# DNBSEQ-G50RS
# High-throughput Sequencing Set User Manual

Catalog number and name

1000016959, DNBSEQ-G50RS High-throughput Sequencing Set（FCL SE50）

1000016961, DNBSEQ-G50RS High-throughput Sequencing Set（FCL SE100）

1000016963, DNBSEQ-G50RS High-throughput Sequencing Set（FCL PE50）

1000016965, DNBSEQ-G50RS High-throughput Sequencing Set（FCL PE100）

Kit version: V3.0 (Note: Mixed use of reagent components from different batches is strictly prohibited)

User manual version: A1

## Revision History

| Version | Date | Summary of change |
|---|---|---|
| A1 | December 2019 | • Added a new section “Attention”.<br>• Updated the equations used to calculate library input.<br>• Added the “Revision History”. |
| A0 | August 2019 | • The first version. |

**Table of Contents**

# 1 Introduction

This manual explains how to perform sequencing using the DNBSEQ-G50RS 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-G50RS High-throughput Sequencing Set is specifically designed for DNA or RNA sequencing on MGISEQ-200RS or DNBSEQ-G50RS. 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 basecalling 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 sequencing read length. For example, a PE100 cycle run performs reads of 100cycles (2 × 100) for a total of 200 cycles. At the end of the insert sequencing run, an extra 10 cycles of index read can be performed, 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 |
|---|---|---|---|---|---|
| SE50 | 50 | — | 10 | 50+10 | 70 |
| SE100 | 100 | — | 10 | 100+10 | 120 |
| PE50 | 50 | 50 | 10 | 100+10 | 120 |
| PE100 | 100 | 100 | 10 | 200+10 | 220 |

### 1.5 Sequencing Time

**Table 1-2：Sequencing time for each read length (hours)**

| Time (hours) | SE50 | SE100 | PE50 | PE100 |
|---|---|---|---|---|
| Single flow cell | 14.5 | 21.3 | 26.5 | 50.6 |
| Data analysis | 0.5 | 1.2 | 1.2 | 2.4 |

### 1.6 Attention

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

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

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.

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

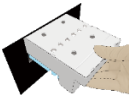
## 2 Sequencing Workflow

Make DNB: use DNB preparation kit for making DNB

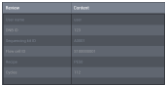
Prepare a new flow cell：remove the flow cell from package and inspect to ensure the flow cell is intact

Prepare a new reagent cartridge: inspect and thaw the reagent cartridge and then load and mix the necessary reagents

Load the flow cell: place the flow cell on the stage of the sequencer



Load the reagent cartridge into the sequencer



Follow the instructions to enter sequencing information and start the run

Monitor the sequencing run from the control software interface

Perform device maintenance when sequencing is completed

# 3 Library preparation

## 3.1 Insert Size Recommendation

This sequencing set is compatible with the libraries prepared by MGI Library Prep Kits.

Library recommendation for insert size:

For SE50/ SE100/PE50, the size distribution of inserts is preferred to be centered around 160-170 bp. For PE100, the size distribution of inserts is preferred to be centered around 280 bp.

## 3.2 Library 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 and each Make DNB reaction requires 40 fmol library.

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.

## 3.3 Calculate the required amount of ssDNA library

The required volume of ssDNA library is determined by the required library amount (fmol) and library concentration quantified in 3.2. The volume of each Make DNB reaction is 100 μL and the required library input for each Make DNB reaction is calculated as flowed:

ssDNA library input (μL) = 40 fmol / library concentration (fmol/μL)

Note:

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

Calculate the required ssDNA library for each Make DNB reaction and fill it in Table 3-1 as V.

ⓘ **Note**:

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

## 3.4 Prepare reagents for the make DNB

Remove libraries, Make DNB Buffer, Make DNB Enzyme Mix I, Low TE Buffer and Stop DNB Reaction Buffer from storage. Thaw reagents for approximately 0.5 hours on ice. After thawing, mix reagents using a vortex mixer

for 5 seconds, centrifuge briefly and place them on ice.

### 3.5 Making DNB

- After the make DNB reaction, the 100 μL DNB making reaction is loaded to one lane on the sequencing flow cell.

- Take 0.2 mL PCR 8-tube strip or PCR tubes. Prepare reaction mix following Table 3-1 below.

**Table 3-1：Make DNB reaction 1**

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

- V represents variable sample volume as determined in section 3.2. Mix gently by vortex and spin down for 5 seconds using the mini centrifuge. Place the mix into a PCR machine and start the reaction. PCR machine settings are described in Table 3-2:

**Table 3-2：DNB reaction condition 1**

| 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 hold on ice.

