STOmics MGI

Part No.: H-020-000926-00

| Cat No. | Product model |
| --- | --- |
| 940-001895-00 | T7 STO FCL PE75 |

# Stereo-seq Visualization Reagent Set

## DNBSEQ-T7RS

## Instructions for Use

Version: 3.0

Leading Life Science Innovation

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CE Research Use Only

Wuhan MGI Tech Co., Ltd.

# About the instructions for use

This instructions for use is applicable to DNBSEQ-T7RS Stereo-seq Visualization Reagent Set. The instructions for use version is 3.0.

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# Revision history

| Version | Date | Description |
|---|---|---|
| 3.0 | January 20, 2025 | Add FF V1.3 library sequencing |
| 2.0 | June 24, 2024 | Update product name. |
| 1.0 | April 22, 2024 | Initial release |

# About the visualization set

| Catalog number | Name | Model | Version |
|---|---|---|---|
| 940-001895-00 | DNBSEQ-T7RS Stereo-seq Visualization Reagent Set | T7 STO FCL PE75 | V1.0 |

# Contents

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# Chapter 1 Introduction

This instructions for use explains how to perform sequencing by using the DNBSEQ-T7RS Stereo-seq Visualization Reagent Set and includes instructions on sample preparation, Flow Cell preparation, visualization kit storage, the sequencing protocol and device maintenance.

## 1.1 Applications

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 stereo-seq visualization set is intended to be used for scientific research only and cannot be used for clinical diagnosis.

## 1.2 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.

## 1.3 Data analysis

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

## 1.4 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, 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.

Tips
- 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 (dark reaction cycle is from 7 to 9), 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.
- Among the maximum cycles of each sequencing, the additional 20 cycles are reserved for resuming a stopped sequencing run, or for a customized run.
- PE means Pair-end sequencing.

**Table 1 Sequencing cycle**

| Library type | Read Length | Read 1 read length | Read 2 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 |

## 1.5 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 |

Tips
- 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.

## 1.6 Precautions and warnings

- This product is for research use only. Please read the manual carefully before use.
- Ensure that you are familiar with the SOP&Attention of all the laboratory apparatus to be used.
- Avoid direct skin and eye contact with any samples and reagents. Do not swallow. Please wash with plenty of water immediately and go to the hospital if this happens.
- All the samples and waste materials should be disposed of according to relevant laws and regulations.
- This product is for one sequencing run only and cannot be reused.
- The components and packages are batched separately. Keep the components in the packages until use and do not remove them. Mixed use of reagent components from different batches is not recommended.
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- The components and packages are batched separately. Keep the components in the packages until use and do not remove them. Mixed use of reagent components from different batches is not recommended.
- Do not use expired products.

# Chapter 2 Main components and user-supplied equipment, reagents and consumables

## 2.1 Main components

**Table 3 DNBSEQ-T7RS Stereo-seq Visualization Reagent Set (T7 STO FCL PE75)**
**Catalog number: 940-001895-00**

| Component | Cap color | Spec&quantity | Transportation temperature | Storage temperature | Expiration date |
|---|---|---|---|---|---|
| **DNBSEQ-T7RS Sequencing Flow Cell (T7 STO FCL PE75) Catalog number: 940-001901-00** | | | | | |
| Sequencing Flow Cell (T7-2 FCL) | / | 1 EA | 2 ℃ to 8 ℃ | 2 ℃ to 8 ℃ | 10 months |
| **DNBSEQ OneStep DNB Make Reagent Kit V4.0 (T7 STO) Catalog number: 940-001890-00** | | | | | |
| Low TE Buffer | | 480 μL/tube×1 tube | -80 ℃ to -15 ℃ | -25 ℃ to -15 ℃ | 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) Catalog number: 940-001888-00** | | | | | |
| DNB Load Buffer I | | 300 μL/tube×1 tube | -80 ℃ to -15 ℃ | -25 ℃ to -15 ℃ | 12 months |
| DNB Load Buffer II | | 150 μL/tube×1 tube | | | |
| Micro Tube 0.5 mL (empty) | | 1 tube | | | |
| Post Load Plate (T7 STO FCL PE75) | / | 1 EA | | | |

| Component | Cap color | Spec&quantity | Transportation temperature | Storage temperature | Expiration date |
|---|---|---|---|---|---|
| **DNBSEQ-T7RS Stereo-seq Visualization Reagent Kit (T7 STO FCL PE75) Catalog number: 940-001892-00** | | | | | |
| dNTPs Mix II |  | 2.73 mL/tube×1 tube | -80 ℃ to -15 ℃ | -25 ℃ to -15 ℃ | 12 months |
| dNTPs Mix |  | 2.29 mL/tube×1 tube | | | |
| Sequencing Enzyme Mix II |  | 3.43 mL/tube×1 tube | | | |
| 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) Catalog number: 940-001904-00** | | | | | |
| Washing Cartridge | / | 1 EA | below 40 ℃ | 0 ℃ to 30 ℃ | 12 months |

Tips

- Mixed use of reagent components from different batches is not recommended.
- The components and packages are batched separately.
- Keep the components in the packages until use and do not take out them.

## 2.2 User-supplied equipment, reagent and consumables

Tips

- Avoid making and loading DNBs using filtered pipette tips.
- It is highly recommended that pipettes and tips of the suggested brands and catalog numbers be used. Using other brands may yield negative results.

**Table 4 Self-prepared equipment and consumables**

| Type | Name | Recommended brand | Catalog number |
|---|---|---|---|
| Equipment | Qubit 4.0 Fluorometer | Thermo Fisher | Q33226 |
| | Thermal cycler | Bio-Rad | / |
| | MPC2000 96-well plate centrifuge | Major Laboratory Supplier (MLS) | / |
| | Pipette | Eppendorf | / |
| | Electronic pipette | Labnet | FASTPETTEV-2 |
| | Mini centrifuge | MLS | / |
| | Vortex mixer | MLS | / |
| | 2 ℃ to 8 ℃ Refrigerator | MLS | / |
| | -25 ℃ to -15 ℃ Freezer | MLS | / |
| | Ice machine | MLS | / |
| | DNB Loader MGIDL-T7RS | MGI | 900-000134-00 |
| Reagent | Qubit ssDNA Assay Kit | Thermo Fisher | Q10212 |
| | Qubit dsDNA HS Assay Kit | Thermo Fisher | Q32851/Q32854 |
| | 2 M NaOH solution | Aladdin | S128511-1L |
| | 100% Tween-20 | BBI | A600560-0500 |
| | 5 M NaCl solution | SIGMA | S5150-4L |
| | 75% Ethanol | MLS | / |

| Type | Name | Recommended brand | Catalog number |
|---|---|---|---|
| Consumables | Power dust remover | MATIN | M-6318 |
| | Sterile pipette tip (box) | AXYGEN | / |
| | 5 mL Sterile pipette tip (box) | AXYGEN | / |
| | 200 μL wide-bore, non-filtered pipette tips | AXYGEN | T-205-WB-C |
| | 200 μL wide-bore, non-filtered pipette tips | MGI | BI-200K-H |
| | Qubit assay tubes | Thermo Fisher | Q32856 |
| | 0.2 mL PCR 8-strip tube | AXYGEN | / |
| | 1.5 mL microcentrifuge tube | AXYGEN | MCT-150-C |
| | Ice box | MLS | / |
| | 100 mL Serological pipet | CORNING | 4491 |
| | 25 mL Serological pipet | CORNING | 4489 |
| | 10 mL Serological pipet | CORNING | 4488 |
| | 15 mL Sterile tube | SARSTEDT | 60.732.001 |
| | Microfiber clean wiper | DUSTFREE TECHNOLOGY CO.,LTD | LJ618180B1 |
| | 5 mL Transport tubes | AXYGEN | / |
| | Lint-free paper | MLS | / |
| | Ziplock bag | MLS | / |
| | T7 Sequencing Cartridge (no Reagent) | MGI | 940-001611-00 |
| | T7 Cleaning Cartridge (no Reagent) | MGI | 940-001612-00 |
| | Post Load Plate (no Reagent) | MGI | 940-001610-00 |

