DNBSEQ-T7RS

High-throughput Sequencing Set User Manual

Catalog number and name:

1000016105, DNBSEQ-T7RS High-throughput Sequencing Set (FCL PE100)

Set version: V1.0

User manual version: A0

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Revision History

Version	Date	Summary of cahnge
A0	December 2019	• The first version

Table of Contents

1 Introduction

This manual explains how to perform sequencing using the DNBSEQ-T7RS High-throughput Sequencing Set and includes instructions on sample preparation, flow cell preparation, sequencing kit storage, the sequencing protocol and device maintenance.

1.1 Applications

DNBSEQ-T7RS High-throughput Sequencing Set is specifically designed for DNA or RNA sequencing on DNBSEQ-T7RS. This sequencing set is intended to be used for scientific research only and cannot be used for clinical diagnosis.

1.2 Sequencing Technology

This sequencing 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 high accuracy sequencing information.

1.3 Data Analysis

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

1.4 Sequencing Read Length

In the sequencing run, the number of sequencing cycles depends on the sequencing read length. A PE100 cycle run performs reads of 100 cycles from each end, for a total of 200 (2 × 100) cycles. At the end of the insert sequencing run, an extra 10 cycles of index read can be carried out, if required.

Table 1-1：Sequencing Cycle

Sequencing read length	Read 1 read length	Read 2 read length	Barcode read length	Total read length	Maximum cycles
PE100	100	100	10	200+10	220

Table 1-2：DNBSEQ-T7RS PE100 Theoretical Sequencing time (h)

Sequencing pattern	Single flow cell/multiple flow cell	Make DNB	Load DNB

Sequencing time	22	1	2

2 Sequencing Sets and Consumables Required but not Provided

2.1 List of Sequencing Set Components

Table 2-1：DNBSEQ-T7RS High-throughput Sequencing (FCL PE100) Cat. 1000016105

Sequencing kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-T7RS Sequencing Flow Cell Catalog number: 100016269	Sequencing Flow Cell (T7 FCL)	1 EA	RT
DNBSEQ DNB Make Reagent Kit Catalog number: 1000016115	Low TE Buffer	960 μL×1tube	-25℃~-15℃
	Make DNB Buffer	400 μL×1tube	
	Make DNB Enzyme Mix I	800 μL×1tube	
	Make DNB Enzyme Mix II（LC）	80 μL×1tube	
	Stop DNB Reaction Buffer	400 μL×1tube	
DNBSEQ-T7RS DNB Load Reagent Kit Catalog number: 1000016114	DNB Load Buffer I	300 μL×1tube	-25℃~-15℃
	DNB Load Buffer II	150 μL×1tube	
	Micro Tube 0.5mL（Empty）	1tube	
	DNBSEQ-T7 Post Load Plate	1 EA	
DNBSEQ-T7RS High-throughput Sequencing Kit (FCL PE100) Catalog number: 1000016111	dNTPs Mix II	4.50 mL×3tube	-25℃~-15℃
	dNTPs Mix IV	5.00 mL×1tube	
	Sequencing Enzyme Mix	4.75 mL×2tube	
	MDA Reagent	4.20 mL×1tube	
	MDA Enzyme Mix	0.60 mL×1tube	
	Sequencing Reagent Cartridge	1 EA	
	Transparent Sealing film	2 sheets	
DNBSEQ-T7RS Cleaning Reagent Kit (FCL PE100) Catalog number: 1000016120	Washing Cartridge	1 EA	0℃~-30℃

2.2 Equipment and Consumables Required but not Provided

Table 2-2：Equipment and Consumables Required but not Provided

Equipment and consumables	Recommended brand	Catalog number
Qubit® 3.0 Fluorometer	Thermofisher	Q33216
PCR machine	Bio-Rad	/
MPC2000 96-well plate centrifuge	/	/
Pipette	Eppendorf	/
Electronic pipette	Labnet	FASTPETTEV-2
Mini centrifuge	Major Laboratory Supplier (MLS)	/
Vortex mixer	MLS	/
2~8℃ refrigerator	MLS	/
-18~-25℃ freezer	MLS	/
Qubit® ssDNA Assay Kit	Thermo Fisher	Q10212
2 M NaOH solution	Aladdin	S128511-1L
100%Tween-20	BBI	A600560-0500
5 M NaCl solution	SIGMA	S5150-4L
75% Ethanol	/	/
Power Dust remover	MATIN	M-6318
Sterile pipette tip(box)	AXYGEN	/
5 mL Sterile pipette tip(box)	AXYGEN	/
200 μL Wide-Bore Pipette Tips	AXYGEN	T-205-WB-C
Qubit Assay Tubes	Thermo Fisher	Q32856
0.2 mL PCR 8-tube strip	AXYGEN	/
1.5 mL Eppendorf	AXYGEN	MCT-150-C
Ice rack	MLS	/
100 mL Serological pipet	CORNING	4491
25 mL Serological pipet	CORNING	4489
10 mL Serological pipet	CORNING	4488
25 mL Sterile tube	SARSTEDT	60.9922.243
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	/

3 Sequencing Workflow

1	Make DNB: use DNB Make Reagent Kit to make DNB
2	Load DNB: load DNB into the flow cell using DNB Load Reagent Kit on MGIDL-T7 loader
3	Prepare a new sequencing kit： inspect, thaw the reagent kit and then load and mix the required reagents, as well as fill the pure water container.
4	Sequencing
5	Data analysis

4 Library Preparation

4.1 Insert Size Recommendation

This sequencing set is compatible with the libraries prepared by MGI Library Prep Kits. Library refers to single stranded circular DNA (ssDNA).

