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Applicable sets/kits

- ATOPlex HIV-1 Library Preparation Set (16 RXN)

Name	Component		Cat. No.
ATOPlex HIV-1 Amplification Kit	ATOPlex HIV-1 Drug Resistance Primer Pool		940-000722-00
	RT-PCR Buffer		
	RT-PCR Enzyme Mix		
MGIEasy Fast PCR-FREE FS DNA Library Prep Set (16 RXN) (Cat. No.: 940-000019-00)	MGIEasy Fast PCR-FREE FS DNA Library Prep Kit	20× Elute Enhancer Fast FS Buffer Fast FS Enzyme Ligation Enhancer Fast Ligation Buffer Ad Ligase	940-000017-00
	MGIEasy UDB PF Adapter Kit	UDB Adapters	940-000018-00
	MGIEasy DNA Clean Beads	TE Buffer DNA Clean Beads	1000005278

- ATOPlex HIV-1 Library Preparation Set (96 RXN)

Name	Component		Cat. No.
ATOPlex HIV-1 Amplification Kit × 6	ATOPlex HIV-1 Drug Resistance Primer Pool		940-000722-00
	RT-PCR Buffer		
	RT-PCR Enzyme Mix		
MGIEasy Fast PCR-FREE FS DNA Library Prep Set (96 RXN) (Cat. No.: 940-000021-00)	MGIEasy Fast PCR-FREE FS DNA Library Prep Kit	20× Elute Enhancer Fast FS Buffer Fast FS Enzyme Ligation Enhancer Fast Ligation Buffer Ad Ligase TE Buffer	940-000020-00
	MGIEasy UDB PF Adapter Kit A	UDB Adapters A	940-000023-00
	MGIEasy DNA Clean Beads × 2	TE Buffer DNA Clean Beads	1000005278
MGIEasy DNA Clean Beads	TE Buffer DNA Clean Beads		1000005278

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- DNBSEQ OneStep DNB Make Reagent Kit (OS-DB) (Cat. No.: 1000026466)

Name	Specifications
Low TE Buffer	300 µL/tube × 1 tube
Make DNB Buffer (OS DB)	80 µL/tube × 1 tube
Make DNB Enzyme Adapters Mix I (OS)	160 µL/tube × 1 tube
Make DNB Enzyme Mix II (OS)	16 µL/tube × 1 tube
Stop DNB Reaction Buffer	100 µL/tube × 1 tube

- Sequencing sets

Name	Cat. No.
DNBSEQ-G99RS High-throughput Sequencing Set (G99 SM FCL PE150)	940-000410-00
DNBSEQ-G99RS High-throughput Sequencing Set (G99 FCL PE150)	940-001269-00

Recommended consumables

Name	Cat. No.	Brand
250 µL automated filter tips	1000000723	MGI
0.2 mL 96 well full-skirt PCR plate	091-000165-00	
1.3 mL 96 deep-well plate	1000004644	

RT-PCR multiplex amplification (manually)

Preparing samples

Prepare the total RNA samples, including plasma and serum. It is recommended to use high-quality genomic RNA samples with the CT value less than or equals to 32. The sample amount is recommended to be 20 µL, but should not exceed 20.5 µL.

Tips

Use a buffer that does not contain EDTA to dissolve RNA samples.

Performing RT-PCR multiplex amplification

1. Take out the following reagents from ATOPlex HIV-1 Amplification Kit. Prepare the RT-PCR multiplex amplification mixture on ice:

Reagent	Volume (µL)
RT-PCR Buffer	25
RT-PCR Enzyme Mix	2.5
ATOPlex HIV-1 Drug Resistance Primer Pool	2
Nuclease-Free Water	0.5
Total	30

2. Vortex the mixture, briefly centrifuge it, and transfer it into a new 0.2 mL PCR tube.
3. Add 20 µL RNA sample to the PCR tube. The total volume reaches 50 µL.
4. Pipette the mixture in the PCR tube for 10 times, briefly centrifuge it, and collect the mixture at the bottom of the tube.

