

Making DNBs

Loading DNBs

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Overview

This guide is used to provide guidance for the use of DNBSEQ-G400RS sequencing reagents and sequencer.

Optional reagent set information is as follows:

| Catalog number | Model | Name |
|----------------|------------------|---|
| 1000016941 | SE50 | DNBSEQ-G400RS High-throughput Sequencing Reagent Set (SE50) |
| 1000016943 | SE100 | DNBSEQ-G400RS High-throughput Sequencing Reagent Set (SE100) |
| 1000016950 | PE100 | DNBSEQ-G400RS High-throughput Sequencing Reagent Set (PE100) |
| 1000016952 | PE150 | DNBSEQ-G400RS High-throughput Sequencing Reagent Set (PE150) |
| 1000016946 | SE400 | DNBSEQ-G400RS High-throughput Sequencing Reagent Set (SE400) |
| 940-000151-00 | PE200 | DNBSEQ-G400RS High-throughput Sequencing Reagent Set (PE200) |
| 1000016998 | SE50 (Small RNA) | DNBSEQ-G400RS High-throughput Sequencing Reagent Set (SE50) (Small RNA) |
| 1000016978 | FCS SE100 | DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set (FCS SE100) |
| 1000016980 | FCS PE100 | DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set (FCS PE100) |

| Catalog number | Model | Name |
|----------------|-----------------|--|
| 1000016982 | FCS PE150 | DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set (FCS PE150) |
| 940-000152-00 | FCS PE300 | DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set (FCS PE300) |
| 940-000238-00 | LV SM FCL PE150 | DNBSEQ-G400RS High-throughput Sequencing Set |
| 1000020834 | / | cPAS Barcode Primer 3 Reagent Kit |
| 1000014048 | / | cPAS Barcode Primer 4 Reagent Kit |

Tips

- DNBSEQ-G400RS High-throughput Sequencing Set (LV SM FCL PE150)(Cat. No.: 940-000238-00) is only manufactured in Latvia.
- The DNBSEQ-G400RS High-throughput Rapid Sequencing Set (FCS PE300) must be used with the sequencer control software in version 1.5.0.1283 and above, and with the BaseCall in version 1.4.0.257 and above.
- The DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE400) must be used with the script in version V1.7.1.03 and above.



Making DNBs

Recommended library insert size

| Library type | Recommended library insert distribution | Main insert size fragment |
|--------------|---|---------------------------|
| ssDNA | 20 bp to 800 bp | within ± 100 bp |

DNA library concentration and amount requirement

| Library type | Library concentration (fmol/ μ L) |
|--|---------------------------------------|
| General libraries (WGS, WES, RNASeq...) | ≥ 2 |
| Small RNA libraries | ≥ 3 |
| PCR free libraries | ≥ 3.75 |

Tips

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

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

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 MGI library preparation kit, then the requirements of the kit should be followed.

Calculating the number of make DNB reactions

| Loading system | Number of sample types to be loaded | DNB Volume (μ L)/lane | | Make DNB reaction (μ L)/lane | | Required number of make DNB reactions/flow cell | |
|----------------|-------------------------------------|----------------------------|-------------------|-----------------------------------|-------------------|---|-----|
| | | FCS PE300 | Other read length | FCS PE300 | Other read length | FCL | FCS |
| DNBSEQ-G400RS | 1 | 45 | 50 | 90 | 100 | 2 | 1 |
| MGIDL-200H | 1-4 | 22.5 | 25 | 45 | 100 | 1-4 | 1-2 |

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Calculating the required amount of ssDNA libraries

The required volume of ssDNA libraries is determined by the required library amount (fmol) and library concentration.

| Library type | Volume of 90 μ L DNB reaction (μ L) | Volume of 45 μ L DNB reaction (μ L) | Volume of 100 μ L DNB reaction (μ L) |
|---------------------|--|--|---|
| General libraries | V=40 fmol/C | V=20 fmol/C | V=40 fmol/C |
| Small RNA libraries | V=60 fmol/C | V=30 fmol/C | V=60 fmol/C |
| PCR free libraries | V=75 fmol/C | V=37.5 fmol/C | V=75 fmol/C |

Tips

- If there are any special requirements or specifications for the library preparation kit, then the requirements of the kit should be followed.
- All samples should be considered potentially infectious and should be handled in accordance with relevant national and local regulations.
- C in the table represents the concentration of libraries (fmol/ μ L).

Making DNBs

Tips

You can thaw the flow cell and sequencing reagent cartridge before making DNBs. For specific thaw times, refer to “*Preparing reagent cartridge*” on page 10.

1. Prepare libraries and reagents according to the table below:

| Component | Step 1) | Step 2) | Step 3) |
|--------------------------|---|---|-------------------------|
| Libraries | / | / | |
| Make DNB Enzyme Mix I | Thaw the reagent for approximately 0.5 hours on ice | Mix the reagents by using a vortex mixer for 5 seconds. | Place on ice until use. |
| Low TE Buffer | | | |
| Make DNB Buffer | Thaw reagents at room temperature for approximately 0.5 hours | centrifuge briefly | |
| Stop DNB Reaction Buffer | | | |

Tips

When you prepare DNBs for FCS PE300, use Make DNB rapid Enzyme Mix II instead of Make DNB Enzyme Mix.

2. Take out a 0.2 mL 8-strip tube or PCR tubes. Prepare Make DNB reaction mixture 1 according to the table below:

Tips

Add reagents in the order listed in the table below.

| Component | Making DNBs for FCS PE300 | | Making DNBs for others |
|---------------------|-----------------------------------|-----------------------------------|------------------------------------|
| | Volume of 90 μ L DNB reaction | Volume of 45 μ L DNB reaction | Volume of 100 μ L DNB reaction |
| Low TE Buffer | 20-V | 10-V | 20-V |
| Make DNB Buffer | 20 | 10 | 20 |
| ssDNA libraries | V | V | V |
| Total volume | 40 | 20 | 40 |

3. Mix the reagents by using a vortex mixer for 5 seconds. Centrifuge the mixture for 5 seconds by using a mini spinner, and place it on ice until use.
4. Place the mixture 1 into a thermal cycler and start the primer hybridization reaction. Thermal cycler settings are shown in the table below:



Tips

When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.

| Temperature | Heated lid (105 °C) | 95 °C | 65 °C | 40 °C | 4 °C |
|-------------|---------------------|-------|-------|-------|------|
| Time | On | 1 min | 1 min | 1 min | Hold |

- Remove Make DNB Enzyme Mix II (LC) from storage and place it on ice. Centrifuge briefly for 5 seconds and hold on ice.
- Take the PCR tube out of the thermal cycler when the temperature reaches 4 °C. Centrifuge briefly for 5 seconds, place the tube on ice.
- Prepare and add Make DNB reaction mixture 2 into the Make DNB reaction 1 of step 6 according to the table below.

| Component | Making DNBs for FCS PE300 | | Making DNBs for others |
|------------------------------|------------------------------|------------------------------|-------------------------------|
| | Volume of 90 µL DNB reaction | Volume of 45 µL DNB reaction | Volume of 100 µL DNB reaction |
| Make DNB rapid Enzyme Mix II | 40 µL | 20 µL | / |
| Make DNB Enzyme Mix II (LC) | 1.6 µL | 0.8 µL | 4 µL |
| Make DNB Enzyme Mix I | / | / | 40 µL |

- Mix the reaction mixture thoroughly by using a vortex mixer and centrifuge it for 5 seconds by using a mini spinner.
- Place the mixture 2 into a thermal cycler and start the Rolling Circle Amplification (RCA) reaction according to the following conditions.

