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· Release date: June 2023

Applicable set/kit

Name	Cat. No.	Brand
MGIEasy Fast FS Library Prep Module	940-000029-00 (16 RXN)/ 940-000027-00 (96 RXN)	MGI
MGIEasy Fast UDB PF Adapter Kit	1000022800 (16 RXN)/ 1000022802 (96 RXN)	
MGIEasy DNA Clean Beads	1000005278 (8 mL)/ 1000005279 (50 mL)	
DNBSEQ One Step DNB Make Reagent Kit	1000026466 (4 RXN)	
DNBSEQ-G99RS High-throughput Sequencing Set (G99 SM FCL PE150)	940-000410-00	

Applicable consumables

Name	Cat. No.	Brand
0.5 mL SC Micro Tube	1000001558	MGI
2 mL SC Micro Tube	1000001553	
96-well Deep-well Plate	1000004644	
96-well PCR Plate	1000012059	
Break-away 8 Strips PCR Tubes and Caps	100-000016-00	

Applicable device/software

Model No.	Cat. No.	Brand
MGISP-100RS	900-000206-00	MGI
DNBSEQ-G99ARS	900-000609-00	
FTAT-miniloader	510-003170-00	
MTB-Explorer Software	970-000385-00 (Use together with bioinformatics analysis server)	

Preparing libraries

Intended use

It is only applicable to use the DNA samples extracted from cultured mycobacterium tuberculosis. The recommended extraction kit is MGIEasy MicrobiomeDNA Extraction Kit (Cat. No.: 1000027955) or equivalents.

Preparing samples

1. Prepare the sample mixture by performing one of the following operations:
   - Take out no less than 10 ng of the gDNA and add TE Buffer to bring the final volume to 48 µL.
   - According to specific requirements, take out 1.1 ng to 110 ng of the gDNA and use TE Buffer to bring the final volume to 48 µL.

Tips

In a library preparation run, the initial input volume of the gDNA should be the same.

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2. Transfer 48 µL of the sample mixture to an 8-strip PCR tube and place the tube on ice. Ensure that no bubbles exist at the bottom of the tube and that no liquid remains on the tube wall.

Tips

- If the number of samples is 4 or 8, one 8-strip PCR tube is required.
- If the number of samples is 16, two 8-strip PCR tubes are required.
- If the number of samples is no greater than 4, 8 or 16, fill the rest of the tubes with water. Prepare the reagents and consumables according to the number of samples.

Preparing reagents

1. Take out all the reagents from the MGIEasy Fast FS Library Prep Module. Shake the Fast FS Enzyme to mix it thoroughly, centrifuge briefly, and place it on ice. Equilibrate other reagents to room temperature, mix them thoroughly with a vortex mixer, centrifuge briefly. Place them on ice for use.
2. According to the number of samples, add reagents into corresponding tubes according to the following table.

Reagent	Tube	Volume (µL)		
		4 RXN	8 RXN	16 RXN
Fast FS Buffer	0.5 mL SC micro tube	65	105	215
Fast FS Enzyme	0.5 mL SC micro tube	30	45	105
Ligation Enhancer	0.5 mL SC micro tube	12	18	55
Fast Ligation Buffer	0.5 mL SC micro tube	143	225	450
Ad Ligase	0.5 mL SC micro tube	33	50	100
PCR Enzyme Mix	2 mL SC micro tube	300	470	955

Tips

When preparing library of 16 samples by using the Library Prep Module (16 RXN), you need to transfer the PCR Enzyme Mix to a 2 mL SC micro tube. For other reagents, use the original tubes in the MGIEasy Fast FS Library Prep Module to prepare the library.

3. Prepare 1x Elute Enhancer, En-TE and En-Beads according to the following tables.

Tips

- Take out the DNA Clean Beads at least 30 minutes in advance to equilibrate to room temperature and mix it thoroughly by vortexing.
- Store 1x Elute Enhancer at room temperature, and store En-TE and En-Beads at 4 ℃. All reagents should be used for up to 7 days.

