Part No.: H-020-000494-00

DNBSEQ-T10×4RS

High-throughput Sequencing Set

User Manual

Version: 2.0

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Qingdao MGI Tech Co., Ltd.

About the user manual

This user manual is applicable to DNBSEQ-T10×4RS High-throughput Sequencing Set. The manual version is 2.0 and the set version is V1.0. The main control software version matched in this manual is DNBSEQ-T10×4RS×1.2.0.237.

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Figures in this user manual are all illustrations. The content might be slightly different from the set, please refer to the set purchased.

Revision history

Version	Date	Description
2.0	March 6, 2023	• Update components of the kit • Update the operation of 6.3.1 and 6.3.2 • Update the part of troubleshooting
1.0	May 31, 2022	Initial release

About the reagent set

Cat. No.	Name	Version
940-000078-00	DNBSEQ-T10×4RS High-throughput Sequencing Set (FCL PE100) (Standard Product)	1.0
940-000100-00	DNBSEQ-T10×4RS High-throughput Sequencing Set (FCL PE150) (Standard Product)	1.0

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Chapter 1 Introduction

This user manual describes how to perform sequencing by using DNBSEQ-T10×4RS High-throughput Sequencing Set and includes instructions regarding DNB (DNA Nanoball) preparation, slide preparation, components of sequencing reagent sets, storage conditions and use methods, sequencing protocols, and device maintenance.

Since the device processes a large number of samples, it is recommended to arrange sequencing in advance through LIMS (Laboratory Information Management System), confirm the current number of sequencing samples, and complete DNB making, DNB quantification, DNB pooling, DNB loading, sequencing and other processes.

1.1 Intended use

This product is a universal reagent set for the sequencing of DNA or RNA libraries, and is used with a genetic sequencer (DNBSEQ-T10×4RS) and an automatic 4-channel DNB loader (MGIDL-T20RS) to complete high-throughput sequencing and obtain sample sequence information. This reagent set is for research use only, and cannot be used for clinical diagnosis.

1.2 Sequencing principle

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

1.3 Data analysis

During sequencing, the control software automatically runs the basecall software for analysis, and outputs the sequencing data to the designated location for secondary analysis.

1.4 Sequencing read length

Sequencing read length determines the number of sequencing cycles for a given sequencing run. For example, a PE150 run performs reads of 150 cycles from each end, for a total of 300 cycles. At the end of the sequencing run, an extra 10 cycles of barcode read can be performed, if required. At this time, if the end cycle process mode is enabled, 151 cycles are sequenced in read 1 and read 2, respectively, but only the first 150 cycles of read 1 and read 2 will be written to FASTQ.

Table 1 Sequencing cycles

Sequencing read length	PE100	PE150
Read 1 read length	100	150
Read 2 read length	100	150
Barcode read length	10	10
Total read length	200+10	300+10
Maximum number of cycles	220	320

1.5 Sequencing time

Table 2 Theoretical sequencing time (hours)

Sequencing read length	PE100	PE150
Multi-slide sequencing time	<96	<96
DNB making time	2	2
DNB loading time	2	2

NOTE
- The multi-slide sequencing time refers to the time from the start of sequencing to the completion of sequencing of 8 slides, including sequencing preparation, read 1 sequencing, read 2 synthesis, read 2 sequencing, barcode sequencing, and device maintenance.
- DNB preparation, DNB loading and writing FASTQ do not require a sequencer, so time spent in these steps are not counted in the sequencing time. It takes 8 h to 10 h for a slide to use a single standard node to write FASTQ on a dedicated server.

1.6 Warnings and precautions

- This product is for research use only. Please read the user manual of the product carefully before use.
- Before experiment, be sure to be familiar with and master the operation methods and precautions of various devices to be used.
- Direct contact with skin and eyes should be avoided for all samples and reagents. Do not swallow. Once this happens, immediately rinse with large amounts of water and go to the hospital in time.
- All samples and wastes should be disposed of in accordance with relevant regulations.
- This product is for single use and shall not be reused.
- Do not use expired products.

Chapter 2 Components of the reagent set and user-supplied consumables

2.1 Components of sequencing reagent set

Table 3 DNBSEQ-T10×4RS High-throughput Sequencing Set FCL PE100 (Standard Product) V1.0 (Cat. No.: 940-000078-00)

Component information	Specification	Storage temperature	Transportation temperature
DNBSEQ-T10×4RS sequencing slide Cat. No.: 930-000038-00			
Sequencing slide (T10×4RS FCL)	8 EA	0 to 30 ℃	0 to 30 ℃
DNBSEQ-T10×4RS high-throughput sequencing kit (Package 1) (FCL PE100/PE150) Cat. No.: 1000027925			
Wash Reagent 2	7330 mL/bottle×1	Below 8 ℃	Below 8 ℃
DIPSEQ Sequencing Reagent	7620 mL/bottle×1		
DNBSEQ-T10×4RS high-throughput sequencing kit (Package 2) (FCL PE100) Cat. No.: 1000027926			
DIPSEQ Image Reagent	6200 mL/bottle×2	-25 ℃ to -15 ℃	Below -15 ℃

Component information	Specification	Storage temperature	Transportation temperature
Bottle cap with pipette	4 EA/bag×1	-25 ℃ to -15 ℃	Below -15 ℃
Hose Barb Socket	1 EA/bag×2		
DNBSEQ-T10×4RS high-throughput sequencing kit (Package 3) (FCL PE100/PE150) Cat. No.: 1000027928			
Make DNB Buffer	12.5 mL/tube×1	-25 ℃ to -15 ℃	Below -15 ℃
Stop DNB Reaction Buffer	12.5 mL/tube×1		
DIPSEQ Make DNB Enzyme Mix I	21 mL/tube×1		
DIPSEQ Make DNB Enzyme Mix II	1 mL/tube×1		
DNB Loading Buffer I	12 mL/tube×1		
DNB Loading Buffer II	10 mL/tube×1		
Phi29 DNA polymerase (HC)	240 µL/tube×1		
DNBSEQ-T10×4RS high-throughput sequencing kit (Package 4) (FCL PE100/PE150) Cat. No.: 1000027929			
MDA Enzyme Mix	5 mL/tube×2	-25 ℃ to -15 ℃	Below -15 ℃
Sequencing Enzyme Mix	54 mL/bottle×2		
DIPSEQ dNTPs Mix	54 mL/bottle×1		
dNTPs Mix II	108 mL/bottle×1		
DIPSEQ Regeneration Reagent	2700 mL/bottle×1		
Block Reagent	135 mL/bottle×1		
AD153 Barcode Primer 2	135 mL/bottle×1		
DNB Loading Reagent Cartridge V4.0	2 EA		
MDA Reagent Cartridge V4.0	2 EA		
DNBSEQ-T10×4RS high-throughput sequencing kit (Package 5) (FCL PE100/PE150) Cat. No.: 1000027930			
DIPSEQ Regeneration Buffer	5170 mL/bottle×1	Below 8 ℃	Below 8 ℃
Wash Reagent 2	3000 mL/bottle×1		
Wash Reagent 2	1800 mL/bottle×1		
DIPSEQ Sequencing Reagent 2 (Hot)	2590 mL/bottle×1		
DIPSEQ Sequencing Reagent 2 (Cold)	2590 mL/bottle×1		
2 L cleaning bottle	1 EA		

Table 4 DNBSEQ-T10×4RS High-throughput Sequencing Set (FCL PE150) (Standard Product) V1.0 (Cat. No.: 940-000100-00)

Component information	Specification	Storage temperature	Transportation temperature
DNBSEQ-T10×4RS sequencing slide Cat. No.: 930-000038-00			
Sequencing slide (T10×4RS FCL)	8 EA	0 to 30 ℃	0 to 30 ℃
DNBSEQ-T10×4RS high-throughput sequencing kit (Package 1) (FCL PE100/PE150) Cat. No.: 1000027925			
Wash Reagent 2	7330 mL/bottle×1	Below 8 ℃	Below 8 ℃
DIPSEQ Sequencing Reagent	7620 mL/bottle×1		
DNBSEQ-T10×4RS high-throughput sequencing kit (Package 2)(FCL PE150) Cat. No.: 1000027927			
DIPSEQ Image Reagent	7500 mL/bottle×2	-25 ℃ to -15 ℃	Below -15 ℃
Bottle cap with pipette	4 EA/bag×1	-25 ℃ to -15 ℃	Below -15 ℃
Hose Barb Socket	1 EA/bag×2		
DNBSEQ-T10×4RS high-throughput sequencing kit (Package 3) (FCL PE100/PE150) Cat. No.: 1000027928			
Make DNB Buffer	12.5 mL/tube×1	-25 ℃ to -15 ℃	Below -15 ℃
Stop DNB Reaction Buffer	12.5 mL/tube×1		
DIPSEQ Make DNB Enzyme Mix I	21 mL/tube×1		
DIPSEQ Make DNB Enzyme Mix II	1 mL/tube×1		
DNB Loading Buffer I	12 mL/tube×1		
DNB Loading Buffer II	10 mL/tube×1		
Phi29 DNA polymerase (HC)	240 μL/tube×1		
DNBSEQ-T10×4RS high-throughput sequencing kit (Package 4) (FCL PE100/PE150) Cat. No.: 1000027929			
MDA Enzyme Mix	5 mL/tube×2	-25 ℃ to -15 ℃	Below -15 ℃
Sequencing Enzyme Mix	54 mL/bottle×2		
DIPSEQ dNTPs Mix	54 mL/bottle×1		
dNTPs Mix II	108 mL/bottle×1		
DIPSEQ Regeneration Reagent	2700 mL/bottle×1		

Component information	Specification	Storage temperature	Transportation temperature
Block Reagent	135 mL/bottle×1	-25 ℃ to -15 ℃	Below -15 ℃
AD153 Barcode Primer 2	135 mL/bottle×1		
DNB Loading Reagent Cartridge V4.0	2 EA		
MDA Reagent Cartridge V4.0	2 EA		
DNBSEQ-T10×4RS high-throughput sequencing kit (Package 5) (FCL PE100/PE150) Cat. No.: 1000027930			
DIPSEQ Regeneration Buffer	5170 mL/bottle×1	Below 8 ℃	Below 8 ℃
Wash Reagent 2	3000 mL/bottle×1		
Wash Reagent 2	1800 mL/bottle×1		
DIPSEQ Sequencing Reagent 2 (Hot)	2590 mL/bottle×1		
DIPSEQ Sequencing Reagent 2 (Cold)	2590 mL/bottle×1		
2 L cleaning bottle	1 EA		

NOTE
- When performing dual-indexed sequencing of FCL PE100 and FCL PE150, in addition to the corresponding high-throughput sequencing set, the DNBSEQ-T10×4RS high-throughput sequencing kit (FCL Dual Index) (Cat. No.: 1000027931) is also required. If there is any question, contact the technical support.
- The specification of sequencing slide is 8 pieces per box. Slides are protected by vacuum packaging. After the vacuum packaging is unpacked, slides must be stored in a nitrogen cabinet and used up as soon as possible. About the validity period, refer to the packaging.

Table 5 Abbreviations for DNBSEQ-T10×4RS high-throughput sequencing reagents

Full name	Abbreviation
DIPSEQ Regeneration Buffer	RB
Wash Reagent 2	WB2
DIPSEQ Sequencing Reagent 2 (Hot)	Hot
DIPSEQ Sequencing Reagent 2 (Cold)	Cold
DIPSEQ Sequencing Reagent	SB2
DIPSEQ Image Reagent	IR
DIPSEQ Regeneration Reagent	RR
DIPSEQ Block Reagent	BR
AD153 Barcode Primer 2	BP2

2.2 User-supplied equipment, reagents and consumables

NOTE Unless otherwise specified, the description of water used in this user manual is divided into laboratory-grade water and pure water. Laboratory-grade water refers to RO water. Pure water refers to ultra-pure water or Milli-Q water (18.2 MΩ·cm). The reference water source is the Milli-Q water purifier in the table below.

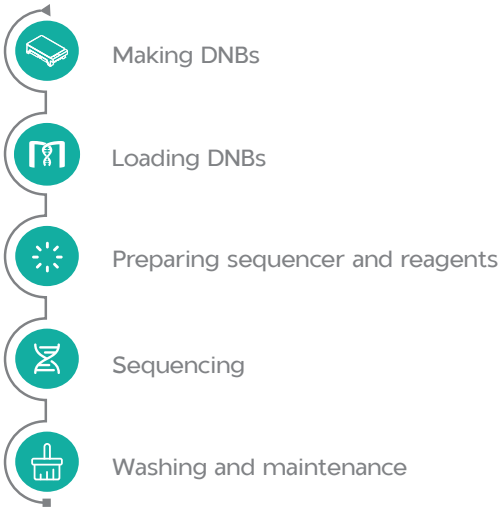
Table 6 Self-provided equipment, reagents and consumables

Type	Name	Recommended brand	Cat. No.
Equipment	Qubit 4.0 fluorometer	Thermo Fisher	Q33226
	MGISP-960 high-throughput automated sample preparation system	MGI	900-000152-00 (Configuration 7)
	Fluorescence microplate reader	BMG LABTECH	FLUOstar Omega
	Automated liquid handling workstation	Hamilton	STARlet
	PCR thermocycler	Bio-Rad	None
	Dishwasher	None	None
	MPC2000 96-well plate centrifuge (plate throwing machine)	DHS Life Science&Technology Co., Ltd.	None
	Pipette	Eppendorf	None
	Electronic pipette	Labnet	FASTPETTEV-2
	A set of manual single-channel pipettes (0.1 µL-5 mL)	Eppendorf	Research plus basic
	Plasma cleaner	Dongguan Sindin	SPV-100
	Nitrogen cabinet	HASUC	HSD240D
	Oven	Boxun	GZX-9140MBE
	Water purifier	Milli-Q	Advantage A10
	Barcode label printer	Toshiba	B-FV4T-TS14
	Mini centrifuge	None	None
	Vortex mixer	None	None
	2 ℃ to 8 ℃ refrigerator	None	None

Type	Name	Recommended brand	Cat. No.
Equipment	-18 ℃ to -25 ℃ freezer	None	None
Reagents	75% ethanol	None	None
	2 M NaOH	Aladdin	S128511-1L
	PBS PH 7.4 (1×)	Thermo Fisher	10010031
	TE Buffer PH 8.0	AMBION	AM9858
	Brightener	Finish rinse aid	None
	Sodium percarbonate	Macklin	S830224-500g
	Qubit ssDNA Assay Kit	Thermo Fisher	Q10212
	Qubit-iT OliGreen ssDNA Assay Kit	Invitrogen	O11492
Consumables	High-purity nitrogen, pressure reducing valve, fixing bracket	None	None
	UV-star 96-well microplate	GREINER BIO-ONE	655801
	Hard-shell low-profile thin-wall 96-well skirted PCR plates	BIO-RAD	1000004492
	Qubit Assay tubes	Thermo Fisher	Q32856
	200 μL wide-bore tip	AXYGEN	T-205-WB-C
	Power dust remover (air tank)	MATIN	M-6318
	5 mL centrifuge tube	Sangon	F611888
	100 mL disposable pipette	CORNING	4491
	25 mL disposable pipette	CORNING	4489
	10 mL disposable pipette	CORNING	4488
	50 mL centrifuge tube	CORNING	430829
	15 mL centrifuge tube	CORNING	430791
	Sterile tips, boxed	AXYGEN	None
	5 mL sterile tips, boxed	AXYGEN	None
	0.2 mL PCR 8-strip tube	AXYGEN	None
	Ice box	AXYGEN	None
	Dust-free cloth	DUSTFREE TECHNOLOGY CO.,LTD	LJ618180B1
	Dust-free paper	KIMTECH	34155

Type	Name	Recommended brand	Cat. No.
Consumables	Medical syringe for single use, 50 mL	None	None
	Washing bottle	None	None
	Tin foil	None	None
	Sealing film	PARAFILM	PM996
	Peelable aluminum film	AGILENT	24210-001
	20 L waste container	hs-science	/
	60 L waste container	hs-science	/
	250 µL automated tips	MGI	1000000723
	50 µL Hamilton tips	Hamilton	235831
	Single-well reagent reservoir	AXYGEN	RES-SW96-HP
	96-well deep-well plate	DN-Biotech	7350504

Chapter 3 Sequencing workflow



Chapter 4 Making DNBs

This chapter describes how to use DNB making reagents to make DNBs from sample libraries on the automated liquid handling workstation including Hamilton and MGISP-960 (Configuration 7).

