DNBSEQ-G400RS High-throughput Rapid Sequencing Set User Manual

Catalog number and name

1000016978, DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS SE100)

1000016980, DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE100)

1000016982, DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE150)

Set version: V1.0 (Note: Mixed use of reagent components from different batches is strictly prohibited)

User manual version: A0

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Table of Contents

1 Introduction

This manual explains how to perform sequencing using the DNBSEQ-G400RS High-throughput Rapid Sequencing Set and includes instructions on sample preparation, Flow Cell preparation, sequencing kit storage, the sequencing protocol and device maintenance.

As compared to DNBSEQ-G400RS High-throughput Sequencing Set, this set is faster and more efficient, which can finish sequencing within a short time. It is more suitable for samples with less data requirement and high urgency, especially for tumor detection.

1.1 Applications

DNBSEQ-G400RS High-throughput Rapid Sequencing Set is specifically designed for DNA sequencing on MGISEQ-2000RS or DNBSEQ-G400RS. 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 PE150 cycle run performs reads of 150cycles (2 × 150) for a total of 300 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
PE150	150	150	10	300+10	320

1.5 Sequencing Time

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

Time (hours)	PE100	PE150
Single Flow Cell	24.9	35.4
Dual Flow Cell	25.0	35.6
Data analysis	1.0	1.2

2 Sequencing Workflow

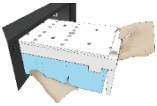
Make DNB: use DNB preparation kit for making DNB

Load DNBs: place sample tubes on the MGIDL-200 or the sequencer

Prepare a new Flow Cell：remove the Flow Cell from package and inspect to ensure the Flow Cell is intact

Prepare a new reagent kit：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 PE100, the size distribution of inserts is preferred to be centered around 280 bp. For PE150, the size distribution of inserts is preferred to be centered around 400 bp.

3.2 Library Requirement

We recommend 40 fmol ssDNA library for each reaction. Perform ssDNA library quantitation using Qubit® ssDNA Assay Kit and Qubit® Fluorometer. And the concentration of ssDNA library is more than 2 fmol/μL. Otherwise, the amount of ssDNA library needed is determined by the following equation.

volume needed (μL)=N*330 *40 /(1000*1000*C)

N represents the number of nucleotides around peak in library QC gel. C represents the concentration of ssDNA library (ng/μL).

If there are special requirements for the specifications of the library kit, then the requirements for the specifications of the kit will be taken.

3.3 Sample Safety

All samples should be considered to contain potentially infectious agents 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 Make DNB

The DNB volume required for each lane of the flow cell varies depending on the DNB loader. Note that all 2 lanes must be the same sample if sequencer is selected as the loader. Two different DNB samples can be loaded into 2 different lanes if MGIDL-200RS or MGIDL-200H are used. If you need to load various samples in 2 different lanes, it is recommended to use the MGIDL-200RS or MGIDL-200H. The volume of one make DNB reaction is 100 μL. The required number of DNB reactions for different loading systems is illustrated in Table 3-1:

Table 3-1：The required number of the make DNB reaction

Loading lanes on a Flow Cell	The required number of make DNB reaction	Loading system
1-2	1	Sequencer MGIDL-200RS MGIDL-200H

- Take 0.2 mL PCR 8-tube strip or PCR tubes. Prepare reaction mix following Table 3-2 below.

Table 3-2：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 3-3:

Table 3-3：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.

ⓘ **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 3-4：Make DNB reaction mix 2

Component	volume（μL）
Make DNB Enzyme Mix I	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 3-5：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

3.7.1 Sequencer DNB loading

- Take 0.5 mL microfuge tubes and add reagents following table 3-6 below.

Table 3-6：DNB loading mix 1

Component	Volume（μL）
DNB Load Buffer II	32
Make DNB Enzyme Mix II（LC）	1
DNB	100

- 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.

3.7.2 MGIDL-200H DNB loading

- Take 0.5 mL microfuge tubes and add reagents following table 3-6 below.

Table 3-7：DNB loading mix 2

Component	Volume（μL）
DNB Load Buffer II	8
Make DNB Enzyme Mix II（LC）	0.25
DNB	25

- 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, this DNB loading mix is the usage of one lane

- If loading DNB with MGIDL-200H, please refer to the “MGIDL-200H Portable DNB Loader Quick Start Guide” for details on loading operation.