# Chapter 3 Sequencing workflow

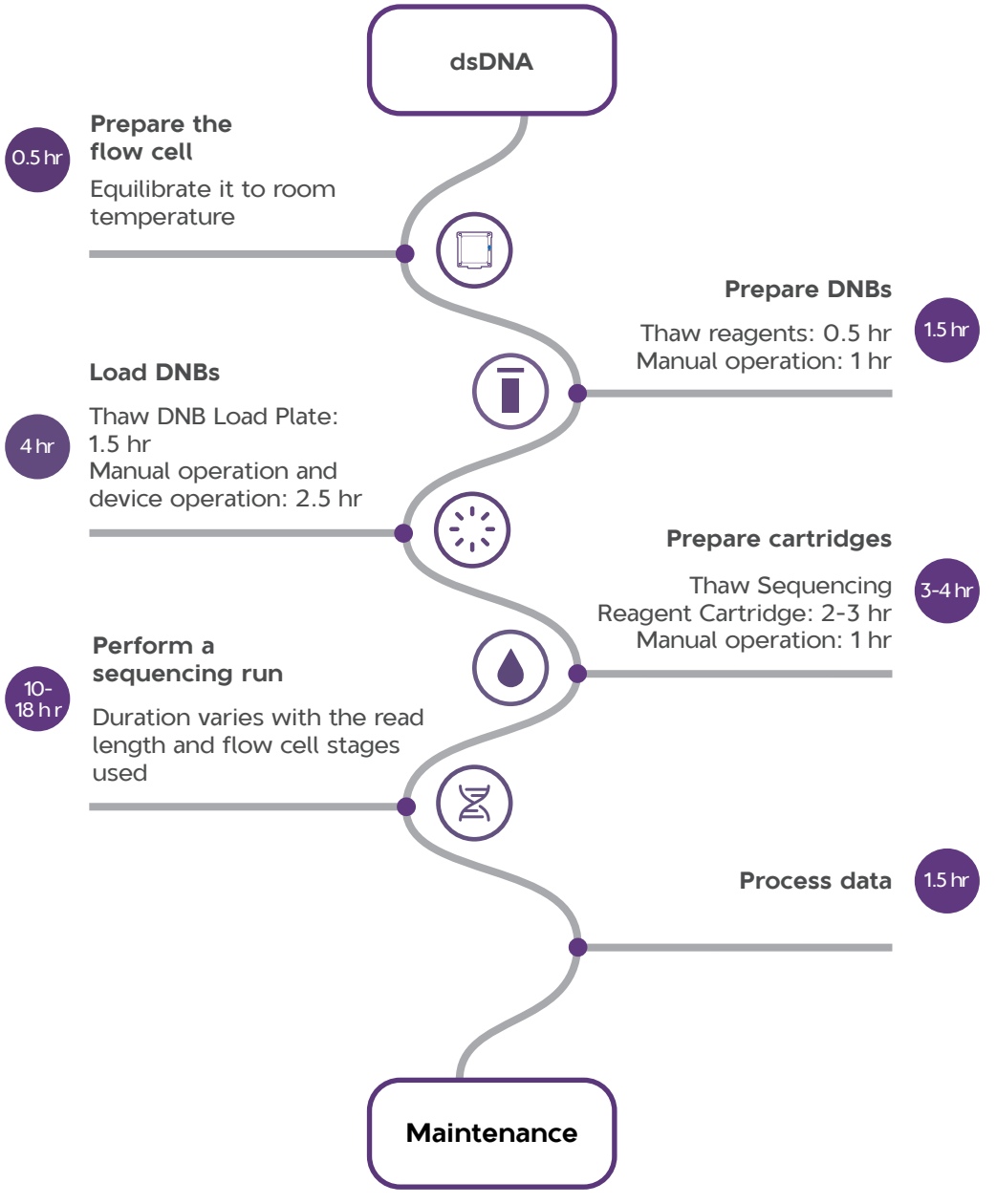


**Figure 1 Sequencing workflow**

Tips The manual operation duration mentioned above is for reference only. The actual duration may vary with your proficiency level.

# Chapter 4 Making DNB

## 4.1 Recommended library insert size

The stereo-seq visualization reagent 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.

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

**Table 5 Recommended insert size and theoretical throughput for each flow cell**

| Library type | Read length | Suggested insert distribution (bp) | Data output (M) |
|---|---|---|---|
| OMNI FFPE | PE25+62 | 150 to 1000 | 4000 |
| FF V1.3 | PE50+100 | 200 to 600 | 3500 |

## 4.2 Library concentration and amount requirement

**Table 6 Library Requirement**

| Library | Library concentration |
|---|---|
| OMNI FFPE dsDNA | ≥ 3 ng/μL |
| FF V1.3 dsDNA | ≥ 20 fmol/μL |

Tips

- If the library concentration is unknown, the DNA library concentration (ng/μL) should be quantified using dsDNA HS Assay Kit with the Qubit 4.0 Fluorometer.
- Use the equation below to convert the concentration of the ssDNA library from ng/μL to fmol/μL:

$$C(\text{fmol}/\mu\text{L}) = \frac{3030 \times C(\text{ng}/\mu\text{L})}{N \times 2}$$

  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 special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.

## 4.3 Library pooling

### 4.3.1 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. 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.

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

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.

### 4.3.2 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 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 be not pooled together; otherwise, it is impossible to assign the reads correctly.

## 4.4 Making DNB

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

### 4.4.1 Preparing DNB

#### 4.4.1.1 Calculating the required amount of dsDNA library

- 270 µL of DNB is required to load one flow cell. One DNB making reaction can make 100 µL of DNBs.
- The required dsDNA library volume to make 100 µL of DNBs are shown in the table below.

Tips C1 represents the OMNI FFPE library concentration (ng/µL) in *4.2 Library concentration and amount requirement on Page 10*. C2 represents the FF V1.3 library concentration (fmol/µL) in *4.2 Library concentration and amount requirement on Page 10*.

**Table 7 Required dsDNA volume per 100 µL reaction**

| Sample 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×270 (μL).
- The number of 100 μL DNB making reactions is equal to round (V/100)+1.(for example: If V=80, it requires one 100 μL of DNB making reaction; If V=120, it requires two 100 μL DNB making reactions).

### 4.4.1.2 Preparing reagents for DNB making

Perform the steps below:

1. Place the library 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 0.5 hr.

**Table 9 Reagent preparation 2**

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

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

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

### 4.4.1.3 Making DNB

Perform the steps below:

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

   **Tips** 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 *4.4.1.1 Calculating the required amount of dsDNA library on Page 11*.

**Table 10 Make DNB Reaction Mixture 1**

| Component | Cap color | Volume (μL) |
|---|---|---|
| Low TE Buffer | | 20-V |
| STO Make DNB Buffer | | 20 |
| dsDNA libraries | / | V |
| **Total volume** | | 40 |

2. Mix Make DNB reaction mixture 1 thoroughly by using a vortex mixer. Centrifuge it for 5 sec 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 |
|---|---|
| Heated lid (105 ℃ ) | On |
| 95 ℃ | 3 min |
| 40 ℃ | 3 min |
| 4 ℃ | 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.

   **Tips**
   - Do not keep the 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 ℃ . 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 by using a vortex mixer for 5 sec and centrifuge briefly for 5 sec.

   Tips 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 Make DNB Reaction Mixture 2 into Make DNB Reaction Mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer. Centrifuge it for 5 sec.
8. Place the tubes into a thermal cycler for the next reaction. The conditions are shown in the table below.

   Tips
   - 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 ℃ or as close as possible to 35 ℃ .

   ▪ RCA conditions for FF V1.3 library:

**Table 13 RCA (Rolling Circle Amplification) Conditions for FF V1.3 library**

| Temperature | Time |
|---|---|
| Heated lid (35 ℃ ) | On |
| 30 ℃ | 25 min |
| 4 ℃ | Hold |

- RCA conditions for OMNI FFPE library:

**Table 14 RCA Conditions for OMNI FFPE library**

| Temperature | Time |
|---|---|
| Heated lid (35 ℃ ) | On |
| 30 ℃ | 30 min |
| 4 ℃ | Hold |

9. When the temperature reach 4 ℃, 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.

Tips
- 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 4 ℃ and perform sequencing within 48 hr.

## 4.4.2 Quantifying DNB

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 *Appendix 1 Qubit ssDNA assay kit on Page 53*. The minimum DNB concentration required for sequencing is 8 ng/µL. For details, refer to *Appendix 1 Qubit ssDNA assay kit on Page 53*.

Tips
- If the concentration is lower than 8 ng/µL, refer to *9.1 Low DNB concentration on Page 49* for details.
- If there are more than 8 samples to quantify, it is recommended to quantify in batches to avoid inaccurate DNB quantification as a result of fluorescence quenching.
- To ensure sequencing quality, it is recommended that you pool and load DNBs 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.

## 4.4.3 DNB pooling

Tips Use normal pipette tips to aspirate the required volume of each DNB and use wide bore tips to mix.

### 4.4.3.1 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 DNB for sample A.

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

.....

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

### 4.4.3.2 Calculating the total relative amount (V) for all samples

V = A1 + B1 + ... + H1

### 4.4.3.3 Calculating the DNB volume needed for each sample

Each FCL flow cell requires 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

# Chapter 5 Loading DNB

## 5.1 Preparing Post Load Plate and buffer

### 5.1.1 Thawing the Post Load Plate

Perform the steps below:

1. Take Post Load Plate out of DNBSEQ-T7RS DNB Load Reagent Kit.
2. Thaw Post Load Plate. Choose the method that best suits you:
   - Thaw it in a water bath at room temperature for 1.5 hr.
   - Thaw it in a 2 ℃ to 8 ℃ refrigerator at least 12 hr in advance.
3. When Post Load Plate is thoroughly thawed, place it in a 2 ℃ to 8 ℃ refrigerator until use.
4. Gently invert the Post Load Plate to mix it 5 times and then centrifuge for 1 min before use.

### 5.1.2 Preparing the DNB loading reagents

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 0.5 hr.
3. Mix the reagent by using a vortex mixer for 5 sec. Centrifuge briefly and place on ice until use.

**Tips** If crystal precipitation is visivle in DNB Load Buffer II, vigorously mix the reagent with 1 to 2 min of continuous vortex to re-dissolve the precipitate before use.

### 5.1.3 Preparing the 0.1 M NaOH reagent

Prepare 0.1 M NaOH according to the procedure described in *8.2.1 Preparing washing reagents on Page 44*. Each Load Plate requires at least 4 mL of 0.1 M NaOH.

## 5.2 Preparing the sequencing flow cell

Perform the steps below:

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

   Tips Do not open the outer plastic package at this time.

2. Balance the flow cell at room temperature for at least 30 min, but no longer than 24 hr.
3. Unwrap the outer package before use and start DNB loading.

   Tips
   - If the flow cell can not be used within 24 hr after being placed in room temperate and the outer plastics package is intact, the flow cell can be placed back in 2 ℃ to 8 ℃ for storage. But the switch between room temperature and 2 ℃ to 8 ℃ must not exceed 3 times.
   - If the outer plastic package has been opened but the flow cell can not be used immediately. Store the flow cell at room temperature and use it within 24 hr. If exceeding 24 hr, it is not recommended to 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 and free of debris.
5. Clean the back of the flow cell using dust remover.

## 5.3 Preparing DNB loading mixture

Tips It is important to prepare a fresh DNB loading mixture right before the DNB loading.

Perform the steps below:

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

   Tips DNB in the above table refers to the pooled DNB in *4.4.3 DNB pooling on Page 15*.

**Table 15 DNB loading mixture**

| Adding order | Component | Cap color | volume (μL) |
|---|---|---|---|
| 1 | DNB | / | 270 |
| 2 | DNB Load Buffer II |  | 90 |
| 3 | Make DNB Enzyme Mix II (OS-V4.0) |  | 1 |

2. Combine components and mix by gently pipetting 8 times by using a wide-bore, non-filtered pipette tip. Immediately load the DNB loading mixture after mixing.

   Tips Do not centrifuge, vortex, or shake the tube.

## 5.4 DNB loading

Perform the steps below:

1. Ensure that the compartment doors of MGIDL-T7RS are closed and start the device.
2. Enter the password and select **Log in** to go to the main interface.
3. Select **A** or **B** to continue. See the figure below.

