Complete GENOMICS™
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DNBSEQ-E25RS & DNBSEQ-E25ARS System Guide

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

Complete Genomics, Inc.

About this guide

CG intends to provide this product solely for research use.

This guide is applicable to Genetic Sequencer (DNBSEQ-E25RS & DNBSEQ-E25ARS) and DNBSEQ-E25RS High-throughput Sequencing Set. The guide version is 3.0 and the software version is V1 and above.

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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.

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Revision history

	Date	Version
Revision	February 20, 2024	3.0
	September 25, 2023	2.0
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Contents

01

Safety

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

Conventions used in this guide

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

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

General safety

DANGER

- Ensure that the device is operated under the conditions specified in this guide. Otherwise, it may cause altered experimental results, device malfunction, or even personal injury.
- Ensure that the components of the device are completely installed before operation. Otherwise, it may result in personal injury.
- Maintain the device by following the instructions described in this guide to ensure best performance. Otherwise, it may result in device malfunction or even personal injury.
- Do not operate the device in the presence of flammable or explosive liquids, vapors, or gases. Otherwise, it may result in device damage, or even personal injury.
- Do not operate the device during maintenance or transportation.

WARNING

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

CAUTION

- Only the peripheral devices and consumables specified by the manufacturer can be used.
- If you have maintenance questions that are not mentioned in this guide, contact CG Technical Support.
- The device has been verified before delivery. If serious deviation occurs during use, contact CG Technical Support for calibration.
- Ensure that you are familiar with the operation of all the laboratory apparatus to be used.

Electrical safety

DANGER

- Ensure that the device is properly grounded, and the grounding resistance meets the requirements. Failure to do so may result in altered experimental results, electric leakage, or even electric shock.
- Do not remove the device cover and expose the inner components. Otherwise, electric shock may be caused.

WARNING

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

CAUTION

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

Mechanical safety

DANGER

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

Components safety

WARNING

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

CAUTION

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

WARNING

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

Symbols

Packaging

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

Symbol	Name	Description
	This way up	Indicates the correct upright position of the crated unit for transport and/or storage
	Fragile, handle with care	Indicates a device that can be broken or damaged if not handled carefully
	Keep dry	Indicates a device that needs to be protected from moisture
	Stacking limit by number	Indicates the maximum number of identical transport packages/items which may be stacked on the bottom package
	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

Symbol	Name	Description
	Humidity limitation	Indicates the range of humidity to which the device can be safely exposed
	Atmospheric pressure limitation	Indicates the range of atmospheric pressure to which the device can be safely exposed

Device

The following table describes symbols on the device:

Symbol	Name	Description
	General warning sign	Signifies a general warning
	Warning; biological hazard	Biological hazard warning
	Caution; hot surface	Indicates that the marked item can be hot and should not be touched without taking proper safety precautions
	Warning; dangerous voltage	Indicates hazards arising from dangerous voltages
	Protective earth	Indicates the terminal of a protective earth (ground) electrode
	"ON" (power)	Indicates the main power supply is on
	"OFF" (power)	Indicates the main power supply is off

Label

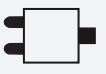
The following table describes symbols on the label:

Symbol	Name	Description
FOR RESEARCH USE ONLY Not for use in diagnostic procedures	/	Indicates a device that is for research use only, and cannot be used for clinical diagnosis
	Manufacturer	Indicates the name and address of the device manufacturer
	Date of manufacture	Indicates the date when the device was manufactured
	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
 Electrical Safety E115643	NRTL Listing and Certification Mark	Used to designate conformance to nationally recognized product safety standards. The mark bears the name and/or logo of the testing laboratory, product category, safety standard to which conformity is assessed and a control number
	Catalog number	Indicates the manufacturer's catalog number so that the device can be identified
	Use by date	Indicates the date after which the device is not to be used
	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified
	Keep away from sunlight	Indicates a device that needs protection from light sources

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

Computing module

The following table describes symbols on the computing module:

Symbol	Title	Description
	Headphone port	Reserved port.
	USB port 3.0	Connects to USB device.
	Network port	Connects to the main unit and other networks. The main unit connects to the port marked with No.2 by using the network cable.
	USB port	Connects to USB device.
	Adapter port	Connects to the adapter.
	DP port	Reserved port.
	HDMI port	Reserved port.

System guide

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

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

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02

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 functionalized 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:

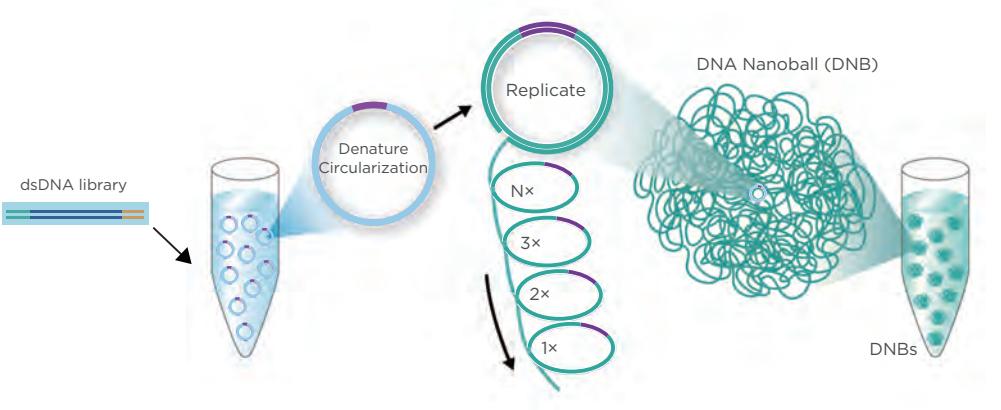

Figure 1 **Making DNBs**

The following figure demonstrates how to load DNBs:

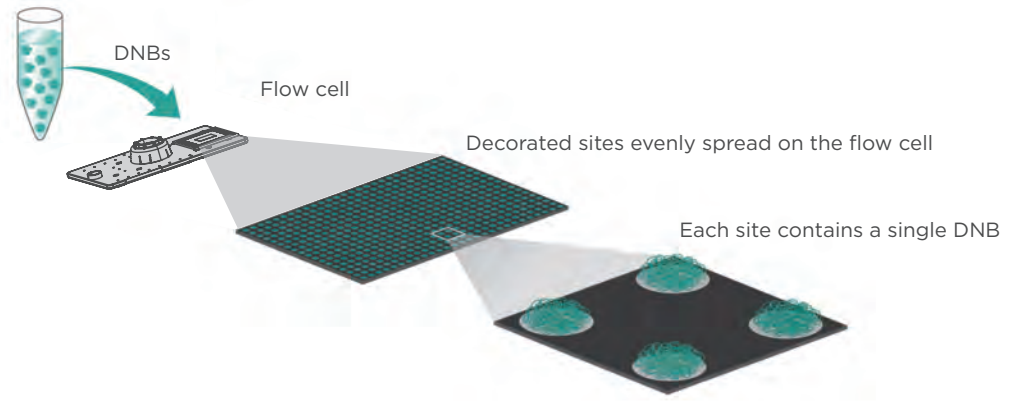

Figure 2 Loading DNBs

The DNBs and sequencing reagents are pumped into the sequencing flow cell through the device. Each DNB combines with different types of signal proteins, which then react with signal reagents to generate self-luminescent signals. These signals are gathered by the signal gathering module and then transferred to digital signals that are transmitted to and processed by the computing module to acquire the nucleotide sequence of the samples to be tested according to the type of self-luminescent signals and combinations.

Sequencer overview

Structural composition

The genetic sequencer consists of the main unit and computing module. The main unit includes the main unit module, housing module, display module, power supply module, control module, and embedded software.

The following table describes the function of each component:

Component		Description
Main unit	Main unit module	Stores reagents, loads DNBs, collects data, and so on.
	Housing module	Provides stable support and protects sensitive components from light sources.
	Display module	Shows control interface on the device and relevant information, and provides a human-computer interaction interface.
	Power supply system	Provides the power supply for the device and computing module.
	Control module	Controls the integrated system to complete communications between the main unit module and the computing module, and data storage. It includes motor module, temperature control module and imaging module.
	Embedded software	Works with hardware, controls the running of the device, and detects the signal on the sequencing flow cell.
Computing module		Processes raw data to acquire nucleotide sequence, and performs bioinformatics analysis.

Basic components

Front view

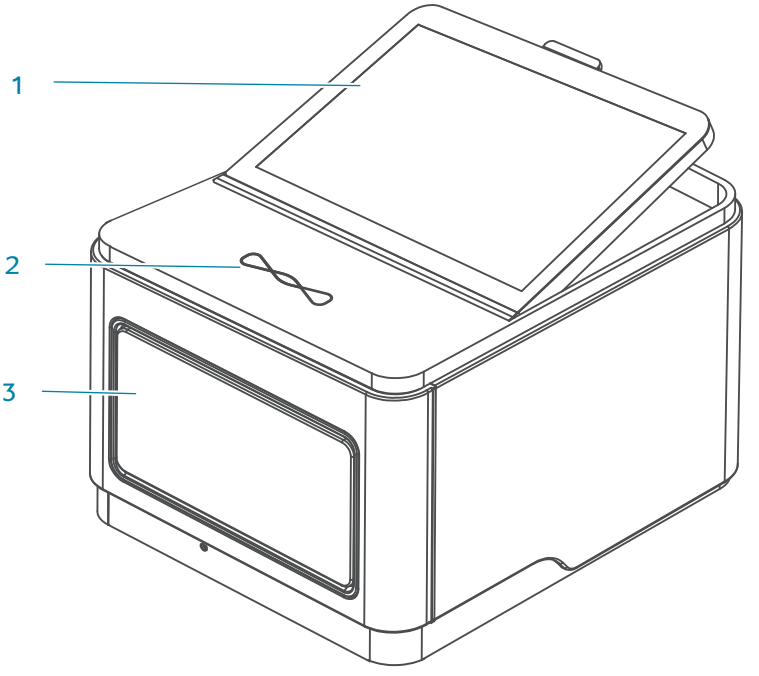

Figure 3 Front view

No.	Name	Description
1	Touch screen monitor	Facilitates on-screen operation and displays information. It is hinged at the base and can be tilted up for easy viewing.
2	Status indicator	When on, displays the current status of the device: • Green: the device is running. • Blue: the device is in standby status. • Yellow: a warning message is displayed, but the device keeps running. • Red: an error occurred and the device stops running.
3	Reagent compartment	Holds the reagent cartridge and sequencing flow cell.

Back view

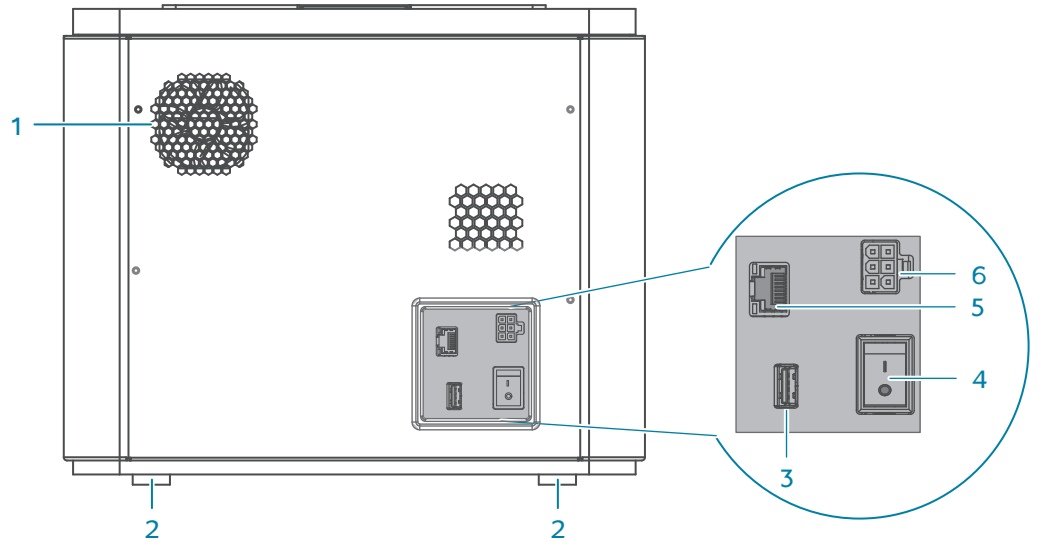

Figure 4 Back view

No.	Name	Description
1	Ventilation outlet	Exhausts air from the device.
2	Fixed feet	Support the main unit to ensure stability.
3	USB port	Connects to the QR scanner.
4	Power switch	Powers the device on and off: • Switch to the | position to power the device on. • Switch to the ◯ position to power the device off.
5	Network port	Connects the device to the computing module.
6	Power port	Connects to the power cord.

Reagent compartment

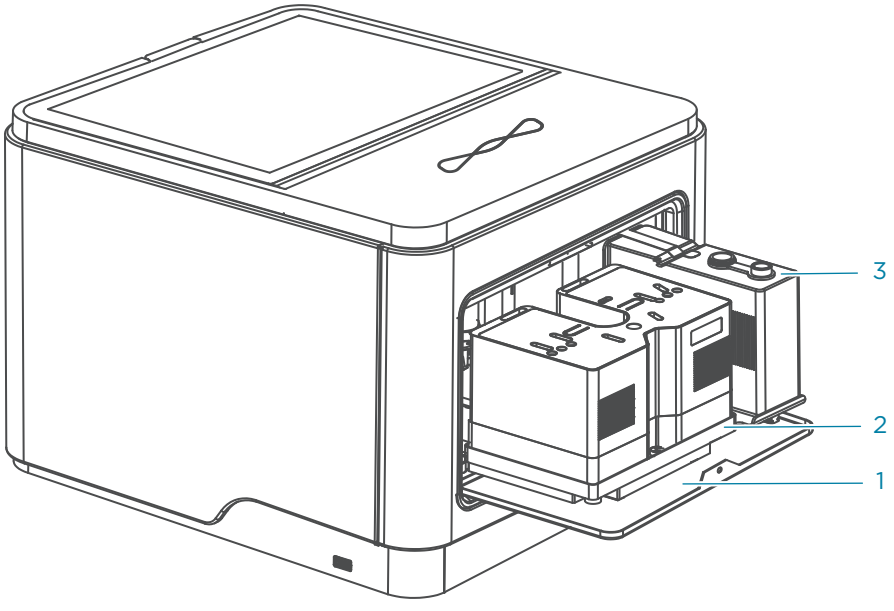

Figure 5 Reagent compartment

No.	Name	Description
1	Reagent compartment door	Opens automatically. It must be closed manually.
2	Cartridge rack	Loads flow cell, reagent cartridge and waste container.
3	Waste container	Collects waste during sequencing.

Computing module

DNBSEQ-E25RS computing module

Front view

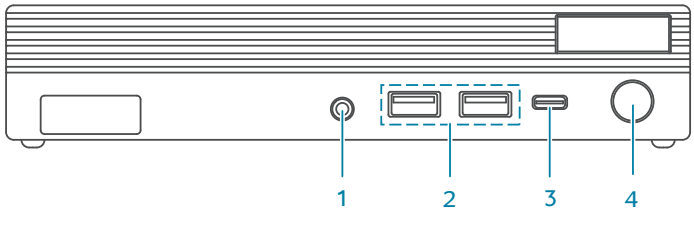

Figure 6 Front view

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 less than 3 m (118 inches).

No.	Name	Description
1	Headphone port	Reserved port.
2	USB port	Connects to USB device.
3	Type-C port	Connects to Type-C device.
4	Power button	Used to power on or off the computing module.

Rear view

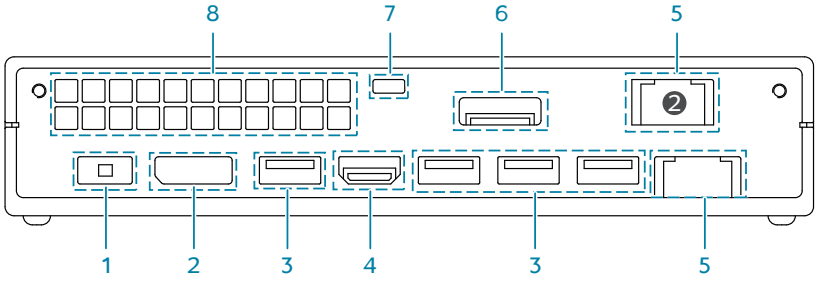

Figure 7 Rear view

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 less than 3 m (118 inches).

No.	Name	Description
1	Adapter port	Connects to the adapter.
2	DP port	Reserved port.
3	USB port	Reserved port.
4	HDMI port	Reserved port.
5	Network port	Connects to the main unit and other networks. The main unit connects to the port marked with No. 2 by using the network cable.
6	Video port	Reserved port.
7	Security lock slot	Connects to the security lock.
8	Ventilation outlet	Ventilates the device.

DNBSEQ-E25ARS computing module

Front view

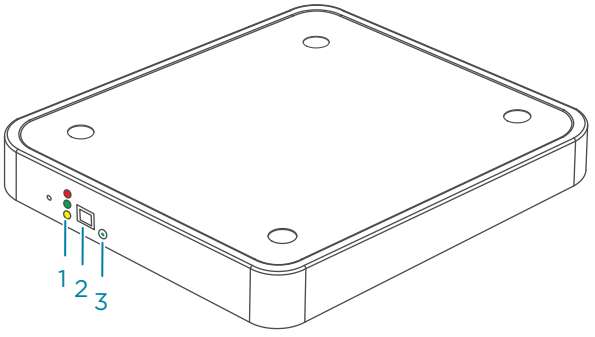

Figure 8 Front view

No.	Name	Description
1	Status indicator	Displays the running status of the device. • Green: the device is running. • Yellow: the device is in standby status. • Red: an error occurred and the device stops running.
2	Power switch	Powers the device on or off. The computing module is required to be powered on when the device is in use.
3	Reset button	Press to reset the computing module.

Side view

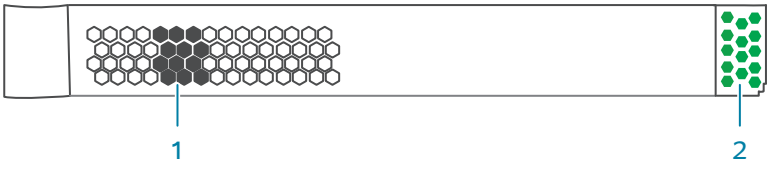

Figure 9 Side view

No.	Name	Description
1	Air inlet	Air enters into the device through this inlet.
2	Ventilation outlet	Exhausts air from the device.

Back view

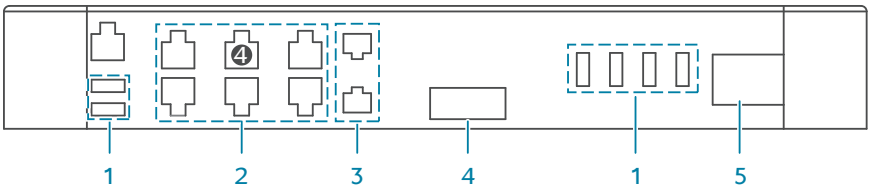

Figure 10 Rear view

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 less than 3 m (118 inches).

No.	Name	Description
1	USB port	USB device connection.
2	RJ45 port	For connecting the main unit to other networks. Connect the network cable to port No. 4.
3	SFP+ port	Fiber optics cable connection.
4	VGA port	Reserved monitor connection.
5	Power port	Power cord connection.

Control software

Overview

The system control software initiates the communication protocol through physical ports to coordinate with the hardware to control gas lines, fluidics lines, temperature, and mechanical 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 in performing various processes on the device.

Login interface

Two types of accounts are supported by the control software: administrator account and user account. They differ in ability of conducting selected functions. For functions that can only be conducted by administrator account, it will be noted where applicable. For security purposes, please change the password regularly.

Account type	Default username	Default password
Administrator account	admin	123456
User account	user	123

When the device is powered on, the login interface is displayed.

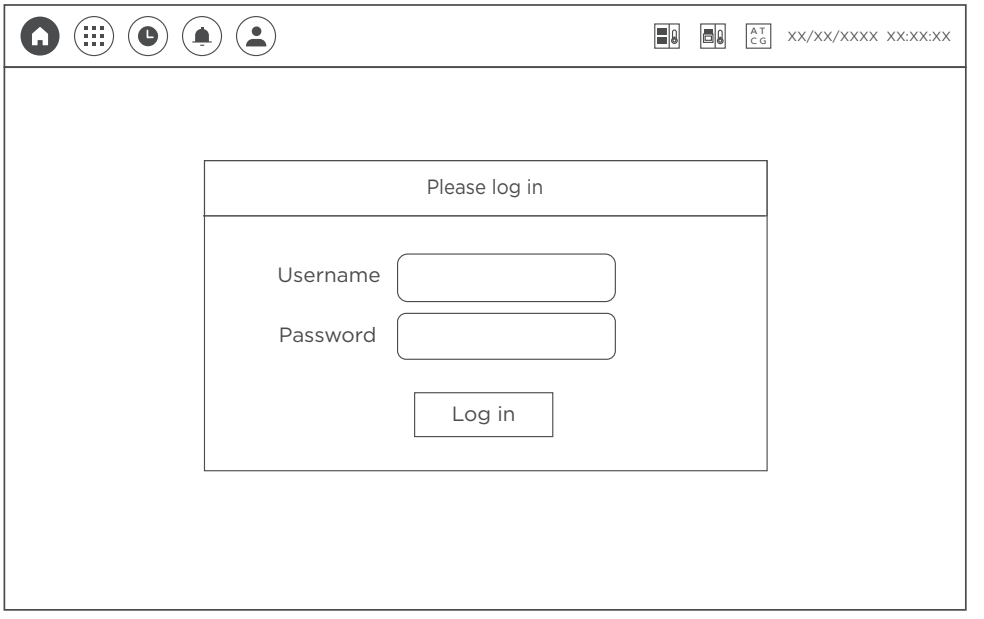

Figure 11 Login interface

Main interface

After successful login, the main interface is displayed.