3.7.2 MGIDL-200RS DNB loading

- Take a new PCR 8-tube strip and add reagents following Table 3-8,

Table 3-8：DNB loading mix 3

Component	Volume（μL）
DNB Load Buffer II	16
Make DNB Enzyme Mix II（LC）	0.5
DNB	50

Combine components to create DNB loading mix 2 and mix by gently wide bore pipetting 5-8 times. Do not centrifuge, vortex, or shake the tube.

- After creating DNB loading mix 2, place the tubes in the labeled positions of the MGIDL-200RS.

Figure 3-1：Place the loading samples

- Load the Flow Cell with DNBs according to step 6.5 Loading the Flow Cell.

Note：

Before loading DNB, perform a wash as described in the "DNB Loader MGIDL-200RS User Manual".

- After loading DNB, remove the Flow Cell, place at room temperature for 30 mins, and immediately place it on the sequencer stage for use.

① **Note：**

Do not move the Flow Cell when loading DNB The Flow Cell, after being placed at room temperature, should be used immediately.

4 Prepare the sequencing cartridge

- Remove the Sequencing Reagent Cartridge from -20℃. and thaw in a room temperature water bath until thawed. Store cartridges at 2-8℃ storage until use (or thaw cartridges 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.

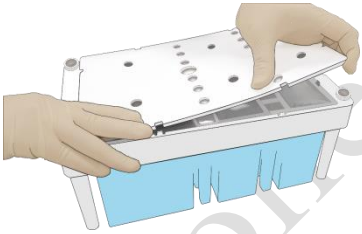


Figure 4-1 : Open and clean the cartridge

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

- Remove Sequencing Enzyme Mix from -20℃ storage and place at 4 °C 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）

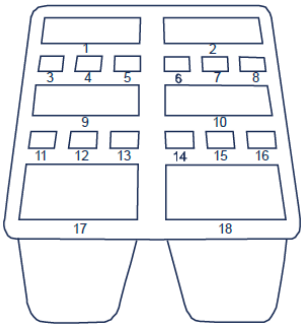


Figure 4-3：Well position

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-G400RS FCS PE100	dNTPs Mix	1.400
DNBSEQ-G400RS FCS PE150	dNTPs Mix	1.900

➢ #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-G400RS FCS PE100	dNTPs Mix II	2.800
DNBSEQ-G400RS FCS PE150	dNTPs Mix II	3.800

- #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	1#、2#well volume (mL)
DNBSEQ-G400RS FCS PE100	Sequencing Enzyme Mix	1.400
DNBSEQ-G400RS FCS PE150	Sequencing Enzyme Mix	1.900

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

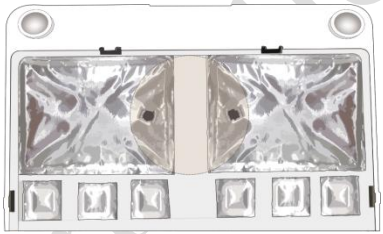


Figure 4-4：Seal with the loading well

- 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.

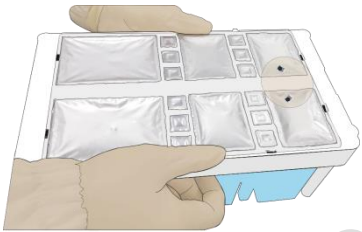


Figure 4-5：Mix reagents after loading

- # 15 well（see Figure 4-3）：The following instruction are only for PE kits.
  Add 500 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 on 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.

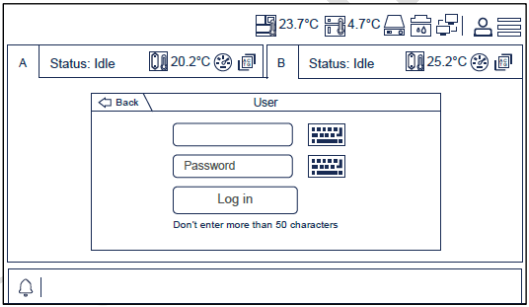


Figure 6-1 : Log-in interface

- See the interface below.

