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Applicable sets/kits

- MGIEasy Fast FS DNA Library Prep Set (16 RXN Cat. No.: 940-000029-00) (96 RXN Cat. No.: 940-000027-00)

Name	Component	Cat. No.
MGIEasy Fast FS DNA Library Prep Module	Fast FS Buffer Fast FS Enzyme Fast Ligation Buffer Ad Ligase Ligation Enhancer 20× Elute Enhancer PCR Enzyme Mix	940-000028-00 (16 RXN) 940-000031-00 (96 RXN)
MGIEasy UDB Primers Adapter Kit	UDB Adapter UDB PCR Primer	1000022800 (16 RXN) 1000022802 (96 RXN)
MGIEasy DNA Clean Beads	DNA Clean Beads TE Buffer	1000005278 (8 mL) 1000005279 (50 mL)

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

Component	Quantity
Low TE Buffer	300 µL × 1 tube
Make DNB Buffer (OS DB)	80 µL ×1 tube

Component	Quantity
Make DNB Enzyme Mix I (OS)	160 µL × 1 tube
Make DNB Enzyme Mix II (OS)	16 µL ×1 tube
Stop DNB Reaction Buffer	100 µL ×1 tube

- 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

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Recommended consumables

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

Preparing DNA libraries

Preparing samples

1. The starting amount of each gDNA sample is recommended to be 110 ng for DNA library preparation. Add TE Buffer to the sample until the total volume reaches 48 µL.

Tips

- If there are specific requirements, the starting amount can be adjusted within the range of 1.1 ng to 220 ng. Add TE buffer to the sample until the total volume reaches 48 µL.
- Only one starting amount can be used for each library preparation process.

2. Transfer 48 µL of each sample to a break-away 8-strip PCR tube, 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.
- For 4 or 8 samples, one set of 8-strip tubes is needed. For 16 samples, 2 sets of 8-strip tubes are needed.
- If the number of samples is less than 4, 8, or 16, fill in the remaining tubes with water. Prepare the reagents and consumables according to the actual number of samples.

Preparing reagents

1. Take out all reagents from MGIEasy Fast FS DNA Library Prep Module. 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. Prepare the reagents according to the table below:

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

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Tips

When preparing libraries for 16 samples by using the MGIEasy Fast FS DNA Library Prep Set (16 RXN), manually transfer PCR Enzyme Mix to a 2 ml PCR SC micro tube, and directly use the tubes of other reagents from the MGIEasy Fast FS DNA Library Prep Set for automatic library preparation.

3. Prepare 1x Elute Enhancer, En-TE, and En-Beads. All the solutions should be used within 7 days.

Tips

1× Elute Enhancer should be stored at room temperature, and En-TE and En-Beads should be placed at a 4 °C refrigerator.

- 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 En-Beads and DNA Clean Beads. Transfer them into two new 2 mL SC micro tubes according to the table below. Mark the tubes with En-Beads and DNA Clean Beads respectively, and cap the tubes.

Component	Consumable	Volume (μL)		
		4 RXN	8 RXN	16 RXN
En-Beads	2 mL SC micro tubes	350	700	1300
DNA Clean Beads		500	1000	1800

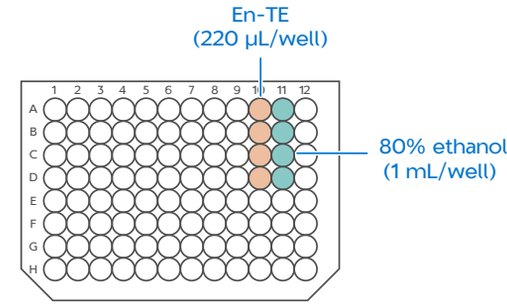
5. Prepare 25 mL of 80% ethanol.

Tips

Use the 80% ethanol immediately after preparation.

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

- For 4 RXN



Add the following reagents:

- Add 220 μL of En-TE to the first four wells in column 10.
- Add 1 mL of 80% ethanol to the first four wells in column 11.

