Vector and Microorganism Identification Package (RNA workflow + DNBSEQ-G50RS) Quick Start Guide

• Part No.: H-020-000865-00
• Version: 2.0
• Release date: October 2024

Applicable sets/kits

Name		Cat. No.
MGIEasy Fast RNA Library Prep Set (16 RXN) Cat. No.: 940-000890-00	MGIEasy Fast RNA Library Preparation Kit	940-000887-00
	MGIEasy UDB Primers Adapter Kit	1000022800
	MGIEasy DNA Clean Beads	940-001176-00
MGIEasy Fast RNA Library Prep Set (96 RXN) Cat. No.: 940-000889-00	MGIEasy Fast RNA Library Preparation Kit	940-000888-00
	MGIEasy UDB Primers Adapter Kit B	1000022802
	MGIEasy DNA Clean Beads	940-001174-00
DNBSEQ OneStep DNB Make Reagent Kit (OS-DB)		1000026466
(Optional) Standard Library Reagent (PCR Product) V4.0, (UDB57-64)		1000027585
DNBSEQ-G50RS High-throughput Rapid Sequencing Set (FCS SE100)		940-001639-00
DNBSEQ-G50RS High-throughput Sequencing Set (FCL SE100)		940-001631-00
Vector and Microorganism Identification Software		970-000331-00

Recommended consumables

Name	Cat. No.	Brand
0.5 mL SC micro tube	1000001558	MGI
2 mL SC micro tube	1000001553	MGI
250 µL automatic tip with filter	1000000723	MGI
1.3 mL U-bottom deep-well plate	1000004644	MGI
Hard-shell thin-wall 96-well skirted PCR plate	091-000165-00	MGI
Break-away 8-strip PCR Tubes and Caps	100-000016-00	MGI

Preparing RNA libraries

Preparing samples

1. The starting amount of each sample is recommended to be 100 ng for RNA library preparation. Add nuclease-free water to the sample until the total volume reaches 10 µL.

Tips

- If there are specific requirements, the starting amount can be adjusted within the range of 10 ng to 1 µg. Supplement the sample with nuclease-free water to 10 µL.
- Use Agilent 2100 Bioanalyzer to perform quality control of extracted total RNA sample. RIN value should be ≥ 7. If RIN < 7, appropriately increase the RNA input and the number of PCR cycles in the library preparation.
- RNA integrity: 1.8 ≤ OD260/OD280 ≤ 2.0, OD260/OD230 ≥ 2.

2. Transfer 10 µL of each sample to Hard-shell thin-wall 96-well skirted PCR plates. When preparing 8 samples, transfer the RNA samples to the first column of the PCR plate; when preparing 16 samples, transfer the RNA samples to columns 1 and 2 of the PCR plate. If the number of samples is less than 16, fill in the remaining tubes with Nuclease-free water. Place it on ice for further use.

• Part No.: H-020-000865-00
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Preparing reagents

1. Take out all reagents from MGIEasy Fast RNA Library Preparation Kit. Thoroughly mix the enzyme by reversing it several times, centrifuge briefly, and then place it on ice for further use. Take out buffer components and thaw them at room temperature. Centrifuge briefly and place them on ice for further use.

Tips

Use Second Strand Buffer (with dNTP) for library preparation.

2. Take out the DNA clean beads from the MGIEasy DNA clean beads reagent cartridge 30 min prior to the experiment, thoroughly mix them by using a vortex mixer, and then centrifuge briefly.
3. The amount of adapter used in Adapter Ligation depends on the amount of RNA input. Dilute the UDB Adapters by using TE Buffer according to the following table. Vortex 3 times for 3 s each, centrifuge briefly, and place them on ice for further use.

Total RNA (ng)	UDB Adapter	
	Dilution Ratio	Input after Dilution (μL)
201-2500	No dilution	5
51-200	5	5
10-50	10	5

4. Take out 1 or 2 strips of PCR 8-strip tubes and caps (MGI, Cat. No.: 100-000016-00). For 8 RNA samples, transfer 8 sets of UDB PCR Primer Mix to one strips of 8-strip PCR tubes. For 16 RNA samples, transfer 16 sets of UDB PCR Primer Mix to 2 strips of 8-strip PCR tubes, 8 μL per tube.

