Part No.: H-T-083

Cat No	Product model
940-000409-00	G99 SM FCL SE100/PE50
940-000410-00	G99 SM FCL PE150
940-000520-00	G99 SM App-C FCL SE100
940-000413-00	G99 SM App-C FCL PE150
940-000417-00	G99 SM FCL SE400
940-000415-00	G99 SM FCL PE300

High-throughput Sequencing Set

DNBSEQ-G99RS

User Manual

Version: 5.0

Leading Life Science Innovation

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Wuhan MGI Tech Co., Ltd.

About the user manual

This user manual is applicable to DNBSEQ-G99RS High-throughput Sequencing Set. The manual version is 5.0 and the set version is 1.0.

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*Unless otherwise informed, this StandardMPS sequencing reagent is not available in Germany, UK, Sweden, and Switzerland.

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Revision history

Version	Date	Description
5.0	April 2024	Update operation diagram.
4.0	October 2023	Upgrade Make DNB Enzyme Mix V
3.0	July 2023	• Add G99 SM FCL PE300 • Update interface diagram
2.0	February 2023	Add G99 SM FCL SE400 and G99 SM App-C FCL SE100.
1.0	August 2022	The first version.

Sequencing kit

Catalog number	Name	Model	Version
940-000409-00	DNBSEQ-G99RS High-throughput Sequencing Set	G99 SM FCL SE100/PE50	1.0
940-000410-00	DNBSEQ-G99RS High-throughput Sequencing Set	G99 SM FCL PE150	1.0
940-000520-00	DNBSEQ-G99RS High-throughput Sequencing Set	G99 SM App-C FCL SE100	1.0
940-000413-00	DNBSEQ-G99RS High-throughput Sequencing Set	G99 SM App-C FCL PE150	1.0
940-000417-00	DNBSEQ-G99RS High-throughput Sequencing Set	G99 SM FCL SE400	1.0
940-000415-00	DNBSEQ-G99RS High-throughput Sequencing Set	G99 SM FCL PE300	1.0

Contents

Chapter 1 Introduction

This manual describes how to perform sequencing using DNBSEQ-G99RS High-throughput Sequencing Set, Includes instructions regarding sample preparation,making DNB, flow cell preparation, DNB loading, Sequencing Reagent Cartridge preparation, sequencing preprocessing, sequencing protocol, and device maintenance.

1.1 Applications

DNBSEQ-G99RS High-throughput Sequencing Set is specifically designed for DNA or RNA sequencing on DNBSEQ-99RS or DNBSEQ-99ARS. This sequencing set is intended to be used for scientific research only, Which cannot be used for clinical diagnosis.

1.2 Sequencing technology

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

1.3 Data analysis

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

1.4 Sequencing read length

Sequencing read length will determine the number of sequencing cycles for a given sequencing run. For example, a PE150 cycle run performs reads of 150 cycles (2×150) for a total of 300 cycles. At the end of the insert sequencing run, one or two extra 10 cycles of **barcode** read can be performed, if required.

Table 1 Sequencing cycle

Sequencing read length	Read 1 read length	Read 2 read length	Barcode read length	Total read length	Maximum cycles
SE100	100	/	10	100+10	132
PE50	50	50	10	50+50+10	132
PE150	150	150	10	150+150+10	332
SE400	400	/	10	400+10	432
PE300	300	300	10	300+300+10	632

1.5 Sequencing time and analysis time

Tips

- The sequencing time (Single flow cell/Dual flow cells) in the table above is the time required from loading 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.
- The time in the table above is measured for single barcode.
- The time in the table above is average value. Actual run time may vary among various sequencers.

Table 2 FCL Sequencing time and analysis time for each read length (hours)

Type	Read length	Sequencing time	Analysis time
Single flow cell	SE100	4.2	0.05
	PE50	5.2	0.05
	PE150	11.8	0.05
	SE400	18.2	0.05
	PE300	27.5	0.1
Dual flow cell	SE100	4.4	0.1
	PE50	5.4	0.1
	PE150	12.0	0.1
	SE400	18.4	0.1
	PE300	27.8	0.2

1.6 Attention

- This product is restricted for research use only, please read the manual carefully before use.
- Make sure that you are familiar with the user manual&attention of all the laboratory apparatus to be used.
- Avoid direct skin and eye contact with any samples and reagents. Don't swallow. Please wash with plenty of water immediately and get medical aid if this happened.
- 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.
- The components are separate from the batch of the kit, do not remove the components, keep them in the box until use. Mixing of reagent components between different batches is strictly prohibited.
- Do not use expired products.

Chapter 2 Main components and usersupplied equipment, reagents and consumables

2.1 Main components

Tips It is worth reminding that:

- A sequencing set includes a sequencing flow cell, a Sequencing Reagent Cartridge and reagents for sequencing.
- Sequencing reagent cartridge can be stacked for storage, and it is recommended that the number of stacked layers should not exceed three.
- To perform SE35, SE50 or PE50 sequencing, please use the High-throughput Sequencing Set (G99 SM FCL S100/PE50).
- To perform PE100 sequencing, please use the High-throughput Sequencing Set (G99 SM FCL PE150).
- The High-throughput Sequencing Set (G99 SM FCL SE400) can not be used to perform PE sequencing.

Table 3 High-throughput Sequencing Set (G99 SM FCL SE100/PE50)
Catalog number: 940-000409-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G99 Sequencing Flow Cell	/	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Low TE Buffer		100 μL×1 tube			
Make DNB Buffer		20 μL×1 tube			
Make DNB Enzyme Mix I		40 μL×1 tube			
Make DNB Enzyme Mix II (LC)		13 μL×1 tube			
Stop DNB Reaction Buffer		50 μL×1 tube			
DNB Load Buffer II		50 μL×1 tube			
MDA Enzyme Mix		0.125 mL×1 tube			
MDA Reagent		1.0 mL×1 tube			
FTAT premixed compaction block	/	1 EA			
Micro Tube 0.5 mL (Empty)		1 tube			
Sequencing Reagent Cartridge	/	1 EA			

Table 4 High-throughput Sequencing Set (G99 SM FCL PE150)
Catalog number: 940-000410-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G99 Sequencing Flow Cell	/	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Low TE Buffer		100 μL×1 tube			
Make DNB Buffer		20 μL×1 tube			
Make DNB Enzyme Mix I		40 μL×1 tube			
Make DNB Enzyme Mix II (LC)		13 μL×1 tube			
Stop DNB Reaction Buffer		50 μL×1 tube			
DNB Load Buffer II		50 μL×1 tube			
MDA Enzyme Mix		0.125 mL×1 tube			
MDA Reagent		1.0 mL×1 tube			
FTAT premixed compaction block	/	1 EA			
Micro Tube 0.5 mL (Empty)		1 tube			
Sequencing Reagent Cartridge	/	1 EA			

Table 5 High-throughput Sequencing Set (G99 SM App-C FCL SE100)
Catalog number: 940-000520-00

Component	Cap color	Spec&quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G99 Sequencing Flow Cell	/	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Low TE Buffer		100 µL×1 tube			
App-C Make DNB Buffer		20 µL×1 tube			
Make DNB Enzyme Mix I		40 µL×1 tube			
Make DNB Enzyme Mix II (LC)		13 µL×1 tube			
Stop DNB Reaction Buffer		50 µL×1 tube			
DNB Load Buffer II		50 µL×1 tube			
FTAT premixed compaction block	/	1 EA			
Micro Tube 0.5 mL (Empty)		1 tube			
Sequencing Reagent Cartridge	/	1 EA			

Table 6 High-throughput Sequencing Set (G99 SM App-C FCL PE150)
Catalog number: 940-000413-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G99 Sequencing Flow Cell	/	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Low TE Buffer		100 μL×1 tube			
App-C Make DNB Buffer		20 μL×1 tube			
Make DNB Enzyme Mix I		40 μL×1 tube			
Make DNB Enzyme Mix II (LC)		13 μL×1 tube			
Stop DNB Reaction Buffer		50 μL×1 tube			
DNB Load Buffer II		50 μL×1 tube			
MDA Enzyme Mix		0.125 mL×1 tube			
MDA Reagent		1.0 mL×1 tube			
FTAT premixed compaction block	/	1 EA			
Micro Tube 0.5 mL (Empty)		1 tube			
Sequencing Reagent Cartridge	/	1 EA			

Table 7 High-throughput Sequencing Set (G99 SM FCL SE400)
Catalog number: 940-000417-00

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G99 Sequencing Flow Cell	/	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Low TE Buffer		100 µL×1 tube			
Make DNB Buffer		20 µL×1 tube			
Make DNB Enzyme Mix V		70 µL×1 tube			
Make DNB Enzyme Mix II (LC)		13 µL×1 tube			
Stop DNB Reaction Buffer		50 µL×1 tube			
DNB Load Buffer II		50 µL×1 tube			
FTAT premixed compaction block	/	1 EA			
Micro Tube 0.5 mL (Empty)		1 tube			
Sequencing Reagent Cartridge	/	1 EA			

Table 8 High-throughput Sequencing Set (G99 SM FCL PE300)
Catalog number: 940-000415-00

Component	Cap color	Spec&quantity	Storage temperature	Transportation temperature	Expiry date
DNBSEQ-G99 Sequencing Flow Cell	/	1 EA	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	12 months
Low TE Buffer		100 μL×1 tube			
Make DNB Buffer		20 μL×1 tube			
Make DNB High-efficiency Enzyme Mix V		40 μL×1 tube			
Make DNB Enzyme Mix II (LC)		13 μL×1 tube			
Stop DNB Reaction Buffer		50 μL×1 tube			
DNB Load Buffer II		60 μL×1 tube			
MDA Enzyme Mix		0.125 mL×1 tube			
MDA Reagent		1.0 mL×1 tube			
FTAT premixed compaction block	/	1 EA			
Micro Tube 0.5 mL (Empty)		1 tube			
Sequencing Reagent Cartridge	/	1 EA			

Table 9 DNBSEQ-G99 Cleaning Reagent Kit (G99 FCL)
Catalog number: 940-000624-00

Component	Spec&quantity	Storage temperature	Transportation temperature	Expiry date
Washing Cartridge	1 EA	0 ℃ to 30 ℃	below 40 ℃	12 months

2.2 User-supplied equipment, reagent and consumables

Tips

- Avoid making and loading DNBs by the pipette tips with filter. It is necessary to use the pipette tips with recommended brands and catalog number.
- Recommended brands and catalog number are suggested for other consumables..

Table 10 Self-prepared equipment and consumables

Type	Equipment	Recommended brand	Catalog number
Equipment	Qubit 4 Fluorometer	Thermo Fisher	Q33226
	Mini spinner	Major Laboratory Supplier (MLS)	/
	Vortex mixer	MLS	/
	Thermal cycler	Bio-Rad	/
	Graduated cylinder, 500 mL	MLS	/
	Pipette, 20 μL	Eppendorf or equivalent	/
	Pipette, 200 μL	Eppendorf or equivalent	/
	Pipette, 1000 μL	Eppendorf or equivalent	/
	2 ℃ to 8 ℃ refrigerator	MLS	/
	-25 ℃ to -15 ℃ freezer	MLS	/
	Ice bucket	MLS	/
	Electronic pipette	Labnet	FASTPETTEV-2
	Portable DNB Loader	MGI	510-003170-00
Reagent	2 M NaOH solution	MLS	/
	Qubit ssDNA Assay Kit	Thermo Fisher	Q10212
	Water, laboratory-grade	MLS	/

Type	Equipment	Recommended brand	Catalog number
Consumables	Qubit Assay Tubes	Thermo Fisher	Q32856
	0.5 mL Thin Wall PCR Tubes with Flat Cap, Clear, Nonsterile (PCR-05-C)	AXYGEN	10011-830
	Canned air duster	MATIN	M-6318
	Sterile pipette tip(various types)	AXYGEN	/
	Sterile 200 µL wide-bore non-filtered pipette tip	AXYGEN	T-205-WB-C
	0.2 mL PCR 8-tube strip, 0.2 mL	AXYGEN	/
	Sterile microcentrifuge tube, 1.5 mL	AXYGEN	MCT-150-C
	Disposable gloves, powder-free	MLS	/
	KimWipes	MLS	/
	Low-lint cloth	MLS	/

Chapter 3 Sequencing workflow

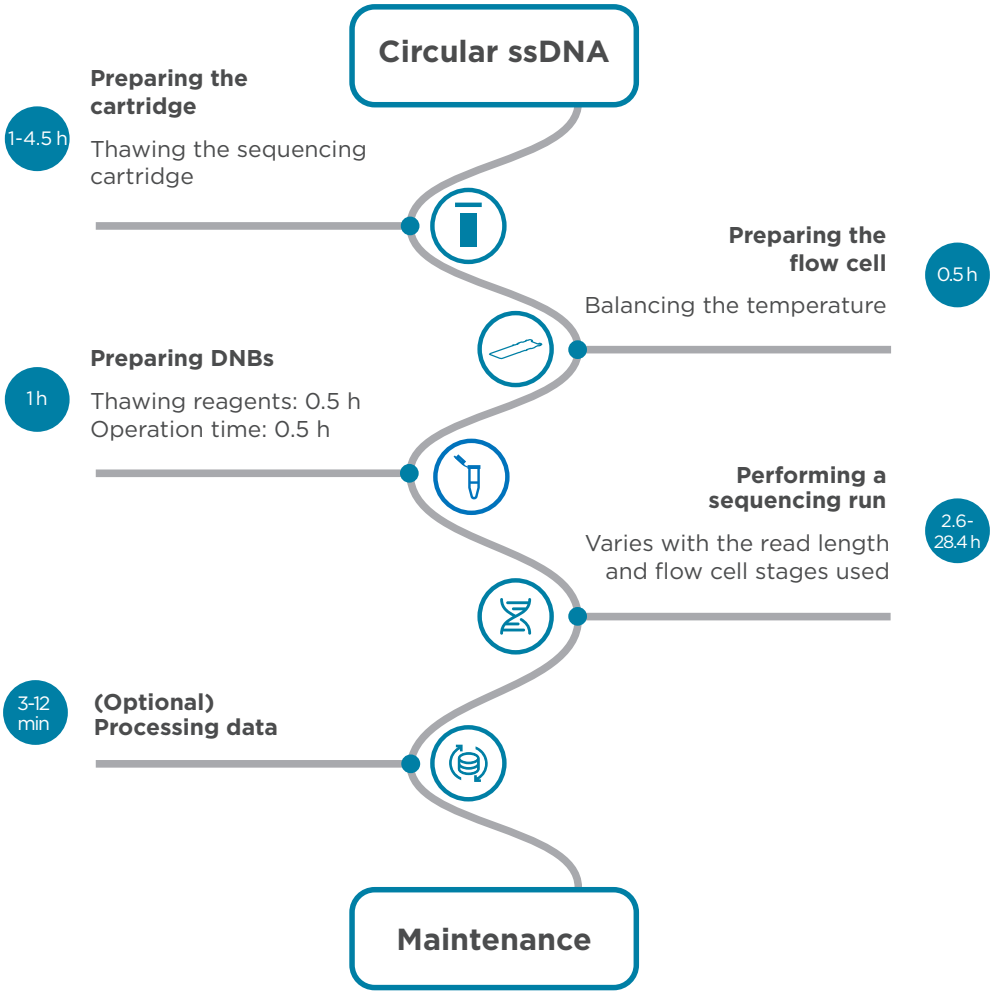

Figure 1 Sequencing workflow

Chapter 4 Making DNB

4.1 Insert size recommendation

Recommended library insert size: The size distribution of inserts should be between 200 to 700 bp, with the main band centered within ±100 bp.

Tips
- Select sequencing sets according to the insert size and the required data output.
- Average data output will vary with different library types and applications.
- If there are special requirements or specifications of the library preparation kit, the requirements of the kit should be followed.

Table 11 Recommended insert size

Product model	Suggested insert distribution (bp)	Data output (Gb/FC)
G99 SM FCL SE100/PE50	200 to 400	8.0
G99 SM FCL PE150	300 to 500	24.0
G99 SM App-C FCL SE100	300 to 500	8.0
G99 SM App-C FCL PE150	300 to 500	24.0
G99 SM FCL SE400	400 to 600	32.0
G99 SM FCL PE300	400 to 700	48.0

4.2 Library concentration and amount requirement

Tips If the library concentration is unknown, it is recommended to perform ssDNA library quantitation (ng/μL) using Qubit ssDNA Assay Kit and Qubit 4 fluorometer. Use the equation below to convert the concentration of the ssDNA library from ng/μL to fmol/μL.

$$C\ (fmol/\mu L) = 3030 \times C\ (ng/\mu L) / N$$

N represents the number of nucleotides (total library length including the adapter).

Table 12 Library requirement

Library type	Library concentration
MGI PCR libraries	≥2 fmol/μL
MGI PCR free libraries	≥3.75 fmol/μL
Third-party PCR libraries	≥3 fmol/μL
Third-party PCR free libraries	≥3.75 fmol/μL

4.3 Making DNB

Tips
- Mixed use of reagent components from different batches is strictly prohibited.
- Avoid making and loading DNBs by the pipette tips with filter. It is necessary to use the pipette tips with recommended brands and catalog number.

DNB making protocols are listed in the sections listed below. Select the appropriate one according to the sequencing sets used.

- *Making DNB for G99 SM FCL SE100, G99 SM FCL PE50 and G99 SM FCL PE150 on Page 16.*
- *Making DNBs for G99 SM App-C FCL SE100 and G99 SM App-C FCL PE150 on Page 19.*
- *Making DNB of G99 SM FCL SE400 on Page 21.*
- *Making DNB of G99 SM FCL PE300 on Page 26.*

4.3.1 Making DNB for G99 SM FCL SE100, G99 SM FCL PE50 and G99 SM FCL PE150

4.3.1.1 Preparing reagents for DNB making

Perform the following steps:

1. Place the library on ice until use.
2. Take out Low TE Buffer, Make DNB Buffer and Stop DNB Reaction Buffer from storage and thaw reagents at room temperature.
3. Thaw Make DNB Enzyme Mix I for approximately 30 minutes on ice.
4. After thawing, mix reagents thoroughly by using a vortex mixer for 5 seconds. Centrifuge briefly and place it on ice until use.

4.3.1.2 Calculating the required amount of ssDNA library

- The required volume of ssDNA libraries is determined by the required library amount (fmol) and library concentration quantified in *Library concentration and amount requirement on Page 14*.

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

Table 13 Required amount of ssDNA libraries

Library type	Volume (μL)
MGI PCR libraries	V=20 fmol/C
MGI PCR free libraries	V=37.5 fmol/C

- Calculate the required ssDNA libraries for each Make DNB reaction and fill it in *Make DNB reaction mix 1 on Page 17* as V.

4.3.1.3 Making DNB

1. Take out 0.2 mL PCR 8-tube strip or PCR tubes. Prepare Make DNB reaction mix 1 according to the table below for different library types.

Tips
- V represents variable sample volume as determined in *Calculating the required amount of ssDNA library on Page 16.*
- Keep Low TE Buffer on ice after use. It may be used for DNB dilution.

Table 14 Make DNB reaction mix 1

Component	Cap color	Volume (μL)
Low TE Buffer		10-V
Make DNB Buffer		10
ssDNA libraries	/	V
Total volume		20

2. Mix the reaction mixture thoroughly by using a vortex mixer for 5 seconds, centrifuge for 5 seconds and place it on ice until use.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown following the table below:

Table 15 Primer hybridization reaction conditions

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

4. Take out the Make DNB Enzyme Mix II (LC) from storage and place it on ice. Centrifuge briefly for 5 seconds and hold on ice.

   Tips
   - Do not place Make DNB Enzyme Mix II (LC) at room temperature.
   - Avoid holding the tube for a prolonged time.

5. Take the PCR tube out of the thermal cycler when the temperature reaches 4 ℃ .
6. Centrifuge briefly for 5 seconds, place the tube on ice and prepare the Make DNB reaction mix 2 following the table below:

Table 16 Make DNB reaction mix 2

Component	Cap color	Volume (µL)
Make DNB Enzyme Mix I		20
Make DNB Enzyme Mix II (LC)		2
Total volume		22

7. Add all the Make DNB reaction mix 2 into the Make DNB reaction 1. Mix thoroughly by 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 following the table below:

   Tips
   - As some thermal cyclers 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 ℃ or the temperature closest to 35 ℃ .

Table 17 RCA (Rolling circle amplication) conditions

Temperature	Time
Heated lid (35 ℃)	On
30 ℃	20 min
4 ℃	Hold

8. Immediately add 10 µL Stop DNB Reaction Buffer once the temperature reaches 4 ℃. Mix gently by pipetting 8 times using a wide-bore non-filtered pipette tip.

