MGISEQ-2000RS

High-throughput Sequencing Set (stLFR) User Manual

Catalog number and name

1000011545, MGISEQ-2000RS High-throughput Sequencing Set（stLFR）

Note: Mixed use of reagents from different batches is strictly prohibited

Control Software: 1.1.0.106 and later versions, base call 1.0.3.174 and later versions

User manual version: A0

Kit version: V1.0

MGI 华大智造

Table of Contents

1 Introduction

This manual explains how to perform sequencing using the MGISEQ-2000RS 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

MGISEQ-2000RS 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 only and cannot be used for clinical diagnosis.

1.2 Sequencing Technology

This sequencing set utilizes DNBseq™ technology. A sequencing run starts with the hybridization of a DNA anchor, then a fluorescent probe is attached to the DNA Nanoball (DNB) using combinatorial probe anchor sequencing (cPAS) chemistry. Finally, the high-resolution imaging system captures the fluorescent signal. After digital processing of the optical signal, the sequencer generates high quality and high accuracy sequencing information.

1.3 Data Analysis

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

1.4 Sequencing Read Length

In the sequencing run, the number of sequencing cycles depends on sequencing read length. 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 index read is carried out.

Table 1-1：Sequencing cycle

Sequencing Kit	Read 1 read length	Read 2 read length	Barcode read length	Total read length	Maximum cycles
stLFR	100	100	42	200+42	252

1.5 Sequencing Time

Table 1-2：Theoretical sequencing time (h)

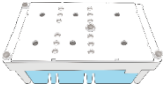
Time (hours)	PE100+42
Single flow cell	52.2
Dual flow cell	53.2
Data analysis	1.5

2 Sequencing Workflow

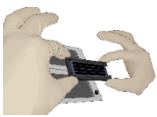
Make DNB: use DNB preparation kit for making DNB

Load DNB: place sample tubes on the MGIDL-200 or MGISEQ-2000RS sequencer

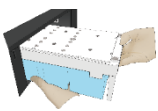
Prepare a new flow cell：remove the flow cell from package and inspect to ensure the flow cell is intact



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



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



Load the reagent kit into the sequencer

Follow the instructions to enter sequencing information and start the run

Monitor the sequencing run from the control software interface

Perform device maintenance when sequencing is completed

3 Library preparation

3.1 Insert Size Recommendation

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

Library recommendation for insert size: the size distribution of inserts is preferred to be centered around 200-1500 bp. If there are special requirements for the specifications of the library kit, then the requirements for the specifications of the kit will be taken.

3.2 Library Requirement

The concentration of dsDNA library is more than 1.5 ng/μL. Perform dsDNA library quantitation using Qubit® dsDNA HS Assay Kit and Qubit® Fluorometer. The amount of dsDNA library input is determined by the quantitation result.

Note: input volume (μL) =20 ng/C

C represents the concentration of dsDNA library (ng/μL).

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

3.3 Sample Safety

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

3.4 Prepare reagents for the make DNB

Remove libraries, stLFR Make DNB Buffer, Make DNB Enzyme Mix III, Low TE Buffer and Stop DNB Reaction Buffer from storage. Thaw reagents for approximately 0.5 hours on ice. After thawing, mix reagents using a vortex mixer for 5 seconds, centrifuge briefly and place back on ice.

3.5 Make DNB

The total volume of make DNB reaction is 80 μL. Each lane on the flow cell requires 40 μL (MGIDL-200) or 50 μL (MGISEQ-2000RS). The required number of make DNB reaction is shown in Table 3-1:

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

Lanes to be loaded with the same sample	The required number of make DNB reaction	Loading system
4	3	MGISEQ-2000RS
3	2	MGIDL-200
1-2	1	MGIDL-200

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

Table 3-2：Make DNB reaction 1

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

- Mix by vortex and then spin down for 5 seconds using the mini centrifuge. Place the mixture into a PCR machine and start the reaction. PCR machine settings are described in Table 3-3:

Table 3-3：DNB reaction condition 1

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

- Remove the Make DNB Enzyme Mix IV from storage and place on ice. Centrifuge briefly for 5 seconds and hold on ice.

① **Note：**

Do not place Make DNB Enzyme Mix IV 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 seconds and add the following mixture on ice:

Table 3-4：Make DNB reaction 2

Component	volume（μL）
Make DNB Enzyme Mix III	32
Make DNB Enzyme Mix IV	3.2

- Mix by vortex and then spin down for 5 seconds using the mini centrifuge. Place the mixture immediately into

a PCR machine and start the reaction. PCR machine settings are described in Table 3-5 below:

Table 3-5：DNB reaction conditions 2

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

Note：

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

- Add 16 μL Stop DNB Reaction Buffer immediately when the temperature reaches 4□. Mix gently by pipetting 5-8 times using wide bore tip. Do not vortex or pipette vigorously. Store DNB at 4□ and perform sequencing within 48 hours.

