Revision	August 01, 2025	B
Initial release	April 02, 2025	A

Contents

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01

Safety

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

Conventions used in this guide

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

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

General safety

DANGER

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

WARNING

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

CAUTION

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

Electrical safety

DANGER

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

WARNING

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

CAUTION

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

Mechanical safety

DANGER

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

Components safety

WARNING

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

CAUTION

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

Biological safety

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

WARNING

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

Symbols

Packaging

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

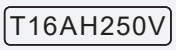
Symbol	Name	Description
	This way up	Indicates the correct upright position of the crated unit for transport and/or storage
	Fragile, handle with care	Indicates a device that can be broken or damaged if not handled carefully
	Keep dry	Indicates a device that needs to be protected from moisture

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

Device

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

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

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

Label

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

Symbol	Name	Description
	Model number	Indicates the model number or type number of a product
	Manufacturer	Indicates the name and address of the device manufacturer
	Date of manufacture	Indicates the date when the device was manufactured
	Serial number	Indicates the manufacturer's serial number so that a specific device can be identified
	Consult instructions for use	Indicates the need for the user to consult the instructions for use
	Catalog number	Indicates the manufacturer's catalog number (Cat. No.) so that the device can be identified

Symbol	Name	Description
	Use by date	Indicates the date after which the device is not to be used
	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified
	Keep away from sunlight	Indicates a device that needs protection from light sources
	Do not re-use	Indicates a component or reagent that is intended for one 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

Device overview

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

Intended use

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

This device is a sequencing instrument that measures optical and electronic signals of the reporting molecules, which decode the sequence information of a DNA or RNA fragment. This is accomplished through 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 one another. This improves the accuracy of signal processing.

The following figure demonstrates how to make DNBs:

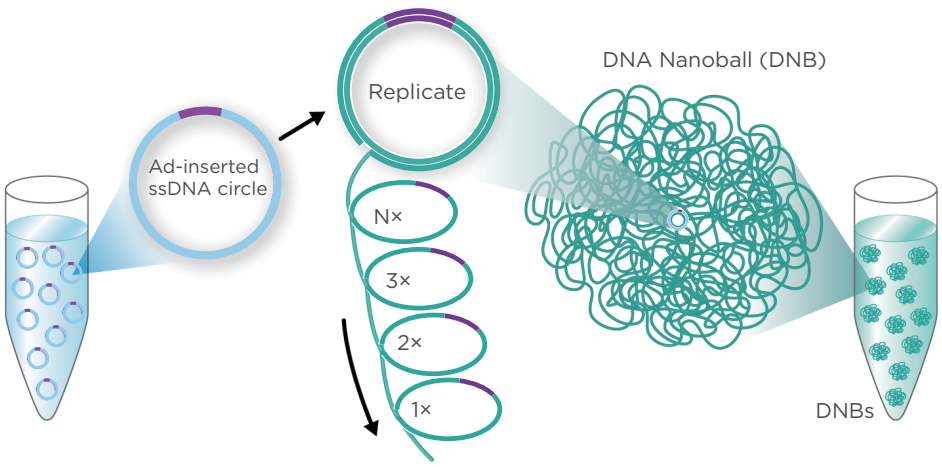


Figure 1 **Making DNBs**

The following figure demonstrates how to load DNBs:

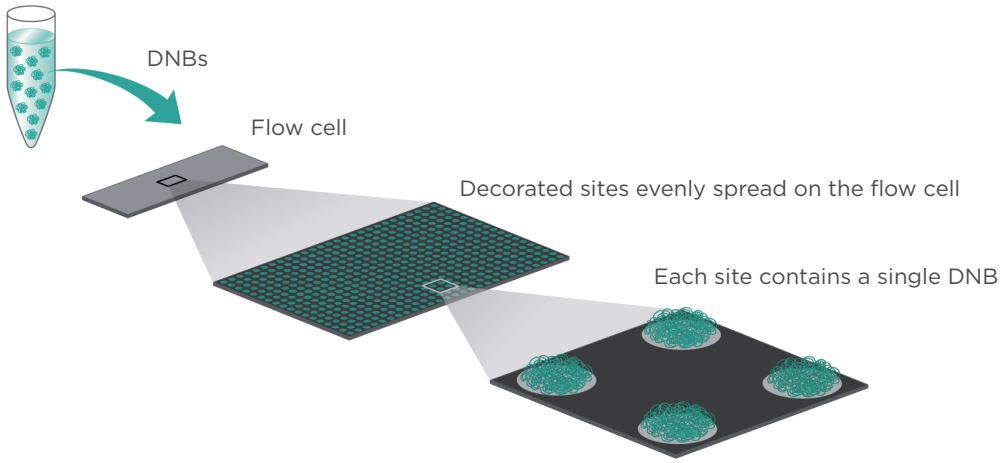


Figure 2 Loading DNBs

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

Sequencer overview

Structural composition

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

The following table describes the function of each component:

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

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

Basic components

Front view

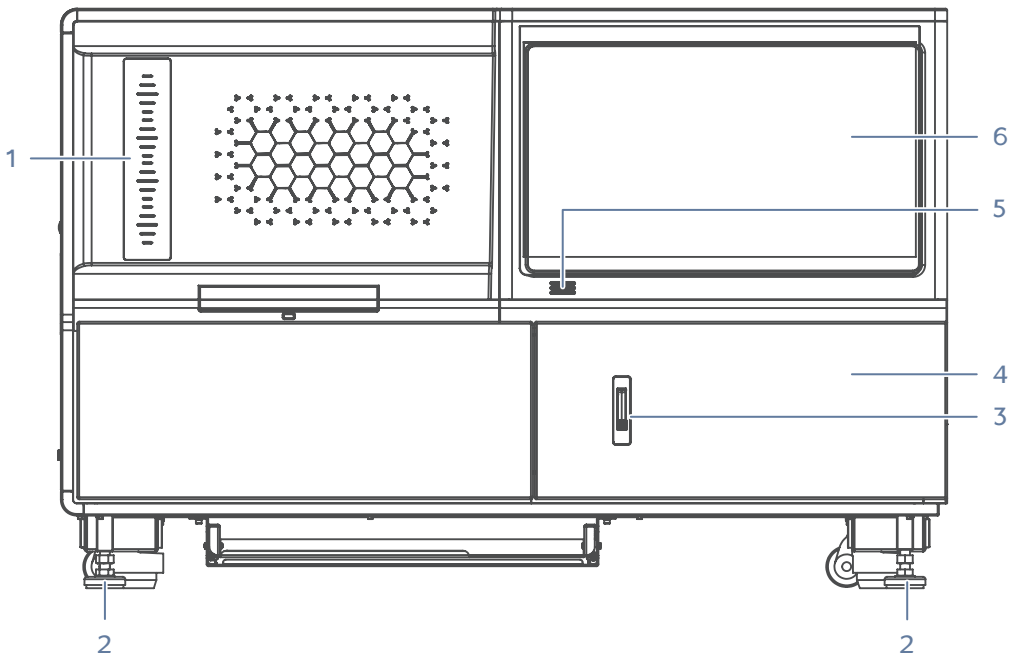


Figure 3 Front view

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

Back view

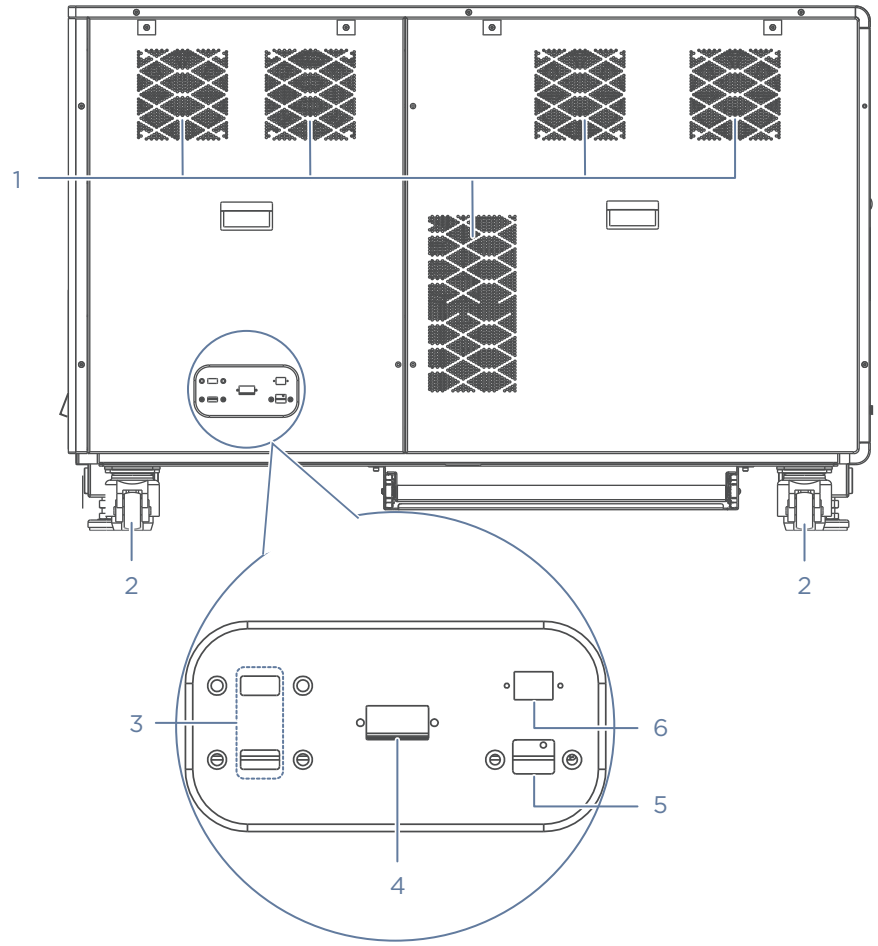


Figure 4 Back view

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

No.	Name	Description
1	Ventilation outlet	Ventilates the device.
2	Wheel	Used for moving the device.
3	USB 3.0 port	Connects to USB devices such as the keyboard, mouse, and scanner.
4	USB port	Connects to USB devices such as the keyboard, mouse, and scanner.

No.	Name	Description
5	Network port	Reserved for future use.
6	Network port	Connects to the network.

Left view

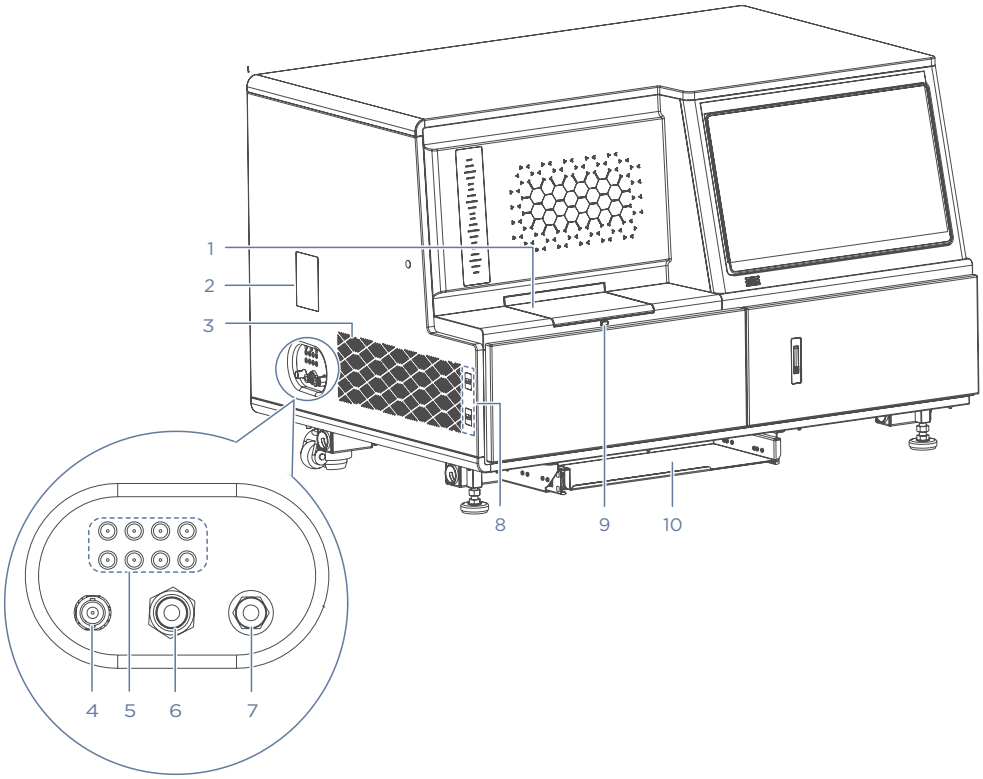


Figure 5 Left view

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

No.	Name	Description
1	Flow cell compartment	Holds flow cells and controls the temperature for biochemical reactions.
2	Window	Allows you to observe the status of the fluidics system and negative pressure gauge through the window.
3	Ventilation inlet	Exhausts air from the device.

No.	Name	Description
4	Level sensor port	Connects the waste level sensor in the waste container.
5	External cleaning module port	Connects the device to the external cleaning module.
6	Condensed water port	Dispenses the condensed water from the cooling system to the waste container via the condenser tube.
7	Waste port	Connects the waste tube to dispense the waste to the waste container.
8	USB 3.0 port	Connects to USB devices.
9	Button on the flow cell compartment door	Press to open the flow cell compartment door.
10	Keyboard drawer	Holds the keyboard and mouse.

Right view

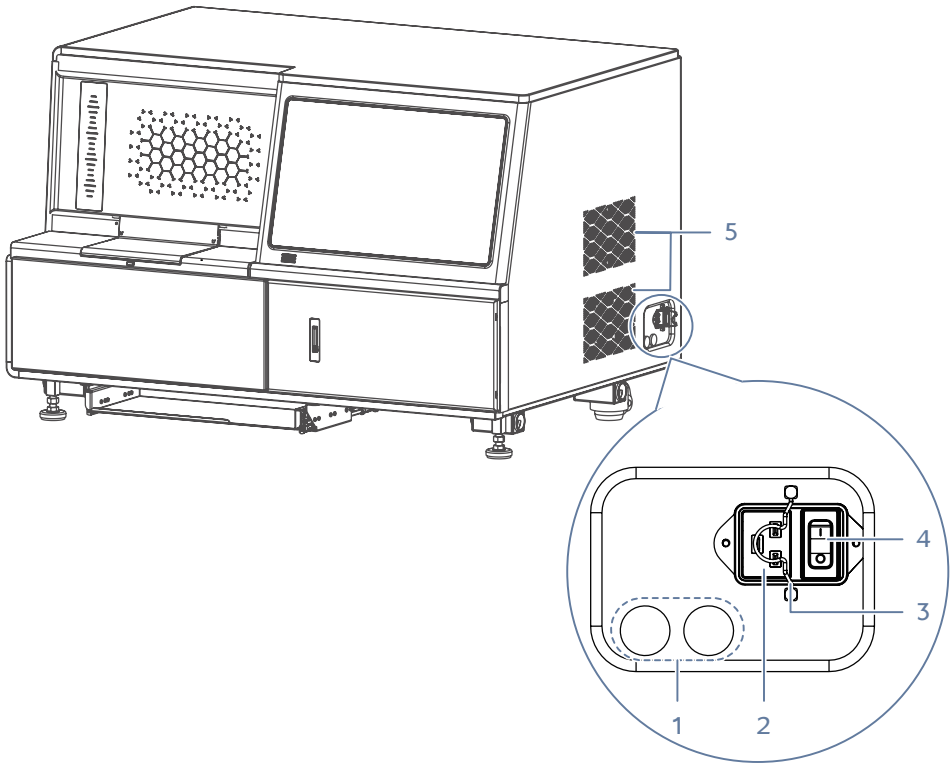


Figure 6 Right view

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

No.	Name	Description
1	Fuse seat	Location of installed fuses.
2	Power supply port	Connects the device to the power supply.
3	Power cord hook	Prevents power cord from moving.
4	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.
5	Ventilation inlet	Ventilates the device.

Flow cell compartment

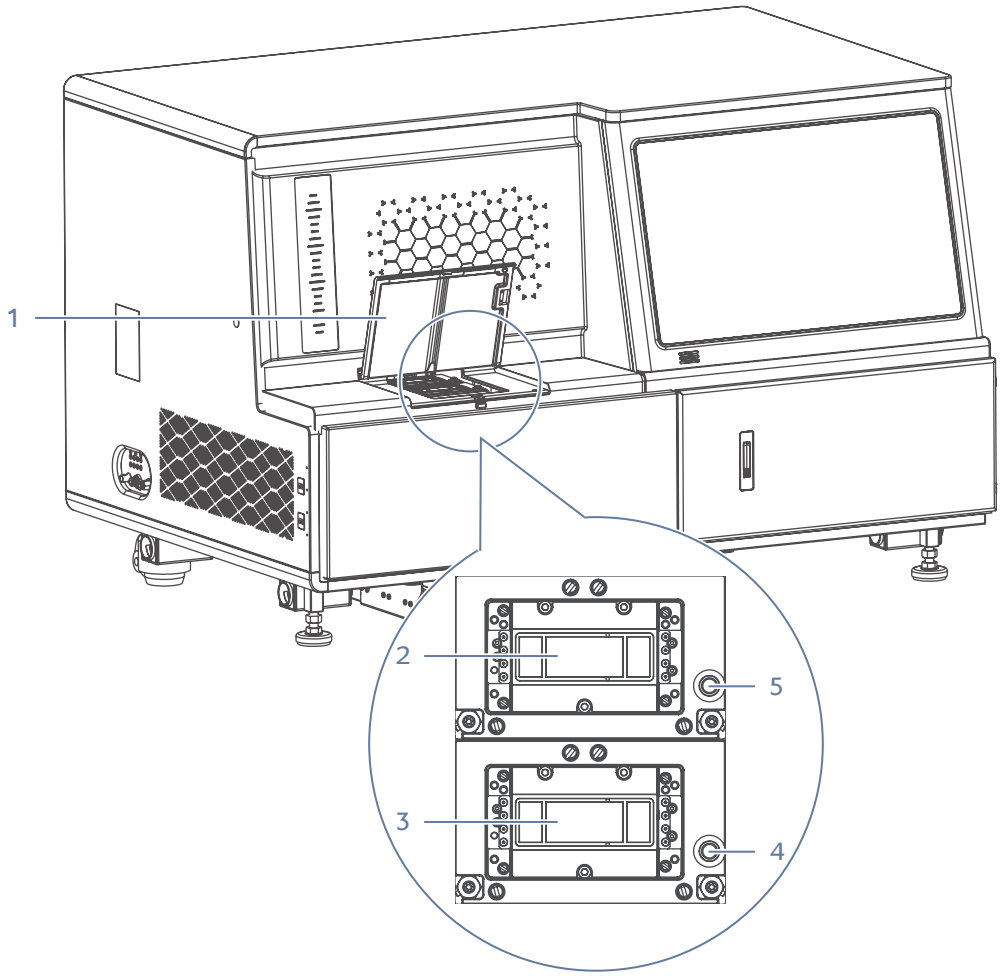


Figure 7 Flow cell compartment

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

Reagent compartment

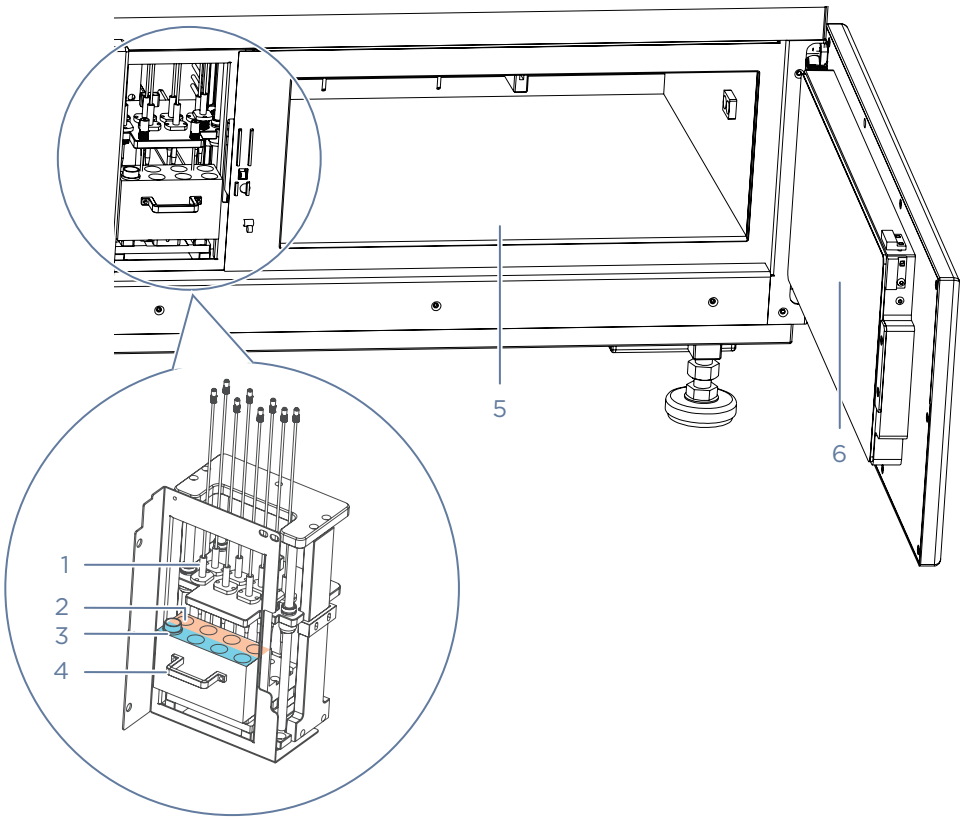


Figure 8 Reagent compartment

No.	Name	Description
1	DNB needles	Aspirates DNBs from the DNB tube.
2	DNB tube B rack	Holds DNB tube B.
3	DNB tube A rack	Holds DNB tube A.
4	Handle	Used to pull out the tube rack.
5	Reagent compartment	Holds the reagent cartridge at appropriate temperatures: • Reagent cartridge A is placed on the left. It provides the required reaction mixture for flow cell A. • Reagent cartridge B is placed on the right. It provides the required reaction mixture for flow cell B.

No.	Name	Description
6	Reagent compartment door	Press the button on the latch of the door, and pull the pop-up ring to open the door.

External cleaning module

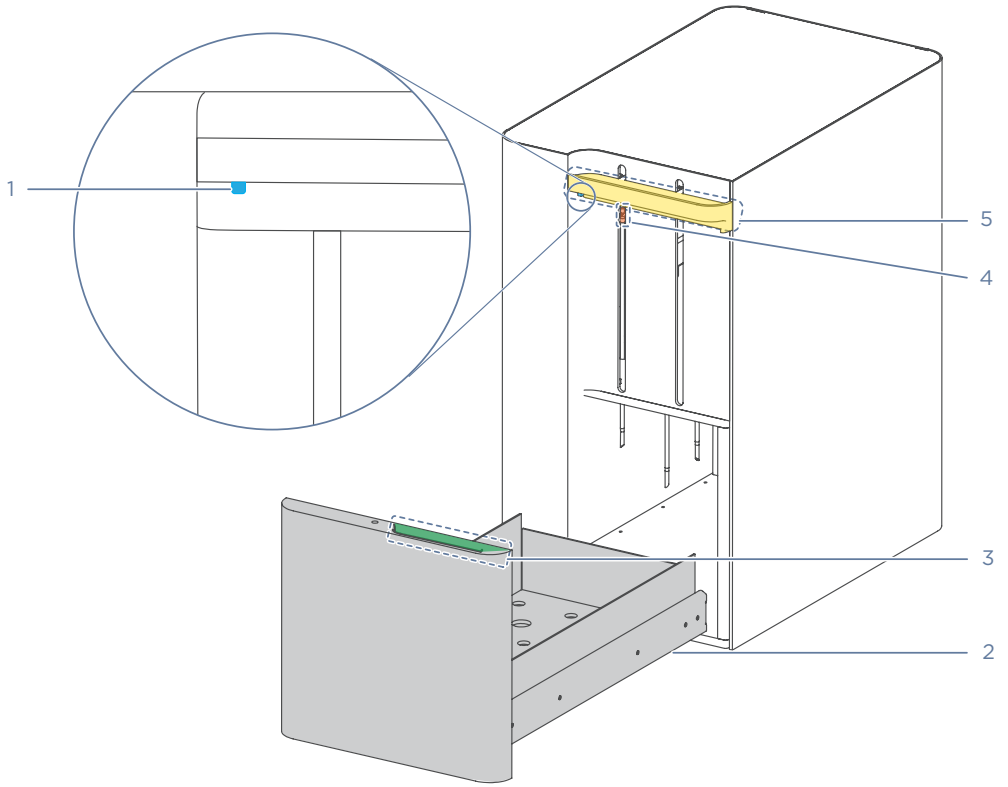


Figure 9 External cleaning module

No.	Name	Description
1	Slider	Unlocks the crossbar.
2	Washing cartridge compartment	Holds the washing cartridge. • The washing cartridge A is placed on the left. • The washing cartridge B is placed on the right.
3	Groove	Used to pull out the washing cartridge compartment.
4	Reagent needle	Aspirate reagents.
5	Crossbar	Used to move the reagent needle up or down manually.

Control software

Overview

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

The following table describes the function of each 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.
Software running	Monitors the status of the system components.

Self-test

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

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

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

2. Follow the on-screen instructions or the solutions that are mentioned in *Sequencer FAQs on Page 148*.
3. Perform the self-test again:
   - Select > > .
   - Select > .

   If the problems persist, contact CG Technical Support.

Main interface

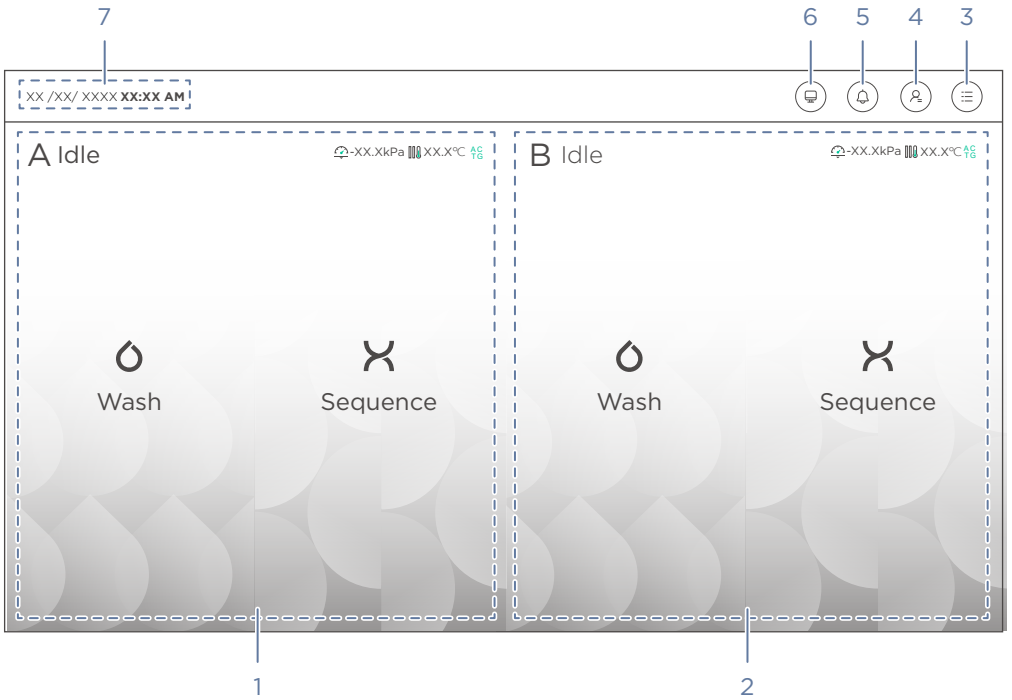


Figure 10 Main interface

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

No.	Item	Description	
1	Flow cell A operation area	Indicates the status of stage A and provides wash and sequencing options.	
2	Flow cell B operation area	Indicates the status of stage B and provides wash and sequencing options.	
3		Select to view the function menu. The function menu provides the following functions:	
			Select to view the logs.
			Select to change settings.
			Select to perform maintenance.
			Select to check the system information.
			Select to view the Help page.
			Select to lock the screen.
			Select to restart the system.
			Select to shut down the system.
4		Select to log in to the system.	
5		The notification icon indicates: • Blue: the device is operating normally. • Flashing yellow: a warning notification has appeared. • Flashing red: an error occurred. General information, warnings, or error messages are displayed on the right of the icon.	

No.	Item	Description	
6		Select to view the following status:	
			Displays the connection status of ZLIMS.
			Displays the usage percentage of the waste container.
			Displays the usage percentage of the disk.
			Displays the temperature of the sequencer.
			Displays the temperature of the refrigerator.
			Displays the inner humidity of the sequencer.
7	Date and time	Displays the current, local date and time.	

The following table describes the function of the icons and buttons in the flow cell A operation area (No. 1 in the table above) and flow cell B operation area (No. 2 in the table above):

Item	Description
A/B	Indicates either flow cell A operation area or flow cell B operation area.
Status	Displays the status of the selected operation area.
	Temperature of the flow cell stage is normal.
	Temperature of the flow cell stage is outside the normal range.
	Negative pressure is normal.
	Negative pressure is outside the normal range.
	Basecalling is connected.
	Errors occurred in the basecalling connection.
	The Basecall software is processing image data. This icon is dynamic.
Wash	Select to set the wash type, and perform the relevant operations by following the on-screen instructions. For details, refer to *Wash on Page 132*.

Item	Description
Sequence	Select to set sequencing parameters, and perform a sequencing run by following the on-screen instructions. For details, refer to *Sequencing on Page 49*.

Log interface

You can view the log in this interface.

Select > to open the log interface.

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

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

System settings interface

You can change system settings in this interface.

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

1. Log in to your account. For details, refer to *Logging in to the control software on Page 46*.
2. Select > .

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

Item	Description
	Select to exit the system settings interface and return to the previous interface.
	Select to save the settings.
Language	Select to change the language of the software. Changes take effect after you restart the system.
Upload	Select **Upload enabled** to upload the data to the specified server.
Fastq	Select to configure the FASTQ parameters.
Others	Includes the following functions: • Select **Play guided animation** to display the guided animation. • Select to change the wait time before the screen locks automatically. • Move the slider to change the buzzer volume.

Maintenance interface

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

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

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

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

Item		Description
		Select to exit the system maintenance interface and return to the previous interface.
System maintenance		Select to discharge the residual liquid in all fluidics lines to the waste container. The fluidics line that is being emptied is highlighted.
		Select to perform a hardware self-test. When the test is finished, a notification and the results will be displayed on the screen.
		Select to clear all sequencing run history data, except for the data from the most recent run.
Recipe settings		Create or delete the customized sequencing recipe.

Item		Description
Export data		• Select a data type and export the data to the specified directory of the external storage device. • Select **Upload data** and upload the data to the specified server.
Script settings		Modify the script.
Barcode	**Barcode import**	Import the barcode file that is saved in the external storage server or hard drive to the device.
	Barcode export	Export the barcode template that is saved in the device to the external storage server or hard drive.
User management		Select to add or delete users, modify user information, or reset passwords.
Device life cycle		Select to view the device life cycle.

The user management interface in Maintenance is as follows:

Use the user account or administrator account to log in to the software.

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

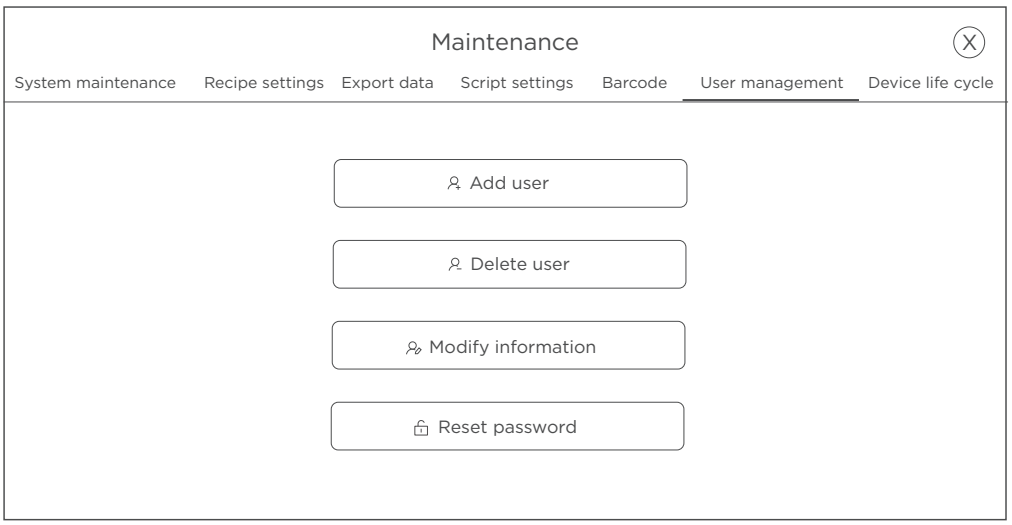


Figure 11 User management interface

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

Item	Description
	Select to add users.
	Select to delete users.
	Select to modify users' passwords.
	Select to reset a password.

Shutdown or restart interface

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

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

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

About interface

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

To open the About interface, select  >  .

DNB loader overview

Overview

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

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

Basic components

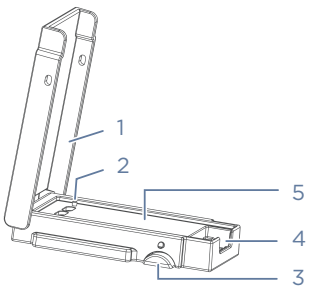


Figure 12 Side view

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

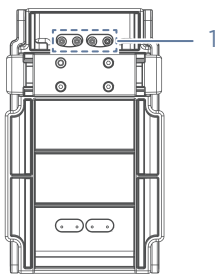


Figure 13 Back view

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

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03

Sequencing sets overview

This chapter describes the sequencing sets information.

Introduction

This section describes the sequencing sets, sequencing run times, and data output. The flow cell type described is FCL (Flow Cell Large) with four lanes per flow cell.

Available sequencing sets

Table 1 Available sequencing set list

Cat. No.	Model	Name	Version	Theoretical data output (Gb/lane)
940-001250-00	CM App-D FCL PE150	DNBSEQ-G800RS CoolMPS High-throughput Sequencing Reagent Set	V1.0	135
940-001733-00	CM App-D FCL SE600	DNBSEQ-G800RS CoolMPS High-throughput Sequencing Reagent Set	V1.0	240

List of sequencing set components

Table 2 DNBSEQ-G800RS CoolMPS High-throughput Sequencing Set (CM App-D FCL PE150)
Cat. No.: 940-001250-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Expiration date
DNBSEQ-G800RS FCL RS Sequencing Flow Cell **Cat. No.: 930-000095-00**					
DNBSEQ-G800 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C	12 months
DNBSEQ-G800RS High-throughput Sequencing Kit (CM App-D FCL PE150) **Cat. No.: 940-001255-00**					

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Expiration date
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
App Make DNB Buffer		100 µL/tube×1 tube			
Make DNB Rapid Enzyme Mix III		200 µL/tube×1 tube			
Make DNB Enzyme Mix II (HF+LC)		25 µL/tube×1 tube			
Stop DNB Reaction Buffer		100 µL/tube×1 tube			
DNB Load Buffer I		200 µL/tube×1 tube			
DNB Load Buffer II		200 µL/tube×1 tube			
Sequencing Enzyme Mix II		5.78 mL/tube×2 tubes			
Micro Tube 0.5 mL (Empty)		4 EA			
Dye Mix I		5.60 mL/tube×1 tube			
Dye Mix II		4.90 mL/tube×1 tube			
dNTPs Mix II		6.16 mL/tube×2 tubes			
Inactive MDA Reagent		3.50 mL/tube×1 tube			
MDA Enzyme Mix II		0.23 mL/tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			
Cleaning Reagent Kit (CM App-D FCL PE150) Cat. No.: 940-001252-00					
Washing cartridge	/	1 EA	0 °C to 30 °C	No higher than 40 °C	12 months

Table 3 DNBSEQ-G800RS High-throughput Sequencing Set (CM App-D FCL SE600)
Cat. No.: 940-001733-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Expiration date
DNBSEQ-G800RS FCL RS Sequencing Flow Cell **Cat. No.: 930-000095-00**					
DNBSEQ-G800 FCL Sequencing Flow Cell	/	1 EA	-25 °C to -15 °C	-80 °C to -15 °C	12 months
DNBSEQ-G800RS CoolMPS High-throughput Sequencing Kit (CM App-D FCL SE600) **Cat. No.: 940-001734-00**					
Low TE Buffer		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
App Make DNB Buffer II		150 µL/tube×1 tube			
Make DNB Rapid Enzyme Mix IIIII		220 µL/tube×1 tube			
Make DNB Enzyme Mix II (HF+LC)		25 µL/tube×1 tube			
Stop DNB Reaction Buffer		100 µL/tube×1 tube			
DNB Load Buffer I		200 µL/tube×1 tube			
DNB Load Buffer II		200 µL/tube×1 tube			
Micro Tube 0.5 mL		4 EA			
Dye Mix I		16.8 mL/tube×1 tube			
Dye Mix II		14.7 mL/tube×1 tube			
dNTPs Mix II		22.2 mL/tube×1 tube			
Sequencing Enzyme Mix II		28.0 mL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Expiration date
Cleaning Reagent Kit (CM App-D FCL SE600) Cat. No.: 940-001732-00					
Washing cartridge	/	1 EA	0 °C to 30 °C	No higher than 40 °C	12 months

Sequencing read length

Sequencing read length determines the number of sequencing cycles for a given sequencing run. One sequencing cycle equates to one base pair of sequence data. For example, a PE150 cycle run performs reads of 150 cycles (2×150) for a total of 300 cycles or 300 bases sequenced. At the end of the sequencing run, an extra 10 cycles or 20 cycles of barcode read can be performed to aid in identifying a specific library, if required.