   Tips
   - Do not vortex, shake the tube or pipette vigorously.
   - It is very important to mix DNB gently using a wide-bore non-filtered pipette tip.
   - Store DNB at 4 ℃ and perform sequencing within 48 hours.

9. For the next step, refer to *Quantifying DNB on Page 29*.

4.3.2 Making DNBs for G99 SM App-C FCL SE100 and G99 SM App-C FCL PE150

4.3.2.1 Preparing reagents for DNB making

Perform the steps below:

1. Place the library on ice until use.
2. Take out Low TE Buffer, App-C Make DNB Buffer and Stop DNB Reaction Buffer from storage and thaw reagents at room temperature.
3. Thaw Make DNB Enzyme Mix I for approximately 30 minutes on ice.
4. After thawing, mix reagents thoroughly by using a vortex mixer for 5 seconds. Centrifuge briefly and place it on ice until use.

4.3.2.2 Calculating the required amount of ssDNA libraries

- The required volume of ssDNA libraries is determined by the required library amount (fmol) and library concentration quantified in *Library concentration and amount requirement on Page 14*.

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

Table 18 Required amount of ssDNA libraries

Library type	Volume (μL)
Third-party PCR libraries	V=30 fmol/C
Third-party PCR free libraries	V=37.5 fmol/C

- Calculate the required ssDNA libraries for each Make DNB reaction and fill it in *Make DNB reaction mix 1 on Page 20* as V.

4.3.2.3 Making DNBs

Perform the steps below:

1. Take out 0.2 mL PCR 8-tube strip or PCR tubes. Prepare Make DNB reaction mix 1 according to the table below for different library types.

   Tips
   - V represents variable sample volume as determined in *Calculating the required amount of ssDNA libraries on Page 19*.
   - Keep Low TE Buffer on ice after use. It may be used for DNB dilution.

Table 19 Make DNB reaction mix 1

Component	Cap color	Volume (μL)
Low TE Buffer		10-V
App-C Make DNB Buffer		10
ssDNA libraries	/	V
Total volume		20

2. Mix the reaction mixture thoroughly by using a vortex mixer for 5 seconds, centrifuge for 5 seconds and place it on ice until use.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown following the table below:

Table 20 Primer hybridization reaction conditions

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

4. Take out the Make DNB Enzyme Mix II (LC) from storage and place it on ice. Centrifuge briefly for 5 seconds and hold on ice.

   Tips
   - Do not place Make DNB Enzyme Mix II (LC) at room temperature.
   - Avoid holding the tube for a prolonged time.

5. Take the PCR tube out of the thermal cycler when the temperature reaches 4 ℃ .
6. Centrifuge briefly for 5 seconds, place the tube on ice and prepare the Make DNB reaction mix 2 following the table below:

Table 21 Make DNB reaction mix 2

Component	Cap color	Volume (μL)
Make DNB Enzyme Mix I		20
Make DNB Enzyme Mix II (LC)		2
Total volume		22

7. Add all the Make DNB reaction mix 2 into the Make DNB reaction 1. Mix thoroughly by 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 following the table below:

   Tips
   - As some thermal cyclers 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 ℃ or the temperature closest to 35 ℃ .

Table 22 RCA conditions

Temperature	Time
Heated lid (35 ℃)	On
30 ℃	20 min
4 ℃	Hold

8. Immediately add 10 µL Stop DNB Reaction Buffer once the temperature reaches 4 ℃. Mix gently by pipetting 8 times using a wide-bore non-filtered pipette tip.

   Tips
   - Do not vortex, shake the tube or pipette vigorously.
   - It is very important to mix DNB gently using a wide-bore non-filtered pipette tip.
   - Store DNB at 4 ℃ and perform sequencing within 48 hours.

9. For the next step, refer to *Quantifying DNB on Page 29*.

4.3.3 Making DNB of G99 SM FCL SE400

4.3.3.1 Preparing reagents for DNB making

Perform the following steps:

1. Place the library on ice until use.
2. Take out Make DNB Buffer, Low TE Buffer and Stop DNB Reaction Buffer from storage and thaw reagents at room temperature.
3. Thaw Make DNB Enzyme Mix V for approximately 30 minutes on ice.
4. After thawing, mix reagents using a vortex mixer for 5 seconds. Centrifuge briefly and place on ice until use.

4.3.3.2 Calculating the required amount of ssDNA library

- The required volume of ssDNA libraries is determined by the required library amount (ng or fmol) and library concentration quantified in *Library concentration and amount requirement on Page 14*. C1 represents the library concentration (ng/μL). C2 represents the library concentration (fmol/μL). n represents the mass of library.

  Tips
  - The input amount of short tandem repetitive amplicon library is recommended to be 5 ~ 10ng.
  - If there are special requirements or specifications of the library preparation kit, then the requirements of the kit should be followed.

Table 23 Required amount of ssDNA libraries

Library type	Volume (μL)
Short tandem repetitive amplicon library	V = n / C1
Non-Short tandem repetitive amplicon library	V = 60 fmol / C2

- Calculate the required ssDNA libraries for each Make DNB reaction and fill it in *Make DNB reaction mix 1 on Page 23 or Make DNB reaction mix 1 on Page 25* as V.

4.3.3.3 Making DNB

Tips This section includes two DNB preparation protocols, Short tandem repetitive amplicon library and Non-Short tandem repetitive amplicon library. Select a appropriate one according to needs.

- Perform the steps below for Short tandem repetitive amplicon library:

1. Take out 0.2 mL PCR 8-tube strip or PCR tubes. Prepare Make DNB reaction mix 1 according to the table below for different library types.

   Tips V represents variable sample volume as determined in *Calculating the required amount of ssDNA library on Page 22*.

Table 24 Make DNB reaction mix 1

Component	Cap color	Volume (μL)
Low TE Buffer		10-V
Make DNB Buffer		10
ssDNA libraries	/	V
Total volume		20

2. Mix the reaction mixture thoroughly by using a vortex mixer for 5 seconds, centrifuge for 5 seconds and place it on ice until use.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown following the table below:

Table 25 Primer hybridization reaction conditions

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

4. Take out the Make DNB Enzyme Mix II (LC) from storage and place it on ice. Centrifuge briefly for 5 seconds and hold on ice.

   Tips
   - Do not place Make DNB Enzyme Mix II (LC) at room temperature.
   - Avoid holding the tube for a prolonged time.

5. Take the PCR tube out of the thermal cycler when the temperature reaches 4 ℃ .
6. Centrifuge briefly for 5 seconds, place the tube on ice and prepare the Make DNB reaction mix 2 following the table below:

Table 26 Make DNB reaction mix 2

Component	Cap color	Volume (μL)
Make DNB Enzyme Mix V		30
Make DNB Enzyme Mix II (LC)		2
Total volume		32

7. Add all the Make DNB reaction mix 2 into the Make DNB reaction 1. Mix thoroughly by 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 following the table below:

   Tips
   - As some thermal cyclers 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 ℃ or the temperature closest to 35 ℃ .

Table 27 RCA conditions

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

8. Immediately add 10 µL Stop DNB Reaction Buffer once the temperature reaches 4 ℃. Mix gently by pipetting 8 times using a wide-bore non-filtered pipette tip.

   Tips
   - Do not vortex, shake the tube or pipette vigorously.
   - It is very important to mix DNB gently using a wide-bore non-filtered pipette tip.
   - Store DNB at 4 ℃ and perform sequencing within 48 hours.

9. For the next step, refer to *Quantifying DNB on Page 29*.

- Perform the steps below for Non-Short tandem repetitive amplicon library:

1. Take out 0.2 mL PCR 8-tube strip or PCR tubes. Prepare Make DNB reaction mix 1 according to the table below for different library types.

   Tips
   - V represents variable sample volume as determined in *Calculating the required amount of ssDNA library on Page 22*.
   - Keep Low TE Buffer on ice after use. It may be used for DNB dilution.

Table 28 Make DNB reaction mix 1

Component	Cap color	Volume (μL)
Low TE Buffer		10-V
Make DNB Buffer		10
ssDNA libraries	/	V
Total volume		20

2. Mix the reaction mixture thoroughly by using a vortex mixer for 5 seconds, centrifuge for 5 seconds and place it on ice until use.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown following the table below:

Table 29 Primer hybridization reaction conditions

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

4. Take out the Make DNB Enzyme Mix II (LC) from storage and place it on ice. Centrifuge briefly for 5 seconds and hold on ice.

   Tips
   - Do not place Make DNB Enzyme Mix II (LC) at room temperature.
   - Avoid holding the tube for a prolonged time.

5. Take the PCR tube out of the thermal cycler when the temperature reaches 4 ℃ .
6. Centrifuge briefly for 5 seconds, place the tube on ice and prepare the Make DNB reaction mix 2 following the table below:

Table 30 Make DNB reaction mix 2

Component	Cap color	Volume (μL)
Make DNB Enzyme Mix V		15
Make DNB Enzyme Mix II (LC)		2
Total volume		17

7. Add all the Make DNB reaction mix 2 into the Make DNB reaction 1. Mix thoroughly by 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 following the table below:

   Tips
   - As some thermal cyclers 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 ℃ or the temperature closest to 35 ℃ .

Table 31 RCA conditions

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

8. Immediately add 10 µL Stop DNB Reaction Buffer once the temperature reaches 4 ℃. Mix gently by pipetting 8 times using a wide-bore non-filtered pipette tip.

   Tips
   - Do not vortex, shake the tube or pipette vigorously.
   - It is very important to mix DNB gently using a wide-bore non-filtered pipette tip.
   - Store DNB at 4 ℃ and perform sequencing within 48 hours.

9. For the next step, refer to *Quantifying DNB on Page 29.*

4.3.4 Making DNB of G99 SM FCL PE300

4.3.4.1 Preparing reagents for DNB making

Perform the following steps:

1. Place the library on ice until use.
2. Take out Make DNB Buffer, Low TE Buffer and Stop DNB Reaction Buffer from storage and thaw reagents at room temperature.
3. Thaw Make DNB High-efficiency Enzyme Mix V for approximately 30 minutes on ice.

4. After thawing, mix reagents using a vortex mixer for 5 seconds. Centrifuge briefly and place on ice until use.

4.3.4.2 Calculating the required amount of ssDNA library

- The required volume of ssDNA libraries is determined by the required library amount (fmol) and library concentration quantified in *Library concentration and amount requirement on Page 14*.

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

Table 32 Required amount of ssDNA libraries

Library type	Volume (μL)
MGI PCR libraries	V=30 fmol/C
MGI PCR free libraries	V=37.5 fmol/C

- Calculate the required ssDNA libraries for each Make DNB reaction and fill it in *Make DNB reaction mix 1 on Page 27* as V.

4.3.4.3 Making DNB

Perform the steps below:

1. Take out 0.2 mL PCR 8-tube strip or PCR tubes. Prepare Make DNB reaction mix 1 according to the table below for different library types.

   Tips V represents variable sample volume as determined in *Calculating the required amount of ssDNA library on Page 27*.

Table 33 Make DNB reaction mix 1

Component	Cap color	Volume (μL)
Low TE Buffer		10-V
Make DNB Buffer		10
ssDNA libraries	/	V
Total volume		20

2. Mix the reaction mixture thoroughly by using a vortex mixer for 5 seconds, centrifuge for 5 seconds and place it on ice until use.

3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown following the table below:

Table 34 Primer hybridization reaction conditions

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

4. Take out the Make DNB Enzyme Mix II (LC) from storage and place it on ice. Centrifuge briefly for 5 seconds and hold on ice.

Tips
- Do not place Make DNB Enzyme Mix II (LC) at room temperature.
- Avoid holding the tube for a prolonged time.

5. Take the PCR tube out of the thermal cycler when the temperature reaches 4 ℃ .
6. Centrifuge briefly for 5 seconds, place the tube on ice and prepare the Make DNB reaction mix 2 following the table below:

Table 35 Make DNB reaction mix 2

Component	Cap color	Volume (μL)
Make DNB High-efficiency Enzyme Mix V		20
Make DNB Enzyme Mix II (LC)		0.8
Total volume		20.8

7. Add all the Make DNB reaction mix 2 into the Make DNB reaction 1. Mix thoroughly by 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 following the table below:

Tips
- As some thermal cyclers 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 ℃ or the temperature closest to 35 ℃ .

Table 36 RCA conditions

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

8. Immediately add 10 µL Stop DNB Reaction Buffer once the temperature reaches 4 ℃. Mix gently by pipetting 8 times using a wide-bore non-filtered pipette tip.

   Tips
   - Do not vortex, shake the tube or pipette vigorously.
   - It is very important to mix DNB gently using a wide-bore non-filtered pipette tip.
   - Store DNB at 4 ℃ and perform sequencing within 48 hours.

9. For the next step, refer to *Quantifying DNB on Page 29*.

4.4 Quantifying DNB

Perform the steps below:

1. When DNB making is completed, take 2 µL of DNBs, and use the Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify the DNBs. For details, refer to *Instructions for using Qubit to quantify the DNBs on Page 87*.

   Tips
   - 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 of libraries prepared by customers is lower than that specified in the table above, refer to *Low DNB concentration on Page 63*.

Table 37 DNB concentration standard

Model	DNB concentration
G99 SM FCL SE100/PE50	≥8 ng/μL
G99 SM FCL PE150	
G99 SM APP-C FCL SE100	
G99 SM APP-C FCL PE150	
G99 SM FCL SE400	
G99 SM FCL PE300	≥12 ng/μL

2. If the concentration exceeds 40 ng/μL, the DNBs should be diluted with Low TE Buffer according to the following table.

   Tips The DNBs for SE400 or PE300 sequencing can not be diluted if the DNB concentration exceeds 40 ng/μL.

Table 38 Scheme for DNB concentration dilution

Model	DNB concentration after diluting
G99 SM FCL SE100/PE50	20 ng/μL
G99 SM FCL PE150	
G99 SM APP-C FCL SE100	
G99 SM APP-C FCL PE150	

3. For the next step, refer to *Loading DNBs by DL-G99 on Page 44*.

Chapter 5 Preparing the flow cell

Perform the steps below:

1. Remove the flow cell box from the sequencing set.

   Tips Do not open the outer plastic package at this moment.

2. Place the flow cell at room temperature for 30 minutes to 24 hours.
3. Unwrap the outer plastic package before use.

Tips
- If the flow cell can not be used within 24 hours after being placed in room temperature and the outer plastics package is intact, the flow cell can be placed back in -25 ℃ to -15 ℃ for storage. But the switch between room temperature and -25 ℃ to -15 ℃ must not exceed 3 times.
- If the outer plastic package has been opened but the flow cell is not used immediately, store the flow cell at room temperature and use within 24 hours. If 24 hours is exceeded, it is not recommended that you use the flow cell.

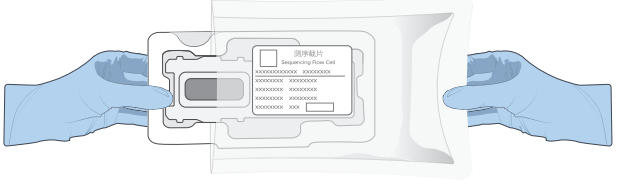

Figure 2 Unwrapping the outer package

4. Take the flow cell out of the inner package and inspect it to ensure that the flow cell is intact.
5. Clean the back of the flow cell by using a canned air duster.

Chapter 6 Preparing the sequencing reagent cartridge

- Follow steps 1 through 7 to prepare the Sequencing Reagent Cartridge for SE sequencing.
- Follow steps 1 through 10 to prepare the Sequencing Reagent Cartridge for PE sequencing.
- The MDA mixture (MDA, Multiple displacement amplification) needs to be added into MDA well if you perform PE sequencing. If prepared reagent cartridges are not used immediately, refer to *Reagent kit storage rules on Page 73*.
- A sequencing reagent cartridge can only be primed up to twice.

Perform the steps below:

1. Remove the Sequencing Reagent Cartridge from storage.

2. Thaw the cartridge in a water bath at room temperature or in a 2 ℃ to 8 ℃ refrigerator. The approximate time to thaw is listed in the following table. Store the cartridge in a 2 ℃ to 8 ℃ refrigerator until use.

Table 39 Approximate thaw time for various models

Model	Method		
	Water bath at room temperature (hours)	Refrigerator at 2℃ to 8℃ overnight then water bath at room temperature (hours)	Refrigerator at 2℃ to 8℃ (hours)
G99 SM FCL SE100/PE50	2.0	0.5	8.0
G99 SM FCL PE150	3.0	0.5	14.0
G99 SM App-C FCL SE100	2.0	0.5	8.0
G99 SM App-C FCL PE150	3.0	0.5	14.0
G99 SM FCL SE400	4.0	0.5	16.0
G99 SM FCL PE300	4.5	0.5	21.0

3. Invert the cartridge 5 times to mix before use.
4. Wipe any water condensation on the cartridge cover and wells with a Kimwipes tissue.
5. Use the Puncher to pierce the M1, M2, M3, M4 wells of the cartridge with the pre-mixed reagents.

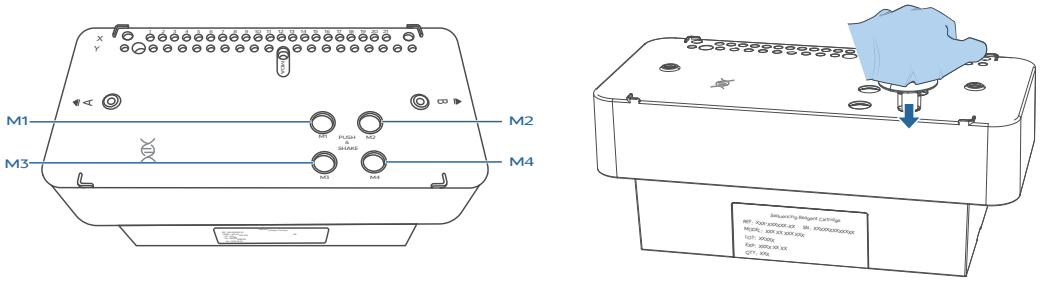

Figure 3 Piercing the M1, M2, M3, M4 wells of the Sequencing Reagent Cartridge

6. Shake the cartridge vigorously 20 times in an up-and-down motion and 20 times in a clockwise and counterclockwise direction. Ensure that reagents are fully mixed.

Tips Failure to mix adequately will affect the results of the experiment.

7. Pierce the seal of MDA well by using a clean 1 mL sterile pipette tip. The position of MDA well is on *Adding MDA mixture on Page 33*.

   Tips The G99 SM FCL SE100/G99 SM App-C FCL SE100/G99 SM FCL SE400 Sequencing Reagent Cartridge is now ready for use. For the next step, refer to *Performing a sequencing run on Page 34*.

8. For PE sequencing, take out MDA Reagent from storage.
9. Add 125 µL of MDA Enzyme Mix to the MDA Reagent tube with a 200 µL pipette and invert the tube 6 times to mix the reagents.

   Tips When using MDA Enzyme Mix, do not touch the tube wall. The heat from your hand can affect the enzyme activity.

10. Add all the mixture to MDA well.

    Tips
    - Transfer the mixture carefully to prevent the mixture from spilling out of the reagent tube.
    - The G99 SM FCL PE50/G99 SM FCL PE150/G99 SM App-C FCL PE150/G99 SM FCL PE300 Sequencing Reagent Cartridge now ready for use. For the next step, refer to *Performing a sequencing run on Page 34*.

