• Part No.: H-020-001040-00
• Version: 2.0
• Release date: October 2024
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Application scope

DNBSEQ-G400RS FluoXpert optional reagent sets for staining:

Cat. No.	Model	Name
940-002132-00	DNBSEQ-G400 SS 6P	FluoXpert mIF Set
940-002133-00	DNBSEQ-G400 SL 6P	FluoXpert mIF Set
940-002134-00	DNBSEQ-G400 DL 6P	FluoXpert mIF Set
940-002221-00	DNBSEQ-G400 SS 24P	FluoXpert mIF Set
940-002219-00	DNBSEQ-G400 SL 24P	FluoXpert mIF Set
940-002218-00	DNBSEQ-G400 DL 24P	FluoXpert mIF Set

Tips

Each reagent set contains one FluoXpert Multiplex Immunofluorescence Reagent Kit (5 rxn/kit) and five staining flow cells.

DNBSEQ-G400RS FluoXpert optional reagent sets for sequencing:

Cat. No.	Model	Name
940-001343-00	G400 FCL SE50	DNBSEQ-G400RS High-throughput Sequencing Reagent Set
940-001613-00	G400 sRNA FCL SE50	DNBSEQ-G400RS High-throughput Sequencing Reagent Set
940-001368-00	G400 FCL SE100	DNBSEQ-G400RS High-throughput Sequencing Reagent Set
940-001361-00	G400 FCL PE50	DNBSEQ-G400RS High-throughput Sequencing Reagent Set
940-001344-00	G400 FCL PE100	DNBSEQ-G400RS High-throughput Sequencing Reagent Set
940-001356-00	G400 FCL PE150	DNBSEQ-G400RS High-throughput Sequencing Reagent Set
940-001804-00	G400 App-D FCL SE50	DNBSEQ-G400RS High-throughput Sequencing Reagent Set
940-001803-00	G400 App-D FCL SE100	DNBSEQ-G400RS High-throughput Sequencing Reagent Set
940-001781-00	G400 App-D FCL PE50	DNBSEQ-G400RS High-throughput Sequencing Reagent Set
940-001806-00	G400 App-D FCL PE100	DNBSEQ-G400RS High-throughput Sequencing Reagent Set
940-001801-00	G400 App-D FCL PE150	DNBSEQ-G400RS High-throughput Sequencing Reagent Set

Introduction

This guide provides guidance for the use of DNBSEQ-G400RS FluoXpert system for multiplex immunofluorescence (mIF) staining and sequencing.

WARNING

The Sequencing Sets hereof are intended only for research use and should not be used for clinical diagnosis.

Preparing for staining

Logging in to the control software

Tips

Different control software is used for mIF staining and sequencing. To switch between these two functions, close the current control software before starting the other one in the FluoXpert program.

1. Power the device on.
2. Log in to the computer with the account *zebra* and the password *123*.
3. Click (FluoXpert) to initialize the device. Switch between the icons to enter different modes and perform different operations:

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- Select (Sequencer) to perform sequencing operations.
- Select (Proteo) to perform mIF staining operations.

4. Select in the main interface.
5. Log in to the control software with the username and password according to the table below:

Account type	User name	Password
User	user	Password123
Administrator	research	Admin123

Performing a pre-run wash

For detailed wash protocol and steps, refer to *Preparing for a wash on Page 6*.

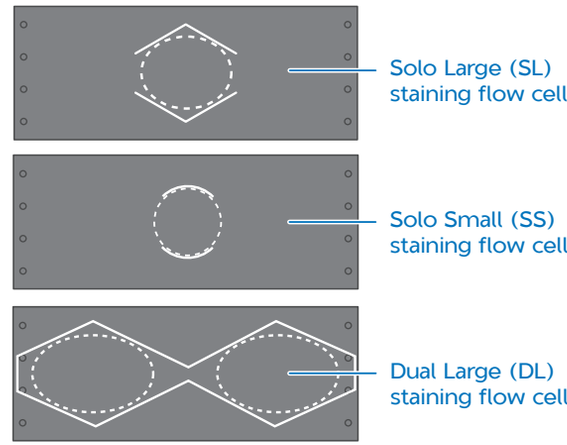
Preparing samples

Sample pretreatment

1. Cut tissue sections from paraffin-embedded sample blocks. The recommended section thickness is 3 μm to 5 μm.
2. Float the sections in a 40 °C water bath until they are flattened out and pick up the sections onto FluoXpert slides.

Tips

Tissue sections must be attached to the designated area on the front side of the flow cell as shown below.



3. Dry the flow cell at 42 °C overnight or bake at 65 °C for 1 h.
4. Place the flow cell vertically in xylene and incubate for 3 h to deparaffinize the tissue sections. Incubate the flow cell in different concentrations of ethanol (absolute ethanol, 95% ethanol, 70% ethanol, 50% ethanol, and purified water) sequentially for 20 min each to rehydrate the tissue sections.
5. Place the rehydrated flow cell in a staining jar containing alkaline antigen retrieval solution and incubate it at 95 °C for 20 min to perform antigen retrieval. Cool the staining jar at room temperature for 40 min.
6. Transfer the flow cell to PBS buffer. Store at 4 °C for short-term storage.

Tips

It is recommended that you complete staining within two weeks.

7. (Optional) Autofluorescence quenching: Immerse the flow cell in 3% hydrogen peroxide solution and expose it to 50 W LED light for 45 min. Replace the hydrogen peroxide solution and expose the flow cell to the LED light for another 45 min. Rinse the flow cell twice with PBS buffer and store it in PBS buffer until use.
8. Place the flow cell in a staining jar containing permeabilization Buffer (0.5% Triton X-100 in PBS buffer) and incubate it at room temperature for 15 min to perform permeabilization. Rinse the flow cell twice with PBS buffer and store it in PBS buffer until use.

Tips

This step is mandatory for the 24-plex kit.

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Preparing & loading the reagent cartridge

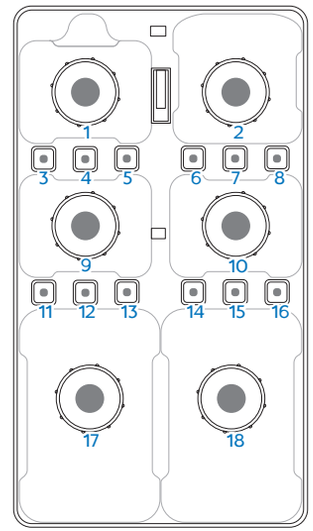
1. Remove the reagent cartridge from storage.
2. Take the primary antibodies out of the storage and mix them well.

   The primary antibody diluent in different wells for different kits:

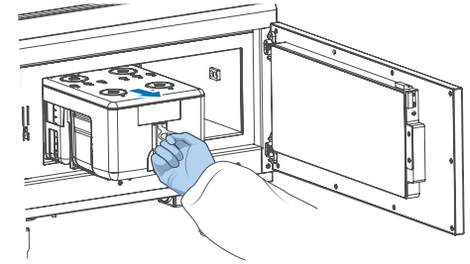
   - 6-plex kit: The reagent tubes for well No. 3, No. 4, No. 5, and No. 6 in this reagent cartridge contain 2.6 mL of primary antibody diluent for each.
   - 24-plex kit: The reagent tubes for well No. 3, No. 4, No. 5, No. 6, No. 7, No. 8, No. 11, No. 12, No. 13, No. 14 and No. 15 in this reagent cartridge contain 2.6 mL of primary antibody diluent for each. The reagent tube for well No. 16 contains 2.6 mL of Nucleus dye in primary antibody diluent.
3. Calculate the required volume of primary antibody based on the dilution ratio. Pierce the primary antibody reagent tubes with pipette tips, add appropriate primary antibodies, and mix well by pipetting.

Tips

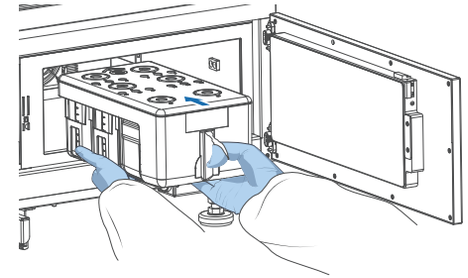
- Each numbered reagent tube can contain up to one rabbit and one mouse primary antibody. Two primary antibodies from the same species (such as two rabbit antibodies or two mouse antibodies) should not be mixed into one tube. For the 24-plex kit, only one rabbit primary antibody can be added into No. 16. Primary antibodies from other species should not be contained in this well.
- It is recommended that you optimize the dilution ratios in pilot experiments based on the user manual of corresponding primary antibody reagent. For example, for a 1:1,000 dilution, add 2.6 µL of primary antibody.