Tips

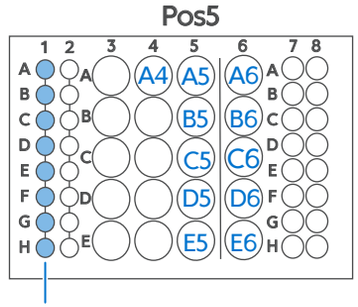
If the samples are loaded on one flow cell, ensure that barcodes corresponding to the libraries are base-balanced.
Otherwise, the following options are recommended:

- Take 30 ng of Standard library reagent (PCR product) V4.0, (UDB57~64, Cat. No.: 1000027585) alone to make DNBs. Mix the DNBs prepared from the test library with those from the standard library at a mass ratio of 4:1 to make DNBs for sequencing.
- When preparing libraries, mix different UDB Primer Mixes and synchronize the barcode split file on the sequencer. For details, contact the technical support.

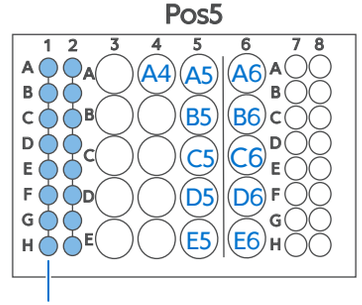
5. Take out the following reagents from the kit, repack into nine 0.5 mL SC micro tubes (MGI, Cat. No.: 1000001558) and two 2 mL SC micro tubes (MGI, Cat. No.: 1000001553) based on the number of samples required for library preparation (8 RXN or 16 RXN) and the insert size required for library preparation (200 bp or 270 bp) according to the following table and figure. After preparation, place them on ice for use. Place the DNA Clean Beads at room temperature before use.

Reagent	Consumable	Location	Reagent input for different sample volume (μL)			
			200 bp		270 bp	
			16 RXN	16 RXN	8 RXN	16 RXN
Fragmentation Buffer	0.5 mL SC micro tube	Pos5 A4	65	130	65	130
RT Buffer	0.5 mL SC micro tube	Pos5 A5	55	110	55	110
RT Enzyme Mix	0.5 mL SC micro tube	Pos5 A6	11	22	11	22
Second Strand Buffer (with dNTP)	0.5 mL SC micro tube	Pos5 B5	240	480	240	480
Second Strand Enzyme Mix	0.5 mL SC micro tube	Pos5 B6	42.3	84.6	42.3	84.6
Ligation Buffer	0.5 mL SC micro tube	Pos5 C5	235	470	235	470

Reagent	Consumable	Location	Reagent input for different sample volume (μL)			
			200 bp		270 bp	
			16 RXN	16 RXN	8 RXN	16 RXN
DNA Ligase	0.5 mL SC micro tube	Pos5 C6	14.8	30.4	14.8	30.4
UDB Adapter	0.5 mL SC micro tube	Pos5 D5	55	110	55	110
PCR Enzyme Mix	0.5 mL SC micro tube	Pos5 D6	240	480	240	480
DNA Clean Beads-1	2.0 mL SC micro tube	Pos5 E5	300	620	360	720
DNA Clean Beads-2	2.0 mL SC micro tube	Pos5 E6	400	780	390	780



UDB PCR Primer Mix (8 RXN)



UDB PCR Primer Mix (16 RXN)

6. Prepare 8 mL (8 RXN) or 16 mL (16 RXN) 80% ethanol by using absolute ethanol and Milli-Q water.

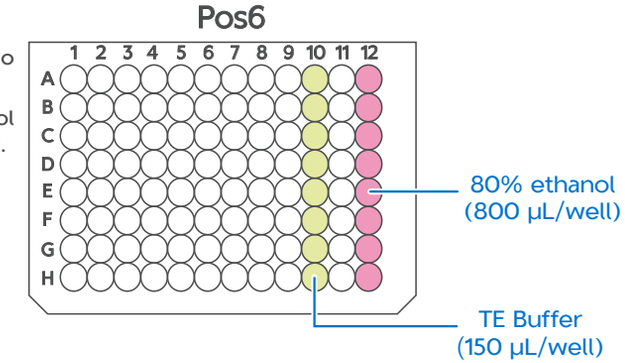
Tips

The 80% ethanol should be used immediately after preparation.