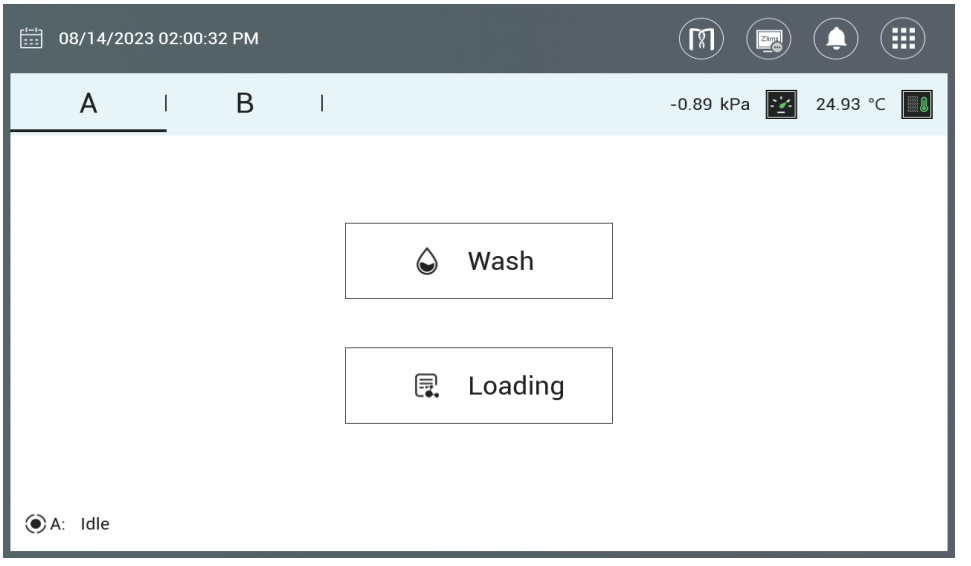


**Figure 2 MGIDL-T7RS selection interface**

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

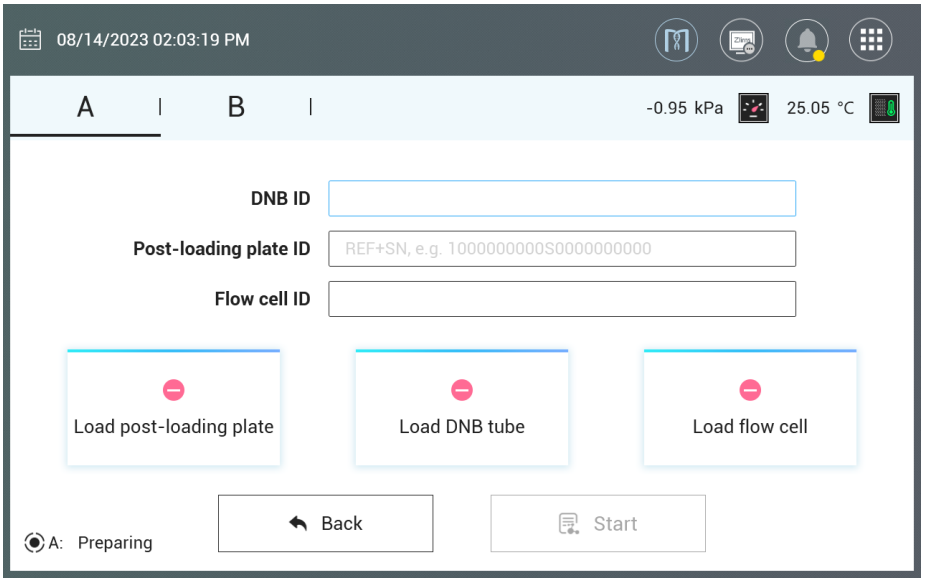


**Figure 3 MGIDL-T7RS information input interface**

5. Open the loading compartment door.
6. Select the text box next to **DNB ID**, and enter the DNB information in the text box.

   Tips 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, and a screen message will confirm that the DNB tube is loaded.

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

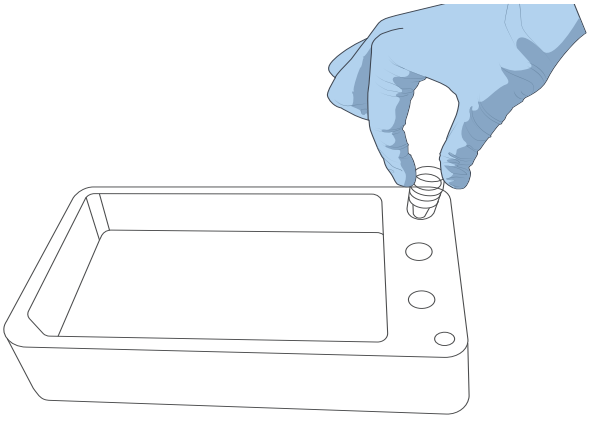

**Figure 4 Placing DNB tube**

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

   Tips
   - 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 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.

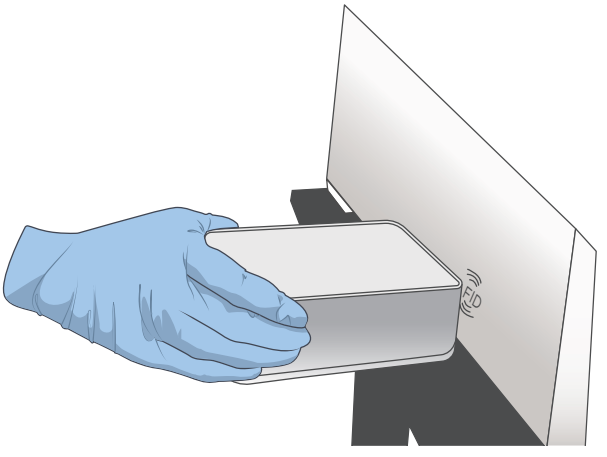

**Figure 5 RFID scanning area of Post Load Plate**

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

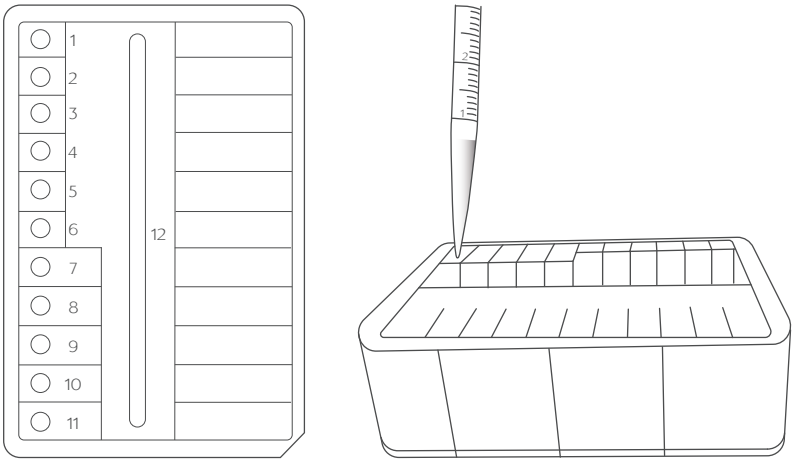


**Figure 6 Adding 4 mL of 0.1 M NaOH into well No. 11**

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

**Figure 7 Placing Post Load Plate**

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

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

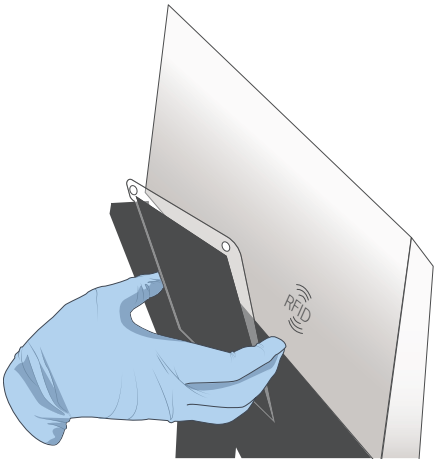


**Figure 8 Scanning the Flow cell ID**

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. Gently press the edges of the flow cell down. See the figure below.

Tips Ensure that all four sealing gaskets are on the four corners of the flow cell.

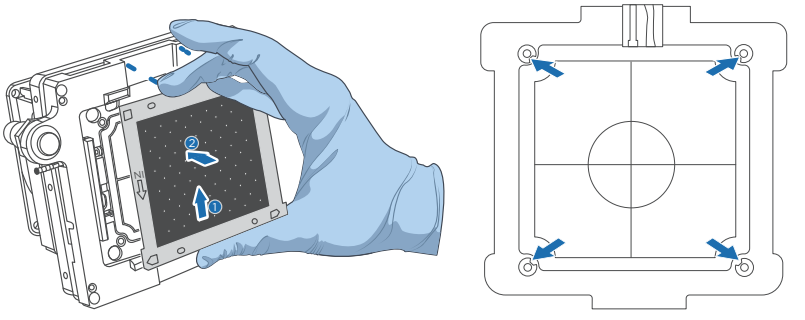

**Figure 9 Flow cell locating**

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.

**Tips**

- 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.

**WARNING** When using the canned air duster to remove the dust on the back of the flow cell, avoid blowing air into the inlet holes.

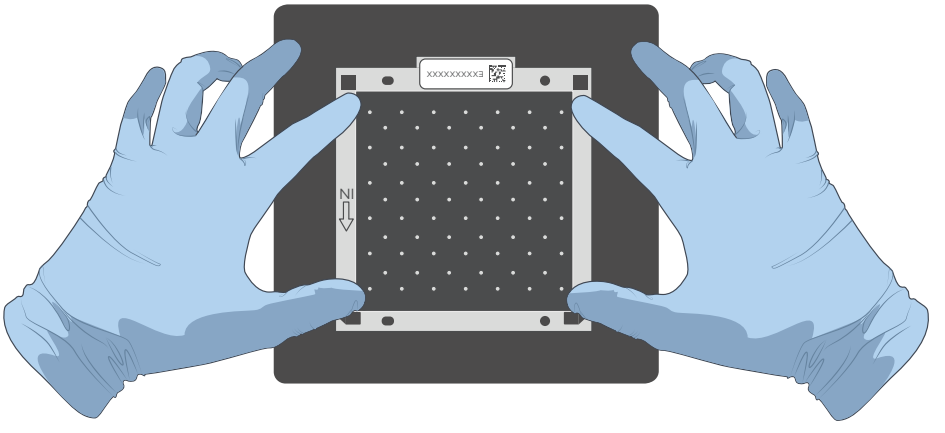

**Figure 10 Flow cell loaded**

14. Close the loading compartment door.

15. Select **Start**, confirm that Post Load Plate model in the pop-up window is correct, and then select **Yes** to start loading. Flow cell loading starts as shown in the figure below.

