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

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

Figure 30 RFID scanning area of DNB Load Plate

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

Figure 31 Placing DNB Load Plate

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

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

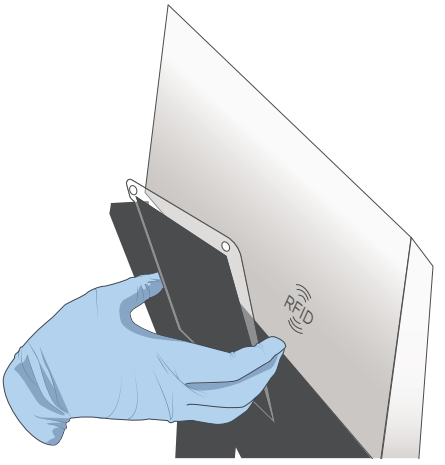

Figure 32 **Scanning the Flow cell ID**

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

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

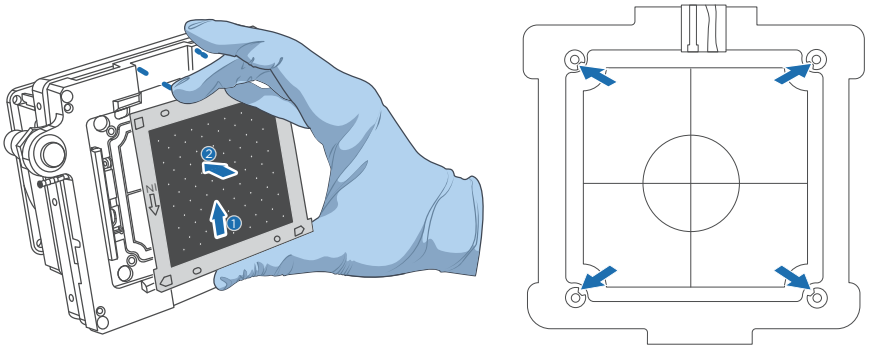

Figure 33 **Flow cell locating**

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

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

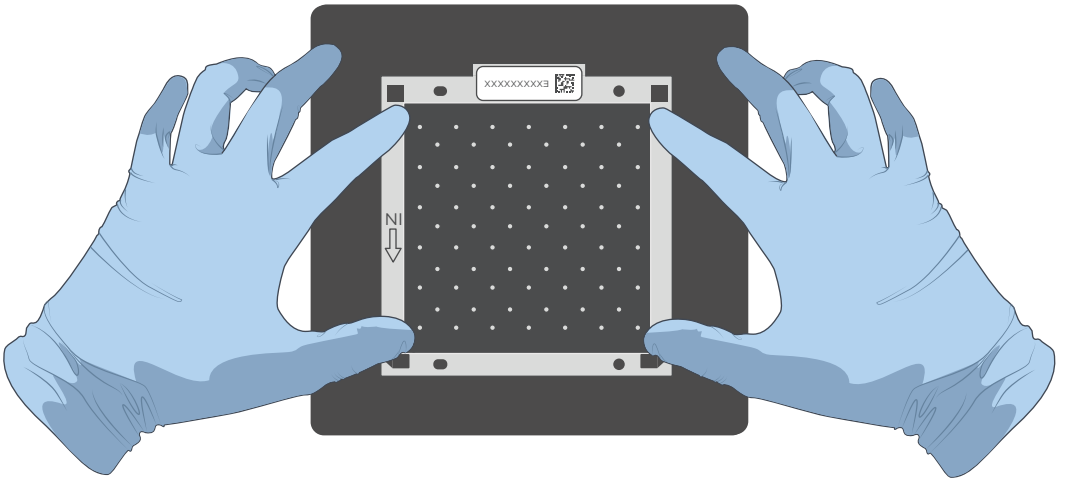

Figure 34 Flow cell loaded

14. Close the loading compartment door.

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

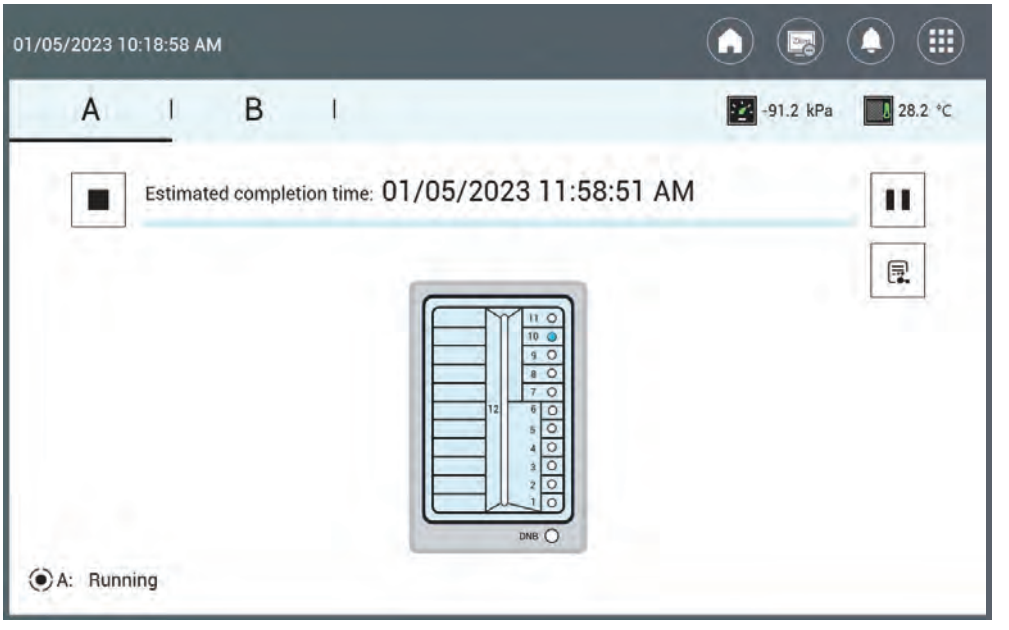

Figure 35 DL-T7RS flow cell loading interface

16. The process takes around two hours. When the screen is shown as *Figure 36 on Page 76*, the flow cell loading is complete.

- Do not open the loading compartment door during loading as it will stop the loading process.
- Do not bump, move, vibrate or impact the device during loading as it may cause inaccurate results.
- Do not place other instruments such as a centrifuge or vortex on the same bench where the loader is placed. Other instruments may cause vibrational interference to the loader.
- Pay special attention to the LED status indicator, icons, and prompts. If errors occur, a message appears on the screen. Follow the prompt to troubleshoot and fix the problem. For information about the troubleshooting, refer to *FAQs on Page 127*. If the problem persists, contact CG Technical Support.

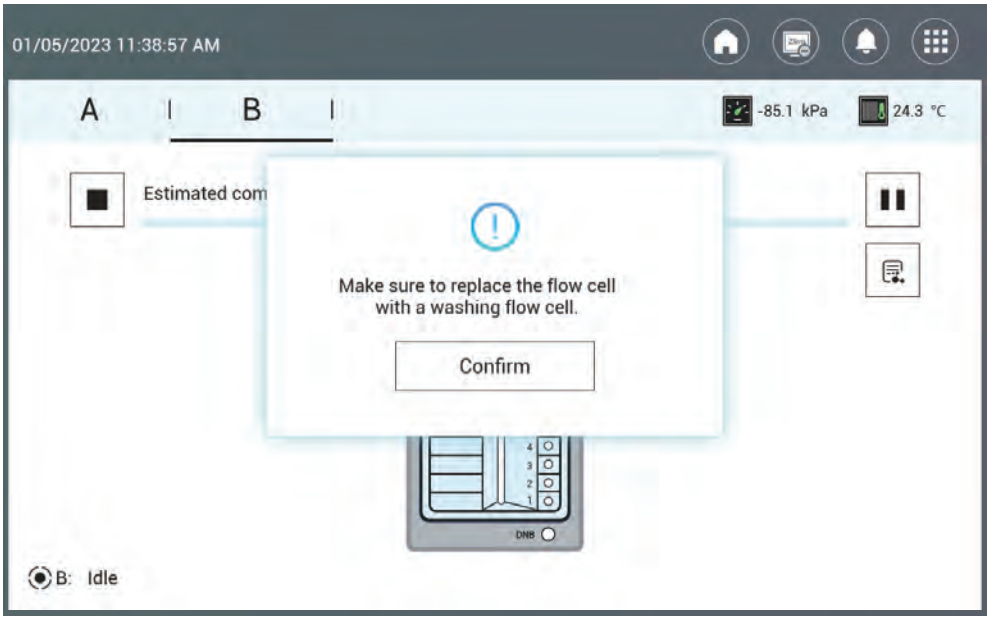

Figure 36 DL-T7RS flow cell loading complete status interface

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

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

18. When the loading is complete, install the washing flow cell onto the flow cell stage and press the flow cell attachment button. Close the flow cell compartment door. Select **Confirm** as shown in *Figure 36 on Page 76*.