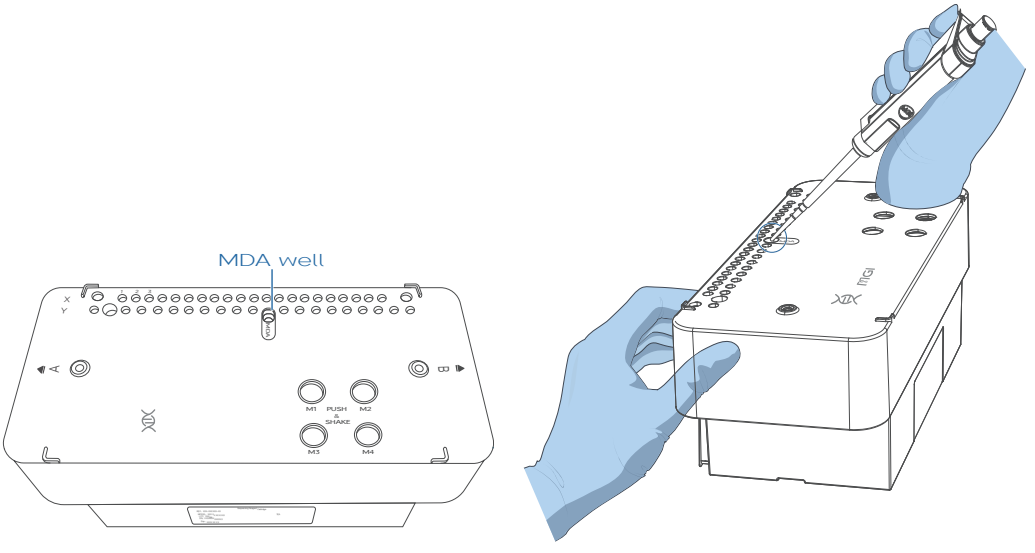

Figure 4 Adding MDA mixture

Chapter 7 Performing a sequencing run

7.1 Checking before sequencing

Perform the steps below:

1. Select in operation A according to your requirement. If both A and B are required, select .

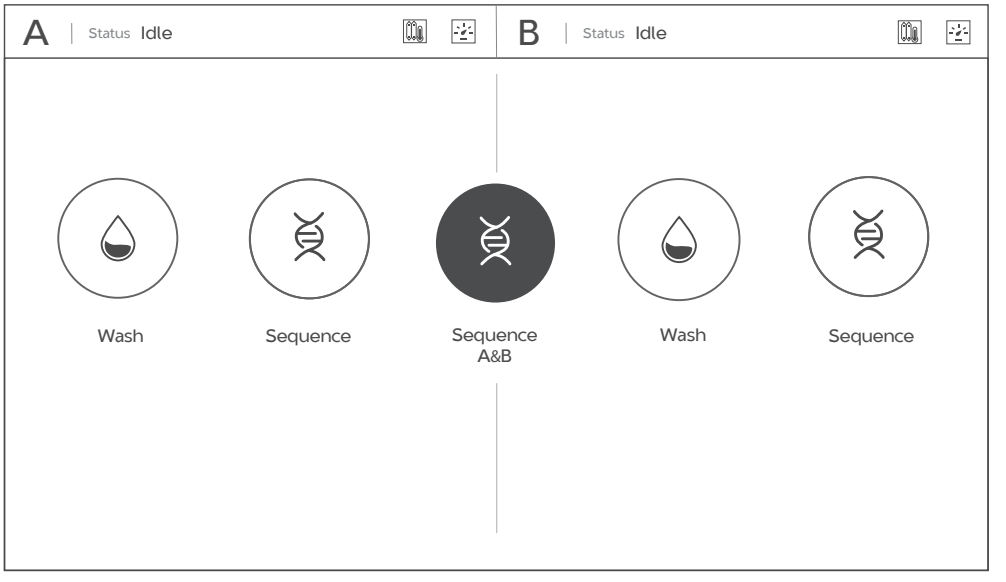

Figure 5 New sequence selection interface

2. The main interface is as below:The system automatically checks the Disk space, Sensor, Optical system and Incubation system one by one.

Tips
- Ensure that the liquid level in the waste container is lower than the upper limit line. If not, empty the waste container.
- If any part of this check fails, refer to *An error occurs before washing on Page 74*.

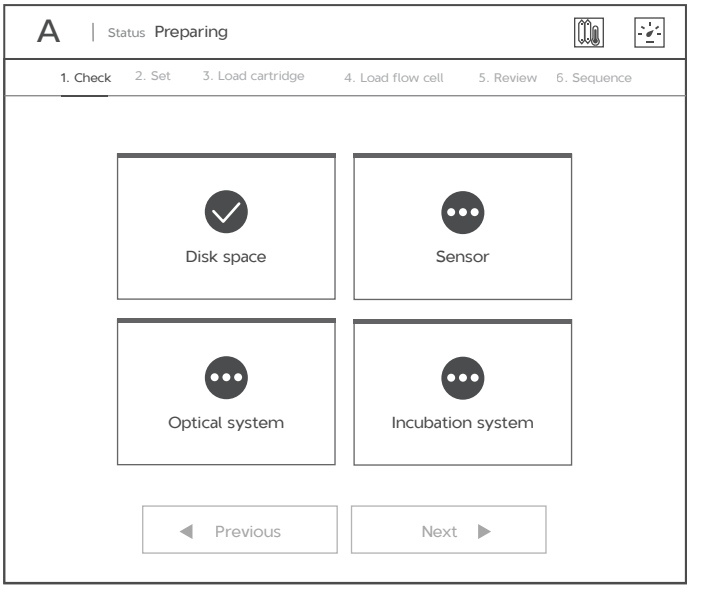

Figure 6 Check interface

3. After the check is completed, select **Next**.

7.2 Setting the sequencing parameters

Choose one of the following workflow types:

- **Sequence Only**: Testing general script.
- **Sequence & Transmission**: After general sequencing, upload data to the server for bioinformatic analysis.
- **BBS** (Bioanalysis By Sequencing): Test Barcode first, and then upload data to the specified node for bioinformatic analysis.

Tips The settings of **Sequence & Transmission** and **BBS** can only be performed on DNBSEQ-G99ARS.

For information on setting parameters, refer to:

- *Setting sequence only parameters on Page 36.*
- *Setting sequence & transmission parameters on Page 38.*
- *Setting BBS parameters on Page 40.*

7.2.1 Setting sequence only parameters

Perform the steps below:

1. Select **Sequence Only** workflow type, and **BBS** will default to No. Select the **DNB ID** box and enter the DNB ID using the on-screen keyboard.

   Tips When naming a DNB ID, use only letters, numbers, "+", "-" and "_".

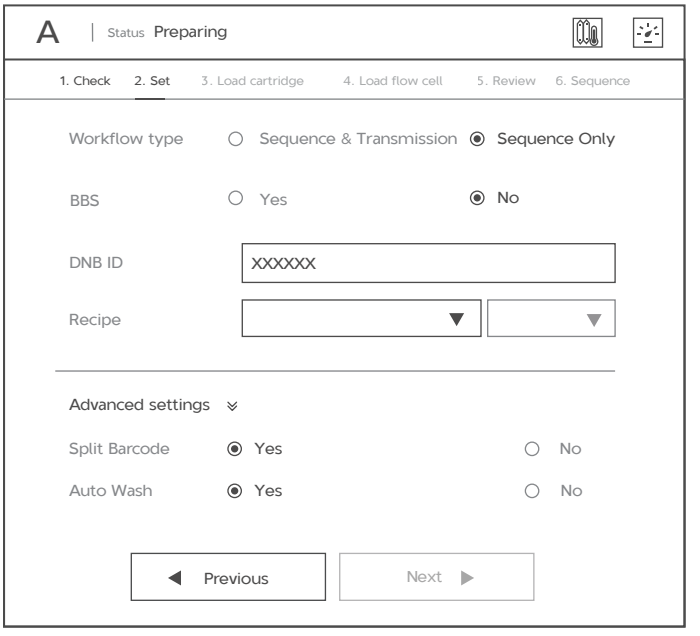

Figure 7 Selecting a workflow type

2. Select the sequencing recipe from the **Recipe** list. There are default sequencing runs (for example, SE100+10(Default)) and a user-customized run (Customize).

   Tips For other recipes(such as SE35, SE50, PE50, PE100 and so on) not in the recipe list, select **Customize** from the **Recipe** list. For information on customizing a recipe, refer to *Important interfaces for customizing a run on Page 76*.

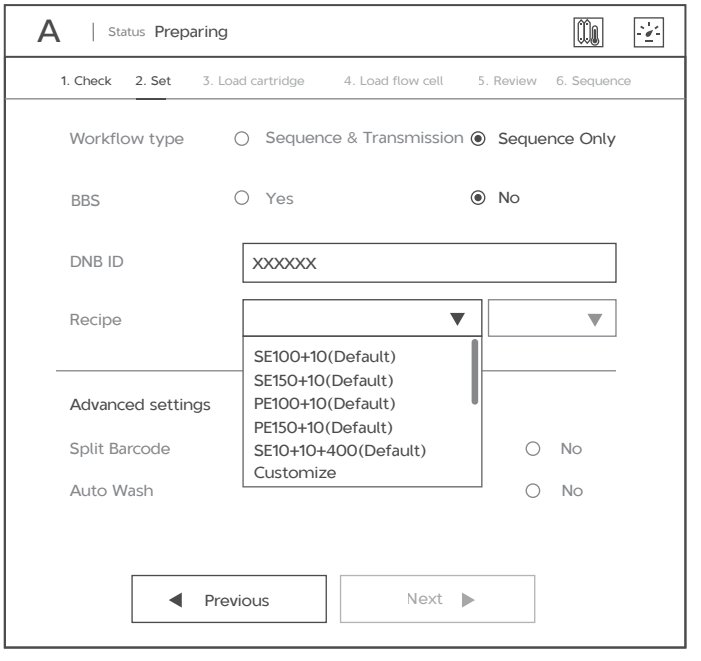

Figure 8 Selecting a sequencing recipe

3. Select a barcode range sequence from the list of barcode ranges next to the **Recipe** list.

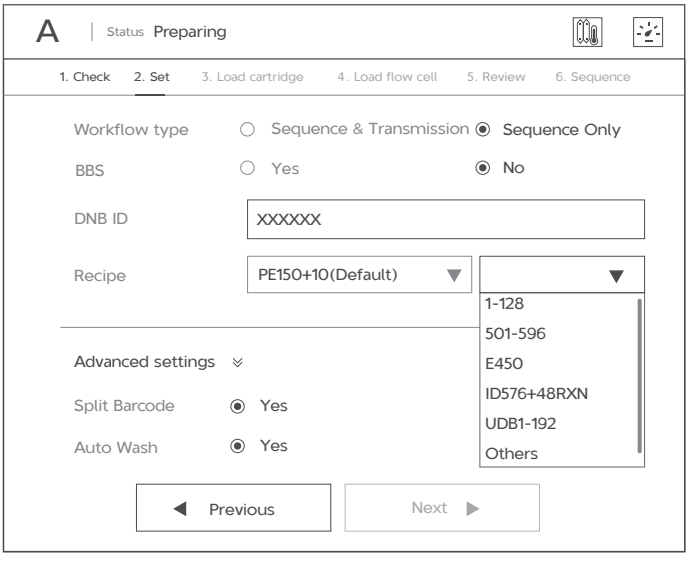

Figure 9 Selecting a barcode range

4. In Advanced settings, select either **Yes** or **No** for **Split Barcode** and **Auto Wash**, according to your needs. Yes is the default for both settings.

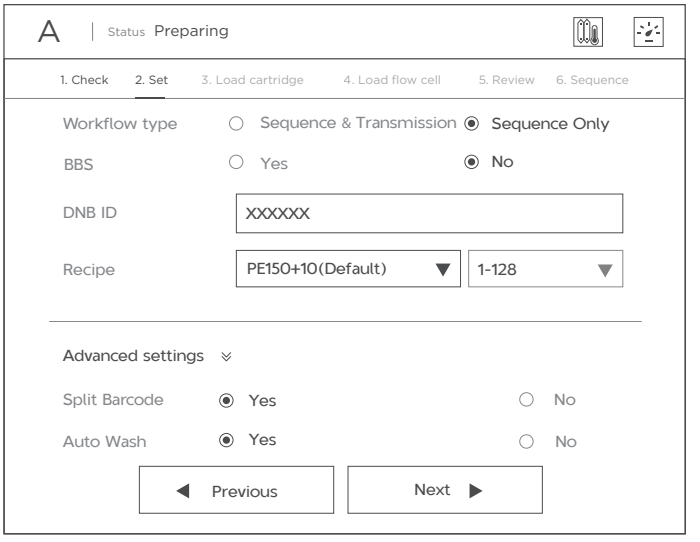

Figure 10 Advanced settings

5. Select **Next**, and for the next step, refer to *Loading the reagent cartridge on Page 41.*

7.2.2 Setting sequence & transmission parameters

Perform the steps below:

1. Select **Sequence & Analysis** workflow type. Select **No** for BBS.

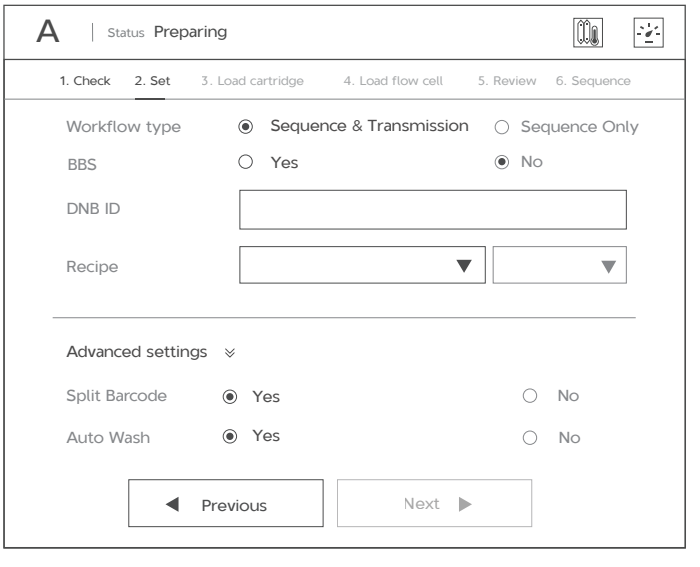

Figure 11 Sequence & Analysis workflow type

2. Select the **DNB ID** box and enter the DNB ID using the on-screen keyboard. Select the sequencing recipe from the **Recipe** list.

   Tips For other recipes(such as SE35, SE50, PE50, PE100 and so on) not in the recipe list, select **Customize** from the **Recipe** list. For information on customizing a recipe, refer to *Important interfaces for customizing a run on Page 76*.

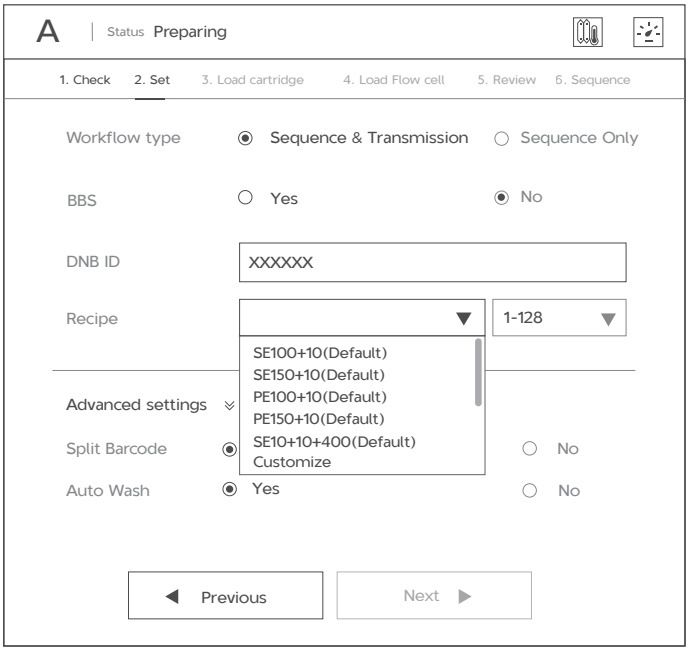

Figure 12 Entering DNB ID and selecting the sequencing recipe

For information on barcode sequence and advanced settings, refer to *Selecting a barcode range on Page 37 and Advanced settings on Page 38*.

3. Select **Next**, and for the next step, refer to *Loading the reagent cartridge on Page 41*.

7.2.3 Setting BBS parameters

Perform the steps below:

1. Select **Sequence** & **Analysis** workflow type. Select **Yes** for BBS and fill in the BBS box next to **Yes** for data analysis. For example, *10,110*, means that the data analysis will be performed at the 10th cycle of read1 and the 10th cycle of read2 for a BBS PE100 sequencing.

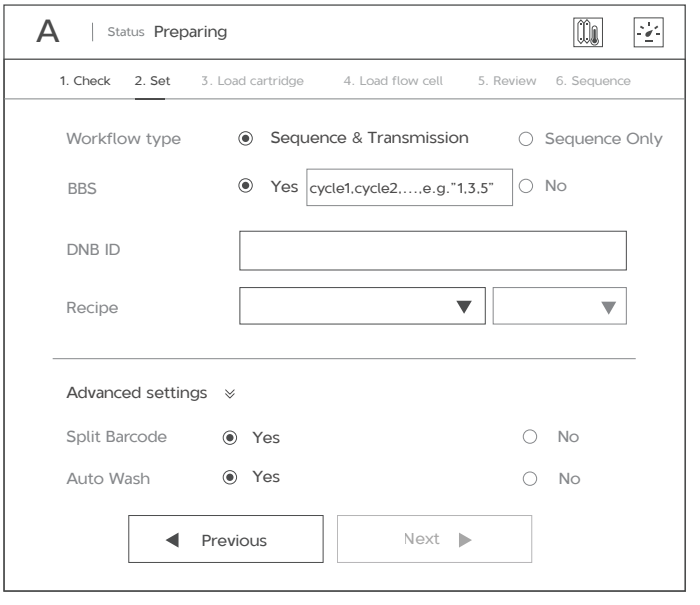

Figure 13 Selecting BBS sequencing type

2. Select the **DNB ID** box and enter the DNB ID using the on-screen keyboard. Select a sequencing recipe from the **Recipe** list.

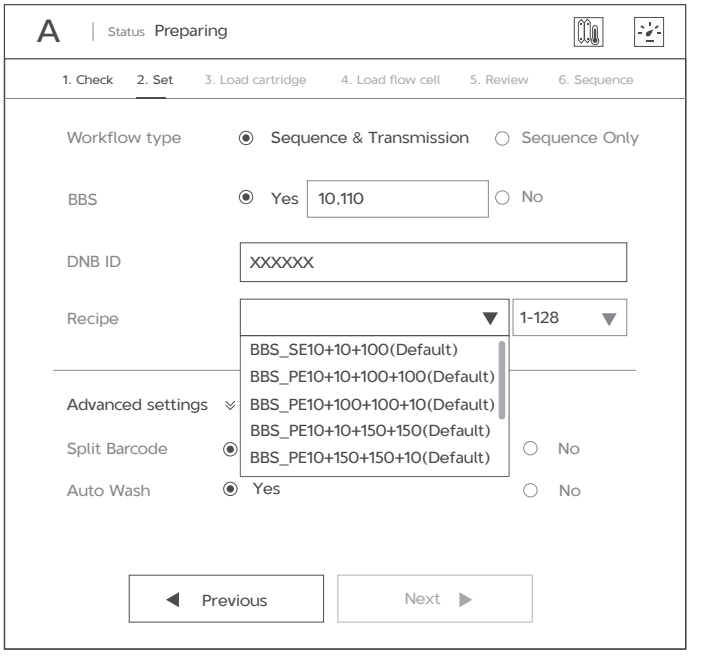

Figure 14 Entering DNB ID and selecting BBS recipe

For information on barcode range and advanced settings, refer to *Selecting a barcode range on Page 37 and Advanced settings on Page 38.*

3. Select **Next**, and for the next step, refer to *Loading the reagent cartridge on Page 41.*

7.3 Loading the reagent cartridge

Perform the steps below:

1. Slide the Sequencing Reagent Cartridge into the reagent compartment until it stops.

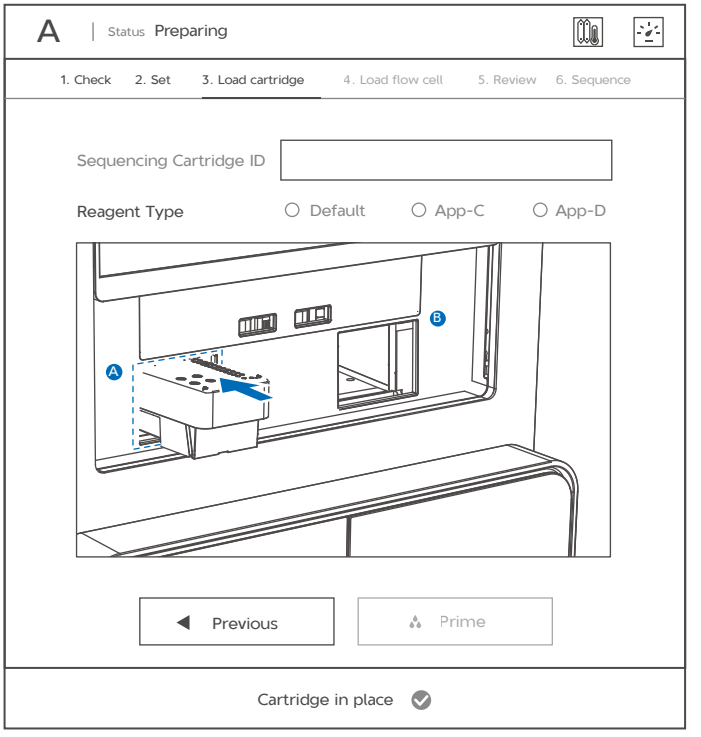

Figure 15 Loading the sequencing cartridge

The RFID (Radio Frequency Identification) scanner will automatically identify the sequencing cartridge ID.