Table 4 Sequencing cycle

Sequencing read length	Read1 read length	Read2 read length	Barcode read length	Dual barcode read length	Maximum cycles
PE150	150	150	10	10	322
SE600	600	--	10	10	621

To ensure sequencing quality, when Read1 and Read2 sequencing are completed, the sequencer will automatically perform one more cycle for correction. For example, for PE150 dual barcode sequencing, Read1 length is 150, Read2 length is 150, barcode read length is 10 and dual barcode read length is 10, plus 1 correction cycle for Read1 and 1 correction cycle for Read2 (barcode does not require correction). The total cycle number of this sequencing is 322.

Sequencing time

Table 5 FCL Sequencing time and analysis time for each read length (h)

Type		
	PE150	SE600
Single flow cell	48	86
Dual flow cells	48	86
Data analysis (Single flow cell)	0.8	1.5
Data analysis (Dual flow cells)	0.8	1.5

- FCL represents DNBSEQ-G800 FCL Sequencing Flow Cell. Each FCL has four lanes, each lane with 580 million (M) DNB binding sites.
- The sequencing time (Single flow cell/Dual flow cells) in the table above includes the time required from post loading prime to sequencing completion. The data analysis time includes the time required for barcode demultiplexing (if **Split barcode** is selected) and FASTQ files output when sequencing is completed.
- The time in the table above is measured for dual barcode.
- The time in the table above is the average time. The actual run time may vary slightly among individual sequencers.

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04

Getting Started

This chapter describes sequencing preparations.

User-supplied equipment and consumables

Before using the device, prepare the following equipment:

Table 6 User-supplied equipment list

Equipment	Recommended brand
Qubit fluorometer	Thermo Fisher
Mini spinner	General lab supplier
Vortex mixer	General lab supplier
Thermal cycler	Bio-Rad
Refrigerator, 2 °C to 8 °C (36 °F to 46 °F)	General lab supplier
Freezer, -25 °C to -15 °C (-13 °F to 5 °F)	General lab supplier
Ultra-pure water machine	General lab supplier
Graduated cylinder, 500 mL	General lab supplier
Ice bucket	General lab supplier
Pipette, 20 µL	Eppendorf or equivalent
Pipette, 200 µL	Eppendorf or equivalent
Pipette, 1000 µL	Eppendorf or equivalent
Electronic pipette	Intergra or equivalent
Ultrasonic Cleaner	CG

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

WARNING Tips are disposable consumables. Do not reuse them.

Table 7 Recommended reagent/consumable list

Reagent/Consumable	Recommended brand	Purpose
2 M NaOH	General lab supplier	Diluting to 0.1 M for washing reagents
75% ethanol	General lab supplier	Device maintenance
0.05% Tween-20+1 M NaCl	General lab supplier	Device maintenance
Sterile pipette tip (boxed)	AXYGEN	Pipetting for diluting and loading wash and loading reagents
Sterile 200 µL wide-bore, non-filtered pipette tip	MGI Cat. No.: 091-000355-00	Mixing DNBs
Qubit ssDNA Assay Kit	General lab supplier	Library and DNB QC
Qubit Assay Tubes	Thermo Fisher	Library and DNB QC
Sterile PCR 8-strip tube, 0.2 mL	AXYGEN	Making DNB reaction mixture
Sterile centrifuge tube, 1.5 mL	AXYGEN	Diluting NaOH and library
Sterile Microcentrifuge tube, 2.0 mL	SARSTEDT	Washing the DNB Loading needle
Sterile Centrifuge tube, 5 mL	General lab supplier	Reagent mix
Sterile Centrifuge tube, 10 mL	General lab supplier	Reagent mix
Sterile Centrifuge tube, 15 mL	General lab supplier	Reagent mix
Canned air duster	MATIN	Cleaning
Disposable gloves, powder-free	General lab supplier	General purpose
KimWipes tissues	VWR	Cleaning
Low-lint cloth	General lab supplier	Cleaning
Laboratory-grade water	General lab supplier	/
Disposable pipette	CORNING	/

Preparing the device

Powering on the device

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

Perform the following steps:

1. Connect the device to the main power supply.
2. (Optional) If a UPS is prepared, connect the device to the main power supply by using the UPS power cord.
3. Turn the power switch of the device to the | position.
4. 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 account *Zebra* and password *123*.
3. Select (user) in the main interface.
4. Log in to the control software with the username *Research* and password *Admin123*.

Performing pre-run checks

Before each sequencing run, perform the following checks:

- Check whether the remaining storage drive space is greater than 4.6 TB. If the remaining space is insufficient, clear history data according to *Reviewing parameters on Page 85*.
- Check the waste container, and fix any problems before sequencing:
  - If the waste level approaches 80% of the maximum volume of the waste container, empty the waste container.

    For details, refer to *Maintaining the waste container on Page 143*.
  - If the waste container icon turns  , empty the waste container.

    For details, refer to *Maintaining the waste container on Page 143*.
  - If the float of the waste level sensor is not properly placed at the lower position of the waste container, clean and move the sensor to the lower position.
  - If any problem occurs other than those listed above, restart the sequencer control software.

  If the problem persists, contact CG Technical Support.
- Check whether the environmental temperature and humidity meet the requirements in *Device specifications on Page 203*. Ensure that the temperature fluctuates within the specified range throughout the sequencing and that the humidity is constant.

Performing a regular wash

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

For details, refer to *Performing a regular wash (~60 min) on Page 136.*

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05

Sequencing

This chapter describes the sequencing workflow, sequencing and analysis, and post-sequencing procedures. Read and follow the instructions to ensure correct operations.

Workflow

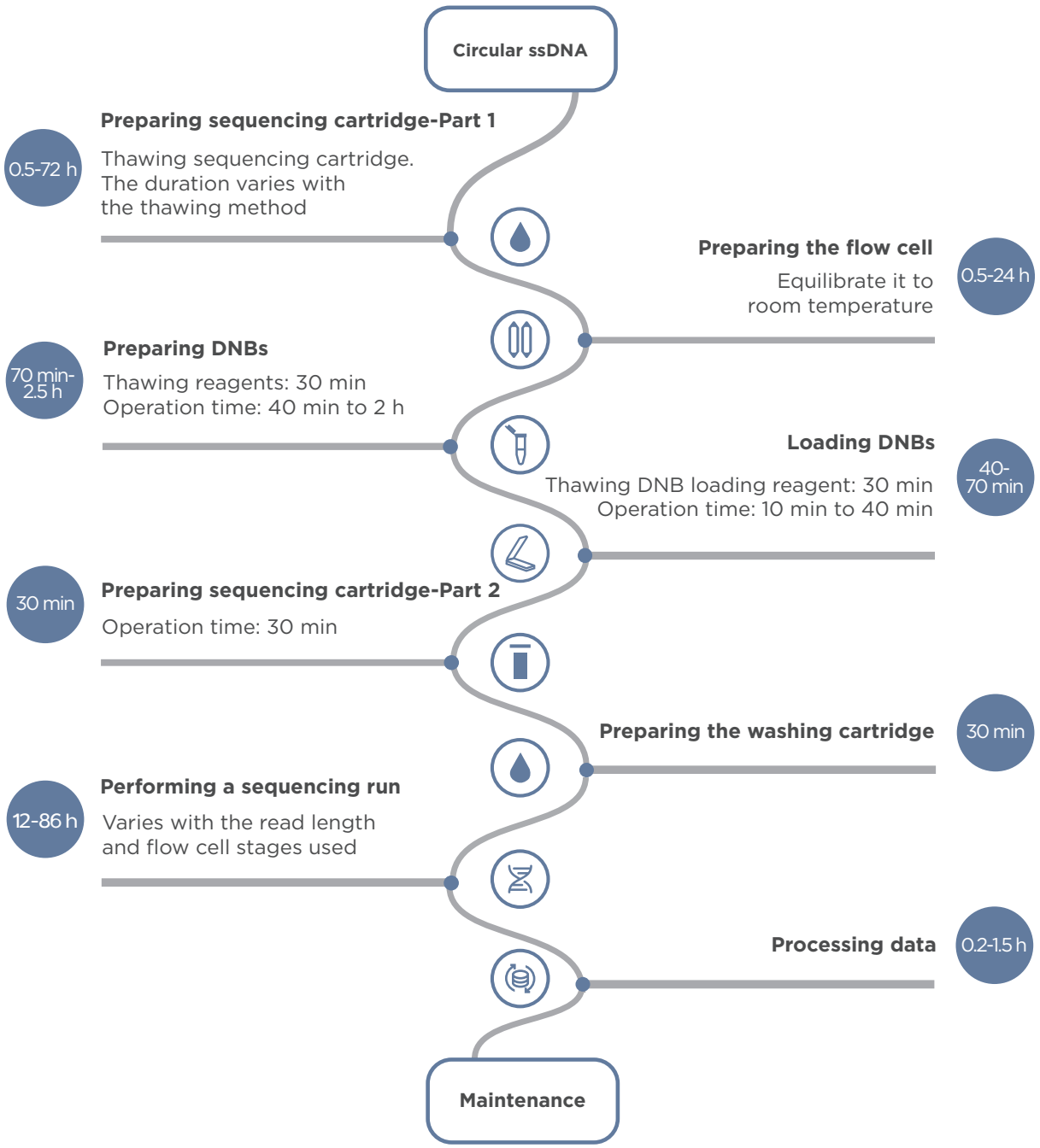


Figure 14 **Sequencing workflow**

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, a mask, gloves, and shoe covers) when using the device.
- If you accidentally splash reagents or waste liquids on the skin or into eyes, immediately flush the affected area with large amounts of water, and seek medical aid immediately.
- When disposing of expired reagents, waste liquids, waste DNBs, and consumables, comply with local regulations.

Preparing the Sequencing Reagent Cartridge-Part 1

Perform the following steps:

1. Remove the Sequencing Reagent Cartridge from storage.
2. Thaw the cartridge according to the following table. Once it is completely thawed, store it in a 2 °C to 8 °C refrigerator until use.

Table 8 Approximate thawing time for various sequencing kits

Model	Method		
	Water bath at room temperature (h)	Refrigerator at 2 °C to 8 °C overnight, then water bath at room temperature (h)	Refrigerator at 2 °C to 8 °C (h)
CM App-D FCL PE150	5.0	2.0	48.0
CM App-D FCL SE600	8.0	4.0	72.0

Preparing the flow cell

Perform the following steps:

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

   Do not open the outer plastic package yet.

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

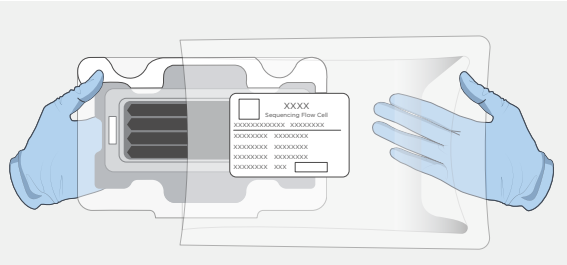


Figure 15 Unwrapping the outer plastic package

- If the flow cell is not used within 24 h after being placed at room temperature with the outer plastic packaging intact, the flow cell can be returned to storage at -25 °C to -15 °C. The freeze-thaw cycle between room temperature and the condition at -25 °C to -15 °C must not exceed 3 cycles.
- 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 h. If the storage limit of 24 h is exceeded, it is not recommended that you use the flow cell.

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

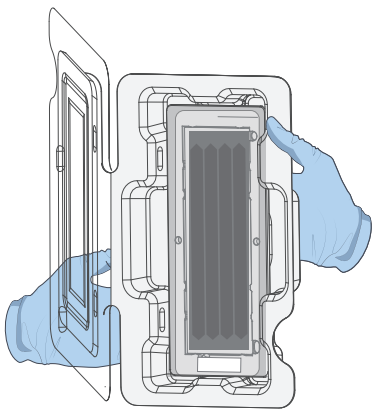


Figure 16 Checking the flow cell

Preparing DNBs

Recommended library insert size

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

Table 9 Conversion kit information

Kit name	Brand	Cat. No.	Reference document
DNBSEQ Universal Library Conversion Kit	CG	940-000963-00 (16 RXN)	DNBSEQ Universal Library Conversion Kit User Manual
DNBSEQ OneStep Library Conversion Kit (Third Party)	CG	940-001648-00	Dual-barcode PCR or PCR-free libraries with TruSeq or Nextera adapter

For instructions on using the kit, refer to the relevant Instructions for Use. For more details, contact CG Technical Support.

The recommended size distribution of inserts for FCL PE150 ranges between 200 bp and 400 bp, the recommended size distribution of inserts for FCL SE600 ranges between 600 bp and 1000 bp.

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

Table 10 Recommended library insert size

Model	Recommended library insert distribution (bp)
CM App-D FCL PE150	200 to 400
CM App-D FCL SE600	600 to 1000

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

DNA library concentration and amount requirements

DNB preparation starts from either a circular ssDNA library or dsDNA library with a recommended insert size between 200 bases and 1000 bases.

CAUTION For SE600 sequencing, DNB preparation starts solely from a circular ssDNA library. Please ask FAS for librabry preparation instruction

ssDNA library

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

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

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

- 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 Circular ssDNA library concentration requirement

Library type	Library concentration	Library required for each Make DNB reaction mixture
PCR libraries	≥ 2.5 fmol / µL	20 fmol
PCR-free libraries	≥ 3.75 fmol / µL	30 fmol

dsDNA library

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

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

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

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

The initial dsDNA library concentration should be no less than 4 fmol/µL.

Making DNBs from ssDNA

- Mixed use of reagent components from different batches is not recommended.
- Avoid making and loading DNBs with filtered pipette tips. It is recommended that you use the pipettes and tips from recommended brands and catalog numbers.

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. Remove the reagents from storage according to your model:
   - For PE150, remove Low TE Buffer, App Make DNB Buffer, Make DNB Rapid Enzyme Mix III, and Stop DNB Reaction Buffer from storage and thaw the reagents for approximately 30 min.
   - For SE600, remove Low TE Buffer, App Make DNB Buffer II, Make DNB Rapid Enzyme Mix IIIII and Stop DNB Reaction Buffer from storage. Thaw the reagents for approximately 30 min.
3. Mix the reagents by using a vortex mixer for 5 s. Centrifuge briefly and place on ice until use.

Selecting DNB loading method

Each sequencing flow cell has 4 lanes, and different DNBs can be loaded into different lanes. Select one of the following methods for loading DNBs onto the sequencing flow cell.

- Using the sequencer to load DNBs
- Using DL-200H to load DNBs

Table 12 Required number of make DNB reactions for each flow cell

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

If you need to prepare 100 µL of make DNB reaction, double the volumes of the library and reagents based on those for preparing 50 µL of make DNB reaction.

Calculating the required amount of ssDNA libraries

The required volume of ssDNA libraries is determined by the required library amount (fmol) and library concentration quantified in *DNA library concentration and amount requirements on Page 53*.

- If there are any special requirements or specifications for the CG library preparation kit, then the requirements of the kit should be followed.
- All samples should be considered potentially infectious and should be handled in accordance with relevant national and local regulations.
- *c* in the following table represents the concentration of libraries (fmol/μL).

Table 13 Required amount of ssDNA libraries

Library type	Volume of 50 μL DNB reaction (μL)
PCR libraries	V = 20 fmol / *c*
PCR-free libraries	V = 30 fmol / *c*

Calculate the required ssDNA libraries for each Make DNB reaction and fill it in *Table 17 on Page 56* as *V*.

Making DNBs (FCL PE150)

Perform the following steps:

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

Table 14 Make DNB reaction mixture 1

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

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

Table 15 Primer hybridization reaction conditions

Temperature	Time
65 °C (Heated lid)	On
60 °C	2 min
40 °C	1 min
4 °C	Hold

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

   - Do not keep Make DNB Enzyme Mix II (HF+LC) at room temperature.
   - Do not hold the tube to avoid enzyme inactivation caused by high temperature.

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

Table 16 Make DNB reaction mixture 2

Component	Cap color	Volume for 50 µL DNB reaction (µL)
Make DNB Rapid Enzyme Mix III		20
Make DNB Enzyme Mix II (HF+LC)		2

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

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

Table 17 RCR (Rolling Circle Replication) conditions

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

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

Component	Cap color	Volume for 50 µL DNB reaction (µL)
Stop DNB Reaction Buffer		10

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

Making DNBs (FCL SE600)

Perform the following steps:

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

Table 18 Make DNB reaction mixture 1 (SE600)

Component	Cap color	Volume for 50 µL DNB reaction (µL)
Low TE Buffer		8 - V
App Make DNB Buffer II		12
ssDNA libraries	/	V
Total volume	/	20

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

Table 19 Primer hybridization reaction conditions

Temperature	Time
65 °C (Heated lid)	On
60 °C	2 min
40 °C	1 min
4 °C	Hold

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

   - Do not keep Make DNB Enzyme Mix II (HF+LC) at room temperature.
   - Do not hold the tube to avoid enzyme inactivation caused by high temperature.

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

Table 20 Make DNB reaction mixture 2 (SE600)

Component	Cap color	Volume for 50 µL DNB reaction (µL)
Make DNB Rapid Enzyme Mix IIIII		22.5
Make DNB Enzyme Mix II (HF+LC)		2

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

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

Table 21 RCA conditions (SE600)

Temperature	Time
35 °C (Heated lid)	On
30 °C	100 min
4 °C	Hold

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

Component	Cap color	Volume for 50 µL DNB reaction (µL)
Stop DNB Reaction Buffer		8

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

Making DNBs from dsDNA library

Selecting a DNB make reagent kit

If you use CG Library Prep Kit to prepare PCR libraries or third-party libraries, you can directly use CG DNBSEQ OneStep DNB Make Reagent Kit V4.0 to make DNBs without the need for phosphorylation and circularization. Use the corresponding kit according to the following table:

Table 22 DNBSEQ OneStep DNB Make Reagent Kit list

Kit name	Brand	Cat. No.	Applicable library
DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode)	CG	940-001750-00	Dual-barcode PCR or PCR-free libraries with CG adapter

Calculating the required amount of dsDNA libraries

The required volume of dsDNA libraries is determined by the required library amount (fmol) and library concentration quantified in *dsDNA library on Page 54*.

- For details on the one-step DNB making method, refer to the DNBSEQ OneStep DNB Make Reagent kit User Manual. Notably, the volume of Make DNB mixture for this platform is 50 µL, so the library input and volumes of reagents for Make DNB mixture should be reduced by half.
- If there are any special requirements or specifications for the CG library preparation kit, then the requirements of the kit should be followed.
- All samples should be considered potentially infectious and should be handled in accordance with relevant national and local regulations.
- *c* in the following table represents the concentration of libraries （fmol/µL).

Table 23 Required amount of dsDNA libraries

Library type	Product name	Volume of 50 µL DNB reaction (µL)
CG	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode)	$V = 90\ \text{fmol} / c$
CG	DNBSEQ OneStep DNB Make Reagent Kit (Third Party*)	$V = 90\ \text{fmol} / c$

* DNBSEQ OneStep DNB Make Reagent Kit (Third Party) is compatible with third-party dsDNA libraries that include either TruSeq or Nextera adapters.

Quantifying DNBs

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

Table 24 DNB concentration standard

Model	DNB concentration
CM App-D FCL PE150	≥8 ng/µL
CM App-D FCL SE600	≥25 ng/µL

- For PE150, if the concentration of libraries prepared by customers is lower than 8 ng/µL, refer to *Q: What should I do if the DNB concentration is low? on Page 155* for details. If the concentration exceeds 40 ng/µL, the DNBs should be diluted to 20 ng/µL with DNB Load Buffer I.
- For SE600, if the concentration is lower than 25 ng/µL, re-make DNBs.
- If there are too many samples in a single test, it is recommended that you quantify in batches to avoid inaccurate DNB quantification due to fluorescence quenching.

Loading DNBs

Loading DNBs by using the sequencer

Preparing reagents

Perform the following steps:

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

   If crystal precipitation is visible in DNB Load Buffer II, vigorously mix the reagent for 1 to 2 min by using a vortex mixer to re-dissolve the precipitation before use.
3. Take out four 0.5 mL Micro Tubes from the sequencing kit. Label the tubes clearly with the lane number, and add the following reagents into each tube.

Table 25 DNB loading mixture 1

Component	Volume (µL)/lane
DNB Load Buffer II	25
Make DNB Enzyme Mix II (HF+LC)	0.5
DNBs	50
Total volume	75.5

4. Gently pipette 8 times to mix DNB loading mixture 1 by using a wide-bore, non-filtered pipette tip. Place the mixture at 2 °C to 8 °C until use.

   - Do not centrifuge, vortex, or shake the tube.
   - Prepare a fresh DNB loading mixture 1 immediately before the sequencing run.
   - Each lane requires 75.5 µL of DNB loading mixture 1.

Loading DNBs

Perform the following steps:

1. Open the reagent compartment door and pull out the tube rack by using the handle.

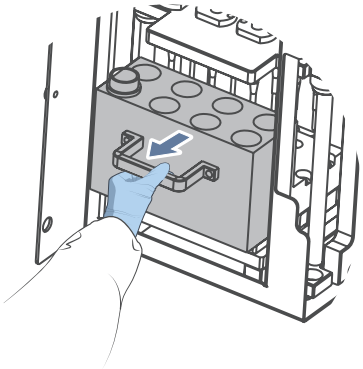


Figure 17 Pulling out the tube rack

2. Gently lift the DNB loading needle with one hand while removing the cleaning reagent tube with the other hand. Load the sample tube prepared in *Preparing reagents on Page 63*. Slowly lower the DNB loading needle until the tip reaches the bottom of the tube.

   Perform this step if you load DNBs on the sequencer. If not, use an empty tube in place of the sample tube.

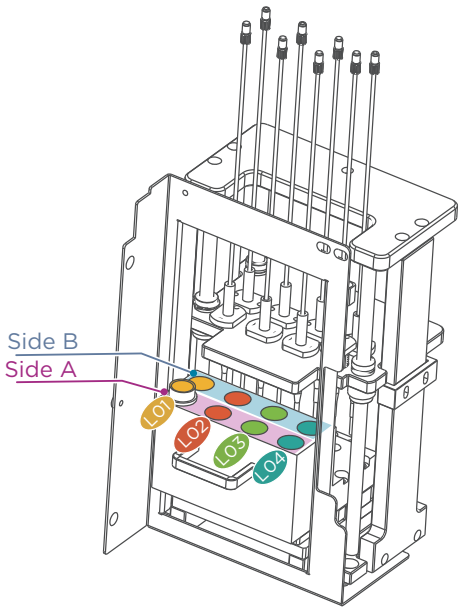


Figure 18 Loading the DNB tube

3. Close the reagent compartment door.

Loading DNBs by using DL-200H

Preparing the flow cell and reagents

Perform the following steps:

1. Take out the flow cell from -25 °C to -15 °C storage and place it at room temperature for 1 h to 24 h before using.
2. Take out 4 new 0.5 mL Micro Tubes and add the reagents shown in the table below:

Table 26 DNB loading mixture 2

Component	Cap color	Volume (µL)
DNB Load Buffer II		12.5
Make DNB Enzyme Mix II (HF+LC)		0.25
DNBs	/	25
Total volume	/	37.75

3. Gently pipette 8 times to mix DNB loading mixture 2 by using a wide-bore, non-filtered pipette tip. Place the mixture at 2 °C to 8 °C until use.

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

Loading DNBs

Ensure that DL-200H is properly maintained and that the sealing gasket of DL-200H is clean and properly maintained. If necessary, perform maintenance according to *DL-200H and sealing gasket maintenance on Page 145*.

Perform the following steps:

1. Install the sealing gasket and the flow cell.

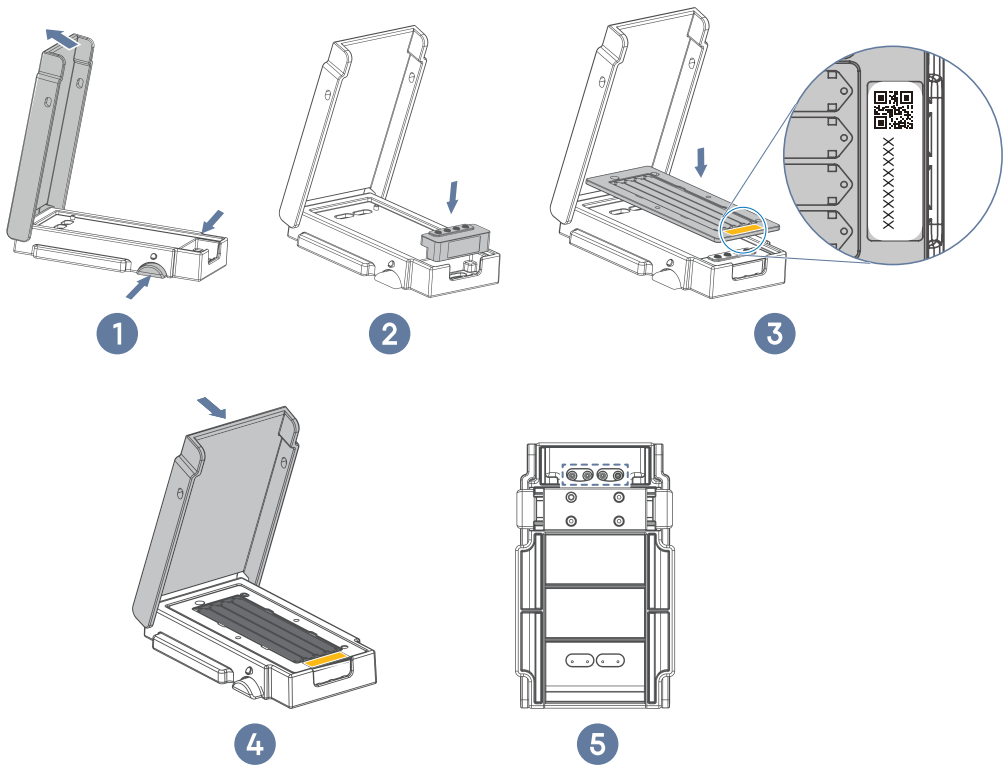


Figure 19 Installing sealing gasket and flow cell

1) Squeeze the latches and open the cover as shown above.
2) Place a clean sealing gasket into the groove and ensure that the gasket surface is even.
3) Align the holes of the flow cell with the alignment pins of the device and place the flow cell on it.

   - Ensure that the label of the flow cell is facing up and in the same position as the sealing gasket.
   - For the label location on the flow cell, refer to the area marked in yellow in *Figure 19 on Page 66* .

4) Close the cover and ensure that it is securely closed.
5) With the back of the DL 200H facing up, verify that the fluidics inlets align with the holes of the sealing gasket and ensure that the holes are clean.

2. Load DNBs by DL-200H, starting from Lane No. 1 and ending with Lane No. 4.

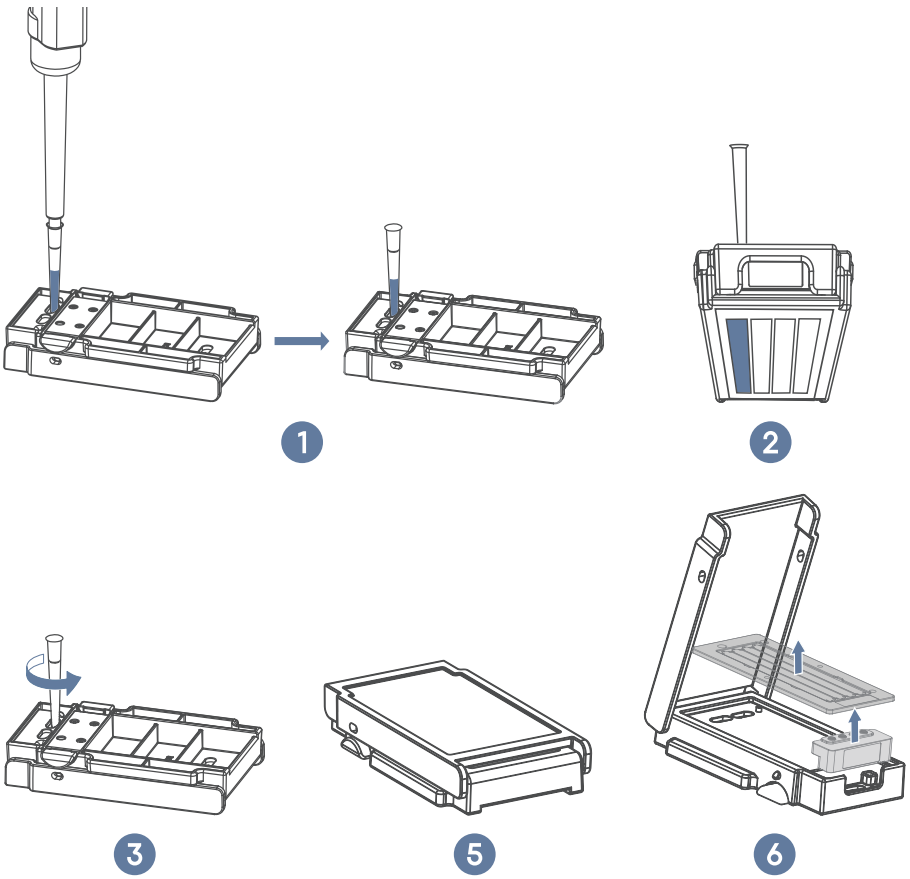


Figure 20 Loading DNBs by using DL-200H

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

   - Do not press the plunger of the pipette.
   - Do not touch or move the tip when ejecting. Doing so may bring bubbles into the flow cell.

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

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

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

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

4) Repeat steps 1) through 3) to load the DNBs to the rest of the lanes of the flow cell sequentially.

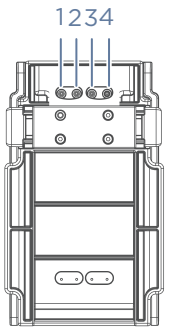


Figure 21 Lane order of DNB loading

5) Place the DL-200H on the bench with the front facing up. Incubate the flow cell at room temperature for 10 min after loading.

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

3. After the DNB loading process has completed, immediately transfer the flow cell to the sequencer for sequencing. After you have prepared the Sequencing Reagent Cartridge, perform the sequencing run according to *Performing a sequencing run on Page 77.*

Preparing the Sequencing Reagent Cartridge-Part2

Sequencing enzyme and dNTP mixes are provided in different tubes and are packaged together with the Sequencing Reagent Cartridge. Before the sequencing run starts, an appropriate amount of sequencing enzyme and dNTP mixture needs to be added to well No. 18 of the Sequencing Reagent Cartridge. Dye Mix I needs to be added to well No. 9 and Dye Mix II needs to be added to well No. 10. If prepared reagent cartridges are not used immediately, refer to *Q: What rules should I follow if I need to store reagent kit temporarily? on Page 156*.

1. Invert the cartridge 3 times to mix before use.
2. Wipe any water condensation from the cartridge cover and wells surround with a KimWipes tissue.

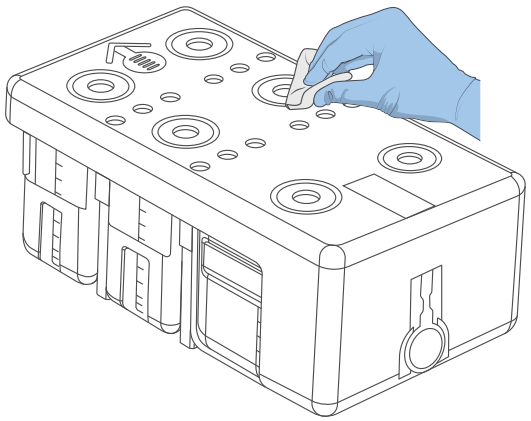


Figure 22 Wiping cartridge cover

Well positions are shown in the figure below.

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

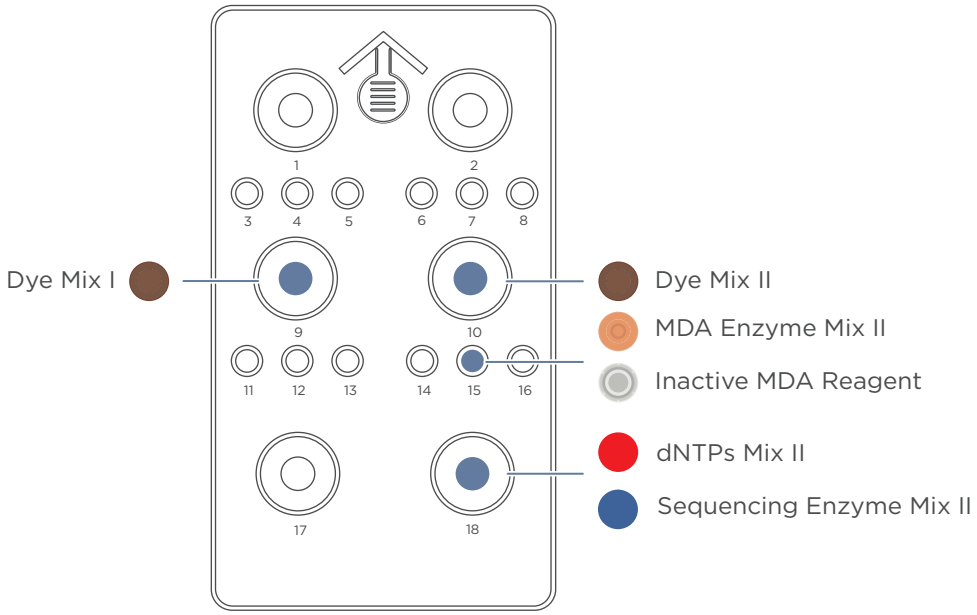


Figure 23 Well positions (PE150)

Well No. 1 is empty.

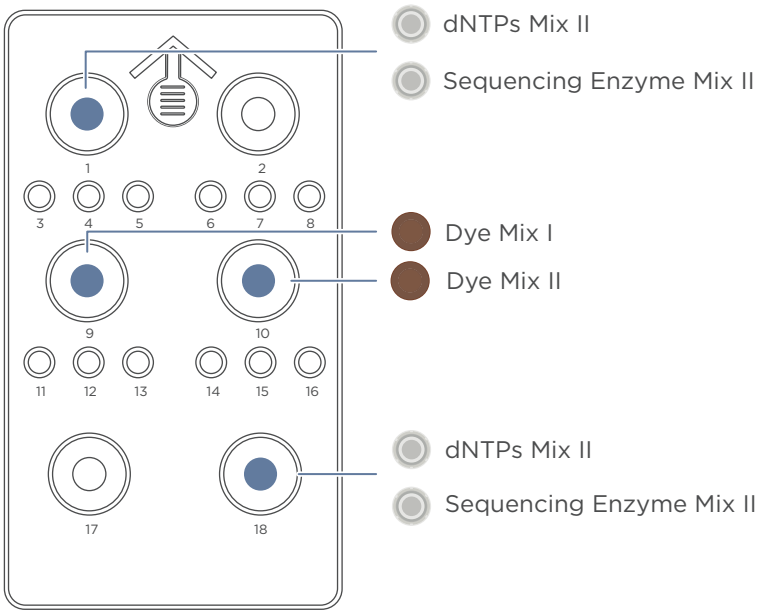


Figure 24 Well positions (SE600)

3. Remove dNTPs Mix II from -25 °C to -15 °C storage 1 h in advance and thaw it at room temperature. After it has thawed, store it at 2 °C to 8 °C until use. Vortex the reagent thoroughly by using a vortex mixer and centrifuge it for 5 s.

4. Remove Dye Mix I, Dye Mix II, and Sequencing Enzyme Mix II from storage at -25 °C to -15 °C. Place them on ice or store at 2 °C to 8 °C until use. Invert the reagents 6 times before use.

   Do not vortex Dye Mix I or Dye Mix II.

5. Pierce the seals in the center of wells No. 9, No. 10, and No. 18 to make a hole approximately 2 cm in diameter by using a 1 mL sterile pipette tip.

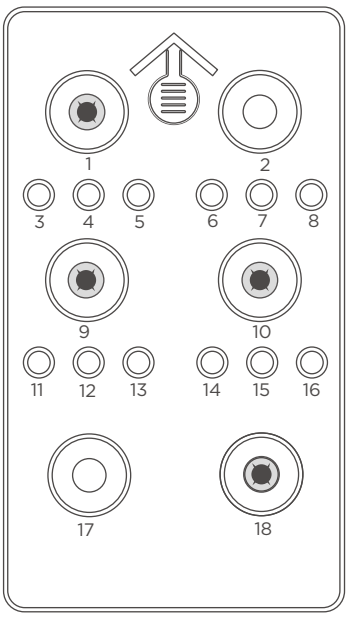


Figure 25 Piercing the seal of cartridge

6. Invert Dye Mix I 6 times and add it into well No. 9 by using a pipette according to the following table.

   When transferring Dye Mix I, operate carefully to prevent Dye Mix I from spilling out of the reagent tube.