4. Open the reagent compartment door and slowly remove the cartridge from the compartment.



5. Moisten a KimWipes tissue with laboratory-grade water and use it to wipe the bottom and sides of the compartment to keep it clean and dry.
6. Slide the cartridge into the compartment by following the direction printed on the cover until it stops.



7. Close the reagent compartment door and tap **Next**.

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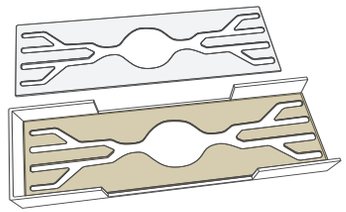
Preparing & loading the flow cell

1. Open the flow cell compartment door.
2. Peel off the release film from the FluoXpert coverslip inside the fixture, and set it aside.



3. Remove the slide and blot the excess water on the back and around the tissue sections.

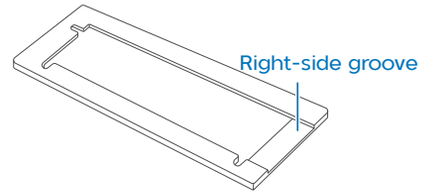
Tips

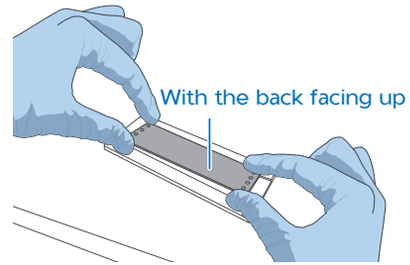
Keep the tissue section moist.

4. Use the fixture to align the inverted slide with tissue section facing towards the coverslip, and place the slide on it. Press gently to affix them evenly to assemble the flow cell.
5. Place the positioning frame with the back facing up, ensure that the groove is on the right side. Remove the flow cell from the fixture and gently slide it into the positioning frame at an angle of 30 degrees.

Tips

To keep the tissue moist, transfer the mounted flow cell to the flow cell stage within 2 min.

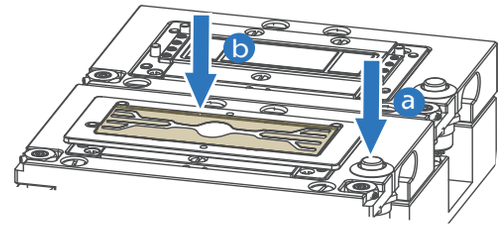




6. Press the flow cell attachment button. Press the left and right sides of the flow cell on the stage at the same time to ensure that the flow cell is properly seated on the stage.

Tips

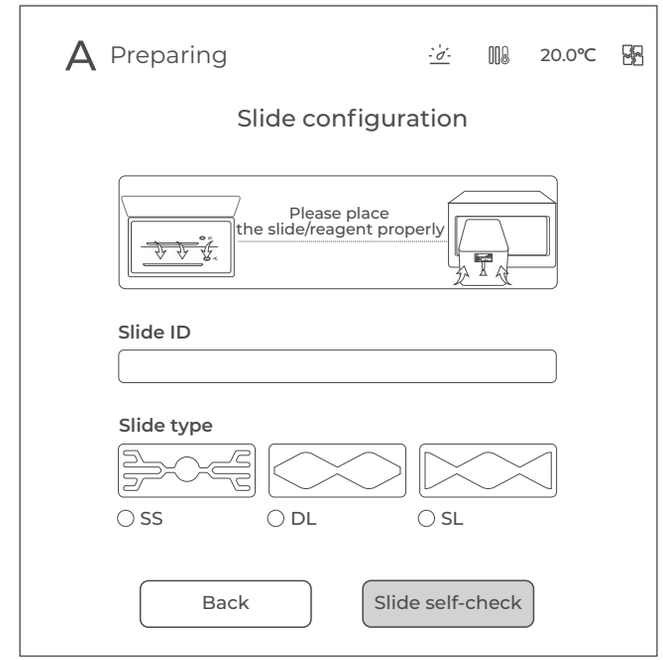
The flow cell is fragile, handle it with caution.



7. Ensure that the negative pressure is within the range of -80 kPa to -90 kPa.
8. Close the flow cell compartment door and tap **Next**.

Performing multiplex immunofluorescence staining

Slide self-check



1. Tap **Staining** in the main interface.
2. Tap the box below **Slide ID** to enter slide name and relevant information, and select the appropriate slide type.
3. Tap **Slide self-check** to perform a self-check.

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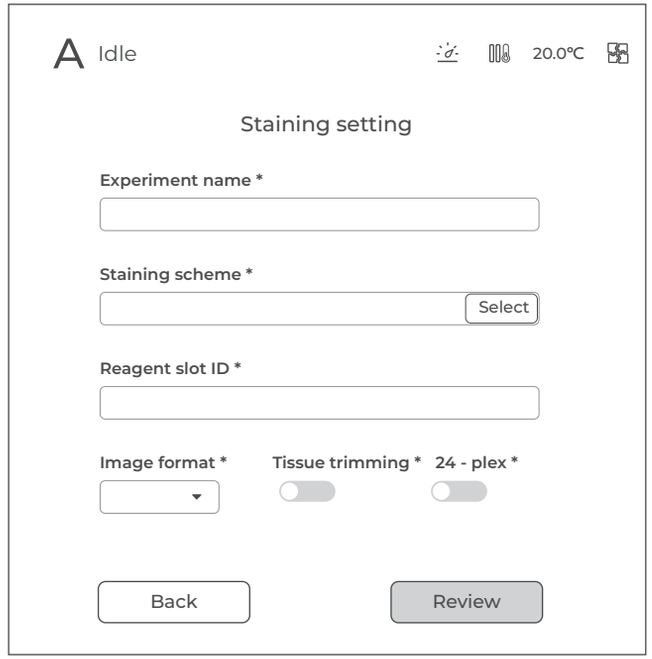
4. Once the self-check is completed, open the flow cell compartment door to ensure that all reagents flow into the flow cell and check whether the images are clear and the imaging area is correct. If many bubbles are visible, ensure that the flow cell inlet and outlet are properly aligned with the stage, and adjust before proceeding.

Selecting staining parameters

1. When the self-check is completed, tap **Staining setting** in the interface.

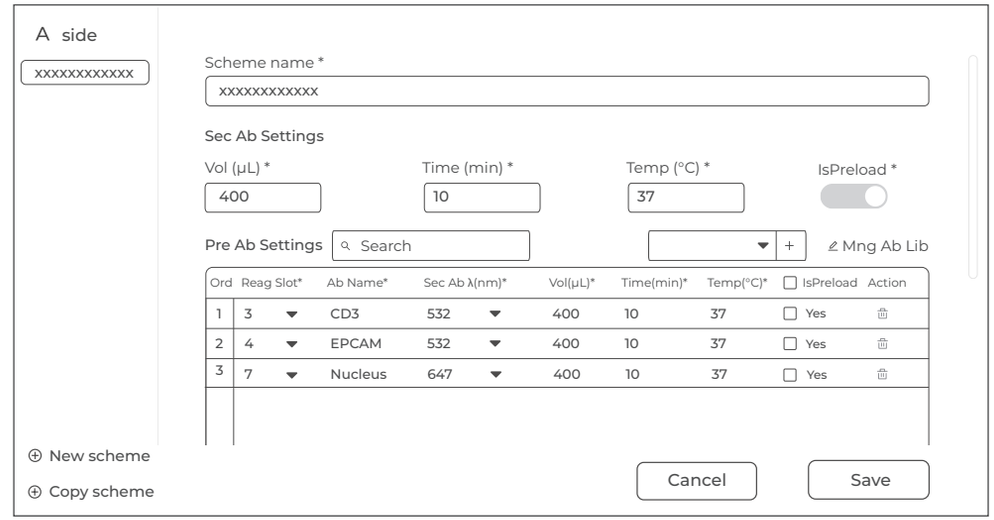


2. Enter the experiment name.
3. Tap **Select** next to the **Staining scheme** box and select the desired staining scheme.
   - Tap ⊕ next to **New scheme** or **Copy scheme** to create new staining schemes or copy the current one.
   - Tap **Mng Ab Lib** to edit the antibody library.
   - Tap **IsPreload** if the reagent cartridge is being used for the first time or the pipeline has been cleaned.
   - Edit primary antibody slot numbers, names, and wavelengths as needed.