Tips Enter the sequencing reagent cartridge ID manually if the RFID scanner fails to identify the ID. The form of the sequencing reagent cartridge ID is"REF-SN".

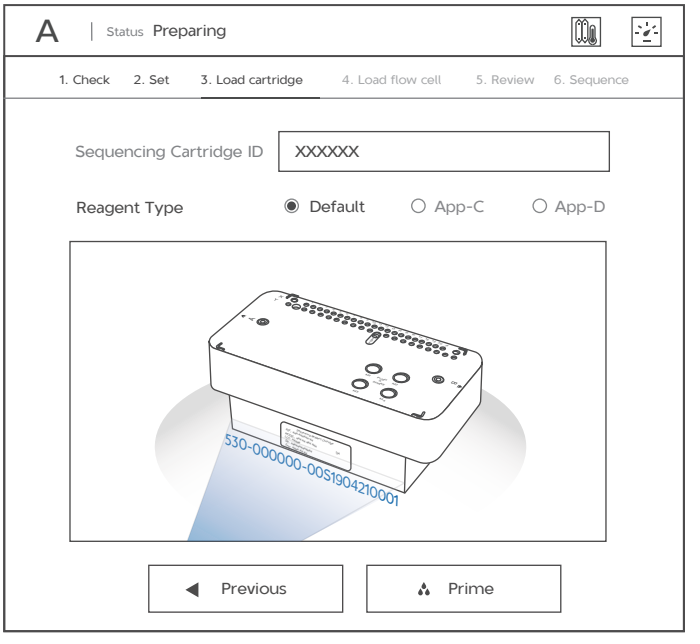

Figure 16 Scanning Sequencing Reagent Cartridge ID

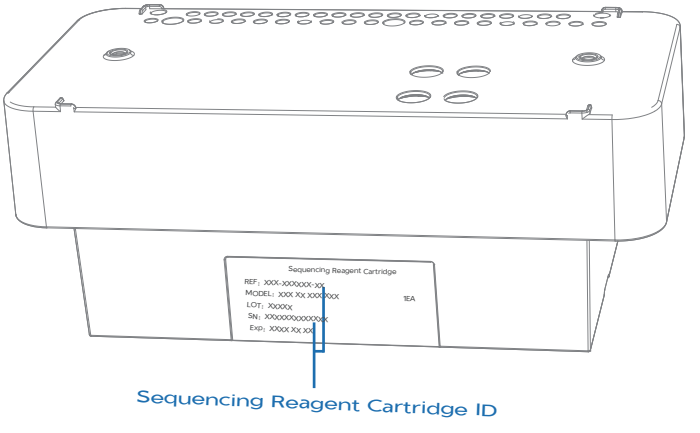

Figure 17 Location of Sequencing Reagent Cartridge ID

2. Select **Prime**.
3. Select **Yes** to start priming. The priming process takes about 2 minutes. If pumping failure occurs during priming, contact Technical Support.

Tips

- If a returning to the main interface option is provided after priming but before sequencing starts, follow the on-screen instructions to resolve the problem before operation. Directly return to the main interface may result in sequencing failure.
- A sequencing reagent cartridge can perform priming up to 2 times.

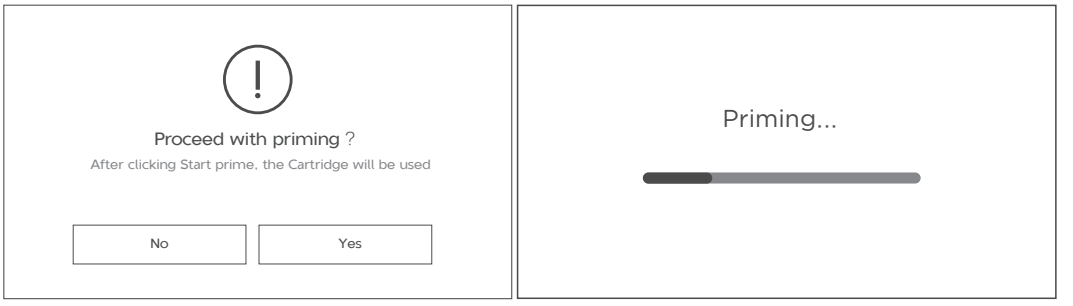

Figure 18 Confirming prime interface

7.4 Loading DNBs by DL-G99

7.4.1 Preparing reagents

Perform the steps below:

1. Remove DNB Load Buffer II from storage and thaw the reagents on ice for approximately 30 minutes.
2. After thawing, mix the reagents using a vortex mixer for 5 seconds. Centrifuge briefly and place on ice until use.

   Tips If crystal precipitation is found in DNB Load Buffer II, vigorously mix the reagent for 1 to 2 minutes using a vortex mixer to re-dissolve the precipitation before use.
3. Remove the Micro Tube 0.5 mL (Empty) from the sequencing set and add the following reagents:

Table 40 DNB loading mixture

Component	Volume (µL)
DNB Load Buffer II	7.0
Make DNB Enzyme Mix II (LC)	1.0
DNBs	21.0
Total Volume	29.0

4. Combine the components and mix by gently pipetting 8 times using a wide-bore, non-filtered pipette tip. Place the mixture at 4 °C until use.

   Tips
   - Do not centrifuge, vortex, or shake the tube.
   - Prepare a fresh DNB loading mixture immediately before the sequencing run.
   - Each FCL requires 10 µL of DNB loading mixture.

7.4.2 Loading DNBs

Perform the steps below:

1. Hold the loader with one hand, and open the cover with the other hand.

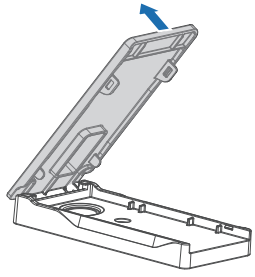

Figure 19 Opening the cover

2. Place the flow cell into the loader, and ensure that the QR code is facing up. Close the cover.

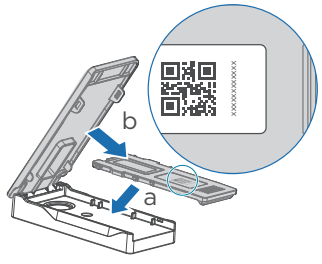

Figure 20 Placing the flow cell

3. Place the loader on the laboratory bench with the back facing up.

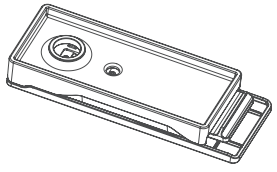

Figure 21 Placing the loader with back facing up

4. Aspirate 10 μL of DNB loading mixture by using a 200 μL non-filtered sharp pipette tip, and vertically insert the tip into inlet A as shown in the following figure:

   Tips Use a 200 μL non-filtered sharp tip but not a wide-bore tip in this loading method.

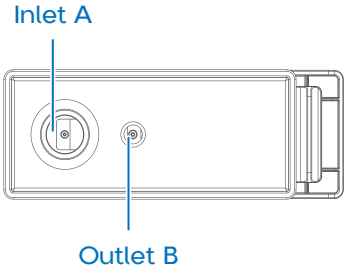

Figure 22 Inlet and outlet of loader

5. Fix the tip with one hand, press the tip ejector on the pipette to unload the tip with the other hand, and observe the liquid level in the tip:
   - If the liquid level drops automatically, the DNB loading mixture will automatically flow into the flow cell, skip step 6.
   - If the liquid level does not drop, continue to step 6.

   Tips
   - During DNB library loading, do not press the button of the pipette.
   - Do not rotate the tip or move the flow cell during the loading process.

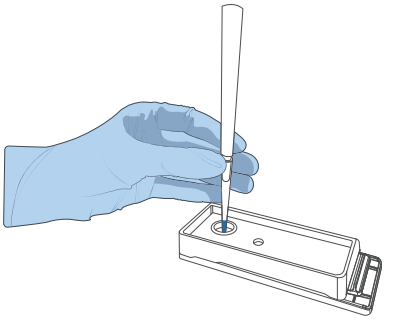

Figure 23 Loading DNBs by using DL-G99

6. (Optional) If the liquid level does not drop, perform the following steps:
   1) Leave the tip with DNB loading mixture in inlet A.
   2) Adjust the aspirate volume to 2 μL and pipette a new 200 μL non-filtered pipette tip.
   3) Hold the new empty tip with one hand and gently insert it into outlet B while pressing the button down with the other hand.

4) Gently release the button and remove the tip in inlet B after the liquid level of the tip at outlet A drops.

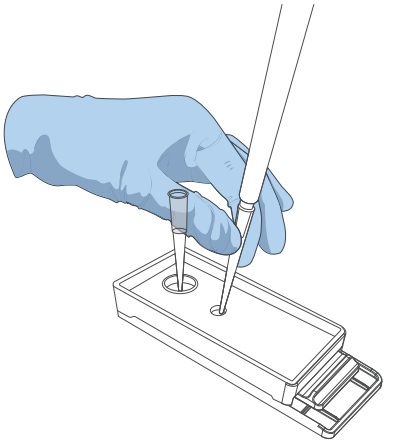

Figure 24 Loading DNBs by using DL-G99

7. When the liquid level in the pipette tip stops dropping, remove the pipette tip in inlet A.
8. Turn the loader upside down, open the cover, remove the flow cell, and transfer it to the sequencer immediately.

7.5 Loading flow cell

Perform the following steps:

1. Insert the flow cell into the flow cell compartment after priming is finished. The RFID scanner will automatically identify the flow cell ID.

   Tips Enter the flow cell ID manually if the RFID scanner fails to recognize the ID.

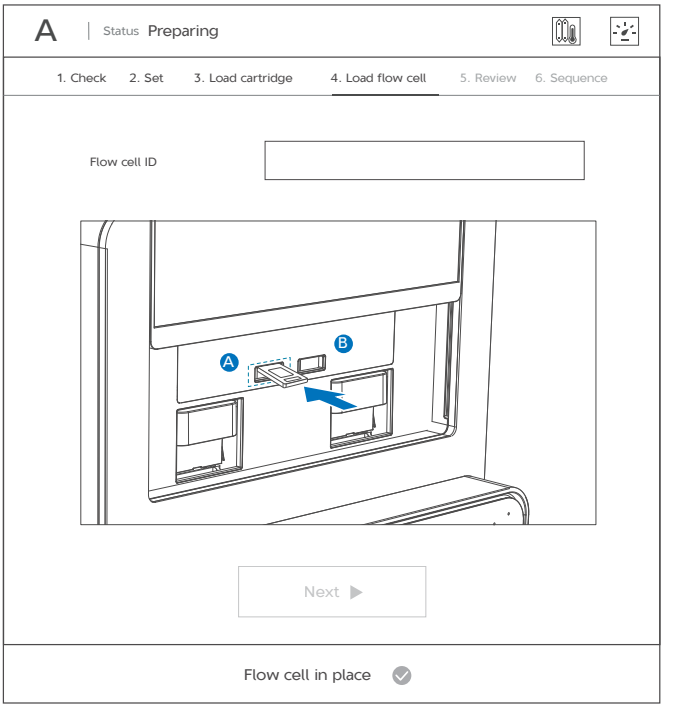

Figure 25 Loading flow cell

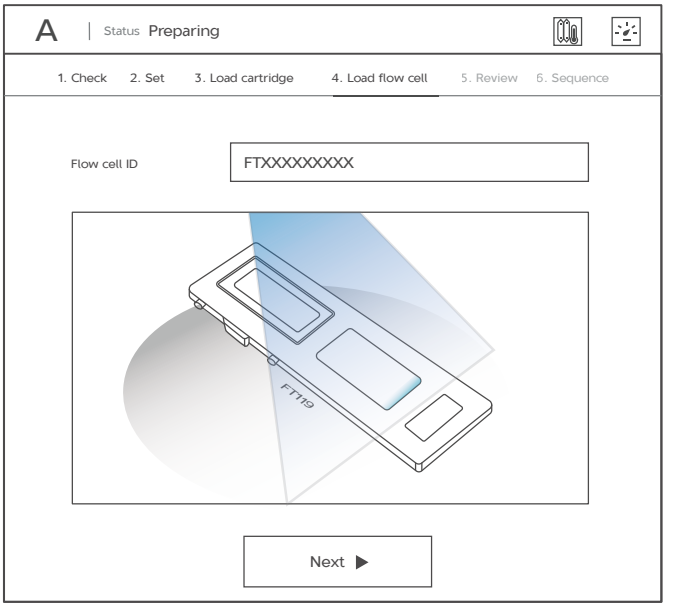

Figure 26 Scanning flow cell ID

2. Select **Next**.

Tips
- If the flow cell is not attached properly, use a canned air duster to blow the dust off the flow cell stage and the back of the flow cell. If there are crystals on the surface of the stage, wipe it gently with a damp Kimwipes tissue and then let it air-dry to ensure that the flow cell can be firmly attached to the stage.
- Do not move the flow cell after it has been loaded. Otherwise, it may cause misalignment between the flow cell inlet and outlet and the gasket.

7.6 Reviewing parameters

Review the parameters and ensure that all information is correct. If not, select to modify the information.

Tips The workflow type cannot be modified.

To ensure sequencing quality, when sequencing of Read1 and Read2 is completed, the sequencer will automatically perform another cycle for calibration. For example, for PE150 sequencing, the length of read1 is 150, the length of read2 is 150, and the length of barcode is 10. Adding 1 correction cycle for Read1 and 1 correction cycle for Read2 (barcode does not need to be corrected), the total number of sequencing cycles is 312.

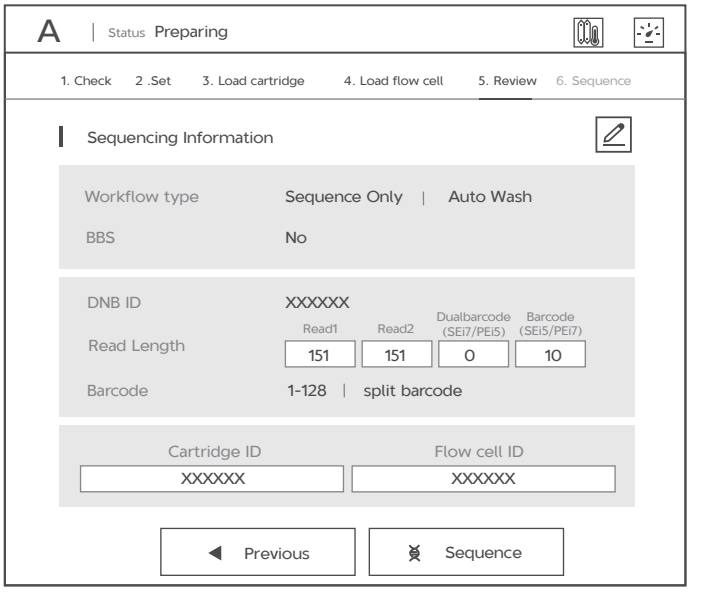

Figure 27 Reviewing information

7.7 Starting sequencing

Perform the steps below:

1. After confirming that the information is correct on *Reviewing parameters on Page 49,* select **Sequence**, and select **Yes** in the pop-up dialog box to start sequencing.

Figure 28 Confirming sequencing interface

2. When sequencing has started, the following interface will appear.

Tips

- Do not bump, move, vibrate, or impact the device during sequencing, as it may cause inaccurate sequencing results.
- Pay special attention to the LED status bar or the on-screen instructions. If errors occur, troubleshoot the problem by following the instructions and this guide. If errors persist, contact Technical Support.

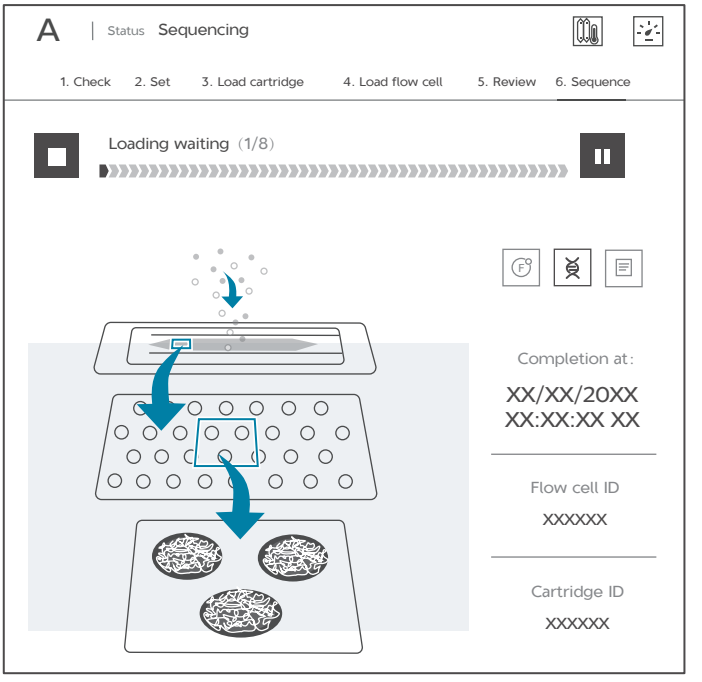

Figure 29 Sequencing interface

While real-time sequencing progress appears in the Sequencing interface, you can still operate the device if needed.

3. After sequencing finished, refer to *Performing a manual wash (~20 min) on Page 54* to perform step 1 to step 3.

7.8 Data access

For detailed information, please refer to *DNBSEQ-G99 Series Genetic Sequencer Software Guide*. After clicking to start sequencing, the sequencing results generated by the control software will appear in D drive.

1. The data folder named after the flow cell ID, mainly contains pictures and data generated during the instrument operation (such as metrics).
2. The Result folder named after the flow cell ID, mainly contains Bioinfo file and FASTQ file.

Chapter 8 Device Maintenance

8.1 Wash instruction

Two wash types are available, depending on sequencer conditions:

Table 41 Wash types

Wash type	Cartridge type	Process time (minutes)	Description
Automatic wash	Sequencing Reagent Cartridge	26	If **Auto wash** is selected in advanced settings before sequencing starts, the system will automatically perform the wash after each sequencing.
Manual wash	Sequencing Reagent Cartridge	20	If **Auto Wash** is unselected in advanced settings before sequencing starts or if sequencing fails, perform a manual wash within 12 hours after sequencing.
Deep wash	Washing cartridge	30	• If the sequencer is to be idle or powered off for more than 7 days, perform an automatic wash and deep wash before powering off and after powering on. • Under normal use, perform a deep wash every month. Tips Normal use means that the sequencing interval of each flow cell stage is less than 7 days, and sequencing and automatic wash are performed smoothly each time.

8.2 Preparing washing reagents

Prepare the washing reagents according to information in the table below:

Table 42 Washing reagent: 0.1M NaOH

Reagent name	Volume (mL)	Final concentration
2 M NaOH	50	0.1 M
Laboratory-grade water	950	N/A
Total volume	1000	
Shelf life	1 month at 4℃	

8.3 Preparing washing cartridge

Tips Washing cartridge for manual wash can be ordered as needed (DNBSEQ-G99 Cleaning Reagent Kit. Catalog No. 940-000624-00).

- Pierce the seals of MDA well and NaOH well using a 1 mL sterile tip.

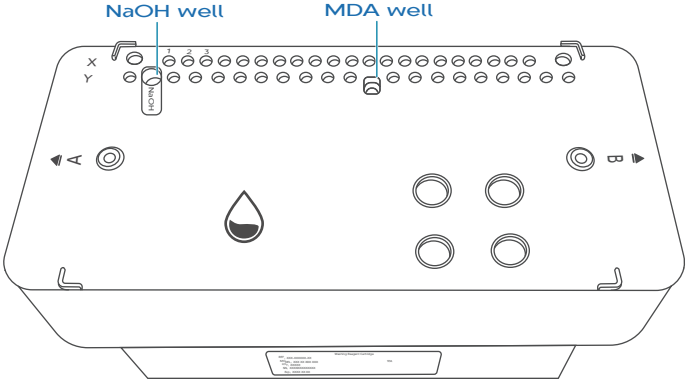

Figure 30 Position of MDA and NaOH wells

- Fill the washing cartridge with washing reagent according to the information in the following table below:

Table 43 Reagents to be added to washing cartridge

Well position	Washing reagent	Volume (mL)
NaOH well	Washing reagent: 0.1 M NaOH	7.5

8.4 Performing a wash

8.4.1 Sequencer automatic wash (~26 min)

If **Auto wash** is selected in setting sequence parameters, the sequencer will perform an automatic wash after sequencing is completed.