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Tips

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

5. Place the PCR tube in the PCR device and start a reaction according to the conditions in the table below:

Temperature	Time	Cycle number
105 ℃ (Heated lid)	On	/
50 ℃	30 min	1
94 ℃	3 min	
94 ℃	30 s	42
58 ℃	45 s	
72 ℃	2 min	
72 ℃	5 min	1
12 ℃	Hold	

6. After the reaction is completed, take out the PCR tube and place it on ice until use.

RT-PCR product purification

Preparing samples

1. Vortex the 50 μL RT-PCR products three times for 3 seconds each time, and then briefly centrifuge them to collect the liquid at the bottom of the PCR tube.
2. Transfer the RT-PCR products to a 0.2 mL 96 well full-skirt PCR plate. Ensure that no bubble exists at the bottom of wells of the PCR plate, no liquid exists on the inner walls. Place the PCR plate on ice until use.

Tips

When the number of the RT-PCR products is less than 96, arrange the products in rows A to H sequentially until all products are placed. Prepare the consumables for the number of 96 samples.

Preparing reagents

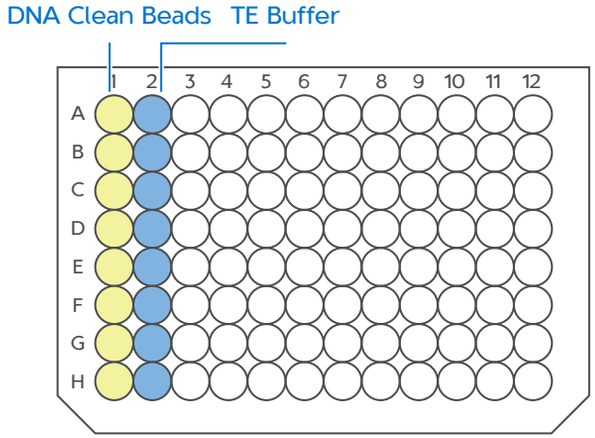
1. Take out the following reagents from MGIEasy DNA Clean Beads. Mix them thoroughly by using a vortex mixer.

Reagent	Container	Volume
DNA Clean Beads	1.3 mL 96 deep-well plate	1 mL
TE Buffer	1.3 mL 96 deep-well plate	450 μL

Tips

Take out DNA Clean Beads 30 min in advance and put them at room temperature. Vortex them thoroughly before using them.

2. Take a new 1.3 mL 96 deep-well plate and pipette the volumes listed in the table below into the wells of the plate. Seal the plate and mark one side of the well plate as “DNA Clean Beads” and “TE Buffer”.



Tips

When the number of samples is less than 96, add the reagents to the corresponding wells in the PCR plate based on the actual arrangement of the RT-PCR products. For example,

- When the samples are distributed within A1-A12, add the reagents to wells A1-A2 in the PCR plate.
- When the samples are distributed in A1-A12, B1-B12, add the reagents to wells A1-A2, B1-B2 in the PCR plate.

3. Prepare 50 mL of 80% ethanol by using absolute ethanol and Milli-Q water.

Tips

Use 80% ethanol immediately after preparation.

4. Take out a new 1.3 mL 96 deep-well plate. Add 80% ethanol to the plate, with 350 µL per well. Seal the plate and mark one side of the well plate as “80% Ethanol”.

Initializing MGISP-960RS

Tips

The software version of MGISP-960RS should be V1.8.0.323 or above and the hardware version should be 2-MGISP-960 and 7-MGISP-960.

1. Turn on the computer and the desktop is displayed. Double-click the icon on the control software to open the software.
2. Select **Real** and click **Create**.
3. Click **User Entry** to open the main interface.
4. Click **Initialize** on the top of the interface to start initializing.

   You will be prompted after a successful initialization.

Performing a pre-clean

Tips

Perform a pre-clean before conducting experiments every day.

1. Click the menu button on the left of the interface, and select **Pre-post clean**.
2. From the Clean list, select **Pre-clean**, and click **Start**.
3. Perform the required operations by following the on-screen instructions. Then, click **Continue**.

   The UV lamp and air filter start working.

Tips

The ultraviolet radiation is harmful to the human body, do not open the door after the pre-clean starts.