Library recommendation for insert size:

The size distribution of inserts should be between 100-500 bp, with the main band centered within ±100bp. If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.

Table 4-1：Suggested Insert Size*

Sequencing kit	Suggested insert size distribution, bp	Data output**, Tb/flow cell
DNBSEQ-T7RS FCL PE100	200 ~ 400	~ 1.0

*Consider the insert size and the required data output when selecting a sequencing kit.

**Average data output will vary with different library types and applications.

4.2 Library concentration and amount requirement

Library requirement is subject to the corresponding library preparation kit user manual. For general libraries, the ssDNA library concentration should be ≥ 2 fmol/μL. If the library concentration is unknown, it is recommended to perform ssDNA library quantitation (ng/μL) using Qubit® ssDNA Assay Kit and Qubit® Fluorometer. Use the following equation to convert the concentration of the ssDNA library from ng/μL to fmol/μL.

Concentration (fmol/μL) = 3030 * Concentration (ng/μL) / N

N represents the number of nucleotides (total library length including the adaptor).

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

The DNBSEQ-T7RS sequencer can simultaneously perform sequencing of 4 flow cells and each flow cell can theoretically produce 5000M reads. For PE100 sequencing, one flow cell can produce 1 Tb data in theory.

The samples that can be pooled together are determined by the data output required for specific applications, sequencing read length, barcodes and other information. It is recommended to quantify DNB before DNB pooling.

Example 1:

Human Whole-genome Sequencing (WGS): When performing PE100 sequencing, 8 samples on each flow cell is recommended. For sequencing with special requirements regarding sequencing depth, sample pooling number can be increased or reduced appropriately.

Sequencing read length	Pooling number*	Theoretical average data output of each sample	Theorical data output range of each sample
PE100	8 samples with different barcode	120 Gb	108 Gb – 132 Gb

* Assuming the difference of sample pooling is within ±10%.

For other applications, the samples that can be pooled together are determined by the total data output of each flow cell, required data output of each sample and the possible error between sample pooling.

Example 2:

Assuming one application requires 50 Gb data for each sample and the sample pooling difference is within ±10%. When performing PE100 sequencing, the calculation is shown in the table below:

Sequencing read length	Each sample requires	Pooling number	Theorical data output range of each sample
PE100	50 Gb	18 samples with different barcode	50 Gb – 61 Gb

The maximum number of sample pooling = Total data output of one flow cell*(1-pooling difference)/ Required data for the application:

= 1000 Gb*(1-10%)/ 50 Gb

= 18 samples

4.4 Make DNB

4.4.1 Prepare Reagents for DNB Making

Place the library on ice until use. Remove Make DNB Buffer, Low TE Buffer and Stop DNB Reaction Buffer from

storage and thaw reagents at room temperature. Thaw Make DNB Enzyme Mix I for approximately 0.5 hours on ice. After thawing, mix reagents using a vortex mixer for 5 seconds, centrifuge briefly and place on ice until use.

Note:

Mixed use of reagent components from different batches is strictly prohibited.

4.4.2 Calculate the Required Amount of ssDNA Library

Make DNB separately for each sample. The volume of each Make DNB reaction is 100 μL and the ssDNA input is 40 fmol. The required amount of ssDNA library is determined by the library concentration quantified in section 4.2. The required loading volume for one flow cell is 270 μL.

Note:

All samples should be considered potentially infectious and should be handled in accordance with relevant national regulations.

4.4.3 Make DNB

Take a 0.2 mL 8-tube strip or PCR tubes and prepare the reaction mix on ice following the table below. The following table only gives illustration of one make DNB reaction. The required number of make DNB reactions is determined by the actual application.

Table 4-2：Make DNB Reaction 1

Component	Volume (μL)
ssDNA libraries	V *
Low TE Buffer	20-V
Make DNB Buffer	20
Total Volume	40

*: V=40 fmol / ssDNA Concentration (fmol/μL)

- Mix the Make DNB reaction 1 thoroughly by vortexing and centrifuge for 5 seconds using a mini centrifuge. Place the mix into a PCR machine and start the primer hybridization reaction. PCR machine settings are described in the table below:

Table 4-3：Primer Hybridization Reaction Conditions

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

- Remove the Make DNB Enzyme Mix II(LC) from storage and place on ice. Centrifuge briefly for 5 s and place on ice until use.

Note：

Do not place Make DNB Enzyme Mix II（LC）at room temperature and avoid holding the tube for a prolonged time .

- Take the PCR tube out of the PCR machine when the temperature reaches 4℃. Centrifuge briefly for 5 s, then place the tube on ice and add the following reagents to the tube.