① **Note：**

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

3.6 Quantify DNB

When the Make DNB is completed, use Qubit® ssDNA Assay Kit and Qubit® Fluorometer to quantify the DNB. Sequencing requires the DNB concentration to be above 6 ng/μL. If the concentration is lower than 6 ng/μL, make a new DNB preparation.

Note：

- As DNB is viscous, it is recommended to take 2 μL for quantification. If the number of samples is large, it is recommended to quantify in batches to avoid inaccurate DNB quantification due to fluorescence quenching.
- If the concentration exceeds 40 ng/μL, the DNB need to be diluted to 20 ng/μL with DNB Load Buffer I for loading.

3.7 DNB loading and processing

3.7.1 MGISEQ-2000RS DNB loading

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

Table 3-6：DNB loading mix 1

Component	volume（μL）
DNB Load Buffer II	64
Make DNB Enzyme Mix IV	2.5
DNB	200

- Gently pipette the DNB loading mix 1 5-8 times using wide bore tip. Do not centrifuge, vortex, or vigorously pipette the mixture. Place the mixture at 4□ until use.

Note：

Prepare a fresh DNB loading mix before the sequencing run.

3.7.2 MGIDL-200 DNB loading

- Take a new PCR 8-tube strip and add reagents following Table 3-7:

Table 3-7：DNB loading mix 2

Component	volume（μL）
DNB Load Buffer II	12.8
Make DNB Enzyme Mix IV	0.5
DNB	40

- Gently pipette the DNB loading mix 2 5-8 times using wide bore tip. Do not centrifuge, vortex, or vigorously pipette the mixture.

- Place the tubes containing DNB loading mix 2 in the labeled positions of MGIDL-200.

Figure 3-1：Place the loading samples

- Load the flow cell with DNB following chapter 6.

Note：

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

- When loading is completed, remove the flow cell and place at room temperature for 30 min, then immediately place it on the MGISEQ-2000RS for use.

① **Note：**

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

4 Prepare the sequencing kit

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

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

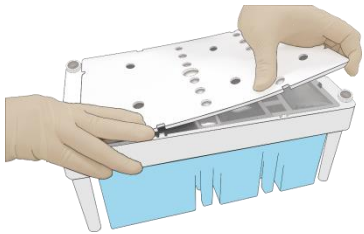


Figure 4-1：Open and clean the cartridge

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

- Remove Sequencing Enzyme Mix from storage and place at 4°C until use.

- Pierce the seal of well No.1 and No.2 to make a hole with the diameter less than 1 cm using a sterile tip (see Figure 4-2):

Figure 4-2：Pierce the seal on the cartridge

- Well No.1（see Figure 4-3）

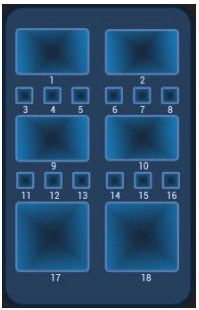


Figure 4-3：Well position

Take a pipette with the appropriate volume range and add reagents to well No.1 following Table 4-1:

Table 4-1：dNTPs Mix loading

Sequencing kit	dNTPs Mix name	Loading volume (mL)
MGISEQ-2000 stLFR PE100	dNTPs Mix	2.000

- Well No.2（see Figure 4-3）

Take a pipette with the appropriate volume range and add reagents to well No.2 following Table 4-2:

Table 4-2：dNTPs Mix II loading

Sequencing kit	dNTPs Mix II name	Loading volume (mL)
MGISEQ-2000 stLFR PE100	dNTPs Mix II	1.700

- Well No.1 and No.2（see Figure 4-3）

Take a pipette with the appropriate volume range and add reagents to well No.1 and No.2 following Table 4-3:

Table 4-3：Sequencing Enzyme Mix loading

Sequencing kit	Sequencing Enzyme Mix name	Well No.1 volume (mL)	Well No.2 volume (mL)
MGISEQ-2000 stLFR PE100	Sequencing Enzyme Mix	2.000	1.700

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

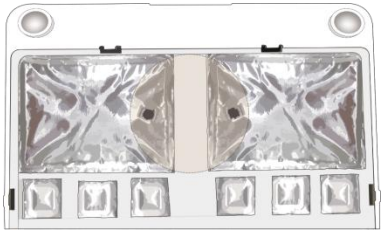


Figure 4-4：Seal the loading well

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

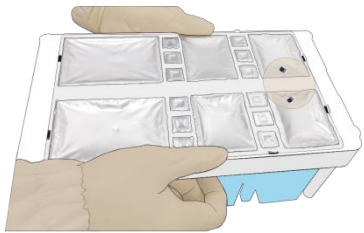


Figure 4-5：Mix reagents after loading

- Well No.15（see Figure 4-3）: Add 500 μL of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette. Vortex for 5 seconds to mix thoroughly and then add the mixture to well No.15. When adding the mixture, make sure there are no bubbles at the bottom of the tube.