① **Note：**

**Do not place Make DNB Enzyme Mix II（LC）at room temperature and avoid holding the tube in such a way as to heat the contents.**

- Take the PCR tube out of the PCR machine after the reaction enters the hold phase at 4℃. Centrifuge briefly for 5 s, place the tube on ice and prepare the Make DNB reaction mix 2.

**Table 3-3：Make DNB reaction mix 2**

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

- Add all of the Make DNB reaction mix 2 into the Make DNB reaction 1. Mix gently by vortex, centrifuge for 5 s using mini centrifuge and place tubes into PCR machine for the next reaction. The conditions are shown in Table 3-5 below:

**Table 3-4：DNB reaction conditions 2**

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

Note:

Recommend to set temperature of the heated lid to 35℃ or the temperature closest to 35℃.

- Add 20 μL Stop DNB Reaction Buffer immediately after the reaction enters cold hold at 4℃. Mix Gently by wide bore pipetting 5-8 times. Do not vortex or shake the tube. Store DNB at 4℃ and perform sequencing within 48 hours.

① **Note:**

**It is very important to mix DNB gently by wide bore pipetting. Do not centrifuge, vortex, or shake the tube.**

### 3.6 Quantify DNB

After the make DNB is completed, 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 preparation.

Note:

- 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.

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

### 3.7 Load DNB

- After the make DNB reaction, the 100 μL DNB making reaction is loaded to one lane on the sequencing flow cell.
- Take 0.5 mL microfuge tubes and add reagents following table 3-6 below.

**Table 3-5：DNB loading mix1**

| Component | volume（μL） |
|---|---|
| DNB Load Buffer I | 50 μL |
| DNB Load Buffer II | 50 μL |
| Make DNB Enzyme Mix II（LC） | 1 μL |
| DNB | 100 μL |

- Combine components to create DNB loading mix 1 and mix by gently wide bore pipetting 5-8 times. Do not centrifuge, vortex, or shake the tube. Place the mixture at 4℃ until use.

Note:

Prepare a fresh DNB loading mix before the sequencing run.

## 4 Prepare the sequencing cartridge

- Remove the Sequencing Reagent Cartridge from -20℃. and thaw in a room temperature water bath until thawed. Store cartridge s at 2-8℃ storage until use (or thaw cartridge s in 2-8℃ fridge one day in advance). Invert the tube 3 times before use.

- Open the cartridge cover and wipe any water condensation with lint-free paper.

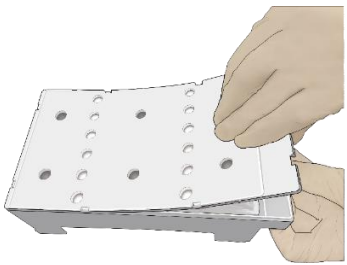


**Figure 4-1：Open and clean the cartridge**

- Remove dNTPs Mix III and dNTPs Mix II from -20℃ storage, 1h in advance to thaw at room temperature, and place at 4 ℃ until use.

- Remove Sequencing Enzyme Mix from -20℃ storage and place at 4 ℃ until use.

Note:

See the name of Sequencing Enzyme Mix for each sequencing read length in Chapter “List of set components”.

- Pierce the seal to make a hole 1cm or less in diameter using a sterile tip at the #1 and #2 well (see Figure 4-2):

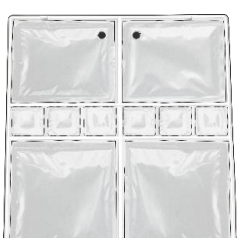


**Figure 4-2：Pierce the seal on the cartridge**

- #1 well（see Figure 4-3）


**Figure 4-3：Well position**

Take a pipette with the appropriate volume range and add reagents to the #1 well according to the following table:

**Table 4-1：dNTPs Mix loading**

| Sequencing Kit | Reagent name | Loading volume (mL) |
|---|---|---|
| DNBSEQ-G50RS FCL SE50 | dNTPs Mix III | 0.400 |
| DNBSEQ-G50RS FCL SE100 | dNTPs Mix III | 0.500 |
| DNBSEQ-G50RS FCL PE50 | dNTPs Mix III | 0.500 |
| DNBSEQ-G50RS FCL PE100 | dNTPs Mix III | 0.800 |

- #2 well（see Figure 4-3）

Take a pipette with the appropriate volume range and add reagents to the #2 well according to the following table:

**Table 4-2：dNTPs Mix II loading**

| Sequencing Kit | Reagent name | Loading volume (mL) |
|---|---|---|
| DNBSEQ-G50RS FCL SE50 | dNTPs Mix II | 0.800 |
| DNBSEQ-G50RS FCL SE100 | dNTPs Mix II | 1.000 |
| DNBSEQ-G50RS FCL PE50 | dNTPs Mix II | 1.000 |
| DNBSEQ-G50RS FCL PE100 | dNTPs Mix II | 1.600 |

- #1 and #2 well（see Figure 4-3）

Take a pipette with the appropriate volume range and add reagents to #1 and #2 well according to the following table:

**Table 4-3：Sequencing Enzyme Mix loading**

| Sequencing Kit | Reagent name | Loading volume (mL) |
|---|---|---|
| DNBSEQ-G50RS FCL SE50 | DNA Sequencing Enzyme Mix | 0.400 |
| DNBSEQ-G50RS FCL SE100 | DNA Sequencing Enzyme Mix | 0.500 |
| DNBSEQ-G50RS FCL PE50 | DNA Sequencing Enzyme Mix | 0.500 |
| DNBSEQ-G50RS FCL PE100 | DNA Sequencing Enzyme Mix | 0.800 |

- Seal the loading well with the transparent sealing film. Do not cover the center of the well to avoid blocking the sampling needle.

**Figure 4-4：Seal with the loading well**

- Place the cartridge horizontally on the table, hold both sides of the cartridge with both hands. Move it clockwise 10-20 times, and then counterclockwise 10-20 times. Make sure that you see the vortex to ensure reagents are fully mixed.

**Figure 4-5：Mix reagents after loading**

- # 15 well（see Figure 4-3）: The following instruction are only for PE cartridges.
  Add 200 μL of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette. Vortex for 5 s, mix thoroughly and then add the mixture to the #15 well. When adding the mixture, make sure there are no bubbles at the bottom of the tube.

ⓘ **Note：**

**When using MDA Enzyme Mix, do not touch the wall of the tube to prevent influences the enzyme activity!**

## 5 Prepare a flow cell

- Remove the sequencing flow cell from storage.

- Unwrap the outer package.

**Figure 5-1：Unwrap the outer package**

- Remove the flow cell from the inner package and inspect to ensure the flow cell is intact.

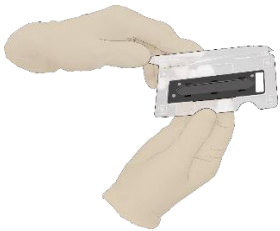


**Figure 5-2：Inspect the flow cell**

# 6 Sequencing

## 6.1 Enter the main interface

- Enter the user name "user" and password "123", click "Log in" to enter the main interface.

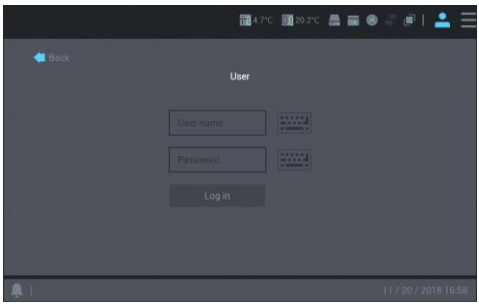



**Figure 6-1：Log-in interface**

- See the interface below.

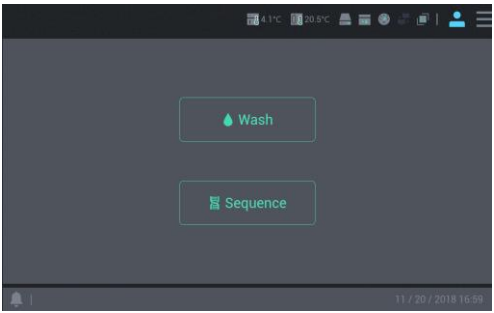



**Figure 6-2：Main interface**

### 6.2 Load the samples

- Click the “Sequence” option on the interface to enter the following interface:

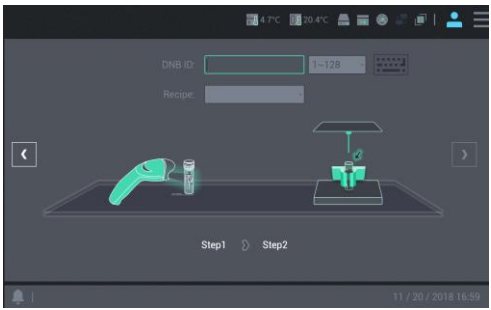



**Figure 6-3：DNB loading interface**

- Move the cursor to the blank area next to the "DNB ID" and enter the library name or number.