Table 4-4：Make DNB reaction 2

Component	volume (μL)
Make DNB Enzyme Mix I	40
Make DNB Enzyme Mix II（LC）	4

- Mix thoroughly by vortexing, centrifuge for 5 s using mini centrifuge and place tubes into PCR machine for the next reaction. The conditions are shown in the table below:

Table 4-5：Rolling Circle Amplification Conditions

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

- Immediately add 20 μL Stop DNB Reaction Buffer once the temperature reaches 4 ℃. Mix gently by pipetting 5-8 times using a wide bore tip. Do not vortex, shake the tube or pipette vigorously.

Note:

It is very important to mix DNB gently using a wide bore pipette tip. Do not centrifuge, vortex, pipette vigorously or shake the tube. Store the DNB at 4℃ and perform sequencing within 48 hours.

Note:

① As some PCR machines are slow in temperature adjustment, when the heated lid is being heated or cooled, the sample block may remain at room temperature and the procedure is not performed. For these types of PCR machines, pre-heating of the heated lid is required to ensure that the heated lid is at working temperature during the DNB reaction.

② It is recommended to set the temperature of the heated lid to 35℃ or the temperature closest to 35℃.

4.5 Quantify DNB and Pooling

4.5.1 Quantify DNB

When the DNB making is complete, use Qubit® ssDNA Assay Kit and Qubit® Fluorometer to quantify the DNB. Sequencing requires the DNB concentration to be above 8 ng/μL. If the concentration is lower than 8 ng/μL, make a new DNB.

Note:

① Because DNB is viscous, it is recommended to take 2 μL for quantification. If the number of samples is large, it is recommended to quantify in batches to avoid inaccurate DNB quantification due to fluorescence quenching.

② If the concentration exceeds 40 ng/μL, the DNB need to be diluted to 20 ng/μL with DNB Load Buffer I before loading.

③ The DNB can be stored at 4℃ and sequencing should be performed within 48 hours

4.5.2 DNB Pooling

Calculate the DNB pooling volume of each sample according to the required data output and DNB concentration. The total DNB volume required for each flow cell is 270 μL. According to DNB concentration, take the pooling of 8 samples as an example, if each sample requires the same data output, please follow the instructions below to calculate the pooling volume of each sample:

1. Assuming the DNB concentrations of the 8 samples are C1, C2……C8, calculate the sum of concentration reciprocals:

Sum of concentration reciprocals = 1/C1+1/C2+……+1/C8

2. Calculate the Parameter B:

Parameter B = 270/ Sum of concentration reciprocals

3. Calculate the pooling volume of each sample:

Pooling volume of sample 1: Parameter B /C1

Pooling volume of sample 2: Parameter B /C2

.....

Pooling volume of sample 8: Parameter B /C8

5 Load DNB

5.1 Prepare the Post Load Plate and Buffers

5.1.1 Thaw the Post Load Plate

Remove the Post Load Plate from the DNBSEQ-T7RS DNB Load Reagent Kit and thaw in a room temperature water bath for 1-2 h. When the Post Load Plate is thoroughly thawed, place in 2-8°C refrigerator until use (or thaw in 2-8°C refrigerator one day in advance). Gently invert the Post Load Plate to mix for 5 times to mix and then centrifuge for 1 min.

5.1.2 Prepare the DNB Loading Reagents

Remove DNB Load Buffer II from the DNBSEQ-T7RS DNB Load Reagent Kit and thaw reagents in room temperature water bath for approximately 0.5 hours. After thawing, mix reagents using a vortex mixer for 5 seconds, centrifuge briefly and place on ice until use. If crystal precipitation is found in DNB Load Buffer II, vigorously mix the reagent with 1-2 min of continuous vortexing to re-dissolve the precipitate before use.

- Take a 0.5 mL microfuge tube and add reagents following the table below.

Table 5-1：DNB Loading Mix

Component	volume (μL)
DNB Load Buffer II	90
DNB*	270
Make DNB Enzyme Mix II（LC）	1

*: DNB in the above table refers to the pooled DNB in 4.5.2.

- Gently pipette the DNB loading mix for 5-8 times using a wide bore tip. Do not centrifuge, vortex, vigorously pipette or shake the tube. Place the mixture at 4°C until use.

Note：

Prepare a fresh DNB loading mix before the sequencing run.

5.2 Prepare the Sequencing Flow Cell

- Remove the flow cell from the DNBSEQ-T7RS sequencing flow cell box.
- Unwrap the outer package.
- Remove the flow cell from the inner package and inspect if the flow cell is intact.
- Clean the back of the flow cell using dust remover.

5.3 DNB Loading

When starting the MGIDL-T7 for the first time, the compartment doors need to be closed.

