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## 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 |
| DNBSEQ-G99RS 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 filter tips | 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 column 1 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.

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## Preparing reagents

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

Tips

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

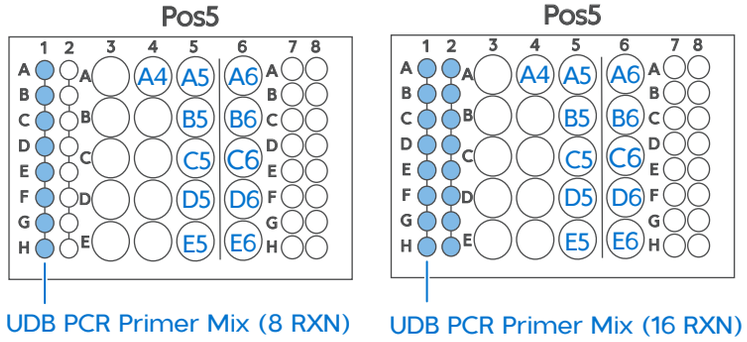
2. Take out the DNA clean beads from the MGIEasy DNA clean beads reagent cartridge 30 min before experiment, thoroughly mix them by using a vortex mixer, and then centrifuge shortly.
3. The amount of adapter used in Adapter Ligation depends on the amount of RNA input. Dilute the UDB Adapters by using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

| Reagent | Consumables | Location | Reagent input for different sample volume (μL) | | | |
|---|---|---|---|---|---|---|
| | | | 200 bp | | 270 bp | |
| | | | 16 RXN | 16 RXN | 8 RXN | 16 RXN |
| DNA Clean Beads-2 | 2.0 mL SC micro tube | Pos5 E6 | 400 | 780 | 390 | 780 |




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

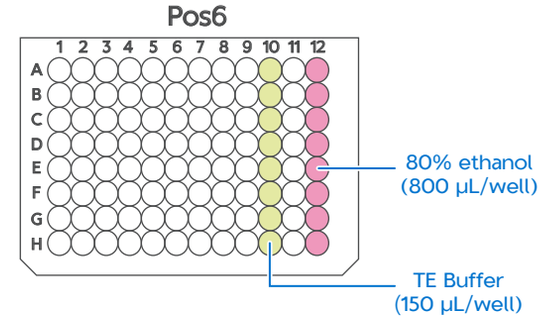
Tips

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:

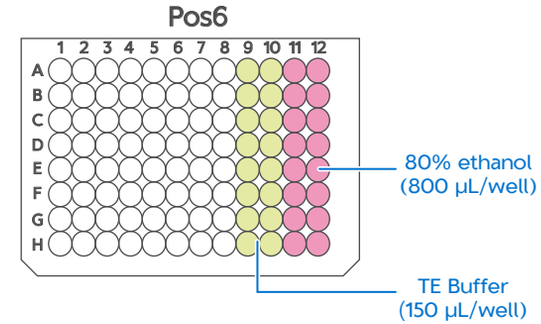
8 RXN:

- 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

Before operation, ensure that the scripts and PCR programs are imported successfully.

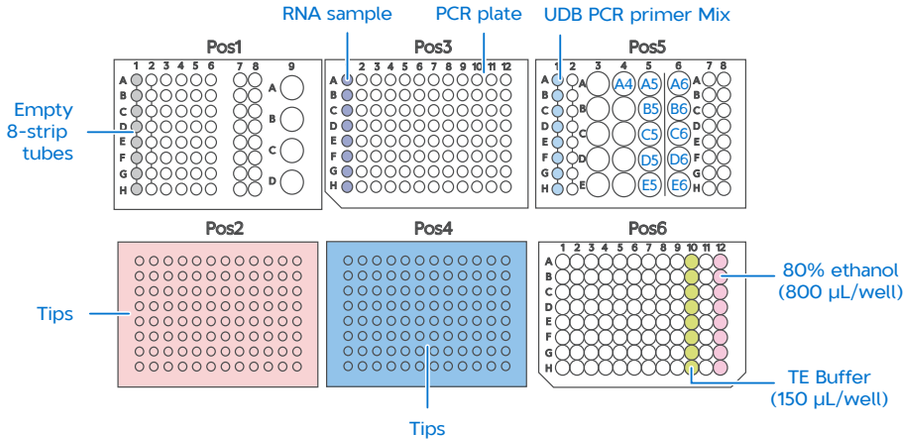
2. Mix DNA Clean Beads thoroughly by using a vortex mixer and centrifuge them briefly before placing consumables.

Tips

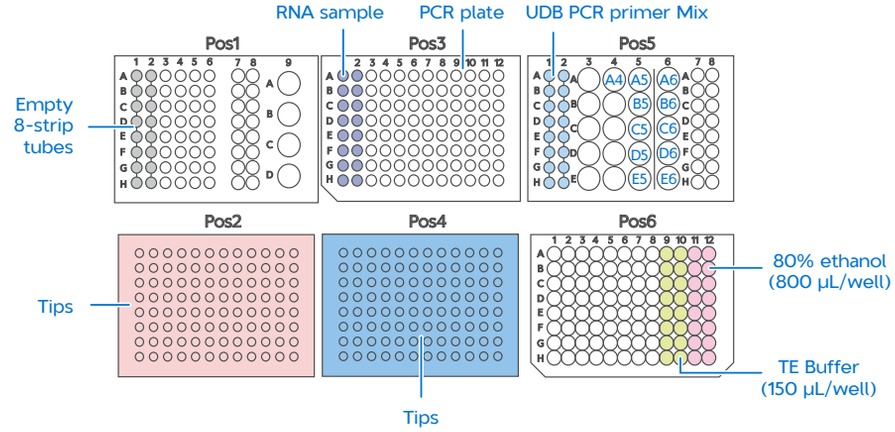
Ensure that no bubble exists at the bottom of other tubes, no liquid exists on the inner wall, and all tubes are uncapped.