Tips

- For 6-plex kits, Nucleus is in slot 7 by default. The wavelength of secondary antibody should be chosen as follows: 532 nm for rabbit primary antibodies, 647 nm secondary antibodies for mouse primary antibodies.
- For 24-plex kits, Nucleus is in slot 16 by default.

Tap **Save** to save the settings.



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4. Tap the box below **Reagent slot ID** to enter reagent cartridge ID.
5. Tap the box below **Image format** to select output image type.
6. Toggle **Tissue trimming** and/or **24-plex** buttons as needed.
   - To trim tissue or crop extra background/flow channel, toggle the **Tissue trimming** button.
   - If a 24-plex kit is used, enter Nucleus information and toggle the **24-plex** button to take effect.
7. Tap **Review**.

Performing staining

1. Review all items to ensure that the information is correct.
2. Tap **Staining** to perform staining.
3. Preview stained images in the FluoXpert staining software interface during staining.
4. After staining is completed, tap **Finish** in the staining interface, and remove the reagent cartridge and flow cell according to on-screen instructions.

Performing elution

1. To perform other operations for the stained flow cell, tap **Elution** in the main interface.
2. After elution is completed, tap **Finish** in the elution interface.

Performing a multiplex immunofluorescence wash

Preparing for a wash

1. Select the wash protocol according to specific situations.

Wash protocol	Description	Washing reagent
Pre-run wash (~15 min)	• Before mIF staining • When changing primary antibodies before the kit is used up, washing is required after staining • If reagents are used up after mIF staining, washing is required after staining	For details, refer to step 3 in *Preparing for a wash on Page 19*
Maintenance wash (~94 min)	• After mIF staining and before sequencing • After sequencing and before mIF staining	For details, refer to step 3 in *Preparing for a wash on Page 19*

Tips

If there are leftover reagents (each kit contains reagents for 5 slides) after staining and no sequencing is performed, no washing is required. Store the reagent kit in the reagent compartment and use it within two weeks.

2. Prepare the washing flow cell.

Tips

Used flow cells for sequencing can be used as washing flow cells.

3. Prepare washing reagent. For details, refer to step 3 in *Preparing for a wash on Page 19*.

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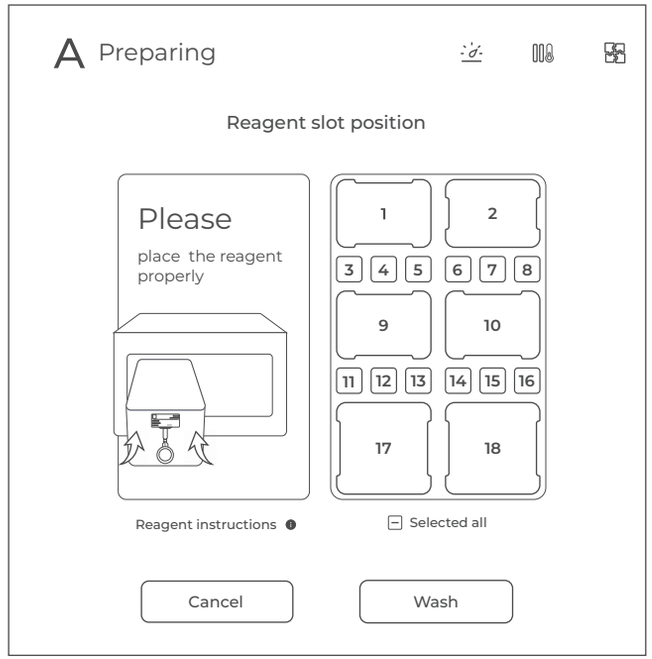
Performing a wash



1. Tap **Wash** in the main interface.
2. Select the reagent slot position that requires washing. Tap **Selected all** to select or deselect all positions.
3. Tap **Wash** to perform wash.
4. After the wash is completed, tap **Finish** in the wash interface.

Checking staining data

After sequencing starts, the sequencing results generated by the control software will be saved to the D drive of the computer.

- The FX_Data folder contains raw data in subfolders named after the experiments.
- The FX_Result folder contains stitched images that can be processed in third-party image analysis software or MGI pathology image analysis software FluoXpert Vision.

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Preparing for sequencing

Logging in to the control software

Tips

Different control software is used for sequencing and mIF staining. To switch between these two functions, close the current control software before starting the other one.

1. Power the device on.
2. Log in to the computer with the account *zebra* and the password *123*.
3. Select in the main interface.
4. Log in to the control software with the account information provided below:

Account type	User name	Password
User	user	Password123
Administrator	research	Admin123

Checking remaining space of the storage drive

Check whether the remaining space of the storage drive is greater than 4.6 TB. If the remaining space is insufficient, clear the history data by tapping **Device maintenance** > **Clear history data**.

Checking the waste container

1. Empty the waste container when the waste level approaches two thirds of the maximum volume of the waste container.
2. Empty the waste container when the waste container icon turns to .
3. If the float of the waste level sensor is not properly placed at the lower position of the waste container, clean and move the sensor to the lower position.
4. If any problem occurs other than those listed above, restart the sequencer control software.
5. If the problem persists, contact the technical support.

Performing a pre-run wash

Before each sequencing run, perform a pre-run wash to flush impurities out and to empty the fluidics line. For details, refer to *Performing a wash for the sequencer on Page 19*.

Preparing the Sequencing Reagent Cartridge-Part 1

1. Remove the Sequencing Reagent Cartridge from storage.
2. Thaw the cartridge in a water bath at room temperature until completely thawed (or thaw in a 2 °C to 8 °C refrigerator 1 to 2 days in advance). The approximate time to thaw is listed in the following table. Store in a 2 °C to 8 °C refrigerator until use.

Approximate thawing time for various sequencing cartridges			
Model	Method		
	Water bath at room temperature (h)	Refrigerator at 2 °C to 8 °C overnight, then water bath at room temperature (h)	Refrigerator at 2 °C to 8 °C (h)
FCL SE50	2.0	0.5	24.0
sRNA FCL SE50	2.0	0.5	24.0
FCL SE100	3.0	1.0	24.0
FCL PE50	3.0	1.0	24.0
FCL PE100	4.0	2.0	36.0
FCL PE150	5.0	2.5	48.0
App-D FCL SE50	2.0	0.5	24.0
App-D FCL SE100	3.0	1.0	24.0
App-D FCL PE50	3.0	1.0	24.0
App-D FCL PE100	4.0	2.0	36.0
App-D FCL PE150	5.0	2.5	48.0

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Preparing the flow cell

Remove the box containing the flow cell from storage and take out the flow cell. Place the flow cell at room temperature for 30 min to 24 h. Unwrap the outer plastic packaging before use. Take the flow cell out from the inner packaging and inspect it to ensure the flow cell is intact and clean, without scratches.

Tips

- If the flow cell is not used within 24 h after being placed at room temperature and the outer plastic packaging is intact, the flow cell can be returned to -25 ℃ to -15 ℃ for storage. The number of freeze-thaw events must not exceed 3 cycles.
- If the outer plastic packaging has been opened but the flow cell cannot be used immediately, store the flow cell at room temperature and use it within 24 h. It is not recommended that you use the flow cell after 24 h.

Preparing DNBs

Recommended library insert size

DNB preparation starts from a circular ssDNA library. The size distribution of inserts should be between 20 bp and 500 bp, with the main insert size fragment centered within ±100 bp.

