· Part No.: H-020-000845-00 · Release date: December 2023
· Version: 1.0




## Applicable sets/kits

- MGIEasy Microorganism Fast RNA Library Prep Set (16 RXN Cat. No.: 940-000107-00) (96 RXN Cat. No.: 940-000108-00)

| Name | Component | Cat. No. |
|---|---|---|
| MGIEasy RNA Library Prep Kit (16 RXN/96 RXN) | Fragmentation Buffer<br>RT Buffer<br>RT Enzyme Mix<br>Second Strand Buffer<br>Second Strand Enzyme Mix<br>ERAT Buffer<br>ERAT Enzyme Mix<br>Ligation Buffer<br>DNA Ligase<br>PCR Enzyme Mix<br>PCR Primer Mix | 1000005274 (16 RXN)<br>1000005276 (96 RXN) |
| MGIEasy DNA Adapters-16 (Tubes) Kit/MGIEasy DNA Adapters-96 (Plate) Kit | DNA Adapters | 1000005284 (16 RXN)<br>1000005282 (96 RXN) |
| MGIEasy DNA Clean Beads | DNA Clean Beads<br>TE Buffer | 1000005278 (8 mL)<br>1000005279 (50 mL) |
| DNBSEQ OneStep DNB Make Reagent Kit (OS-SB) | Low TE Buffer<br>Make DNB Buffer (OS-SB)<br>Make DNB Enzyme Mix I (OS)<br>Make DNB Enzyme Mix II (OS)<br>Stop DNB Reaction Buffer | 1000020563 |

- DNBSEQ-G99RS High-throughput Sequencing Set (G99 SM FCL SE100/PE50) (Cat. No.: 940-000409-00)

| Component | Quantity |
|---|---|
| DNBSEQ-G99 Sequencing Flow Cell | 1 EA |
| Low TE Buffer | 100 µL × 1 tube |
| Make DNB Buffer | 20 µL ×1 tube |
| Make DNB Enzyme Mix I | 40 µL × 1 tube |
| Make DNB Enzyme Mix II (LC) | 13 µL ×1 tube |
| Stop DNB Reaction Buffer | 50 µL ×1 tube |
| DNB Load Buffer II | 50 µL ×1 tube |
| MDA Enzyme Mix | 0.125 mL × 1 tube |
| MDA Reagent | 1.0 mL × 1 tube |
| FTAT premixed compaction block | 1 EA |
| Micro Tube 0.5 mL (Empty) | 1 tube |
| Sequencing Reagent Cartridge | 1 EA |

· Part No.: H-020-000845-00 · Release date: December 2023
· Version: 1.0




## Recommended consumables

| Name | Cat. No. | Brand |
|---|---|---|
| 0.5 mL SC micro tube | 1000001558 | MGI |
| 2 mL SC micro tube | 1000001553 | |
| 250 µL automatic filter tips | 1000000723 | |
| 1.3 mL U-bottom deep-well plate | 1000004644 | |
| Hard-shell thin-wall 96-well skirted PCR plate | 1000012059 | |
| Break-away 8-strip PCR Tubes and Caps | 100-000016-00 | |

## 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.
- Only one starting amount can be used for each library preparation process.

2. Transfer 10 µL of each sample to break-away 8-strip PCR tubes, and place the tube on ice until use.

Tips

- Ensure that no bubble exists at the bottom of the tube and no liquid exists on the inner wall.
- If the number of samples is less than 16, fill in the remaining tubes with water. Prepare the reagents and consumables according to 16 samples.

### Preparing reagents

1. Take out all reagents from MGIEasy RNA Library Prep Kit. Mix Fast FS Enzyme thoroughly, briefly centrifuge it, and place it on ice until use. Thaw the rest buffers at room temperature, mix them thoroughly, briefly centrifuge them, and place them on ice until use.
2. Preparing reagents:
   - When preparing RNA libraries by using MGIEasy RNA Library Prep Kit (16 RXN), directly use the reagents for automatic library preparation.
   - When preparing RNA libraries by using the MGIEasy RNA Library Prep Kit (96 RXN), prepare the reagents according to the table below before automatic library preparation:

| Name | Component | Consumable | Volume (µL) |
|---|---|---|---|
| MGIEasy RNA Library Prep Kit (96 RXN) | Fragmentation Buffer | 0.5 mL PCR SC micro tube | 105 |
| | RT Buffer | | 105 |
| | RT Enzyme Mix | | 28 |
| | Second Strand Buffer | | 490 |
| | Second Strand Enzyme Mix | | 88 |

3. Take out DNA Clean Beads from MGIEasy DNA Clean Beads, mix them thoroughly, and transfer 1350 µL of the beads to a 2 mL PCR SC micro tube. Mark the tube as “DNA Clean Beads 1”.
4. Prepare 10 mL of 80% ethanol.