Tips

If you use the same thermal cycler of step 4, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions. It is recommended to use another thermal cycler.

| RCA conditions of making DNBs for FCL PE300 | | | |
|---|--------------------|--------|------|
| Temperature | Heated lid (35 °C) | 30 °C | 4 °C |
| Time | On | 15 min | Hold |

| RCA conditions of making DNBs for others | | | |
|--|--------------------|--------|------|
| Temperature | Heated lid (35 °C) | 30 °C | 4 °C |
| Time | On | 25 min | Hold |

- Take the PCR tube out of the thermal cycler immediately when the temperature reaches 4 °C and place the tube on ice.
- According to different situations, add Stop DNB Reaction Buffer to the tube and pipette for 5 to 8 times to mix the reagents gently by using a wide-bore tip.

Tips

When mixing the reagents, slowly aspirate the DNBs and hover the tip above the liquid surface, then slowly dispense DNBs drop by drop to avoid bubbles.

| Component | Making DNBs for FCS PE300 | | Making DNBs for others |
|--------------------------|------------------------------|------------------------------|-------------------------------|
| | Volume of 90 µL DNB reaction | Volume of 45 µL DNB reaction | Volume of 100 µL DNB reaction |
| Stop DNB Reaction Buffer | 10 µL | 5 µL | 20 µL |

- Store DNBs for FCS PE300 at 2 °C to 8 °C and use them within 4 hours.
Store DNBs of other read length at 2 °C to 8 °C and use them within 48 hours.

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Store the DNBs at the temperature mentioned above. Before you use DNBs, pipette for 5 to 8 times to mix the DNBs gently by using a wide-bore tip.

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 seconds, 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 seconds, 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 seconds, and centrifuge briefly. Place them at room temperature away from direct sunlight for 2 minutes, 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 3 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.
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.

Tips

- If the amount of DNB is large, it is recommended to quantify in batches to avoid inaccurate quantification of DNB concentration due to fluorescence quenching.
- The qualified concentration of DNBs is 8 ng/μL. If the concentration is unqualified, the DNB needs to be re-prepared.

9. (Optional) If the concentration exceeds 40 ng/μL, the DNBs should be diluted to 20 ng/μL by using Low TE Buffer (for FCS PE300) or DNB Load Buffer I (for other models) according to the table below:

| Model | Component | Storage conditions | Storage time |
|-----------|-------------------|--------------------|--------------|
| FCS PE300 | Low TE Buffer | 4 °C | ≤4 h |
| Others | DNB Load Buffer I | 4 °C | ≤48 h |

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Select an appropriate system to load DNBs according to the table below:

| Loading system | Conditions | |
|----------------|---------------------------------|--|
| | Same DNBs loaded onto all lanes | Different DNBs loaded onto different lanes |
| DNBSEQ-G400RS | √ | × |
| MGIDL-200H | √ | √ |

Preparing the flow cell

Take the flow cell box out of storage (-25°C to -15 °C) and remove the flow cell plastic packaging from the box. Place the plastic packaging at room temperature for 1 hour to 24 hours. Unwrap the outer plastic packaging before use. Take the flow cell out from the inner packaging and ensure that the flow cell is intact.

Tips

- If the flow cell is not used within 24 hours after being placed at room temperature and the outer plastics packaging is intact, the flow cell can be placed back in -25 °C to -15 °C for storage. The number of freeze-thaw events must not exceed 3 times.
- 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 hours. If 24 hours is exceeded, it is not recommended to use the flow cell.

Loading DNBs by the sequencer

1. Prepare reagents according to the table below:

| Model | Component | Step 1) | Step 2) | Step 3) |
|-----------|--------------------|---|--|------------------------|
| FCS PE300 | DNB Load Buffer IV | Thaw the reagents on ice for approximately 30 min | Mix the reagents by using a vortex mixer for 5 seconds, centrifuge briefly | Place on ice until use |

| Model | Component | Step 1) | Step 2) | Step 3) |
|-----------------------------|--------------------|---|--|------------------------|
| Models except for FCS PE300 | DNB Load Buffer II | Thaw the reagents on ice for approximately 30 min | Mix the reagents by using a vortex mixer for 5 seconds, centrifuge briefly | Place on ice until use |

Tips

When loading DNBs by the sequencer for FCS PE300, ensure that the control software version is 1.5.0.1283 or above, and the BaseCall software version is 1.4.0.257 or above.

2. Take out a 0.5 mL Micro Tube from the sequencing kit and add the following reagents according to different sequencing read length.

Tips

The total volume in the table below is for one flow cell.

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| Component | DNB loading mixture (for FCS PE300) | DNB loading mixture (for other models) | |
|-----------------------------|-------------------------------------|--|-----------------|
| | FCS volume (μL) | FCL volume (μL) | FCS volume (μL) |
| DNB Load Buffer IV | 45 | / | / |
| DNB | 90 | 200 | 100 |
| DNB Load Buffer II | / | 64 | 32 |
| Make DNB Enzyme Mix II (LC) | / | 2 | 1 |
| Total volume | 135 | 266 | 133 |

3. Combine components and mix by gently pipetting 5 to 8 times by using a wide-bore, non-filtered pipette tip. Place the mixture at 2 °C to 8 °C until use.



Prepare a fresh DNB loading mixture immediately before use.

Loading DNBS by MGIDL-200H



For detailed operations, refer to *MGIDL-200H Portable DNB Loader Quick Start Guide*.

1. Prepare reagents according to the table below:

| Model | Component | Step 1) | Step 2) | Step 3) |
|-----------|--------------------|---|--|------------------------|
| FCS PE300 | DNB Load Buffer IV | Thaw the reagents on ice for approximately 30 min | Mix the reagents by using a vortex mixer for 5 seconds, centrifuge briefly | Place on ice until use |
| Others | DNB Load Buffer II | | | |

2. Take out a new 8-strip tube. Prepare DNB loading mixture according to the table below:



The total volume in the table below is for one flow cell.

| Component | DNB loading mixture (FCS PE300) | DNB loading mixture (Other models) | |
|-----------------------------|---------------------------------|------------------------------------|---------------------|
| | Volume for FCS PE300 (μL) | Volume for FCL (μL) | Volume for FCS (μL) |
| DNB Load Buffer IV | 22.5 | / | / |
| DNB | 45 | 100 | 50 |
| DNB Load Buffer II | / | 32 | 16 |
| Make DNB Enzyme Mix II (LC) | / | 1 | 0.5 |
| Total volume | 67.5 | 133 | 66.5 |

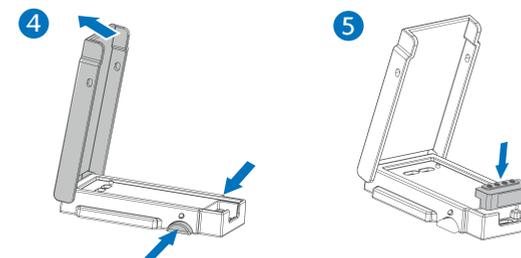
3. Mix gently by pipetting 5 to 8 times by using a wide-bore, non-filtered pipette tip.



