



Part No.: SOP-013-B01-086

High-throughput Sequencing Set

DNBSEQ-G400RS

User Manual

Version: 7.0

Leading Life Science Innovation

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About the User Manual

This User Manual is applicable to DNBSEQ-G400RS High-throughput Sequencing Set (stLFR). The manual edition is 7.0 and the set version is V1.1.

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Revision history

Version	Date	Description
7.0	August 2022	<ul style="list-style-type: none">• Changed the template of User Manual• Updated the Catalog number of flow cell for PE100 and PE150• Updated the sale statement
6.0	February 2022	<ul style="list-style-type: none">• Updated the company logo• Updated the sales statement• Changed the Transport Temperature to -80 °C to -15 °C• Changed operation diagrams
A4	December 2020	<ul style="list-style-type: none">• Updated the logo, website and email address of MGI• Added the temperature for transportation• Updated the part of sequencing reagent cartridge figures
A3	July 2020	Updated the version of control software and base call
A2	May 2020	Changed the Storage Temperature of flow cell to -25 °C to -15 °C
A1	January 2020	<ul style="list-style-type: none">• Detailed the description of DNB loading on MGIDL-200H• Updated the information about adding and mixing dNTPs and enzyme in the chapter of "Prepare the sequencing cartridge"• Added the solution for crystal precipitation in DNB Load Buffer II• Moved the chapter "Sequencing Sets and Consumables Required but not Provided" to the front of the chapter "Sequencing Workflow"• Added support for dual barcode sequencing• Updated the Control Software to 1.5.0.1280 and later versions• Updated the figures of sequencer interface• Added the section of "Attention"• Added the "Revision History"
A0	August 2019	Initial release

Sequencing set

Catalog number	Name	Model	Version
1000016984	DNBSEQ-G400RS High-throughput Sequencing Set	stLFR FCL PE100	V1.1

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Chapter 1 Introduction

This manual describes how to perform sequencing using the DNBSEQ-G400RS High-throughput Sequencing Set (stLFR) and includes instructions on sample preparation, flow cell preparation, sequencing kit storage, the sequencing protocol and device maintenance.

1.1 Applications

DNBSEQ-G400RS High-throughput Sequencing Set (stLFR) is specifically designed for stLFR library sequencing on MGISEQ-2000RS. This sequencing set is intended to be used for scientific research, which 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 highly accurate sequencing information.

1.3 Data analysis

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

1.4 Sequencing read length

Sequencing read length determines the number of sequencing cycles for a given sequencing run. For example, a PE100 cycle run performs reads of 100 cycles (2×100) for a total of 200 cycles. At the end of the insert sequencing run, an extra 42 cycles of barcode read is performed.


Table 1 Sequencing cycle

Sequencing type	Read 1 read length	Read 2 read length	Barcode read length	Dual barcode read length	Total read length	Maximum cycles
Single Barcode	100	100	42	/	200+42	252
Dual Barcode	100	100	42	10	200+52	252

1.5 Sequencing time

Table 2 Theoretical sequencing time (hours)

Time	PE100+42	PE100+52
Single flow cell	52.2	54.0
Dual flow cells	53.2	55.0
Data analysis	1.5	3.3

-  **Tips**
- The sequencing time in the table above is 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 complete.
 - The time in the table above is theoretical and the actual run time may vary among various sequencing instruments.

1.6 Attention

- This product is for research use only, please read the manual carefully before use.
- Ensure that you are familiar with the SOP & Attention of all the laboratory apparatus to be used.
- Avoid direct skin and eye contact with any samples and reagents. Don't swallow. When it happens, please rinse with plenty of water immediately and go to the hospital.
- All the samples and waste materials should be disposed of according to relevant laws and regulations.
- This product is for one sequencing run only and cannot be reused.

- Mixed use of reagent components from different batches of kits is not recommended.
- Do not use expired products.