Table 27 Reagent preparation for well No. 9

Model	Dye Mix I loading volume (mL)
FCL PE150	5.60
FCL SE600	16.80

7. Invert Dye Mix II 6 times and add it into well No. 10 by using a pipette according to the following table.

   When transferring Dye Mix II, operate carefully to prevent Dye Mix II from spilling out of the reagent tube.

Table 28 Reagent preparation for well No. 10

Model	Dye Mix II loading volume (mL)
FCL PE150	4.90
FCL SE600	14.70

8. Mix dNTPs Mix II by using a vortex mixer for 5 s and centrifuge it briefly. Invert Sequencing Enzyme Mix II 6 times. Add dNTPs Mix II and Sequencing Enzyme Mix II into a 50 mL sterile tube by using a pipette according to the following table.

Table 29 Reagent preparation for well No. 18

Model	dNTPs Mix II loading volume (mL)	Sequencing Enzyme Mix II loading volume (mL)
FCL PE150	12.32	11.56
FCL SE600	14.72	13.80

Table 30 Reagent preparation for well No. 1

Model	dNTPs Mix II loading volume (mL)	Sequencing Enzyme Mix II loading volume (mL)
FCL SE600	7.04	6.6

9. Invert the tube 6 times to mix the reagents in the tube and add the reagents into well No. 1 and well No. 18.

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

10. Seal the loading wells No. 1, No. 9, No. 10, and No. 18 with the provided transparent sealing films.

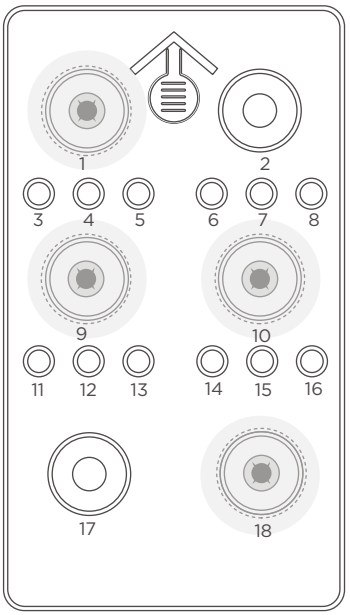


Figure 26 Sealing the loading wells of the cartridge

11. Press the film around the well with your finger, ensuring that the well is tightly sealed and that no air bubbles exist between the film and cartridge surface.

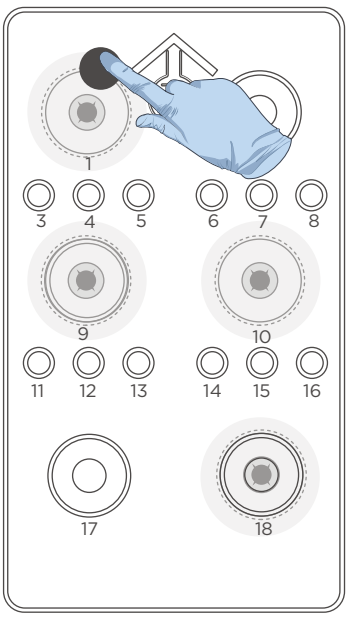


Figure 27 Sealing the loading wells of the cartridge tightly

12. Lift the cartridge horizontally and hold both sides of the cartridge with both hands. Shake the cartridge 20 times in a clockwise and counterclockwise direction until the color of the reagent in the upper level is the same as that in the bottom level in wells No. 9 and No. 10. Ensure that reagents are fully mixed.

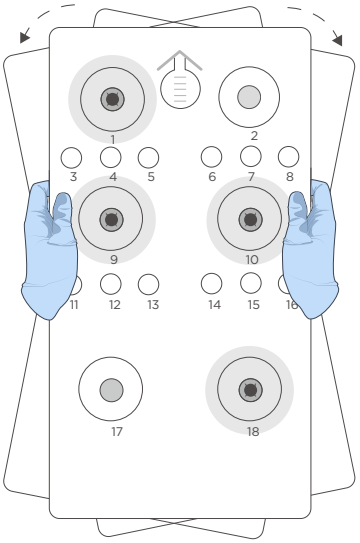


Figure 28 Mixing reagents after loading

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

- Do not reuse the waste seals.
- To avoid cross contamination, ensure that the surface around wells No. 1, No. 9, No. 10, and No. 18 is clean.

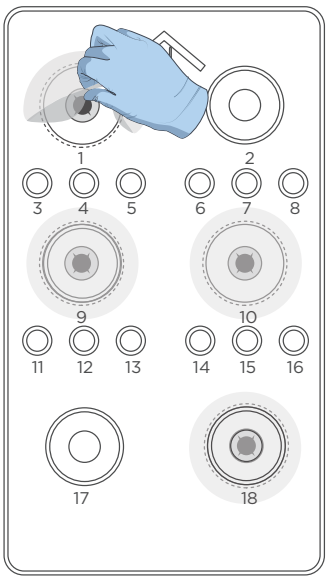


Figure 29 Removing the seal from cartridge

14. Gently tap the cartridge on the bench to reduce air bubbles in the reagents.

    - The FCL SE600 Sequencing Reagent Cartridge is now ready for use.
    - For the next step, refer to *Performing a sequencing run on Page 77*.

15. Perform the following steps for PE sequencing:

    1) Pierce the seal of well No. 15 by using a 1 mL sterile pipette tip.
    2) Add 230 µL of MDA Enzyme Mix II to the Inactive MDA Reagent tube with a 1 mL pipette.

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

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

       - When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.
       - The FCL PE150 Sequencing Reagent Cartridge is now ready for use.

Preparing the washing cartridge

CAUTION

- The washing cartridge is required for sequencing and must be used in pairs with the Sequencing Reagent Cartridge. Ensure that the washing cartridge used matches the corresponding Sequencing Reagent Cartridge.
- The washing cartridge and reagents used for sequencing can be subsequently used as the washing cartridge for a single maintenance wash without changing reagents.

Perform the following steps:

1. Shake the washing cartridge taken out from the corresponding sequencing kit vigorously clockwise 10 times, and then counterclockwise 10 times. Ensure that washing reagents are fully mixed.
2. Spray a small amount of 75% ethanol on the surface of each well. Use a KimWipes tissue or a low-lint cloth to dry.

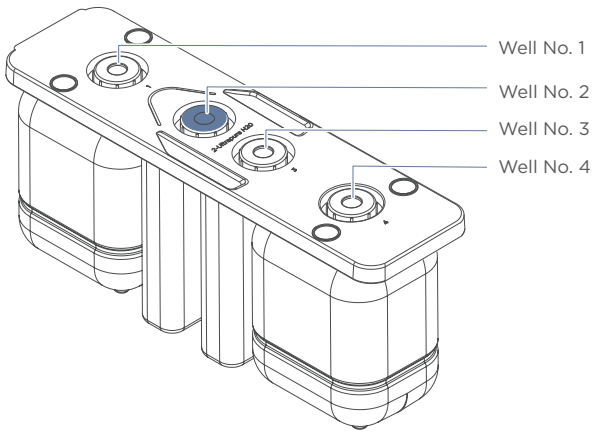


Figure 30 Washing cartridge

3. Pierce the seals in the center of well No. 2 and well No. 3 to make holes approximately 2 cm in diameter by using a 1 mL sterile pipette tip.
4. Add 200 mL of Laboratory-grade water into well No. 2, and add 100 mL of 0.1 M NaOH into well No. 3. Preparing 0.1 M NaOH according to the table below:

Table 31 Preparing 0.1 M NaOH

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

Performing a sequencing run

Loading the Sequencing Reagent Cartridge

Perform the following steps:

1. Open the reagent compartment door.
2. Moisten KimWipes tissue or a low-lint cloth with laboratory-grade water. Use it to wipe the bottom and inner walls of the compartment to keep it clean and dry.

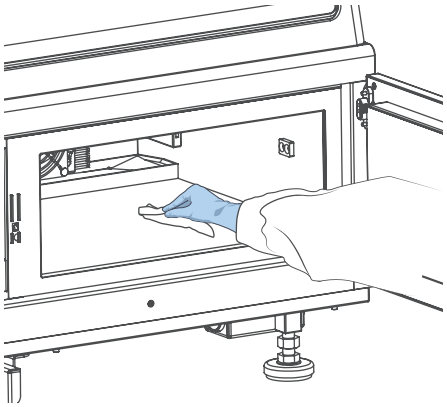


Figure 31 **Maintaining the reagent compartment**

3. Slide the reagent cartridge into the compartment gently by following the direction printed on the cover until it stops.

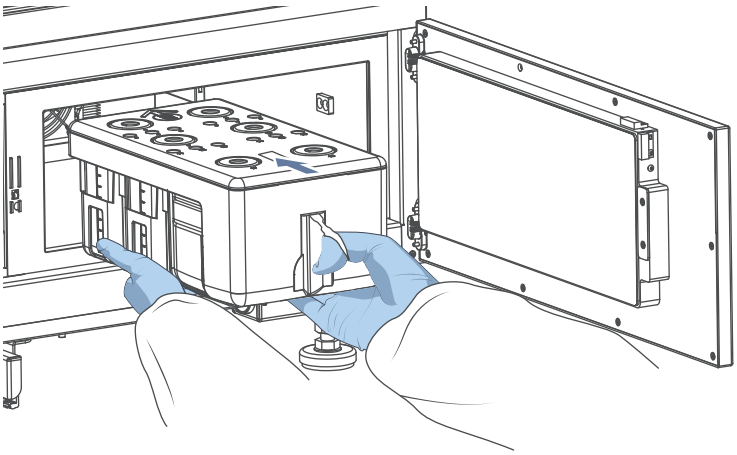


Figure 32 **Sliding a new Sequencing Reagent Cartridge into the reagent compartment**

4. Close the reagent compartment door.

Loading the flow cell

The canned air duster can be replaced by a low-lint cloth moistened with 75% ethanol to remove dust in the following steps.

Perform the following steps:

1. Open the flow cell compartment door.
2. Use a canned air duster to remove the dust on the flow cell stage.

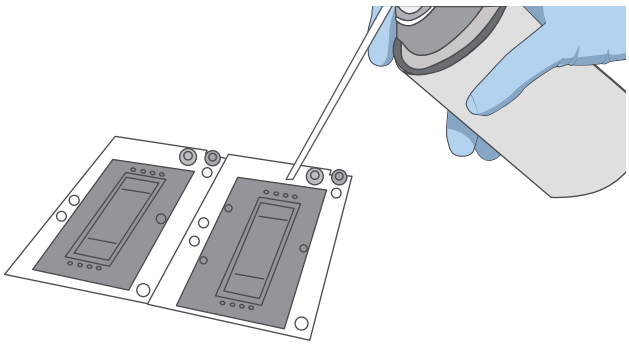


Figure 33 Cleaning the flow cell stage

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

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

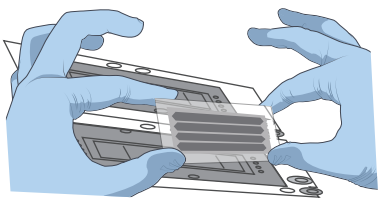


Figure 34 Loading the flow cell

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

The flow cell is fragile. Handle it with care.

7. Ensure that the negative pressure, depending on site altitude, is within the range of -80 kPa to -99 kPa before continuing.

Table 32 Negative pressure ranges for different altitudes

Site altitude	Upper limit	Lower limit
0 m to 500 m	-80 kPa	-99 kPa
500 m to 1500 m	-75 kPa	-95 kPa
1500 m to 2500 m	-60 kPa	-80 kPa
2500 m to 3500 m	-55 kPa	-70 kPa

If the negative pressure is abnormal, refer to *Q: What should I do if abnormal negative pressure appears during flow cell attachment? on Page 156*.

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

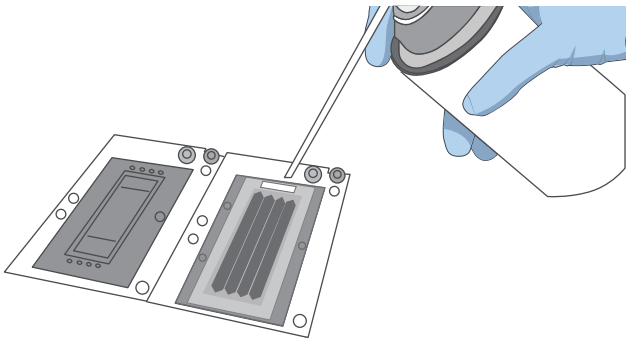


Figure 35 Cleaning the flow cell

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

CAUTION

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

Loading the washing cartridge

Perform the following steps:

1. Slide the crossbar of the external cleaning module to the top to raise the needle.

   i Do not raise the needle if either stage A or stage B is running.

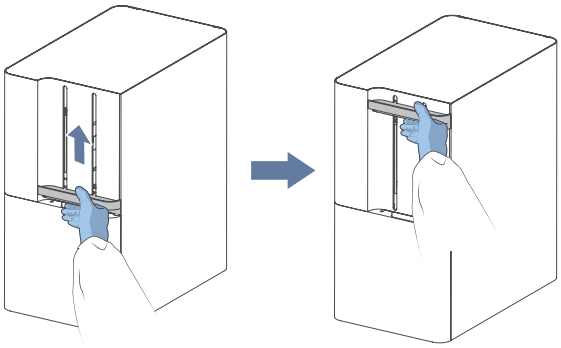


Figure 36 Sliding the crossbar to the top

2. Hold the groove and pull the washing cartridge compartment out until you encounter resistance.

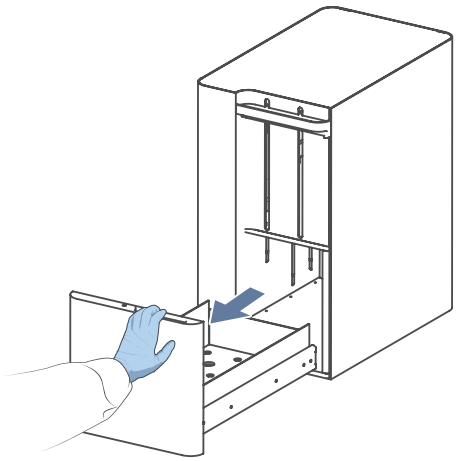


Figure 37 Pulling out the washing cartridge compartment

3. Hold the prepared Washing cartridge by the edges with both hands, place it into the compartment, and push the compartment in until it stops moving.

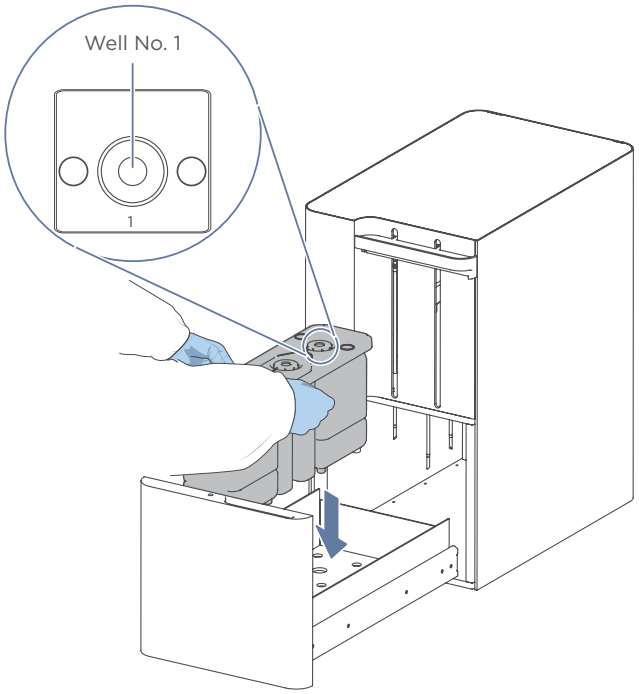


Figure 38 Placing the Washing cartridge into the compartment

4. Slide the circle slider to the right until it stops moving.

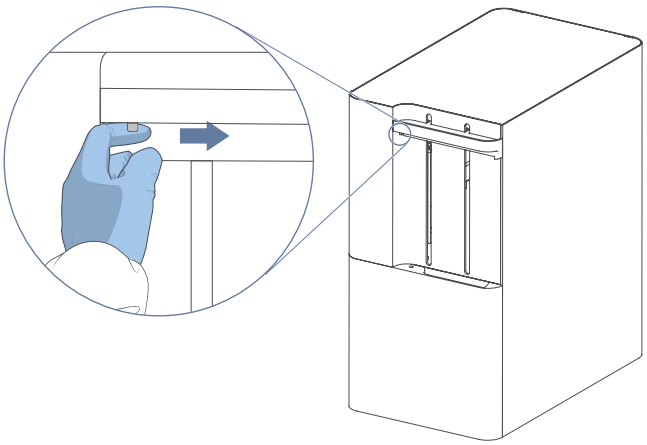


Figure 39 Sliding the circle slider to the right

5. Slide the crossbar to the bottom.

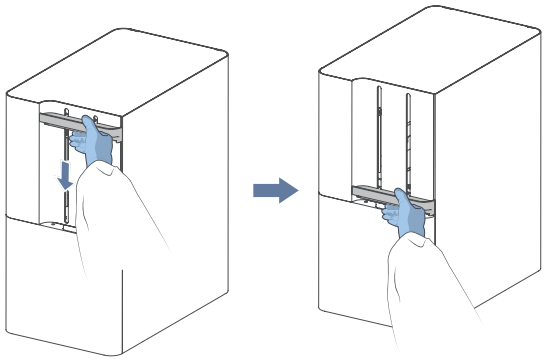


Figure 40 Sliding the crossbar to the bottom

Entering run information

Perform the following steps:

1. In the main interface, select **Sequence**. The following interface is displayed.

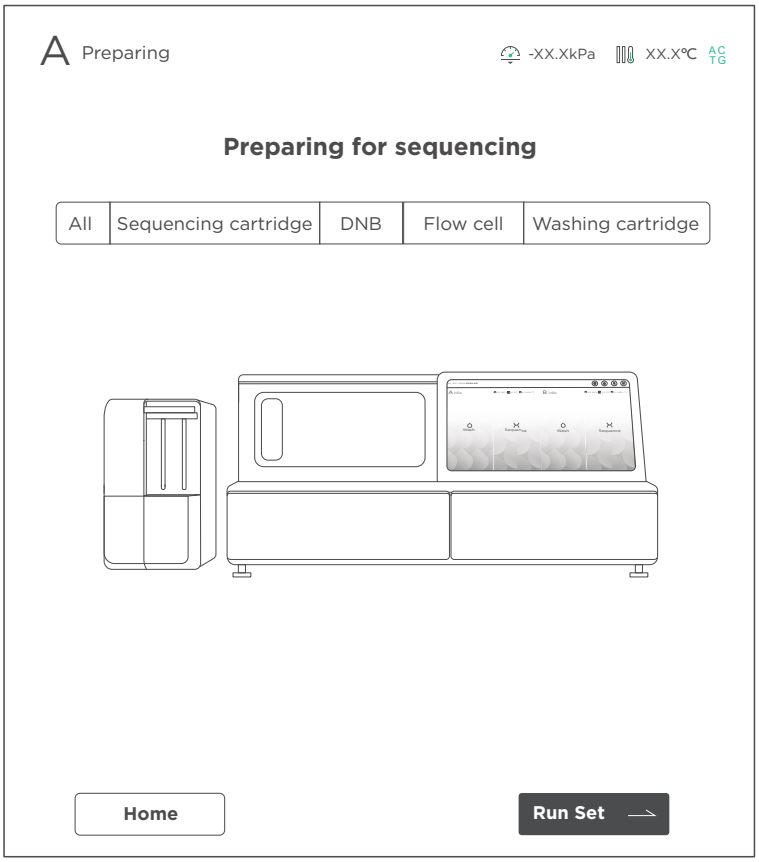


Figure 41 Loading sequencing material

2. Load sequencing materials according to the video guide in the interface.
3. Select **Run Set** to open the Run setting interface:

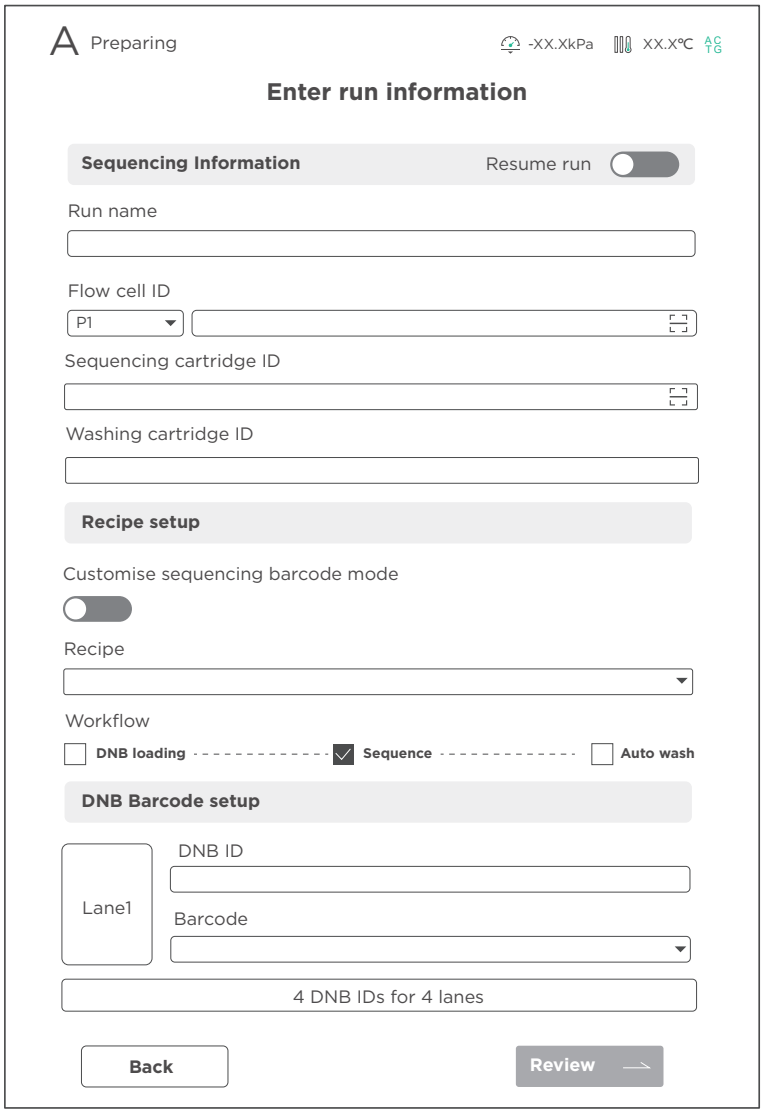


Figure 42 Run setting interface

4. In the Run setting interface, keep the **Resume run** at status. Enter a run name.
5. Select on the **Flow cell ID** box to scan the ID on the flow cell or manually enter the flow cell ID by using the on-screen keyboard.

   When entering the ID manually, only letters, numbers and a middle bar (-) are allowed. Otherwise, an ID error appears and the operation cannot continue.

6. Select on the **Sequencing cartridge ID** box to scan the ID on the Sequencing Reagent Cartridge. If ID is not scanned automatically, use a handheld scanner to scan the 2D Barcode on labels, or manually enter the sequencing cartridge SN by using the on-screen keyboard.

   - There are two labels on the sequencing cartridge. Both labels contain the 2D Barcodes to scan with handheld scanner, on top left of the labels.
   - When entering the SN manually, only letters, numbers and an underscore "_" are allowed. Otherwise, an ID error appears and the operation cannot continue.

7. Select the **Washing cartridge ID** box, and use a handheld scanner to scan the 2D Barcode on the prepared washing cartridge, or manually enter the washing cartridge SN by using the on-screen keyboard.

   When entering the SN manually, only letters, numbers and an underscore "_" are allowed. Otherwise, an ID error appears and the operation cannot continue.

8. Select an appropriate recipe from the **Recipe** list.

   - There is no predefined sequencing recipe in the **Recipe** list. For first-timers, select **Customize** from the **Recipe** list to create recipes for use. These recipes can be subsequently selected from the **Recipe** list.
   - After you select the recipe from the **Recipe** list, the recipe will be automatically selected in the **Flow cell ID** list.
   - If you want to customize the recipe, select **Customize** from the Recipe list. For details, refer to *Instructions for customizing a run on Page 167.*

9. (Optional) For a dual barcode sequencing run, set **Double-index barcode** to the right if Barcode and Dual barcode need to be combined into a dual barcode file.
10. Select **DNB loading** in the process bar if you want to load DNBs by using the sequencer. If not, leave it in blank.

    Do not select **Auto wash** in the process bar.

11. Enter DNB ID(s) and select the barcode range.

    - Select **4 DNB IDs for 4 lanes** and enter the DNB ID of 4 lanes if the DNB ID of four lanes is not the same.
    - Select **No barcode** from the **DNB ID** box if you do not want to split the barcode.
    - If the **DNB ID** box is left in blank, the barcode will be split by default.

    1) To set a same DNB ID for 4 lanes, select the DNB ID box and Barcode box of Lane1, and enter the DNB ID and barcode range manually by using the on-screen keyboard.

2) To set different DNB IDs for 4 lanes, select **4 DNB IDs for 4 lanes**. Select the DNB ID boxes and Barcode boxes of **Lane1** to **Lane4**, and enter the DNB ID and barcode range of lane 1 to lane 4 manually by using the on-screen keyboard.

12. Select **Review**.

Reviewing parameters

To ensure sequencing quality, when Read1 and Read2 sequencing is completed, the sequencer will automatically perform another cycle for calibration. For example, for PE150 sequencing, the length of Read1 is 150, the length of Read2 is 150, and the length of barcode is 10. Adding 1 correction cycle for Read1 and 1 correction cycle for Read2 (barcode does not need to be corrected), the total number of sequencing cycles is 312.

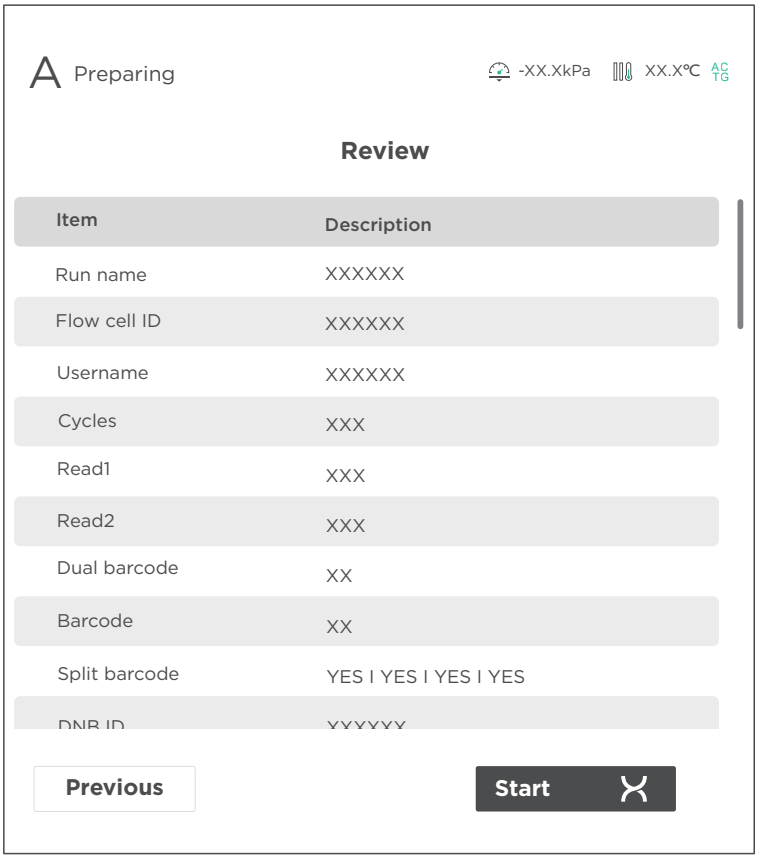


Figure 43 **Reviewing information**

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

- If you find errors, select **Previous** to return to the previous interface and reset.

- If all parameters are correct, select **Start**. The software automatically checks the available storage drive space:
  - If the storage space is sufficient, confirm the information in the pop-up dialog box. Select **Yes** to start the sequencing run.
  - If the storage space is insufficient, perform the following steps:
    a. In the pop-up dialog box, select the data that you want to delete and then select **Clear history data**.
    b. When the storage space icon turns green, select **Previous** to return to the parameter review interface, and then select **Start**.

Starting sequencing

Perform the following steps:

1. When sequencing has started, wait a few minutes (11 min for loading by the sequencer or 1 min for loading by the DL-200H) before you open the flow cell compartment door to check the flow cells, ensure that DNBs or reagents are flowing through the flow cell, and close the compartment door.

   If DNBs or reagents are not flowing through the flow cell, fix the problems before you restart sequencing. For details, refer to *Q: What should I do if pumping failure occurs during DNB loading and sequencing? on Page 157.*

   CAUTION
   - Do not bump, move, vibrate, or impact the device during sequencing. Because it may cause inaccurate sequencing results.
   - If malfunctions related to fluidics lines (for example, bubbles) occur during sequencing, fix the problems before you restart sequencing. For details, refer to *Sequencer FAQs on Page 148.*
   - Pay special attention to the LED status indicator or the on-screen instructions. If errors occur, troubleshoot the problem by following the instructions and this guide. If errors persist, contact CG Technical Support.

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

Table 33 Sequencing interface description

Item	Description
Run name	Shows this sequencing run name.
Expected end time	Shows the expected end time for sequencing.

Item	Description
Phase	Shows the current phase of sequencing.
Step	Shows the current step and total sequencing steps.
	Select this button and select **Yes** in the confirmation dialog box to stop sequencing.
	Select to pause sequencing. Select  to resume the sequencing that has been paused.
	Select to view the run name, expected end time, phase, and step.
	Select a QC value graph from the QC type list to assess the sequencing quality.
	Select this button to open the Review interface and check the sequencing information.
	After imaging of the first base is completed, select this button to open the first base report.
	Select to view the summary report after the sequencing is completed.
	Select to raise the needles after pausing the sequencing.
	Select to finish the sequencing and return to the main interface.

When the interface shows that the device is processing data or is in idle status, it means that the sequencing has finished.

(Optional) Continuing a stopped sequencing run

Perform the following steps:

- Resuming a run is only applicable to the flow cell on the sequencer whose run is not completed.
- The run cannot be resumed if it is stopped in post-loading, PE synthesis, cleavage, barcode synthesis, or dual barcode synthesis.

1. In the Run setting interface, set the **Resume run** button to status. The following interface is displayed.

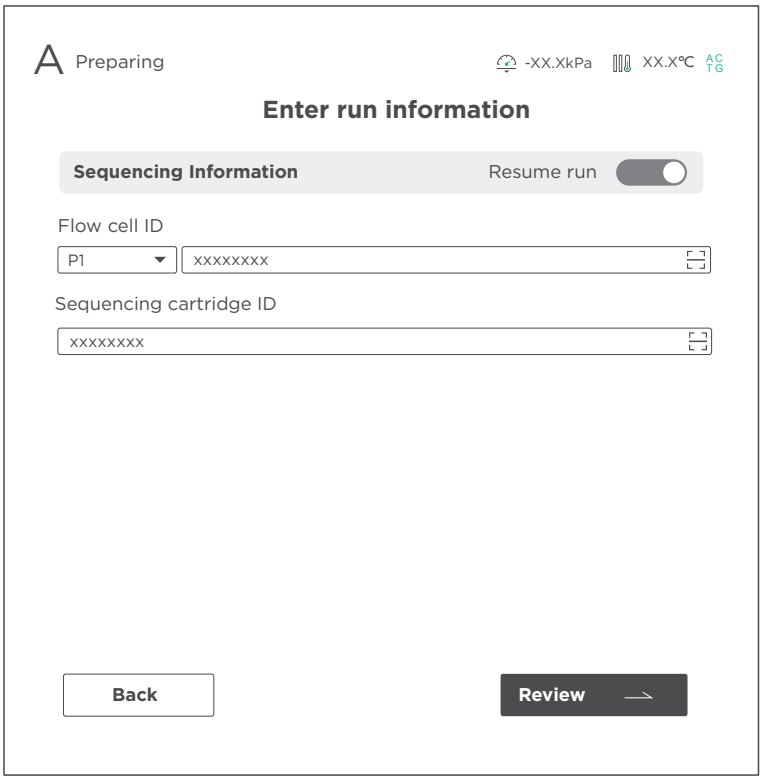


Figure 44 Resuming the sequencing run

2. Use either method below to enter the flow cell ID and the sequencing cartridge ID:

   - The flow cell ID and the sequencing cartridge ID entered should be the same as the IDs of the paused sequencing run.
   - When entering the ID manually, only letters, numbers, and middle bar "-" are allowed. Otherwise, an ID error appears and the operation cannot continue.

   - Select the **Flow cell ID** box and **Sequencing Cartridge ID** box and manually enter the flow cell ID and sequencing cartridge ID by using the on-screen keyboard.
   - Select to scan the ID on the flow cell and the Sequencing Reagent Cartridge.

3. Select **Review**. Proceed to *Reviewing parameters on Page 85.*

(Optional) Performing a maintenance wash

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

For details, refer to *Performing a maintenance wash (~100 min) on Page 138.*

Disposing of the Sequencing Reagent Cartridge and flow cell

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

Perform the following steps:

1. Wear protective equipment.
2. Open the flow cell compartment and remove the flow cell:
   1) Hold the flow cell by the edges with one hand to prevent the flow cell from falling into the device, and to prevent damage to the flow cell.
   2) Press the flow cell attachment button with the other hand to release the flow cell.
   3) Remove the flow cell.
3. Open the reagent compartment door, pull out the Sequencing Reagent Cartridge by using the pull ring, and remove the cartridge. Move up the base, and remove the DNB tubes.
4. Empty the remaining solution in the Sequencing Reagent Cartridge and tube into an appropriate waste container.
5. Dispose of the tube, flow cell, and Sequencing Reagent Cartridge in accordance with local regulations and safety standards of your laboratory.

(Optional) Powering the device off

CAUTION

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

Perform the following steps:

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

06

Sequencing data

This chapter describes the sequencing output data.

Sequencing output files

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

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

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

Exporting data

Running data, sequencing results, and logs can be exported from the sequencer to an external storage device according to your needs.

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

Perform the following steps:

1. In the maintenance interface, select **Export data**.
2. Select the data type.
   - If you select **Running data** or **Sequencing results**, select the file type that you need:
     - Running data file types: image, metrics of each cycle.
     - Sequencing result file types: FASTQ, metrics, report, and others of each flow cell.

       Others refers to files except for FASTQ file and report.
   - If you select **Logs**, select the date range for the logs that you want to export.

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

5. (Optional) If you want to upload the data to a storage server or to the server where the ZLIMS software is installed, set the **Upload data** button to status.

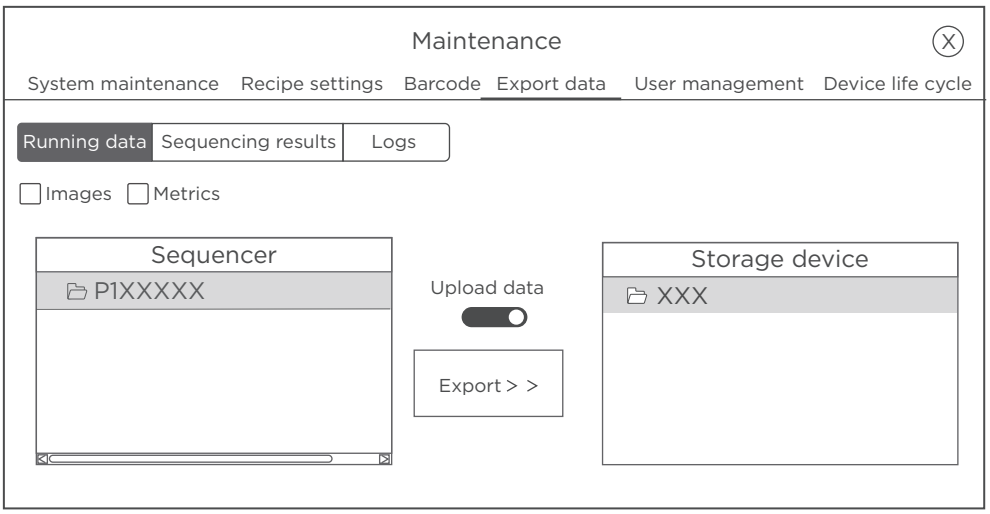


Figure 45 **Export data interface**

Summary report

Report parameter overview

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

Table 34 Parameters for Tab1 in the summary report

Parameter	Description
SoftwareVersion	Version of BasecallLite. Ensure that the version of BasecallLite is the officially released version.
TemplateVersion	Version of summary report template.
Reference	The species category of the sample. When the species category is unknown or when the category is not E. coli, the reference will be indicated as NULL.
CycleNumber	The total cycle of the sequencing run (not including the extra cycles, but including barcode, regardless of whether the barcode is split or not).
ChipProductivity (%)	Flow cell productivity. The yield of the flow cell is estimated by the following formula: $ChipProductivity = \frac{ValidFovNumber \times ESR}{ImageArea} \times 100\%$
ImageArea	The total number of FOVs (field of view) in a lane. The system reads the total number of FOVs from the *QC.csv* file under the metrics directory generated by the basecall software.
TotalReads (M)	Reads included in the FASTQ file (Reads after filtering).
MappedReads (M)	Number of reads mapped to the reference genome. For PE sequencing, a mapped read implies that both Read1 and Read2 are mapped to the reference genome.
Q30 (%)	The percentage of bases with a quality score of no less than 30. A base with a quality score of 30 implies that the chances of this base being called incorrectly are 1 in 1000.
Q40 (%)	The percentage of bases with a quality score of no less than 40. A base with a quality score of 40 implies that the chances of this base being called incorrectly are 1 in 10,000.