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Performing RT-PCR product purification

1. Set Solution and Script in the Run Wizard interface of MGISP-960RS according to the table below:

Solution	**JB-A09-157 MGI HIV-1 Drug Resistance Sequencing Package_RV1.0_SV1.0**
Script	**1.ATOPlex_RNA_HIVDR_PCR_Purification_step1_EN.py**

Tips

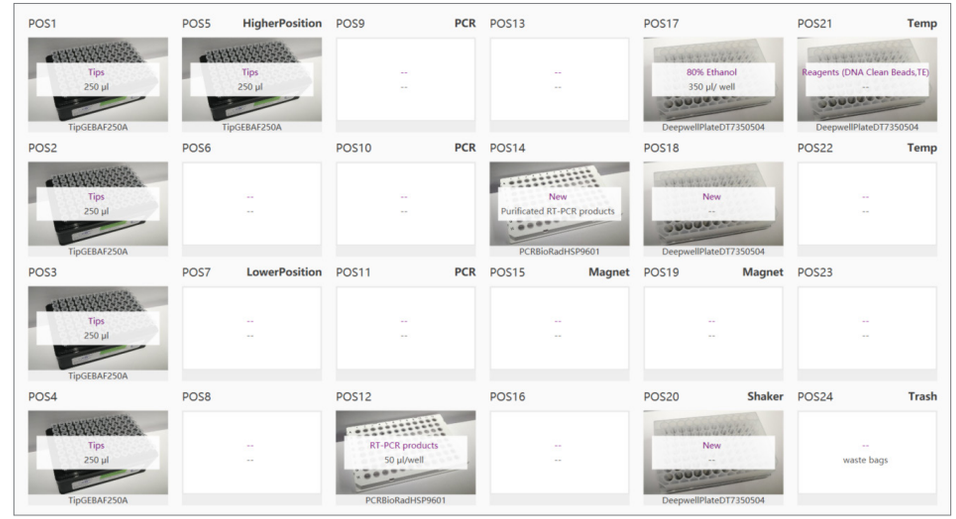
Ensure that the automation script and PCR program have been properly installed according to *MGISP-960 MGI HIV-1 Drug Resistance Sequencing Package User Manual* before running the script.

2. Mix DNA Clean Beads and EN-Beads thoroughly by using a vortex mixer and centrifuge them briefly.

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. Open the door of MGISP-960RS. Place the consumables according to the figure and the table below:



Name	Consumables	Position
New tips	250 µL automated filter tips	POS1 to POS5
RT-PCR products	0.2 mL 96 well full-skirt PCR plate	POS12
Purificated RT-PCT products	0.2 mL 96 well full-skirt PCR plate	POS14
80% ethanol	1.3 mL 96 deep-well plate	POS17
New deep-well plate	1.3 mL 96 deep-well plate	POS18 and POS20
DNA Clean Beads and TE	1.3 mL 96 deep-well plate	POS21

4. Close the door of the device.
5. Click **Run**.
6. After the process is completed, take out the RT-PCR products at POS14. The volume should be 30 µL for each well.

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7. Click **Continue** to end the process.
8. Quantify the RT-PCR products by using Qubit® dsDNA HS Assay Kit or Quant-iT PicoGreen dsDNA Assay Kit. The product concentration should be greater than or equals to 2 ng/μL.

Stoppoint

The RT-PCR products can be stored in a -20 °C refrigerator and should be used in two weeks.

9. Dispose of the discarded PCR plates, deep-well plates, and waste bag to the designated waste area.
10. (Optional) If no experiment will be conducted on the day, clean the device with pure water and 75% ethanol. Then, perform a post-clean.

Preparing Fast PCR-FREE library

Preparing samples

1. The starting amount of each sample is recommended to be 215 ng for Fast PCR-FRE library preparation. Add TE Buffer to the sample until the total volume reaches 48 μL. If the starting amount is less than 215 ng, put all of the sample for library preparation and add TE Buffer to the sample until the total volume reaches 48 μL.
2. Transfer 48 μL of total volume to a 0.2 mL 96 well full-skirt PCR plate.

Tips

- Ensure that no bubble exists at the bottom of the plate and no liquid exists on the inner walls.
- When the number of samples is less than 96, arrange the products in rows A to H sequentially until all products are placed. Prepare the consumables for the number of 96 samples.

