Complete GENOMICS™
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DNBSEQ-T7RS Stereo-seq Visualization Reagent Set

Instructions for Use

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

Complete Genomics, Inc.

Part No.: H-020-000928-00

About the Instructions for Use

CG intends to provide this product solely for research use.

This Instructions for Use is applicable to DNBSEQ-T7RS Stereo-seq Visualization Reagent Set. The Instructions for Use version is 3.0 and the set version is 1.0.

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Revision history

	Date	Version
Add FF V1.3 library sequencing	January 20, 2024	3.0
Update product name	June 11, 2024	2.0
Initial release	April 10, 2024	1.0

Contents

01

Visualization Set overview

This chapter describes the sequencing sets information.

Application

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

DNBSEQ-T7RS Stereo-seq Visualization Reagent Set is compatible with Stereo-seq FF V1.3 and Stereo-seq OMNI FFPE libraries constructed using the Stereo-seq 16 Barcode Library Preparation Kit (Cat. No.: 111KL160). This visualization set is intended to be used for scientific research only and cannot be used for clinical diagnosis.

Sequencing technology

This stereo-seq visualization set utilizes DNBSEQ technology. A sequencing run starts with the hybridization of a DNA anchor, then a fluorescent probe is attached to the DNA Nanoball (DNB) using combinatorial probe anchor sequencing (cPAS) chemistry. Finally, the high-resolution imaging system captures the fluorescent signal. After digital processing of the optical signal, the sequencer generates high-quality and accurate sequencing information.

Data analysis

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

Sequencing read length

Sequencing read length determines the number of sequencing cycles for a given sequencing run. For example, the PE25+62 cycle run for Stereo-seq OMNI FFPE libraries performs Read1 of 25 cycles and Read2 of 62 cycles (dark reaction cycle is from 7 to 9), for a total of 87 cycles. At the end of the insert sequencing run, an extra 10 cycles of barcode read can be performed, if required.

Table 1 Sequencing cycle

Library type	Read length	Read1 read length	Read2 read length	Barcode read length	Total read length	Maximum cycles
OMNI FFPE	PE25+62	25	62 (dark reaction cycle is from 7 to 9)	10	26+63+10	192
FF V1.3	PE50+100	50 (dark reaction cycle is from 26 to 40)	100	10	51+101+10	192

To ensure sequencing quality, when Read1 and Read2 sequencing is complete, the sequencer will automatically perform one more cycle for correction. For example, for PE25+62 Single Barcode sequencing, Read1 length is 25, Read2 length is 62, Barcode read length is 10, plus 1 correction cycle for Read1 and 1 correction cycle for Read2 (barcode does not require correction). The total number of sequencing cycles is 99.

Sequencing time

Table 2 Theoretical sequencing time (hr)

Library type	Read length	Single Flow Cell	Four Flow Cells	DNB preparation	DNB loading
OMNI FFPE	PE25+62	10.0 to 11.0	11.0 to 12.0	1	2.5
FF V1.3	PE50+100	16.0 to 17.0	17.0 to 18.0	1	2.5

- 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.
- The time in the table above is theoretical. The actual run time may vary among various sequencers.

Biological safety

- Reagents and waste chemicals may cause personal injury through skin, eye, or mucosal contact. Follow the safety standards of your laboratory and wear protective equipment (such as a laboratory coat, protective glasses, a mask, gloves, and shoe covers) when using the kit.
- If you accidentally splash reagents or waste liquids on your skin or into your eyes, immediately flush the affected area with large amounts of water and seek medical aid immediately.
- When disposing of expired reagents, waste liquids, waste samples, and consumables, comply with local regulations.
- Use and store the reagents according to the Instructions For Use. Failure to do so may negatively impact performance.
- Check the expiration date of all reagents before use. Using expired reagents may cause inaccurate results.

List of visualization set components

Table 3 DNBSEQ-T7RS Stereo-seq Visualization Reagent Set (T7 STO FCL PE75)
Cat. No.: 940-001889-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Expiration date
DNBSEQ-T7RS Sequencing Flow Cell (T7 STO FCL PE75) Cat. No.: 940-001902-00					
Sequencing Flow Cell (T7-2 FCL)	/	1 EA	2 °C to 8 °C	2 °C to 8 °C	10 months
DNBSEQ OneStep DNB Make Reagent Kit V4.0 (T7 STO) Cat. No.: 940-001891-00					
Low TE Buffer		480 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
STO Make DNB Buffer		200 µL/tube×1 tube			
Make DNB Enzyme Mix I (OS-V4.0)		400 µL/tube×1 tube			
Make DNB Enzyme Mix II (OS-V4.0)		20 µL/tube×1 tube			
Stop DNB Reaction Buffer		200 µL/tube×1 tube			
DNBSEQ-T7RS DNB Load Reagent Kit (T7 STO FCL PE75) Cat. No.: 940-001894-00					
DNB Load Buffer I		300 µL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
DNB Load Buffer II		150 µL/tube×1 tube			
Microcentrifuge Tube 0.5 mL (Empty)		1 tube			
DNB Load Plate (T7 STO FCL PE75)	/	1 EA			
DNBSEQ-T7RS Stereo-seq Visualization Reagent Kit (T7 STO FCL PE75) Cat. No.: 940-001893-00					
dNTPs Mix		2.29 mL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
dNTPs Mix II		2.73 mL/tube×1 tube			

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Expiration date
Sequencing Enzyme Mix II		3.43 mL/tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
MDA Reagent		4.20 mL/tube×1 tube			
MDA Enzyme Mix II		0.60 mL/tube×1 tube			
MDA Block Reagent		4.05 mL/tube×1 tube			
MDA Block Component		0.45 mL/tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (T7 STO FCL PE75) Cat. No.: 940-001903-00					
Washing Cartridge	/	1 EA	0 °C to 30 °C	below 40 °C	12 months

User-supplied equipment and consumables

Before using the kit, 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

Equipment	Recommended brand
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:

Tips are disposable consumables. Do not reuse them.

Table 5 Recommended reagent/consumable list

Reagent/Consumable	Recommended brand	Purpose
2 M NaOH	General lab supplier	Instrument washes
5 M NaCl	General lab supplier	Instrument washes
Tween-20	Sigma-Aldrich, Cat. No.: P7949	Performing a maintenance wash
Sterile pipette tip (various types)	General lab supplier	Pipetting and loading solutions
Sterile 200 µL wide-bore, non-filtered pipette tip	AXYGEN, Cat. No.: T-205-WB-C	Mixing DNBs
Qubit HS dsDNA Assay Kit	Thermo Fisher	Library QC
Qubit ssDNA Assay Kit	Thermo Fisher	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, Cat. No.: 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™ cloth	VWR	Cleaning
Low-lint cloth	General lab supplier	Cleaning
Laboratory-grade water	General lab supplier	/
Ziplock bag	General lab supplier	Storing loaded flow cell

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02

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

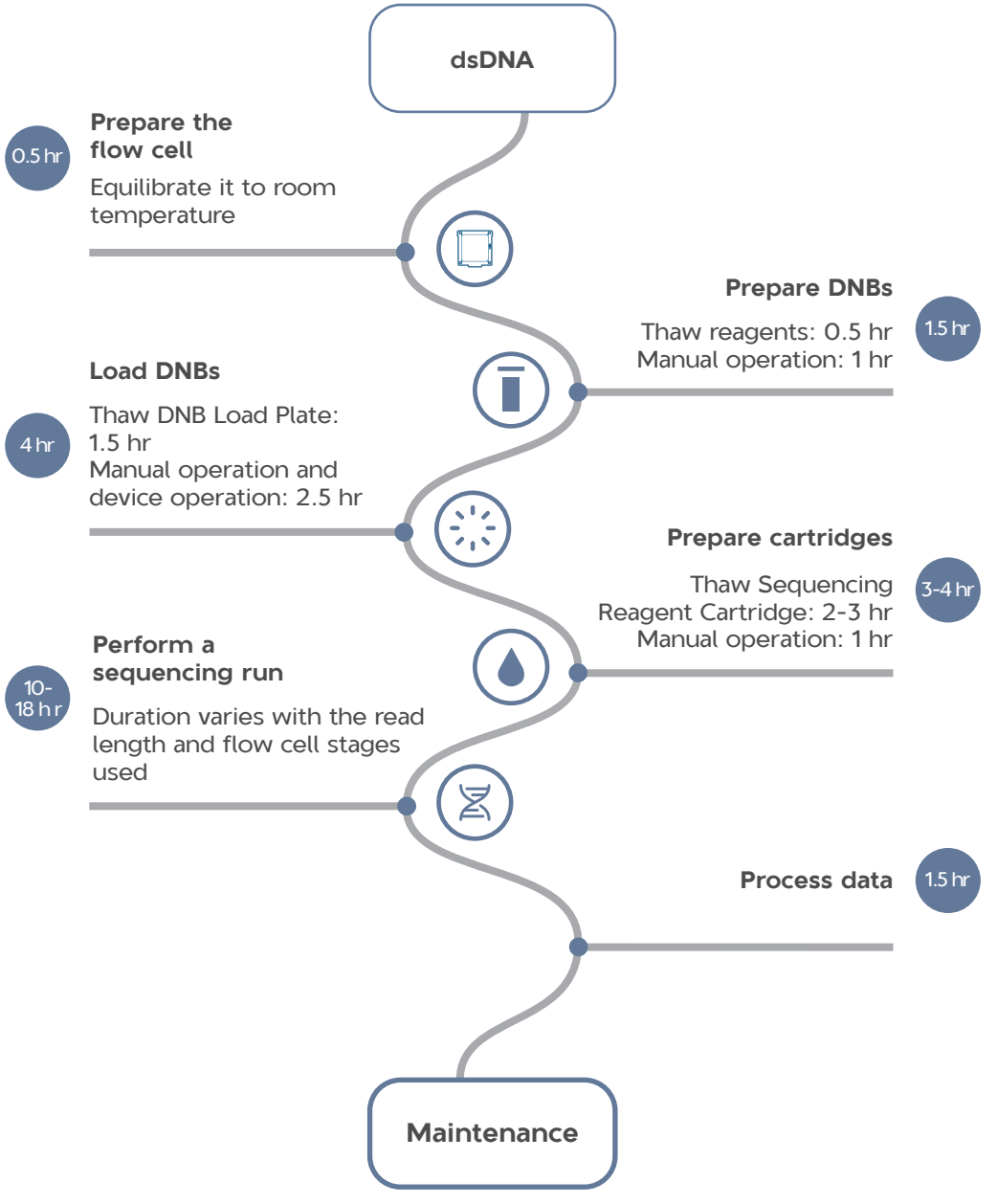

Figure 1 Sequencing workflow

- 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 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 visualization set is compatible with libraries constructed using the Stereo-seq OMNI Transcriptomics Set for FFPE or the Stereo-seq Transcriptomics Sets for FF V1.3. The recommended size distribution of OMNI FFPE inserts ranges between 150 bp and 1000 bp, with the main insert size fragment centered within ±100 bp. The recommended size distribution of FF V1.3 inserts ranges between 200 bp and 600 bp, with the main insert size fragment centered within ±100 bp.

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

Table 6 Recommended insert size and theoretical throughput for each flow cell

Library type	Reading length	Recommended library insert distribution (bp)	Data output (M/Flow Cell)
OMNI FFPE	PE25+62	150 to 1000	4000
FF V1.3	PE50+100	200 to 600	3500

DNA library concentration and amount requirement

The concentration of the OMNI FFPE dsDNA library should be no less than 3 ng/μL and the concentration of the FF V1.3 dsDNA library should be no less than 20 fmol/μL.

- If the library concentration is unknown, it is recommended that you perform dsDNA library quantitation (ng/µL) using Qubit HS dsDNA Assay Kit and the Qubit Fluorometer.

$$\text{C(fmol/µL)} = \frac{\text{3030 x C(ng/µL)}}{\text{N x 2}}$$

  N represents the average number of basepairs within the DNA fragments (the lengths of which includes associated adapter sequences). C (ng/µL) represents the DNA library concentration

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

Library pooling

Number of samples that can be pooled together

The sequencer can simultaneously perform sequencing of 4 flow cells. 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 6 on Page 11*. This is because variation in pooling and the fact that not all barcodes will generate the same amount of data output from the same amount of DNBs can cause insufficient data output for some samples.

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

Example: If the total data output is 3500 M, and 380 M is required for each sample, then a maximum of 8 samples is recommended to be pooled for each flow cell.

Expected pooling variations are within ±10%.