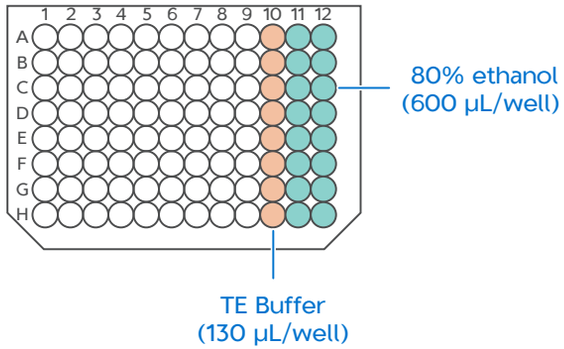
· Part No.: H-020-000845-00 · Release date: December 2023
· Version: 1.0




Tips

Use the 80% ethanol immediately after preparation.

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




## Preparing libraries on MGISP-100RS

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

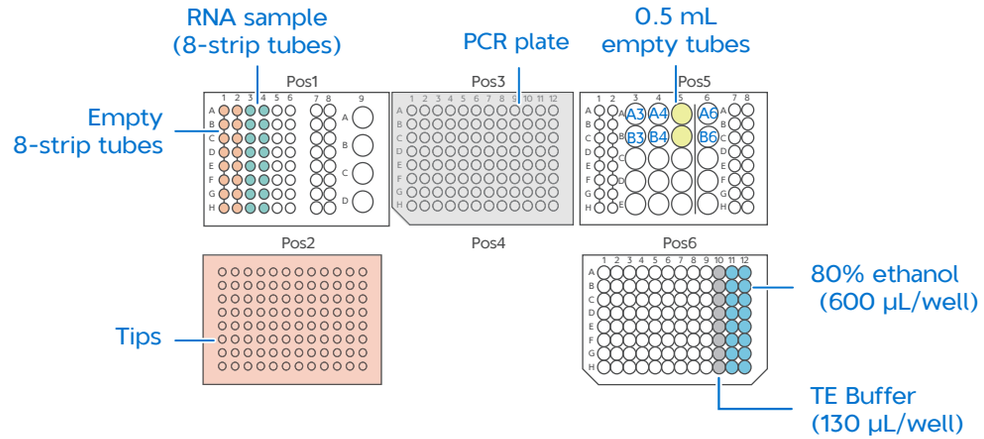
| | |
|---|---|
| Solution | JB-A06-074 MGIEasy RNA Library Prep Kit PathoRNAseq_RV3.1_SV2.0 |
| Script | 2.MGIEasy_RNA_Library_Prep(150bp_PathoRNAseq)_step2.py |

2. Mix DNA Clean Beads and 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 figure below:




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

| Well | Reagent |
|---|---|
| A3 | RT Buffer |
| A4 | RT Enzyme Mix |
| A6 | Fragmentation Buffer |
| B3 | Second Strand Buffer |
| B4 | Second Strand Enzyme Mix |
| B6 | DNA Clean Beads 1 |

5. Click **Run** to start the process.

Tips

The entire process is about 2 hours and 45 minutes. You can pause or resume it as needed while running.

6. After the process is completed, take out the PCR product from Pos 1. The total volume should be 40 µL. Then, place the PCR product in a -20 °C refrigerator.

· Part No.: H-020-000845-00 · Release date: December 2023
· Version: 1.0




7. Take out the PCR plate from Pos 3 and seal it with sealing film. Mark the plate as “Pos 3”.

Tips

The PCR plate recovered from Pos 3 is used in Pos 3 in the next procedure.

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

# Preparing DNA libraries

## Preparing samples

Take out the PCR product. Mix it thoroughly. centrifuge it, and place it on ice until use.

Tips

Ensure that no bubble exists at the bottom of the tubes and no liquid exists on the inner wall.

## Preparing reagents

1. Take out all reagents from MGIEasy RNA Library Prep Kit. Mix Fast FS Enzyme thoroughly, briefly centrifuge it, and place it on ice until use. Thaw Fast FS Buffer and Fast Ligation Buffer at room temperature, mix them thoroughly, briefly centrifuge them, and place them on ice until use.
2. Preparing reagents:
   - When preparing RNA libraries by using the MGIEasy RNA Library Prep Kit (16 RXN), direct use the reagents for automatic library preparation.
   - When preparing RNA libraries by using the MGIEasy RNA Library Prep Kit (96 RXN), prepare the reagents according to the table below before performing the automatic library preparation:

| Name | Component | Consumable | Volume (μL) |
|---|---|---|---|
| MGIEasy RNA Library Prep Kit (96 RXN) | ERAT Buffer | 0.5 mL PCR SC micro tube | 143 |
| | ERAT Enzyme Mix | | 64 |
| | Ligation Buffer | | 475 |
| | DNA Ligase | | 42 |
| | PCR Enzyme Mix | | 500 |
| | PCR Primer Mix | | 110 |