Tips

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

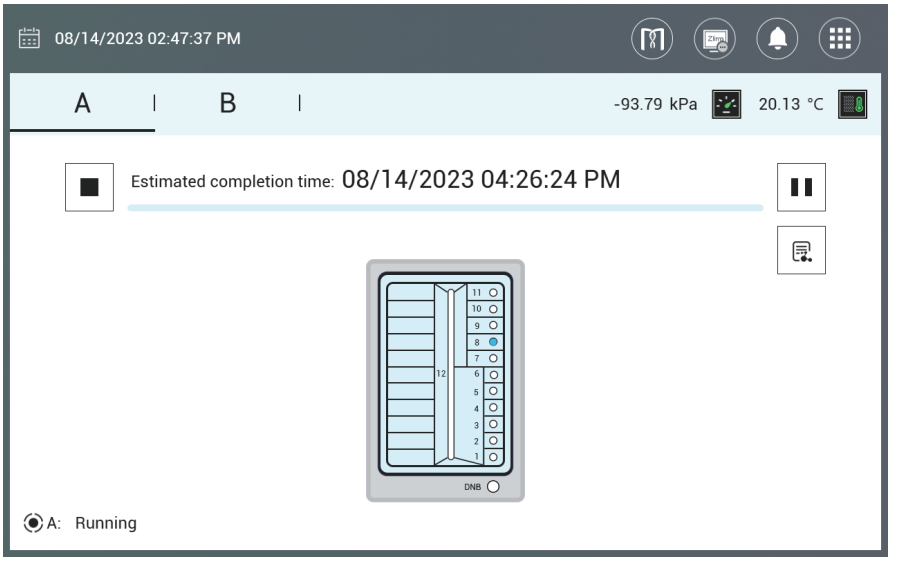


**Figure 11 MGIDL-T7RS flow cell loading interface**

16. The process takes approximately 2.5 hr. When flow cell loading is complete, the screen appears as shown in Figure 12.

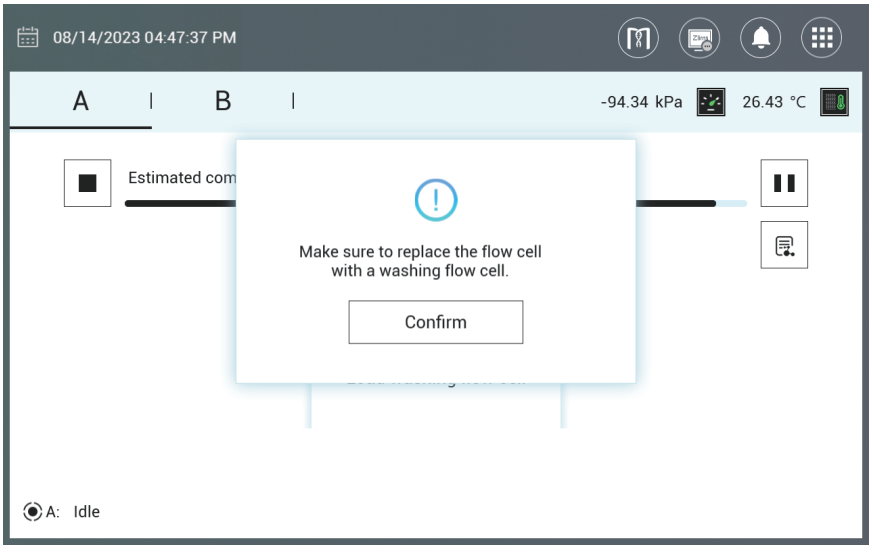


**Figure 12 MGIDL-T7RS flow cell loading complete status interface**

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

Tips

- If sequencing cannot be performed immediately, put the loaded flow cell in a clean zip bag and store it at 2 ℃ to 8 ℃ 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 12 on Page 25*.

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

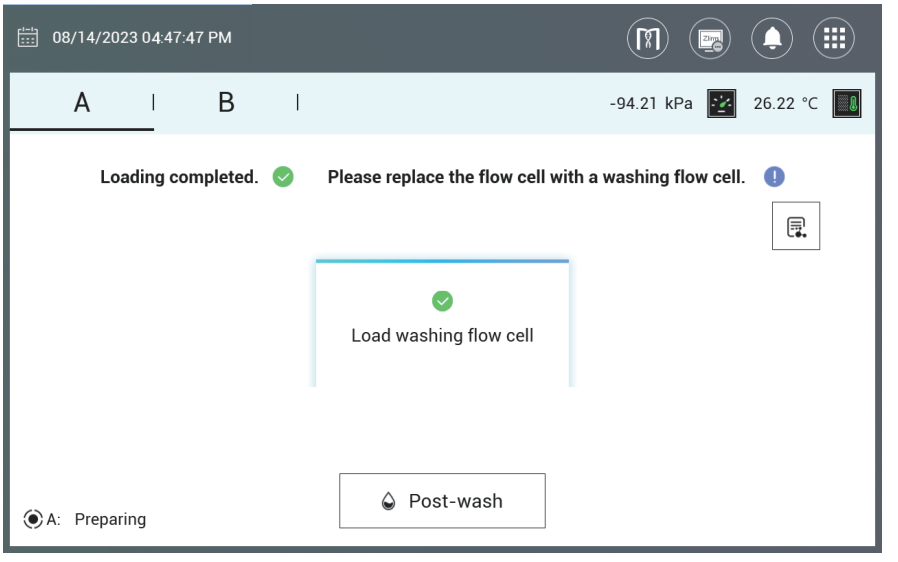


**Figure 13 MGIDL-T7RS post-wash interface**

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

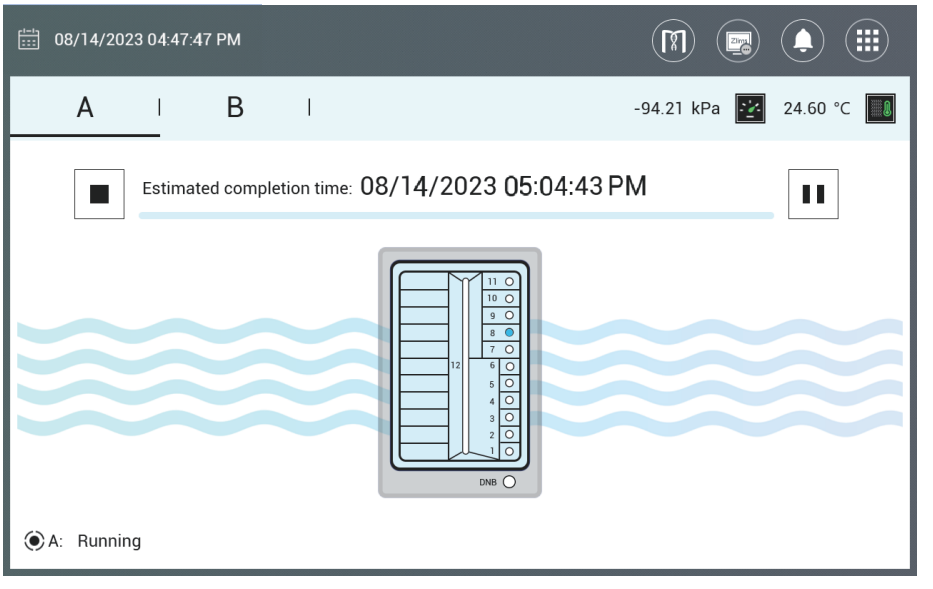


**Figure 14 MGIDL-T7RS wash interface**

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

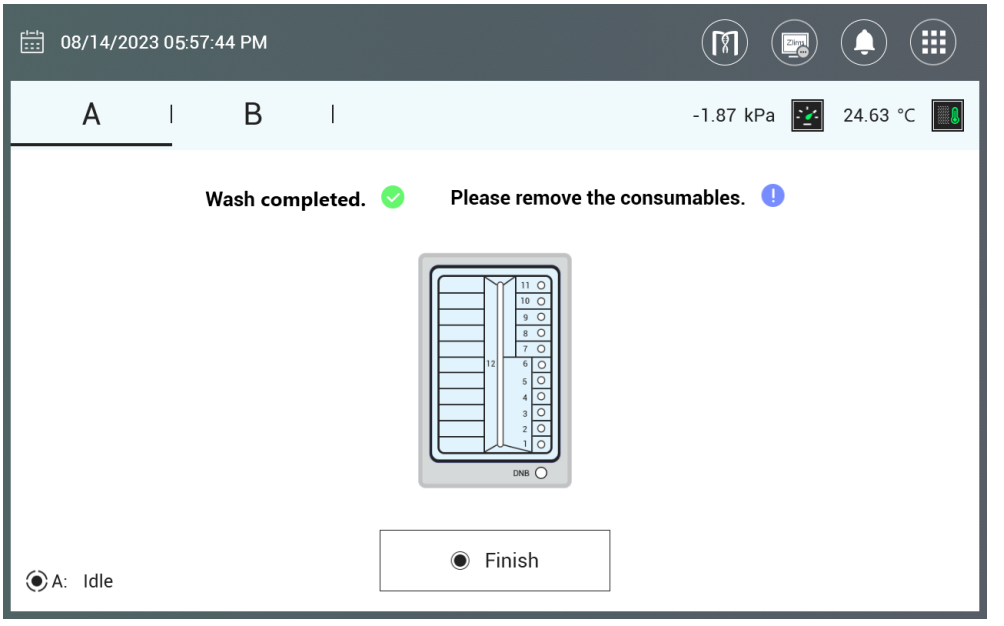


**Figure 15 MGIDL-T7RS wash complete status interface**

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 Post Load Plate into an appropriate waste container.
24. Dispose of the waste and DNB tube.

# Chapter 6 Preparation before sequencing

## 6.1 Preparing the Sequencing Reagent Cartridge

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, an appropriate amount of sequencing enzyme and dNTP mix must be added to well No. 9 and well No. 10 of the Sequencing Reagent Cartridge. Furthermore, MDA Block Reagent and MDA Block Component must be added to well No. 15. The MDA mixture (MDA, Multiple displacement amplification) must be added to well No. 8. If prepared reagent cartridges are not used immediately, refer to *9.3 Reagent kit storage on Page 50*.

