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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 | 20× Elute Enhancer<br>Fast FS Buffer<br>Fast FS Enzyme<br>Ligation Enhancer<br>Fast Ligation Buffer<br>Ad Ligase<br>PCR Enzyme Mix | 940-000028-00 (16 RXN)<br>940-000031-00 (96 RXN) |
| MGIEasy UDB Primers Adapter Kit | UDB Adapter<br>UDB PCR Primer | 1000022800 (16 RXN)<br>1000022802 (96 RXN) |
| MGIEasy DNA Clean Beads | DNA Clean Beads<br>TE Buffer | 1000005278 (16 RXN)<br>1000005279 (96 RXN) |

- 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 |
| 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-G50RS High-throughput Seqencing Set (FCS SE100) (Cat. No.: 1000019860)

| Name | Component | Cat. No. |
|---|---|---|
| DNBSEQ-G50RS Rapid Sequencing Flow Cell | Sequencing Flow Cell | 1000020209 |
| DNBSEQ-G50RS High-throughput Sequencing Kit (FCL SE50/FCS SE100) | Low TE Buffer<br>Make DNB Buffer<br>Make DNB Enzyme Mix I<br>Make DNB Enzyme Mix II (LC)<br>Stop DNB Reaction Buffer<br>DNB Load Buffer I<br>DNB Load Buffer II<br>Micro Tube 0.5mL (Empty)<br>dNTPs Mix III<br>dNTPs Mix II<br>Sequencing Enzyme Mix<br>Sequencing Reagent Cartridge<br>Transparent sealing film | 1000019849 |

- DNBSEQ-G50RS High-throughput Sequencing Set (FCL SE100) (Cat. No.: 1000019856)

| Name | Component | Cat. No. |
|---|---|---|
| DNBSEQ-G50RS Rapid Sequencing Flow Cell | Sequencing Flow Cell | 1000020208 |

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| Name | Component | Cat. No. |
|---|---|---|
| DNBSEQ-G50RS High-throughput Sequencing Kit (FCL SE100) | Low TE Buffer<br>Make DNB Buffer<br>Make DNB Enzyme Mix I<br>Make DNB Enzyme Mix II (LC)<br>Stop DNB Reaction Buffer<br>DNB Load Buffer I<br>DNB Load Buffer II<br>Micro Tube 0.5mL (Empty)<br>dNTPs Mix III<br>dNTPs Mix II<br>Sequencing Enzyme Mix<br>Sequencing Reagent Cartridge<br>Transparent sealing film | 1000019850 |

- CPAS Barcode Primer 4 Reagent Kit (Cat. No.: 1000014048)

| Component | Quantity |
|---|---|
| AD153 Barcode Primer 4 | 3.5 mL × 1 tube |

# 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.

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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 | 0.5 mL PCR SC micro tube | 30 | 45 | 105 |
| Ligation Enhancer | 0.5 mL PCR SC micro tube | 12 | 18 | 55 |
| Fast Ligation Buffer | 0.5 mL PCR SC micro tube | 143 | 225 | 450 |
| Ad Ligase | 0.5 mL PCR SC micro tube | 33 | 50 | 100 |
| PCR Enzyme Mix | 2 mL PCR SC micro tube | 300 | 470 | 955 |

**Tips**

When preparing libraries for 16 samples by using MGIEasy Fast FS DNA Library Prep Set (16 RXN), manually transfer the 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 1× 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, while En-TE and En-Beads should be stored at a 4 °C refrigerator.

- 1× Elute Enhancer

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

- En-TE

| Component | Volume (μL) |
|---|---|
| 1× Elute Enhancer | 8 |
| TE Buffer | 3992 |
| **Total volume** | 4000 |

- EN-Beads

| Component | Volume (μL) |
|---|---|
| 1× 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 then 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 | 2 mL SC micro tubes | 500 | 1000 | 1800 |

5. Prepare 25 mL of 80% ethanol.

**Tips**

Use the 80% ethanol immediately after preparation.