4.1 Library requirements

This kit is applicable to libraries prepared by using DNB making kit of MGI. Conventional libraries are single-stranded circular DNA (ssDNA).

4.1.1 Library insert size

The recommended library insert size ranges between 100 bp and 500 bp, with main band within ±300 bp.

If special requirements of library insert size are written in the user manual of the library prep kit, it shall prevail.

Table 7 Recommended library insert size

Kit model	FCL PE100	FCL PE150
Recommended library insert size (bp)	200 to 400	300 to 500
Applicable library type	WGS	WGS
Average number of reads (M/slide)	45000	30000
The amount of data output (TB/slide)	About 9	About 9

NOTE The amount of data output is for reference only, and it varies among different libraries and applications.

4.1.2 Library concentration

- For PCR libraries, the initial ssDNA library concentration is required to be not less than 0.6 ng/μL.
- For PCR-Free libraries, the concentration of the initial ssDNA library is required to be not less than 0.6 ng/μL. However, for libraries with a concentration of less than 0.8 ng/μL, it is recommended to use the Qubit ssDNA Assay Kit for re-quantification.
- If the concentration is unknown, it is recommended to use the Qubit ssDNA Assay Kit and Qubit Fluorometer for quantification to obtain the actual library concentration. As for a large amount of

samples to be tested in a single run, the fluorescence microplate reader named FLUOstar omega can be used to determine the library concentration.

Approximate conversion formula between fmol and ng:

Concentration (fmol/μL) = 3030×concentration (ng/μL)/N

N represents the average number of nucleotides in the library.

The summation formula of *N* is:

N=main-band insert size of fragment+84 (MGI single-barcode library)

4.2 Pooling protocol

4.2.1 Calculating the number of pooling samples

The amount of samples that can be pooled together is determined by the required data output for each sample, the sequencing read length, sequencing barcode type and other requirements determine

In both PE100 and PE150 sequencing, a single slide can theoretically produce 9 TB of data. According to application needs. PE100 or PE150 is selected.

NOTE After DNB preparation, it is recommended to quantify DNB first and then pool DNB. It is not recommended to pool libraries with different main-band insert sizes together.

Since there are deviations in the amount of data output produced by each barcode, the pooling bias is currently measured by using the coefficient of variation (CV). If CV of 15% is taken as the standard to calculate, it is recommended that the total amount of data required does not exceed 80% of the theoretical data output, which is equivalent to 7.2 TB.

Below are some examples:

- For human whole-genome sequencing (WGS), each sample has a sequencing depth of 30× and requires at least 90 GB of data. It is recommended to pool 80 samples per slide.
- If there are special requirements of sequencing depth, the number of pooling samples can be appropriately increased or decreased. For example, in 40× WGS, the recommended number of pooling samples is 60.

Table 8 Example for calculating the number of pooling samples

Sequencing read length	PE100	PE150
Requirement for each sample	90 GB	90 GB
The number of pooling samples	80	80
Theoretical output range for each sample	80 GB to 140 GB	80 GB to 140 GB

NOTE The above results assume that the pooling bias of the sample is within ±15%.

- For other applications, the number of pooling samples is calculated according to the total data output of a slide, the required data output of each sample, and the possible bias between sample pooling, and then adjusted according to the calculated value.

  For example, if an application requires each sample to output 200 GB at PE150 sequencing, and the bias of sample pooling is within ±15%, calculate the size according to the follows:

  The maximum number of pooling samples

  = Total data output of a slide×(1-pooling coefficient of variation, CV)/required data output of application

  = 9000 GB×(1-15%)/200 GB

  =38

4.2.2 Verifying the base balance for barcode

Check the barcodes of the samples to be pooled to ensure that these samples do not contain the same barcode. The best sequencing quality can be obtained only when the base content is relatively balanced in each cycle. Therefore, it is recommended that the relative content of A, C, G, and T bases in each cycle is not less than 12.5%.

If the relative content of a certain base is between 5% and 12.5%, samples can be sequenced with a certain risk.

If the relative content of a certain base is lower than 5%, it is not recommended to start DNB pooling, and the pooling protocol needs to be re-established.

NOTE This set has a large amount of data output for a single slide and a large number of pooling samples. Therefore, in WGS sequencing, there is generally no base imbalance.

4.3 Library normalization

4.3.1 Calculating the required amount of ssDNA library

NOTE All samples are regarded as potentially infectious items, and operations must be performed in accordance with relevant national standards.

The calculation is as follows:

- For this sequencing set, DNB needs to be prepared separately for each sample, and the volume of the preparation system shall be 50 μL.
- The required amount of each ssDNA library is 6 ng. This amount is suitable for 30× data output of standard human PCR-free WGS samples. The exact amount required can be adjusted according to the type of library and sequencing.
- The library concentration is required to be greater than 0.6 ng/μL. According to the formula: V=6 ng/ssDNA concentration (ng/μL), the maximum volume of one DNB prepared for each sample is 10 μL.
- For this sequencing set, the volume required to load a sequencing slide is 4000 μL, which contains DNB and DNB Loading Buffer I/II.

4.3.2 Library normalization

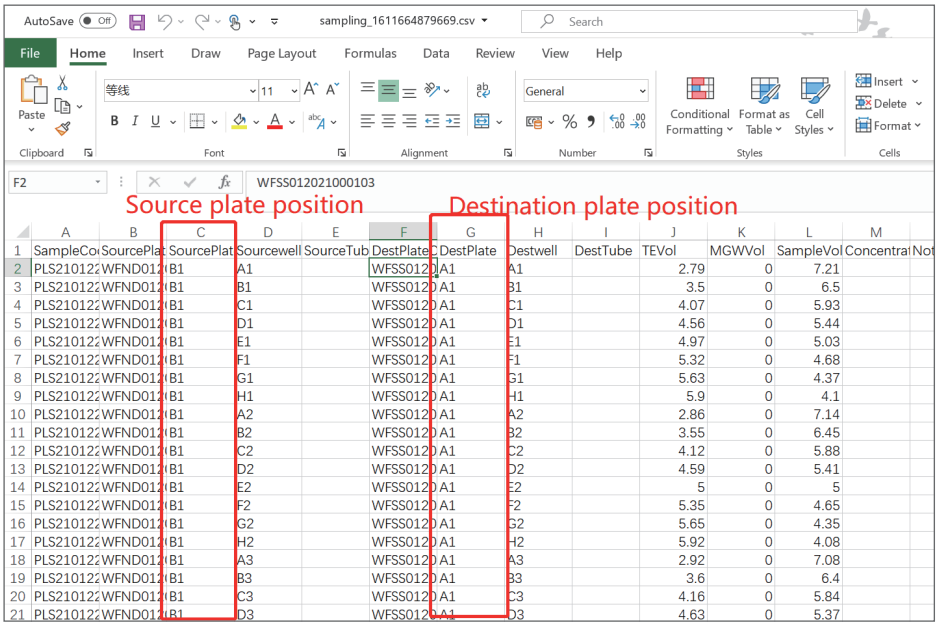
If Hamilton is used for library normalization, the user should be familiar with and master the operation methods and precautions of this device. This manual only describes the operations in normal circumstances.

Perform the following steps:

1. Power on the Hamilton device, start the device, and perform a pre-clean.
2. According to the table below, calculate the required amount of ssDNA library and 1×TE Buffer, and fill the data in an EXCEL file.

Table 9 Library normalization system

Component	Volume (μL)
ssCircle DNA library	V
1×TE Buffer	10-V



	A	B	C	D	E	F	G	H	I	J	K	L	M
1	SampleCo	SourcePla	SourcePlat	Sourcewell	SourceTub	DestPlate	DestPlate	Destwell	DestTube	TEVol	MGWVol	SampleVol	Concentra
2	PLS210122	WFND012	B1	A1		WFSS0120	A1	A1		2.79	0	7.21	
3	PLS210122	WFND012	B1	B1		WFSS0120	A1	B1		3.5	0	6.5	
4	PLS210122	WFND012	B1	C1		WFSS0120	A1	C1		4.07	0	5.93	
5	PLS210122	WFND012	B1	D1		WFSS0120	A1	D1		4.56	0	5.44	
6	PLS210122	WFND012	B1	E1		WFSS0120	A1	E1		4.97	0	5.03	
7	PLS210122	WFND012	B1	F1		WFSS0120	A1	F1		5.32	0	4.68	
8	PLS210122	WFND012	B1	G1		WFSS0120	A1	G1		5.63	0	4.37	
9	PLS210122	WFND012	B1	H1		WFSS0120	A1	H1		5.9	0	4.1	
10	PLS210122	WFND012	B1	A2		WFSS0120	A1	A2		2.86	0	7.14	
11	PLS210122	WFND012	B1	B2		WFSS0120	A1	B2		3.55	0	6.45	
12	PLS210122	WFND012	B1	C2		WFSS0120	A1	C2		4.12	0	5.88	
13	PLS210122	WFND012	B1	D2		WFSS0120	A1	D2		4.59	0	5.41	
14	PLS210122	WFND012	B1	E2		WFSS0120	A1	E2		5	0	5	
15	PLS210122	WFND012	B1	F2		WFSS0120	A1	F2		5.35	0	4.65	
16	PLS210122	WFND012	B1	G2		WFSS0120	A1	G2		5.65	0	4.35	
17	PLS210122	WFND012	B1	H2		WFSS0120	A1	H2		5.92	0	4.08	
18	PLS210122	WFND012	B1	A3		WFSS0120	A1	A3		2.92	0	7.08	
19	PLS210122	WFND012	B1	B3		WFSS0120	A1	B3		3.6	0	6.4	
20	PLS210122	WFND012	B1	C3		WFSS0120	A1	C3		4.16	0	5.84	
21	PLS210122	WFND012	B1	D3		WFSS0120	A1	D3		4.63	0	5.37	

Figure 1 Library normalization table

3. Place the library input and output plates, pipette tips(Hamilton, Cat. No.: 235831) and TE deep-well plate(DN-Biotech, Cat. No.: 7350504) in the corresponding positions in Hamilton according to the figure below.

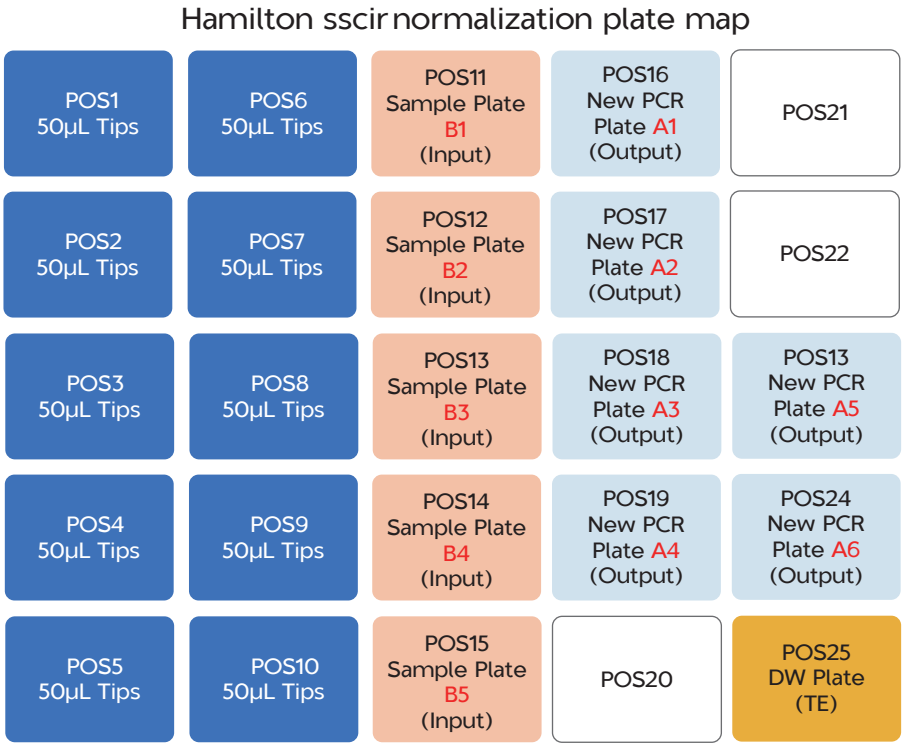


Figure 2 Hamilton library normalization plate map (single device)

NOTE
- The library plate map should correspond to the library normalization table.
- For a plate with normalized libraries, 150 μL of TE Buffer is needed to be added into A1 to H1, respectively.

4. Import the library normalization table, start the Hamilton run control software, and complete library preparation according to relevant operation guides of Hamilton.
5. Seal the normalized library plate with sealing film, mix the library with a vortex mixer, centrifuge quickly for 5 s to 10 s, and place it in a 2 ℃ to 8 ℃ refrigerator for further use.
6. Seal the used TE deep-well plate with sealing film, place the remaining tips on the table back into the tip box and clean the table.

NOTE The TE deep-well plate can be reused within a day.

4.4 Making DNBs

The following DNB making steps are based on automated liquid handling workstations including Hamilton (Cat. No.: 960-000107-00) and MGISP-960 (configuration 7). Please refer to the corresponding operation guide for specific operations.

4.4.1 Preparing DNB making reagents

Perform the following steps:

1. Take out Make DNB Buffer, Stop DNB Reaction Buffer, DIPSEQ Make DNB Enzyme Mix I and DIPSEQ Make DNB Enzyme Mix II from Package 3 at -25 ℃ to -15 ℃ .
2. DIPSEQ Make DNB Enzyme Mix II shall be briefly centrifuged and placed on ice until use. The other three reagents are thawed at room temperature for about 1 h to 2 h.
3. After thawing, use a vortex mixer to mix them for 5 s to 10 s, centrifuge briefly and place them on ice until use.

NOTE Mixed use of reagents from different batches is not recommended.

4.4.2 Dispensing DNB making reagents

Perform the following steps:

1. Ensure that four reagents to be dispensed are prepared according to the table below.

Table 10 DNBSEQ-T10×4RS high-throughput sequencing set package 3

Kit	Component
Package 3 Cat. No.: 1000027928 Storage temperature: -25 ℃ to -15 ℃	Make DNB Buffer
	DIPSEQ Make DNB Enzyme Mix I
	DIPSEQ Make DNB Enzyme Mix II
	Stop DNB Reaction Buffer

2. Take a new 1.3 mL 96-well deep-well plate (DN-Biotech, Cat. No.: 7350504), mark it as "Make DNB Reagent", and place it on ice.
3. Dispense the four reagents into a deep-well plate as shown in the figure below. Refer to *Table 11 on Page 17* for DNB making volume.

