Front view

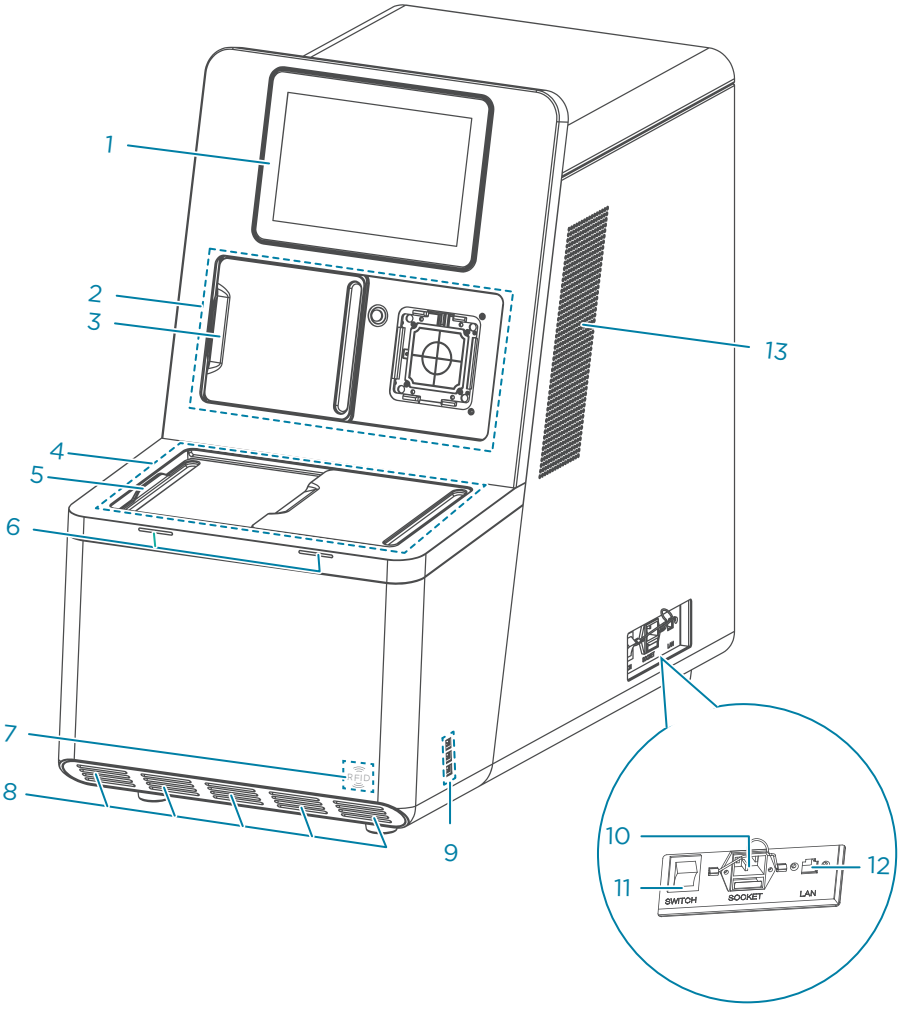

Figure 12 **Front view of the DNB loader**

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

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

Back view

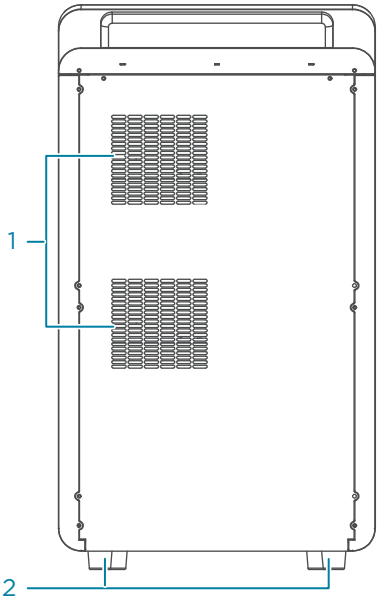

Figure 13 Back view of the DNB loader

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

Flow cell stage A

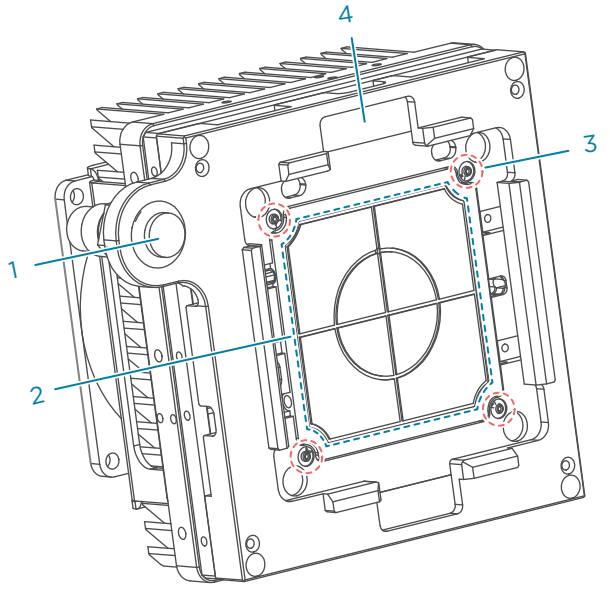

Figure 14 Flow cell stage A

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

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

Plate tray unit

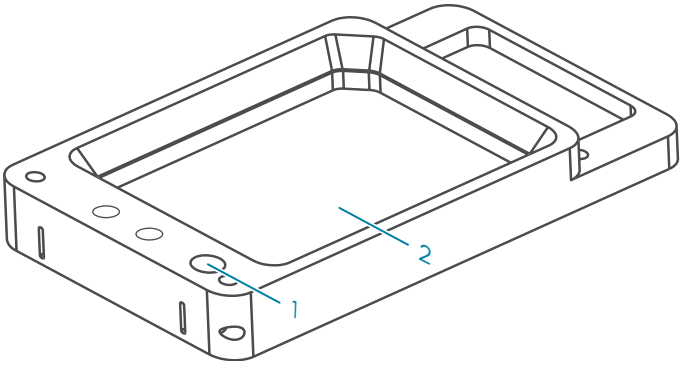

Figure 15 Plate tray unit

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

Control software

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

The following table describes the function of each functional module:

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

Self-test interface

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

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

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

2. Follow the on-screen instructions or the solutions that are mentioned in *FAQs on Page 127*.
3. Perform a self-test again:
   - Select > **Maintenance** > **Self-test**.
   - Select > **Restart**.

If the problems persists, contact CG Technical Support.

Main interface

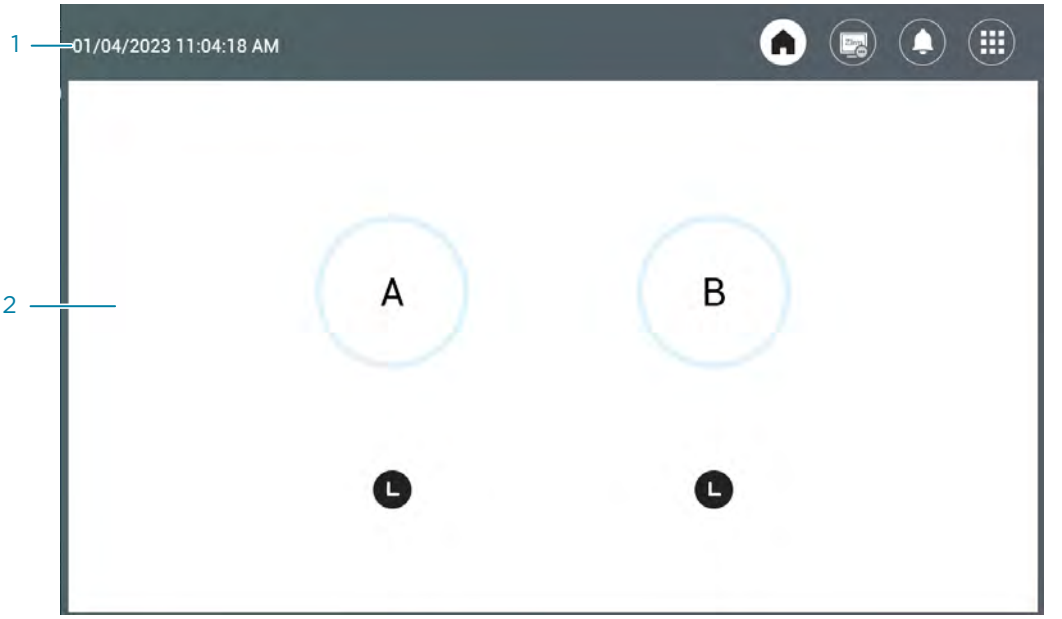

Figure 16 **Main interface**

The main interface includes the following areas.

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

Operation area

Item	Description
A	Flow cell stage name.

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

Main interface A

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

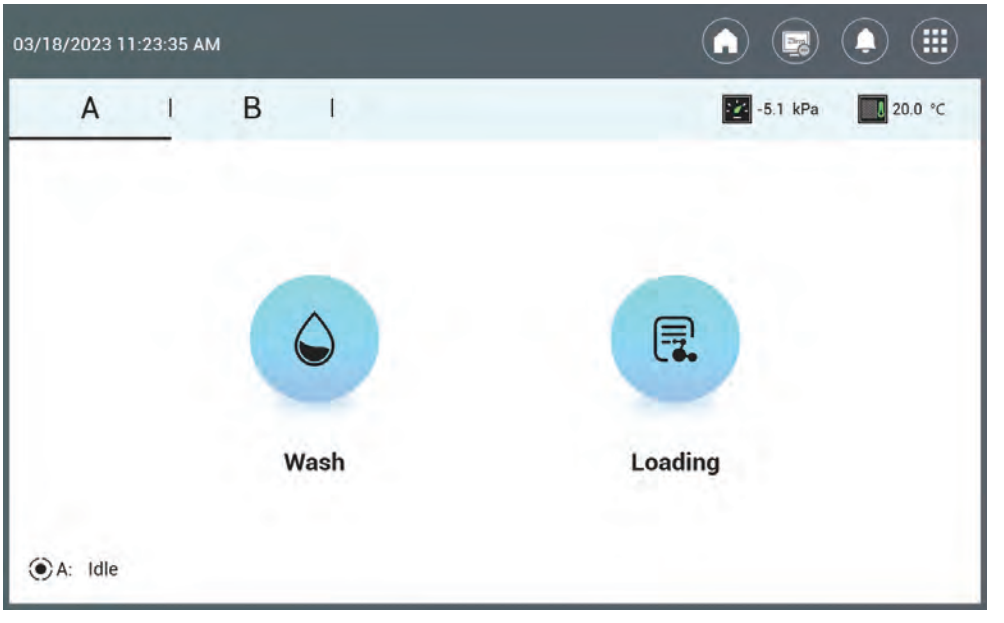

Figure 17 Main interface A

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

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

Item	Description
	Negative pressure is outside the normal range.