7. Take out a deep-well plate (MGI, Cat. No.: 1000004644) and add TE buffer and 80% ethanol into the deep-well plate according to the figure below:

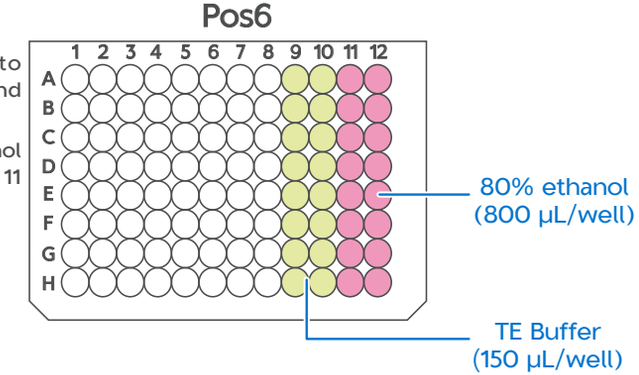
8 RXN:

- Add 150 μL of En-TE into each well of column 10.
- Add 800 μL of 80% ethanol into each well of column 12.



16 RXN:

- Add 150 μL of En-TE into each well of column 9 and 10.
- Add 800 μL of 80% ethanol into each well of column 11 and 12.



Preparing libraries on MGISP-100RS

1. Set **Solution** and **Script** in the Run Wizard interface of MGISP-100RS according to the table below:

Solution	JB-A06-101 MGIEasy Fast RNA Library Prep Set_RV1.0_SV5.0
Script	MGIEasy Fast RNA Library Prep Set (8RXN)_RV1.0_SV5.0.py or MGIEasy Fast RNA Library Prep Set (16RXN)_RV1.0_SV5.0.py

Tips

Ensure that the scripts and PCR programs are imported successfully before operation.

2. Mix DNA Clean Beads thoroughly by using a vortex mixer and centrifuge them briefly before placing consumables.

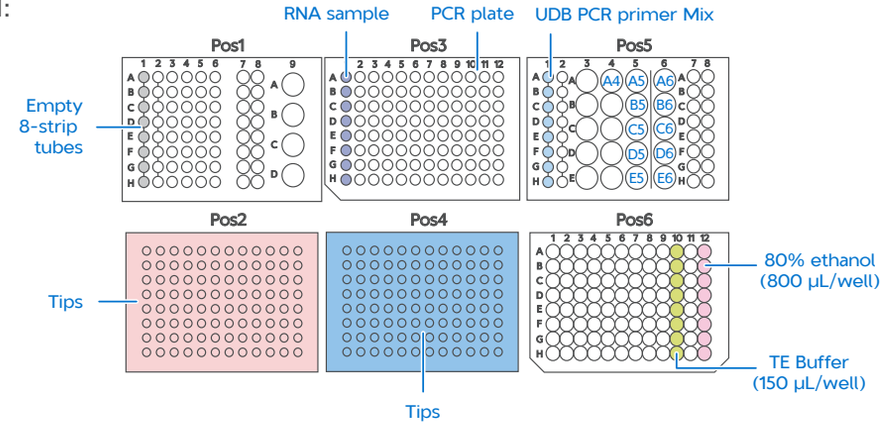
Tips

Ensure that no bubble exists at the bottom of other tubes, no liquid exists on the inner wall, and all tubes are uncapped.

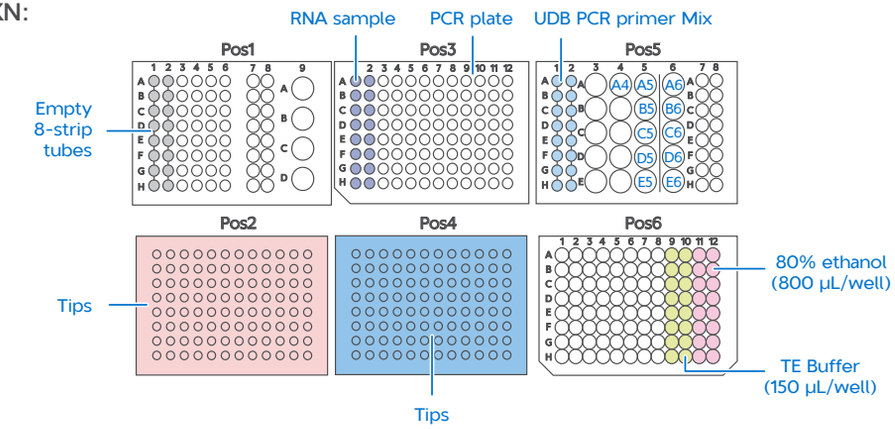
3. Place the 96-well PCR plate in a plate centrifuge and centrifuge briefly. Ensure that no bubbles exist at the bottom of the tube and no liquid remains on the side wall.