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

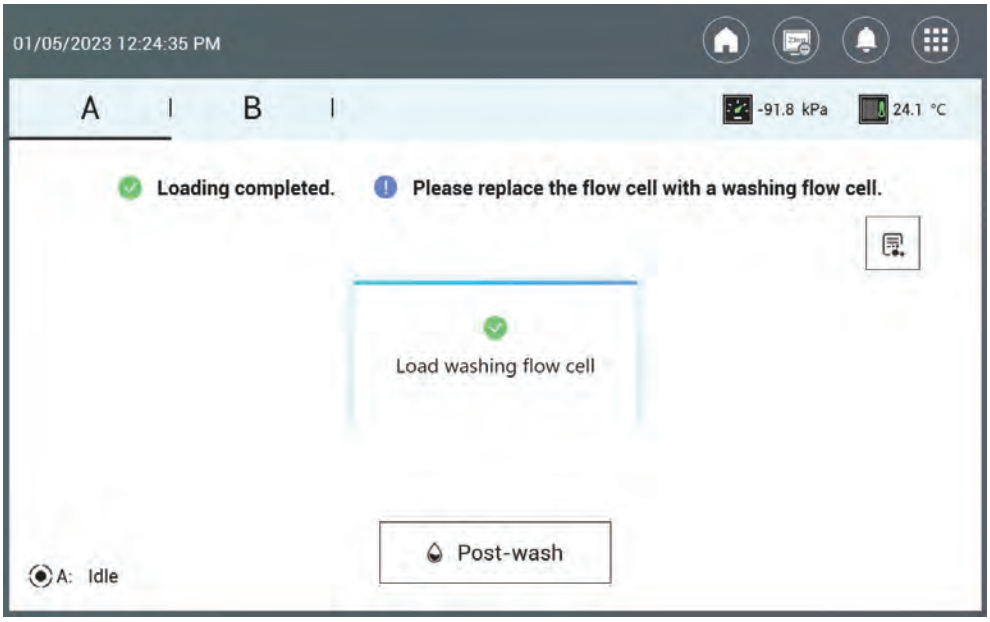

Figure 37 DL-T7RS post-wash interface

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

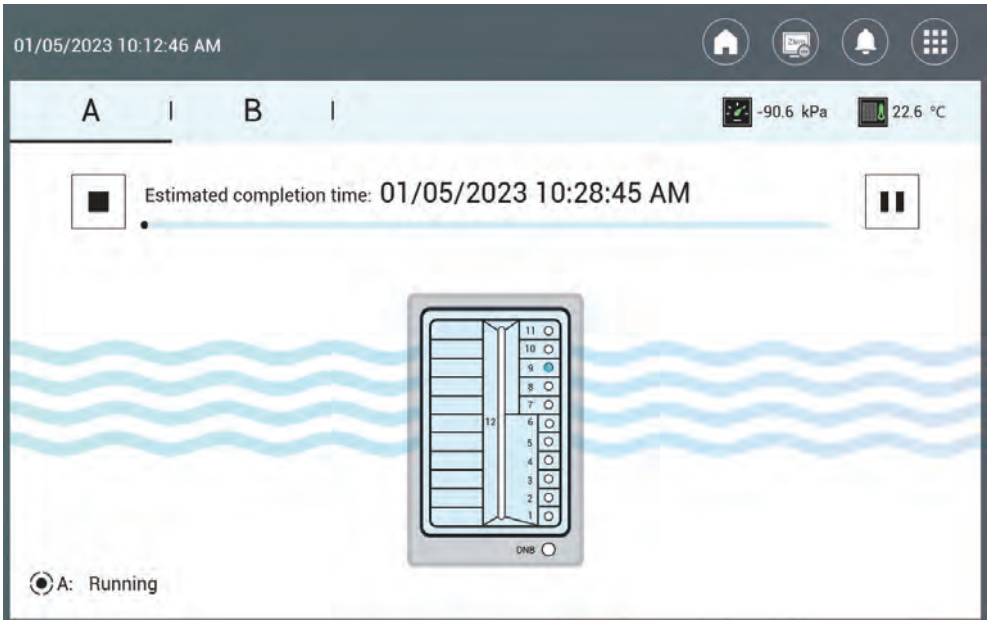

Figure 38 DL-T7RS wash interface

When the screen is shown as the figure below, the wash is complete.

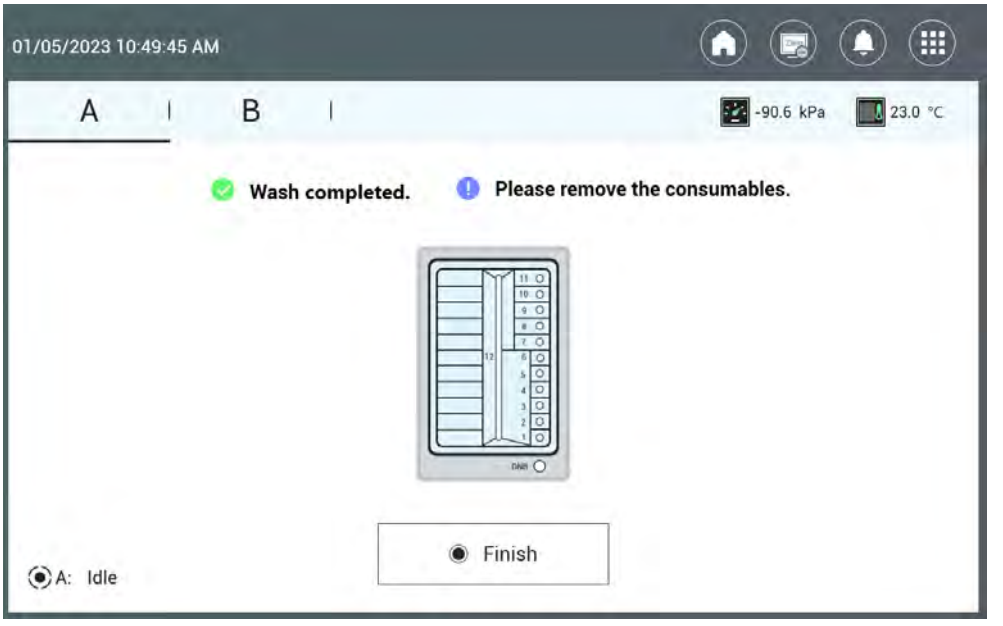

Figure 39 DL-T7RS wash complete status interface

21. After completing the loading process by selecting **Finish**, another flow cell loading process can be performed for another load plate.
22. Remove the washing flow cell and store it at room temperature.
23. Empty any remaining washing solution in the DNB Load Plate (T7 FCL PE150) into an appropriate waste container.
24. Dispose of the waste and DNB tube.

Preparation before sequencing

CAUTION If prepared cartridges are not used immediately, refer to *Q: What rules should I follow if I need to store a reagent kit temporarily? on Page 135* for details.

Preparing the Sequencing Reagent Cartridge

Sequencing Enzyme Mix and dNTP Mix are provided in different tubes and packaged together with the Sequencing Reagent Cartridge. Before the sequencing run starts, an appropriate amount of sequencing enzyme and dNTP mix needs to be added to well No. 9 and well No. 10 of the Sequencing Reagent Cartridge. Furthermore, the MDA mixture (MDA, Multiple displacement amplification) needs to be added to well No. 8 if performing PE (Pair-end) sequencing.

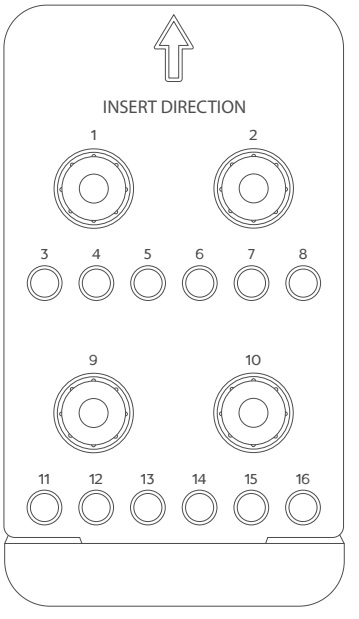

Figure 40 Sequencing cartridge wells

Perform the following steps:

1. Take the Sequencing Reagent Cartridge out of the DNBSEQ-T7RS High-throughput Sequencing Kit.
2. Thaw the Sequencing Reagent Cartridge. The approximate time to thaw is listed in the following table. Store in a 2 °C to 8 °C refrigerator until use. Choose the method that best suits your situation:

Table 28 Approximate thaw times for various sequencing kits

Model	Method		
	Water bath at room temperature (hours)	Refrigerator at 2 °C to 8 °C overnight then water bath at room temperature (hours)	Refrigerator at 2 °C to 8 °C (hours)
FCL PE100	2.5	1.5	24.0
FCL PE150	3.0	2.0	24.0
stLFR FCL PE100	2.5	1.5	24.0

3. Invert the cartridge three times to mix before use.
4. Shake the cartridge vigorously clockwise 20 times, and then counterclockwise 20 times. Ensure that reagents are fully mixed.

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

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

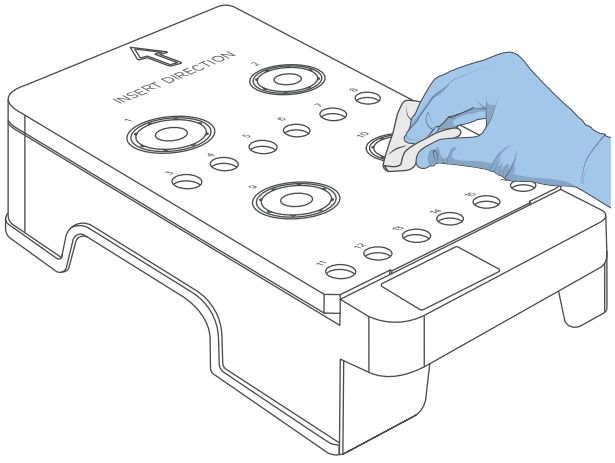

Figure 41 Wiping cartridge cover

6. Take the dNTPs Mix IV (or dNTPs Mix V) and dNTPs Mix II out of the sequencing kit and thaw them at room temperature.
7. Invert the dNTPs Mix IV (or dNTPs Mix V) and dNTPs Mix II 6 times. Gently tap the tube on the bench to bring the liquid to the bottom. Place them on ice until use.

