DNBSEQ-G400 High-throughput Sequencing Set User Manual



Catalog number	Mod	Specification
1000016966	FCL SE35	45 Cycles/Set
1000016967	FCL SE50	60 Cycles/Set
1000016968	FCL SE100	110 Cycles/Set
1000016969	FCL PE50	110 Cycles/Set
1000016970	FCL PE100	210 Cycles/Set
1000016971	FCL PE150	310 Cycles/Set

Kit version: V3.1 (Note: Mixed use of reagent components from different batches is strictly prohibited)

User manual version: A1



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1 Introduction

This manual explains how to perform sequencing using the DNBSEQ-G400 High-throughput Sequencing Set on DNBSEQ-G400. It includes instructions on sample preparation, flow cell preparation, sequencing kit storage, the sequencing protocol and device maintenance.

1.1 Intended Use

DNBSEQ-G400 High-throughput Sequencing Set is specifically designed for DNA or RNA sequencing on DNBESQ-G400. As a general reagent for the sequencing reaction system, it is used with gene sequencing instrument aiming to obtain sample sequence information through high-throughput sequencing process.

1.2 Sequencing Technology

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

1.3 Data Analysis

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

1.4 Sequencing Read Length

In the sequencing run, the number of sequencing cycles depends on sequencing read length. For example, a PEIS0 cycle run performs reads of 150cycles (2 × 150) for a total of 300 cycles. At the end of the insert sequencing run, an extra 10 cycles of barcode read can be carried out, if required.

-					
Sequencing	Read 1 read	Read 2 read	Barcode	Total read	Maximum
read length	length	length	read length	length	cycles
SE35	35		10	35+10	55
SE50	50		10	50+10	70
SE100	100		10	100+10	120
PE50	50	50	10	100+10	120
PE100	100	100	10	200+10	220
PE150	150	150	10	300+10	320

Table 1-1: Sequencing cycle

1.5 Sequencing Time

Time (hours)	SE35	SE50	SE100	PE50	PE100	PE150
Single flow cell	11.6	14.8	23.9	27.3	50.0	70.4
Dual flow cell	12	15.2	24.3	28.1	51.0	72.6
Data analysis	0.3	0.4	0.8	0.8	1.5	2.3

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

2 Sequencing Workflow



Make DNB: use DNB preparation kit for making DNB



Load DNBs: place sample tubes on the MGIDL-200 or 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 cartridge: inspect and thaw the reagent

cartridge and then load and mix the necessary reagents



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



Load the Reagent cartridge into the sequencer



Follow the instructions to enter sequencing information and start the run



Monitor the sequencing run from the control software interface



Perform device maintenance when sequencing is completed

3 Library preparation

3.1 Insert Size Recommendation

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

Library recommendation for insert size:

For SE50, the size distribution of inserts is preferred to be centered around 160-170 bp. For PE100, the size distribution of inserts is preferred to be centered around 280 bp. For PE150, the size distribution of inserts is preferred to be centered around 400 bp.

3.2 Library Requirement

We recommend 40 fmol ssDNA library for each reaction. Perform ssDNA library quantitation using Qubit® ssDNA Assay Kit and Qubit® Fluorometer. And the concentration of ssDNA library is more than 2 fmol/µL. Otherwise, the amount of ssDNA library needed is determined by the following equation. volume needed (µL)=N*330 *40 /(1000*1000*C)

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

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

3.3 Sample Safety

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

3.4 Prepare reagents for the make DNB

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

3.5 Make DNB

DNB can be directly loaded by the sequencer, but all 4 lanes must be the same sample. If you need to load various samples in 4 different lanes, it is recommended to use the MGIDL-200 instrument.

3.5.1 Define number of DNB making reaction

After the make DNB reaction, 50 μL of the 100 μL DNB making reaction is loaded to each lane on the sequencing flow cell. The required number of DNB reactions for different loading systems is illustrated in Table 3-1:

Loading lanes on a flow cell	The required number of make DNB reaction	Loading system	
4	2	Sequencer	
3	2	MGIDL-200	
1-2	1	MGIDL-200	

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

3.5.2 Making DNB

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

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

Table 3-2: Make DNB reaction 1

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

Temperature	Time
Heated lid (105°C)	On
95°C	1 min
65°C	1 min
40°C	1 min
4℃	Hold

Table 3-3: DNB reaction condition 1

Remove the Make DNB Enzyme Mix II(LC) from storage and place on ice. Centrifuge briefly for 5 s and hold on ice.