4. Place the consumables according to the figure below:

8 RXN:



16 RXN:



5. Click **Run** to start the process. In the pop-up windows, set the fragmentation condition (**270 bp** is recommended), PCR cycles, and sample pre-processing method (**Other**), and then select **Continue**.

Insert size	Fragmentation condition	Fragmentation duration
200 bp	94 ℃	6 min
270 bp	87 ℃	6 min

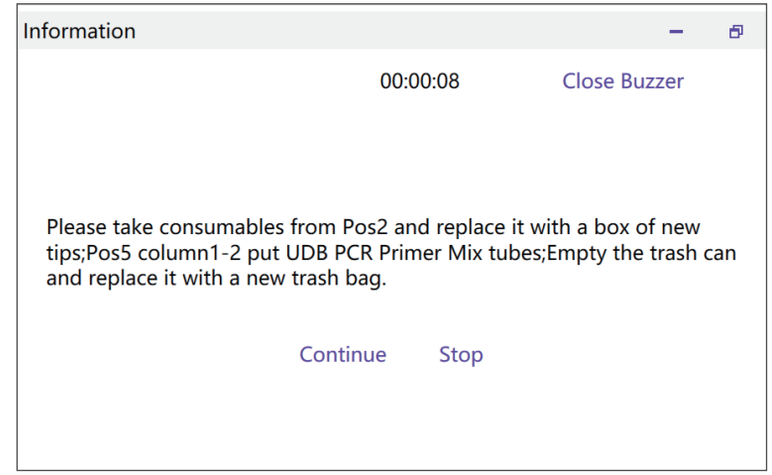
Total RNA (ng)	PCR cycles
10	16-18
50	15-16
100	14-15
200	13-14
1000	11-12

6. If the number of RNA samples is 16, after running continuously for 2 h, the ligation reaction proceeds in the PCR device, and a new operation deck arrangement figure is displayed. And you will be prompted to perform a list of operations, as shown in the following figure. Take out the consumables from Pos2 and replace them with a box of new tips.

   If the number of RNA samples is 8, no need to replace consumables or reagents in the whole process.

Tips

It is recommended that you complete the steps by following the on-screen instructions within 10 min.



7. After about 4 h to 4 h 40 min (Based on the sample number, insert size, and RCA time), the workflow completes. Take out the PCR product from Pos1 column1 (8 RXN) or Pos1 column1 and column2 (16 RXN), in a volume of 30 µL/well.
8. Quantify them with dsDNA Fluorescence Assay Kits. The required yield for PCR products is no less than 3 ng/µL.

Tips

The PCR products can be stored in a -20 ℃ freezer temporarily.

9. (Optional) If no experiment will be conducted on the day, clean the operation deck with pure water and 75% ethanol, and perform a post-clean. Place the discarded sample tubes, reagent tubes, deep-well plates, and waste bag in the designated waste area.

• Part No.: H-020-000865-00
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Making OS DNBs

Preparing samples

Tips

Ensure that the data output of each sample is ≥ 20 M reads. If the samples are loaded on one flow cell, ensure that barcodes corresponding to the libraries are base-balanced.

1. Prepare all the libraries in equal quantities and transfer them into a 0.2 mL PCR tube. Mix the tube thoroughly by using a vortex mixer. Take 30 ng of the mixed library to make DNBs. The volume should be less than or equal to 20 μL.

Tips

If the total volume is less than 20 μL, add TE Buffer to the sample until the total volume reaches 20 μL.

