Labnics

DNA Analyzer

NDA-100

Index

1. Introduction

DNA Analyzer NDA-100 offers 100 bp long single-end reads and 150 bp long paired-end reads for versatile genomic analysis. This analyzer utilizes self-luminescence to generate light, minimizing noise and enhancing signal clarity. It features an integrated sequencing flow cell that employs microfluidics for efficient sample processing. It is equipped with a 12.1-inch high-resolution LCD touchscreen for user-friendly operation.

2. Features

- No signal interference
- Decreased duplicates for accuracy
- High repeatability
- Integrated CMOS Module
- Reduced index hopping

3. Specifications

Model	NDA-100
Read Lengths	100 bp long single-end reads with 25 million effective reads 150 bp long paired-end reads with 25 million effective reads 100 bp long reads with 20 million effective reads 150 bp long reads with 20 million effective reads
Operating Temperature	15° C to 30° C
Relative Humidity	20% RH to 80% RH
Atmospheric Pressure	70 kPa to 106 kPa
Storage Temperature	-20° C to50° C
Maximum Sound Pressure	15% RH to 85% RH
Shell Protection Grade	65 dBA
Touch Screen	IPX0
Touch Screen Size	LCD Touch Screen
Touch Screen Resolution	12.1 inch
Computing Module	1280 × 800
Power	CPU: i7-10700(8 Core) Internal Storage: 64G SSD: 256GB SSD + 2T HDD
Dimensions (L × W × H)	Rated Voltage and Power 24V DC, 5A
Weight	348 × 312 × 257 mm

4. Applications

DNA Analyzer is used for accurate genetic sequencing and rapid pathogen detection. It is employed in research laboratories, clinical diagnostics, and public health settings.

5. Operations

5.1 Amplifying RT-PCR manually

5.1.1 Sample requirements

- This package applies to the total RNA extracted from multiple sample types, including influenza strains, swabs, and so on.
- It is recommended to use the high-quality total RNA sample with its Ct
- value is no greater than 32 and set the initial input to 10 PL.

5.1.2 Consumable Information

- 0.2 mL PCR tube
- Icebox
- Pipettes and applicable filtered tips

5.1.3 Performing the RT-PCR amplification process

1. Take out the following reagents from the MGI Easy Respiratory Microorganisms Genome Amplification Kit. Prepare the RT-PCR reaction mixture on ice, mix it thoroughly, and transfer it to a new 0.2 mL PCR tube.

RT-PCR reaction mixture	
Reagent	**Volume(μl)**
Flu A/B Primer Pool	2
Flu Control Primer	2
RT-PCR buffer	25
RT-PCR Enzyme Mix	2.5
Nuclease-Free Water	8.5
Total Volume	**40**

2. Transfer 10 pL of RNA sample into the PCR tube and the total volume is 50Pl.
3. Mix the mixture by pipetting 10 times and centrifuge it briefly to collect the solution to the bottom of the tube.

Note:

- Do not vortex the mixture
- It is recommended to add a negative control sample to each batch of experiments. Add 10 pL of nuclease-free water into a single-tube reaction to reptace the RNA sample. The negative control can be involved in the whole process to evaluate whether there is a risk of cross-contamination during each experiment. If the negative control in the analysis report is negative for the influenza virus, the risk of cross-contamination is low. Otherwise, the risk of cross-contamination is high
- Place the PCR tube into the thermal cycler for the next reaction according to the following conditions.

Temperature	Time	Cycle
Heated lid(105°C)	On	1
45°C	30 min	
55°C	15 min	
95°C	3 min	
95°C	30 S	5
55°C	30 S	
68°C	3 min	
95°C	30 S	38
64°C	30 S	
68°C	3 min	
68°C	5 min	1
12°C	Hold	

Note:

- Set the number of amplification cycles according to the Ct value of the sample. If the Ct value of the sample is greater than 20 but less than or equal to 32.it is recommended to use 38 cycles.
- Perform RT-PCR amplification reaction in the post-PCR area.