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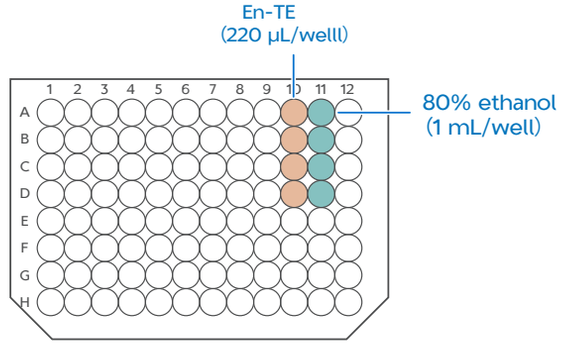
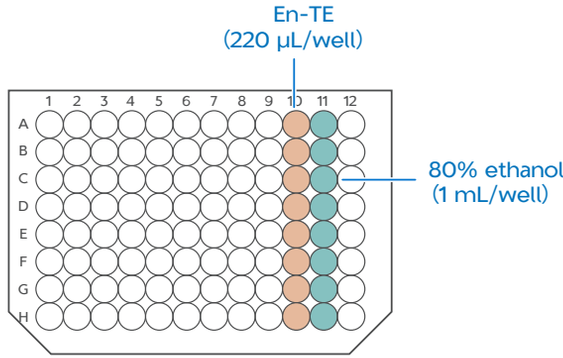
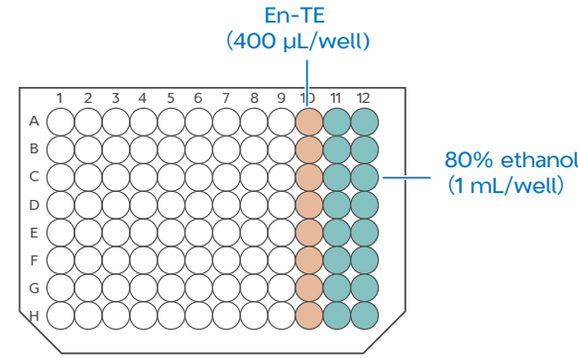
6. Take out a U-bottom deep-well plate and add the reagents according to the figures below:
   - For 4 RXN:




     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.
   - For 8 RXN:




     Add the following reagents:
     - Add 220 µL of En-TE to each well of column 10.
     - 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 every well of column 10.
     - Add 1 mL of 80% ethanol to every well of columns 11 and 12.
7. Calculate the adapter dilution factor (diluted by using TE Buffer) according to the actual gDNA amount and the table below. For the gDNA 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 | 35 | 55 | 100 |
| 10 | 5x | 35 | 55 | 100 |
| 5 | 10x | 35 | 55 | 100 |
| 2.5 | 15x | 35 | 55 | 100 |
| 1 | 45x | 35 | 55 | 100 |

8. Transfer PCR Primer into one set (for 4 or 8 RXN) or two sets (for 16 RXN) of 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.

## Preparing libraries on MGISP-100RS

1. Set **Solution** and **Script** in the Run Wizard interface of MGISP-100RS according to the table below:

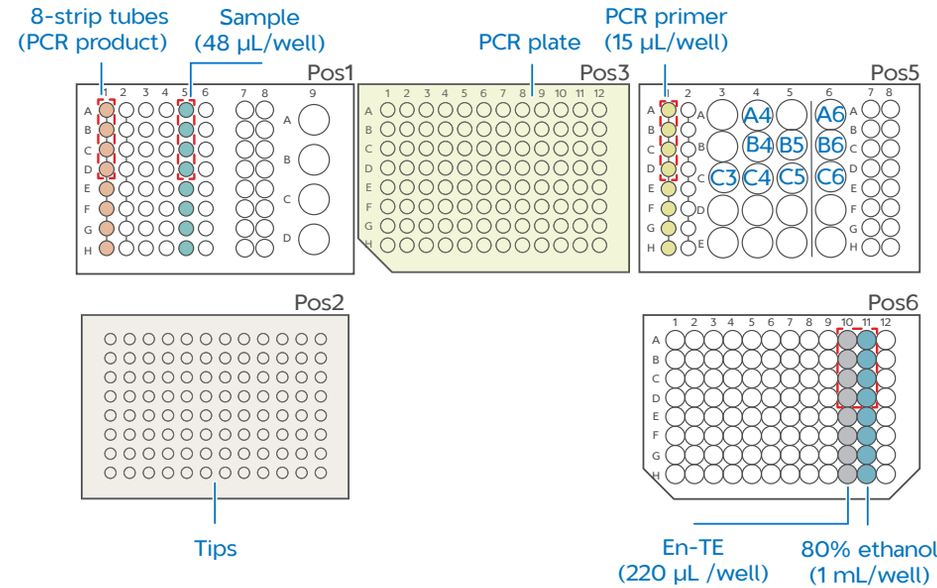
| | |
|---|---|
| Solution | **JB-A06-079 MGIEasy Fast FS DNA Library Prep Set_RV1.0_SV2.0** |
| Script | **Fast_PCR_DNA_Library_SP100_4&8RXN.py**<br>**Fast_PCR_DNA_Library_SP100_16RXN.py** |