Start the MGIDL-T7 program. Enter the user name "user" and password "123", click "Log in" to enter the main interface, see Figure 5-1:

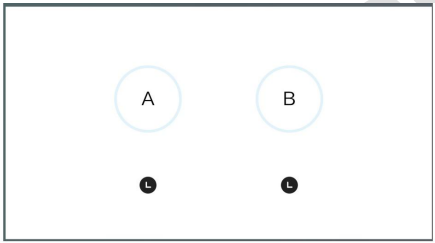

Figure 5-1：MGIDL-T7 Main Interface

- Click on “A” or “B” to continue the operation, see Figure 5-2：

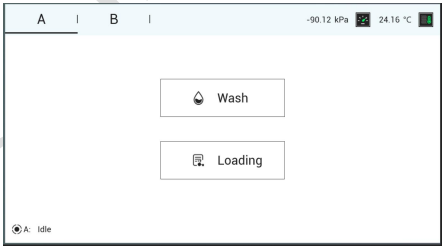

Figure 5-2：MGIDL-T7 Selection Interface

- Click on “Loading” and enter the information input interface, see Figure 5-3:

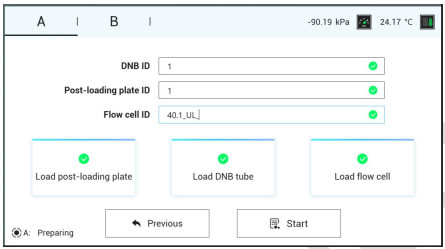

Figure 5-3: MGIDL-T7 Information Input Interface

- Open the loading compartment door
- Click on the text box behind “DNB ID” (see Figure 5-3), align the 0.5mL micro tube containing DNB loading mix to the RFID scanning area and ID information will appear in the text box. Place the 0.5mL micro tube containing DNB loading mix into the DNB tube hole (see Figure 5-4), the screen will prompt that the DNB tube is loaded.
- Click on the text box behind “Post-loading plate ID” (see Figure 5-3), align the post load plate to the RFID scanning area and ID information will appear in the text box. Remove the seal of the post load plate, add 4 mL of 0.1M NaOH into well No.11 (see Figure 5-4). Refer to section “9.3 Prepare wash reagents” for the preparation of 0.1M NaOH. Place the prepared post load plate on the plate tray of MGIDL-T7 (see Figure 5-4), the screen will prompt that the post load plate is loaded.

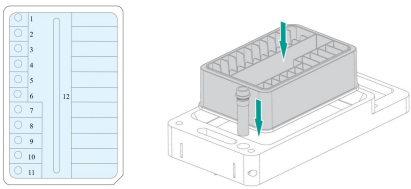

Figure 5-4 Post-loading Plate

- Click on the text box behind “Flow cell ID” (see Figure 5-3), align the flow cell to the RFID scanning area and ID information will appear in the text box. Hold both sides of the flow cell, upwardly align the locating bulge on the flow cell to the locating groove on the flow cell stage. Gently press down the edges of the flow cell (see Figure 5-5).

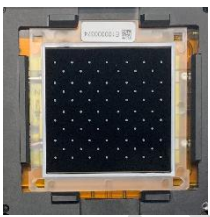

Figure 5-5 Flow Cell

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

ⓘ **Note：**

DO NOT press or touch the glass cover of the flow cell to avoid flow cell damage or fingerprints and impurities left on the glass surface. DO NOT move the flow cell after installing the flow cell onto the stage, or it may cause the sealing gaskets to misalign with holes of the fluidics line. If flow cell attachment fails, gently wipe the back of the flow cell and flow cell stage with microfiber clean wiper moistened with 75% ethanol, then clean with a dust remover.

- Close the loading compartment door.
- Click the “Start” button and select “Yes” as shown in Figure 5-6:

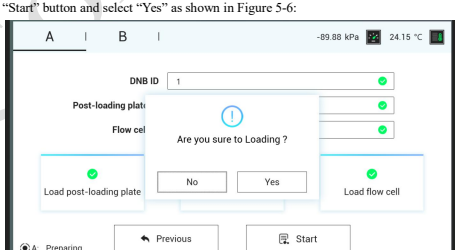

Figure 5-6: MGIDL-T7 loading Confirmation Dialog Box

- Flow cell loading starts as shown in Figure 5-7:

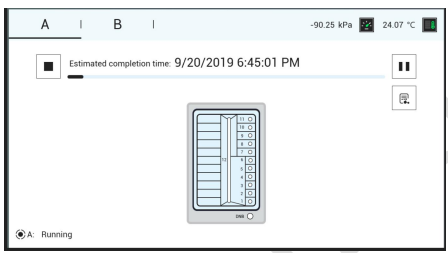

Figure 5-7: MGIDL-T7 Flow Cell Loading Interface

- When the screen is shown as Figure 5-8, flow cell loading is complete. The process will take around 1hour and 40 min.

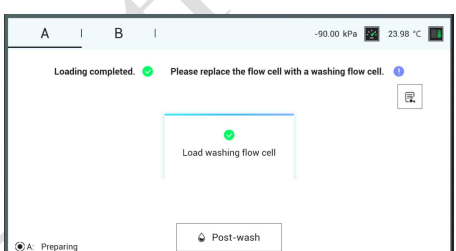

Figure 5-8: MGIDL-T7 Flow Cell Loading Complete Status Window

- Press the Flow Cell attachment button and remove loaded flow cell from the stage. The flow cell is now ready for sequencing. If sequencing cannot be performed immediately, store the loaded flow cell in 4℃ until use.