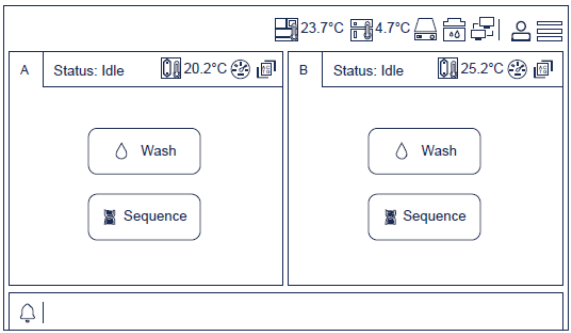


Figure 6-2：Main interface

6.2 Load the DNBs

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

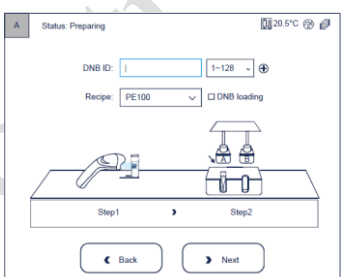


Figure 6-3：DNBs loading interface

- Click on the ⊕ on the right of the "DNB ID" and the two lane information appears.

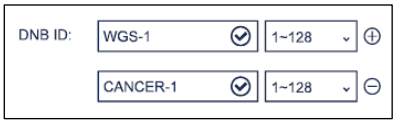


Figure 6-4：DNBs and information selection interface

- Move the cursor to the blank area next to the "DNB ID" and enter the library name or number.
- Pull the drop-down menu on the left of ⊕ and select the barcode sequence of different lanes.
- 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.

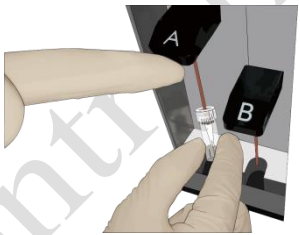


Figure 6-5：Load the DNBs tube

Note：

If the DNB is loaded using the sequencer, perform this step. If not, skip this step.

- Close the reagent compartment door.

6.3 Select sequencing parameters

- Select the sequencing recipe in the “Recipe” drop-down menu, one-click sequencing run (PE150、PE100) and user-customized run (Customize).

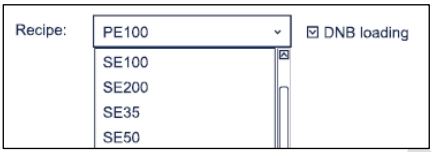


Figure 6-6 : Select sequencing solutions

- If you choose one-click sequencing and the DNB is loaded on the sequencer, check the "DNB loading" (such as Figure 6-6). Otherwise leave it blank and then go to the next step 6.4. If you choose "Customize", continue performing the following steps.

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

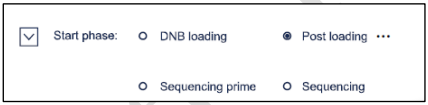


Figure 6-7 : Select the step to start sequencing

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

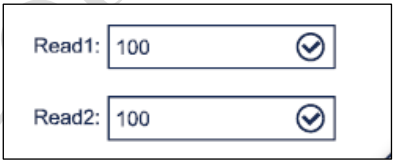


Figure 6-8 : Choose the read length

- 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.

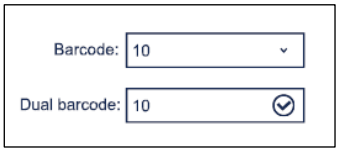


Figure 6-9 : Select the barcode length

- Select the lane for barcode demultiplexing.

Figure 6-10 : Barcode demultiplexing on different lanes

- Select the dark reaction for any position of read length in read 1 or 2 , please do not enter any number if no dark reactions are required.

Dark reaction: only chemical reaction without optical information capture

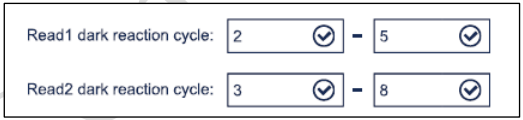


Figure 6-11 : Select the dark reaction

- 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.

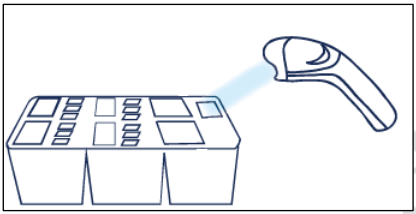


Figure 6-12：Reagent cartridge information entry interface

- 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.

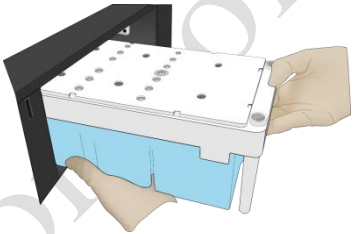


Figure 6-13：Remove cleaning cartridge

- 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 6-14 : 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.