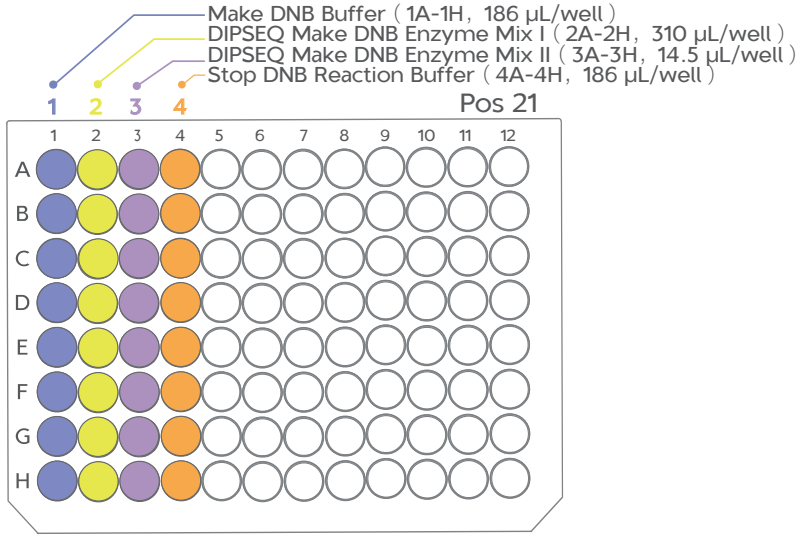


Figure 3 Make DNB Reagent well map

NOTE DIPSEQ Make DNB Enzyme Mix II should be stored at 2 ℃ to 8 ℃ after use.

Table 11 DNB making volume for a single sample

Component	Volume
Make DNB Buffer	10 μL
DIPSEQ Make DNB Enzyme Mix I	20 μL
DIPSEQ Make DNB Enzyme Mix II	1 μL
Stop DNB Reaction Buffer	10 μL

4. After dispensing, seal the plate with film and centrifuge quickly to prevent bubbles from forming during dispensing, which may affect the accuracy of subsequent aspiration.

4.4.3 Making DNBs

Perform the following steps:

1. Before using this application for the first time, install the application scripts and PCR program according to *MGISP-100&MGISP-960 Application Script Installation Instructions*.
2. Start the MGISP-960 software and log in.
3. Tap ☰ > **Run Wizard** to enter the Run Wizard interface.
4. Click the drop-down list of **Solution** and select **JB-A10-002MGIEasy FS PCR-Free DNA Library Prep**.
5. Click the drop-down list of **Script** and select **4.T10 DNB_Making_for_FS_PCRFree_Library_step1_50ul_RV1.0SV1.0.**
6. Place consumables according to the Make DNB Reagent plate map. The concrete requirement is as below:
   - POS1-3, POS5: tip boxes (MGI, Cat. No.: 1000000723)
   - POS11: normalized library plates
   - POS13-14, POS16: 96-well skirted PCR plates (BIO-RAD, Cat. No. 1000004492)
   - POS21: the dispensed Make DNB Reagent plates

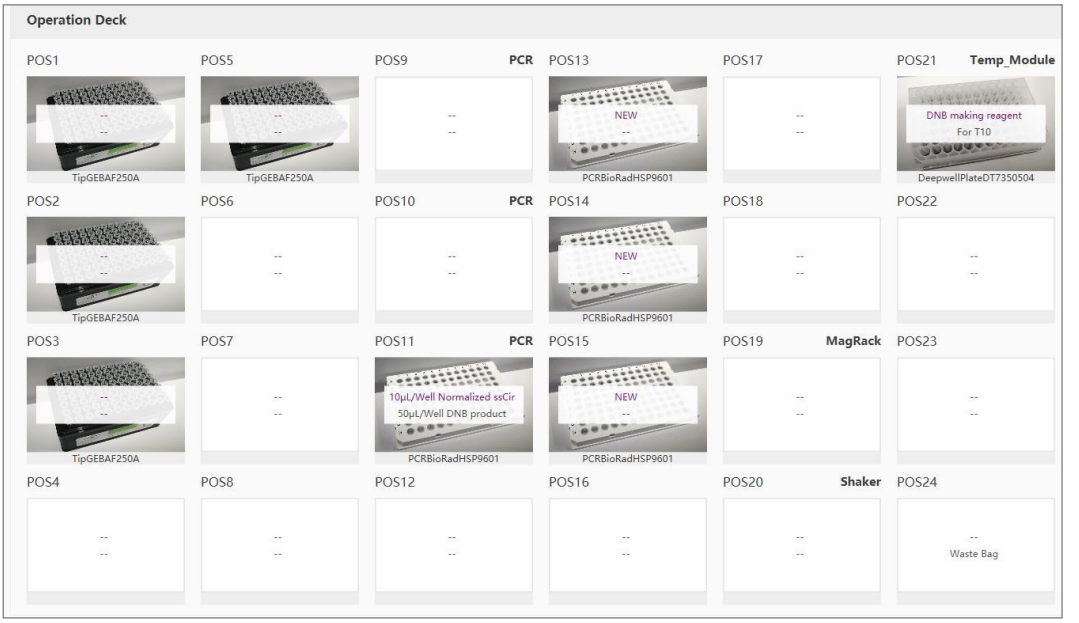


Figure 4 Make DNB reagent plate map

NOTE Place the Make DNB Reagent plates on the ice box first. When the temperature of MGISP-960 operation table reaches 8 ℃, place these plates on MGISP-960.

7. After confirming the placement, click **Start**. Click **Start** in the pop-up window.
8. In the pop-up window, click the drop-down list of **RCA_time**, select **15 min** and click **Continue**. The run takes about 1 h.
9. At the end of the run, remove the DNB product from POS11, seal it and mark it as "DNB" on the plate.
10. According to the experimental needs, quantify the concentration of DNB product. For details, refer to *4.5 Quantifying DNB concentration and eligibility criteria on Page 19*.

    If the product is not used temporarily, store it in a refrigerator at 2 ℃ to 8 ℃, in which the product could be stored for 7 days at most.

11. If no experiment is to be conducted on the day, use laboratory-grade water and 75% ethanol to clean the surface of the device and perform a post-clean.

4.5 Quantifying DNB concentration and eligibility criteria

4.5.1 Preparing and dispensing quantitative reagents

Perform the following steps:

1. For first use, take out the OliGreen package from the refrigerator and thaw it at room temperature in dark conditions.
2. After thawing, mix and centrifuge quickly for 10 s until use. After use, store the OliGreen package at 2 ℃ to 8 ℃ .
3. Pipette 24875 μL of TE Buffer and 125 μL of dye solution into a new 50 mL centrifuge tube, and mix the tube by vortexing.

NOTE
- OliGreen dye solution should be prepared as required and should not be stored overnight.
- The volume of dye solution is the amount within 2 plates of DNB (192 samples) for quantification. If there are more than 2 plates, please refer to the following table for calculation.

Table 12 DNB dye preparation

Reagent	Quant-iT OliGreen ssDNA	1×TE Buffer
Volume for single reaction (μL)	1	199
Total volume (μL)	(N×50+25)×1	(N×50+25)×199

- The single-well deep-well plate has a certain dead volume, and the minimum volume required for TE quantification is 30 mL, which needs to be calculated by referring to the following table.

Table 13 Volume required for 1×TE quantification

Reagent	1×TE Buffer
The minimum volume of deep-well plate (mL)	30
Total volume (mL)	30+(N-2)×10×1.2

N(>2) is the total number of DNB plates to be quantified.

4. Add the prepared dye solution to the single-well reagent reservoirs (AXYGEN, Cat. No.: RES-SW96-HP) for a total of 25 mL.
5. Dispense the 1×TE into single-well reagent reservoirs for a total of 30 mL.

4.5.2 Automated pipetting on MGISP-960

Perform the following steps:

1. Click the drop-down list of **Script**, and select **5.Quantification_for_1_4_Plates_SV1.0-singlewell_step2**.
2. Place the DNB plate, OliGreen dye solution plate, 1×TE plate, microplate (GREINER BIO-ONE, Cat. No.: 655801) and tips according to the following figure.

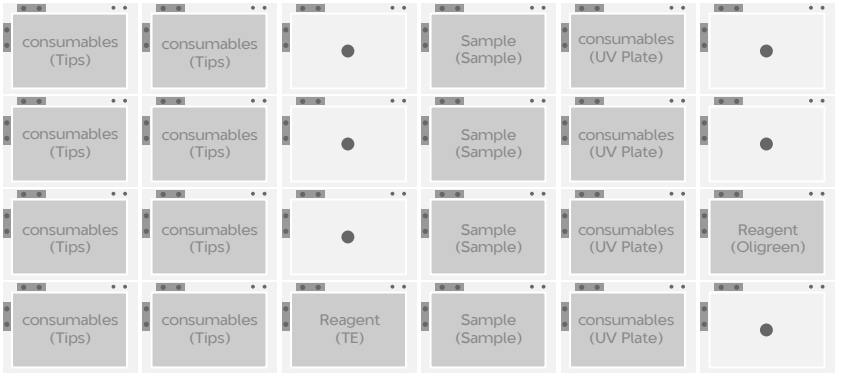


Figure 5 Automated pipetting plate map

3. Click **Start**, and select the number of plates to be quantified in the pop-up window.
4. After the program, take out the microplate and store it in dark conditions for 5 min before BMG quantification. After sealing the DNB plate, store it in a refrigerator at 2 ℃ to 8 ℃ .

NOTE The aspiration volume of DNB, 1×TE, dye solution is 2 μL, 98 μL and 100 μL, respectively. The total volume is 200 μL.

4.5.3 Preparing standards and quantifying with BMG microplate reader

NOTE The dye solution and TE used for standards preparation should be those in DNB quantitative reagents.

1. Aspirate 294 μL of TE Buffer and 6 μL of nucleic acid standard, mix them thoroughly. Place the mix into a 0.5 mL centrifuge tube, then pipette 200 μL from the centrifuge tube and add it to the empty well A12 of the microplate.
2. Aspirate TE Buffer and add it to 7 wells including B12, C12, D12, E12, F12, G12 and H12, with 100 μL per well.
3. Aspirate 100 μL of the mix from A12 with a pipette and add it to well B12. After mixing well by pipetting up and down, aspirate 100 μL from B12, transfer it to C12, and mix by pipetting up and down. According to the gradient dilution method, repeat the above steps until the mix from well F12 is transferred to well G12 with the mix being pipetted up and down. When gradient dilution is finished, discard the excess 100 μL of the mix.

   NOTE The amount of the nucleic acid standard in well H12 is 0, which is a negative control.

4. Add 100 μL of the dye solution prepared for DNB quantification to wells A12 to H12, mix thoroughly and store the plate in dark conditions for 5 min.

Table 14 Standard curve

BMG standard (ng, total amount)	FL strength
200	A
100	B
50	C
25	D
12.5	E
6.25	F
3.125	G
0	H

5. Place the standard quantitative plate and the DNB quantitative plate prepared in the previous step into the BMG device to start quantification.
6. Open the DNB quantification form, and fill in the absorbance value of the standards and DNB absorbance value read by BMG into the corresponding position of the form, and the form will automatically calculate the DNB concentration in each well position.

4.5.4 Eligibility criteria

The acceptable range for DNB concentration is from 8 ng/μL to 40 ng/μL.

- If the concentration is less than 8 ng/μL, perform BMG quantification again, preferably with the same batch of quantitative reagents.
- If the criteria is still unfulfilled, do not perform pooling and prepare again. If the criteria is fulfilled, record the remeasured concentration.

4.6 DNB pooling

4.6.1 Manually calculating the DNB pooling volume

Taking 80 pooling samples as an example. The recommended calculation method of pooling volume is as follows:

1. Select 80 sample combinations with qualified concentrations according to the standard of "Pooling protocol", calculate the average and standard deviation (SD) of DNB concentration, and calculate the standard value *A* according to the following formula:

   $$A = \text{Average} - 2\text{SD}$$

2. According to the calculated standard value *A*, the volume of other DNBs to be pooled is converted according to the volume, and the DNB with volume higher than 48 μL is placed in as 48 μL. The pooling volume is as follows:

   Assuming that the DNB concentrations of the 80 samples are C1, C2…C80, respectively, the pooling volumes of each sample are:

   The pooling volume of sample 1 is: Parameter A/C1×48

   The pooling volume of sample 2 is: Parameter A/C2×48

   The pooling volume of sample 3 is: Parameter A/C3×48

   …

   The pooling volume of sample 80 is: Parameter A/C80×48

   NOTE When SD/Average is higher than 25%, the above method may cause slightly lower pooling volume. At this time, contact the technical support.

4.6.2 DNB pooling in Hamilton

Perform the following steps:

1. According to *4.6.1 Manually calculating the DNB pooling volume on Page 22*, calculate the sample volume of each DNB and fill out the following table.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	SampleCode	SourcePlateCode	SourcePlate	Sourcewell	SourceTube	DestPlateCode	DestPlate	Destwell	DestTube	TEVol	MGWVol	SampleVol	Concentration	Note
2	YY210116104	UNP210117005	B1	A1		BRMW0120200001TE	A1	A1		0	0	36.82		
3	YY210116105	UNP210117005	B1	B1		BRMW0120200001TE	A1	B1		0	0	36.71		
4	YY210116106	UNP210117005	B1	C1		BRMW0120200001TE	A1	C1		0	0	36.63		
5	YY210116107	UNP210117005	B1	D1		BRMW0120200001TE	A1	D1		0	0	36.54		
6	YY210116108	UNP210117005	B1	E1		BRMW0120200001TE	A1	E1		0	0	36.45		
7	YY210116109	UNP210117005	B1	F1		BRMW0120200001TE	A1	F1		0	0	36.37		
8	YY210116110	UNP210117005	B1	G1		BRMW0120200001TE	A1	G1		0	0	36.26		
9	YY210116111	UNP210117005	B1	H1		BRMW0120200001TE	A1	H1		0	0	36.18		
10	YY210116112	UNP210117005	B1	A2		BRMW0120200001TE	A1	A1		0	0	36.71		
11	YY210116113	UNP210117005	B1	B2		BRMW0120200001TE	A1	B1		0	0	36.63		
12	YY210116114	UNP210117005	B1	C2		BRMW0120200001TE	A1	C1		0	0	36.54		
13	YY210116115	UNP210117005	B1	E2		BRMW0120200001TE	A1	E1		0	0	36.37		
14	YY210116116	UNP210117005	B1	F2		BRMW0120200001TE	A1	F1		0	0	36.26		
15	YY210116117	UNP210117005	B1	G2		BRMW0120200001TE	A1	G1		0	0	36.18		
16	YY210116118	UNP210117005	B1	H2		BRMW0120200001TE	A1	H1		0	0	36.09		
17	YY210116119	UNP210117005	B1	A3		BRMW0120200001TE	A1	A1		0	0	36.63		
18	YY210116120	UNP210117005	B1	B3		BRMW0120200001TE	A1	B1		0	0	36.54		
19	YY210116124	UNP210117005	B1	C3		BRMW0120200001TE	A1	C1		0	0	36.45		
20	YY210116125	UNP210117005	B1	D3		BRMW0120200001TE	A1	D1		0	0	36.37		
21	YY210116126	UNP210117005	B1	E3		BRMW0120200001TE	A1	E1		0	0	36.26		

Figure 6 DNB pooling table

2. Power on the Hamilton device, start the device, and perform a pre-clean.
3. Place the required DNB plates, deep-well plates and tips according to the figure below.