Recommended library insert size and data output		
Model	Recommended library insert distribution (bp)	Data output (Gb/flow cell)
FCL SE50	50 to 230	75 to 90
sRNA FCL SE50	20 to 60	75 to 90
FCL SE100	200 to 400	150 to 180
FCL PE50	100 to 300	150 to 180
FCL PE100	200 to 400	300 to 360
FCL PE150	300 to 500	450 to 540
App-D FCL SE50	50 to 230	75 to 90
App-D FCL SE100	200 to 400	150 to 180
App-D FCL PE50	100 to 300	150 to 180
App-D FCL PE100	200 to 400	300 to 360
App-D FCL PE150	300 to 500	450 to 540

Calculating the number of make DNB reactions

Loading system	Minimum number of required Make DNB reactions/ flow cell	Make DNB reaction (μL)	DNB Volume (μL)/lane
DNBSEQ-G400RS FluoXpert	2	100	50
MGIDL-200H	2	50	25

DNA library concentration and amount requirements

Library type	Minimum library concentration	Volume of 100 μL DNB reaction (μL)	Volume of 50 μL DNB reaction (μL)
PCR	2 fmol/μL	V=40 fmol/C	V=20 fmol/C
PCR-free	3.75 fmol/μL	V=75 fmol/C	V=37.5 fmol/C
Small RNA	3 fmol/μL	V=60 fmol/C	V=30 fmol/C
App PCR	3 fmol/μL	V=60 fmol/C	V=30 fmol/C
App PCR-free	3.75 fmol/μL	V=75 fmol/C	V=37.5 fmol/C

Tips

- If the library concentration is unknown, it is recommended that you perform ssDNA library quantitation (ng/μL) by using Qubit® ssDNA Assay Kit and Qubit Fluorometer. Use the equation below to convert the concentration of the ssDNA library from ng/μL to fmol /μL:

$$C(\text{fmol}/\mu\text{L}) = \frac{3030 \times C(\text{ng}/\mu\text{L})}{N}$$

N represents the number of nucleotides (average library length including the adapter) as determined by fragment size analysis.

- If there are any special requirements or specifications for the library preparation kit, then the requirements of the kit should be followed.
- *C* in the following table represents the concentration of libraries (fmol/μL).

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Making DNBs

Tips

- App Make DNB Buffer can be used to make DNBs for both MGI and App libraries.
- Mixed use of reagent components from different batches is not recommended.
- For transferring or mixing DNBs, use the wide-bore, non-filtered pipette tips.
- For other reagents, use a proper pipette tip according to the actual situation. It is recommended that you use the pipette tips from recommended brands and catalog numbers.

1. Place the libraries on ice until use.
2. Remove Low TE Buffer, Make DNB Buffer or App Make DNB Buffer and Stop DNB Reaction Buffer from storage and thaw the reagents at room temperature.
3. Remove Make DNB High-efficiency Enzyme Mix I from storage and thaw the reagent for approximately 30 min on ice.
4. Mix the reagents using a vortex mixer for 5 s. Centrifuge briefly and place on ice until use.
5. Take out a 0.2 mL 8-strip tube or PCR tubes. Prepare Make DNB reaction mixture 1 according to different ssDNA libraries.

Make DNB reaction mixture 1 for MGI libraries		
Component	Volume of 100 µL DNB reaction (µL)	Volume of 50 µL DNB reaction (µL)
Low TE Buffer	20 - V	10 - V
Make DNB Buffer	20	10
ssDNA libraries	V	V
Total Volume	40	20

Make DNB reaction mixture 1 for App libraries		
Component	Volume of 100 µL DNB reaction (µL)	Volume of 50 µL DNB reaction (µL)
Low TE Buffer	20 - V	10 - V
App Make DNB Buffer	20	10
ssDNA libraries	V	V
Total Volume	40	20

6. Mix the reaction mixture thoroughly by using a vortex mixer. Centrifuge for 5 s by using a mini centrifuge, and place it on ice until use.
7. Place the mixture into a thermal cycler and start the primer hybridization reaction. The thermal cycler settings are shown in the table below.

Primer hybridization reaction conditions					
Temperature	Heated lid (105 °C)	95 °C	65 °C	40 °C	4 °C
Time	On	1 min	1 min	1 min	Hold

8. Remove Make DNB Enzyme Mix II (LC) from storage and place on ice. Centrifuge briefly for 5 s and hold on ice.

Tips

- Do not keep Make DNB Enzyme Mix II (LC) at room temperature.
- Avoid holding the tube for a prolonged time.

9. Remove the PCR tube from the thermal cycler when the temperature has reached 4 °C .
10. Centrifuge briefly for 5 s, place the tube on ice, and prepare Make DNB reaction mixture 2 according to the table below.

Make DNB reaction mixture 2		
Component	Volume of 100 µL DNB reaction (µL)	Volume of 50 µL DNB reaction (µL)
Make DNB High-efficiency Enzyme Mix I	40	20
Make DNB Enzyme Mix II (LC)	4	2
Total Volume	44	22

11. Add all of Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer and centrifuge for 5 s by using a mini centrifuge.

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12. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below.

RCA (Rolling Circle Amplification) conditions			
Temperature	Heated lid (35 °C)	30 °C	4 °C
Time	On	25 min	Hold

13. Immediately add Stop DNB Reaction Buffer when the temperature reaches 4 °C. The volume of Stop DNB Reaction Buffer is shown in the table below. Mix gently by pipetting 8 times by using a wide-bore, non-filtered pipette tip.

Volume of Stop DNB Reaction Buffer		
Component	Volume of 100 µL DNB reaction (µL)	Volume of 50 µL DNB reaction (µL)
Stop DNB Reaction Buffer	20	10

Tips

- It is very important to mix DNBs gently by using a wide-bore, non-filtered pipette tip. Do not centrifuge, vortex, or shake the tube.
- Store DNBs at 2 °C to 8 °C and perform sequencing within 48 h.

Quantifying DNBs

Preparing Qubit working solution

1. Take out the Qubit ssDNA Reagent, Qubit ssDNA Standard #1 and Qubit ssDNA Standard #2 from the Qubit ssDNA Assay Kit. Mix these components with a vortex mixer for 5 s, centrifuge briefly and place them at room temperature until use.

Tips

Thaw Qubit ssDNA Reagent away from direct sunlight and mix thoroughly before use.

2. Prepare Qubit working solution according to the following table.

Component	Volume (µL)
Qubit ssDNA Buffer	199× (N+1)
Qubit ssDNA Reagent	1× (N+1)

3. Mix the working solution thoroughly with a vortex mixer for 5 s, and centrifuge briefly. Add 190 µL of working solution into 2 standard test tubes, and add 198 µL of working solution into the DNB test tube.
4. Add 10 µL of Qubit ssDNA Standard #1 and Qubit ssDNA Standard #2 to 2 standard test tubes respectively, and add 2 µL of prepared DNBs to DNB test tube.
5. Mix these tubes thoroughly with a vortex mixer for 5 s, and centrifuge briefly. Place them at room temperature away from direct sunlight for 2 min, and start quantification.

Tips

During operation, avoid direct contact between the outer wall of the test tube and other objects to prevent the temperature of the tube wall from being too high or too low, which may affect the concentration values.

Calculating DNB concentration

Take Qubit Fluorometer as an example. a is a test room for placing test tubes. b is a touch screen for operation and result display.

1. Tap **Oligo** > **ssDNA** > **Read standard value**, and start testing.
2. Place the standard #1 test tube in A, close the lid, tap **Read standard value**, and take it out after finishing.
3. Place the standard #2 test tube in A, close the lid, and tap **Read standard value**.
4. After testing, tap **Run Sample**, set the volume to 10 µL, and the concentration unit to ng/µL.

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5. Tap **Read tube**. The concentration is required to range between 19.9 ng/μL and 20 ng/μL. Otherwise, repeat step 2 to step 5.
6. Remove the standard #2 test tube, reset the volume to 2 μL and the concentration unit is ng/μL.
7. Put the sample test tube in, close the lid, and tap **Read test tube**. At this time, the sample concentration is displayed on the screen.
8. Repeat step 7, and test the remaining samples.

Loading DNBs

- Using the sequencer to load DNBs

  All lanes in the flow cell must be loaded with the same DNBs.
- Using MGIDL-200H to load DNBs

  Different DNBs can be loaded into different lanes.

Tips

If you load DNBs by the sequencer, prepare the sequencing cartridge first according to *Preparing the Sequencing Reagent Cartridge-Part 2 on Page 14*, then proceed to the loading process.

Loading DNBs by the sequencer

1. Prepare reagents according to the table below.

Tips

If crystal precipitation is visible in DNB Load Buffer II, vigorously mix the reagent for 2 min by using a vortex mixer to re-dissolve the precipitation before use.