Chapter 2 Sequencing sets and self-prepared consumables

2.1 List of sequencing set components

Table 3 DNBSEQ-G400RS High-throughput Sequencing Set (stLFR FCL PE100)
Catalog number: 1000016984



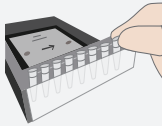
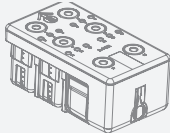
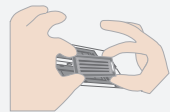
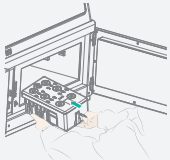
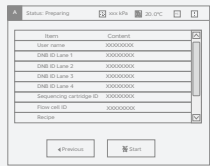
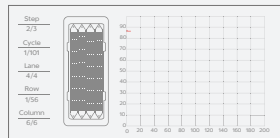

Component	Spec & quantity	Storage temperature	Transportation temperature
DNBSEQ-G400RS Sequencing flow cell Catalog number: 940-000625-00			
Sequencing flow cell	1 EA	-25°C to -15°C	-80°C to -15°C
DNBSEQ-G400RS High-throughput Sequencing Kit (stLFR FCL PE100) Catalog number: 1000016983			
Low TE Buffer	300 µL×1 tube	-25 °C to -15 °C	-80 °C to -15 °C
stLFR Make DNB Buffer	100 µL×1 tube		
Make DNB Enzyme Mix III	200 µL×1 tube		
Make DNB Enzyme Mix IV	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	1 tube		
dNTPs Mix	1.10 mL×2 tube		
dNTPs Mix II	1.80 mL×1 tube		
Sequencing Enzyme Mix	4.00 mL×1 tube		
MDA Reagent	3.50 mL×1 tube		
MDA Enzyme Mix	0.60 mL 1 tube		
Sequencing Reagent Cartridge	1 EA		
Transparent sealing film	2 sheets		

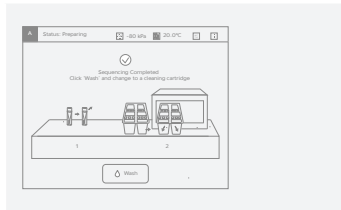
2.2 Self-prepared equipment and consumables

Table 4 Self-prepared equipment and consumables

Equipment and consumables	Recommended brand	Catalog number
Qubit 3.0 fluorometer	Thermo Fisher	Q33216
Mini centrifuge	Major Laboratory Supplier (MLS)	/
Vortex mixer	MLS	/
Thermal cycler	Bio-Rad	/
Pipette	Eppendorf	/
2 °C to 8 °C refrigerator	MLS	/
-25 °C to -15 °C freezer	MLS	/
Qubit dsDNA HS assay kit	Thermo Fisher	Q32851
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	A600560-0500
5 M NaCl solution	SIGMA	S5150-4L
2 M NaOH solution	Aladdin	S128511-1L
0.2 mL PCR 8-tube strip	AXYGEN	/
1.5 mL Microcentrifuge tube	AXYGEN	MCT-150-C
2.0 mL Cryotube	SARSTEDT	72.609.003
Ice box	MLS	/
5 mL Tube	SARSTEDT	60.558.001