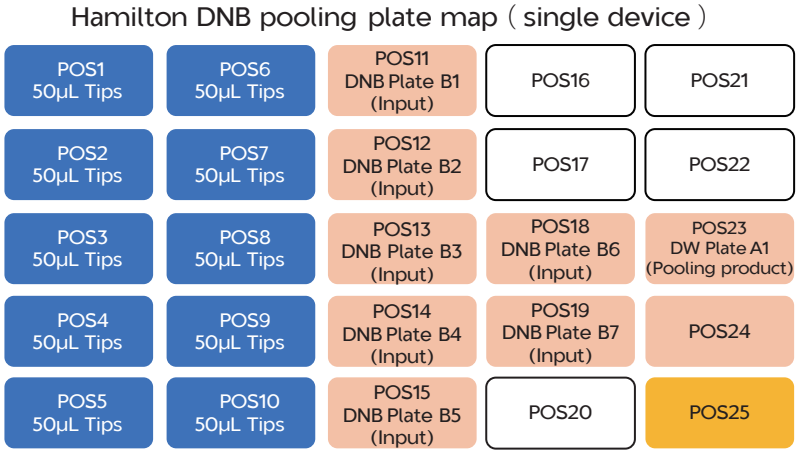


Figure 7 Hamilton DNB pooling plate map

4. Start the Hamilton run control software and complete DNB making according to Hamilton's relevant operation guides.
5. Seal the pooled deep-well plate with sealing film and place it in a refrigerator at 2 ℃ to 8 ℃ until use.
6. Place the remaining clean tips on the table back to the tip box and clean the operation table.

Chapter 5 Loading DNBs

This chapter describes how to use DNB loading reagents to load DNB onto the slide on the 4-channel DNB loader MGIDL-T20RS.

NOTE
- A DNB loader can load 1 to 4 sequencing slides at a time. One DNB loading reagent cartridge can support up to 4 sequencing slides loaded simultaneously. To save time, it is recommended to load 4 sequencing slides at a time. If loading separately is required, contact the technical support.
- Before the experiment, it is necessary to be familiar with and master the operation methods and precautions of the loader and third-party devices.
- Regarding the use of MGIDL-T20RS automatic DNB loader, this manual only provides routine operation steps. For details, refer to *MGIDL-T20RS Automatic DNB Loader User Manual*.

5.1 Preparing DNB loading reagent cartridge and buffer

Perform the following steps:

1. After completing DNB pooling, remove the 96-well deep-well plate. Next, combine the DNB in the same column of wells of the deep-well plate into a new 5 mL centrifuge tube, and mark the DNB number, date and other information on the DNB tube.

   NOTE During DNB sampling, slowly aspirate the liquid with a sharp-mouth pipette tip. After sampling all DNBs, mix gently and thoroughly by using a wide-bore pipette tip.

2. Take out the DNB Loading Buffer I, DNB Loading Buffer II and DNB loading reagent cartridge from the kits 3 and 4, and thaw them at room temperature for about 2 hours before use. Before using the DNB loading reagent cartridge, slowly rotate it horizontally 10 times with the sealing film facing up to mix the reagents and prevent the sealing film of the reagent cartridge from breaking.
3. Prepare 0.1 M NaOH. For details, refer to *6.3.1 Preparing sequencing reagents on Page 39*. When washing with NaOH, it is recommended to add it to 2/3 of the well height of the washing cartridge.
4. Take out the 5 mL centrifuge tube and add the reagents in the sequence as shown in the table below.

Table 15 DNB loading mixture

Component	Volume (μL)
DNB	V
DNB Loading Buffer I	3000-V
DNB Loading Buffer II	1000
Select one reagent according to the notes below this table:	
• DIPSEQ Make DNB Enzyme Mix II	25
• Phi29 DNA polymerase (HC)	20

NOTE
- DNB refers to the DNB sample that has been pooled. *V* refers to the volume after pooling.
- Phi29 DNA polymerase (HC) in Box 3 should be used in DNB loading mixture for special circumstances such as significant base distribution separation. Please contact technical support before use.

5. Slowly mix the DNB loading mixture for 5 to 8 times with a 1 mL wide-bore pipette tip. Do not centrifuge, shake or vigorously pipette in the process.
6. The DNB loading mixture needs to be prepared and used immediately. After preparation, it should be placed at 2 ℃ to 8 ℃ for 0.5 h at most.

NOTE It is recommended to prepare the system after completing *Page 30 "Priming"*.

5.2 Preparing sequencing slides

Perform the following steps:

1. Take out the vacuum-packed slide from the reagent set, open the vacuum package, and take out the slide.

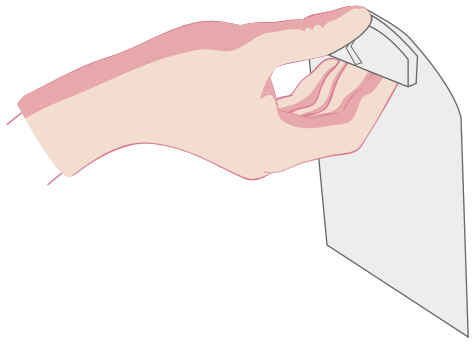


Figure 8 Taking out slide

NOTE When taking out the slide, hold the upper edge of the handle with the thumb and the lower edge of the handle with other fingers. In the process, avoid touching other areas on the front of the slide, avoid blowing air, and avoid bumping and contamination.

2. Check whether the appearance of the slide is complete and whether the QR code label is clear.
3. Place the slides back into the slide box and place the box in a nitrogen cabinet for further use.

5.3 Preparing the glass cover plate

Perform the following steps:

1. Remove the dried cover plate from the oven.
2. Turn on the Plasma power supply and open the door, place the glass cover plate into the Plasma chamber with the sealing ring facing up, and close the Plasma door.
3. Enter the password "8888" of the operator account to enter the interface, tap **Automatic mode**, and set the discharge power to 400 W, and the time to 600 s.

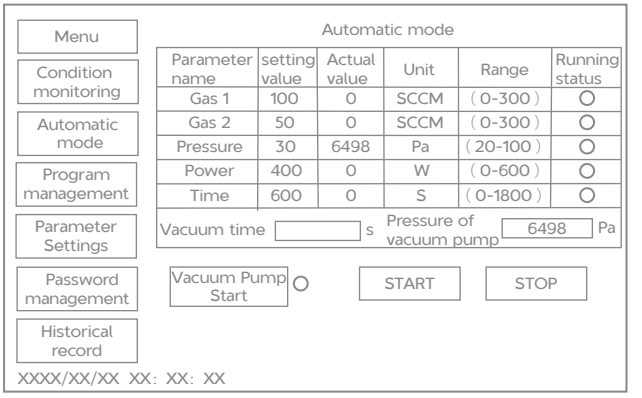


Figure 9 Automatic mode interface

4. Click **Vacuum Pump Start** to start the vacuum pump.
5. Click **Start**. The vacuum pump button is green when running, and pink when the chamber is discharged.

NOTE The vacuum pump must be turned on first, otherwise it will not work.

6. After the run is over, click **Vacuum Pump Stop** to turn off the vacuum pump.

7. Open the Plasma door, take out the cover plate, and set aside until use.

NOTE
- Cover plates conditioned with plasma should not be exposed to air for a prolonged time. If it is not used within 10 minutes, it is recommended to leave it in the Plasma chamber. In order to avoid recontamination, if it has not been used for more than half an hour, it must be reprocessed.
- The cover plate can be reused. For the specific washing method, refer to *Page 35 "Washing the glass cover plate"* .

5.4 Loading DNBs

5.4.1 Starting the loader and software

Perform the following steps:

1. Press the power button on the right, and press the computer button on the left IPC (Industrial Personal Computer) to turn on the power.
2. Double-click the control software icon of **MGIDL-T20RS** on the computer desktop to start the software.
3. The window is displayed prompting whether to run the self-test. Click **Run**, and the system starts the self-test.

   After the self-test is successful, the system enters the main interface.

4. Click ⋮ in the upper right corner, click **Login**, enter the username "genomics", password "genomics", and click **Login**.

5. Click ☰ >**Loading** on the upper left corner to enter the main interface of loading.

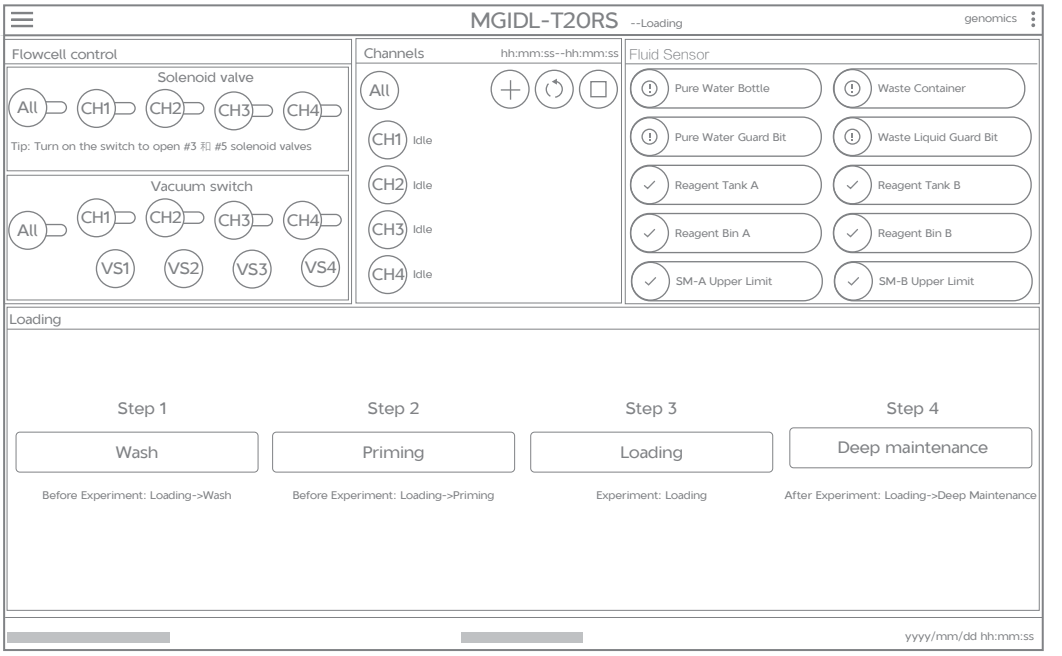


Figure 10 Loading interface

5.4.2 Washing

Perform the following steps:

1. Confirm that the washing cover plate has been taken out.
2. In the experiment interface, select the required channel and click **Wash**. Click **Yes** in the pop-up window.
3. Follow the interface prompts to confirm whether the liquid level of the pure water bottle is lower than the lower limit of the liquid level, and whether the liquid level of the waste container is higher than the upper limit of the liquid level.

   - If the liquid level of the pure water bottle is lower than the lower limit of the liquid level, open the door of the pure water bottle and add pure water until the liquid level exceeds the lower limit.
   - If the liquid level of the waste container is higher than the upper limit of the liquid level, replace the waste container.

   After completion, click **OK**.

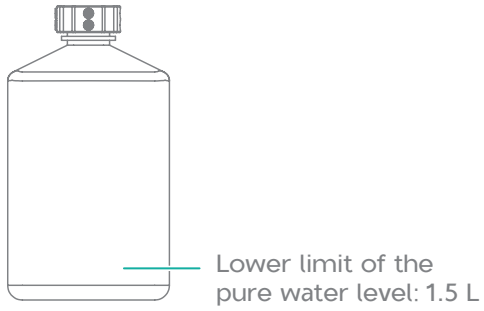


Figure 11 Lower limit of pure water level

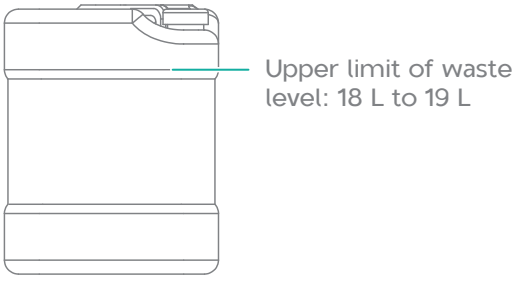


Figure 12 Upper limit of waste level

4. Place the pure water washing cartridge into the reagent cabinet A on the left, and add 4 mL to 5 mL of pure water to DNB washing centrifuge tubes. Close all doors, click **OK**, and the device starts washing.

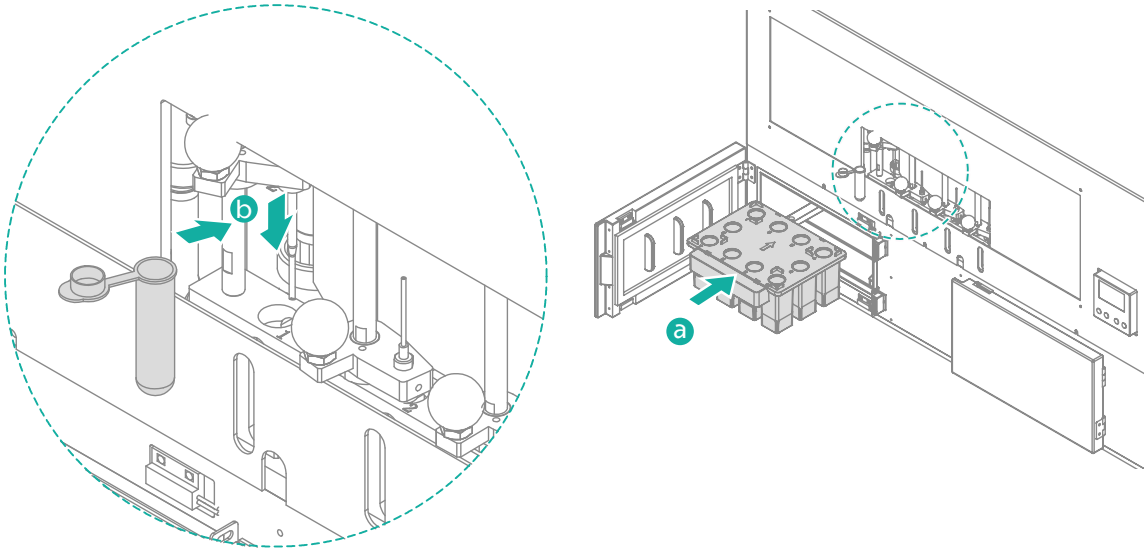


Figure 13 Loading DNB washing centrifuge tubes and pure water washing cartridge

5.4.3 Priming

1. After washing is completed, remove the pure water washing cartridge and ensure that the plastic cover plate used for deep wash has been removed.
2. Confirm required channels and click **Prime**. Click **Yes** in the pop-up window.
3. Place the DNB Loading Reagent Cartridge V4.0 into the reagent cabinet A, check the pure water bottle and waste container on the right side of the device, and click **OK**.

   NOTE When placing the loading reagent cartridge, remove the cover plate and wipe off the residual condensation on the cartridge surface.
4. Add 4 mL to 5 mL of pure water to DNB washing centrifuge tubes, close all cabinet doors and click **OK**.
5. After priming is completed, wipe the waste that flows out of the No.1 valve port with a dust-free paper. After completion, click **OK**.

5.4.4 Loading

Perform the following steps:

1. Click (+), and scan the code to enter the information about the slide, cover plate, DNB and reagent. If the code cannot be scanned, use the keyboard to enter the information. After entering the information, click **Save**.
2. Click **Loading** in the experiment interface, and select **Yes** in the pop-up dialog box.
3. Follow the interface prompts to perform the following steps:

   1) Open the loading compartment door and place the DNB tube.
   2) Open the cabinet door of pure water bottle and check the liquid level of the pure water bottle.

      If the liquid level of the pure water bottle is lower than the lower limit of the liquid level, add pure water until the liquid level exceeds the lower limit.

   3) Check the liquid level of waste container.

      If the liquid level of the waste container is higher than the upper limit of the liquid level, replace the waste container and click **OK**.