Icon and button area

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

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

Log interface

You can view log information in this interface.

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

The following table describes control functions in the log interface:

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

Settings interface

You can change system settings in this interface.

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

The following table describes control functions in the settings interface:

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

Maintenance interface

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

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

The following table describes the maintenance menu:

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

Shutdown or restart interface

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

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

About interface

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

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

Loading interface

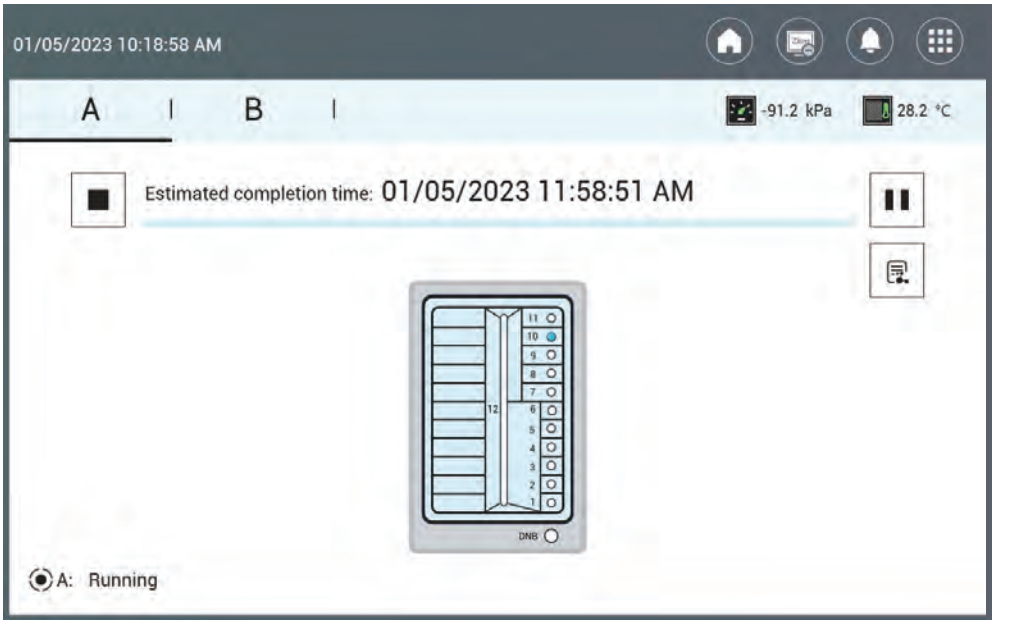

Figure 18 **DL-T7RS flow cell loading interface**

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

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

CG-ZTRON-LITE overview

Introduction

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

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

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

Figure 19 CG-ZTRON-LITE

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

Technical specifications

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

Bandwidth requirement

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

03

Sequencing sets overview

This chapter describes the sequencing sets information.

Introduction

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

Available sequencing set list

Table 1 Sequencing set

Catalog number	Model	Name	Version	Theoretical Data output (GB)
940-000838-00	FCL PE100	DNBSEQ-T7RS High-throughput Sequencing Set	V1.0	1160
940-000836-00	FCL PE150	DNBSEQ-T7RS High-throughput Sequencing Set	V1.0	1740
940-000840-00	stLFR FCL PE100	DNBSEQ-T7RS High-throughput Sequencing Set	V1.0	800

Sequencing read length

Sequencing read length determines the number of sequencing cycles for a given sequencing run. One sequencing cycle equates to one base pair of sequence data. For example, a PE100 cycle run performs reads of 100 cycles (2×100) for a total of 200 cycles or 200 bases sequenced. At the end of the sequencing run an extra 10 cycles or 20 cycles of barcode read can be performed to aid in identifying a specific library, if required. For stLFR (single-tube Long Fragment Read) libraries, at the end of the sequencing run, an extra 42 or 52 cycles of barcode read will be performed.

Table 2 Sequencing cycle

Sequencing read length	Read1 length	Read2 length	Barcode read length	DualBarcode read length	Maximum cycles
PE100	100	100	10	10	240
PE150	150	150	10	10	340
stLFR PE100	100	100	42	10	272

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

Sequencing time

Table 3 Theoretical sequencing time (hours)

Model	Single flow cell	Four flow cells	DNB preparation	DNB loading
FCL PE100	15.0 to 16.0	16.0 to 20.0	1	2
FCL PE150	21.0 to 23.0	23.0 to 28.0	1	2
stLFR FCL PE100	20.0 to 21.0	23.5 to 24.5	1	2

- Sequencing run time for a single flow cell and four flow cells only refer to the time elapsing from the “start” to the “finish” of the sequencing run. The time used for DNB preparation, DNB loading and Write FQ is not included. Write FQ for a single flow cell will take approximately 1.5 hours.
- Two flow cells can be loaded with DNBs concurrently by using one DNB loader. The total time is approximately two hours.
- The time in the table above is the average value. The actual run time may vary slightly among individual sequencers.
- Sequencing run time includes the time for the single barcode (10 cycles) sequencing, except for the stLFR PE100 where the time for 42+10 barcode cycles run is included.

User-supplied equipment and consumables

Before using the device, prepare the following equipment:

Table 4 User-supplied equipment list

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

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

Table 5 Recommended reagent/consumable list

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

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

WARNING Tips are disposable consumables, do not reuse them.

Recommended laboratory-grade water types include:

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

Preparing the pure water container

Perform the following steps:

1. Ensure that the pure water volume is sufficient.

   WARNING Insufficient pure water volume will result in sequencing failure.