Parameter	Description
SplitRate (%)	The proportion of FASTQ data that can be split according to barcodelist. This indicator is obtained from the *BarcodeStat.txt* file, and the split results are included in *Sequencestat.txt*. The Split Rate is counted from the filtered reads only.
Lag/Runon	• Lag: For a given DNB with m copies of DNA fragments, while sequencing at cycle i, n copies of DNA fragments react at i-1 cycle, the Lag is defined as n/m. • Runon: For a DNB with m copies of DNA fragments, while sequencing at cycle i, n copies of DNA fragments react at i+1 cycle, the runon is defined as n/m. • Lag1 (%) is the slope of the Lag curve for the 1st strand sequencing. • Lag2 (%) is the slope of the Lag curve for the 2nd strand sequencing. • Runon1 (%) is the slope of the runon curve for the 1st strand sequencing. • Runon2 (%) is the slope of the runon curve for the 2nd strand sequencing.
ESR (%)	Effective spot rate. Percentage of effective spots after filtering in the flow cell. $ESR = \frac{allreads}{SpotNum \times Effective\ FOV} \times 100\%$
MappingRate (%)	The ratio of mapped reads to total reads. The indicator is defined as follows: $MappingRate = \frac{MappedReads}{TotalReads} \times 100\%$
AvgErrorRate (%)	After the mapping analysis of TotalReads, the error rate of the number of reads mapped to the reference genome. AvgErroRate (%) is defined as follows: $AvgErrorRate(\%) = \frac{TotalMismatchBaseNumber}{MappedReadsNumber \times ReadLength} \times 100\%$
AvgErrorRate!N (%)	The average error rate after removing the mismatches caused by call N.

Parameter	Description
MaxOffsetX/ MaxOffsetY	During the sequencing process, the basecall program will output the Offset value of each FOV in channel A compared with the standard template in cycle 1. The two values are based on the results of channel A base fluorescent imaging.
InitialOffsetX/ InitialOffsetY	The offset value of A base fluorescent channel in cycle 1.
RecoverValue (A)/ RecoverValue (C)/ RecoverValue (G)/ RecoverValue (T)	The ratio of second-strand signal to first-strand signal. This indicator is only for PE sequencing.
RecoverValue (AVG)	The average ratio of second-strand signal to first-strand signal of 4 (ACGT) bases. This indicator is only for PE sequencing.

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

Table 35 Parameters for Tab2 in the summary report

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

Diagrams in summary report

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

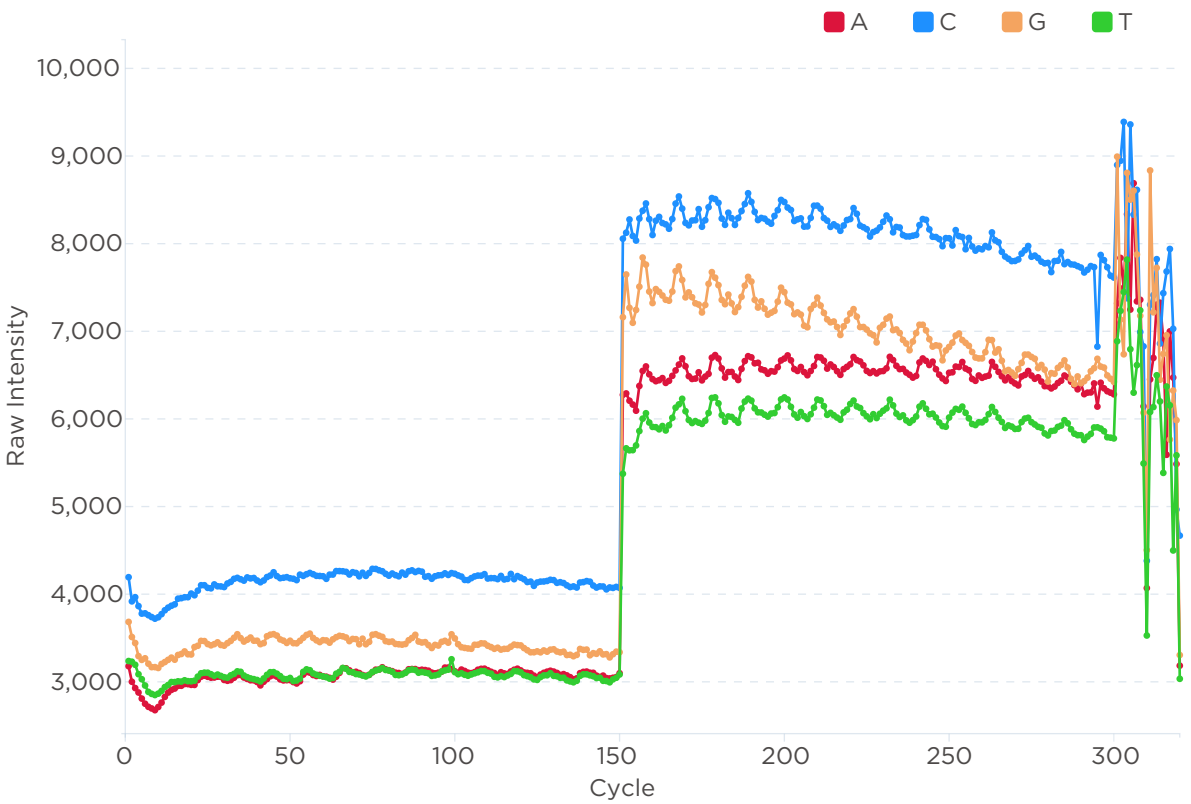


Figure 46 Raw Intensity

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

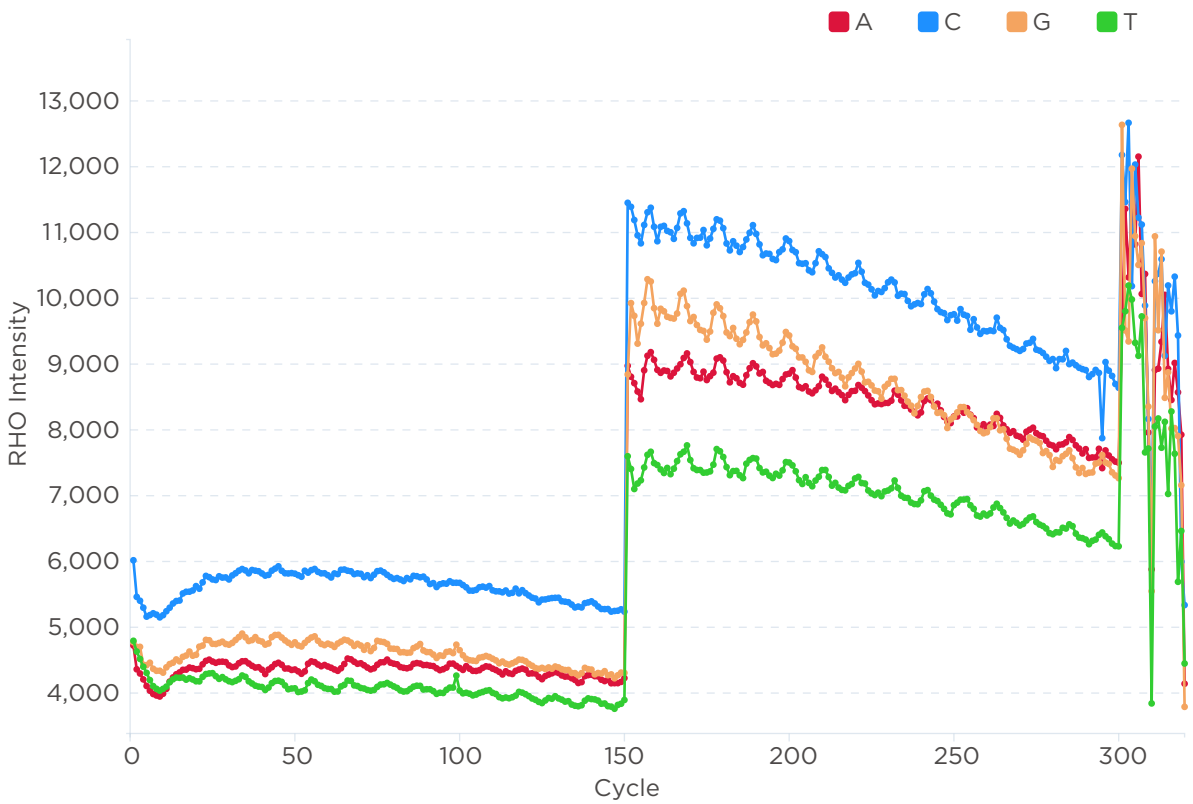


Figure 47 RHO Intensity

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

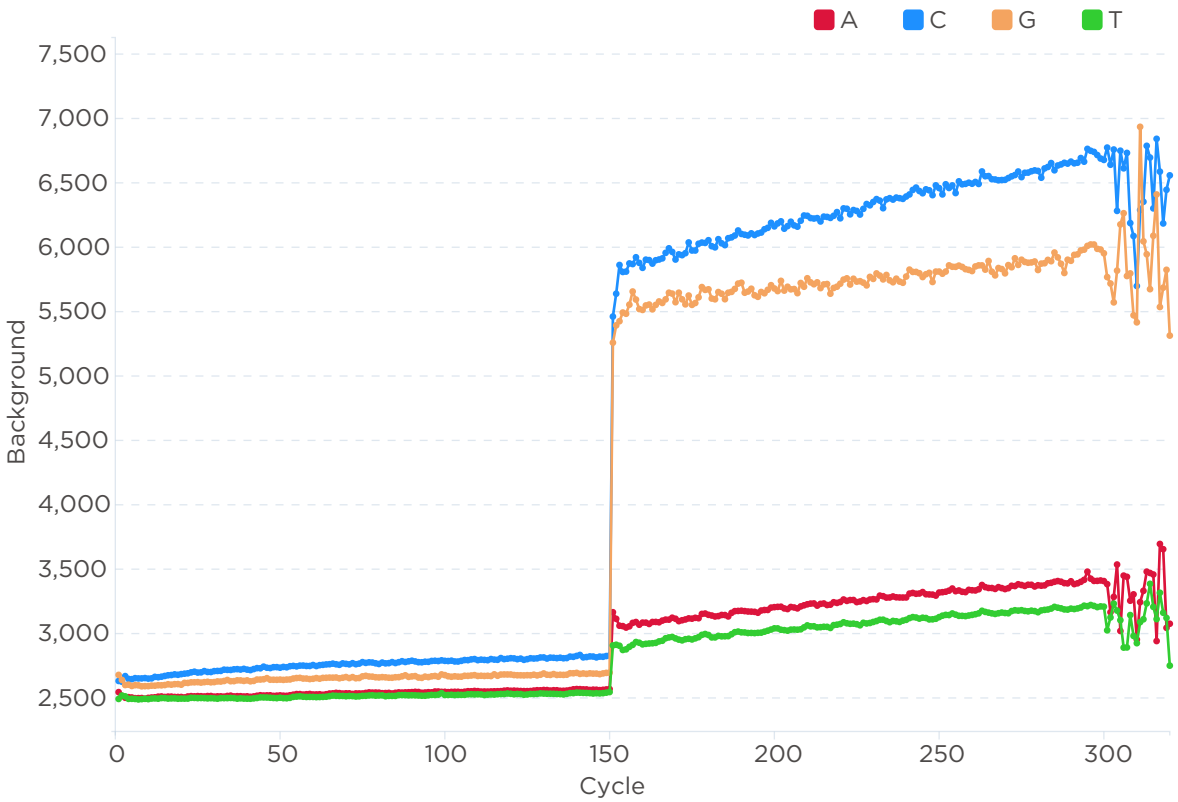


Figure 48 Background

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

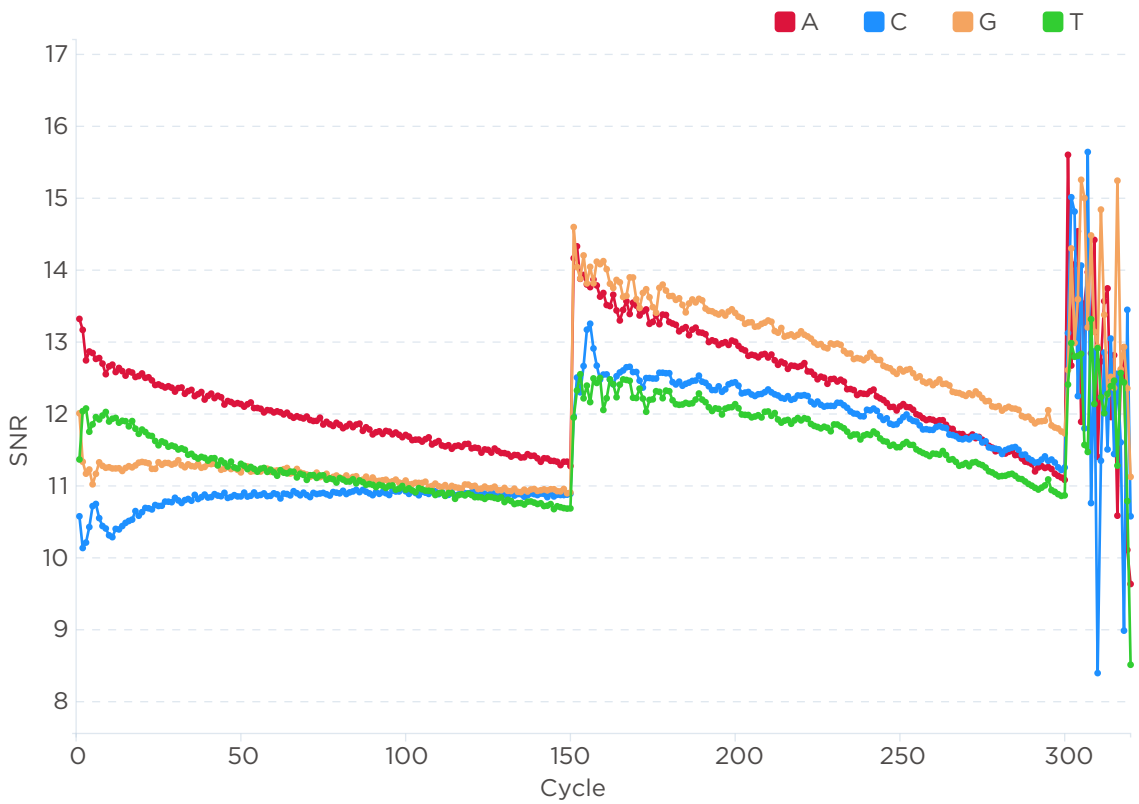


Figure 49 SNR

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

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

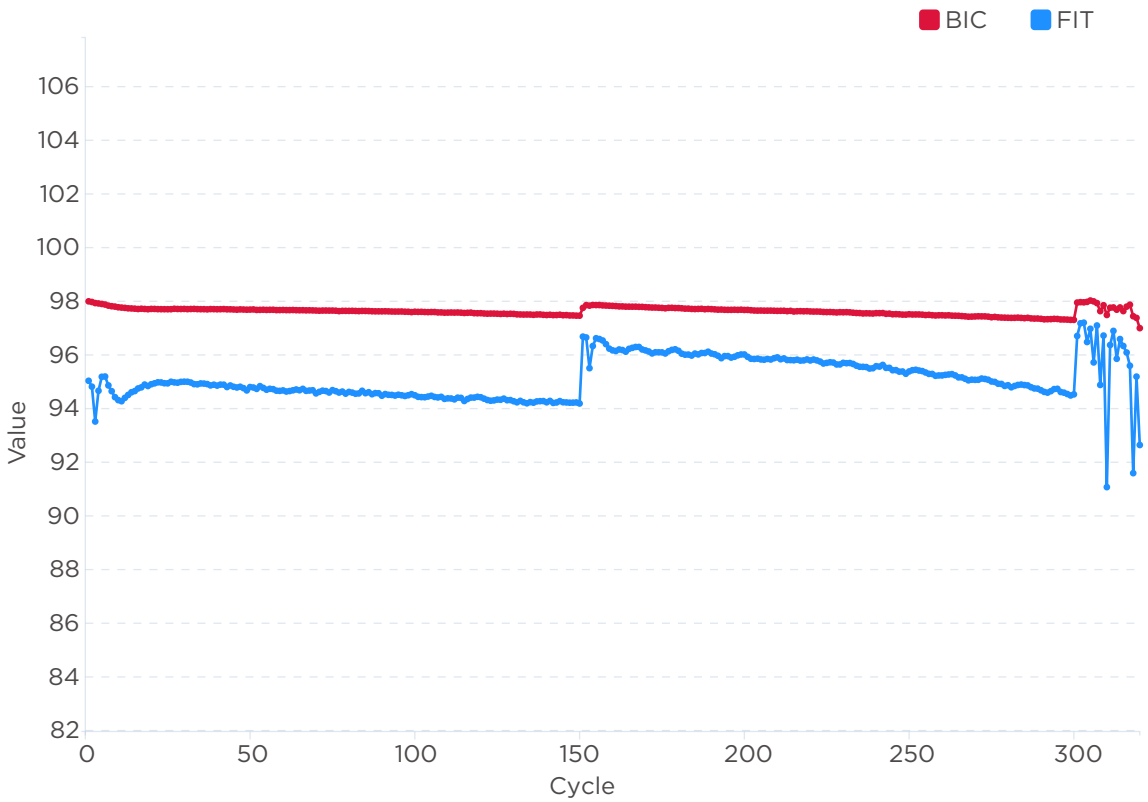


Figure 50 **BIC And FIT**

X axis	Cycle
Y axis	BIC and FIT Value: • BIC (Basecall Information Content): Percentage of spots that can be used for basecalling. • FIT (Crosstalk Fit Score): Discrete degree of the signals of A/T/C/G bases.

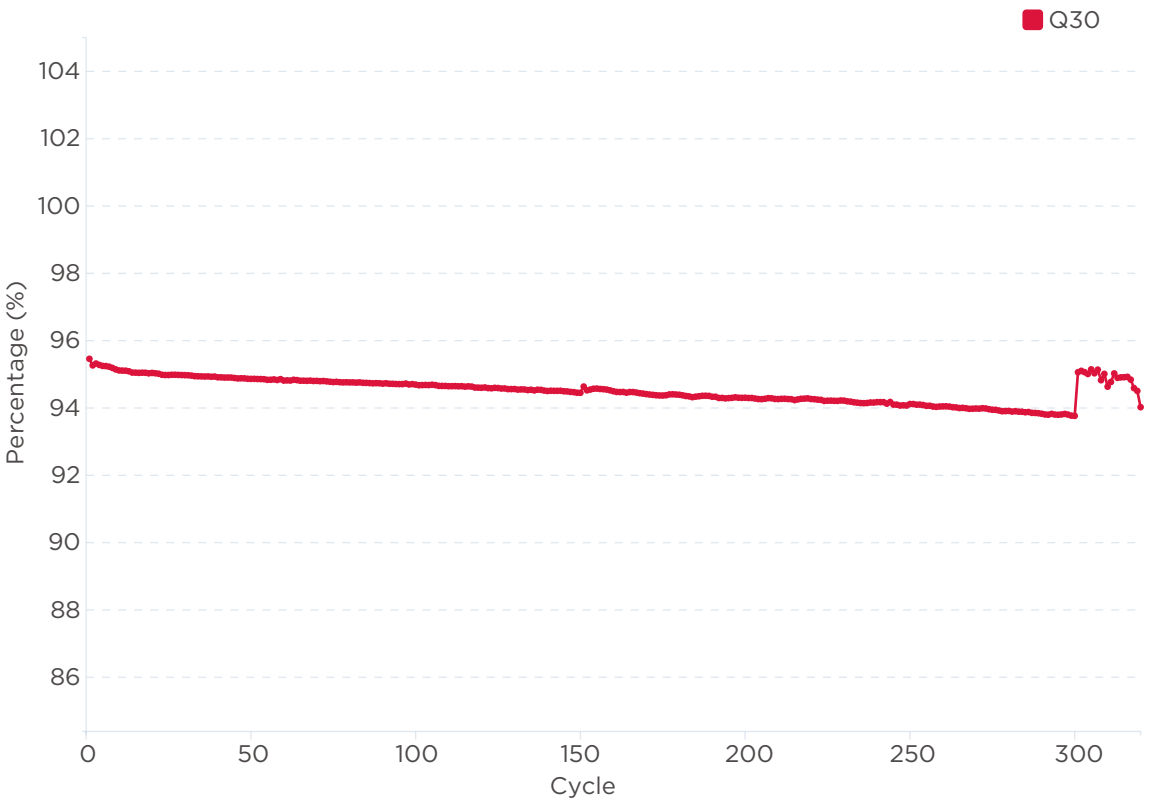


Figure 51 **Unfiltered Q30**

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

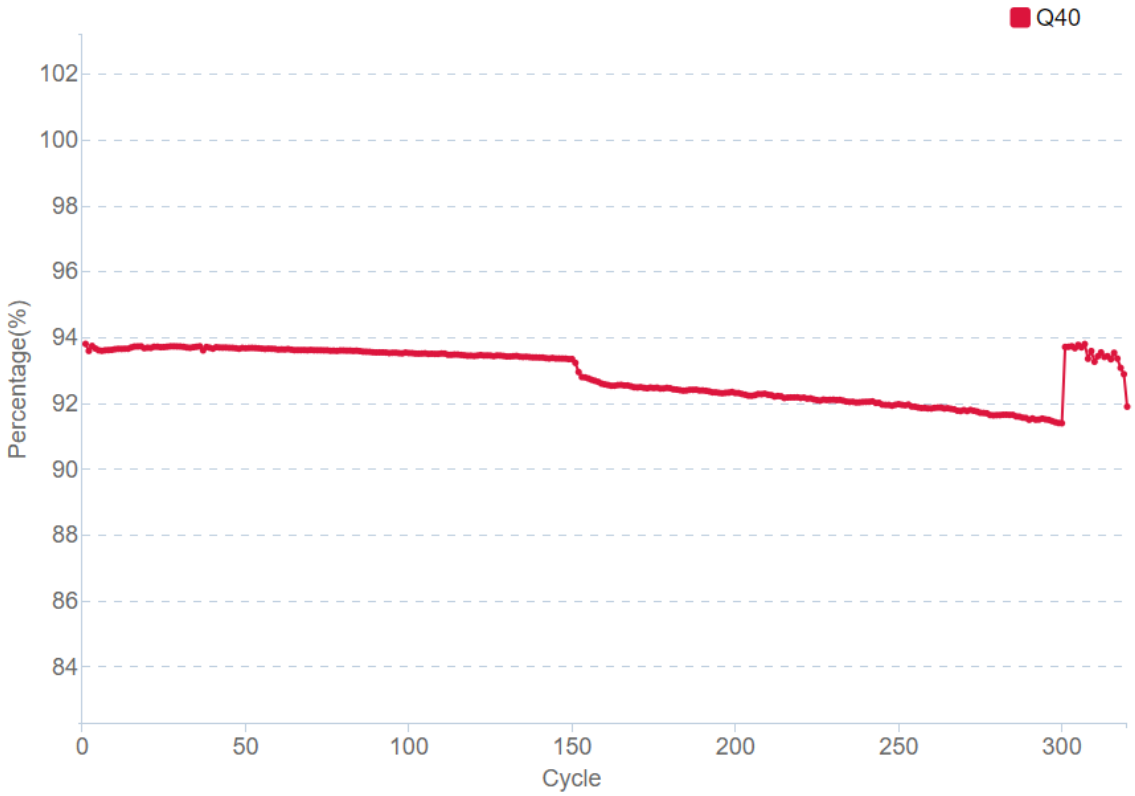


Figure 52 **Unfiltered Q40**

X axis	Cycle
Y axis	Percentage (%): The percentage of bases with quality score no less than 40 in each cycle before filtering.

