

# DNBSEQ-G400RS

## High-throughput Sequencing Set (stLFR) User Manual



Catalog number and name

1000016984, DNBSEQ-G400RS High-throughput Sequencing Set (stLFR FCL PE100)

Control Software: 1.5.2.1298 and later versions

Base call: 1.0.7.84 and later versions

Set version: V1.1

User manual version: A3

## Revision History

Version	Date	Summary of change
A3	July 2020	<ul style="list-style-type: none"><li>• Updated the version of control software and base call</li></ul>
A2	May 2020	<ul style="list-style-type: none"><li>• Changed the Storage Temperature of flow cell to -25°C~-15°C</li></ul>
A1	January 2020	<ul style="list-style-type: none"><li>• Detailed the description of DNB loading on MGIDL-200H.</li><li>• Updated the information about adding and mixing dNTPs and enzyme in the chapter of "Prepare the sequencing cartridge".</li><li>• Added the solution for crystal precipitation in DNB Load Buffer II.</li><li>• Moved the chapter "Sequencing Sets and Consumables Required but not Provided" to the front of the chapter "Sequencing Workflow".</li><li>• Added support for dual barcode sequencing.</li><li>• Updated the Control Software to 1.5.0.1280 and later versions</li><li>• Updated the figures of sequencer interface</li><li>• Added the section of "Attention".</li><li>• Added the "Revision History".</li></ul>
A0	August 2019	<ul style="list-style-type: none"><li>• The first version</li></ul>

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## 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 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

Sequencing read length will determine the number of sequencing cycles for a given sequencing run. For example, a PE100 cycle run performs reads of 100 cycles ( $2 \times 100$ ) for a total of 200 cycles. At the end of the insert sequencing run, an extra 42 cycles of index read is performed.

**Table 1-1: 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 1-2: Theoretical sequencing time (h)

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

Notes:

- ① The sequencing time 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 Attention

- 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.
- 5) This product is for one sequencing run only and cannot be reused.
- 6) Do not use expired products.

## 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 (stLFR FCL PE100)

Catalog number: 1000016984

Sequencing Kit	Component	Spec & Quantity	Storage Temperature
DNBSEQ-G400RS Sequencing flow cell Catalog number: 1000016985	Sequencing flow cell	1 EA	-25°C~-15°C
DNBSEQ-G400RS High-throughput Sequencing Kit (stLFR FCL PE100) Catalog number: 1000016983	Low TE Buffer	300 μL×1tube	-25°C~-15°C
	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 EA	
Transparent sealing film	2 sheets		

## 2.2 Equipment and Consumables Required but not Provided

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

Equipment and consumables	Recommended brand	Catalog number
Qubit <sup>®</sup> 3.0 Fluorometer	Thermo Fisher	Q33216
Mini centrifuge	Major Laboratory Supplier (MLS)	/
Vortex mixer	MLS	/
PCR machine	Bio-Rad	/
Pipette	Eppendorf	/
2~8°C refrigerator	MLS	/
-25~-15°C refrigerator	MLS	/
Qubit <sup>®</sup> dsDNA HS Assay Kit	Thermo Fisher	Q32851
Qubit <sup>®</sup> 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	BBi	A600560-0500
5M NaCl solution	SIGMA	S5150-4L
2M NaOH solution	Aladdin	S128511-1L
0.2mL PCR 8-tube strip	AXYGEN	/
1.5mL Eppendorf	AXYGEN	MCT-150-C
Ice rack	MLS	/
5 mL Tube	SARSTEDT	60.558.001

### 3 Sequencing Workflow



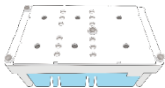
Make DNB: use DNB preparation kit for making DNB



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



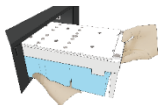
DNB loading: load the DNB onto sequencing flow cell



Prepare a new reagent 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





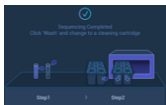
Start sequencing: follow the instructions to enter sequencing information and start the run



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 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 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  $\geq 1.5$  ng/ $\mu$ L. Perform dsDNA library quantitation (ng/ $\mu$ L) using Qubit<sup>®</sup> dsDNA HS Assay Kit and Qubit<sup>®</sup> Fluorometer. The amount of dsDNA library input is determined by the quantitation result.

Note: input volume ( $\mu$ L) = 20 ng/C

C represents the concentration of dsDNA library (ng/ $\mu$ L).

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 4-2 as V.

① **Note:**

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

### 4.3 Make DNB

#### 4.3.1 Prepare reagents for the make DNB

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

- When using the sequencer to load DNBs, all 4 lanes must be the same DNB. Each lane requires at least 50  $\mu$ L DNBs.
- When using the MGIDL-200RS to load DNBs, 4 different DNBs can be loaded onto 4 different lane. Each lane requires at least 40  $\mu$ L DNBs.
- When using the MGIDL-200H to load DNBs, 4 different DNBs can be loaded onto 4 different lane. Each lane requires 25  $\mu$ L DNBs.