Prepare a fresh DNB loading mixture immediately before use.

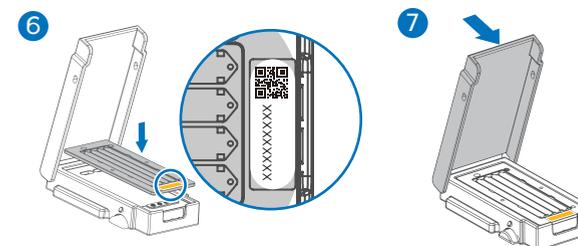
4. Squeeze the latches and open the cover.

5. Place a clean sealing gasket into the groove and ensure that the gasket surface is even.

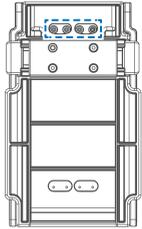


6. Align the holes of the flow cell with the alignment pins of the device and place the flow cell on it.

7. Close the cover and ensure that the cover is securely closed.

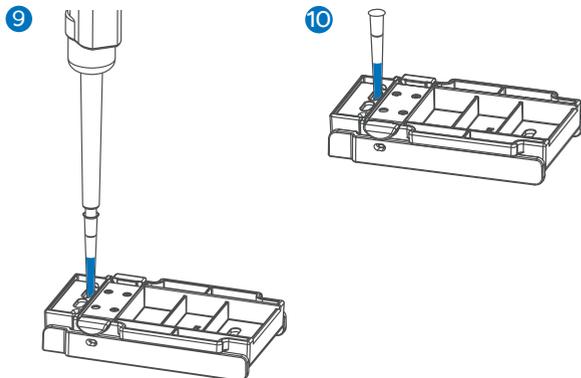


8. Place the device on the laboratory bench with the back facing up, and verify that the fluidics inlets align with the holes of the sealing gasket and ensure that the holes are clean.



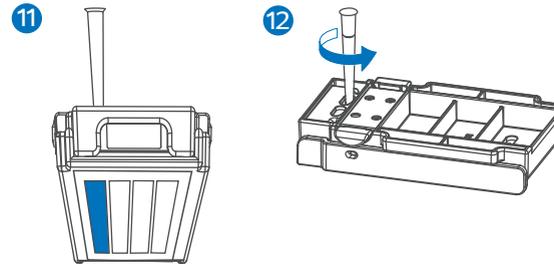
9. Aspirate 30 μ L of DNB loading mixture with a wide-bore, non-filtered pipette tip, and insert the tip into the fluidics inlet.

10. Eject the tip from the pipette. DNBs automatically flow into the flow cell.



11. Lift up the device, but do not tilt it (keep it parallel to the bench), and verify that the DNBs flow through the flow cell.

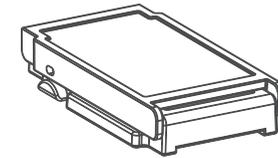
12. Ensure that all DNBs flow into the flow cell. Hold the device and rotate the tip counterclockwise to remove it.



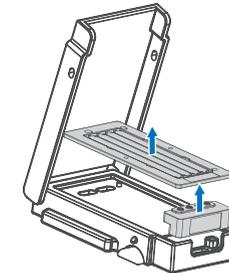
13. Repeat step 9 to 12 to load the DNBs to the rest of the lanes of the flow cell.

14. Place MGIDL-200H on the bench with the front facing up. According to the model, perform one of the following:

- For FCS PE300, it is recommended to keep the flow cell in MGIDL-200H and place at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 60 to 90 minutes for the DNB loading process,
- For other sequencing models, wait 30 minutes for the DNB loading process.



15. Open the cover, take out the flow cell, and transfer it to the sequencer for sequencing. Take out the sealing gasket for wash.



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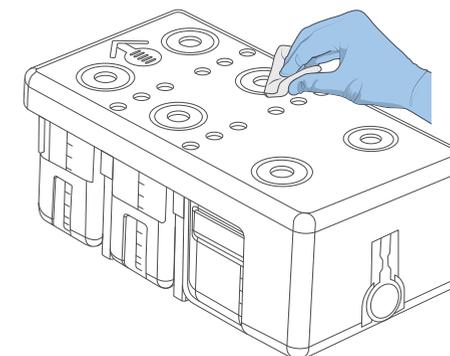
1. Remove the Sequencing Reagent Cartridge from storage.
2. Thaw the reagent. The approximate method and time to thaw is listed in the following table. Ensure that the reagents are thawed thoroughly.

| 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 |
| FCL SE100 | 2.0 | 0.5 | 24.0 |
| FCL SE400 | 8.0 | 3.0 | 48.0 |
| FCL PE100 | 3.0 | 1.5 | 36.0 |
| FCL PE150 | 5.0 | 2.0 | 48.0 |
| FCL PE200 | 6.0 | 3.5 | 48.0 |
| FCS SE100 | 1.0 | 0.5 | 24.0 |
| FCS PE100 | 2.0 | 0.5 | 36.0 |
| FCS PE150 | 3.0 | 1.5 | 36.0 |
| FCS PE300 | 6.0 | 3.5 | 48.0 |

3. 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 reagents are fully mixed, especially for the reagents in wells No. 9 and No. 10.

Tips

- Presence of dark green crystals in well No. 10 is normal due to crystallization of reagent materials in this well. When the cartridge is thawed, mix the reagents in the cartridge thoroughly and the crystals will dissolve. Sequencing quality will not be affected.
 - Since the reagent cartridge of PE300 is full, ensure that the reagent is mixed thoroughly before use.
4. Wipe any water condensation on the cartridge cover and well surround with a Kimwipes tissue.



5. Remove dNTPs Mix and dNTPs Mix II from storage 1 hour in advance and thaw at room temperature. Store at 2 °C to 8 °C or place on ice until use.
6. Remove the Sequencing Enzyme Mix from storage, store at 2 °C to 8 °C or place on ice until use.
7. Remove reagents from storage according to your model:
 - PE sequencing:
Remove MDA Reagent from storage, store at 2 °C to 8 °C or place on ice until use.
 - FCL SE50 (Small RNA) Sequencing or FCL SE400 sequencing:

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Remove Wash Buffer For Small RNA Sequencing from storage and thaw at room temperature. Store at 2 °C to 8 °C or place on ice until use.

■ Dual barcode SE sequencing:

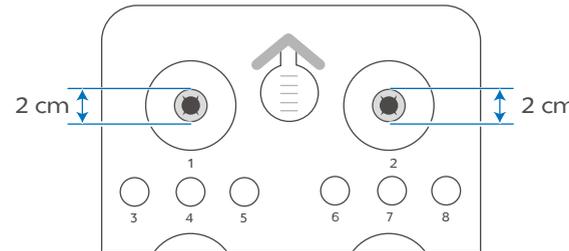
Take out the cPAS AD153 Barcode Primer 4 from the cPAS Barcode Primer 4 Reagent Kit and thaw at room temperature. Store at 2 °C to 8 °C or place on ice until use.

