• Part No.: H-020-000864-00
• Version: 2.0
• Release date: October 2024

Applicable sets/kits

Name		Cat. No.
MGIEasy Fast FS DNA Library Prep Set V2.0 (16 RXN), Cat. No.: 940-001193-00	MGIEasy Fast FS Library Prep Module V2.0	940-001197-00
	MGIEasy UDB Primers Adapter Kit	1000022800
	MGIEasy DNA Clean Beads	940-001176-00
MGIEasy Fast FS DNA Library Prep Set V2.0 (96 RXN) Cat. No.: 940-001194-00	MGIEasy Fast FS Library Prep Module V2.0	940-001195-00
	MGIEasy UDB Primers Adapter Kit B	1000022802
	MGIEasy DNA Clean Beads	940-001174-00
DNBSEQ OneStep DNB Make Reagent Kit (OS-DB)		1000026466
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 DNA libraries

Preparing samples

1. The starting amount of each gDNA sample is recommended to be 110 ng for DNA library preparation. Add TE Buffer to the sample until the total volume reaches 48 µL.

Tips

- If there are specific requirements, the starting amount can be adjusted within the range of 1.1 ng to 220 ng. Add TE buffer to the sample until the total volume reaches 48 µL. Only one starting amount can be used for each library preparation process.
- It is recommended that you use high quality genomic DNA (1.8 ≤ OD260/OD280 ≤ 2.0, OD260/OD230 ≥ 1.7) for fragmentation.
- Any residual impurities (such as metal chelators or other salts) in the gDNA sample may adversely affect the efficiency of the fragmentation step and the fragment size.

2. Transfer 48 µL of each sample to a break-away 8-strip PCR tube, and place the tube on ice until use. Cap the tubes of the 8-tube strip, and mix it 3 times, 3 s each, by using a vortex mixer. Ensure that no bubbles exist at the bottom of the tube and no liquid remains on the tube wall. Place it on ice for further use.

Tips

- For 4 or 8 samples, one set of 8-strip tubes is needed. For 16 samples, 2 sets of 8-strip tubes are needed.
- If the number of samples is less than 4, 8, or 16, fill in the remaining tubes with water. Prepare the reagents and consumables according to the actual number of samples.

• Part No.: H-020-000864-00
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Preparing reagents

1. Take out the UDB Adapter from the MGIEasy UDB Primers Adapter Kit and dilute the UDB Adapter in a 0.5 mL SC micro tube according to the table below, depending on the starting volume.

Manual DNA input (ng)	Automated DNA input (ng)	Adapter dilution factor	Volume of diluted UDB Adapter (μL)		
			4 RXN	8 RXN	16 RXN
200	220	/	≥ 35	≥ 52	≥ 100
100	110	2×			
50	55	4×			
25	27.5	8×			
10	11	20×			
5	5.5	40×			
1	1.1	100×			

2. Take out all reagents from MGIEasy Fast FS Library Prep Module V2.0. Mix Fast FS Enzyme II thoroughly, centrifuge briefly, and place it on ice until use. Thaw other components at room temperature, mix them thoroughly, centrifuge briefly, and place them on ice until use.
3. Prepare the reagents according to the table below:

Component	Consumable	Volume (μL)		
		4 RXN	8 RXN	16 RXN
Fast FS Buffer II	0.5 mL SC micro tube	65	105	Original kit tube
Fast FS Enzyme II	0.5 mL SC micro tube	25	45	Original kit tube
Ligation Enhancer	0.5 mL SC micro tube	25	25	Original kit tube
Fast Ligation Buffer	0.5 mL SC micro tube	125	225	Original kit tube
Ad Ligase	0.5 mL SC micro tube	25	45	Original kit tube
PCR Enzyme Mix	0.5 mL SC micro tube	125	225	Original kit tube
UDB Adapter	0.5 mL SC micro tube	35	52	100

Tips

- When preparing libraries for 16 samples by using the MGIEasy Fast FS DNA Library Prep Set V2.0 (16 RXN), there is no need to transfer reagents. Take out the corresponding reagents from the kit, thaw, mix, centrifuge, and place them on ice for further use. The original reagent tubes can be directly placed on MGISP-100RS for use.
- Do not vortex the Fast FS Enzyme II, invert it 10 times to mix thoroughly, and flick the bottom gently to ensure that no residual reagent is left at the bottom. Insufficient mixing may affect the fragmentation effect, please strictly follow the instructions.