8.4.2 Performing a manual wash (~20 min)

Perform the following steps:

1. Select **Finish** after sequencing is completed.

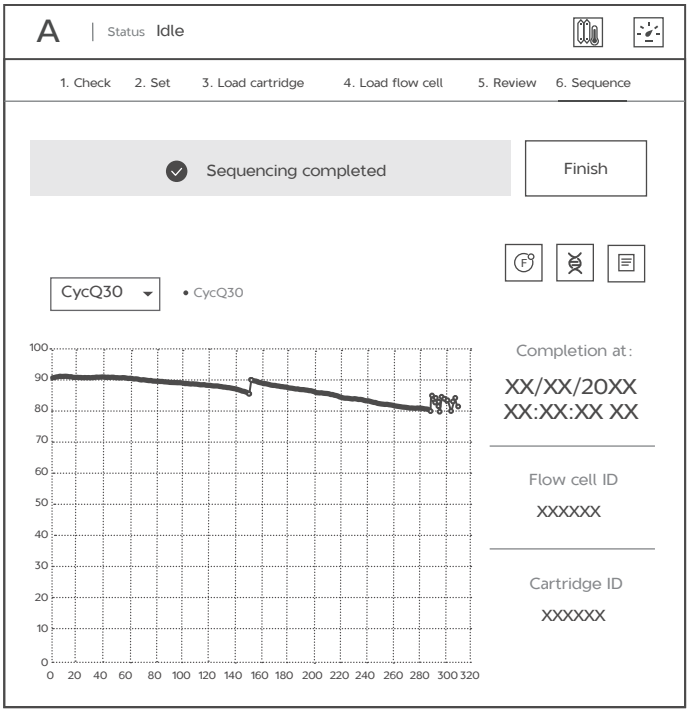

Figure 31 Sequencing completed interface

2. Remove the flow cell and Sequencing Reagent Cartridge after the auto-sliding screen moves up. Pour out the waste and clean the waste container, and then put the waste container into the waste compartment.

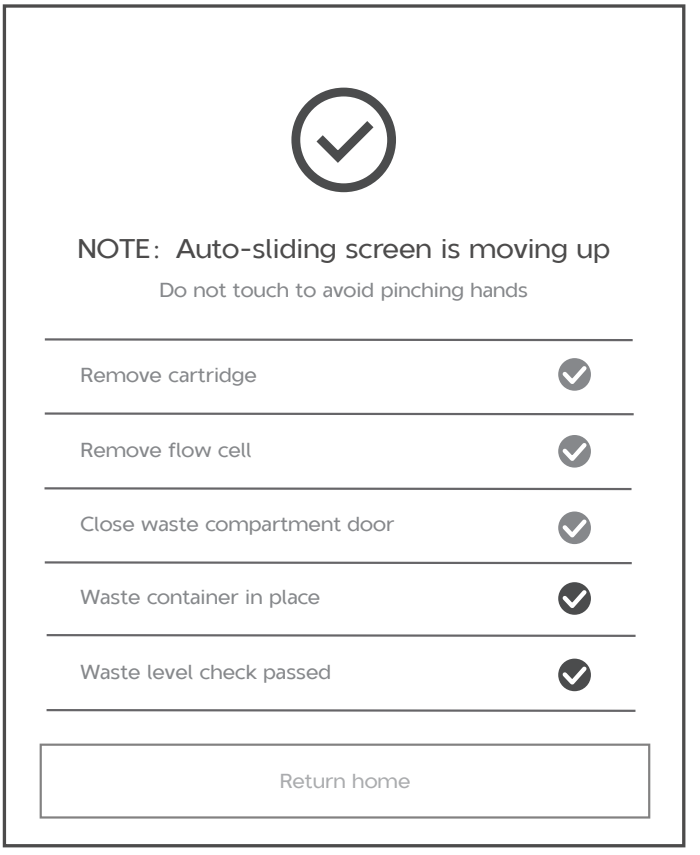

Figure 32 Return home interface

3. Select **Return home** after all items are completed.

4. Select  .

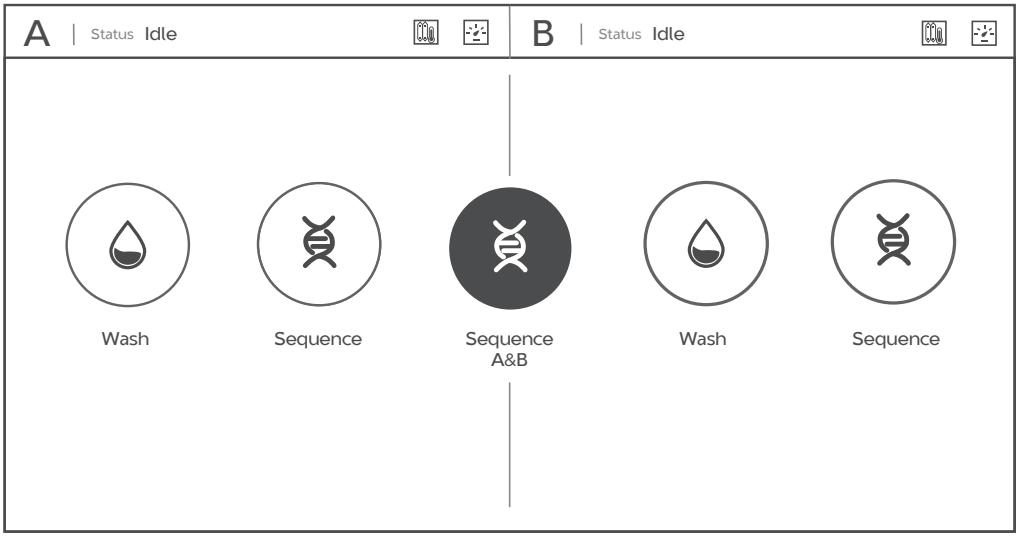

Figure 33 Main interface

5. Placing the sequencing reagent cartridge without doing automatic wash.

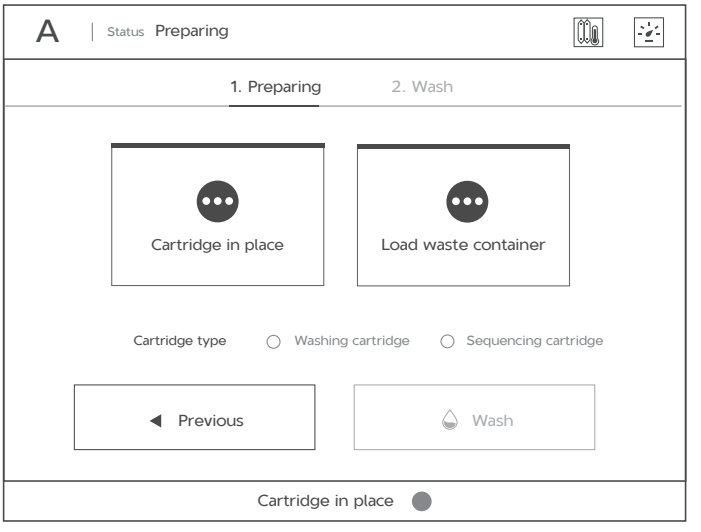

Figure 34 Placing the sequencing reagent cartridge

6. (Optional) Check the waste container.

Tips
- If the waste container is in place and the waste level is under the limit, skip this step.
- If the waste container is not in place or the waste level is over the limit, the waste container door will automatically pop open. Pour out the waste and clean the waste container. Then put the waste container into the waste compartment and close the waste compartment door.

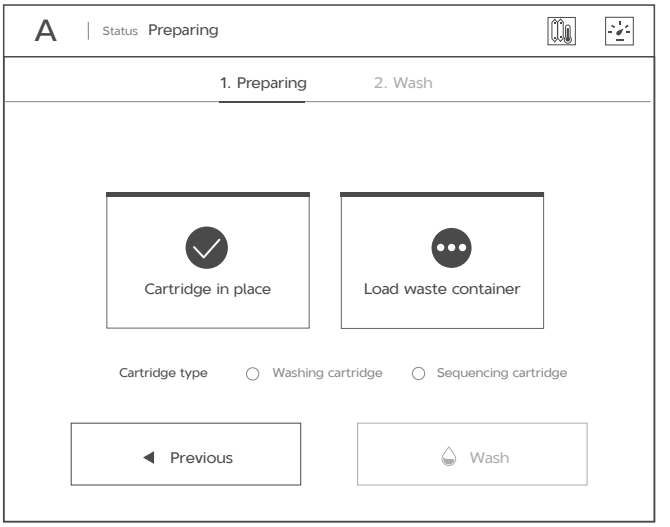

Figure 35 Check waste container

7. Select **Wash**.

Tips Select the sequencing cartridge type manually if the system fails to identify the type of cartridge.

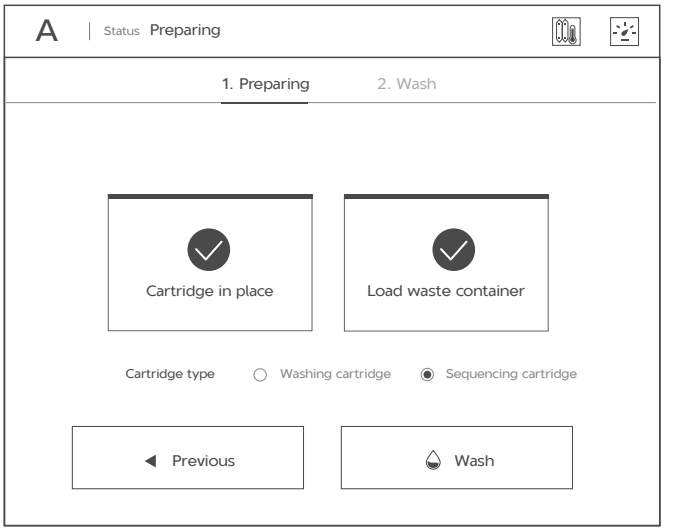

Figure 36 Check completed

8. Select **Yes** to start washing.

Figure 37 Confirming washing interface

9. Select **Finish** after washing is completed.

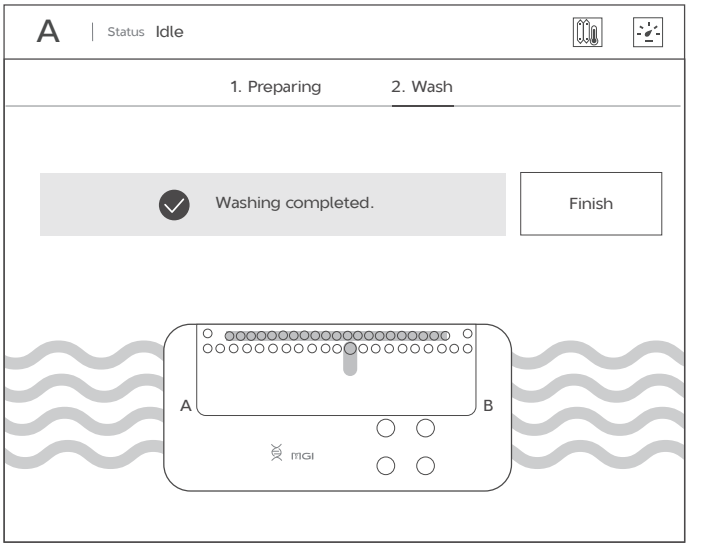

Figure 38 Washing completed interface

10. Remove the sequencing reagent cartridge. Pour out the waste and clean the waste container, and then put the waste container into the waste compartment.

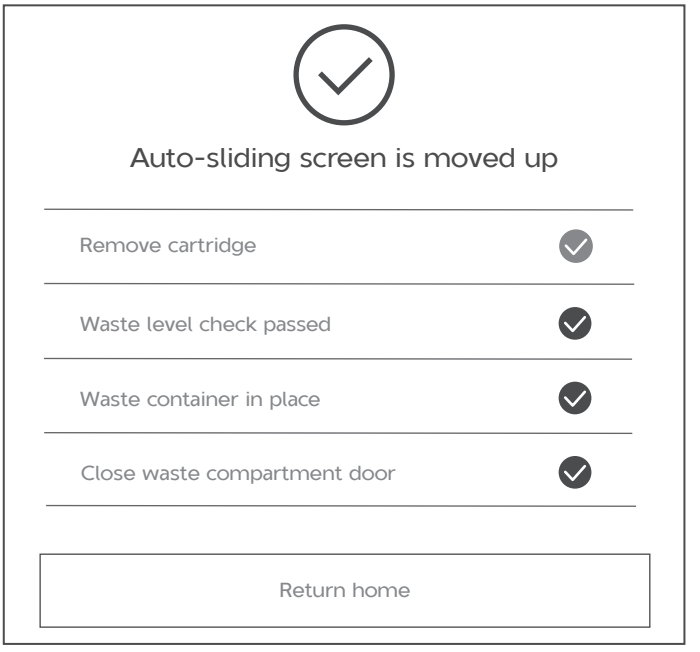

Figure 39 Removing the sequencing reagent cartridge

11. Select **Return home**.

8.4.3 Performing a deep wash (~30 min)

Perform the following steps using washing cartridge:

1. Select .

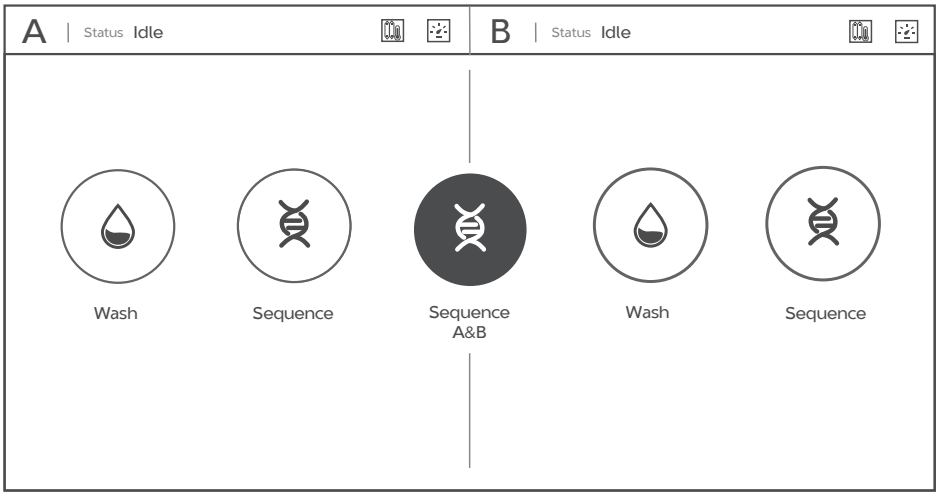

Figure 40 Main interface

2. Placing washing cartridge.

Tips
- A used sequencing reagent cartridge without doing automatic wash can be used for manual wash.
- The system will automatically identify the type of Cartridge for manual wash.

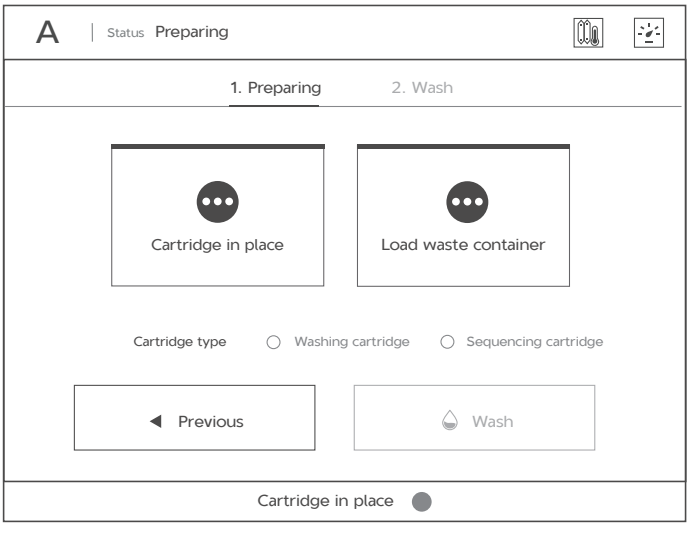

Figure 41 Placing washing cartridge

3. (Optional) Check the waste container.

   Tips
   - If the waste container is in place and the waste level is under the limit, skip this step.
   - If the waste container is not in place or the waste level is over the limit, the waste container door will automatically pop open. Pour out the waste and clean the waste container. Then put the waste container into the waste compartment and close the waste compartment door.

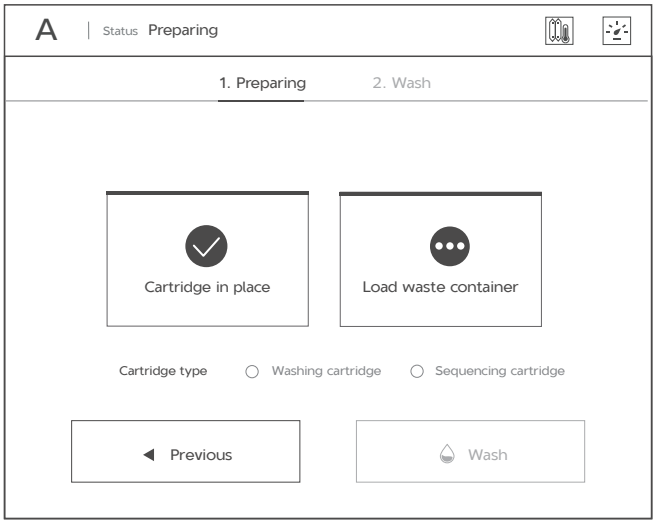

Figure 42 Check waste container

4. Select **Wash**.

   Tips Select the washing cartridge type manually if the system fails to identify the type of cartridge.

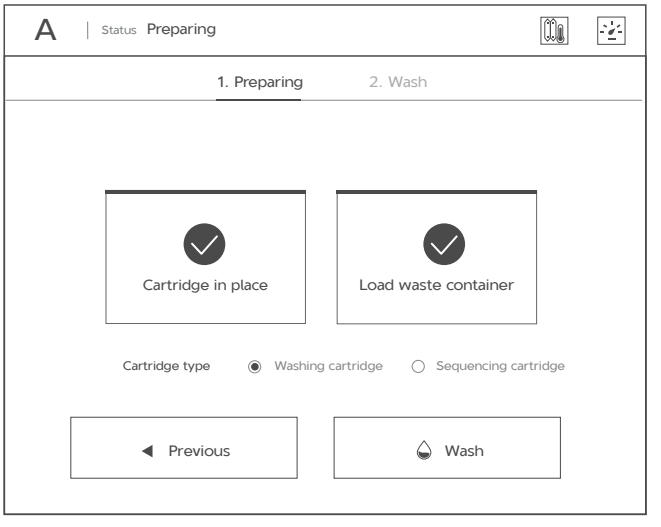

Figure 43 Check completed

5. Select **Yes** to start washing.

Figure 44 Confirming washing interface

6. Select **Finish** after washing is completed.

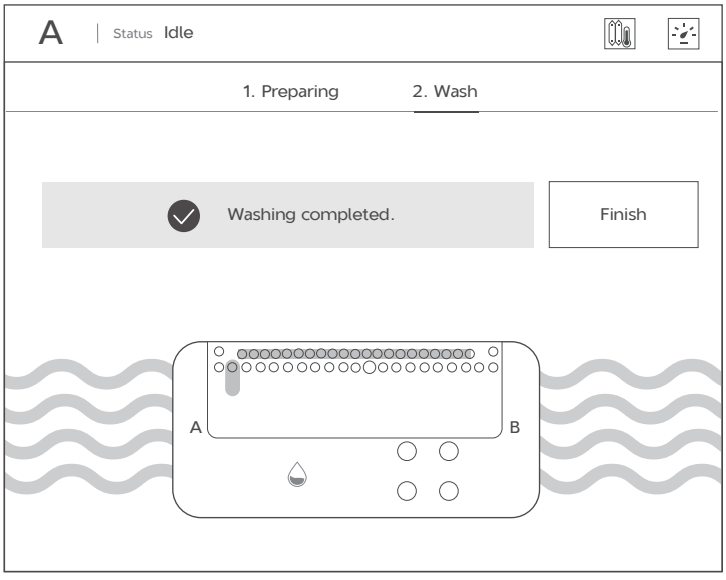

Figure 45 Washing completed interface

7. Remove the washing cartridge. Pour out the waste and clean the waste container, and then put the waste container into the waste compartment.