- 1x Elute Enhancer

Component	Volume (µL)
20x Elute Enhancer	2.5
Nuclease-Free Water	47.5
Total volume	50

- En-TE

Component	Volume (µL)
1x Elute Enhancer	8
TE Buffer	3992
Total volume	4000

- En-Beads

Component	Volume (µL)
1x Elute Enhancer	15
DNA Clean Beads	1485
Total volume	1500

4. Mix the En-Beads and DNA Clean Beads thoroughly, respectively. Transfer them into two different 2 mL SC tubes and cap the tubes.

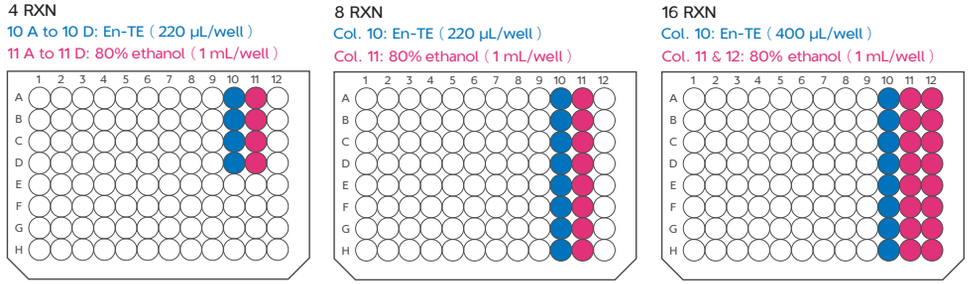
Reagent	Consumables	Volume (µL)		
		4 RXN	8 RXN	16 RXN
En-Beads	2 mL SC micro tube	350	700	1300
DNA Clean Beads	2 mL SC micro tube	500	1000	1800

5. Prepare 25 mL of the 80% ethanol by using Milli-Q water.

Tips

The 80% ethanol should be used immediately after preparation.

6. Take out a 96-well deep-well plate and add reagents according to the following figures.



7. Use TE Buffer in the kit to dilute the adapter. Confirm the dilution times according to the following table. If the dilution times are more than once, ensure that the volume of the diluted adapter mixture is no less than 100 µL. According to the input volume in the following table, transfer the diluted adapter mixture to a clean 0.5 mL SC micro tube, vortex and centrifuge the tube briefly, and place the tube on ice for use.

Tips

The quality and volume of the adapter may affect the efficiency of the library preparation and quality of the library.

gDNA (ng)	MGI adapter Dilution times	Input volume to 0.5 mL SC micro tubes after dilution (µL)		
		4 RXN	8 RXN	16 RXN
50 to 200	No dilution	35	55	100
25	2 x	35	55	100
10	5 x	35	55	100
5	10 x	35	55	100
2.5	15 x	35	55	100
1	45 x	35	55	100

8. Transfer the PCR Primer into one 8-strip PCR tube (4 RXN or 8 RXN) or two 8-strip tubes (16 RXN). If the number of samples is 4, you only need to transfer the PCR Primer to the first 4 tubes of the 8-strip PCR tube. The volume for each tube should be no less than 15 µL.

Tips

The 8-strip tube is the break-away PCR 8-strip tube.

Running MGISP-100RS software

1. Set the solution as **JB-A06-079 MGIEasy Fast FS DNA Library Prep Set_RV1.0_SV2.0** in the Run wizard interface of MGISP-100RS.

Tips

Before operation, ensure that the automation script and the PCR program have been imported successfully.

2. Before placing consumables, thoroughly mix the DNA Clean Beads with a vortex mixer and centrifuge it briefly.

Tips

Ensure that no bubbles exist at the bottom of the tube, that no liquid remains on the wall, and that all the tubes are uncapped.