A Note:

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

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

Table 3-4: Make DNB reaction mix 2

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

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

Temperature	Time
Heated lid (35°C)	On
30°C	25 min
4°C	Hold

Table 3-5: DNB reaction conditions 2

Note:

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

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

A Note:

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

3.6 Quantify DNB

After the make DNB is completed, use Qubit® ssDNA Assay Kit and Qubit® Fluorometer to quantify the DNB. Sequencing requires the DNB concentration to be above 8 ng/µL. If the concentration is lower than 8 ng/µL, make a new DNB preparation.

Note:

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

Buffer I for loading.

3.7 Load DNB

3.7.1 Sequencer DNB loading

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

Component	volume (µL)
DNB Load Buffer II	64
Make DNB Enzyme Mix II (LC)	2
DNB	200

Table 3-6: DNB loading mix1

Combine components to create DNB loading mix 1 and mix by gently wide bore pipetting 5-8 times. Do not centrifuge, vortex, or shake the tube. Place the mixture at 4°C 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	16
Make DNB Enzyme Mix II (LC)	0.5
DNB	50

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

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



Figure 3-1: Place the loading samples

> Load the flow cell with DNBs according to step "6.5 Loading the flow cell".

Note:

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

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

A Note:

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

4 Prepare the sequencing cartridge

- Remove the Sequencing Reagent cartridge from -20°C and thaw in a room temperature water bath until thawed. Store cartridges at 2-8°C storage until use (or thaw cartridges in 2-8°C fridge one day in advance). Invert the tube 3 times before use.
- > Open the cartridge cover and wipe any water condensation with lint-free paper.



Figure 4-1: Open and clean the cartridge

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

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

Note:

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

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



Figure 4-2: Pierce the seal on the cartridge

> #1 well (see Figure 4-3)



Figure 4-3: Well position

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

Sequencing kit	Reagent name	Loading volume (mL)
DNBSEQ-G400 FCL SE35	dNTPs Mix	0.600
DNBSEQ-G400 FCL SE50	dNTPs Mix	0.700
DNBSEQ-G400 FCLSE100	dNTPs Mix	1.100
DNBSEQ-G400 FCL PE50	dNTPs Mix	1.100
DNBSEQ-G400 FCL PE100	dNTPs Mix	1.800
DNBSEQ-G400 FCL PE150	dNTPs Mix	2.400

Table 4-1: dNTPs Mix loading

> #2 well (see Figure 4-3)

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

Table 4-2:	dNTPs Mix II	loading
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Sequencing kit	Reagent name	Loading volume (mL)
DNBSEQ-G400 FCL SE35	dNTPs Mix II	0.500
DNBSEQ-G400 FCL SE50	dNTPs Mix II	0.600
DNBSEQ-G400 FCL SE100	dNTPs Mix II	0.900
DNBSEQ-G400 FCL PE50	dNTPs Mix II	0.900
DNBSEQ-G400 FCL PE100	dNTPs Mix II	1.500
DNBSEQ-G400 FCL PE150	dNTPs Mix II	2.100

> #1 and #2 well (see Figure 4-3)

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

Coguenoing kit	Deggent name	#1 well volume	#2 well volume
Sequencing kit Reagent hame		(mL)	(mL)
DNBSEQ-G400 FCL SE35	Sequencing Enzyme Mix	0.600	0.500
DNBSEQ-G400 FCL SE50	Sequencing Enzyme Mix	0.700	0.600
DNBSEQ-G400 FCL SE100	Sequencing Enzyme Mix	1.100	0.900
DNBSEQ-G400 FCL PE50	Sequencing Enzyme Mix	1.100	0.900
DNBSEQ-G400 FCL PE100	Sequencing Enzyme Mix	1.800	1.500
DNBSEQ-G400 FCL PE150	Sequencing Enzyme Mix	2.400	2.100

Table 4-3: Sequencing Enzyme Mix loading

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



Figure 4-4: Seal with the loading well

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



Figure 4-5: Mix reagents after loading

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

A Note:

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

5 Prepare a flow cell

- > Remove the sequencing flow cell from storage.
- > Unwrap the outer package.