Chapter 3 Sequencing workflow

	<p>Making DNB: use DNB preparation reagents to make DNB.</p>																		
	<p>Preparing a new flow cell: take out the flow cell from package and inspect to ensure the flow cell is intact.</p>																		
	<p>Loading DNB: load the DNB onto sequencing flow cell.</p>																		
	<p>Preparing a new reagent cartridge: inspect and thaw the reagent cartridge and then load and mix the necessary reagents.</p>																		
	<p>Loading the flow cell: place the flow cell on the stage of the sequencer.</p>																		
	<p>Loading the reagent cartridge into the sequencer.</p>																		
 <table border="1"> <thead> <tr> <th>Item</th> <th>Content</th> </tr> </thead> <tbody> <tr> <td>User Name</td> <td>XXXXXXXXXX</td> </tr> <tr> <td>DNB ID Lane 1</td> <td>XXXXXXXXXX</td> </tr> <tr> <td>DNB ID Lane 2</td> <td>XXXXXXXXXX</td> </tr> <tr> <td>DNB ID Lane 3</td> <td>XXXXXXXXXX</td> </tr> <tr> <td>DNB ID Lane 4</td> <td>XXXXXXXXXX</td> </tr> <tr> <td>Reagent Cartridge ID</td> <td>XXXXXXXXXX</td> </tr> <tr> <td>Flow cell ID</td> <td>XXXXXXXXXX</td> </tr> <tr> <td>Stage</td> <td></td> </tr> </tbody> </table>	Item	Content	User Name	XXXXXXXXXX	DNB ID Lane 1	XXXXXXXXXX	DNB ID Lane 2	XXXXXXXXXX	DNB ID Lane 3	XXXXXXXXXX	DNB ID Lane 4	XXXXXXXXXX	Reagent Cartridge ID	XXXXXXXXXX	Flow cell ID	XXXXXXXXXX	Stage		<p>Starting sequencing: follow the User Manual to enter sequencing information and start the run.</p>
Item	Content																		
User Name	XXXXXXXXXX																		
DNB ID Lane 1	XXXXXXXXXX																		
DNB ID Lane 2	XXXXXXXXXX																		
DNB ID Lane 3	XXXXXXXXXX																		
DNB ID Lane 4	XXXXXXXXXX																		
Reagent Cartridge ID	XXXXXXXXXX																		
Flow cell ID	XXXXXXXXXX																		
Stage																			
	<p>Sequencing: monitor the sequencing run from the control software interface.</p>																		
	<p>Data analysis: the sequencer will automatically split barcode (if Split barcode is selected) and output FASTQ files when sequencing is complete.</p>																		




Device maintenance: perform device maintenance when sequencing is complete.

Chapter 4 Making DNB


4.1 Insert size recommendation

- This sequencing set is compatible with the stLFR libraries prepared by MGI stLFR Library Prep Kit.
- Recommended library insert size: The size distribution of inserts is preferred to be centered around 200 to 1500 bp.

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

4.2 Library requirement

- The concentration of dsDNA library should be no less than 1.5 ng/ μ L.

 **Tips** • If the library concentration is unknown, it is recommended to perform dsDNA library quantitation (ng/ μ L) using Qubit dsDNA HS Assay Kit and Qubit 3.0 Fluorometer. Use the following equation below to calculate the input volume of dsDNA library.

Input volume (μ L) = $20 \text{ ng} / C$, C represents the concentration of dsDNA library (ng/ μ L).

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

4.3 Making DNB

4.3.1 Preparing reagents for DNB making

Perform the steps below:

1. Take out libraries, Low TE Buffer, stLFR Make DNB Buffer and Stop DNB Reaction Buffer from freezer. Thaw reagents for approximately 0.5 hours at room temperature.
2. Take out Make DNB Enzyme Mix III and thaw it for approximately 0.5 hours on ice.
3. After thawing, mix reagents using a vortex mixer for 5 seconds. Centrifuge them briefly and place on ice until use.

4.3.2 Calculating the number of DNB reaction system

The MGISEQ-2000RS sequencing flow cell contains 4 lanes. Three options for DNBs to be loaded onto the flow cell:

- Using the sequencer
All 4 lanes must be the same DNB. Each lane requires at least 50 μL DNBs.
- Using the MGIDL-200RS
4 different DNBs can be loaded onto 4 different lanes. Each lane requires at least 40 μL DNBs.
- Using the MGIDL-200H
4 different DNBs can be loaded onto 4 different lanes. Each lane requires 25 μL DNBs.