3. Take out DNA Clean Beads from MGIEasy DNA Clean Beads, mix them thoroughly, and prepare the beads according to the table below:

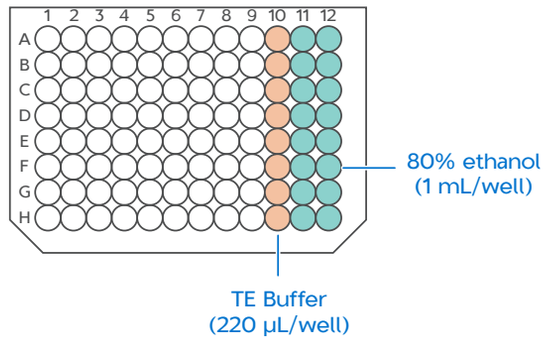
| Component | Consumable | Volume (μL) |
|---|---|---|
| DNA Clean Beads 2 | 2 mL PCR SC micro tube | 1000 |
| DNA Clean Beads 3 | | 1150 |

4. Prepare 20 mL of 80% ethanol.

Tips

Use the 80% ethanol immediately after preparation.

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




6. Take out DNA Adapters from MGIEasy DNA Adapters-16 (Tubes) Kit. Dilute them with TE Buffer according to the table below. Place the diluted adapters into break-away 8-strip PCR tubes, with 10 µL per tube.

| Total RNA (ng) | Dilution factor |
|---|---|
| < 10 | 100 |
| 10~50 | 20 |
| 51~200 | 10 |
| 201~1000 | 5 |

## Preparing libraries on MGISP-100RS

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

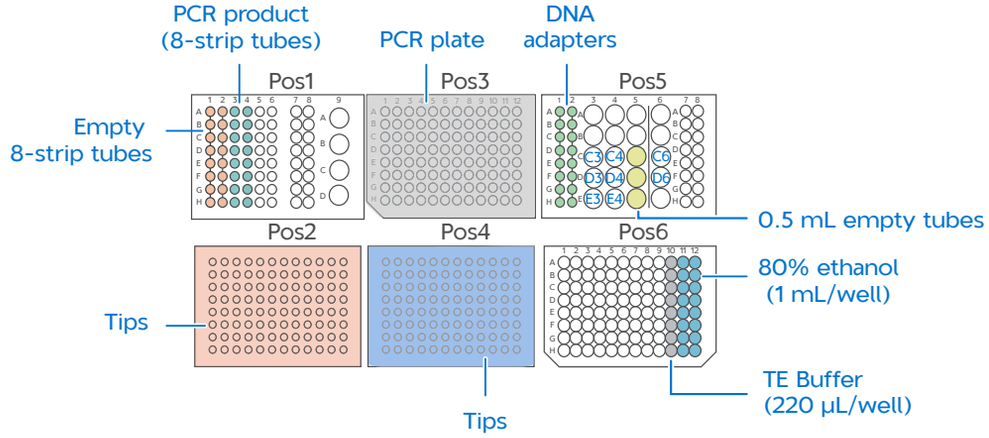
| | |
|---|---|
| Solution | JB-A06-074 MGIEasy RNA Library Prep Kit PathoRNAseq_RV3.1_SV2.0 |
| Script | 3.MGIEasy_RNA_Library_Prep(150bp_PathoRNAseq)_step3.py |

2. Mix DNA Clean Beads and 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 PCR plate from step 7 of *Preparing libraries on MGISP-100RS on Page 3* into Pos 3, and then remove the film. Place the consumables according to the figure below:



· Part No.: H-020-000845-00 · Release date: December 2023
· Version: 1.0




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

| Well | Reagent |
|---|---|
| C3 | ERAT Buffer |
| C4 | ERAT Enzyme Mix |
| C6 | DNA Clean Beads 2 |
| D3 | Ligation Buffer |
| D4 | DNA Ligase |
| D6 | DNA Clean Beads 3 |
| E3 | PCR Enzyme Mix |
| E4 | PCR Primer Mix |

5. Click **Run**. Select the required PCR cycles according to the table below, leave **Value** blank, and click **Continue** to start the automatic library preparation.

| Total RNA (ng) | Required cycles (≥ 300 ng) |
|---|---|
| < 10 | 20 |
| 10 | 17–18 |
| 50 | 15–16 |
| 200 | 13–14 |
| 1000 | 11–12 |

Tips

The entire process is about 4 hours. You can pause or resume it as needed in the experiment process.