4. Prepare 1× Elute Enhancer, En-TE, and En-Beads. All the solutions should be used within 7 days.

Name	Component	Volume (μL)	Total volume (μL)	Storage requirements	Validity period
1× Elute Enhancer	20× Elute Enhancer	2	40	Room temperature	7 d
	Nuclease-Free Water	38			
En-TE	1× Elute Enhancer	5.4	2700	2 °C ~8 °C	7 d
	TE Buffer	2694.6			
En-Beads	1× Elute Enhancer	24	2400	2 °C ~8 °C	7 d
	DNA Clean Beads	2376			

5. Mix the En-Beads, transfer them into two new 2 mL SC micro tubes according to the table below, and cap the tubes.

Component	Consumable	Volume (μL)		
		4 RXN	8 RXN	16 RXN
En-Beads	2 mL SC micro tubes	500	1000	1000 × 2 tubes

6. Prepare 15 mL of 80% ethanol.

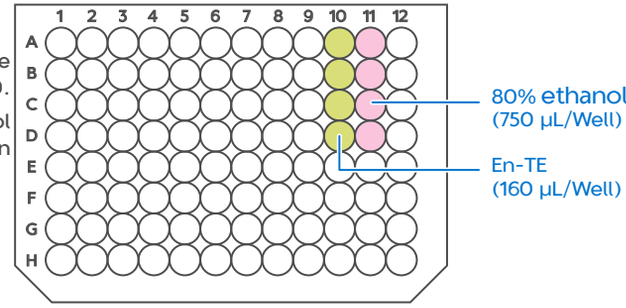
Tips

Use the 80% ethanol immediately after preparation.

7. Take out a U-bottom deep-well plate and add the reagents according to the figures below:

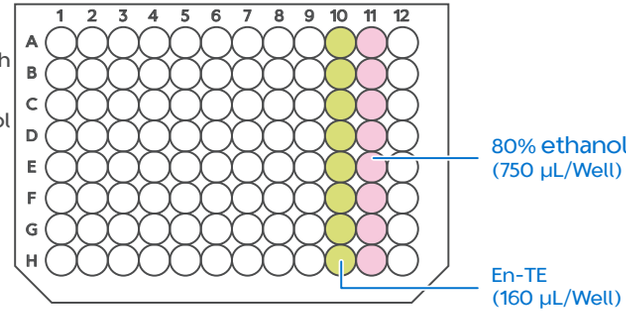
4 RXN:

- Add 160 µL of En-TE to the first four wells in column 10.
- Add 750 µL of 80% ethanol to the first four wells in column 11.



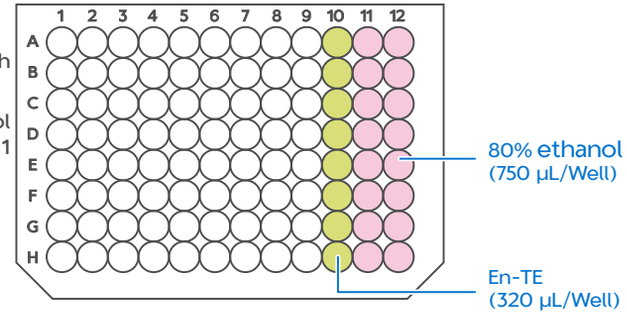
8 RXN:

- Add 160 µL of En-TE to each well of column 10.
- Add 750 µL of 80% ethanol to each well of column 11.



16 RXN:

- Add 320 µL of En-TE to each well of column 10.
- Add 750 µL of 80% ethanol to each well of column 11 and 12.