- Open the reagent compartment door, gently lift the sampling needle with one hand, remove the cleaning reagent tube with the other hand, load the sample tube, then slowly lower the sampling needle tip reaches the bottom of the tube.

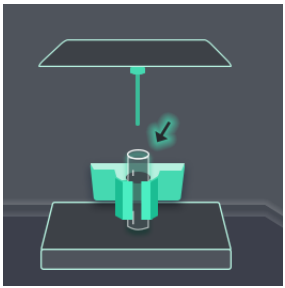


**Figure 6-4：Load the DNB tube**

- Close the reagent compartment door.

### 6.3 Select sequencing parameters

- Select the sequencing recipe in the “Recipe” drop-down menu, one-click sequencing run (SE50, SE100, PE50, PE100, etc.), and user-customized run (Customize).


**Figure 6-5：Select sequencing solutions**

- In the beginning, please select a step to start the sequencing run.


**Figure 6-6：Select the step to start sequencing**

- Select the read length. For example, with PE100 enter 100 for read 1 and 100 for read 2.

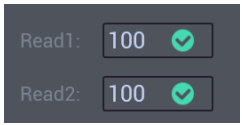



**Figure 6-7：Choose the read length**

- Select the barcode length from 6 or 10. If it is dual barcode sequencing, you need to fill in the length of the Dual barcode. Leave the dual barcode blank if it is a single barcode sequencing run.


**Figure 6-8：Select the barcode length**

- Select the lane for barcode demultiplexing.

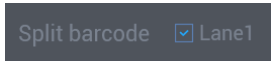



**Figure 6-9：Barcode demultiplexing on different lanes**

- Select the dark reaction for any position of read length in read 1 or 2.

Dark reaction: only chemical reaction without optical information capture

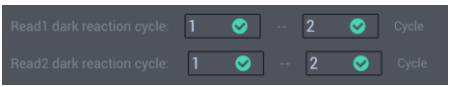



**Figure 6-10：Select the dark reaction**

- Click “Confirm”

### 6.4 Load the reagent cartridge

- Move the cursor to the “Reagent ID” blank, enter the cartridge information manually or using the barcode scanner to scan the cartridge barcode.

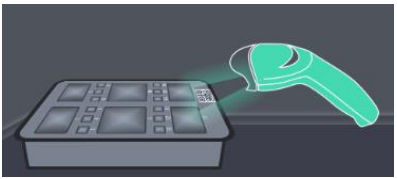


**Figure 6-11：Reagent cartridge information entry interface**

- Open the reagent compartment door. Hold the handle of the cleaning cartridge 1 with one hand, place the other hand underneath the cartridge for support, and slowly remove it from the compartment.

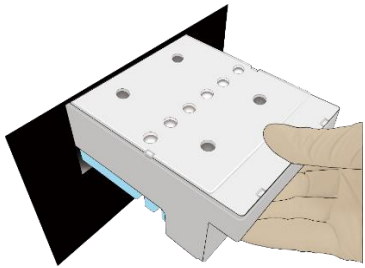


**Figure 6-12：Remove cleaning cartridge**

- Moisten dust-free paper or a dust-free cloth with laboratory-grade water and use it to wipe the bottom and sides of the compartment to keep it clean and dry.

**Figure 6-13：Maintain the reagent compartment**

- Hold the handle of the reagent cartridge with one hand and place the other hand underneath for support. Slide the new cartridge into the compartment following the direction printed on the cover until it stops. Check that the reagent cartridge is in the correct position and close the reagent compartment door.

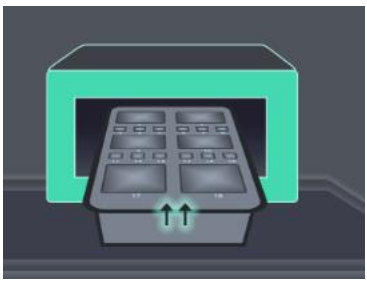


**Figure 6-14：Slide the new reagent cartridge into the reagent compartment**

### 6.5 Loading the flow cell

- Open the flow cell compartment door, press one side of flow cell used for washing, and press the flow cell attachment button with the other hand. After the vacuum is released, remove the flow cell for washing from the stage.

- Use dust remover to remove the dust on the flow cell stage and the back of the flow cell. If there are impurities on the stage surface, please gently wipe it with wet dust-free paper to ensure that the flow cell can be held properly.