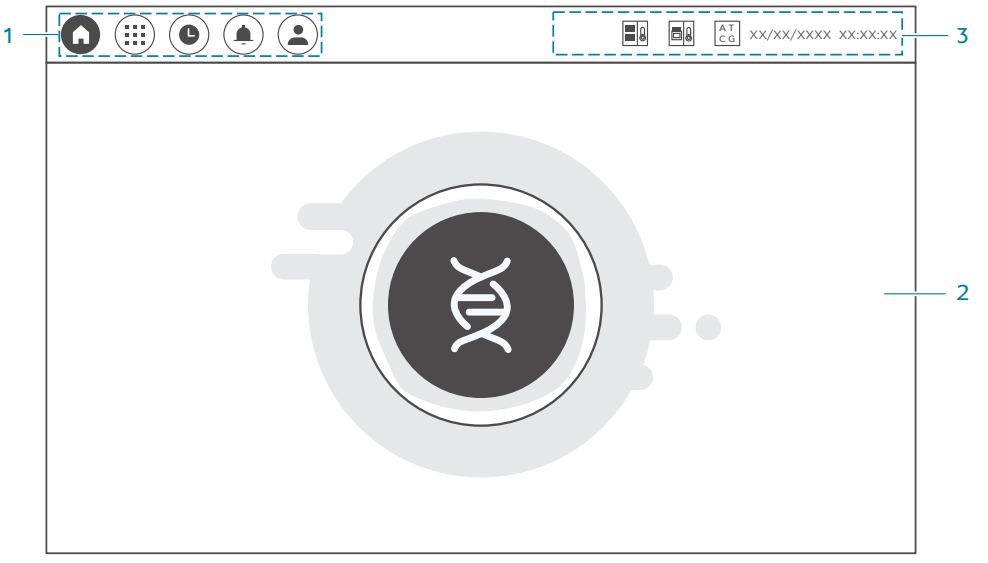

Figure 12 Main interface

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

No.	Name	Description
1	Menu area	Select to view the logs, change settings, lock the screen, shut down or restart the system, and check the system information.
2	Operation area	Provides sequencing options.
3	Status area	Indicates the status of critical device components.

Menu area

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

Item	Description
	Select to enter the main interface and perform a sequencing workflow.
	Select to view other available functions, including **Settings**, **About**, and **Shutdown**. The **Settings** interface is available to administrator accounts only.
	Select to enter the history review interface.
	Select to check the logs.

Item	Description
	Select to check the current account or log out of the current account.

Operation area

The area is used to display sequencing workflow. Select  to start sequencing.

Status area

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

Item	Description
	The computing module is being connected. This icon is dynamic.
	The computing module is connected.
	The computing module is disconnected.  Connect the computing module again after 10 or more seconds, and the status becomes “connected” or “connecting”.
xxxx/xx/xx xx:xx:xx	Displays date and time.
	Indicates the device temperature status as follows: • Green: the temperature is normal. • Red: the temperature is outside the normal range.
	Indicates the ambient temperature status as follows: • Green: the temperature is normal. • Red: the temperature is outside the normal range.

Log interface

You can view the log information in this interface.

To open the log interface, select  in the menu area, and double-click a single item to view details.

The following table describes the information of icons in the interface:

Item	Description
Time	Displays operation time.

Item	Description
Category	Displays the operation logs, warning logs, and error logs.
Details	Displays operation results.

History review interface

You can review the history in this interface.

To open the history review interface, select in the menu area, and select **History review**.

The following table describes control functions in the interface:

Item	Description
Task	Displays the number of the workflow.
Status	Includes the following status: • **Completed**: sequencing is successfully completed. • **Stopped**: sequencing is manually stopped. • **Unsuccessful**: sequencing exits abnormally, or the device does not generate the report in a specified time. • **Running**: sequencing is in progress, and the data cannot be viewed.
Size	Displays the data size.
Report	Select **View** corresponding to a specific task to check the report.
Details	Select **View** corresponding to a specific task to check the parameters.
Delete	Delete the selected history data.
Refresh	Refresh the history data.
Export	Export the selected history data.

Settings interface

Only the administrator account can access this interface.

You can change system settings such as time, language and parameters in this interface.

To open the settings interface, select in the menu area, and select **Settings**.

The following table describes control functions in the interface:

Item	Description
Time	Select to set the system time and lock screen time.
Language	Select to set the language.
Barcode parameters	Select to import and configure barcode parameters.

About interface

You can view the computing module information, device information, and system information in this interface.

To open the About interface, select in the menu area, and select **About**.

In this interface, you can check the following information:

Information type	Description
Computing module information	Displays disk space.
Device information	Displays disk space and update records.
System information	Displays system release version number, full version number, serial number, and manufacturer information.

Shutdown interface

You can shut down the system in this interface.

To open the shutdown interface, select in the menu area, and select **Shutdown**.

- For user account, select , select **Shutdown**, and select **Regular shutdown**.
- For administrator account, to transport the device, select , select **Shutdown**, and select **Device packing**.

  For device packing, the flow cell should be kept in the cartridge rack.

- To shut down the computing module, select , select **Shutdown**, and select **CM shutdown**.

User information interface

You can view the current account information and switch account in this interface.

To open the user information interface, select  in the menu area, and select **User information**.

To log out of the current account, select **Log out**. The system returns to the login interface.

03

Sequencing sets overview

This chapter describes the sequencing sets information.

Introduction

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

Available sequencing set list

Table 1 Available sequencing set list

Catalog number	Model	Name	Version	Theoretical data output (GB)
940-000894-00	FCL SE100	DNBSEQ-E25RS High-throughput Sequencing Set	1.0	About 2.5
940-000891-00	FCL PE150	DNBSEQ-E25RS High-throughput Sequencing Set	1.0	About 7.5
940-000895-00	App-C FCL SE100	DNBSEQ-E25RS High-throughput Sequencing Set	1.0	About 2
940-000901-00	App-C FCL PE150	DNBSEQ-E25RS High-throughput Sequencing Set	1.0	About 6

Sequencing read length and cycle

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. If required, an extra 10 cycles or 20 cycles of barcode read can be performed to aid in identifying a specific library.

Table 2 Sequencing cycle

Sequencing read length	Read 1 length	Read 2 length	Barcode read length	Dual barcode read length	Maximum cycles
SE100	100	--	10	10	121
PE150	150	150	10	10	322
App-C FCL SE100	100	--	10	10	121
App-C FCL PE150	150	150	10	10	322

Selected barcode file for FCL SE100/FCL PE150 is pre-imported for your convinience, For App-C sequencing, you need to customize and import the barcode file. For details, refer to *Instructions for importing a barcode file on Page 89.*

To ensure sequencing quality, when Read 1 and Read 2 sequencing are completed, the sequencer will automatically perform one more cycle for correction. For example, for PE150 dual barcode sequencing, Read 1 length is 150, Read 2 length is 150, barcode read length is 10 and dual barcode read length is 10, plus 1 correction cycle for Read 1 and 1 correction cycle for Read 2 (barcode does not require correction). The total cycle number of this sequencing is 322.

Sequencing time

Table 3 Theoretical sequencing time

Model	Sequencing time (hours)	Analysis time (minutes)
FCL SE100	5.5	10 to 20
FCL PE150	20.5	15 to 30
App-C FCL SE100	5.5	10 to 20
App-C FCL PE150	20.5	15 to 30

- The time in the table above is measured for single barcode.
- The time in the table above is average value. The actual run time might vary slightly among individual sequencers.

User-supplied equipment and consumables

Before using the device, prepare the following equipment:

Table 4 User-supplied equipment list

Equipment	Recommended brand
Freezer, -25 °C to -15 °C	General lab supplier
Refrigerator, 2 °C to 8 °C	General lab supplier
Pipette, 10 µL	Eppendorf or equivalent
Pipette, 100 µL	Eppendorf or equivalent
Pipette, 200 µL	Eppendorf or equivalent

Equipment	Recommended brand
Pipette, 1000 µL	Eppendorf or equivalent
Electronic pipette	Intergra or equivalent
Vortex mixer	General lab supplier
Qubit Fluorometer	Thermo Fisher
Thermal cycler	Bio-Rad or equivalent
Mini centrifuge	General lab supplier
Pointed-tip tweezers	General lab supplier

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

Table 5 Recommended reagent/consumable list

Reagent/Consumable	Recommended brand	Purpose
Dust-free paper	General lab supplier	Cleaning
Qubit ssDNA Assay Kit	General lab supplier	Library and DNB QC
Qubit dsDNA HS Assay Kit	General lab supplier	Library QC
Qubit Assay Tubes	Thermo Fisher Catalog number: Q32856	Library and DNB QC
Thin-wall, clear PCR tubes, 0.5 mL	AXYGEN Catalog number: 10011-830	DNB QC
Sterile pipette tip (various types)	General lab supplier	Pipetting for mixing and loading reagents
Sterile 200 µL wide-bore, non-filtered pipette tip	MGI Catalog number: 091-000355-00	Mixing DNBs
Sterile PCR 8-strip tube, 0.2 mL	Thermo Fisher	Making DNB reaction mixture
Sterile microcentrifuge tube, 1.5 mL	VWR Catalog number: 20170-038	Making DNB loading mixture
Ice box	General lab supplier	Storing crushed ice for making DNBs and DNB loading mixture

WARNING Tips are disposable consumables. Do not reuse them.

04

Sequencing

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

Workflow

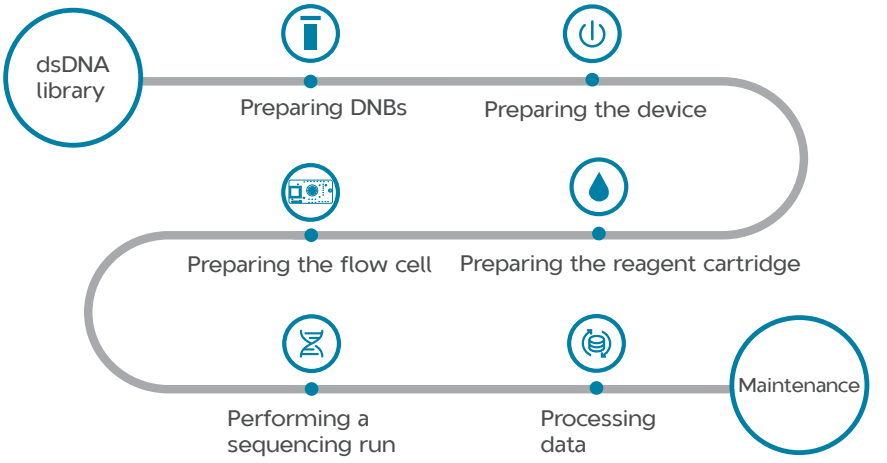

Figure 13 Sequencing workflow

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

Preparing DNBs

Recommended library insert size

This sequencing set is compatible with the dsDNA libraries with 5' end phosphate group prepared by CG Library Prep Kits. If third-party library preparation kits are used, it is recommended that you use the following conversion options:

Kit name	Brand	Cat. No.
DNBSEQ Universal Library Conversion Kit	CG	940-000963-00 (16 RXN)
DNBSEQ App Library Circularization Kit	CG	940-000914-00

For details, please contact CG Technical Support.

For general dsDNA libraries, the recommended insert size ranges between 200 bp and 500 bp, with the main insert size fragment centered within ±100 bp.

- Average data output will vary with different library types and applications.
- If there are any special requirements or specifications for the CG library preparation kit, then the requirements for the kits should be followed.

Table 6 Recommended insert size

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

DNA library concentration and amount requirements

- DNA library concentration requirement

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

$$c\ (fmol/\mu L) = 1515 \times c\ (ng/\mu L) / N$$

N represents the 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.

  - For library with MGI adapter, the initial dsDNA library concentration is required to be no less than 2 fmol/μL.
  - For App library, the initial dsDNA library concentration is required to be no less than 5 fmol/μL.

- DNA library amount requirement
  - For sequencing with library with MGI adapter, making a DNB loading mixture needs 40 fmol of library.
  - For App-C sequencing, making a DNB loading mixture needs 100 fmol of library.

    If there are any special requirements or specifications for the CG Library Prep Kit, then the requirements for the kit should be followed.

Making DNBs

- Mixed use of reagent components from different batches is not recommended.
- Use the wide-bore, non-filtered pipette tips to make, mix and load DNBs.
- Each kit is sufficient to make DNBs for 4 sequencing runs.

Perform the following steps:

1. Place the dsDNA library on ice until use.
2. Use different sequencing sets according to different sequencing recipes:
   - For SE100 sequencing, use the DNBSEQ-E25RS High-throughput Sequencing Set (FCL SE100).
   - For PE150 sequencing, use the DNBSEQ-E25RS High-throughput Sequencing Set (FCL PE150).
   - For App-C SE100 sequencing, use the DNBSEQ-E25RS High-throughput Sequencing Set (App-C FCL SE100).
   - For App-C PE150 sequencing, use the DNBSEQ-E25RS High-throughput Sequencing Set (App-C FCL PE150).
3. Thaw Low TE Buffer, Make DNB Buffer (OS-V2.0-SB) or Make DNB Buffer (OS-V2.0-DB) or Make DNB Buffer (OS-App-V4.0), Conversion Enzyme, Make DNB Enzyme Mix I (OS) and Stop DNB Reaction Buffer on ice for 30 minutes.

   - For SE100 sequencing or PE150 sequencing, Make DNB Buffer (OS-V2.0-SB) is applicable to single-barcode library.
   - For SE100 sequencing or PE150 sequencing, Make DNB Buffer (OS-V2.0-DB) is applicable to dual-barcode library.
   - Make DNB Buffer (OS-App-V4.0) and Conversion Enzyme are only applicable to App-C sequencing.
4. Mix all the reagents by using a vortex mixer for 5 seconds. Centrifuge briefly and place on ice until use.
5. Calculate the library input according to the following formula.

   Library input V(μL)=Insert size(bp)×330×2×40 fmol/c(ng/μL)/10^6

   In the formula, the insert size represents the average library length, including the adapter, as determined by fragment size analysis. *c* represents the library concentration. 330 represents the average molecular weight of base (330 g/mol). The value of *V* obtained from the above equation will be used in *Table 7 on Page 35*.
6. Use a 0.2 mL 8-strip tube or PCR tubes to prepare the Make DNB reaction mixture 1 according to the following table:

Table 7 Make DNB reaction mixture 1 (library with MGI adapter)

Component	Volume (μL)
Low TE Buffer	20-V
Make DNB Buffer	20
dsDNA library	V
Total volume	40

The type of Make DNB Buffer depends on the buffer taken out in step 3.

Table 8 Make DNB reaction mixture 1 (App library)

Component	Volume (μL)
Low TE Buffer	20-V
Make DNB Buffer	20
dsDNA library	V
Conversion Enzyme	0.5
Total volume	40.5

The type of Make DNB Buffer depends on the buffer taken out in step 3.

7. Mix Make DNB reaction mixture 1 thoroughly by using a vortex mixer, centrifuge it for 5 seconds. Place the mixture into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown in the table below:

Table 9 Primer hybridization reaction conditions (library with MGI adapter)

Temperature	Time
105 °C (Heated lid)	On
95 °C	3 min
57 °C	3 min
4 °C	Hold

Table 10 Primer hybridization reaction conditions (App library)

Temperature	Time
105 °C (Heated lid)	On
37 °C	5 min
95 °C	3 min
40 °C	3 min
4 °C	Hold

8. Place Make DNB Enzyme Mix II (OS) on ice, centrifuge briefly for 5 seconds and then place it on ice until use.

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

9. Remove the tube out of the thermal cycler when the temperature reaches 4 °C. Centrifuge briefly for 5 seconds and place the tube on ice. Add the following reagents to the tube:

Table 11 Make DNB reaction mixture 2

Component	Volume (μL)
Make DNB Enzyme Mix I (OS)	40
Make DNB Enzyme Mix II (OS)	4

10. Add all the Make DNB reaction mixture 2 into the Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer and centrifuge it for 5 seconds. Place the tube into the thermal cycler for the next reaction. The conditions are shown in the table below:

    - When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, preheating 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 12 RCR (Rolling Circle Replication) conditions

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

11. Remove the tube out of the thermal cycler when the temperature reaches 4 °C, add 20 µL of Stop DNB Reaction Buffer to the tube, and gently pipette the reagent 8 times to mix it by using a wide-bore, non-filtered pipette tip.

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

Quantifying DNBs

Perform the following steps:

When DNB making is completed, take out 2 µL of DNBs, and use Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify the DNBs. For details, refer to *Instructions for using Qubit to quantify DNBs on Page 101*.

- If the concentration is between 4 ng/µL and 40 ng/µL, the DNB meets requirements for sequencing.
- If the concentration is less than 4 ng/µL or more than 40 ng/µL, refer to *Q: What should I do if DNB concentration does not meet requirements? on Page 86* for details.

Preparing the device

Powering the device on

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 one end of the power cord to the power port on the device and the other end to the main supply.
2. Connect one end of the power cord to the power port on the computing module and the other end to the main supply.
3. Connect the main unit to the computing module with a network cable.

4. (Optional) If a UPS is prepared, connect one end of the UPS power cord to the device and the other end to the main supply.
5. Turn the power switch of the device to the | position.
6. Power on the computing module. The status indicators light up.

Logging in to the control software

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

Perform the following steps:

1. Ensure that the device is powered on.
2. Log in to the control software with the username and password. The default username is **admin** while the password is **123456**.

Performing pre-run checks

Ensure that the environmental temperature and humidity meet the requirements in *Device specifications on Page 107*. Ensure that any temperature fluctuations are within the specified range throughout the sequencing and that the humidity is constant.

Preparing the sequencing reagent cartridge

- For single-end sequencing, prepare the reagent cartridge according to steps 1 to 10 and step 12.
- For pair-end sequencing, prepare the reagent cartridge according to steps 1 to 12.
- Signal Protein 1, Signal Protein 2 and Signal Protein Buffer are provided in different tubes and are packaged together with the Sequencing Reagent Cartridge. Before the sequencing run starts, an appropriate amount of Signal Protein 1, Signal Protein 2 and Signal Protein Buffer needs to be mixed together and added into the MSP well (MSP, Mixture of Signal Protein) of the Sequencing Reagent Cartridge.
- If you perform pair-end sequencing, an appropriate amount of MDA Enzyme Mix and MDA T-Reagent needs to be mixed together and added into the MDA well (MDA, Multiple Displacement Amplification).
- If prepared reagent cartridges are not used immediately, refer to *Q: What rules should I follow if I need to store an open or thawed reagent kit temporarily? on Page 86*.

Perform the following steps:

1. Place the reagent cartridge upright as the following figure with the label facing up. The well information is shown as the following figure.

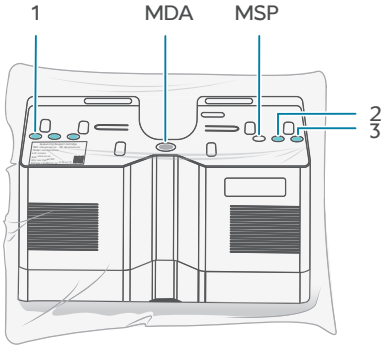

Figure 14 Placing the reagent cartridge upright

2. Thaw the reagent cartridge and Signal Protein Buffer according to the following table, which lists approximate thawing times. Choose the method that best suits your situation.

Model	Method	
	In a refrigerator at 2 °C to 8 °C (hours)	At room temperature from 15 °C to 25 °C (hours)
FCL SE100	6	3.5 to 4.5
FCL PE150	10	4.5 to 5
App-C FCL SE100	6	3.5 to 4.5
App-C FCL PE150	10	4.5 to 5

- The reagent cartridge is completely thawed when there is no sound of cracked ice during shaking.
- Keep other components at -25 °C to -15 °C if the reagent cartridge is thawed overnight.
- Do not thaw the reagent cartridge in a water bath.

3. Thaw Signal Protein 1, and Signal Protein 2 by placing them on ice for about 10 minutes. Thaw DNB Load Buffer II on ice until use.
4. After thawing the reagents, determine if there is ice in the cartridge by shaking the cartridge. If there is a sound of cracked ice, place the reagent cartridge at room temperature until no ice exists and use dust-free paper to remove condensation from the reagent cartridge.

- Do not use the cartridge and transfer it to the designated container immediately when its packaging bulges or breaks, or when the reagents instead of condensation are leaking out of it.
- The reagents are leaking out of the reagent cartridge when the liquid is in color or leaks out of the bottom, the bottom cover in particular, of the reagent cartridge and when the amount of liquid is large enough to moisten the whole bottom part of the reagent cartridge.
- The flowing liquid is condensation when it exists on the sides or corners of the cartridge.

5. Hold the two sides of the cartridge with two hands. Invert it 20 times and gently tap it on a flat surface 10 times. Invert it 10 times and gently tap it on a flat surface 10 times again.