3. Place the 96-well PCR plate in a plate centrifuge and centrifuge briefly. Ensure that no air bubbles exist at the bottom 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** recommended), PCR cycles, and sample pre-processing method (**Other**), and then select **Continue**.

| Insert size | Fragmentation condition | Fragmentation duration |
|---|---|---|
| 200 bp | 94 °C | 6 min |
| 270 bp | 87 °C | 6 min |

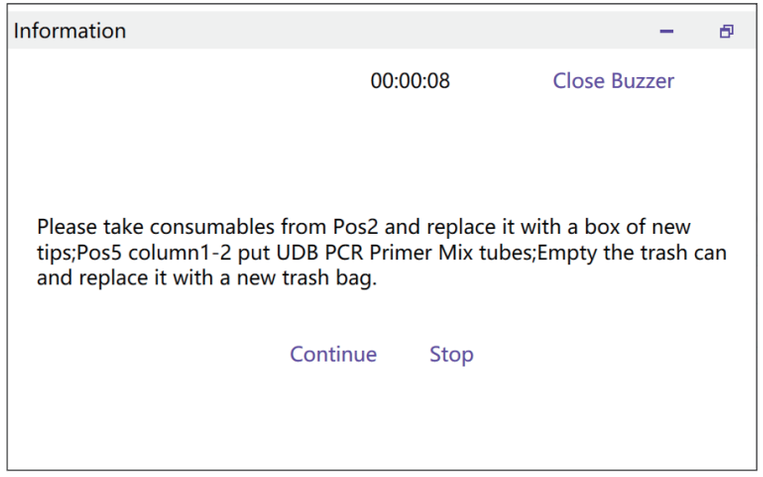
| Total RNA ( ng ) | PCR cycles |
|---|---|
| 10 | 16-18 |
| 50 | 15-16 |
| 100 | 14-15 |
| 200 | 13-14 |
| 1000 | 11-12 |

6. If the number of RNA samples is 16, after running continuously for 2 h, the ligation reaction is proceeding 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 it 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

We recommend 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 column 1 (8 RXN) or Pos1 columns 1 and 2 (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 °C 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.

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## Making OS DNBs

### Preparing samples

Tips

Ensure that the data output of each sample is no less than 20 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-SB) | 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 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

DNBs can be placed at a 2 °C to 8 °C refrigerator for up to 48 h.

9. Take out 2 µL of DNBs and quantify the library by using Qubit dsDNA HS Assay Kit or Quant-iT PicoGreen dsDNA Assay Kit. The concentration should be no less than 8 ng/µL.

Tips

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

## Performing sequencing

### Preparing the sequencing reagent cartridge

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

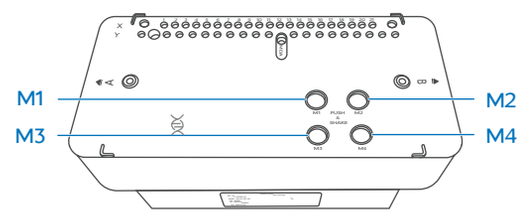
Tips

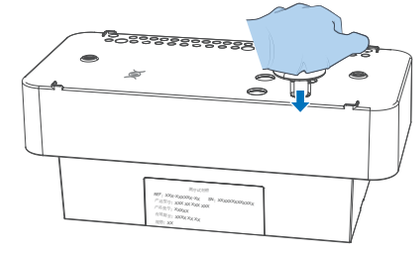
- The flow cell should not be placed at room temperature for over 24 h.
- Do not open the outer plastic package of the flow cell at this time.

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.

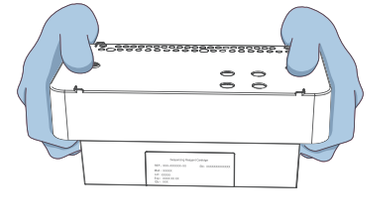
| Model | Method | | |
|---|---|---|---|
| | Water bath at room temperature (h) | Refrigerator at 2 °C to 8 °C overnight. and then water bath at room temperature (h) | Refrigerator at 2 °C to 8 °C (h) |
| G99 FCL SE100/PE50 | 2.0 | 0.5 | 8.0 |

3. Invert the cartridge 5 times to mix the reagents. Remove the package of the cartridge and wipe the water condensation with a KimWipes tissue.
4. Press the cartridge wells M1, M2, M3, and M4 with a pressing tool.



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 clean 1 mL pipette tip.



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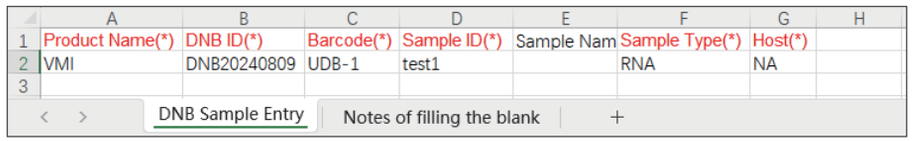
## Inputting sample information

1. Launch the Chrome™ browser, enter the following IP address, 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.




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** field. 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.

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## Sequencing

1. Tap 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 this DNB ID is consistent with that of input in ZLIMS Lite.

5. Select **SE100+10** in the **Recipe** box. Tap the dropdown box on the right 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 select **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 to thaw. Mix it thoroughly by using a vortex mixer and 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 2 °C to 8 °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, the auto-sliding screen moves up automatically. Insert the flow cell into the flow cell compartment, and the system will automatically identify the ID.

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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  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*.