   For information on pure water consumption, refer to *Filling the pure water container on Page 84*. For information on maintaining the pure water container, refer to *Maintaining the pure water container on Page 123*.

2. Connect the pure water container to the device.

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

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

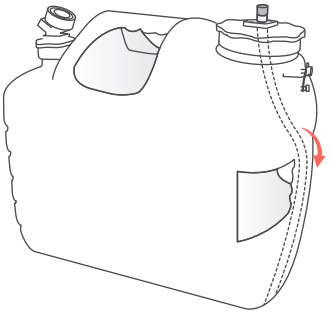

Figure 20 Inserting the pure water tube

2) Secure the fixing plate and lid.

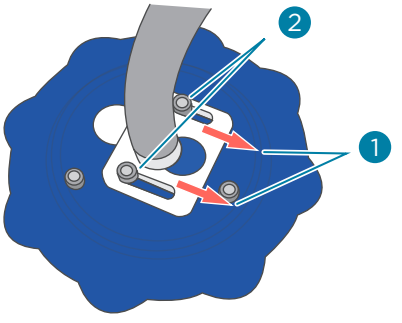

Figure 21 Securing the fixing plate and lid

3) Open the airway.

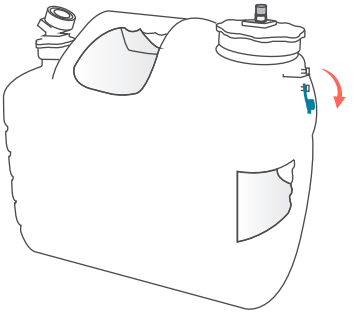

Figure 22 Opening the airway

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

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

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

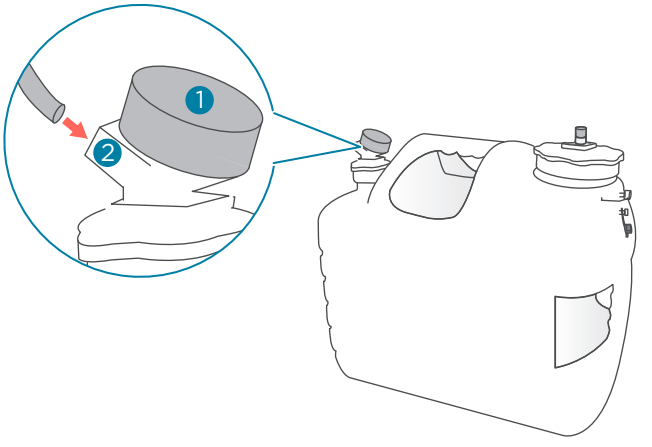

Figure 23 Adding pure water during sequencing

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

Preparing the waste container

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

For information on estimating the space and replacing the waste container, refer to *Replacing the waste container on Page 124*.

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04

Sequencing

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

Workflow

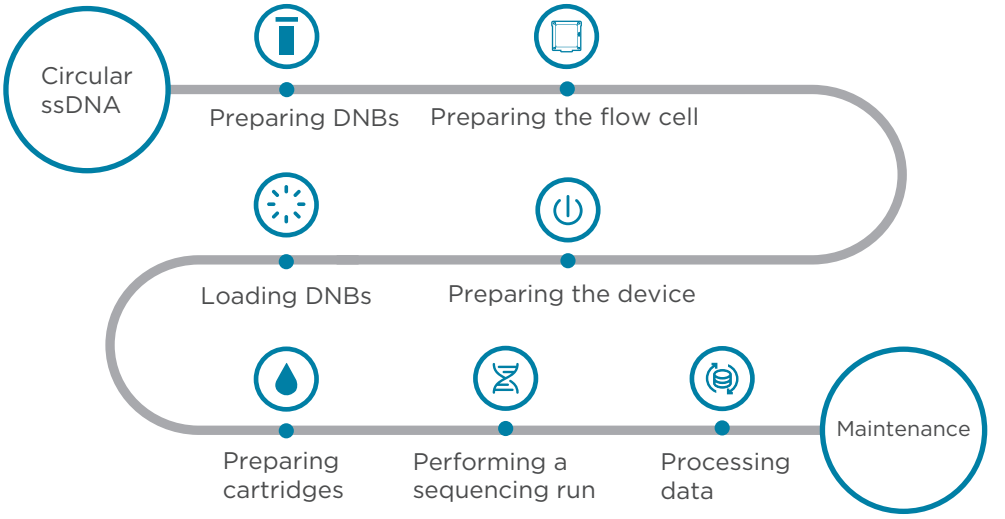

Figure 24 Sequencing workflow

- 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

The sequencing set is compatible with the libraries prepared by CG Library Prep Kits and the stLFR libraries prepared by CG stLFR Library Prep Kit. If third-party library preparation kits are used, please contact CG Technical Support for conversion options.

- For stLFR libraries, the recommended size distribution of inserts ranges between 200 bp and 1500 bp.

- For other libraries, the recommended size distribution of inserts ranges between 20 bp and 800 bp, with the main insert size fragment centered within ±100 bp.

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

Table 6 Recommended library insert size and applications

Model	Recommended library insert distribution (bp)	Applications
FCL PE100	200 to 400	WGS, WES, RNAseq, Single Cell
FCL PE150	300 to 500	WGS, WES, RNAseq
stLFR FCL PE100	200 to 1500	stLFR

DNA library concentration and amount requirement

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

  $$C\ (fmol/\mu L) = 3030 \times C\ (ng/\mu L) / N$$

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

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

Table 7 Circular ssDNA library concentration requirement

Library type	Library concentration
General libraries	≥3 fmol/μL
PCR free libraries	≥3.75 fmol/μL
stLFR libraries	≥1.9 ng/μL

Library pooling

Number of samples that can be pooled together

The sequencer can simultaneously perform sequencing of 4 flow cells. For PE100 sequencing, one flow cell can produce 1 TB of data in theory. The number of samples that can be pooled together for each flow cell depends on the required data output, read length, and specific application.