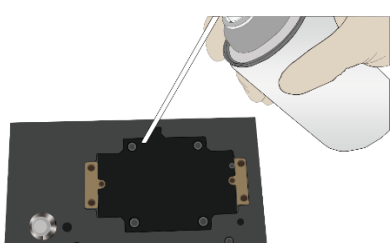


**Figure 6-15：Clean the flow cell stage**

- Press the flow cell attachment button.

- Take out a new flow cell or the loaded flow cell. There are two alignment holes on the left side and one hole on the right side. The label is on the right. Hold the flow cell by the edges with both hands.

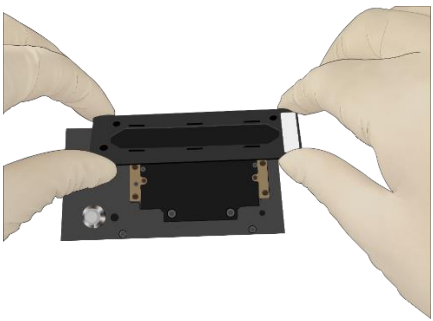


**Figure 6-16：Load the flow cell**

- Align the holes on the flow cell with the locating pins on the flow cell stage. Gently slide the flow cell to keep the flow cell aligned with the pin. Press the left and right sides of the flow cell on the stage at the same time to ensure the flow cell is properly seated on the stage.

ⓘ **Note：**

**The flow cell is fragile, please use caution when handling the flow cell**

- Use a dust remover to remove the dust on the flow cell surface and close the flow cell compartment door.

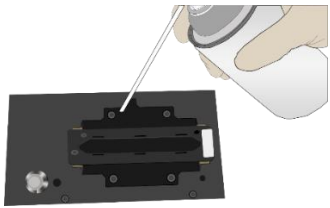


**Figure 6-17：Clean the flow cell**

- Click “Next”, the device will automatically enter the flow cell ID; if automated entry does not work, move the cursor to the “Flow cell ID” blank and manually enter the ID.

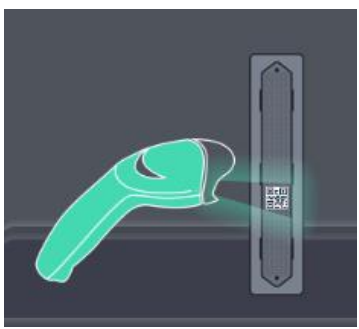


**Figure 6-18：Flow cell information entry interface**

- Click “Next”

### 6.6 Review parameters

Review the run parameters to ensure that all information is correct.

| Review | Content |
| --- | --- |
| User name | user |
| DNB ID | 123 |
| Sequencing kit ID | A0001 |
| Flow cell ID | S100000001 |
| Recipe | Customize |
| Cycles | 212 |

**Figure 6-19：Review information**

### 6.7 Start sequencing

- After confirming that the information is correct, click “Start”.

- The system will display the dialog box “Start the sequencing.” Click “Yes” to start sequencing.


**Figure 6-20：Confirm sequencing interface**

- Once sequencing has started, immediately open the flow cell compartment door to ensure that DNB (or reagents) are flowing through the flow cell.

# 7 Device Maintenance

## 7.1 Terminology and Definition

**Table 7-1：Wash Solution**

| Wash type | Description |
|---|---|
| Full wash | Step 1 - Maintenance wash, Step 2 – Regular wash. |
| Maintenance wash | Procedure: Cleaning cartridge 4 → Cleaning cartridge 3 → Cleaning cartridge 2<br>To remove residual reagents and proteins in the pipeline, reducing risk of blockage. |
| Regular wash | Procedure: Cleaning cartridge 1 → Air Prime<br>To remove residual reagents, reducing risk of cross-contamination. |

## 7.2 Wash instruction

- When the following interface appears, you can perform a wash.

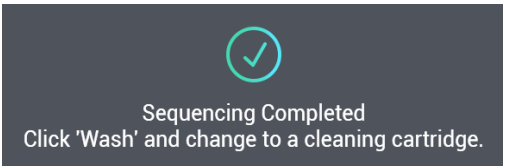



**Figure 7-1：Wash interface**

- After the sequencing is completed, the device needs to be washed within 24 hours.
- A Full Wash is required if the sequencer was used for a PE run. A regular wash is sufficient for an SE run.
- After a full wash is completed, if the device has been idle for more than 12 hours, perform a regular wash again before use.
- After an engineer performs system maintenance, perform a regular wash.
- After replacing the tubing, sampling needles, or other accessories exposed to the reagents, perform a full

wash.

- If the sequencer is to be powered off for more than 7 days, perform a maintenance wash before powering off and after powering on.

- If the sequencer has been idle for seven days or longer, perform a full wash prior to sequencing.