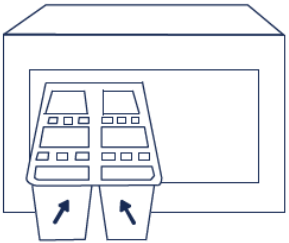


Figure 6-15 : Slide the new reagent cartridge into the reagent compartment

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.

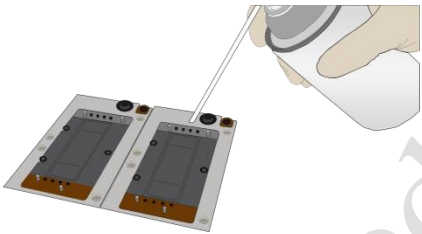


Figure 6-16：Clean the Flow Cell stage

- 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 6-17：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 at a 45° angle to the upper left corner (45° to the upper right corner when loading the Flow Cell on the MGIDL-200) 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.

① **Note：**

The Flow Cell is fragile, please use caution when handling the Flow Cell

- Ensure that the negative pressure is in the range of -80 ~ -99 kPa.

- Use a dust remover to remove the dust on the Flow Cell surface and close the Flow Cell compartment door.

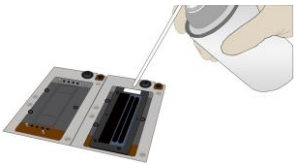


Figure 6-18：Clean the Flow Cell

- 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.

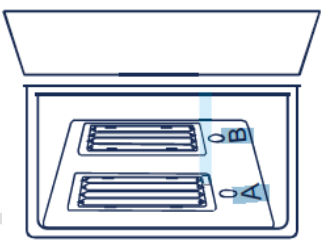


Figure 6-19：Flow Cell information entry interface

- Click “Next”

6.6 Review parameters

Review the run parameters to ensure that all informations are correct.

Item	Content
User name	user
DNB ID Lane1	WGS-1 | 1~128
DNB ID Lane2	CANCER-1 | 1~128
Sequencing cartridge ID	T0001
Flow cell ID	F300001234
Recipe	PE150
Start phase	Post loading
Cycles	312

Figure 6-20：Review information

6.7 Start sequencing

- After confirming that the informations are correct, click “Start”.

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

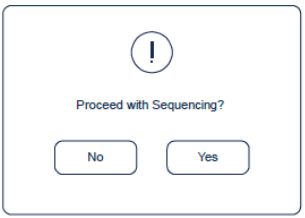


Figure 6-21：Confirm sequencing interface

- 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 To remove residual reagents and proteins in the pipeline, reducing risk of blockage. Procedure: Cleaning cartridge 1 → Air Prime
Regular wash	To remove residual reagents, reducing risk of cross-contamination.

7.2 Wash instruction

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

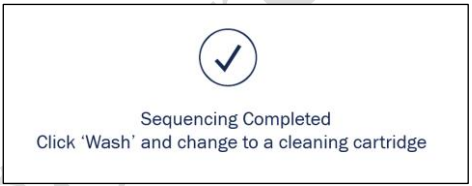


Figure 7-1：Wash interface

- 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 either A) a PE run or B) a DNB loading/post-load. 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 cyrotube 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 cyrotube 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, and it must use the FCL Flow Cell.

- Select regular wash from the drop-down menu to start the regular wash which takes about 50 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, and it must use the FCL Flow Cell.
- Select the maintenance wash from the drop-down menu to start the maintenance wash which takes about 25 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 25 minutes.

Figure 7-4 : Maintenance wash end interface

- When the interface appears as figure 7-5, click “Yes” to lift the needle and replace the cleaning cartridge.

Figure 7-5 : Maintenance wash end interface

- Use cleaning cartridge 2 and continue the maintenance wash which takes around 25 minutes.

Figure 7-6 : Maintenance wash end interface

- When the interface appears as Figure 7-6, the maintenance wash ends.

7.5.3 Full wash procedures

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

8 Troubleshooting

8.1 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 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 the engineer.

8.3 Bubbles

- Replace the used Flow Cell and inspect the pump.

- If the problem persists, please contact the engineer.

8.4 Impurities

- Perform a full wash on MGIDL-200 and the sequencer.

- If the problem persists after a full wash, please contact the engineer.

