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Overview

Applicable set/kit

Name	Cat. No.	Brand
MGIEasy Signature Identification Library Prep Kit (48 RXN)	940-000343-00	MGI
MGIEasy Signature Identification Library Prep Kit (576 RXN)	940-001338-00	
DNBSEQ-G99RS High-throughput Sequencing Set (G99 SM FCL SE400)	940-000417-00	

Recommended consumables

Name	Required quantity for DNA samples	Required quantity for blood card samples	Cat. No.	Brand
250 µL automated pipette filter tips	1 box	1 box	1000000723	MGI
1.3 mL U-bottom deep-well plate	1 EA	1 EA	1000004644	
0.2 mL 96-well skirted PCR plate	1 EA	1 EA	091-000165-00	
Break-away 8-strip PCR tubes and caps	3 Sets × 8 (Sample quantity ≤ 8) 6 Sets × 8 (Sample quantitiy > 8)	2 Sets × 8 (Sample quantity ≤ 8) 4 Sets × 8 (Sample quantity > 8)	100-000016-00	
0.5 mL SC micro tube, PCR-RT	2 EA	2 EA	1000001558	
2 mL SC micro tube, PCR-RT	2 EA	2 EA	1000001553	

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Applicable device/software

- Solution A

Model	Cat. No.	Brand
MGISP-100RS	900-000070-00	MGI
MGIDL-G99RS	510-003112-00	
DNBSEQ-G99ARS	900-000560-00	
DNA Signature Identification Software	970-000139-00 (installed on the bioinformatics analysis server)	

- Solution B

Model	Cat. No.	Brand
MGISP-100RS	900-000070-00	MGI
MGIDL-G99RS	510-003112-00	
DNBSEQ-G99RS	900-000561-00	
DNA Signature Identification System	900-000440-00	

Preparing libraries for signature identification

Preparing samples

MGISP-100RS supports the library preparation of 1 to 16 DNA samples or blood card samples. Prepare the samples according to the sample type:

- DNA samples: Dilute the samples to a recommended concentration of 0.15 ng/μL to 1.5 ng/μL. Transfer 10 μL of each sample to one of the break-away 8-strip PCR tubes.

Tips

  - When the number of samples is no more than 8, you need 1 set of 8-strip tubes.
  - When the number of samples is no less than 9, you need 2 sets of 8-strip tubes.

- Blood card samples: Add the blood cards with the diameter of 1 mm to 1.2 mm to the PCR plate at Pos3 in order. If the environment is dry, add 1 μL of TE buffer to the wells first. When adding the blood cards, ensure that the blood cards are always at the bottom of the wells.

Tips

  - When the number of samples is no more than 8, add the samples to Well 3A to Well 3H.
  - When the number of samples is no less than 9, add the samples to Well 3A to Well 3H and Well 4A to Well 4H.

Preparing reagents

1. Take out PCR Primer Pool, PCR Block, PCR Enzyme Mix, PCR Dual Barcode Primer, Clean Buffer, and TE Buffer from the MGIEasy Signature Identification Library Prep Kit. Thaw, mix, centrifuge, and place them on ice until use.
2. Take out DNA Clean Beads 30 minutes in advance and place them at room temperature.

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3. Take out the consumables according to the table below. Mark them according to the reagent classifications and prepare the reagents using a pipette with filter. Mix, centrifuge, and place the reagents on ice until use.