8. Take out the primer from the MGIEasy UDB Primers Adapter Kit, mix and centrifuge it thoroughly, and then transfer the adapter (10 µL/well) into the 8-strip tubes.

Tips

- For 4 samples, transfer the primer into the tubes in the first 4 wells in column 1.
- The 8-strip tubes are separated from the Break-away PCR Plate.

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-A06-116 MGIEasy Fast FS DNA Library Prep_RV2.0_SV4.0**
Script	• Fast_FS_Library_Prep_4RXN.py • Fast_FS_Library_Prep_8RXN.py • Fast_FS_Library_Prep_16RXN.py

Tips

Ensure that the automation script and PCR program have been installed before running the script.

2. Mix the EN-Beads thoroughly by using a vortex mixer and centrifuge them briefly before placing consumables.

Tips

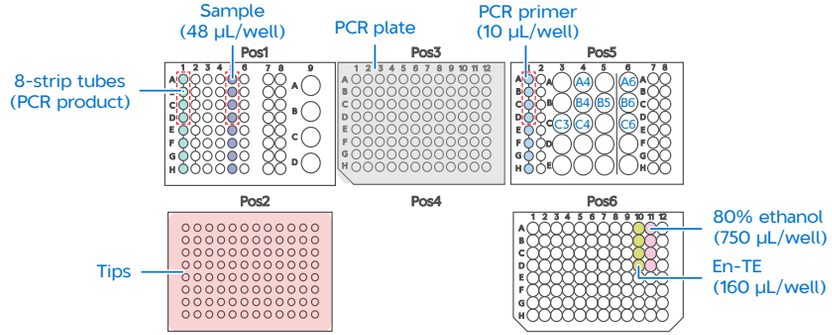
Ensure that no bubble exists at the bottom of other tubes, no liquid exists on the inner wall, and all tubes are uncapped.

3. Place the consumables according to the figures below:

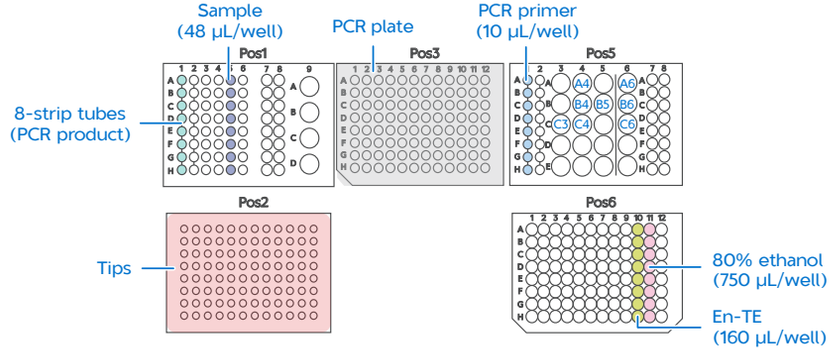
Tips

- For 4 samples, add reagents or samples to the tubes in the first four wells in the 8-strip tube and in column 1 of the deep-well plate. The libraries will be collected in the tubes in the first four wells in column 1 of the deep-well plate.
- Before the operation, the En-Beads should be thoroughly mixed by using a vortex mixer and then centrifuge briefly. For the other reagents, ensure that no bubbles exist at the bottom of the tubes and no liquid remains on the tube wall.

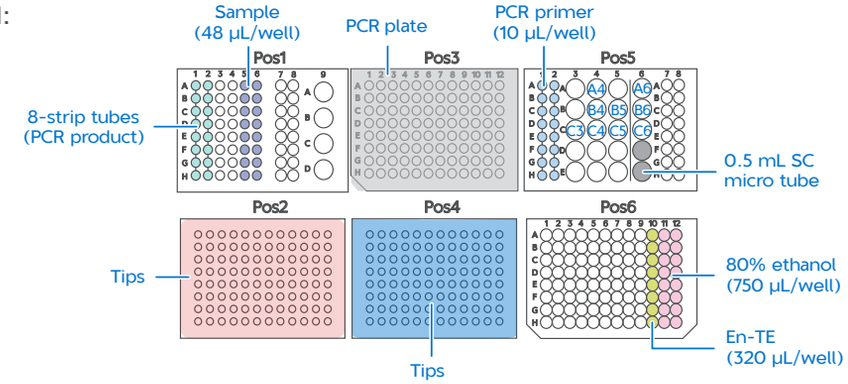
4 RXN:



8 RXN:



16 RXN:



Add the reagents to wells of Pos 5 according to the table below:

Well	Reagent
A4	Fast FS Buffer II
A6	Fast FS Enzyme II
B4	Fast Ligation Buffer
B5	Ligation Enhancer
B6	Ad Ligase
C3	UDB Adapter
C4	En-Beads
C5	En-Beads (16RXN)
C6	PCR Enzyme Mix
D6	0.5 mL tube (16RXN)
E6	0.5 mL tube (16RXN)

• Part No.: H-020-000864-00
• Version: 2.0
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4. Click **Run** to start Fast FS Library Preparation process. Follow the prompts to select fragmentation time and PCR cycle number, and then click **Continue**.

Manual DNA input (ng)	Automated DNA input (ng)	Fragmentation time (min)	Required cycles
200	220	12	4
100	110	14	5
50	55	16	6
25	27.5	16	7
10	11	18	9
5	5.5	20	10
1	1.1	22	12

Tips

The entire process takes around 2 h 40 min to 3 h 30 min You can pause or resume it as needed in the experiment process.

5. After the process is completed, take out the PCR product from Pos1_Column1 or Pos1_Column1 and Pos1_Column2. The volume should be 30 µL. Then cap the 8-strip PCR tubes.
6. Quantify the PCR product by using dsDNA Fluorescence Assay Kits. The product concentration should be no less than 3 ng/µL.

Stoppoint

The PCR product can be stored in a -20 °C freezer.

7. (Optional) If no experiment will be conducted on the day, clean the device with pure water and 75% ethanol. Then, perform a post-clean.

Tips

Place the discarded sample tubes, reagent tubes, deep-well plates, and waste bag in the designated waste area.

Making OS DNBs

Preparing samples

Tips

Ensure that the data output of each sample is ≥ 10 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 °C (Heated lid)	On
95 °C	3 min
40 °C	3 min
4 °C	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 °C (Heated lid)	On
30 °C	25 min
4 °C	Hold

Tips

It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C .

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 for 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

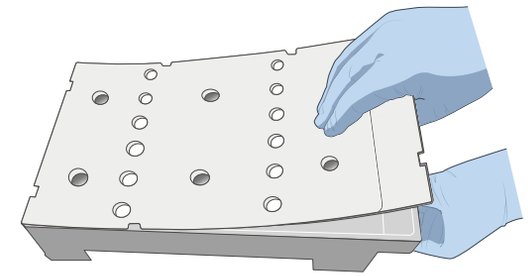
1. Select the sequencing recipe based on different scenarios:

Tips

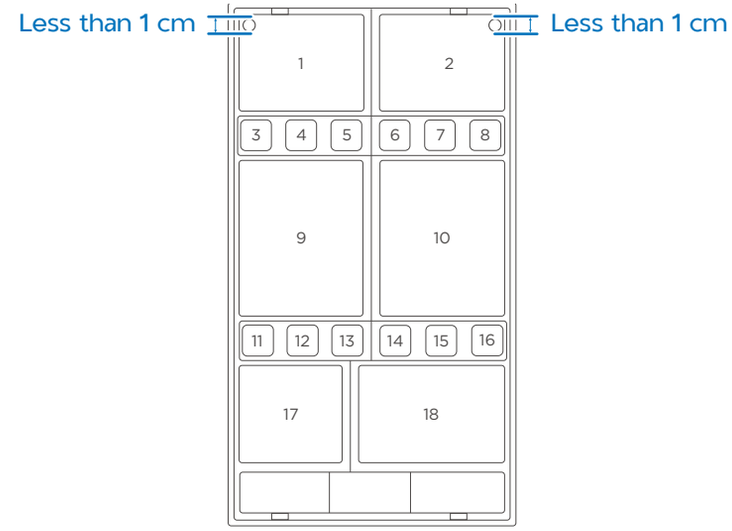
If the samples are loaded on one flow cell, ensure that barcodes corresponding to the libraries are base-balanced.