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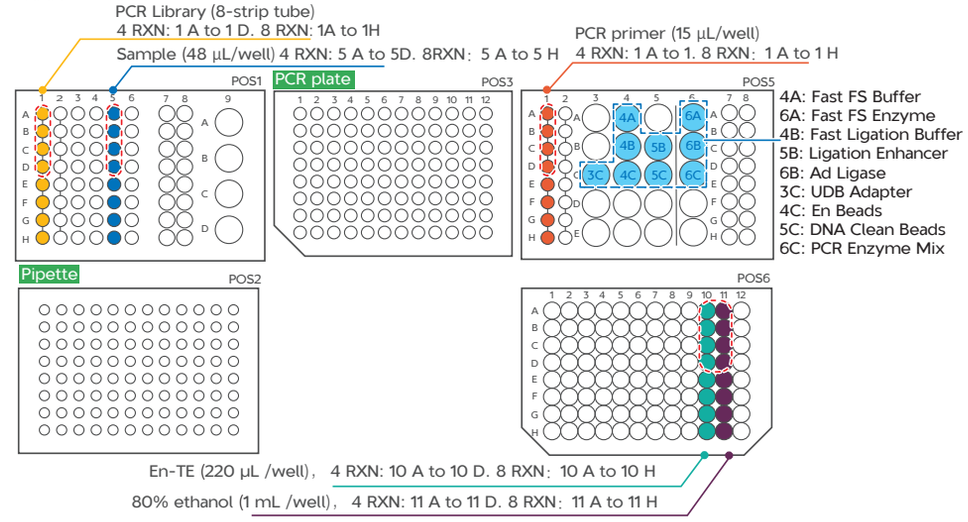
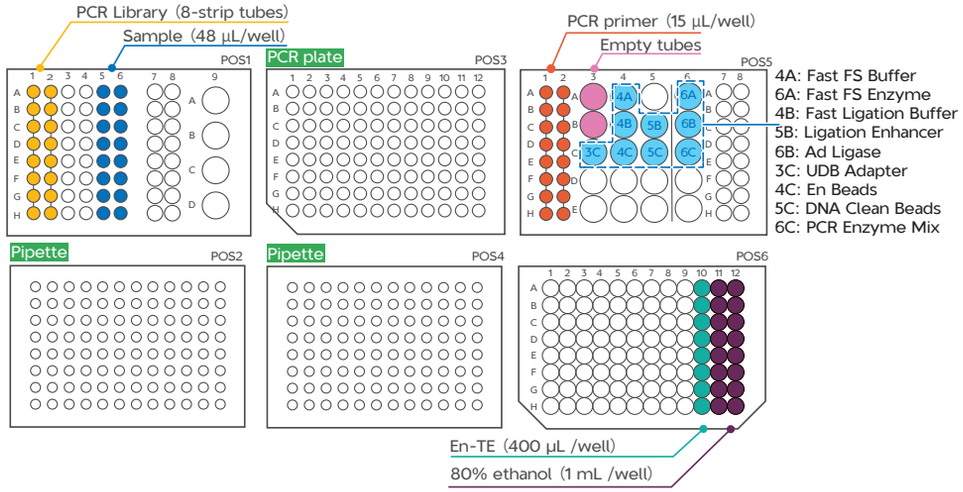
3. Place the consumables according to the following figures.

4 RXN & 8 RXN



16 RXN



Tips

- If the number of samples is 4, only fill the first 4 tubes of 8-strip PCR tubes and deep-well plate. Library products will be collected in the first 4 tubes of the 8-strip PCR tube.
- After the first use of Ligation Enhancer, store it at 10 °C to 30 °C and protect it from light.

4. Click **Run**. If the running script is for 4 RXN & 8 RXN, the Sample_Num window is displayed. Select the sample number according to requirements.