Reagent classification	Name	Automatic reagent volume	Specification
The first-round PCR Reaction Solution Mix	PCR Primer Pool	6 μL × (Sample quantity+2)	0.5 mL SC micro tube
	PCR Enzyme Mix	12.5 μL × (Sample quantity+2)	
The second-round PCR Reaction Solution Mix	PCR Block	3.6 μL × (Sample quantity+2)	0.5 mL SC micro tube
	PCR Enzyme Mix	15 μL × (Sample quantity+2)	
	TE Buffer	6.6 μL × (Sample quantity+2)	
DNA Clean Beads	DNA Clean Beads	46 μL × (Sample quantity+3)	2 mL SC micro tube
TE Buffer	TE Buffer	Sample quantity<7	2 mL SC micro tube
		80 μL × (Sample quantity+2)	
		Sample quantity ≥7: 720 μL	

4. Aspirate PCR Dual Barcode Primer F (2.4 μL for each sample) and PCR Dual Barcode Primer R (2.4 μL for each sample) according to "Appendix B PCR Dual Barcode Primer Strategies" of *MGIEasy Signature Identification Library Prep Kit User Manual*. Add the aspirated reagents to the 8-strip tubes in Well A to Well H successively (or directly add 4.8 μL of PCR Dual Barcode Prime Mix for each sample. Cap the tubes and mark them. Mix, centrifuge, and place the tubes on ice until use.

Tips

- When the number of samples is no more than 8, you need 1 set of break-away 8-strip PCR tubes.
- When the number of samples is no less than 9, you need 2 sets of break-away 8-strip PCR tubes.

5. Prepare 25 mL of 80% ethanol with Milli-Q water.

Tips

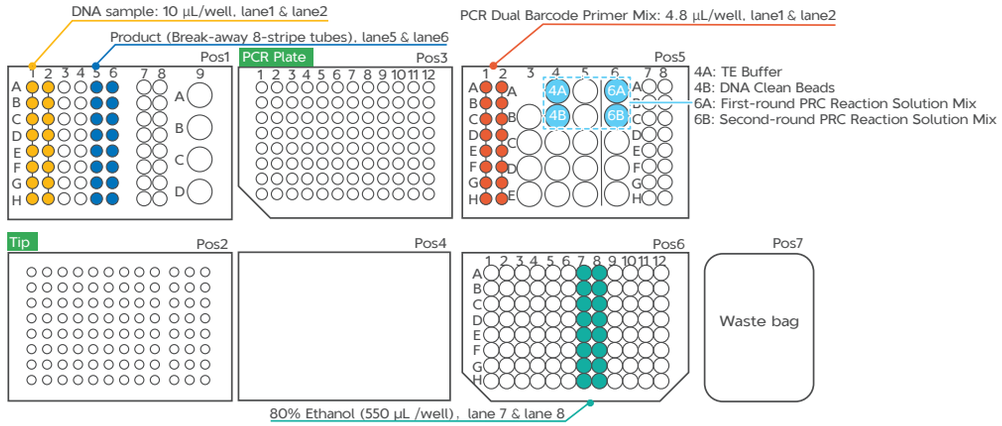
Use 80% ethanol immediately after preparation.

6. Take out a deep-well plate.
   - When preparing libraries for DNA samples, add 550 μL of 80% ethanol to each of Well 7A to Well 7H and Well 8A to Well 8H according to the number of samples.
   - When preparing libraries for blood card samples, add 550 μL of 80% ethanol to each of Well 7A to Well 7H and Well 8A to Well 8H according to the samples. Further add 145 μL of Clean Buffer to Well 9A to Well 9H and Well 10A to Well 10H respectively.
7. Mix DNA Clean Beads with the vortex mixer thoroughly, and then centrifuge them briefly.