Figure 5-1: Unwrap the outer package

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



Figure 5-2: Inspect the flow cell

6 Sequencing

6.1 Enter the main interface

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

		2	23	7°C 🔒 4.7°C 🖕		31 A 🗏
Α	Status: Idle	🚺 20.2°C 🚱 👼	в	Status: Idle	01 25	.2°C 🛞 🗊
	C Bad	Password Log in Don't enter more tha	n 50 d	naracters		
Ą						

Figure 6-1: Log-in interface

> See the interface below.

E	₽23.7°C 🔐 4.7°C 🛁 🔂 🛃 名 🧮
A Status: Idle	B Status: Idle 🚺 25.2°C 🚱 🗐
🛆 Wash	🖉 Wash
QI	

Figure 6-2: Main interface

6.2 Load the DNBs

> Click the "Sequence" option on the interface to enter the following interface:

A	Status: Preparing
	DNB ID: 1-128 → ⊕ Recipe: PE 100 → □ DNB loading
	Step1 Step2
	C Back Next

Figure 6-3: DNBs loading interface

 \succ Click on the \oplus on the right of the "DNB ID" and the four lane information appears.

DNB ID:	WGS	⊘ 1~128 ~ ⊕
	RNA	⊘ 501~596 ↓ ⊖
	WGS	⊘ 1~128 、 ⊖
	RNA	⊘ 501~596 ↓ ⊖

Figure 6-4: DNBs and information selection interface

- > Move the cursor to the blank area next to the "DNB ID" and enter the library name or number.
- > Pull the drop-down menu on the left of \oplus and select the barcode sequence of different lanes.
- > Open the reagent compartment door, gently lift the sampling needle with one hand, remove the

cleaning reagent tube with the other hand, load the sample tube, then slowly lower the sampling needle tip reaches the bottom of the tube.



Figure 6-5: Load the DNBs tube

Note:

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

> Close the reagent compartment door.

6.3 Select sequencing parameters

Select the sequencing recipe in the "Recipe" drop-down menu, one-click sequencing run (PEI50, SE50, etc.), and user-customized run (Customize).

Recipe:	PE100	~	DNB loading
	SE100		
	SE200	h	
	SE35		
	SE50		
	SE50_sR		
	Customize	⊠	

Figure 6-6: Select sequencing solutions

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

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



Figure 6-7: Select the step to start sequencing

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

Read1:	100	\bigotimes
Read2:	100	\bigotimes

Figure 6-8: Choose the read length

Select the barcode length from 6 or 10.

Barcode:	10 ~	
Dual barcode:		

Figure 6-9: Select the barcode length

> Select the lane for barcode demultiplexing.

Split barcode:	☑ Lane1	☑ Lane2	⊠ Lane3	⊡ Lane4

Figure 6-10: Barcode demultiplexing on different lanes

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

Dark reaction: only chemical reaction without optical information capture

Read1 dark reaction cycle:	2 ⊘ - 5 ⊘
Read2 dark reaction cycle:	3 🔗 - 8 🔗

Figure 6-11: Select the dark reaction

Click "Confirm"

6.4 Load the reagent cartridge

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



Figure 6-12: Reagent cartridge information entry interface

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



Figure 6-13: Remove cleaning cartridge

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



Figure 6-14: Maintain the reagent compartment

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



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

6.5 Loading the flow cell

- Open the flow cell compartment door, press one side of flow cell used for washing, and press the flow cell attachment button with the other hand. After the vacuum is released, remove the flow cell for washing from the stage.
- Use dust remover to remove the dust on the flow cell stage and the back of the flow cell. If there are impurities on the stage surface, please gently wipe it with wet dust-free paper to ensure that the flow cell can be held properly.



Figure 6-16: Clean the flow cell stage

Press the flow cell attachment button.

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



Figure 6-17: Load the flow cell

Align the holes on the flow cell with the locating pins on the flow cell stage. Gently slide the flow cell at a 45° angle to the upper left corner (45° to the upper right corner when loading the flow cell on the MGIDL-200) to keep the flow cell aligned with the pin. Press the left and right sides of the flow cell on the stage at the same time to ensure the flow cell is properly seated on the stage.



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

- Ensure that the negative pressure is in the range of -80 ~ -99 kPa.
- Use a dust remover to remove the dust on the flow cell surface and close the flow cell compartment door.