5. Select the stop time according to the following table and click **Continue**.

Initial input (ng)	Automation input (ng)	PE150 stop time (min)
200	220	12
100	110	12
50	55	12
5	3.5	8
1	1.1	8

6. Select PCR cycles according to the following table. Do not fill in the blank under any value. After all the conditions are selected, click **Continue**. The experiment starts.

gDNA(ng)	Required cycles for different outputs	
	300 ng	1 µg
⩾ 100	3 to 4	5 to 6
50	5 to 6	6 to 7
5	7 to 9	10 to 11
1	11 to 12	13 to 15

Tips

The whole workflow takes about 3 h 10 min to 3 h 50 min. During the workflow, you can pause or resume the experiment according to requirements.

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7. After the workflow is completed, take out the PCR library (the volume is 30 µL) in the first and second column at POS1.
8. Use Qubit dsDNA HS Assay Kit or Quant-iT PicoGreen dsDNA Assay Kit to quantify the library. For details, refer to the relevant quantification kit operation guide.

Tips

The PCR library concentration should be no less than 1 ng/µL.

Stop point

You can store the PCR library at -20 °C in the freezer.

9. (Optional) Use Mili-Q water and 75% ethanol to clean the surface of the device and perform a post-clean.
10. Dispose of the used sample tubes, deep-well plates, PCR plates, and waste bags to the designated waste area.

Making OS DNBs

Tips

Ensure that the library complies with the base balance principle when using the same flow cell to perform the sequencing.

Preparing samples

1. Add the library in a new 0.2 mL PCR tube and mix the library at specific rate according to the requirement of sequencing data. The mixed DNA library quality is 20 ng and the volume is no more than 10 µL.

Tips

When the total volume is less than 10 µL, add TE Buffer to bring the total volume to 10 µL.

2. Take out the following reagents from the DNBSEQ One-step DNB Prep Module (OS-DB) and prepare the reaction mixture on ice.

Component	Volume (µL)
Mixed library	V
TE Buffer	10-V
Make DNB Buffer (OS-DB)	10
Total volume	20

3. Vortex the PCR tube and centrifuge briefly to collect the mixture at the bottom of the tube.
4. Place the PCR tube into the PCR machine and run the program as follows:

Temperature	Time
105 °C (heated lid)	on
95 °C	3 min
40 °C	3 min
4 °C	hold

5. After the reaction is completed, place the tube on ice, and add the following reagents. The total volume of the mixture in the PCR tube is 42 µL.

Component	Volume (µL)
Make DNB Enzyme Mix I	20
Make DNB Enzyme Mix II (OS)	2
Total volume	22

6. Vortex the PCR tube and centrifuge briefly to collect the solution to the bottom of the tube.
7. Place the PCR tube into the PCR machine and perform the reaction as follows.

Tips

Setting the temperature of the heated lid to 35 °C or as close as possible to 35 °C is recommended.

Temperature	Time
35 ℃ (heated lid)	on
30 ℃	20 min
4 ℃	hold

8. Immediately add 10 μL of the Stop DNB Reaction Buffer to the PCR tube once the reaction is completed. Mix them gently by pipetting 5 to 8 times with a wide-bore tip.

Tips

Do not shake or vortex the tube.

Stop point

You can store the DNBs at 4 ℃ and use the DNBs to perform sequencing within 48 hours.

9. Take out 2 μL of the DNBs and use Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify the DNBs. For details, refer to the relevant operation guide. The final DNBs concentration should be no less than 8 ng/μL.

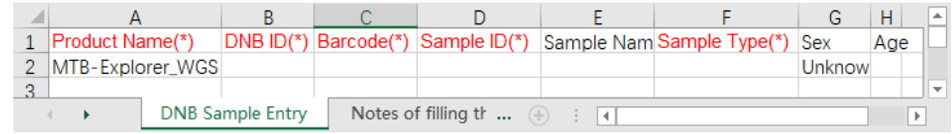
Tips

If the DNBs concentration is greater than 40 ng/μL, dilute the DNB mixture to 20 ng/μL with TE Buffer.