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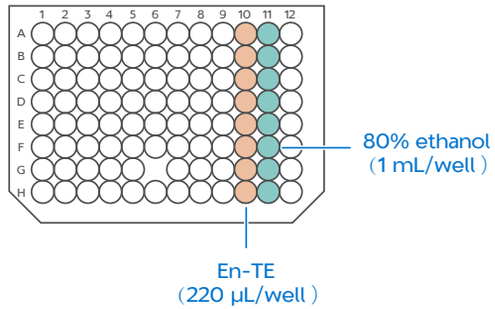
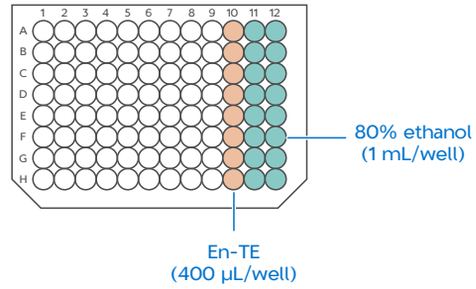
- For 8 RXN



Add the following reagents:

- Add 220 µL of En-TE to each well of column 11.
- Add 1 mL of 80% ethanol to each well of column 11.

- For 16 RXN



Add the following reagents:

- Add 400 µL of En-TE to each well of column 10.
- Add 1 mL of 80% ethanol to each well of columns 11 and 12.

7. Calculate the adapter dilution factor (diluted by using TE Buffer from the kit) according to the actual gDNA amount and the table below. For the gDNA sample with the amount ranging from 50 ng to 200 ng, no dilution is required. For other gDNA samples that require dilution, transfer the adapter solution to a new 0.5 mL SC micro tube, mix the adapter solution thoroughly, centrifuge it, and place the tube on ice until use.

Tips

The quality and quantity of adapters affect library preparation efficiency and library quality.

gDNA (ng)	MGI adapter	Volume (µL) of the transferred adapter solution		
	Dilution factor	4 RXN	8 RXN	16 RXN
50~200	No dilution	35	55	100
25	2x			
10	5x			
5	10x			
2.5	15x			
1	45x			

8. Transfer PCR Primer into one set (for 4 or 8 RXN) or two sets (for 16 RXN) of break-away 8-strip tubes.

Tips

For 4 samples, transfer PCR Primer into the tubes in the first 4 wells in column 1, with a minimum volume of 15 µL of PCR Primer per tube.

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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-A06-079 MGIEasy Fast FS DNA Library Prep Set_RV1.0_SV2.0
Script	Fast_PCR_DNA_Library_SP100_4&8RXN.py Fast_PCR_DNA_Library_SP100_16RXN.py

Tips

Ensure that the automation script and PCR program have been installed before running the script.

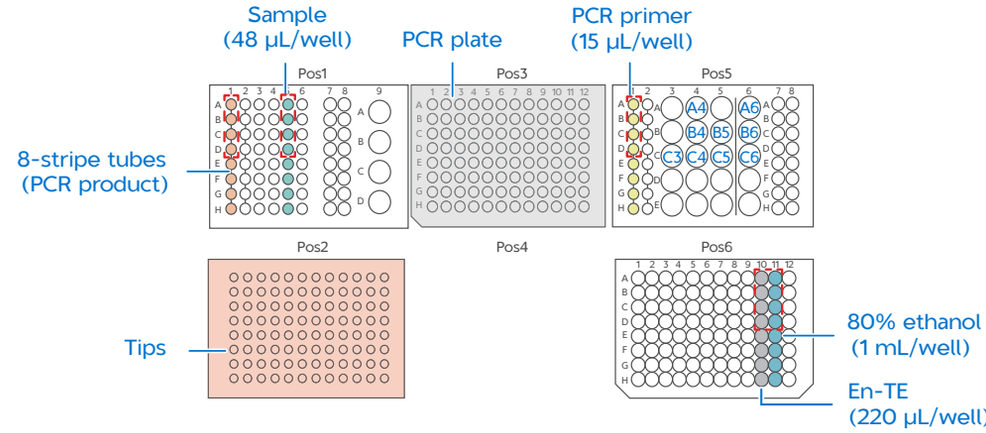
2. Mix DNA Clean Beads and EN-Beads thoroughly by using a vortex mixer and centrifuge them briefly before placing consumables.