5.2 Purifying the RT-PCR Product

5.2.1 Preparing the device

- Start the computer and MGISP-100RS. Double-click the icon of MGISP-100RS on the desktop to run the software.
- Select Real. Click Create and click User Entry to enter the initialization interface.
- Click Initialize to start initialization.
- After successful initialization, a prompt will appear on the interface.
- Perform a pre-clean according to the following steps:
  1. Click Pre-post > pre-clean > Start.
  2. Follow the prompts to complete operations. Click continue.

Note:

- It is recommended to perform automation with MGISP-100RS OF V1,0 and above.
- Before using the device, ensure that the application scripts and PCR Programs have been imported.

5.2.2 Preparing the consumables

Prepare the following consumables and reagents for automation before the experiment:

- 250 pL automated filtered tips
- 1.3 mL 96-welt deep-well plate
- Break-away PCR 8-strip tubes and the caps (hereafter referred to as 8-strip tubes)
- 2 mL SC micro tube, PCR-PT
- MGIEasy DNA Clean Beads
- Absolute ethanol
- Milli-Q water

5.2.3 Preparation of samples

Transfer 50 pL of RT-PCR product into a new 8-strip tube. Ensure that no bubbles exist at the bottom of the tube, and no liquid remains on the tube watt. Place the product on ice until use.

Note:

One 8-strip tube is needed for 8 samples and two tubes for 16 samples.

If the number of samples is less than 8 or 16, fill the empty welts with Milli-Q water and prepare reagents and consumables for 8 or 16 samples.

5.2.4 Preparing of reagents

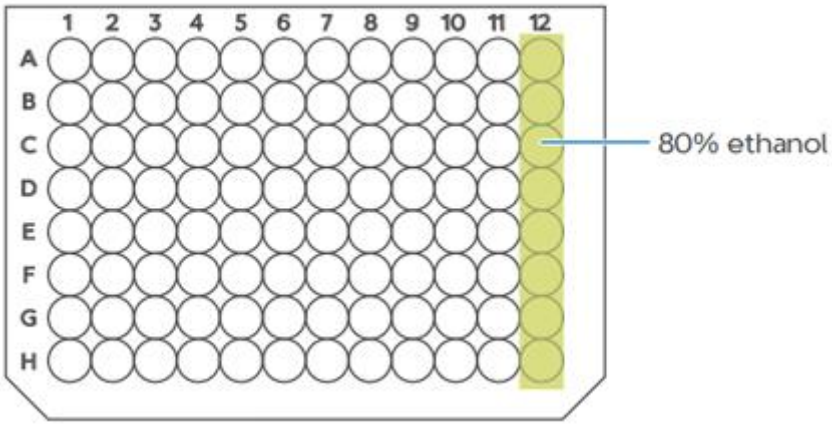
1. Take out the following reagents from the MGIEasy DNA Clean Beads Kit. Vortex and mix them thoroughly.

Reagents	Volume(μl)	
	8 RXN	16 RXN
DNA Clean Beads	380	660
TE Buffer	500	900

2. Take out the DNA Clean Beads at room temperature for 30 minutes in advance. and mix it thoroughly before using it.
3. Transfer DNA Beads and TE Buffer into two different 2 ml SC micro tubes according to the table above. Then close the tube caps and mark them as 'DNA Beads' Beads and 'TE'' respectively.
4. Use absolute ethanol and Milli-Q water to prepare 25 ml of 80% ethanol.
5. Take out a 96-well deep well plate. Add reagents according to the following figure.
   8 RXN: 450 μL/Well
   16RXN:750 μL/Well

Note:

Use the prepared 80% ethanol immediately after preparation

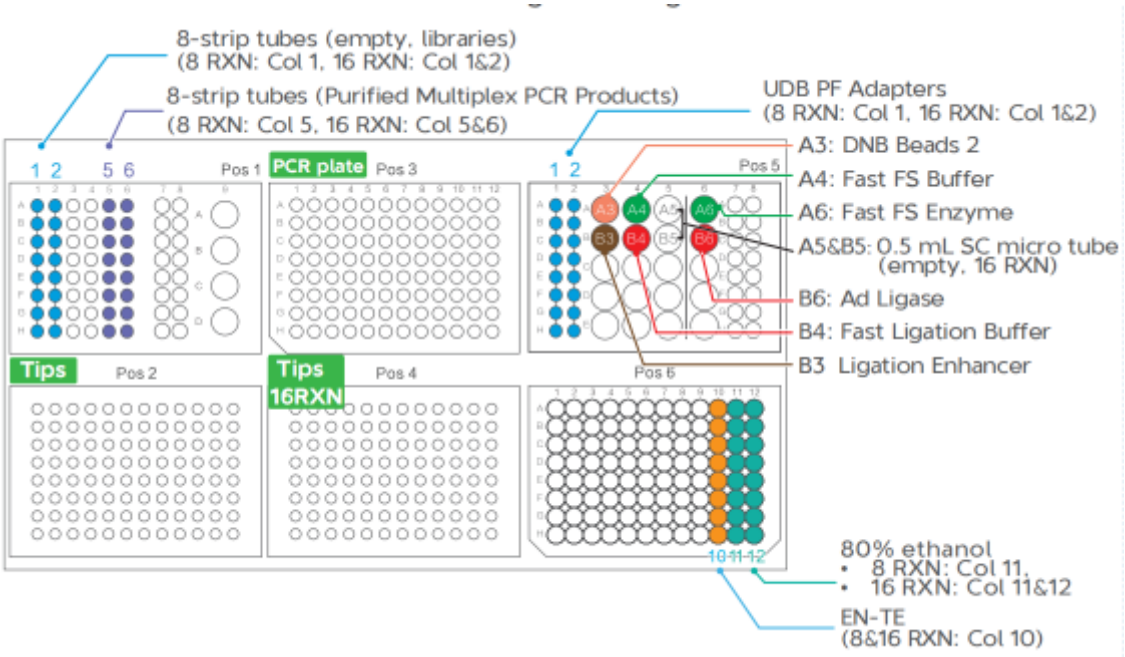


5.2.5 Performing the RT-PCR Purification Process

1. Configure the solution and script in the run wizard interface of MGISP-100RS according to the following table:

Solution	JB-A06-087 MGI Respiratory Microorganisms genome sequencing pacakage_ RV.0_SV1/0
Script	1.ATOPlex_RNA_Respmulti_Purification_step1.py

2. Thoroughly mix the DNA clean Beads with vortex mixer and centrifuge them briefly before placing reagents and consumables.
3. Place the consumables according to the figure below.



4. Confirm the placement. close the door and click **RUN**.
5. After the library preparation, follow the prompts to take out the libraries from pos1. The volume of the products is 25μl.
6. Click '**Continue'** to end the process.

7. Quantify the products according to the instructions of the Qubit dsDNA HS Assay kit or Quanti-It Pico Green DsDNA Assay Kit. The required concentration should be no less than 0.8ng/ μl.
8. Dispose of the used sample tubes, PCR Plates, deep well plates, and waste bags to the designated waste area.
9. (Optional) If you will not conduct any experiments on the day. Use laboratory-grade WATER ANDF 75% ethanol to clean the surface of the device and perform a post-clean.

Note:

- Before using the device, ensure that the application scripts and PCR program have been imported.
- Ensure that no bubbles exist at the bottom of the tube, that no liquid remains on the tube wall, and that all tube caps are open.
- This concentration requirement does not apply to the purified product of the negative control sample.
- Store the libraries in a -20°C freezer.

5.3 Making of DNBs

1. Prepare the NDA-100 high-throughput sequencing set (FCL PE150) according to the following table.

Reagent kit	Component
NDA-100 High-throughput sequencing set (FCL PE150)	Low TE Buffer
	Make a DNA Buffer (OS-V2. O-DB)
	Make a DNA Buffer (OS-V2. O-SB)
	Make DNB Enzyme Mix I (OS)
	Make DNB Enzyme Mix II(OS)
	Stop DNB Reaction Buffer
	DNB load Buffer II
	Signal Protein 1
	Signal Protein 2
	Signal Protein Buffer

Note

- Do not use filtered pipette tips for making and loading DNBs.
- Each kit is sufficient to make DNB for 4 sequencing runs.