Note:

Loaded flow cell must be used within one week.

- When the loading is completed, install the washing flow cell onto the flow cell stage and press the flow cell attachment button.
- Click “Post-wash” and select “Yes” to start MGIDL-T7 wash (see Figure 5-9), which will take around 20 min.

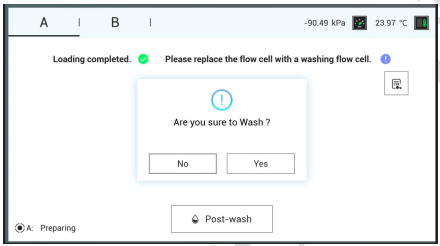

Figure 5-9: MGIDL-T7 Post-wash Confirmation Interface

- MGIDL-T7 wash starts, see figure 5-10:

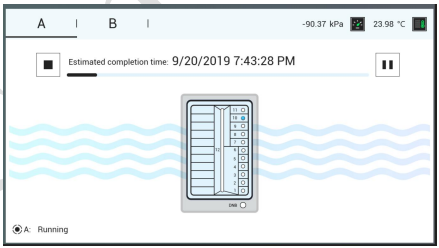

Figure 5-10：MGIDL-T7 Wash Interface

- When the screen is shown as Figure 5-11, the wash is completed. Click “Finish” and another flow cell

loading can be performed.

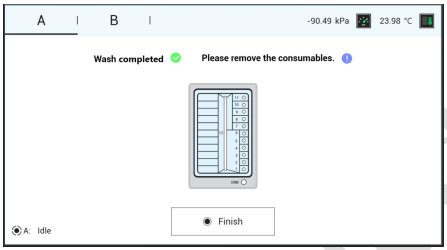

Figure 5-11: MGIDL-T7 Wash Complete Status Window

6 Preparation Before Sequencing

6.1 Prepare the PE100 Sequencing Cartridge

- Remove the Sequencing Reagent Cartridge from the DNBSEQ-T7RS High-throughput Sequencing Kit. Thaw in a room temperature water bath for 3-4 hours until thoroughly thawed. Store at 2-8℃ until use (or thaw cartridge in 2-8℃ fridge one day in advance). Shake the cartridge vigorously in all directions for 10-20 times until no visible layers can be seen in the cartridge, especially for well No.1 and No.2.

  Note:

  If dark green crystal appears in well No.1, it is precipitation of raw materials of the reagent in well No.1. This is a normal phenomenon. When the cartridge is thawed, mix the reagents in the cartridge well and the crystals will dissolve. Sequencing quality will not be affected.

- Before using the cartridge, dNTPs Mix IV, dNTPs Mix II and Sequencing Enzyme Mix need to be added into wells No.9 and No.10. The mixture of MDA Enzyme Mix and MDA Reagent will need to be added into well No.8. Well positions are shown in Figure 6-1.
- Remove dNTPs Mix IV and dNTPs Mix II from the sequencing kit and thaw at room temperature. After thawing, mix reagents using a vortex mixer. Invert the Sequencing Enzyme Mix for 4-6 times to mix. Centrifuge briefly and place on ice until use.
- Open the kit cover and wipe any water condensation with lint-free paper. Spray 75% ethanol on the surface of cartridge seal and clean the seal with lint-free paper. Pierce the seal at the edge of well No.9 and No.10 to make a hole around 2 cm in diameter using 1 mL sterile tip.

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix IV into a new 15 mL or 25 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix IV in that tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.9:

Table 6-1：PE100 sequencing cartridge well No.9 reagent loading

Sequencing kit	dNTPs Mix IV loading volume	Sequencing Enzyme Mix loading volume
PE100	5.0 mL	5.0 mL

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix II into a new 25 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix II in that tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.10:

Table 6-2：PE100 sequencing cartridge well No.10 reagent loading

Sequencing kit	dNTPs Mix II loading volume	Sequencing Enzyme Mix loading volume
PE100	13.5 mL	4.5 mL

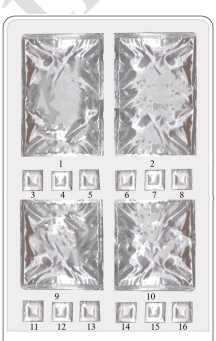

Figure 6-1: Well Position

- Seal the loading wells of well No.9 and No.10 with the transparent sealing film. Do not cover the center of the well to avoid blocking the sampling needle.
- Place the cartridge horizontally on the table, and hold both sides of the cartridge with both hands. Shake it clockwise 10-20 times, and then counterclockwise 10-20 times, until the reagent color in well No.9 is even. Make sure that you see the vortex to ensure reagents are fully mixed.
- Pierce the seal of well No.8 using 1 mL sterile tip. Add 600 μL of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette. Invert the tube for 4-6 times to mix the reagents, then add the mixture to well No.8. When adding the mixture, make sure there are no bubbles at the bottom of the tube. The PE100 sequencing cartridge is now ready to be used.

ⓘ **Note:**

When using MDA Enzyme Mix, do not touch the wall of the tube to prevent influencing the enzyme activity.