2. Take out the following reagents from DNBSEQ OneStep DNB Make Reagent Kit (OS-DB) to prepare the following reaction solution.

Component	Volume (μL)
Mixed library	V
TE Buffer	20-V
Make DNB Buffer (OS-DB)	20
Total volume	40

3. Vortex and briefly centrifuge the PCR tube to collect the reaction solution at the bottom of the tube.
4. Place the PCR tube in a PCR device and start a reaction according to the conditions in the table below:

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

5. After the reaction is completed, take out the PCR tube, place it on ice, and add the following reagents:

Component	Volume (μL)
Make DNB Enzyme Mix I	40
Make DNB Enzyme Mix II (OS)	4
Total volume	44

6. Vortex and briefly centrifuge the PCR tube to collect the reaction solution at the bottom of the tube.
7. Place the PCR tube in a PCR device and start a reaction according to the conditions in the table below. The total reaction volume should be 84 μL.

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

Tips

It is recommended to set the temperature of the heated lid to 35 ℃ or as close as possible to 35 ℃ .

8. After the reaction is completed, add 20 µL of Stop DNB Reaction Buffer to the PCR tube. Pipette the solution 10 times with a wide-bore pipette tip to mix the solution thoroughly.

Tips

Do not vortex or shake the tube or pipette the solution vigorously.

Stoppoint

The DNBs can be placed at 4 °C for up to 48 h.

9. Take out 2 µL of the DNBs and quantify it by using Qubit® ssDNA Assay Kit. The concentration of the DNBs should not be less than 8 ng/µL.

Tips

If the concentration exceeds 40 ng/µL, dilute the DNBs to the concentration of 20 ng/µL with DNB Load Buffer I.

Performing sequencing

Preparing the DNB loading mixture

1. Take out DNB Load Buffer I and DNB Load Buffer II from DNBSEQ-G50RS High-throughput Sequencing Set and place it at room temperature for at least 30 min.
2. Mix the buffers thoroughly by using a vortex mixer for 5 s, centrifuge them briefly, and then place them on ice until use.
3. Take out a new 0.5 mL SC micro tube. Add the reagents to the tube according to the following table:

DNB volume (µL)	DNB Load Buffer I (µL)	DNB Load Buffer II (µL)	Make DNB Enzyme Mix II (LC) (µL)
100	50	50	1

4. Mix DNB loading mixture 10 times by using a wide-bore pipette tip to mix it thoroughly. Then, place it at 4 °C until use.

Tips

- Use the DNB loading mixture immediately after preparation.
- Do not centrifuge, vortex, or pipette the DNB loading mixture vigorously.

Preparing the sequencing reagent cartridge

1. Select the sequencing recipe based on different scenarios:

Tips

If the samples are loaded on one flow cell, ensure that barcodes corresponding to the libraries are base-balanced.

- When the number of RNA libraries is less than or equal to 4, select FCS SE100.
- When the number of RNA libraries is less than or equal to 20, select FCL SE100.

2. Take out the sequencing reagent cartridge, thaw it in a water bath at room temperature for about 3 h to 4 h, and place in a 2 °C to 8 °C refrigerator until use.
3. Take out dNTPs Mix III and dNTPs Mix II one hour in advance, thaw them at room temperature, and place them at 4 °C until use.

• Part No.: H-020-000865-00
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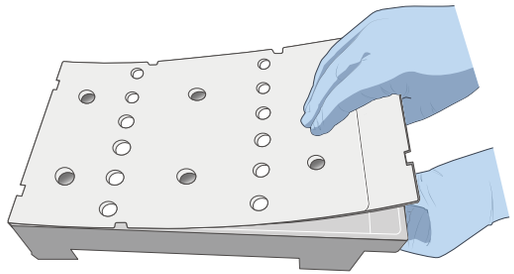
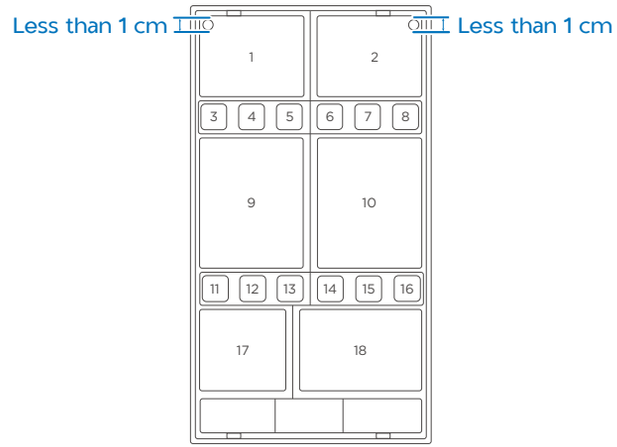
4. Invert the cartridge 3 times before use. Shake the cartridge vigorously up and down 20 times, and then left and right 20 times. Ensure that the reagents are thoroughly mixed, especially the reagents in well No. 17 and No. 18.
5. Open the cartridge cover and wipe the water condensation with a dust-free paper.