5.3.1 Preparing of samples

1. The recommended initial library preparation input for each sample is 215ng. Add TE Buffer to bring the final volume to 48μl.
    - If the volume of purified product ranges from 150ng to 215ng. use all the products for library preparation and add TE Buffer for a total volume of 48 μl.
    - If the purified product volume in the negative control sample is less than 150ng. Use all the products and add TE Buffer for a total volume 0of 48 μl.

2. Respectively transfer 48 µl of each sample into the prepared 8-strip tubes. Ensure that no bubbles exist at the bottom of the tube and that no liquid remains on the tube wall. place them on ice for further use.

Note
One 8-strips tube is needed for 8 samples and two tubes for 16 samples.
If the number of samples is less than 8 or 16. fill the empty wells with Milli Q water and prepare reagents and consumables for 8 or 16 samples.

5.3.2 Preparing of reagents

1. Prepare 1× Elute Enhancer, En-TE and EN-Beads. All reagents can be used within 7 days

Reagent	Component	Volume (µl)	Storage condition
1×Elute Enhancer	20×Elute Enhancer,	1.5	Room Temperature
	Nuclease-Free water	28.5	
	Total Volume	30	
EN-TE	1×Elute Enhancer,	5.4	4°C
	TE Buffer	2694.6	
	Total Volume	2700	
EN-Beads	1×Elute Enhancer	20	4°C
	DNA Clean Beads	1980	
	Total Volume	2000	

2. Mix the EN-Beads thoroughly. Transfer it into a 2.0ml SC microtube. Close the tube cap and mark it as'' DNA Beads2''

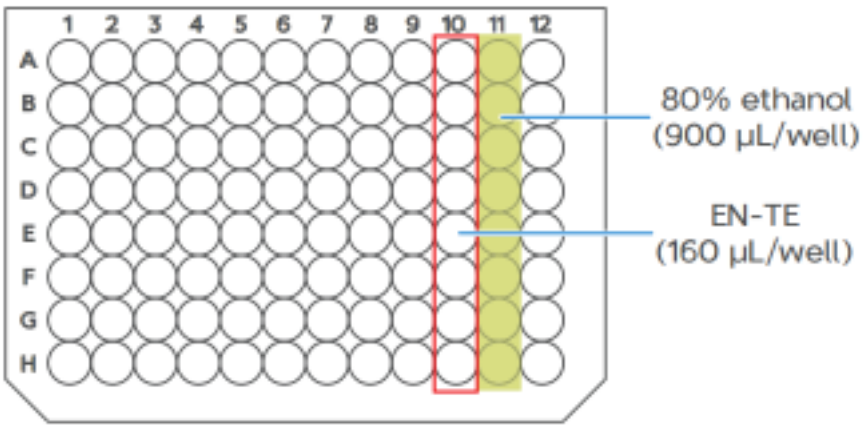
Reagent	Volume(µl)	
	8 RXN	16 RXN
En-Beads	950	1800

3. Take out of the following reagents. thaw them and mix the reagents thoroughly. centrifuge shortly and place them on ice until use. Take out five (8 RXN) or seven (16 or RXN) 0.5ml SC microtubes and dispense library prep reagents into them.

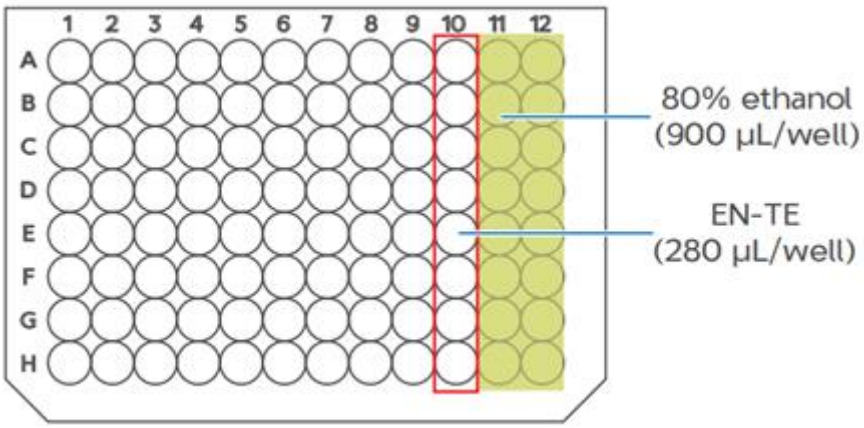
Reagent	Volume (µl)	
	8 RXN	16 RXN
Fast FS Buffer	102	200
Fast FS Enzyme	47.5	110
Ligation Enhancer	25	48
Fast Ligation Buffer	220	430
Ad Ligase	45	105