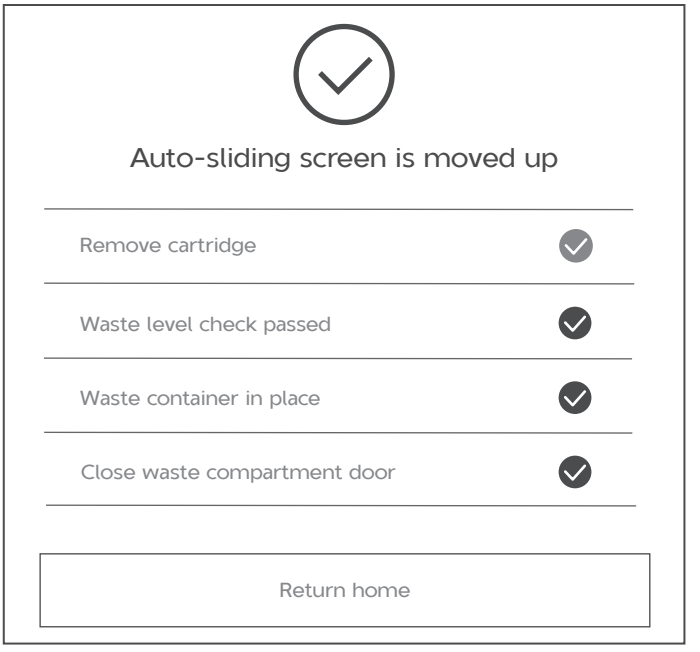

Figure 46 Removing washing cartridge

8. Select **Return home**.

Chapter 9 Troubleshooting

9.1 Low DNB concentration

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

- Check if the kit has expired.
- Check if the library meets the requirements.
- Make a new DNB preparation. You can order DNBSEQ DNB Make Reagent Kit (Catalog No. 1000016115) to make new DNBs. If DNB concentration still does not meet the requirements after a new sample preparation, please contact technical support.

Tips This DNB Make Reagent Kit is only suitable for G99 SM FCL SE100/PE50 and G99 SM FCL PE150 sequencing.

9.2 For PE sequencing run, forgot to add reagent into MDA well

MDA Enzyme is required to make the second strand template for PE sequencing. When preparing the Sequencing Reagent Cartridge, the appropriate amounts of MDA Enzyme Mix and MDA Reagent need be added to MDA well. If you forgot to add the reagent into MDA well when performing the sequencing run, this can be resolved by performing the following steps, as long as the sequencing run is at the read1 sequencing phase.

Perform the following steps:

1. Stop the run: select ■ at any sequencing cycle within read1, and select **Yes** when you are prompted as shown below:

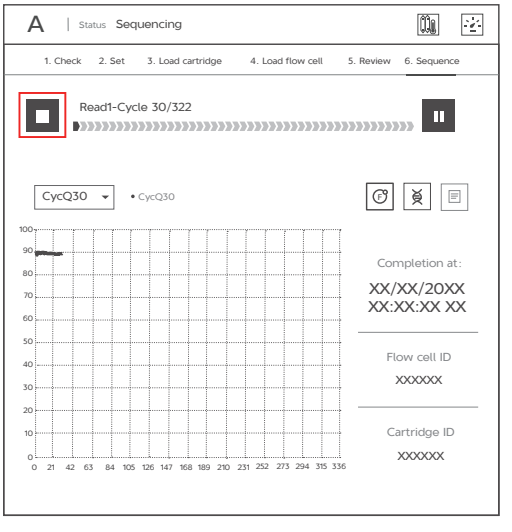

Figure 47 Selecting the sequencing stage to stop

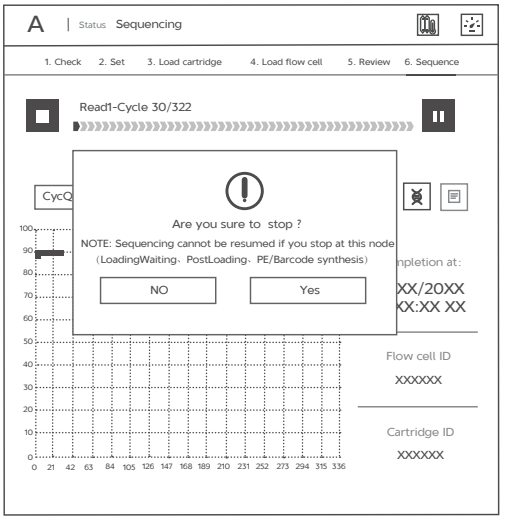

Figure 48 Confirming to stop the run

2. Remove the Sequencing Reagent Cartridge and Flow Cell: Select **Finish**. When the sequencing run is stopped, remove the Sequencing Reagent Cartridge and Flow Cell after the reagent compartment door slides up. Finally, select **Return home** as shown below:

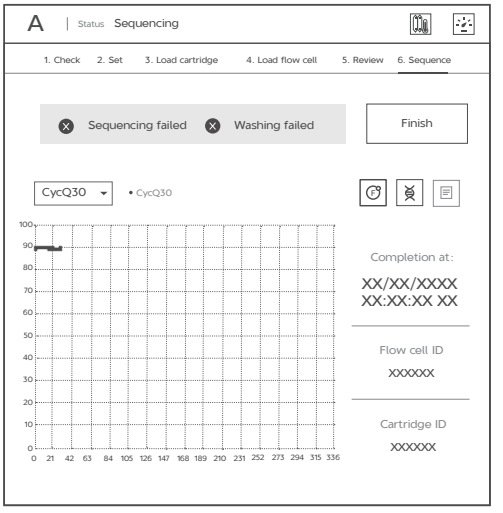

Figure 49 Selecting Finish

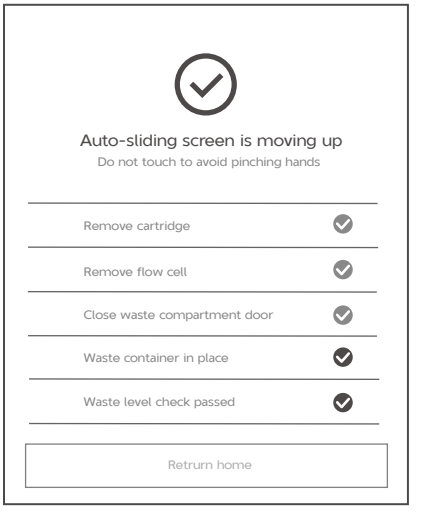

Figure 50 Removing Sequencing Reagent Cartridge and flow cell

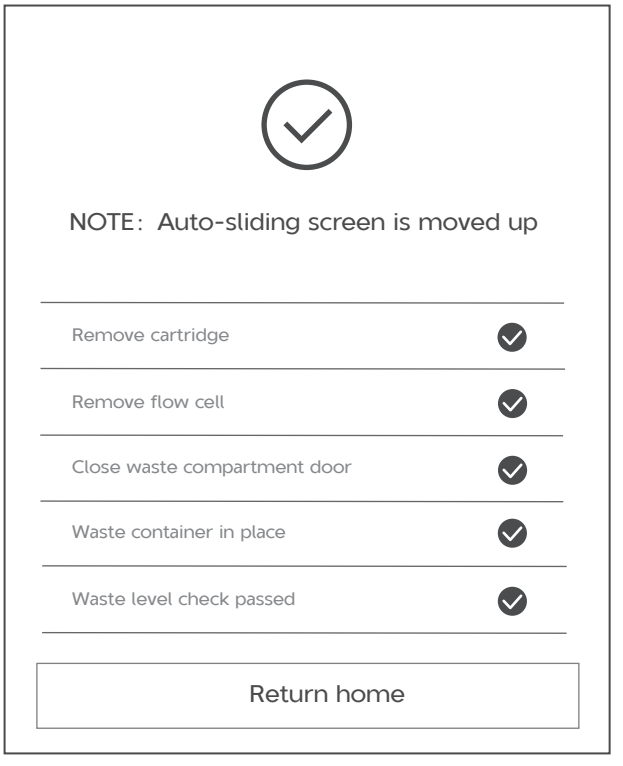

Figure 51 Selecting Return home

3. Add MDA mixture to the Sequencing Reagent Cartridge: Add 125 µL of MDA Enzyme Mix to the MDA Reagent tube with a 200 µL pipette. Mix well and transfer all mixture into MDA well.

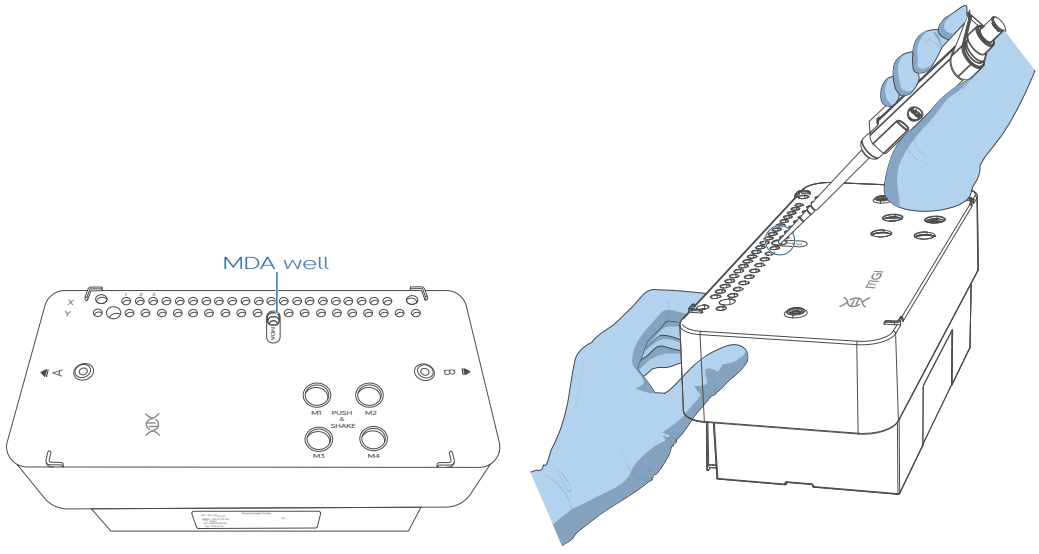

Figure 52 Adding MDA mixture

4. Check before resume sequence: select , then select and the system will perform checking before resume sequence. Select **Next** after the check has completed.

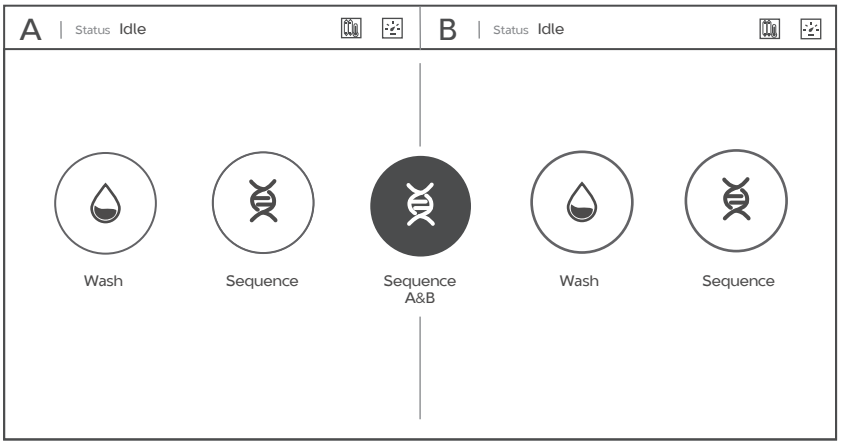

Figure 53 Main interface

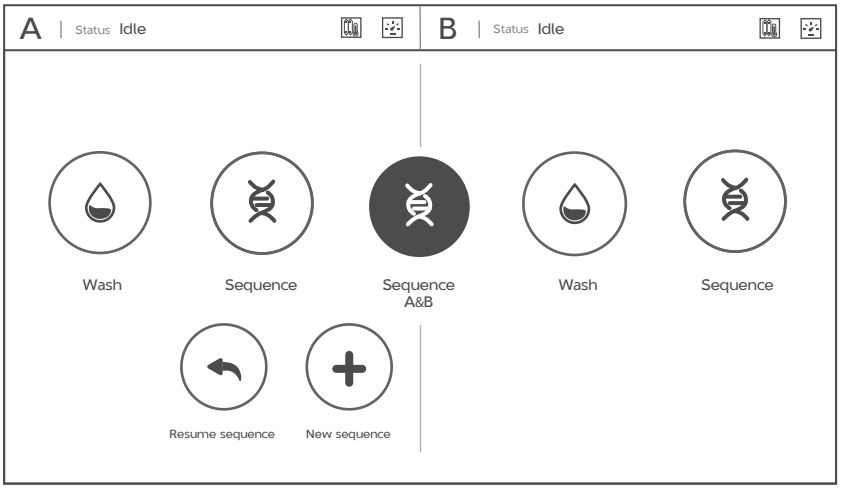

Figure 54 Resume sequence interface

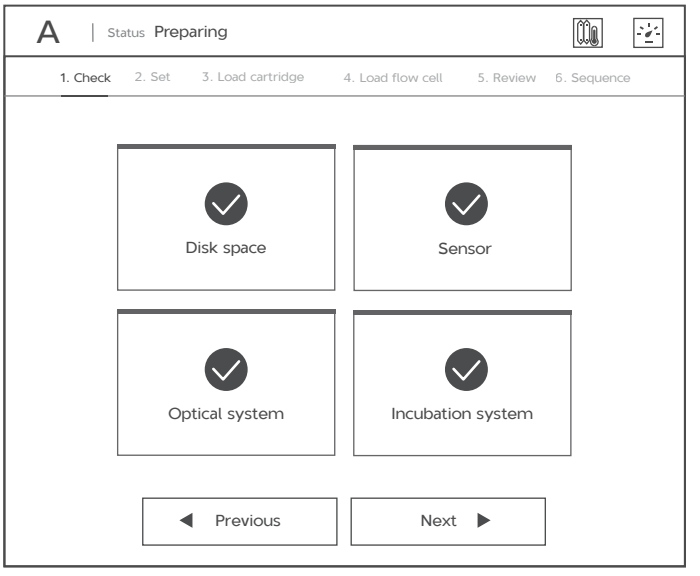

Figure 55 Checking completed

5. Resume sequence: Put the Sequencing Reagent Cartridge back to the sequencer and select **Prime** to perform priming. After priming is completed, insert the Flow Cell and select **Next** and confirm that all information is correct, select **Sequence** to resume the sequencing run as shown below:

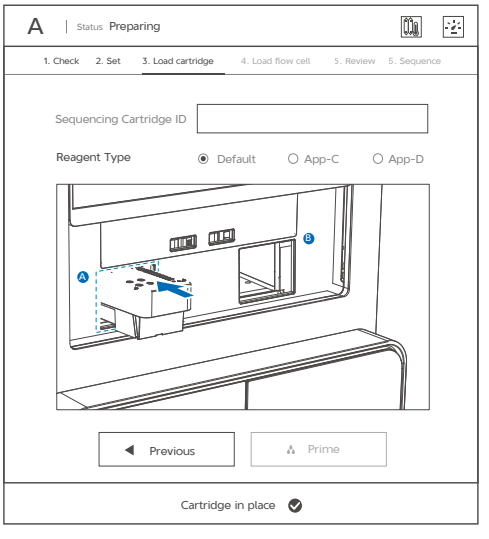

Figure 56 Placing cartridge

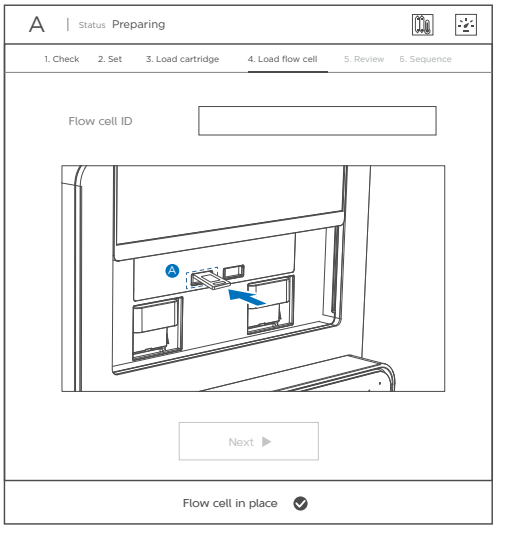

Figure 57 Placing flow cell

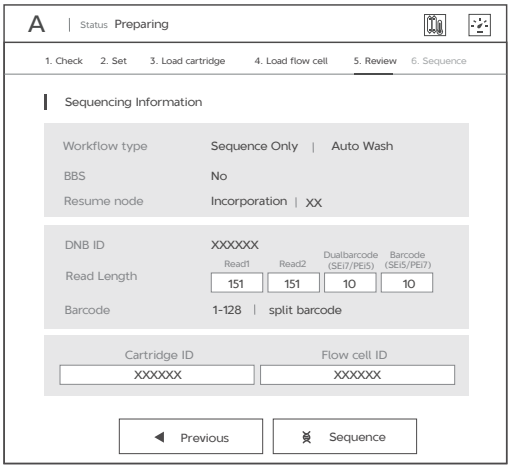

Figure 58 Confirming information

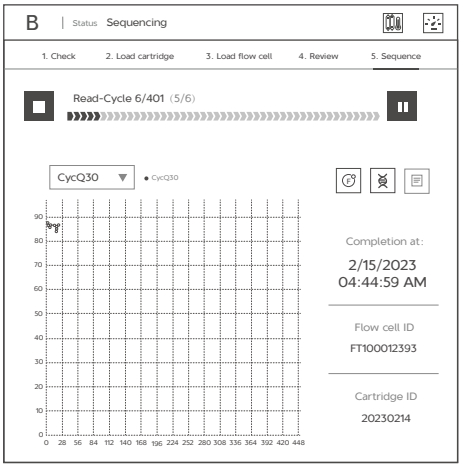

Figure 59 Starting resuming sequencing

9.3 Resume a stopped sequencing run

If you want to resume a stopped sequencing run, only the run that was stopped during read1, read2 or barcode sequencing phase can be resumed.

Tips A sequencing reagent cartridge can only be resumed twice.

Perform the following steps:

1. Stop the run: select ■ at any sequencing cycle within read1, and select **Yes** when you are prompted as shown below:

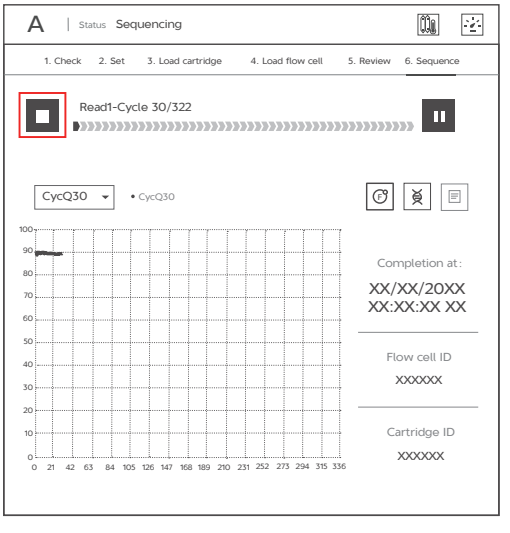

Figure 60 Selecting the sequencing stage to stop

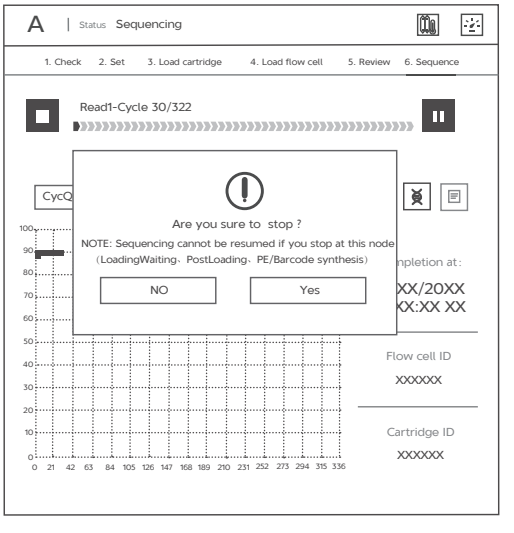

Figure 61 Confirming to stop the run

2. Remove the Sequencing Reagent Cartridge and Flow Cell: Select **Finish**. When the sequencing run is stopped, remove the Sequencing Reagent Cartridge and Flow Cell after the reagent compartment door slides up. Finally, select **Return home** as shown below:

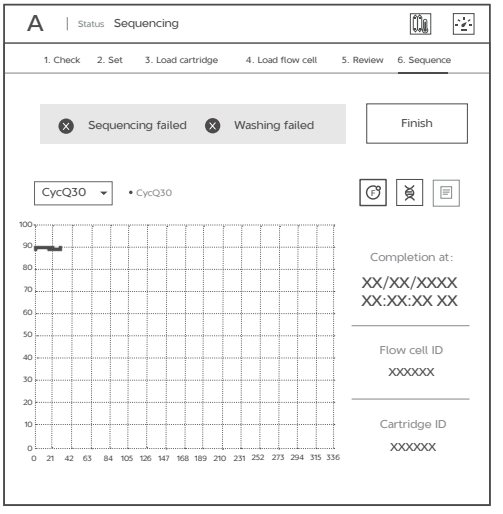

Figure 62 Selecting Finish

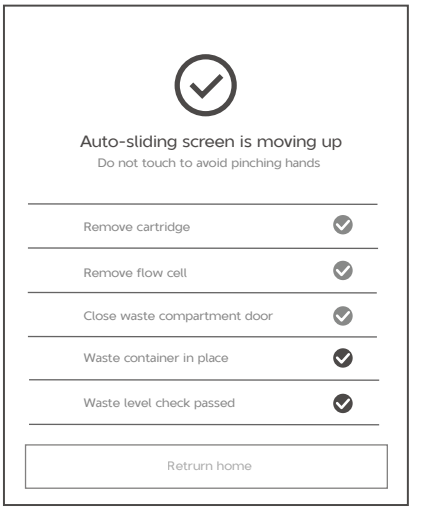

Figure 63 Removing Sequencing Reagent Cartridge and flow cell

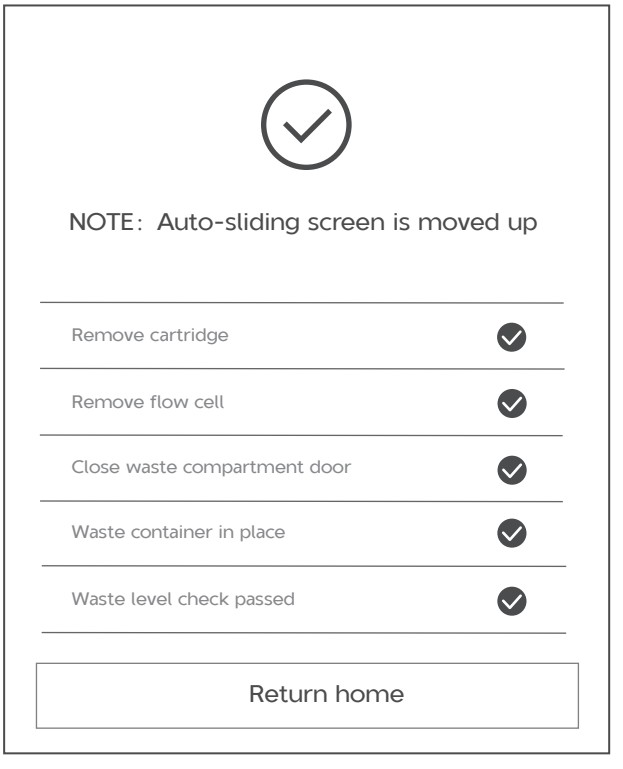

Figure 64 Selecting Return home

3. Check before resume sequence: select , then select and the system will perform checking before resume sequence. Select **Next** after the check has completed.