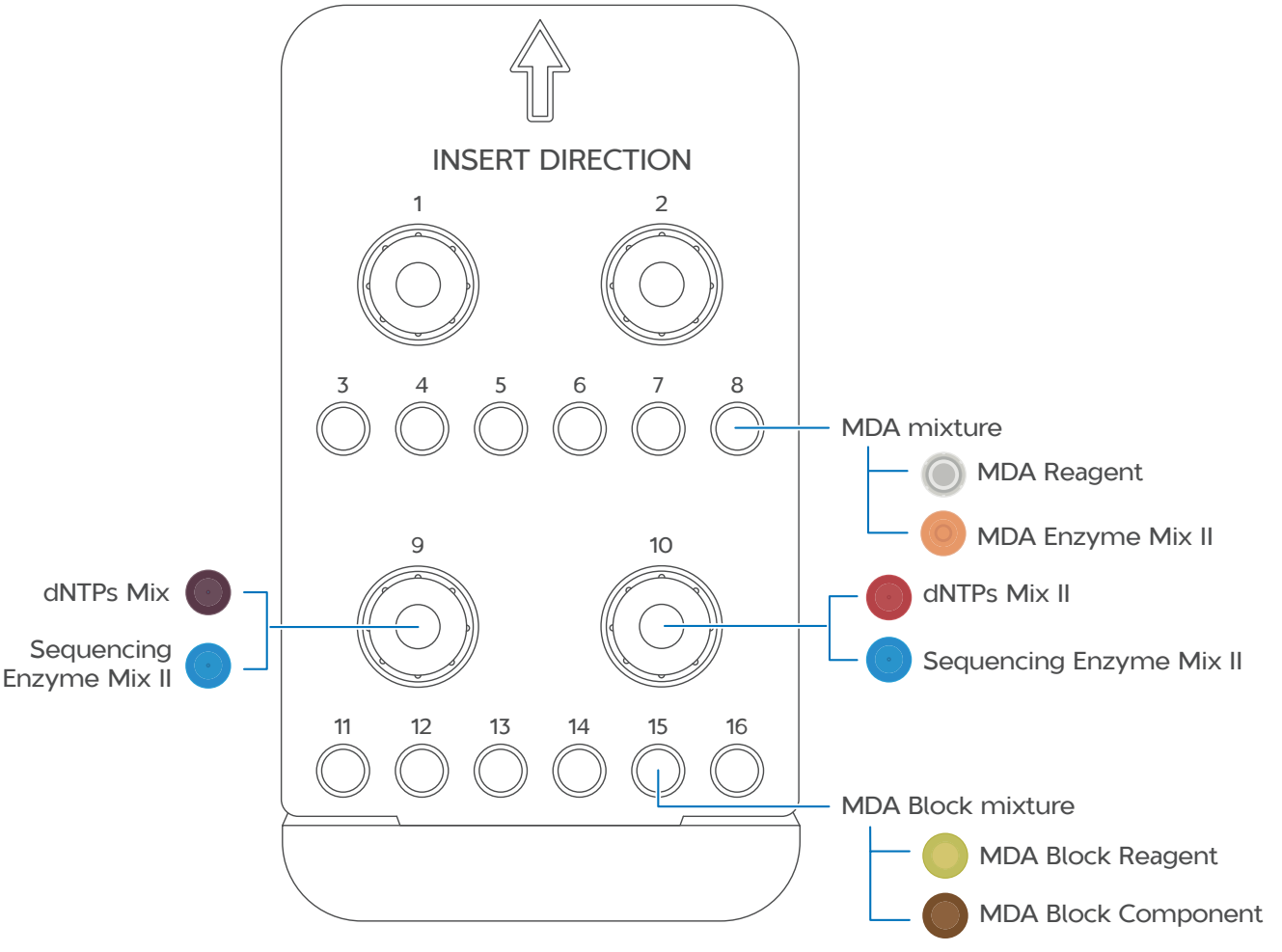


**Figure 16 Sequencing Reagent Cartridge wells**

Perform the following steps:

1. Take the Sequencing Reagent Cartridge out of DNBSEQ-T7RS High-throughput Sequencing Reagent Kit.
2. Thawing the cartridge. Perform the following steps according to different situations:
   - Thaw it in a water bath at room temperature for 2 to 3 hr.
   - Thaw it in 2 ℃ to 8 ℃ refrigerator one day in advance.
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 any water condensation on the cartridge cover and around the well with a Kimwipes tissue.

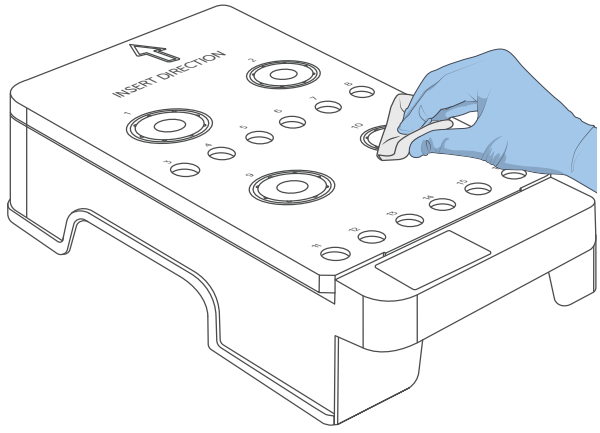


**Figure 17 Wiping cartridge cover**

6. Take dNTPs Mix, dNTPs Mix II, and MDA Block Reagent out of DNBSEQ-T7RS High-throughput Sequencing Reagent Kit and thaw them at room temperature.

**Table 16 Reagent preparation 3**

| Component | Cap color |
|---|---|
| dNTPs Mix | |
| dNTPs Mix II | |
| MDA Block Reagent | |

7. After thawing, invert the 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 High-throughput Sequencing Reagent Kit and thaw them at room temperature of 20 ℃ to 25 ℃.

   Tips To prevent refreezing, do not keep MDA Block Component on ice or at room temperature below 19 ℃ .

**Table 17 Reagent preparation 4**

| Component | Cap color |
|---|---|
| MDA Block Component |  |

9. After thawing, invert the 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 High-throughput Sequencing Reagent Kit. Invert Sequencing Enzyme Mix II 6 times and place it on ice until use.
11. Pierce the seal in the center of wells No. 9 and No. 10 to make a hole around 2 cm in diameter by using a 1 mL sterile 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 18 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 19 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 over the cartridge.
16. Lift the cartridge horizontally, hold both sides of the cartridge with both hands. Shake it clockwise 20 times, and then counterclockwise 20 times. Ensure that reagents are fully mixed.

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.

    Tips
    - 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 by using a 1 mL sterile tip. 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 all of the mixture into well No. 15.

**Table 20 Reagent preparation for well No. 15**

| Component | Cap color |
|---|---|
| MDA Block Component | |
| MDA Block Reagent | |

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

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

**Table 21 Reagent preparation for well No. 8**

| Component | Cap color |
|---|---|
| MDA Enzyme Mix II | |
| MDA Reagent | |

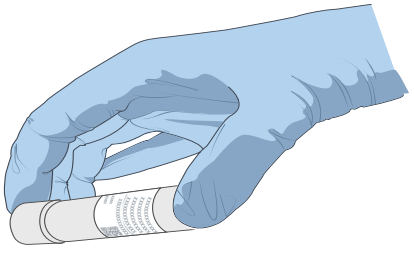

**Figure 18 MDA mixture**

## 6.2 Preparing the washing cartridge

Perform the steps below:

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 tissue. Pierce either side of well No. 2 by using a 1 mL sterile tip.

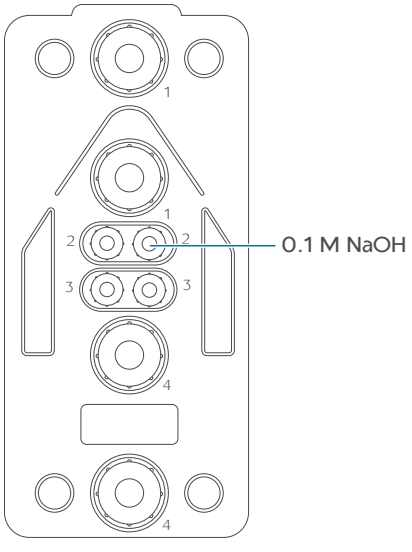


**Figure 19 Washing Cartridge**

3. Add 45 mL of 0.1 M NaOH into well No. 2 through the pierce by using an electronic pipette. Refer to *8.2.1 Preparing washing reagents on Page 44* for the preparation of 0.1 M NaOH.

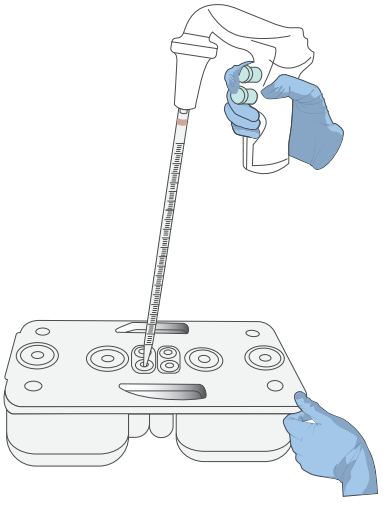

**Figure 20 Adding 0.1 M NaOH to Washing Cartridge**

## 6.3 Filling the pure water container

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

Tips

- 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.
- Refer to *H-020-000157-00 DNBSEQ-T7RS Genetic Sequencer User Manual* for the preparation of the water container.

**Table 22 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 |

# Chapter 7 Sequencing

## 7.1 Loading the reagent cartridge

Perform the steps below:

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

   Tips 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.