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 not be 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 for a more balanced base composition in the barcode.

- It is also important to note that two or more samples with an identical barcode should not be pooled together; otherwise, it is impossible to assign the read correctly.

Making DNBs

- Mixed use of reagent components from different batches is not recommended.
- For transferring or mixing DNBs, use wide-bore, non-filtered pipette tips.
- For preparing other reagents, use a proper pipette tip according to the step.

Calculating the required amount of dsDNA libraries

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

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

The required dsDNA library volume is shown in the table below.

- If there are any special requirements or specifications for the library preparation kit, then the requirements of the kits should be followed.
- *C1* represents the OMNI FFPE library concentration (ng/µL) in *DNA library concentration and amount requirement on Page 11*. *C2* represents the FF V1.3 library concentration (fmol/µL) in *DNA library concentration and amount requirement on Page 11 DNA library concentration and amount requirement on Page 11.*

Table 7 Volume of dsDNA libraries per 100 µL reaction

Library type	Required dsDNA volume: V (µL)
OMNI FFPE dsDNA	V = 60 ng / C1
FF V1.3 dsDNA	V = 400 fmol / C2

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 10 on Page 15*.

- 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.

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. Take the following reagents out of DNBSEQ OneStep DNB Make Reagent Kit and thaw them at room temperature.

Table 8 Reagent preparation 1

Component	Cap color
Low TE Buffer	
STO Make DNB Buffer	
Stop DNB Reaction Buffer	

3. Take the Make DNB Enzyme Mix I (OS-V4.0) out of DNBSEQ OneStep DNB Make Reagent Kit and thaw it on ice for approximately 30 min.

Table 9 Reagent preparation 2

Component	Cap color
Make DNB Enzyme Mix I (OS-V4.0)	

4. Mix the reagents using a vortex mixer for 5 sec. Centrifuge briefly and place on ice until use.

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

Making DNBs

Perform the following steps:

1. Take out a 0.2 mL PCR tube. Prepare Make DNB Reaction Mixture 1 according to the table below.

The following table illustrates the volume used for one DNB reaction. The required number of make DNB reactions is determined by the actual application as described in *Calculating the required amount of dsDNA libraries on Page 13.*

Table 10 Make DNB Reaction Mixture 1

Component	Cap color	Volume (µL)
Low TE Buffer		20 – V
STO Make DNB Buffer		20
dsDNA library	/	V
Total volume		40

2. Mix the reaction mixture thoroughly using a vortex mixer, centrifuge for 5 sec using a mini spinner, 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

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

4. Take Make DNB Enzyme Mix II (OS-V4.0) out of DNBSEQ OneStep DNB Make Reagent Kit and place it on ice. Centrifuge briefly for 5 sec, and place on ice until use.

   - Do not keep Make DNB Enzyme Mix II (OS-V4.0) 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. Centrifuge briefly for 5 sec and place the tube on ice.
6. Prepare Make DNB Reaction Mixture 2 on ice according to the table below. Mix Make DNB Reaction Mixture 2 using a vortex mixer for 5 sec and centrifuge briefly for 5 sec.

   Do not discard Make DNB Enzyme Mix II (OS-V4.0) after you finish this step; it will be used in DNB loading operations.

Table 12 Make DNB Reaction Mixture 2

Component	Cap color	Volume (µL)
Make DNB Enzyme Mix I (OS-V4.0)		40
Make DNB Enzyme Mix II (OS-V4.0)		2

7. Add all of Make DNB Reaction Mixture 2 into Make DNB Reaction Mixture 1. Mix the reaction mixture thoroughly using a vortex mixer, centrifuge for 5 sec using a mini spinner.
8. Place the tubes into the thermal cycler for the Rolling Circle Amplication (RCA) reaction. The conditions are shown in the table below.

- The reaction procedures for FF V1.3 library and FFPE library are different. Select the corresponding procedure according to actual needs.
- 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.

- RCA conditions for FF V1.3 library:

Table 13 RCA conditions for FF V1.3 library

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

- RCA conditions for OMNI FFPE library:

Table 14 RCA conditions for OMNI FFPE library

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

9. When the temperature reach 4 °C, immediately add 20 µL of Stop DNB Reaction Buffer into the PCR tube. Mix gently by pipetting 8 times using a wide-bore, non-filtered pipette tip.

   - It is very important to use a wide-bore, non-filtered pipette tip.
   - Do not centrifuge, vortex, or shake the tube.
   - Store the DNBs at 4 °C and perform sequencing within 48 hr (about 2 days).

Table 15 Volume of Stop DNB Reaction Buffer

Component	Cap color	DNB reaction (µL)
Stop DNB Reaction Buffer		20

Quantifying DNBs and pooling

Quantifying DNBs

When DNB production is complete, take 2 µL of DNBs, and use the Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify the DNBs. The DNB concentration should be no less than 8 ng/µL. For details, refer to *Instructions for using Qubit to quantify the DNBs on Page 63.*

- If there are more than 8 samples to quantify, it is recommended that you quantify in batches to avoid inaccurate DNB quantification as a result of fluorescence quenching.
- If the concentration of libraries prepared is lower than the specified minimum DNB concentration, refer to *Q: What should I do if the DNB concentration is low? on Page 56.*
- To ensure sequencing quality, it is recommended that you pool and load DNBs as soon as possible. If sequencing for 4 flow cells is performed simultaneously, you can make the DNBs together.

DNB pooling

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

The 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 stereo-seq visualization reagent kit.

Calculating the relative amount for each sample

Assuming that 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 samples

V = A1 + B1 + ... + H1

Calculating the DNB volume needed for each sample

For each FCL flow cell 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

Preparing flow cell

Perform the following steps:

1. Take the flow cell out of the DNBSEQ-T7RS Sequencing Flow Cell box.

   Do not open the outer plastic package at this time.

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

   - If the flow cell is not used within 24 hr after being placed at room temperature and the outer plastic package is intact, the flow cell can be returned to 2 °C to 8 °C for storage. But the switch between room temperature and 2 °C to 8 °C must not exceed 3 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 hr. If 24 hr is exceeded, it is not recommended that you use this flow cell.

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

Loading DNBs

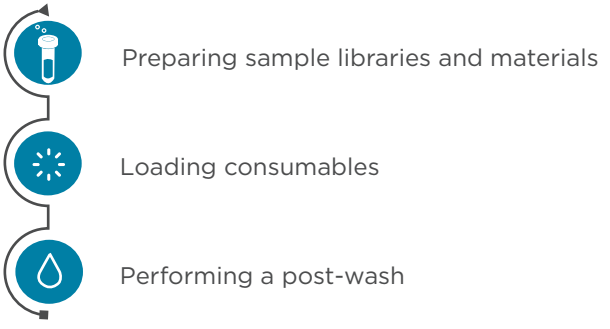

Figure 2 DNB loading workflow

Preparing DNB Load Plate

Perform the following steps:

1. Take out DNB Load Plate.
2. Thaw DNB Load Plate.
   - Thaw it in a water bath at room temperature for 1.5 hr.
   - Thaw it in a 2 °C to 8 °C refrigerator at least 12 hr in advance.
3. When 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 5 times and then centrifuge for 1 min before use.

Preparing DNB Load Buffer II

Perform the following steps:

1. Take DNB Load Buffer II out of DNBSEQ-T7RS DNB Load Reagent Kit.
2. Thaw the reagent in a water bath at room temperature for approximately 30 min.
3. Mix the reagent using a vortex mixer for 5 sec. Centrifuge briefly and place on ice until use.

If crystallized precipitation is visible in DNB Load Buffer II, vigorously mix the reagent for 1 to 2 min 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 a Wash on Page 49*. Each DNB Load Plate requires at least 4 mL of 0.1 M NaOH.

Preparing DNB loading mixture

Perform the following steps:

1. Take microcentrifuge tube 0.5 mL (empty) out of DNBSEQ-T7RS DNB Load Reagent Kit and add the following components in order.

   - DNB in the table below refers to the pooled DNBs in *DNB pooling on Page 17*.
   - Prepare a fresh DNB loading mixture within 10 min before DNB loading.

Table 16 DNB loading mixture

No.	Component	Cap color	Volume (µL)
1	DNB	/	270
2	DNB Load Buffer II		90
3	Make DNB Enzyme Mix II (OS-V4.0)		1

Performing 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 **Login** to go to the main interface.

3. Select **A** or **B** to continue. See the figure below.

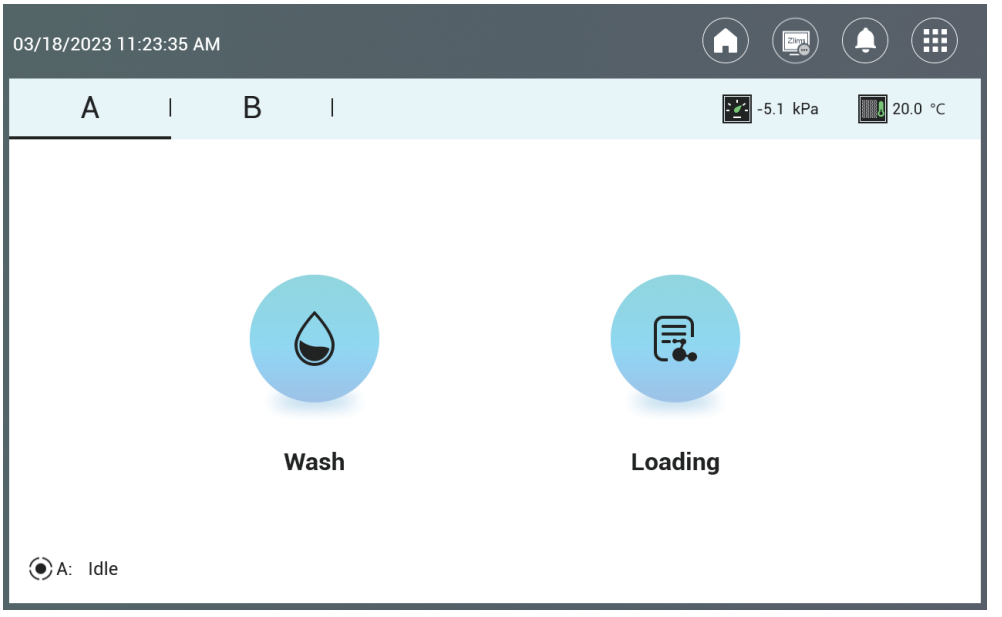

Figure 3 DL-T7RS selection interface

4. Select **Loading** to go to the information input interface. See the figure below.

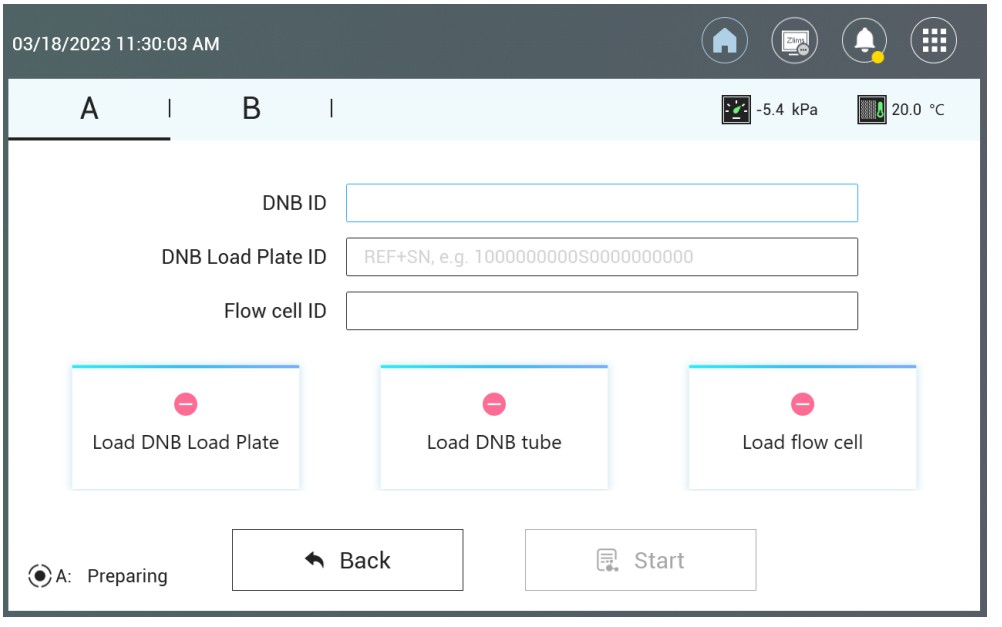

Figure 4 DL-T7RS information input interface

5. Open the loading compartment door.

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

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

7. Place the microcentrifuge tube 0.5 mL containing DNB loading mixture into the DNB tube hole, and a screen message will confirm that the DNB tube is loaded.