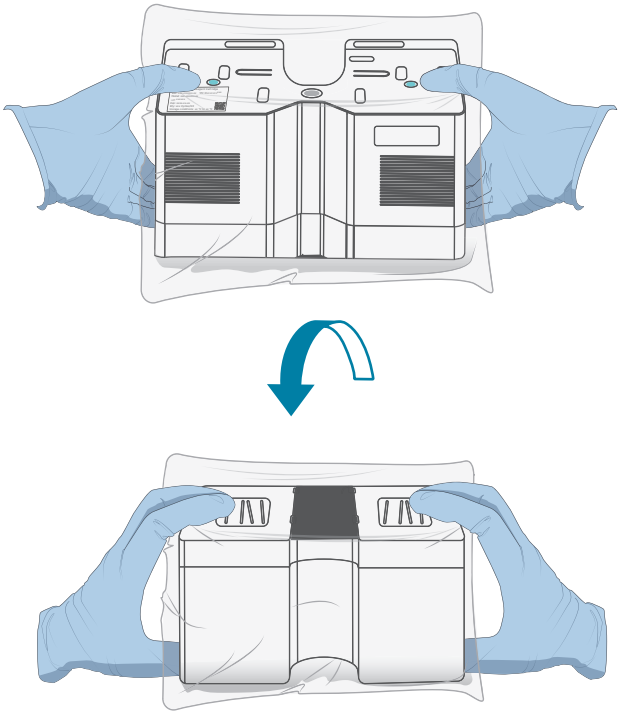

Figure 15 Inverting the reagent cartridge

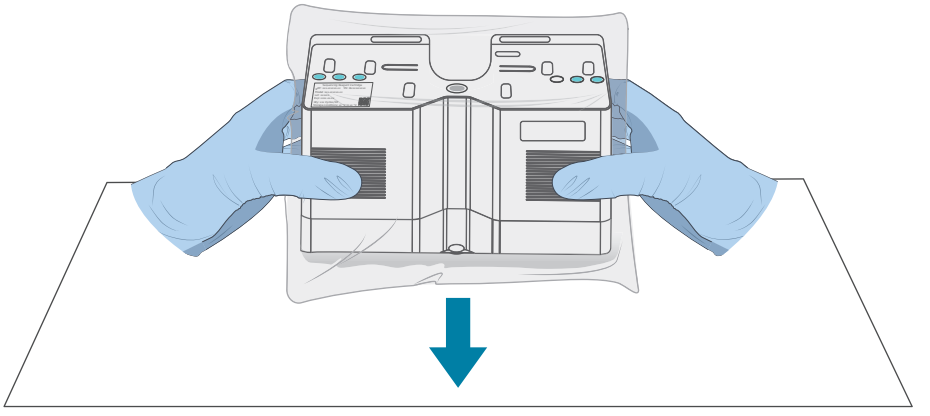

Figure 16 Tapping the reagent cartridge on a flat surface

6. Hold the reagent cartridge upright and swing downward 10 times to bring the reagents on the sides of the walls to the bottom of their respective wells. Cut and remove the outer packaging.
7. Use a vortex mixer to mix the Signal Protein 1 and Signal Protein 2 for 5 seconds. Centrifuge them briefly for 5 seconds and place them on ice until use.
8. According to the following table, add an appropriate amount of Signal Protein 1 and Signal Protein 2 to the tube containing the Signal Protein Buffer to make the Signal Protein Mixture.

	FCL SE100	FCL PE150	App-C FCL SE100	App-C FCL PE150
Signal Protein 1	15 µL	31.5 µL	15 µL	31.5 µL
Signal Protein 2	10 µL	21 µL	10 µL	21 µL
Signal Protein Buffer	10 mL	21 mL	10 mL	21 mL

9. Secure the cover on the tube containing the Signal Protein Mixture and invert it 10 to 15 times to mix thoroughly. To avoid bubble formation, do not vortex the Signal Protein Mixture vigorously.

10. Place the reagent cartridge on a flat surface as shown in the following figure. Place the funnel over the MSP well and add the Signal Protein Mixture in the tube into the MSP well.

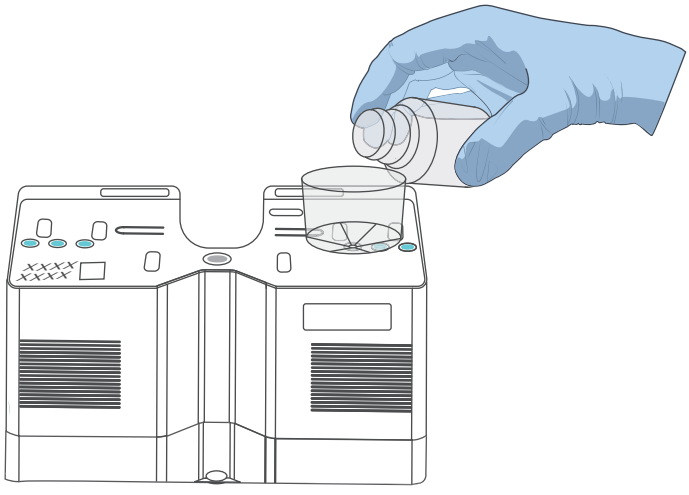

Figure 17 Adding the Signal Protein Mixture into MSP well

11. (Optional) For pair-end sequencing, perform the following steps:

    1) Take out MDA T-Reagent and MDA Enzyme Mix.
    2) Invert the MDA Enzyme Mix to mix it and then centrifuge it briefly.
    3) Transfer 50 µL of MDA Enzyme Mix to the tube containing MDA T-Reagent to make the MDA Mixture. Pipette the reagent 10 to 15 times to mix it without vortexing vigorously to prevent bubble formation.
    4) Use a clean tip to pierce the MDA well, and transfer the MDA Mixture into the MDA well.

    The reagent cartridge containing the MDA Mixture should be loaded within 20 minutes. Failure to do so might affect the sequencing quality.

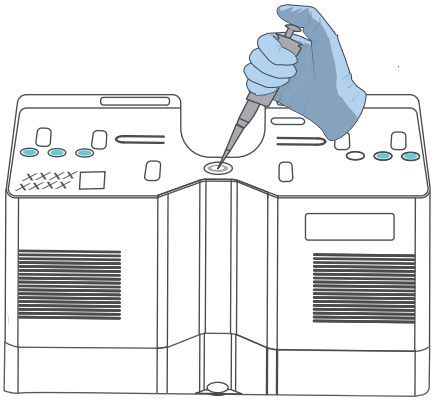

Figure 18 Adding MDA mixture into MDA well

12. Place the reagent cartridge on a flat surface as shown in the following figure. Use a pair of pointed-tip tweezers to remove the stoppers in wells No. 1, No. 2, and No. 3 and discard them in a designated container.

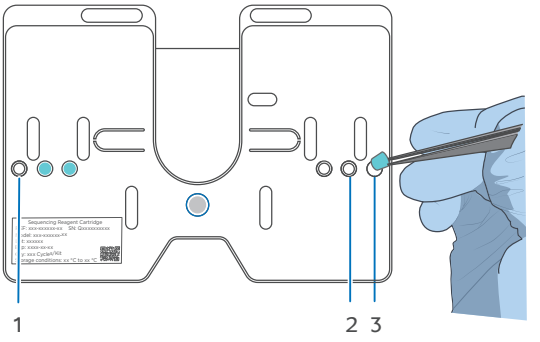

Figure 19 Removing the stoppers in wells No. 1, No. 2, and No. 3

Preparing the flow cell

Perform the following steps:

1. Remove the flow cell box from storage, and remove the flow cell from the box.

   Do not open the outer plastic packaging yet.

2. Unwrap the outer plastic packaging before use, and use the flow cell within 24 hours.

Performing a sequencing run

Selecting a recipe

Perform the following steps:

1. Select to enter the customization interface.

   The compartment door opens automatically and the rack slides out.

   Do not close the compartment door manually before sequencing starts.

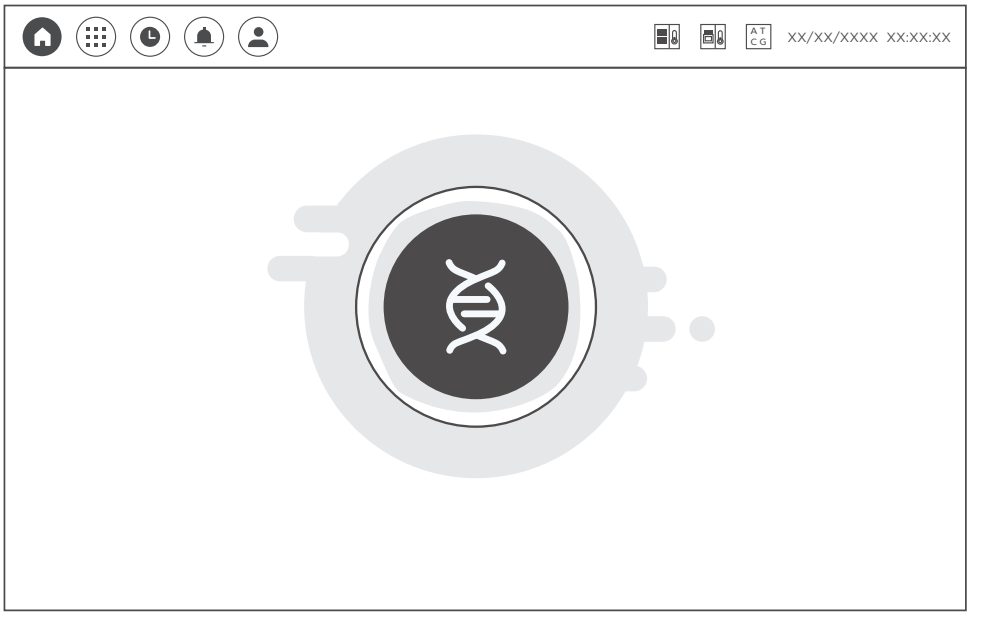

Figure 20 Main interface

2. Select the **Recipe** list and select a recipe such as PE150.

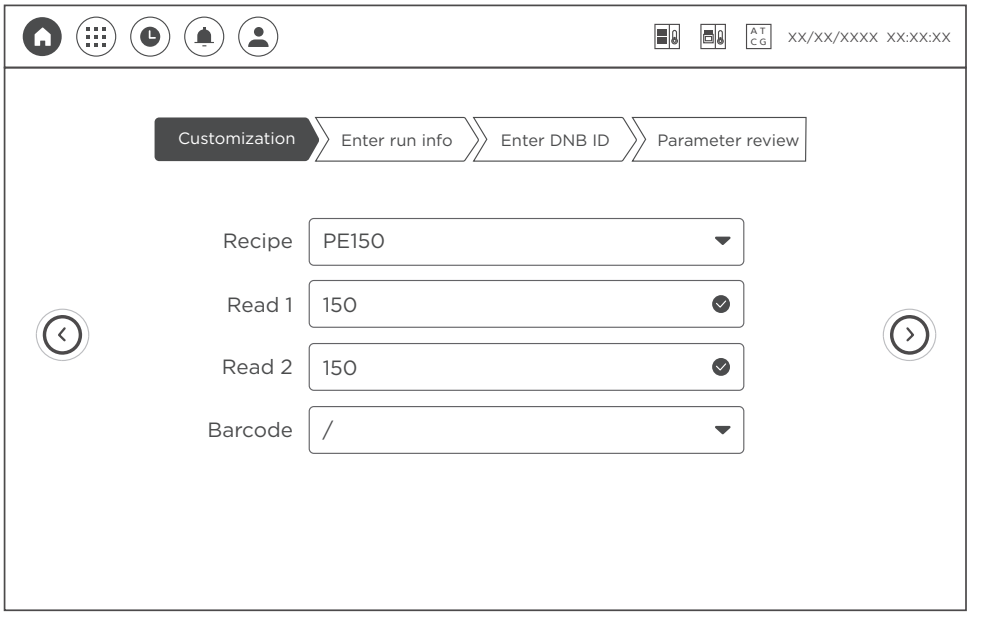

Figure 21 Selecting a recipe

3. Select the **Read 1** box and use the on-screen keyboard to enter the read length of Read 1.

   For any recipe, the entered read length cannot be greater than the largest read length the recipe can support. Reduce the read length manually if required.

4. Select the **Read 2** box and use the on-screen keyboard to enter the read length of Read 2.

   - For single-end recipes, the Read 2 box cannot be edited.
   - For pair-end recipes, such as PE150, Read 1 and Read 2 are 150 by default.
   - Reduce the read length manually if required.

5. Select the **Barcode** list to select the required barcode recipe.

   Four barcode recipe options are listed below:

Item	Description
MGI UDBA	Supports dual-barcode sequencing. Compatible with MGI UDB Primers Adapter Kit A.
MGI PFA	Supports dual-barcode sequencing. Compatible with MGI UDB PF Adapter Kit A.
MGI Single	Supports single-barcode sequencing. Compatible with MGI single-barcode Adapter Kit.
/	No barcode sequencing.

   If customizing barcode recipe is required, refer to *Importing a barcode file on Page 91.*

6. Ensure that the required recipe is selected, and select  .

   If you select  , a message appears stating that **the loaded consumables shall be discarded once offloaded**.

   - To cancel the sequencing, select **Yes**. The device returns to the main interface and starts to offload.
   - To continue the sequencing, select **No**.

Loading the flow cell, reagent cartridge and waste container

Perform the following steps:

1. Enter information for the **Flow cell ID, Throughput** and **Expiration date** boxes on the left by scanning the QR code on the plastic package of the sequencing flow cell or manually with the on-screen keyboard.

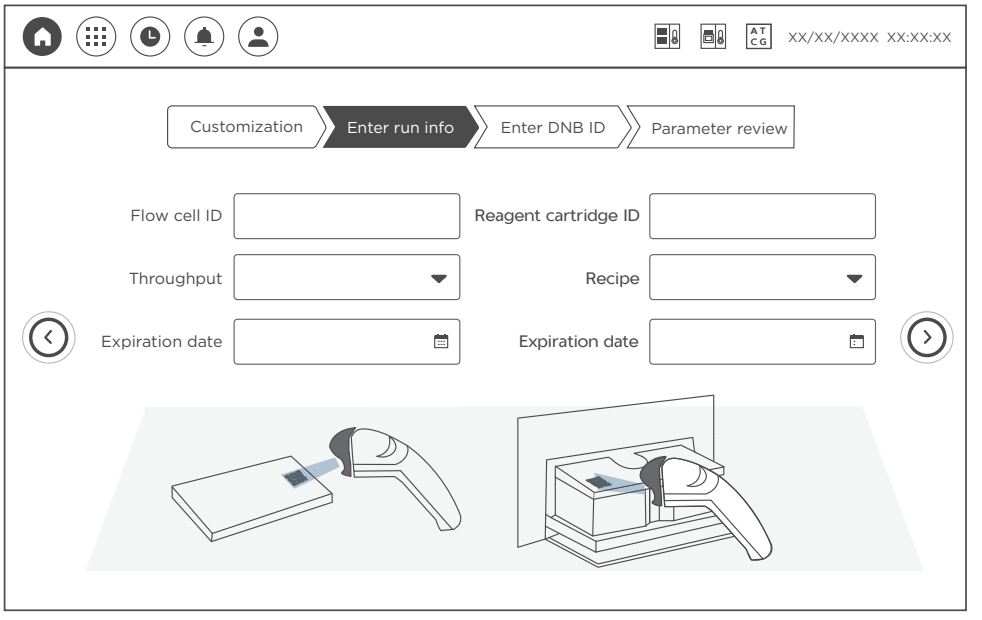

Figure 22 **Scanning the QR code of the flow cell**

2. Open the flow cell package and confirm that the flow cell is intact. Confirm that the scanned ID is the same as the ID on the flow cell.

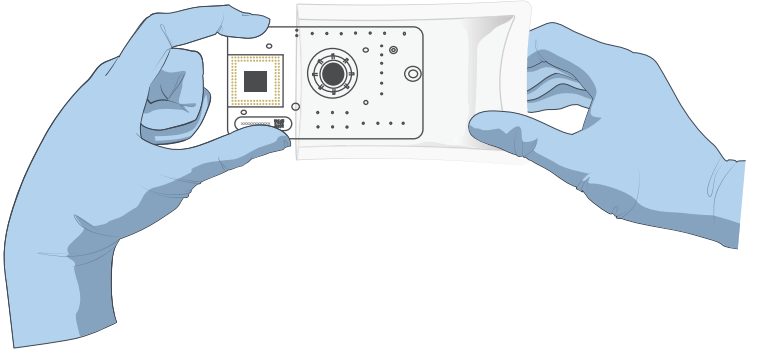

Figure 23 **Checking the flow cell**

To prevent contamination when holding the flow cell, do not touch small wells on the flow cell.

3. After the rack pops up, install the flow cell onto the rack by pinching the rotary valve in the flow cell and aligning the wells in the flow cell with the positioning columns on the rack.

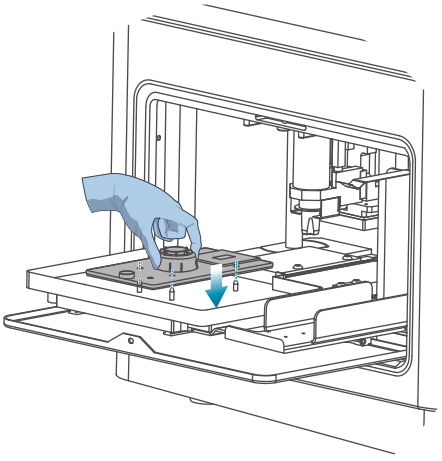

Figure 24 Placing the flow cell

4. Enter information for the **Reagent cartridge ID, Recipe** and **Expiration date** boxes on the right by scanning the QR code on the reagent cartridge or manually with the on-screen keyboard.

   When manually entering the information for the **Recipe** box, enter PE150 for pair-end sequencing and SE100 for single-end sequencing.

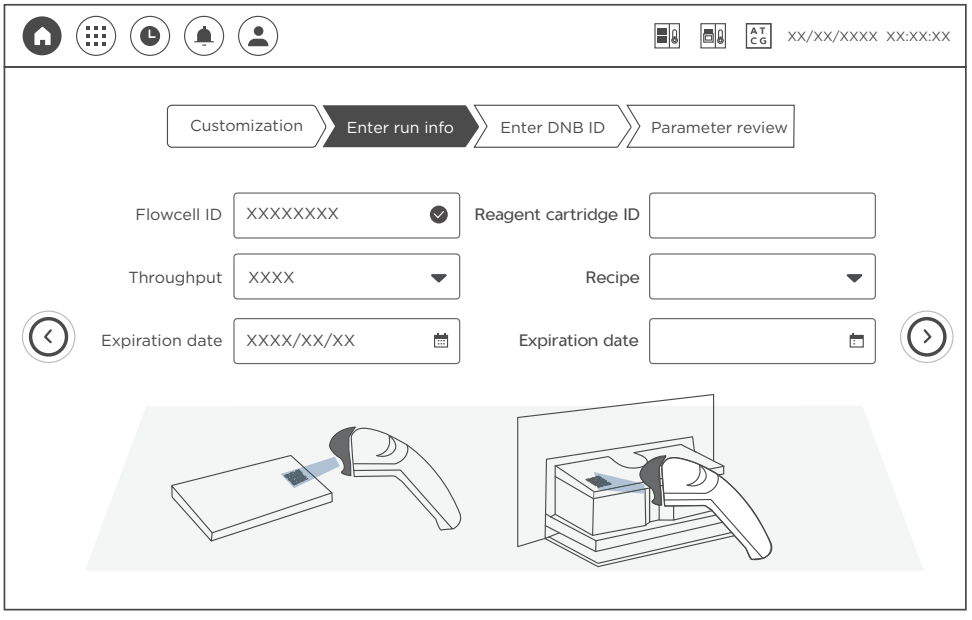

Figure 25 Scanning the QR code of the reagent cartridge

5. Slowly and carefully remove the bottom cover in the middle of the reagent cartridge and ensure that 21 rubber stoppers are present in the wells on the bottom of the reagent cartridge.

   If rubber stoppers fall off the cartridge or into the bottom cover, refer to *Q: What should I do if the rubber stopper at the bottom of the reagent cartridge falls off or tilts? on Page 86.*

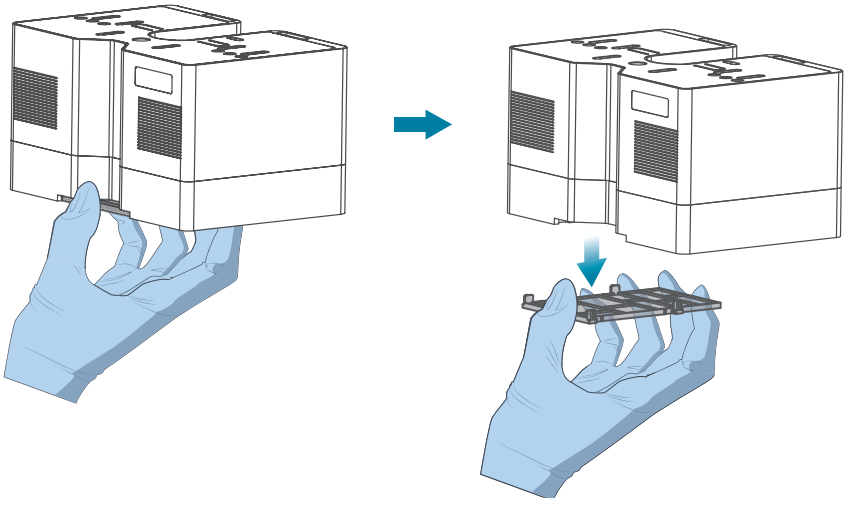

Figure 26 Taking off the bottom cover

6. Align the reagent cartridge with the positioning columns on the rack and place it over the flow cell. Keep the reagent cartridge horizontal in the process.

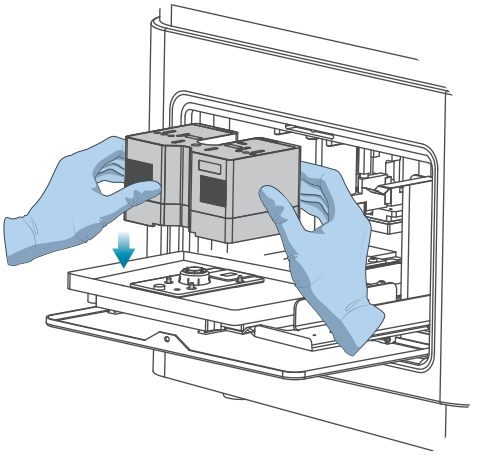

Figure 27 Placing the reagent cartridge

If you want to place the reagent cartridge again, ensure that rubber stoppers at the bottom of the reagent cartridge do not fall off the cartridge or tilt.