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



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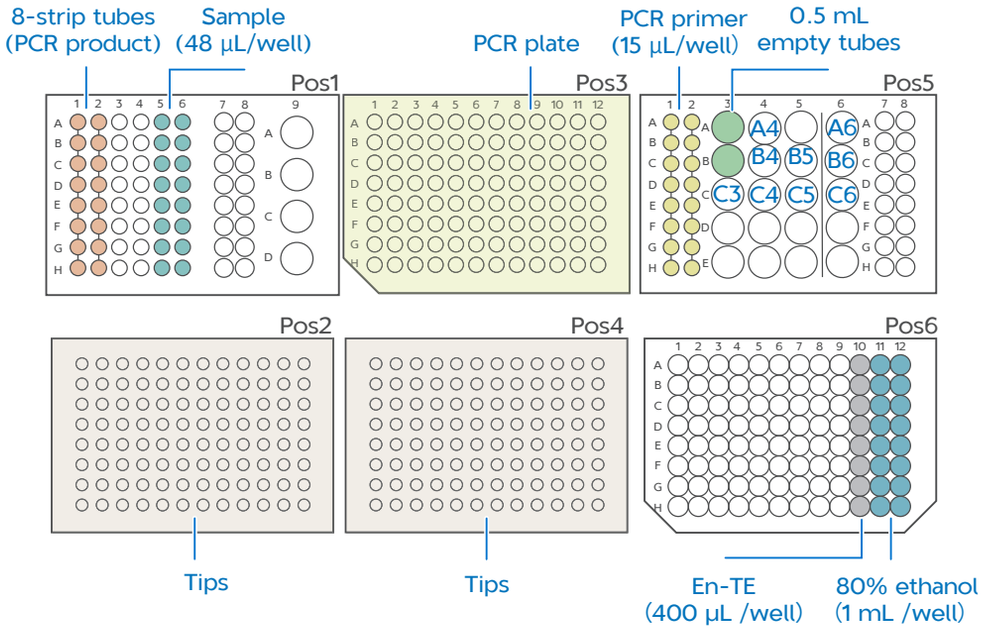
- For 16 RXN




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 ℃ to 30 ℃ and away from light.

4. Tap **Run**. If the 4&8 RXN script runs, the Sample_Num window will pop 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, tap **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 based on your requirements, leave **Value** blank, and tap **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 of 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 concentration should be no less than 0.8 ng/µL.

**Stoppoint**

The PCR product can be placed 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 operation deck with pure water and 75% ethanol. Then. perform a post-clean.

# Making OS DNBs

## Preparing samples

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 25 ng of the mixed library to make DNBs. The volume should be no less than or equal to 20 µL.
2. Take out Make DNB Buffer (OS-DB) from DNBSEQ OneStep DNB Make Reagent Kit (OS-DB) to prepare the following reaction solution:

| Component | Volume (µL) |
|---|---|
| Mixed library | 20 |
| Make DNB Buffer (OS-DB) | 20 |
| **Total volume** | 40 |

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 | 40 |
| Make DNB Enzyme Mix II (OS) | 4 |
| **Total volume** | 44 |

6. Vortex and briefly centrifuge the PCR tube to collect the reaction solution at the bottom of the tube.

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7. Place the PCR tube in a PCR device and start a reaction according to the conditions in the table below. The total reaction volume should be 84 µL.

| Temperature | Time |
|---|---|
| 35 ℃ (Heated lid) | On |
| 30 ℃ | 25 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 20 µL of Stop DNB Reaction Buffer to the PCR tube. Pipette the solution 5 to 8 times with 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 placed at 4 ℃ for up to 48 hours.

