# DNBSEQ-G400RS
# High-throughput Sequencing Set User Manual

Catalog number and name:

1000016941, DNBSEQ-G400RS High-throughput Sequencing Set（FCL SE50）

1000016943, DNBSEQ-G400RS High-throughput Sequencing Set（FCL SE100）

1000016950, DNBSEQ-G400RS High-throughput Sequencing Set（FCL PE100）

1000016952, DNBSEQ-G400RS High-throughput Sequencing Set（FCL PE150）

1000016946, DNBSEQ-G400RS High-throughput Sequencing Set（FCL SE400）

1000016955, DNBSEQ-G400RS High-throughput Sequencing Set（FCL PE200）

1000016998, DNBSEQ-G400RS High-throughput Sequencing Set (Small RNA FCL SE50)

Set version: V3.1

1000011532, CPAS Barcode Primer 3 Reagent Kit

Kit version: V1.0

User manual version: A1

## Revision History

| Version | Date | Summary of change |
|---|---|---|
| A1 | December 2019 | • Updated the equations used to calculate library input.<br>• Updated the information about adding and mixing dNTPs and enzyme in the chapter of “Prepare the sequencing cartridge”.<br>• Added information for “Dark green crystals in well No.10” and “Library amount less than 40 fmol” in the chapter of “Troubleshooting”.<br>• Moved the chapter “Sequencing Sets and Consumables Required but not Provided” to the front of the chapter “Sequencing Workflow”.<br>• Added the section of “Attention”.<br>• Added the “Revision History”. |
| A0 | August 2018 | • The first version. |

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# 1 Introduction

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

## 1.1 Applications

DNBSEQ-G400RS High-throughput Sequencing Set is specifically designed for DNA or RNA 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

Sequencing read length will determine the number of sequencing cycles for a given sequencing run. For example, a PE150 cycle run performs reads of 150 cycles (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 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 |
| SE400 | 400 | —— | 10 | 400+10 | 420 |
| PE100 | 100 | 100 | 10 | 200+10 | 220 |
| PE150 | 150 | 150 | 10 | 300+10 | 320 |
| PE200 | 200 | 200 | 10 | 400+10 | 420 |

### 1.5 Sequencing Time

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

| Time (hours) | SE50 | SE100 | SE400 | PE100 | PE150 | PE200 |
|---|---|---|---|---|---|---|
| Single flow cell | 14.2 | 24.3 | 103.5 | 46.5 | 67.4 | 100.0 |
| Dual flow cell | 15.0 | 26.2 | 103.5 | 50.3 | 72.4 | 108.0 |
| Data analysis | 1.0 | 1.5 | 5.0 | 3.3 | 5.2 | 5.0 |

Notes:

① The sequencing time (Single flow cell/Dual flow cell) in the table above includes the time required from Post loading prime to sequencing completion. The data analysis time includes the time required for barcode demultiplexing (if Split barcode is selected) and FASTQ files output when sequencing is completed.

② The time in the table above is theoretical and actual run time may vary among various sequencing instruments.

### 1.6 Sequencing Time

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 Sets and Consumables Required but not Provided

### 2.1 List of sequencing set components

Table 2-1: DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE50)　Catalog number: 1000016941

| Sequencing Kit | Component | Spec & Quantity | Storage Temperature |
|---|---|---|---|
| DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985 | Sequencing Flow Cell | 1 EA | RT |
| DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE50) Catalog number: 1000016940 | Low TE Buffer | 300 μL×1 tube | -25℃~-15℃ |
| | Make DNB Buffer | 100 μL×1 tube | |
| | Make DNB Enzyme Mix I | 200 μL×1 tube | |
| | Make DNB Enzyme Mix II（LC） | 25 μL×1 tube | |
| | Stop DNB Reaction Buffer | 100 μL×1 tube | |
| | DNB Load Buffer I | 200 μL×1 tube | |
| | DNB Load Buffer II | 200 μL×1 tube | |
| | Micro Tube 0.5mL（Empty） | 1 tube | |
| | dNTPs Mix | 0.80 mL×1tube | |
| | dNTPs Mix II | 0.70 mL×1tube | |
| | Sequencing Enzyme Mix | 1.60 mL×1tube | |
| | Sequencing Reagent Cartridge | 1 EA | |
| | Transparent sealing film | 2 sheets | |

Table 2-2: DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE100)　Catalog number: 1000016943

| Sequencing Kit | Component | Spec & Quantity | Storage Temperature |
|---|---|---|---|
| DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985 | Sequencing Flow Cell | 1 EA | RT |
| DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE100) Catalog number: 1000016942 | 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） | 1 tube | |
| | dNTPs Mix | 1.20 mL×1tube | |
| | dNTPs Mix II | 1.00 mL×1tube | |
| | Sequencing Enzyme Mix | 2.30 mL×1tube | |
| | Sequencing Reagent Cartridge | 1 EA | |
| | Transparent sealing film | 2 sheets | |

Table 2-3: DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE400) Catalog number: 1000016946

| Sequencing Kit | Component | Spec & Quantity | Storage Temperature |
|---|---|---|---|
| DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985 | Sequencing Flow Cell | 1 EA | RT |
| DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE400) Catalog number: 1000016944 | Low TE Buffer | 300 μL×1 tube | -25℃~-15℃ |
| | Make DNB Buffer | 100 μL×1 tube | |
| | Make DNB Enzyme Mix I | 200 μL×1 tube | |
| | Make DNB Enzyme Mix II（LC） | 25 μL×1 tube | |
| | Stop DNB Reaction Buffer | 100 μL×1 tube | |
| | DNB Load Buffer I | 200 μL×1 tube | |
| | DNB Load Buffer II | 200 μL×1 tube | |
| | Micro Tube 0.5mL（Empty） | 1 tube | |
| | dNTPs Mix | 4.10 mL×1tube | |
| | dNTPs Mix II | 12.20 mL×1tube | |
| | Sequencing Enzyme Mix | 8.30 mL×1tube | |
| | Wash Buffer For Sequencing | 2.90 mL×1tube | |
| | Sequencing Reagent Cartridge | 1 EA | |
| | Transparent sealing film | 6 sheets | |
| DNBSEQ-G400RS High-throughput Sequencing Refill Kit (FCL SE400) Catalog number: 1000016945 | Sequencing Reagent V4.0 | 200.0 mL×1bottle | -25℃~-15℃ |
| | Regeneration Reagent | 200.0 mL×1bottle | |
| | Image Reagent V2.0 | 200.0 mL×1bottle | |
| | Wash Reagent 1 V2.0 | 200.0 mL×1bottle | |
| | Wash Reagent 2 | 200.0 mL×1bottle | |