7. Ensure that the cover of the waster container is open, place the waste container on the rack according to the direction shown in the figure below, and ensure that it fits into the bent metal clip.

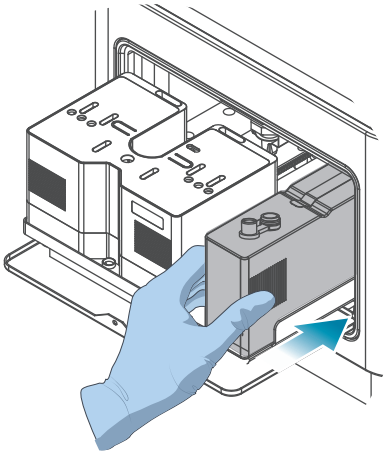

Figure 28 Placing the waste container

The waste container is in place when the positioning clamp aligns exactly with the groove on the waste container.

8. After placing the waste container, select ›, and select **Yes**. The rack automatically retracts into the compartment and the device presses the components tightly. Select › to enter the next step.

CAUTION Do not manually close the compartment door.

Loading DNBs

Perform the following steps:

1. Enter information for the **DNB ID** box by scanning the QR code on the sample tube or manually with the on-screen keyboard.

CAUTION Returning to the customization interface and selecting ‹ to cancel sequencing will cause the loaded reagent cartridge and flow cell to become inoperative.

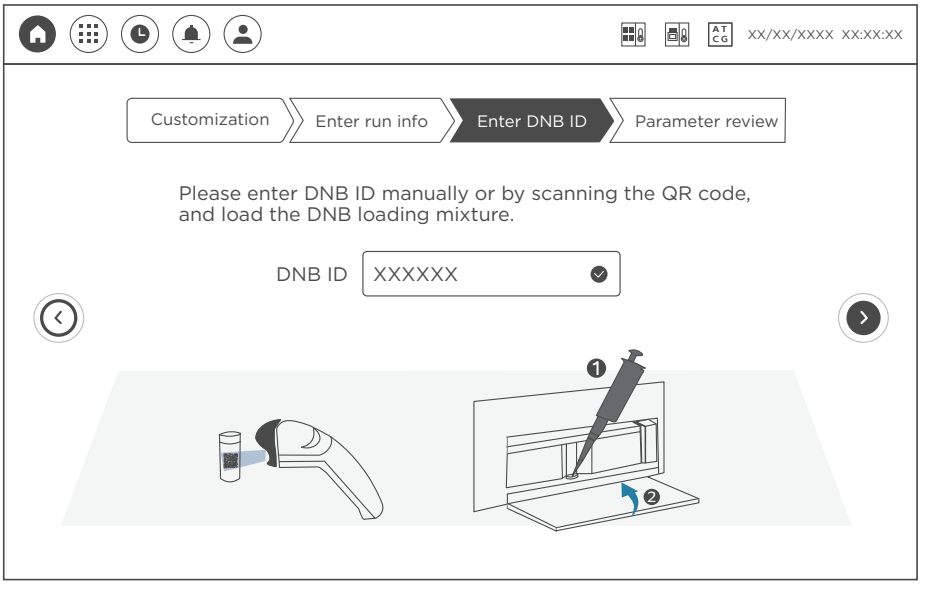

Figure 29 **Entering DNB ID**

2. Prepare the DNB loading mixture.

   1) Use a vortex mixer to mix DNB Load Buffer II thoroughly for 5 seconds, and centrifuge it briefly. Place it on ice until use.

      If crystals appear in DNB Load Buffer II, vortex it vigorously by using a vortex mixer for 1 to 2 minutes until the precipitation dissolves. Centrifuge it briefly before use.

   2) Add 34 µL of DNB Load Buffer II to the PCR tubes containing DNBs to make the DNB loading mixture.

      The DNB loading mixture should be used immediately after preparation.

Table 13 **DNB loading mixture**

Component	Volume (µL)
DNB Load Buffer II	34
DNB	102
Total volume	136

   3) Gently pipette up and down 8 times to mix the DNB loading mixture by using a wide-bore, non-filtered pipette tip.

      Do not centrifuge, vortex, or shake the tube.

3. Add all the DNB loading mixture into the DNB loading well by using a wide-bore pipette tip. Go to the next step when no bubbles exist in the well.

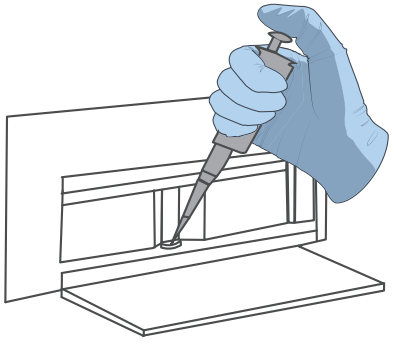

Figure 30 Adding DNB loading mixture

To prevent bubbles from forming, put the wide-bore pipette tip against the inner wall of the DNB loading well to ensure that the mixture flows into the well slowly.

4. Push the compartment door back to close it.
5. Select to enter the next step.

Reviewing parameters

Review all information.

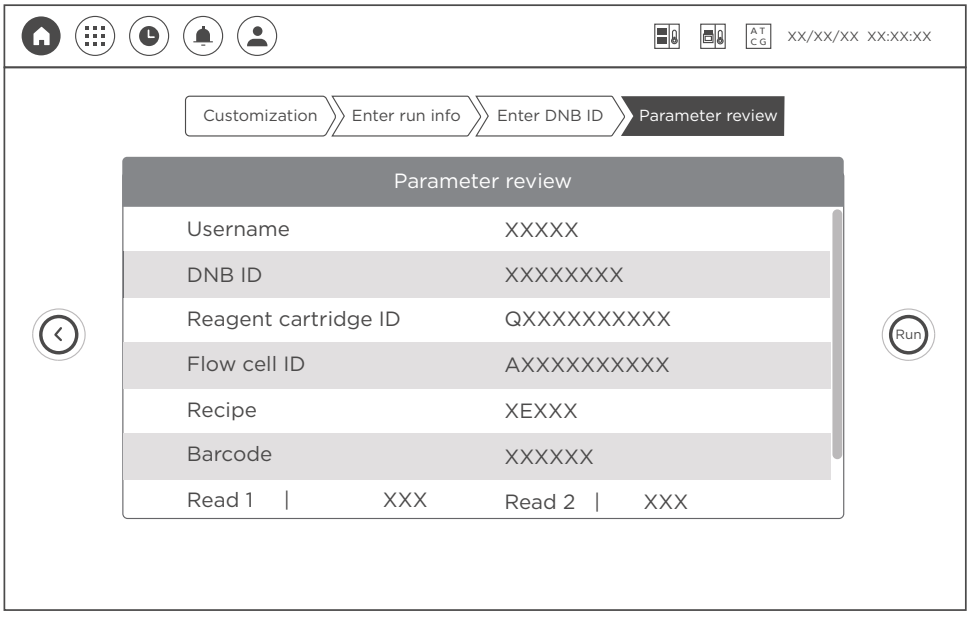

Figure 31 Reviewing parameters

- If the information is incorrect, select ⟨ to return to the previous interface and modify the information.

  CAUTION Returning to the customization interface and selecting ⟨ to cancel sequencing will cause the loaded reagent cartridge and flow cell to become inoperative.

- If the information is correct, ensure that the computing module is connected and the compartment door is closed. Select Run, and select **Yes** to start sequencing.

Sequencing

CAUTION

- Before sequencing, check whether the compartment door is closed. If it is open, ensure that no barrier is in the door and close the door.
- During sequencing, do not operate the door to avoid affecting the sequencing result.
- Do not impact or move the device. Remove vibration-producing equipment around the device during sequencing. Otherwise, inaccurate results or even damages to the device might occur.
- Pay attention to the icons, status indicators, and pop-up dialog boxes on the screen. In the event that abnormalities occur, check the problematic parts according to the prompts. If problems persist, contact CG Technical Support.

When sequencing starts, the DNB loading interface is displayed.

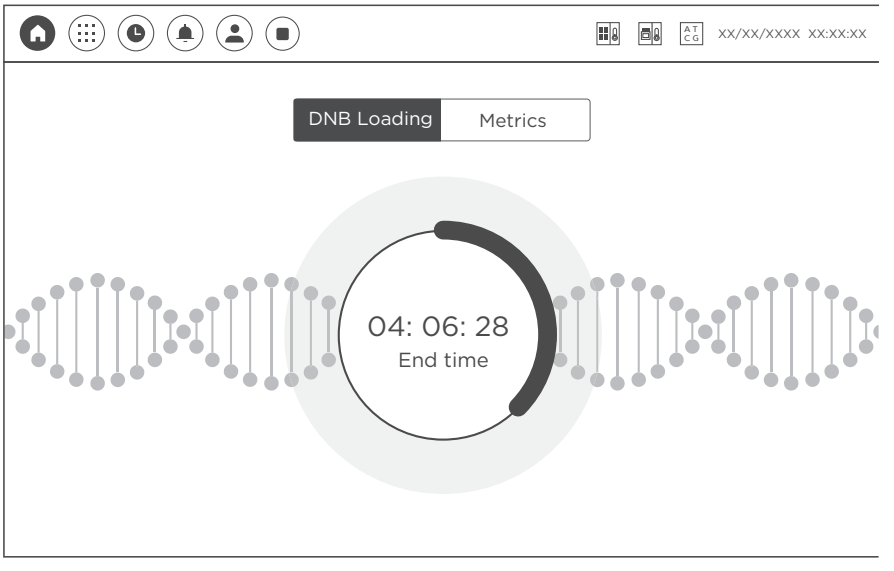

Figure 32 DNB loading interface

The following table describes the controls in the interface:

Item	Description
⏹	Select to end sequencing in advance. ⚠ CAUTION Sequencing cannot be resumed after ending, so please operate with care.
DNB loading	Displays the current sequencing phase, such as **DNB loading** and **progress (current cycles/total cycles)**.
Progress	Displays the sequencing progress as a percentage.
Time remaining	Displays the remaining time of sequencing. Select to view **End time**.
End time	Displays the estimated ending time of sequencing. Select to view **Time remaining**.
Metrics	Displays sequencing quality. In **Metrics**, select the drop-down list of **Sequencing metrics** and select the required indicator to view the graph.

After the first cycle is completed, the first base report is displayed.

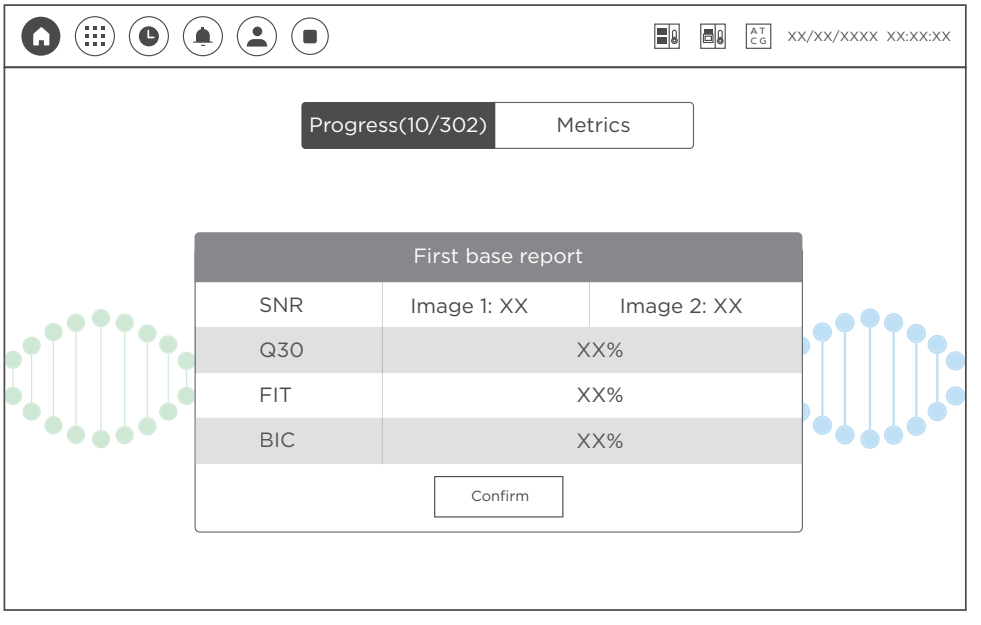

Figure 33 First base report

After all cycles are completed, wait for the report as prompted.

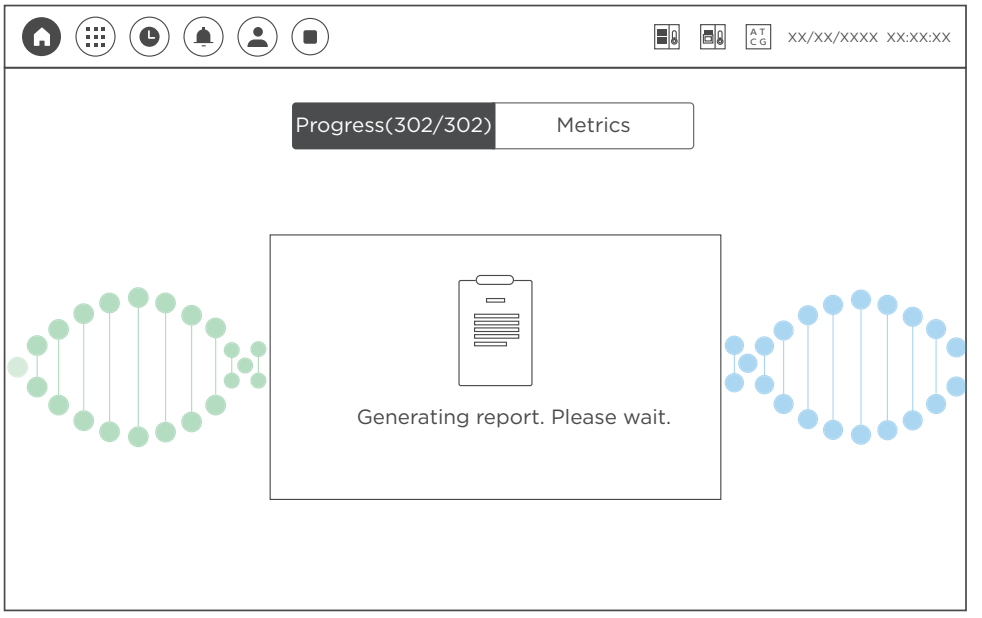

Figure 34 Report generation interface

- If the report is generated, the report is displayed automatically. Close the report and select ✓ to enter the sequencing completion interface.
- If no report is generated, ✓ and **Task exception** are displayed. Select ✓ to enter the sequencing completion interface. Check the possible causes of the failure.
  - If failure results from the device problem, contact CG Technical Support to maintain it.
  - If failure results from improper operation (for example, the computing module is disconnected), perform the sequencing run again by following the required procedure.

Sequencing is completed when the following interface is displayed:

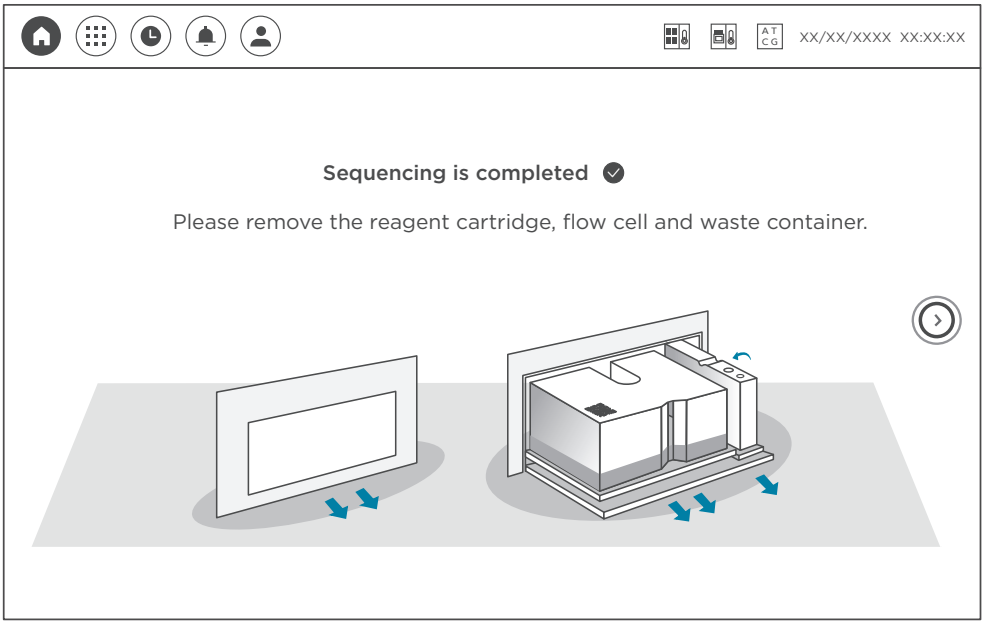

Figure 35 Sequencing completion interface

Disposing of the flow cell, reagent cartridge and waste container

CAUTION

- When removing the flow cell and the reagent cartridge after sequencing, ensure that the flow cell is firmly attached to the reagent cartridge and keep the reagent cartridge horizontal to prevent waste spills from causing biological contamination.
- After sequencing and before shutting down the sequencer, check whether the reagent cartridge and waste container are removed to avoid damages to components.

Perform the following steps:

1. Remove the flow cell and the reagent cartridge. In the process, ensure that the flow cell is firmly attached to the reagent cartridge and keep the reagent cartridge horizontal to prevent waste spills from causing biological contamination.

When removing the reagent cartridge and flow cell, immediately transfer them to a container to catch reagents that may leak out of them.

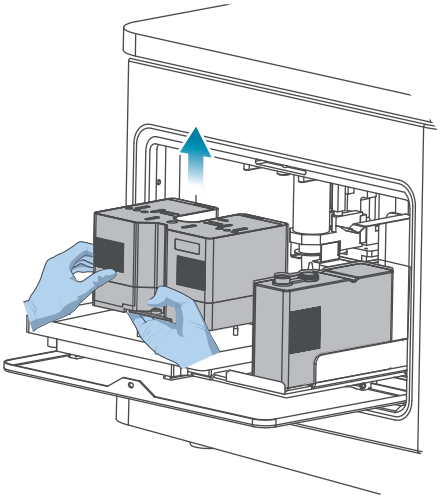

Figure 36 Removing the flow cell and reagent cartridge

2. Close the cover of the waste container, slightly raise up the waste container and remove it from the rack. Transfer it to the designated container.

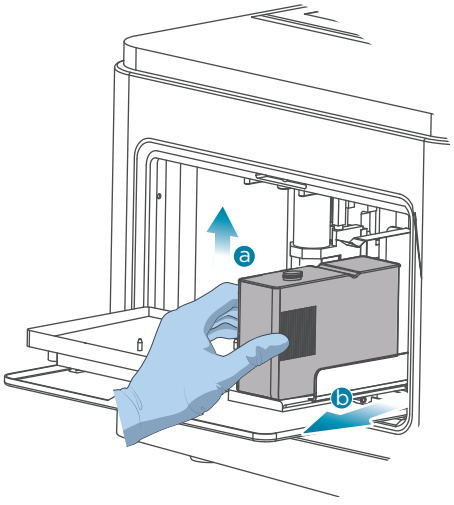

Figure 37 Removing the waste container

3. Push the compartment door back to close it.
4. Select  to return to the main interface.
5. Dispose of the tube, flow cell, and reagent cartridge in accordance with local regulations and safety standards for your laboratory.

(Optional) Powering off the device

CAUTION

- Power off the device and disconnect the power cord if you do not plan to use the device for an extended period of time.
- Before you power off the device, ensure that the sequencing run is completed, the control software is shut down, and the compartment door is closed. Failure to do so might damage the control software.

Perform the following steps:

1. Select , select **Shutdown**, and select **Regular shutdown**.
2. Turn the power switch to the ◯ position.
3. Disconnect the device power cord from the main power supply or the UPS.
4. Press the button to power off the computing module.
5. Disconnect the power cord of the computing module from the main supply socket or UPS.

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05

Sequencing data

This chapter describes the sequencing output data.

Sequencing output files

During sequencing, 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 are saved automatically.

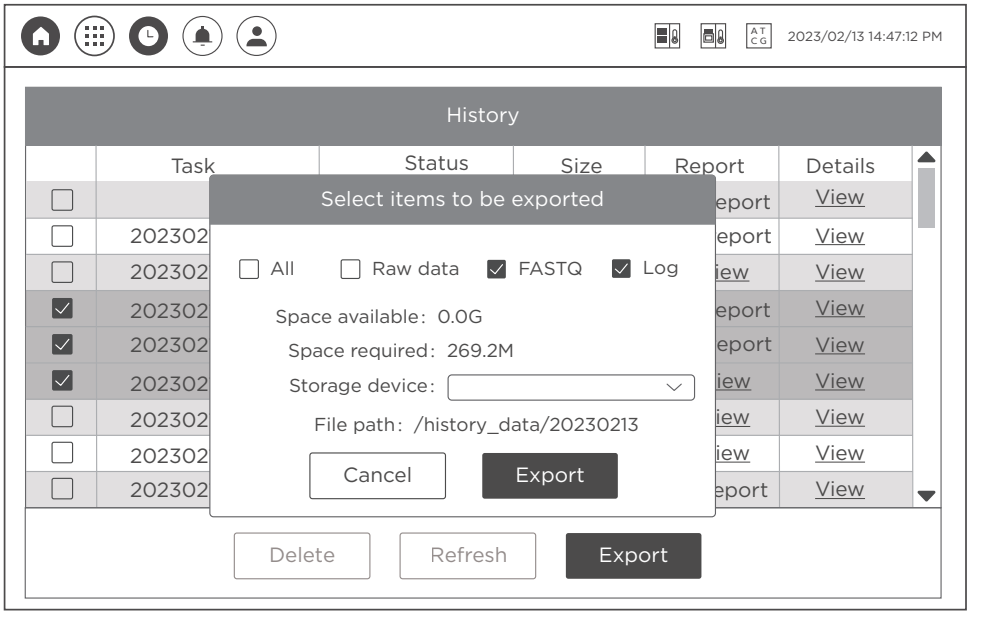
Exporting data

You can export running data, sequencing results and logs from the sequencer to an external storage device, such as a USB storage drive, according to your needs.