   Be sure to remove the tube lid to avoid damage to the DL-T7RS.

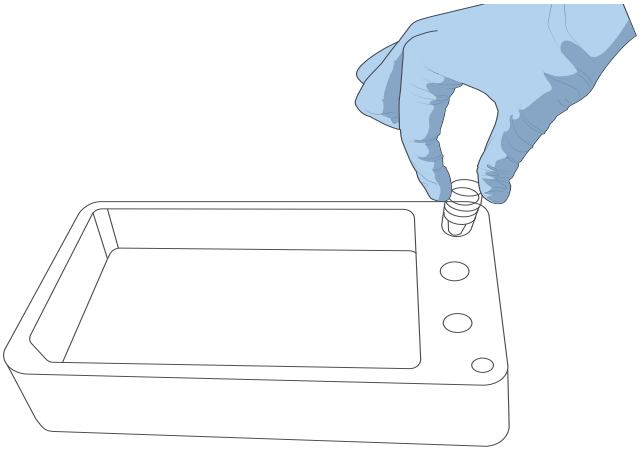

Figure 5 Placing DNB tube

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

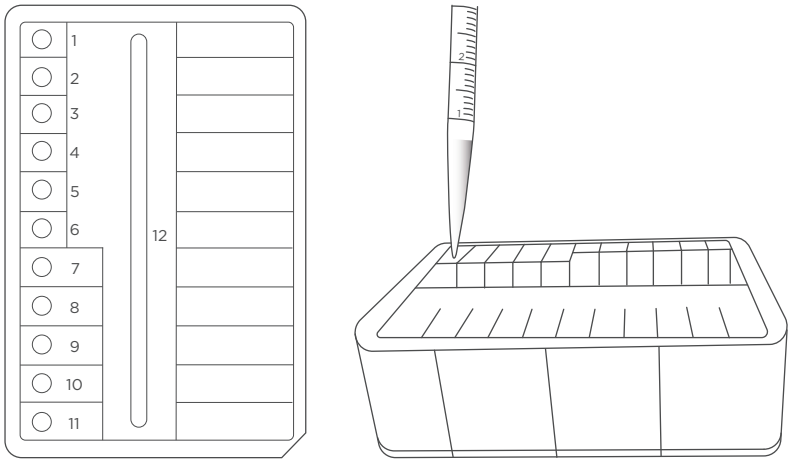

Figure 6 Adding 4 mL of 0.1 M NaOH into well No. 11

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

Figure 7 RFID scanning area of DNB Load Plate

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

Figure 8 Placing DNB Load Plate

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

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

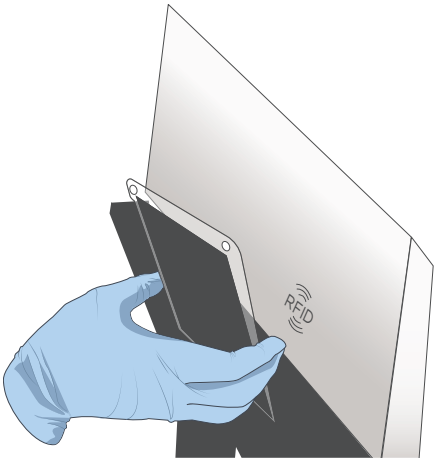

Figure 9 Scanning the Flow cell ID

12. Orient the flow cell upwards by holding the sides of the flow cell. Align the locating protrusion on the flow cell to the locating groove on the flow cell stage. Gently press the edges of the flow cell down. See the figure below.

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

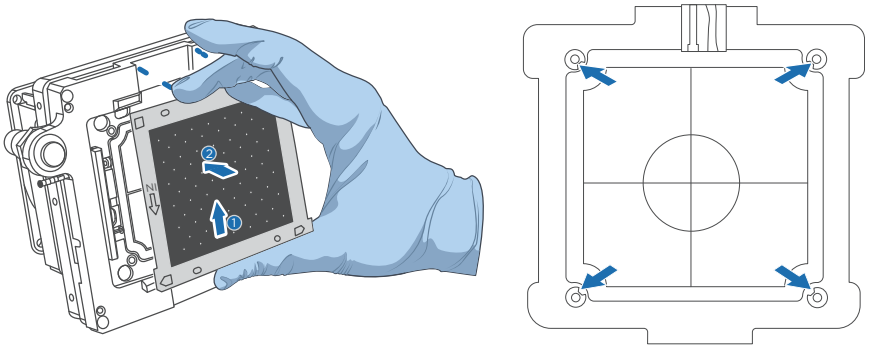

Figure 10 Flow cell locating

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 show that the flow cell is loaded.

- Remove any dust on both sides of the flow cell with a canned air duster.
- To prevent damage to the flow cell, do not press or touch the glass cover of 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 the 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. Remove any dust from the flow cell with a canned air duster.

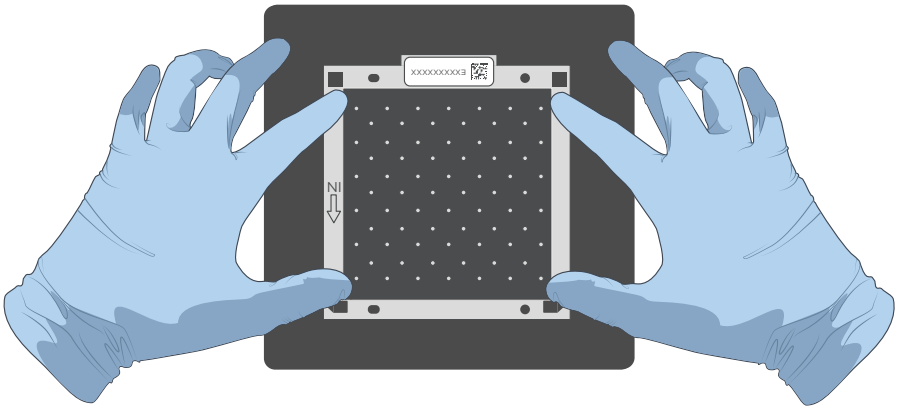

Figure 11 **Flow cell loaded**

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 figure below.

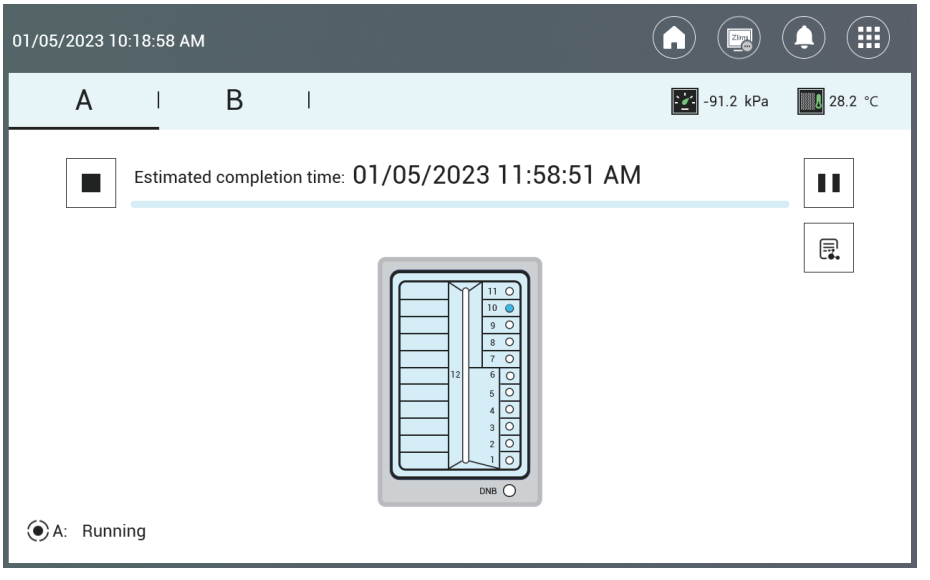

Figure 12 **DL-T7RS flow cell loading interface**

16. The process takes approximately 2 hr. When flow cell loading is complete, the screen appears as shown in Figure 13.

    - Do not open the loading compartment door during loading. Doing so will stop the loading process.
    - Do not bump or move the device during loading. Doing so may cause inaccurate results.
    - Do not place other instruments such as a centrifuge or vortex on the same bench that the loader is on. 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 prompts to troubleshoot and fix the problem. For information about the troubleshooting, refer to *FAQs on Page 55*. If the problem persists, contact CG Technical Support.

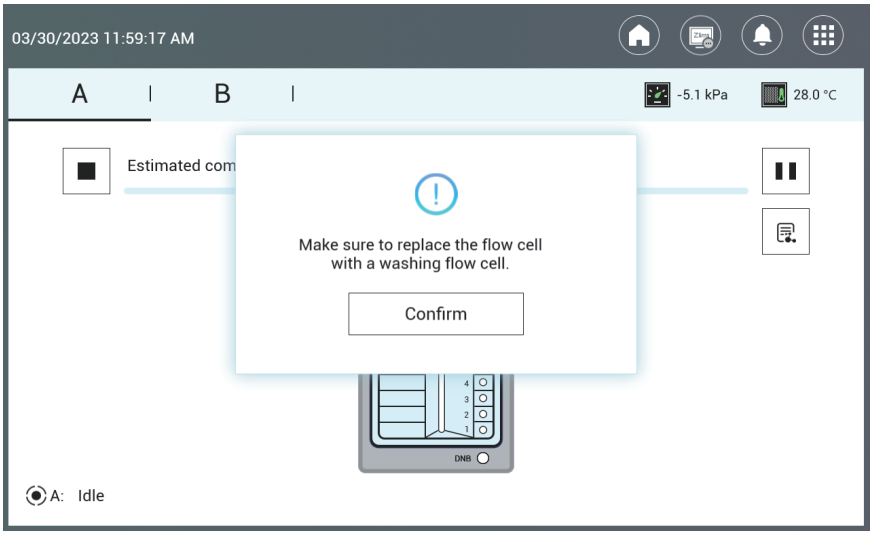

Figure 13 DL-T7RS flow cell loading complete status interface

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 Ziplock bag and store it at 2 °C to 8 °C until use.
    - The maximum storage time for a loaded flow cell is 48 hr.