6. Gently pierce two holes with its diameter smaller than 1 cm at the edge of Wells 1 and 2 by using a new 1 mL pipette tip.



7. Take out a 1 mL pipette and add the reagents according to the tables below:
   - FCS SE100

Name	Volume (mL)	
	Well No. 1	Well No. 2
dNTPs Mix III	0.320	/
dNTPs Mix II	/	0.560
Make DNA Enzyme Mix	0.320	0.280

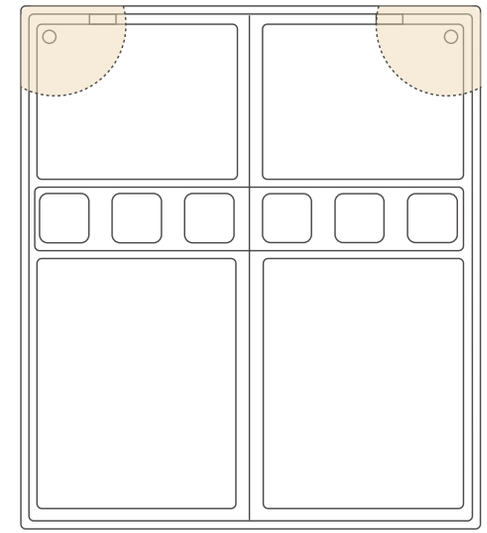
   - FCL SE100

Name	Volume (mL)	
	Well No. 1	Well No. 2
dNTPs Mix III	0.440	/
dNTPs Mix II	/	0.760
Make DNA Enzyme Mix	0.440	0.380

8. Cover the holes with transparent sealing films.

Tips

Do not cover the center of the holes to avoid interfering with the reagent needle.



9. Hold both sides of the cartridge with both hands and shake it 20 times in a clockwise and counterclockwise direction.

Tips

- It is not recommended that you shake the cartridge violently.
- To prevent the reagent from spilling out of the cartridge, do not tilt it or shake it up and down.

Inputting sample information

1. Launch the Chrome™ browser, type the following address in the address bar, and press the **Enter** key:

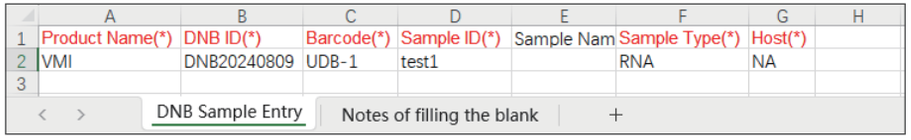
   127.0.0.1

2. Enter the username *lite* and password *lite123456*, and tap **Login**.
3. Click **Sequence + Analysis** on the Home page to open New Sequencing + Analysis page.
4. Select **VMI** or set analysis product to **VMI**, select **Import the Sample ID**, and tap **New**.

Tips

The import method is used as an example. For details, refer to *Vector and Microorganism Identification Software User Manual*.

5. Tap **Excel Template** or **CSV Template** in the sequencing + analysis import window to download the sample template in *.xlsx* or *.csv* format.
6. Open the template, fill in the worksheet, and save it to the designated directory.