Remove libraries, Low TE Buffer, stLFR Make DNB Buffer, Make DNB Enzyme Mix III 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 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 total volume of make DNB reaction is 80  $\mu$ L. Each lane on the flow cell requires 50  $\mu$ L (MGISEQ-2000RS or DNBSEQ-G400RS) or 40  $\mu$ L (MGIDL-200) or 25  $\mu$ L (MGIDL-200H). The required number of make DNB reaction is shown in Table 4-1:

**Table 4-1: The required number of make DNB reactions for each flow cell**

Loading system	DNB loading volume ( $\mu\text{L}$ )/Lane	Make DNB reaction ( $\mu\text{L}$ )	The required number of make DNB reaction /flow cell
Sequencer	50	80	3
MGIDL-200RS	40	80	2-4
MGIDL-200H	25	80	2-4

**4.3.3 Make DNB**

- Take 0.2 mL PCR 8-tube strip or PCR tubes. Prepare reaction mix following Table 4-2 below:

**Table 4-2: Make DNB reaction mix 1**

Component	volume ( $\mu\text{L}$ )
Library DNA	V
Low TE Buffer	16-V
stLFR Make DNB Buffer	16
Total Volume	32

- Mix gently by vortexing and centrifuge for 5 seconds using a mini centrifuge. Place the mix into a PCR machine and start the reaction. PCR machine settings are shown in the table below:

**Table 4-3: DNB reaction condition 1**

Temperature	Time
Heated lid (105°C)	On
95°C	3 min
40°C	3 min
4°C	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°C. Centrifuge briefly for 5 seconds and add the following mixture on ice:

**Table 4-4: Make DNB reaction mix 2**

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

- 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 4-5: DNB reaction conditions 2**

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

**Note:**

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

- Immediately add 16 μL Stop DNB Reaction Buffer once the temperature reaches 4°C. Mix gently by pipetting 5-8 times using a wide bore pip. Do not vortex, shake the tube, or pipette vigorously.

① **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°C and perform sequencing within 48 hours.**

**Note:**

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.

#### 4.4 Quantify DNB

When the make DNB is complete, take 2  $\mu\text{L}$  DNB and use Qubit<sup>®</sup> ssDNA Assay Kit and Qubit<sup>®</sup> Fluorometer to quantify the DNB. Sequencing requires a minimum DNB concentration of 6 ng/ $\mu\text{L}$ .

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/ $\mu\text{L}$ , the DNBs should be diluted to 20 ng/ $\mu\text{L}$  with DNB Load Buffer I before loading.
- ③ Store DNB at 4°C and perform sequencing within 48 hours.

## 5 Prepare a Flow Cell

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

## 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 microtube and add the following reagents (see Table 6-1 below):

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

Component	volume ( $\mu\text{L}$ )
DNB Load Buffer II	64
Make DNB Enzyme Mix IV	2.5
DNB	200
Total Volume	266.5

- 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.

Notes:

- ① Prepare a fresh DNB loading mix immediately before the sequencing run.
- ② Each flow cell requires 266.5  $\mu$ L 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 ( $\mu$ L)
DNB Load Buffer II	12.8
Make DNB Enzyme Mix IV	0.5
DNB	40
Total Volume	53.3

- 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 53.3  $\mu$ L 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. Holding the flow cells by edges, 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 container (such as 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 ( $\mu\text{L}$ )
DNB Load Buffer II	8
Make DNB Enzyme Mix IV	0.31
DNB	25
<b>Total volume</b>	<b>33.31</b>

- 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  $\mu\text{L}$  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  $\mu\text{L}$  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.

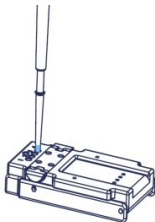


Figure 6-2: Load samples using MGIDL-200H

① **Note:**

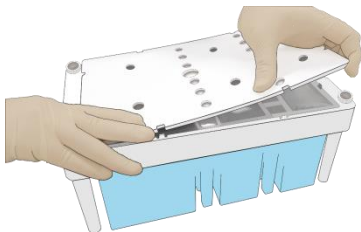
**Do not move the flow cell during loading procedure. After being placed at room temperature, the flow cell should be used immediately.**

## 7 Prepare the sequencing cartridge

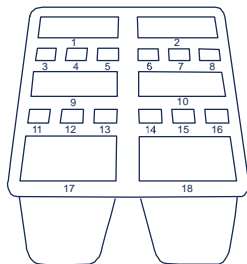
Remove the Sequencing Reagent Cartridge from storage. Thaw in a room temperature water bath until completely thawed (or thaw in 2-8°C fridge one day in advance). Store at 2-8°C storage until use. The flow cell can be removed from -25~-15°C and place at room temperature at this point. Invert the cartridge 3 times to mix before use. Vigorously shake the cartridge in all directions 10-20 times until no visible layers can be seen in the cartridge, especially for reagents in well No.9 and No.10.