■ Dual barcode PE sequencing:

Take out the cPAS AD153 Barcode Primer 3 from the cPAS Barcode Primer 3 Reagent Kit and thaw at room temperature. Store at 2 °C to 8 °C or place on ice until use.

8. Prepare reagents for well No. 1:

- ① 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 new 1 mL sterile pipette tip.



- ② Ensure that dNTPs Mix is thawed thoroughly.
- ③ Mix dNTPs Mix for 5 seconds by using a vortex mixer and centrifuge briefly before use.
- ④ Add dNTPs Mix into a new 5 mL or 15 mL sterile tube according to the table below.

| Model | Well No. 1 | |
|----------------------|----------------|----------------------------|
| | dNTPs Mix (mL) | Sequencing Enzyme Mix (mL) |
| FCL SE50 | 0.700 | 0.700 |
| FCL SE50 (small RNA) | 0.700 | 0.700 |
| FCL SE100 | 1.100 | 1.100 |
| FCL SE400 | 4.000 | 4.000 |
| FCS SE100 | 0.800 | 0.800 |
| FCL PE100 | 1.800 | 1.800 |
| FCL PE150 | 2.400 | 2.400 |
| FCL PE200 | 3.800 | 3.800 |
| FCS PE100 | 1.400 | 1.400 |
| FCS PE150 | 1.900 | 1.900 |
| FCS PE300 | 3.800 | 3.800 |

- ⑤ Invert Sequencing Enzyme Mix 4 to 6 times before use.
- ⑥ Add Sequencing Enzyme Mix into the dNTPs Mix in the same tube according to the table above.
- ⑦ Invert the tube 4 to 6 times to mix the reagents before use.
- ⑧ Add dNTPs Mix and Sequencing Enzyme into well No. 1.

Tips

When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.

9. Prepare reagents for well No. 2:

- ① Ensure that dNTPs Mix II is thawed thoroughly.
- ② Mix dNTPs Mix II for 5 seconds by using a vortex mixer and centrifuge briefly before use.
- ③ Add dNTPs Mix II into a new 5 mL, 15 mL, or 25 mL sterile tube according to the table below.

| Model | Well No. 2 | |
|----------|-------------------|----------------------------|
| | dNTPs Mix II (mL) | Sequencing Enzyme Mix (mL) |
| FCL SE50 | 0.600 | 0.600 |

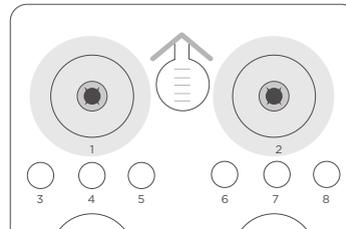
| Model | Well No. 2 | |
|----------------------|-------------------|----------------------------|
| | dNTPs Mix II (mL) | Sequencing Enzyme Mix (mL) |
| FCL SE50 (small RNA) | 0.600 | 0.600 |
| FCL SE100 | 0.900 | 0.900 |
| FCL SE400 | 12.000 | 4.000 |
| FCS SE100 | 1.600 | 0.800 |
| FCL PE100 | 1.500 | 1.500 |
| FCL PE150 | 2.100 | 2.100 |
| FCL PE200 | 5.700 | 3.800 |
| FCS PE100 | 2.800 | 1.400 |
| FCS PE150 | 3.800 | 1.900 |
| FCS PE300 | 5.700 | 3.800 |

- ④ Invert Sequencing Enzyme Mix 4 to 6 times before use.
- ⑤ Add Sequencing Enzyme Mix into the dNTPs Mix II in the same tube according to the table above.
- ⑥ Invert the tube 4 to 6 times to mix the reagents before use.
- ⑦ Add dNTPs Mix II and Sequencing Enzyme into well No. 2.

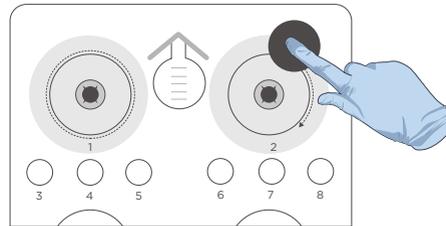
Tips

When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube.

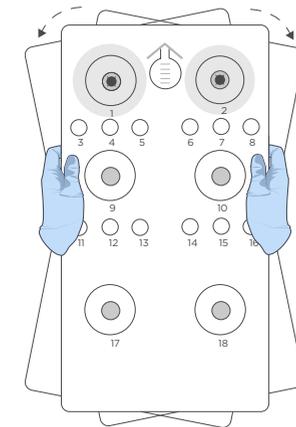
10. Seal loading well No. 1 and No. 2 with transparent sealing films.



11. Press the film with your finger around the well. Ensure that the well is tightly sealed and that no air bubbles exist between the film and cartridge surface, so that the reagents would not flow over the cartridge.



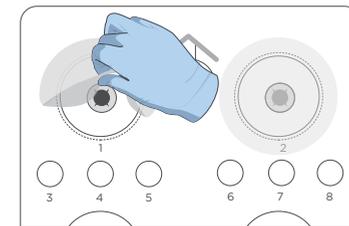
12. Lift the cartridge horizontally, hold both sides of the cartridge with both hands. Shake the cartridge 20 times in a clockwise and counterclockwise direction. Ensure that the reagents are fully mixed.



13. Carefully remove the seals from the loading wells after fully mixing.

Tips

- Do not reuse the used sealing film.
- Ensure that the surface around wells No. 1 and No. 2 is clean to avoid cross contamination.



14. It is recommended to handle reagent in well No.10 according to the following steps:

Making DNBS

Loading DNBS

Preparing reagent cartridge

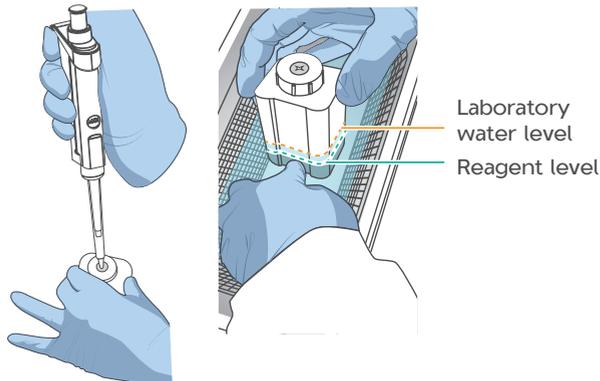
Preparing for sequencing run

Starting sequencing

Performing a post run wash

Checking sequencing data

- 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 to 5 minutes.

- 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.

15. Gently tap the cartridge on the bench to reduce air bubbles in the reagents.



Tips

The FCL SE50/FCL SE100/FCS SE100 sequencing reagent cartridge for single barcode is now ready for use.

17. Add reagents according to the model. When adding the reagent, ensure that no bubbles exist at the bottom of the tube.