It is recommended that you format the external storage device, such as a USB storage drive before use. The device only supports a USB storage drive in the FAT32 or NTFS format but does not support a USB storage drive in the exFAT format.

Perform the following steps:

1. Insert the external storage device into the sequencer.
2. In the history review interface, select the check box to the left of the required task and select **Export**.



3. Select the data type:
   - If you select **Raw data**, **FASTQ** or **Log**, the file of the corresponding type will be exported.

- Raw data file type: images
- FASTQ file type: analysis report
- Log: operation, running and warning information

- If you select **All**, files of all three data types will be exported.

4. Select **Export**. The data will be exported to the path: */history_data/xxxxxxxxx*.

xxxxxxxxx represents the date when the sequencing run is performed.

Summary report

Report parameter overview

The following table describes parameters for Tab1 of summary report:

Table 14 Parameter description for Tab1 of the summary report

Parameter	Description
BaseCall Ver	Version of BaseCall. Ensure that the basecall application is in the official release version
Template Ver	Version of summary report template
Machine Ver	Version of the machine
Machine ID	Serial number of the sequencer
Flow cell ID	Flow cell ID that you enter
Reagent ID	Serial number of the reagent cartridge
DNB ID	DNB ID that you enter
Start Sequence Time	The time at which the sequencing started
Barcode Name	The name of the barcode recipe
Dual-Barcode Name	The name of the barcode recipe
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)

Parameter	Description
ActivePixelsNum(M)	The number of effective pixels
TotalReads(M)	Reads included in the FASTQ file (Reads after filtering)
AvgDuplicationRate(%)	The average duplication rate AvgDuplicationRate(%) is defined as the following: $\text{AvgDuplicationRate(\%)} = 1 - \frac{\text{Unique reads}}{\text{Total reads}}$
LoadingRate(%)	The success ratio of loading
ESR(%)	Effective spot rate. Percentage of effective spots after filtering in the flow cell
Q30(%)	The percentage of bases with quality score no less than 30. A base with a quality score of 30 implies that the chances of this base called incorrectly are 1 in 1000.
Lag/Runon	• Lag1 (%) is the slope of the Lag curve for the 1st strand sequencing. • Lag2 (%) is the slope of the Lag curve for the 2nd strand sequencing. • Runon1 (%) is the slope of the runon curve for the 1st strand sequencing. • Runon2 (%) is the slope of the runon curve for the 2nd strand sequencing.
RecoverValue	The ratio of second strand signal to first strand signal. This indicator is only for PE sequencing.
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.

Diagrams in summary report

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

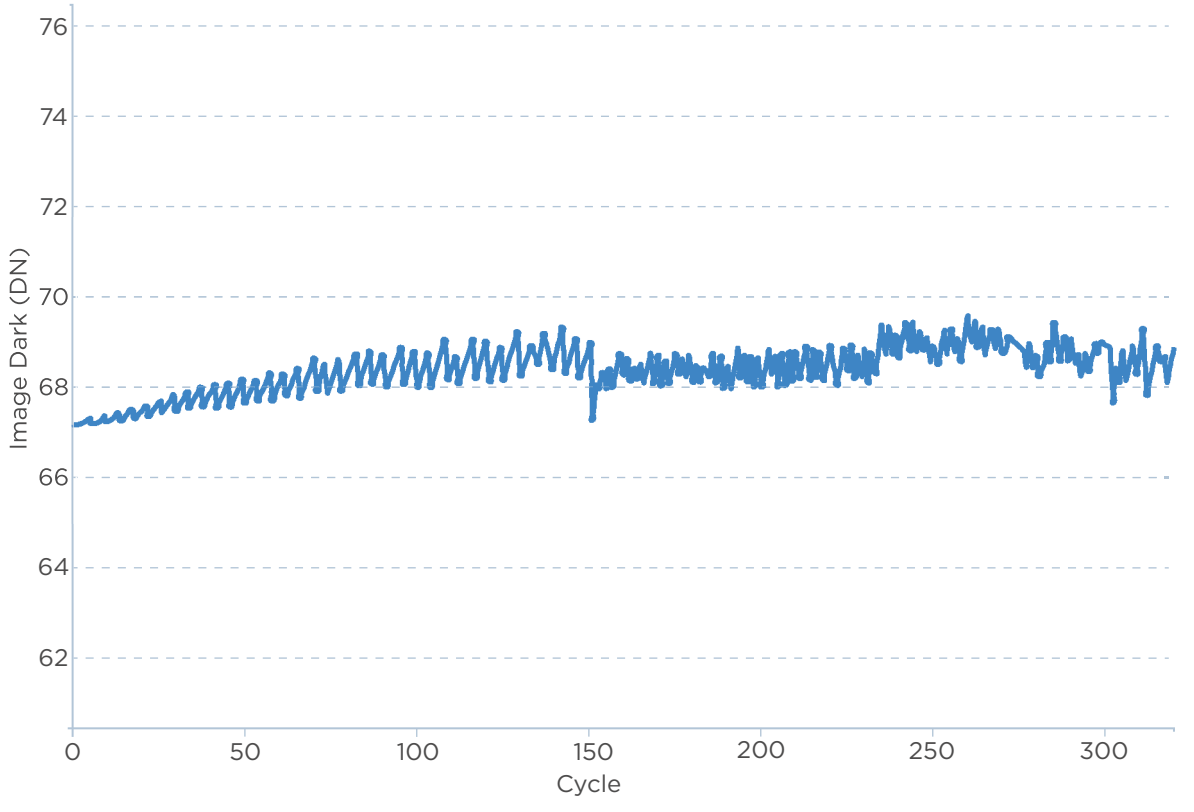


Figure 38 ImgDark

X axis	Cycle
Y axis	Image Dark (DN): the average background value of flow cell before the signal reagents flow into the flow cell in each cycle.

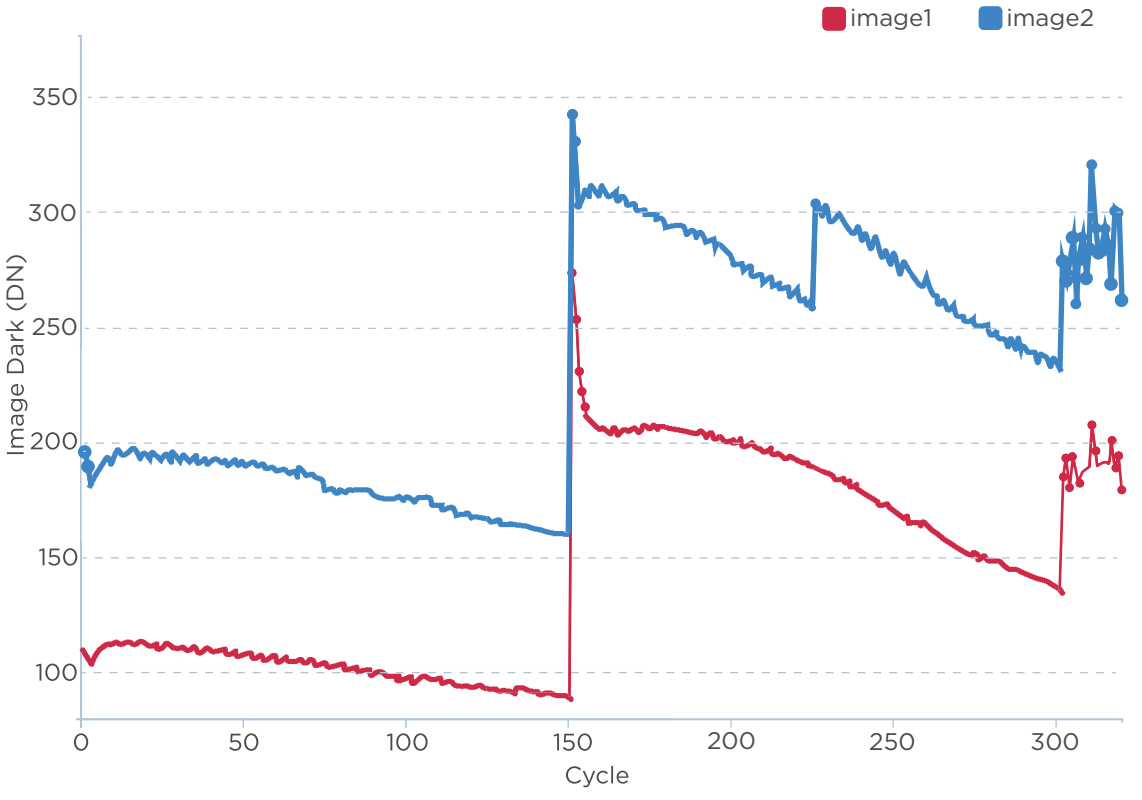


Figure 39 **ImgSignal**

X axis	Cycle
Y axis	Image Signal (DN): the average signal value of die1 and die2 after preliminary treatment in each cycle.

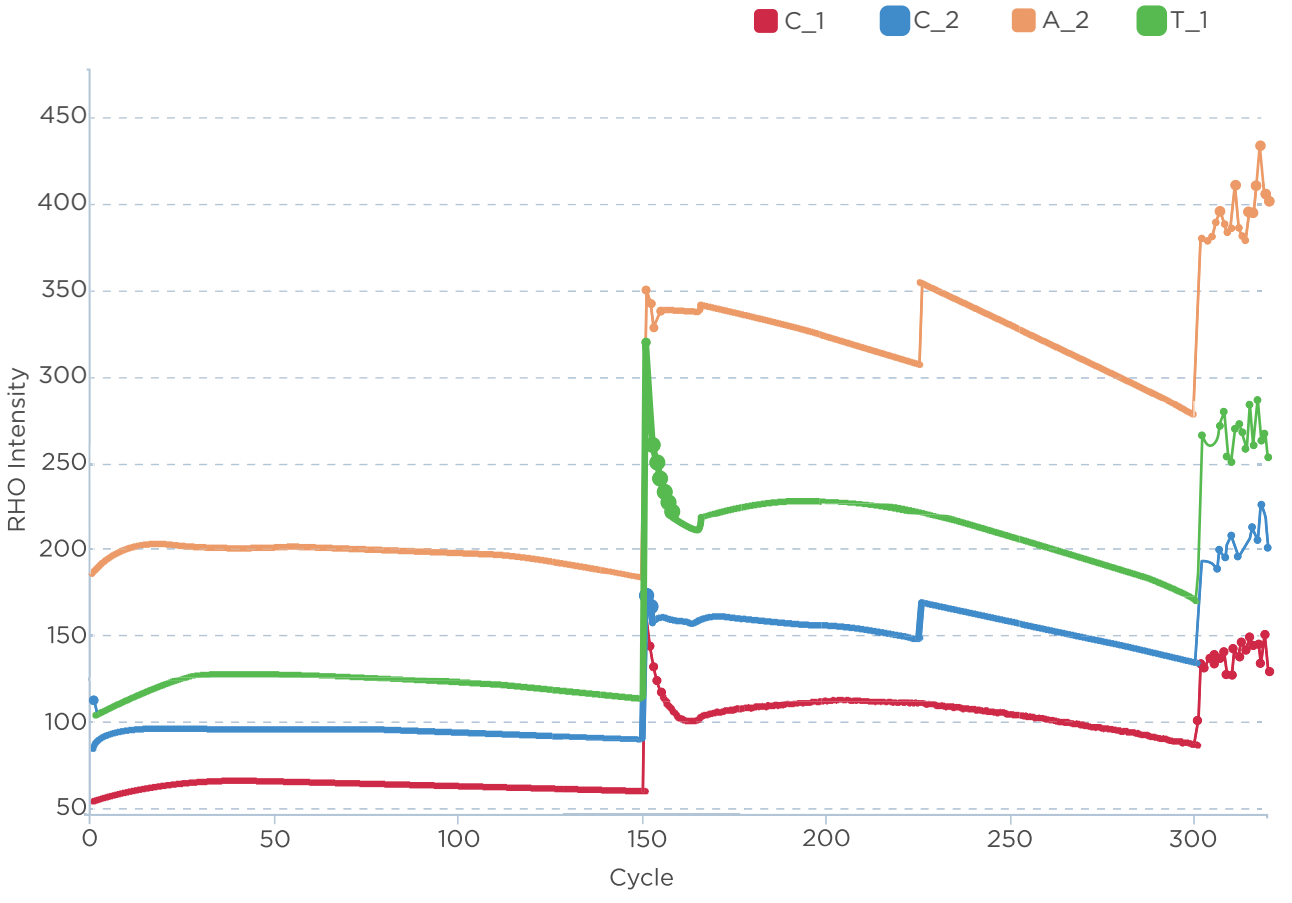


Figure 40 RHO Intensity

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

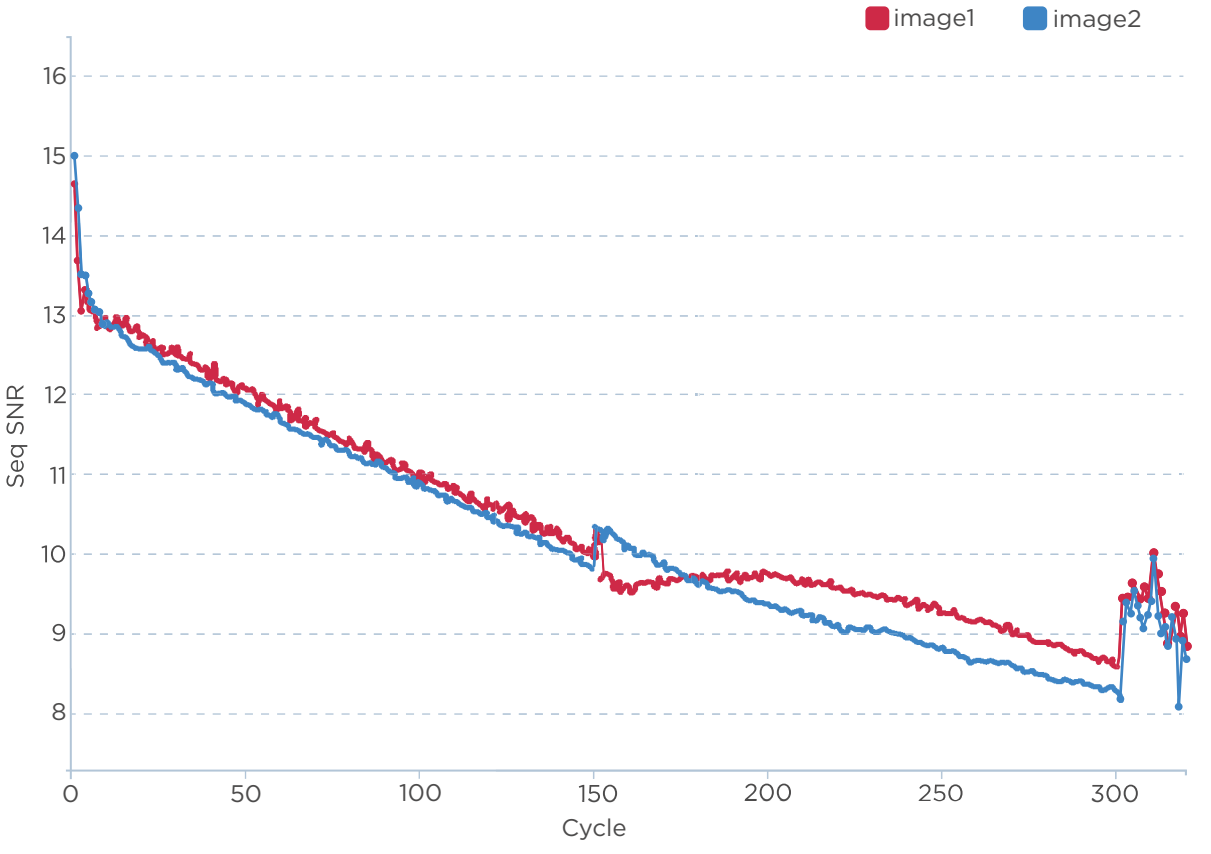


Figure 41 SeqSNR

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

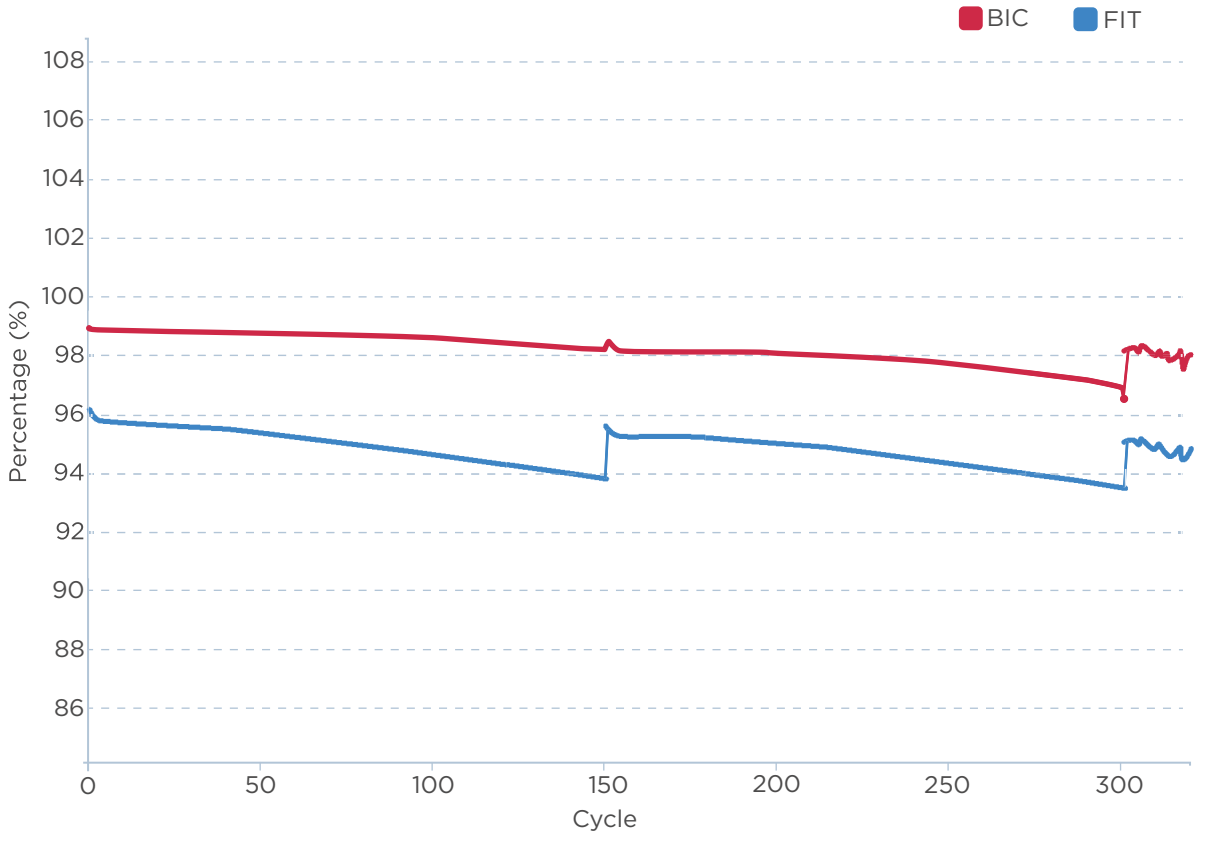


Figure 42 **BIC And FIT**

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

A, T, C, and G represent the four base types.