18. When 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 13.

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

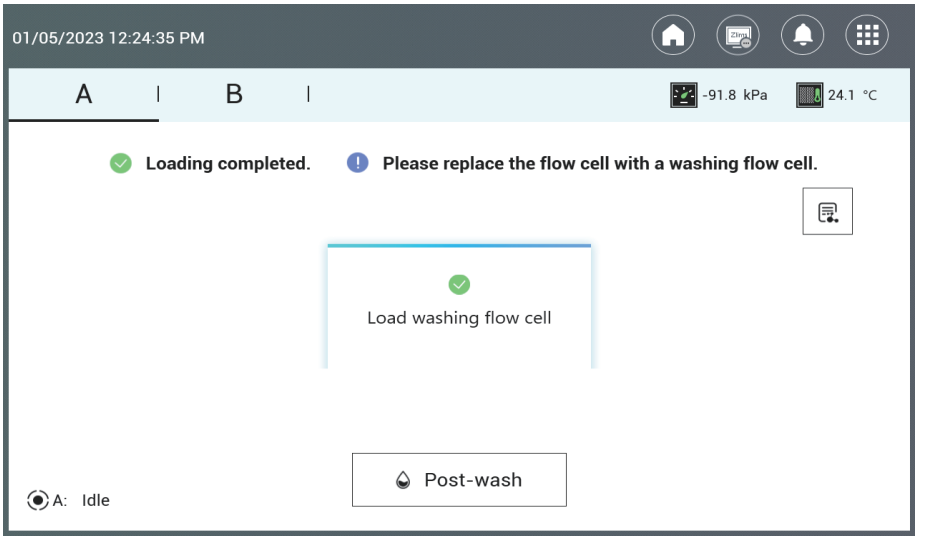

Figure 14 DL-T7RS post-wash interface

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

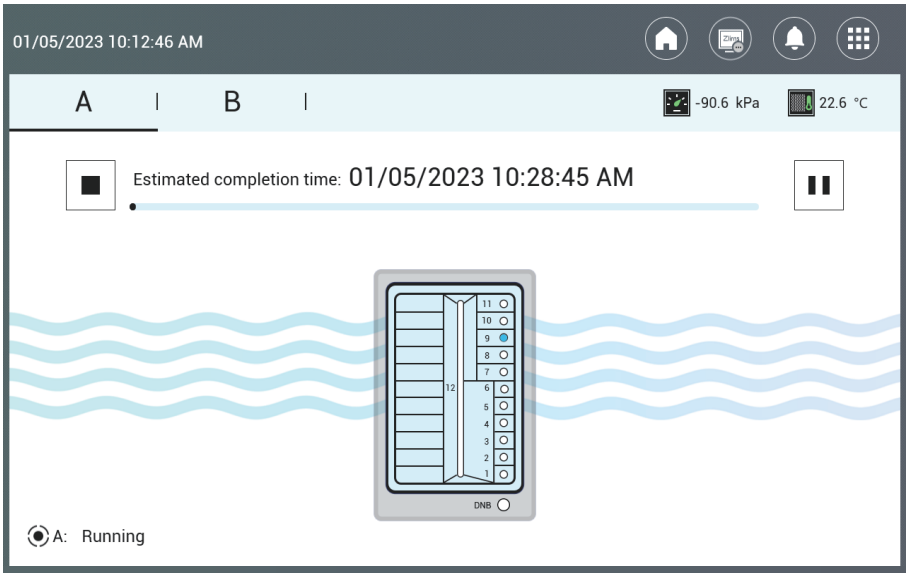

Figure 15 DL-T7RS wash interface

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

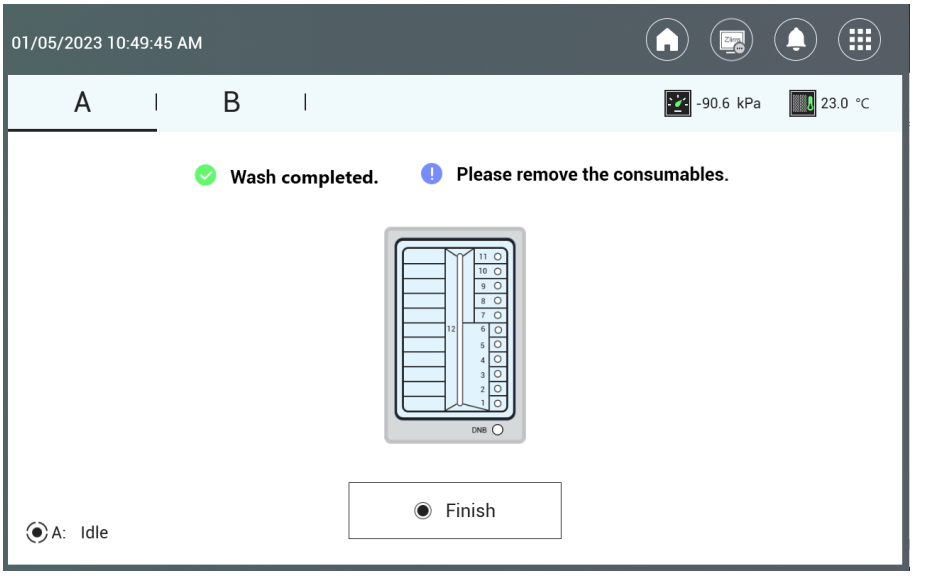

Figure 16 DL-T7RS wash complete status interface

21. Select **Finish** to complete the loading process, and 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 into an appropriate waste container.
24. Dispose of the waste and DNB tube.

Preparing the Sequencing Reagent Cartridge

The Sequencing Enzyme Mix II , dNTPs Mix, and dNTPs Mix II are provided in different tubes and are packaged together with the Sequencing Reagent Cartridge. Before the sequencing run starts, add an appropriate amount of sequencing enzyme and dNTP mix to well No. 9 and well No. 10 of the Sequencing Reagent Cartridge. Furthermore, MDA Block Reagent and MDA Block Component must to be added to well No. 15, and the MDA mixture (MDA, Multiple displacement amplification) must to be added to well No. 8. If prepared reagent cartridges are not used immediately, refer to *Q:What rules should I follow if I need to store a reagent kit temporarily? on Page 60.*

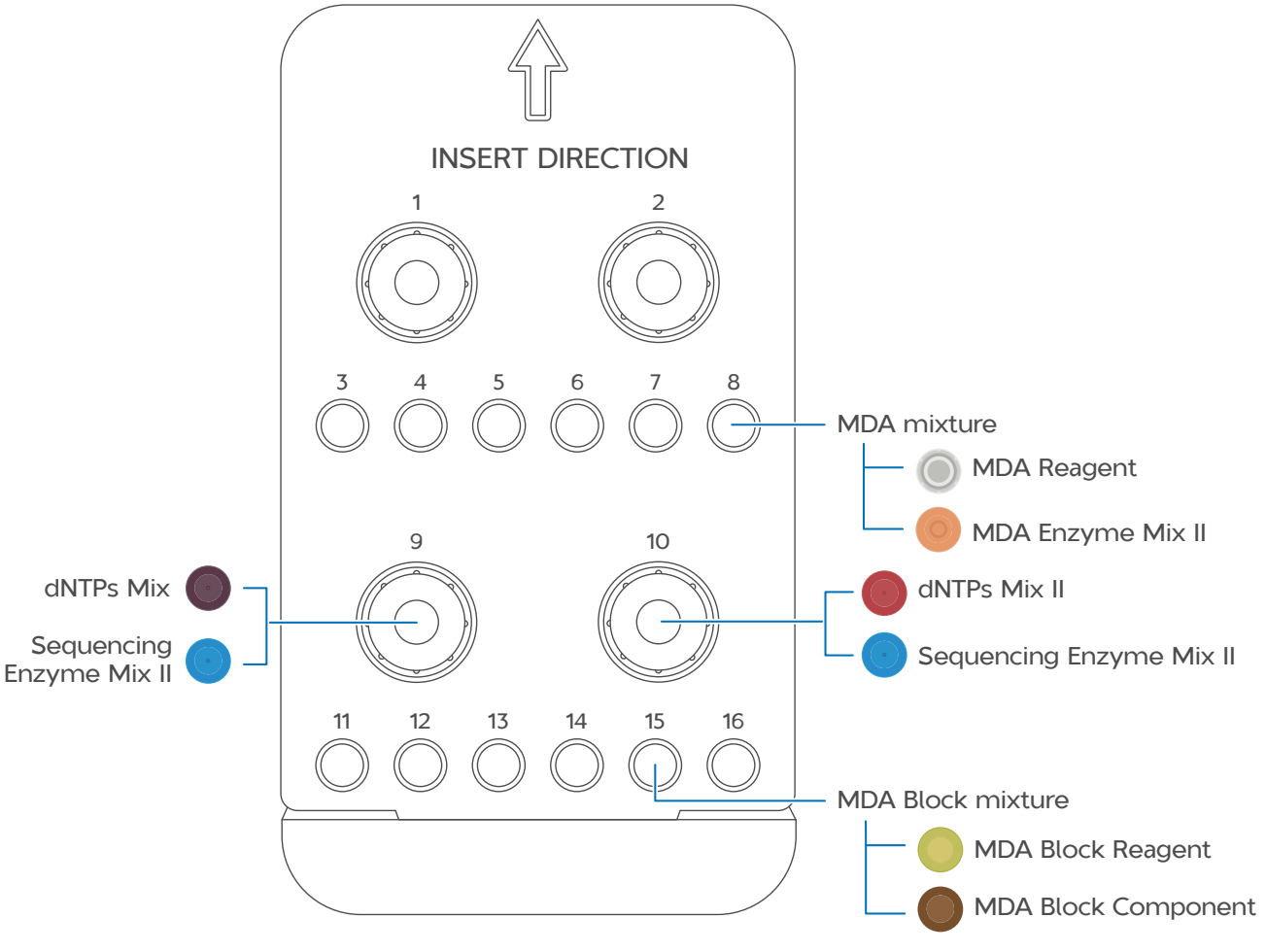

Figure 17 Sequencing Reagent Cartridge wells

Perform the following steps:

1. Take the Sequencing Reagent Cartridge out of DNBSEQ-T7RS High-throughput Sequencing Reagent Kit.
2. Thaw in a water bath at room temperature until completely thawed (or thaw in a 2 °C to 8 °C refrigerator 1 day in advance). The approximate time to thaw is listed in the following table. Store in a 2 °C to 8 °C refrigerator after thawinguntil use.

Table 17 Approximate thaw times for various sequencing cartridges

Model	Method		
	Water bath at room temperature (hr)	Refrigerator at 2°C to 8°C overnight then water bath at room temperature (hr)	Refrigerator at 2°C to 8°C (hr)
T7 STO FCL PE75	2.0 to 3.0	1.5	24.0

3. Invert the cartridge 3 times to mix before use.
4. Shake the cartridge vigorously clockwise 20 times, and then counterclockwise 20 times. Ensure that the reagents are fully mixed.
5. Wipe away any water condensation on the cartridge cover and around the well with a KimWipes cloth.

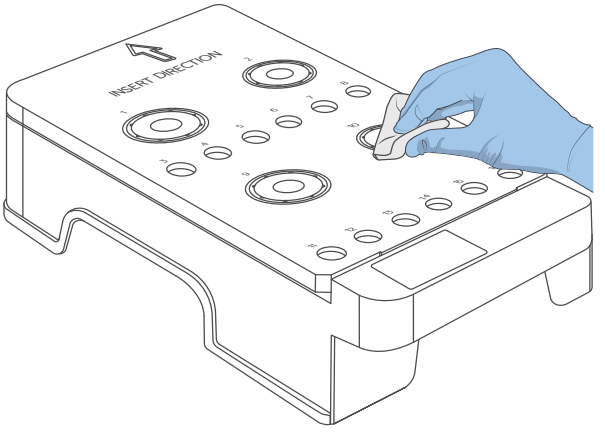

Figure 18 Wiping cartridge cover

6. Take dNTPs Mix, dNTPs Mix II, and MDA Block Reagent out of DNBSEQ-T7RS Stereo-seq Visualization Reagent Kit and thaw them at room temperature.

Table 18 Reagent preparation 3

Component	Cap color
dNTPs Mix	
dNTPs Mix II	
MDA Block Reagent	

7. After thawing, invert dNTPs Mix, dNTPs Mix II, and MDA Block Reagent 6 times. Gently tap the tube on the bench to bring the liquid to the bottom. Place them on ice until use.
8. Take MDA Block Component out of DNBSEQ-T7RS Stereo-seq Visualization Reagent Kit and thaw them at room temperature of 20 °C to 25 °C.

   To prevent refreezing, do not keep MDA Block Component on ice or at room temperature below 19 °C to avoid refreezing.

Table 19 Reagent preparation 4

Component	Cap color
MDA Block Component	

9. After thawing, invert MDA Block Component 6 times. Gently tap the tube on the bench to bring the liquid to the bottom.
10. Take Sequencing Enzyme Mix II out of DNBSEQ-T7RS Stereo-seq Visualization Reagent Kit. Invert Sequencing Enzyme Mix II 6 times and place it on ice until use.
11. Pierce the seals in the center of wells No. 9 and No. 10 to make a hole approximately 2 cm in diameter using a 1 mL sterile pipette tip.
12. Take out a pipette with the appropriate volume range. Add dNTPs Mix and Sequencing Enzyme Mix II into well No. 9 according to the table below:

Table 20 Reagent preparation for well No. 9

Model	Volume (mL)	
	dNTPs Mix	Sequencing Enzyme Mix II
T7 STO FCL PE75	2.290	2.290

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

Table 21 Reagent preparation for well No. 10

Model	Volume (mL)	
	dNTPs mix II	Sequencing Enzyme Mix II
T7 STO FCL PE75	2.730	1.140

14. Seal the loading wells No. 9 and No. 10 with the transparent sealing film.
15. Press the film around the well with your finger. 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 out of the cartridge.
16. Lift the cartridge horizontally, hold both sides of the cartridge with both hands. Shake the cartridge 20 times in a clockwise and counterclockwise direction. Ensure that the reagents are fully mixed.
17. Gently tap Sequencing Reagent Cartridge on the bench to reduce air bubbles in the reagents.
18. Carefully remove the seals from the loading wells after fully mixing.

    - Do not reuse the used sealing film.
    - To prevent cross contamination, ensure that the surface around wells No. 9 and No. 10 is clean.

19. Pierce the seal of well No. 15 using a 1 mL sterile tip.
20. Add 450 µL of MDA Block Component to MDA Block Reagent tube with a 1 mL pipette. Invert the tube 6 times to mix the reagents. Add the mix into well No. 15.