ⓘ **Note：**

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

5 Prepare the flow cell

- Refer to the "MGISEQ-2000RS High-throughput Sequencing Set User Manual" for details.

6 Sequencing

6.1 Enter the main interface and place the sample

- Refer to the "MGISEQ-2000RS High-throughput Sequencing Set User Manual" for details.

6.2 Select sequencing parameters

- Select the sequencing recipe in the "Recipe" drop-down menu where contains one-click sequencing run (PE150, SE50, etc.) and user-customized run (Customize).

- Select "Customize", continue performing the following steps.

- In the beginning, please select a step to start the sequencing run. If DNB will be loaded using MGISEQ-2000RS, select "DNB loading".

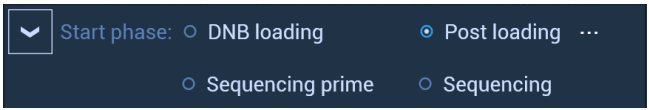


Figure 6-1：Select the step to start sequencing

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

Figure 6-2：Select the read length

- Select the barcode length as 42, leave the other barcode length blank.

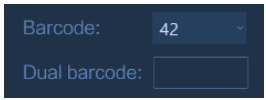


Figure 6-3：Select the barcode length

- Select do not execute barcode demultiplexing.

Figure 6-4：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).

Dark reaction: only perform chemical reactions without optical information collection.

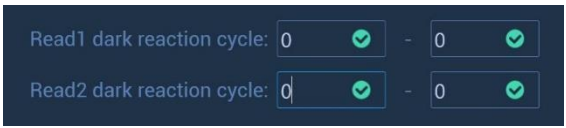


Figure 6-5：Select the dark reaction

- Click “Confirm”

6.3 Load the reagent cartridge and flow cell

- Refer to the “MGISEQ-2000RS High-throughput Sequencing Set User Manual” for details.

6.4 Review parameters

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

6.5 Start sequencing

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

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

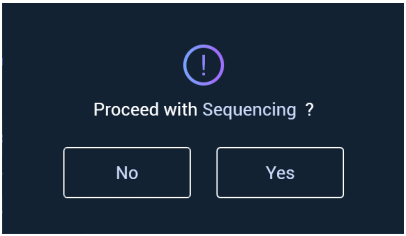


Figure 6-6：Confirm sequencing interface

- Once sequencing starts, 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.

7 Device Maintenance and Troubleshooting

- Refer to the “MGISEQ-2000RS High-throughput Sequencing Set User Manual” for details.

8 Equipment and Consumables Required but not Provided

Table 8-1：Equipment and consumables Required but not Provided

Equipment and consumables	Recommended brand	Catalog number
Qubit® 3.0 Fluorometer	Thermofisher	Q33216
Mini centrifuge	/	/
Vortex mixer	/	/
PCR machine	Bio-Rad	/
Pipette	Eppendorf	/
2~8□ refrigerator	/	/
-18~-25□ refrigerator	/	/
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
Tween-20	Sangon Biotech	A600560-0500
NaCl	/	/
NaOH	Sinopharm	10019719
0.2mL PCR 8-tube strip	AXYGEN	/
1.5mL Eppendorf	AXYGEN	MCT-150-C
Ice rack	MLS	/

9 List of kit components

Table 9-1：List of kit components 1

Product	Sequencing kit	Component	Spec & Quantity	storage temperature
MGISEQ-2000RS High-throughput Sequencing Set (stLFR) (PE100) Catalog number: 1000011545	MGISEQ-2000RS Sequencing flow cell Catalog number： 1000008403	Sequencing flow cell	1	RT (0-30□)
	MGISEQ-2000RS High-throughput Sequencing Kit (stLFR) (PE100) Catalog number： 1000011546	Low TE Buffer	300 μL×1tube	-25□~-15□
		stLFR Make DNB Buffer	100 μL×1tube	
		Make DNB Enzyme Mix III	200 μL×1tube	
		Make DNB Enzyme Mix IV	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	1tube	
		dNTPs Mix	1.10 mL×2tube	
		dNTPs Mix II	1.80 mL×1tube	
		Sequencing Enzyme Mix	4.00 mL×1tube	
		MDA Reagent	3.50 mL×1tube	
		MDA Enzyme Mix	0.60 mL×1tube	
		Sequencing Reagent Cartridge	1	
		transparent sealing film	2 sheets	

MGI WeChat

Contact information

MGI Tech Co., Ltd
Address: Main Building and Second floor of No.11 Building, Beishan Industrial Zone, Yantian District, Shenzhen, 518083, Guangdong, China
E-mail: MGI-service@genomics.cn
Website：www.mgitech.cn