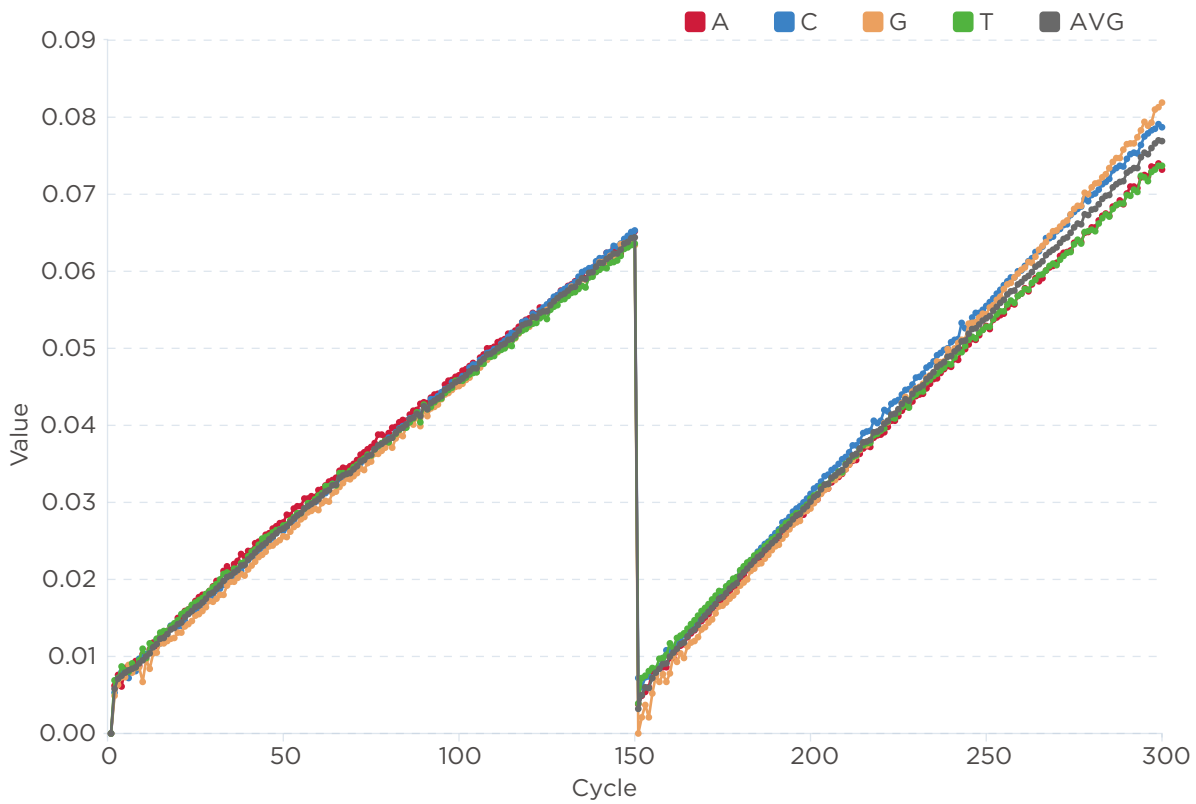


Figure 53 Runon

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

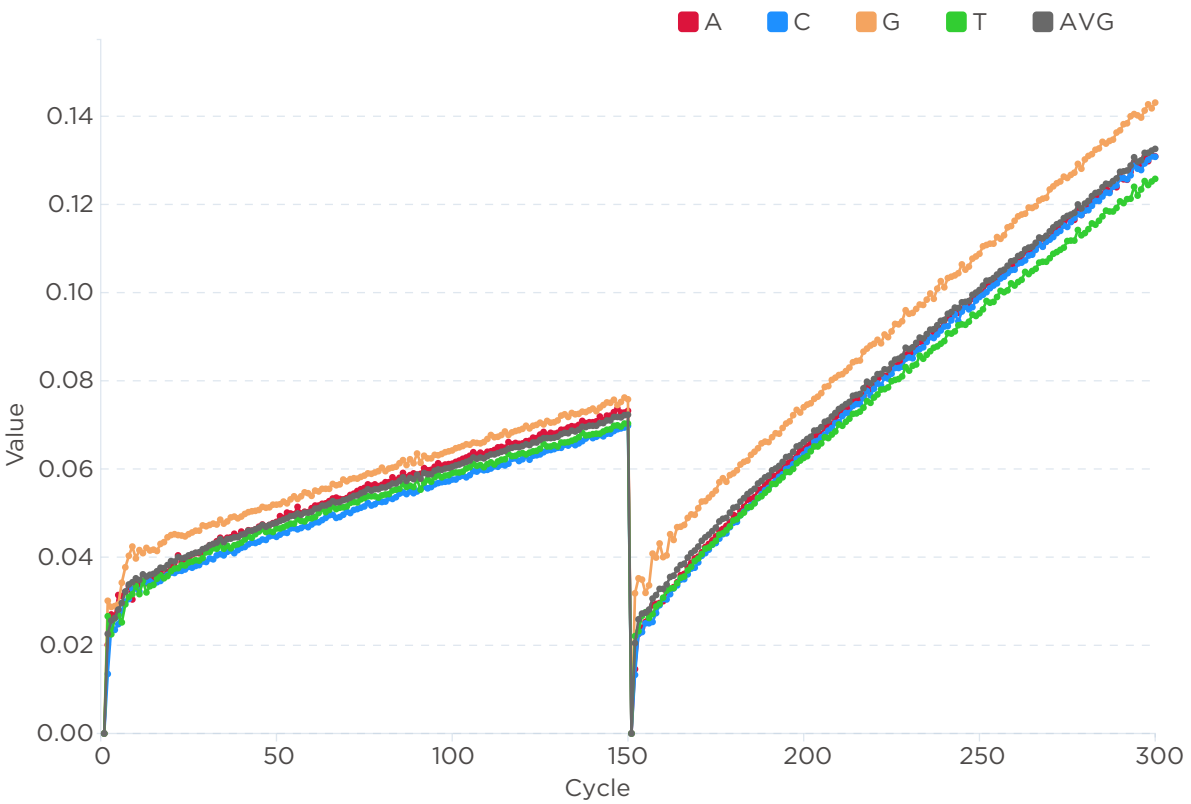


Figure 54 **Lag**

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

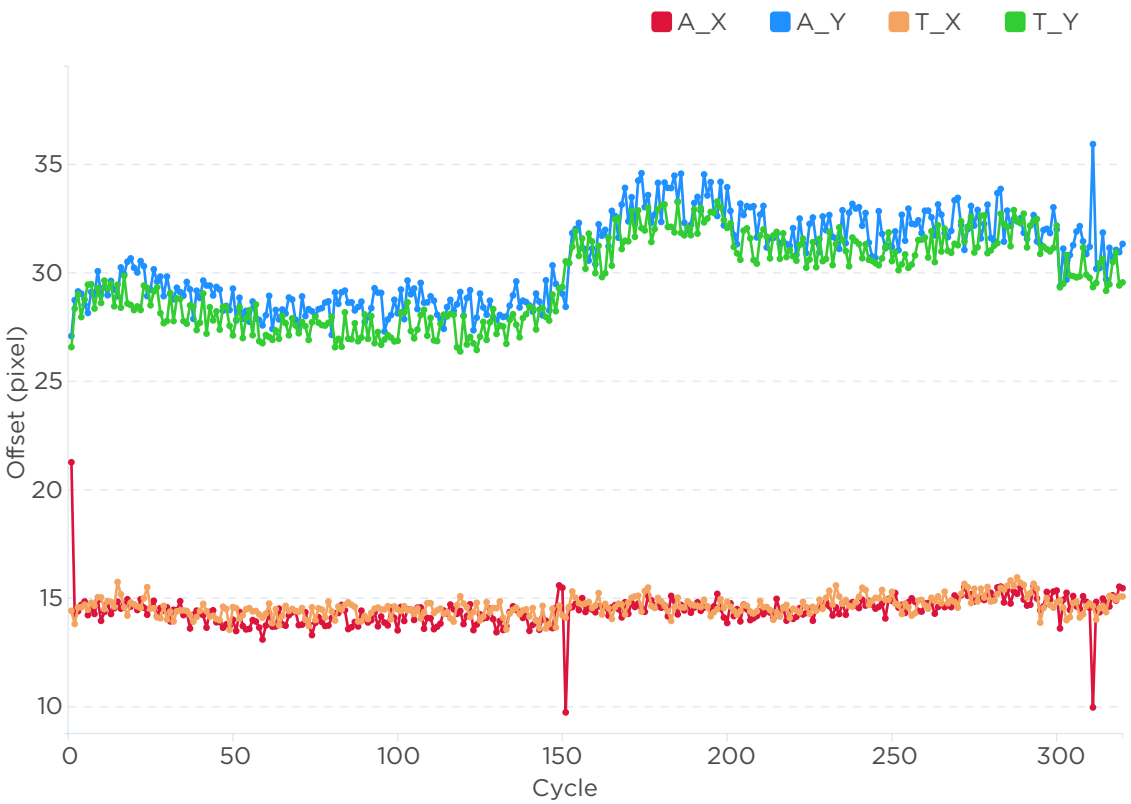


Figure 55 Offset

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

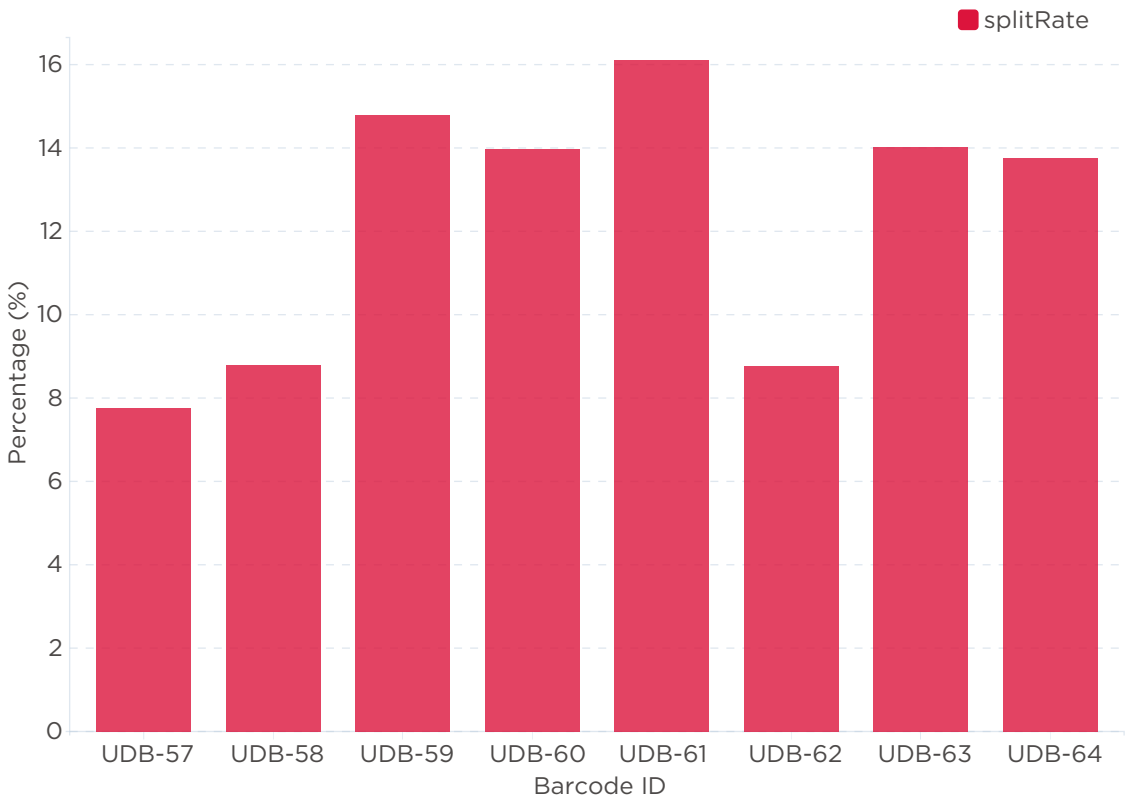


Figure 56 **Barcode Split Rate**

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

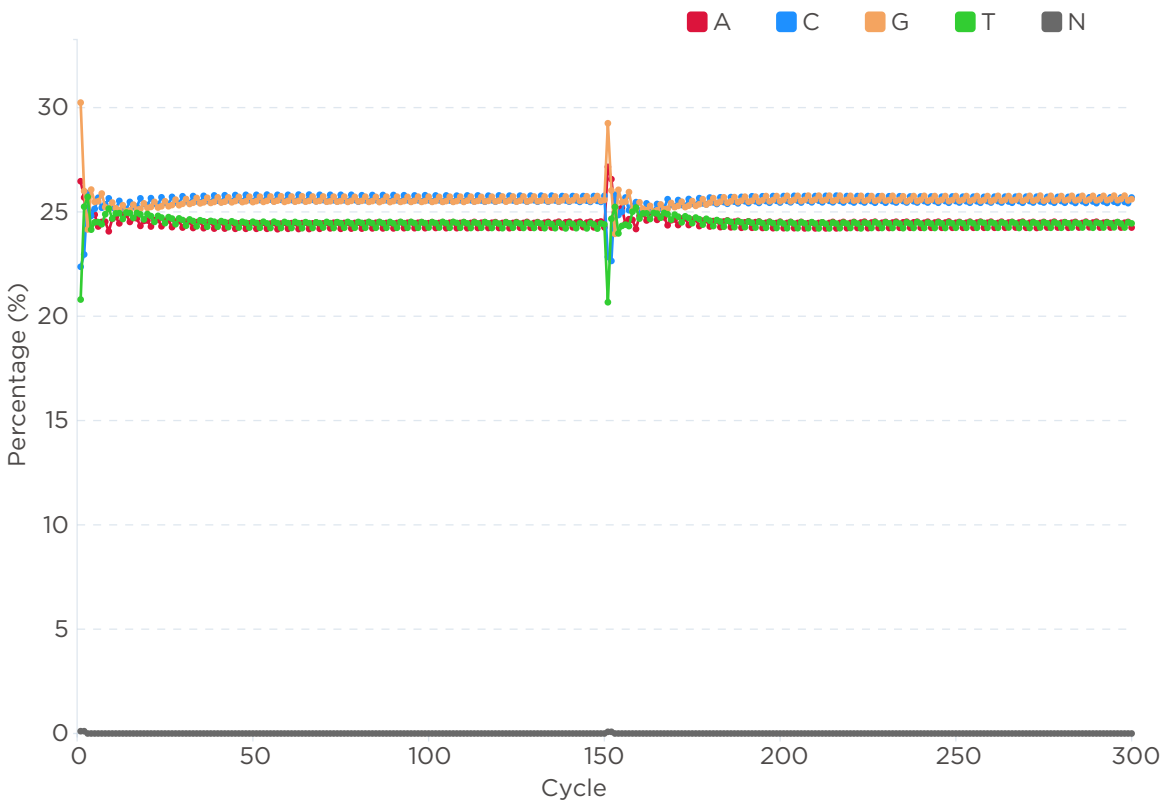


Figure 57 Bases Distribution

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

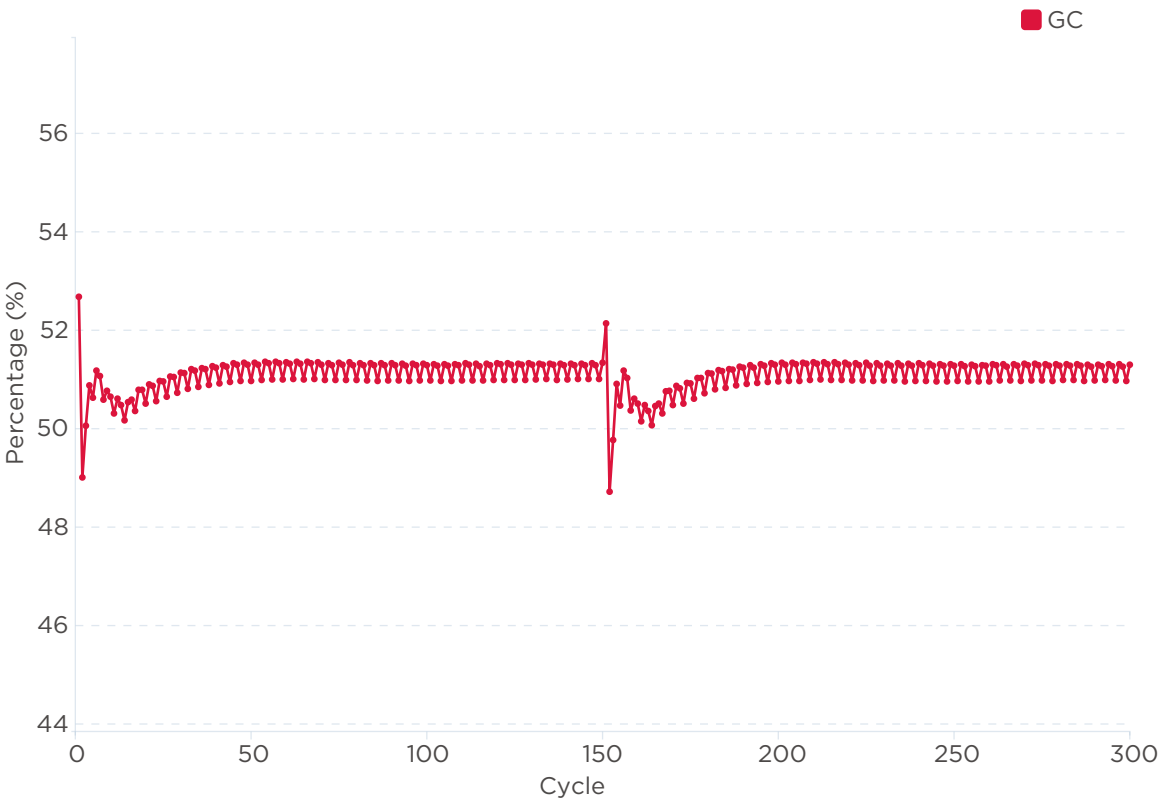


Figure 58 GC Distribution

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

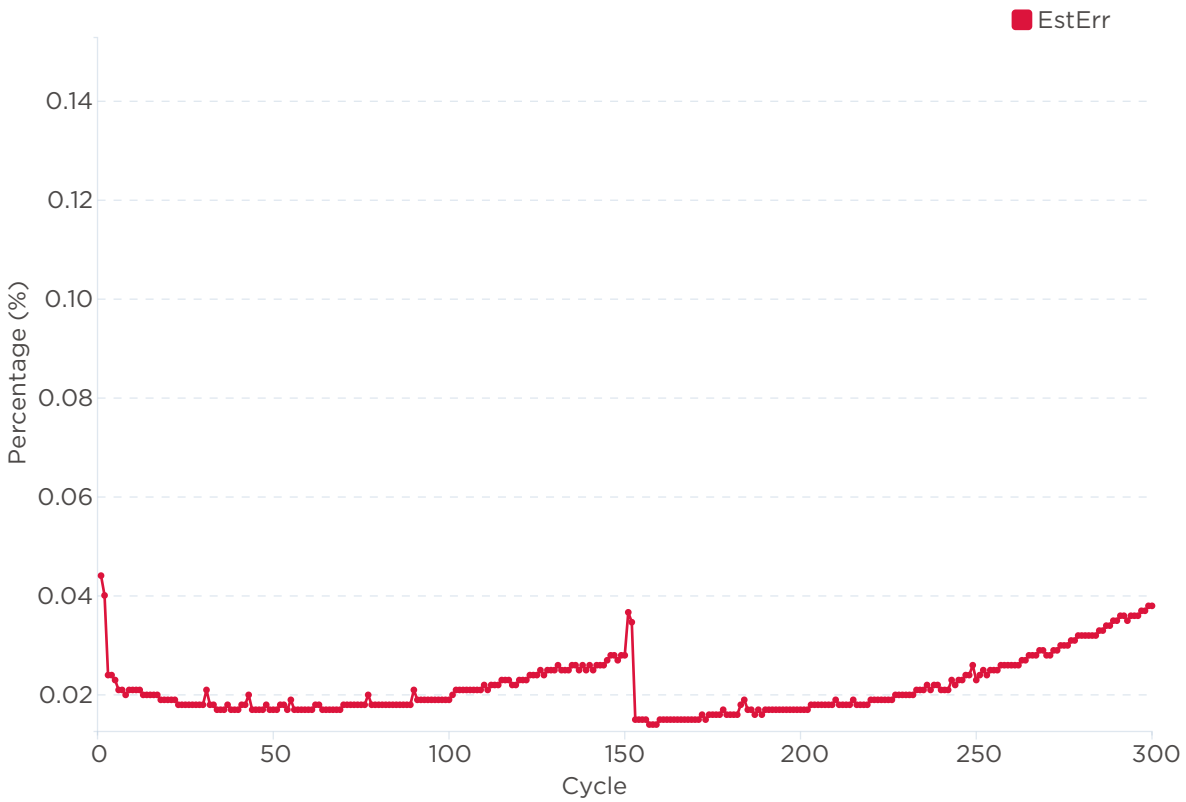


Figure 59 Estimated Error Rate

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

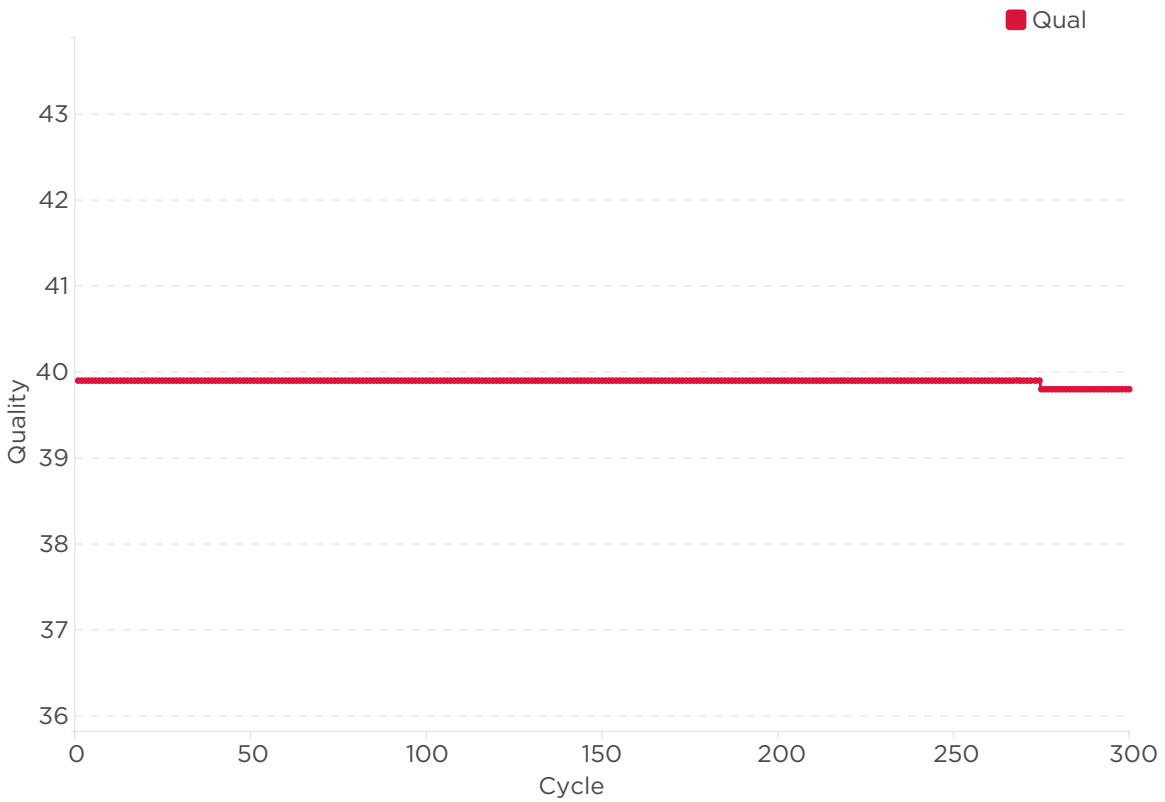


Figure 60 Average Quality Distribution

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

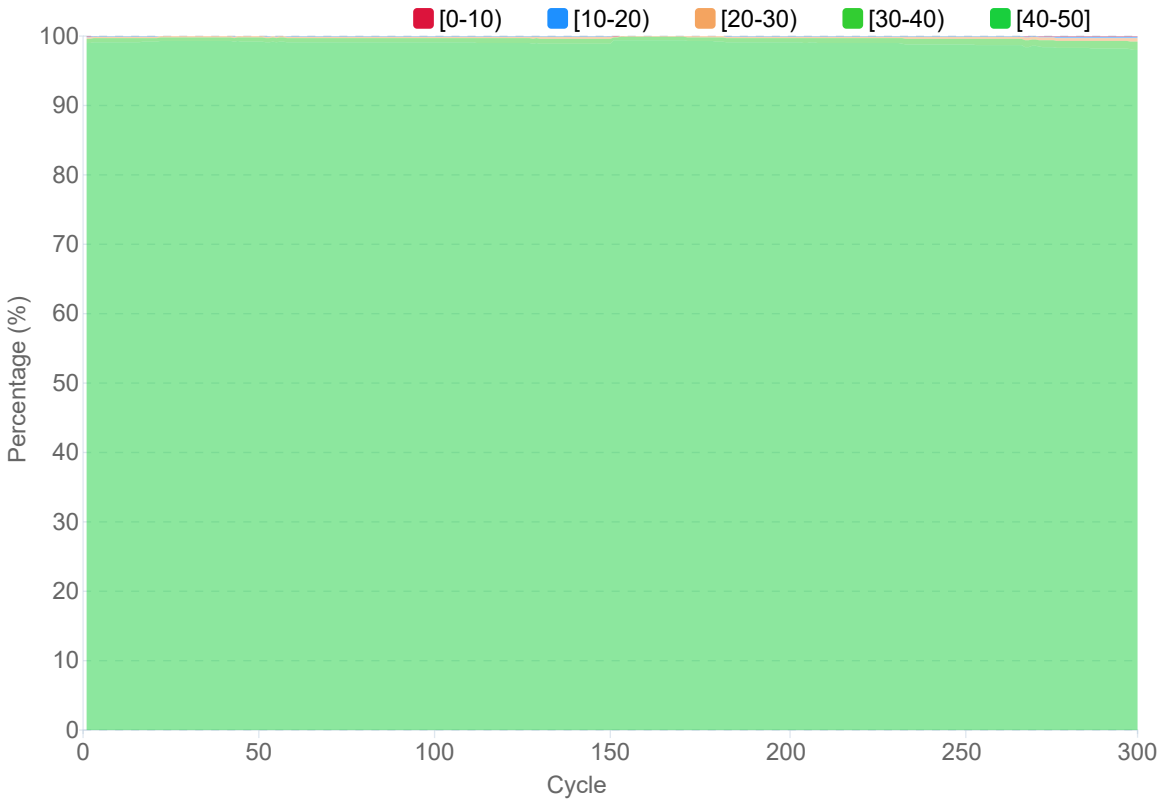


Figure 61 **Quality Proportion Distribution**

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

Other reports

Table 36 Other report descriptions

Name	Description
XXXXXXXX_L0X.heatmapReport.html	It contains information of each FOV in the lane generated during sequencing, including AvgQ30, offset_x, offset_y, lag1, lag2, runon1, and runon2.
XXXXXXXX_L0X.bestFovReport.html	The summary of the best FOV and basecall information during the entire sequencing run.
XXXXXXXX_L0X.allCycleHeatmap.html	Information in each FOV of every cycle, including LoadedDNB, Offset, Signal, Background, RHO, SNR, Q30, BIC, Fit, A-T, G-C, Lag, and Runon.

XXXXXXXX represents the flow cell ID. *L0X* represents the lane number.

Data processing

Introduction

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

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

The two Write FASTQ methods are described below.

Write FASTQ on sequencer automatically

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

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

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

Write FASTQ on sequencer manually

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

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

Preparation before writing FASTQ manually

Perform the following steps:

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

   Also ensure that the number of Metrics files for one lane is consistent with the total cycle number.

   For example, set sequencing parameters with the following assumptions:

   - Sequencing run: PE150+10
   - Length of Read1: 150
   - Length of Read2: 150
   - Length of Barcode: 10
   - Total cycles = 150+150+10+2= 312

   When checking the Metrics file path, ensure that the expected number of Metrics files is 312:

   D:/Result/workspace/P1XXXXXX/L0X/Metrics

3. Rename the original FASTQ folder. For example, rename *P10000XXXX* to *P10000XXXX_old*, or *L01 rename* to *L01_old*.

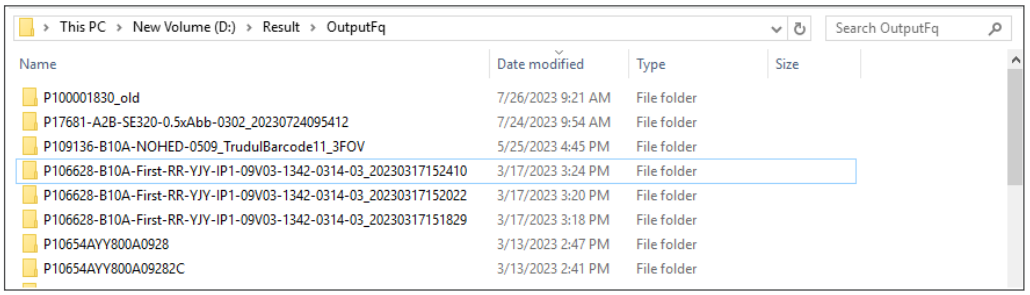


Figure 62 Renaming the FASTQ folder

4. Prepare the barcode file that you need to write FASTQ manually.

   For details, refer to *Instructions for importing barcode on Page 159.*

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

BasecallLite (Litecall) write FASTQ manually

Perform the following steps:

1. Open the *C:\BasecallLite\Config* folder to confirm the location of the *Client.ini* file.

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

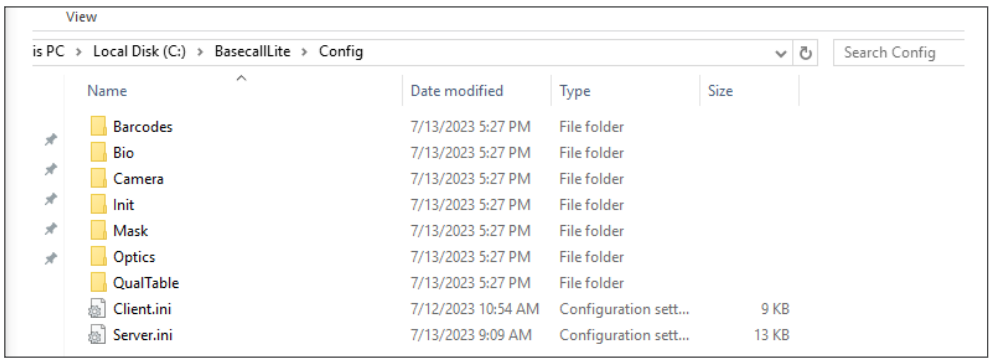


Figure 63 Location of Client.ini file

2. Select the *Client.ini* file, and right-click **Edit with Notepad++** to open the file.

   The following table lists the parameters in the file:

Table 37 Parameter settings descriptions

Parameter settings	Description
Cycle	This parameter indicates the number of cycles. Cycle=r[read1cycle number]e1r[read2cycle number]e1b[dualbarcode cycle number]b[barcode cycle number]. e1 means end cycle process mode. Assumptions: • PE100+10(101+101+10), Cycle=r100e1r100e1b10 • PE100+10+10(101+101+10+10), Cycle=r100e1r100e1b10b10 • SE50+10(51+10), Cycle=r50e1b10
SubmitImages	This parameter indicates writing FASTQ from *.cal* file.
CalFilePath	This parameter describes the *.cal* file storage path.
Filter	This parameter indicates whether to write FASTQ with filter or not.
BarcodeType	This parameter indicates the barcode type. If BarcodeType=0, users define the barcode and users need to change the barcode file path. BarcodeType=1, 501-596, 10 bp, mismatch is 1. BarcodeType=2, 1-128, 6 bp, mismatch is 1. BarcodeType=3, 1-128, 10 bp, mismatch is 2.
BarcodeFile	This parameter describes the barcode file storage path. You need to enter the barcode file path here if you use a user-defined barcode or if the barcode type is 0. *i* Comments are displayed in green in the file. You can refer to the comments to modify the relevant parameters.
Split	This parameter indicates whether to split the barcode. It is applicable to single barcode.
Reverse	This parameter indicates whether the barcode is reverse complementary completely. For example, if the barcode (AAATTTGGGC) is reverse complementary, the sequencing result is for the barcode (GCCCAAATTT). It is applicable to single barcodes.
Mismatch	This parameter represents the number of mismatch bases. It is applicable to single barcodes.

Parameter settings	Description
DualbarcodeSplit	This parameter indicates whether to split the barcode and whether one barcode can be split separately. It is applicable to dual barcodes. For example, if barcodes are split and one barcode can be split separately, then DualbarcodeSplit= (true, true).
DualbarcodeReverse	This parameter indicates whether barcodes are reverse complementary. For example, if the barcode (AAATTTGGGC) is reverse complementary, the sequencing result is for the barcode (GCCCAAATTT). It is applicable to dual barcodes. For example, if barcodes are reverse complementary completely, then DualbarcodeReverse= (true, true).
DualbarcodeMismatch	This parameter represents the number of mismatch bases. It is applicable to dual barcode.

3. Edit the file according to the following steps:
   1) Modify the number of cycles.
   2) Ensure that **SubmitImages** is **false**.
   3) Change the *.cal* File path.
   4) Set **Filter** as **true** if FASTQ is written with filter and as **false** if FASTQ is not written with filter.
   5) Change the barcode type as needed.
   6) If barcode file path needs to be changed, set the barcode type to 0 and change the barcode file path.
   7) Set **Split** as **true** if barcode is split, **Reverse** as **true** if the single barcode is reverse completely, and set the number of mismatch bases.

      This part is applicable to the single barcode.
   8) Set **DualbarcodeSplit** as **(true, true)** if barcode is split and a barcode can be split separately, **DualbarcodeReverse** as **(true, true)** if the two barcodes are reverse completely, and set the number of mismatch bases. This part is applicable to only the dual barcode.

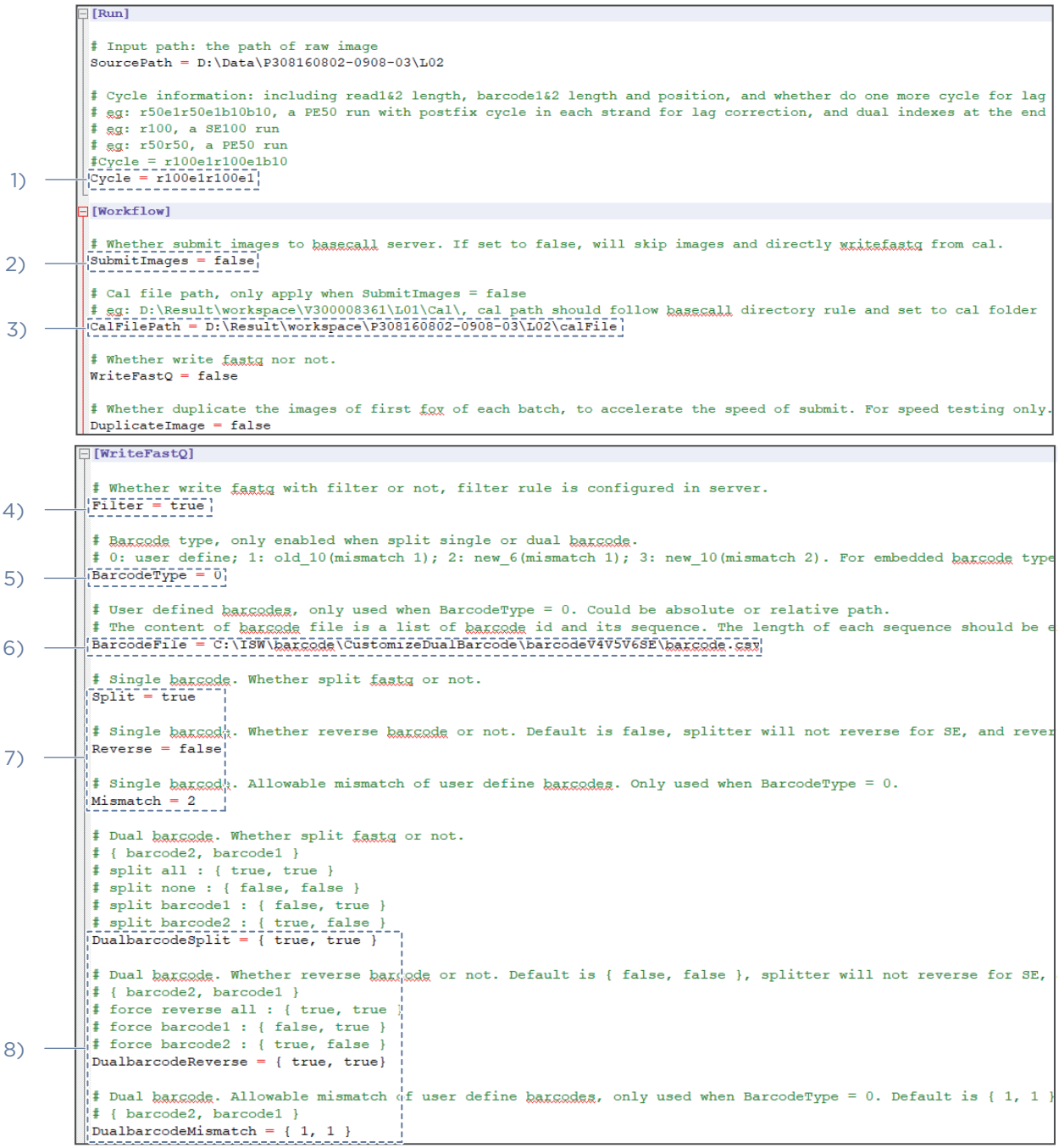


Figure 64 Changing *.cal* file path and barcode splitting parameters

4. Click **Save** and close the *Client.ini* file.

5. Use either method below to run the write FASTQ program.
   - Method 1
     a. Open the Task Manager, select the **Services** tab, and start LiteCall.

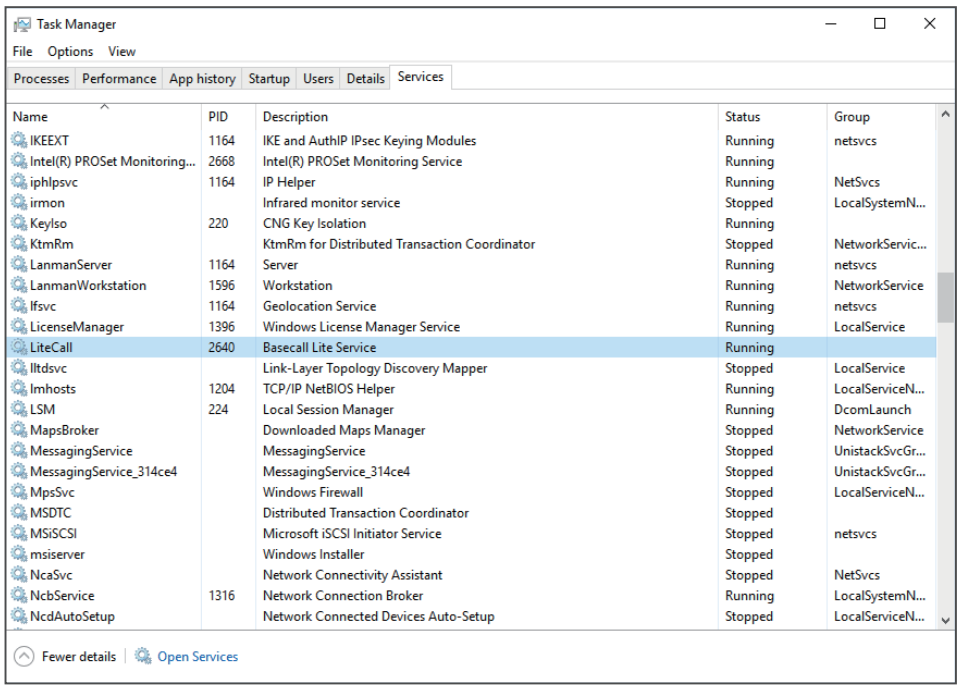


Figure 65 Starting LIteCall service

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

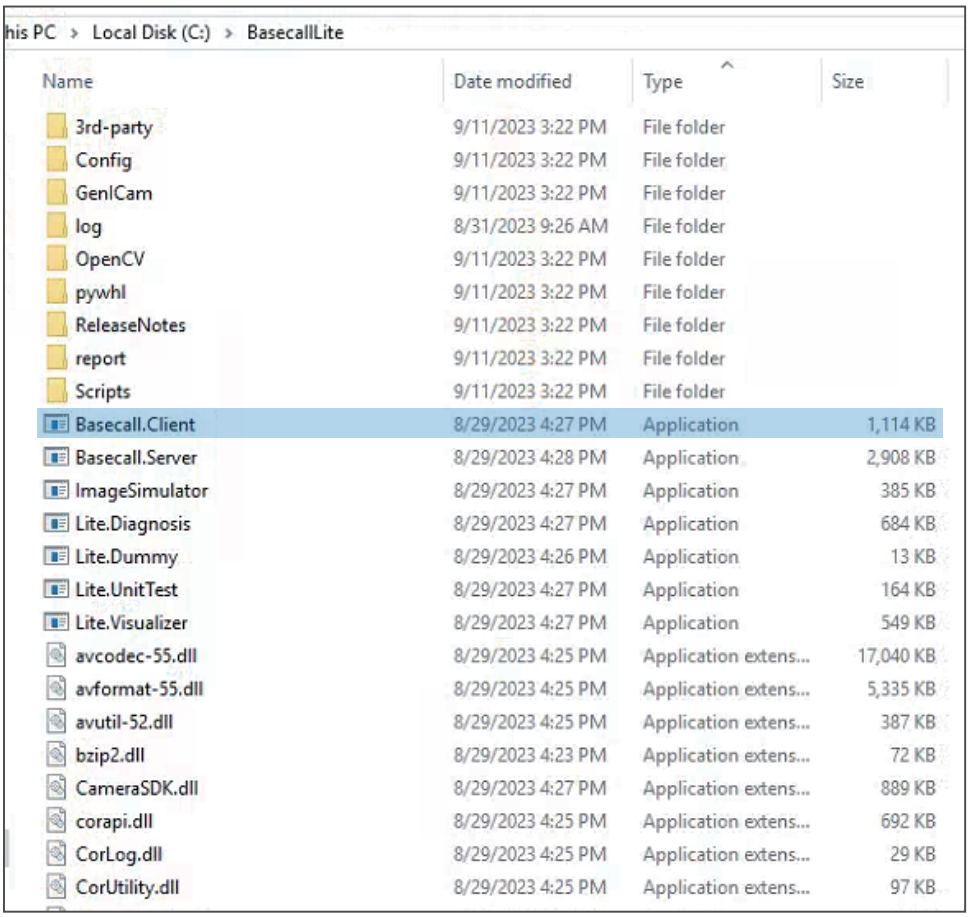


Figure 66 Opening the Basecall.Client.exe program

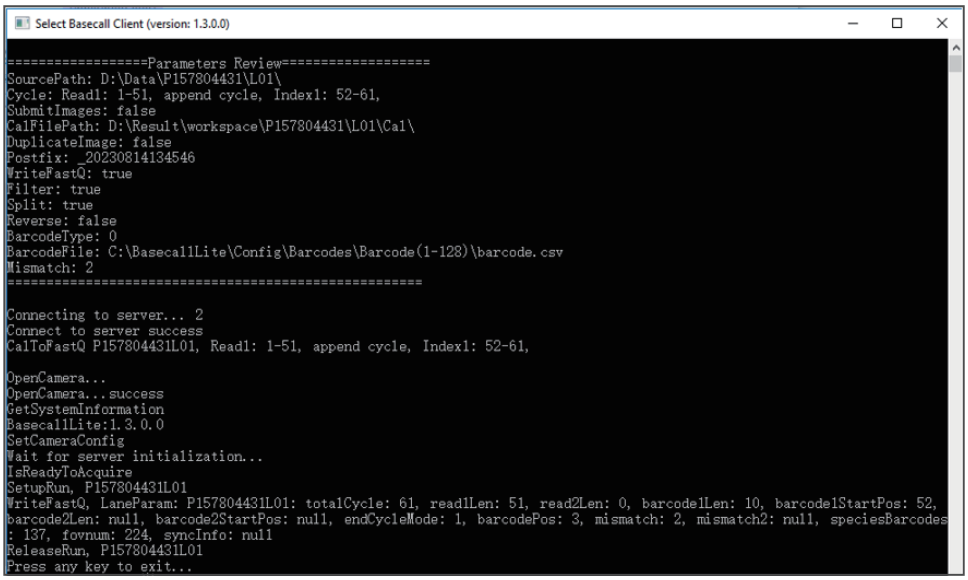


Figure 67 Starting the Basecall.Client.exe program

- Method 2

  By using this method, you can view the progress of the write FASTQ process in the *Server.exe* program.