4) Open the door of the gas-liquid separation bottle cabinet and check the liquid level of the gas-liquid separation bottle.

   If the liquid level of the gas-liquid separation bottle is higher than the upper limit of the liquid level, pour the liquid in the bottle into the container designated by the laboratory, and dispose of the waste according to the requirements of the laboratory, local laws and regulations, and place the emptied gas-liquid separation bottle back into the cabinet.

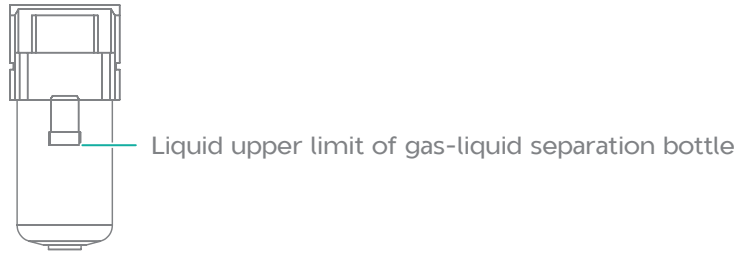


Figure 14 Upper limit of the liquid level of the gas-liquid separation bottle

5) Close all cabinet doors and click **OK**.

4. Follow the interface prompts to place slides on all selected slide stages:

   1) Open the slide stage cover.
   2) Hold the handle of the slide and align the edges of the slide with the edges of the positioning posts on the slide stage in the direction shown (the handle and label are facing down).
   3) Gently place the slide on the slide stage, ensuring that the edge of the slide is tangent to the edge of the positioning post and that it is fully seated on the slide stage.

   NOTE After placing the slides, do not move the slides, otherwise the adsorption may not be successful, or the slides may be damaged when the cover plate is being installed.

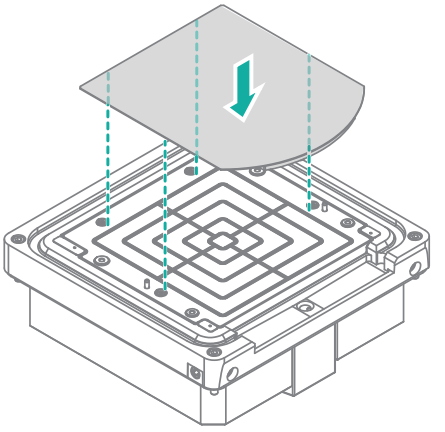


Figure 15 Placing a slide

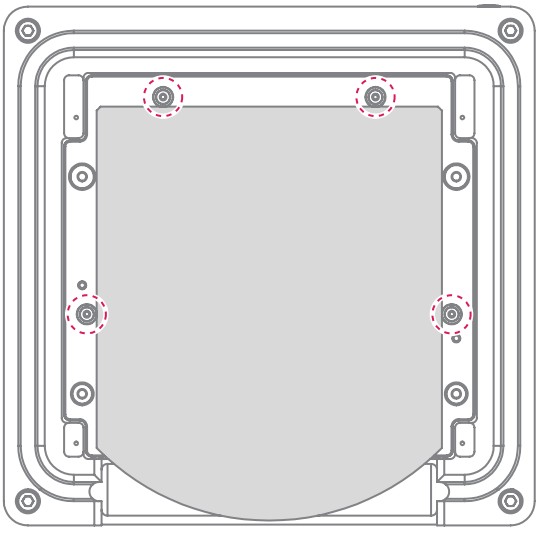


Figure 16 Aligning the slide with positioning posts

4) Click **OK** to adsorb the slides.
5) Confirm again that the slides are correctly installed and click **OK**.
6) (Optional) If the slide is not adsorbed, an exception will be prompted on the interface. Remove the slide within time limit, and clean the slide stage and the back of the slide with a dust-free cloth dipped in 75% ethanol. Do not touch or press the front surface of the slide to avoid damaging the slide or leaving fingerprints and impurities on the glass surface.

   After cleaning, install the slide again, click the vacuum switch of the corresponding channel in the experiment interface, and click **OK** after completion.

NOTE If the slide is accidentally dropped and broken, please handle it with care to avoid scratches.

5. Follow the interface prompts to install glass cover plates for all selected slide stages:

   1) Clean the glass cover plate before use and refer to *4.1.1 Library insert size on Page 10* for specific operations.
   2) Align the positioning holes of the cover with the positioning pins. Ensure that that the side with the sealing ring is facing down, and slowly place it into the slide stage in parallel.
   3) Using a wrench with a set torque (45 cN·m), in ascending alphabetical order (as shown in the figure below), tighten all the screws for fixing the cover plate one turn at first, but do not completely tighten them.

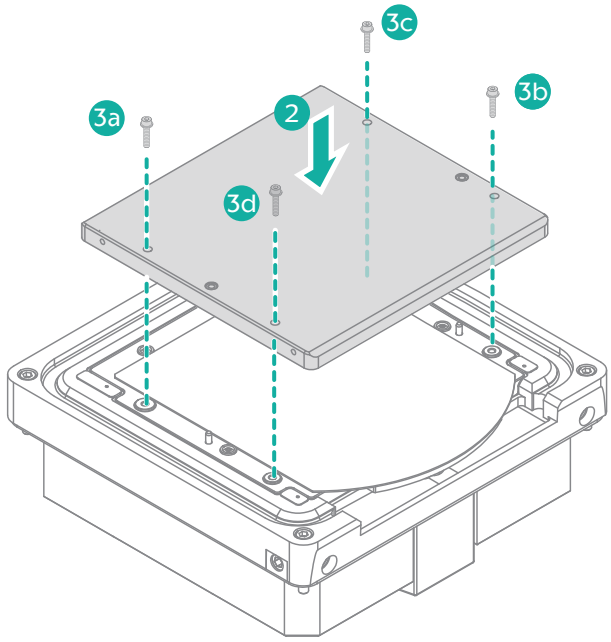


Figure 17 Installing the glass cover plate

   4) After all screws are tightened one turn, use a torque wrench to fully tighten them.
   5) Touch the spacer of the screw with hands, or press the four corners of the glass cover plate lightly to check for looseness. After confirming that all the screws are tightened, click **OK**.

      If after tightening the screws, the cover plate and screws are loosened and cause fluid leakage, refer to *Troubleshooting in Page 38 of MGIDL-T20RS Automated DNB loader User Manual*.

   6) Close the slide stage cover.

6. Click **OK**, and the device starts loading.
7. After loading, remove the glass cover plate and slide according to the interface prompts:

1) Open the slide stage cover.
2) Remove all screws on the glass cover plate in ascending alphabetical order (as shown above), and click **OK**.
3) Confirm that all screws have been removed, and click **OK**.
4) Remove the glass cover plate and click **OK**.
5) Hold the handles and take down the slide, then throw the waste on the slide into a medical waste bin or waste well in front of the stage. Place the slide into a wet slide box with WB2. About the wet slide box, refer to *7.1 Preparing slides on Page 43*. Click **OK** after completion.
6) (Optional) If it is prompted in the interface that the automatic unloading of the slide fails, click the vacuum switch of the corresponding channel in the experiment interface, manually take down the slide, and click **OK**.

   The removed slides can be used for sequencing. If they are not in use, empty the WB2 in the wet slide box, and pour about 800 mL of new WB2 into the box. Place the slides into the wet slide box, close the lid, store the box in a 2 °C to 8 °C refrigerator, and use it within a week.

   NOTE It is recommended to replace the WB2 in the wet slide box after each run.

8. Wipe the slide stage with a dust-free cloth dipped in 75% ethanol and wait for it to dry naturally.

5.4.5 Performing deep maintenance

Perform the following steps:

1. Click **Deep Maintenance** > **Yes**.
2. Place the NaOH washing cartridge into the reagent cabinet A on the left, open the door of the pure water bottle, and check the liquid level of the pure water bottle. After the inspection, add 4.5 mL of 0.1 M NaOH to the DNB washing centrifuge tube, and place the centrifuge tube in the loading compartment. After completion, click **OK**.

   NOTE Ensure that the plastic washing cover plate is installed.

3. Take out the NaOH washing cartridge from the reagent cabinet A on the left, replace it with a pure water washing cartridge, and replace the DNB washing tube with a pure water washing tube according to the prompts. After completion, click **OK**.

NOTE
- After washing the used washing cartridge with pure water for 3 to 5 times, it can be used in the subsequent washing process. It is recommended to replace it after three months of continuous use.
- Add 0.1 M NaOH reagent to 2/3 of the well height of the washing cartridge. About the preparation method of 0.1 M NaOH, refer to *Page 55 "Preparing washing reagents"* .

5.4.6 Washing the glass cover plate

Perform the following steps:

1. The used cover plate is rinsed with RO water, and then placed in the dishwasher with 3 g of sodium percarbonate and 5 mL of brightener.
2. After washing, take out the cover plate. Hold the backside of the cover plate and rinse it with pure water for at least one minute in turn to clean four inlet wells and outlet wells, and two slots. Ensure that the front surface is not touched and no contamination is caused.
3. Place the washed cover plate into an oven, set the temperature to 37 ℃ , and dry for more than 5 hours.

NOTE
- It is recommended to set the washing time to a minimum of 90 minutes without drying.
- For areas with hard water, the dishwasher inlet should use RO water or water in higher grade.
- During the use of a new dishwasher, there may be plastic particles attached to the glass cover plate, so the new dishwasher needs to be run empty for several times. The washed cover plate should be rinsed with RO or higher-grade pure water before being dried and stored in an oven, and placed on a special cover rack.
- If the washed cover is not in use, the cleaned and dried cover plate can be stored in the oven, but it is better to turn off the power of the oven to avoid accelerated aging of the rubber ring of the cover plate.

Chapter 6 Preparing the sequencer and reagents

This chapter describes how to prepare the sequencer and reagents, including checking the completeness of reagent set, thawing, adding and mixing reagents, installing reagent bottles, and maintenance.

6.1 Sequencing instructions

- This sequencer supports up to 8 slides for sequencing at the same time, supports libraries prepared by DNB making kits of MGI or customized libraries, and supports both single-end and paired-end sequencing. A single imager supports simultaneous sequencing of up to 2 slides.
- It is recommended to run 8 slides with the same sequencing library type and sequencing type during each run.
- This sequencer supports the sequencing of 1 to 8 slides, but less than 8 slides will increase the cost of sequencing, so it is generally not recommended.
- The main control software version matched in this manual is DNBSEQ-T10×4RS×1.2.0.237.
- The biochemical and imager parameters of PE100 and PE150 sequencing will not shift automatically with the selection of sequencing types, and need to be manually confirmed according to the user manual. Use PE100 or PE150 sequencing set according to sequencing type requirements. The two sequencing types cannot be performed at the same time.
- The illustrations in the user manual of this set are for reference only and do not fully represent the actual production.

6.2 Preparing the sequencer

Perform the following steps:

1. Clean the surface of the biochemical platform. Use laboratory-grade water to dissolve the salt crystals in the spilled tray of the reaction slot. Clean the crystals of the biochemical platform with a dust-free cloth dipped in laboratory-grade water.
2. Clean the robot grippers. Wipe and clean the grippers of the four-axis and six-axis robot with a dust-free cloth dipped with water to remove the crystals on the grippers.

3. Clean the photoelectric sensor and scanner. Ensure that the photoelectric sensor and scanner on the four-axis robot are clean. If they are not clean, wipe them gently with a dust-free cloth dipped in a small amount of pure water.

Figure 18 Photoelectric sensor

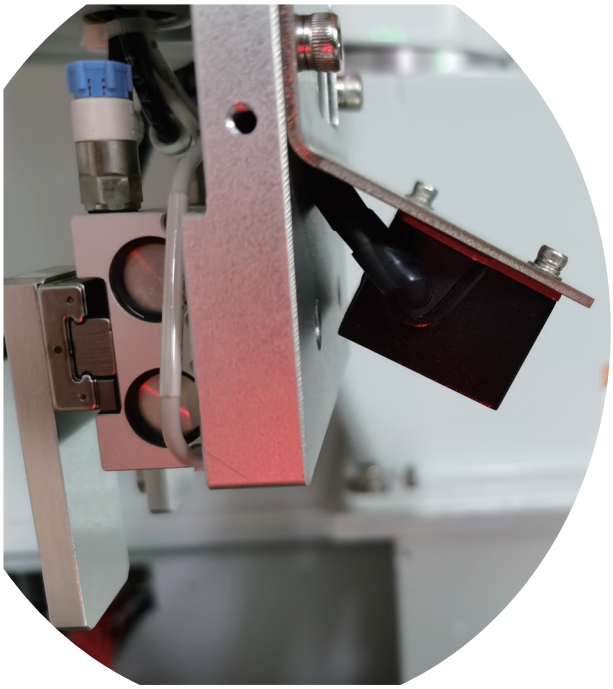


Figure 19 Scanner

4. Clean the imager's slide stage and the Image Reagent needles. Use a dust-free cloth dipped in water to clean the slide stage and the Image Reagent needles.

NOTE In order to avoid damage to the imager's objective lens due to improper operation, the technical support is required for maintenance.

5. Clean the waste collector in front of the imager. Add a small amount of laboratory-grade water to the crystal in the Image Reagent (IR) waste collector in front of the imager. After dissolving, use the special pumping button on the biochemical platform near the waste container to pump it to the waste container.
6. Perform the liquid wash and lens wash.

   For washing rules and operations, refer to *8.2 Washing rules on Page 54*.

7. Ensure that the waste container is replaced and that the connector is connected.

   NOTE
   - Regarding the replacement of the waste container, under normal circumstances, the total volume of waste reagent and washing waste generated after each run is about 50 L, so the standard 60 L waste container needs to be replaced after each round of sequencing and washing.
   - Before transferring the waste container, carefully unscrew the container cover device for connection, cover the double container's cover, and use a trailer to transfer. Please pay attention to personal protection. The waste container should be handled by two people together if necessary.

8. After the above inspection steps are completed, tap the touch screen of the sequencer, double-click **BioPlatform**, and open the biochemical platform software. The software automatically starts to initialize.

   NOTE There are two BioPlatform icons on the desktop. BioPlatform-100%Liquid is suitable for PE100 sequencing, and BioPlatform-70%Liquid is suitable for PE150 sequencing. Ensure that you click the proper BioPlatform icon.

9. After the initialization is completed, confirm whether the current configuration environment is consistent with the selected sequencing type. After completion, click **OK** to enter the main interface.

   NOTE If the operating environment is not displayed in the format of "Sequencing Type+Production", contact the technical support.

10. Click **Run** to enter the biochemical platform operation interface.

11. Return to the desktop and double-click the icon of **DipseqRunnerClient** to launch the imager software.
12. Select all software applications in the tab of each imager successively, click **Start**, and the status is **Running**.

    To stop the program, click **Stop**, and the status is **Not Running**.

    NOTE
    - Do not close any software while the imaging task is in progress.
    - The basecall software must not be closed before each run is completed.

13. Return to the desktop and double-click the icon of **DNBSEQ-T10×4RS** to start the user interface program.
14. After the software is initialized, log in with the user account. If the login is canceled, the account is the guest account by default.
15. After logging in, check the status of each module in the interface.

    If it is unavailable, it means that the module is not connected. Please refer to the previous steps to restart all the software.

    If problems still exist, contact the technical support.

16. Click **System Settings** > **Imager Settings** to set the row and column parameters of the imager and save them. Except that the upload path parameters will be different, it should be consistent with the following.

    - The corresponding imaging area of PE100 reagent set is set to "row 1-90, column 1-90".
    - The corresponding imaging area of the PE150 reagent set is set to "row 1-63, column 1-90".