Table 22 Reagent preparation for well No. 15

Component	Cap color
MDA Block Component	
MDA Block Reagent	

21. Pierce the seal of well No. 8 using a 1 mL sterile tip.
22. Add 600 µL of MDA Enzyme Mix II to MDA Reagent tube with a 1 mL pipette and invert the tube 6 times to mix the reagents. Add all of the MDA mixture into well No. 8. When adding the MDA mixture, ensure that there are no bubbles at the bottom of the tube.

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

Table 23 Reagent preparation for well No. 8

Component	Cap color
MDA Enzyme Mix II	
MDA Reagent	

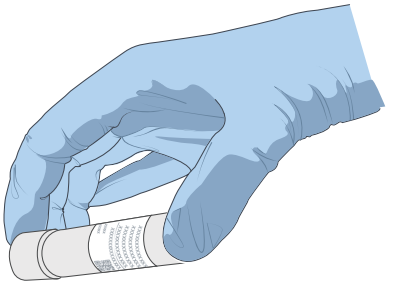

Figure 19 MDA mixture

Preparing the Washing Cartridge

Perform the following steps:

1. Shake the cartridge clockwise 5 to 10 times, and then counterclockwise 5 to 10 times to ensure that the reagents are fully mixed.

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

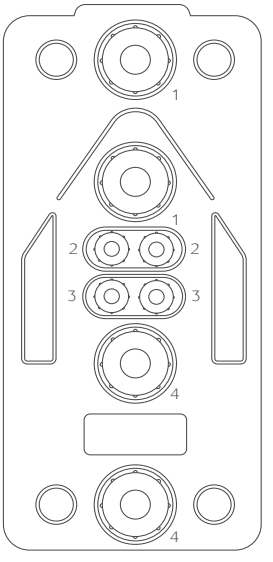

Figure 20 Washing Cartridge

3. Add 45 mL of 0.1 M NaOH into well No. 2 through the pierce using an electronic pipette. Refer to *Preparing washing reagents on Page 49*.

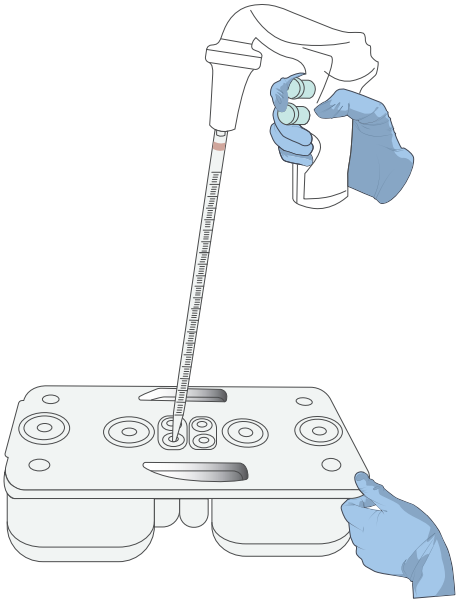

Figure 21 Adding 0.1 M NaOH to Washing Cartridge

Filling the pure water container

Fill the pure water container with laboratory-grade water according to the table below.

- Check whether the water level in the pure water container is sufficient. If the water level is insufficient, the sequencing will fail. Replenish the pure water reservoir regularly and ensure that the air vent of the pure water container remains unobstructed.
- The pure water will be used in sequencing so it must be kept clean. Renew 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 microfiber cloths. Rinse the container with fresh pure water 3 times.

Table 24 Pure water consumption (L)

Model	1 flow cell	2 flow cells	3 flow cells	4 flow cells
T7 STO FCL PE75	2.5	4.5	7.0	9.0

Performing a sequencing run

Entering DNB ID

Perform the following steps:

1. Open the reagent compartment door and clean the inner walls with a KimWipes cloth 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.

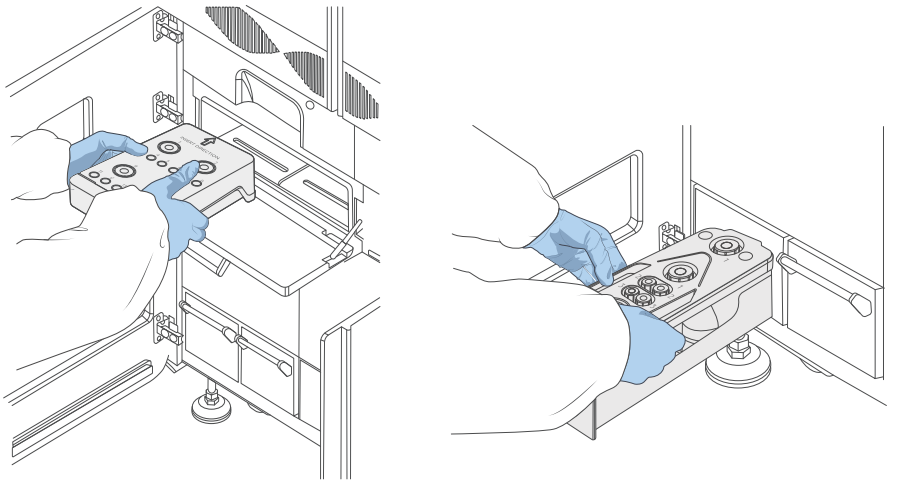

Figure 22 Loading the cartridges

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 **Login** to go to the main interface.

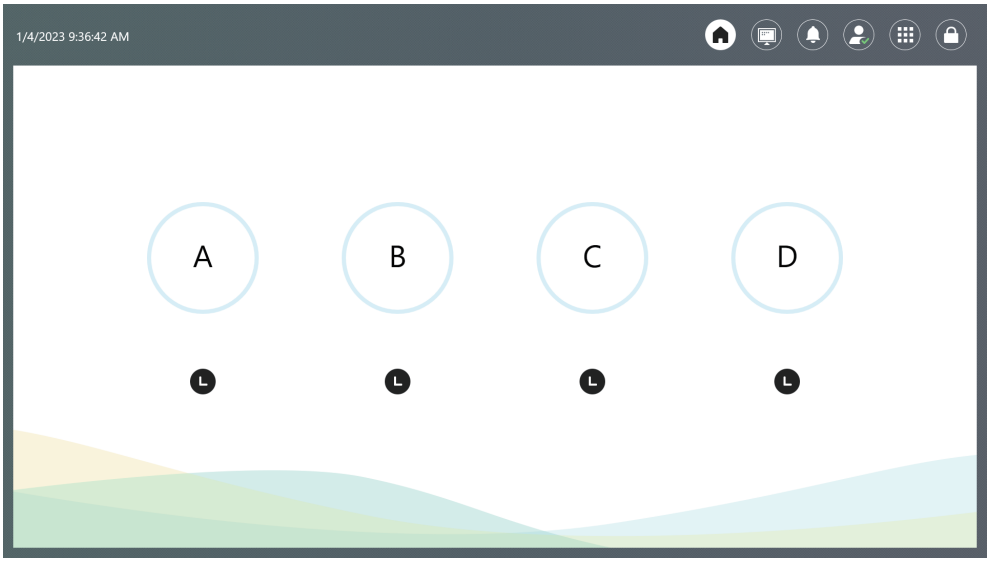

Figure 23 DNBSEQ-T7RS main interface

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**.

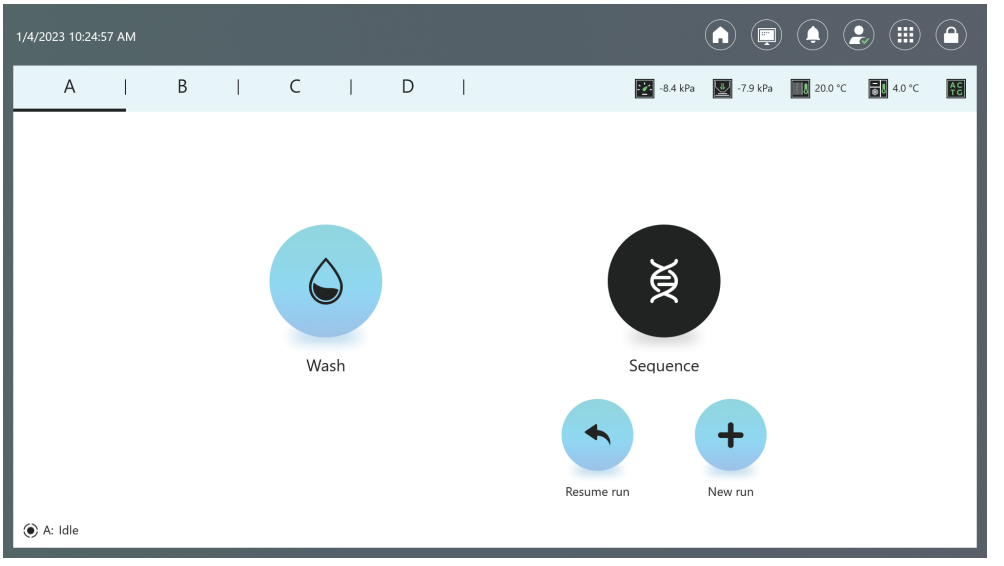

Figure 24 DNBSEQ-T7RS selection interface

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 press the flow cell drive control button to load the flow cell into the device.

WARNING

- When using the canned air duster to remove the dust from the back of the flow cell, avoid blowing air into the inlet holes.
- If the flow cell accidentally falls to the floor and breaks, handle with care to prevent personal injury.

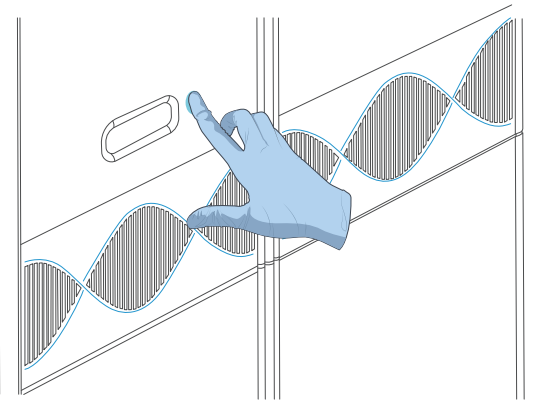

Figure 25 Flow cell drive

Sequencing parameters

Perform the following steps:

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

- If scanning fails, input the cartridge ID with the on-screen keyboard.
- Ensure that the ID format is correct when you input the ID manually. Otherwise, you will be informed that the ID is incorrect and the procedure cannot continue.
- The cartridge ID consists of the catalog number (REF on the label) and serial number (SN on the label). When inputting the ID manually, input the special characters in the catalog number as well.