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



Figure 6-19: Flow cell information entry interface

Click "Next"

6.6 Review parameters

Review the run parameters to ensure that all 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 6-20: Review information

6.7 Start sequencing

- > After confirming that the information is correct, click "Start".
- > The system will display the dialog box "Start the sequencing." Click "Yes" to start sequencing.



Figure 6-21: Confirm sequencing interface

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

7 Device Maintenance 7.1 Terminology and Definition

Wash type	Description
Full wash	Step 1 - Maintenance wash, Step 2 – Regular wash.
Maintenance wash	Procedure: Cleaning cartridge 4 \rightarrow Cleaning cartridge 3 \rightarrow Cleaning cartridge 2 To remove residual reagents and proteins in the pipeline, reducing risk of blockage.
Regular wash	Procedure: Cleaning cartridge1 → Air Prime To remove residual reagents, reducing risk of cross-contamination.

Table 7-1: Wash Solution

7.2 Wash instruction

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



Figure 7-1: Wash interface

- > After the sequencing is completed, the device needs to be washed within 24 hours.
- A Full Wash is required if the sequencer was used for either A) a PE run or B) a DNB loading/postload. 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 sequencer has been idle for seven days or longer, perform a full wash prior to sequencing.
- > If impurities are found on the flow cell, perform a full wash.

7.3 Prepare wash reagents

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

Table 7-2: Wash reagents preparation (1)

Reagent	Volume
100% Tween-20	0.5 mL
Laboratory-grade water	999.5 mL

Prepare 1M NaCl + 0.05% Tween-20 following the table below (Can be used for up to 28 days if stored at 4°C).

Reagent	Weight/Volume
5M NaCl solution	200 mL
100% Tween-20	0.5 mL
Laboratory-grade water	799.5 mL

Table 7-3: Wash reagent preparation (2)

> Prepare 0.1M NaOH following the table below (valid for 28 days if stored at 4°C).

Table 7-4: Wash reagent preparation (3)

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

7.4 Wash cartridge

- An empty cleaning cartridge and washing flow cell for a full wash are provided together with the device.
- Wash the cleaning cartridge prior to refilling it with cleaning reagents. Replace cleaning reagents after 20 uses.
- Used flow cells from previous runs can be used as washing flow cells. Each flow cell can be used for up to 20 full washes.

- Wash cleaning cartridge 1: Take a clean cleaning cartridge and a 0.5 mL cryotube, add laboratory-grade water to the cryotube and cleaning cartridge (all wells) to a final 90% volume and mark it as the cleaning reagent cartridge 1.
- Wash cleaning cartridge 2: Take a clean cleaning cartridge and a 0.5 mL cryotube, add laboratory-grade water to the cryotube and cleaning cartridge (all wells) to a final 90% volume and mark it as the cleaning reagent cartridge 2.
- Wash cleaning cartridge 3: Take a clean cleaning cartridge and a 0.5 mL cryotube, add 50 mL 0.1M NaOH into large wells, 6 mL 0.1M NaOH into small wells and 400 µL 0.1M NaOH to 0.5mL cryotube. Mark it as the cleaning reagent cartridge 3.
- Wash cleaning cartridge 4: Take a clean cleaning cartridge and a 0.5 mL cryotube, add 50 mL 0.05% Tween-20 solution into large wells, 6 mL 1M NaCl + 0.05% Tween-20 solution to No.15 well, 400 μL 1M NaCl + 0.05% Tween-20 solution to 0.5mL cryotube and 6 mL 0.05% Tween-20 solution to the rest of the wells. Mark it as the cleaning reagent cartridge 4.

Note: Large wells are No. 1, 2, 9, 10, 17, 18 Small wells are No. 3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16

7.5 Wash procedures

7.5.1 Regular wash

- Use cleaning cartridge 1. Open the reagent compartment door. Hold the handle of the cleaning cartridge 1 with one hand and place the other hand underneath the cartridge 1 for support. Slide it into the reagent compartment slowly following the direction printed on the cartridge cover until it stops. Close the reagent compartment door.
- > Click the wash button on the interface.
- Place the flow cell for washing.
- Select regular wash from the drop-down menu to start the regular wash which takes about 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.