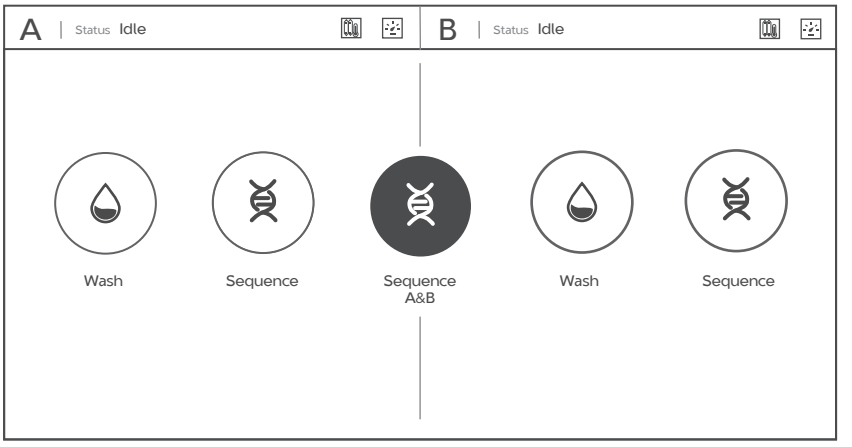

Figure 65 Main interface

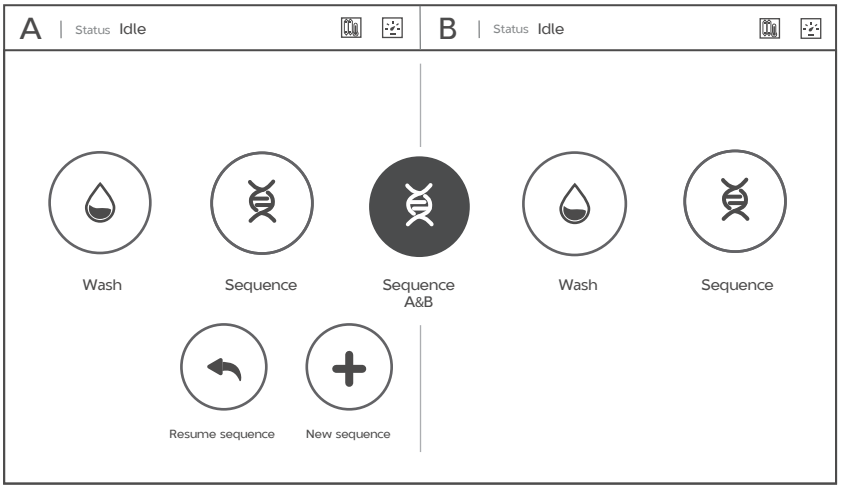

Figure 66 Resume sequence interface

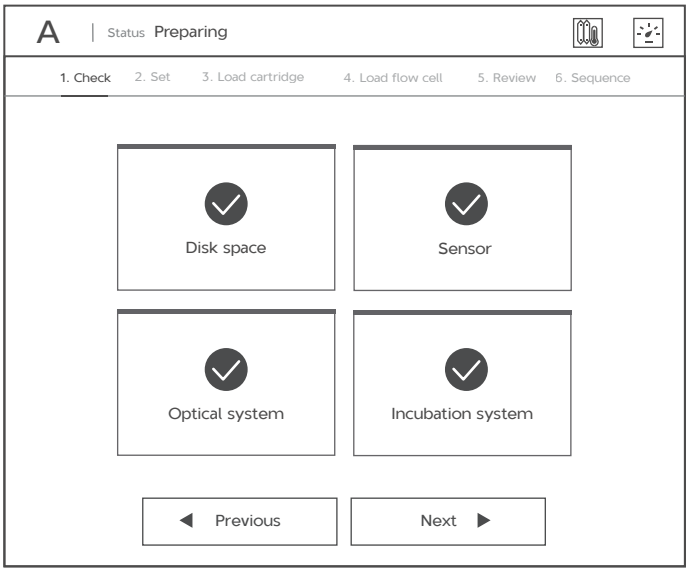

Figure 67 Checking completed

4. Resume sequence: Put the Sequencing Reagent Cartridge back to the sequencer and select **Prime** to perform priming. After priming is completed, insert the Flow Cell and select **Next** and confirm that all information is correct, select **Sequence** to resume the sequencing run as shown below:

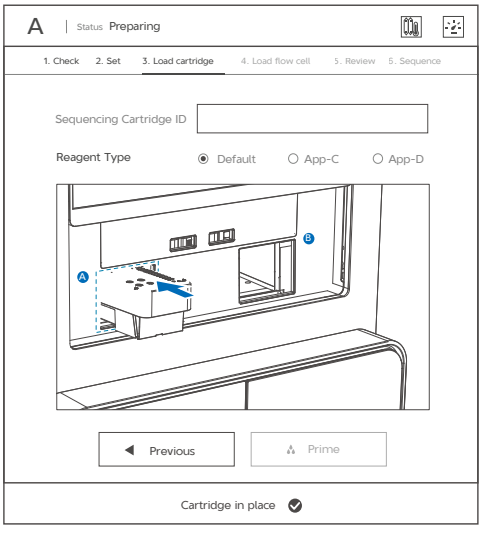

Figure 68 Placing cartridge

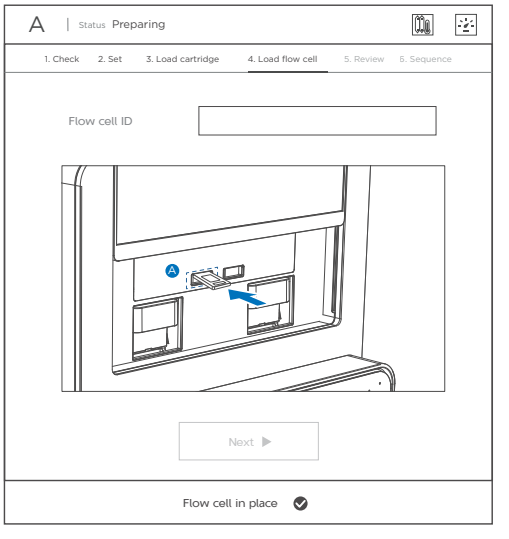

Figure 69 Placing flow cell

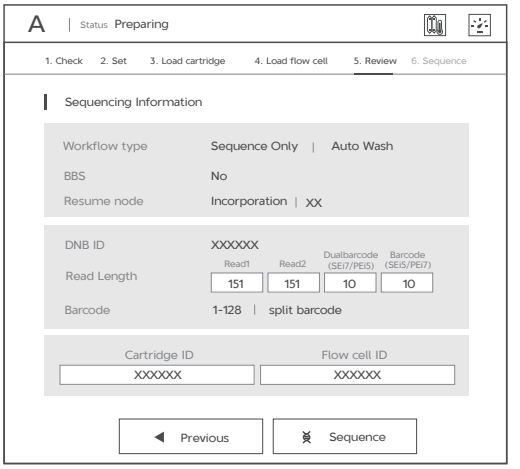

Figure 70 Confirming information

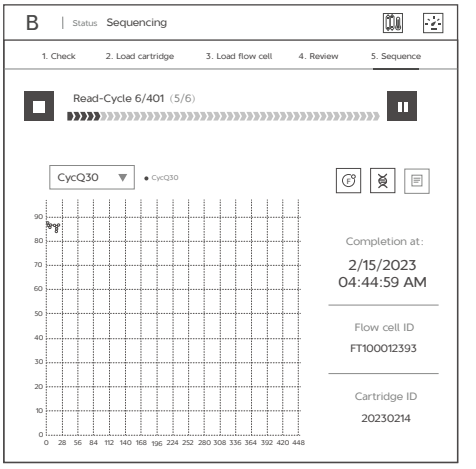

Figure 71 Starting resuming sequencing

9.4 Reagent kit storage rules

- If a cartridge has been thawed without pressing M1, M2, M3, M4 wells and cannot be used within 24 hours, the cartridge can be frozen and thawed at most one additional time. Or, store the cartridge at 4 ℃, and use it within 24 hours. Mix the reagents in the cartridge following the instructions in *Preparing the sequencing reagent cartridge on Page 31* before use.
- If the reagents of M1, M2 and M3, M4 have been added into the cartridge (the cartridge has been prepared but cannot be used immediately), store it at 4 ℃ and use it within 24 hours. Mix the reagents in the cartridge following instruction in *Preparing the sequencing reagent cartridge on Page 31* before use.

9.5 An error occurs before washing

Perform the following steps:

1. If an error message occurs after selecting wash, select **Confirm**.

Figure 72 Error message

2. Select , if the following alarm appears, perform the step 3.

Figure 73 Error alarm

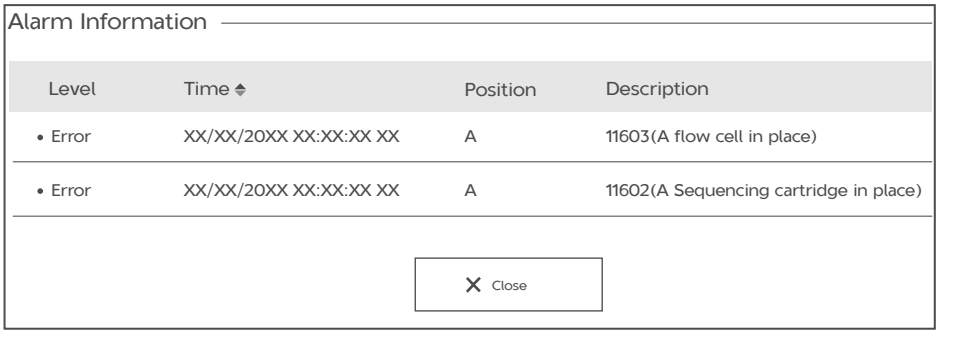

Figure 74 Alarm information

3. Select **Close** to close alarm information. Select > **Maintenance** > **Tools**. Select **Auto-sliding screen** and **Screen Up**, then remove the Sequencing Reagent Cartridge and Flow Cell.

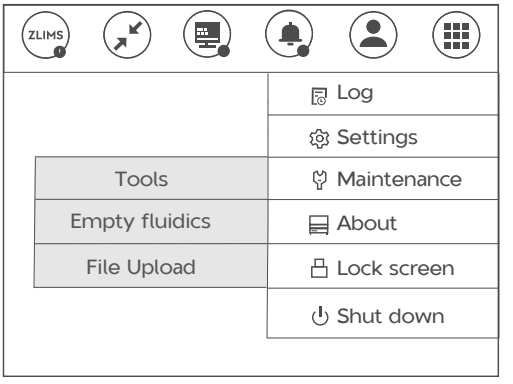

Figure 75 Maintenance menu

Figure 76 Maintenance operation interface

4. Select **Close**.

9.6 Abnormal negative pressure appears during flow cell attachment

When the negative pressure appears in yellow, the negative pressure is abnormal, try the steps below:

1. Gently wipe the stage surface of flow cell stage with a damp Kimwipes tissue or a low-lint cloth and blow the stage using a canned air duster and ensure that no dust is present.

2. Blow the back of the flow cell using a canned air duster to ensure no dust is present.
3. If the problem persists, contact Technical Support.

9.7 Impurities appear in the original sequencing image

If impurities appear, try the steps below:

1. Moisten a Kimwipes tissue with 75% ethanol and use it to wipe the flow cell stage, and perform a deep wash on the sequencer according to *Performing a deep wash (~30 min) on Page 60*.
2. If the problem persists after a full wash, contact Technical Support.

Chapter 10 Important interfaces for customizing a run

This section describes how to customize a sequencing run in the following situations:

- When read length(s) in Read1 and/or Read2 are not the same as those predefined in the **Recipe** list.
- When barcode length(s) in Barcode and/or DualBarcode are not the same as those predefined in the **Recipe** list.
- The recipe you want is not within the predefined recipe list.
- Dark reaction cycles are required in Read1 and/or Read2 sequencing.

10.1 Customize a recipe interface

In the main interface, select **Sequence**. The **Customize** recipe is displayed:

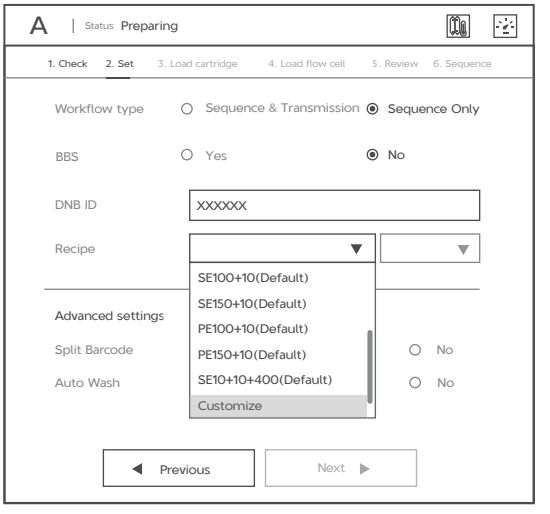

Figure 77 Customize recipe

10.2 Customize interface

After you select **Customize** from the **Recipe** list, the Customize interface is displayed:

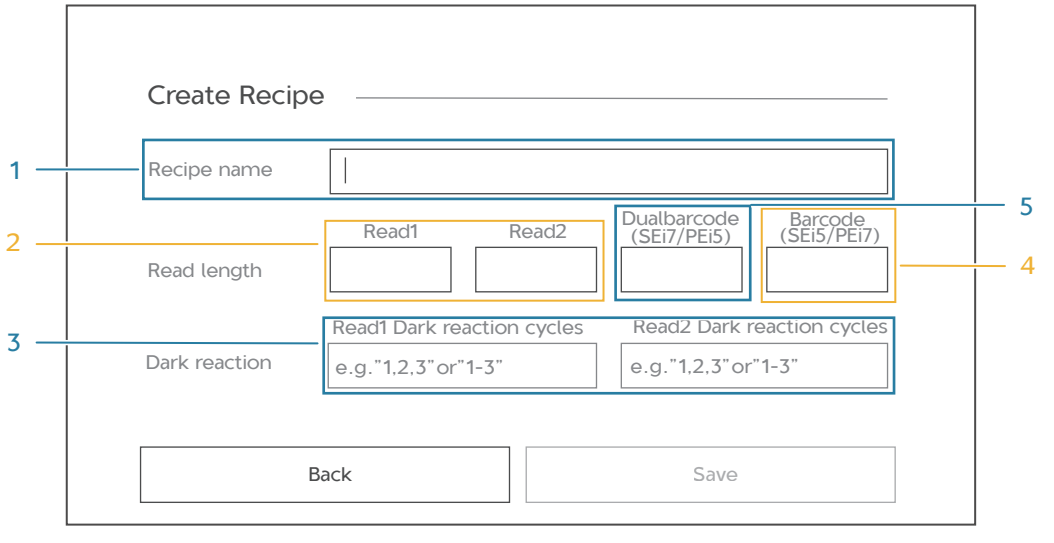

Figure 78 Customize interface

The following table describes the function of buttons and areas in the interface:

Table 44 Parameters introduction of a customize sequencing for MGI adapter library

No.	Item	Description
1	**Recipe name**	Write a name for a sequencing run
2	**Read1/Read2**	Customize read1 and (or) read2 length for a sequencing run
3	**Read1 dark reaction cycle/Read2 dark reaction cycle**	Customize dark reaction range in Read1 and (or) Read2
4	**Barcode**	Customize Barcode length for PE single barcode sequencing or customize Dualbarcode length for SE dualbarcode sequencing.
5	**Dualbarcode**	Customize Dualbarcode length for PE dualbarcode sequencing or customize Barcode length for SE single barcode sequencing.

Table 45 Parameters introduction of a customize sequencing for App adapter library

No.	Item	Description
1	**Recipe name**	Write a name for a sequencing run
2	**Read1/Read2**	Customize read1 and (or) read2 length for a sequencing run
3	**Read1 dark reaction cycle/Read2 dark reaction cycle**	Customize dark reaction range in Read1 and (or) Read2
4	**SEi7/PEi5**	Customize Barcode length of i7 adapter for App library SE sequencing or customize Barcode length of i5 adapter for App library PE sequencing.
5	**SEi5/PEi7**	Customize Barcode length of i5 adapter for App library SE sequencing or customize Barcode length of i7 adapter for App library PE sequencing.

10.3 Barcode (not predefined) interface

If you want to perform sequencing without using a predefined barcodes list, perform the following steps:

1. Select **Others** from the barcode range list next to the first **Recipe** box.

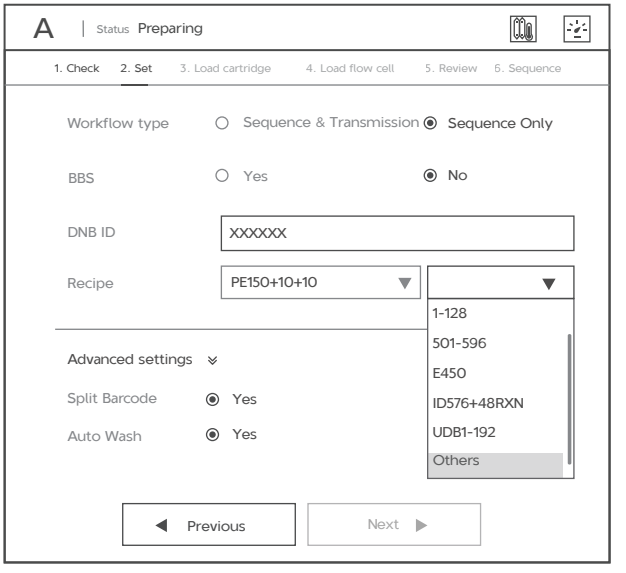

Figure 79 Selecting Others

2. Select next to the **Barcode file**.

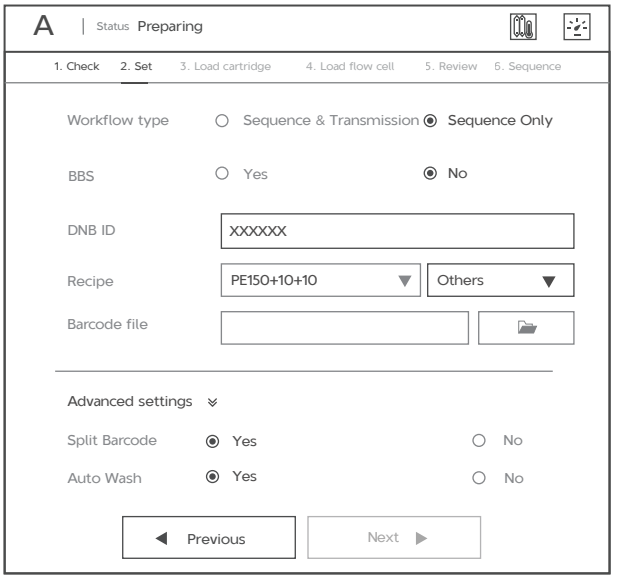

Figure 80 Configuring customize settings

3. Select the barcode file previously imported.

10.4 Examples of customized run

Tips Ensure that you are aware of the following information:

- Before starting the customizing run, confirm that the customized barcode files are already imported into the sequencer.
- Ensure that the total number of sequencing cycles including Read1, Read2, Barcode, Dualbarcode, and Dark Cycle is less than the maximum sequencing cycles for a given sequencing set as defined in *FCL Sequencing time and analysis time for each read length (hours) on Page 2*.
- Dark reaction cycle: A sequencing cycle in which the chemical reaction is performed, but with no imaging. Therefore, the output FASTQ file will not contain the dark cycle information. For example, for FCL PE150 sequencing, if cycle 2-10 for Read1 are dark cycles, the total cycles in the FASTQ file for Read1 is 141.