8. Take the Sequencing Enzyme Mix out of the DNBSEQ-T7RS High-throughput Sequencing Kit. Invert the Sequencing Enzyme Mix 6 times and place it on ice until use.
9. Pierce the seal in the center of well No. 9 and No. 10 to make a hole around 2 cm in diameter by using a 1 mL sterile tip.
10. Take out a pipette with the appropriate volume range. Add the dNTPs Mix IV (or dNTPs Mix V) and Sequencing Enzyme Mix into well No. 9 according to the table below:

Table 29 Reagent preparation for well No. 9

Model	dNTPs Mix IV volume (mL)	dNTPs mix V volume (mL)	Sequencing enzyme mix volume (mL)
FCL PE100	/	2.760	2.760
FCL PE150	/	3.740	3.740
stLFR FCL PE100	5.400	/	5.400

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

Table 30 Reagent preparation for well No. 10

Model	dNTPs mix II volume (mL)	Sequencing enzyme mix volume (mL)
FCL PE100	8.280	2.760
FCL PE150	11.220	3.740
stLFR FCL PE100	14.700	4.900

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

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

16. Pierce the seal of well No. 8 by using a 1 mL sterile tip.

17. Add 600 µL of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette and invert the tube 6 times to mix the reagents.

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

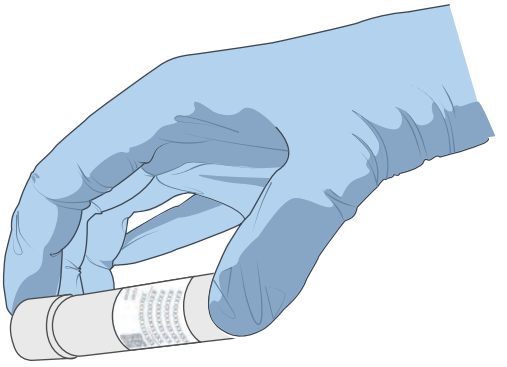

Figure 42 MDA mixture

18. Add the MDA mixture to well No. 8. When adding the MDA mixture, ensure that there is no bubble at the bottom of the tube.
19. Gently tap the Sequencing Reagent Cartridge on the bench to reduce air bubbles in the reagents.

Preparing the Washing Cartridge

Perform the following steps:

1. Shake the cartridge clockwise 10 times, and then counterclockwise 10 times to ensure the reagents are fully mixed.

2. Clean the foil seal on the wells with a Kimwipes tissue. Pierce either side of well No. 2 by using a 1 mL sterile tip.

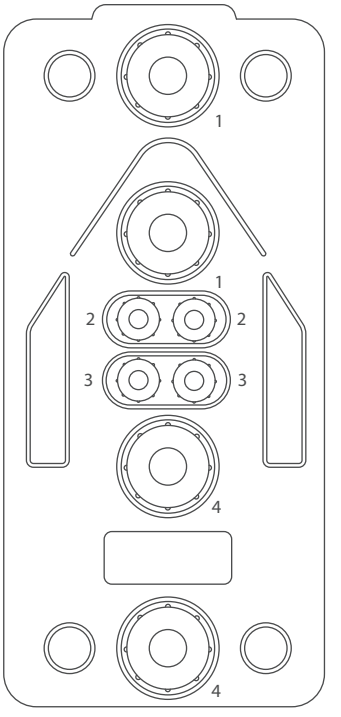

Figure 43 Washing Cartridge

3. Add 45 mL of 0.1 M NaOH into well No. 2 through the pierce by using an electronic pipette. Refer to *Preparing washing reagents on Page 117* for the preparation of 0.1 M NaOH.

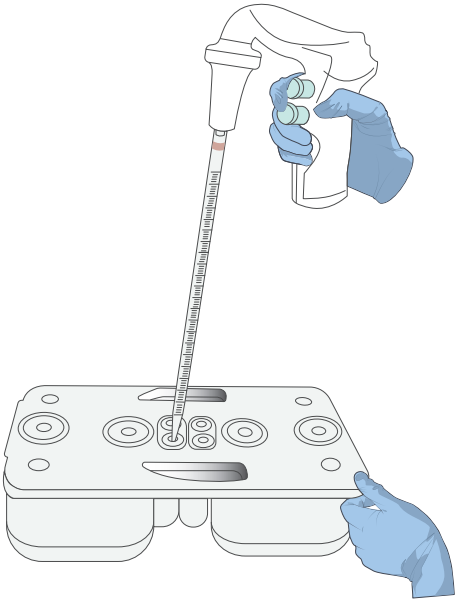

Figure 44 Washing Cartridge added 0.1 M NaOH

Filling the pure water container

Fill the pure water container with laboratory-grade water according to *Table 31 on Page 85*.

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

Table 31 Pure water consumption (L)

Model	1 flow cell	2 flow cells	3 flow cells	4 flow cells
FCL PE100	3.0	6.0	9.0	12.0
FCL PE150	4.5	9.0	13.5	18.0
stLFR FCL PE100	3.5	7.0	10.5	14.0

Performing a sequencing run

Loading the cartridges

Perform the following steps:

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

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

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

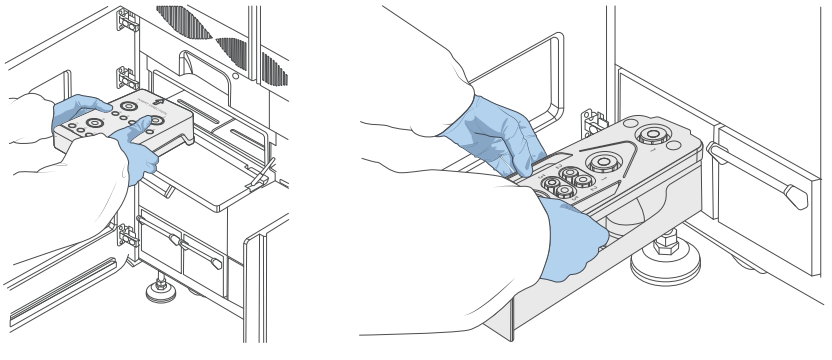

Figure 45 Loading the cartridges

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

Entering sequencing interface

Enter the user name and password. Select **Log in** to enter the main interface.

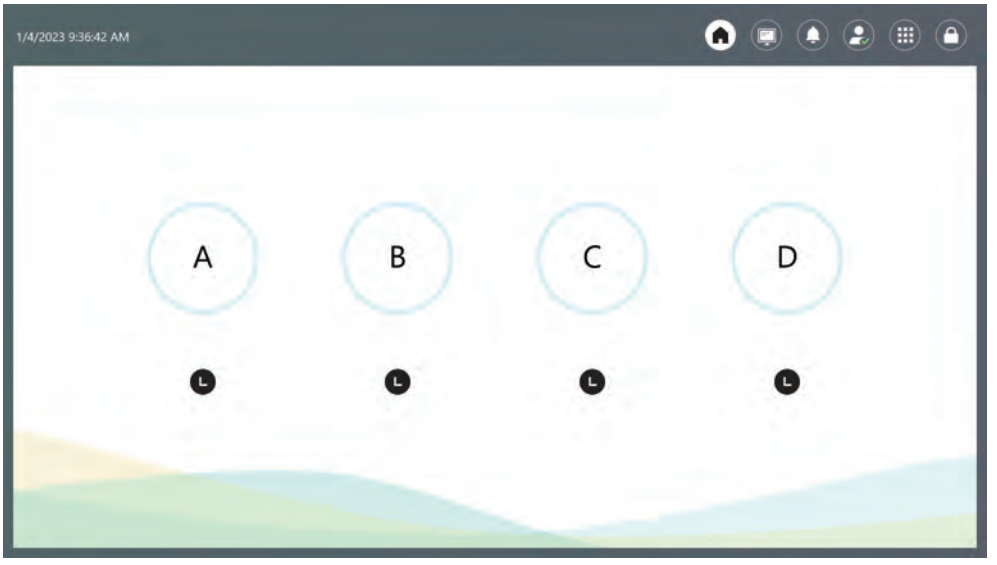

Figure 46 DNBSEQ-T7RS main interface

Loading the flow cell

Perform the following steps:

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

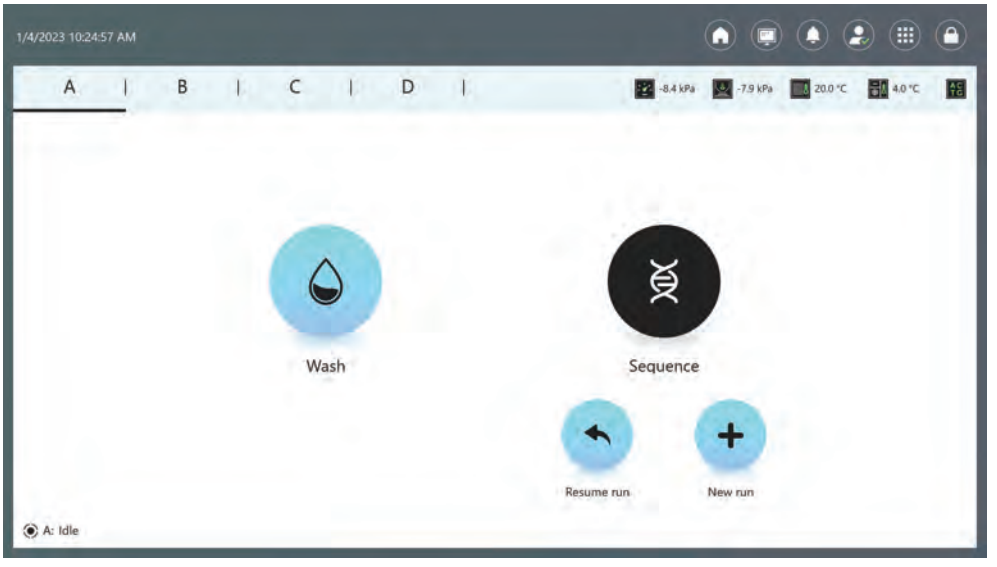

Figure 47 DNBSEQ-T7RS selection interface

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

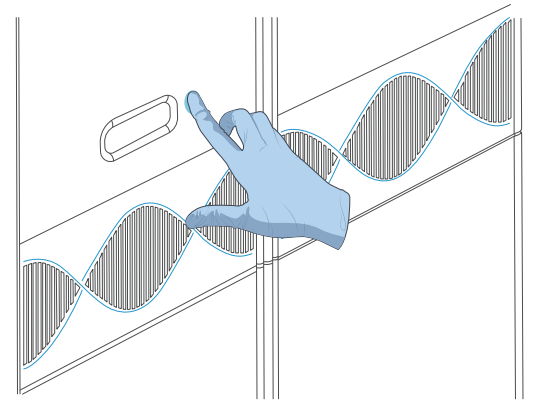

Figure 48 Flow cell drive

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

Sequencing parameters

Perform the following steps:

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

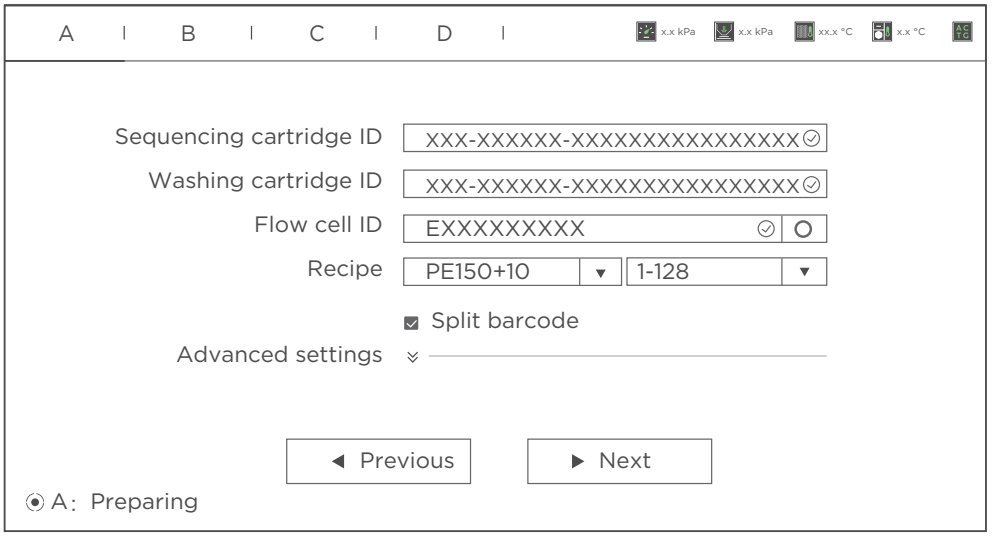

Figure 49 DNBSEQ-T7RS sequencing parameters

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

If a customized recipe is required, select **Customize** from the **Recipe** list. For information on recipe customization, refer to *Instructions for customizing a run on Page 145*.