	A	B	C	D	E	F	G	H
1	Product Name(*)	DNB ID(*)	Barcode(*)	Sample ID(*)	Sample Nam	Sample Type(*)	Host(*)	
2	VMI	DNB20240809	UDB-1	test1		RNA	NA	
3								

DNB Sample Entry | Notes of filling the blank | +

Tips

- A field with an asterisk (*) is required, and others are optional.
- Cells in Excel template cannot be merged, and no spaces are allowed before or after the contents in each cell.
- Some fields include drop-down options, and other fields require manual input. The inputting method varies among the fields.
- **DNB ID**: The DNB ID should include both letters and numbers. It cannot be the same as the DNB ID that already exists in ZLIMS Lite.
- **Barcode**: When one sample ID corresponds to multiple barcodes, the multiple barcodes are separated by half-width commas (,). For multiple consecutive barcodes without letters, a tilde (~) can be used.
- **Sample ID**: It should be a combination of letters and numbers and be unique for identifying the sample.
- **Sample Name**: The same name indicates the same sample. If both the sample names and sample types are the same, the data will be merged for analysis.
- For the vector and microorganism identification analysis pipeline, fill in *NA* in the **Host** column. The software will perform filtering based on the host information obtained from the vector identification results.

7. Return to the sequencing + analysis import interface, tap **Choose File**. Select the completed worksheet in the pop-up box and tap **Upload**. The New Sequence + Analysis page returns to view after importing the DNB sample information.
8. After reviewing the sample information, tap **Save**, and then tap **OK** in the pop-up window.

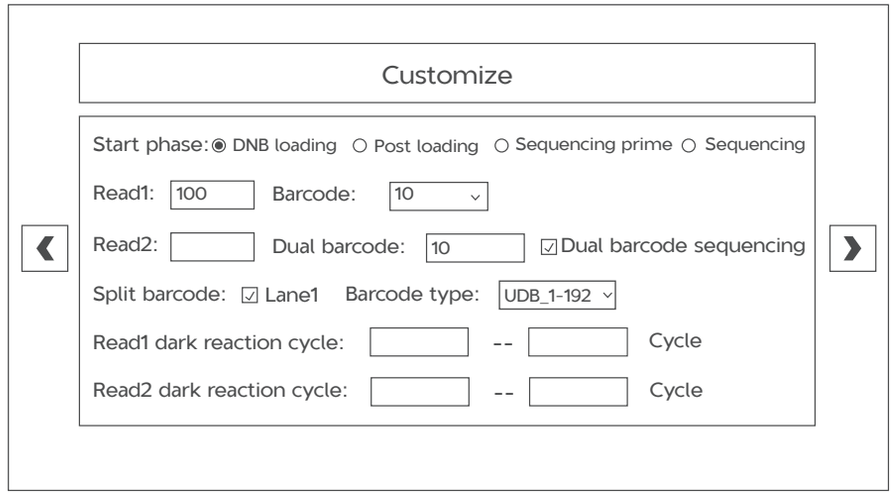
Sequencing

1. In the main interface of the control software, tap in the upper-right corner to open the login interface.
2. Enter the username *user* and password *Password123*. Tap **Login**.
3. Tap **Sequence** and enter DNB ID in the **DNB ID** box.

Tips

Ensure that DNB ID is consistent with that of input in ZLIMS.

4. Select **Customize**, and set the cycle and barcode according to the figure below.



5. Place the tube that contains the DNB loading mixture into the tube bracket of the reagent compartment, and tap ›.
6. Take out the prepared reagent cartridge, scan the reagent cartridge ID by using a scanner or manually enter the ID.
7. Push the sequencing cartridge into the reagent compartment and close the compartment door, and tap ›.
8. Scan the flow cell ID of by using a barcode scanner or manually input the flow cell ID.
9. Press the attachment button on the flow cell stage to install the flow cell and close the door of the compartment, and tap ›.
10. After reviewing all information, tap **Sequence** and select **Yes** when you are prompted **Proceed with sequencing ?**.

    The interface displays real-time sequencing progress, and you can operate the device if needed.
11. After the sequencing is completed, the analysis software automatically starts the analysis.

Viewing and downloading the report

1. In the main interface of ZLIMS, tap the number under **Report Today** to open the Analysis Report page.
2. Set the query term in the Query area and tap to locate the sample. Tap the link in the **Analysis type** column.
3. Tap in the **Report** column to view the report.

Tips

- You can tap to download the reports and results of all samples in the same batch.
- For details about software operation, refer to *Vector and Microorganism Identification Software User Manual*.