WARNING

Wash Buffer For Small RNA Sequencing and Wash Buffer For Sequencing for SE400 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. Dispose of the waste according to local regulations.

- Small RNA sequencing
 - a. Remove Wash Buffer For Small RNA Sequencing from storage.
 - b. Mix Wash Buffer For Small RNA Sequencing by using a vortex mixer for 5 seconds.
 - c. Pierce the seal of well No. 7 by using a 1 mL sterile pipette tip.
 - d. Add 4.50 mL of Wash Buffer For Small RNA Sequencing into well No. 7.
- FCL SE400 sequencing
 - a. Remove Wash Buffer For Sequencing from storage.
 - b. Mix Wash Buffer For Sequencing by using a vortex mixer for 5 seconds.
 - c. Pierce the seal of well No. 7 by using a 1 mL sterile pipette tip.
 - d. Add 2.70 mL of Wash Buffer For Sequencing into well No. 7.

Making DNBS

Loading DNBS

Preparing reagent cartridge

Preparing for sequencing run

Starting sequencing

Performing a post run wash

Checking sequencing data

- PE sequencing



Tips

When using MDA Enzyme Mix or MDA Enzyme Mix II (For the name of the reagent, refer to the actual name on the label of the reagent tube), do not touch the wall of the tube. The heat from your hands may affect the enzyme activity.

- Pierce the seal of well No. 15 by using a 1 mL sterile pipette tip.
 - Add 500 μ L of MDA Enzyme Mix or MDA Enzyme Mix II to the MDA Reagent tube with a 1 mL pipette.
 - Invert the tube 4 to 6 times to mix the reagents.
 - Add the mixture to well No. 15. When adding the mixture, ensure that no bubbles appear at the bottom of the tube.
- Dual barcode SE sequencing
 - Take out the 1 μ M AD153 Barcode Primer 4.
 - Mix the 1 μ M cPAS AD153 Barcode Primer 4 using a vortex mixer for 5 seconds.
 - Pierce the seal of well No. 4 by using a sterile tip.

- Add 2.90 mL of the 1 μ M AD153 Barcode Primer 4 into well No. 4.

- Dual barcode PE sequencing
 - Take out the 1 μ M AD153 Barcode Primer 3.
 - Mix the 1 μ M cPAS AD153 Barcode Primer 3 using a vortex mixer for 5 seconds.
 - Pierce the seal of well No. 4 by using a sterile tip.
 - Add 2.90 mL of the 1 μ M AD153 Barcode Primer 3 into well No. 4.

Making DNBs

Loading DNBs

Preparing reagent cartridge

Preparing for sequencing run

Starting sequencing

Performing a post run wash

Checking sequencing data

Preparing for sequencing run

Logging in to the control software

1. Power the device on.
2. Log in to the computer with the default account. The password is "123".
3. Tap  in the main interface.
4. Log in to the control software with the username and password according to the table below:

| Version | Username | Password |
|-------------------|----------|-------------|
| ECR7.0 or earlier | user | 123 |
| ECR7.0 and later | user | Password123 |

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

Empty the waste container when:

- the waste level approaches two thirds of the maximum volume of the waste container
- the waste container icon turns to 

To maintain the waste container, perform the following steps:

1. Wear protective equipment.
2. Remove the lid without tubes from the waste container.
3. Pour the waste into an appropriate waste container, and dispose of the waste according to local regulations and safety standards of your laboratory.
4. Add sufficient laboratory-grade water into the waste container, attach the lid back onto the container if necessary, and gently swirl it until all inner walls are cleaned.
5. Pour the laboratory-grade water into an appropriate waste container.
If necessary, repeat step 4 to 5.
6. Clean the surface and opening of the waste container with a 75% ethanol wipe. Ensure that no waste remains in the container.
7. Attach the lid back onto the waste container.

Performing a pre-run wash

Before each sequencing run, select a wash type. For detailed wash type and operations, refer to "Performing a wash for sequencer" on page 21.

Cleaning flow cell stage

To clean the flow cell stage, you need the following materials: washing flow cell, low-lint cloth, 75% ethanol, and canned air duster.

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 carefully blow particulate matter and dust from the surface of the flow cell stage until cleaned.
If crystals appear on the surface of the stage, wipe it gently with a low-lint cloth moistened with 75% ethanol and let it air-dry.

Making DNBS

Loading DNBS

Preparing reagent cartridge

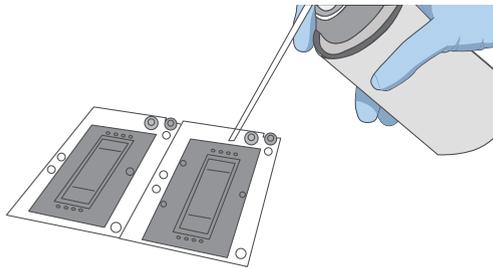
Preparing for sequencing run

Starting sequencing

Performing a post run wash

Checking sequencing data

Do not wipe the vacuum inlet and vacuum attachment slot to prevent 75% ethanol from entering the holes and damaging the device.



Making DNBs

Loading DNBs

Preparing reagent cartridge

Preparing for sequencing run

Starting sequencing

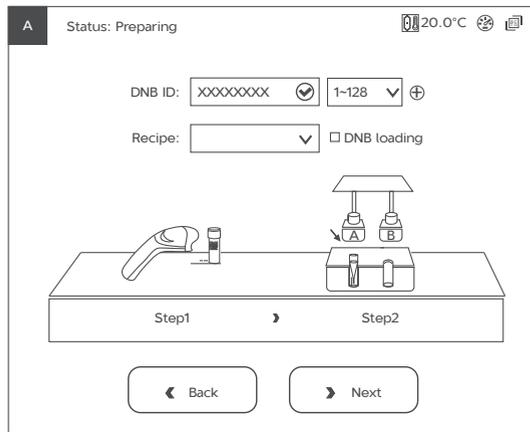
Performing a post run wash

Checking sequencing data

Starting sequencing

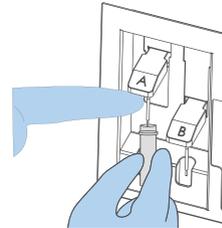
Selecting sequencing parameters

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



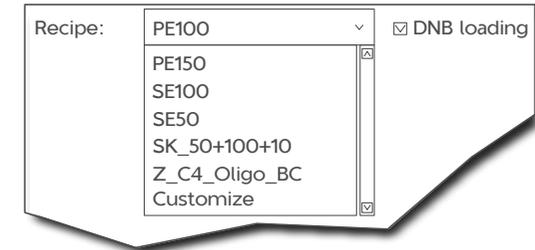
2. Tap the **DNB ID** box, enter the DNB ID manually by using the on-screen keyboard. Select a barcode range of different lanes from the list next to the DNB ID box.
Tap **+** to add a line of DNB ID if needed.
3. If you load DNBs by using the sequencer, perform the following steps:

- ① Open the reagent compartment door. Gently lift the DNB loading needle and remove the cap of a 0.5 mL Micro Tube that is used to load DNBs.
- ② Load the tube into the tube rack, slowly lower the DNB loading needle until the tip reaches the bottom of the tube.