- When the number of DNA libraries is less than or equal to 8, select FCS SE100.
- When the number of DNA libraries is less than or equal to 40, 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.
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

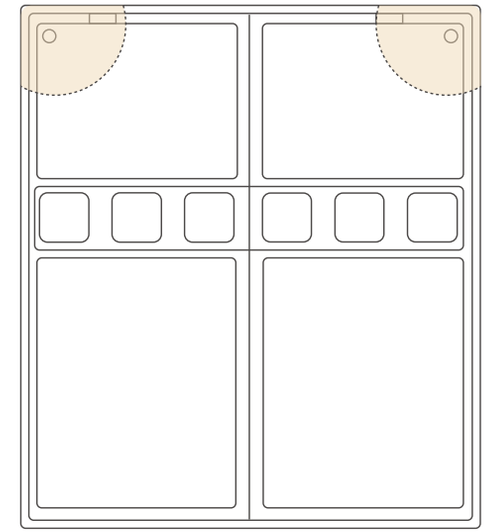
   - FCL SE100

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:

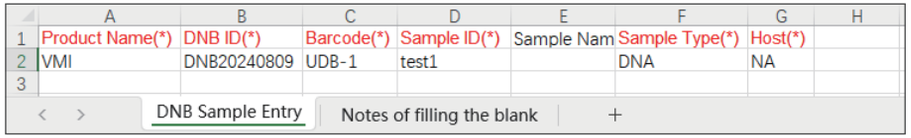
   127.0.0.1

2. Enter the username *lite* and password *lite123456*, and tap **Login**.
3. Click **Sequencing + Analysis** on the Home page to open the New Sequence + 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		DNA	NA	
3								

DNB Sample Entry | Notes of filling the blank | +

Tips

- A field with an asterisk (*) is required, and others are optional.
- Cells in the 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.
- **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.

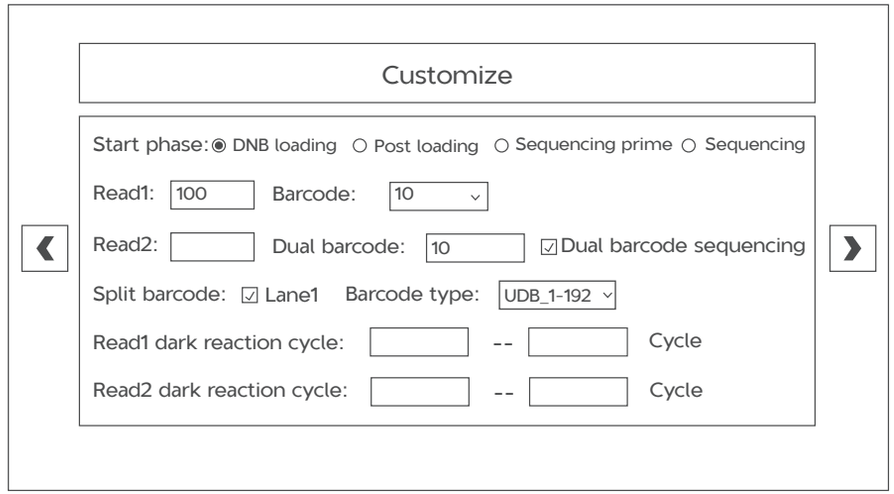
Sequencing

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 on the upper left of the report page to download the reports and results of all samples in the same batch.
- For details on software operations, refer to *Vector and Microorganism Identification Software User Manual*.