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

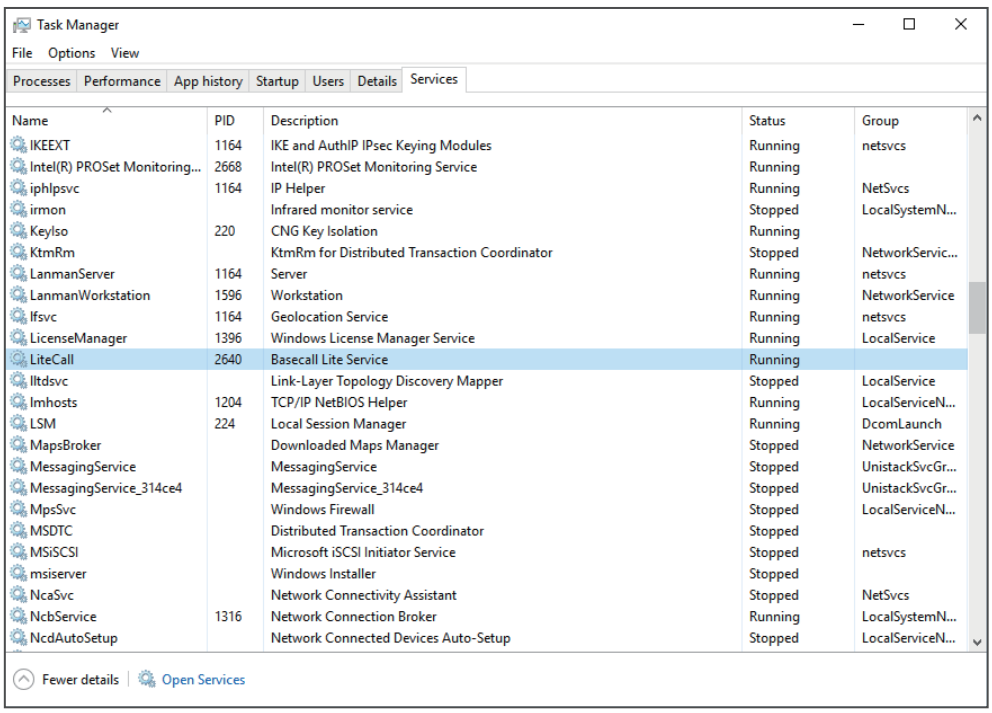


Figure 68 Stopping the LiteCall service

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

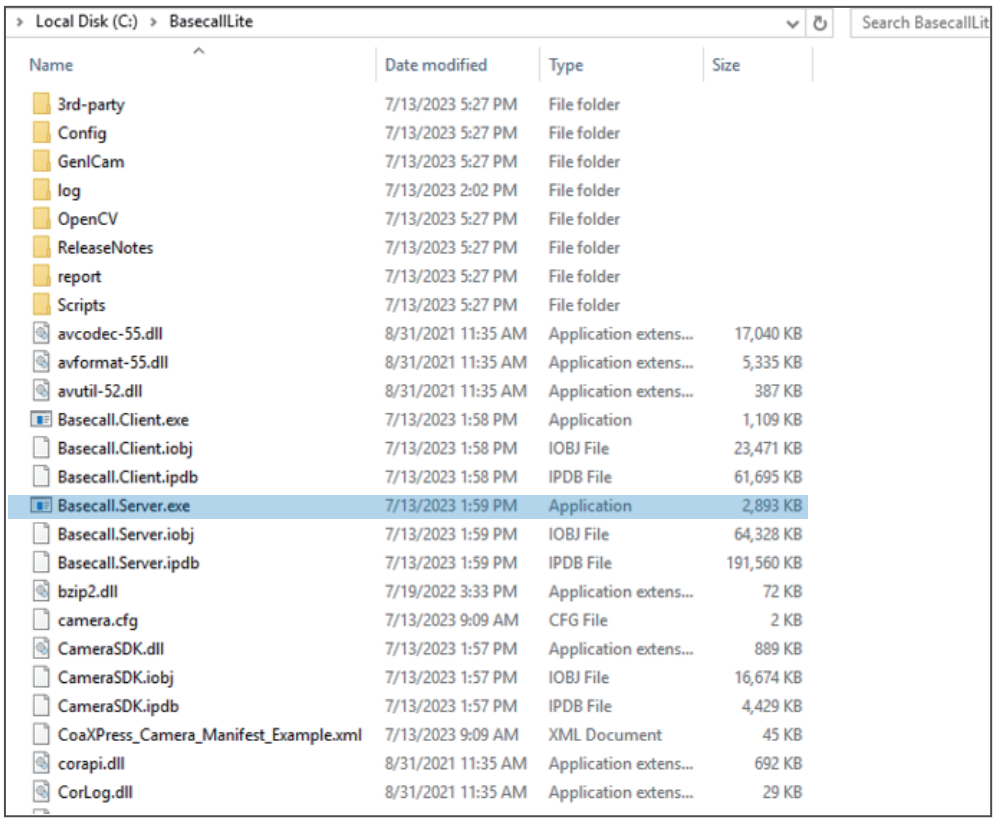


Figure 69 Opening the Basecall.Servicer.exe

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

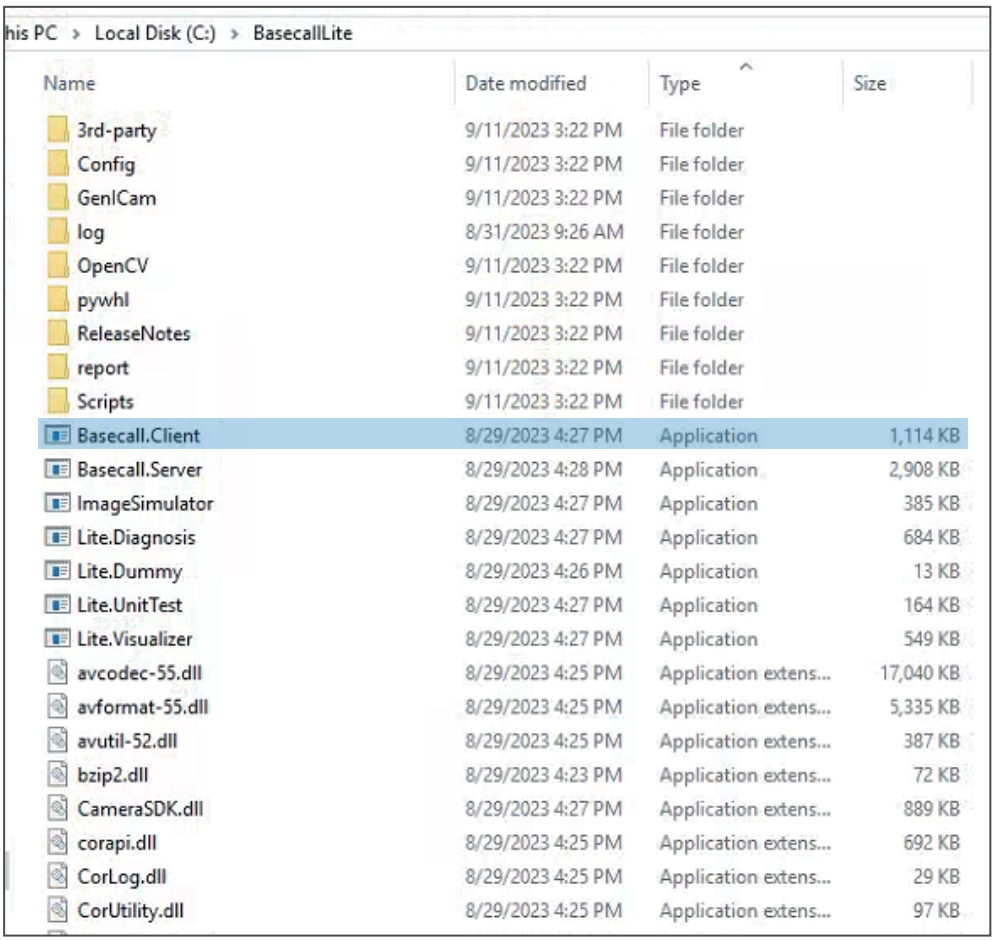


Figure 70 Opening the Basecall.Client.exe

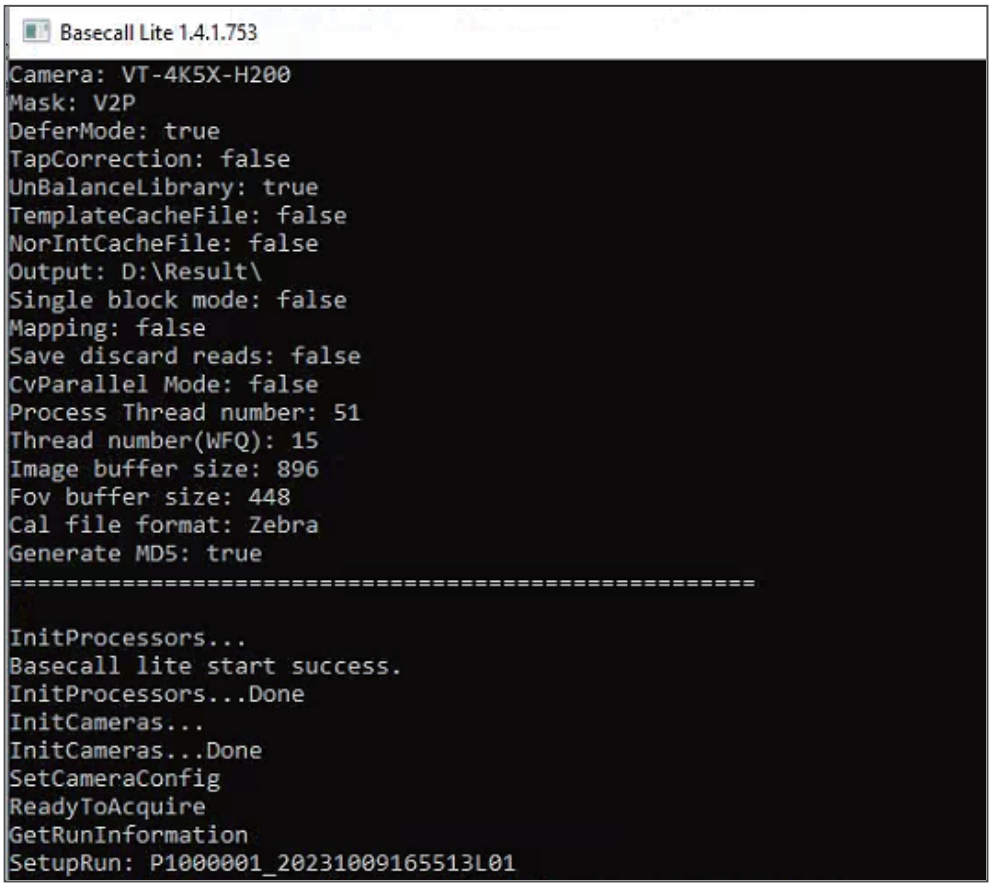


Figure 71 Starting the Basecall.Server.exe

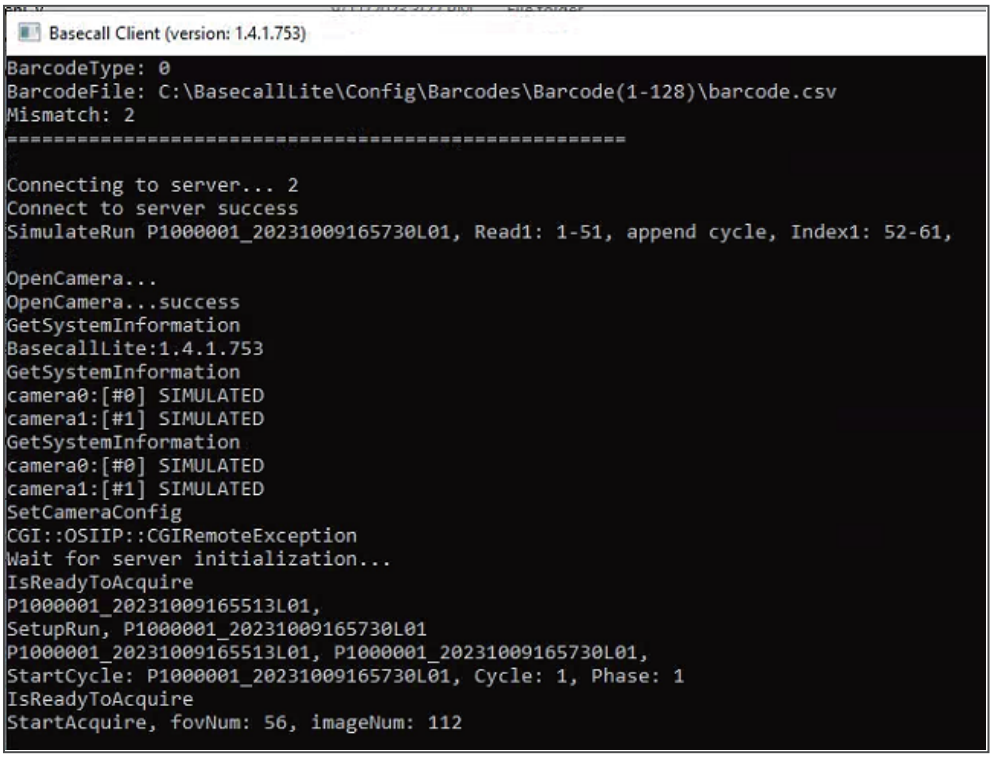


Figure 72 **Starting the Basecall.Client.exe**

Example of parameter setting (PE150+10+8)

Perform the following steps:

1. Set sequencing parameters with the following assumptions:
   - Sequencing run: PE150+10+8
   - Length of Read1: 150
   - Length of Read2: 150
   - Length of Dual barcode: 10 (both length of barcode 1 and length of barcode 2 are 10)
   - Length of barcode1: 8

- *CalFilePath*: *D:\Result\workspace*\P150060903*L02\calFile*\

```
Cycle = r100e1r100e1

[Workflow]

# Whether submit images to basecall server. If set to false, w
SubmitImages = false

# Cal file path, only apply when SubmitImages = false
# eg: D:\Result\workspace\V300008361\L01\Cal\, cal path should
CalFilePath = D:\Result\workspace\P150060903\L02\calFile
```

Figure 73 Parameter settings

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

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

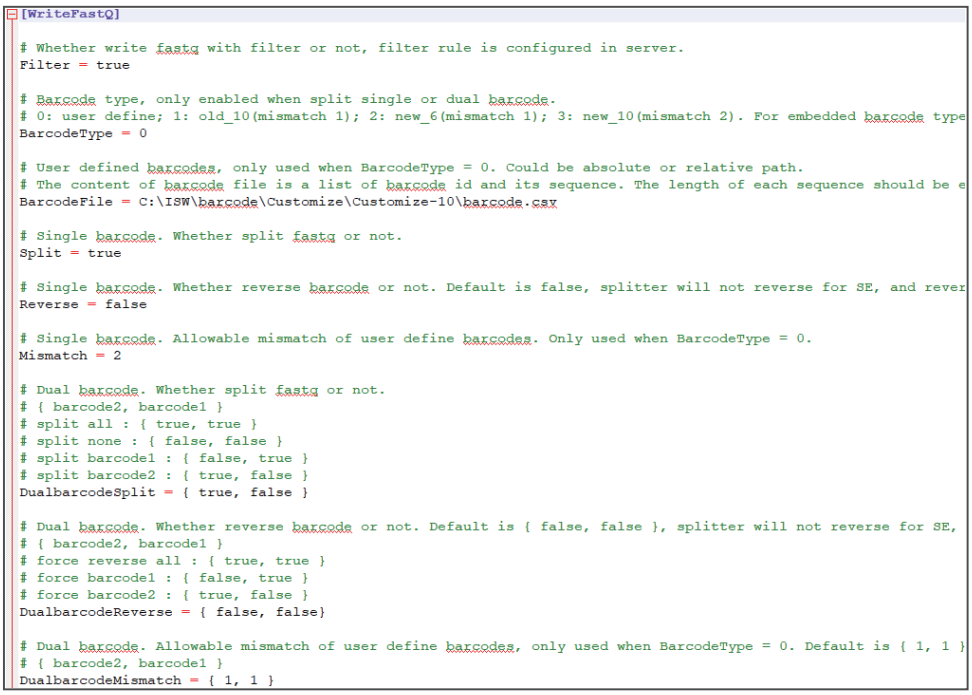
     BarcodeFile path:

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

```
[WriteFastQ]

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

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

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

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

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

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

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

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

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

Figure 74 Splitting both barcode1 and barcode2

- Splitting barcode2 only:



```
[WriteFastQ]

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

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

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

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

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

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

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

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

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

Figure 75 Splitting barcode2 only

- Splitting barcode1 only:

```
[WriteFastQ]

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

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

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

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

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

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

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

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

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

Figure 76 Splitting barcode1 only

3. Set **SaveDiscardedReads** as **true**.

```
[WriteFastQ]

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

# Use in  global filter
FilterReadLen = 100

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

Figure 77 Setting the SaveDiscardedReads

07

Device maintenance

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

DANGER

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

WARNING

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

Service plan

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

Sequencer maintenance

Wash

Wash type

- Laboratory-grade water includes 18 Megohm (MΩ) water, Milli-Q water, Super-Q water, or similar molecular biology-grade water.
- Sequencing cartridge and washing cartridge are from the sequencing set you purchase.
- Empty High-throughput Washing Cartridges are delivered with the device, which are used to hold the waste liquid during wash.
- Empty External Washing Cartridges are delivered with the device, which can be used to hold the washing reagents during wash and should be prepared before use.
- Wash manifold is also delivered with the device, only use washing manifold for the washing procedures.

There are three different wash types based on sequencer conditions:

Table 38 Wash types

Wash type	Required washing reagent	Description
Regular wash (~60 min)	Laboratory-grade water	Perform a regular wash every 7 days under the following circumstances: • If the device is intended to be idle for over 14 days
Maintenance wash (~100 min)	0.05% Tween-20+1 M NaCl or WB1, 0.1 M NaOH, and laboratory-grade water	Perform a maintenance wash under the following circumstances: • Within 24 h after a sequencing run. • Before sequencing, if the device has not been used for over 7 days. • After device overhaul or when impurities are discovered.
Ultra deep wash (~12 h)	0.05% Tween-20+1 M NaCl or WB1, 0.1 M NaOH, and laboratory-grade water	Perform an ultra deep wash under the following circumstances: • The first use of DNBSEQ-G800RS. • Before sequencing, if the device has not been used for over 14 days. • After the sequencer maintenance is performed by an engineer. This includes, but is not limited to the replacement of pipelines, sample needles, and other accessories exposed to reagents.

Preparing for wash

Preparing washing reagents

Prepare the washing reagents according to table below:

Table 39 Preparing 0.1 M NaOH

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

Table 40 Preparing 0.05% Tween-20**+**1 M NaCl

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

If the reagent is made of powder, it needs to be filtered by using a 0.22 μm membrane before use.

Preparing the External Washing Cartridge

Empty External Washing Cartridges are provided with the device. To purchase a new one, order it by the catalog number according to *Order information on Page 211.*

Perform the following steps:

1. Spray a small amount of 75% ethanol on the surface of each well. Use a KimWipes tissue or a low-lint cloth to dry.

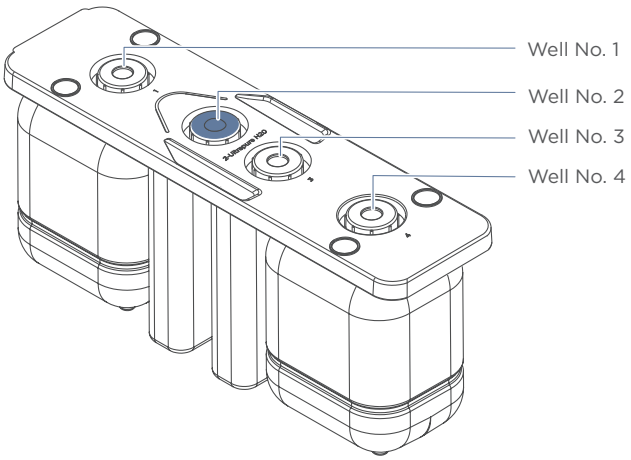


Figure 78 External Washing cartridge

2. Pierce the seals in the center of well No. 1, well No. 2, and well No. 3 to make holes approximately 2 cm in diameter by using a 1 mL sterile pipette tip.
3. Add reagents into the washing cartridge according to the following table:

Wash selection	Washing reagent	Volume (mL)	Well No.
Regular wash	Laboratory water	200	2
Maintenance wash	Laboratory water	200	2
	0.1 M NaOH	100	3
	0.05% Tween-20+1 M NaCl	200	1
Ultra deep wash	Laboratory water	1600	1
	0.1 M NaOH	300	3
	0.05% Tween-20+1 M NaCl	300	2

For ultra deep wash, the volume of washing reagents and well positions differ from other washing types. Mixed use of washing cartridges is not recommended.

Preparing DNB loading needle washing tubes

Prepare an empty 0.5 mL sterile microcentrifuge tube for washing the DNB loading needle.

Preparing wash manifold

Each wash manifold, stored at room temperature or at a 2 °C to 8 °C refrigerator, can be used repeatedly.

CAUTION

- Ensure the wash manifold is loaded and used only after the self-check is complete and the objective lens is stationary.
- Sequencing is not allowed during wash. When using the wash manifold for a wash on stage A or stage B, the other stage cannot be used for sequencing.

- A used sequencing flow cell cannot be used as a washing flow cell.
- Remove the wash manifold promptly once the wash is complete.

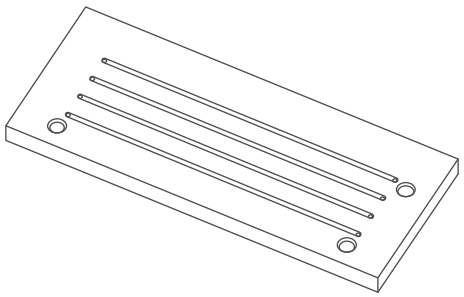


Figure 79 Wash manifold

Performing a wash

Performing a regular wash (~60 min)

Perform the following steps:

1. Before sequencing, log in to the software, and then select **Wash**.

   Or after sequencing, select **Yes** when you are prompted to wash.
2. Place the wash manifold, DNB tube(s) into the sequencer, and place the corresponding cartridges into the corresponding compartments according to the video guide in the interface.
3. Select **Wash set**.

4. Select **Regular wash** from the **Wash type** list.

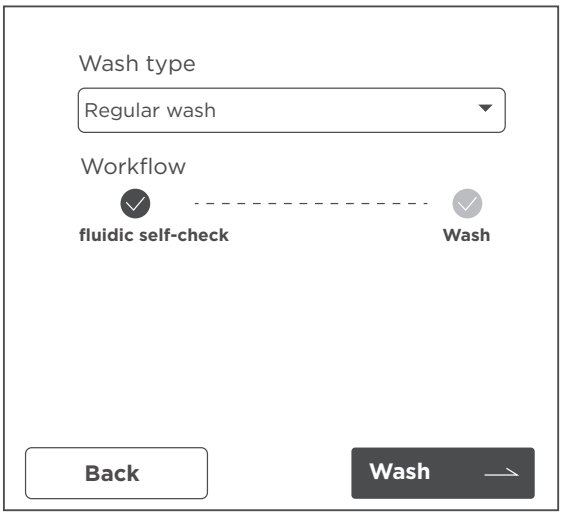


Figure 80 Selecting the wash type

5. (Optional) Select or cancel **fluidic self-check**.
6. Select **Wash** on the bottom of the interface. After confirming the information in the pop-up dialog box, select **Yes**.

   If the fluidic self-check succeeds, wash will begin automatically.

   - Select ⏸ to pause wash, and select it again to resume wash.
   - Select ■ and then select **Yes** in the pop-up dialog box to stop wash.

   If the fluidic check fails, replace the flow cell and try the fluidic check again. If the problem persists after several attempts, contact CG Technical Support.
7. When wash is completed, select ⏏ to open the Preparing material unloading interface.
8. Remove the washing materials according to the video guide in the interface.

   CAUTION Remove the wash manifold promptly once the wash is complete.
9. Select **Home** to return to the main interface.
10. Store the wash manifold at room temperature.

    A used sequencing flow cell cannot be used as a washing flow cell.
11. Dispose of DNB tube(s) according to local regulations and safety standards of your laboratory.
12. Empty the High-throughput Washing Cartridge and wash it with laboratory-grade water, and let it air-dry.

It is recommended that you dispose of the High-throughput Washing Cartridge according to local regulations and safety standards of your laboratory after it has been used 20 times.

Performing a maintenance wash (~100 min)

Perform the following steps:

1. Before sequencing, log in to the software, and then select **Wash**.

   Or after sequencing, select **Yes** when you are prompted to wash.
2. Place the wash manifold, DNB tube(s) into the sequencer, and place the corresponding cartridges into the corresponding compartments according to the video guide in the interface.
3. Select **Wash set**.
4. Select **Maintenance wash** from the **Wash type** list.

Figure 81 Selecting the wash type

5. (Optional) Select or cancel **fluidic self-check**.
6. Select **Wash** on the bottom of the interface. After confirming the information in the pop-up dialog box, select **Yes**.

   If the fluidic check succeeds, wash will begin automatically.

   - Select ⏸ to pause wash, and select it again to resume wash.
   - Select ■ and then select **Yes** in the pop-up dialog box to stop wash.

   If the fluidic check fails, replace the flow cell and try the fluidic check again. If the problem persists after several attempts, contact CG Technical Support.

7. When wash is completed, select ⊏ to open the Preparing material unloading interface.
8. Remove the washing materials according to the video guide in the interface.

   CAUTION Remove the wash manifold promptly once the wash is complete.
9. Select **Home** to return to the main interface.
10. Store the wash manifold at room temperature.

    A used sequencing flow cell cannot be used as a washing flow cell.
11. Dispose of the DNB tubes according to local regulations and safety standards of your laboratory.
12. Empty the High-throughput Washing Cartridge and wash it with laboratory-grade water, and let it air-dry.

    It is recommended that you dispose of the High-throughput Washing Cartridge according to local regulations and safety standards of your laboratory after it has been used 20 times.

Performing an ultra deep wash (~12 h)

Perform the following steps:

1. Before sequencing, log in to the software, and then select **Wash**.

   Or after sequencing, select **Yes** when you are prompted to wash.
2. Place the wash manifold, DNB tube(s) into the sequencer, and place the corresponding cartridges into the corresponding compartments according to the video guide in the interface.
3. Select **Wash set**.

4. Select **Ultra deep wash** from the **Wash type** list.

Figure 82 Selecting the wash type

5. (Optional) Select or cancel **fluidic self-check**.
6. Select **Wash** on the bottom of the interface. After confirming the information in the pop-up dialog box, select **Yes**.

   If the fluidic check succeeds, wash will begin automatically.

   - Select ⏸ to pause wash, and select it again to resume wash.
   - Select ■ and then select **Yes** in the pop-up dialog box to stop wash.

   If the fluidic check fails, replace the flow cell and try the fluidic check again. If the problem persists after several attempts, contact CG Technical Support.
7. When wash is completed, select ⏏ to open the Preparing material unloading interface.
8. Remove the washing materials according to the video guide in the interface.

   ⚠ **CAUTION** Remove the wash manifold promptly once the wash is complete.
9. Select **Home** to return to the main interface.
10. Store the wash manifold at room temperature.

    ℹ A used sequencing flow cell cannot be used as a washing flow cell.
11. Dispose of the DNB tubes according to local regulations and safety standards of your laboratory.
12. Empty the High-throughput Washing Cartridge and wash it with laboratory-grade water, and let it air-dry.

It is recommended that you dispose of the High-throughput Washing Cartridge according to local regulations and safety standards of your laboratory after it has been used 20 times.

For ultra deep wash, the volume of washing reagents and well positions differ from other washing types. Mixed use of washing cartridges is not recommended.

Reusing the High-throughput Washing Cartridge

CAUTION It is not recommended that you use the Sequencing Reagent Cartridge as a High-throughput Washing Cartridge.

High-throughput Washing Cartridges are used to hold the waste liquid during wash and can be reused.

After each use, empty the High-throughput Washing Cartridge and rinse it with the pure water until no obvious crystals or mold can be found.

Replace a High-throughput Washing Cartridge after it has been used for 20 times or every 6 months.

To purchase a new, empty High-throughput Washing Cartridge, order it by the catalog number according to *Order information on Page 211.*

Weekly maintenance

WARNING Wear a laboratory coat, a mask, and gloves before maintenance.

Clearing the historical data in the storage drive

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

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

Powering cycle the device

Perform the following steps:

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

Maintaining the power supply

Perform the following steps:

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

Cleaning the flow cell stage

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

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

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

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

Perform the following steps:

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

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

Maintaining the waste container

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

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

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

Perform the following steps:

1. Wear protective equipment.
2. Remove only the lid from the waste container.
3. Pour the waste into an appropriate waste container, and dispose of the waste according to local regulations and safety standards of your laboratory.
4. Add sufficient laboratory-grade water into the waste container, reattach the lid if necessary, and gently swirl the container until all inner walls are cleaned.
5. Pour the laboratory-grade water into an appropriate waste container.

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

Monthly maintenance

Maintaining the device

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

Perform the following steps:

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

Checking and cleaning the cooling fan

Perform the following steps:

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

Annual maintenance

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

Software maintenance

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

Storage and transportation

- Store the device according to the environment requirements in this guide.
- If you require 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 requirement in this guide. Dispose of the end-of-life device according to local regulations. However, if it is confirmed that the device is still functioning safely and effectively after maintenance, continue to use the device.

DL-200H and sealing gasket maintenance

WARNING

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

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

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

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08

FAQs

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

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

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

Sequencer FAQs

Q: What should I do if FIT Value is less than 70 during sequencing (DNBs loaded by the sequencer) ?

Perform the following steps:

1. Perform and ultra deep wash. For details, refer to *Performing an ultra deep wash (~12 h) on Page 139*.
2. Restart the device.

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

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

Perform the following steps:

1. Ensure that the power cord is plugged in.
2. Check whether fuses have blown. If so, contact CG Technical Support.

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

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

Perform the following steps:

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

3. Restart the device.

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

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

To resolve the issue, perform the following steps:

1. Check whether the flow cell attachment button is pressed.
2. Check the flow cell stage for dust, debris, or damage. Clean the flow cell stage. For details, refer to *Cleaning the flow cell stage on Page 142.*

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

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

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

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

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

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

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

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

To resolve the issue, perform the following steps:

1. Remove the flow cell, check the back of the flow cell and the stage for dust, batting, or crystals, and so on.
2. Clean the flow cell stage, and place the flow cell back onto the stage.
3. Check whether the reagent is pumped out or not. If not, add or replace the reagent.
4. If air bubbles appear in the reagent cartridge, tap the surface of the cartridge until the air bubbles disappear.

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

The waste level sensor alarms when the waste container is full, the level sensor is not installed properly, or when a software error occurs. For details, refer to *Performing pre-run checks on Page 47.*

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

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

To resolve the issue, perform the following steps:

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

Reagent FAQs

Q: What should I do if the library amount is inadequate (less than 20 fmol)?

If the library amount is less than 20 fmol but no less than 12 fmol, try 30 µL of Make DNB reaction mixture. It must be noted that 30 µL of Make DNB reaction mixture may cause data loss and lower sequencing quality than expected. When the library amount is adequate, 50 µL of Make DNB reaction mixture is still required.

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

Calculating the required amount of ssDNA libraries

The required volume of ssDNA libraries is determined by the required library amount (fmol) and library concentration quantified in *DNA library concentration and amount requirements on Page 53*. The volume of each Make DNB reaction mixture is 30 µL and the required library input for each Make DNB reaction mixture is calculated as follows:

- 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 41 Required amount of ssDNA libraries

Library type	Volume of 30 µL DNB reaction (µL)
APP-D general libraries	V=12 fmol/c
APP-D PCR-free libraries	V=18 fmol/c

Calculate the required ssDNA libraries for each Make DNB reaction mixture and fill it *Table 45 on Page 151* as *V*.

Making DNBs (PE150)

Perform the following steps:

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

Table 42 Make DNB reaction mixture 1 (PE150)

Component	Cap color	Volume (µL)
Low TE Buffer		6 – *V*
App Make DNB Buffer		6
ssDNA libraries	/	V
Total volume	/	12

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

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

Table 43 Primer hybridization reaction conditions

Temperature	Time
65 °C (Heated lid)	On
60 °C	2 min
40 °C	1 min
4 °C	Hold

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

   - Do not keep Make DNB Enzyme Mix II (LC) at room temperature.
   - To prevent enzyme inactivation caused by high temperatures, do not hold the tube.

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

Table 44 Make DNB reaction mixture 2 (PE150)

Component	Cap color	Volume (µL)
Make DNB Rapid Enzyme Mix III		12
Make DNB Enzyme Mix II (HF+LC)		1.2

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

Table 45 RCR conditions (PE150)

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

10. Immediately add 6 µL of Stop DNB Reaction Buffer when the temperature reaches 4 °C. Mix gently by pipetting 8 times by using a wide-bore, non-filtered pipette tip.

Table 46 Volume of Stop DNB Reaction Buffer (PE150)

Component	Cap color	Volume (µL)
Stop DNB Reaction Buffer		6

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

11. Proceed to *Quantifying DNBs on Page 62*.

Making DNBs (SE600)

Perform the following steps:

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

Table 47 Make DNB reaction mixture 1 (SE600)

Component	Cap color	Volume (µL)
Low TE Buffer		4.8 – *V*
App Make DNB Buffer II		7.2
ssDNA libraries	/	V
Total volume	/	12

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

Table 48 Primer hybridization reaction conditions

Temperature	Time
65 °C (Heated lid)	On
60 °C	2 min
40 °C	1 min
4 °C	Hold

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

   - Do not keep Make DNB Enzyme Mix II (LC) at room temperature.
   - To prevent enzyme inactivation caused by high temperature, do not hold the tube.

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

Table 49 Make DNB reaction mixture 2 (SE600)

Component	Cap color	Volume (µL)
Make DNB Rapid Enzyme Mix IIIII		13.5
Make DNB Enzyme Mix II (HF+LC)		1.2

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

Table 50 RCR conditions (SE600)

Temperature	Time
35 °C (Heated lid)	On
30 °C	100 min
4 °C	Hold

10. When the temperature reaches 4 °C, immediately add 4.8 µL of Stop DNB Reaction Buffer. Mix gently by pipetting 8 times by using a wide-bore, non-filtered pipette tip.

Table 51 Volume of Stop DNB Reaction Buffer (SE600)

Component	Cap color	Volume (µL)
Stop DNB Reaction Buffer		4.8

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

11. Proceed to *Quantifying DNBs on Page 62.*

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

When the DNB concentration is lower than that specified in *Table 27 on Page 62,* perform the following steps until the problem is resolved:

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

Q: What should I do if dark green crystals appear in well No. 17?

That dark green crystals appear in well No. 17 is normal due to crystallization of reagent materials in this well. At this time, thaw the cartridge and mix the reagents in the cartridge well to dissolve the crystals. Sequencing quality will not be affected.

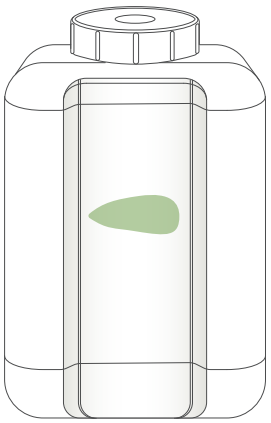

Figure 83 **Dark green crystals in well No. 17**

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

- If a kit has been thawed (not including dNTPs II) and cannot be used within 24 h, it can be frozen and thawed at most one time.
- If a kit has been thawed (including dNTPs II) but cannot be used immediately, store it at 2 °C to 8 °C. It is strongly recommended that you use it within 24 h. Mix the reagents in the cartridge according to *Preparing the Sequencing Reagent Cartridge-Part2 on Page 69* before use.
- If dNTPs Mix II, Dye Mix I, or Dye Mix II, and Sequencing Enzyme Mix II have been added into the cartridge but the cartridge cannot be used immediately, store it at 2 °C to 8 °C and use it within 24 h. Mix the reagents in the cartridge according to *Preparing the Sequencing Reagent Cartridge-Part2 on Page 69* before use.
- If dNTPs Mix II, Dye Mix I, or Dye Mix II, and Sequencing Enzyme Mix II have been added into the cartridge, the cartridge has been loaded and the reagent needles have started aspiration, but the cartridge cannot be used immediately, the cartridge must be sealed with foil or plastic wrap. Store the cartridge at 2 °C to 8 °C, and use it within 24 h. Gently mix the reagents in the cartridge before use. To prevent reagent contamination when mixing, be careful not to spill any reagent from the needle holes.

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

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

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

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

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

1. Check if the reagent needles move properly. If not, slowly lower the reagent needles manually until the tip reaches the bottom of the tube and start sequencing. If the reagent needles have moved down, perform one of the following steps:
   - If DNBs in the sample tube have not been aspirated, remove the flow cell, check for impurities in the sealing gasket, and remove dust with a canned air duster. Restart the sequencer software, re-load the flow cell according to *Loading the flow cell on Page 78*, and start sequencing.
   - If DNBs in the sample tube have been aspirated partially or completely, remove the flow cell and store at 4 °C. Restart the sequencer software, perform a sequencer wash. Re-make DNBs, re-load the flow cell according to *Loading the flow cell on Page 78*, and start sequencing.
2. If the problem persists, contact CG Technical Support.

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

If impurities appear, perform the following steps:

1. Moisten a KimWipes tissue with 75% ethanol and use it to wipe sealing gaskets on the flow cell stage, and perform a maintenance wash on the sequencer according to *Performing a maintenance wash (~100 min) on Page 138*.
2. If the problem persists after a maintenance wash, contact CG Technical Support.

