· Part No.: H-020-000844-00 Release date: September 2024
· Version: 2.0

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
High-throughput Sequencing Set (G99 FCL SE100/PE50)		940-001268-00
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 to 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-020-000844-00 Release date: September 2024
· Version: 2.0

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 the other components at room temperature, mix them thoroughly, briefly centrifuge them, 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 μL	105 μL	Original kit tube
Fast FS Enzyme II	0.5 mL SC micro tube	25 μL	45 μL	Original kit tube
Ligation Enhancer	0.5 mL SC micro tube	25 μL	25 μL	Original kit tube
Fast Ligation Buffer	0.5 mL SC micro tube	125 μL	225 μL	Original kit tube
Ad Ligase	0.5 mL SC micro tube	25 μL	45 μL	Original kit tube
PCR Enzyme Mix	0.5 mL SC micro tube	125 μL	225 μL	Original kit tube
UDB Adapter	0.5 mL SC micro tube	35 μL	52 μL	100 μL

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. Mix Fast FS Enzyme II by inverting 10 times and flicking the bottom gently, ensure that no residual reagent is left at the bottom. Insufficient mixing may affect the fragmentation effect, please strictly follow the instructions.

· Part No.: H-020-000844-00 Release date: September 2024
· Version: 2.0

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 days
	Nuclease-Free Water	38			
En-TE	1× Elute Enhancer	5.4	2700	2 °C ~8 °C	7 days
	TE Buffer	2694.6			
En-Beads	1× Elute Enhancer	24	2400	2 °C ~8 °C	7 days
	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.

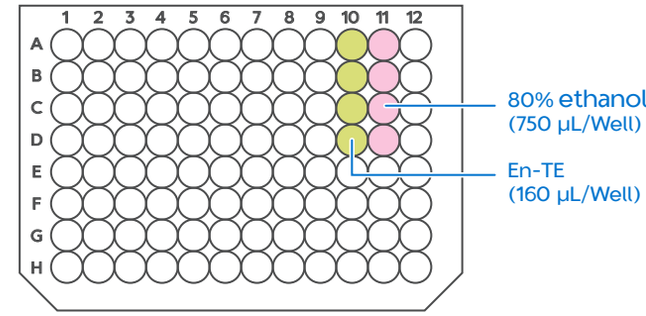
Tips

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:

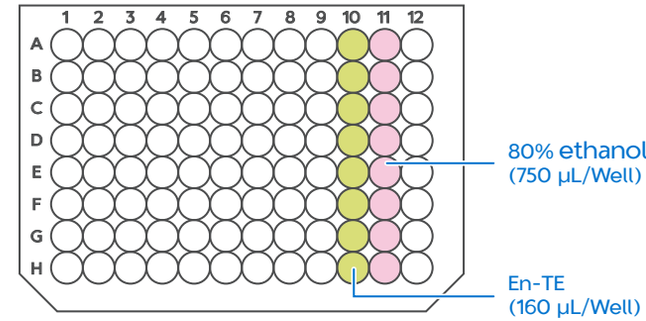
4 RXN:

- 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.



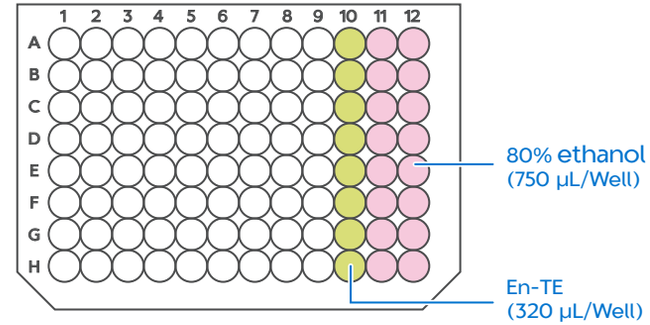
8 RXN:

- Add 160 μL of En-TE to each well of column 11.
- 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 columns 11 and 12.



· Part No.: H-020-000844-00 Release date: September 2024
· Version: 2.0

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

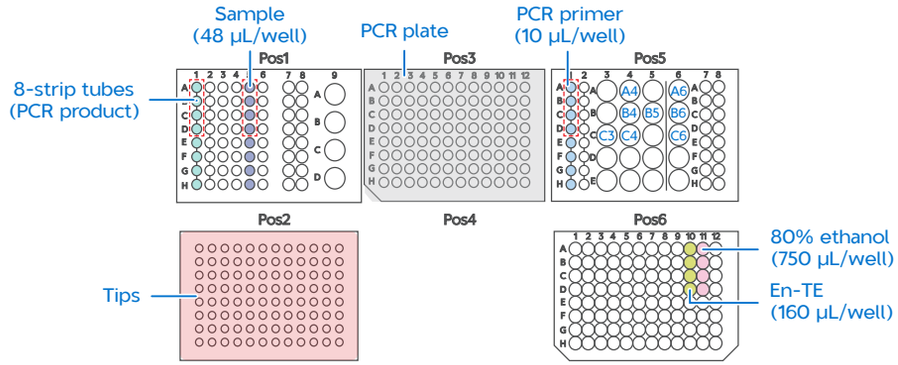
Ensure that no bubble exists at the bottom of other tubes, no liquid exists on the inner wall, and all tubes are uncapped.