- ③ Tap the **DNB loading** box in the DNB ID entry interface.
4. Select an appropriate recipe from the **Recipe** list.

If you want to customize the recipe, select **Customize** from the **Recipe** list, and configure settings in the Customize interface. For details, refer to *DNBSEQ-G400 & DNBSEQ-G400RS Genetic Sequencer Software Guide*.



5. Tap **Next**.

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.

Making DNBs

Loading DNBs

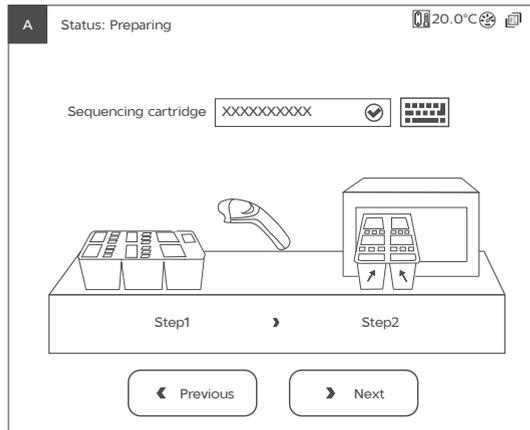
Preparing reagent cartridge

Preparing for sequencing run

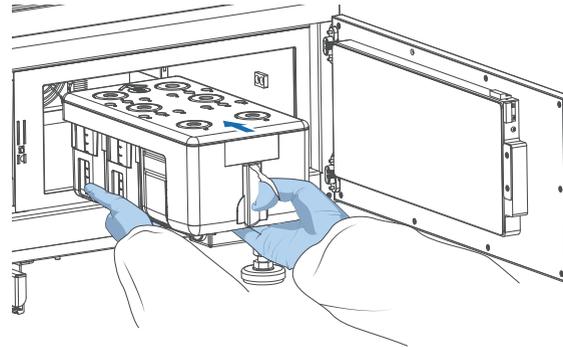
Starting sequencing

Performing a post run wash

Checking sequencing data



- 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.
- Slide the prepared sequencing reagent 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.

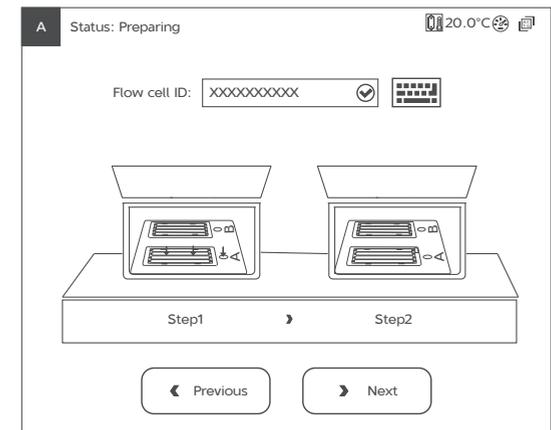
- Close the reagent compartment door and tap **Next**.

Loading the flow cell

- 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.

- Select the flow cell according to your DNB loading method:
 - If you load DNBs by the sequencer, take the new flow cell out from the inner packaging and inspect to ensure the flow cell is intact.
 - If you load DNBs by MGIDL-200H, take out the flow cell that is loaded with DNBs.

- The flow cell ID can be entered automatically by the internal barcode scanner. If automated entry does not work, move the cursor to the **Flow cell ID** box and enter the ID manually.



Making DNBs

Loading DNBs

Preparing reagent cartridge

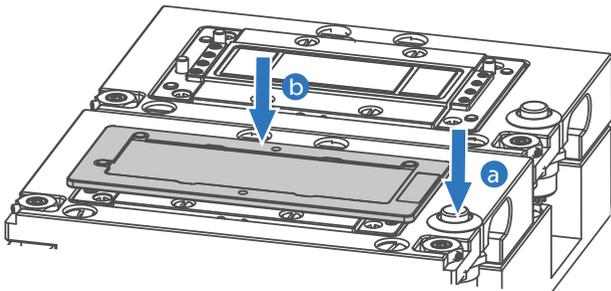
Preparing for sequencing run

Starting sequencing

Performing a post run wash

Checking sequencing data

- Ensure that two alignment holes are on the left side and one hole is on the right side. Ensure that the flow cell label is facing up and on the right. Hold the edge of the flow cell with both hands.
- Align the holes on the flow cell with the alignment 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 pins.
- 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.

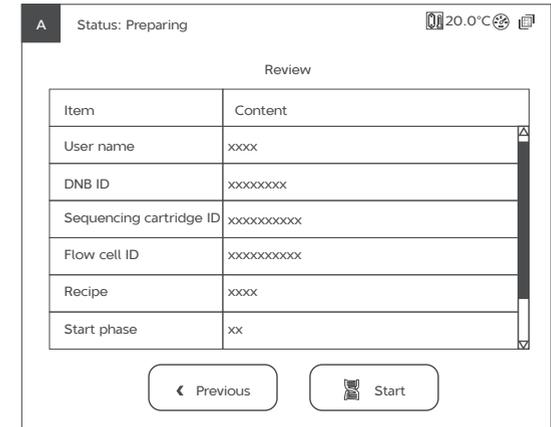


Tips

- The flow cell is fragile. Ensure that the flow cell touches the positioning post lightly. If there is excessive friction with the alignment pins when pressing the flow cell, the flow cell may be broken.
 - Do not touch the flow cell surface when loading the flow cell. If there are impurities on the flow cell surface, use a canned air duster to remove the dust on it.
- Ensure that the negative pressure is within the range of -80 kPa to -99 kPa before you start the sequencing. If negative pressure is abnormal, solve the problem by following instructions in *section 10.2 of DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set User Manual*.
 - Close the flow cell compartment door. Tap **Next**.

Starting the sequencing run

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



Tips

- To ensure sequencing quality, when Read1 and Read2 sequencing is complete, the sequencer will automatically perform one more cycle for correction. For example, for PE100 dual barcode sequencing, Read1 length is 100, Read2 length is 100, barcode read length is 10 and dual barcode read length is 10, plus 1 correction cycle for Read1 and 1 correction cycle for Read2 (barcode does not require correction). The total cycle number of this sequencing is 222.
- Tap **Start** and tap **Yes** when prompted to begin sequencing.



- 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, or place equipment that is subject to vibration around the device, as it may cause inaccurate sequencing results.

Making DNBs

Loading DNBs

Preparing reagent cartridge

Preparing for sequencing run

Starting sequencing

Performing a post run wash

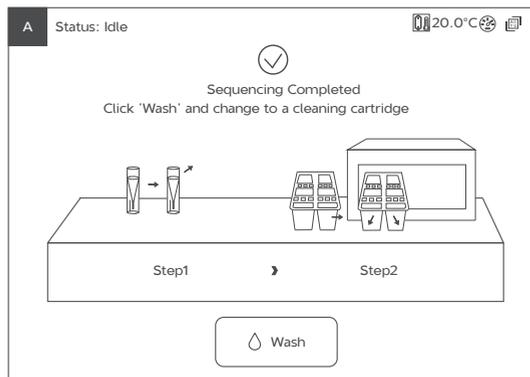
Checking sequencing data

Performing a post-run wash

Performing a wash for sequencer

Preparing for a wash

- When the sequencing is completed and the interface below appears, perform a wash. The device needs to be washed within 24 hours.