Table 5 The required number of make DNB reactions for each flow cell

Loading system	DNB volume (μL) / lane	Make DNB reaction (μL)	The required number of make DNB reaction / flow cell
Sequencer	50	80	3
MGIDL-200RS	40	80	2 to 4
MGIDL-200H	25	80	2 to 4

4.3.3 Making DNB

Perform the following steps:

1. Take 0.2 mL PCR 8-tube strip or PCR tubes. Prepare reaction mix according to the volumes in the following table:

 **Tips** V represents variable sample volume as determined in 4.2 Library requirement on Page 8.

Table 6 Make DNB reaction mix 1

Component	Volume (μL)
Low TE Buffer	16 - V
stLFR Make DNB Buffer	16
Library DNA	V
Total Volume	32

2. Mix reagents using a vortex mixer for 5 seconds, centrifuge briefly. Place the mixture into a thermal cycler and start the reaction. Thermal cycler settings are shown in the table below:

Table 7 DNB reaction condition 1

Temperature	Time
Heated lid (105 °C)	On
95 °C	3 min
40 °C	3 min
4 °C	Hold

3. Take Make DNB Enzyme Mix IV out of freezer and place it on ice. Centrifuge it briefly for 5 seconds and hold it on ice.



- Tips**
- Do not place Make DNB Enzyme Mix IV at room temperature.
 - Do not hold the tube for a prolonged time.

4. Take the PCR tube out of the thermal cycler when the temperature reaches 4 °C. Centrifuge briefly for 5 seconds and add reagent following the table below:

Table 8 Make DNB reaction mix 2

Component	Volume (µL)
Make DNB Enzyme Mix III	32.0
Make DNB Enzyme Mix IV	3.2

5. Mix reagents using a vortex mixer for 5 seconds, centrifuge briefly, and place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:




- Tips**
- As some thermal cycler 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 thermal cyclers, pre-heating of the heated lid is required to ensure the heated lid is at working temperature during the DNB reaction.
 - It is recommended to set the temperature of the heated lid to 35 °C or as close as possible to 35 °C .

Table 9 DNB reaction conditions 2


Temperature	Time
Heated lid (35 °C)	On
30 °C	30 min
4 °C	Hold

6. Immediately add 16 μL Stop DNB Reaction Buffer once the temperature reaches 4 °C. Mix gently by pipetting 5 to 8 times using a wide bore tip.

-  **Tips**
- Be sure to use a wide bore pipette tip to mix DNB gently using a wide bore pipette tip. Do not centrifuge, vortex, or pipette vigorously.
 - Store DNB at 4 °C and perform sequencing within 48 hours.

4.4 Quantifying DNB

When DNB making is complete, take 2 μL DNB and use Qubit ssDNA Assay Kit and Qubit 3.0 Fluorometer to quantify the DNB.

-  **Tips**
- Sequencing requires a minimum DNB concentration of 6 ng/ μL .
 - If there are too many samples in a single test, it is recommended to quantify in batches to avoid inaccurate DNB quantification due to fluorescence quenching.
 - If the concentration exceeds 40 ng/ μL , the DNBs should be diluted to 20 ng/ μL with DNB Load Buffer I before loading.


Chapter 5 Preparing a flow cell

Refer to *DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set User Manual* for details.

Chapter 6 Loading DNB

Perform the steps below:

1. Take out DNB Load Buffer II from freezer and thaw reagents on ice for approximately 0.5 hours.
2. After thawing, mix reagents using a vortex mixer for 5 seconds, centrifuge briefly and place them on ice until use.

-  **Tips** Vigorously mix the reagents for 1 to 2 minutes of continuous vortex to redissolve the precipitate before use when crystal precipitation is found in DNB Load Buffer II.

6.1 Loading DNB in Sequencer

Perform the steps below:


1. Take out the 0.5 mL microfuge tube and add the reagents in the table below.

-  **Tips** Each flow cell requires 266.5 μ L DNB loading mix 1.


Table 10 DNB loading mix 1

Component	Volume (μ L)
DNB Load Buffer II	64.0
Make DNB Enzyme Mix IV	2.5
DNB	200.0
Total Volume	266.5

2. Combine components and mix by gently pipetting 5 to 8 times by using a wide bore tip. Place the mixture at 4 °C until use.

-  **Tips**
- Do not centrifuge, vortex, or shake the tube.
 - Prepare a fresh DNB loading mix 1 immediately before the sequencing run.