Starting sequencing + analysis

Inputting the sample

1. Double-click the icon on the desktop of the server to enter the Login page.
2. Input the username and password, and click **Login**.
3. Click **Sequencing + Analysis** on the Home page. The New Sequencing + Analysis page is displayed.
4. Select **MTB-Explorer_WGS** as the analysis product. Select a method to input the DNB sample according to requirements. For example, select **Import the Sample ID** and click **New**.
5. Click **Excel template** or **CSV template** to download a template in *.xlsx* or *.csv*.
6. Open the template, input information in **DNB Sample Entry** sheet, and then click **Save**.



Tips

- The field with a red asterisk (*) is required, and other fields are optional.
- Cells in the Excel sheet cannot be merged. No spaces or special characters should exist before and after the content in each cell.
- Some fields include drop-down options, and other fields require manual input. The formats used with the fields are different.
- **DNB ID**: A DNB ID should be unique for identifying the sample and include both letters and numbers .
- **Barcode**: When one sample ID corresponds to multiple barcodes, the multiple barcodes are separated by halfwidth commas (,). For multiple consecutive barcodes, a tilde (~) can be used.
- **Sample ID**: A sample ID should include both letters and numbers, and can be the same as a sample ID already existing in the system.
- **Sample Name**: If both sample names and sample types are the same, data of the samples is merged to be analyzed.

7. On the Import Sequencing + Analysis page, click **Choose File**. Choose the Excel file in the pop-up dialog box and click **Upload.**

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8. After the DNB sample information is entered, return to the New Sequencing + Analysis page, click **Save**, and then click **OK** in the pop-up window.

Preparing the flow cell

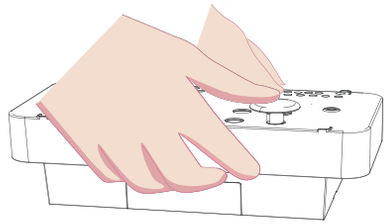
Take out the flow cell at least 30 minutes, but no more than 24 hours in advance to equilibrate to room temperature for loading DNBs.

Tips

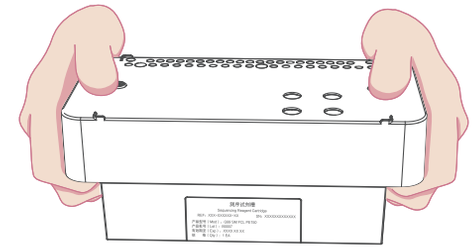
Do not unwrap the vacuum package at this moment.

Preparing the sequencing cartridge

1. Take out the sequencing cartridge. Thaw the sequencing cartridge in a water bath at room temperature for 3 to 4 hours or at 2 ℃ to 8 ℃ in a refrigerator a day before. After thawing, store the sequencing cartridge at 2 ℃ to 8 ℃ in a refrigerator for future use.
2. Unwrap the package and wipe the condensation water on the lid and the well with a dust-free wipe.
3. Use the pressing tool to press the wells M1, M2, M3, M4.



4. According to the signs on the cartridge, hold both A and B sides of the cartridge with both hands, shake to mix it thoroughly for 10 to 20 times, and ensure that reagents inside are fully mixed. Gently tap the cartridge to remove bubbles in the reagents.
5. Add 125 µL of the Make MDA Mix into the MDA tube by using a pipette with 200 µL measurement range. Shake and vortex to mix it thoroughly for 4 to 6 times.
6. Pierce the MDA well on the cartridge with the pipette and slowly add all the reagents into the MDA well. Ensure that reagents do not overflow to other wells when adding the reagents.



Starting sequencing

1. Input the username *user* and password *123*, and click **Login** to enter the main interface.
2. Choose side **A** or side **B** in idle status to perform the sequencing. Click **Sequence** and select **New Sequence**. If you need to perform sequencing on both sides, click **Sequence A&B**.
3. After clicking **New Sequencing**, the waste compartment door will be opened automatically. Perform a pre-run check according to the following steps:
   ① Place the waste container and close the compartment door.
   ② Check if the waste container is installed correctly according to the on-screen tips.
   ③ Empty the waste container or ensure that the waste level is under the maximum value of the waste container before sequencing.
4. Click **Next** after self-check and input the sequencing information.