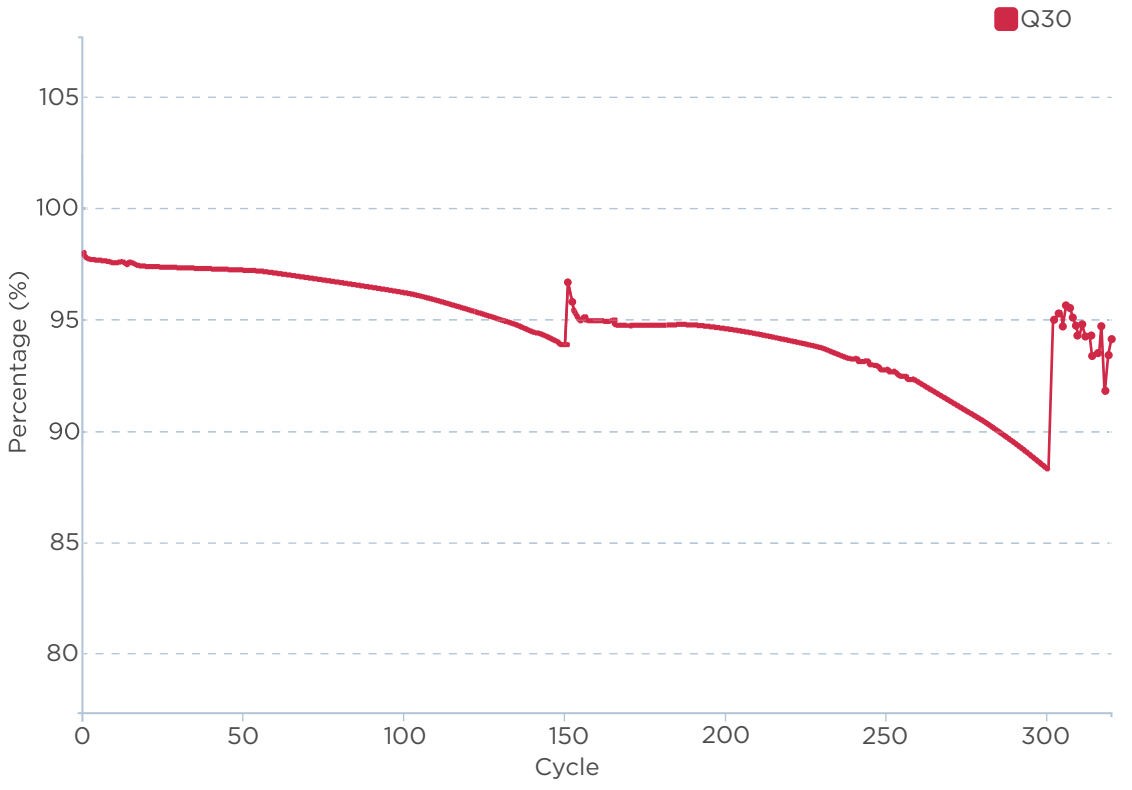


Figure 43 **Unfiltered Q30 Rate**

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

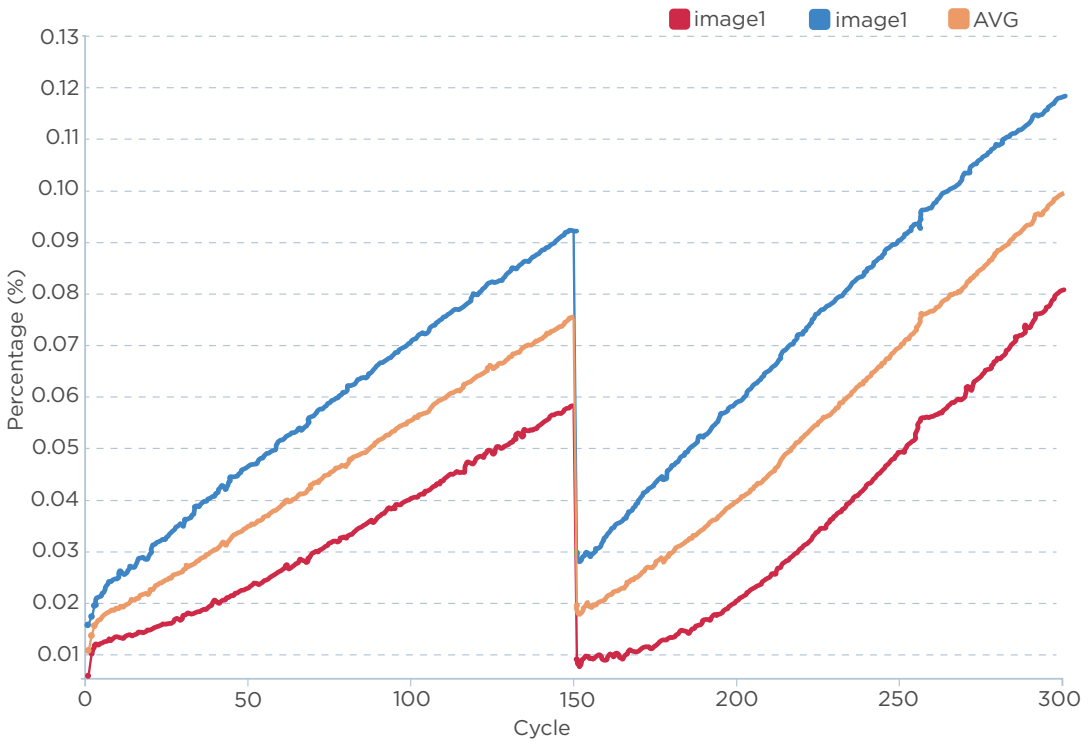


Figure 44 **Runon**

X axis	Cycle
Y axis	Percentage (%): Runon value for each cycle. 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*.

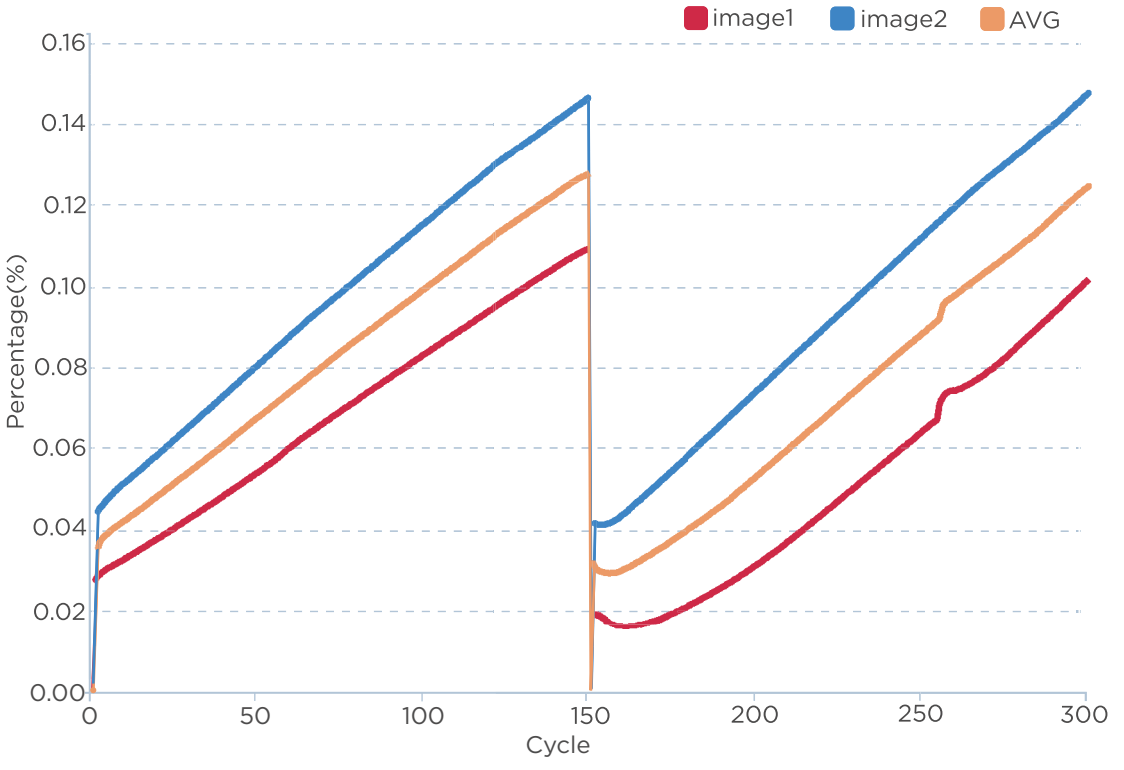


Figure 45 Lag

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

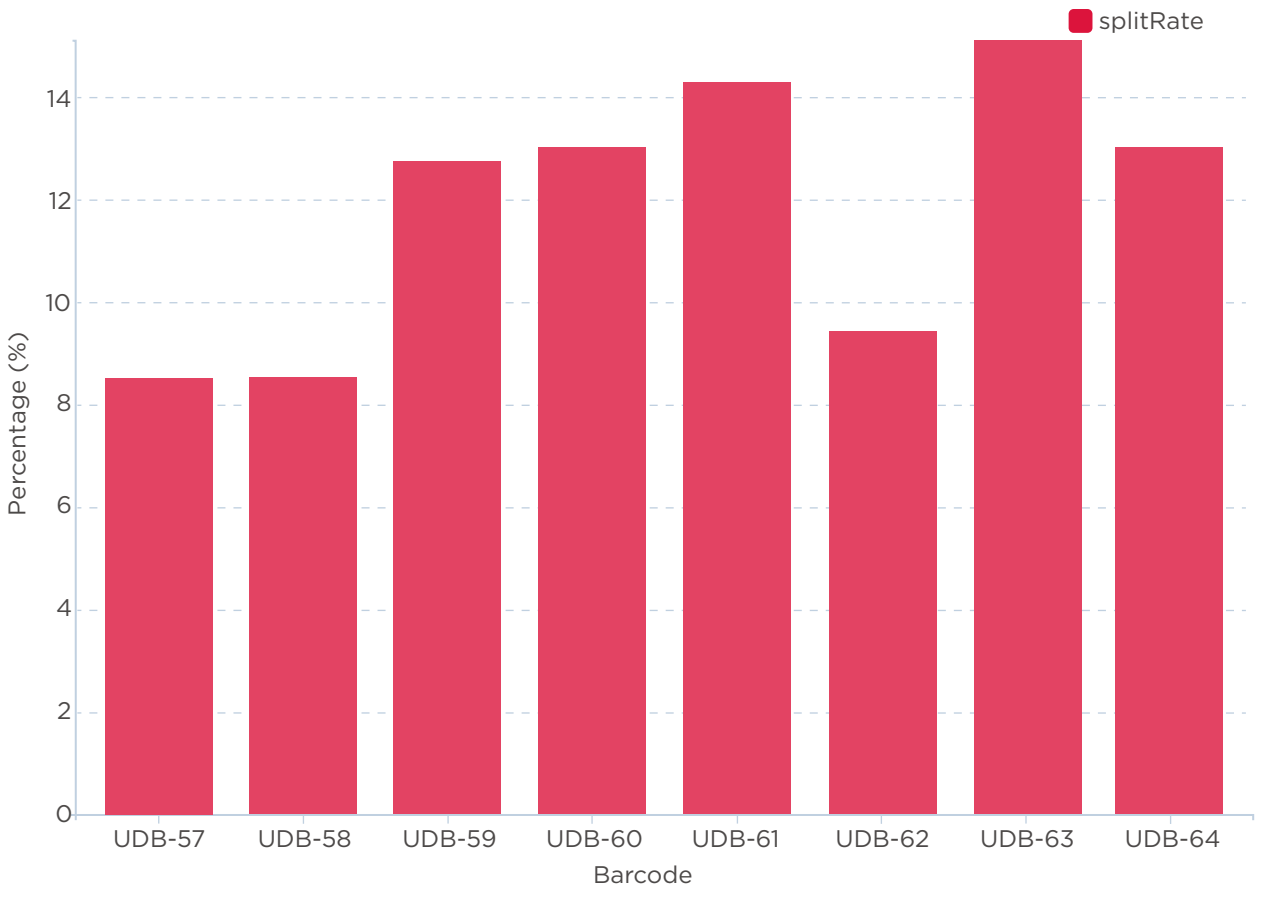


Figure 46 **Barcode Split Rate**

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

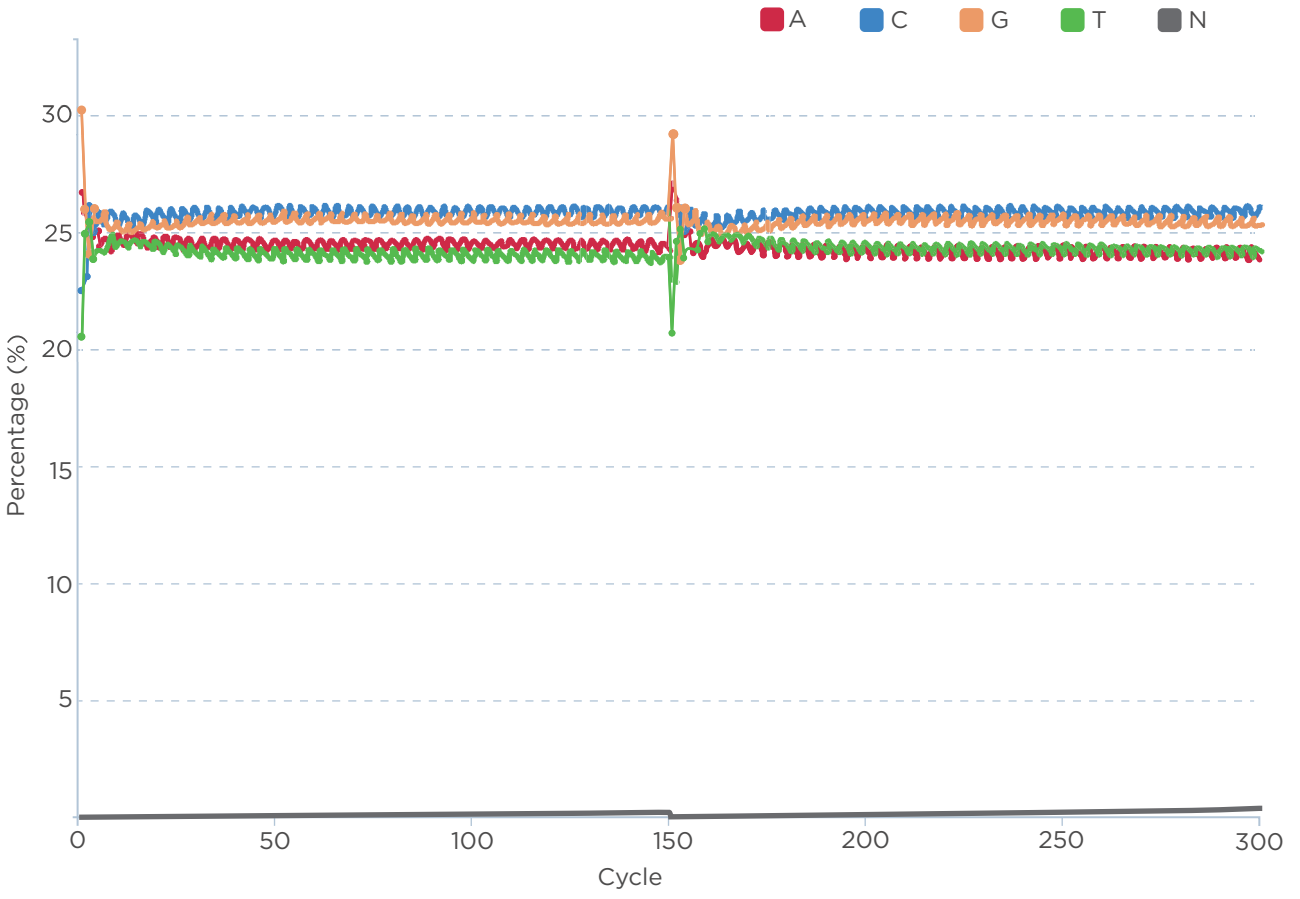


Figure 47 Base Distribution

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

- A, T, C, and G represent the four base types.
- N means that the base type cannot be identified.

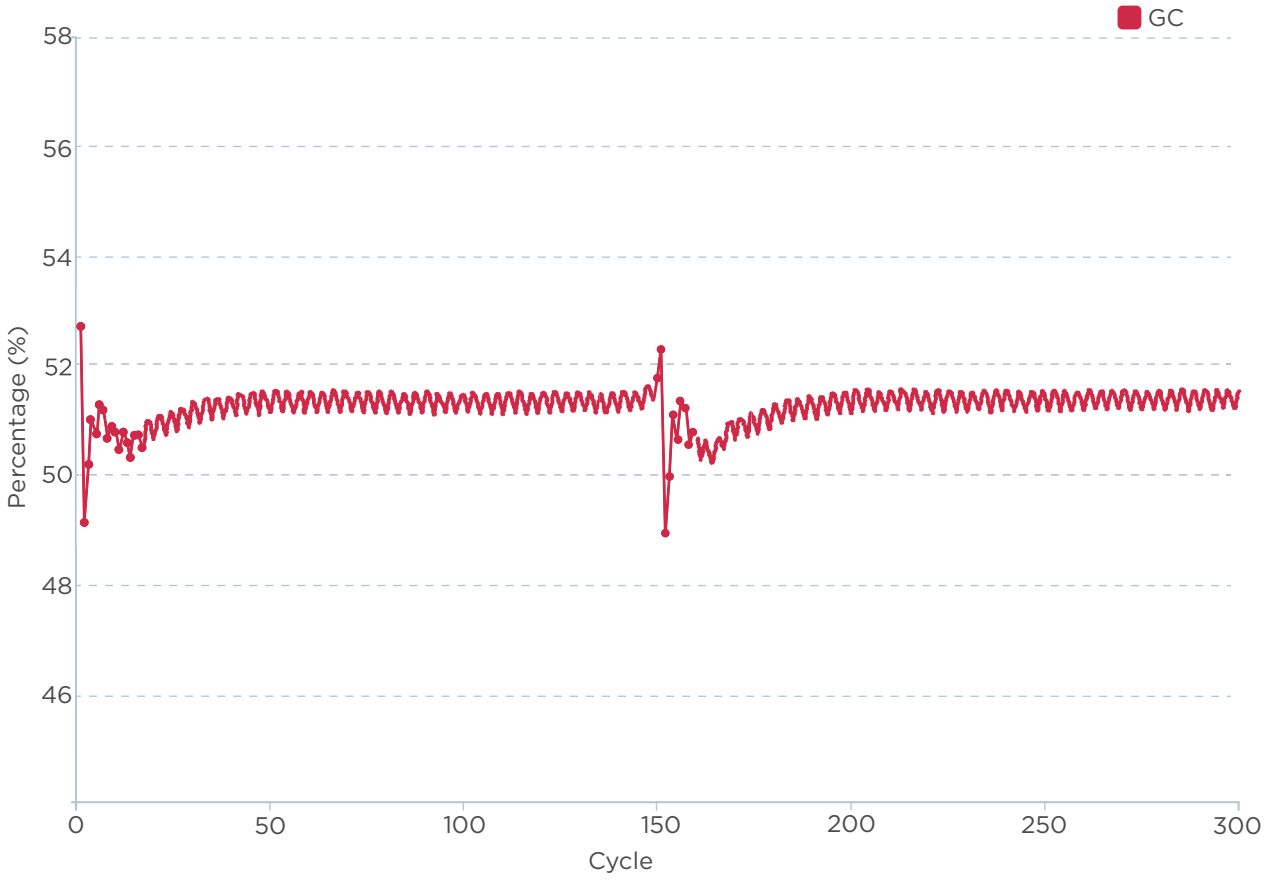


Figure 48 GC Distribution

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

G and C represent the two base types.