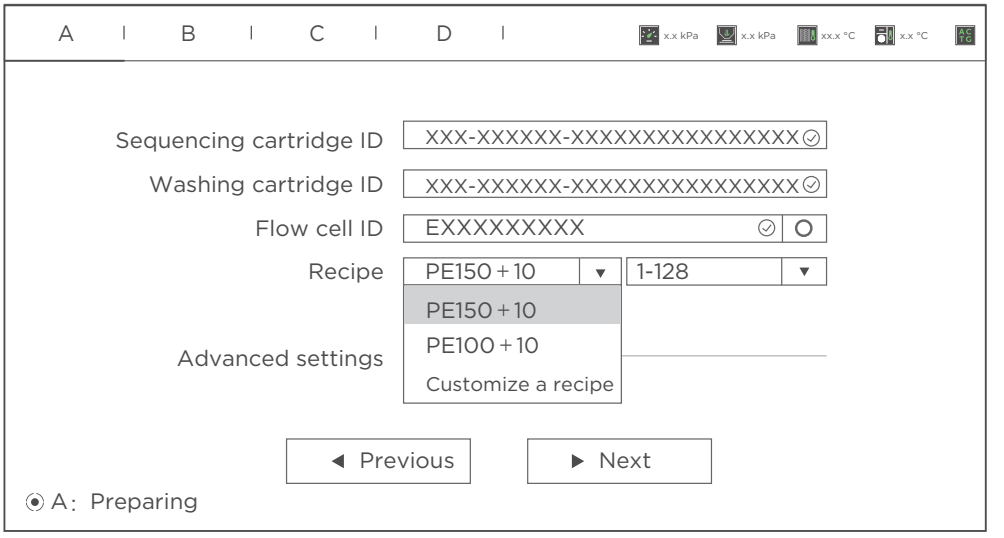

Figure 50 Set the sequencing recipe

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

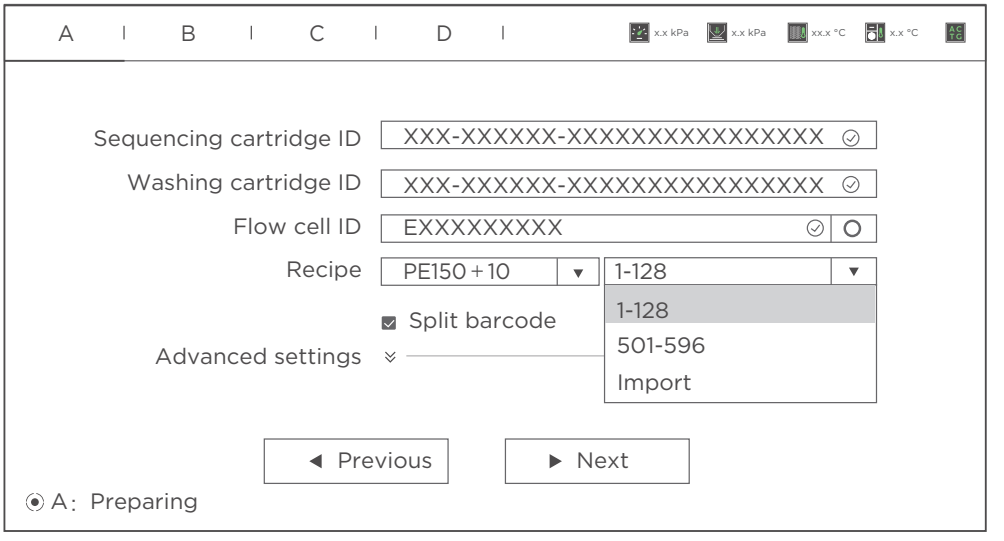

Figure 51 Set the barcode sequence

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

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

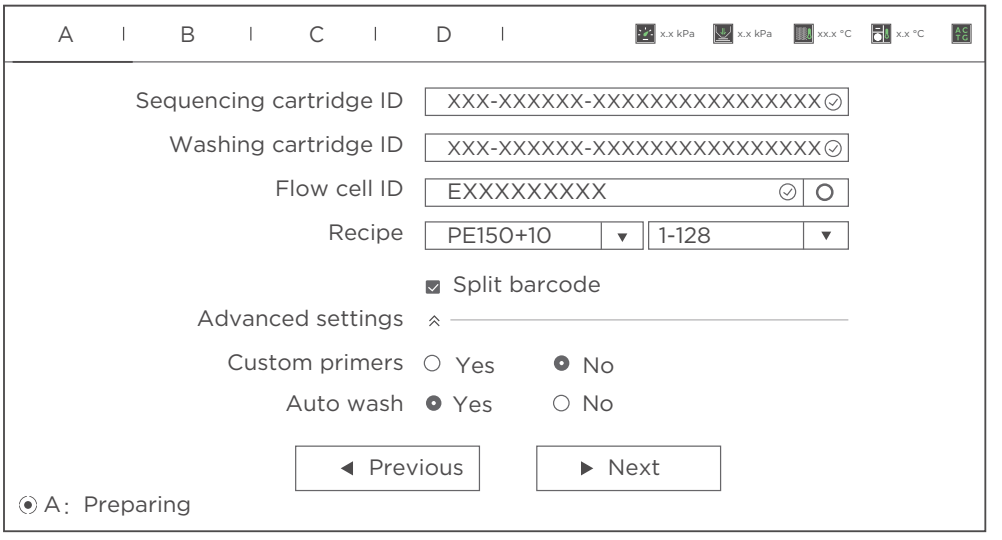

Figure 52 DNBSEQ-T7RS advanced settings

6. Select **Next**.

Reviewing parameters

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

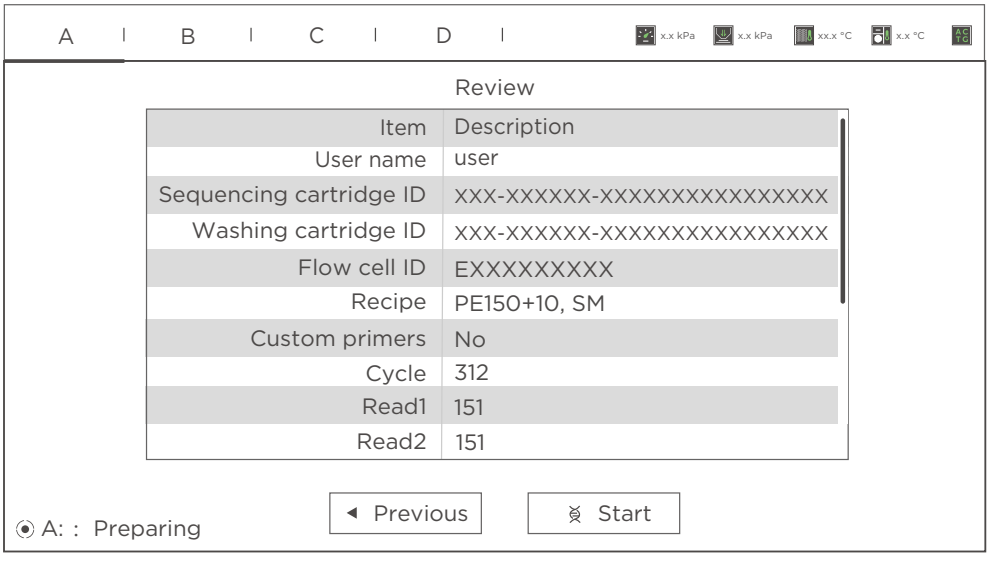

Figure 53 Reviewing information

Starting sequencing

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

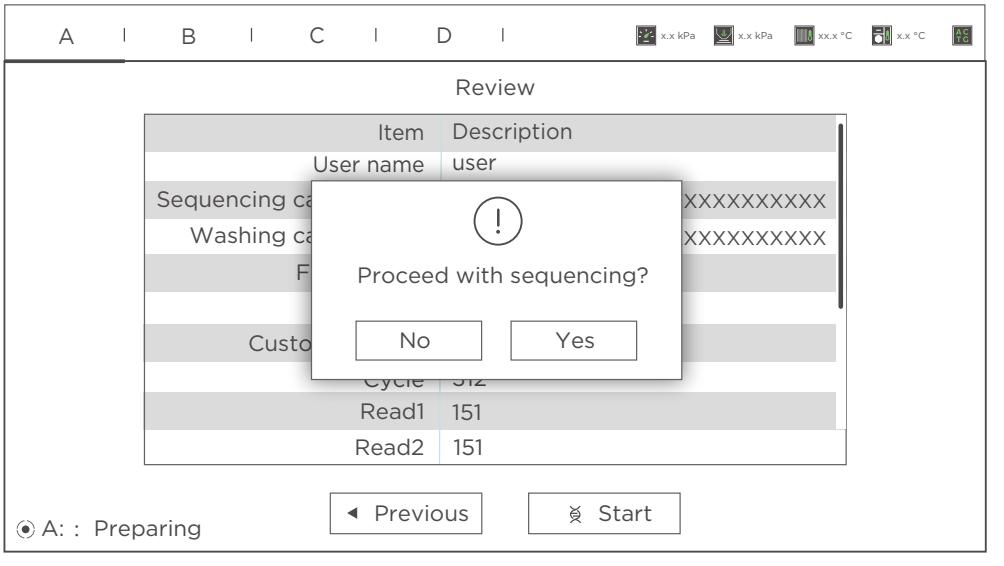

Figure 54 **Confirm sequencing interface**

When the following screen appears, the sequencing has begun.

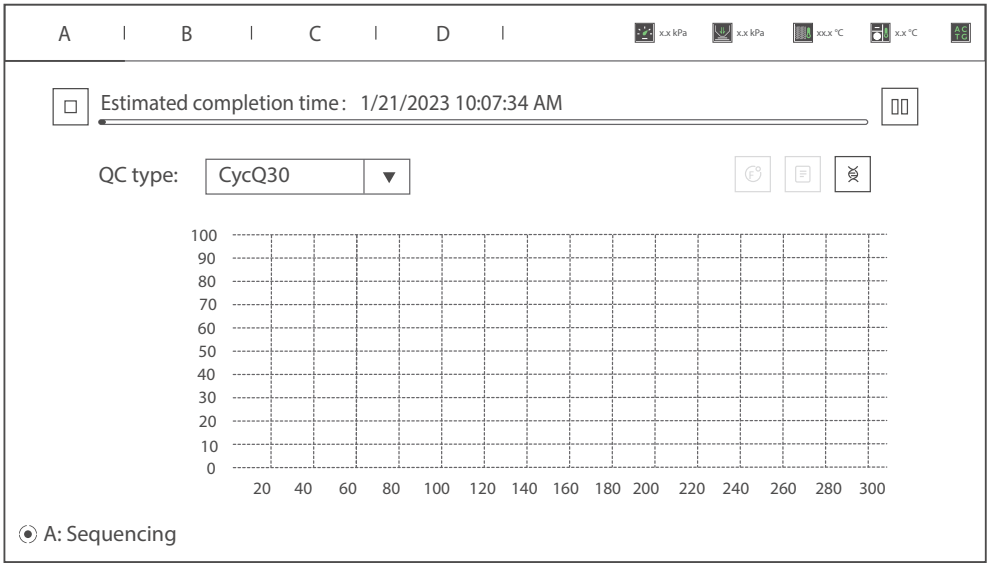

Figure 55 **Sequencing start interface**

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

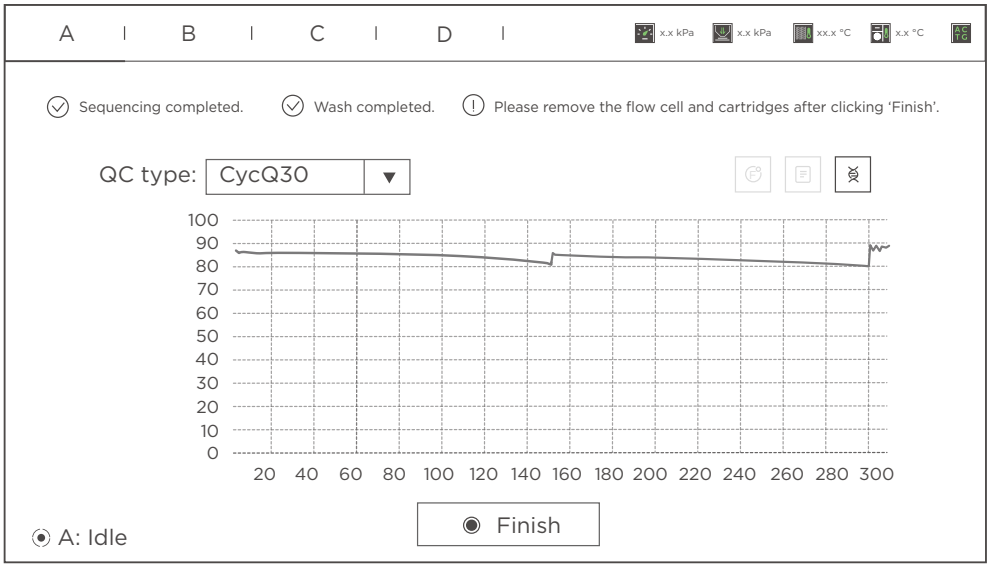

Figure 56 Sequencing and wash complete interface

CAUTION

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

Automatic post-wash

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

During troubleshooting, you can set **Auto wash** to **No** when necessary and perform a wash manually right after troubleshooting. For information on how to perform a wash manually, refer to *Wash procedures on Page 119*.

Disposing of cartridges and flow cells

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

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

> For information on reusing cartridges and flow cells, refer to *Reusing the washing flow cell, washing cartridge, and washing plate on Page 121*.

(Optional) Powering the devices off

CAUTION

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

Powering the sequencer off

Perform the following steps:

1. Select > **Shut down**.
2. Turn the power switch to the OFF position.

Powering the DNB loader off

Perform the following steps:

1. Select > **Shut down**.
2. Turn the power switch to the ◯ position.

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05

Sequencing data

This chapter describes the sequencing output data.

Sequencing output files

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

Folder structure

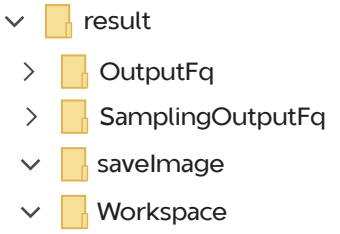

Figure 57 *result* folder of BCS or BIS

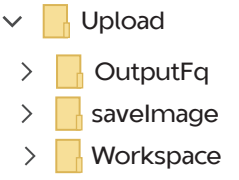

Figure 58 *Upload* folder of CG-ZTRON-LITE

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

When CG-ZTRON-LITE is connected:

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

No.	Description
result	Sequencing result folder for algorithm software
OutputFq	FASTQ and report folder. In this folder, a folder named with the flow cell ID is produced, and Bioinfo file is generated in it promptly after the sequencing run starts. Writing FASTQ is finished when the summary report is generated.

No.	Description
SamplingOutputFq	Summary report folder
saveimage	Images folder
Workspace	Intermediate files. Only cal and metrics files are saved by default.

File type description

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

Summary report

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

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

Report parameter review

The following table describes critical report parameters:

Parameter	Description
SoftwareVersion	Version of BasecallLite. Ensure that the BasecallLite is in the official release version.
TemplateVersion	Version of summary report template
Reference	The species category of the sample. When the species category is unknown or when the category is not Ecoli, the reference will be indicated as NULL.
CycleNumber	The total cycle of the sequencing run (not including the extra cycles, but including barcode regardless of whether the barcode is split or not)
ChipProductivity(%)	Flow cell productivity. The yield of the flow cell is estimated by the following formula: $$\textbf{ChipProductivity} = \frac{\textbf{ValidFovNumber} \times \textbf{ESR}}{\textbf{ImageArea}} \times \textbf{100\%}$$
ImageArea	The total number of FOVs in a lane. The system reads the total number of FOVs from the *QC.csv* file under the metrics directory generated by the Basecall software.
TotalReads(M)	Reads included in the FASTQ file (Reads after filtering)
Mappedreads(M)	Number of reads mapped to the reference genome. For PE sequencing, a mapped read implies that both Read1 and Read2 are mapped to the reference genome.
Q30(%)	The percentage of bases with a quality score ≥30. A base with a quality score of 30 implies that the chances that this base called incorrectly are 1 in 1000.
SplitRate(%)	The proportion of FASTQ data that can be split according to barcode sequences. This indicator is obtained from the *BarcodeStat.txt* file, and the split results are included in *Sequencestat.txt*. The split rate is counted from the filtered reads only.