6. After the process is completed, take out the PCR library from Pos 1. The total volume should be 30 µL.
7. Quantify the PCR product by using Qubit® dsDNA HS Assay Kit or Quant-iT PicoGreen dsDNA Assay Kit. The product concentration should be no less than 2.5 ng/µL.

Stoppoint

The PCR product can be placed in a -20 °C refrigerator.

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

# Making OS DNBs

## Preparing samples

Tips

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 20 ng of the mixed library to make DNBs. The volume should be no less than or equal to 10 µL. If the 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 |

· Part No.: H-020-000845-00 · Release date: December 2023
· Version: 1.0




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

- The total volume should be 42 μL.
- 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 5 to 8 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 4 °C refrigerator for up to 48 hours.

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.

# Sequencing

## Preparing the sequencing reagent cartridge

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

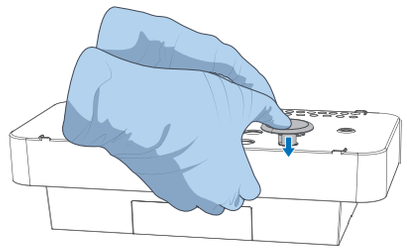
Tips

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

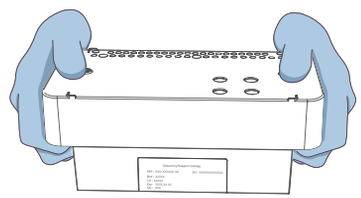
· Part No.: H-020-000845-00 · Release date: December 2023
· Version: 1.0




2. Take out the sequencing cartridge and thaw it in a water bath at room temperature for 3 to 4 hours or in a 2 ℃ to 8 ℃ refrigerator one day in advance. After thawing, place the cartridge in the 2 ℃ to 8 ℃ refrigerator until use.
3. Remove the package of the cartridge, open the cartridge cover, and wipe the water condensation with a dust-free paper.
4. Press the cartridge wells M1, M2, M3, and M4 with a pressing tool.



5. Hold the A and B sides of the cartridge with both hands by following the marks A and B on the cartridge, and invert the cartridge for 10 to 20 times to mix the reagents thoroughly. Pierce the MDA well with a clean 1 mL pipette tip. Then, gently tap the cartridge against the desktop to remove bubbles.



## Importing samples

1. Tap the ZLIMS icon on the desktop of the sequencer to open the login page of ZLIMS Lite.
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** (for example), and tap **New**.
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.

| Product Name(*) | DNB ID(*) | Barcode(*) | Sample ID | Sample Name | Sample Type(*) | Host (*) |
|---|---|---|---|---|---|---|
| VMI | DNB20221101 | 1 | test1 | | RNA | NA |

DNB Sample Entry | Notes of filling the blank

· Part No.: H-020-000845-00 · Release date: December 2023
· Version: 1.0




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.

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. 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**.
4. After tapping **Sequence**, the system will automatically open the waste container compartment door. Check if the waste container is in place by following the on-screen prompts. After checking, close the waste container compartment door. The device automatically starts to check.

Tips

Empty the waste container in the waste container compartment before starting sequencing.

5. After the check is completed, tap **Next** to open the setting parameters interface. select **Sequencing & Analysis**. Input the DNB ID in the **DNB ID** box.

Tips

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

6. Select **SE100+10** in the **Recipe** box. Tap the dropdown box on the right and select **1-128**.

Tips

For instructions on importing the customized barcode **1-128**, refer to relevant the user manual.

7. Select **Yes** for both **Split Barcode** and **Auto Wash**. Tap **Next**. The auto-sliding screen moves up automatically.
8. Push the sequencing cartridge into the reagent compartment. The system will automatically scan the cartridge ID and display it in the **Reagent cartridge** box.

Tips

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

9. Tap **Prime** and select **Yes** in the pop-up window. The system automatically closes the auto-sliding screen and starts priming.
10. Load the DNBs into the flow cell:
    ① Take out DNB Load Buffer II and that it on ice for approximately 30 minutes to thaw. Mix it thoroughly by using a vortex mixer and for 5 seconds, briefly centrifuge it, and place it on ice until use.
    ② Take out a new 0.2 mL tube 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 5 to 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&MGIDLG99RS Portable DNB Loader Quick Start Guide*.
11. 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 scan the ID.

Tips

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

12. Tap **Next** to review the information.
13. 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.
14. After the sequencing is completed, tap **Finish**. The auto-sliding screen moves up. Then take out the reagent cartridge and flow cell, and tap **Return Home**.
15. After the sequencing is completed, the analysis software automatically starts the analysis.

## Viewing and downloading the report

Tips

You can tap  to download the reports and results of all samples in the same batch.

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.