Do not pool more samples if their total data output is larger than 90% of the theoretical data output as described in *Table 8 on Page 53*. This is due to variation in pooling and the fact that not all barcodes will generate the same amount of the data output from the same amount of DNBs.

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

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

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

- Example 2: stLFR human sample

  When using the PE100 sequencing kit, if the required sequencing depth is 40×, then 6 samples are recommended to be pooled for each flow cell.

- Example 3: 50G are required for each sample

  When using the PE100 sequencing kit, if 50G are required for each sample, then 20 samples are recommended to be pooled for each flow cell.

- Example 4: Pooling samples with various applications

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

  Expected pooling variation are within ±10%.

Table 8 Examples of various sample pooling

Model	Minimum data for each sample (GB)	Pooling sample number	Theoretical data output range for each sample (GB)
FCL PE100	100	10	104 to 127
stLFR FCL PE100	120	6	120 to 146
FCL PE100	50	20	52 to 63
FCL PE150	50	23 RNAseq	51 to 62
	100	4 WGS	102 to 122

Verifying the base balance for barcode

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

Making DNBs

- Mixed use of reagent components from different batches is not recommended.
- Avoid making and loading DNBs with filtered pipette tips. It is highly recommended that pipettes of the suggested brands and catalog numbers be used. Using other brands may yield negative results.

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

- *Making DNBs for FCL PE100 on Page 54*
- *Making DNBs for FCL PE150 on Page 57*.
- *Making DNBs for stLFR FCL PE100 on Page 60*.

Making DNBs for FCL PE100

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. Take the Low TE Buffer, Make DNB Buffer and Stop DNB Reaction Buffer out of the DNBSEQ-T7 DNB Make Reagent Kit (FCL PE100) and thaw the reagents at room temperature.
3. Take out Make DNB Enzyme Mix I and thaw it on ice for approximately 0.5 hours.
4. Mix all the reagents by using a vortex mixer for five seconds. Centrifuge briefly and place on ice until use.

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

Calculating the required amount of ssDNA libraries

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

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

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

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

Table 9 Volume of ssDNA libraries for FCL PE100

Library type	Required ssDNA volume: V (µL)	
	100 µL DNB reaction	50 µL DNB reaction
General libraries	V = 60 fmol / C	V = 30 fmol / C
PCR free libraries	V = 75 fmol / C	V = 37.5 fmol / C

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

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

Calculate the required ssDNA libraries for each Make DNB reaction. The value of *V* obtained from the above equation will be used in *Table 10 on Page 55*.

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

  For example:

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

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

Making DNBs

Perform the following steps:

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

Table 10 Make DNB reaction mixture 1 for FCL PE100

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

2. Mix Make DNB reaction mixture 1 thoroughly by using a vortex mixer, centrifuge it for five seconds, and place it on ice until use.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown in the table below:

Table 11 Primer hybridization reaction conditions for FCL PE100

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

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

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

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

Table 12 Make DNB reaction mixture 2 for FCL PE100

Component	Volume of 100 µL DNB reaction (µL)	Volume of 50 µL DNB reaction (µL)
Make DNB Enzyme Mix I	40	20
Make DNB Enzyme Mix II (LC)	4	2

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

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

Table 13 RCR (Rolling Circle Replication) conditions for FCL PE100

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

9. Immediately add Stop DNB Reaction Buffer when the temperature reaches 4 °C. The volume of Stop DNB Reaction Buffer is shown in the table below. Mix gently by pipetting 8 times by using a wide-bore, non-filtered pipette tip.

Table 14 Volume of Stop DNB Reaction Buffer for FCL PE100

Component	Volume of 100 µL DNB reaction (µL)	Volume of 50 µL DNB reaction (µL)
Stop DNB Reaction Buffer	20	10

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

Making DNBs for FCL PE150

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. Take the Low TE Buffer, Make DNB Buffer and Stop DNB Reaction Buffer out of DNBSEQ-T7RS DNB Make Reagent Kit (FCL PE150) and thaw the reagents at room temperature.
3. Take out Make DNB Rapid Enzyme Mix II and thaw it on ice for approximately 0.5 hours.
4. Mix all the reagents by using a vortex mixer for five seconds. Centrifuge briefly and place on ice until use.

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

Calculating the required amount of ssDNA libraries

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

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

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

Table 15 Volume of ssDNA libraries for FCL PE150

Library type	Required ssDNA volume: V (µL)
General libraries	V = 60 fmol / C
PCR free libraries	V = 75 fmol / C

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

$$V = a / b \times 300 \ (\mu L)$$

Calculate the required ssDNA libraries for each Make DNB reaction. The value of *V* obtained from the above equation will be used in *Table 16 on Page 58*.