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Instructions for importing barcode

Preparing a barcode file

Ensure that the barcode file meets the following requirements:

- The barcode file to be imported should be named “barcode.csv”. In the imported directory, only one “*barcode.csv*” file is available.
- It is recommended that you use the “Notepad++” program to open the barcode file. Barcode ID and barcode data in the file should be separated by a comma.
- The barcode file should not contain blank lines, full-width characters, or Chinese characters. The barcode data should include no fewer than two bases.
- Barcode data should be unique, and barcode ID and barcode data should not be empty.
- Barcode data of a dual barcode file should not contain any characters other than “A”, “T”, “C”, “G”, and “N”.
- Barcode data of a single barcode file should not contain any characters other than “A”, “T”, “C”, and “G”.
- For dual barcode sequencing, dual barcode sequencing and single barcode sequencing will be performed in order. The meaning of Barcode and Dual barcode for different libraries is shown in the following table:

Library type	Dual barcode	Barcode
MGI library	Barcode1 (PE/SE)	Barcode2 (PE/SE)
App library	i5 (PE)/i7 (SE)	i5 (SE)/i7 (PE)

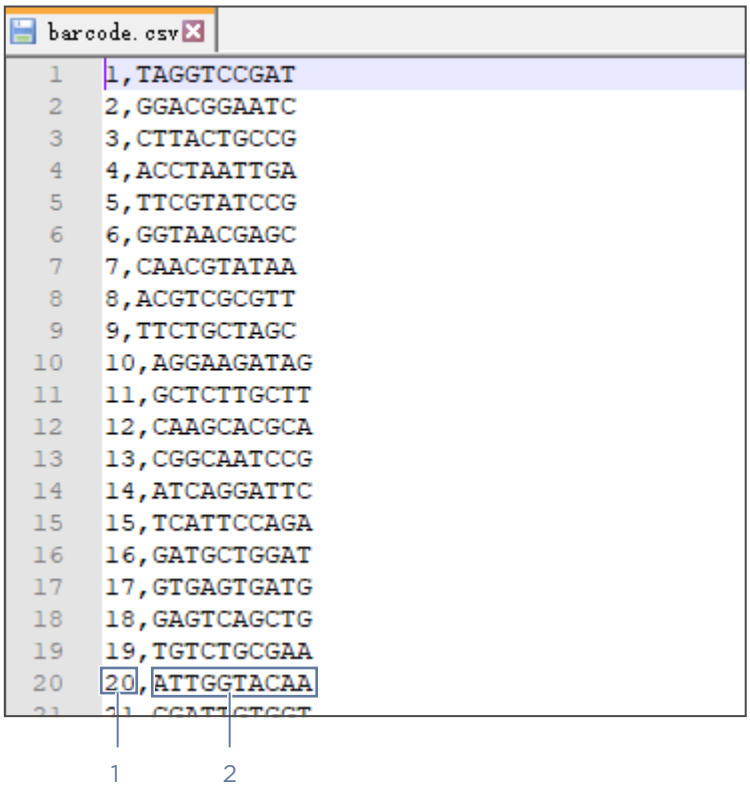

Figure 84 **Single barcode file**

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

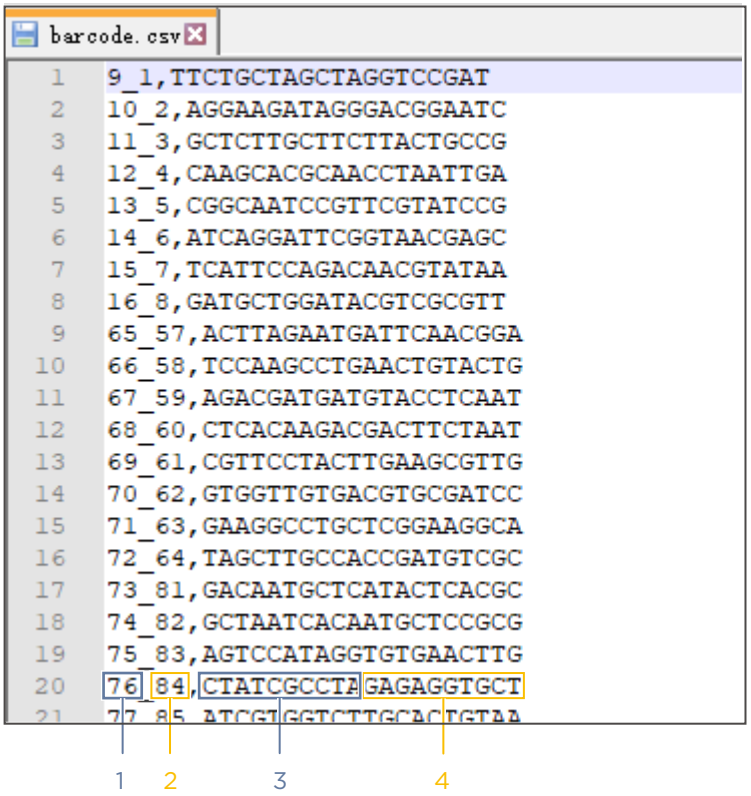

Figure 85 **Dual barcode file**

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

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

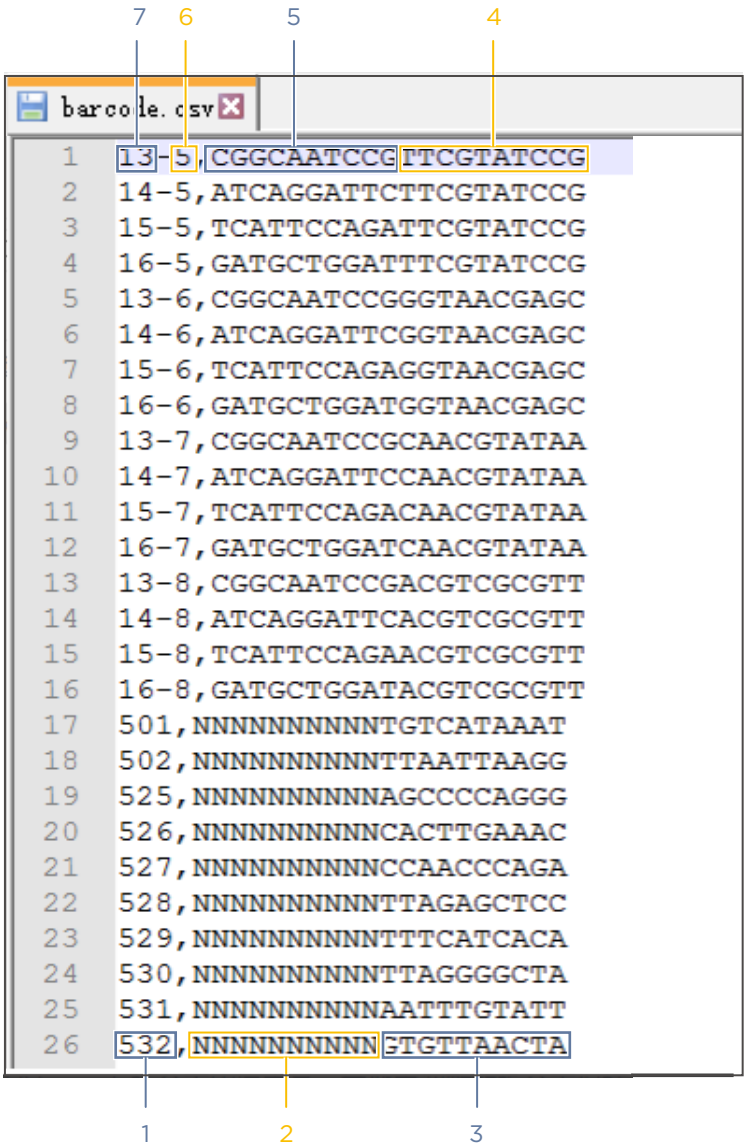

Figure 86 Single and dual barcode file1

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

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

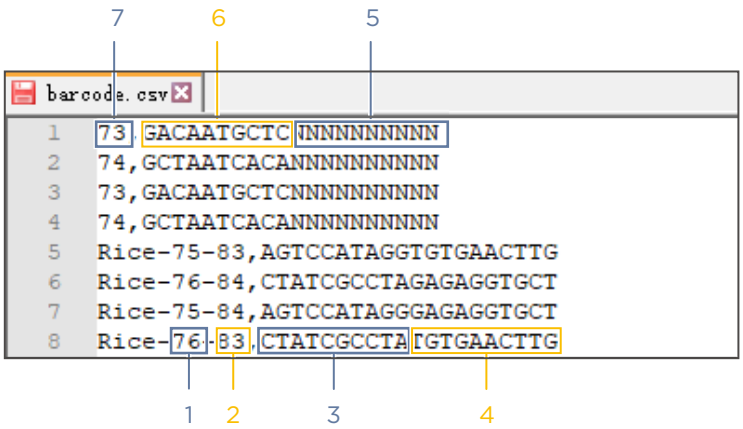

Figure 87 Single and dual barcode file2

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

Importing a barcode file

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

Perform the following steps:

1. Obtain an external storage device (for example, a USB storage drive), and create a folder in the root directory. Ensure that the folder name is in English. Copy the prepared "*barcode.csv*" file to the folder.
2. In the maintenance interface, select **Barcode**.
3. Select **Import**.
4. Select a barcode type.
   - To import a single barcode file, select **Barcode**.
   - To import a dual barcode file, select **Dual barcode**. Ensure that barcode 1 and barcode 2 have been combined in the dual barcode file.

Ensure that the barcode file type is consistent with the barcode type.

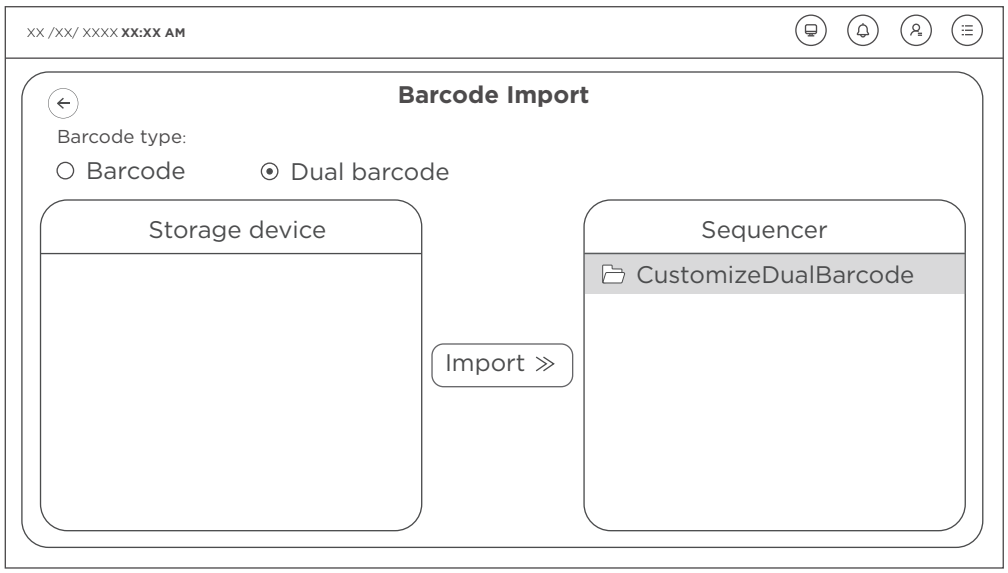

Figure 88 Barcode import interface

5. Select the export and import directories.

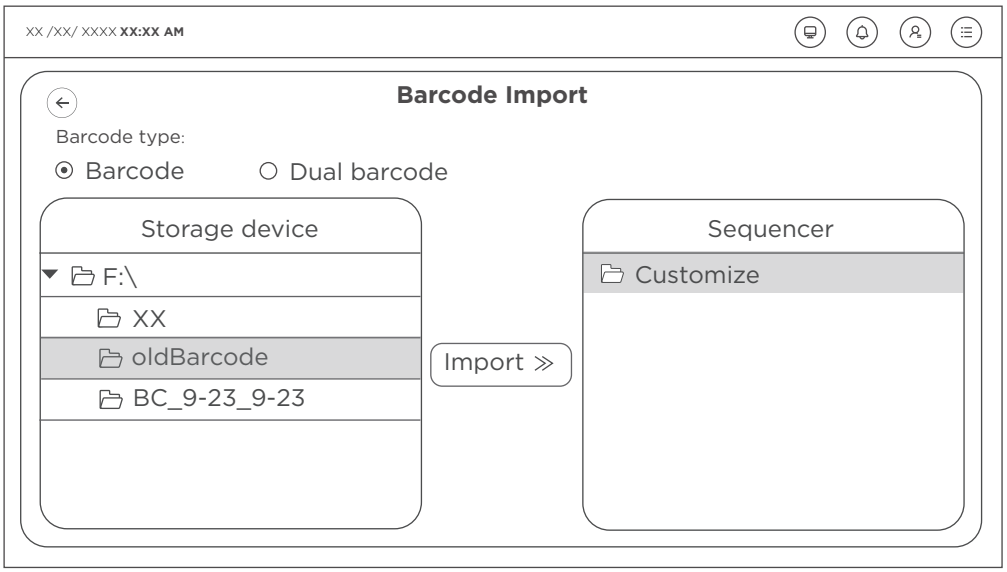

Figure 89 Importing a single barcode file

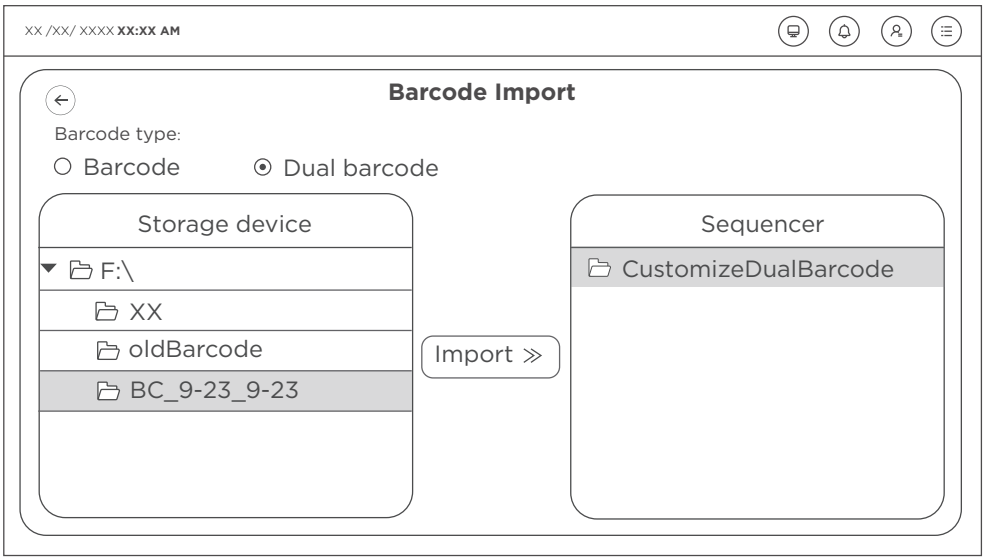

Figure 90 Importing a dual barcode file

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

     C:/ISW/Barcode/Dualbarcode
   - Single barcode file

     C:/ISW/Barcode/Barcode

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Instructions for customizing a run

Introduction

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

- When read length(s) in Read2 and/or Read1 are not the same as those predefined in the **Recipe** list.
- For a single barcode sequencing run, the barcode sequences are not within the predefined barcode list.
- All are dual barcode sequencing runs.
- Dark reaction cycles are required in Read1 and/or Read2 sequencing.
- Dark reaction cycles are required in Barcode and/or Dual barcode sequencing.
- If unexpected problems occur during DNB loading, Post loading, Sequence priming, or Sequencing, after fixing the problem, you can continue the sequencing run.

Important interfaces for customizing a run

Use either method below to open the Create recipe interface:

- In the main interface, select **Sequence > Run Set** to open the Run setting interface. Select **Customize** from the **Recipe** list to open the Create recipe interface.
- In the main interface, select > > **Recipe settings** to open the Recipe settings interface. Select **Create recipe** to open the Create recipe interface.

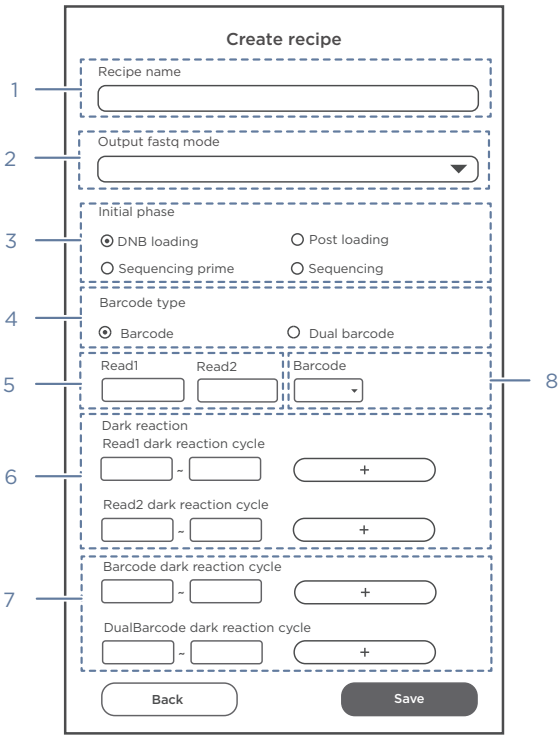


Figure 91 Create recipe interface-single barcode

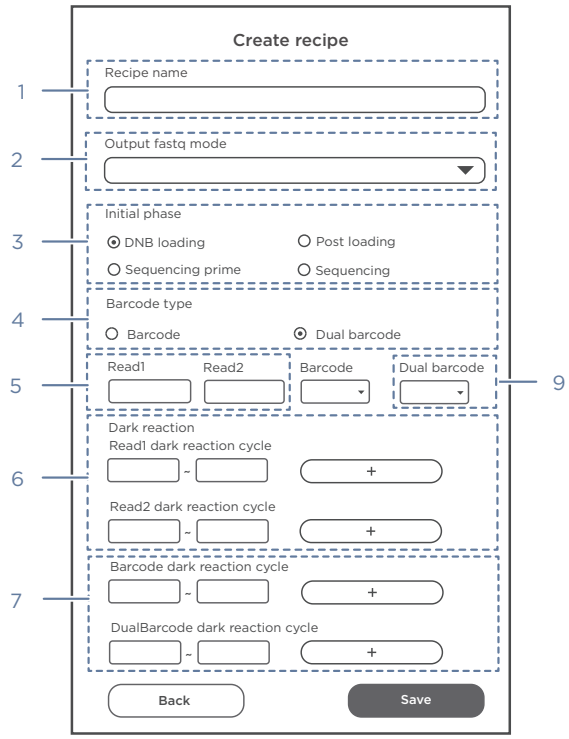
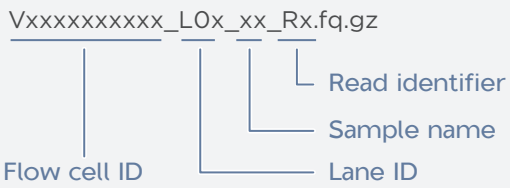
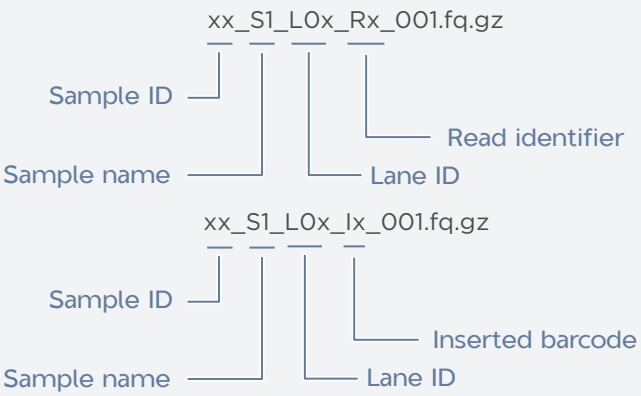


Figure 92 Create recipe interface-dual barcode

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

No.	Item	Description
1	**Recipe name**	Enter a name for the customized recipe
2	**Output fastq mode**	Select a mode to determine whether to generate the FASTQ file separately. The format of the file name varies. • If you select **Create fastq file for insert only**, the control software will not generate the FASTQ file separately. The format of the FAST file name is illustrated as follows:  • If you select **Create fastq files for insert and barcode**, the control software will generate the FASTQ file separately. The formats of the FAST file name are illustrated as follows: 

No.	Item	Description
3	**Initial phase**	Select a start phase for a sequencing run. • **DNB loading**: if you want to load DNBs by the sequencer, select **DNB loading**. • **Post loading**: if you have loaded DNBs by DL-200H, select **Post loading**. • **Sequencing prime**: if the "Post loading" process has been performed on this flow cell already, select **Sequencing prime**, and the sequencing run will start at this step. This is not common in the sequencing procedure. • **Sequencing**: if the sequencing run stops before the first cycle imaging step with the error message of "Imaging failed", restart the sequencing run by selecting **Sequencing**.
4	**Barcode type**	Select a barcode type for a sequencing run. • If you select **Barcode**, you need to set the length in the box below **Barcode**. • If you select **Dual barcode**, you need to set the length in the boxes below **Barcode** and **Dual barcode**.
5	**Read1/Read2**	Customize Read1 and (or) Read2 length for a sequencing run
6	**Read1/Read2 dark reaction cycle**	Customize dark reaction range in Read1 and (or) Read2
7	**Barcode/DualBarcode dark reaction cycle**	Customize dark reaction range in Barcode and (or) DualBarcode
8	**Barcode**	Customize barcode length for a sequencing run
9	**Dual barcode**	Customize dual barcode length for a sequencing run

Examples of customized run

Ensure that the barcode file meets the following requirements:

- Before starting the customizing run, confirm that the customized barcode files are already imported into the sequencer. If not, refer to *Instructions for importing barcode on Page 159* to import the customized barcode.
- Ensure that the total number of sequencing cycles including Read1, Read2, barcode, dual barcode, and dark cycle is less than the maximum sequencing cycles for a given sequencing kit, as defined in *Table 7 on Page 36*.
- The maximum read length for both Read1 and Read2 should be no more than that specified in the sequencing kit. For example, if PE150 is used, the maximum customized Read1 length and Read2 length should be no greater than 150.
- When you perform a dual barcode sequencing run, it is recommended that you use identical settings for the sequencing parameters in both stage A and stage B.
- Dark reaction cycle: A sequencing cycle in which the chemical reaction is performed, but with no imaging. Therefore, the output FASTQ file will not contain the dark cycle information. For example, for FCL PE150 sequencing, if cycle 2-10 for Read1 are dark cycles, the total cycles in the FASTQ file for Read1 is 141.
- The barcodes for 4 different lanes within a flow cell can be different, but barcode lengths for 4 different lanes within a flow cell have to be the same.
- If you need to split the barcode, select a barcode file such as **1~128** or **501~596** in the **Barcode** box. If you do not want to split the barcode, select **No barcode** in the **Barcode** box.
- **Double-index barcode** will be displayed in the Run setting interface if the customized recipe is set for dual barcode sequencing and will not be displayed if the customized recipe is set for single barcode sequencing.
- If you want to combine Barcode and Dual barcode into one dual barcode file for dual barcode sequencing, set **Double-index barcode** to the status in the Run setting interface.

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

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

Example 1

Assumptions are as follows:

- Sequencing run: PE150+10
- DNB loading: sequencer

- Length of Read1: 120
- Length of Read2: 140
- Length of Barcode: 10
- Length of Dual barcode: 0
- Split barcode: Yes. Barcode (1~128)
- Total cycles = 120+140+10+2= 272
- Select a PE150 kit

Perform the following steps:

1. Use either method below to open the Create recipe interface:
   - In the main interface, select **Sequence > Run Set** to open the Run setting interface. Select **Customize** from the **Recipe** list to open the Create recipe interface.
   - In the main interface, select > > **Recipe settings** to open the Recipe interface. Select **Create recipe** to open the Create recipe interface.
2. Set the recipe as shown in the following figure.

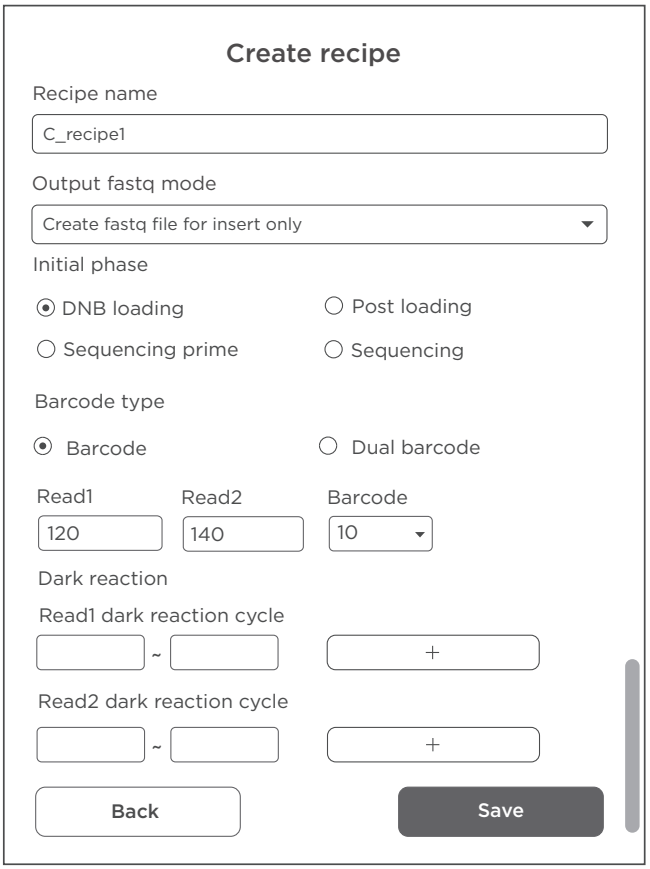


Figure 93 Setting the recipe

3. Click **Save**.
4. Set the run according to the following figure.

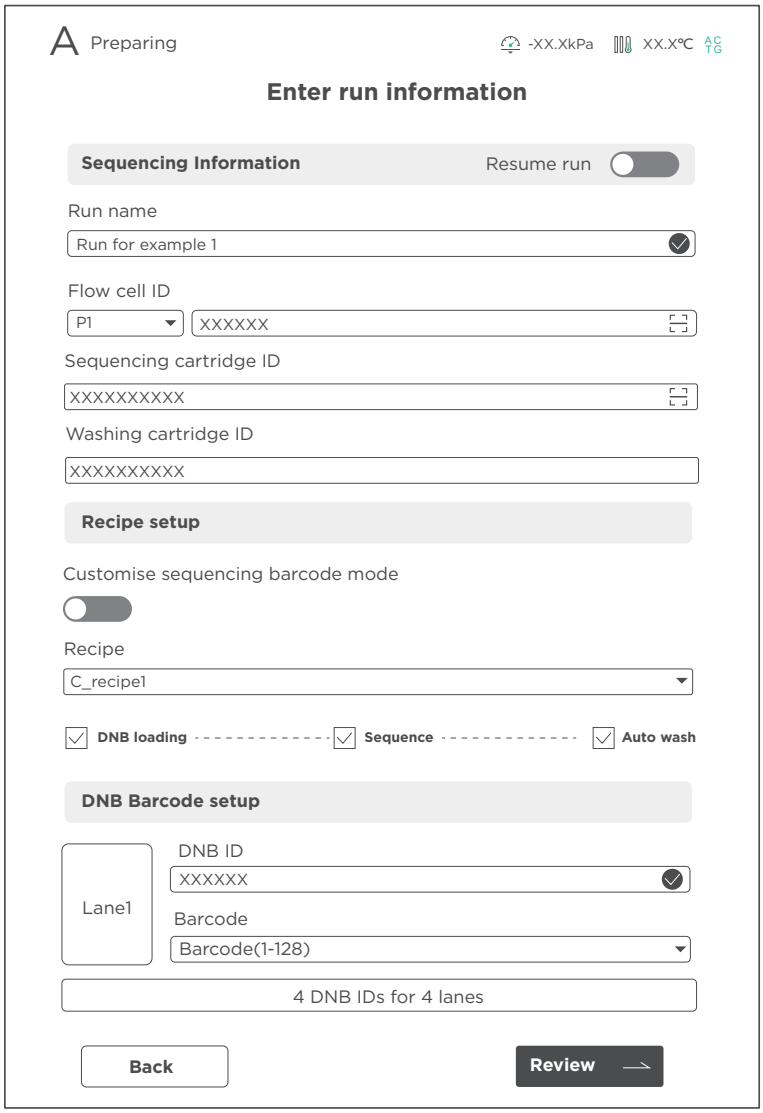


Figure 94 Setting the run

Example 2 (10×3’ RNAseq)

Assumptions are as follows:

- Sequencing run: PE100+8+8
- DNB loading: sequencer
- Length of Read1: 28
- Length of Read2: 100
- Length of Barcode: 8

- Length of Dual barcode: 8
- Split barcode: Yes, Barcode (1-64)
- Total cycles = 28+100+8+8+2= 146
- Select a PE150 kit

Perform the following steps:

1. Use either method below to open the Create recipe interface:
   - In the main interface, select **Sequence > Run Set** to open the Run setting interface. Select **Customize** from the **Recipe** list to open the Create recipe interface.
   - In the main interface, select > > **Recipe settings** to open the Recipe interface. Select **Create recipe** to open the Create recipe interface.
2. Set the recipe as shown in the following figure.

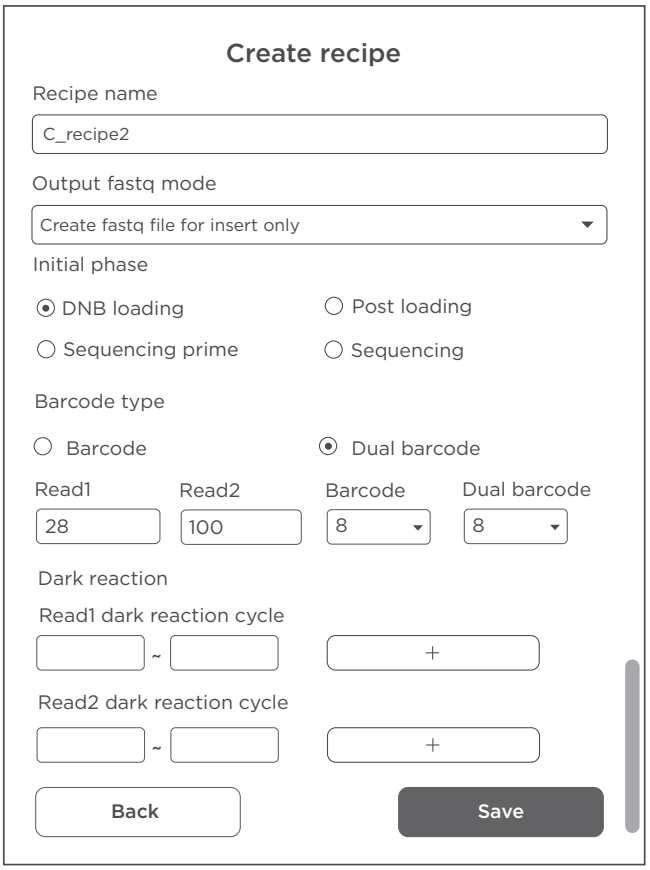


Figure 95 Setting the recipe

3. Click **Save**.

4. Set the run according to the following figure.

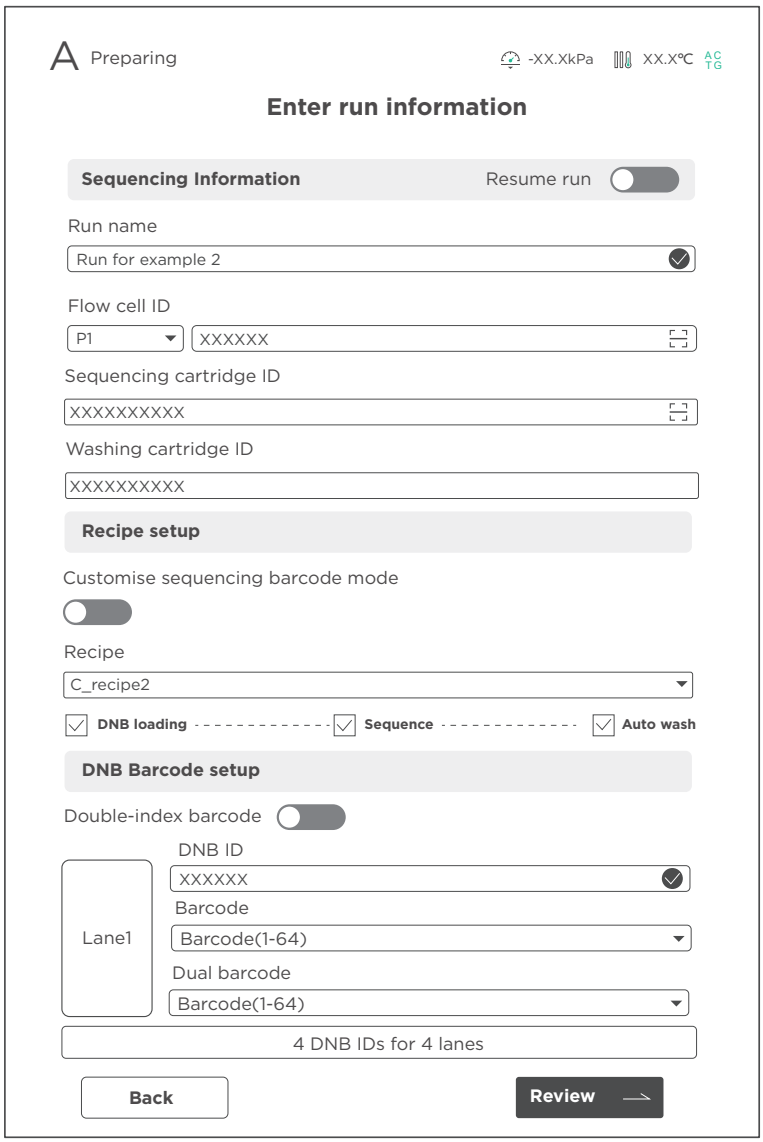


Figure 96 Setting the run

Example 3 (10×5’ RNAseq)

Assumptions are as follows:

- Sequencing run: PE150+8+8
- DNB loading: sequencer
- Length of Read1: 150
- Length of Read2: 150
- Length of Barcode: 8

- Length of Dual barcode: 8
- Split barcode: No
- Total cycles = 150+150+8+8+2= 318
- Select a PE150 kit

Perform the following steps:

1. Use either method below to open the Create recipe interface:
   - In the main interface, select **Sequence > Run Set** to open the Run setting interface. Select **Customize** from the **Recipe** list to open the Create recipe interface.
   - In the main interface, select > > **Recipe settings** to open the Recipe interface. Select **Create recipe** to open the Create recipe interface.
2. Set the recipe as shown in the following figure.

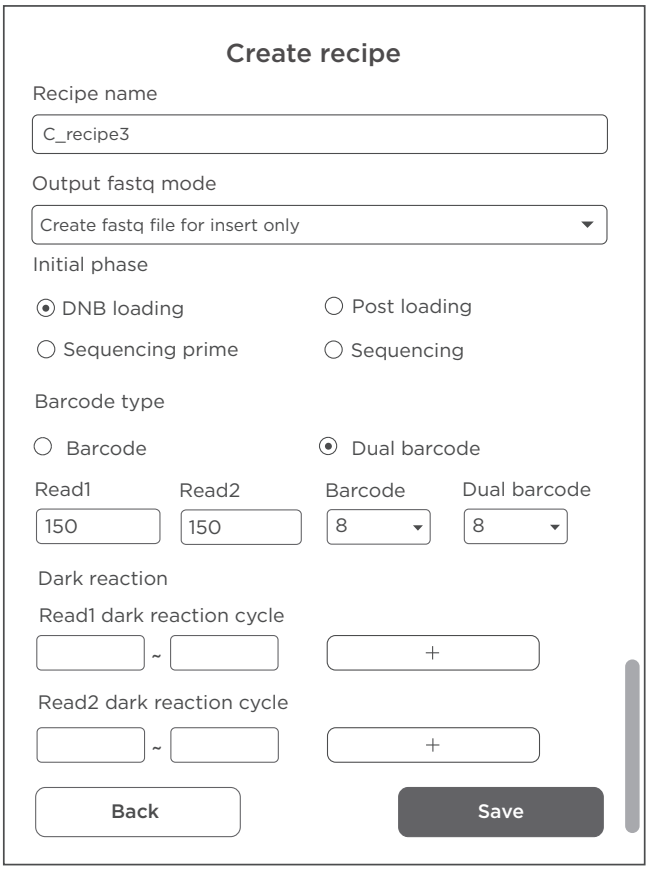


Figure 97 Setting the recipe

3. Click **Save**.

4. Set the run according to the following figure.

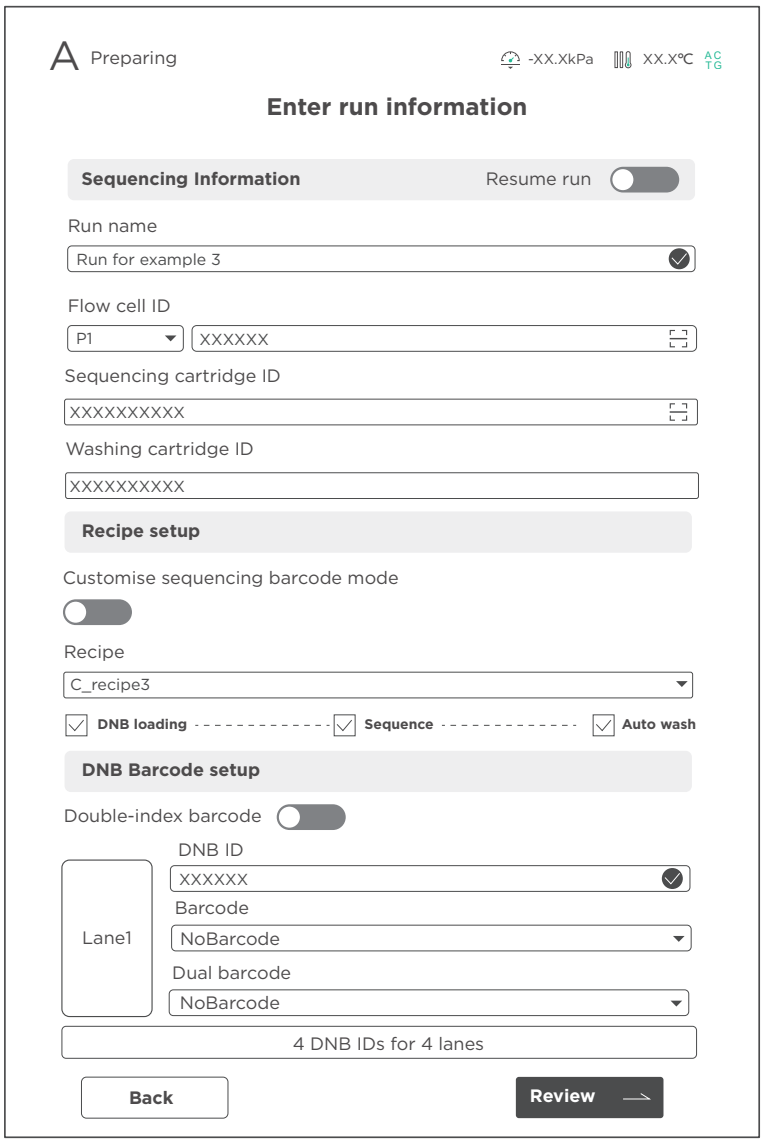


Figure 98 Setting the run

2. Length of the single barcode is not 10

Assumptions are as follows:

- Sequencing run: PE150+8
- DNB loading: DL-200H
- Length of Read1: 150
- Length of Read2: 150

- Length of Barcode: 8
- Length of Dual barcode: 0
- Split barcode: Yes, Barcode (1~64)
- Total cycles = 150+150+8+2= 310
- Select a PE150 kit

Perform the following steps:

1. Use either method below to open the Create recipe interface:
   - In the main interface, select **Sequence > Run Set** to open the Run setting interface. Select **Customize** from the **Recipe** list to open the Create recipe interface.
   - In the main interface, select > > **Recipe settings** to open the Recipe interface. Select **Create recipe** to open the Create recipe interface.
2. Set the recipe as shown in the following figure.