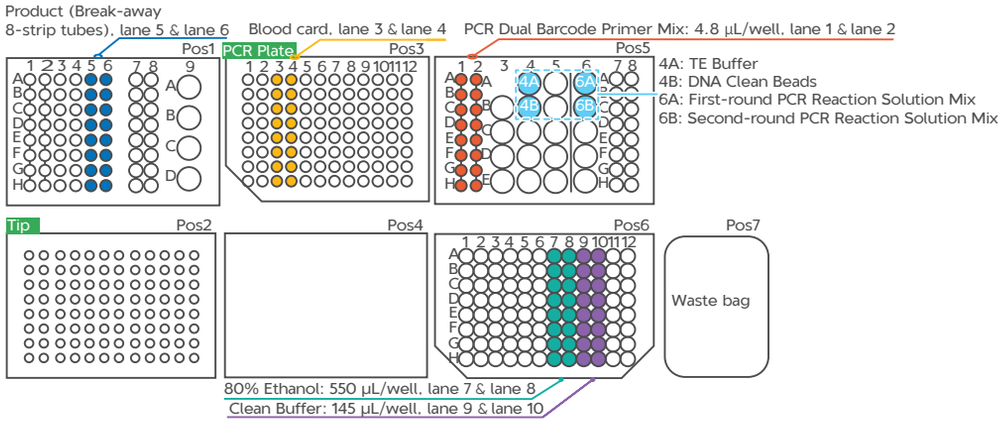
Tips

Before placing the consumables, ensure that no bubble exists at the bottoms of all tubes, no liquid resides on the inner wall, and all tubes are uncapped.

8. Place the consumables according to the figures below:
   - DNA sample:



   - Blood card sample:



Preparing libraries on MGISP-100RS

1. Set **Solution** to **JB-A06-067 MGIEasy Signature Identification Library Prep Kit_RV2.1_SV3.0** on the MGISP-100RS Run Wizard interface.
2. Select the script:
   - DNA sample: **1.Library preparation DNA.py**
   - Blood card sample: **2.Library preparation Card.py**

Tips

Ensure that the automation script and PCR program have been input before running the script. For specific operations, refer to the relevant user manuals.

3. Ensure that all consumables and reagents are placed in correct positions and all SC micro tubes and PCR tubes are uncapped before closing the door of MGISP-100RS.
4. Tap **Run** and a window pops up. Then select the desired number of samples on the pop-up window.
5. Select the required cycle number in the PCR_CycleNum window. Tap **Continue** to start automatic library preparation.

Tips

- If the sample concentration is low, increase the cycle number accordingly.
- The entire process is expected to run for 3 to 4 hours. You can pause or resume it as needed while running.
- If you prepare libraries for blood card samples, a window will pop up after running for 20 to 25 minutes. Follow the on-screen prompts to add the standard references, and then click **Continue**.

6. After the process is completed, follow the prompts in the pop-up window to take out the second-round PCR product from Pos1. The volume in each tube should be 21 µL. Then cap the 8-strip PCR tubes.
7. Quantify the second-round PCR product by using Qubit dsDNA Fluorescence Assay Kit. The product concentration should be no less than 4 ng/µL.

Stoppoint

The second-round PCR product can be stored in a -20 ℃ 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 device with pure water and 75% ethanol. Then, perform a post-clean. For specific operations, refer to the *MGISP-100RS DNA Sequencing Library Preparation System User Manual*.

Making DNBs

Circularization

1. Mix the second-round PCR product to 500 ng, place the product to a new 0.2 mL PCR tube, and add TE Buffer to the tube to 48 µL. Perform the denaturation reaction according to the table below:

Temperature	Time
105 ℃ (Heated lid)	On
95 ℃	5 min

2. After the reaction is completed, place the PCR tube on ice immediately for 3 minutes, and briefly centrifuge it. Then, add the following reagents:

Component	Volume (µL)
T Buffer	6
S Enzyme	1
Total volume	7

3. Vortex the solution 3 times for 3 seconds each time, and then centrifuge the tube briefly. Collect the reaction solution at the bottom of the tube. Perform the circularization pretreatment reaction according to the table below:

Temperature	Time
45 ℃ (Heated lid)	On
37 ℃	10 min
4 ℃	Hold

4. After the reaction is completed, centrifuge the PCR tube briefly and place it on ice. Then add the following reagents according to the table below:

Component	Volume (µL)
Splint Buffer	5.6
DNA Rapid Ligase	0.5
Total volume	6.1

5. Vortex the solution 3 times for 3 seconds each time and then centrifuge the tube briefly. Collect the reaction solution at the bottom of the tube. Perform the single-stranded circularization reaction according to the table below:

Temperature	Time
45 ℃ (Heated lid)	On
37 ℃	30 min
4 ℃	Hold

6. After the reaction is completed, centrifuge the PCR tube briefly, place it on ice, and proceed to the next reaction immediately.