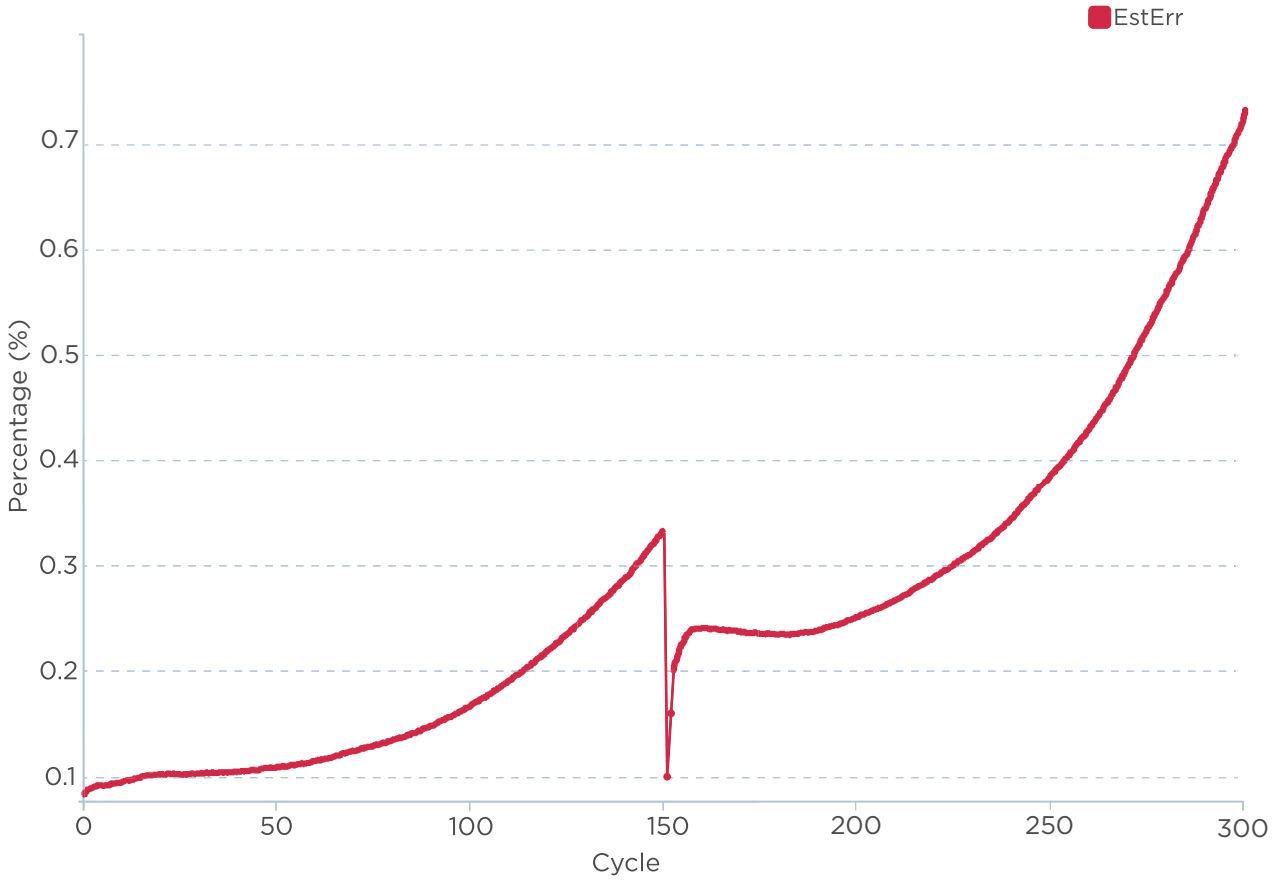


Figure 49 **Error Rate Estimation**

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

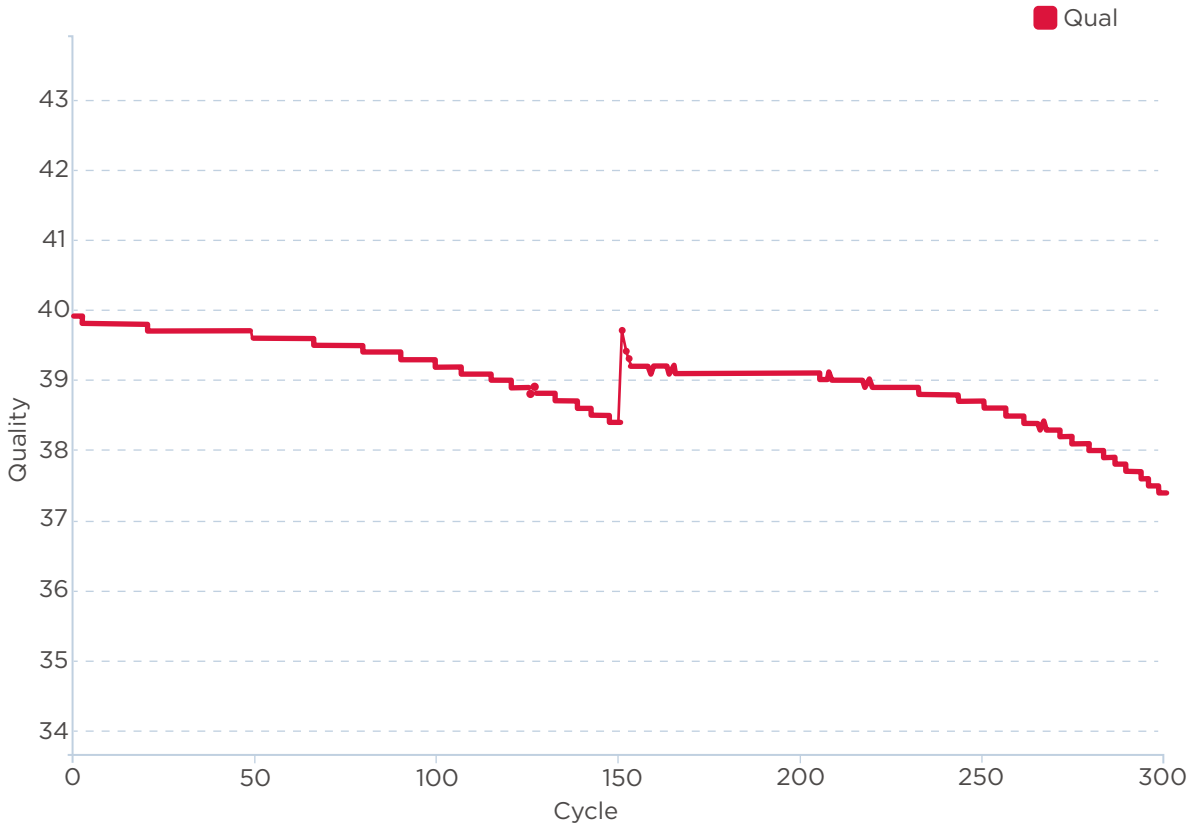


Figure 50 **Average Quality Distribution**

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

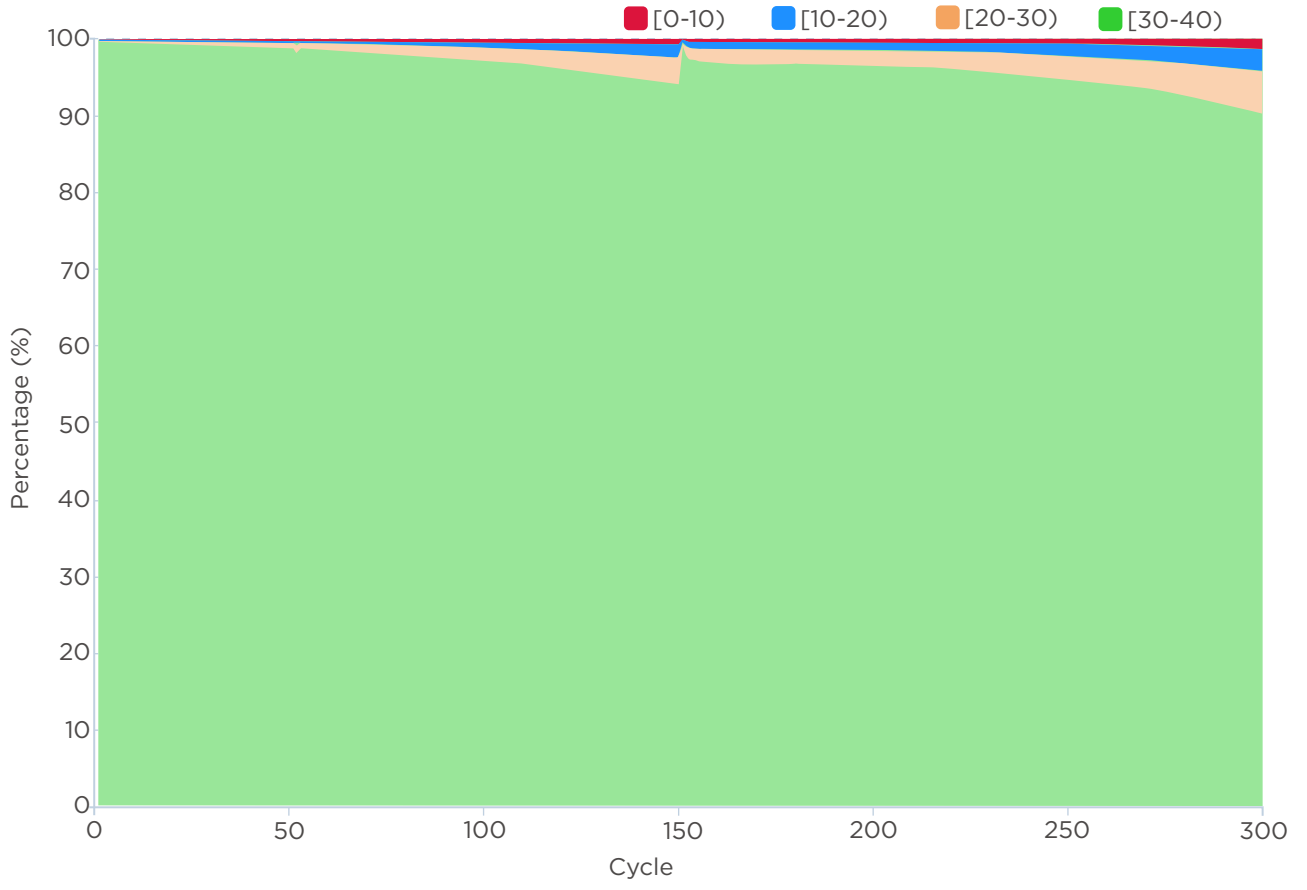


Figure 51 **Quality Proportion Distribution**

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

Data processing

Introduction

The sequencer processes the image files to generate a base call at each position of the read and saves the base sequence information in the FASTQ format. The FASTQ file and report file are generated by utilizing the split rate obtained by barcode analysis.

During a sequencing run, the control software will automatically generate *cal*. files in real time by the specific basecall application. After the sequencing run is completed, the basecall application will generate FASTQ files based on *cal*. files from all FOVs automatically (termed Write FASTQ on sequencer automatically).

Details on how the computing module automatically writes FASTQ on the sequencer are provided below.

Write FASTQ on sequencer automatically

After sequencing has started, the sequencing results generated by the control software are saved automatically. Metrics can be viewed in the sequencing interface.

After the sequencing ends, the basecall application will automatically write FASTQ files based on *cal*. files, and generate a summary report.

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06

Device maintenance

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

DANGER

- Ensure that the device is powered off before cleaning or disinfecting. Failure to do so might cause personal injury.
- Do not spray the wash solutions or disinfectants into the device during cleaning or disinfecting. Doing so might damage the device.

WARNING

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

Weekly maintenance

WARNING Wear a laboratory coat, a mask, and gloves before performing the following steps.

Maintaining the disk space

After powering off or restarting the device, the device retains raw images of the recent three sequencing runs, sequencing results of the recent twenty sequencing runs, and logs of the recent month. Therefore, it is necessary to regularly back up the required historical data to the peripheral storage devices.

During sequencing, before the device presses the reagent cartridge, the device automatically checks whether the disk space is sufficient for this sequencing run. If there is insufficient disk space, a message is displayed. To delete data, perform the following steps:

1. Select in the menu area, and select **History review**.
2. Select the check box next to the data that you want to delete, and select **Delete**.
3. Select to return to the sequencing interface and continue sequencing.

Maintaining the power supply

Perform the following steps:

1. Periodically check the power cord and cables. Ensure that they are connected correctly and are in good condition. If you need new cables, contact CG Technical Support.
2. Ensure that the area around the power supply is dry and free of moisture.

Cleaning the reagent cartridge rack

Perform cleaning and maintenance for the reagent cartridge rack before each use. Failure to do so might affect the sequencing result.

Wear protective gloves when cleaning the reagent cartridge rack. Dust, lint, and other particulate matters can affect the sequencing result.

Prepare the following tools and solutions to clean the reagent cartridge rack:

- Dust-free cloth
- laboratory-grade water

Perform the following steps:

1. Check for dust, debris, damage, and particulate matters on the surface of the reagent cartridge rack.
2. Wipe the rack with a dust-free cloth moistened with laboratory-grade water, and then let the rack air-dry.

Monthly maintenance

The low-lint cloth should keep moist without droplets.

Wipe the surface and the screen of the device with a cloth moistened with laboratory-grade water. Ensure that the surface is free of samples, reagents, blood, or potential biological contaminants.

Do not use detergent that could react with the device or sequencing reagents, which may cause danger.

Annual maintenance

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

Maintaining the software

Contact CG Technical Support to update and maintain the software.

Storage and transportation

- Store the device according to the environment requirements in this guide.
- If you want to move or transport the device, contact CG Technical Support.

Disposal of the device

The service life of this device is 5 years, which is determined by the simulated service life evaluation method. For the date of manufacture, refer to the label on the device. Perform the maintenance according to the requirements in this guide. Dispose of the end-of-life device according to local regulations. However, if it is confirmed that the device is still functioning safely and effectively after maintenance, continue to use the device.

07

FAQs

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

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

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

Sequencer FAQs

Q: What should I do if error messages related to the system appear?

When a system failure message appears, a problem may have occurred during communications between the device and the computing module.

To solve the problem, restart the device.

If the problem persists, contact CG Technical Support.

Q: What should I do if error messages related to the motor module appear during initialization, loading and offloading?

When a message appears indicating that the motor module failed, a problem may have occurred during communications or in the motor module.

Perform the following steps until the problem is solved:

1. Close the message box and try to continue the sequencing again.
2. Restart the device and try a new sequencing run without using consumables.
3. If the problem persists, contact CG Technical Support.

Q: What should I do if error messages related to the temperature control module appear during sequencing?

When a message appears indicating that temperature control module failed, errors may have occurred during communications, or in the flow cell or the temperature control module.

Perform the following steps to check the cause:

1. Use another flow cell and try a new sequencing run without using consumables.
2. Restart the device and try a new sequencing run without using consumables.

If the problem persists, contact CG Technical Support.

Q: What should I do if error messages related to the camera appear during sequencing?

When a message appears indicating that camera failed, errors may have occurred during communications, or in the flow cell or the imaging module.

Perform the following steps to check the cause:

1. Load the flow cell again.
2. Use another flow cell and try a new sequencing run without using consumables.
3. Restart the device and try a new sequencing run without using consumables.

If the problem persists, contact CG Technical Support.

Q: What should I do if error messages related to software update appear?

When a message appears indicating that the software failed to update, errors may have occurred during software updates and may occur in the update package or disk.

Perform the following steps until the problem is solved:

1. Update the software again.
2. Download the update package again and update the software.
3. Check whether error occurs in the disk.
4. If the problem persists, contact CG Technical Support.

Q: What should I do if error messages related to images appear?

When a message appears indicating that images were not found, errors may have occurred in insufficient storage space, the disk or camera.

Perform the following steps until the problem is solved:

1. Check whether the storage space is insufficient and if so, delete the raw data.
2. Restart the device and try a new sequencing run without using consumables.
3. Identify the cause of the imaging error based on the camera error code.
4. If the problem persists, contact CG Technical Support.

Reagent FAQs

Q: What should I do if DNB concentration does not meet requirements?

When the DNB concentration does not meet requirements specified in *Quantifying DNBs on Page 37* , perform the following steps:

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

Q: What rules should I follow if I need to store an open or thawed reagent kit temporarily?

- If a reagent kit has been thawed but is not used within 24 hours of thawing, you can re-freeze and thaw it only one more time.
- If a reagent cartridge has been thawed, but it cannot be used in time, store it in a refrigerator at 2 °C to 8 °C and use it within 24 hours. Before use, mix the reagent cartridge according to *Preparing the sequencing reagent cartridge on Page 38*.
- If the Signal Protein Mixture has been added into the cartridge, but it cannot be used in time, store the cartridge at room temperature and use it within 2 hours.
- If the MDA Mixture has been added into the cartridge, but it cannot be used in time, store it at room temperature and use it within 20 minutes.

Q: What should I do if the rubber stopper at the bottom of the reagent cartridge falls off or tilts?

When the rubber stopper at the bottom of the reagent cartridge falls off or tilts, but the reagent does not leak out of the cartridge, perform the following steps:

1. Keep the reagent cartridge horizontal.
2. Locate the well whose rubber stopper falls off or tilts.
3. Use a pair of pointed-tip tweezers to hold the rubber stopper, align it with the well and tighten the rubber stopper.
4. Tighten all rubber stoppers.

What should I do if the blue rubber stopper in the well of the reagent cartridge sinks into the well or falls off?

- When the blue rubber stopper in the well of the reagent cartridge sinks into the well, perform the following steps:
  1) Place the reagent cartridge on a level surface.
  2) Locate the blue rubber stoppers that sank into the wells.
  3) Use a pair of pointed-tip tweezers to clip the blue rubber stoppers up and bring it level with other stoppers.
- When the blue rubber stopper in the well of the reagent cartridge falls off, perform the following steps:
  1) Place the reagent cartridge on a level surface.
  2) Locate the blue rubber stopper that fell off.
  3) Wash the rubber stopper with laboratory-grade water.
  4) Use a pair of pointed-tip tweezers to hold the rubber stopper, align it with the well, and tighten the rubber stopper to bring it level with other stoppers.

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Instructions for importing a barcode file

Only the administrator account can access the Settings interface to import a barcode file.

Preparing a barcode file

Ensure that the barcode file meets the following requirements:

- The barcode file to be imported should be named in English in the text format (*.txt*).
- It is recommended that you use the Notepad application to open the barcode file. Barcode ID and barcode sequence in the file should be separated by a space.
- The barcode file should not contain blank lines, full-width characters, or Chinese characters. The barcode sequence should include no fewer than two bases.
- Barcode sequence should be unique. Barcode ID and barcode sequence should not be empty.
- Barcode sequence of a dual barcode file should not contain any characters other than “A”, “T”, “C”, and “G”.
- Barcode sequence of a single barcode file should not contain any characters other than “A”, “T”, “C”, and “G”.
- The total length of each barcode should be 8, 10, 16 or 20. When the total length is 16, the length of the Barcode 1 and Barcode 2 is 8 respectively. When the total length is 20, the length of the Barcode 1 and Barcode 2 is 10 respectively.
- If the mismatch number is not written in the file, it will be displayed as “1” by default in the interface.
- When the total length of each barcode is 16 or 20, the mismatch number cannot be modified in the interface.

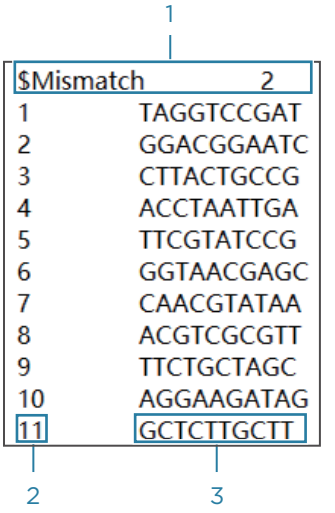

Figure 52 Single barcode file

No.	Name	Description
1	Mismatch	Corresponds to data of **Mismatch** in the **Customizing barcode parameters** interface
2	Barcode ID	/
3	Barcode sequence	Indicates the **Length** of the **Barcode 1** in the **Customizing barcode parameters** interface

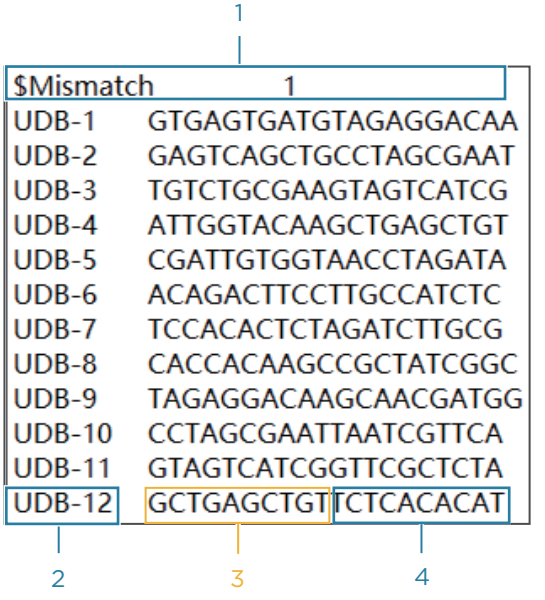

Figure 53 Dual barcode file

No.	Name	Description
1	Mismatch	Corresponds to data of **Mismatch** in the **Customizing barcode parameters** interface
2	Barcode ID	/
3	Barcode sequence	Shows the bases and **Length** of the **Barcode 1** in the **Customizing barcode parameters** interface
4	Barcode sequence	Shows the bases and **Length** of the **Barcode 2** in the **Customizing barcode parameters** interface

Importing a barcode file

- It is recommended that you format an external storage device, such as a USB drive, before use. The device only supports a USB storage drive in the FAT32 or NTFS format but does not support the exFAT format.
- Up to 5 customized barcode recipes can be configured for each user. Each barcode recipe is associated with a barcode document in text format *(.txt)* and mismatch number.

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 is named as "barcode". Copy the prepared "*barcode.txt*" file to the folder.
2. In the settings interface of the device, select **Barcode parameters**.

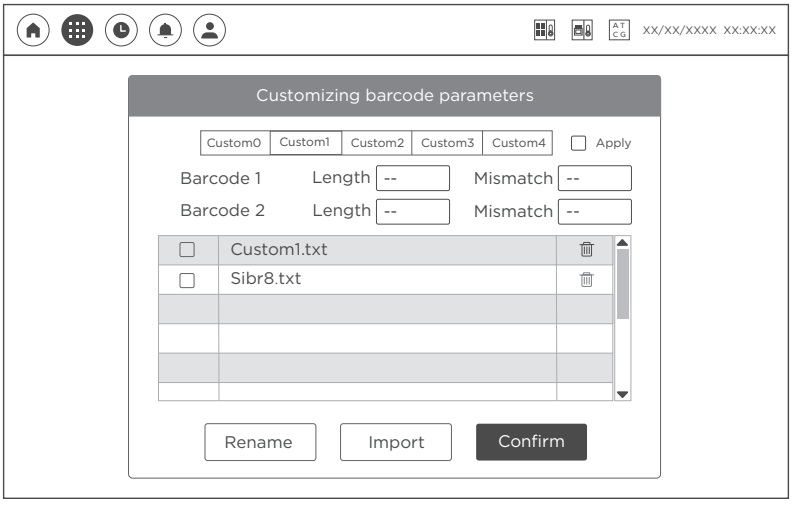

Figure 54 Barcode parameters interface

3. Select **Custom0**, and select **Rename** to name the barcode recipe.
4. Select **Import** to import the barcode document from the external storage device to the computing module. After the document is imported successfully, it appears in the list.

   By default, the barcode file is imported to the directory: *Computingmodule/Home/Documents/Basecall/Basecall/Barcode*
5. Select the required document in the list. **Length** information and **Mismatch** information are automatically filled in according to the barcode file. Only **Mismatch** information can be modified under special circumstances.

   To delete the barcode document, select  .
6. Select a barcode document and select **Confirm** to associate the document with corresponding barcode recipe.
7. Select **Apply**. At this time, the barcode recipe is available in the barcode recipe interface.

Instructions for customizing a run

Introduction

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

- When read length(s) in Read 1 and/or Read 2 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.
- For a dual barcode sequencing run, the barcode sequences are not within the predefined barcode list.

Important interfaces for customizing a run

Customizing barcode parameters

In the main interface, after you select , select **Settings**, and select **Barcode parameters**, the customizing barcode parameters interface is displayed:

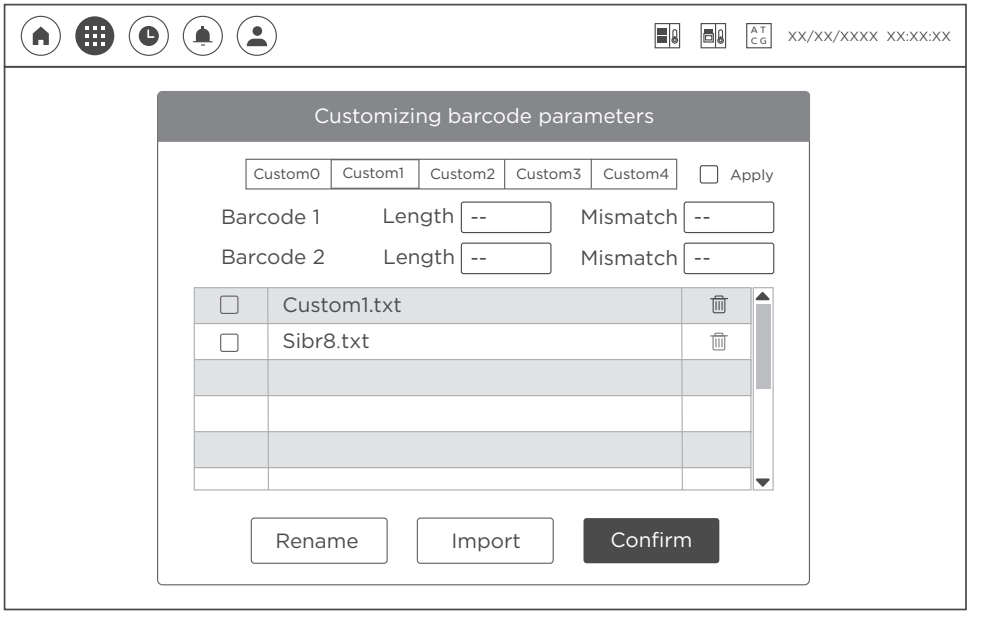

Figure 55 Barcode recipe

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

No.	Item	Description
1	**Custom0/Custom1/Custom2/ Custom3/Custom4**	Indicates the name of the barcode recipe.
2	**Length/Mismatch** for **Barcode 1**	Indicates customized number of bases of the first barcode and mismatch number for a sequencing run.
3	**Length/Mismatch** for **Barcode 2**	Indicates customized number of bases of the second barcode and mismatch number for a sequencing run.