Preparing reagents

1. Prepare 1× Elute Enhancer, En-TE, and En-Beads by using the following reagents from MGIEasy Fast PCR-FREE FS DNA Library Prep Set.

Tips

1× Elute Enhancer should be stored at room temperature, and En-TE and En-Beads should be placed at a 4 °C refrigerator. Use all solutions within 7 days.

- 1× Elute Enhancer

Component	Volume (μL)
20× Elute Enhancer	8.5
Nuclease-Free Water	161.5
Total volume	170

- En-TE

Component	Volume (μL)
1× Elute Enhancer	30
TE Buffer	14970
Total volume	15000

- EN-Beads

Component	Volume (μL)
1× Elute Enhancer	130
DNA Clean Beads	12870
Total volume	13000

2. Prepare 50 mL of 80% ethanol by using absolute ethanol and Milli-Q water.

Tips

Use 80% ethanol immediately after preparation.

3. Take two new 1.3 mL 96 deep-well plates. Add En-TE and 80% ethanol to wells of the plates respectively. Seal the plates and mark them.

Reagent	Volume (μL)
En-TE	150
80% ethanol	850

4. Take out the reagents according to the table below and thaw them. Mix each reagent thoroughly, centrifuge them, and place them on ice until use. Then, take out a 1.3 mL 96 deep-well plate to aliquot the following reagents. Seal the plate, mark on one side of the well plate as “reagents for library preparation”, and place it at 4 °C until use.

Tips

- Before aliquoting the reagents, mix DNA Clean Beads and EN-Beads thoroughly by using a vortex mixer and centrifuge them briefly. Ensure that no bubble exists at the bottom of other tubes, no liquid exists on the inner walls.
- When the number of samples is less than 96, add the reagents from the deep-well plate to the corresponding wells in the PCR plate based on the arrangement of the RT-PCR products. For example,
  - When the samples are distributed within A1 to A12, add the reagents to wells A1 to A7 in the PCR plate.
  - When the samples are distributed in A1-A12, B1-B12, add the reagents to wells A1-A7, B1-B7 in the PCR plate.

Well	Reagent	Volume (μL)/Well
Column 1	Fast FS buffer	150
Column 2	Fast FS Enzyme	70
Column 3	Fast Ligation buffer	350
Column 4	Ad Ligase	70
Column 5	Ligation Enhancer	35
Column 6	En-Beads	800
Column 7	En-Beads	800

5. Use the adapter plate or transfer some of the adapters to a new 96-well PCR plate based on the number of samples, with 10 μL per well. Seal the plate.

Tips

Arrange the adapters on the plate corresponding to the arrangement of the 48 μL samples.

Preparing libraries on MGISP-960RS

1. Set **Solution** and **Script** in the Run Wizard interface of MGISP-960RS according to the table below:

Solution	**JB-A09-157 MGI HIV-1 Drug Resistance Sequencing Package_RV1.0_SV1.0**
Script	**2.ATOPlex_FastFS_PCRFree_DNA_Library_Prep_Step2_EN.py**