6.2 Prepare the Washing Cartridge

- Shake the cartridge clockwise 5-10 times, and then counterclockwise 5-10 times to ensure reagents are fully mixed.
- Spray 75% ethanol on the surface of cartridge seal and clean the seal with lint-free paper. Pierce either of the well No.2 using 1 mL sterile tip (see Figure 6-2 for well position).
- Add 45 mL of 0.1M NaOH into well No.2 through the pierce using electronic pipette. Refer to section "9.3 Prepare wash reagents" for the preparation of 0.1M NaOH.

Figure 6-2 Washing Cartridge

6.3 Fill the Pure Water Container

Fill up the pure water container with laboratory-grade water and open the air vent of the container.

Table 6-3：Pure Water Consumption

Sequencing kit	Pure water consumption/flow cell
DNBSEQ-T7RS FCL PE100	3.0 L

① **Note:**

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 pure water tube. Wipe and clean the surfaces with new microfiber clean wipers. Rinse the container with fresh pure water 3 times.

7 Load the Reagent Cartridge

7.1 Maintain the Compartment

Open the reagent compartment door and clean the inner walls with microfiber clean wiper or lint-free paper moistened with laboratory-grade water. Keep the compartment clean and dry.

① **Note：**

Be careful not to be scratched by the sampling needle above when cleaning the inner walls of the compartment.

7.2 Load the Reagent Cartridge

Place the sequencing cartridge into the upper low-temperature compartment and place the washing cartridge into the lower room-temperature compartment.

7.2 Close the Doors

Close the doors of both low-temperature compartment and room-temperature compartment, and then close the door of the reagent compartment.

8 Start Sequencing

8.1 Enter the Program

- Enter the user name "user" and password "123", click "Log in" to enter the main interface (see Figure 8-1):

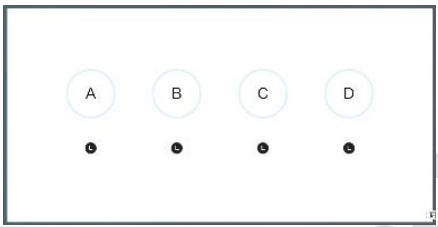

Figure 8-1：DNBSEQ-T7 Main Interface

8.2 Load the flow cell

- Select A/B/C/D respectively according to sequencing demand. Click on “Sequencing” and select “New run” (see Figure 8-2).

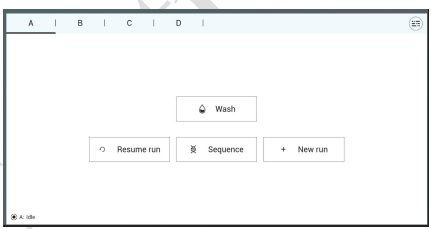

Figure 8-2：DNBSEQ-T7 Selection Interface

- Clean the loaded flow cell with a dust remover to ensure no visible dust on the surface and back of the flow cell. Put the flow cell on the flow cell drive, and tap the flow cell drive control button to withdraw the flow cell drive.

8.3 Sequencing Parameters

- RFID can automatically read the ID information on the sequencing cartridge, washing cartridge and flow cell and display the information on corresponding text box (see Figure 8-3). If the reader fails, information can be entered manually.

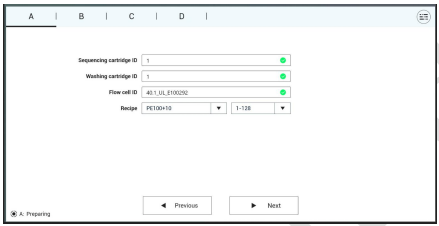

Figure 8-3：DNBSEQ-T7 sequencing parameters

- Click on the "▼" behind the "Recipe". Select the appropriate sequencing recipe and barcode sequence in the drop-down menu.

8.4 Review parameters

- Click "Next" to review the parameters and ensure that all information is correct (see Figure 8-4):

Figure 8-4：Review information

8.5 Start Sequencing

- After confirming that all the information is correct, click “Start” and select “Yes” (see Figure 8-5):

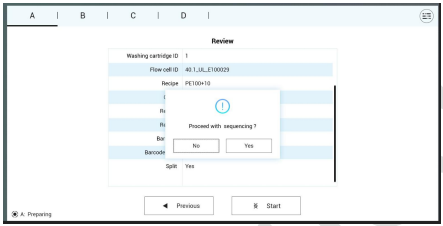

Figure 8-5：Confirm Sequencing Interface

- When the screen appears as shown in Figure 8-6, the sequencing is started. In general, a PE100 sequencing run will take around 22 hours.

Figure 8-6：Sequencing Starts Interface

- When the screen appears as shown in Figure 8-7, the sequencing and wash process for this PE100 run are completed.