Model	Component	Step 1)	Step 2)	Step 3)
FCL SE50, sRNA FCL SE50, FCL SE100, FCL PE50, FCL PE100, FCL PE150, App-D FCL SE50, App-D FCL SE100, App-D FCL PE50, App-D FCL PE100, App-D FCL PE150	DNB Load Buffer II	Thaw the reagents on ice for approximately 30 min	Mix the reagents by using a vortex mixer for 5 s, centrifuge briefly	Place on ice until use

2. Take out a 0.5 mL microcentrifuge tube from the sequencing kit and add the following reagents according to different sequencing read lengths.

DNB loading mixture 1		
Model	Component	Volume (μL)
FCL SE50, sRNA FCL SE50, FCL SE100, FCL PE50, FCL PE100, FCL PE150, App-D FCL SE50, App-D FCL SE100, App-D FCL PE50, App-D FCL PE100, App-D FCL PE150	DNB Load Buffer II	64
	Make DNB Enzyme Mix II (LC)	2
	DNBs	200
	Total Volume	266

3. Combine components and mix by gently pipetting 8 times by using a wide-bore, non-filtered pipette tip. Place the mixture at 2 °C to 8 °C until use.

Tips

- Do not centrifuge, vortex, or shake the tube.
- Prepare a fresh DNB loading mixture 1 immediately before the sequencing run.
- Each FCL requires 266 μL of DNB loading mixture 1.

4. Open the reagent compartment door.
5. Gently lift the DNB loading needle with one hand, remove the cleaning reagent tube with the other hand, load the prepared sample tube, and slowly lower the DNB loading needle until the tip reaches the bottom of the tube.

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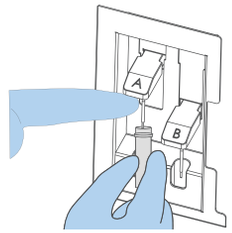


6. Close the reagent compartment door.
7. Select the **DNB loading** box in the DNB ID entry interface. After you prepare the Sequencing Reagent Cartridge, load the flow cell and perform sequencing according to *Starting sequencing on Page 17*.

Loading DNBs by MGIDL-200H

1. Prepare reagents according to the table in step 1 in *Loading DNBs by the sequencer on Page 12*.
2. Take out a new 1.5 mL Sterile microcentrifuge tube or 0.5 mL Sterile microcentrifuge tube and add the reagents shown in the table below according to different read lengths:

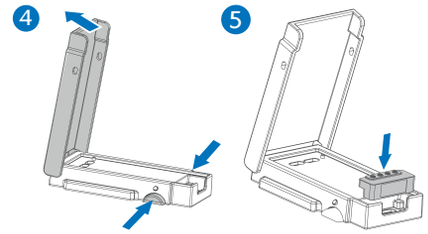
DNB loading mixture 2		
Model	Component	Volume (μL)
FCL SE50, sRNA FCL SE50, FCL SE100, FCL PE50, FCL PE100, FCL PE150, App-D FCL SE50, App-D FCL SE100, App-D FCL PE50, App-D FCL PE100, App-D FCL PE150	DNB Load Buffer II	8
	Make DNB Enzyme Mix II (LC)	0.25
	DNBs	25
	Total Volume	33.25

3. Combine components and mix by gently pipetting 8 times by using a wide-bore, non-filtered pipette tip. Place the mixture at 2 ℃ to 8 ℃ until use.

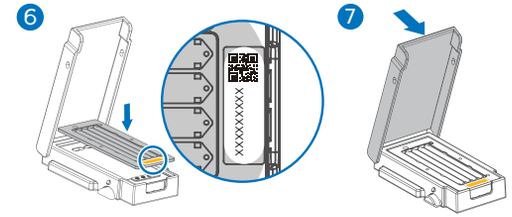
Tips

- Do not centrifuge, vortex, or shake the tube.
- Prepare a fresh DNB loading mixture 2 immediately before the sequencing run.
- Each lane requires at least 30 μL of DNB loading mixture 2.

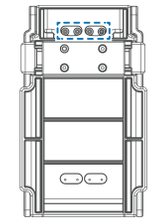
4. Squeeze the latches and open the cover.
5. Place a clean sealing gasket into the groove and ensure that the gasket surface is even.



6. Align the holes of the flow cell with the alignment pins of the device and place the flow cell on it.
7. Close the cover and ensure that the cover is securely closed.



8. With the back of the DL 200H facing up, verify that the fluidics inlets align with the holes of the sealing gasket and ensure that the holes are clean.



9. Aspirate 30 μL of DNB loading mixture 2 with a wide-bore, non-filtered pipette tip, and insert the tip into the fluidics inlet.
10. Eject the tip from the pipette. DNBs will automatically flow into the flow cell.

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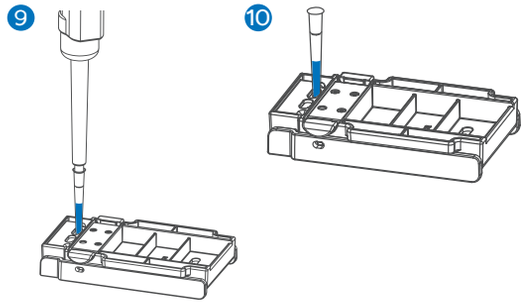
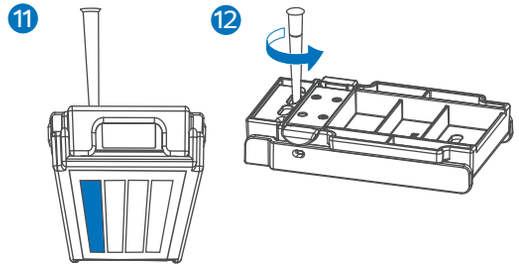
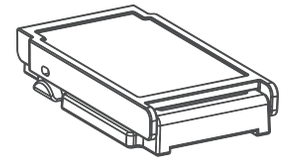


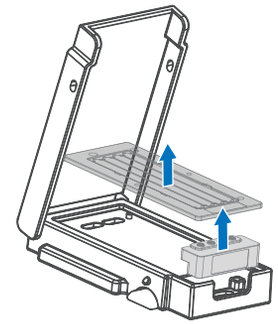
11. Lift up the DL 200H, but do not tilt it (keep it parallel to the bench), and check whether the DNBs flow through the flow cell.
12. Ensure that all DNBs flow into the flow cell, hold the device and rotate the tip counterclockwise to remove it.



13. Repeat step 9 to 12 to load the DNBs to the rest of the lanes of the flow cell.
14. Place MGIDL-200H on the bench with the front facing up. wait 30 min for the DNB loading process.



15. Open the cover and take out the flow cell and the sealing gasket.



Preparing the Sequencing Reagent Cartridge-Part 2

1. Invert the cartridge 3 times to mix before use. Shake the cartridge vigorously up and down 20 times, and then left and right 20 times. Ensure that the reagents are thoroughly mixed.
2. Wipe any water condensation from the cartridge cover and wells surround with a KimWipes tissue.
3. Remove dNTPs Mix and dNTPs Mix II from -25 ℃ to -15 ℃ storage 1 h in advance and thaw at room temperature. Store at 2 ℃ to 8 ℃ until use.
4. Remove Sequencing Enzyme Mix II from -25 ℃ to -15 ℃ storage and place on ice until use.
5. Remove reagents from storage according to your model:
   - For sRNA FCL SE50 Sequencing, remove Wash Buffer for Small RNA Sequencing from storage and thaw at room temperature. Store at 2 ℃ to 8 ℃ until use.
   - For PE sequencing:
     a. Remove MDA Block Reagent from storage packaging and thaw it at room temperature until use.
     b. Remove MDA Block Component from storage packaging and thaw it at room temperature of 20 ℃ to 30 ℃ until use. After thawing, invert it 6 times.

     Tips

     Do not keep MDA Block Component below 20 ℃ and avoid refreezing.

     c. Remove Inactive MDA Reagent from storage and place on ice until use.