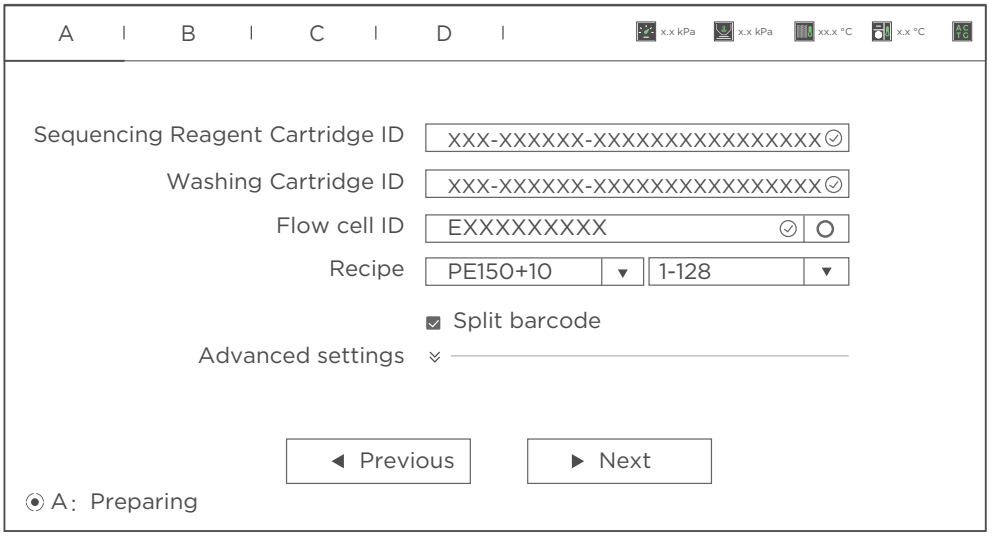

Figure 26 DNBSEQ-T7RS sequencing parameters

2. Select the first ▼ next to Recipe and select Customize a recipe in the sequencing parameters interface.

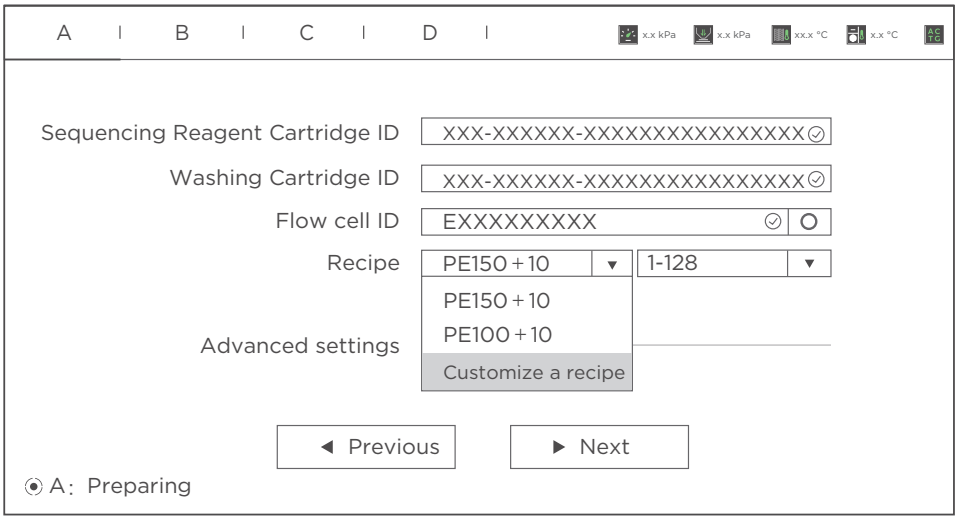

Figure 27 Selecting Customize a recipe

3. Fiil in the **Customize a recipe** as follows, and select **Save:**

- You only need to customize the script when sequencing this read length for the first time. Next time, you can directly select the recipe name you created for this recipe.
- For OMNI FFPE library sequencing: the length of Read1 is 25, the length of Read2 is 62 (dark cycle is 7 to 9), and the length of barcode is 10.
- For FF V1.3 library sequencing: the length of Read1 is 50 (dark cycle is 26 to 40), the length of Read2 is 100, and the length of barcode is 10.

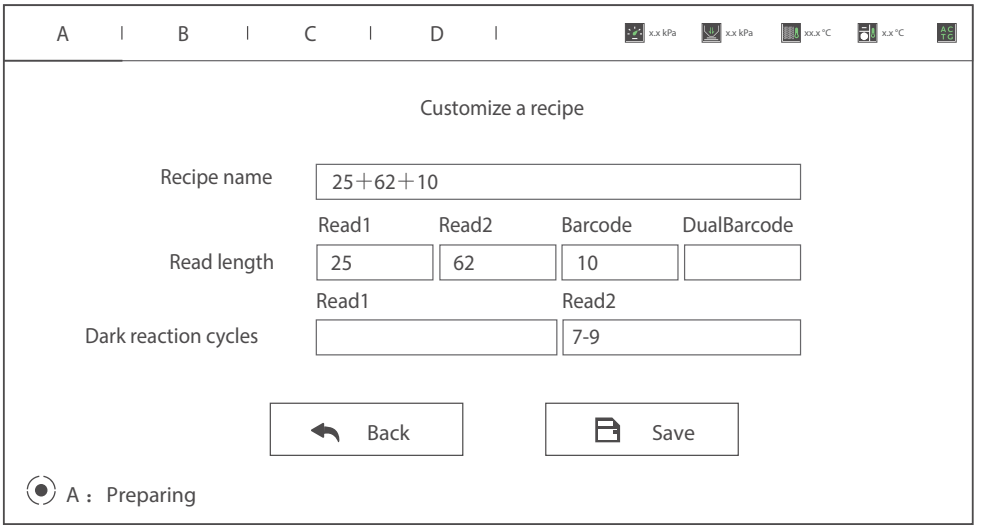

Figure 28 Configuring customized settings for OMNI FFPE

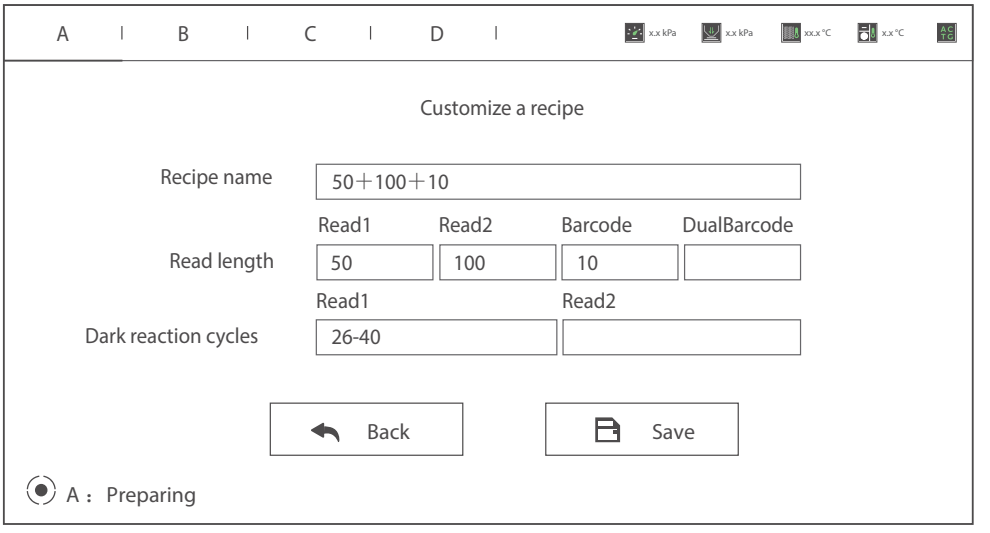

Figure 29 Configuring customized settings for FF V1.3

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

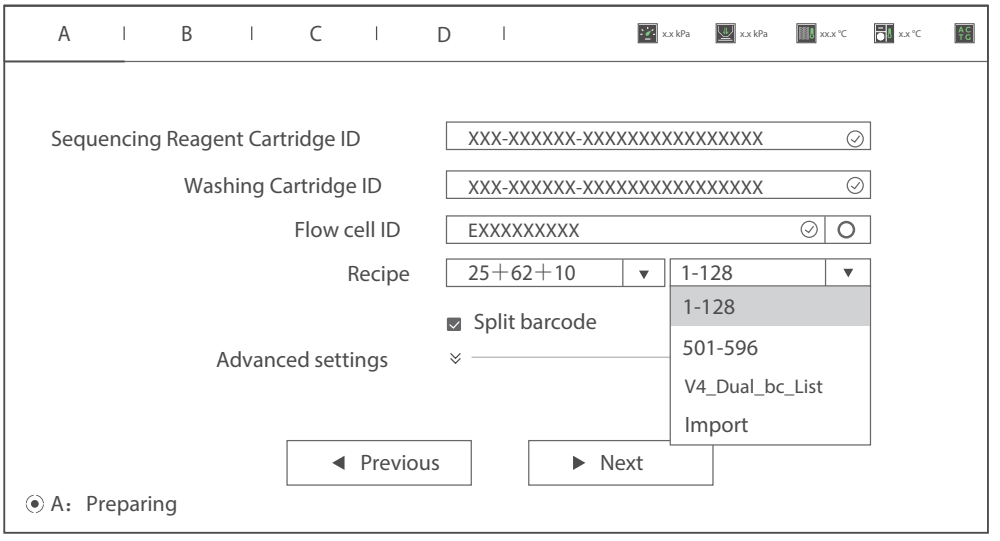

Figure 30 Set the barcode sequence

5. Select » next to **Advanced settings** to go to the interface as shown in the figure below. You can indicate whether an auto wash is to be performed. To enable auto wash, select the Yes option for Auto wash.

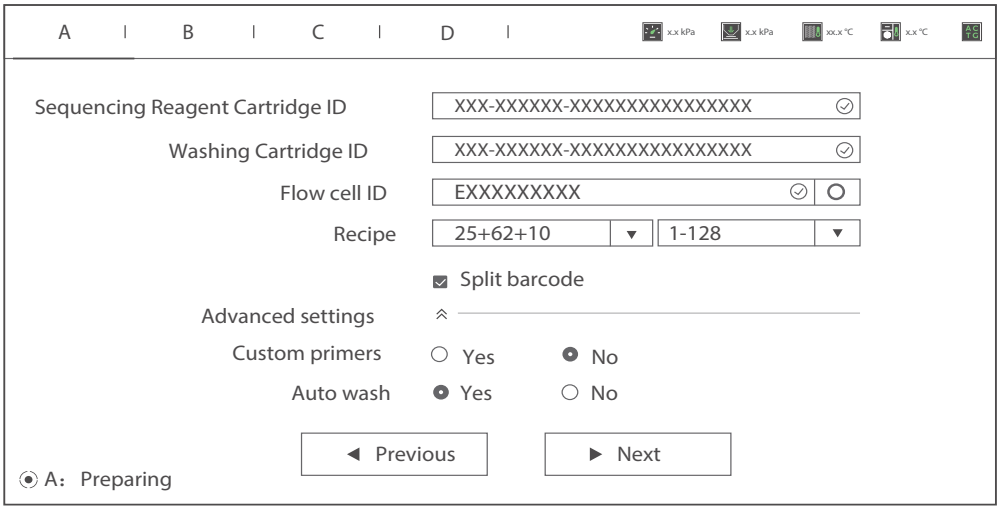

Figure 31 DNBSEQ-T7RS advanced settings

6. Select **Next**.

Reviewing parameters

Review the parameters and ensure that all information is correct.

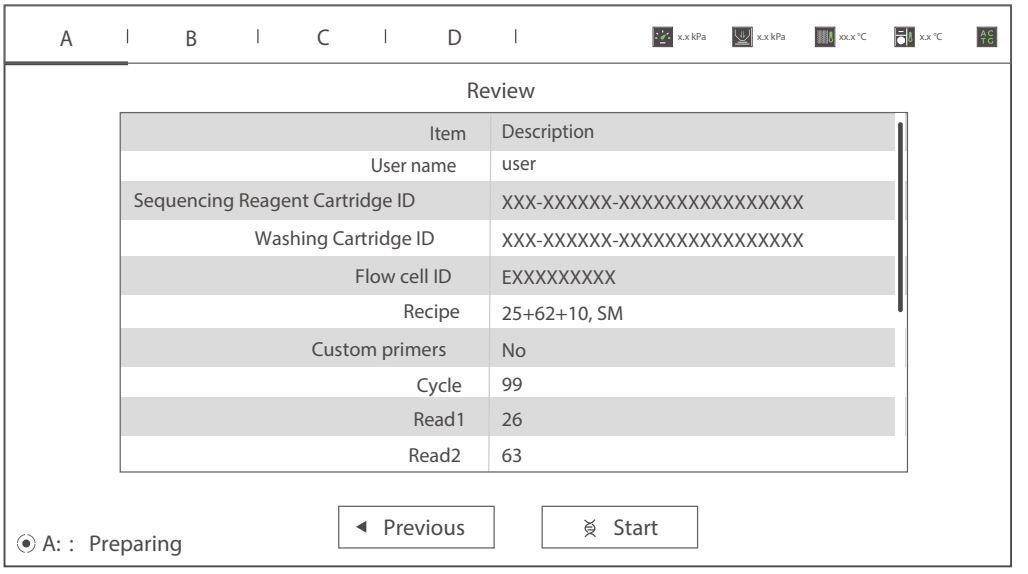

Figure 32 Reviewing information of OMNI FFPE

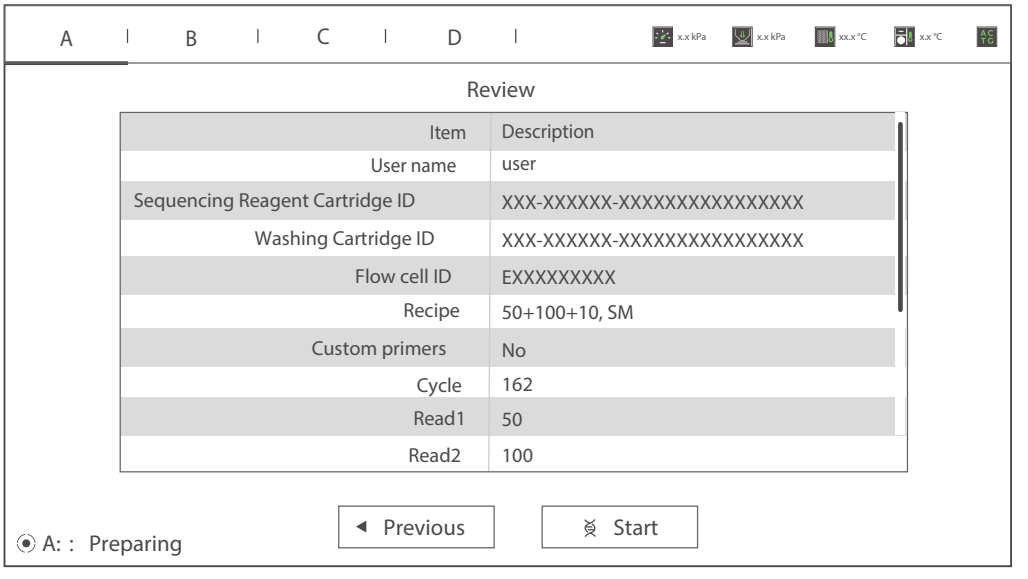

Figure 33 Reviewing information of FF V1.3

Starting sequencing

Perform the following steps:

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

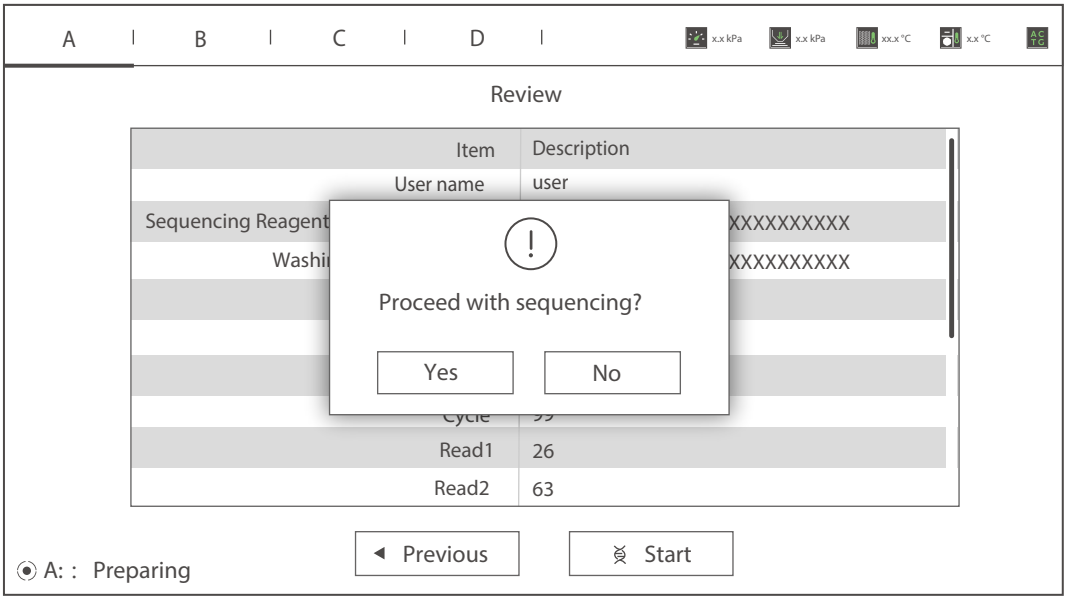

Figure 34 Confirming sequencing interface

2. When the following screen appears, the sequencing has begun.

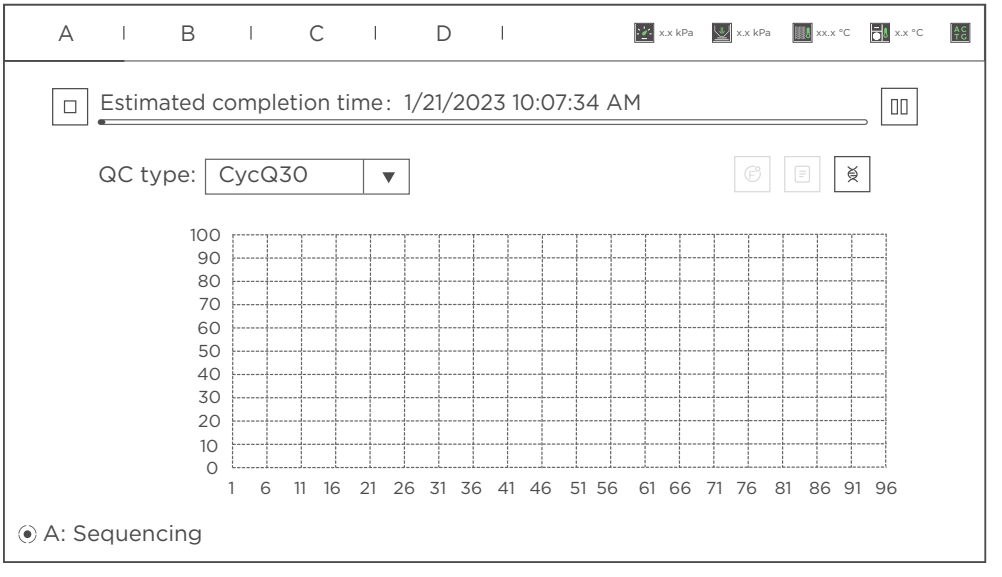

Figure 35 Sequencing start interface

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

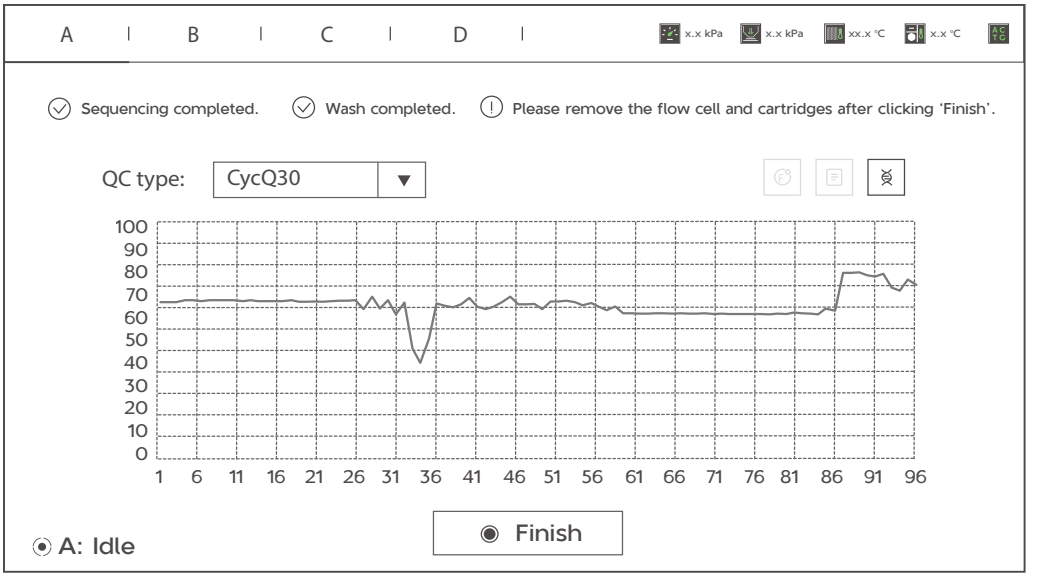

Figure 36 Sequencing and wash complete interface

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 or move the device during loading. Doing so 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 on the screen and in 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 immediately after troubleshooting. For details about how to perform a wash manually, refer to *Wash procedures on Page 52.*

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.

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03

Device maintenance

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

DANGER

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

WARNING

- It is not recommended that you use other disinfectants or wash solutions except for those specified 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 25 Wash type introduction

Equipment	Wash type	Cartridge type	Process time (min)	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	DNB Load Plate (no Reagent)*	20	• The device is used for the first time. • The device has not been used for 7 days or longer. • Impurities are visible 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 (min)	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	T7 Sequencing Cartridge (no Reagent) and T7 Cleaning Cartridge (no Reagent)*	40	• The device is used for the first time. • The device has not been used for 7 days or longer. • Impurities are visible in the device or flow cell. • Tubing, sampling needles, or other accessories exposed to the reagents were replaced.

DNB Load Plate (no Reagent), T7 Sequencing Cartridge (no Reagent) and T7 Cleaning Cartridge (no Reagent) should be purchased additionally. For detailed purchasing information, refer to *Order information on Page 67.*

Preparing a Wash

Preparing washing reagents

Prepare the washing reagents according to the table below.

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

Table 26 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 27 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

The top view of the loader washing plate is shown below.

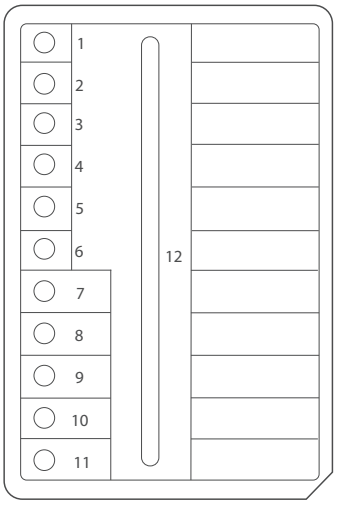

Figure 37 DNB Load Plate (no Reagent)

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

Prepare the loader washing plate using DNB Load Plate (no Reagent) according to the table below:

Table 28 Loader washing plate preparation

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:

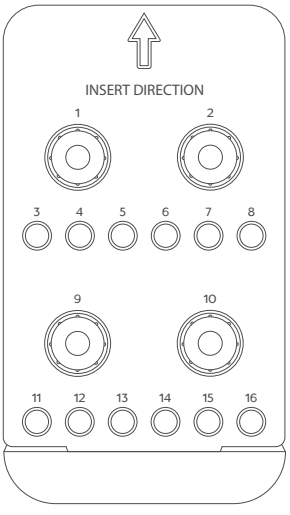

Figure 38 T7 Sequencing Cartridge (no Reagent)

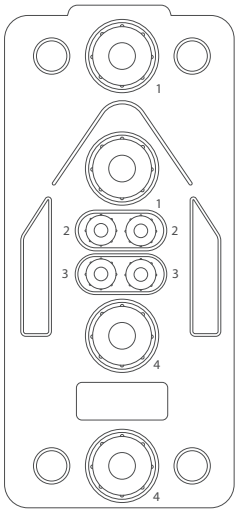

Figure 39 T7 Cleaning Cartridge (no Reagent)

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

Table 29 Washing cartridges preparation

Cartridge type	Well position	Washing reagent	Volume (mL)
T7 Sequencing Cartridge (no Reagent)	All	NA	NA
T7 Cleaning Cartridge (no Reagent)	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

Used sequencing flow cells 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 min)

Perform the following steps:

1. Start the loader, enter the password, and then select **Login** to go to 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.

   i 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 min.
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 min)

To prevent cross contamination, perform a wash to remove the remaining reagents from the fluidics lines and flow cell stages.

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 7 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.
2. Start the sequencer. Enter the user name and password, select **Login** to go to the main interface

3. Select **Wash**. Press the flow cell drive control button to install a washing flow cell. Press the flow cell drive control button again to load the washing flow cell into the device.
4. Place the prepared T7 Sequencing 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 T7 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 min.
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 refilling with fresh washing reagents, used washing plates must be cleaned 3 to 5 times with laboratory-grade water.
- After cleaning 3 to 5 times with laboratory-grade water, used DNB load plates can be used as washing plates.

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04

FAQs

This chapter describes frequently asked questions about the reagents.

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

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

Reagent FAQs

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

When DNB concentration is lower than 8 ng/μL, perform the following steps:

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

Q: What should I do if I forget to add reagent into well No. 8 for PE sequencing run?

MDA Enzyme is required to make the second-strand template for PE sequencing. When preparing the Sequencing Reagent Cartridge, the appropriate amounts of MDA Enzyme Mix and MDA Reagent must be added to well No. 8. If MDA mixture was not added to well No. 8 before starting the sequencing run, this can be resolved by performing the following steps if the sequencing run is in the sequencing phase of Read1:

1. Pause the run: Select the pause button ⏸ in the sequencing interface and select **Yes** when prompted.
2. Lift the needle:
    1) Select the stop button ■ and select **Yes** when prompted.
    2) Select **Finish**.
3. Fill well No. 8 of the Sequencing Reagent Cartridge:
    1) Open the reagent compartment door and take out the Sequencing Reagent Cartridge.
    2) Prepare the MDA mixture by adding the appropriate amount of MDA Enzyme Mix into the MDA Reagent tube.
    3) Mix thoroughly and transfer all solution into well No. 8. as described in *Preparing the Sequencing Reagent Cartridge on Page 29*.