2. Mix DNA Clean Beads and EN-Beads thoroughly by using a vortex mixer and centrifuge them briefly.

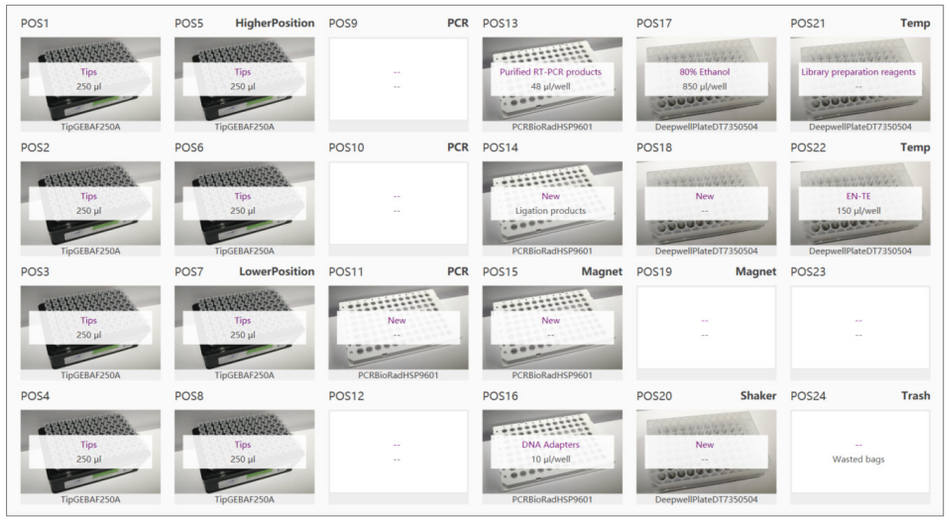
Tips

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

3. Open the door of MGISP-960RS. Place the consumables according to the figure and the table below:

Tips

Remove the sealing films of the deep-well plate or PCR plate before running the program.



Name	Consumables	Position
New tips	250 µL automated filter tips	POS1 to POS8
New PCR plates	0.2 mL 96 well full-skirt PCR plate	POS11, POS14, and POS15
Homogenized RT-PCR products	0.2 mL 96 well full-skirt PCR plate	POS13
UDB Adapters	0.2 mL 96 well full-skirt PCR plate	POS16
80% ethanol	1.3 mL 96 deep-well plate	POS17
New deep-well plate	1.3 mL 96 deep-well plate	POS18 and POS20
Reagents for library preparation	1.3 mL 96 deep-well plate	POS21
EN-TE	1.3 mL 96 deep-well plate	POS22

4. Close the door of the device.
5. Click **Run**.
6. After the process is completed, take out the Fast PCR-FREE product from POS14. The volume should be 30 µL for each well.
7. Click **Continue** to end the process.
8. Quantify the Fast PCR-FREE product by using Qubit dsDNA HS Assay Kit or Quant-iT PicoGreen dsDNA Assay Kit. The product concentration should be greater than or equals to 0.8 ng/µL.

Stoppoint

The Fast PCR-FREE product can be stored in a -20 ℃ refrigerator.

9. Dispose of the discarded PCR plates, deep-well plates, and waste bag in the designated waste area.
10. (Optional) If no experiment will be conducted on the day, clean the device with pure water and 75% ethanol. Then, perform a post-clean.

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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 libraries in equal quantities and transfer them into a 0.2 mL PCR tube. Mix the libraries thoroughly by using a vortex mixer. Take 15 ng of the mixed library to make DNBs. The volume should be equal to or more than or 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), and prepare the 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 the 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	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 the PCR device and start a reaction according to the conditions in the table below:

Temperature	Time
35 ℃ (Heated lid)	On
30 ℃	20 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 10 μL of Stop DNB Reaction Buffer to the PCR tube. Pipette up and down 5 to 8 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 ℃ refrigerator for up to 48 hours.

9. Take out 2 μL of the DNBs and quantify it by using Qubit ssDNA Assay Kit. The concentration of the DNBs should be no less than 8 ng/μL.

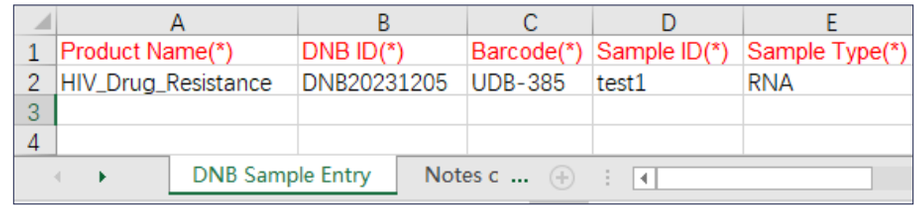
Tips

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

Sequencing and analysis

Importing the sample information

1. Tap the ZLIMS icon on the desktop of the sequencer to open the login page of MGI ZLIMS.
2. Enter the username (**lite**) and password (**lite123456**), and tap **Login** to open the Home page.
3. Tap **Sequencing + Analysis** on the Home page to open the New Sequence + Analysis page.
4. Select **HIV_Drug_Resistance** or set analysis product to **HIV_Drug_Resistance**, select **Import the Sample ID (**using this method as an example**) to import DNB sample information**, 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.