- If impurities are found on the flow cell, perform a full wash.

### 7.3 Prepare wash reagents

- Prepare 0.05% Tween-20 following the table below (Can be used for up to 28 days if stored at 4°C)

**Table 7-2：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 (Can be used for up to 28 days if stored at 4°C).

**Table 7-3：Wash reagent preparation（2）**

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

### 7.4 Wash the cleaning cartridge

- An empty cleaning cartridge and washing flow cell for a full wash are provided together with the device.

- Wash the cleaning cartridge prior to refilling it with cleaning reagents. Replace cleaning reagents after 20 uses.

- Used flow cells from previous runs can be used as washing flow cells. Each flow cell can be used for up to 20 full washes.

- Wash cleaning cartridge 1: Take a clean cleaning cartridge and a 0.5 mL cryotube, add laboratory-grade water to the cryotube and cleaning cartridge (all wells) to a final 90% volume and mark it as the cleaning reagent cartridge 1.

- Wash cleaning cartridge 2: Take a clean cleaning cartridge and a 0.5 mL cryotube, add laboratory-grade water to the cryotube and cleaning cartridge (all wells) to a final 90% volume and mark it as the cleaning reagent cartridge 2.

- Wash cleaning cartridge 3: Take a clean cleaning cartridge and a 0.5 mL cryotube, add 50 mL 0.1M NaOH into large wells, 6 mL 0.1M NaOH into small wells and 400 μL 0.1M NaOH to 0.5mL cryotube. Mark it as the cleaning reagent cartridge 3.
- Wash cleaning cartridge 4: Take a clean cleaning cartridge and a 0.5 mL cryotube, add 50 mL 0.05% Tween-20 solution into large wells, 6 mL 1M NaCl + 0.05% Tween-20 solution to No.15 well, 400 μL 1M NaCl + 0.05% Tween-20 solution to 0.5mL cryotube and 6 mL 0.05% Tween-20 solution to the rest of the wells. Mark it as the cleaning reagent cartridge 4.

Note:

Large wells are No. 1, 2, 9, 10, 17, 18

Small wells are No. 3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16

### 7.5 Wash procedures

#### 7.5.1 Regular wash

- Use cleaning cartridge 1. Open the reagent compartment door. Hold the handle of the cleaning cartridge 1 with one hand and place the other hand underneath the cartridge 1 for support. Slide it into the reagent compartment slowly following the direction printed on the cartridge cover until it stops. Close the reagent compartment door.

- Click the wash button on the interface.
- Place the flow cell for washing.
- Select regular wash from the drop-down menu to start the regular wash which takes about 30 minutes.
- If you perform the regular wash only, observe the status of the washing flow cell in this step. If you see many bubbles, continue the wash. If not, stop the wash, replace the flow cell and start the wash. If you perform the regular wash after the maintenance wash, skip this step.

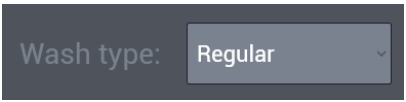



**Figure 7-2：Select the wash type**

- When the interface appears as the figure below, the regular wash ends.


**Figure 7-3：Regular wash end interface**

**7.5.2 Maintenance wash**

- Use cleaning cartridge 4. Open the reagent compartment door. Hold the handle of the cleaning cartridge 4 with one hand and place the other hand underneath for support. Slide it to the reagent compartment slowly following the direction printed on the cartridge cover until it stops. Close the reagent compartment door.
- Click the wash button on the interface.

- Place the flow cell for washing.
- Select the maintenance wash from the drop-down menu to start the maintenance wash which takes about 15 minutes.
- Observe the status of flow cell for wash in this step. If you see many bubbles, continue the wash. If not, stop the wash, replace the flow cell and start the wash.
- When the interface appears as Figure 7-4, click “Yes” to lift the needle and replace the cleaning cartridge.
- Use cleaning cartridge 3 and continue the maintenance wash which takes around 15 minutes.

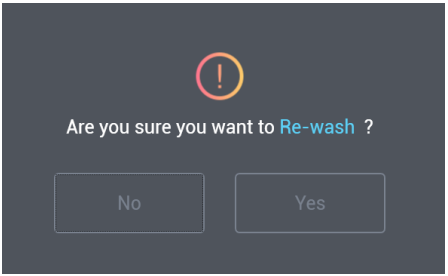



**Figure 7-4：Maintenance wash end interface**

- When the interface appears as figure 7-4, click “Yes” to lift the needle and replace the cleaning cartridge.
- Use cleaning cartridge 2 and continue the maintenance wash which takes around 15 minutes.
- When the interface appears as Figure 7-4, the maintenance wash ends.