You can refer to the following setting examples for your customized run.

10.4.1 Read1/Read2 lengths are not the same as those predefined in the Recipe list

Assumptions are as follows:

- Sequencing run: PE120+140+10
- Length of read1: 120
- Length of read2: 140
- Length of barcode: 10
- Length of Dualbarcode: 0
- Split barcode: Yes
- Total cycles = 120+1+140+1+10 = 272
- Select a PE150 set

The Customize interface is set as follows:

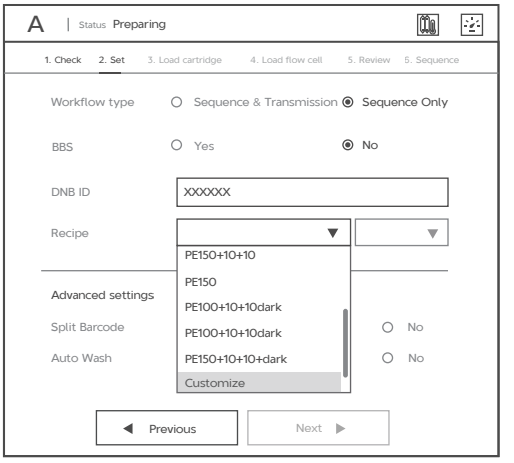

Figure 81 Selecting Customize

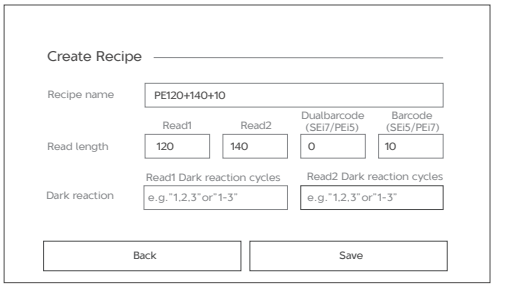

Figure 82 Configuring customize settings for example 1

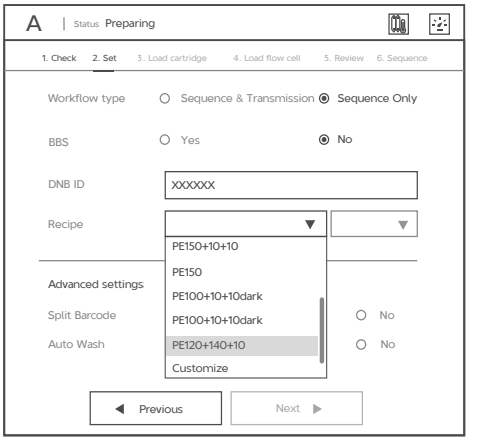

Figure 83 Selecting PE120+140+10

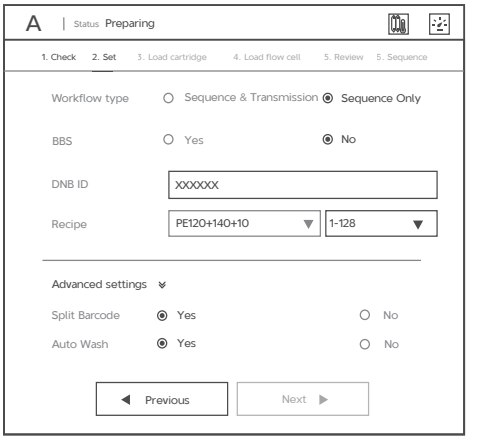

Figure 84 Selecting barcode type and split strategy for example 1

10.4.2 Read1 lengths are not the same as those predefined in the Recipe list

Assumptions are as follows:

- Sequencing run: SE75+10
- Length of read1: 75
- Length of read2: 0
- Length of barcode: 0
- Length of Dualbarcode: 10
- Total cycles = 75+1+10 = 86

- Select a SE100 set

The Customize interface is set as follows:

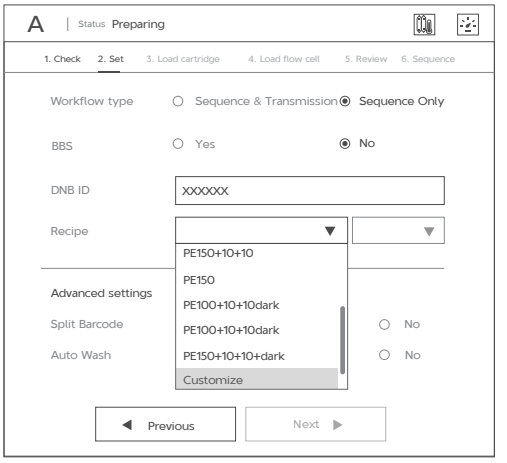

Figure 85 Selecting Customize

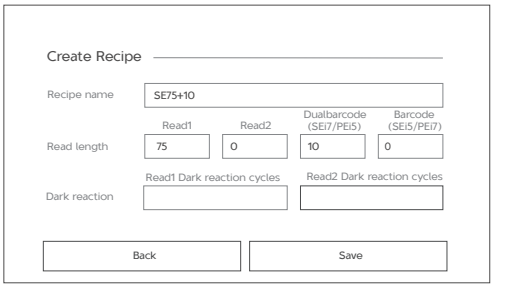

Figure 86 Configuring customize settings for example 4

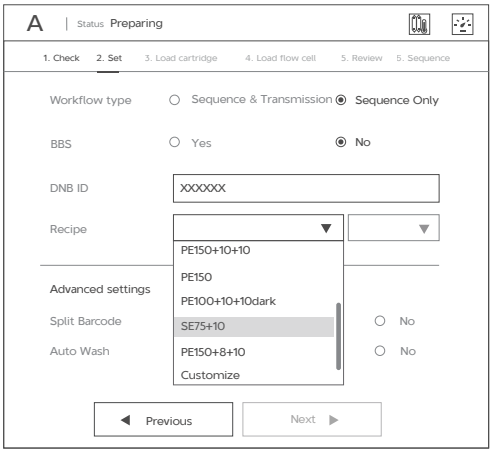

Figure 87 Selecting PE150+8+8DR

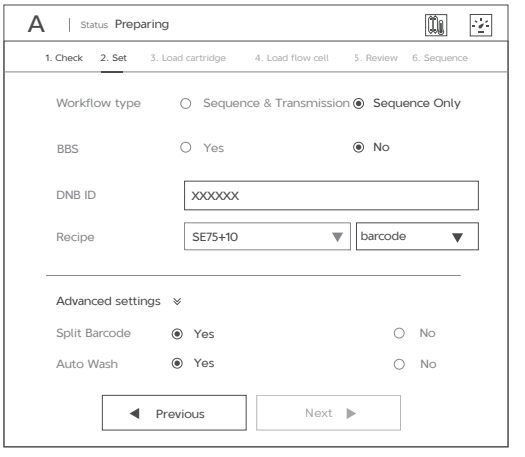

Figure 88 Selecting barcode type and split strategy for example 4

10.4.3 Length of the single barcode is not 10 for PE sequencing

Assumptions are as follows:

- Sequencing run: PE150+8
- Length of read1: 150
- Length of read2: 150
- Length of barcode: 8

- Length of Dualbarcode: 0
- Split barcode: Yes
- Total cycles = 150+1+150+1+8 = 310
- Select a PE150 set

The Customize interface is set as follows:

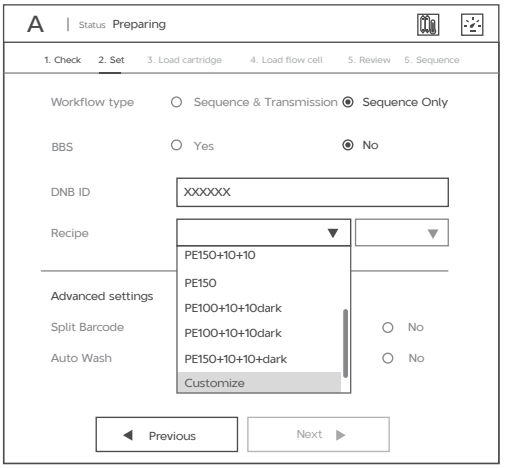

Figure 89 Selecting Customize

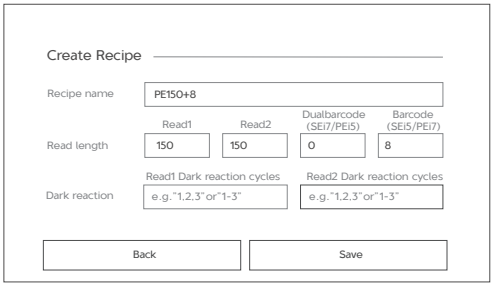

Figure 90 Configuring customize settings for example 2

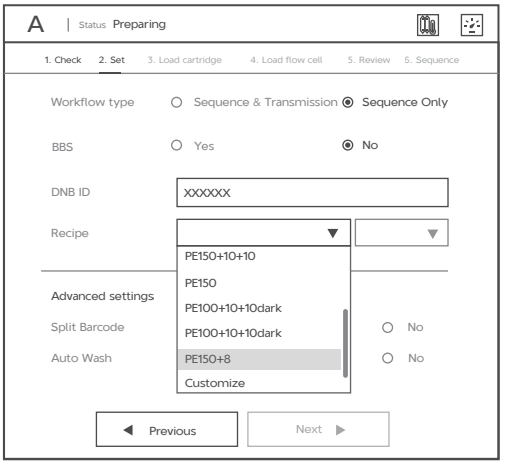

Figure 91 Selecting PE150+8

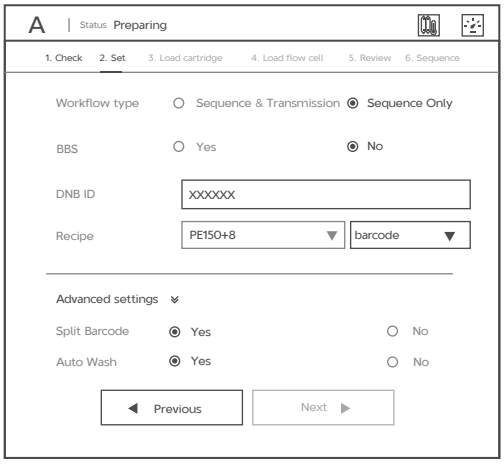

Figure 92 Selecting barcode type and split strategy for example 2

10.4.4 A dual barcode sequencing run

Assumptions are as follows:

- Sequencing run: PE150+8+10
- Length of read1: 150
- Length of read2: 150

- Length of barcode: 10
- Length of Dualbarcode: 8
- Split barcode: Yes
- Total cycles = 150+1+150+1+8+10 = 320
- Select a PE150 set

The Customize interface is set as follows:

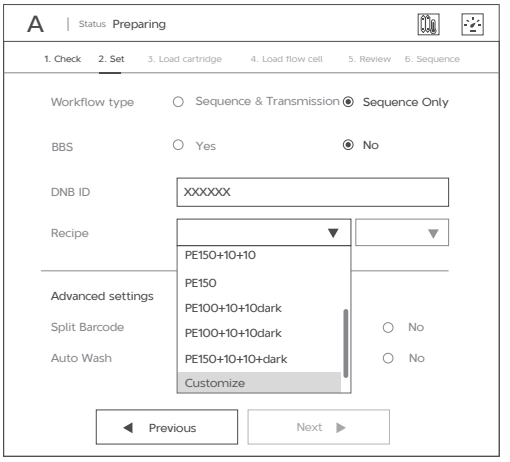

Figure 93 Selecting Customize

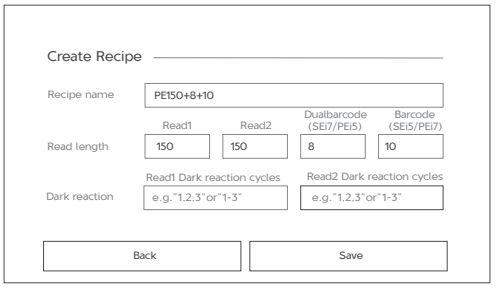

Figure 94 Configuring customize settings for example 3

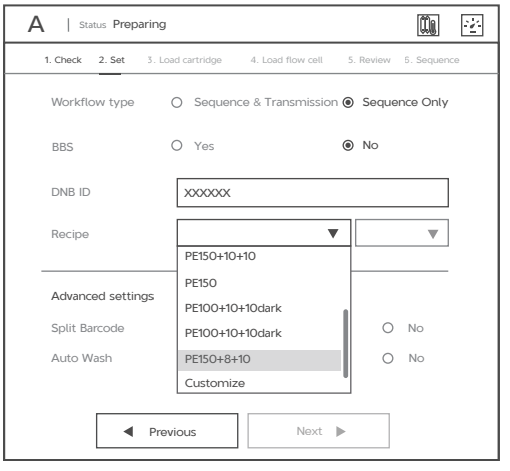

Figure 95 Selecting PE150+8+10

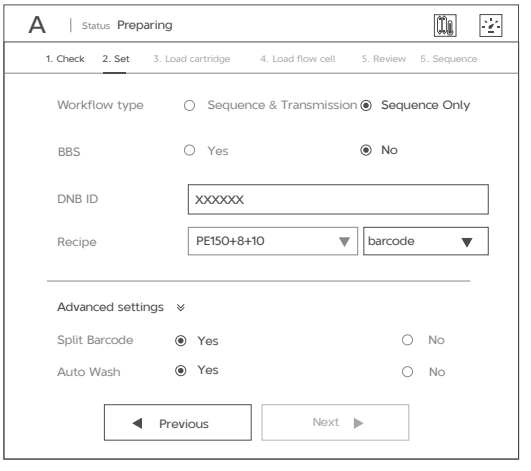

Figure 96 Selecting barcode type and split strategy for example 3

These parameters can be set in both sides. It is recommended that you use identical settings for the sequencing parameters in both sides.

10.4.5 Dark reaction cycles are required in read1 and/or read2 sequencing

Assumptions are as follows:

- Sequencing run: PE150+8+8
- Length of read1: 150
- Length of read2: 150
- Length of barcode: 8
- Length of Dualbarcode: 8
- Dark cycles: From cycle-2 to cycle-10, cycle-22 to cycle-30 in read1 and cycle-16 to cycle-20, cycle-30 to cycle-40 in read2.
- Total cycles = 150+1+150+1+8+8 = 318
- Select a PE150 set

The Customize interface is set as follows:

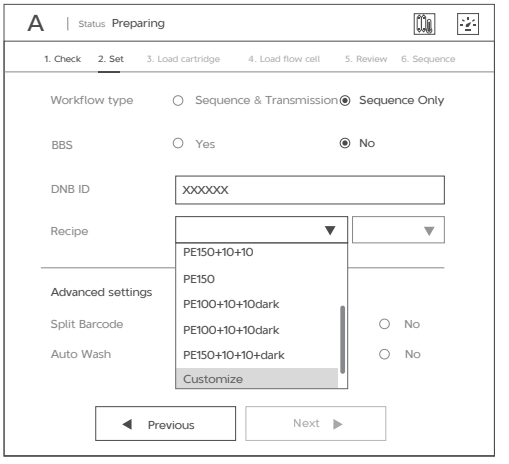

Figure 97 Selecting Customize

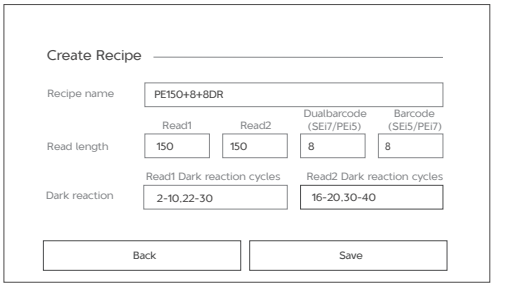

Figure 98 Configuring customize settings for example 4

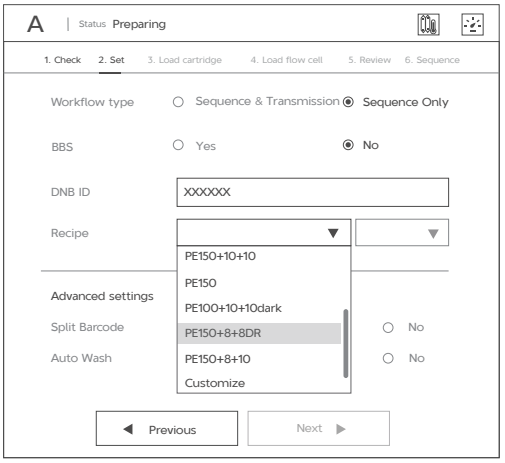

Figure 99 Selecting PE150+8+8DR

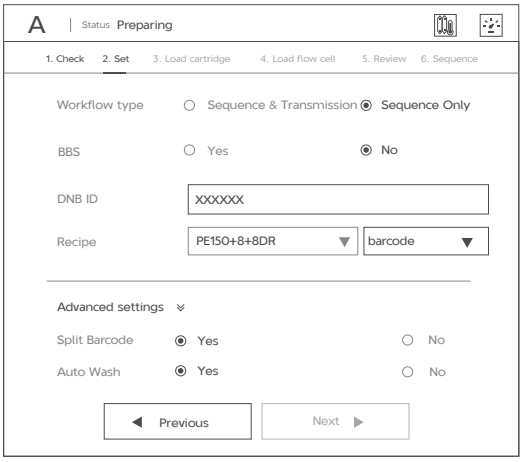

Figure 100 Selecting barcode type and split strategy for example 4

Appendix 1 Instructions for using Qubit to quantify the DNBs

Tips
- Working solution should be used within 30 minutes after preparation.
- Avoid touching the wall of tapered detection tubes.
- No Bubbles in detection tubes.

Perform the steps below:

1. Prepare the Qubit working solution by diluting the Qubit ssDNA Reagent 1:200 in Qubit ssDNA Buffer. Use a clean plastic tube each time you prepare Qubit working solution. Do not mix the working solution in a glass container.

Tips The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit working solution, and each sample tube requires anywhere from 180 - 199 µL.

Prepare sufficient Qubit working solution to accommodate all standards and samples.

For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~ 200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit reagent plus 1990 µL of Qubit Buffer).

2. Add 190 µL of Qubit working solution to each of the tubes used for standards.
3. Add 10 µL of each Qubit standard to the appropriate tube, then mix by vortexing 3 to 5 seconds. Be careful not to create bubbles.
4. Set up the required number of 0.5-mL tubes for standards and samples. The Qubit ssDNA Assay requires 2 standards.

Tips
- Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit assay tubes (Cat. No. Q32856) or Axygen PCR-05-C tubes (Part No. 10011-830).
- Number of Qubit test tubes needed are the number of samples plus 2 standards tubes. For example, if you have 3 samples, you will need 5 tubes.

5. Label the tube lids. Do not label the side of tube.
6. Prepare the solutions used for standards and sample tests according to the table below.

/	S1 (μL)	S2 (μL)	D1 (μL)	D2 (μL)	D3 (μL)
Working solution	190	190	198	198	198
S1 (0 ng/μL)	10	/	/	/	/
S2 (20 ng/μL)	/	10	/	/	/
Sample (μL)	/	/	2	2	2
Total volume	200	200	200	200	200

Label the tube lids.

7. Mix tubes by using a vortex mixer, centrifuge briefly for 5 seconds, then incubate at room temperature for 2 minutes.
8. Proceed instructions in section "Reading standards and samples" of relevant Qubit user guide; follow the procedure appropriate for your instrument.

Appendix 2 Manufacturer

Manufacturer	Wuhan MGI Tech Co., Ltd.
Address	Building B13, No.818, Gaoxin Avenue, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China
	Building 24, Stage 3.1, BioLake Accelerator, No.388, 2nd Gaoxin Road, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China
E-mail	MGI-service@mgi-tech.com
Website	www.mgi-tech.com

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