6.2 Loading DNB in MGIDL-200RS

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

Perform the steps below:

1. Take out a new PCR 8-tube strip and add the reagents in the table below.



-  **Tips** Each lane requires at least 53.3 μ L DNB loading mix 2.

Table 11 DNB loading mix 2

Component	Volume (μL)
DNB Load Buffer II	12.8
Make DNB Enzyme Mix IV	0.5
DNB	40.0
Total Volume	53.3

- Combine components and mix by gently pipetting 5 to 8 times using a wide bore tip. Place the mixture at 4 °C until use.
 -  **Tips**
 - Do not centrifuge, vortex, or shake the tube.
 - Prepare a fresh DNB loading mix 2 immediately before the sequencing run.
- Place the tubes containing DNB loading mix 2 in the labeled positions of MGIDL-200RS.

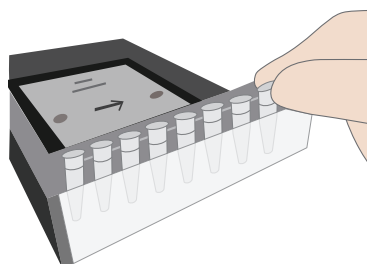




Figure 1 Placing the loading samples

- Press the flow cell attachment button, hold the flow cells by the 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.
- Select the desired loading recipe from the drop-down list and start DNB loading.
- After DNB loading, take out the flow cell and place it in a PE glove or a plastic bag at room temperature for 30 minutes, then immediately place it on the sequencer for use.
 -  **Tips** Refer to *MGIDL-200RS User Manual* for details on loading operation.

6.3 Loading DNB in MGIDL-200H

 **Tips** For wash before DNB loading and loading operation, please refer to *MGIDL-200H Quick Start Guide*.

Performing the steps below:


1. Take out a new PCR 8-tube strip and add the reagents in the table below.

 **Tips** Each lane requires 30 μL DNB loading mix 3.

Table 12 DNB loading mix 3


Component	Volume (μL)
DNB Load Buffer II	8.00
Make DNB Enzyme Mix IV	0.31
DNB	25.00
Total volume	33.31

2. Combine components and mix by gently pipetting 5 to 8 times using a wide bore tip. Place the mixture at 4 $^{\circ}\text{C}$ until use.

 **Tips**

- Do not centrifuge, vortex, or shake the tube.
- Prepare a fresh DNB loading mix 3 immediately before the sequencing run.

3. Install the sealing gasket and flow cell. Aspirate 30 μL DNB loading mix 3 with a pipette and insert the wide bore tip into the fluidics inlet.

 **Tips**

- 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 onto the flow cell.
- During loading DNB, do not move the wide bore tip or flow cell to prevent bubbles from entering.

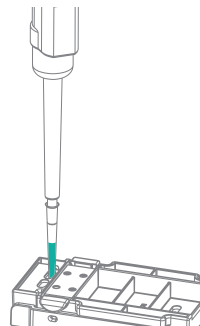


Figure 2 Loading samples using MGIDL-200H


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

 **Tips** Refer to *MGIDL-200H Portable DNB Loader Quick Start Guide* for details on loading operation.

Chapter 7 Preparing the sequencing reagent cartridge

Performing the steps below:

1. Take Sequencing Reagent Cartridge out of freezer.
2. Thaw it in water bath at room temperature until completely thawed, or thaw it at 2 °C to 8 °C in a refrigerator on day in advance. Store it at 2 °C to 8 °C freezer until use.
3. The flow cell can be taken out from -25 °C to -15 °C and placed at room temperature at this point.

 **Tips** After being taken out from -25 °C to -15 °C condition, the flow cell must be placed at room temperature for at least 60 minutes but not exceeding 24 hours before DNB loading.

4. Invert the cartridge 3 times to mix before use.
5. Vigorously shake the cartridge in all directions 10 to 20 times until no visible layers can be seen in the cartridge, especially for reagents in well No.9 and No.10.
6. Wipe any water condensation on the cartridge cover and well surround with lint-free paper. Well positions are shown in the figure below.