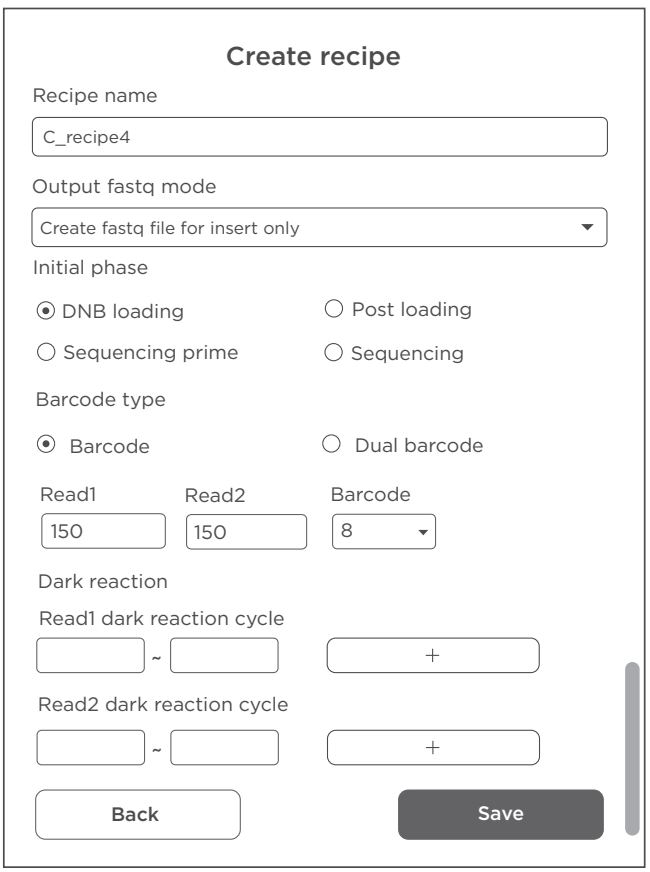


Figure 99 Setting the recipe

3. Click **Save**.

4. Set the run according to the following figure.

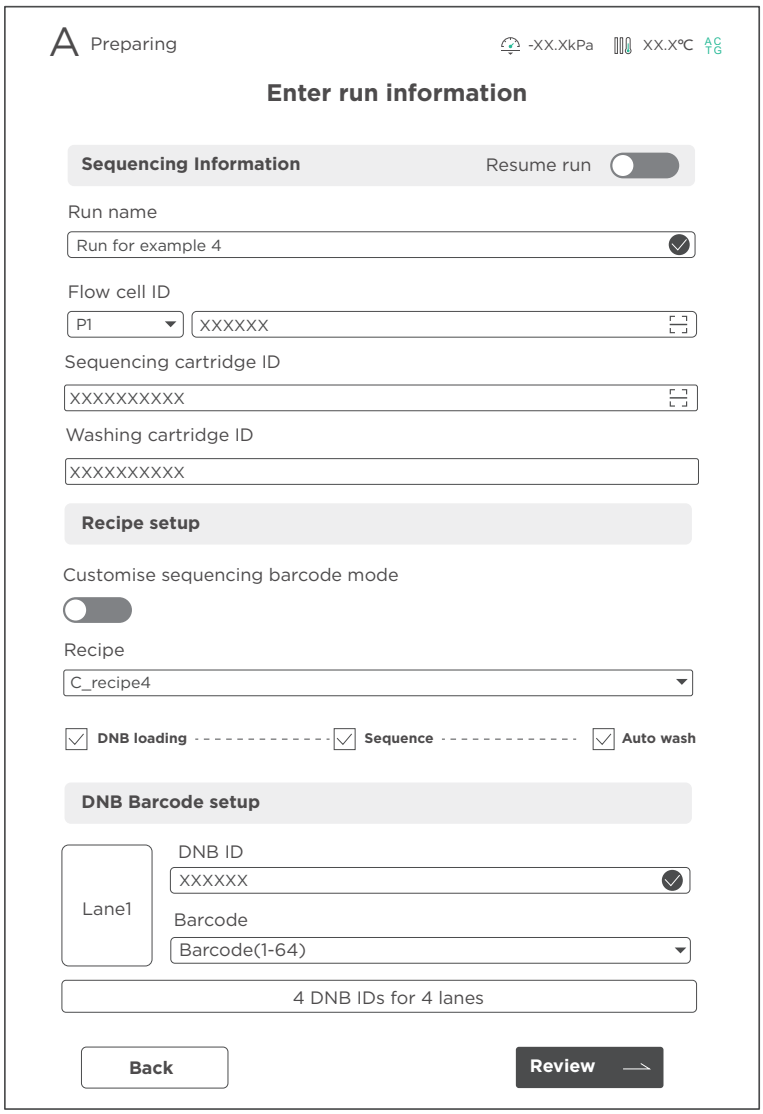


Figure 100 Setting the run

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

Assumptions are as follows:

- Sequencing run: PE150+10+10
- DNB loading: DL-200H
- Length of Read1: 150
- Length of Read2: 150

- Length of Barcode: 10
- Length of Dual barcode: 10
- Split barcode: Yes, Barcode (1~128)
- Split dual barcode: Yes, Barcode (1~128)
- Total cycles = 150+150+10+10+2= 322
- Select a PE150 kit

Perform the following steps:

1. Use either method below to open the Create recipe interface:
   - In the main interface, select **Sequence > Run Set** to open the Run setting interface. Select **Customize** from the **Recipe** list to open the Create recipe interface.
   - In the main interface, select > > **Recipe settings** to open the Recipe interface. Select **Create recipe** to open the Create recipe interface.
2. Set the recipe as shown in the following figure.

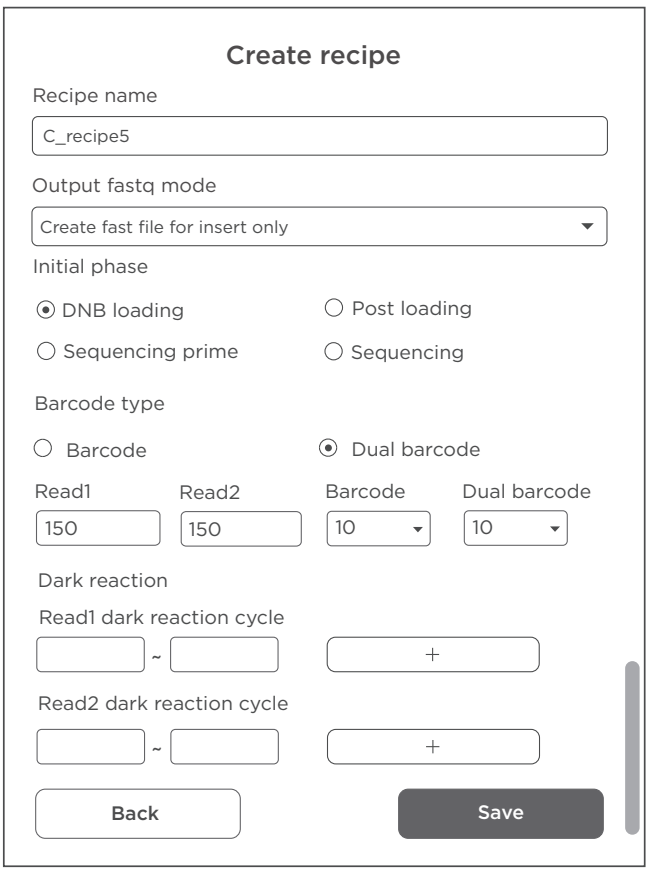


Figure 101 Setting the recipe

3. Click **Save**.

4. Set the run according to the following figure.

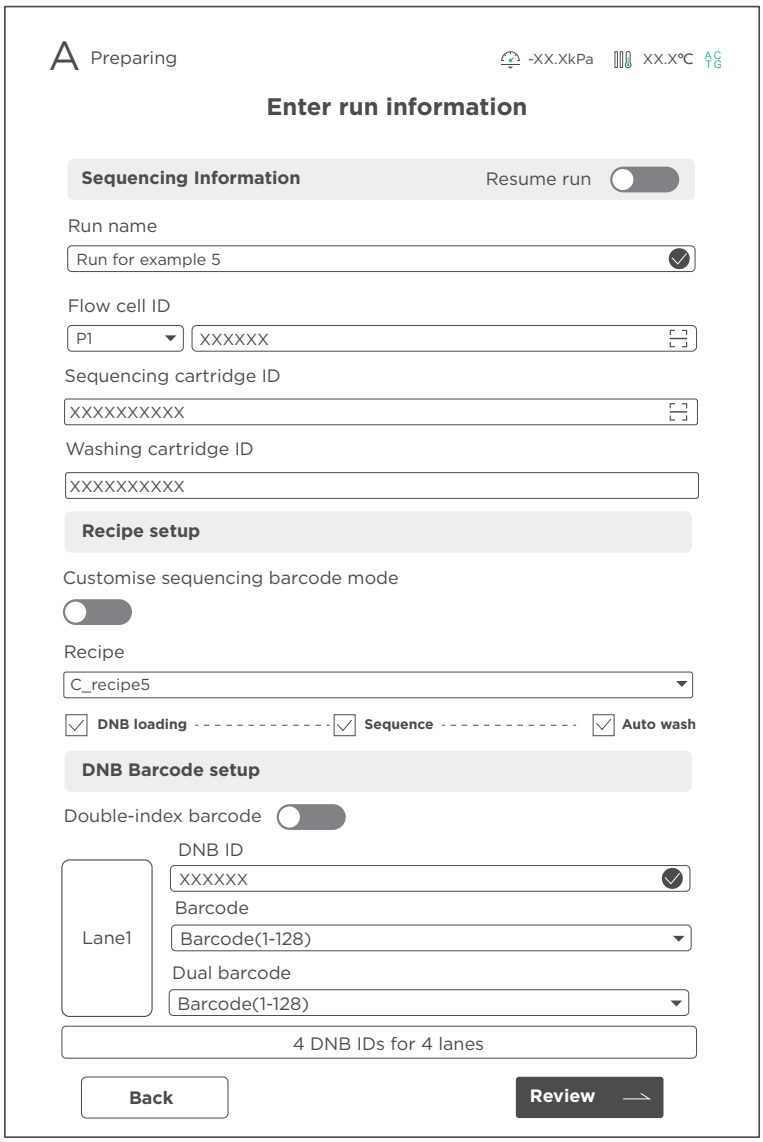


Figure 102 Setting the run

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

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

Assumptions are as follows:

- Sequencing run: PE150+8+8
- DNB loading: DL-200H
- Length of Read1: 150
- Length of Read2: 150
- Length of Barcode: 8
- Length of Dual barcode: 8
- Split barcode: Yes, Barcode (1~64)
- Split dual barcode: Yes, Barcode (1~64)
- Total cycles = 150+150+8+8+2= 318
- Select a PE150 kit

Perform the following steps:

1. Use either method below to open the Create recipe interface:
   - In the main interface, select **Sequence > Run Set** to open the Run setting interface. Select **Customize** from the **Recipe** list to open the Create recipe interface.
   - In the main interface, select  >  >**Recipe settings** to open the Recipe interface. Select **Create recipe** to open the Create recipe interface.

2. Set the recipe as shown in the following figure.

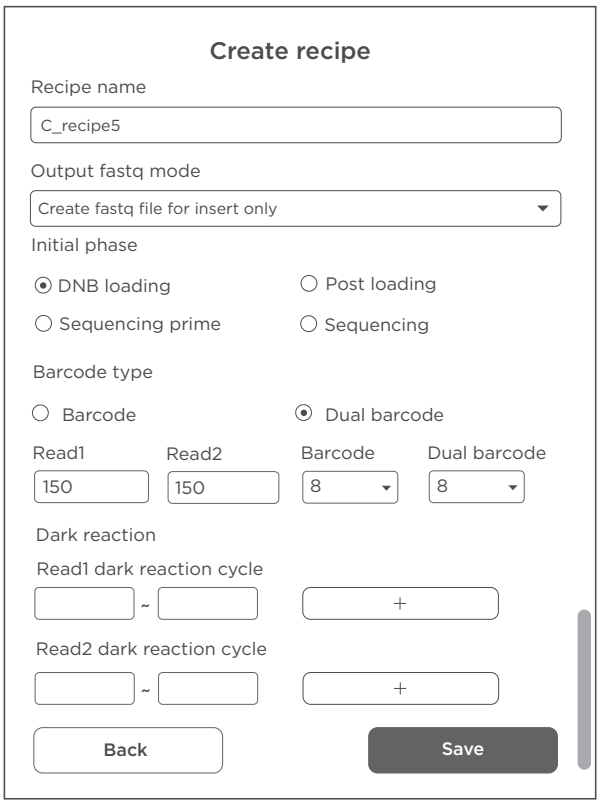


Figure 103 Setting the recipe

3. Click **Save**.

4. Set the run according to the following figure.

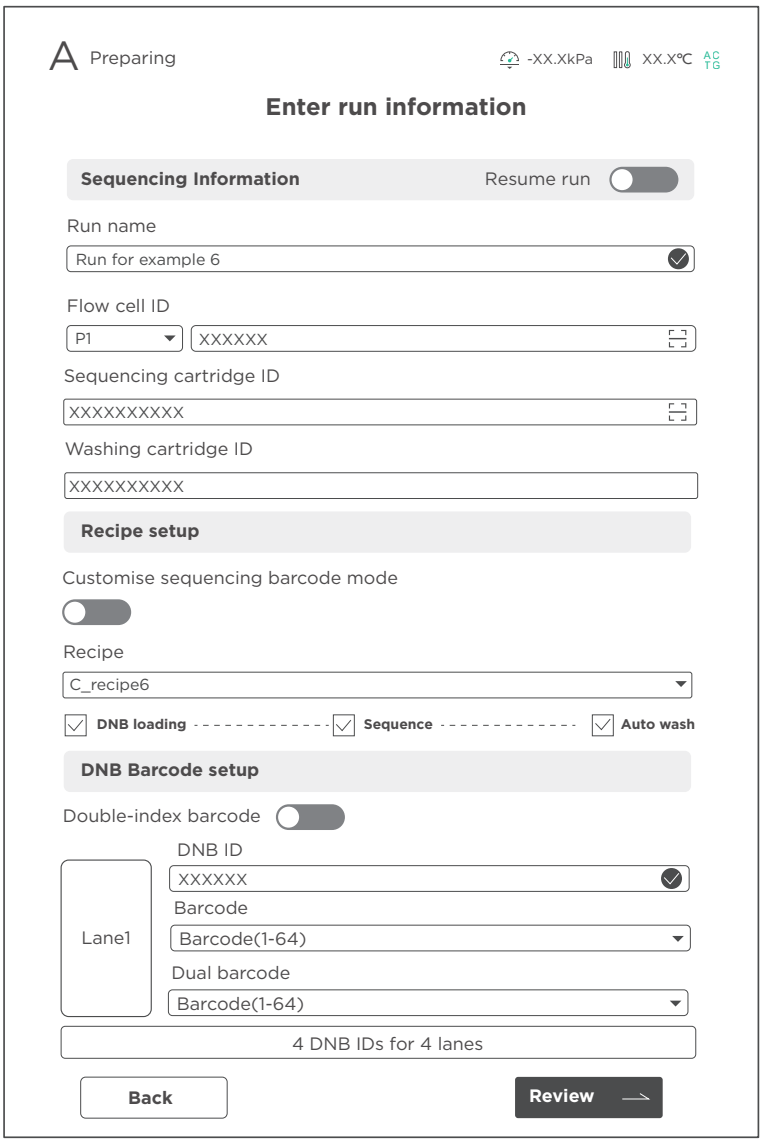


Figure 104 Setting the run

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

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

Assumptions are as follows:

- Sequencing run: PE150+8+8
- DNB loading: sequencer
- Length of Read1: 150
- Length of Read2: 150
- Length of Barcode: 8
- Length of Dual barcode: 8
- Lane1 and Lane4 require barcode splitting, Lane2 and Lane3 do not require barcode splitting
- Dark cycles: From cycle-2 to cycle-10 in Read1 and cycle-16 to cycle-20 in Read2.
- Total cycles = 141+145+8+8+2= 304
- Select a PE150 kit

Perform the following steps:

1. Use either method below to open the Create recipe interface:
   - In the main interface, select **Sequence > Run Set** to open the Run setting interface. Select **Customize** from the **Recipe** list to open the Create recipe interface.
   - In the main interface, select > > **Recipe settings** to open the Recipe interface. Select **Create recipe** to open the Create recipe interface.

2. Set the recipe as shown in the following figure.

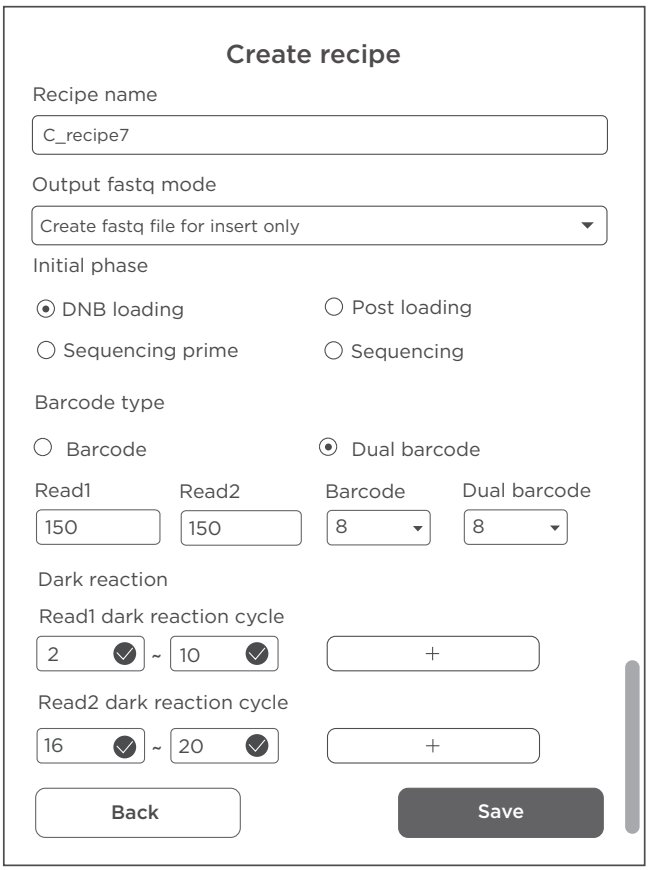


Figure 105 Setting the recipe

3. Click **Save**.

4. Set the run according to the following figure.

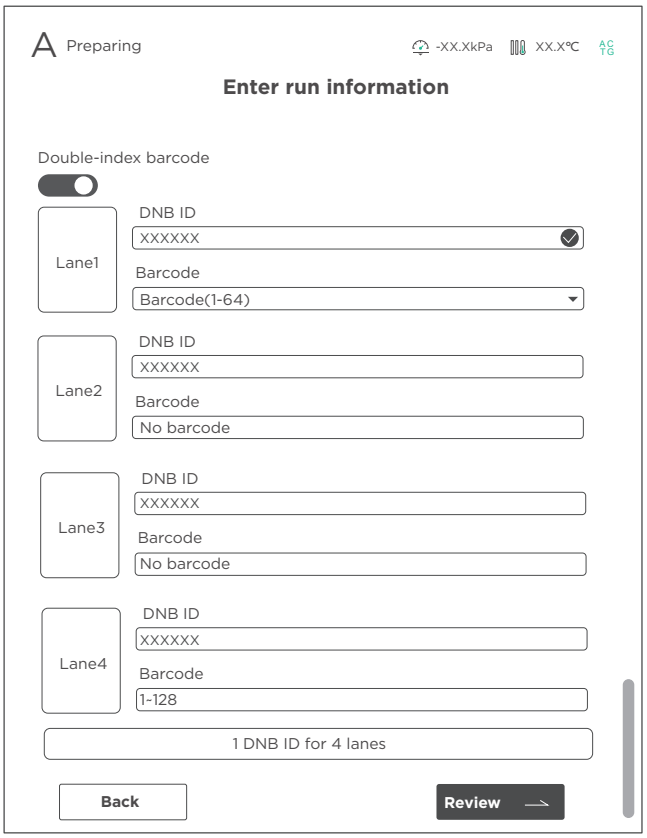


Figure 106 Setting the run

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

6. Dark reaction cycles are required in Barcode and/or Dual barcode sequencing (10X ATAC library)

Assumptions are as follows:

- Sequencing run: PE49+50+24+8
- DNB loading: sequencer
- Length of Read1: 50
- Length of Read2: 49
- Length of Barcode: 24
- Length of Dual barcode: 8
- Split barcode: Yes, Barcode (1~128)

- Split dual barcode: Yes, Barcode (1~128)
- Dark cycles: From cycle-1 to cycle-8 in Dual barcode.
- Total cycles = 50+49+16+8+2= 125
- Select a PE150 kit

Perform the following steps:

1. Use either method below to open the Create recipe interface:
   - In the main interface, select **Sequence > Run Set** to open the Run setting interface. Select **Customize** from the **Recipe** list to open the Create recipe interface.
   - In the main interface, select > > **Recipe settings** to open the Recipe interface. Select **Create recipe** to open the Create recipe interface.
2. Set the recipe as shown in the following figure.

   For 10X ATAC library, select **Create fastq file for insert and barcode** from the **Output fastq mode** list.

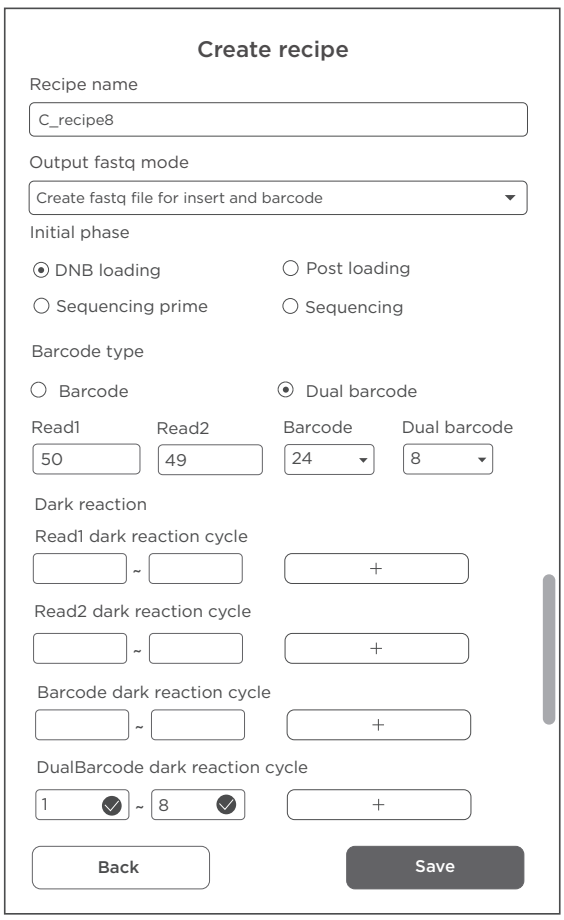


Figure 107 Setting the recipe

3. Click **Save**.
4. Set the run according to the following figure.

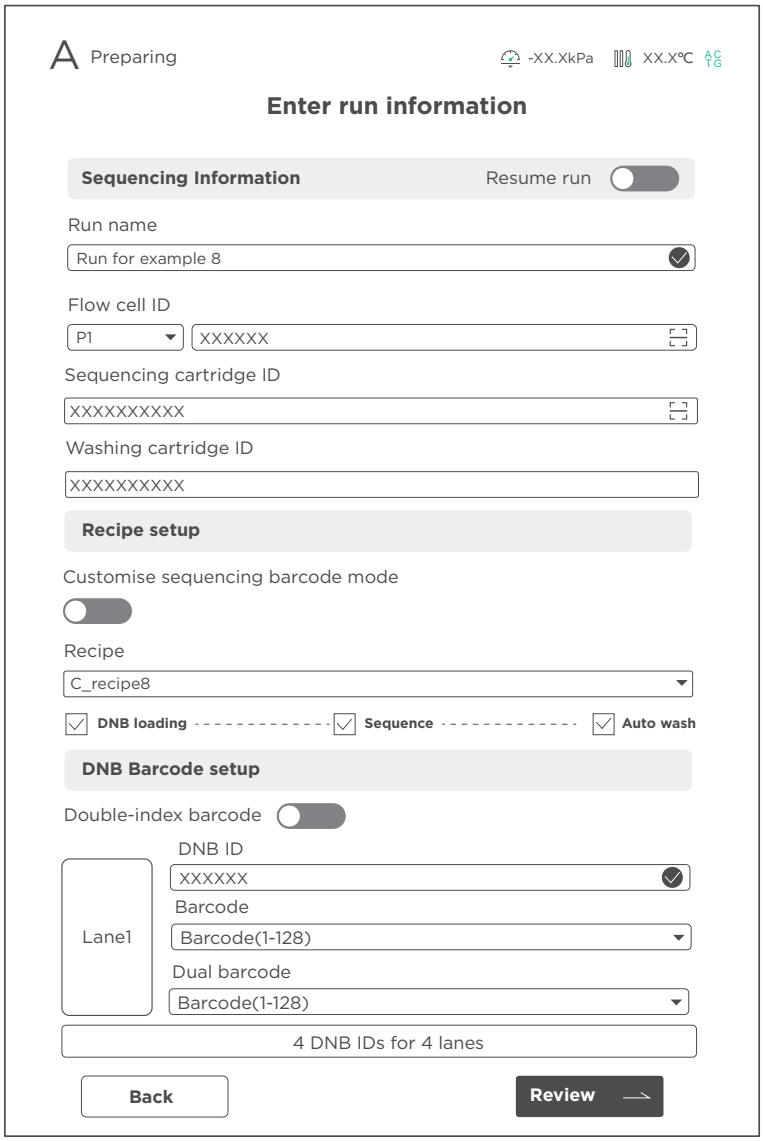


Figure 108 Setting the run

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

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Instructions for using Qubit to quantify the DNBs

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

Perform the following steps:

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

   The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit working solution, and each sample tube requires 180 µL to 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 2 mL of working solution (10 µL of Qubit reagent plus 1990 µL of Qubit Buffer).
2. Add 190 µL of Qubit working solution to each tube used for standards.
3. Add 10 µL of each Qubit standard to the appropriate tube, and mix by vortexing 3 to 5 s. Be careful not to create bubbles.
4. Set up the required number of 0.5-mL tubes for standards and samples. The Qubit ssDNA Assay requires 2 standards.

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

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

Table 52 Working solution

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

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

Instructions for performing dual-stage circular sequencing

This section applies to when the FASTQ file is required to be written to a server instead of to a sequencer.

Recommended server configuration

- Operating system: CentOS Linux 7.5
- Processor: Intel Xeon Gold 6240 CPU@2.60GHz ×2
- Storage space: More than 4.6 TB
- Network bandwidth: no less than 1000 Mbit/s
- Hard disk drive: 7200 revolutions per minute
- Control software: 1.0
- LiteCall: V2.1
- Samba: 4.7

Mapping a network drive of server

The dual-stage circular sequencing is performed by the sequencer connecting to Linux Samba.

It is recommended that you contact CG Technical Support to complete network connection, deployment of dual-stage circular sequencing, and parameter settings.

Perform the following steps:

1. Ensure that Samba is installed and configured on the Linux system. Enter the username and password to realize Samba share.
2. Ensure that the sequencer and the server are in the same Local Area Network (LAN).
3. Create a batch file on the sequencer.
   1) Create a TXT file and change the suffix of *txt* in the name to *bat*.
   2) Copy the content below to the file.

      net use H: \\10.176.2.41\share1 zlims /user:localhost\zlims

Item	Description
H:	Drive name
\\10.176.2.41	IP address for Samba share
\share1	Name of Samba share
zlims	Password to access to Samba share
user:localhost\zlims	Username to access to Samba share. "zlims" should be modified.

4. Create a task.
   1) Enter *task* in the search box on the desktop and select **Task Scheduler**.
   2) Select **Task Scheduler Library** in the menu on the left and right-click **Create Task...**.
   3) In the **General** tab, set the name. Select **Change User or Group...** > **Advanced...** > **SYSYTEM** > **OK**.
   4) In the **Triggers** tab, select **New...**, click the drop-down list of **Begin the task** and select **At log on**. Select **OK**.
   5) In the **Actions** tab, select **New...** > **Browse.** Select the batch file created in step 3, and then select **OK**.
5. After the task is created, in the Task Scheduler interface, right-click the task name and select **Run**.
6. Double-tap **This PC** on the desktop.
7. On the **Computer** tab, select the new network drive in the **Network Locations**.

   If error messages appear when accessing the drive, enter *run* in the search box on the desktop and enter \\remote IP address. Enter the username and password in the pop-up dialog box.

8. Enter the shared folder path in the folder box.
   - Folder path format: \\server IP\shared folder name
   - Example of folder path: \\172.28.35.152\Share
9. Select **Reconnect at sign-in** and select **Finish**.
10. In the pop-up dialog box, enter the server access username and password, select **Remember my credentials** as needed, and select **OK**.
11. Double-tap **This PC** on the desktop, and open the shared folder mapped above.
12. Create, modify, and delete a test file in this folder.
13. Check whether the server is connected and whether you can read and write files in the shared folder.

Starting a dual-stage circular sequencing

The stage A and stage B within the sequencer can be operated independently under dual-stage circular sequencing mode.

Before starting the dual-stage circular sequencing mode, self-check must be performed to check the authorization and available disk space of the network drive. If the two conditions are not met, error messages will appear and sequencing will be stopped.

Several precautions are listed below.

- Dual-stage circular sequencing mode is enabled when:
  - Open **This PC**, and a new network drive is mapped in the **Network Locations**. Double-click the drive, and you can successfully create a **Result** folder under the drive.
  - You will not be prompted in the control software that the remote disk cannot be accessed.
- While stage A and stage B started sequencing together, assuming that stage A finished the sequencing ahead of stage B, stage A could start the regular wash and new sequencing run.
- If stage A needs to start a new sequencing run while stage B is still in the process of sequencing, then stage B sequencing process must be paused in order to load a new sample, new Sequencing Reagent Cartridge, and new flow cell into stage A of the sequencer. This pause can only be done when the imaging is paused.

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Instructions for splitting barcode

Manual barcode splitting

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

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

C:\BasecallLite\Config

This section uses the following conditions as examples:

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

Preconditions:

- SubmitImages = false
- *.cal*FilePath is set correctly:

  D:\Result\workspace\P150060903\L02\calFile

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

Table 53 Splitting Barcode and Dual barcode

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

Table 54 Splitting dual barcode only

Parameter setting	Description
Cycle = r100e1r100e1b10b8	Enter the complete sequencing recipe.
BarcodeFile =	Enter Barcodelist of 10 bp for splitting Dual barcode only.

Parameter setting	Description
DualbarcodeSplit DualbarcodeSplit = { true, false}	Set the first DualbarcodeSplit to true, and set the second one to false.
DualbarcodeMismatch = { 1, 1 }	Set the first mismatch only

Table 55 Splitting barcode only

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

The entering order of Cycle (sequencing read length) is: Read1 length, e1, Read2 length, e1, barcode2 length, barcode1 length. If there is no extra one cycle for calibration, remove e1. Barcode2 refers to dual barcode. If no dual barcode is needed, remove the barcode2 length.

Automatic barcode splitting

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

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

1. In the main interface, select **Sequence > Run Set** to enter the Run setting interface.
2. Select a barcode range from the **Barcode** box, for example, **1~128**, or **501~596**.

   Select **4 DNB IDs for 4 lanes** if the DNB ID or barcode range is not the same for 4 lanes.

   If you select **No barcode** from the list, and no barcode file is selected in the **Barcode type** list, the barcode will not be split by the sequencer. For information on customizing a run, refer to *Instructions for customizing a run on Page 167.*

This section uses the following conditions as examples:

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

Splitting Barcode and Dual barcode

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

C:\Log

Log example:

|ISW->LITE| generateFastQ: LaneParam: S200032456L01: totalCycle: 220, read1Len: 101, read2Len: 101, barcode1Len: 8, barcode1StartPos: 213, barcode2Len: 10, barcode2StartPos: 203, endCycleMode: 3, barcodePos: 3, mismatch: 1, mismatch2: 1, speciesBarcodes: 104

Table 56 Expected parameter passing for splitting Barcode and Dual barcode

Expected parameter passing	Description
totalCycle: 220	Total read length 220
read1Len: 101	Read1 length 101
read2Len: 101	Read2 length 101
barcode1Len: 8	The barcode read length
barcode1StartPos: 213	The first cycle of the barcode
barcode2Len: 10	The dual barcode read length
barcode2StartPos: 203	The first cycle of the dual barcode
endCycleMode: 3	Both Read1 and Read2 have an extra cycle for calibration
barcodePos: 3	The sequencing order is: 1. Insert sequencing 2. Barcode sequencing
mismatch: 1	Fault tolerance of the barcode
mismatch2: 1	Fault tolerance of the dual barcode
speciesBarcodes: 104	The number of barcodes in Barcodelist

Splitting Dual barcode only

You can check whether the barcode is split successfully through logs. You can find logs in the following path:

C:\Log

Log example:

|ISW->LITE| generateFastQ: LaneParam: S200032456L01: totalCycle: 220, read1Len: 101, read2Len: 101, barcode1Len: 10, barcode1StartPos: 203, barcode2Len: null, barcode2StartPos: null, endCycleMode: 3, barcodePos: 3, mismatch: 1, mismatch2: 1, speciesBarcodes: 104

Table 57 Expected parameter passing for splitting Dual barcode only

Expected parameter passing	Description
totalCycle: 220	Total read length 220
read1Len: 101	Read1 length 101
read2Len: 101	Read2 length 101
barcode1Len: 10	The barcode read length that needs to be split, that is, the read length for the dual barcode
barcode1StartPos: 203	The first cycle of barcode that needs to be split, that is, the first cycle of the dual barcode
barcode2Len: null	If you want to split the dual barcode only, the value should be null
barcode2StartPos: null	If you want to split the dual barcode only, the value should be null
endCycleMode: 3	Both Read1 and Read2 have an extra cycle for calibration
barcodePos: 3	The sequencing order is: 1. Insert sequencing 2. Barcode sequencing
mismatch: 1	Fault tolerance of the barcode
mismatch2: 1	Fault tolerance of the dual barcode
speciesBarcodes: 104	The number of barcodes in Barcodelist

Splitting Barcode only

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

C:\Log

Log example:

|ISW->LITE| generateFastQ: LaneParam: S200032456L01: totalCycle: 220, read1Len: 101, read2Len: 101, barcode1Len: 8, barcode1StartPos: 213, barcode2Len: null, barcode2StartPos: null, endCycleMode: 3, barcodePos: 3, mismatch: 1, mismatch2: 1, speciesBarcodes: 104

Table 58 Expected parameter passing for splitting Barcode only

Expected parameter passing	Description
totalCycle: 220	Total read length 220
read1Len: 101	Read1 length 101
read2Len: 101	Read2 length 101
barcode1Len: 8	The barcode read length that needs to be split, that is, read length for the barcode
barcode1StartPos: 213	The first cycle of barcode that needs to be split, that is, the first cycle of the barcode
barcode2Len: null	If you want to split the barcode only, the value should be null
barcode2StartPos: null	If you want to split the barcode only, the value should be null
endCycleMode: 3	Both Read1 and Read2 have an extra cycle for calibration
barcodePos: 3	The sequencing order is: 1. Insert sequencing 2. Barcode sequencing
mismatch: 1	Fault tolerance of the barcode
mismatch2: 1	Fault tolerance of the dual barcode
speciesBarcodes: 104	The number of the barcodes in Barcodelist

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Device specifications

CAUTION

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

Item	Description
Laser classification of the device	Class 1 laser product
Dimensions	1086 mm × 756 mm × 710 mm (42.8 inches × 29.8 inches × 28 inches)
Net weight	Approximately 220 kg (485 lb.)
Touch screen	• Type: LCD • Size: 19.5 inches (49.3 cm) • Resolution: 1920 × 1080 pixels
Power	• Supply voltage: 100 V to 240 V~ (10% tolerance) • Transient over-voltage category:II • Frequency: 50/60 Hz • Rated power: 1200 VA
Fuse specification	T16AH250V
Maximum sound pressure level	75 dB(A)
Degrees of protection provided by enclosures (IP Code)	IPX0
Operating environment requirements	• Temperature: 19 °C to 30 °C (66 °F to 86 °F) indoor used only • Relative humidity: 20% to 80%, non-condensing • Atmospheric pressure: 70 kPa to 106 kPa • Maximum altitude: 3000 m (9843 ft)

Item	Description
Transportation/Storage environment requirements	• Temperature: -20 °C to 50 °C (-4 °F to 122 °F) • Relative humidity: 15% to 90%, non-condensing • Atmospheric pressure: 70 kPa to 106 kPa • Maximum altitude: 3000 m (9843 ft)
SBC Configuration	• Operating system: Windows10 21H2 LTSC • Processor: Intel(R) Xeon Gold 5318Y CPU @ 2.10GHz *2 / AMD EPYC 7713 64-Core 2.00GHz • RAM: 256GB DDR4 3200MHz • System Disk: 480G SSD • Data Disk: 14T NVMe SSD • Network : 10-Gigabit Ethernet
Accompanying items	Refer to the packing list

Compliance information

The device complies with the following standards:

Item	Standard
Safety requirements	• UL 61010-1 Safety Requirements For Electrical Equipment For Measurement, Control, And Laboratory Use - Part 1: General Requirements • CAN/CSA-C22.2 NO. 61010-1-12 + GI1 + GI2 (R2017) + A1 Safety Requirements For Electrical Equipment For Measurement, Control, And Laboratory Use - Part 1: General Requirements (Tri-National Standard, With UL 61010-1 And ANSI/ISA-61010-1 (82.02.01) • UL 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 • 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 (Adopted IEC 61010-2-010:2019, Fourth Edition, 2019-02, With Canadian Deviations) • UL 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 • 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 (Adopted IEC 61010-2-081:2019, Third Edition, 2019-02, With Canadian Deviations) • IEC 60825-1 Safety Of Laser Products - Part 1: Equipment Classification And Requirements

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Research use only

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

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Order information

Cat. No.	Model	Name	Version
940-001250-00	CM App-D FCL PE150	DNBSEQ-G800RS CoolMPS High-throughput Sequencing Reagent Set	V1.0
940-001733-00	CM App-D FCL SE600	DNBSEQ-G800RS CoolMPS High-throughput Sequencing Reagent Set	V1.0
510-003751-00	/	DNBSEQ-G800RS High-throughput Washing Cartridge	/
510-003707-00	/	DNBSEQ-G800RS External Washing Cartridge	/
510-003139-00	/	V2L Gasket (Sterile)	/
900-000218-00	DL-200H	Portable DNB Loader	/

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