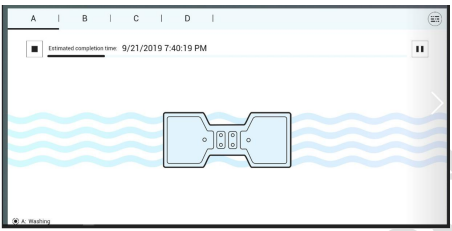

Figure 8-7：DNBSEQ-T7 Sequencing Complete Interface

9 Device Maintenance

9.1 Terminology and Definition

Table 9-1：Wash Methods

Wash type	Description
MGIDL-T7 Automatic wash	When the loading is completed, replace the flow cell with a used flow cell and click “Wash”. The loader will automatically perform the wash without the need to change the post load plate.
DNBSEQ-T7RS Automatic wash	After each sequencing run, the system will automatically perform a wash.

MGIDL-T7 Manual wash	Perform a wash manually under the following conditions: 1. The device is used for the first time 2. The device has not been used for 7 days or longer 3. Impurities are found in the device or flow cell.
DNBSEQ-T7RS Manual wash	Perform a wash manually under the following conditions: 1. The device is used for the first time 2. The device has not been used for 7 days or longer 3. Impurities are found in the device or flow cell.

9.2 Maintenance Rules

- When the loading is completed on the MGIDL-T7, an automatic wash can be performed.
- After each sequencing run, the DNBSEQ-T7RS will automatically perform a wash.
- After MGIDL-T7 or DNBSEQ-T7RS maintenance by an engineer, perform a wash manually.
- After replacing the tubing, sampling needles, or other accessories exposed to the reagents, perform a wash manually.
- If the device has been idle for more than a week, perform a wash manually before use.
- If impurities are found on the flow cell and other affecting factors have been excluded, perform a wash manually.

9.3 Prepare Wash Reagents

- Prepare 0.05% Tween-20 following the table below (valid for 28 days if stored at 4°C)

Table 9-2：Wash Reagents Preparation（1）

Reagent	Volume
100% Tween-20	0.5 mL
Laboratory-grade water	999.5 mL

- Prepare 1M NaCl + 0.05% Tween-20 following the table below (valid for 28 days if stored at 4°C).

Table 9-3：Wash Reagent Preparation（2）

Reagent	Volume
5M NaCl solution	200 mL
100% Tween-20	0.5 mL
Laboratory-grade water	799.5 mL

- Prepare 0.1M NaOH following the table below (valid for 28 days if stored at 4°C).

Table 7-4：Wash Reagent Preparation（3）

Reagent	Volume
2M NaOH solution	50 mL
Laboratory-grade water	950 mL

9.4 The Cleaning Kit

- An empty washing cartridge and washing flow cell for a full wash are provided together with the device.
- The washing plate and washing cartridge must be cleaned and refilled with fresh washing reagents every time before use. Replace the washing plate and washing cartridge after three months of continuous use.
- Flow cells from previous runs can be used as washing flow cells. Each flow cell can be used 3 times.
- Prepare the MGIDL-T7 washing plate: take a clean and empty post-load plate (see Figure 9-1), add 4 mL 0.1 M NaOH into well No.11, 4 mL 1M NaCl + 0.05% Tween-20 into well No.10, 4 mL laboratory-grade water into well No.9 and 20 mL laboratory-grade water int well No.12.

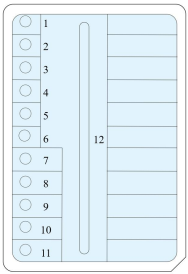

Figure 9-1: MGIDL-T7 Washing Plate

- DNBSEQ-T7RS washing cartridge 1: A clean and empty sequencing cartridge (see Figure 9-2).

Figure 9-2: DNBSEQ-T7RS Washing Cartridge 1

- Prepare DNBSEQ-T7RS washing cartridge 2: Take a clean and empty sequencing cartridge, add 45 mL 0.1 M NaOH into either of the well No.2, and 45 mL 1M NaCl + 0.05% Tween-20 into either of the well No.3.

Figure 9-3: DNBSEQ-T7RS Washing Cartridge 2

9.5 Wash Procedures

9.5.1 MGIDL-T7 Automatic Wash

- When “Loading completed” appears on the screen, the flow cell loading is completed and an automatic wash can be performed
- Press the flow cell attachment button and wait until the negative pressure is released. Remove the loaded flow cell from the stage for safekeeping.
- Take out the washing flow cell and place it on the flow cell stage. Press the flow cell attachment button and press down the flow cell to ensure the flow cell is securely attached to the stage.
- As shown in Figure 9-4, click “Post-wash” and select “Yes” to begin the MGIDL-T7 wash, which will take around 20 min.

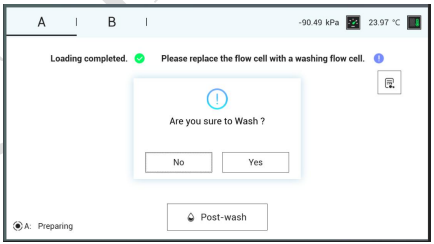

Figure 9-4: MGIDL-T7 Post-wash Confirmation Interface

- When the screen shown in Figure 9-5 appears, the wash is completed. Click “Finish” and another flow cell loading can be performed.