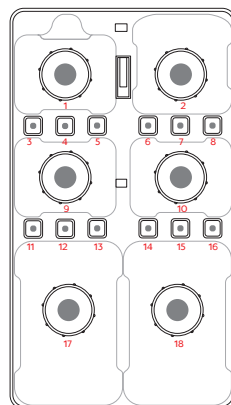


Figure 3 Well positions

7. Take dNTPs Mix and dNTPs Mix II out of freezer 1 hour in advance to thaw it at room temperature.

8. Take Sequencing Enzyme Mix and MDA Enzyme out of freezer and store them at 4 °C until use.
9. Pierce the seal in the center of well No.1 and No.2 to make a hole around 2 cm in diameter using a 1 mL sterile tip.

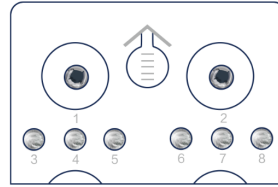


Figure 4 Piercing the seal on the cartridge

10. Add reagents using a pipette with an appropriate volume range according to 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 4 to 6 times to mix the reagents in the tube before adding all of them into well No.1.

- Tips**
- Mix dNTPs Mix using a vortex mixer for 5 seconds and centrifuge briefly before use.
 - Invert Sequencing Enzyme Mix 4 to 6 times before use. Do not mix the Dye Mix by vortex mixer.
 - When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.

Table 13 Well No.1 reagent loading

Model	dNTPs Mix loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
stLFR FCL PE100	2.000	2.000

11. Add reagents using a pipette with an appropriate volume range according to 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 4 to 6 times to mix the reagents in the tube before adding all of them into well No.2.

- Tips**
- Mix dNTPs Mix II using a vortex mixer for 5 seconds and centrifuge briefly before use.
 - Invert Sequencing Enzyme Mix 4 to 6 times before use. Do not mix the Dye Mix by vortex mixer.
 - When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.

Table 14 Well No.2 reagent loading

Model	dNTPs Mix II loading volume (mL)	Sequencing Enzyme Mix loading volume (mL)
stLFR FCL PE100	1.700	1.700

- Seal the loading wells of well No.1 and No.2 with the transparent sealing film.

 **Tips** Do not cover the center of the well to avoid blocking the sampling needle.

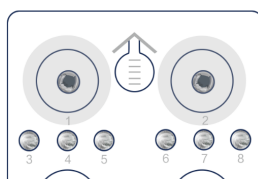


Figure 5 Sealing the loading wells of cartridge

- Press the film with finger around the round cap. Make sure to seal tightly and no bubbles between the film and cap. Ensure the reagents would not overflow from the cartridge.

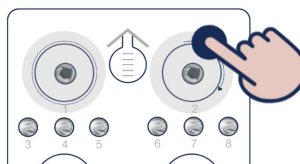


Figure 6 Sealing the loading wells of cartridge tightly

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

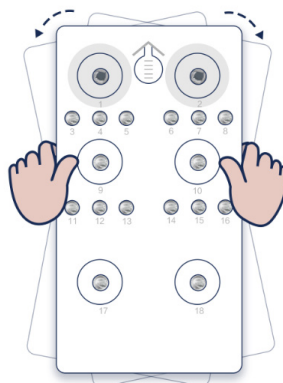




Figure 7 Mixing reagents after loading

- Pierce the seal of well No.15 using a 1 mL sterile tip.
- Add 500 μ L of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette. Invert the tube 4 to 6 times to mix the reagents.

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

- Add the mixture to well No.15. When adding the mixture, make sure there are no bubbles at the bottom of the tube.

 **Tips** When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.

- Take out the seal of loading wells from the cartridge carefully after fully mixing. Make sure the well No.1 and No.2 clean around avoiding a cross contamination. The sequencing cartridge is now ready for use.