Wash type:	Regular	~

Figure 7-2: Select the wash type

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



Figure 7-3: Regular wash end interface

7.5.2 Maintenance wash

- Use cleaning cartridge 4. Open the reagent compartment door. Hold the handle of the cleaning cartridge 4 with one hand and place the other hand underneath for support. Slide it to the reagent compartment slowly following the direction printed on the cartridge cover until it stops. Close the reagent compartment door.
- > Click the wash button on the interface.
- Place the flow cell for washing.
- Select the maintenance wash from the drop-down menu to start the maintenance wash which takes about 25 minutes.
- Observe the status of flow cell for wash in this step. If you see many bubbles, continue the wash. If not, stop the wash, replace the flow cell and start the wash.

- When the interface appears as Figure 7-4, click "Yes" to lift the needle and replace the cleaning cartridge.
- > Use cleaning cartridge 3 and continue the maintenance wash which takes around 25 minutes.



Figure 7-4: Maintenance wash [1] end interface

- When the interface appears as figure 7-5, click "Yes" to lift the needle and replace the cleaning cartridge.
- > Use cleaning cartridge 2 and continue the maintenance wash which takes around 25 minutes.



Figure 7-5: Maintenance wash [2] end interface

> When the interface appears as Figure 7-6, click "No" to end the maintenance wash.



Figure 7-6: Maintenance wash end interface

7.5.3 Full wash procedures

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

8 Troubleshooting

8.1 Low DNB concentration

- > Check if the cartridge has expired.
- > Check if the library meets the requirements.
- If DNB concentration still does not meet the requirements after a new sample preparation, please contact the field service engineer.

8.2 Abnormal negative pressure

- Gently wipe the stage surface with a damp, lint-free paper or a lint-free cloth, and blow the stage with a power dust remover and ensure no dust is left.
- > Blow the back of the flow cell with a dust remover to ensure no dust is left.
- > If these solutions cannot solve the problem, please contact the engineer.

8.3 Bubbles

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

8.4 Impurities

- > Perform a full wash on MGIDL-200 and the sequencer.
- > If the problem persists after a full wash, please contact the engineer.

8.5 Pump fails

- MGIDL-200 and sequencer: remove the flow cell, check if there are impurities in sealing gasket and remove the dust with the dust remover. Place the flow cell following the instruction and start the pump again.
- > Check if the Sequencing Flow Cell move properly.
- > If the sampling needles cannot move properly, restart sequencing software.
- > If the problem persists, please contact the engineer.

8.6 Reagent cartridge storage

- If the cartridge has been thawed (including dNTPs) and cannot be used within 24 hours, it can be frozen and thawed at most once.
- If the cartridge has been thawed (including dNTPs) but cannot be used immediately, store it at 4°C and use it within 24 hours.
- If dNTPs and enzyme have been added to the cartridge, the kit has been prepared but cannot be used immediately, store it at 4 °C and use it within 24 hours.
- If dNTPs and enzyme have been added to the kit, the cartridge has been prepared and the cartridge 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.

8.7 Post loading fail

- If Post loading fails, but prime step has been performed, in this condition please re-start from the Post loading.
- > Start from the chapter 6 "Sequencing" and re-load the Flow Cell.
- > When selecting 6.3 sequencing parameters, choose progamme "Customize".
- Select "Post loading" and click "...".



Figure 8-1: Select re-start Post loading

- If starts from the Post loading prime, select "Prime" as Figure 8-1, otherwise if starts from the step Post loading, don't select "Prime".
- > Other steps please follow the chapter 6 "Sequencing" in this manual.

9 Equipment and Consumables Required but not Provided

Equipment and consumables	Recommended brand	Catalog number
Qubit® 3.0 Fluorometer	Thermofisher	Q33216
Mini centrifuge	Major Laboratory Supplier (MLS)	/
Vortex mixer	MLS	/
PCR machine	Bio-Rad	/
Pipette	Eppendorf	/
2℃~8℃ refrigerator	MLS	/
-25°C~-15°C 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	/

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

10 List of set components

Product	Sequencing kit	Component	Spec & Quantity	Storage Temperature
	Package I	DNBSEQ-G400 Sequencing Flow Cell	1	RT(0°C~30°C)
		Low TE Buffer	300 µL×1 tube	
	Package II	Make DNB Buffer	100 µL×1 tube	
		Make DNB Enzyme Mix I	200 µL×1 tube	
DNBSEQ-G400		Make DNB Enzyme Mix II (LC)	25 μL×1 tube	
High-throughput		Stop DNB Reaction Buffer	100 µL×1 tube	
Sequencing Set		DNB Load Buffer I	200 µL×1 tube	
(FCL SE35)		DNB Load Buffer II	200 µL×1 tube	-25°C~-15°C
1000016966		Micro Tube 0.5mL(Empty)	1 tube	
1000010700		dNTPs Mix	0.70 mL×1 tube	
		dNTPs Mix II	0.60 mL×1 tube	
		Sequencing Enzyme Mix	1.40 mL×1 tube	
		Sequencing Reagent Cartridge	1	
		transparent sealing film	2 sheets	