17. The imager-related configuration requirements of PE100 and PE150 are different. Before sequencing, if changing sequencing type is required, contact the technical support.

6.3 Preparing and installing sequencing reagent bottles

6.3.1 Preparing sequencing reagents

NOTE Please reserve time for thawing the kits.

Perform the following steps:

1. Take out the bottled Sequencing Image Reagent in box 2 stored at -25 ℃ to -15 ℃ from the reagent kit as well as the DIPSEQ Regeneration Reagent in box 4.
2. Thaw the above two reagents in a water bath at room temperature for 6 to 8 hours. After completion, store them in a 2 ℃ to 8 ℃ refrigerator until use. Or, place them in a 2 ℃ to 8 ℃ refrigerator for 2 to 3 days in advance to thaw it.
3. Thaw the bottled DIPSEQ dNTPs Mix, dNTPs Mix II, Block Reagent and AD153 Barcode Primer 2 in box 4 in a water bath at room temperature for 3 hours before sequencing.

   NOTE For dual-barcode sequencing, take out AD153 Barcode Primer 3 (BP3) and Block Reagent (FCL Dual Index) from DNBSEQ-T10×4 RS High-throughput Sequencing Kit (FCL Dual Index) (Cat. No: 1000027931), thaw them and add Block Reagent (FCL Dual Index) into the bottle with Block Reagent.
4. After they are completely thawed, shake the bottled reagents vigorously back and forth for 10 to 20 times until there is no visible stratification in the reagents, and store them in a refrigerator at 2 ℃ to 8 ℃ until use.
5. Before sequencing, take out the thawed bottled DIPSEQ dNTPs Mix and dNTPs Mix II, unscrew the bottle caps, remove the sealing film on the bottle, and wipe off the condensed water on the bottles with dust-free paper.
6. Unscrew the caps of the DIPSEQ Sequencing Reagent 2 (Hot) and DIPSEQ Sequencing Reagent 2 (Cold) and remove the sealing film on the bottle.
7. Pour the DIPSEQ dNTPs Mix into the DIPSEQ Sequencing Reagent 2 (Hot) and pour the dNTPs Mix II into the DIPSEQ Sequencing Reagent 2 (Cold). Take out two bottles of Sequencing Enzyme Mix stored at -25 ℃ to -15 ℃ and add each of them into DIPSEQ Sequencing Reagent 2 (Hot) and DIPSEQ Sequencing Reagent 2 (Cold).

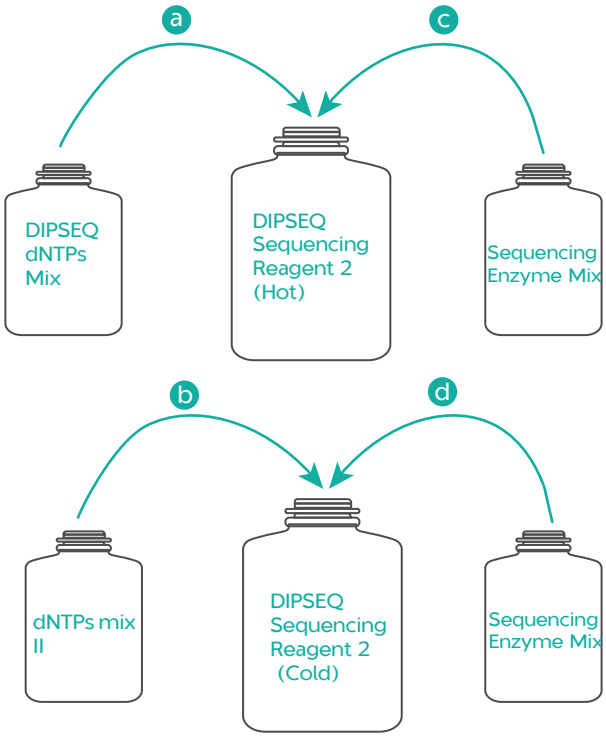


Figure 20 Preparing reagents

8. Seal the bottle mouth with parafilm, tighten the caps and shake these bottles vigorously for 10 to 20 times.

   NOTE Parafilm can prevent condensation and other contamination, but it should be fixed on the mouth to avoid falling into the bottle with the reagent needles.

9. Wrap the prepared DIPSEQ Sequencing Reagent (Hot) with tin foil and store it away from direct sunlight.
10. Take out the attached pure water bottle and fill the bottle with 2 L of pure water.

6.3.2 Installing reagent bottles

Perform the following steps:

1. Shake the bottled reagents vigorously for 5 to 10 times.
2. Unscrew the caps of the reagent bottles one by one with the cap of the DIPSEQ Regeneration Reagent being the last one to be unscrewed.
3. Place the DIPSEQ Image Reagent into the upper layer of the refrigerator with the bottle's mouth facing outwards, and unscrew the cap.

4. Take out the attached aspiration component for the bottle. Insert the aspirating end into the reagent bottle and tighten the aspirating end.
5. Insert the aspiration end to the two connectors.
6. Pull out the reagent base, place the reagent bottles and pure water bottle into the it in order from the inside to the outside according to the reagent names marked on the reagent base.

<table>
<tr><td>Upper layer</td><td colspan="2">IR
Image reagent
9L Bottle</td><td>IR
Image reagent
9L Bottle</td></tr>
<tr><td rowspan="6">Lower layer</td><td>COLD
Sequencing reagent 2 (Cold) 5L Bottle</td><td rowspan="2">RB
Regeneration buffer
10L Bottle</td><td>RR
Regeneration reagent
5L Bottle</td></tr>
<tr><td rowspan="2">HOT
Sequencing reagent 2 (Hot) 5L Bottle</td><td rowspan="2">WB2
Wash reagent 2
5L Bottle</td></tr>
<tr><td>RO-H2O
Pure water for lens wash
2L Bottle</td></tr>
<tr><td rowspan="3">SB2
Sequencing reagent
10L Bottle</td><td>BR
Block reagent
250mL Bottle</td><td rowspan="3">WB2
Wash reagent 2
10L Bottle</td></tr>
<tr><td>BP2
Barcode primer 2
250mL Bottle</td></tr>
</table>

Figure 21 Placement position and volume of reagents

7. Push the base in and close the door of the refrigerator.
8. Press **DOWN** to lower the needle.

Chapter 7 Sequencing

This chapter describes how to complete read 1 sequencing, MDA on MGIDL-T20RS by using MDA reagent kit, read 2 sequencing, reaction of barcode primer adding, and barcode sequencing.

NOTE
- Before sequencing, it is recommended to fill in the information of the loaded slide into the printed sequencing workflow checklist, and use it throughout the process to standardize the sequencing process.
- After sequencing is completed, the server of the sequencer or a special cluster server starts to write FASTQ and split barcode.

7.1 Preparing slides

Perform the following steps:

1. Fill in the sequencing arrangement on the sequencing workflow checklist.
2. Take out the slides to be loaded, and review the slide information.
3. Place slides into the wet slide box that has been preinstalled with WB2.

NOTE
- WB2 is taken from the 2 L bottle in box 1 of the reagent set.
- Instructions for using the wet slide box are as follows:
  - A wet slide box can hold 8 slides, and the box is filled with WB2 to protect the slides loaded with DNBs for transfer and temporary storage. All slots are connected, and a single slide requires 800 mL of WB2. The higher the number of slides, the less WB2 is required.
  - Before use, empty the reagents in wet slide boxes to a waste container. Rinse the wet slide box with laboratory-grade water and add new WB2.
  - Since the loaded slides may need to be stored for a period of time, it is recommended that a single set of sequencers be equipped with at least 4 wet slide boxes. If there are *N* sets of sequencers, match *2×N+2* wet slide boxes.
  - Wet slide boxes should be placed upright with the latches closed to avoid tipping.

7.2 Creating a new sequencing run

7.2.1 Pre-loading Image Reagent (IR) pipeline

NOTE As in the current software version, pre-loading IR pipeline is not included in the reagent pre-loading, so it is done separately in the maintenance interface.

Perform the following steps:

1. Enter the main page of the user interface, and click **Maintenance**. Click **Imager Maintenance** in the left navigation bar to enter the imager maintenance interface.

2. Check **Synchronize To All**. Click any imager in the imager list and fill in the volume parameters, including the following two parameters:
   - For the IR pipeline pre-loading of a new sequencing run, set the pump IR pipe to 0, which means the volume preloaded into the pipeline by default. But all imagers are different. The volume ranges from about 100 mL to 200 mL.
   - For the pre-loading of reagent pipeline for short-term downtime during sequencing, set the pump IR pipe to 2500. Since the imager reagent is preloaded into the imager by using a four-strip tube, the pre-injection volume of each imager is 10 mL.
3. Click **Pump IR pipe**>**OK**, and the pre-loading of the IR reagents of the four imagers starts. The preloaded IR will be dropped directly into the waste collector of the slide stage.
4. After the screen prompts that the injections of the four imagers are completed, click > to return to the main interface.

7.2.2 Creating a new sequencing task

Perform the following steps:

1. On the main page of the user interface, click **New Sequencing.**

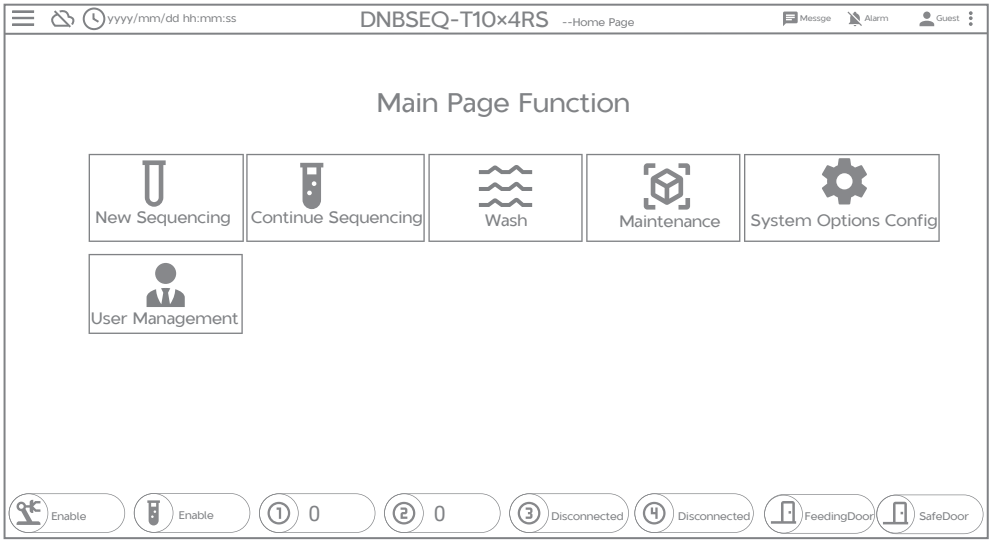


Figure 22 Main page

2. After entering the self-test interface, wait for all modules to complete the self-test. After self-test is completed, click ⟩ to go to the next step.

7.2.3 Entering sequencing information

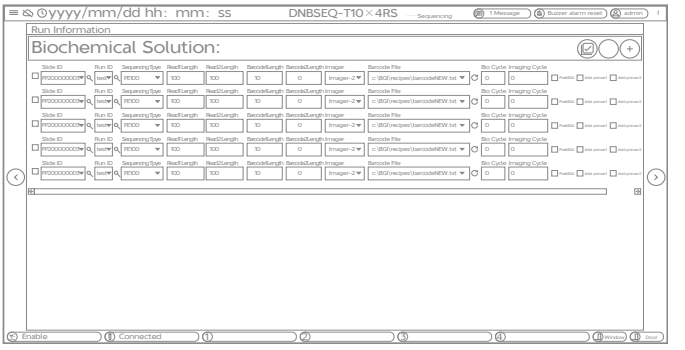
Perform the following steps:

1. Click ⊕ in the upper right corner of the interface, and scan the QR code with the code scanner to enter the slide ID.
2. Click 🔍 next to the slide ID, enter the information of all 8 slides in sequence, and click ⟩ to go to the next step.

   - If the slide ID already exists in the LIMS, the current sequencing information of the slide can be automatically obtained from the LIMS system, and the sequencing information field cannot be modified after it is obtained from the LIMS.
   - If the slide ID does not exist or the query fails, it indicates that the slide is a non-LIMS slide or the LIMS connection is disconnected. For non-LIMS slides, you can choose to manually enter the sequencing task information.

NOTE Instructions for entering slide information:

- When entering slide information manually, slide ID, run ID, sequencing type, imager and barcode file are required options. After selecting the sequencing type, the remaining parameters will be filled in automatically. There is no need to fill in the barcode file for LIMS slides.
- Biochemical cycle and imaging cycle represent real-time cycle numbers, which are automatically updated with sequencing. When loading a new slide, ensure that both cycle numbers are 0. During sequencing, unless the error message clearly indicates that the cycle information needs to be modified, it should not be modified.
- Fields such as PreMDA, Add primer 1, and Add primer 2 are the marks for the completion of the special process in sequencing. After completion, they will be automatically marked as ☑ , and are all unselected when sequencing starts. Slide recall means that the user actively recalls the slides to the recycling area and suspends the sequencing of the slides.
- The standard entry format of sequencing information is as follows (PE100 as an example):



7.2.4 Reviewing information

Perform the following steps:

1. After entering the information review interface, check the complete sequencing information displayed on the interface.
2. After review and confirmation, click ☑ . At this time, the current slide task information is synchronously saved locally on the sequencer and to the LIMS.

If problems are found during the review, click to return to the previous step to modify.

7.2.5 Allocating memory

After the sequencing information is saved and synchronized, the memory allocation window pops up automatically.

Perform the following steps:

1. To allocate memory for all slides, click **Check All**>**Allocate Memory**.

   To allocate memory for a single slide, select the slides requiring memory allocation and click **Allocate Memory**.

2. If the screen prompts that the allocation starts successfully, click **Close**, and the sequencing running interface will be displayed.

   If an error message appears on the screen, follow the prompts to make adjustments.

7.3 Starting sequencing

Perform the following steps:

1. On the sequencing interface, check the 24 reaction slots on the left and the imager's icon on the status bar below to confirm whether there is any incorrectly displayed slide information. The normal state should generally be shown as below. If there is any question, contact the technical support.

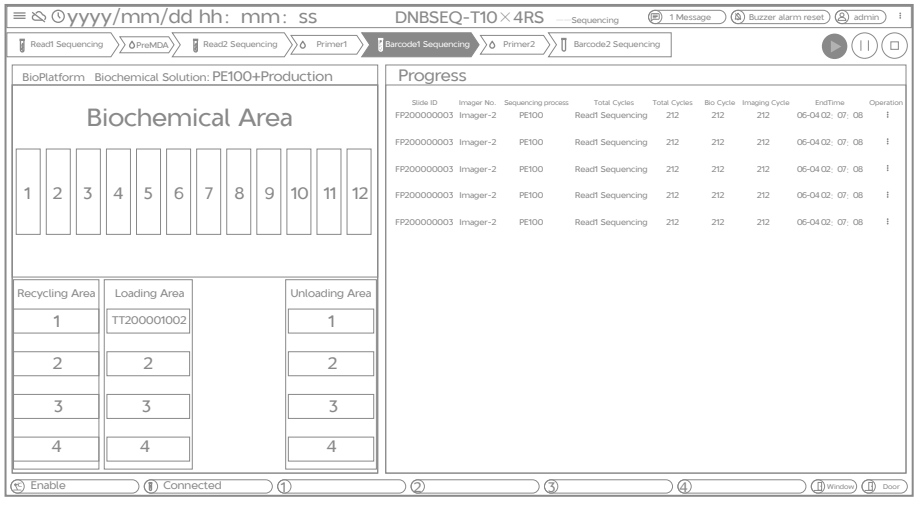


Figure 23 Sequencing interface

2. Access the reagent pre-loading window according to the following two methods:
   - Click **Read1 Sequencing**>**OK**, and wait for the reagent pre-loading window to pop up automatically.
   - Click Running >**Reagent Replacement** at the bottom of the interface to enter the reagent pre-loading window.
3. Select **Sequencing Reagent pre-loading 1**>**Reagent Replacement** to complete the complete reagent pre-loading for a new sequencing run.