4) Insert the filled sequencing cartridge back into the sequencer.

4. Resume the run:

   1) Select **Sequence** > **Resume run** on the main interface.
   2) Clean the loaded flow cell with a canned air duster to ensure that no visible dust exists on the surface and back of the flow cell. Place the flow cell on the flow cell drive, and press the flow cell drive control button to load the flow cell into the device.
   3) Select **Next** to review the parameters and ensure that all parameters are correct.
   4) Select **Start** > **Continue**.

Q: How do I resume a sequencing run?

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

Perform the following steps:

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

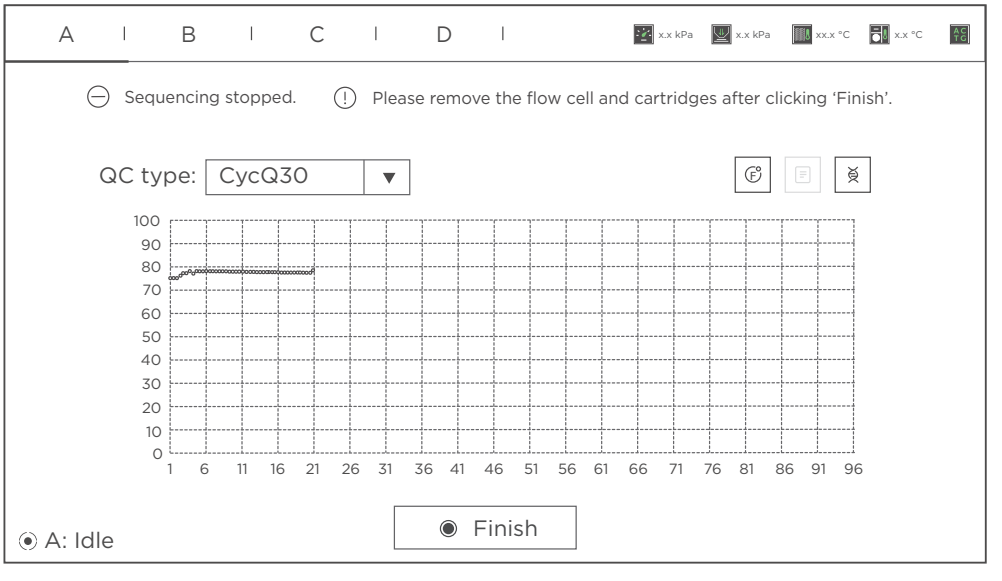

Figure 40 Sequencing stopped interface

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

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

3. Re-load the flow cell:
   1) Remove any dust from the loaded flow cell from the interrupted sequencing run with a canned air duster. Ensure that no visible dust is present on the surface and back of the flow cell.
   2) Place the flow cell on the flow cell drive, and press the flow cell drive control button to load the flow cell into the device.
4. Select **Next** to review the parameters and ensure that all information is correct.

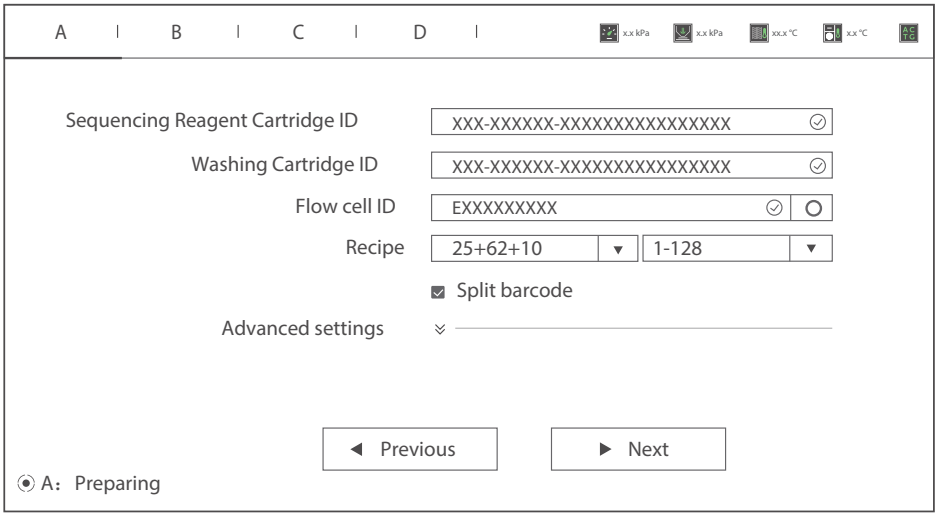

Figure 41 Cartridge ID, flow cell ID interface

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

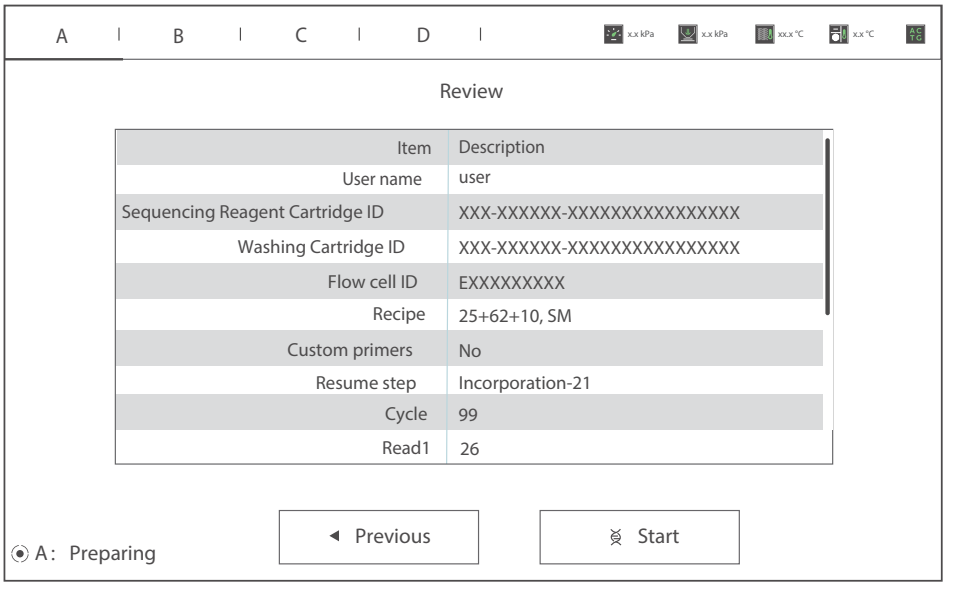

Figure 42 Sequencing parameter interface

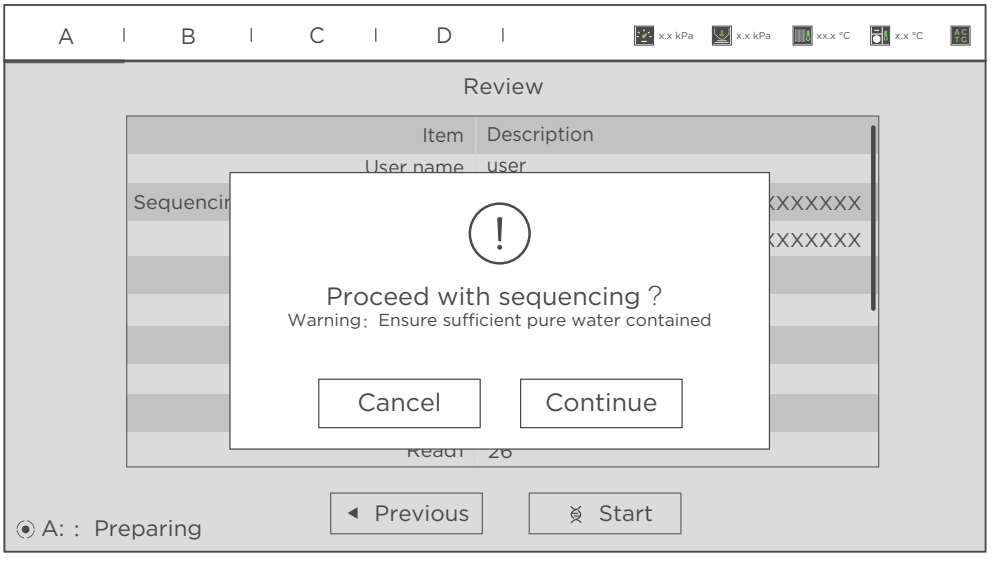

Figure 43 Continue run interface

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

- If a kit has been thawed (including the dNTPs) but cannot be used within 24 hr, it can be frozen and thawed one additional time.
- If a kit has been thawed (including the dNTPs) but cannot be used immediately, store it at 2 °C to 8 °C. It is strongly recommended that you use it within 24 hr. A thawed kit that is stored at 2 °C to 8 °C may still be used within 7 days, although it may compromise sequencing quality. It is not recommended that you use a kit that has been thawed and stored at 2 °C to 8 °C for more than 7 days.
- If the dNTPs and Sequencing Enzyme Mix have been added into the cartridge, for example, the cartridge has been prepared and the needles have punctured the seal but the cartridge cannot be used immediately, the cartridge must be covered with foil or plastic wrap. Store the kit at 2 °C to 8 °C and use it within 24 hr. Gently mix the reagents in the cartridge before use. To prevent reagent contamination when mixing, be careful not to spill any reagent from the needle holes.

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

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

1. Gently wipe the stage surface with a damp KimWipes cloth and remove dust from the stage with a canned air duster. Ensure that no dust is present on the flow cell stage.
2. Remove any dust from the back of the flow cell with a canned air duster.
3. If the problem persists, contact CG Technical Support.

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

Bubbles in DL-T7RS

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

Bubbles in DNBSEQ-T7RS

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

  For information on placing the water tube, refer to *Filling the pure water container on Page 35.*
- Check the reagent needles to ensure that they can immerse fully into the cartridges. Otherwise, restart the sequencing software.
- If the problem persists after a restart, contact CG Technical Support.

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

- Check the pure water volume to ensure that it is sufficient.
- When errors occur on DL-T7RS and DNBSEQ-T7RS:
  - Remove the flow cell and check for dust on the sealing gasket. Remove any dust with a canned air duster.
  - Place the flow cell by following the instructions, and start the pump again.
- Visually confirm that the sampling needles are moving properly. If the sampling needles are not moving properly, restart the control software.
- If the problem persists, contact CG Technical Support.

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

If impurities appear, perform the following steps:

1. Perform a manual wash on DL-T7RS and DNBSEQ-T7RS.
2. If there is still no improvement after a manual wash, prepare washing reagents again according to *Preparing washing reagents on Page 49*, and perform a manual wash again on DL-T7RS and DNBSEQ-T7RS.
3. If the problem persists, contact CG Technical Support.

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Instructions for using Qubit to quantify the DNBs

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

Perform the following steps:

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

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

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

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

   - Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit assay tubes (Cat. No.: Q32856) or Axygen PCR-05-C tubes (Cat. No.: 10011-830).
   - The number of Qubit test tubes needed are the number of samples plus 2 standards tubes. For example, if you have 3 samples, you will need 5 tubes.
5. Label the tube lids. Do not label the side of tube.
6. Prepare the solutions used for standards and sample tests according to the table below.

Table 30 Qubit solutions preparation

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

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

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

Manufacturer information

Manufacturer	Complete Genomics, Inc.
Address	2904 Orchard Parkway, San Jose, CA 95134
Customer service telephone	+1 (888) 811-9644
Customer service Email	US-CustomerService@CompleteGenomics.com
Technical support Email	US-TechSupport@CompleteGenomics.com
Website	www.completegenomics.com

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Order information

Catalog number	Model	Name	Version	Recommended brand
940-001889-00	T7 STO FCL PE75	DNBSEQ Stereo-seq Visualization Reagent Set	V1.0	CG
940-000871-00	/	T7 Sequencing Cartridge (no Reagent)	/	CG
940-000872-00	/	T7 Cleaning Cartridge (no Reagent)	/	CG
940-000873-00	/	DNB Load Plate (no Reagent)	/	CG
900-000697-00	DL - T7RS	DNB Loader DL-T7RS	/	CG

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Acronyms and abbreviations

Item	Description
bp	Base-pair
DNA	Deoxyribonucleic Acid
DNB	DNA Nanoball
dsDNA	double-stranded DNA
ssDNA	single-stranded DNA
FAQ	Frequently Asked Questions
FCL	Flow Cell Large
PE	Paired-end sequencing
QC	Quality Control
RCA	Rolling Circle Amplification
PCR	Polymerase Chain Reaction
dNTP	deoxy-ribonucleoside triphosphate
MDA	Multiple displacement amplification