Table 2-4: DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE100) Catalog number: 1000016950

| Sequencing Kit | Component | Spec & Quantity | Storage Temperature |
|---|---|---|---|
| DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985 | Sequencing Flow Cell | 1 EA | RT |
| DNBSEQ-G400RS High-throughput Sequencing Kit (FCL PE100) Catalog number: 1000016949 | 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） | 1 tube | |
| | dNTPs Mix | 1.90 mL×1tube | |
| | dNTPs Mix II | 1.60 mL×1tube | |
| | Sequencing Enzyme Mix | 3.60 mL×1tube | |
| | MDA Reagent | 3.50 mL×1tube | |
| | MDA Enzyme Mix | 0.60 mL×1tube | |
| | Sequencing Reagent Cartridge | 1 EA | |
| | Transparent sealing film | 2 sheets | |

Table 2-5: DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150) Catalog number: 1000016952

| Sequencing Kit | Component | Spec & Quantity | Storage Temperature |
|---|---|---|---|
| DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985 | Sequencing Flow Cell | 1 EA | RT |
| DNBSEQ-G400RS High-throughput Sequencing Kit (FCL PE150) Catalog number: 1000016951 | 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） | 1 tube | |
| | dNTPs Mix | 1.30 mL×2tube | |
| | dNTPs Mix II | 1.15 mL×2tube | |
| | Sequencing Enzyme Mix | 4.80 mL×1tube | |
| | MDA Reagent | 3.50 mL×1tube | |
| | MDA Enzyme Mix | 0.60mL×1tube | |
| | Sequencing Reagent Cartridge | 1 EA | |
| | Transparent sealing film | 2 sheets | |

Table 2-6: DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE200) Catalog number: 1000016955

| Sequencing Kit | Component | Spec & Quantity | Storage Temperature |
|---|---|---|---|
| DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985 | Sequencing Flow Cell | 1 EA | RT |
| DNBSEQ-G400RS High-throughput Sequencing Kit (FCL PE200) Catalog number: 1000016953 | 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） | 1 tube | |
| | dNTPs Mix | 3.20 mL×2tube | |
| | dNTPs Mix II | 8.30 mL×2tube | |
| | Sequencing Enzyme Mix | 6.10 mL×1tube | |
| | MDA Reagent | 3.50 mL×1tube | |
| | MDA Enzyme Mix | 0.60mL×1tube | |
| | Sequencing Reagent Cartridge | 1 EA | |
| | transparent sealing film | 5 sheets | |
| DNBSEQ-G400RS High-throughput Sequencing Refill Kit (FCL PE200) Catalog number: 1000016954 | Regeneration Reagent | 200.0 mL×1bottle | -25℃~-15℃ |
| | Wash Reagent 1 V2.0 | 200.0 mL×1bottle | |
| | Wash Reagent 2 | 200.0 mL×1bottle | |

Table 2-7: DNBSEQ-G400RS High-throughput Sequencing Set (Small RNA FCL SE50)　Catalog number: 1000016998

| Sequencing Kit | Component | Spec & Quantity | Storage Temperature |
|---|---|---|---|
| DNBSEQ-G400RS Sequencing Flow Cell Catalog number: 1000016985 | Sequencing Flow Cell | 1 EA | RT |
| DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE50) Catalog number: 1000016940 | Low TE Buffer | 300 μL×1 tube | -25℃~-15℃ |
| | Make DNB Buffer | 100 μL×1 tube | |
| | Make DNB Enzyme Mix I | 200 μL×1 tube | |
| | Make DNB Enzyme Mix II（LC） | 25 μL×1 tube | |
| | Stop DNB Reaction Buffer | 100 μL×1 tube | |
| | DNB Load Buffer I | 200 μL×1 tube | |
| | DNB Load Buffer II | 200 μL×1 tube | |
| | Micro Tube 0.5mL（Empty） | 1 tube | |
| | dNTPs Mix | 0.80 mL×1tube | |
| | dNTPs Mix II | 0.7 mL×1 tube | |
| | Sequencing Enzyme Mix | 1.60 mL×1 tube | |
| | Sequencing Reagent Cartridge | 1 EA | |
| | Transparent sealing film | 2 sheets | |
| MGIEasy Wash Buffer For Small RNA Sequencing Catalog number: 1000006387 | Wash Buffer For Small RNA Sequencing | 1.60mL×3tube | -25℃~-15℃ |

Table 2-8: CPAS Barcode Primer 3 Reagent Kit Catalog number: 1000011532

| Product | Sequencing Kit | Component | Spec & Quantity | Storage Temperature |
|---|---|---|---|---|
| Only for double index sequencing (Pair End Sequencing use only) | CPAS Barcode Primer 3 Reagent Kit Catalog number: 1000011532 | 1μM AD153 Barcode Primer 3 Catalog number: 1000011531 | 3.0mL×1tube | -25℃~-15℃ |

## 2.2 Equipment and Consumables Required but not Provided

**Table 2-9：Equipment and consumables Required but not Provided**

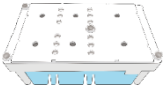
| Equipment and consumables | Recommended brand | Catalog number |
|---|---|---|
| Qubit® 3.0 Fluorometer | Thermofisher | Q33216 |
| Mini centrifuge | Major Laboratory Supplier (MLS) | / |
| Vortex mixer | MLS | / |
| PCR machine | Bio-Rad | / |
| Pipette | Eppendorf | / |
| 2~8℃ refrigerator | MLS | / |
| -18~-25℃ freezer | 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 | / |
| Electronic pipette | Labnet | FASTPETTEV-2 |
| Serological pipet | CORNING | / |
| 5 mL Tube | SARSTEDT | 60.558.001 |
| 10 mL Tube | SARSTEDT | 60.551.001 |
| 15 mL Tube | SARSTEDT | 60.732.001 |
| 25 mL Tube | SARSTEDT | 60.9922.243 |

## 3 Sequencing Workflow

Make DNB: use DNB preparation kit for making DNB

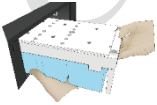
Prepare a new Flow Cell：remove the Flow Cell from package and inspect to ensure the Flow Cell is intact

DNB loading: load the DNB onto sequencing flow cell



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

Load the Flow Cell：place the Flow Cell on the stage of the sequencer



Load the reagent kit into the sequencer

Start sequencing: follow the instructions to enter sequencing information and start the run

Sequencing: monitor the sequencing run from the control software interface

Data analysis: the sequencer will automatically split barcode (if Split barcode is selected) and output FASTQ files when sequencing is completed.