### 7.5.3 Full wash procedures

Step 1 – Maintenance wash, Step 2 – Regular wash. Total time is 70 mins

# 8 Troubleshooting

## 8.1 Low DNB concentration

- Check if the cartridge 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 a field service engineer.

## 8.2 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 power 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 a field service engineer.

## 8.3 Bubbles

- Replace the used flow cell and inspect the pump.
- If the problem persists, please contact a field service engineer.

## 8.4 Impurities

- Perform a full wash on the sequencer.
- If the problem persists after a full wash, please contact a field service engineer.

## 8.5 Pump fails

- Sequencer：remove the flow cell, check if there are impurities in sealing gasket and remove the dust with the dust remover. Place the flow cell following the instruction and start the pump again.
- Check if the sampling needles move properly.
- If the sampling needles cannot move properly, restart sequencing software.

- If the problem persists, please contact a field service engineer.

**8.6 Reagent cartridge storage**

- If the cartridge has been thawed (including dNTPs) and cannot be used within 24 hours, it can be frozen and thawed at most once.

- If the cartridge has been thawed (including dNTPs) but cannot be used immediately, store it at 4℃ and use it within 24 hours.

- If dNTPs and enzyme have been added to the cartridge, ie the cartridge has been prepared but cannot be used immediately, store it at 4 °C and use it within 24 hours.

- If dNTPs and enzyme have been added to the cartridge, ie the cartridge has been prepared and the reagent needles have started aspiration but the cartridge cannot be used in time, the cartridge must be sealed with foil or plastic wrap. Store the cartridge at 4°C and use it within 24 hours.

## 9 Equipment and Consumables Required but not Provided

**Table 9-1：Equipment and consumables Required but not Provided**

| Equipment and consumables | Recommended brand | Catalog number |
|---|---|---|
| Qubit® 3.0 Fluorometer | Thermofisher | Q33216 |
| Mini centrifuge | Major Laboratory Supplier (MLS) | / |
| Vortex mixer | MLS | / |
| PCR machine | Bio-Rad | / |
| Pipette | Eppendorf | / |
| 2~8℃ refrigerator | MLS | / |
| -25℃~-15℃ refrigerator | MLS | / |
| Qubit® ssDNA Assay Kit | Thermo Fisher | Q10212 |
| Power Dust remover | MATIN | M-6318 |
| Sterile pipette tip(box) | AXYGEN | / |
| 200μL Wide-Bore Pipette Tips | AXYGEN | T-205-WB-C |
| Qubit Assay Tubes | Thermo Fisher | Q32856 |
| 100%Tween-20 | MLS | / |
| 5M NaCl solution | MLS | / |
| 2M NaOH solution | MLS | / |
| 0.2mL PCR 8-tube strip | AXYGEN | / |
| 1.5mL Eppendorf | AXYGEN | MCT-150-C |
| Ice rack | MLS | / |

## 10 List of set components

**Table10-1：List of set components 1**

| Product | Sequencing kit | Component | Spec & Quantity | Storage Temperature |
|---|---|---|---|---|
| DNBSEQ-G50RS High-throughput Sequencing Set (FCL SE50) Catalog number: 1000016959 | DNBSEQ-G50RS Sequencing Flow Cell Catalog number: 1000016986 | DNBSEQ-G50 Sequencing Flow Cell | 1 EA | Room Temperature |
| | DNBSEQ-G50RS High-throughput Sequencing Kit (FCL SE50) Catalog number: 1000016958 | Low TE Buffer | 100 μL×1tube | -25℃~-15℃ |
| | | Make DNB Buffer | 50 μL×1tube | |
| | | Make DNB Enzyme Mix I | 100 μL×1tube | |
| | | Make DNB Enzyme Mix II（LC） | 13 μL×1tube | |
| | | Stop DNB Reaction Buffer | 50 μL×1tube | |
| | | DNB Load Buffer I | 300 μL×1tube | |
| | | DNB Load Buffer II | 120 μL×1tube | |
| | | Micro Tube 0.5mL（Empty） | 1tube | |
| | | dNTPs Mix III | 0.50 mL×1tube | |
| | | dNTPs Mix II | 0.90 mL×1tube | |
| | | Sequencing Enzyme Mix | 1.10 mL×1tube | |
| | | Sequencing Reagent Cartridge | 1 EA | |
| | | transparent sealing film | 2 sheets | |