4. Use absolute ethanol and Milli-Q water to prepare 25 ml of 80% ethanol.
5. Taker out a 96-well deep well plate. Add reagents according to the following figure.

8 RXN



16 RXN



6. Take out the adapters from the MGIEasy UDB PF Adapter kit. Mix and centrifuge them thoroughly, then transfer the adapters (10 µl/Well) into the 8-strip tube.

Note:

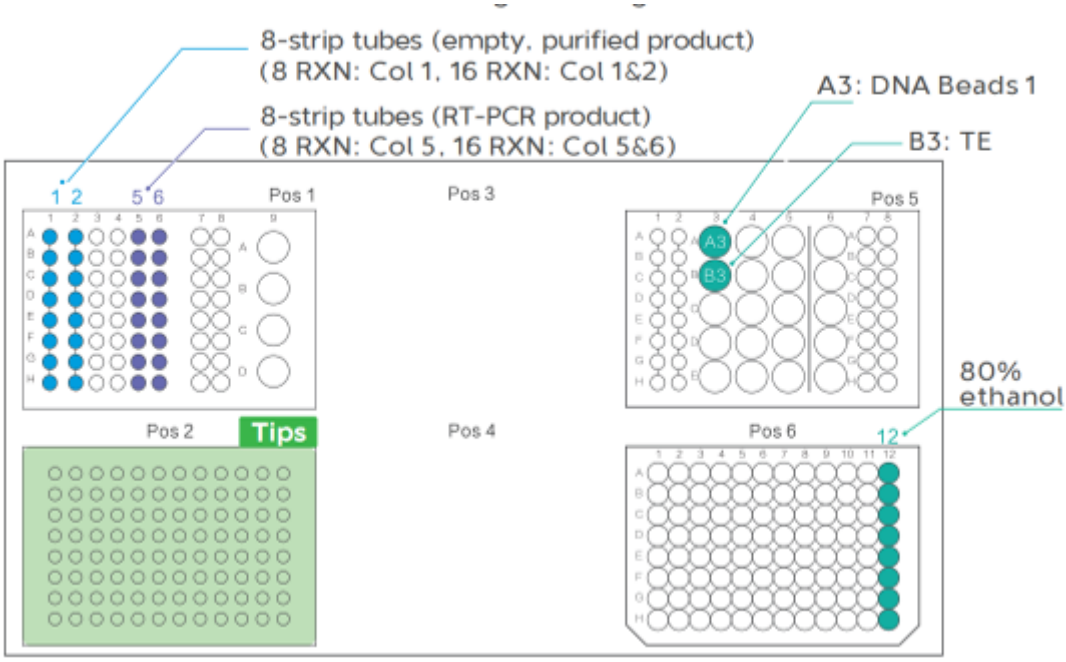
- If you directly purchase the MGIEasy fast PCR-FREE FS Library prep set (16RXN) for 16 RXN library preparation. You can thaw, mix, and centrifuge the reagents in the original tube and directly use them on the MGISP-100RS without dispensing them independently. But 2 additional 0.5 ml SC micro tubes are needed for future use.
- When using 96 RXN reagent kits to perform 16RXN Experiments .2 tubes are not dispensed with reagents.
- Use the 80% ethanol immediately after preparation

5.4 Preparing Fast-PCR-Free Library

1. Configure the Run Wizard interface of MGISP-100RS according to the following table.

Solution	JB-A06-87 MGI Respiratory microorganisms Genome Sequencing Package _RV1.0_SV1.0
Script	1.ATOPlex_RNA_RespMulti_PCR_Purification_Step1.py