Device maintenance: perform device maintenance when sequencing is completed

# 4 Make DNB

## 4.1 Insert Size Recommendation

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

Recommended library insert size:

The size distribution of inserts should be between 20-800 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: Recommended insert size

| Sequencing Kit* | Suggested insert distribution (bp) | Data output (Gb/lane) ** |
|---|---|---|
| DNBSEQ-G400RS FCL SE50 | 50-230 | 18.7-22.5 |
| DNBSEQ-G400RS FCL SE100 | 200-400 | 37.5-45.0 |
| DNBSEQ-G400RS FCL SE400 | 400-600 | 150.0-180.0 |
| DNBSEQ-G400RS FCL PE100 | 200-400 | 75.0-90.0 |
| DNBSEQ-G400RS FCL PE150 | 300-500 | 112.5-135.0 |
| DNBSEQ-G400RS FCL PE200 | 400-600 | 150-180.0 |
| DNBSEQ-G400RS Small RNA FCL SE50 | 20-60 | / |

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

**Average data output will vary with different library type 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 and each Make DNB reaction requires 40 fmol library. For small RNA libraries, the ssDNA library concentration should be ≥ 3 fmol/μL and each Make DNB reaction requires 60 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.

### 4.3 Make DNB

#### 4.3.1 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.

① **Note**:

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

**4.3.2 select the DNB loader**

The DNBSEQ-G400RS sequencing flow cell contains 4 lanes. DNB can be loaded onto the flow cell using the sequencer, the MGIDL-200RS or the MGIDL-200H.

- When using the sequencer to load DNB, all 4 lanes must be the same DNB. Each lane requires 50 μL DNB.
- When using the MGIDL-200RS to load DNB, 4 different DNB can be loaded onto 4 different lane. Each lane requires 50 μL DNB.
- When using the MGIDL-200H to load DNB, 4 different DNB can be loaded onto 4 different lane. Each lane requires 25 μL DNB.

Table 4-2: The required number of make DNB reactions for each DNBSEQ-G400RS flow cell

| Loading system | DNB loading volume (μL)/Lane | Make DNB reaction (μL) | The required number of make DNB reaction /flow cell |
|---|---|---|---|
| Sequencer | 50 | 100 | 2 |
| MGIDL-200RS | 50 | 100 | 2-4 |
| MGIDL-200H | 25 | 100 | 1-4 |

**4.3.3 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 4.2. The volume of each Make DNB reaction is 100 μL and the required library input for each Make DNB reaction is calculated as followed:

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

Note:

For Small RNA libraries, ssDNA library input (μL) = 60 fmol / library concentration (fmol/μL)

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 4-3 as V.

① **Note**:

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

**4.3.4 Make DNB**

- Take a 0.2 mL 8-tube strip or PCR tubes. Prepare reaction mix following Table 4-3 below.

**Table 4-3：Make DNB reaction mix 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 4.3.3. Mix gently 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 shown in the table below:

**Table 4-4：Primer hybridization reaction condition**

| 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 for a prolonged time.**

- Take the PCR tube out of the PCR machine when the temperature reaches 4℃. Centrifuge briefly for 5 s, place the tube on ice and prepare the Make DNB reaction mix 2.

**Table 4-5：Make DNB reaction mix 2**

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

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

**Table 4-6：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, or shake the tube. Store DNB at 4℃ and perform sequencing within 48 hours.**

Notes:

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 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℃.

### 4.4 Quantify DNB

When the make DNB is completed, take 2 μL DNB, use Qubit® ssDNA Assay Kit and Qubit® Fluorometer to quantify the DNB. Sequencing requires a minimum DNB concentration of 8 ng/μL. If the concentration is lower than 8 ng/μL, make a new DNB preparation.

Notes:

① 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 DNBs should be diluted to 20 ng/μL with DNB Load Buffer I before loading.

③ Store DNB at 4℃ and perform sequencing within 48 hours.

## 5 Prepare a Flow Cell

- Unwrap the outer package before use.

**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 DNB Loading

Remove DNB Load Buffer II from storage and thaw reagents on ice 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.

### 6.1 Sequencer DNB loading

- Take a 0.5 mL microfuge tube and add the following reagents (see table 6-1 below).

**Table 6-1：DNB loading mix 1 (for sequencer loading)**

| Component | Volume（μL） |
|---|---|
| DNB Load Buffer II | 64 |
| Make DNB Enzyme Mix II（LC） | 2 |
| DNB | 200 |
| Total Volume | 266 |

- Combine components and mix by gently pipetting 5-8 times using a wide bore tip. Do not centrifuge, vortex, or shake the tube. Place the mixture at 4°C until use.

Notes:

① Prepare a fresh DNB loading mix immediately before the sequencing run.

② Each flow cell requires 266 μL DNB loading mix.

### 6.2 MGIDL-200RS DNB loading

- Take a new PCR 8-tube strip and add the following reagents (see table 6-2 below)

**Table 6-2：DNB loading mix 2 (for MGIDL-200RS loading)**

| Component | Volume（μL） |
|---|---|
| DNB Load Buffer II | 16 |
| Make DNB Enzyme Mix II（LC） | 0.5 |
| DNB | 50 |
| Total volume | 66.5 |

- Combine components and mix by gently pipetting 5-8 times using a wide bore tip. Do not centrifuge, vortex, or shake the tube. Place the mixture at 4°C until use.
- Please refer to the “MGIDL-200RS User Manual” for details on loading operation.

Notes:

① Each lane requires at least 66.5 μL of DNB loading mix.

② Before DNB loading, perform a wash as described in the MGIDL-200RS User Manual.

③ Place the tubes containing DNB loading mix in the labeled positions of MGIDL-200RS (see Figure 6-1).

④ Press the flow cell attachment button, hold the flow cells by edges and align the holes on the flow cells with the locating pins on the flow cell stages. Press the left and right sides of the flow cell on the stage at the same time to ensure that the flow cells are securely seated on the stage.