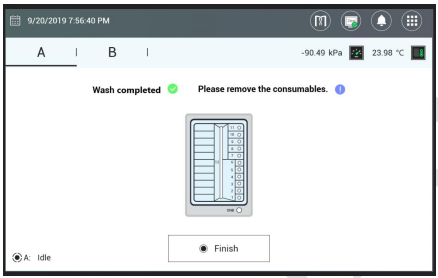

Figure 9-5: MGIDL-T7 Wash Completed Interface

9.5.2 DNBSEQ-T7RS Automatic Wash

- When the sequencing is complete on the DNBSEQ-T7RS, the system will automatically perform a wash.
- When the screen shown in Figure 9-6 appears, the automatic wash on DNBSEQ-T7RS is complete. Automatic washing takes around 30 min.

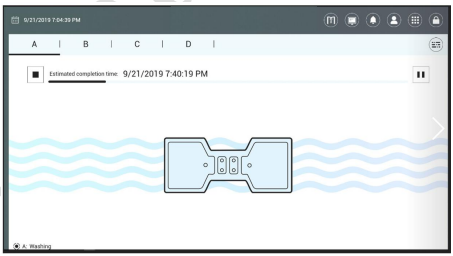

Figure 9-6: DNBSEQ-T7RS Wash Completed Interface

9.5.3 MGIDL-T7 Manual Wash

- Enter the program
- Enter the user name "user" and password "123", click "Log in" to enter the main interface
- Select the side that needs to be washed
- Open the loading compartment door
- Place the washing plate filled with wash reagents into the side that needs to be washed. Close the compartment door.
- Press the flow cell attachment button and wait until the negative pressure is released. Remove the flow cell from the stage. Skip this step if no flow cell is on the stage.
- Take out the washing flow cell and place it on the flow cell stage. Press the flow cell attachment button and press down the flow cell to ensure the flow cell is securely attached to the stage.
- Click “Wash” and select “Yes” to begin the MGIDL-T7 wash, which will take around 20 min.

9.5.4 DNBSEQ-T7RS Manual Wash

- Enter the program. Enter the user name "user" and password "123", click "Log in" to enter the main interface
- Click “Wash”.
- Place the clean and empty DNBSEQ-T7RS washing cartridge 1 into the low-temperature compartment on the side that needs to perform a wash, and then close the low-temperature compartment door.
- Place the DNBSEQ-T7RS washing cartridge 2 filled with wash reagents into the room-temperature compartment on the side that needs to perform the wash, and then close the room-temperature compartment door and the reagent compartment door.
- Press the flow cell drive control button to eject the flow cell drive and install a used flow cell from a previous run. Press the flow cell drive control button again to withdraw the flow cell drive.
- Click “Wash” and select “Yes” to begin the DNBSEQ-T7RS manual wash, which will take around 37 min.

10 Troubleshooting

10.1 Low DNB Concentration

- Check if the kit has expired.
- Check if the library meets the requirements.
- If DNB concentration still does not meet the requirements after a new sample preparation, please contact the engineer.

10.2 Abnormal Negative Pressure

- Gently wipe the stage surface with a damp, lint-free paper or a lint-free cloth, and blow the stage with a dust remover and ensure no dust is left.
- Blow the back of the Flow Cell with a dust remover to ensure no dust is left.

- If these solutions cannot solve the problem, please contact the engineer.

10.3 Bubbles

- Replace the used Flow Cell and inspect the pump.
- If the problem persists, please contact the engineer.

10.4 Impurities

- Perform a manual wash on MGIDL-T7 and DNBSEQ-T7RS.
- If the problem persists after manual wash, please contact the engineer.

10.5 Pump fails

- Check if the pure water volume is sufficient.
- MGIDL-T7 and DNBSEQ-T7RS: remove the flow cell, check if there are impurities in sealing gasket and remove any dust with a dust remover. Place the flow cell following the instruction and start the pump again.
- Check if the sampling needles can move properly.
- If the sampling needles cannot move properly, restart sequencing software.
- If the problem persists, please contact the engineer.

10.6 Reagent kit storage

- If the kit has been thawed (including dNTPs) but cannot be used within 24 hours, it can be frozen and thawed once only.
- If the kit has been thawed (including dNTPs) but cannot be used immediately, store it at 4℃ and use it within 24 hours.
- If dNTPs Mix IV or dNTPs Mix II and enzyme have been added into the cartridge, i.e. the cartridge has been prepared but cannot be used immediately, store it at 4 °C and use it within 24 hours. Re-mix the reagents in the cartridge following the instruction in section 6.1.
- If dNTPs Mix IV or dNTPs Mix II and enzyme have been added into the cartridge, i.e. the cartridge has been prepared and the needles have punctured the seal but the cartridge cannot be used in time, the cartridge must be covered with foil or plastic wrap. Store the kit at 4℃ and use it within 24 hours.

11 Attention

11.1 This product is restricted for research use only, please read the manual carefully before use.

11.2 Make sure that you are familiar with the SOP & Attention of all the laboratory apparatus to be used.

11.3 Avoid direct skin and eye contact with any samples and reagents. Don't swallow. Please wash with plenty of water immediately and go to the hospital when this happened.

11.4 All the samples and waste materials should be disposed according to relevant laws and regulations.

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