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

Making DNBs

Perform the following steps:

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

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

Table 16 Make DNB reaction mixture 1 for FCL PE150

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

2. Mix Make DNB reaction mixture 1 thoroughly by using a vortex mixer. Centrifuge it for five seconds and place it on ice until use.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown in the table below:

Table 17 Primer hybridization reaction conditions for FCL PE150

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

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

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

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

Table 18 Make DNB reaction mixture 2 for FCL PE150

Component	Volume of 90 µL DNB reaction (µL)
Make DNB Rapid Enzyme Mix II	40
Make DNB Enzyme Mix II (LC)	1.6

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

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

Table 19 RCR conditions for FCL PE150

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

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

   - Keep DNBs on ice during the entire operation to prevent DNBs from performing secondary replication.
   - It is very important to mix DNBs gently by using a wide-bore, non-filtered pipette tip. Do not centrifuge, vortex, or shake the tube.
   - This is not a STOP point, immediately go to the next step: *Quantifying DNBs and pooling on Page 63*.

Making DNBs for stLFR FCL PE100

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. Take Low TE Buffer, stLFR Make DNB Buffer and Stop DNB Reaction Buffer out of the DNBSEQ-T7 DNB Make Reagent Kit (stLFR FCL PE100) and thaw the reagents at room temperature.
3. Take out Make DNB Enzyme Mix III and thaw it on ice for approximately 0.5 hours.
4. Mix all the reagents by using a vortex mixer for five seconds. Centrifuge briefly and place on ice until use.

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

Calculating the required amount of dsDNA libraries

270 µL of DNBs is required to load one flow cell for the stLFR FCL PE100. One DNB making reaction can make 80 µL of DNBs. 30 ng dsDNA libraries are needed to make 80 µL of DNBs; Therefore, the volume of stLFR library needed for each 80 µL DNB preparation reaction is defined as follows:

$$V\ (\mu L) = 30\ ng / C$$

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

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

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

Calculate the required dsDNA libraries for each Make DNB reaction. The value of *V* obtained from the above equation will be used in *Table 20 on Page 61*.

The number of the 80 μL DNB making reactions is equal to (V/80)+1 rounded down to the nearest whole number.

Making DNBs

Perform the following steps:

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

Table 20 Make DNB reaction mixture 1 for stLFR FCL PE100

Component	Volume of 80 μL DNB reaction (μL)
Low TE Buffer	16 – V
stLFR Make DNB Buffer	16
dsDNA libraries	V
Total volume	32

2. Mix Make DNB reaction mixture 1 thoroughly by using a vortex mixer, centrifuge it for five seconds, and place it on ice until use.
3. Place the mixture into a thermal cycler, and start the primer hybridization reaction. Thermal cycler settings are shown in the table below.

Table 21 Primer hybridization reaction conditions for stLFR FCL PE100

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

4. Remove Make DNB Enzyme Mix IV from storage and place on ice. Centrifuge briefly for five seconds and hold on ice.

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

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

Table 22 Make DNB reaction mixture 2 for stLFR FCL PE100

Component	Volume of 80 µL DNB reaction (µL)
Make DNB Enzyme Mix III	32
Make DNB Enzyme Mix IV	3.2

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

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

Table 23 RCR conditions for stLFR FCL PE100

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

9. Immediately add 16 µL of Stop DNB Reaction Buffer to the tube when the temperature reaches 4 °C. Mix gently by pipetting 8 times by using a wide-bore, non-filtered 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 the DNBs at 2 °C to 8 °C and perform sequencing within 48 hours.

Quantifying DNBs and pooling

Quantifying DNBs

Perform the following steps:

1. When DNB making is completed, take out 2 μL of DNBs, and use Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify the DNBs. For details, refer to *Instructions for using Qubit to quantify the DNBs on Page 165.*

   - If the concentration of libraries prepared by customers is lower than that specified in the table below, refer to *Q: What should I do if DNB concentration is low? on Page 131* for details.
   - If there are too many samples in a single test, it is recommended to quantify in batches to avoid inaccurate DNB quantification due to fluorescence quenching.

Table 24 DNB concentration standard

Model	DNB concentration
FCL PE100	≥12 ng/μL
FCL PE150	≥5 ng/μL
stLFR FCL PE100	≥6 ng/μL

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

Table 25 DNB dilution buffer and storage conditions

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

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

DNB pooling

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

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

Calculating the relative amount for each sample

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

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

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

.....

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

Calculating the total relative amount (V) for all sample

V = A1 + B1 + ... + H1

Calculating the DNB volume needed for each sample

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

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

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

...

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

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

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

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

...

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

Preparing the flow cell

Perform the following steps:

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

Do not open the outer plastic package yet.

2. Place the flow cell at room temperature for 0.5 hours to 24 hours.
3. Unwrap the outer package before use.

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

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

Preparing the devices

Powering the device on

CAUTION

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

Powering the sequencer on

Perform the following steps:

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

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

Powering the DNB loader on

Perform the following steps:

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

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

Logging in to the control software

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

Perform the following steps:

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

Loading DNBs

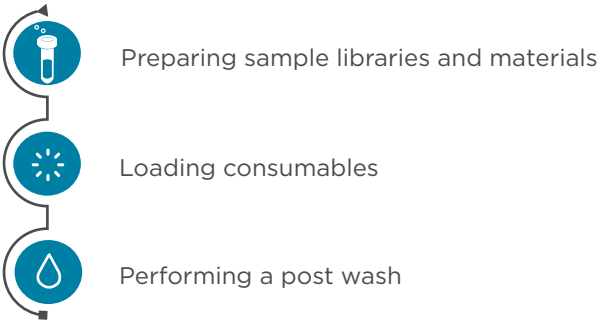

Figure 25 DNB loading workflow

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

- *Preparing DNB Load Plate and buffers for FCL PE100 or stLFR FCL PE100 sequencing on Page 67.*
- *Preparing DNB Load Plate and buffers for FCL PE150 sequencing on Page 68.*