⑤ Select the desired loading recipe from the drop-down list and start DNB loading.

⑥ After DNB loading, remove the flow cell and place it in a PE glove or a plastic bag at room temperature for 30 min, then immediately place it on the sequencer for use.

**Figure 6-1：Place the loading samples**

### 6.3 MGIDL-200H DNB loading

- Take a new PCR 8-tube strip and add the following reagents (see table 6-3 below)

**Table 6-3：DNB loading mix 3 (for MGIDL-200H loading)**

| Component | Volume（μL） |
|---|---|
| DNB Load Buffer II | 8 |
| Make DNB Enzyme Mix II（LC） | 0.25 |
| DNB | 25 |
| Total volume | 33.25 |

- Combine components and mix by gently pipetting 5-8 times using a wide bore tip. Do not centrifuge, vortex, or shake the tube. Place the mixture at 4°C until use.
- Please refer to the "MGIDL-200H Portable DNB Loader Quick Start Guide" for details on loading operation.

Notes:

① Each lane requires 30 μL of DNB loading mix.

② Before DNB loading, clean the device as described in the MGIDL-200H Quick Start Guide.

③ Install the sealing gasket and flow cell. Aspirate 30 μL DNB loading mix with a pipette and insert the wide bore tip into the fluidics inlet (see Figure 6-2). Do not press the control button of the pipette after inserting the tip into the fluidics inlet. Eject the tip from the pipette and the DNB loading mix will automatically flow into the flow cell.

④ After DNB loading, rotate the tips counterclockwise to remove them. Place the device on the bench with the front upward for 30 min before use.

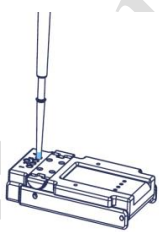


**Figure 6-2：Load samples using MGIDL-200H**

① **Note：**

**Do not move the flow cell during loading procedure. After being placed at room temperature, the flow cell should be used immediately.**

## 7 Prepare the sequencing cartridge

- Remove the Sequencing Reagent Cartridge and the High-throughput Sequencing Refill Kit (only for SE400 and PE200 sequencing) from storage. Thaw in a room temperature water bath until completely thawed. Store at 2-8°C storage until use (or thaw in 2-8°C fridge one day in advance). Invert the cartridge 3 times to mix before use. Shake the cartridge violently in all directions for 10-20 times until no visible layers can be seen in the cartridge, especially for reagents in well No.9 and No.10.

Note:

If dark green crystals appear in well No.10, it is precipitation of raw materials of the reagent in well No.10. 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. See "10.8 Dark green crystals in well No.10" in this manual for details.

- Open the cartridge cover and wipe any water condensation with lint-free paper (see Figure 7-1). Well position is shown in Figure 7-2.

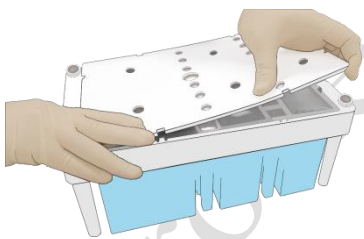


**Figure 7-1：Open and clean the kit**

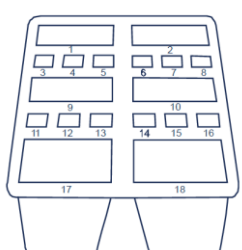



**Figure 7-2：Well position**

- Remove dNTPs Mix and dNTPs Mix II from -20℃ storage 1h in advance and thaw at room temperature. Store at 4°C until use. Mix the reagents using a vortex mixer for 5 seconds and centrifuge briefly before use.
- Remove Sequencing Enzyme Mix from -20℃ storage and place on ice until use. Invert Sequencing Enzyme Mix 4-6 times before use.

### 7.1 Prepare the SE50 and SE100 sequencing cartridge

- Pierce the seal at the edge of well No.1 and No.2 (see Figure 7-2) to make a hole around 2 cm in diameter using 1 mL sterile tip (see Figure 7-3):

**Figure 7-3: Pierce the seal of SE50 or SE100 cartridge**

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix into a new 5 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.1:

**Table 7-1: SE50 and SE100 sequencing cartridge well No.1 reagent loading**

| Sequencing kit | dNTPs Mix loading volume | Sequencing Enzyme Mix loading volume |
|---|---|---|
| SE50 | 0.700 mL | 0.700 mL |
| SE100 | 1.100 mL | 1.100 mL |

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix II into a new 5 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix II in the same tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.2:

**Table 7-2：SE50 and SE100 sequencing cartridge well No.2 reagent loading**

| Sequencing kit | dNTPs Mix II loading volume | Sequencing Enzyme Mix loading volume |
|---|---|---|
| SE50 | 0.600 mL | 0.600 mL |
| SE100 | 0.900 mL | 0.900 mL |

- Seal the loading wells of well No.1 and No.2 with the transparent sealing film. Do not cover the center of the well to avoid blocking the sampling needle:

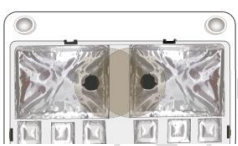


**Figure 7-4：Seal the loading wells of SE50 or SE100 cartridge**

- Place the cartridge horizontally on the table, hold both sides of the cartridge with both hands (see Figure7-5). Shake it clockwise 10-20 times, and then counterclockwise 10-20 times, until the reagent color in well No.1 is uniform. Make sure that you see the vortex to ensure reagents are fully mixed. The SE50 or SE100 sequencing cartridge is now ready for use.

**Figure 7-5：Mix reagents after loading**

### 7.2 Prepare the SE400 sequencing cartridge

- Remove the Wash Buffer For Sequencing from storage and thaw at room temperature.