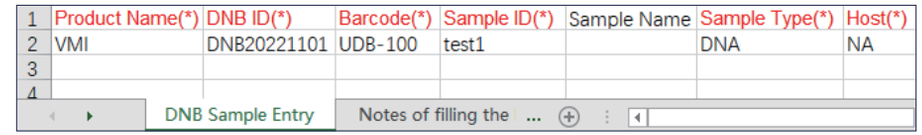
9. Take out 2 µL of the DNBs and quantify it by using Qubit ssDNA Assay Kit. The concentration of the DNBs should not be less than 8 ng/µL.

Tips

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

## Importing samples

1. Click the ZLIMS icon on the desktop of Platform of microorganisms Fast Identification to open the login page of ZLIMS.
2. Enter the username (**lite**) and password (**lite123456**), and click **Login**.
3. Click **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. Click **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.



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Tips

- A field with an asterisk (*) is required, and others are optional.
- Cells in 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, select **Choose File**. Select the completed worksheet in the pop-up box and click **Upload**. The New Sequence + Analysis page returns to view after importing the DNB sample information.
8. After reviewing the sample information, click **Save**, and then click **OK** in the pop-up window.

# Sequencing

## Preparing the DNB loading mixture

1. Take out DNB Load Buffer I and DNB Load Buffer II from DNBSEQ-G50RS High-throughput Sequencing Set and place it at room temperature for at least 30 minutes.
2. Mix the buffers thoroughly by using a vortex mixer for 5 seconds, centrifuge them briefly, and then place them on ice until use.
3. Take out a new 0.5 mL SC micro tube. Add the reagents to the tube according to the following table:

| Loader | DNB volume (μL) | DNB Load Buffer I (μL) | DNB Load Buffer II (μL) | Make DNB Enzyme Mix II (LC) (μL) |
|---|---|---|---|---|
| DNBSEQ-G50RS | 100 | 50 | 50 | 1 |

4. Mix DNB loading mixture for 5 to 8 times by using a wide-bore pipette tip to mix it thoroughly. 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.

## Preparing the sequencing reagent cartridge

1. Select the sequencing recipe based on different scenarios:
   - When the number of DNA libraries is less than or equal to 8, select FCS SE100.

- When the number of DNA libraries is less than or equal to 40, select FCL SE100.

**Tips**

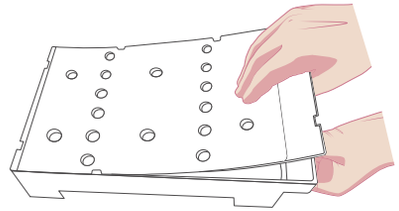
If the samples are loaded on one flow cell, ensure that barcodes corresponding to the libraries are base-balanced.

2. Take out the sequencing reagent cartridge, thaw it in a water bath at room temperature, and place in a 2 ℃ to 8 ℃ refrigerator until use.
3. Take out dNTPs Mix III and dNTPs Mix II one hour in advance, thaw them at room temperature, and place them at a 4 ℃ refrigerator until use. Mix them by thoroughly by using a vortex mixer for 5 seconds and centrifuge them briefly.
4. Invert the cartridge for 3 times. Take out Make DNA Enzyme Mix, place it at a 4 ℃ refrigerator, and mix it thoroughly by inverting it before being added to the cartridge.

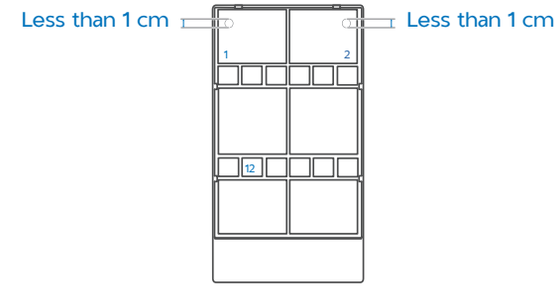
**Tips**

Ensure that no visible layer exists in the reagents, especially in reagents 17 and 18.

5. Open the cartridge cover and wipe the water condensation with a dust-free paper.



6. Gently pierce two holes with its diameter smaller than 1 cm at the edge of Wells 1 and 2 by using a new 1 mL pipette tip.