8.5 Pump fails

- MGIDL-200 and the 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 the 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 s 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.

8.7 Post loading fail

- If Post loading fails,but prime step has been performed, in this condition please re-start from the Post loading.

- Start from the chapter 6 “Sequencing” and re-load the Flow Cell.

- When selecting 6.3 sequencing parameters, choose progamme “Customize”.

- Select “Post loading” and click “…” .

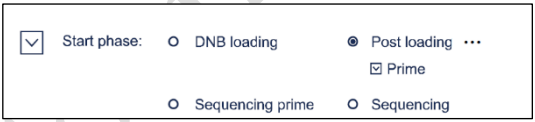


Figure 8-1 ：Select re-start Post loading

- If starts from the Post loading prime, select “Prime” as Figure 8-1, otherwise if starts from the step Post loading, don`t select “Prime”.

- Other steps please follow the chapter 6 “Sequencing” in this manual.

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

Table 10-1：List of set components 1

Product	Sequencing kit	Component	Spec & Quantity	Storage temperature
DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS SE100) Catalog number: 1000016978	DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number：1000016987	Sequencing Flow Cell	1	RT
	DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS SE100) Catalog number：1000016977	Low TE Buffer	300 μL×1tube	-25℃~-15℃
		Make DNB Buffer	100 μL×1tube	
		Make DNB Enzyme Mix I	200 μL×1tube	
		Make DNB Enzyme Mix II（LC）	25 μL×1tube	
		Stop DNB Reaction Buffer	100 μL×1tube	
		DNB Load Buffer I	200 μL×1tube	
		DNB Load Buffer II	200 μL×1tube	
		Micro Tube 0.5mL（Empty）	1tube	
		dNTPs Mix	0.90 mL×1tube	
		dNTPs Mix II	1.70 mL×1tubes	
		Sequencing Enzyme Mix	1.90 mL×1tube	
		Sequencing Reagent Cartridge	1	
		transparent sealing film	2 sheets	

Table 10-2：List of set components 2

Product	Sequencing kit	Component	Spec & Quantity	Storage temperature
DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE150) Catalog number: 1000016982	DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number：1000016987	Sequencing Flow Cell	1	RT
	DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE150) Catalog number：1000016981	Low TE Buffer	300 μL×1tube	-25℃~-15℃
		Make DNB Buffer	100 μL×1tube	
		Make DNB Enzyme Mix I	200 μL×1tube	
		Make DNB Enzyme Mix II（LC）	25 μL×1tube	
		Stop DNB Reaction Buffer	100 μL×1tube	
		DNB Load Buffer I	200 μL×1tube	
		DNB Load Buffer II	200 μL×1tube	
		Micro Tube 0.5mL（Empty）	1tube	
		dNTPs Mix	2.00 mL×1tube	
		dNTPs Mix II	2.00 mL×2tubes	
		Sequencing Enzyme Mix	4.80 mL×1tube	
		MDA Reagent	3.50 mL×1tube	
		MDA Enzyme Mix	0.60 mL×1tube	
		Sequencing Reagent Cartridge	1	
		transparent sealing film	2 sheets	

Table 10-3：List of set components 3

Product	Sequencing kit	Component	Spec & Quantity	Storage temperature
DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE100) Catalog number: 1000016980	DNBSEQ-G400RS Rapid Sequencing Flow Cell Catalog number：1000016987	Sequencing Flow Cell	1	RT
	DNBSEQ-G400RS High-throughput Rapid Sequencing Kit (FCS PE100) Catalog number：1000016979	Low TE Buffer	300 μL×1tube	-25℃~-15℃
		Make DNB Buffer	100 μL×1tube	
		Make DNB Enzyme Mix I	200 μL×1tube	
		Make DNB Enzyme Mix II（LC）	25 μL×1tube	
		Stop DNB Reaction Buffer	100 μL×1tube	
		DNB Load Buffer I	200 μL×1tube	
		DNB Load Buffer II	200 μL×1tube	
		Micro Tube 0.5mL（Empty）	1tube	
		dNTPs Mix	1.50 mL×1tube	
		dNTPs Mix II	1.50 mL×2tubes	
		Sequencing Enzyme Mix	3.10 mL×1tube	
		MDA Reagent	3.50 mL×1tube	
		MDA Enzyme Mix	0.60mL×1tube	
		Sequencing Reagent Cartridge	1	
		transparent sealing film	2 sheets	

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