3. Place the consumables according to the figures below:

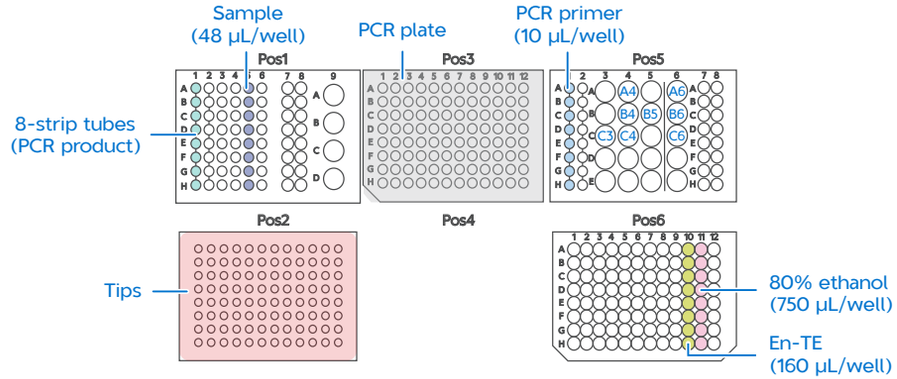
Tips

- 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 shortly. For the other reagents, ensure that no bubbles exist at the bottom of the tubes and no liquid remains on the tube wall.

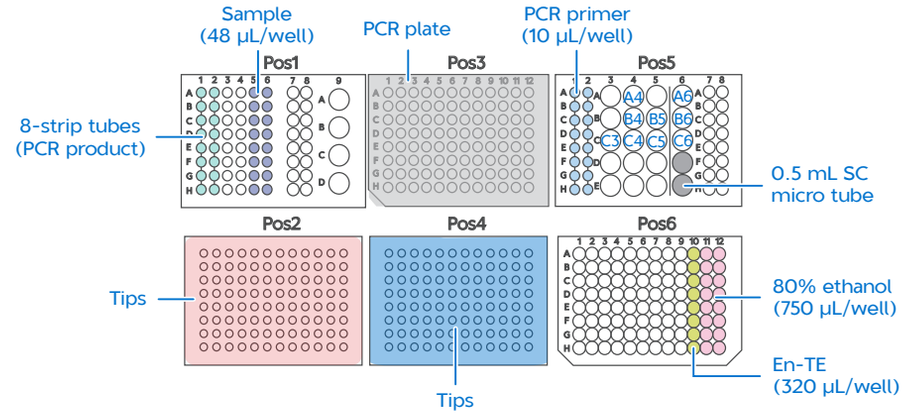
4 RXN:



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)

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**.

· Part No.: H-020-000844-00 Release date: September 2024
· Version: 2.0

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 column 1 or Pos1 columns 1 and 2. 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.

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 10 µL.

Tips

If the total volume is less than 10 µL, add TE Buffer to the sample until the total volume reaches 10 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	10-V
Make DNB Buffer (OS-DB)	10
Total volume	20

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	20
Make DNB Enzyme Mix II (OS)	2
Total volume	22

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:

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

Tips

It is recommended to 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 10 μL of Stop DNB Reaction Buffer to the PCR tube. Pipette up and down 10 times by using 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 stored at a 4 °C refrigerator for up to 48 h.

9. Take out 2 μL of the DNBs and quantify it according to the quantification instructions of Qubit ssDNA Assay Kit. The concentration of the DNBs should not be less than 8 ng/μL.

Tips

If the concentration of the DNBs exceeds 40 ng/μL, dilute the DNBs to a concentration of 20 ng/μL with TE buffer.

· Part No.: H-020-000844-00 Release date: September 2024
· Version: 2.0

Performing sequencing

Preparing the flow cell and sequencing reagent cartridge

1. Take out the flow cell and place it at room temperature for at least 30 min for DNB loading.

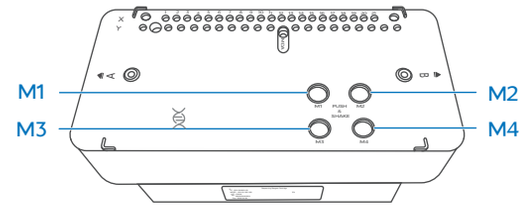
Tips

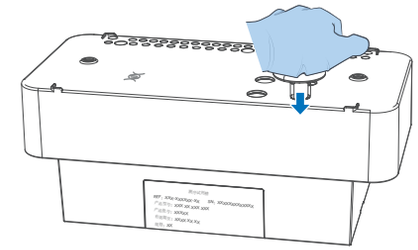
- The flow cell should not be left at room temperature for more than 24 h.
- Do not open the outer plastic package of the flow cell at this time.