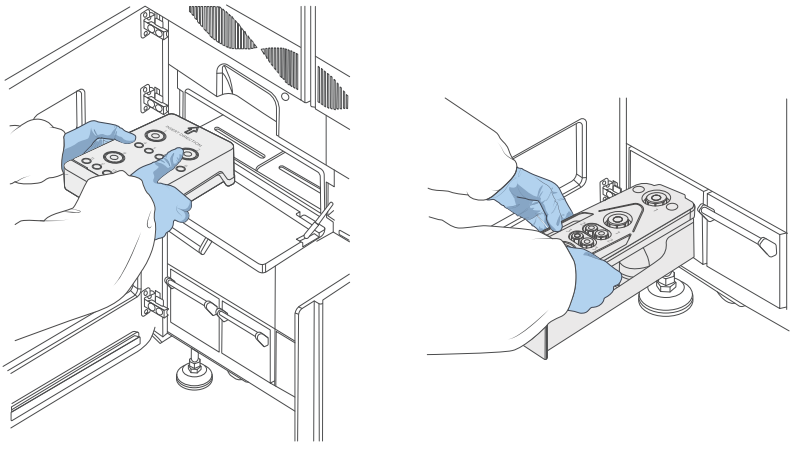

**Figure 21 Loading the cartridges**

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

## 7.2 Entering sequencing interface

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

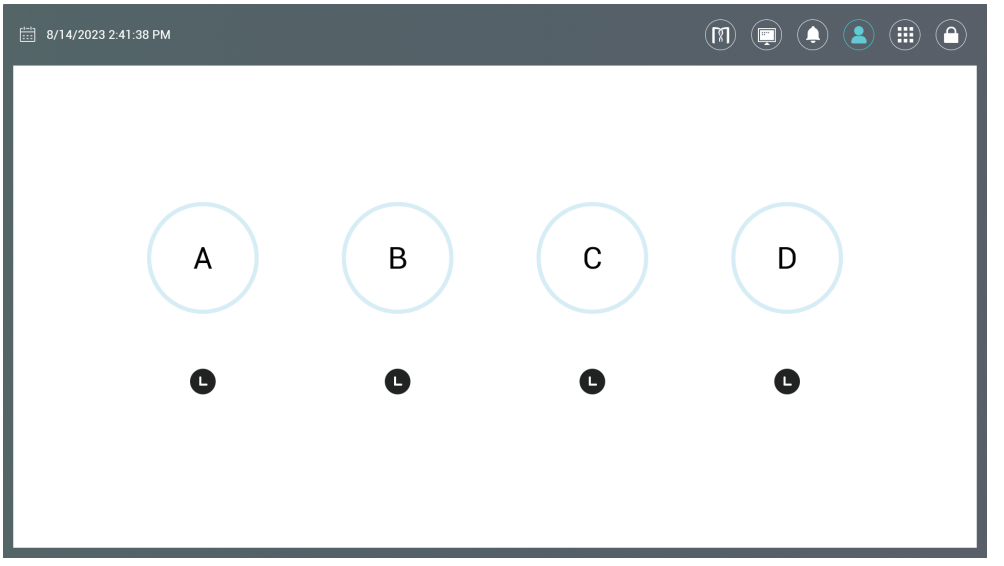


**Figure 22 DNBSEQ-T7RS main interface**

## 7.3 Loading the flow cell

Perform the steps below:

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

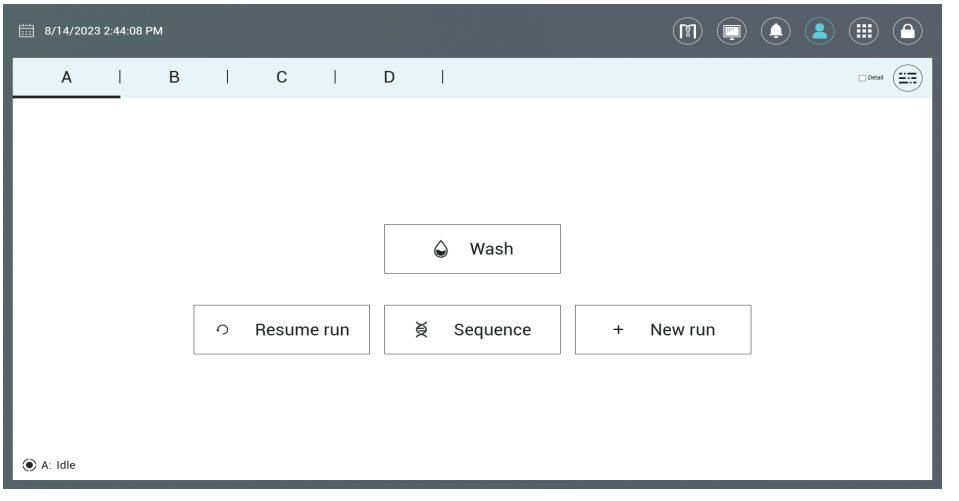


**Figure 23 DNBSEQ-T7RS selection interface**

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.

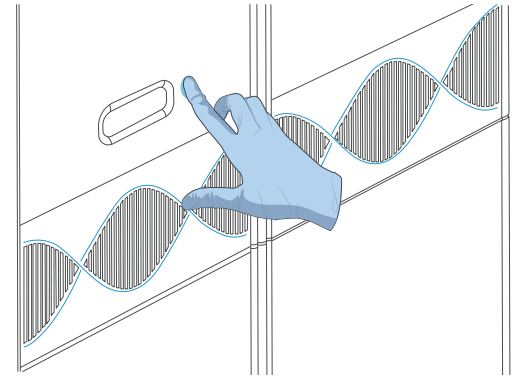

**Figure 24 The flow cell drive**

## 7.4 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.

**Tips**

- 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.

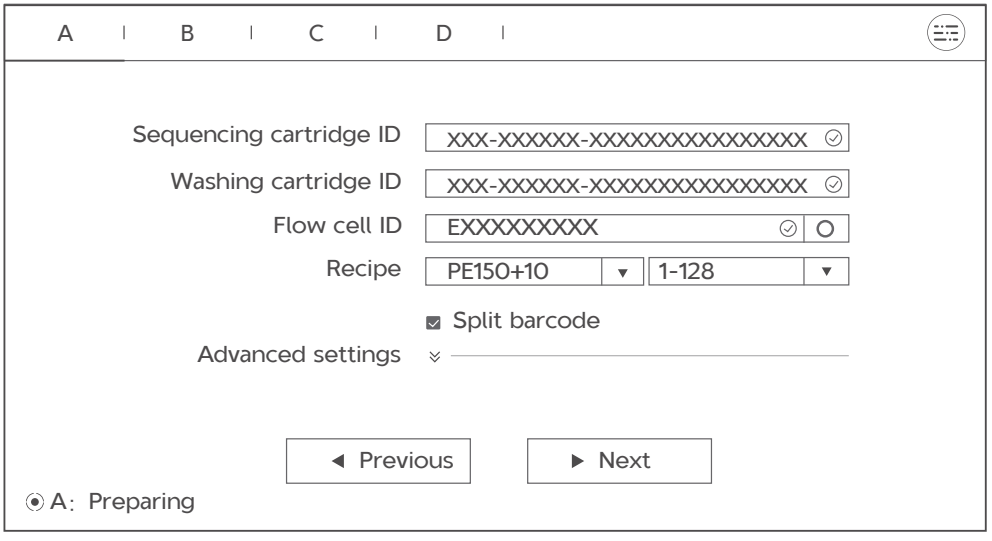


**Figure 25 DNBSEQ-T7RS sequencing parameters**

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

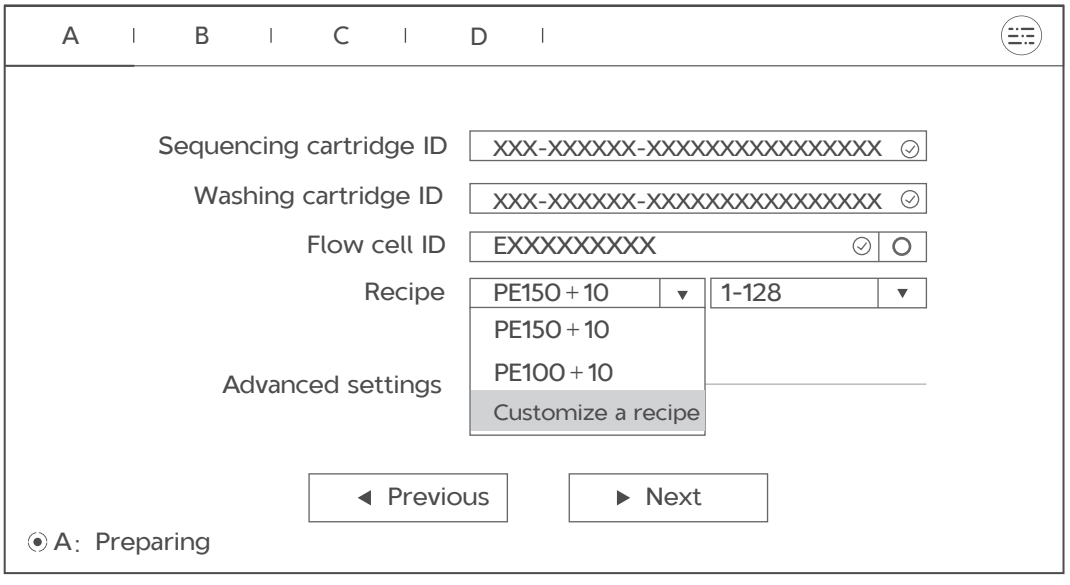


**Figure 26 Selecting Customize a recipe**

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

**Tips**

- 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.