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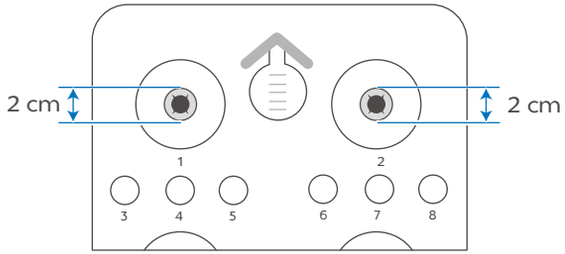
6. Pierce the seals in the center of well No. 1 and No. 2 to make a hole approximately 2 cm in diameter by using a 1 mL sterile pipette tip.



7. Take out a pipette with the appropriate volume range. Add dNTPs Mix into a new 5 mL/ 10 mL sterile tube, and then add Sequencing Enzyme Mix II into the dNTPs Mix in the same tube. Invert the tube 6 times to mix the reagents in the tube before adding all of them into well No. 1.

Tips

- Mix dNTPs Mix for 5 s by using a vortex mixer and centrifuge briefly before use.
- Invert Sequencing Enzyme Mix II 6 times before use.
- When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.

Model	Well No. 1	
	dNTPs Mix (mL)	Sequencing Enzyme Mix II (mL)
FCL SE50	0.400	0.750
sRNA FCL SE50	0.400	0.750
FCL SE100	0.550	1.100
FCL PE50	0.550	1.100
FCL PE100	0.900	1.850
FCL PE150	1.250	2.500
App-D FCL SE50	0.400	0.750
App-D FCL SE100	0.550	1.100
App-D FCL PE50	0.550	1.100
App-D FCL PE100	0.900	1.850
App-D FCL PE150	1.250	2.500

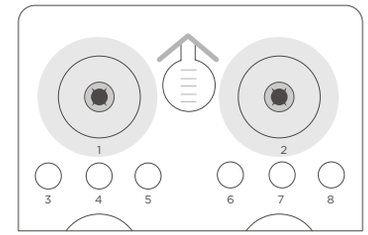
8. Take out a pipette with the appropriate volume range. Add dNTPs Mix II into a new 5 mL/ 10 mL sterile tube, and then add Sequencing Enzyme Mix II into the dNTPs Mix II in the same tube. Invert the tube 6 times to mix the reagents in the tube before adding all of them into well No. 2.

Tips

- Mix dNTPs Mix II for 5 s by using a vortex mixer and centrifuge briefly before use.
- Invert Sequencing Enzyme Mix II 6 times before use.
- When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.

Model	Well No. 2	
	dNTPs Mix II (mL)	Sequencing Enzyme Mix II (mL)
FCL SE50	0.600	0.350
sRNA FCL SE50	0.600	0.350
FCL SE100	0.800	0.500
FCL PE50	0.800	0.500
FCL PE100	1.200	0.750
FCL PE150	1.700	1.050
App-D FCL SE50	0.600	0.350
App-D FCL SE100	0.800	0.500
App-D FCL PE50	0.800	0.500
App-D FCL PE100	1.200	0.750
App-D FCL PE150	1.700	1.050

9. Seal loading well No. 1 and No. 2 with transparent sealing films.



10. Press the film around the well with your finger, and ensure that the well is tightly sealed and that there are no air bubbles between the film and cartridge surface. This ensures that the reagents will not flow over the cartridge.

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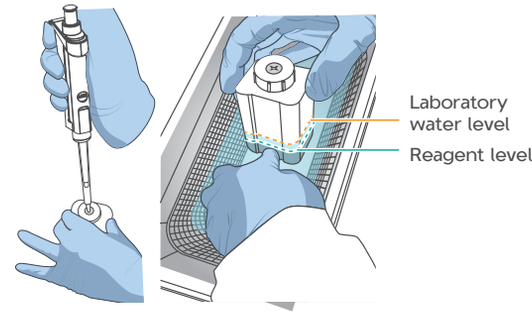
11. Lift the cartridge horizontally and hold both sides of the cartridge with both hands. Shake the cartridge vigorously 20 times in a clockwise and counterclockwise direction. Ensure that the reagents are fully mixed.
12. Carefully remove the sealing films from the loading wells after fully mixing.

Tips

- Do not reuse the used sealing film.
- To prevent cross contamination, ensure that the surface around wells No. 1 and No. 2 is clean.

13. It is recommended that you handle reagent in well No. 10 according to the following steps:
    ① Open the reagent cartridge cover and remove the reagent bottle from well No. 10.
    ② Fill the ultrasonic cleaner with laboratory water. The recommended power for the ultrasonic cleaner is 300 W to 600 W, with a capacity of 10 L to 30 L.
    ③ Pierce the sealing film of the reagent bottle with a pipette tip. Place the reagent bottle into the ultrasonic cleaner, ensure that the water level of the cleaner is above the level of reagent inside the bottle, and avoid water from entering the reagent bottle.



    ④ Start the ultrasonic cleaner and vibrate for 3 min to 5 min.
    ⑤ After completion, remove the reagent bottle and avoid shaking it.
    ⑥ Use a KimWipes tissue to wipe off any moisture on the surface of the reagent bottle.
    ⑦ Place the reagent bottle back into the reagent cartridge and close the cartridge cover.
14. Gently tap the cartridge on the bench to reduce air bubbles in the reagents.

Tips

The FCL SE50/FCL SE100/App-D FCL SE50/App-D FCL SE100 Sequencing Reagent Cartridge is now ready for use. For the next step, refer to *Starting sequencing on Page 17*.

15. Perform the following steps for Small RNA sequencing:

Tips

Wash Buffer for Small RNA Sequencing contains highly concentrated formamide, which may have potential reproductive toxicity. Avoid breathing vapor and wear protective gloves/protective clothing/protective eye mask/ protective mask when using these reagents.

    ① Mix Wash Buffer for Small RNA Sequencing by using a vortex mixer for 5 s and centrifuge briefly before use.
    ② Pierce the seal of well No. 7 and add 4.50 mL of Wash Buffer for Small RNA Sequencing. When adding the reagent, ensure that no bubbles exist at the bottom of the tube.

Tips

The Small RNA FCL SE50 Sequencing Reagent Cartridge is now ready for use. For the next step, refer to *Starting sequencing on Page 17*.

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16. Perform the following steps for PE sequencing:
    ① Pierce the seals of well No. 13 and No. 15 by using a 1 mL sterile pipette tip.
    ② Add 300 µL of MDA Block Component to the MDA Block Reagent tube with a 1 mL pipette. Invert the tube 4 to 6 times to mix the reagents thoroughly. Add the mixture to well No. 13. When adding the mixture, ensure that there are no bubbles at the bottom of the tube.
    ③ Add 500 µL of MDA Enzyme Mix II to the Inactive MDA Reagent tube with a 1 mL pipette. Invert the tube 6 times to mix the reagents. Add all the mixture to well No. 15. When adding the mixture, ensure that there are no bubbles at the bottom of the tube.

Tips

- When using MDA Enzyme Mix II, do not touch the wall of the tube. The heat from your hands may affect the enzyme activity.
- When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.
- The FCL PE50/FCL PE100/FCL PE150/App-D FCL PE50/App-D FCL PE100/App-D FCL PE150 Sequencing Reagent Cartridge is now ready for use. For the next step, refer to *Starting sequencing on Page 17*.

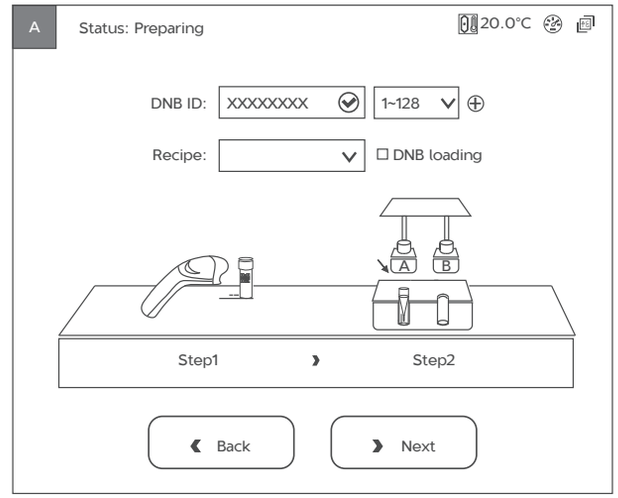
Starting sequencing

Selecting sequencing parameters

1. In the main interface, tap **Sequence** to enter the DNB ID entry interface.

Tips

If a pop-up dialog box indicating that a wash is required appears, perform a wash according to *Performing a wash for the sequencer on Page 19*.