2. Take out the sequencing cartridge and thaw it in a water bath at room temperature or in a 2 °C to 8 °C refrigerator. After thawing, place the cartridge in a 2 °C to 8 °C refrigerator until use.

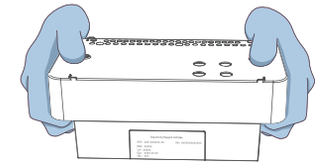
Model	Method		
	Water bath at room temperature (h)	Refrigerator at 2 °C to 8 °C overnight. and then water bath at room temperature (h)	Refrigerator at 2 °C to 8 °C (h)
G99 FCL SE100/PE50	2.0	0.5	8.0

3. Invert the cartridge 5 times to mix before use. Remove the package of the cartridge and wipe the water condensation with a KimWipes tissue.
4. Use the puncher to pierce the M1, M2, M3, M4 wells of the cartridge.





5. Hold sides A and B of the cartridge with both hands by following the marks A and B on the cartridge, and vigorously shake the cartridge up and down 20 times and shake it clockwise and counterclockwise 20 times. Pierce the MDA well with a 1 mL pipette tip.



Inputting sample information

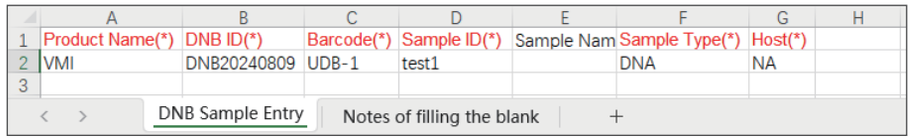
1. Launch the Chrome™ browser, type the following address in the address bar, and press **Enter**:
   - Using the DNBSEQ-G99ARS server: *192.168.1.5*
   - Using the PFI server: *127.0.0.1*
2. Enter the username *lite* and password *lite123456*, and tap **Login**.
3. Tap **Sequencing + Analysis** on the 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** for **Host**. 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.

· Part No.: H-020-000844-00 Release date: September 2024
· Version: 2.0

Sequencing

1. Tap (user icon) in the upper-right corner of the main interface of the control software.
2. Enter the username *user* and password *123*. Tap **Login**.
3. Tap **Sequence** in the flow cell A or B operation area. If you need simultaneously perform sequencing on the flow cell stages A and B, tap **Sequence A&B**. Tap **New Sequence**. The system will automatically perform a self-check.

Tips

Ensure that the waste container is empty before starting sequencing.

4. After the check is completed, tap **Next** to open the setting parameters interface. Select **Sequencing & Transmission** and input the DNB ID in the **DNB ID** box.

Tips

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

5. Select **SE100+10+10** in the Recipe box. Tap the second box next to Recipe and select **UDB_1-192**.
6. Select **Yes** for both **Split Barcode** and **Auto Wash**. Tap **Next**. The auto-sliding screen moves up automatically.
7. Load the sequencing cartridge into the reagent compartment. The system will automatically identify the cartridge ID and display it in the **Reagent cartridge** box.

Tips

If automatic scanning fails, you can manually input the ID.

8. Tap **Prime** and then **Yes** in the pop-up window. The system automatically closes the auto-sliding screen and starts priming.
9. Load the DNBs into the flow cell:
   ① Take out DNB Load Buffer II in advance and place it on ice for approximately 30 min. Mix it thoroughly by using a vortex mixer for 5 s, briefly centrifuge it, and place it on ice until use.
   ② Take out the 0.5 mL tube from the kit and prepare the DNB loading mixture according to the table below.

Component	Volume (μL)
DNB Load Buffer II	7
Make DNB Enzyme Mix II (LC)	1
DNB	21
Total volume	29

   ③ Mix the DNB loading mixture slowly by using a wide-bore pipette tip for 8 times. Then, place it at a 4 °C refrigerator until use.

Tips

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

   ④ Load the DNBs by using a Portable DNB Loader. For specific instructions, refer to *MGIDL-G99 Portable DNB Loader Quick Start Guide*.

10. After the priming is completed, tap **Next**. The auto-sliding screen moves up automatically. Insert the flow cell into the flow cell compartment, and the system will automatically identify the ID.

Tips

If automatic scanning fails, you can manually input the ID.

11. Tap **Next** to review the information.
12. After reviewing all information, tap **Sequence** and select **Yes** to start sequencing. The auto-sliding screen moves down and the Sequencing interface displays on the screen.

    The interface displays real-time sequencing progress, and you can operate the device if needed.
13. After the sequencing is completed, tap **Finish**. The auto-sliding screen moves up. Take out the reagent cartridge and flow cell and tap **Return Home**.
14. 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*.

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