	A	B	C	D	E
1	Product Name(*)	DNB ID(*)	Barcode(*)	Sample ID(*)	Sample Type(*)
2	HIV_Drug_Resistance	DNB20231205	UDB-385	test1	RNA
3					
4					

DNB Sample Entry | Notes c ...

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 Type**: Select **RNA** as the sample type.

7. Return to the sequencing + analysis import window, tap **Choose File**. Select the completed worksheet 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.

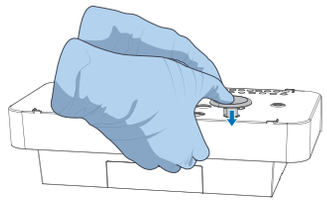
Preparing the flow cell and sequencing reagent cartridge

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

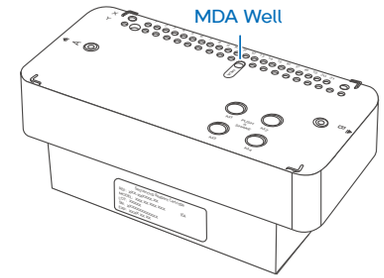
Tips

- The flow cell should not be left at room temperature for more than 24 hours.
- 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 for 3 to 4 hours. After thawing, place the cartridge in the 2 ℃ -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.



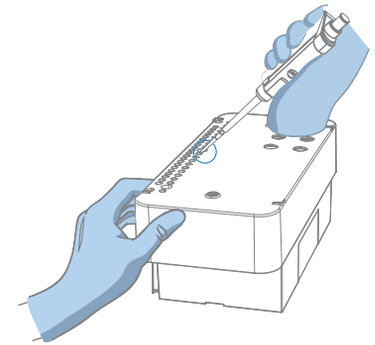
5. Hold the A and B sides of the cartridge with both hands by following the marks A and B on the cartridge, and invert the cartridge for 10 to 20 times to mix the reagents thoroughly. Pierce the MDA well with a 1 mL pipette tip. Then, gently tap the cartridge against the desktop to remove bubbles.



6. Take out MDA reagent and MDA Enzyme Mix from the reagent kit. Transfer 125 µL of MDA Enzyme Mix into MDA reagent tube by using a 200 µL pipette for PE sequencing.
7. Invert the cartridge 6 times to mix the solution thoroughly.
8. Add all solution into the MDA well.

Tips

- When adding MDA Enzyme Mix, ensure that the pipette tip is tilted against one side of the MDA well to avoid bubbles.
- Add MDA Enzyme Mix slowly to prevent it from overflowing into other wells.



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Sequencing and analysis

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 & Transmission** and input the DNB ID in the **DNB ID** box.

Tips

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

6. Select **PE150+10+10** in the Recipe box. Tap the dropdown box and select **UDB (1-480)**.

Tips

For instructions on importing the customized barcode **UDB (1-480)**, refer to the relevant 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 then **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 in advance and place it on ice for approximately 30 minutes. Mix it thoroughly by using a vortex mixer 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.

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11. Take out the flow cell and check it. Pipette 10 μL of DNB loading mixture by using a 100 μL pipette. Gentle transfer the mixture into the inlet of the flow cell and press the release button on the pipette to discharge it. The mixture will automatically flow into the flow cell.

Tips

- Do not press the control button on the pipette.
- During the DNB loading process, do not rotate the pipette tip or move the flow cell to avoid bubbles.

12. 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 scan the ID.

Tips

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

13. Tap **Next** to review the information.
14. After reviewing all information, tap **Sequence** and select **Yes** to start sequencing. The auto-sliding screen moves down and the Sequencing interface is displayed.

    The interface shows the real-time sequencing progress, and you can view the status after refreshing the system.
15. 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**.
16. After the sequencing is completed, the analysis software automatically starts the analysis.

Viewing and downloading the report

1. Click the number under **Report Today** on the Home page. The Analysis Report page is displayed.
2. Set the query term in the **Query** area and click  to locate the sample.
3. Click the batch code under the **Batch Code** column to view project details.
4. Click  in the **Result Path** column to open the result path.
5. Click **Result** to view and download the analysis reports.

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