- Select the wash type according to specific situations.

| Wash type | Description | Time |
|-----------------------------|--|--|
| Full wash 1/ Full wash 2 | <ul style="list-style-type: none"> After the sequencer is used for a PE run or DNB loading. After pipelines, sample needles and other accessories exposed to reagents are replaced by technical support, perform a wash before use. | <ul style="list-style-type: none"> Full wash 1: about 76 min Full wash 2: about 42 min |
| | <ul style="list-style-type: none"> When the sequencer is not in use for more than 7 days, perform a wash before use. When the sequencer is to be powered off for more than 7 days, perform a wash before you power off and after you power on. Impurities are found on the flow cell. | |

| Wash type | Description | Time |
|--------------|--|--------------|
| Regular wash | <ul style="list-style-type: none"> After the sequencer is used for a SE run. The device is not in use for more than 24 hours but less than 7 days after a full wash, perform a wash again before use. After parts not exposed to reagents are replaced by technical support. Other situations except for full wash 1 or full wash 2. | About 48 min |

Tips

- The function of full wash 1 includes that of full wash 2.
 - Full wash 2 needs to be used with the script *StandardMPS_V1.6.1.04* and above. You can check the script version in the summary report.
- Prepare wash reagent and perform different washing steps according to specific wash types.

Making DNBs

Loading DNBs

Preparing reagent cartridge

Preparing for sequencing run

Starting sequencing

Performing a post run wash

Checking sequencing data

Tips

- The regular wash aims to remove residual reagents that could reduce the risk of cross-contamination, and to empty the fluidics line.
- The full wash 1 or 2 aims to remove residual reagents and proteins in the fluidics line that could reduce the risk of blockage.
- Once a week or each time before using the cleaning cartridge, empty the waste in the cartridge, and wash it with ultra-pure water for three times, and let it air-dry before refilling it with cleaning reagents.
- Replace the cleaning cartridge after 20 uses or every half year.
- Powder reagent can be used after being filtered by the 0.22 μm filtered film.
- Store washing reagents in squeeze bottles until use. These reagents are valid for up to 28 days if they are stored at 2 °C to 8 °C .

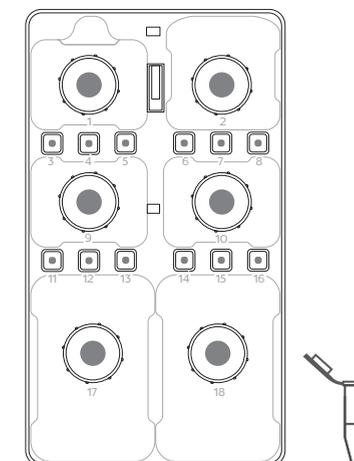
| Wash type | Description | Washing reagent |
|--------------|----------------------|--|
| Regular wash | Cleaning cartridge 1 | More than 95% volume of laboratory-grade water for all wells |

| Wash type | Description | Washing reagent |
|-------------|------------------------|--|
| Full wash 1 | ① Cleaning cartridge 3 | 1. Wash reagent 1 (0.05% Tween-20): 0.5 mL 100% Tween-20+999.5 mL laboratory-grade water |
| | ② Cleaning cartridge 2 | 2. Wash reagent 2 (1 M NaCl+0.05% Tween-20): 200 mL 5 M NaCl +0.5 mL 100% Tween-20+799.5 mL laboratory-grade water |
| | ③ Cleaning cartridge 1 | Wash reagent 3 (0.1 M NaOH): 50 mL 2 M NaOH +950 mL laboratory-grade water |
| Full wash 2 | ① Cleaning cartridge 4 | More than 95% volume of laboratory-grade water for all wells |
| | ② Cleaning cartridge 1 | Wash reagent 4 (0.05% Tween-20+0.03% ProClin300): 0.5 mL 100% Tween-20+0.3 mL 100% ProClin300+999.2 mL laboratory-grade water |

Cleaning cartridge 1

Prepare the washing reagents, cleaning cartridge 1, and DNB loading needle washing tubes according to the figure below:

Cleaning cartridge 1
(More than 95% laboratory-grade water for all wells)



Making DNBS

Loading DNBS

Preparing reagent cartridge

Preparing for sequencing run

Starting sequencing

Performing a post run wash

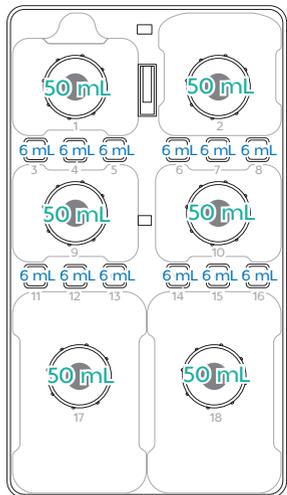
Checking sequencing data

■ Cleaning cartridge 2

Prepare the washing reagents, cleaning cartridge 2, and DNB loading needle washing tubes according to table and figure below:

| Washing reagent 3 (0.1 M NaOH) | |
|--------------------------------|-------------|
| Component | Volume (mL) |
| 2 M NaOH | 50 |
| Laboratory-grade water | 950 |

Cleaning cartridge 2
(0.1 M NaOH)



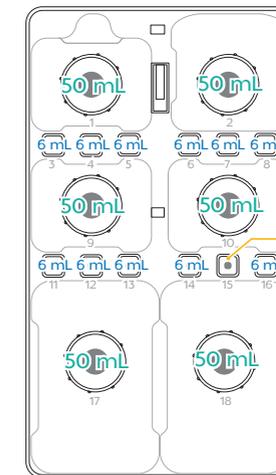
■ Cleaning cartridge 3

Prepare the washing reagents, cleaning cartridge 3, and DNB loading needle washing tubes according to table and figure below:

| Washing reagent 1 (0.05% Tween-20) | |
|------------------------------------|-------------|
| Component | Volume (mL) |
| 100% Tween-20 | 0.5 |
| Laboratory-grade water | 999.5 |

| Washing reagent 2 (1 M NaCl+0.05% Tween-20) | |
|---|-------------|
| Component | Volume (mL) |
| 5 M NaCl | 200 |
| 100% Tween-20 | 0.5 |
| Pure water | 799.5 |

Cleaning cartridge 3
(0.05% Tween-20)



6 mL
1 M NaCl+0.05% Tween-20

1.8 mL
1 M NaCl+0.05% Tween-20

■ Cleaning cartridge 4

Prepare the washing reagents, cleaning cartridge 4, and DNB loading needle washing tubes according to table and figure below:

| Washing reagent 4 (0.05% Tween-20+0.03% ProClin300) | |
|---|-------------|
| Component | Volume (mL) |
| 100 % Tween-20 | 0.5 |
| 100 % ProClin300 | 0.3 |
| Laboratory-grade water | 999.2 |