7. Take out a 1 mL pipette and add the reagents according to the tables below:

- FCS SE100

| Name | Volume (mL) | |
|---|---|---|
| | Well 1 | Well 2 |
| dNTPs Mix III | 0.320 | / |
| dNTPs Mix II | / | 0.560 |
| Make DNA Enzyme Mix | 0.320 | 0.280 |

- FCL SE100

| Name | Volume (mL) | |
|---|---|---|
| | Well 1 | Well 2 |
| dNTPs Mix III | 0.440 | / |
| dNTPs Mix II | / | 0.760 |
| Make DNA Enzyme Mix | 0.440 | 0.380 |

8. Cover the holes with transparent sealing films.

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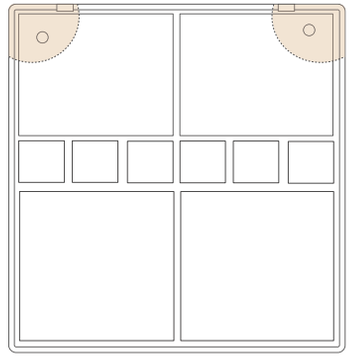
Tips

Do not cover the center of the holes to avoid interfering with the reagent needle.



9. Hold both sides of the cartridge with both hands and invert it for 10 to 20 times to mix the reagents thoroughly.

Tips

Ensure that visible vortex exists and the colors of both layers in reagent in Well 1 are the same.

10. (Optional) Dual barcode sequencing:
    ① Take out CPAS AD153 barcode primer 4 solution from CPAS Barcode Primer 4 Reagent Kit, thaw it at room temperature, and mix it thoroughly for 5 seconds by using a vortex mixer.
    ② Pierce the sealing film of well 12 by using a new 1 mL pipette tip.
    ③ Take out 1.30 mL of AD153 Barcode Primer 4 and add it to well 12.

Tips

Ensure that no bubble exists at the bottom of the well.

## Sequencing

1. In the main infertace of the control software of the sequencer, tap in the upper-right corner to open the login interface.
2. Enter the username (**user**) and password (**123**) and tap **Login**.
3. Tap **Sequence**. Enter DNB ID in the **DNB ID** box.

Tips

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

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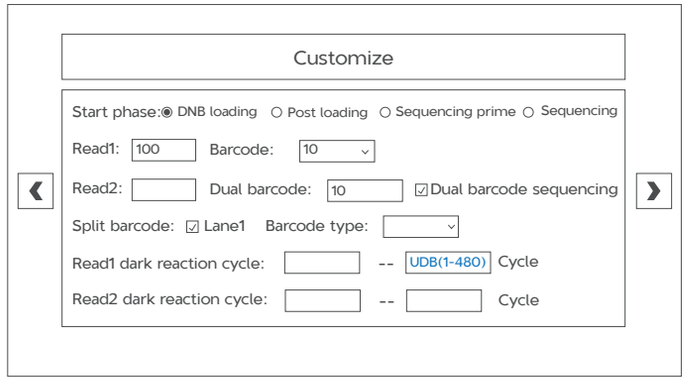
4. Select **Customize**, and set the length and barcode according to the figure below. Then, tap ›.




5. Place the tube that contains the DNB loading mixture into the tube bracket of the reagent compartment.
6. Scan the reagent cartridge ID by using a scanner or manually enter the ID and tap ›.
7. Push the sequencing cartridge into the reagent compartment and close the compartment door.
8. Scan the flow cell ID of by using a barcode scanner or manually input the flow cell ID and tap ›.
9. Press the attachment button on the flow cell stage to install the flow cell and close the door of the compartment.
10. After reviewing all information, tap **Sequence** and select **Yes**.

    The interface displays real-time sequencing progress, and you can operate the device if needed.
11. After the sequencing is completed, the analysis software automatically starts the analysis.

## Viewing and downloading the report

**Tips**

You can click to download the reports and results of all samples in the same batch.

1. In the main interface of ZLIMS, click the number under **Report Today** to open the Analysis Report page.
2. Set the query term in the Query area and click to locate the sample. Click the link in the **Analysis type** column.
3. Click in the **Report** column to view the report.