Enzyme digestion

1. After the single-stranded circularization reaction is completed, add the following reagents to the PCR tube:

Component	Volume (μL)
Digestion Buffer	1.4
Digestion Enzyme	2.6
Total volume	4

2. Vortex the solution 3 times for 3 seconds each time and then centrifuge the tube briefly. Collect the reaction solution at the bottom of the tube. Perform the enzyme digestion reaction according to the table below:

Temperature	Time
45 ℃ (Heated lid)	On
37 ℃	30 min

3. After the reaction is completed, add 7.5 μL of Digestion Stop Buffer to the PCR tube. Vortex the solution 3 times for 3 seconds each time, and then centrifuge the tube briefly. Collect the reaction solution at the bottom of the tube. Transfer all the product of enzyme digestion to a new 1.5 mL centrifuge tube.
4. Add 170 μL of DNA Clean Beads to the product. Mix it thoroughly and then centrifuge it briefly. Incubate the mixture at room temperature for 10 minutes.
5. Place the centrifuge tube on a magnetic rack and place it for 2 to 5 minutes until the solution becomes clear. Use a pipette to remove the supernatant.
6. Add 500 μL of freshly prepared 80% ethanol to wash the beads and tube wall. Wait for 30 seconds. Then remove the supernatant. Air dry the beads until no wetness or glossiness is visible on the beads surface and no beads crack.

Tips

Aspirate all the solution from the bottom of the tube.

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7. Remove the centrifuge tube from the magnetic rack. Add 25 µL of TE Buffer for DNA elution. Mix the solution thoroughly, incubate it at room temperature for 5 minutes, and centrifuge it briefly.
8. Place the centrifuge tube on the magnetic rack for 2 to 5 minutes until the liquid becomes clear. Transfer 23 µL of the supernatant to a new 1.5 mL centrifuge tube.
9. Quantify the product of enzyme digestion and purification by using Qubit ssDNA Assay Kit. The product concentration should be no less than 0.5 ng/µL.

Stoppoint

The product of enzyme digestion and purification can be stored in a -20 ℃ refrigerator.

Making DNBs

1. Take out the reagents from the DNBSEQ-G99RS High-throughput Sequencing Set (G99 SM FCL SE400). Add the product of enzyme digestion and purification according to the sample type. Prepare Make DNB Reaction Solution I according to the table below:

Component	Volume (µL)
Library	V
Low TE Buffer	10-V
Make DNB Buffer	10
Total volume	20

Tips

- The library input volume (V) should be calculated according to the sample type.
  - For DNA samples, V is equal to 10 ng divided by the library concentration.
  - For blood card samples, V is equal to 5 ng divided by the library concentration.
  - For mixed DNA and blood card samples, the library input volumes for DNA samples and blood card samples should be calculated separately. The final volume should be the higher one between the two results.
- If the calculated input volume is greater than 10 µL, add 10 µL.

2. After thorough mixing, perform the reaction according to the table below:

Temperature	Time
105 ℃ (Heated lid)	On
95 ℃	1 min
65 ℃	1 min
40 ℃	1 min
4 ℃	Hold

3. After the reaction is completed, add the following reagents, mix them thoroughly, and centrifugate the solution:

Component	Volume (µL)
Make DNB Enzyme Mix V	30
Make DNB Enzyme Mix II (LC)	2

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4. Place the solution in a PCR thermal cycler and perform the reaction according to the table below:

Temperature	Time
35 ℃ (Heated lid)	On
30 ℃	25 min
4 ℃	Hold

5. After the reaction is completed, add 10 μL of DNB Stop Buffer immediately and slowly pipette the solution with a wide-bore pipette for 5 to 8 times.