 **Tips** Do not use the wasted seals again.

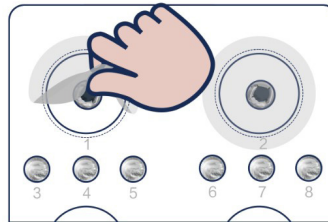


Figure 8 Removing the seal from cartridge after mixing

- Gently tap the cartridge on the bench to reduce air bubbles in the reagents.
- The cartridge for stLFR FCL PE100 sequencing is ready for use now.

Chapter 8 Sequencing

8.1 Entering the main interface and place the sample

Refer to *DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set User Manual* for details.

8.2 Selecting the sequencing parameters

Perform the steps below:

- Select the sequencing recipe in the **Recipe** drop-down menu. There are one-click sequencing run and user-customized run (Customize).

2. Select **Customize**, continue performing the following steps.
3. In the beginning, please select a step to start the sequencing run. If DNBs will be loaded on the sequencer, select **DNB loading**.

Start phase: DNB loading Post loading ...
 Sequencing prime Sequencing
 Prime

Figure 9 Selecting the step to start sequencing

4. Select the read length. Enter 100 for read 1 and 100 for read 2.

Read1: 100 ✓
 Read2: 100 ✓

Figure 10 Selecting the read length

5. Perform the following steps according to different situations:
 - For Single Barcode sequencing, enter 42 for the barcode length and leave the Dual barcode length blank.

Barcode: 42 v
 Dual barcode:

Figure 11 Entering the barcode length

- For Dual Barcode sequencing, enter 42 for the barcode length and 10 for the Dual barcode length.

Barcode: 42 v
 Dual barcode: 10 ✓

Figure 12 Entering the barcode and dual barcode length

6. Perform the following steps according to different situations:
 - For single barcode sequencing, do not execute barcode demultiplexing.

Split barcode: Lane1 Lane2 Lane3 Lane4

Figure 13 Barcode demultiplexing on different lanes

- For Dual Barcode sequencing, click **V** before the **Start phase** in *Figure 9* on *Page 19* to enter the second page of **Customize**. Pull the drop-down menu and select the **1-128** barcode sequence as Dual barcode type. Do not execute barcode demultiplexing.

Figure 14 Dual barcode demultiplexing on different lanes

- Select the dark reaction for any position of read length in read 1 or 2. (stLFR sequencing does not need to perform dark reaction. Skip this step).


 **Tips** Dark reaction means there is only chemical reaction without capturing optical information.

Figure 15 Do not select the dark reaction

- Click **Confirm**.

8.3 Loading the reagent cartridge and flow cell

Refer to *DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set User Manual* for details.

8.4 Reviewing parameters

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

8.5 Starting sequencing

Perform the steps below:

1. After confirming that all information is correct, click **Start**.
2. The system will display the dialog box “Proceed with Sequencing?”. Click **Yes** to start sequencing.

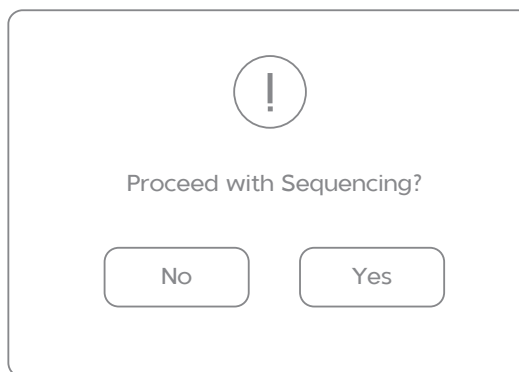


Figure 16 Confirming sequencing interface

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

8.6 Data access

Refer to *MGISEQ-2000 & MGISEQ-2000RS Gene Sequencer Software Operation Guide* for details.

Chapter 9 Device maintenance and troubleshooting

Refer to *DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set User Manual* for details.

Appendix 1 Manufacturer

Manufacturer	MGI Tech Co., Ltd./Wuhan MGI Tech Co., Ltd.
Address	Main Building and Second floor of No. 11 Building, Beishan Industrial Zone, Yantian District, 518083, Shenzhen, Guangdong, P.R.China
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