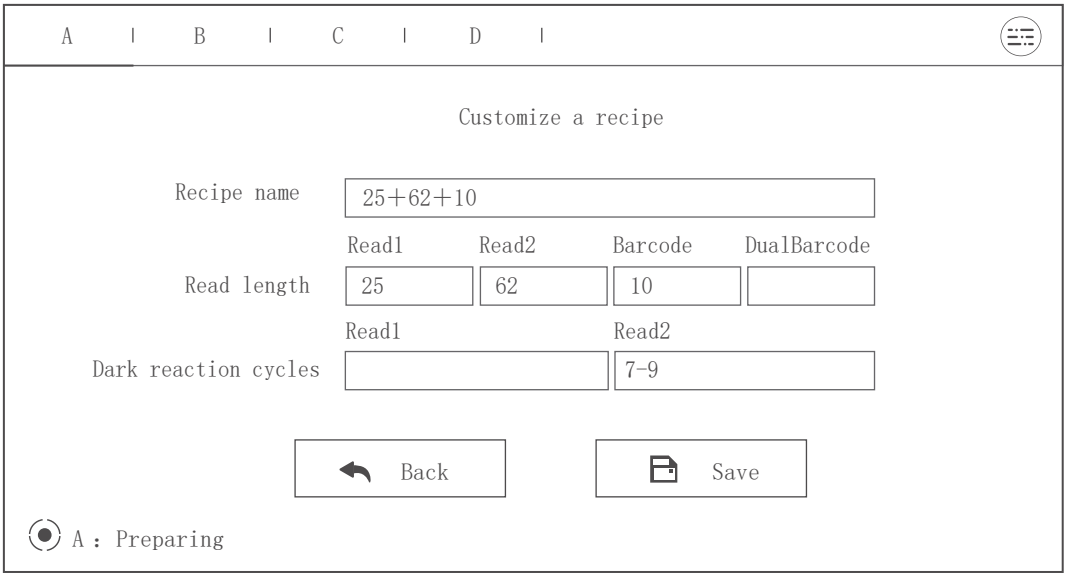


**Figure 27 Configuring customized settings for OMNI FFPE**

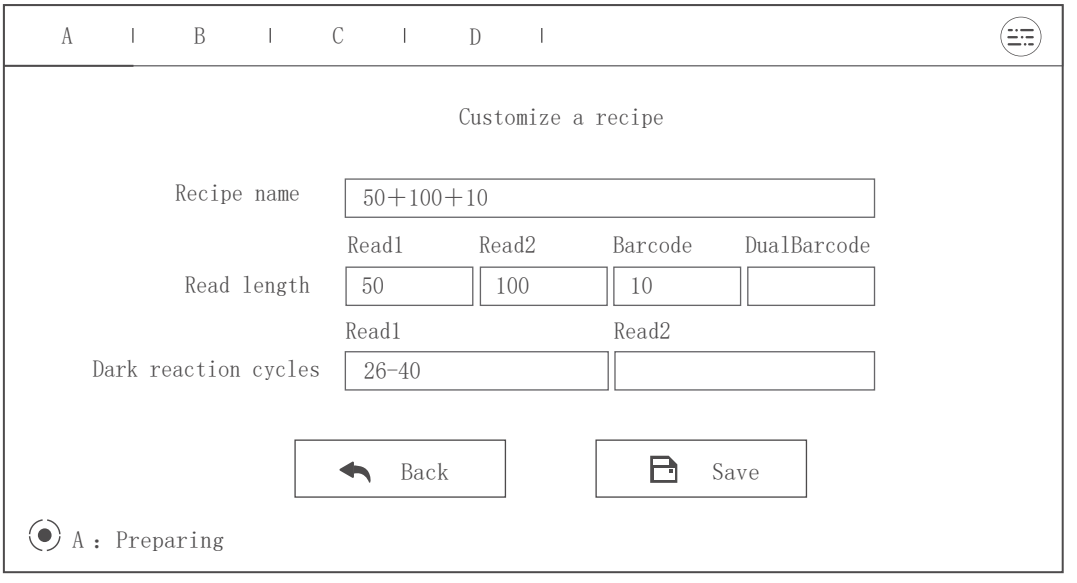


**Figure 28 Configuring customized settings for FF V1.3**

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

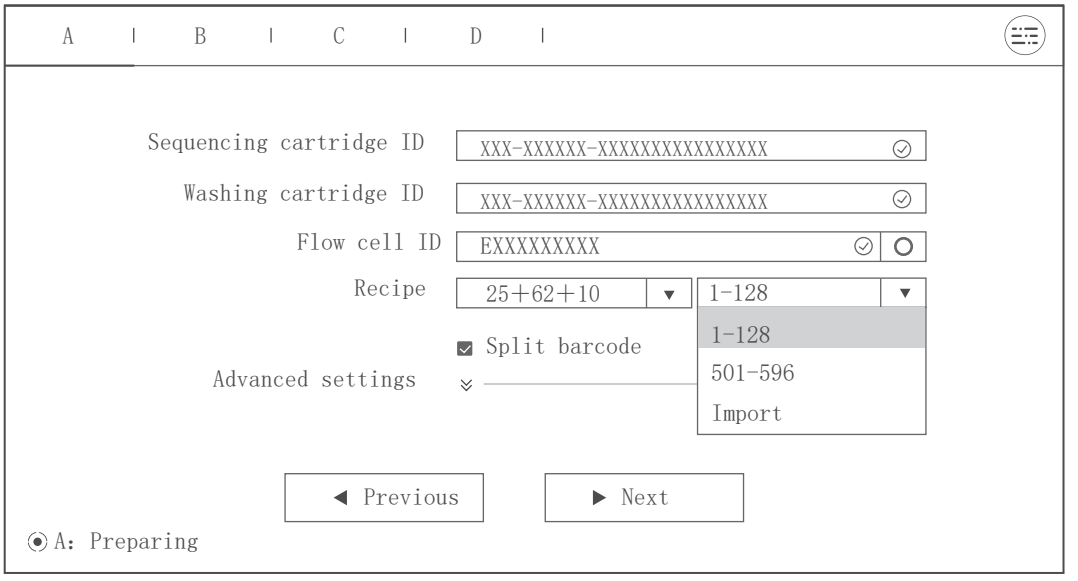


**Figure 29 Set the barcode sequence**

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.

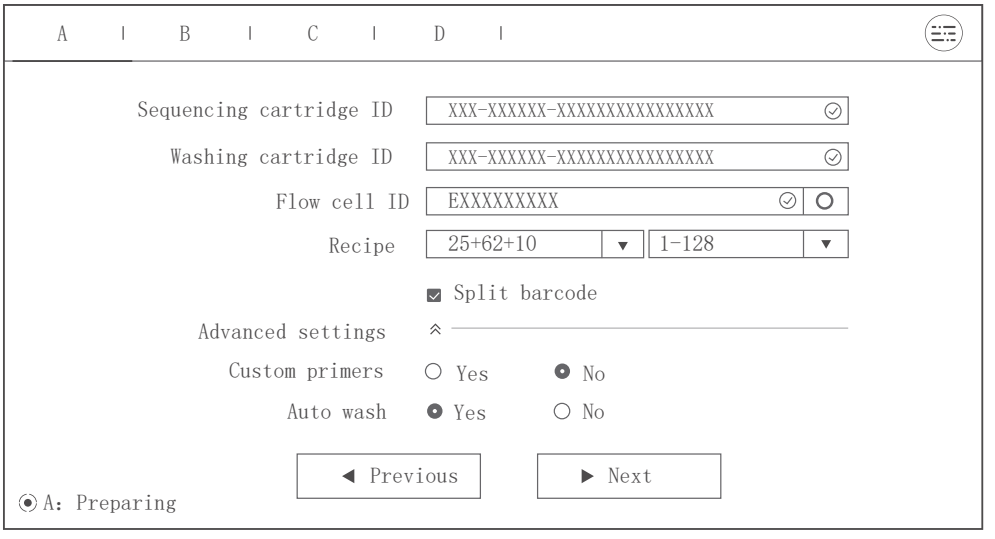


**Figure 30 DNBSEQ-T7RS advanced settings**

6. Select **Next**.

## 7.5 Reviewing parameters

Review the parameters and ensure that all information is correct.

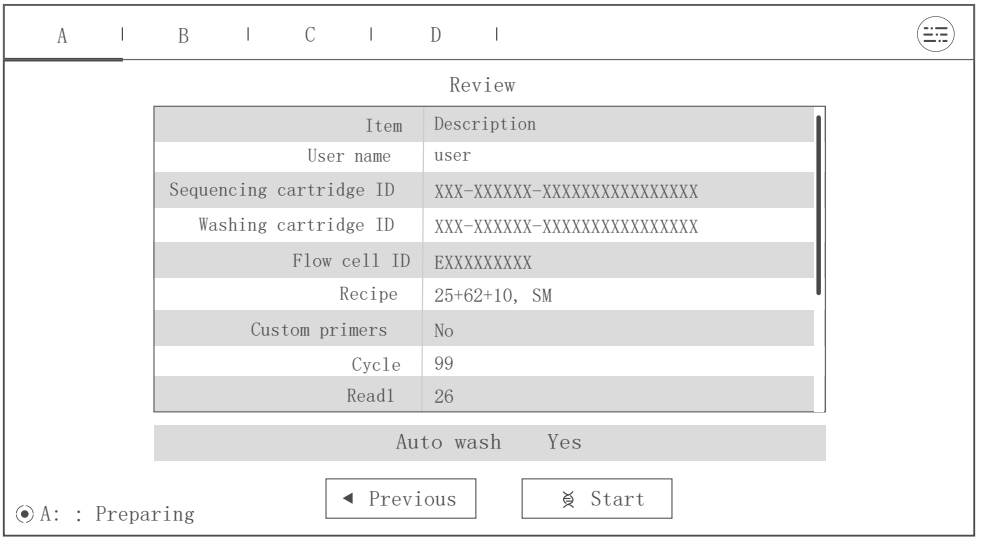


**Figure 31 Reviewing information of OMNI FFPE**

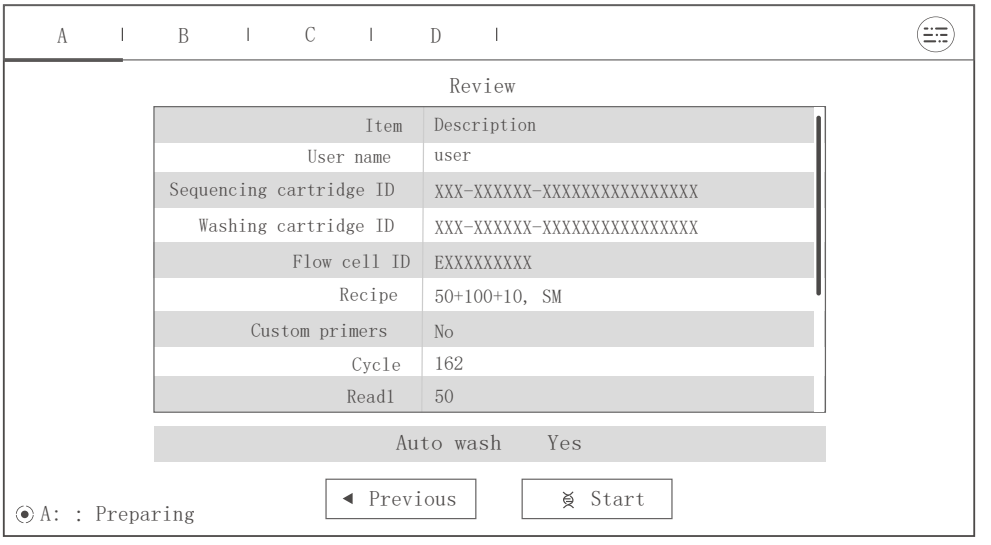


**Figure 32 Reviewing information of FF V1.3**

## 7.6 Starting sequencing

Perform the steps below:

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

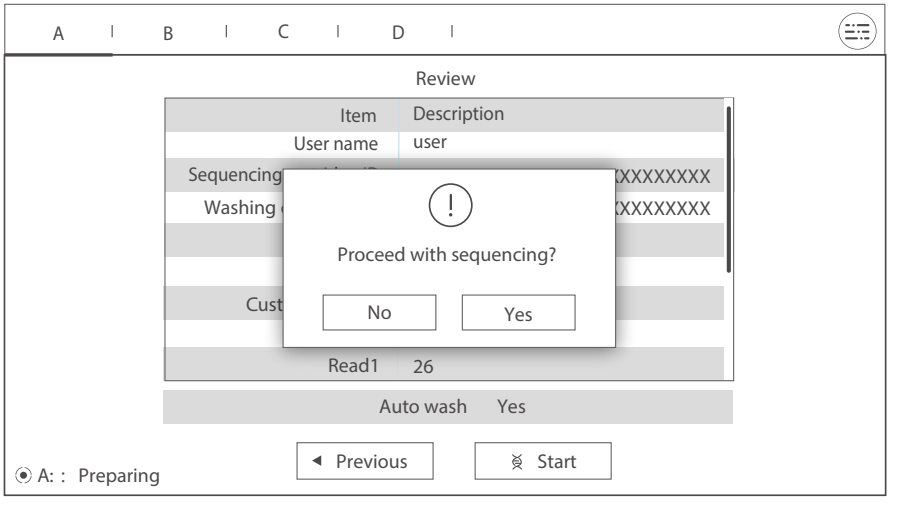


**Figure 33 Confirming sequencing interface**

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

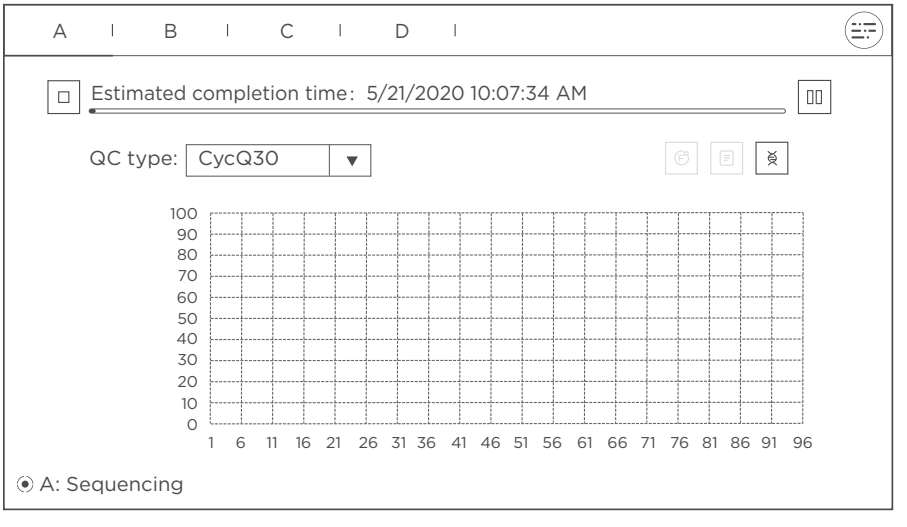


**Figure 34 Sequencing Starts Interface**

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

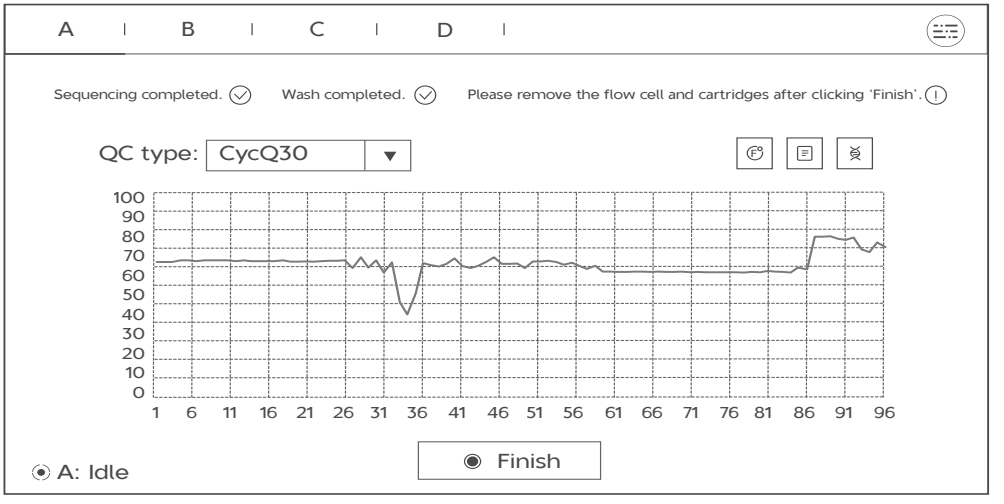


**Figure 35 Sequencing and wash complete interface**

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 guide. If errors persist, contact technical support.

## 7.7 Data access

After clicking to start sequencing, the sequencing results generated by the control software will appear in drive D.

1. The data folder named after the flow cell ID, maily contains pictures and data generated during the instrument operation (such as metrics).
2. The Result folder named after the flow cell ID, maily contains Bioinfo file and FASTQ file.

# Chapter 8 Device maintenance

## 8.1 Wash introduction

**Table 23 Wash type introduction**

| Equipment | Wash type | Cartridge type | Process time (min) | Description |
|---|---|---|---|---|
| Loader | Automatic wash | Post Load Plate | 15 | When DNB loading is complete, the loader will automatically perform the wash without the need to change the Post Load Plate. |
| | Manual wash | Post Load Plate (no Reagent)* | 20 | • The device is used for the first time.<br>• The device has not been used for 7 days or longer.<br>• Impurities are visible in the device or flow cell.<br>• Tubing, sampling needles, or other accessories exposed to the reagents were replaced. |
| 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.<br>• The device has not been used for 7 days or longer.<br>• Impurities are visible in the device or flow cell.<br>• Tubing, sampling needles, or other accessories exposed to the reagents were replaced. |

Tips Post 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 *2.2 User-supplied equipment, reagent and consumables on Page 6.*

## 8.2 Preparing a Wash

### 8.2.1 Preparing washing reagents

Prepare the washing reagents according to the table below.

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

**Table 24 Wash reagent 2: 0.05% Tween+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 ℃ | |

**Table 25 Wash 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 ℃ | |

## 8.2.2 Preparing the loader washing plate

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

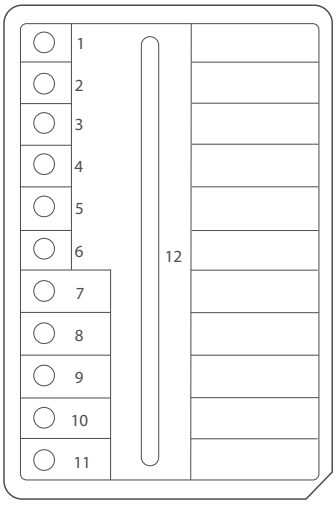


**Figure 36 Post Load Plate (no Reagent)**

Tips

- 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 by using Post Load Plate (no Reagent) according to the table below:

**Table 26 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 |

## 8.2.3 Preparing washing cartridges

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

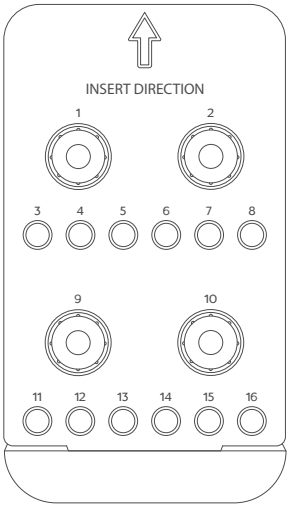


**Figure 37 T7 Sequencing Cartridge (no Reagent)**

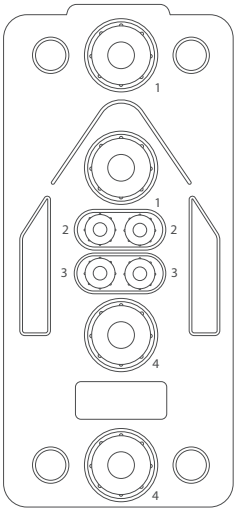


**Figure 38 T7 Cleaning Cartridge (no Reagent)**

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

**Table 27 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 |

## 8.2.4 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.

## 8.3 Wash procedures

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

### 8.3.1 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.

   Tips 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.

### 8.3.2 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.

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

### 8.3.3.1 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.

### 8.3.3.2 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.

### 8.3.3.3 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 cleaned 3 to 5 times with laboratory-grade water, used DNB load plates can be used as washing plates.

# Chapter 9 Troubleshooting

## 9.1 Low DNB concentration

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 Technical Support.

## 9.2 Forget to add reagent into well No. 8

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 *6.1 Preparing the Sequencing Reagent Cartridge on Page 28*.
   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**.

## 9.3 Reagent kit storage

- 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.

## 9.4 Abnormal negative pressure

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 Technical Support.

## 9.5 Bubbles

### 9.5.1 Bubbles in MGIDL-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 Technical Support.

### 9.5.2 Bubbles in DNBSEQ-T7RS

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

  For information on placing the water tube, refer to *6.3 Filling the pure water container on Page 33*.
- 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 Technical Support.

## 9.6 Pump fails

- Check if the pure water volume is sufficient.
- When errors occur on DL-T7RS and DNBSEQ-T7RS:
  - Remove the flow cell and check for is 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 Technical Support.

## 9.7 Impurities

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 *8.2.1 Preparing washing reagents on Page 44*, and perform a manual wash again on DL-T7RS and DNBSEQ-T7RS.
3. If the problem persists, contact Technical Support.

# Appendix 1 Qubit ssDNA assay kit

**Tips**

- Working solution should be used within 0.5 hr following preparation.
- Avoid touching the wall of tapered detection tubes.
- Avoid introducing bubbles in detection tubes.

Perform the steps below:

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.

   **Tips** 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.

   **Tips**

   - Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit assay tubes (Cat. No.: Q32856).
   - 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 standard tubes and sample tubes to be tested according to the table below.

| / | S1 (µL) | S2 (µL) | D1 (µL) | D2 (µL) | D3 (µL) |
|---|---|---|---|---|---|
| working solution | 190 | 190 | 198 | 198 | 198 |
| S1 (0 ng/µL) | 10 | / | / | / | / |
| S2 (20 ng/µL) | / | 10 | / | / | / |
| Sample | / | / | 2 | 2 | 2 |
| Tatal | 200 | 200 | 200 | 200 | 200 |

7. Mix the tubes by 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.

# Appendix 2 Manufacturer

| | |
|---|---|
| Manufacturer | Wuhan MGI Tech Co., Ltd. |
| Address | Building B13, No.818, Gaoxin Avenue, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China |
| | Building 24, Stage 3.1, BioLake Accelerator, No.388, 2nd Gaoxin Road, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China |
| E-mail | MGI-service@mgi-tech.com |
| Website | www.mgi-tech.com |

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