Tips

Do not vortex or pipette the tube vigorously.

Stoppoint

The DNB product can be stored at 4 ℃ for 48 hours.

6. Quantify the DNB product by using the single-stranded DNA quantification reagent. The product concentration should be no less than 8 ng/μL. If the concentration is less than 8ng/uL, remake DNBs.

Sequencing and analyzing

For specific operations of the sequencer, the loader, and the analysis software, refer to the relevant user manuals.

Importing samples

Tips

The following steps should be completed on ZLIMS.

1. Tap on the desktop of the sequencer to open the login page of ZLIMS.
2. Enter the username (**lite**) and password (**lite123456**) and tap **Login**. The Home page of ZLIMS is displayed.
3. Tap **Sequencing + Analysis** on the Home page to go to the new sequencing and analysis page.
4. Select the analysis product **FIS** or **FIS_SNP**.
5. Select a method for inputting DNB sample information. For example, select **Import the Sample ID**. Then click **New**.

Tips

FIS is suitable for analyzing SE400 sequencing data, and FIS_SNP is suitable for analyzing SE50 sequencing data.

6. Tap **Excel Template** or **CSV Template** in the pop-up page to download the sample template in *.xlsx* or *.csv* format.
7. Open the template and fill in the worksheet, then save it to the designated directory.

Product Name(*)	DNB ID(*)	Barcode(*)	Sample ID(*)	Sample Nam	Sample Type(*)	Group
FIS	MGI	1-1	MGI99		DNA	

Tips

- A field with an asterisk (*) is required, and other fields are optional.
- Cells in Excel template cannot be merged, and no spaces or special characters 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 is already in ZLIMS.
- **Barcode**: When one sample ID corresponds to multiple barcodes, the multiple barcodes are separated by commas (,). For multiple consecutive barcodes without letters, a tilde (~) can be used.
- **Sample ID**: The sample ID should include both letters and numbers. It can be the same as the sample ID that is already in ZLIMS.
- **Sample Type**: Select **DNA**.

8. Return to the Sequencing + Analysis page and tap **Choose File**. Then select the completed worksheet and tap **Upload**. The new Sequencing + Analysis page returns to view after importing the DNB sample information.
9. Tap **Save**, and then tap **OK** in the pop-up window.

Preparing the flow cell

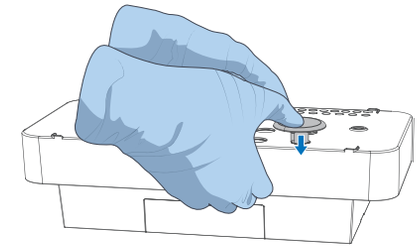
Take out the flow cell for DNB loading and place it at room temperature for at least 30 minutes, but not exceeding 24 hours.

Tips

Do not open the package at this time.

Prepare the sequencing reagent cartridge

1. Take out the sequencing reagent cartridge and place it at room temperature in water bath for 4 hours, or pre-thaw it in a refrigerator at 2 ℃ to 8 ℃ one day ahead. Then, place the sequencing reagent at 2 ℃ to 8 ℃ until use.
2. Tear off the package and wipe the condensate water on the package and well with dust-free paper.
3. Press the M1, M2, M3, and M4 wells on the cartridge with a pressing tool.



4. Hold the A and B sides of the cartridge with both hands by following the marks A and B on the cartridge, and shake it vertically and horizontally for 10 to 20 times to mix the reagents thoroughly. Gently tap the cartridge against the desktop to remove bubbles.

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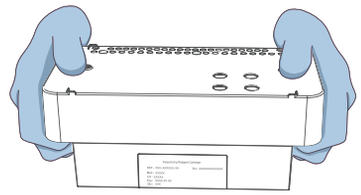


5. Pierce the MDA well on the cartridge with a pipette tip.

Sequencing

1. Tap in the upper-right corner of the main interface the control software of the sequencer. Enter the user name (**user**) and password (**123**). Tap **Login**. The main interface is returned after login.
2. 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**.
3. 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 or ensure that waste container is below the detection line before starting sequencing.