Parameter	Description
Lag/Runon	• Lag1 (%) is the slope of the Lag curve for the first-strand sequencing. Lag2 (%) is the slope of the Lag curve for the second-strand sequencing. • Runon1 (%) is the slope of the runon curve for the first-strand sequencing. • Runon2 (%) is the slope of the runon curve for the second-strand sequencing.
ESR(%)	Effective spot rate. Percentage of effective spots after filtering in the flow cell.
MappingRate (%)	The ratio of mapped reads to total reads. The indicator is defined as the following: $$\textbf{MappingRate} = \frac{\textbf{MappedReads}}{\textbf{TotalReads}} \times \textbf{100\%}$$
AvgErrorRate(%)	After the mapping analysis of TotalReads, the error rate of the number of reads mapped to the reference genome. AvgErroRate(%) is defined as the following: $$\textbf{AvgErrorRate(\%)} = \frac{\textbf{TotalMismatchBaseNumber}}{\textbf{MappedReadsNumber} \times \textbf{ReadLength}} \times \textbf{100\%}$$
AvgErrorRate!N(%)	The average error rate after removing the mismatches caused by call N
RecoverValue(AVG)	The ratio of second-strand signal to first-strand signal. This indicator is only for PE sequencing.
R1 declining	The subtraction of the Q30 value of the penultimate cycle from the Q30 value of the fifth cycle in Read1, indicating the Q30 decline on Read1. This indicator is only for PE sequencing.
R2 declining	The subtraction of the Q30 value of the penultimate cycle from the Q30 value of the fifth cycle in Read2, indicating the Q30 decline on Read2. This indicator is only for PE sequencing.

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

Table 32 Parameter description for Tab2 of the summary report

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

Diagram description

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

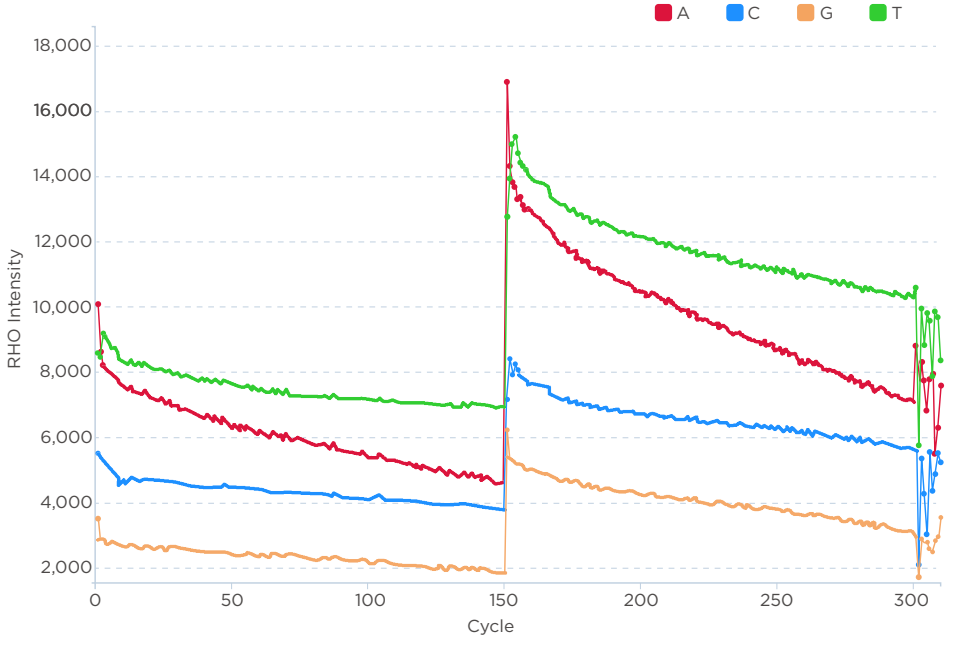

Figure 59 RHO Intensity

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.