- Pierce the seal at the edge of well No.1, No.2, No.7, No.9, No.10, No.17 and No.18 to make a hole around 2 cm in diameter using 1 mL sterile tip (see Figure 7-6):

**Figure 7-6: Pierce the seal of SE400 cartridge**

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix into a new 10 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.1:

**Table 7-3：SE400 sequencing cartridge well No.1 reagent loading**

| Sequencing kit | dNTPs Mix loading volume | Sequencing Enzyme Mix loading volume |
|---|---|---|
| SE400 | 4.000 mL | 4.000 mL |

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix II into a new 20 mL or 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.2:

**Table 7-4：SE400 sequencing cartridge well No.2 reagent loading**

| Sequencing kit | dNTPs Mix II loading volume | Sequencing Enzyme Mix loading volume |
|---|---|---|
| SE400 | 12.00 mL | 4.000 mL |

- Invert to mix the refill reagents 3 times and mix the Wash Buffer For Sequencing using a vortex mixer for 5 seconds. Take a pipette with the appropriate volume range and add reagents following the table below:

**Table 7-5：SE400 refill reagent loading**

| Sequencing kit | Well No. | Reagent name | Loading volume mL |
|---|---|---|---|
| SE400 | 1 | Sequencing Reagent V4.0 | 100.0 |
| | 2 | Sequencing Reagent V4.0 | 100.0 |
| | 17 | Wash Reagent 1 V2.0 | 200.0 |
| | 18 | Wash Reagent 2 | 200.0 |
| | 10 | Image Reagent V2.0 | 200.0 |
| | 9 | Regeneration Reagent | 200.0 |
| | 7 | Wash Buffer For Sequencing | 2.700 |

① **Note：**

① **Please add the refill reagents in strict accordance with the order shown in the table.**

② **After adding one reagent, please change your gloves before adding the next reagent.**

⚠ **Note:**

**Wash Buffer For Sequencing contains highly concentrated formamide which may have potential reproductive toxicity. Avoid breathing steam and wear protective gloves/protective clothing/protective eye mask/protective mask when using. The waste reagent must be discarded according to local and national regulations.**

- Seal the loading wells of well No.1, No.2, No.9, No.10, No.17 and No.18 with the transparent sealing film. Do not cover the center of the well to avoid blocking the sampling needle (see Figure 7-7):

**Table 7-7：Seal the loading wells of SE400 cartridge**

- Place the cartridge horizontally on the table, hold both sides of the cartridge with both hands (see Figure7-5). Shake it clockwise 10-20 times, and then counterclockwise 10-20 times, until the reagent color in well No.1 is uniform. Make sure that you see the vortex to ensure reagents are fully mixed. The SE400 sequencing cartridge is now ready for use.

### 7.3 Prepare the PE100 and PE150 sequencing cartridge

- Pierce the seal at the edge of well No.1 and No.2 (see Figure 7-2) to make a hole around 2 cm in diameter using 1 mL sterile tip (see Figure 7-8):

**Figure 7-8: Pierce the seal of PE100 or PE150 cartridge**

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix into a new 10 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.1:

**Table 7-6：PE100 and PE150 sequencing cartridge well No.1 reagent loading**

| Sequencing kit | dNTPs Mix loading volume | Sequencing Enzyme Mix loading volume |
|---|---|---|
| PE100 | 1.800 mL | 1.800 mL |
| PE150 | 2.400 mL | 2.400 mL |

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix II into a new 5 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix II in the same tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.2:

**Table 7-7：PE100 and PE150 sequencing cartridge well No.2 reagent loading**

| Sequencing kit | dNTPs Mix II loading volume | Sequencing Enzyme Mix loading volume |
|---|---|---|
| PE100 | 1.500 mL | 1.500 mL |
| PE150 | 2.100 mL | 2.100 mL |

- Seal the loading wells of well No.1 and No.2 with the transparent sealing film. Do not cover the center of the well to avoid blocking the sampling needle:

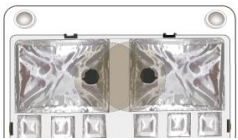


**Figure 7-9：Seal the loading wells of PE100 or PE150 cartridge**

- Place the cartridge horizontally on the table, hold both sides of the cartridge with both hands (see Figure7-5). Shake it clockwise 10-20 times, and then counterclockwise 10-20 times, until the reagent color in well No.1 is

uniform. Make sure that you see the vortex to ensure reagents are fully mixed.

- Pierce the seal of well No.15 using 1 mL sterile tip. Add 500 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.15. When adding the mixture, make sure there are no bubbles at the bottom of the tube. The PE100 or PE150 sequencing cartridge is now ready for use.

① **Note:**

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

### 7.4 Prepare the PE200 sequencing cartridge

- Pierce the seal at the edge of well No.1, No.2, No.9, No.17 and No.18 to make a hole around 2 cm in diameter using 1 mL sterile tip (see Figure 7-10):

**Figure 7-10: Pierce the seal of PE200 cartridge**

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix into a new 10 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.1:

**Table 7-8：PE200 sequencing cartridge well No.1 reagent loading**

| Sequencing kit | dNTPs Mix loading volume | Sequencing Enzyme Mix loading volume |
|---|---|---|
| PE200 | 3.100 mL | 3.100 mL |

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix II into a new 15 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix II in the same tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.2:

**Table 7-9：PE200 sequencing cartridge well No.2 reagent loading**

| Sequencing kit | dNTPs Mix II loading volume | Sequencing Enzyme Mix loading volume |
|---|---|---|
| PE200 | 8.100 mL | 2.700 mL |

- Invert to mix the refill reagents 3 times to mix. Take a pipette with the appropriate volume range and add reagents following the table below:

**Table 7-10：PE200 refill reagent loading**

| Sequencing kit | Well No. | Reagent name | Loading volume mL |
|---|---|---|---|
| PE200 | 17 | Wash Reagent 1 V2.0 | 200.0 |
| | 18 | Wash Reagent 2 | 200.0 |
| | 9 | Regeneration Reagent | 200.0 |

① **Note：**

① **Please add the refill reagents in strict accordance with the order shown in the table.**

② **After adding one reagent, please change your gloves before adding the next reagent.**

- Seal the loading wells of well No.1, No.2, No.9, No.17 and No.18 with the transparent sealing film. Do not cover the center of the well to avoid blocking the sampling needle (see Figure 7-11):

**Table 7-11：Seal the loading wells of PE200 cartridge**

- Place the cartridge horizontally on the table, hold both sides of the cartridge with both hands (see Figure7-5). Shake it clockwise 10-20 times, and then counterclockwise 10-20 times, until the reagent color in well No.1 is uniform. Make sure that you see the vortex to ensure reagents are fully mixed. The SE400 sequencing cartridge is now ready to be used.

- Pierce the seal of well No.15 using 1 mL sterile tip. Add 500 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.15. When adding the mixture, make sure there are no bubbles at the bottom of the tube. The PE200 sequencing cartridge is now ready for use.