   NOTE
   - Sequencing Reagent pre-loading 1 contains the complete pipeline reagent pre-loading, which is used for new sequencing after the sequencer is cleaned.
   - Sequencing Reagent pre-loading 2 is used for short-term downtime of more than 2 hours, to resume the manual reagent pre-loading before sequencing. For example, during sequencing, an error is reported and the downtime lasts for more than 2 hours, or read 2 sequencing is normally performed.
   - To skip or exit the reagent pre-loading step, click **Cancel**.
   - The reagent pre-loading window automatically pops up when switching between sequencing stages or clicking **Start Sequencing**. At this time, ensure that only one of the reagent pre-loadings is performed.
4. After the reagent pre-loading process is completed, the reagent pre-loading window disappears automatically. Click Door , and the two buttons will be automatically unavailable and cannot be clicked.
5. When the maintenance door is unlocked, which means the icon automatically changes to Door , manually open the maintenance door.
6. Manually check the liquid level of all reaction slots except biochemical reaction slots 7, 9, and 12 for obvious problems.
7. After checking that there is no problem, close the maintenance door. Click Door , and the icon changes to Door , which means the maintenance door is locked successfully.

NOTE
- If the sequencing has been started, it will automatically pause. If the door and window are opened successfully, and it will continue automatically if the door and window are closed successfully.
- In PE150 sequencing, the liquid level is about 2/3 of the height of the reaction slot. In PE100 sequencing, the liquid level is about 24 mm away from the opening at the upper end of the reaction slot.

8. Click Window . When it prompts that unlocking is successful and the icon changes to Window , open the feeding window.
9. Take out the slides from the wet slide box and wipe off the handle with a dust-free cloth. Confirm which slot is not color-coded on the recycling area, and gently insert 4 slides into slots. After completion, if the slot turns red, the corresponding slide is detected.
10. After completion, close the feeding window and click Window to lock.
11. After reconfirming that the six-axis robot, biochemical platform and other hardware are connected properly, click ▶ to start sequencing.
    - ➢ To pause sequencing, click ⏸ .
    - ➢ To stop sequencing, click ⏹ .
12. After the slide is scanned successfully, the slide ID is automatically displayed on the interface, and the corresponding slot turns green.
13. After the robot transfers all the four slides in the first round from the recycling area to the loading area and the biochemical area, click Window to open the window and load the other four slides. After completion, click Window to close the window.

NOTE The slides must be handled with gloves. Do not touch the front of the slides during the process, and do not let the front of the slides touch other objects. The slide handle in the reaction slots should face the four-axis robot of the biochemical platform.

14. During running, it is required to observe the status of the sequencer's three-color light and buzzer, and the interface shows the running status in real time.

If there is no failure in the process, read 1 sequencing will be completed for all slides after about 38 hours and the process will automatically switch to preMDA. It takes about 20 minutes for preMDA, and finally all slides will automatically return to the recycling area and reaction slots of the loading area.

If the buzzer beeps for a long time or the three-color light turns red, immediately check the cause of the error.

NOTE
- In the progress area of slide on the interface, both the biochemical cycle and the imaging cycle should be 101.
- Click ⋮ to check the PreMDA status of each slide and all should be selected. Do not modify it manually. If there is any exception, contact the technical support.

15. Open the feeding window and remove all slides from the recycling area and loading area to the wet boxes.
16. Click ☰ > ⊞ to return to the main interface.
17. Click **Maintenance**>**Dip Maintenance**.
18. Select four of the reaction slots in the loading area, and click **Clear Logic Bit** to clear the logical state of the slides in the loading slots that have removed the slides in the loading area.
19. After completion, return to the main interface and refer to *Chapter 8 Washing and maintenance on Page 54* to clean the objective lens and slide stage.

7.4 MDA

NOTE
- This step needs to be performed on MGIDL-T20RS.
- One MGIDL-T20RS can perform MDA of 1 to 4 sequencing slides at a time.
- One MDA reagent cartridge can support up to 4 sequencing slides to perform MDA simultaneously or separately. If MDA is performed separately, the MDA reagent cartridge should be used within 72 h after the MDA enzyme is added to the well, and stored at 2 ℃ to 8 ℃ . To save time, it is recommended to select an MDA that performs 4 sequencing slides at a time.
- During the MDA process, it is necessary to check the water level of the pure water bottle and the waste container, replenish the pure water and replace the waste container in time.
- For the operation methods and precautions of the automated DNB loader and third-party devices, refer to *Page 24 "Loading DNBs"* .

Perform the following steps:

1. Take out the MDA Reagent Cartridge from the box 4 of reagent set package and thaw it at room temperature for about 2 h to 3 h. After thawing, store it in a 2 ℃ to 8 ℃ refrigerator and use within 7 days.
2. Take out the glass cover plate processed by Plasma. For Plasma conditioning, refer to *5.3 Preparing the glass cover plate on Page 26*.
3. Take out MDA enzyme from box 4, and add 5 mL of MDA enzyme into the well with an arrow pointing at as the figure shows below. Mix it thoroughly and store it in a 2 ℃ to 8 ℃ refrigerator until use.

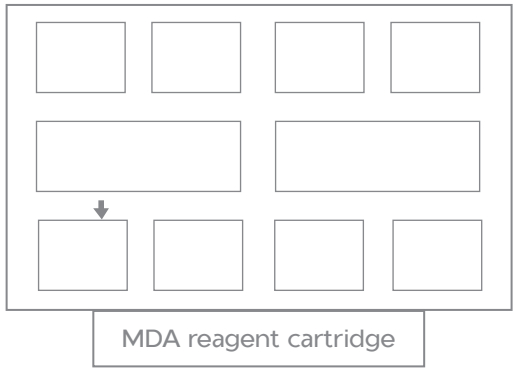


Figure 24 MDA reagent cartridge

4. Open the MGIDL-T20RS application, perform the self-test and log in.
5. Click ☰ >**MDA** to enter the main interface of MDA.
6. Select the required channel and click **Wash**.
7. Place the pure water washing cartridge into the reagent cabinet B on the right to wash the pipeline before MDA.

   NOTE During MDA, the washing cartridge in washing, priming, and reaction slots are placed in the right reagent cabinet B, which is different from the position of the reagents in DNB loading.

8. After washing, confirm the target channel again, place the MDA kit into the reagent cabinet B on the right, and click **Priming**.
9. After priming is completed, click **MDA**, and the whole process lasts about 2.5 h.
10. Place the slide on the slide stage of the first channel. After completion, click **OK** to adsorb the slide.
11. Check whether the slide adsorption position is normal.

- If normal, go to the next step.
- If an error is reported, clean the slide and adsorption platform and then adsorb the slide again.

12. Install the glass cover plate of CH1, tighten all screws, and then click **OK**.
13. Repeat steps 10 to 12 on all selected channels.
14. Continue the MDA reaction as prompted.

NOTE
- Be sure to observe and ensure that all prompts have been acknowledged as the screws need to be tightened for a second time. Otherwise, the MDA process will remain suspended. About 10 min to 15 min after clicking MDA, or 3 min to 5 min after installing the cover of the last channel, a prompt will appear, and the specific time is related to the operation speed.
- Air bubbles during the initial steps of MDA are normal and do not require treatment.

15. After the MDA is completed, follow the prompts to disassemble the slides and place them in a wet slide box for read 2 sequencing.
16. Install the washing cover. According to the prompts of the program, place the 0.1 M NaOH and pure water washing cartridge into the reagent cabinet B for washing. For details, refer to *5.4.5 Performing deep maintenance on Page 34*.
17. Place the glass cover plate in the dishwasher for washing, then place it into the oven to dry. For details, refer to *5.4.6 Washing the glass cover plate on Page 35*.

7.5 Continuing sequencing

Perform the following steps:

1. After MDA is completed, click **Maintenance**>**Imager Maintenance** to perform short-term pre-loading of IR reagents separately. For details, refer to *7.2.1 Pre-loading Image Reagent (IR) pipeline on Page 43*.
2. Click **Continue Sequencing** on the main interface to return to the sequencing running interface.
3. Click **Read2 Sequencing**, and when the reagent pre-loading window pops up, click **Sequencing Reagent pre-loading 2** > **Reagent Replacement** to start the short-term downtime of reagent pre-loading.

4. After pre-loading is completed, perform steps 5 to 12 from *7.3 Starting sequencing on Page 47* to complete liquid level checking and slide loading.
5. The device continues to perform read 2 sequencing, barcode primer adding and barcode sequencing. These steps of sequencing take about 43 h.
6. After the entire sequencing cycle is completed for all the slides, these slides are automatically recalled to the loading area and the recycling area, and the writing FASTQ task of slides is automatically started. At this point, confirm whether both the slide biochemical cycles and imaging cycles are equal to the total sequencing cycles. If so, this sequencing run is completed.

7.6 Maintaining the sequencer

Perform the following steps:

1. Take out all slides to the wet slide box or directly discard them to the designated collection place, and clear the logic bit in the loading area.
2. Click **Stop Sequencing** to exit the sequencing interface.
3. Open the reagent compartment, raise the reagent needles, detach hoses and connectors connecting with the IR bottle according to *Performing a liquid wash and lens wash after long-time shutdown on Page 61.*
4. Take out all the reagents from the compartment, and transfer them to the waste container.
5. Perform the liquid wash and lens wash. For details, refer to *Chapter 8 Washing and maintenance on Page 54*. The entire process of deep wash is expected to take 1.5 h.
6. Perform other routine checkings and washings. For details, refer to steps 1 to 5 from *6.2 Preparing the sequencer on Page 36*. When all is done, dispose of the waste container.
7. Confirm whether the local data can be cleared and whether the engineer has completed the routine maintenance. After confirmation, the next sequencing run can be scheduled.

Chapter 8 Washing and maintenance

8.1 Washing definitions and terminology

NOTE
- The washing of the DNBSEQ-T10×4RS sequencer should be carried out by manually replacing different washing containers according to prompts.
- In the first step of the liquid wash process, selecting the reaction slots and the imagers to be cleaned can complete the liquid wash of the biochemical platform and the imager at the same time.

Washing type	Selection method	Washing definition
General wash	Selecting the method in the wash interface manually	Rinse the residual reagents in the reaction slots and pipelines with pure water.
Deep wash		Use 0.1 M NaOH and pure water to clean residual reagents and impurities in the pipelines to reduce the risk of blockage of pipelines.
Lens wash		Rinse the imager's objective lens with pure water, dissolve and remove the remaining reagents and crystals on the surface of the objective lens, and protect the objective lens and slides.

8.2 Washing rules

Washing protocol	Washing moments
Lens wash	• Within 6 hours after the completion of each sequencing. • During sequencing, bubbles were continuously encountered. • If the device is shut down for a short time (≤12h) before starting sequencing, and the reagent bottle has been installed at this time, the reagent compartment of the sequencer can be used to temporarily store the reagents, and the lens wash can be performed when the machine continues to be used.

Washing protocol	Washing moments
General wash	• Before each sequencing, the time from the deep wash last time is more than 48 hours, but less than 2 weeks. • After the engineer overhauled.
Deep wash	• Within 6 hours after the completion of each sequencing. • Before each sequencing, the time from the deep wash last time is more than two weeks. • After replacing the accessories that contact with the reagents, such as pipelines, reagent needles, and reaction slots. • There are obvious impurities in the original sequencing cycles' images, and other factors have been excluded.

8.3 Preparing washing reagents

Prepare 6 L of 0.1 M NaOH as the washing reagent according to the table below, and store it at 2 ℃ to 8 ℃ after completion. The validity period is 28 days. One deep wash consumes approximately 5 L of 0.1 M NaOH and the rest of washing reagent is used for the deep wash of the loader.

Table 16 Preparing washing reagents

Reagent	Volume (mL)
2 M NaOH solution	50
Pure water	950

8.4 Preparing washing containers and washing slides

Each sequencer is equipped with two sets of washing containers, which are divided into washing container (pure water) and washing container (0.1 M NaOH). The shape is the same, and they are used separately according to the NaOH and water marks on the side.

Each washing container is divided into three parts. The composition, filling amount and the relative direction of the reagent compartment installation are shown in the figure below. Component ① needs to be embedded separately in the upper right corner of component ②.

Before each use, the washing container must be cleaned and checked, and it is recommended to replace the washing container with a new one after 40 times of consecutive uses or every six months. When using the washing container, it is necessary to place it into the reagent compartment first, and then pour the corresponding washing reagent into it. Among them, pour 0.1 M NaOH according to the volume shown in the figure below. The volume of pure water needs to be close to the upper limit of the liquid level. When the above steps are finished, remove and clean the washing container after transferring the residual washing reagent to the waste container.

NOTE 0.1 M NaOH is corrosive, so take care when pouring it out.

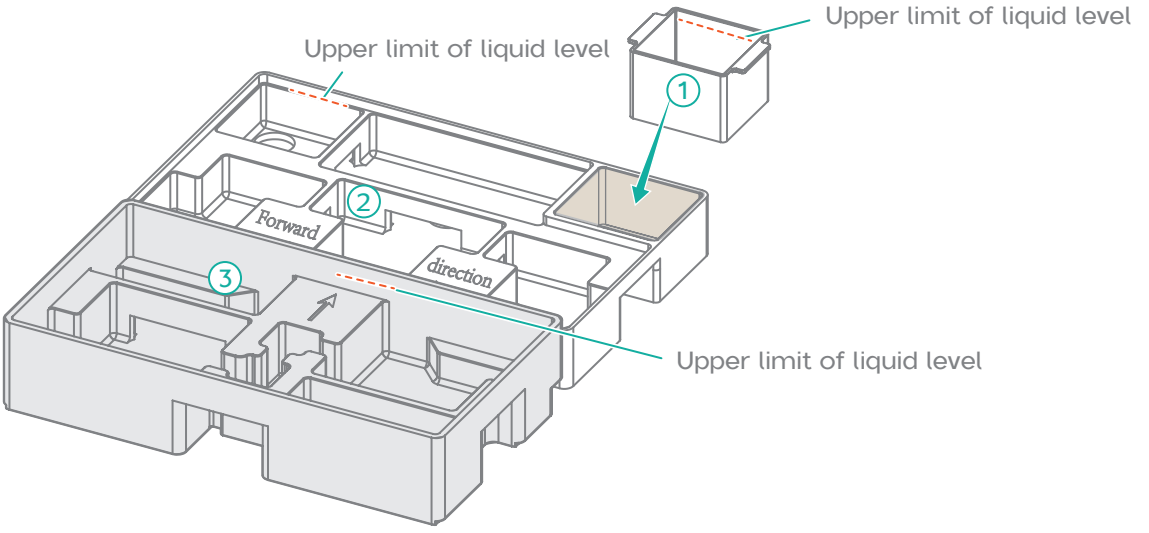


Figure 25 Washing container (pure water)

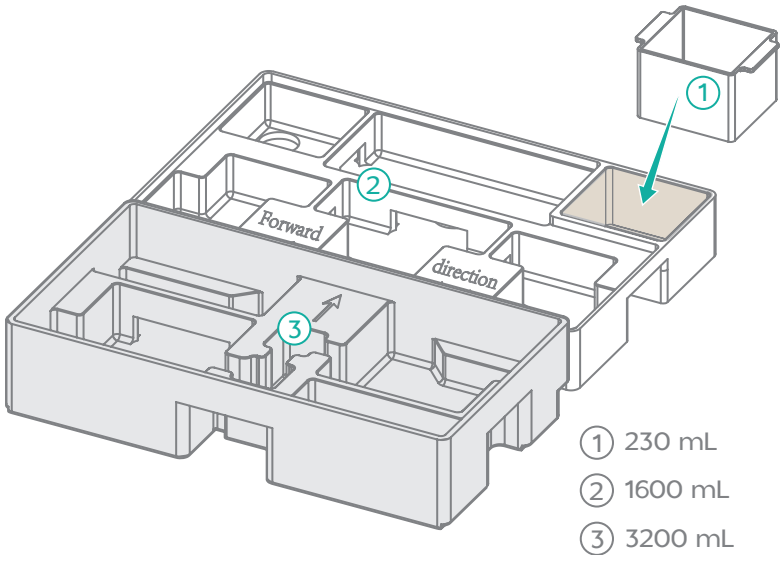


Figure 26 Washing container (0.1 M NaOH)

The washing slides used for the lens wash should be intact and clean. Rinse them with pure water before and after use.