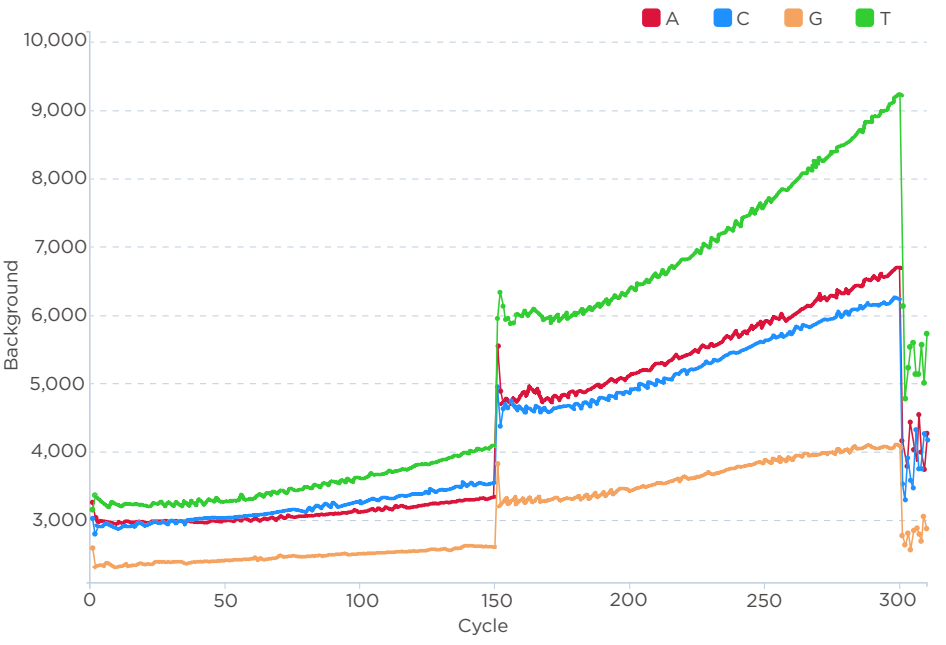

Figure 60 Background

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

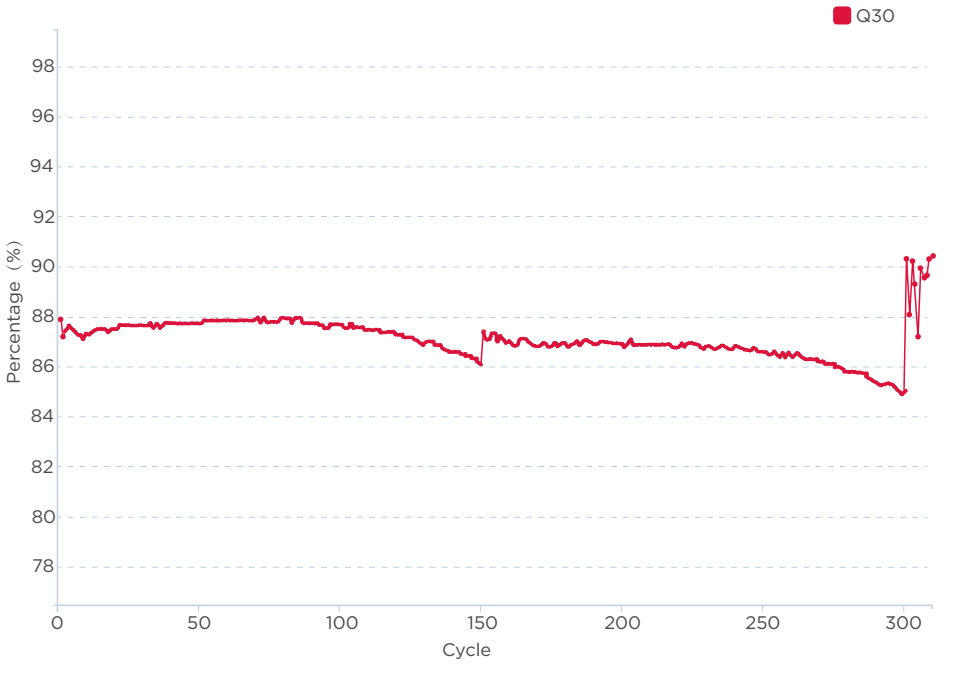

Figure 61 **Unfiltered Q30**

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

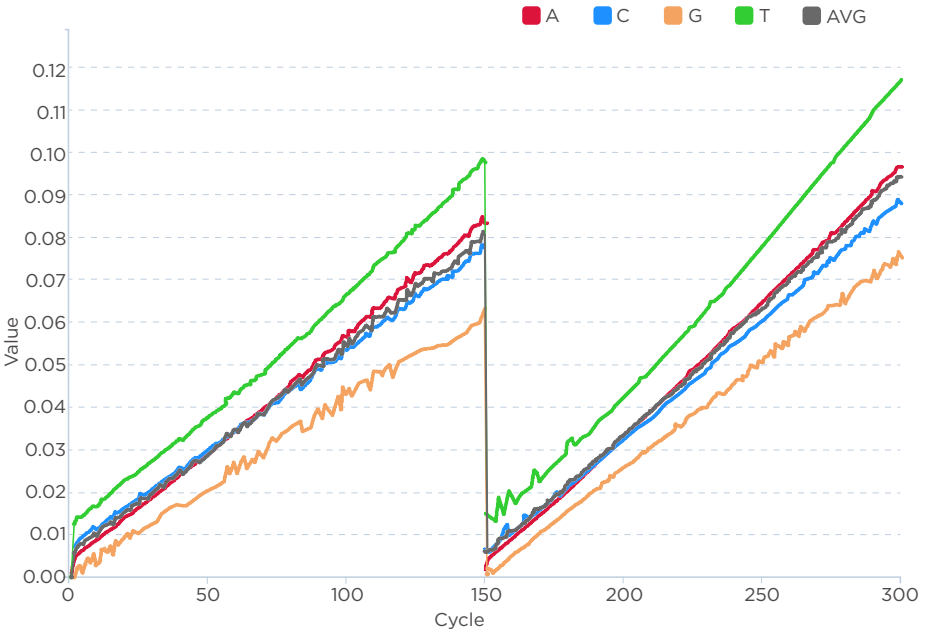

Figure 62 **Runon**

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

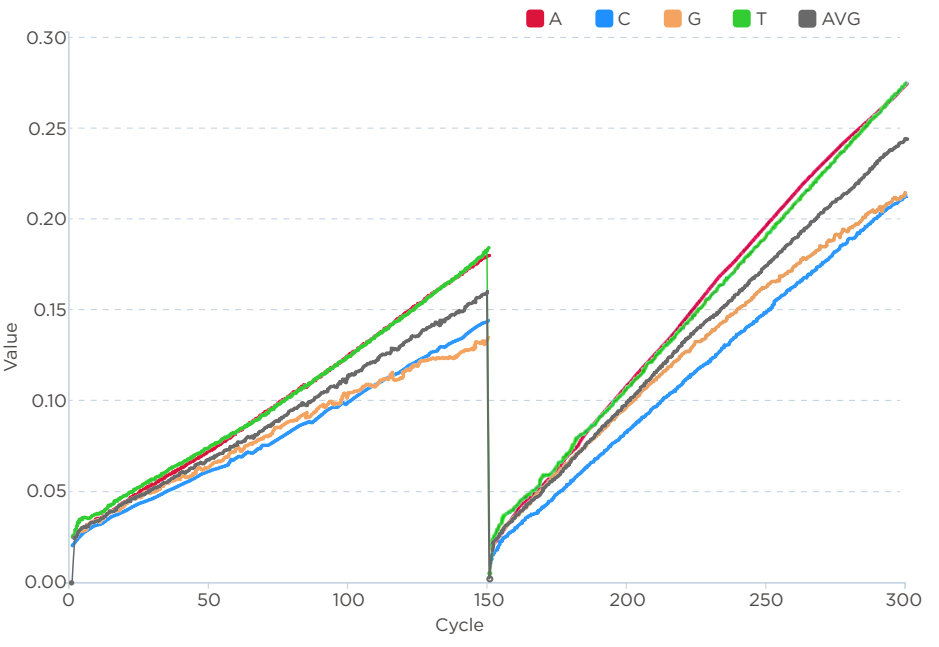

Figure 63 Lag

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

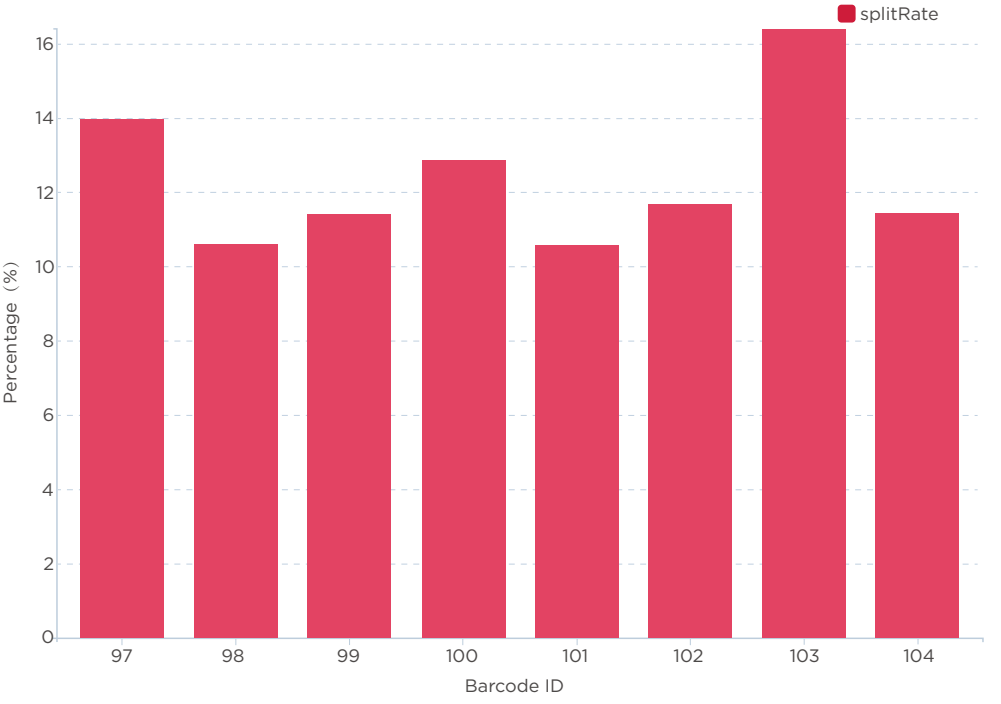

Figure 64 **Barcode Split Rate**

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

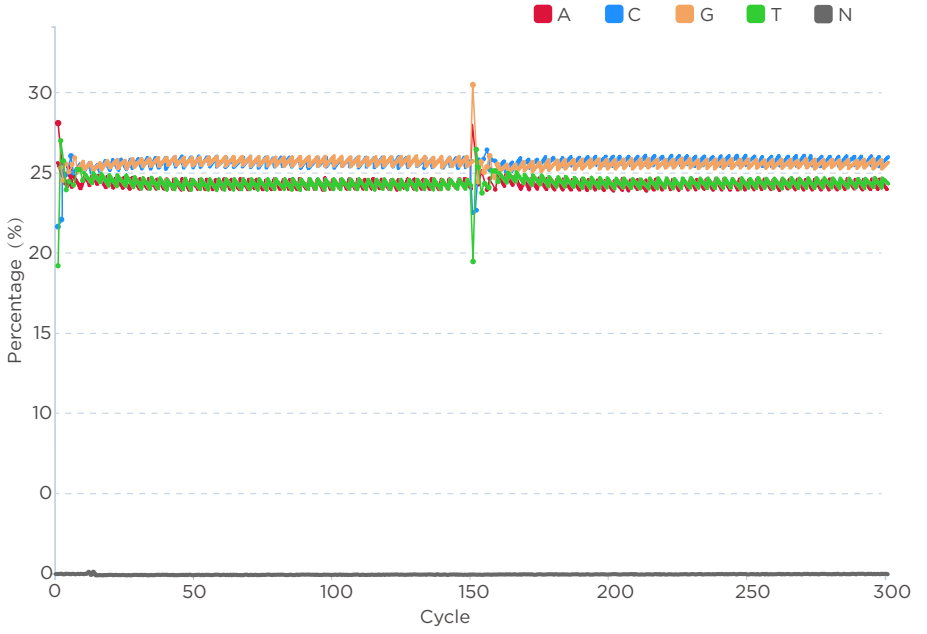

Figure 65 **Bases Distribution**

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

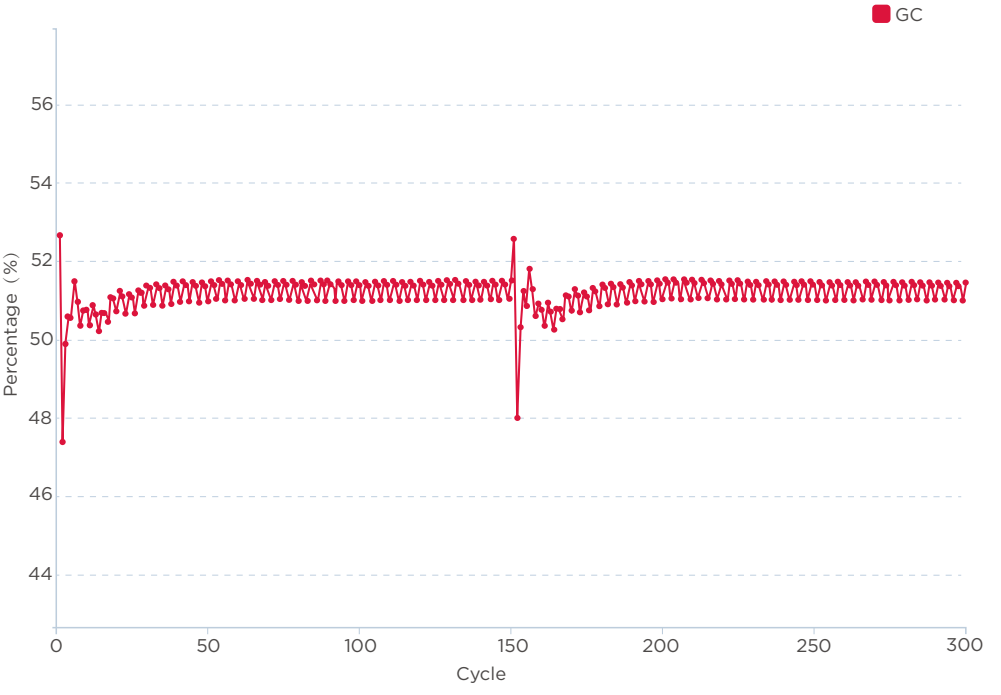

Figure 66 GC Distribution

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

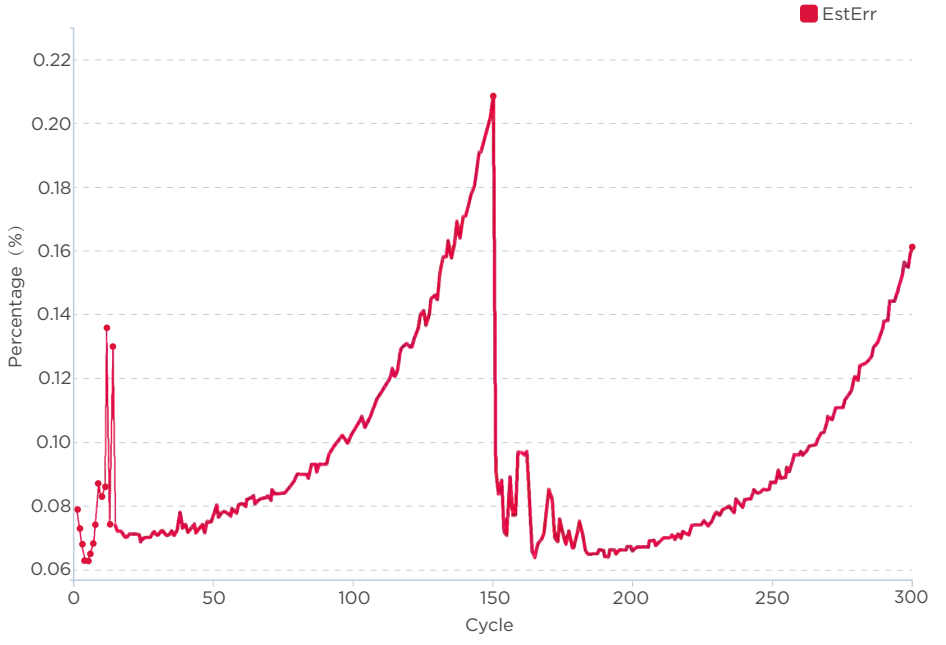

Figure 67 Estimated Error Rate

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

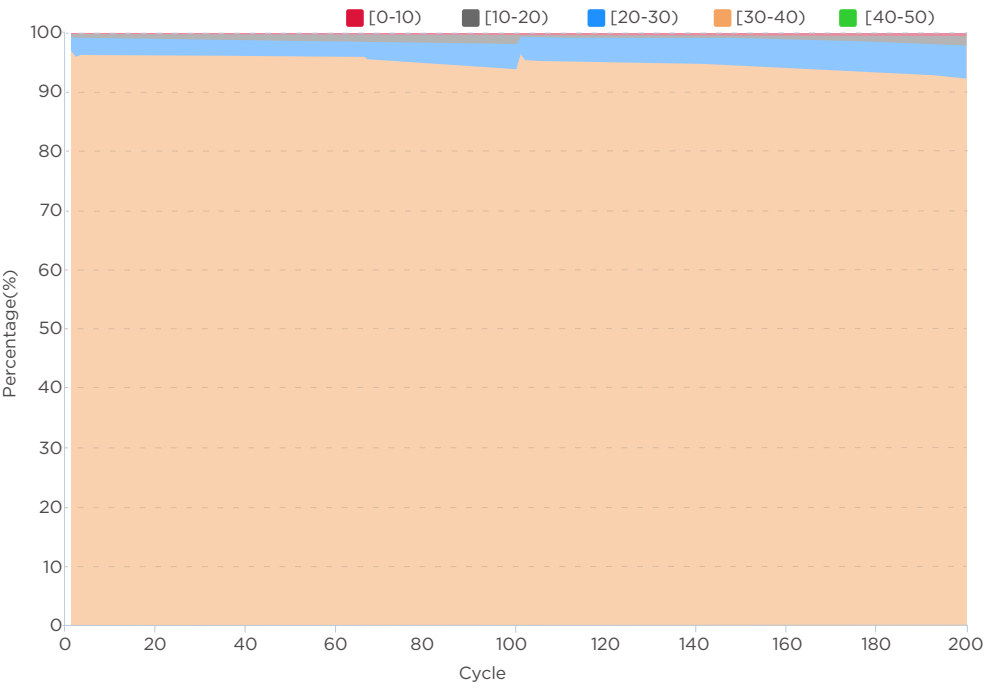

Figure 68 Quality Proportion Distribution

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

Other reports

Table 33 Other report description

Name	Description
XXXXXXXX_L01.heatmapReport.html	Contains information on each FOV in the lane generated during sequencing, including AvgQ30, offset_x, offset_y, lag1, lag2, runon1, and runon2. *XXXXXXXX* represents flow cell ID.
XXXXXXXX_L01.bestFovReport.html	The summary of the best FOV and basecall information during the entire sequencing run.
XXXXXXXX_L01.allCycleHeatmap.html	Information in each FOV of every cycle, including LoadedDNB, Offset, Signal, Background, RHO, SNR (Signal to Noise Ratio), Q30, BIC (Basecall Information Content), Fit, A-T, G-C, Lag, and Runon.

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06

Data Processing

This chapter describes data processing.

CAUTION

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

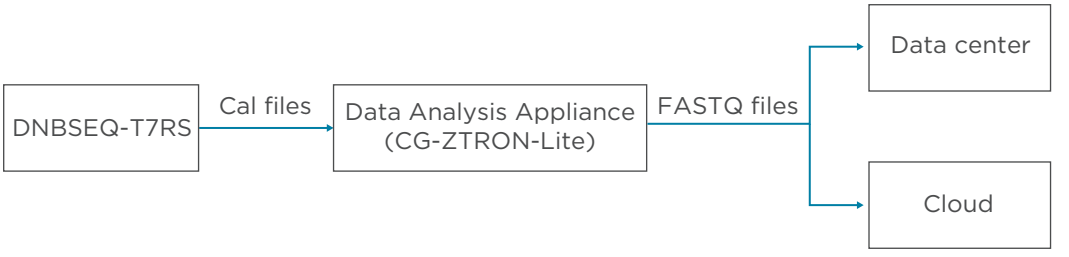

Figure 69 Data processing workflow

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

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

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

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

07

Device maintenance

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

DANGER

- Ensure that the device is powered off before cleaning or disinfecting to avoid personal injury.
- Do not spray the wash solutions or disinfectants into the device during cleaning or disinfecting to avoid device damage.

WARNING

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

Service plan

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

Wash

Wash introduction

Table 34 Wash type introduction

Equipment	Wash type	Cartridge type	Process time (minutes)	Description
Loader	Automatic wash	DNB Load Plate	15	When DNB loading is complete, the loader will automatically perform the wash without the need to change the DNB Load Plate.
	Manual wash	Washing plate	20	• The device is used for the first time. • The device has not been used for 7 days or longer. • Impurities are found in the device or flow cell. • Tubing, sampling needles or other accessories exposed to the reagents were replaced.

Equipment	Wash type	Cartridge type	Process time (minutes)	Description
Sequencer	Automatic wash	Sequencing Reagent Cartridge and Washing Cartridge	40	If **Auto wash** is enabled, the system will automatically perform a wash after each sequencing run.