① **Note：**

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

### 7.5 Prepare the SE50 (Small RNA) sequencing cartridge

- Remove the Wash Buffer For Small RNA Sequencing from storage and thaw at room temperature.

- Pierce the seal at the edge of well No.1 and No.2 (see Figure 7-2) to make a hole around 2 cm in diameter using 1 mL sterile tip (see Figure 7-12):

**Figure 7-12：Pierce the seal of SE50 cartridge**

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix into a new 5 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix in the same tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.1:

**Table 7-11：SE50 sequencing cartridge well No.1 reagent loading**

| Sequencing kit | dNTPs Mix loading volume | Sequencing Enzyme Mix loading volume |
|---|---|---|
| SE50 | 0.700 mL | 0.700 mL |

- Take a pipette with the appropriate volume range and add reagents following the table below. First add dNTPs Mix II into a new 5 mL sterile tube. Then add Sequencing Enzyme Mix into the dNTPs Mix II in the same tube. Invert the tube for 4-6 times to mix the reagents in the tube before adding all of them into well No.2:

**Table 7-12：SE50 sequencing cartridge well No.2 reagent loading**

| Sequencing kit | dNTPs Mix II loading volume | Sequencing Enzyme Mix loading volume |
|---|---|---|
| SE50 | 0.600 mL | 0.600 mL |

- Seal the loading wells of well No.1 and No.2 with the transparent sealing film. Do not cover the center of the well to avoid blocking the sampling needle:

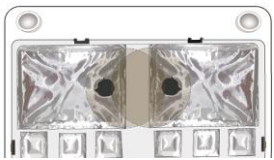


**Figure 7-13：Seal the loading wells of SE50 cartridge**

- Place the cartridge horizontally on the table, hold both sides of the cartridge with both hands (see Figure7-5). Shake it clockwise 10-20 times, and then counterclockwise 10-20 times, until the reagent color in well No.1 is uniform. Make sure that you see the vortex to ensure reagents are fully mixed.

- Mix the Wash Buffer For Small RNA Sequencing using a vortex mixer for 5 seconds and centrifuge briefly before use. Pierce the seal of well No.7 then add 4.50 mL of the Wash Buffer For Small RNA Sequencing. When adding the reagent, make sure there are no bubbles at the bottom of the tube. The SE50 (Small RNA) sequencing cartridge is now ready for use.

⚠ **Note:**

**Wash Buffer For Small RNA Sequencing contains highly concentrated formamide which may have potential reproductive toxicity. Avoid breathing steam and wear protective gloves/protective clothing/protective eye mask/protective mask when using. The waste reagent must be discarded according to local and national regulations.**

### 7.6 Dual barcode sequencing

- Remove the CPAS Barcode Primer 3 (for Pair End Sequencing only) from the CPAS Barcode Primer 3 Reagent Kit and thaw at room temperature. Mix the CPAS Barcode Primer 3 using a vortex mixer for 5 seconds and centrifuge briefly before use.

- Pierce the seal of well No.4 using a sterile tip, then add 2.90 mL of the CPAS Barcode Primer 3. When adding the reagent, make sure there are no bubbles at the bottom of the tube.

## 8 Sequencing

### 8.1 Enter the main interface

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

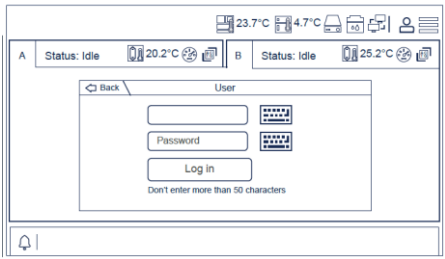



**Figure 8-1：Log-in interface**

- See the interface below.

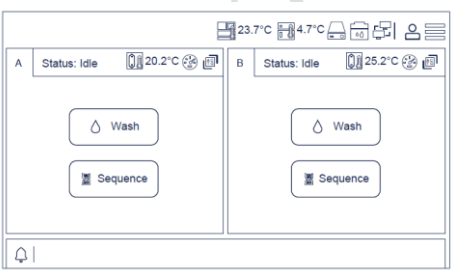



**Figure 8-2：Main interface**

## 8.2 Load the DNBs

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

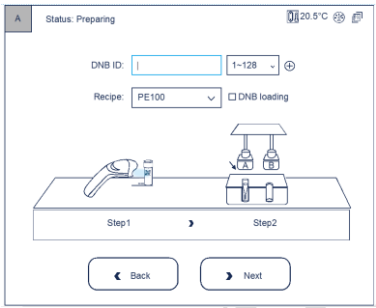



**Figure 8-3：DNBs loading interface**

- Click on the ⊕ on the right of the "DNB ID" and the four lane information will appear.

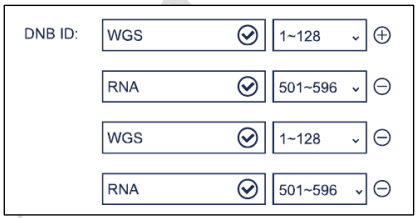



**Figure 8-4：DNBs and information selection interface**

- 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.
- When using the sequencer to load DNB, 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 until the tip reaches the bottom of the tube.

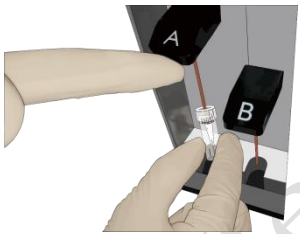



**Figure 8-5：Load the DNBs tube**

- Close the reagent compartment door.

### 8.3 Select sequencing parameters

- Select the sequencing recipe in the “Recipe” drop-down menu. There are one-click sequencing run (PE150, SE50, etc.) and user-customized run (Customize).

Note:

Sequencing solution“SE50_sR”is for Small RNA sequencing.

If for dual barcode sequencing, choose recipe “Customize”

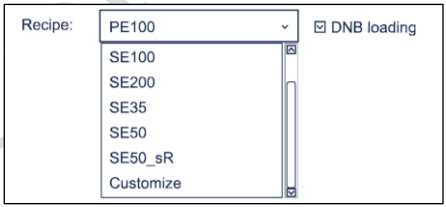



**Figure 8-6：Select sequencing solutions**

- If you choose one-click sequencing and the DNB is loaded on the sequencer, check the “DNB loading” (see

Figure 8-6). Otherwise leave it blank and then go to step 8.4. If you choose “Customize”, continue performing the following steps.