Making DNBs

Loading DNBs

Preparing reagent cartridge

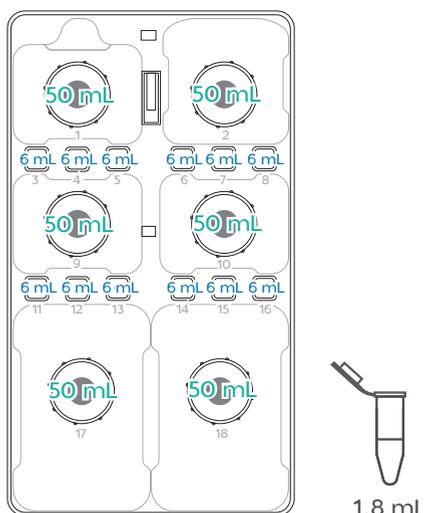
Preparing for sequencing run

Starting sequencing

Performing a post run wash

Checking sequencing data

Cleaning cartridge 4
(0.05% Tween-20+0.03% ProClin300)



1. Prepare the washing flow cell.



Tips
Used flow cells from previous runs can be used as washing flow cells. Each flow Cell can be used for up to 20 full washes.

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.

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 21.

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 (C₂H₄Cl₂), trichloroethylene (C₂HCl₃), chloroform (CHCl₃), and toluene (C₇H₈) to clean MGIDL-200H. Doing so may damage the device.
- It is recommended to replace MGIDL-200H with a new one after using for one year (MGI, Cat. No.: 900-000218-00).
- If you have questions about the compatibility of disinfectants, contact the technical support.

After each DNB loading, perform the following steps:

1. Wipe all sides of the device with a low-lint cloth moistened with 75% ethanol and a low-lint cloth moistened with ultra-pure 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 empty the beaker after wash. Repeat step 3-4 for 2 times.
5. Fill the ultrasonic cleaner tank with ultra-pure water, and wash the sealing gasket in the ultrasonic cleaner tank for about 15 minutes.
6. Repeat step 3-4, place the cleaned sealing gasket into a clean container and let it air-dry.
7. (Optional) Replace with a new sealing gasket (MGI, Cat. No.: 510-000718-00) if any of the following happens:
 - The sealing gasket has been cleaned for 20 times.

Making DNBs

Loading DNBs

Preparing reagent cartridge

Preparing for sequencing run

Starting sequencing

Performing a post run wash

Checking sequencing data

- The sealing gasket has been used for 3 months.
- The pipette tip loosens during loading DNBs.

Checking sequencing data



Tips

For details about the sequencing output data, refer to *DNBSEQ-G400 & DNBSEQ-G400RS Genetic Sequencer Software Guide*.

After sequencing starts, the sequencing results generated by the control software will appear in 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.

Troubleshooting

DNB concentration is low

When the DNB concentration is lower than 8 ng/ μ L, perform the following steps:

1. Check if the DNB preparation reagents have expired.
2. Check if the libraries meet the requirements.
3. Order a new library preparation kit (Cat. No. 1000016115) and make a new DNB preparation. If the DNB concentration still does not meet the requirements after a new sample preparation, contact the technical support.

Abnormal negative pressure appears

When the negative pressure is shown in red, the negative pressure is abnormal. Perform the following steps:

1. Moisten a Kimwipes tissue with 75% ethanol and use it to wipe the stage surface of flow cell stage and dust the stage with a canned air duster. Ensure that no dust is present on the flow cell stage.

2. Moisten a Kimwipes tissue with 75% ethanol and use it to wipe the back of the flow cell and dust it with a canned air duster. Ensure that no dust is present on the flow cell.
3. If the problem persists, contact the technical support.

Bubbles appear during washing

1. Check if the sealing gaskets are broken, or there are any impurities on it.
2. In wash or stand-by status, check the elasticity of the fluidics inlet and outlet blocks, ensure that they can move up and down smoothly.
3. Replace with a new washing flow cell and verify that the bubbles appear in the flow cell.
4. If the problem persists, contact the technical support.

Impurities appear in the original sequencing image

1. Check the quality of the original washing reagent.
2. Check the expiration date of the washing reagent.

3. Check the cleanliness and maintenance status of the cleaning cartridges.
4. Perform a full wash of the sequencer according to the relevant user manuals.
5. If the problem persists, contact the technical support.

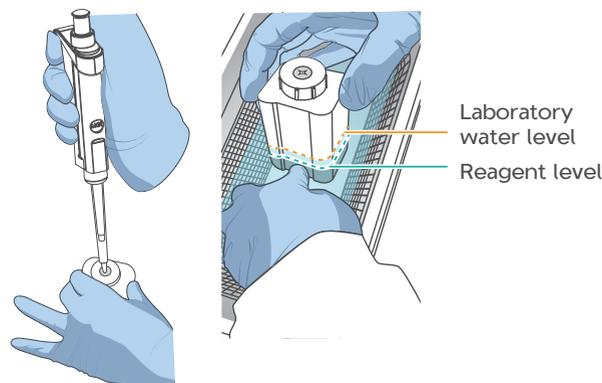
Many bubbles appear during sequencing

A large number of bubbles may be released from the IR reagent in well No. 10 during the liquid pumping process, and the degassing chamber cannot completely remove them. To solve the problem, try the following operations before sequencing:

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 to 5 minutes.
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.

Pumping fails

If liquids cannot be pumped into the flow cell, perform the following steps:

1. If wash process is normal, remove the flow cell from the sequencer and verify that any impurities appear near the sealing gaskets and flow cell inlet and outlet.
2. If any impurities appear, use a canned air duster to blow off the dust. Re-load the flow cell according to “Loading the flow cell” on page 18.
3. If the problem persists, contact the technical support.

If liquids cannot be pumped into the flow cell during sequencing, perform the following steps:

1. Tap , and verify that lint or crystals appear on the back of the flow cell and near the sealing gaskets.
2. Feedback the observed information to the technical support.

Storing a reagent kit temporarily

- If a kit has been thawed (including dNTPs), dNTPs and MDA Enzyme Mix or MDA Enzyme Mix II are not added to the kit, but cannot be used within 24 hours, it can be frozen and thawed at most one more time.
- If a kit has been thawed (including dNTPs) but cannot be used immediately, store it at 2 °C to 8 °C away from light. It is strongly recommended to use it within 24 hours. Mix the reagents in the cartridge by following instructions in “Loading the sequencing reagent cartridge” on page 17 before use.
- If dNTPs and Sequencing Enzyme Mix II have been added into the cartridge, i.e. the cartridge has been prepared but cannot be used immediately, store it at 2 °C to 8 °C and use it within 24 hours. Mix the reagents in the cartridge by following instructions in “Loading the sequencing reagent cartridge” on page 17 before use.
- If dNTPs and Sequencing Enzyme Mix II have been added into the cartridge, i.e.

the cartridge has been prepared and the needles have punctured the seal, but the cartridge cannot be used immediately, the cartridge must be sealed with foil or plastic wrap. Store the cartridge at 2 °C to 8 °C and use it within 24 hours. Gently mix the reagents in the cartridge before use. When mixing, be careful not to spill any reagent from the needle holes to avoid reagent contamination.

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