**Table10-2：List of set components 2**

| Product | Sequencing kit | Component | Spec & Quantity | Storage Temperature |
|---|---|---|---|---|
| DNBSEQ-G50RS High-throughput Sequencing Set (FCL SE100) Catalog number: 1000016961 | DNBSEQ-G50RS Sequencing Flow Cell Catalog number: 1000016986 | DNBSEQ-G50 Sequencing Flow Cell | 1 EA | Room Temperature |
| | DNBSEQ-G50RS High-throughput Sequencing Kit (FCL SE100) Catalog number: 1000016960 | Low TE Buffer | 100 μL×1tube | -25℃~-15℃ |
| | | Make DNB Buffer | 50 μL×1tube | |
| | | Make DNB Enzyme Mix I | 100 μL×1tube | |
| | | Make DNB Enzyme Mix II（LC） | 13 μL×1tube | |
| | | Stop DNB Reaction Buffer | 50 μL×1tube | |
| | | DNB Load Buffer I | 300 μL×1tube | |
| | | DNB Load Buffer II | 120 μL×1tube | |
| | | Micro Tube 0.5mL（Empty） | 1tube | |
| | | dNTPs Mix III | 0.60 mL×1tube | |
| | | dNTPs Mix II | 1.10 mL×1tube | |
| | | Sequencing Enzyme Mix | 1.30 mL×1tube | |
| | | Sequencing Reagent Cartridge | 1 EA | |
| | | transparent sealing film | 2 sheets | |

**Table10-3：List of set components 3**

| Product | Sequencing kit | Component | Spec & Quantity | Storage Temperature |
|---|---|---|---|---|
| DNBSEQ-G50RS High-throughput Sequencing Set (FCL PE50) Catalog number: 1000016963 | DNBSEQ-G50RS Sequencing Flow Cell Catalog number: 1000016986 | DNBSEQ-G50 Sequencing Flow Cell | 1 EA | Room Temperature |
| | DNBSEQ-G50RS High-throughput Sequencing Kit (FCL PE50) Catalog number: 1000016962 | Low TE Buffer | 100 μL×1tube | -25℃~-15℃ |
| | | Make DNB Buffer | 50 μL×1tube | |
| | | Make DNB Enzyme Mix I | 100 μL×1tube | |
| | | Make DNB Enzyme Mix II（LC） | 13 μL×1tube | |
| | | Stop DNB Reaction Buffer | 50 μL×1tube | |
| | | DNB Load Buffer I | 300 μL×1tube | |
| | | DNB Load Buffer II | 120 μL×1tube | |
| | | Micro Tube 0.5mL（Empty） | 1tube | |
| | | dNTPs Mix III | 0.60 mL×1tube | |
| | | dNTPs Mix II | 1.10 mL×1tube | |
| | | Sequencing Enzyme Mix | 1.30 mL×1tube | |
| | | MDA Reagent | 1.40 mL×1tube | |
| | | MDA Enzyme Mix | 0.30 mL×1tube | |
| | | Sequencing Reagent Cartridge | 1 EA | |
| | | transparent sealing film | 2 sheets | |

**Table 10-4：List of set components 4**

| Product | Sequencing kit | Component | Spec & Quantity | Storage Temperature |
|---|---|---|---|---|
| DNBSEQ-G50RS High-throughput Sequencing Set (FCL PE100) Catalog number: 1000016965 | DNBSEQ-G50RS Sequencing Flow Cell Catalog number: 1000016986 | DNBSEQ-G50 Sequencing Flow Cell | 1 EA | Room Temperature |
| | DNBSEQ-G50RS High-throughput Sequencing Kit(FCL PE100) Catalog number: 1000016964 | Low TE Buffer | 100 μL×1tube | -25℃~-15℃ |
| | | Make DNB Buffer | 50 μL×1tube | |
| | | Make DNB Enzyme Mix I | 100 μL×1tube | |
| | | Make DNB Enzyme Mix II（LC） | 13 μL×1tube | |
| | | Stop DNB Reaction Buffer | 50 μL×1tube | |
| | | DNB Load Buffer I | 300 μL×1tube | |
| | | DNB Load Buffer II | 120 μL×1tube | |
| | | Micro Tube 0.5mL（Empty） | 1tube | |
| | | dNTPs Mix III | 0.90 mL×1 | |
| | | dNTPs Mix II | 1.70 mL×1tube | |
| | | Sequencing Enzyme Mix | 1.90 mL×1tube | |
| | | MDA Reagent | 1.40 mL×1tube | |
| | | MDA Enzyme Mix | 0.30 mL×1tube | |
| | | Sequencing Reagent Cartridge | 1 EA | |
| | | transparent sealing film | 2 sheets | |

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