2. Thoroughly mix the DNA-clean beads with a vortex mixer and centrifuge them briefly before placing reagents and consumables.
3. Place the consumables according to the figure below



4. Confirm the placement and close the door. Click **Run**, select sample number **8 or 16** by following the prompt, and click **Continue**.
5. After Purification. follow the prompts to take out the purified RT-PCR products from Pos1. The volume of the product is 30 μl.
6. Click **Continue** to end the process.
7. Quantify the products according to the instructions of the Qubit dsDNA HS Assay Kit or Quanti-It™ Pico Green™ dsDNA Assay Kit. The required concentration should be no less than 5ng/ μl.
8. Dispose of the used sample tubes, PCR Plates, deep well plates, and waste bags to the designated waste area.
9. (Optional) If no experiment is to be conducted on the day. Use laboratory-grade water and 75% Ethanol to clean the surface of the device and perform a post clean.

Note:

- Before using the devices. Ensure that the application scripts and PCR Program have been imported.
- Ensure that no bubbles exist at the bottom of the tube, that no liquid remains on the tube wall, and that all tube caps are open.
- This concentration requirement does not apply to the purified product of the negative control sample.
- The purified RT-PCR Product can be stored at -20°C and should be used within 2 weeks.

5.4.1 Preparing the reagent kits

1. Prepare the reagent kits according to the following table

Reagent Kit	Component
MGIEasy Fast -PCR-FREE FS library prep Module	20× Elute Enhancer
	Fast FS Buffer
	Fast FS Enzyme
	Ligation Enhancer
	Fast Ligation Buffer
	Ad Ligase
MGIEasy DNA Clean Beads	TE Buffer
	DNA Clean beads
MGIEasy UDB PF Adapter kit	UDB Adapters
NDA-100 High-throughput Sequencing set (FCL PE150)	MDA T-Reagent
	MDA Enzyme Mix
	Sequencing Reagent Cartridge
	Waste container
	Funnel
NDA-100 Sequencing Flow cell	Funnel

2. Take out low TE buffer, Make DNA Buffer (OS-V2.0-DB), Make DNB Enzyme Mix I (OS), and stop DNB Reaction Buffer From NDA-100 High throughput sequencing set (FCL PE150) and thaw them on ice for 30 min.
3. After thawing, use a vortex mixer to vortex them for 5 s. centrifuge briefly and place them on ice until use
4. Take out a 0.2 ml 8-strip or PCR tube and prepare the DNB reaction mixture 1 according to the following table.

Component	Volume(µl)
Low TE buffer	20-V
dsDNA Library	V
Make DNB buffer (OS-V2.0-DB)	20
Total Volume	**40**

5. Mix Make DNB reaction mixture 1 thoroughly by vortexing. Centrifuge it for 5 Seconds. Place the tube into a thermal cycler to start the reaction under the following conditions.

Temperature	Time
heated lid (105°C)	On
95°C	3 min
57°C	3 min
4°C	Hold

6. Place make DNB enzyme mix II (OS) on ice, centrifuge briefly for 5 s, and place it on ice until use.
7. Take the tube out of the thermal cycler when the temperature reaches 4°C. Centrifuge briefly for 5 seconds and prepare DNB reaction mixture 2 on ice according to the following table.

Component	Volume(µl)
Make DNB Enzyme Mix I(OS)	40
Make DNB Enzyme Mix II(OS)	4

8. Mix the reagent gently by vortexing, centrifuge for 5 s, and place the tube into a thermal cycler. Start the reaction according to the following conditions.

Temperature	Time
Heated lid (35°C)	on
30°C	25 min
4°C	Hold

9. Take the tube out of the thermal cycler when the temperature reaches 4°C. add 20 µl of stop DNB Buffer to the tube. Use a non-filtered wide bore tip to pipette up and down 8 times to mix the reagent gently.
10. Use the Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify 2 µl of DNBs. The concentration of DNBs should range from 4 to 40ng/ µl. Otherwise, you need to make DNBs again.