8.5 Washing

8.5.1 Performing a liquid wash

Perform the following steps:

1. Enter the sequencer user interface and click **Wash**.
2. Check **Liquid Wash**, and select **Deep wash** or **General wash** according to the requirements of washing rules.

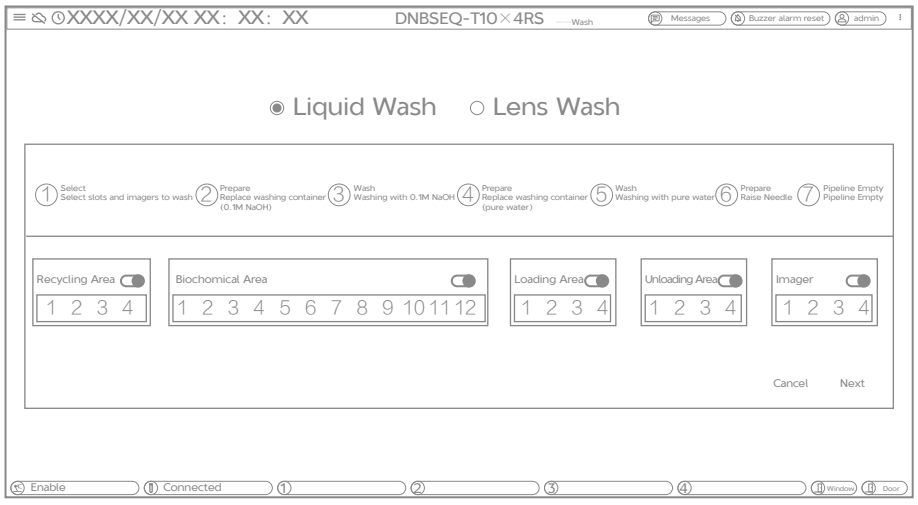


Figure 27 Liquid wash

3. Select the washing range. The optional range includes biochemical fluids (all reaction slots) and 4 imager reagent pipelines, all of which are selected by default.
4. Raise the reagent needles, open the reagent compartment door, replace the original washing container with the required one, and inject the required washing reagent.
5. Replace the connector of the IR reagent bottle to the imager's pipeline with a connector with pure water supply, close the reagent compartment door, and lower the reagent needles.
6. Click **Wash** and wait for the prompt to finish washing.
7. Click **Pipeline Empty** and wait for the prompt to complete the emptying.
8. After completing all the steps as prompted by the process, click **Complete**.

8.5.2 Performing a lens wash

Perform the following steps:

1. Enter the sequencer user interface and click **Wash**.
2. After checking **Lens wash**, select the required imager's objective lens to be cleaned. Normally, all are selected by default.

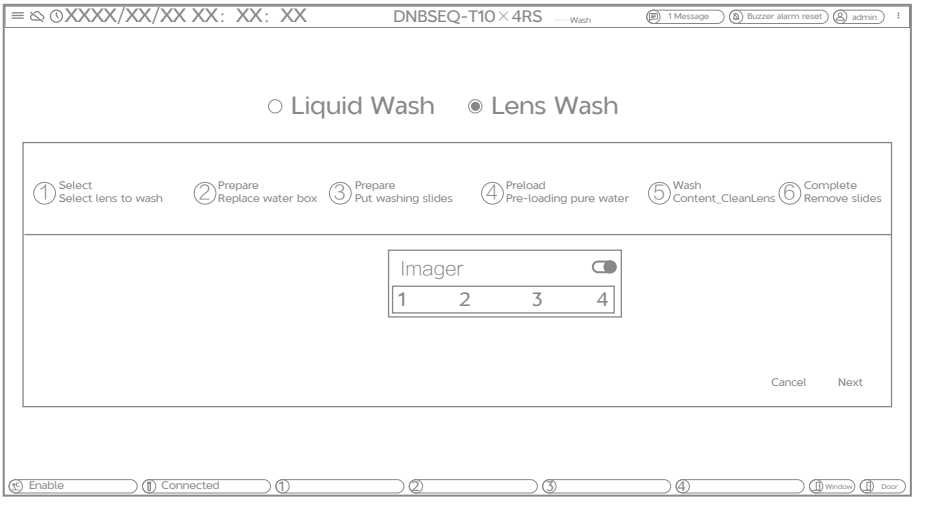


Figure 28 Lens wash

3. Replace the washing container (pure water).

   ➢ If washing is performed before or after sequencing, the preparation of the washing container (pure water) is the same as the liquid wash, and can be shared.

- If washing is to be performed after a temporary downtime in the middle of sequencing and before restarting the machine, use the 2 L pure water bottle installed on the machine, and no additional washing container (pure water) is required.

4. Placing the washing slides. Open the feeding window as prompted, place the washing slides into the recycling cartridge, close the feeding window, and click **Continue**.

   - If washing is performed after a temporary downtime in the middle of sequencing, before restarting the machine, it is required to confirm whether it is already on the sequencing running interface. If so, click **Stop Sequencing** and remove all slides to a wet slide boxes.
   - Collect and clean 1 to 4 used slides in advance as washing slides.

5. Prefill with pure water to ensure that the lens washing pipeline is filled.
6. After completion, click **Next** to start washing the objective lens. At this time, the robot grabs the washing slides to the imager, and the imager's control pipeline injects water into the washing slide. After completion, the pop-up window is displayed prompting that lens wash is completed.
7. Click **Continue**, follow the prompts to open the feeding window, take out all washing slides from the recycling area, and the washing is completed, which takes about 0.5 h.

Chapter 9 Troubleshooting

NOTE This chapter only lists the solutions for common failures. For other problems, contact the technical support.

9.1 Low DNB concentration

Perform the following steps:

1. Use the Qubit ssDNA Assay Kit to perform manual quantitative sampling.
2. Check whether the Make DNB kit is expired or not.
3. Check whether the library meets the requirements or not. If not, try to prepare again.

If it still does not meet the requirements after re-preparation, contact the technical support.

9.2 Abnormal initial sequencing quality

Perform the following steps:

1. Check library quality.
2. Check whether the original image is out of focus or the Heatmap is uneven.
3. Check whether the Make DNB kit is expired or not.
4. Check whether the slides used are expired and whether the storage conditions meet the requirements.
5. Contact the technical support to check whether the MGIDL-T20RS is operating normally.

9.3 Abnormal sequencing run

Most of the abnormal situations in the DNBSEQ-T10×4RS sequencing process can be solved manually by the operator according to the prompts, and the operation will resume without affecting the quality of slide sequencing.

Unless it is an error related to the six-axis robot and the imager platform, the sequencer usually automatically recalls all slides into the loading area and the recycling area, and automatically turns off the heating of the reaction slots.

- Negative pressure failure
  - If the slide is still on the imager, manually retrieve the slide and use a flashlight to assist in checking the slide stage.
  - Wipe lightly with a dipped dust-free cloth to ensure no visible dust or crystals. Never drip water directly onto the slide stage.
  - If the above methods still cannot be solved, and there are three or more repeated errors, contact the technical support.
- Imager's camera reported error: 21275, 21370, 21371, 21372, 21373, 21375

When the error occurs twice or more in the same imager, perform a lens wash. If the error still exists, contact the technical support.

- Hardware error of sequencer

- If the user interface prompts a hardware-related abnormality, recall and store the slide, and contact the technical support. Wait for the problem to be resolved, and reload the slides.

- Sequencer temporary downtime and restart
  - If the device fails, and the issue cannot be resolved temporarily, it is necessary to check the slide recall function of all slides, wait for all slides to be automatically transferred to the recycling slot and the feeding slot, open the feeding window and maintenance door, and return all slides to the wet slide box, that is, the temporary downtime is completed.
  - If the downtime is less than 2 hours, place the slide back in the recycling area and restart directly. If it is longer than 2 hours, refer to *7.2.1 Pre-loading Image Reagent (IR) pipeline on Page 43* to preload the reagent for a short time and then restart.

- Performing a liquid wash and lens wash after long-time shutdown

  Before wash, perform the following steps to detach the hoses and connectors connecting with the IR bottle:

  1) Fold the hose in half close to the connector and pinch it with hands. Detach the hose and connector while pinching the folded hose.
  2) Quickly raise the connector up to 15 cm higher from the bottle cap for 10 seconds to have the liquid inside the hose to flow back into the IR bottle and place the hose and connector on the upper surface of the bottle.
  3) Repeat step 1 and step 2 to detach another hose and connector.
  4) Twist the two hoses around the bottle cap and place the connectors above the bottle cap.

9.4 Temporary storage of the set

- If the components of the set, including DIPSEQ dNTPs Mix and dNTPs Mix II, have been thawed, and the device cannot be used within 7 days, it can be stored in a freezer at -25 ℃ to -15 ℃ for up to two more freeze-thaw cycles.
- If the components of the set have been thawed (including DIPSEQ dNTPs Mix and dNTPs Mix II), and the device can be used within 7 days, it can be temporarily stored at 2 ℃ to 8 ℃ and used within 24 hours. If it cannot be used for more than 24 hours, it can be restored at -25 ℃ to -15 ℃ for up to two more freeze-thaw cycles.
- If the DIPSEQ dNTPs Mix, dNTPs Mix II and Sequencing Enzyme Mix have been added to the sequencing reagent, but cannot be used

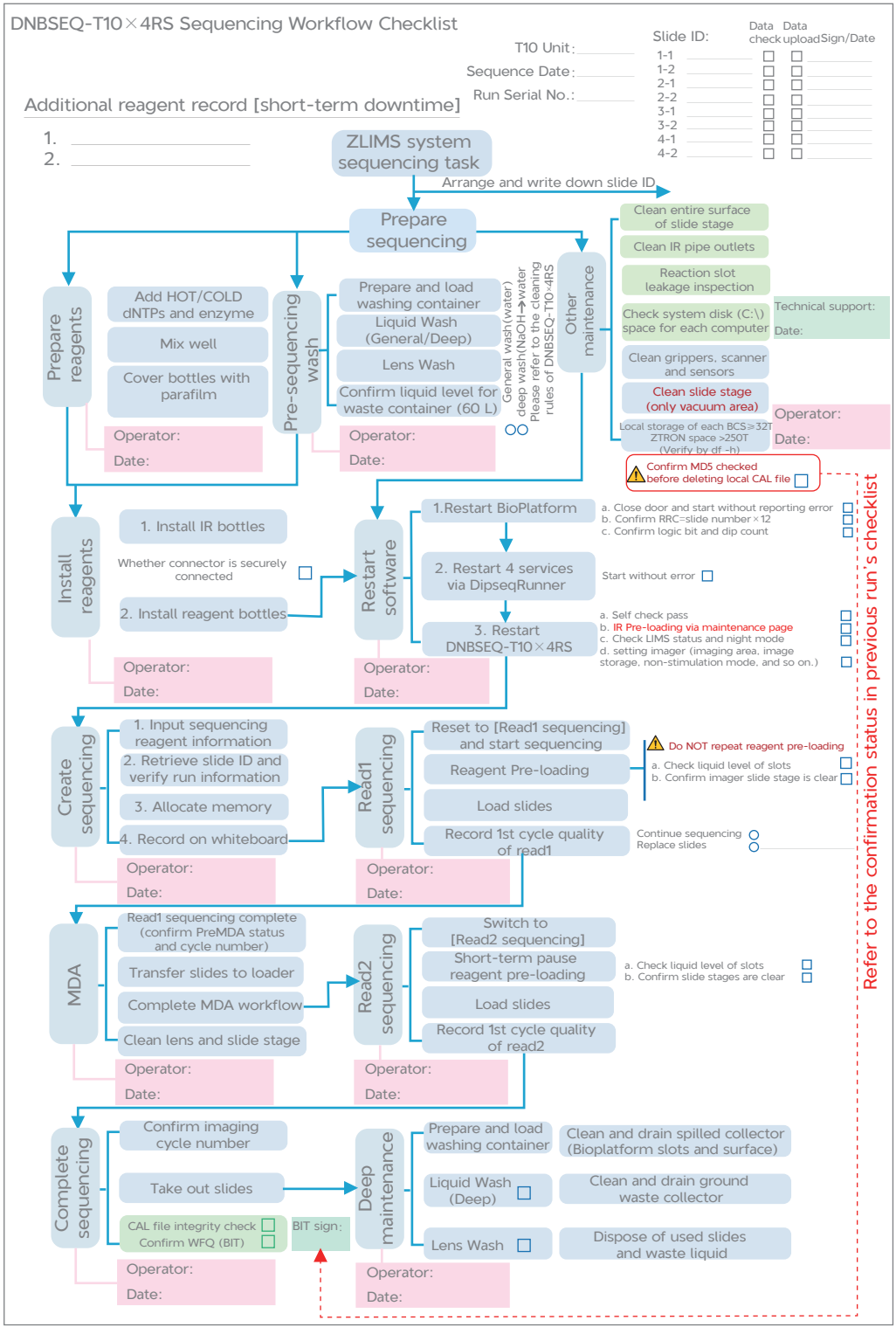
in time, they can be temporarily stored at 2 ℃ to 8 ℃ and used within 24 hours. It can be used within 7 days with risks, but cannot be restored at -25 ℃ to -15 ℃ again.

- If DIPSEQ dNTPs Mix, dNTPs Mix II and Sequencing Enzyme Mix have been added to the sequencing reagent, and installed on the reagent compartment and the needles have been lowered, but at this time, the device cannot be used in time for other reasons, or it is temporarily shut down in the middle of sequencing, with device conditions allowing, it is recommended to use the sealing film to seal the bottle mouth after raising the reagent needles, and then keep it in the reagent compartment of the sequencer for temporary storage, or place it in other laboratory refrigerators at 2 ℃ to 8 ℃ for temporary storage, and use it within 24 hours, which can be loaded within 7 days with risks.
- After sequencing is completed normally, the remaining reagents cannot be used further and must be dumped into the waste container. If the sequencing ends abnormally due to device failure or other reasons, and there are too much residual reagent, please contact the technical support.

Appendix 1 Manufacturer information

Manufacturer	Qingdao MGI Tech Co., Ltd.
Address	Building 4, No. 2, Hengyunshan Road, Qingdao Area, Pilot Free Trade Zone, Shandong, China.
Service hotline	(+86) 4000-688-114
E-mail	MGI-service@mgi-tech.com
Website	www.mgi-tech.com

Appendix 2 Sequencing workflow checklist



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