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For example, select **Sequencing + Analysis** as the sequencing type, select **No** for BBS, and then input the DNB information in the **DNB ID** text box.

Tips

Ensure that the entered DNB ID is exactly the same with the DNB ID entered in the DNB Sample Entry table on ZLIMS.

5. Select **PE150+10+10** in the sequencing recipe drop-down list and select the **UDB 1~480** in the drop-down menu on the right.
6. In the **Advance settings** area, select **Yes** for **Barcode splitting** and **Auto-wash.**
7. Click **Next**. The auto-sliding screen moves up automatically.
8. Push the prepared sequencing cartridge into the cartridge compartment. The system scans the cartridge ID automatically and displays the ID in the **Cartridge ID** text box.

Tips

If the scanning fails, enter the ID manually.

9. Click **Prime** and click **Yes**. The preloading starts. The progress is displayed in the software interface.

Loading DNBs

1. Take out the DNB Load Buffer, thaw on ice for about 30 minutes, and vortex to mix it thoroughly with a vortex mixer. Centrifuge it briefly for 5 seconds, and then place it on ice for future use.
2. Take out a new 0.2 mL SC micro tube and prepare the DNB loading mixture according to the following table.

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

3. Gently pipette the tube 5 to 8 times to mix it thoroughly with a wide-bore pipette. Place the tube on ice at 4 ℃ .

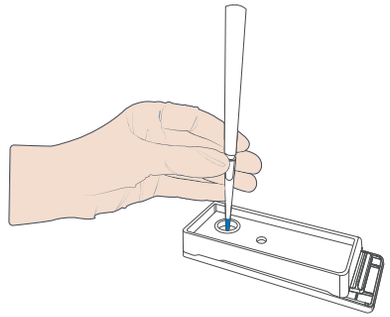
Tips

- The DNB loading mixture should be used immediately after preparation.
- Do not centrifuge, shake or vortex the tube.

4. Take out the O-ring that is installed in the loader by pressing it from the bottom of the loader. Hold the loader with left hand and open the lid. Insert the flow cell into the loader and close the lid.

   Check if the center of the gasket is photic. If yes, the flow cell is installed correctly. Rotate the flow cell and place it on the table.

5. Use a 200 μL pipette tip to aspirate 10 μL of the DNB loading mixture. Insert the pipette into the fluidics inlet of the flow cell vertically. Fix the pipette with left hand, and press the button on the pipette to eject the pipette tip.



Tips

- Do not press the control button of the pipette.
- Do not rotate the pipette tip or move the flow cell when loading DNBs to prevent bubbles from entering the flow cell.
- Do not transfer the DNB library into the flow cell by using the pipette gun.

6. Keep the pipette tip for 3 seconds to let the DNB library flow into the flow cell.
7. Rotate the tip counterclockwise to remove it. Check if the mixture are flowing in the flow cell through observation hole. If the liquid surface is reflective, the liquid flows into the flow cell successfully. Immediately place the flow cell facing up on the sequencer for use.

Placing the flow cell and reviewing the information

1. After the loading is completed, click **Next**. The auto-sliding screen moves up automatically.
2. Insert the prepared flow cell into the flow cell stage. The system scans the flow cell ID automatically.

Tips

If the scanning fails, enter the ID manually.

3. Click **Next**, review the sequencing information. Ensure that all the information is correct, click **Sequence**, and click **Yes** in the pop-up window. The sequencing starts.

Tips

The real-time sequencing progress and steps are displayed on the Software page. After refreshing the Sample Management page on ZLIMS, you can view the status of the sample.

Viewing and downloading the report

1. On the Home page of ZLIMS, click the numbers under **Report Today** to enter the Analysis Task page.
2. Enter a query term in the **Query** area, click [🔍] to search for the sequencing task, and click the link in the **Analysis Type** column.
3. Click [📄] in the **Report** column to view the report.
4. Click [📁] in the **Result File** column to download the result files.

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