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





**Figure 7-1: Open and clean the cartridge**

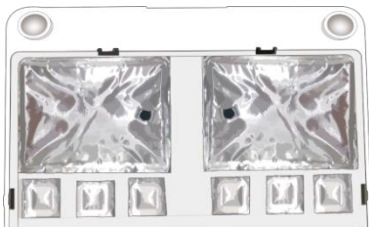


**Figure 7-2: Well position**

Remove dNTPs Mix and dNTPs Mix II from storage 1h in advance to thaw at room temperature, then place at 4°C until use. Mix reagents using a vortex mixer for 5 seconds and centrifuge briefly before use.

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

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



**Figure 7-3: Pierce the seal on the 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 4-6 times to mix the reagents in the tube before adding all of them into well No.1:

**Table 7-1: Well No.1 reagent loading**

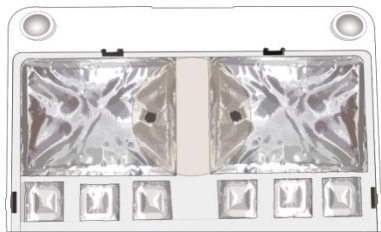
Sequencing kit	dNTPs Mix loading volume	Sequencing Enzyme Mix loading volume
stLFR FCL PE100	2.000 mL	2.000 mL

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

**Table 7-2: Well No.2 reagent loading**

Sequencing kit	dNTPs Mix II loading volume	Sequencing Enzyme Mix loading volume
stLFR FCL PE100	1.700 mL	1.700 mL

Seal the loading wells 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**

Place the cartridge horizontally on the table and 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.



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

Pierce the seal of well No.15 using 1 mL sterile tip. Add 500  $\mu\text{L}$  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 stLFR FCL PE100 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**

## 8 Sequencing

### 8.1 Enter the main interface and place the sample

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

### 8.2 Select sequencing parameters

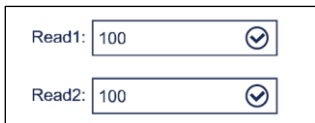
- Select the sequencing recipe in the “Recipe” drop-down menu. There are one-click sequencing run (PE150, SE50, etc.) and user-customized run (Customize).
- Select “Customize”, continue performing the following steps.
- In the beginning, please select a step to start the sequencing run. If DNBs will be loaded on the sequencer, select “DNB loading”.



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

Figure 8-1: Select the step to start sequencing

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



Read1: 100 ✓  
Read2: 100 ✓

Figure 8-2: Select the read length

- For single barcode sequencing, enter 42 for the barcode length and leave the Dual barcode length blank (See Figure 8-3).

Barcode: 42 v

Dual barcode:

**Figure 8-3: Enter the barcode length**

- For dual barcode sequencing, enter 42 for the barcode length and 10 for the Dual barcode length (See Figure 8-4).

Barcode: 42 v

Dual barcode: 10 ✓

**Figure 8-4: Enter the barcode and dual barcode length**

- For single barcode sequencing, do not execute barcode demultiplexing.

Split barcode:  Lane1  Lane2  Lane3  Lane4

**Figure 8-5: Barcode demultiplexing on different lanes**

- For dual barcode sequencing, dual barcode demultiplexing can be performed on the sequencer. Click “V” before the “Start phase” in Figure 8-1 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 (See Figure-8-6):

**Customize**

Small RNA
 Dual barcode sequencing

	Barcode type	<input type="checkbox"/>	Dual barcode type	<input type="checkbox"/>
Lane1	[v]	<input type="checkbox"/>	1-128 [v]	<input checked="" type="checkbox"/>
Lane2	[v]	<input type="checkbox"/>	1-128 [v]	<input checked="" type="checkbox"/>
Lane3	[v]	<input type="checkbox"/>	1-128 [v]	<input checked="" type="checkbox"/>
Lane4	[v]	<input type="checkbox"/>	1-128 [v]	<input checked="" type="checkbox"/>

**Figure 8-6: 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).

Dark reaction: only chemical reaction without optical information capture.

Read 1 dark reaction cycle: [ ] — [ ]

Read 2 dark reaction cycle: [ ] — [ ]

**Figure 8-7: Do not select the dark reaction**

- Click "Confirm"

### 8.3 Load the reagent cartridge and flow cell

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

#### 8.4 Review parameters

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

#### 8.5 Start sequencing

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



**Figure 8-8: 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.

### 9 Device Maintenance and Troubleshooting

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



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■ Contact information

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