Preparing DNB Load Plate and buffers for FCL PE100 or stLFR FCL PE100 sequencing

Preparing DNB Load Plate (T7 FCL PE100 or T7 stLFR FCL PE100)

Perform the following steps:

1. Take out DNB Load Plate. Perform the steps according to the appropriate model:
   - For FCL PE100:

     Take DNB Load Plate (T7 FCL PE100) out of the DNBSEQ-T7RS DNB Load Reagent Kit (FCL PE100).
   - For stLFR FCL PE100:

     Take DNB Load Plate (T7 stLFR FCL PE100) out of the DNBSEQ-T7RS DNB Load Reagent Kit (stLFR FCL PE100).
2. Thaw DNB Load Plate. Choose the method that best suits you:
   - Thaw it in a water bath at room temperature for 1.5 hours.
   - Thaw it in 2 °C to 8 °C refrigerator at least 12 hours in advance.
3. Once DNB Load Plate is thoroughly thawed, place it in a 2 °C to 8 °C refrigerator until use.
4. Gently invert DNB Load Plate to mix it five times and then centrifuge for one minute before use.

Preparing DNB Load Buffer II

Perform the following steps:

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

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

Preparing the 0.1 M NaOH reagent

Prepare 0.1 M NaOH according to the procedure described in *Preparing washing reagents on Page 117*. Each DNB Load Plate (T7 FCL PE100 or T7 stLFR FCL PE100) requires at least 4 mL of 0.1 M NaOH.

Preparing DNB loading mixture

Perform the following steps:

1. Take Micro Tube 0.5 mL (Empty) out of DNBSEQ-T7RS DNB Load Reagent Kit (FCL PE100 or stLFR FCL PE100) and add the following components in order.

   DNB in the table below refers to the pooled DNBs in *DNB pooling on Page 63.*

Table 26 DNB loading mixture for FCL PE100 and stLFR FCL PE100

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

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

   - Do not centrifuge, vortex, or shake the tube.
   - Prepare a fresh DNB loading mixture immediately before the sequencing run.

Preparing DNB Load Plate and buffers for FCL PE150 sequencing

Preparing DNB Load Plate (T7 FCL PE150)

Perform the following steps:

1. Take DNB Load Plate (T7 FCL PE150) out of the DNBSEQ-T7RS DNB Load Reagent Kit (FCL PE150).
2. Thaw DNB Load Plate (T7 FCL PE150) . Choose the method that best suits you:
   - Thaw it in a water bath at room temperature for 1.5 hours.
   - Thaw it in 2 °C to 8 °C refrigerator at least 12 hours in advance.
3. Once DNB Load Plate (T7 FCL PE150) is thoroughly thawed, place it in a 2 °C to 8 °C refrigerator until use.

4. Gently invert the DNB Load Plate (T7 FCL PE150) to mix it five times, and then centrifuge it for one minute before use.

Preparing DNB Load Buffer IV

Perform the following steps:

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

Preparing the 0.1 M NaOH reagent

Prepare 0.1 M NaOH according to the procedure described in *Preparing washing reagents on Page 117*. Each DNB Load Plate (T7 FCL PE150) requires at least 4 mL of 0.1 M NaOH.

Preparing DNB loading mixture

Perform the following steps:

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

   DNB in the table below refers to the pooled DNBs in *DNB pooling on Page 63*.

Table 27 DNB loading mixture for FCL PE150

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

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

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

Perform DNB Loading

Perform the following steps:

1. Ensure that the compartment doors of DL-T7RS are closed and start the device.

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

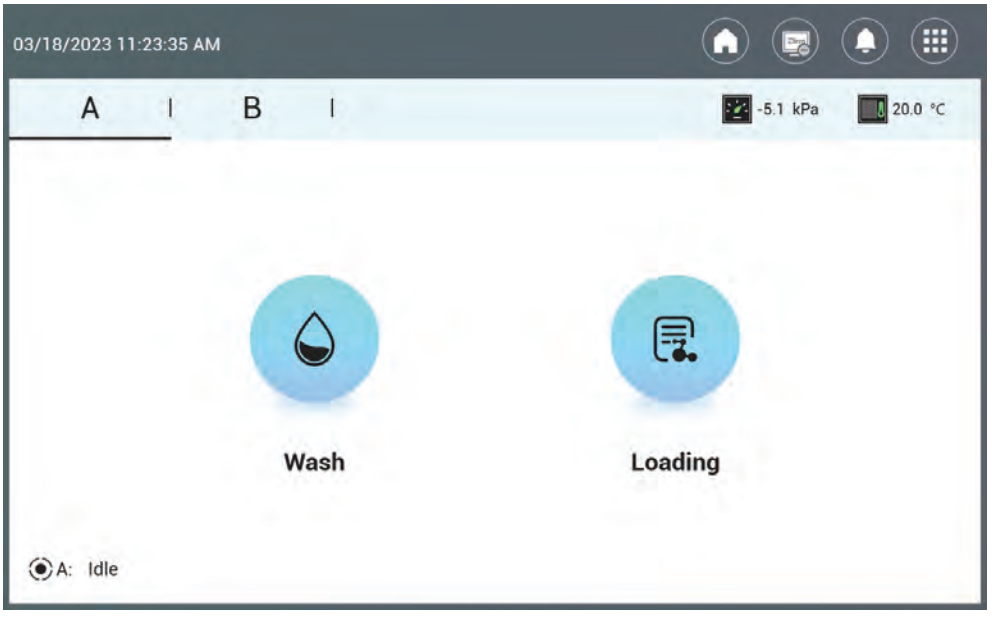

Figure 26 DL-T7RS selection interface

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

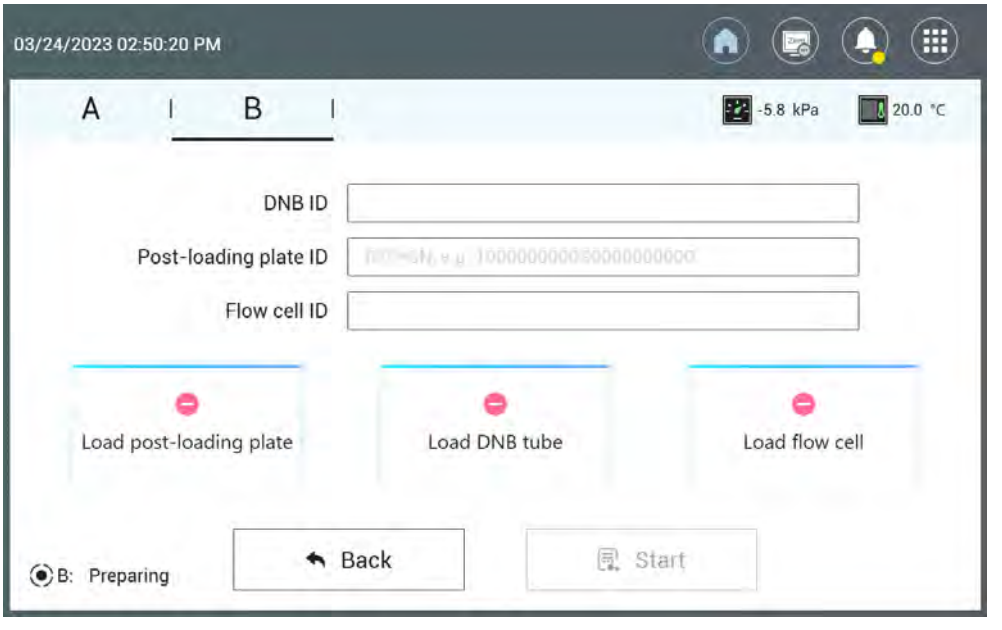

Figure 27 DL-T7RS information input interface

5. Open the loading compartment door.

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

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

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

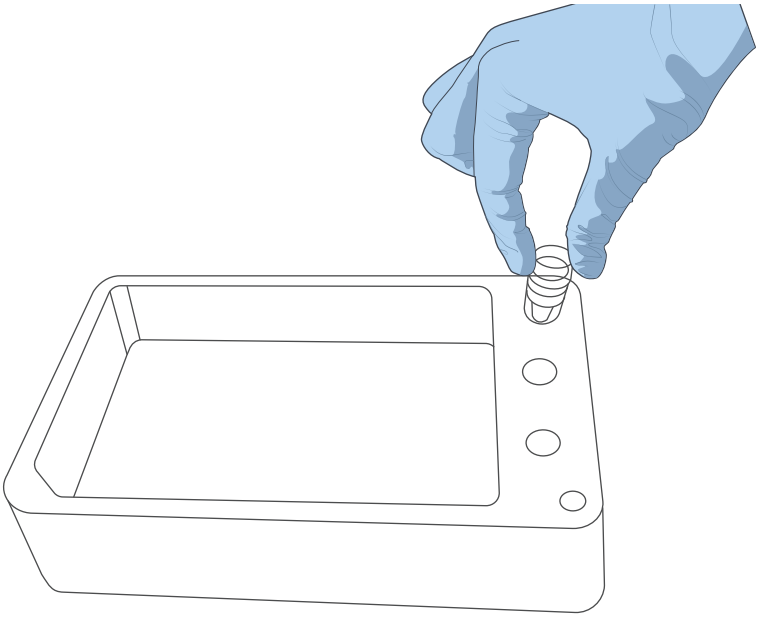

Figure 28 Placing DNB tube

8. Remove the seal of the DNB Load Plate and add 4 mL of 0.1 M NaOH into well No. 11.

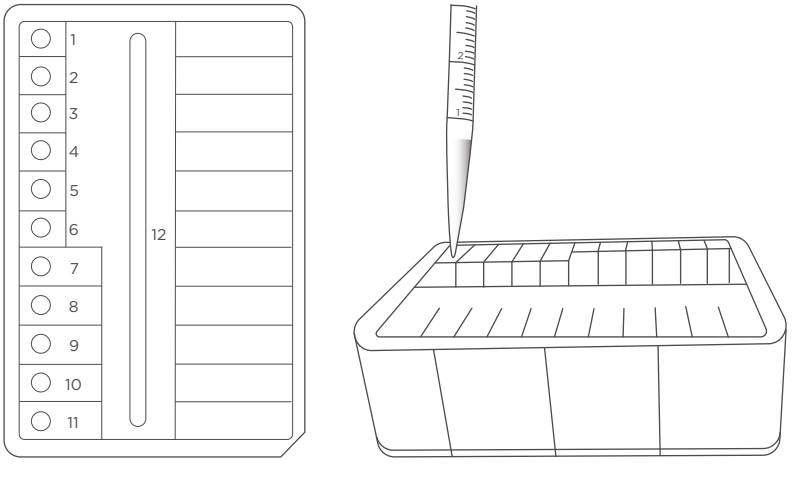

Figure 29 Adding 4 mL of 0.1 M NaOH into well No. 11