4. After the check is completed, tap **Next** to open the setting parameters interface.

   Select **Sequence & Analysis** as the workflow and **No** in the **BBS** box by default. Input the DNB ID in the **DNB ID** box.

Tips

Ensure that the DNB ID filled here is consistent with that input in ZLIMS.

5. Select **SE10+10+400** (for FIS) or **SE50+10+10** (for FIS_SNP) in the **Recipe** box. Then select **ID-Dualbarcode** in the box next to the **Recipe** box.
6. Select **Yes** for both the **Split barcode** and **Auto wash** in the advanced settings.
7. 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 the automatic scanning fails, you can manually enter the ID.

9. Tap **Prime** and then **Yes** in the pop-up window. The system automatically closes the auto-sliding screen and starts prime.

Loading DNBs

1. Take out DNB Loading Buffer II in advance and place it on ice for 30 minutes. Mix it thoroughly by using a vortex mixer, centrifuge it for 5 seconds 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 Loading Buffer II	7
Make DNB Enzyme Mix II (LC)	1
DNB	21
Total volume	29

3. Mix the DNB loading mixture with a wide-bore tip for 5 to 8 times. Then place it at 4 °C until use.

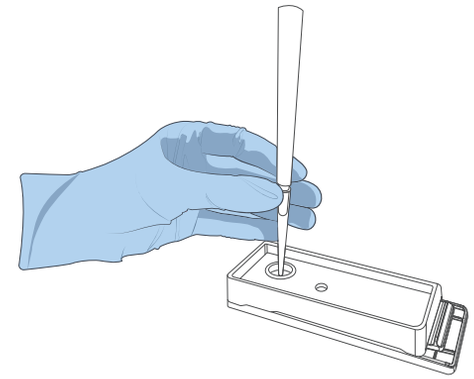
Tips

- Use the DNB loading mixture immediately after preparation.
- Do not centrifuge, vortex, or pipette the DNB loading mixture vigorously.

4. Take out the O-ring that is installed in the loader by pressing it from the bottom of the loader and store it in the packaging. Hold the loader with your left hand and open the cover with the other hand. Install the flow cell into the loader and close the cover.

   Observe the center of the O-ring. If it is transparent, the flow cell is properly installed. Then, flip the loader and place it on a desktop.

5. Aspirate 10 μL of the DNB loading mixture by using a 200 μL and vertically insert the tip into the inlet. Fix the tip with the left hand, press the tip ejector on the pipette to unload the tip with the right hand.



Tips

- During DNB library loading, do not press the push button of the pipette.
- Do not rotate the tip or move the flow cell during the loading process.

6. Place it for 3 seconds and observe the liquid level in the tip. If the liquid level drops automatically, the DNB loading mixture flows into the flow cell successfully.
7. After ensuring that the DNB loading is complete, pull out the tip at the inlet. Turn the loader upside down and immediately transfer the flow cell to the sequencer.

Loading the flow cell and reviewing information

1. After the prime is completed, tap **Next**. The auto-sliding screen moves up automatically.
2. Install the prepared flow cell into the platform, and the system will scan the flow cell ID automatically.

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Tips

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

3. Tap **Next** to go the review interface and review all items in the review interface. After checking that all information is correct, tap **Sequencing**, and then select **Yes** to start sequencing.

Tips

The Sequencing interface displays real-time sequencing progress, and you can operate the device if needed.

Performing an analysis

After the sequencing is completed, the analysis software automatically starts the analysis.

You can tap any number in the **Task Status** area or tap **Task** on the left navigation bar on the Home page of ZLIMS to open the Task page and view the status and task progress.

Viewing and downloading the report

1. 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 analysis report.
4. Tap  in the **Result Path** column to download the analysis report.