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


**Figure 8-7：Select the step to start sequencing**

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

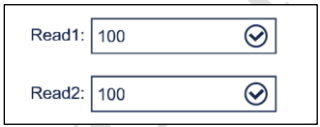



**Figure 8-8：Choose the read length**

- Select the barcode length. For dual barcode sequencing, fill in the length of the Dual barcode. Leave the Dual barcode blank if it is a single barcode sequencing run.

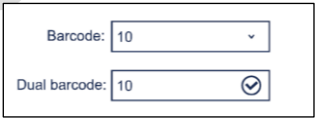



**Figure 8-9：Select the barcode length**

- Select the lane for barcode demultiplexing.


**Figure 8-10：Barcode demultiplexing on different lanes**

- Select the dark reaction for any position of read length in read 1 or 2. If dark reaction is not required, leave the table below blank.
  Dark reaction: only chemical reaction without optical information capture.

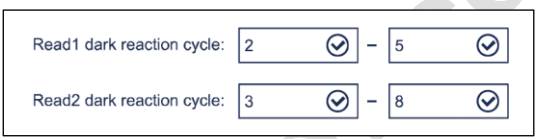



**Figure 8-11：Select the dark reaction**

- Click “Confirm”

### 8.4 Load the reagent cartridge

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

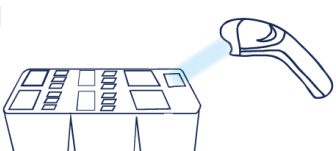


**Figure 8-12：Reagent cartridge information entry interface**

- 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.

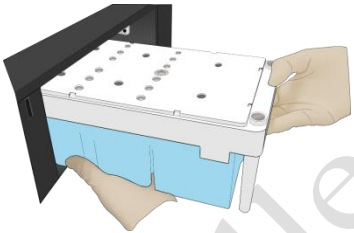


**Figure 8-13：Remove cleaning cartridge**

- 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 8-14：Maintain the reagent compartment**

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

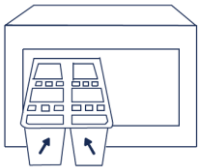


**Figure 8-15：Slide the new reagent cartridge into the reagent compartment**

### 8.5 Load the Flow Cell

- Open the flow cell compartment door, press both sides of the 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.

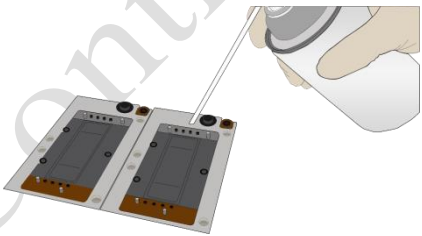


**Figure 8-16：Clean the Flow Cell stage**

- 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.

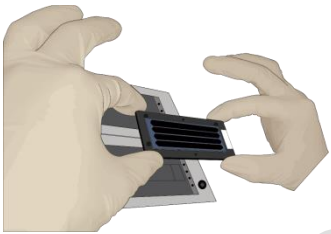


**Figure 8-17：Load the Flow Cell**

- 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-200RS) to keep the flow cell aligned with the pin. Press the flow cell attachment button. 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.**

- Ensure that the negative pressure is within the range of -80 ~ -99 kPa before continuing. If the negative pressure is abnormal, refer to "10.2 Abnormal negative pressure" in this manual for troubleshooting.

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

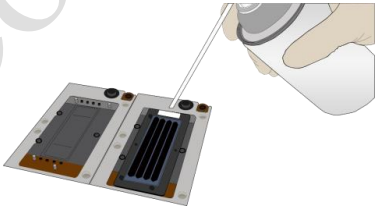


**Figure 8-18：Clean the Flow Cell**

- 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 enter the ID manually.

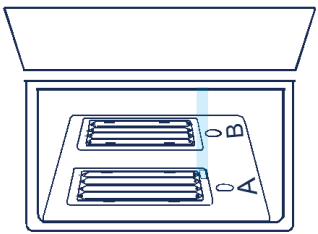



**Figure 8-19：Flow Cell information entry interface**

- Click “Next”

## 8.6 Review parameters

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

| Item | Content |
| --- | --- |
| User name | user |
| DNB ID Lane1 | WGS \| 1~128 |
| DNB ID Lane2 | RNA \| 501~596 |
| DNB ID Lane3 | WGS \| 1~128 |
| DNB ID Lane4 | RNA \| 501~596 |
| Sequencing cartridge ID | AA000012 |
| Flow cell ID | V300001234 |
| Recipe | Customize |
| Start phase | DNB loading |
| Cycles | 222 |
| Read 1 | 100 |
| Read 2 | 100 |
| Dual Barcode | 10 |
| Barcode | 10 |
| Split barcode | Yes \| Yes \| Yes \| Yes |
| Read1 dark reaction | 2 - 5 |
| Read2 dark reaction | 3 - 8 |

**Figure 8-20：Review information**

Note:

To ensure sequencing quality, when read 1 and read 2 sequencing is completed, the sequencer will automatically perform one more cycle for correction. For example, for PE100 dual barcode sequencing, read 1 read length is 100, read 2 read length is 100, barcode read length is 10 and dual barcode read length is 10, plus 1 correction cycle for read 1 and 1 correction cycle for read 2 (barcode does not require correction), the total cycle number of the sequencing is 222.

### 8.7 Start sequencing

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

- The system will display the dialog box “Proceed with Sequencing?”. Click “Yes” to start sequencing.


**Figure 8-21：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.

## 9 Device Maintenance

### 9.1 Terminology and Definition

**Table 9-1：Wash Solution**

| Wash type | Description | Time |
|---|---|---|
| Full wash | Step 1: Maintenance wash → Step 2: Regular wash. | ~ 125 min |
| Maintenance wash | To remove residual reagents and proteins in the pipeline, reducing risk of blockage.<br>Procedure: Cleaning cartridge 4 → Cleaning cartridge 3 → Cleaning cartridge 2 | ~ 75 min |
| Regular wash | To remove residual reagents, reducing risk of cross-contamination.<br>Procedure: Cleaning cartridge 1 → Air Prime | ~ 50 min |

### 9.2 Wash instruction

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

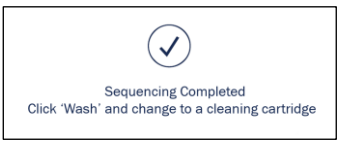



**Figure 9-1：Wash interface**

- When 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.