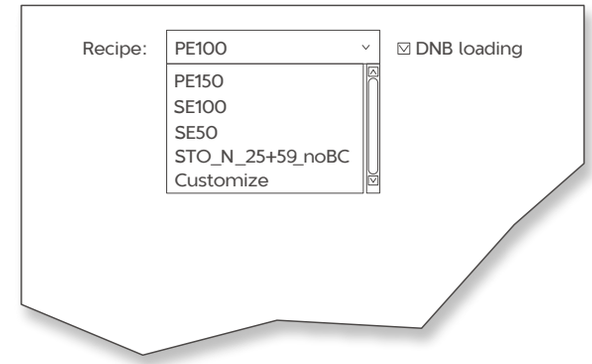


2. Tap the **DNB ID** box, enter the DNB ID manually by using the on-screen keyboard.

   Select a barcode range of different lanes from the list next to the DNB ID box.

   Tap ⊕ to add a row of DNB ID if needed.

3. Select an appropriate recipe from the **Recipe** list. One-click sequencing runs (for example, **SE50**, and so on) and a user-customized run (**Customize**) are available.



Tips

- For Dual Barcode sequencing, select **Customize** from the **Recipe** list, and configure settings.
- STO_xx sequencing recipe is applicable to STOmics libraries, and must be used with the corresponding STOmics reagent set.

4. Tap **Next**.

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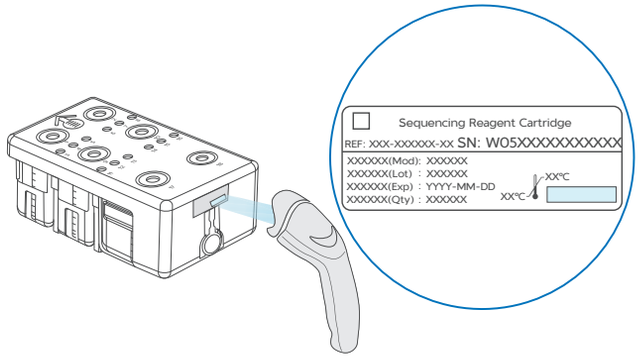
Loading the Sequencing Reagent Cartridge

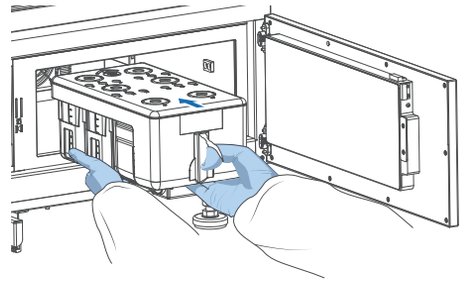
1. Tap the **Sequencing cartridge ID** field, manually enter the cartridge ID according to the SN printed on the cartridge label or use the barcode scanner to scan the cartridge barcode at the lower right corner of the Sequencing Reagent Cartridge label.



CAUTION

Do not scan the QR code in the upper-left corner by mistake.

2. Open the reagent compartment door and slowly remove the cleaning cartridge from the compartment. Moisten a KimWipes tissue with laboratory-grade water and use it to wipe the bottom and sides of the compartment to keep it clean and dry.
3. Slide the cartridge into the compartment by following the direction printed on the cover until it stops.



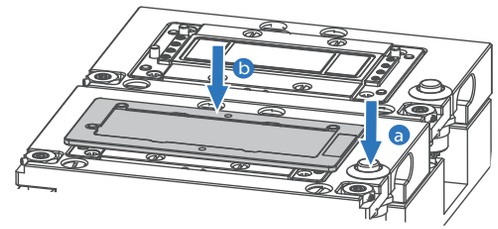
Tips

Reagent cartridge A is placed on the left and reagent cartridge B is placed on the right. Ensure that the loading position of the reagent cartridge is consistent with the placement position of the flow cell.

4. Close the reagent compartment door and tap **Next**.

Loading the flow cell

1. Open the flow cell compartment door. Press both sides of the washing flow cell, and press the flow cell attachment button with the other hand. After the vacuum is released, remove the washing flow cell from the stage.
2. Use a canned air duster to remove the dust on the flow cell stage and the back of the flow cell. If there are impurities on the stage surface, gently wipe the surface with a wet KimWipes tissue to ensure that the flow cell can be held properly.
3. Take out the new flow cell. There are two alignment holes on the left side and one on the right side. The label is on the right. Hold the flow cell by the edges with both hands.
4. Align the holes on the flow cell with the locating pins on the flow cell stage. Gently slide the flow cell at an angle of 45 degrees to the upper-left corner to keep the flow cell aligned with the pin.
5. Press the flow cell attachment button. Press the left and right sides of the flow cell on the stage at the same time to ensure that the flow cell is properly seated on the stage.



Tips

The flow cell is fragile, handle it with caution.

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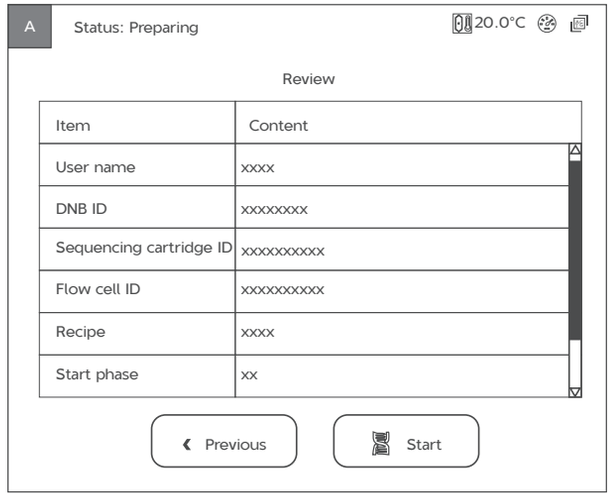
6. Ensure that the negative pressure is within the range of -80 kPa to -99 kPa.
7. Use a canned air duster to remove the dust on the flow cell surface and then close the flow cell compartment door.
8. Tap **Next**. The flow cell ID can be entered using the barcode scanner.

   If automated entry does not work, move the cursor to the **Flow cell ID** box and enter the ID manually.
9. Tap **Next**.

Starting the sequencing run

1. Carefully check each item in the review interface and ensure that each item is correct.



2. Tap **Start** and tap **Yes** when prompted to begin sequencing.
3. Once sequencing has started, immediately open the flow cell compartment door to inspect the flow cells, and ensure that DNBs or reagents are flowing through the flow cell. Close the flow cell compartment door.

Tips

- Do not bump, move, vibrate, or impact the device during sequencing, as it may cause inaccurate sequencing results.
- To ensure sequencing quality, when sequencing of Read1 and Read2 is completed, the sequencer will automatically perform another cycle for calibration. For example, for PE100 sequencing, the length of Read1 is 100, the length of Read2 is 100, the length of barcode is 10. Adding 1 correction cycle for Read1 and 1 correction cycle for Read2 (barcode does not need to be corrected), the total number of sequencing cycles is 212.

Performing a wash

Performing a wash for the sequencer

Preparing for a wash

1. When the sequencing is completed, perform a wash within 24 h. When the following interface appears, tap **Wash** and perform the wash procedures.

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2. Select the wash protocol according to specific situations.

Wash protocol	Wash selection	Cartridge type	Description
Pre-run wash	Regular (54 min)	Cleaning cartridge 1	• After an SE sequencing run. • It has been more than 24 h but less than 7 d since the last maintenance wash. • After the sequencer maintenance is performed by an engineer, which includes the replacement of accessories not exposed to reagents. • Other situations except for maintenance wash.
Maintenance wash	Maintenance (20 min)	Cleaning cartridge 3	• It has been more than 7 d since the last operation, and a pop-up dialog box indicates a wash. • When using the control software for the first time, updating to a new version, or manually clearing data resulting in no records, a pop-up dialog box indicates a wash. • After an PE sequencing run or DNB loading. • After the sequencer maintenance is performed by an engineer. This includes, but is not limited to the replacement of pipelines, sample needles and other accessories exposed to reagents. • If the sequencer is to be powered off for more than 7 d, a wash before being powered off and after being powered on is required. • When impurities are visible on the flow cell,
	Maintenance (20 min)	Cleaning cartridge 2	
	Regular (54 min)	Cleaning cartridge 1	
DNBTube wash (Optional)	DNBTube (5 min)	Cleaning cartridge 1	If you need an extra wash for the DNB loading tubes after pre-wash and maintenance wash.
	DNBTube (5 min)		
	DNBTube (5 min)		

3. Prepare washing reagents, cleaning cartridges, and DNB loading needle washing tubes according to specific wash protocols.