Tips

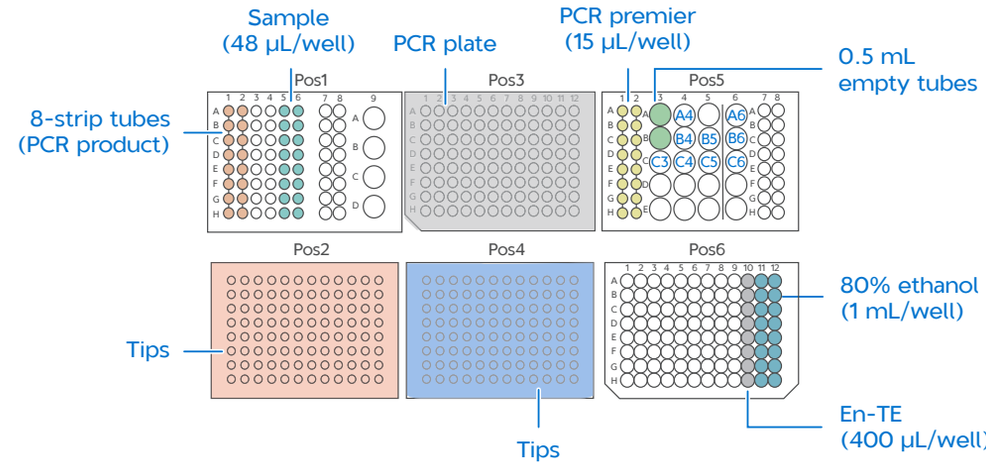
Ensure that no bubble exists at the bottom of other tubes, no liquid exists on the inner wall, and all tubes are uncapped.

3. Place the consumables according to the figures below:

- For 4&8 RXN



- For 16 RXN



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

Well	Reagent
A4	Fast FS Buffer
A6	Fast FS Enzyme
B4	Fast Ligation Buffer
B5	Ligation Enhancer
B6	Ad Ligase
C3	UDB Adapter
C4	En-Beads
C5	DNA Clean Beads
C6	PCR Enzyme Mix

Tips

- For 4 samples, add reagents to the tubes in the first four wells in column 1 of the deep-well plate. The libraries will be collected in the tubes in the first four wells in column 1 of the deep-well plate.
- After first use of Ligation Enhancer, place it at a temperature of 10 °C to 30 °C away from light.

4. Click **Run**. If the 4&8 RXN script runs, the Sample_Num window pops up. Then select the desired number of samples on the pop-up window.
5. Select the fragmentation time based on the starting amount according to the table below. Then, click **Continue**.

Starting amount (ng)	Input amount (ng)	Fragmentation time (min)
200	220	12
100	110	12
50	55	12
5	5.5	8
1	1.1	8

6. Select the required PCR cycles according to the actual requirements, leave **Value** blank, and click **Continue** to start the automatic library preparation.

gDNA (ng)	Required cycles	
	300 ng	1 μg
≥ 100	3-4	5-6
50	5-6	6-7
5	7-9	10-11
1	11-12	13-15

Tips

The entire process is about 3 hours 10 minutes to 3 hours and 50 minutes. You can pause or resume it as needed in the experiment process.

7. After the process is completed, take out the PCR product from Pos 1. The total volume should be 30 μL. Then cap the 8-strip PCR tubes.
8. 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 ng/μL.

Stoppoint

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

9. Place the discarded sample tubes, reagent tubes, 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 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 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-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 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 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 °C refrigerator for up to 48 hours.

9. Take out 2 μL of the DNBs and quantify it according to the quantification instructions of Qubit ssDNA Assay Kit. The concentration of the DNBs should not be 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 TE buffer.

Sequencing

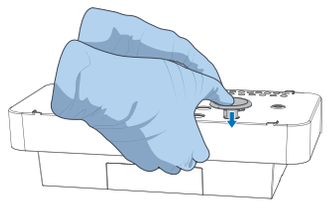
Preparing the flow cell and sequencing reagent cartridge

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

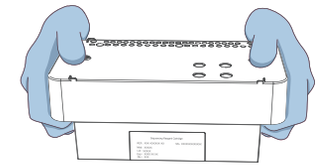
Tips

- 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.



Importing samples

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**.
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		DNA	NA

DNB Sample Entry | Notes of filling the blank

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

Tips

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

6. Select **SE100+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 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.

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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 ℃ 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&MGIDL-G99RS Portable DNB Loader Quick Start Guide*.
11. 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.

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.