### 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 | 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 9-4：Wash reagent preparation（3）**

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

### 9.4 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 every time before refilling it with cleaning reagents. Replace cleaning cartridge after 20 uses or every half year.

- 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 (for DNB loading tube

washing), 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

### 9.5 Wash procedures

#### 9.5.1 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 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 9-2：Select the wash type**

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

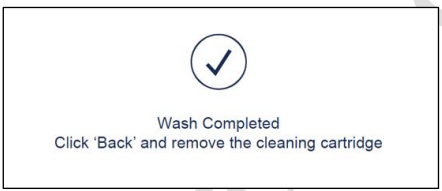



**Figure 9-3：Regular wash end interface**

**9.5.2 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 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 9-4, click "Yes" and the sequencer will automatically lift the sampling

needles. Then open the compartment door and replace the cleaning cartridge.

- Use cleaning cartridge 3 and continue the maintenance wash which takes around 25 minutes.


**Figure 9-4：Maintenance wash [1] end interface**

- When the interface appears as figure 9-5, click “Yes” and the sequencer will automatically lift the sampling needles. Then open the compartment door and replace the cleaning cartridge.


**Figure 9-5：Maintenance wash [2] end interface**

- Use cleaning cartridge 2 and continue the maintenance wash which takes around 25 minutes.
- When the interface appears as Figure 9-6, click “No” to end the maintenance wash.


**Figure 9-6：Maintenance wash end interface**

#### 9.5.3 Full wash procedures

Step 1 – Maintenance wash, Step 2 – Regular wash. Total time is around 125 min.

## 10 Troubleshooting

### 10.1 Low DNB concentration

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

- 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 a field service engineer.

### 10.2 Abnormal negative pressure

When the negative pressure is shown in red, the negative pressure is abnormal, try the following steps:

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

**10.3 Bubbles**

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

**10.4 Impurities**

- Perform a full wash on MGIDL-200RS and the sequencer following the MGIDL-200RS User Manual and "9.5 Wash procedures" in this manual.
- If the problem persists after a full wash, please contact a field service engineer.

**10.5 Pump fails**

If liquids cannot be pumped into the flow cell, or large bubbles appear in the flow cell, try the following steps:

- MGIDL-200RS 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 in "8.5 Load the Flow Cell" 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.

**10.6 Reagent kit storage**

- If the kit has been thawed (including dNTPs) and cannot be used within 24 hours, it can be frozen and thawed at most once.
- If the kit has been thawed (including dNTPs) but cannot be used immediately, store it at 4℃ and use it within 24 hours. Mix the reagents in the cartridge following instruction in "7. Prepare the sequencing cartridge" before use.
- If dNTPs and enzyme have been added into the cartridge, i.e. the cartridge has been prepared but cannot be used immediately, store it at 4℃ and use it within 24 hours. Mix the reagents in the cartridge following instruction in "7. Prepare the sequencing cartridge" before use.

- If dNTPs and enzyme have been added into the cartridge, i.e. the cartridge has been prepared and the sampling 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. Gently mix the reagents in the cartridge before use. When mixing, be careful not to spill any reagent from the needle holes to avoid reagent contamination.

### 10.7 Post loading fails

- If post loading fails, but prime step has been performed, in this condition please re-start from the post loading.
- Start from "8 Sequencing" and re-load the flow cell.
- When selecting "8.3 Select sequencing parameters", choose progamme "Customize".
- Select "Post loading" and click "…".

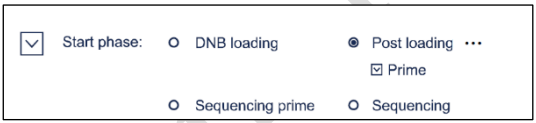



**Figure 10-1: Select re-start Post loading**

- If starts from the Post loading prime, select "Prime" in Figure 10-1. If starts from the step Post loading, don`t select "Prime".
- Other steps please follow "8 Sequencing" in this manual.

### 10.8 Dark green crystals in well No.10

- If dark green crystals appear in well No.10 (see Figure 10-2), it is precipitation of raw materials of the reagent in well No.10. 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.

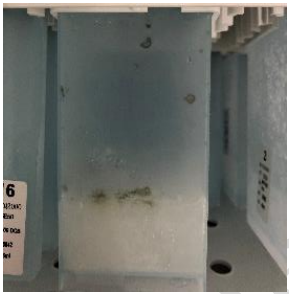


**Figure 10-2：Dark green crystals in well No.10**

### 10.9 Library amount less than 40 fmol

If the library amount is less than 40 fmol (but not less than 24 fmol), 60 μL Make DNB reaction can be tried. It must be noted that 60 μL Make DNB reaction may cause data loss and sequencing quality poorer than expectation. When the library amount is adequate, 100 μL Make DNB reaction is still required.

- 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 4.2. The volume of each Make DNB reaction is 60 μL and the required library input for each Make DNB reaction is calculated as followed:

  ssDNA library input (μL) = 24 fmol / library concentration (fmol/μL)

  Calculate the required ssDNA library for each Make DNB reaction and fill it in Table 10-1 as V.

- Make DNB

  Take a 0.2 mL 8-tube strip or PCR tubes. Prepare reaction mix following the table below:

**Table 10-1：Make DNB reaction mix 1**

| Component | Volume（μL） |
|---|---|
| ssDNA libraries | V |
| Low TE Buffer | 12-V |
| Make DNB Buffer | 12 |
| Total Volume | 24 |

Mix gently 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 shown in the table below:

**Table 10-2：Primer hybridization reaction condition**

| 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 for a prolonged time .**

Take the PCR tube out of the PCR machine when the temperature reaches 4℃. Centrifuge briefly for 5 s, place the tube on ice and prepare the Make DNB reaction mix 2.

**Table 10-3：Make DNB reaction mix 2**

| Component | Volume（μL） |
|---|---|
| Make DNB Enzyme Mix I | 24 |
| Make DNB Enzyme Mix II（LC） | 2.4 |

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

**Table 10-4：Rolling circle amplification conditions**

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

Immediately add 12 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, or shake the tube.**

- Store the DNB at 4°C and perform sequencing within 48 hours. Proceed to “4.4 Quantify DNB”.

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