DNB loading needle wash tube type	Washing reagent	Cartridge type	Washing reagent
DNB loading needle washing tube 3	0.05% Tween-20; 1 M NaCl + 0.05% Tween-20	Cleaning cartridge 3	0.05% Tween-20; 1 M NaCl + 0.05% Tween-20
DNB loading needle washing tube 2	0.1 M NaOH	Cleaning cartridge 2	0.1 M NaOH
DNB loading needle washing tube 1	Laboratory-grade water	Cleaning cartridge 1	Laboratory-grade water

0.05% Tween-20 preparation		
Reagent name	**Volume (mL)**	**Final concentration**
100% Tween-20	0.5	0.05%
Laboratory-grade water	999.5	/
Total Volume	1000	

0.05% Tween-20+1 M NaCl preparation		
Reagent name	**Volume (mL)**	**Final concentration**
100% Tween-20	0.5	0.05%
5 M NaCl solution	200	1 M
Laboratory-grade water	799.5	/
Total Volume	1000	

0.1 M NaOH preparation		
Reagent name	**Volume (mL)**	**Final concentration**
2 M NaOH	50	0.1 M
Laboratory-grade water	950	/
Total Volume	1000	

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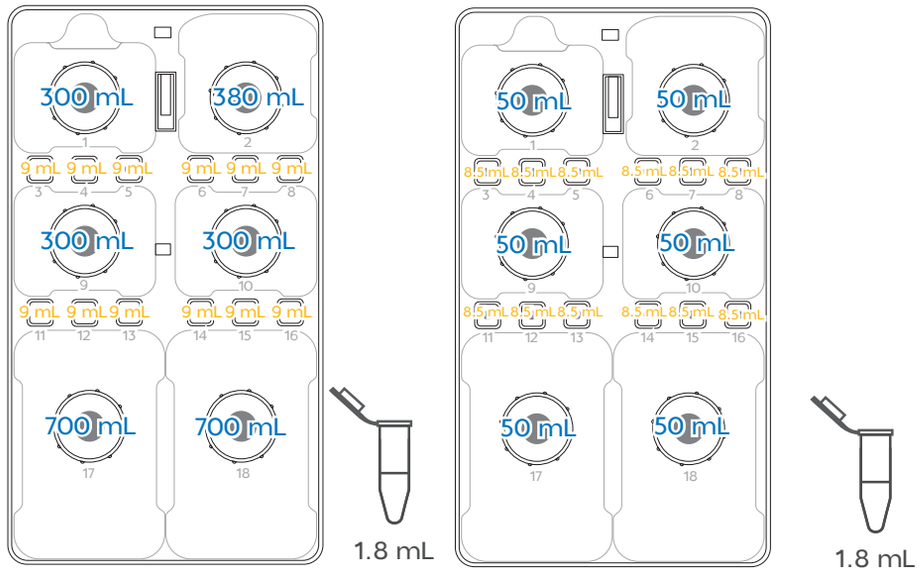
- Cleaning cartridge 1 and 2

Cleaning cartridge 1
(Laboratory-grade water)

Cleaning cartridge 2
(0.1 M NaOH)

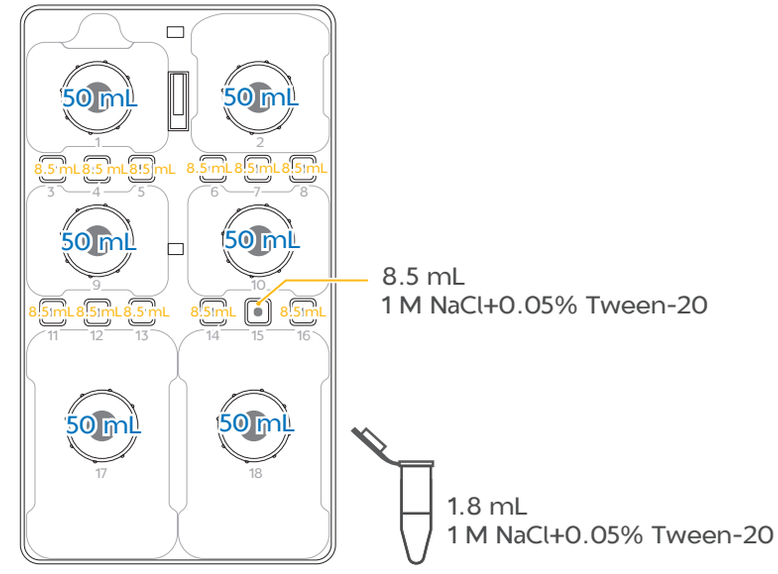


Tips

2.0 mL sterile microcentrifuge tubes are used for DNB loading needle washing.

- Cleaning cartridge 3

Cleaning cartridge 3
(0.05% Tween-20/0.05% Tween-20+1 M NaCl)



4. Prepare the washing flow cell.

Tips

- A used flow cell without physical damage can be used as a washing flow cell.
- Each washing flow cell, stored at room temperature or in a 2 ℃ to 8 ℃ refrigerator, can be reused 20 times.

Performing a wash

1. Take out the prepared cleaning cartridge and washing flow cell according to the selected wash type.
2. Load the washing flow cell on the flow cell stage.

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3. Load the cleaning cartridge into the reagent compartment, and close the compartment door.
4. Tap **Wash** when prompted after the sequencing is completed.
5. Select a wash type from the **Wash type** list, and tap **Yes** when you are prompted.
6. Perform the wash according to the step 3 in *Preparing for a wash on Page 19*.

MGIDL-200H and sealing gasket maintenance

⚠ WARNING

- Do not immerse MGIDL-200H into the liquid for cleaning. Doing so may damage the device.
- Do not use other disinfectants such as dichloroethane ($C_2H_4Cl_2$), trichloroethylene (C_2HCl_3), chloroform ($CHCl_3$), and toluene (C_7H_8) to clean MGIDL-200H. Doing so may damage the device.
- It is recommended that you replace MGIDL-200H (Cat. No.: 900-000217-00) with a new one after using for one year.
- If you have questions about the compatibility of disinfectants, contact the technical support.

After each DNB loading, perform the following steps to maintain MGIDL-200H and the sealing gasket:

1. Wipe all sides of the device with a low-lint cloth moistened with 75% ethanol and a low-lint cloth moistened with ultrapure water.
2. Wipe the device with a low-lint cloth and let it air-dry.
3. Collect the used sealing gasket into a 200 mL beaker.
4. Fill the beaker with ultra-pure water and wash the sealing gasket in the beaker, and then empty the beaker. Repeat the wash twice, for a total of 3 times.
5. Fill the ultrasonic cleaner tank with ultra-pure water, and wash the sealing gasket in the ultrasonic cleaner tank for about 15 min.
6. Repeat step 4, place the cleaned sealing gasket into a clean container and let it air-dry.
7. (Optional) Replace with a new sealing gasket (Cat. No.: 510-000718-00) if any of the following occurs:
   - The sealing gasket has been cleaned 20 times.
   - The sealing gasket has been used for 3 months.
   - The pipette tip loosens during loading DNBs.

Checking sequencing data

After sequencing starts, the sequencing results generated by the control software will be saved to the D drive of the computer.

- The data folder, named after the flow cell ID, mainly contains pictures and data (such as metrics) generated during the device operation.
- The result folder, named after the flow cell ID, primarily contains Bioinfo files and FASTQ files, reports, and *.cal* files.

Research use only

MGI has labeled the product solely for research use only and specified "RS" in the model name which means that it should not be used for clinical diagnosis. Please refer to FDA Guidance, *Distribution of In Vitro Diagnostic Products Labeled for Research Use Only or Investigational Use Only* (Nov. 2013) (available at: *https://www.fda.gov/media/87374/download*). If you have any question, please contact MGI at +86-4000-688-114.