Note:

- Do not take Make DNB Buffer (OS-V2.0-SB) by mistake.
- DsDNA Library input V (µl) =15ng/mixed library concentration (ng/ µl).
- Do not place DNB Enzyme Mix II (OS) at room temperature.
- Avoid Holding the tube for a long time.
- Do not centrifuge the vortex or pipette vigorously.
- Store DNBs at 2 to 8 °C and perform sequencing within 48 hours

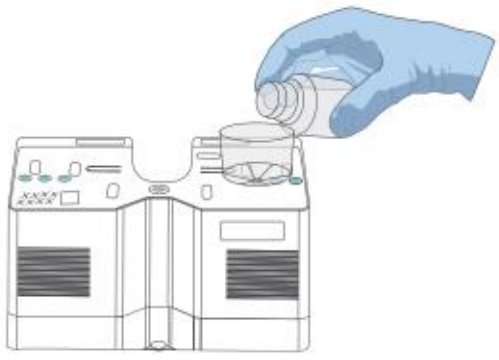
5.5 Preparing the reagent cartridge and flow cell

5.5.1 Preparing the reagent cartridge

1. Place the Reagent cartridge upright (with the label facing upwards) and thaw it in a refrigerator at 2°C to 8°C for 12 hours or at 15°C to 25°C for 4.5 to 5 hours.
2. Thaw signal protein buffer by placing them on ice.
3. Thaw signal protein 1 and signal protein 2 on ice for about 10 minutes.
4. After thawing, shake the cartridge to check whether there is ice in it. If there is a sound of cracked ice, place the reagent cartridge at room temperature until no ice exists.
5. Hold the two sides of the cartridge with two hands. Invert it 20 times and gently tap it on a flat surface 10 times. Invert it 10 times and gently tap it on a flat surface 10 times again.
6. Hold the reagent cartridge upright swing it downward 10 times and remove the outer packaging.
7. Vortex the signal protein1 and signal protein 2 for 5 s. centrifuge them, briefly for 4 seconds or 5 seconds and place them on ice until used.
8. Add an appropriate amount of signal protein 1 and signal protein 2 to the signal protein buffer tube according to the following table.

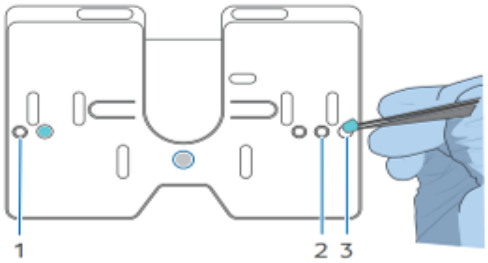
Signal Protein Mixture	
Component	**Volume**
Signal protein 1	31.5µl
Signal Protein 2	21 µl
Signal Protein Buffer	21ml

9. Screw the cap of the signal protein mixture tube and invert the tube 10 to 15 times to mix thoroughly.
10. Place the reagent cartridge on a flat surface as the following figure. Place the funnel over the MSP well and add the signal protein mixture into the MSP Well.



11. Remove the MDA T-Reagent and MDA Enzyme Mix from storage. Invert the MDA Enzyme Mix to ensure proper mixing, then briefly centrifuge it. Transfer 50 µl of the MDA Enzyme Mix to the MDA T-Reagent tube and invert the tube six times to mix thoroughly.
12. Use a clean tip to pierce the MDA well and transfer the MDA mixture into the MDA well.

13. Use the pointed-tip tweezers to remove the stoppers in wells No.1, No. 2, and No. 3. Place these stoppers into the designated container.



Note

- Do not thaw the reagent cartridge in a water bath.
- To prevent bubble formation, avoid stirring the mixture vigorously.
- The reagent cartridge with the MDA mixture should be loaded within 20 min. Failure to do so might affect the sequencing quality.

5.5.2 Preparing the flow cell

Take the flow cell box out of storage and remove the flow cell from the box. Unwrap the outer plastic package before use and use it within 24 hours.