Table10-1: List of set components 1

Product	Sequencing kit	Component	Spec & Quantity	Storage Temperature
	Package I	DNBSEQ-G400 Sequencing Flow Cell	1	RT(0°C~30°C)
	Package II	Low TE Buffer	300 µL×1 tube	
		Make DNB Buffer	100 µL×1 tube	
		Make DNB Enzyme Mix I	200 µL×1 tube	
DNBSEQ-G400		Make DNB Enzyme Mix II (LC)	25 μL×1 tube	
High-throughput		Stop DNB Reaction Buffer	100 µL×1 tube	
Sequencing Set		DNB Load Buffer I	200 µL×1 tube	
(FCL SE50)		DNB Load Buffer II	200 µL×1 tube	-25°C~-15°C
1000016967		Micro Tube 0.5mL (Empty)	1 tube	
1000010707		dNTPs Mix	0.80 mL×1 tube	
		dNTPs Mix II	0.70 mL×1 tube	
		Sequencing Enzyme Mix	1.60 mL×1 tube	
		Sequencing Reagent Cartridge	1	
		transparent sealing film	2 sheets	

Table 10-2: List of set components 2

Product	Sequencing kit	Component	Spec & Quantity	Storage Temperature
	Package I	DNBSEQ-G400 Sequencing Flow Cell	1	RT(0°C~30°C)
	Package II	Low TE Buffer	300 µL×1 tube	
		Make DNB Buffer	100 µL×1 tube	
		Make DNB Enzyme Mix I	200 µL×1 tube	
DNBSEQ-G400		Make DNB Enzyme Mix II (LC)	25 μL×1 tube	
High-throughput		Stop DNB Reaction Buffer	100 µL×1 tube	
Sequencing Set		DNB Load Buffer I	200 µL×1 tube	
(FCL SE100)		DNB Load Buffer II	200 µL×1 tube	-25 °C ~ -15°C
1000016968		Micro Tube 0.5mL (Empty)	1 tube	
1000010700		dNTPs Mix	1.20 mL×1 tube	
		dNTPs Mix II	1.00 mL×1 tube	
		Sequencing Enzyme Mix	2.30 mL×1 tube	
		Sequencing Reagent Cartridge	1	
		transparent sealing film	2 sheets	

Table 10-3: List of set components 3

Product	Sequencing kit	Component	Spec & Quantity	Storage Temperature
	Package I	DNBSEQ-G400 Sequencing Flow Cell	1	RT(0°C~30°C)
DNBSEQ-G400 High-throughput Sequencing Set (FCL PE50) Catalog number: 1000016969	Package II	Low TE Buffer Make DNB Buffer Make DNB Enzyme Mix I Make DNB Enzyme Mix II (LC) Stop DNB Reaction Buffer DNB Load Buffer I DNB Load Buffer II Micro Tube 0.5mL (Empty) dNTPs Mix	300 µL×1 tube 100 µL×1 tube 200 µL×1 tube 25 µL×1 tube 100 µL×1 tube 200 µL×1 tube 1 tube 1 tube	-25°C15°C
		dINTPs Mix II Sequencing Enzyme Mix MDA Reagent MDA Enzyme Mix Sequencing Reagent Cartridge transparent sealing film	1.00 mL×1 tube 2.30 mL×1 tube 3.50 mL×1 tube 0.60 mL×1 tube 1 2 sheets	