	Manual wash	Sequencer Cleaning Cartridge and Cleaning Cartridge	40	• The device is used for the first time. • The device has not been used for 7 days or longer. • Impurities are found in the device or flow cell. • Tubing, sampling needles or other accessories exposed to the reagents were replaced.

Wash preparation

Preparing washing reagents

Prepare the washing reagents according to the table below.

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

Table 35 Washing reagent 2: 0.05% Tween-20+1 M NaCl

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

Table 36 Washing reagent 3: 0.1 M NaOH

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

Preparing the loader washing plate

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

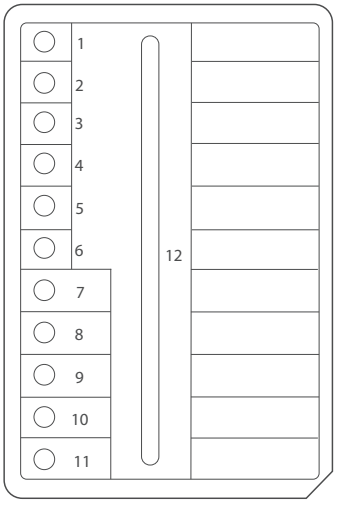

Figure 70 DNB Load Plate (no Reagent)

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

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

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

Preparing washing cartridges

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

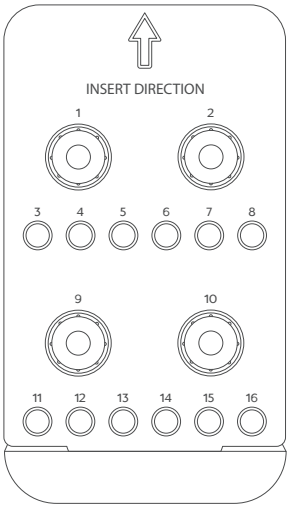

Figure 71 Sequencer Cleaning Cartridge

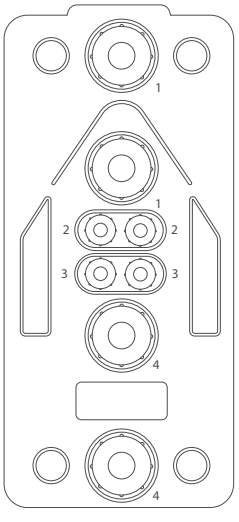

Figure 72 Cleaning Cartridge

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

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

Preparing the washing flow cell

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

Wash procedures

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

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

Perform the following steps:

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

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

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

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

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

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

Perform the following steps:

1. Ensure that the pure water container is filled with at least 4.5 L of laboratory-grade water before performing the wash.

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

2. Start the sequencer. Enter the user name and password, select **Log in** to enter the main interface
3. Select **Wash**. Touch the flow cell drive control button to install a washing flow cell. Touch the flow cell drive control button again to load the washing flow cell into the device.

4. Place the prepared Sequencer Cleaning Cartridge into the sequencing cartridge compartment on the flow cell stage that needs to be washed. Close the sequencing cartridge compartment door.
5. Place the prepared Cleaning Cartridge into the washing cartridge compartment on the flow cell stage that needs to be washed. Then close the washing cartridge compartment and reagent compartment doors.
6. Select **Start** and select **Yes** when prompted to begin the manual wash, which takes approximately 40 minutes.
7. When the wash is complete, select **Finish** to return to the main interface.
8. Remove the washing flow cell, Sequencer Cleaning Cartridge, and Cleaning Cartridge.

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

Washing flow cell

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

Washing cartridge

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

Washing plate

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