5.6 Performing Sequencing

5.6.1 Inputting samples

1. Double-click the ZLIMS icon on the desktop, input the username lite and password, and select **login.**
2. Click **Sequencing+Analysis** in the sample interface and click Sample Template on the pop-up page.
3. Choose **MGI_FluTrack** as the analysis product. Select "**Import the sample ID**" to input the sample information, then click "**New**." In the pop-up window, click on either the **Excel Template** or **CSV Template** to download the DNB sample template in .xlsx or .csv format.
4. Fill in the worksheet DNB sample entry in the template file and save the file.

	A	B	C	D	E	F
1	Product Name(*)	DNB ID(*)	Barcode(*)	Sample ID(*)	Sample Name	Sample Type(*)
2	MGI_Flutrack	test2023	UDB-449,UDB-450	sample1		RNA
3	MGI_Flutrack	test2023	UDB-451,UDB-452	sample2		RNA
4	MGI_Flutrack	test2023	UDB-453,UDB-454	sample3		RNA
5	MGI_Flutrack	test2023	UDB-455,UDB-456	sample4		RNA
6						

DNB Sample Entry | Notes of filling the blank

5. Click **Choose file** to import the prepared file and click **Upload.**
6. Ensure that the uploaded file is correct and click Ok to save the task.
7. Refresh the task management interface and view the status of the DNB sample.

Note:

- It is recommended to perform sequencing with ZLIMS-Lite V2.1 and above on NDA-100 V1.0 and above.
- The Cells with* are require.
- The cells cannot be merged. Blank spaces or special characters are not allowed.
- Different field types correspond to different entering formats.
- DNB ID generally consists of a letter and number, which cannot be repeated with the DNB ID that already exists.
- Sample ID generally consists of letters and numbers, which can be repeated with the sample ID that already exists in the system.
- If the sample name and sample type are both the same, the data will be merged for analysis.

5.6.2 Start Sequencing

1. Enter the username and password in the login interface and click Log in.
2. Click  to enter the customization interface. The compartment door opens automatically, and the rack slides out.
3. Click the **Recipe** list and select **PE150**.
4. Select the **Read 1** box and set the read length of read 1 to 100 with the on-screen Keyboard.
5. Repeat step 4 to configure **Read 2**. Select the Barcode list to click PFAS_PE.
6. Ensure that the required recipe is selected and select.  .
7. Enter flow cell information on the left interface by scanning the QR Code on the package of the flow cell or manually with the on-screen Keyboard.
8. Open the flow cell package to check whether the flow cell is intact and whether the scanned ID is the same as the ID on the flow cell. After confirming, install the flow cell.
9. Take out the prepared reagent cartridge and enter the reagent cartridge information on the right interface by scanning the QR code on the cartridge or manually with the on-screen keyboard.
10. Remove the bottom cover in the middle of the reagent cartridge, align the reagent cartridge with the positioning columns on the rack, and place it over the flow cell.
11. Ensure that the cover of the waste container is open, place the waste container on the rack, and ensure that it fits into the bent metal clip. click  > Yes >  .
12. Enter DNB ID information by scanning the QR code on the sample tube or manually with the on-screen keyboard.
13. Prepare the DNB Loading mixture:
    - Vortex to mix DNB load buffer II thoroughly for 5 s, centrifuge it briefly, and place it on ice until used.
    - Prepare the DNB loading mixture according to the following table.

Component	Volume
DNB Load Buffer	34
DNB	101
Total Volume	136

- Use a non-filtered. Wide-bore tip to pipette up and down 8 times to mix the DNB loading mixture gently.
- Use a wide-bore tip to transfer all the DNB loading mixture into the DNB loading well. Ensure that no bubbles exist in the tube.

14. Close the compartment door and click  to check all the information.
15. If the information is correct, ensure that the computing module is connected.
    Select  > Yes to start sequencing.
    You can check the sample status in the sample management interface of the ZLIMS system.
16. After sequencing, open the compartment door. Remove the flow cell the reagent cartridge, and the waste container. Click  to return to the main interface.

5.7 Viewing the report.

1. In the task management interface of ZLIMS Click the task name to enter the **Analysis Report** interface.
2. View report details on the report page.

Note:
If you need to download any table or graph in the report, click above the table or graph.

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