No.	Item	Description
5	**Apply**	Select to have the barcode recipe available in the barcode list.
6	**Confirm**	Select to associate the barcode document with the barcode recipe.
7	**Rename**	Select to name the customized barcode recipe.
8	**Import**	Select to import the barcode document.

Customization interface

After you select , the customization interface is displayed:

Figure 56 **Customization interface**

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

No.	Item	Description
1	**Recipe**	Select a recipe for a sequencing run.
2	**Read 1/Read 2**	Select to customize Read 1 and (or) Read 2 length for a sequencing run.
3	**Barcode**	Tap the drop-down list to select the required barcode recipe.

Examples of customized run

Ensure that the barcode file meets the following requirements:

- Before starting the customized run, confirm that the customized barcode files have been imported into the sequencer. If they have not been imported, refer to *Instructions for importing a barcode file on Page 89* to import the customized barcode recipe.
- Ensure that the total number of sequencing cycles, including Read 1, Read 2, barcode 1, and barcode 2 is less than the maximum sequencing cycles for a given sequencing kit as defined in *Sequencing read length and cycle on Page 28*.
- The maximum read length for both Read 1 and Read 2 should not be more than that specified in the sequencing kit. For example, if PE150 is used, the maximum customized Read 1 length and Read 2 length should not be greater than 150.

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

1. Read 1/Read 2 lengths are not the same as those predefined in the Recipe list

Assumptions are as follows:

- Sequencing recipe: PE150+10
- Length of Read 1: 120
- Length of Read 2: 140
- Length of Barcode 1: 10
- Length of Barcode 2: 0
- The flow cell requires a non-predefined barcode list
- Total cycles = 120+140+10+0+2= 272
- Select a PE150 kit

The customization interface is set as follows:

Figure 57 Customization interface

2. Length of the single barcode is not 10

Assumptions are as follows:

- Sequencing recipe: PE150+8
- Length of Read 1: 150
- Length of Read 2: 150
- Length of barcode 1: 8
- Length of barcode 2: 0
- Total cycles = 150+150+8+0+2= 310
- Select a PE150 kit

Perform the following steps:

1. Prepare a barcode file named as "Sibr8" according to *Preparing a barcode file on Page 89*.

2. Import the barcode file named as "Sibr8" according to *Importing a barcode file on Page 91*. The **Customizing barcode parameters** interface is displayed as follows:

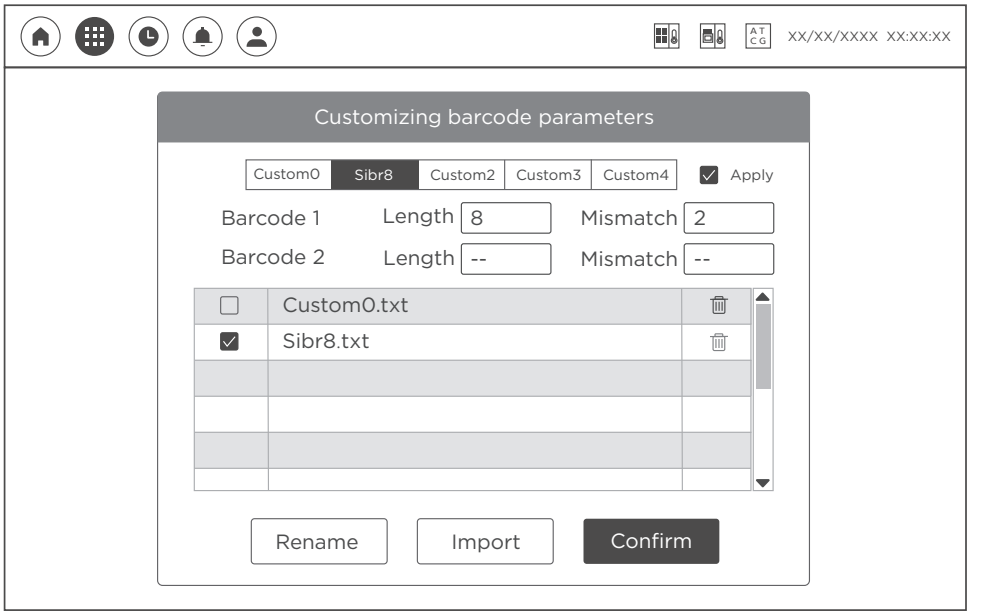

Figure 58 **Customizing barcode parameters interface**

3. Select , and select . Set the customization interface as follows:

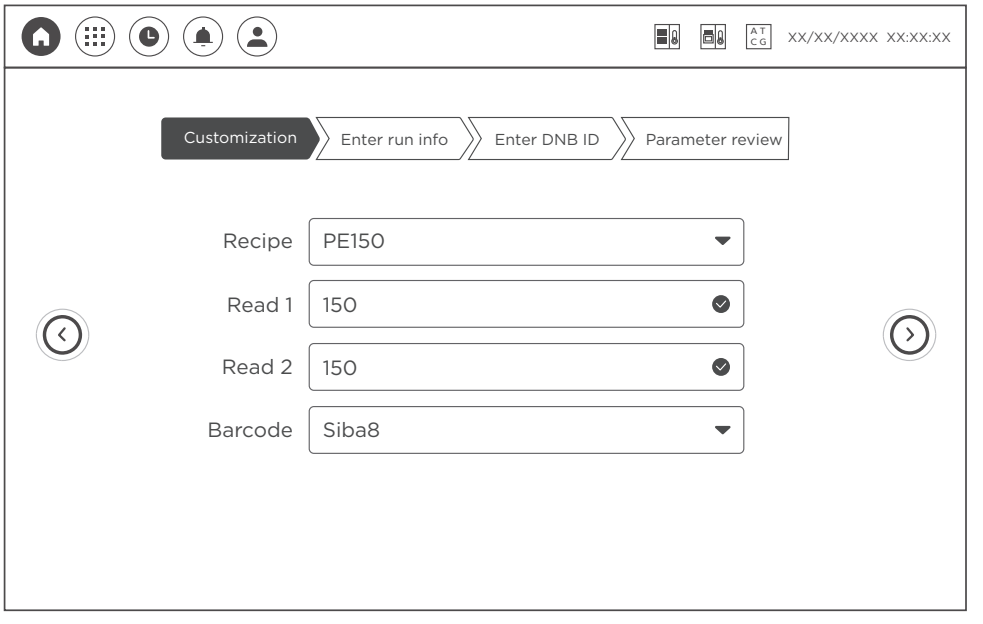

Figure 59 **Setting the customization interface**

3. Length of the Barcode 1 and Barcode 2 are not 10

Assumptions are as follows:

- Sequencing recipe: PE150+8+8
- Length of Read 1: 150
- Length of Read 2: 150
- Length of Barcode 1: 8
- Length of Barcode 2: 8
- Total cycles = 150+150+8+8+2= 318
- Select a PE150 kit

Perform the following steps:

1. Prepare a barcode file named as "Dubr8" according to *Preparing a barcode file on Page 89.*
2. Import the barcode file named as "Dubr8" according to *Importing a barcode file on Page 91.* The **Customizing barcode parameters** interface is displayed as follows:

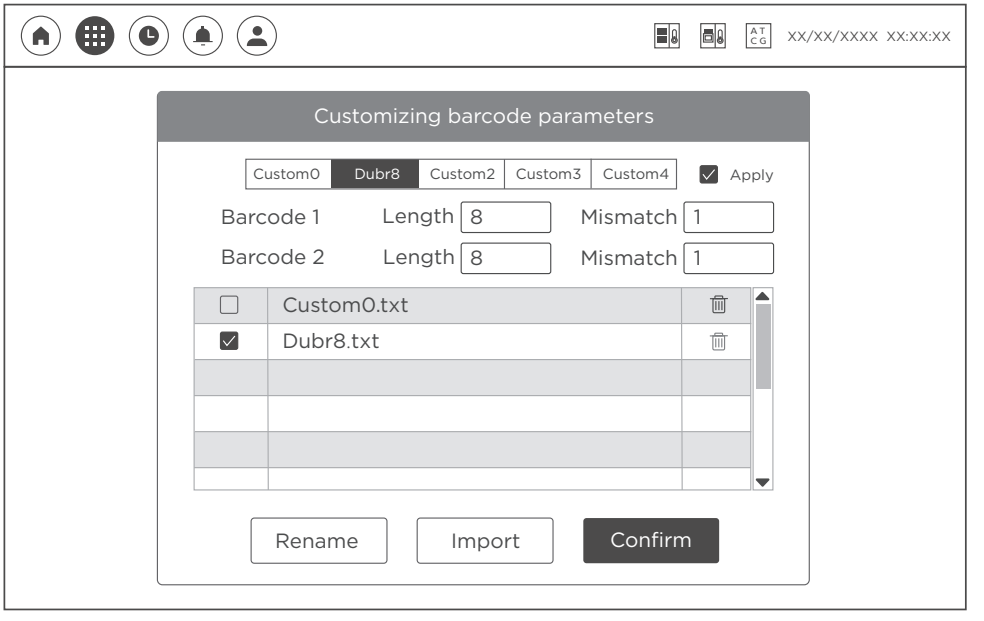

Figure 60 **Customizing barcode parameters interface**

3. Select , and select . Set the customization interface as follows:

Figure 61 Setting the customization interface

Instructions for using Qubit to quantify DNBs

- The working solution should be used within 0.5 hours after preparation.
- Do not touch the wall of selected detection tubes.
- Ensure that no bubbles exist in detection tubes.

Perform the following steps:

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

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

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

   For example, for 8 samples, prepare enough working solution for the samples and 2 standards: 200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit reagent plus 1990 µL of Qubit Buffer).
2. Add 190 µL of Qubit working solution to each of the tubes used for standards.
3. Add 10 µL of each Qubit standard to the appropriate tube, then mix by vortexing 3 to 5 seconds. Please operate with care to prevent bubbles from forming.
4. Prepare a sufficient 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 (Catalog number: Q32856) and AXYGEN PCR-05-C tubes (Catalog number: 10011-830).
   - The number of Qubit test tubes needed corresponds to the number of samples plus 2 standards tubes. For example, if you have 3 samples, you will need 5 tubes.
5. Label the tube lids. Do not label the side of tube.
6. Prepare the solutions used for standards and sample tests according to the following table.

	S1 (µL)	S2 (µL)	D1 (µL)	D2 (µL)	D3 (µL)
Working solution	190	190	198	198	198

	S1 (μL)	S2 (μL)	D1 (μL)	D2 (μL)	D3 (μL)
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 tubes by using a vortex mixer, centrifuge briefly for 5 seconds, and then incubate at room temperature away from direct sunlight for 2 minutes.
8. Refer to the Qubit user manual for instructions on reading standards and samples. Follow the appropriate procedure for your instrument.

List of sequencing set components

A sequencing set includes a sequencing flow cell and a sequencing kit. A sequencing kit includes a Sequencing Reagent Cartridge and reagents for sequencing.

Table 15 DNBSEQ-E25RS High-throughput Sequencing Set (FCL SE100)
Catalog number: 940-000894-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Validity period
DNBSEQ-E25RS Sequencing Flow Cell **Catalog number: 940-000893-00**					
DNBSEQ-E25RS FCL Sequencing Flow Cell	/	1 EA	10 °C to 25 °C	10 °C to 25 °C	6 months
DNBSEQ-E25RS High-throughput Sequencing Kit (FCL SE100) **Catalog number: 940-000897-00**					
Low TE buffer		300 µL/tube×1	-25 °C to -15 °C	-80 °C to -15 °C	6 months
Make DNB Buffer (OS-V2.0-DB)		80 µL/tube×1			
Make DNB Buffer (OS-V2.0-SB)		80 µL/tube×1			
Make DNB Enzyme Mix I (OS)		160 µL/tube×1			
Make DNB Enzyme Mix II (OS)		16 µL/tube×1			
Stop DNB Reaction Buffer		100 µL/tube×1			
DNB Load Buffer II		120 µL/tube×1			
Signal Protein 1		15 µL/tube×1			
Signal Protein 2		10 µL/tube×1			
Signal Protein Buffer		10 mL/tube×1			
Sequencing Reagent Cartridge	/	1 EA			
Waste container	/	1 EA			

Table 16 DNBSEQ-E25RS High-throughput Sequencing Set (FCL PE150)
Catalog number: 940-000891-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Validity period
DNBSEQ-E25RS Sequencing Flow Cell **Catalog number: 940-000896-00**					
DNBSEQ-E25RS FCL Sequencing Flow Cell	/	1 EA	10 °C to 25 °C	10 °C to 25 °C	6 months
DNBSEQ-E25RS High-throughput Sequencing Kit (FCL PE150) **Catalog number: 940-000900-00**					
Low TE buffer		300 µL/tube×1	-25 °C to -15 °C	-80 °C to -15 °C	6 months
Make DNB Buffer (OS-V2.0-DB)		80 µL/tube×1			
Make DNB Buffer (OS-V2.0-SB)		80 µL/tube×1			
Make DNB Enzyme Mix I (OS)		160 µL/tube×1			
Make DNB Enzyme Mix II (OS)		16 µL/tube×1			
Stop DNB Reaction Buffer		100 µL/tube×1			
DNB Load Buffer II		120 µL/tube×1			
Signal Protein 1		31.5 µL/tube×1			
Signal Protein 2		21 µL/tube×1			
Signal Protein Buffer		21 mL/tube×1			
MDA T-Reagent		0.35 mL/tube×1			
MDA Enzyme Mix		0.05 mL/tube×1			
Sequencing Reagent Cartridge	/	1 EA			
Waste container	/	1 EA			

Table 17 DNBSEQ-E25RS High-throughput Sequencing Set (App-C FCL SE100)
Catalog number: 940-000895-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Validity period
DNBSEQ-E25RS Sequencing Flow Cell **Catalog number: 940-000892-00**					
DNBSEQ-E25RS FCL Sequencing Flow Cell	/	1 EA	10 °C to 25 °C	10 °C to 25 °C	6 months
DNBSEQ-E25RS High-throughput Sequencing Kit (App-C FCL SE100) **Catalog number: 940-000899-00**					
Low TE buffer		300 µL/tube×1	-25 °C to -15 °C	-80 °C to -15 °C	6 months
Make DNB Buffer (OS-App)		80 µL/tube×1			
Conversion Enzyme		5 µL/tube×1			
Make DNB Enzyme Mix I (OS)		160 µL/tube×1			
Make DNB Enzyme Mix II (OS)		16 µL/tube×1			
Stop DNB Reaction Buffer		100 µL/tube×1			
DNB Load Buffer II		120 µL/tube×1			
Signal Protein 1		15 µL/tube×1			
Signal Protein 2		10 µL/tube×1			
Signal Protein Buffer		10 mL/tube×1			
Sequencing Reagent Cartridge	/	1 EA			
Waste container	/	1 EA			

Table 18 DNBSEQ-E25RS High-throughput Sequencing Set (App-C FCL PE150)
Catalog number: 940-000901-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Validity period
DNBSEQ-E25RS Sequencing Flow Cell Catalog number: 940-000902-00					
DNBSEQ-E25RS FCL Sequencing Flow Cell	/	1 EA	10 °C to 25 °C	10 °C to 25 °C	6 months
DNBSEQ-E25RS High-throughput Sequencing Kit (App-C FCL PE150) Catalog number: 940-000898-00					
Low TE buffer		300 µL/tube×1	-25 °C to -15 °C	-80 °C to -15 °C	6 months
Make DNB Buffer (OS-App)		80 µL/tube×1			
Conversion Enzyme		5 µL/tube×1			
Make DNB Enzyme Mix I (OS)		160 µL/tube×1			
Make DNB Enzyme Mix II (OS)		16 µL/tube×1			
Stop DNB Reaction Buffer		100 µL/tube×1			
DNB Load Buffer II		120 µL/tube×1			
Signal Protein 1		31.5 µL/tube×1			
Signal Protein 2		21 µL/tube×1			
Signal Protein Buffer		21 mL/tube×1			
MDA T-Reagent		0.35 mL/tube×1			
MDA Enzyme Mix		0.05 mL/tube×1			
Sequencing Reagent Cartridge	/	1 EA			
Waste container	/	1 EA			

Device specifications

CAUTION

- The maximum sound pressure level is measured based on the distance between the position where the device operator stands during normal operation and any position which is one meter from the device and has the maximum sound pressure level.
- Because the temperature and humidity fluctuations influence the accuracy of the 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		
Main unit	Dimensions		348 mm (W)×312 mm (H)×257 mm (D) (13.7 inches×12.3 inches×10.1 inches)
	Net weight		Approximately 15 kg (33.1 lbs)
	Supply voltage		24 VDC, 5 A
Computing module	Dimensions·		348 mm (W)×312 mm (H)×40 mm (D) (13.7 inches×12.3 inches×1.6 inches)
	Net weight		Approximately 5 kg (11 lbs)
	Supply voltage	DNBSEQ-E25RS	20 VDC, 11.5 A
		DNBSEQ-E25ARS	100-240 V~, 50/60 Hz, 300 VA
Touch screen	Type		LCD
	Size		12.1 inches
	Resolution		1280×800 pixels
Maximum sound pressure level	65 dBA		

Item	Description	
Degrees of protection provided by enclosures (IP Code)	IPX0	
Operating environment requirements	Temperature	15 °C to 30 °C (59 °F to 86 °F)
	Relative humidity	20% to 80%, non-condensing
	Atmospheric pressure	70 kPa to 106 kPa
Altitude	3000 m	
Operating environment requirements	Pollution degree	2
Operating environment requirements	Usage scenario	For indoor use
	Temperature and humidity fluctuations influence the accuracy of the experiment results. It is recommended that you install an air conditioning system and a humidifier or dehumidifier in the laboratory.	
Transportation/ storage environment requirements	Temperature	-20 °C to 50 °C (-4 °F to 122 °F)
	Relative humidity	15% to 85%, non-condensing
Accompanying items	Refer to the packing list.	

Compliance information

The device complies with the following standards:

Item	Standard
Electromagnetic Compatibility (EMC)	• IEC 61326-1 Electrical equipment for measurement, control and laboratory use – EMC requirements ~ Part 1: General requirements • FCC Part 15 Subpart B – Radio Frequency Devices Unintentional Radiators
Safety requirements	• UL 61010-1/CSA C22.2#61010-1-12 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use ~ Part 1: General Requirements • UL 61010-2-010/CSA C22.2#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 • UL61010-2-081/CSA C22.2 No.61010-2-081 Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use - Part 2-081: Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes

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Research use only

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

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Manufacturer information

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Customer service telephone	+1 (888) 811-9644
Website	www.completegenomics.com

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Order information

Catalog number	Model	Name	Version	Recommended brand
940-000894-00	FCL SE100	DNBSEQ-E25RS High-throughput Sequencing Set	1.0	CG
940-000891-00	FCL PE150	DNBSEQ-E25RS High-throughput Sequencing Set	1.0	CG
940-000895-00	App-C FCL SE100	DNBSEQ-E25RS High-throughput Sequencing Set	1.0	CG
940-000901-00	App-C FCL PE150	DNBSEQ-E25RS High-throughput Sequencing Set	1.0	CG
900-000702-00	DNBSEQ-E25ARS	Genetic Sequencer	1.0	Advanced Config, CG
900-000703-00	DNBSEQ-E25RS	Genetic Sequencer	1.0	Standard Config, CG

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Acronyms and abbreviations

Item	Description
bp	base pair
BIC	Basecall Information Content
cPAS	Combinatorial Probe-anchor Synthesis
CM shutdown	Shut down the computing module
DNA	Deoxyribonucleic Acid
DNB	DNA Nanoball
dsDNA	double-stranded DNA
EMC	Electromagnetic Compatibility
ESR	Effective Spots Rate
FAQ	Frequently Asked Questions
FCL	Flow Cell Large
FIT	Least square fit to the DNB intensities in 4 color space to represent the overall quality of the clusters
FOV	Field of View
FASTQ	A text-based format for storing both a biological sequence (usually nucleotide sequence) and its corresponding quality scores
HS	High Sensitivity
ID	Identification
LCD	Liquid Crystal Display
MSP	Mixture of Signal Protein
MDA	Multiple Displacement Amplification
PE	Pair-end sequencing
PFA	PCR-Free Set A
QR	Quick Response
QC	Quality Control
RCR	Rolling Circle Replication

Item	Description
RNA	Ribonucleic Acid
RJ45	Registered Jack45
SE	Single-end sequencing
SNR	Signal to Noise Ratio
ssDNA	single-stranded DNA
SFP+	Small Form-factor Pluggables
USB	Universal Serial Bus
UPS	Uninterruptible Power Supply
UDB	Unique Dual Barcode
UDBA	Unique Dual Barcode Set A
VGA	Video Graphics Array

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