Table 10-4: List of set components 4

Product	Sequencing kit	Component	Spec & Quantity	Storage Temperature
	Package I	DNBSEQ-G400 Sequencing Flow Cell	1	RT(0°C~30°C)
DNBSEQ-G400 High-throughput Sequencing Set (FCL PE100) Catalog number: 1000016970	Package II	Low TE Buffer Make DNB Buffer Make DNB Enzyme Mix I Make DNB Enzyme Mix II (LC) Stop DNB Reaction Buffer DNB Load Buffer I DNB Load Buffer II Micro Tube 0.5mL (Empty) dNTPs Mix	300 μL×1 tube 100 μL×1 tube 200 μL×1 tube 25 μL×1 tube 100 μL×1 tube 200 μL×1 tube 1 tube 1,90 mL×1 tube	-25°C~-15°C
		dNTPs Mix II Sequencing Enzyme Mix MDA Reagent MDA Enzyme Mix Sequencing Reagent Cartridge transparent sealing film	1.60 mL×1 tube 3.60 mL×1 tube 3.50 mL×1tube 0.60 mL×1tube 1 2 sheets	

Table 10-5: List of set components 5

Product	Sequencing kit	Component	Spec & Quantity	Storage Temperature
	Package I	DNBSEQ-G400 Sequencing Flow Cell	1	RT(0°C~30°C)
DNBSEQ-G400 High-throughput Sequencing Set (FCL PE150) Catalog number: 1000016971	Package II	Low TE Buffer Make DNB Buffer Make DNB Enzyme Mix I Make DNB Enzyme Mix II (LC) Stop DNB Reaction Buffer DNB Load Buffer II DNB Load Buffer II Micro Tube 0.5mL (Empty) dNTPs Mix	300 µL×1 tube 100 µL×1 tube 200 µL×1 tube 25 µL×1 tube 100 µL×1 tube 200 µL×1 tube 1 tube 1.30 mL×2 tube	-25°C15°C
		Sequencing Enzyme Mix MDA Reagent MDA Enzyme Mix Sequencing Reagent Cartridge	4.80 mL×1 tube 3.50 mL×1tube 0.60 mL×1tube 1	
		transparent sealing film	2 sheets	

Table 10-6: List of set components 6

11 Testing results interpretation

11.1 The following conditions may compromise the results of sequencing:

- > Prolonged storage of the DNB samples
- > Sample contamination
- > Difference in the mixing ratio of libraries containing different molecular barcodes

112 Other factors that might compromise the results include: use of an expired reagent kit, poor accuracy of pipettes, high room temperature, and failure to follow the instructions.

12 Product performance specification

12.1 Accuracy

When tests are performed on reference product Q, the coincidence rate between sequencing results and the known reference sequence should be over 99%.

12.2 Repeatability

Repeat the tests on reference product Q 5 times, CV value of the coincidence rate between sequencing results and the known reference sequence should be no more than 5% (n=5).

12.3 Batch variations

When tests are performed on reference product Q, use sequencing kits from three different batches and repeat the tests 5 times respectively. CV value of the coincidence rate between sequencing results and the known reference sequence should be no more than 5% (n=15).

13 Precautions

13.1 For in-vitro diagnostic use only.

- 13.2 Please read this manual carefully before use, and be sure to understand and sophisticated in device operation and fully aware of the precautions before starting the assay.
- 13.3 All samples and reagents shall be avoided from direct contact with skin and eyes, and forbidden to swallow. When this happens, please immediately wash with lots of clear water and go to hospital for treatment as soon as possible.

13.4 All samples and various wastes are all deemed to have potential contamination, and they shall be treated as pollutants.

14 Literature references

14.1

Dean, F. B. et al. Comprehensive human genome amplification using multipledisplacement amplification.Pr oc. Natl Acad. Sci. USA. 99, 5261–5266 (2002).

14.2

Peters, B.A., et al., Accurate whole genome sequencing and haplotyping from-10 to 20 human cells. Nature 487,190–195(2012).

14.3

Drmanac, R. Nucleic acid analysis by random mixtures of non-overlapping fragments. US patent 7,901 891 (2006).

14.4

Lander, E. S. et al. Initial sequencing and analysis of the human genome. Nature 409, 860-921 (2001).

14.5

Drmanac, R. et al. Human genome sequencing using unchained base reads on self- assembling DNA nanoarrays. Science 327, 78–81 (2010).

15 Contact details

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16 Language edition

For the requirements of Instruction for Use in other languages, please contact Latvia MGI Tech, SIA

17 Release date of the user manual

This manual was released on 2019-12.

18 Key to symbols used





CE MARK

CONSULT INSTRUCTIONS FOR USE

KEEP AWAY FROM SUNLIGHT

KEEP DRY

DO NOT RE-USE

CONTAINS SUFFICIENT FOR NTESTS



MGI WeChat

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