· Part No.: H-020-001026-00
· Version: 1.0
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Overview

This guide is used to provide guidance for the use of DNBSEQ-T7RS sequencing reagents and sequencer.

WARNING

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

Optional reagent set information is as follows:

Catalog number	Model	Name	Version
940-000270-00	FCL SE35	DNBSEQ-T7RS High-throughput Sequencing Set	V2.0
940-000271-00	FCL SE50	DNBSEQ-T7RS High-throughput Sequencing Set	V2.0
940-000272-00	FCL SE100	DNBSEQ-T7RS High-throughput Sequencing Set	V2.0
940-000269-00	FCL PE100	DNBSEQ-T7RS High-throughput Sequencing Set	V3.0
940-000268-00	FCL PE150	DNBSEQ-T7RS High-throughput Sequencing Set	V3.0
1000020834	/	CPAS Barcode Primer 3 Reagent Kit	V2.0
1000014048	/	CPAS Barcode Primer 4 Reagent Kit	V1.0

Getting started

Preparing the flow cell - Part 1

1. Take the flow cell box out of storage and remove the flow cell plastic package from the box.

Tips

Do not open the outer plastic package yet.

2. Place the flow cell at room temperature for 30 min to 24 h.

Preparing the Sequencing Reagent Cartridge -Part 1

1. Remove the Sequencing Reagent Cartridge from the DNBSEQ-T7RS High-throughput Sequencing Reagent Kit.
2. Thaw the Sequencing Reagent Cartridge. The approximate time to thaw is listed in the following table. Store in a 2 °C to 8 °C refrigerator until use. Choose the method that best suits your situation:

Sequencing read length	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)
SE35	1.0	0.5	8.0
SE50	1.0	0.5	10.0
SE100	2.0	0.5	12.0
PE100	2.5	1.0	14.0
PE150	3.0	1.0	18.0

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· Part No.: H-020-001026-00
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Preparing DNB Load Plate

1. Take DNB Load Plate out of the DNBSEQ-T7RS DNB Load Reagent Kit. Thaw DNB Load Plate at 2 °C to 8 °C (at least 12 h in advance) or in a water bath at room temperature (1.5 h) until it is completely thawed before use.
2. Once DNB Load Plate is thoroughly thawed, place it at 2 °C to 8 °C until use.

Preparing DNB Load Buffer

1. Take the DNB Load Buffer II or DNB Load Buffer IV out of the DNBSEQ-T7RS DNB Load Reagent Kit.
2. Thaw the reagent in a water bath at room temperature for approximately 30 min.
3. Mix the reagent by using a vortex mixer for 5 s. Centrifuge briefly and place on ice until use.

Preparing the 0.1 M NaOH reagent

Prepare 0.1 M NaOH according to the procedure described in *Wash preparations on Page 19*. Each DNB Load Plate requires at least 4 mL of 0.1 M NaOH.

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Preparing DNBs

WARNING

Do not use filtered pipette tips in the Make DNB process.

Recommended library insert size

Tips

- The insert size and required data output should be considered when selecting sequencing kits.
- Average data output will vary with different library type and applications.
- If there are any special requirements or specifications for the library preparation kit, then the requirements of the kit should be followed.

Recommended library insert size and applications				
Model	Recommended library insert distribution (bp)	Applications	Data output (M)	Theoretical Data output (Gb)
FCL SE35	50 to 230	NIPT	5800	203
FCL SE50	50 to 230	NIPT, PMSEQ	5800	290
FCL SE100	200 to 400	PMSEQ	5800	580
FCL PE100	200 to 400	WGS, WES, RNAseq, Single Cell	5800	1160
FCL PE150	300 to 500	WGS, WES, RNAseq	5800	1740

Input circular ssDNA library requirement

Circular ssDNA library concentration requirement		
Library type	Library concentration	Applications
General libraries	≥3 fmol/μL	WGS, WES, RNAseq, Single Cell
PCR free libraries	≥3.75 fmol/μL	WGS, WES, RNAseq

Tips

- If the library concentration is unknown, it is recommended to perform ssDNA library quantitation (ng/μL) using the Qubit ssDNA Assay Kit and the Qubit 4.0 Fluorometer. Use the following equation 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 (total library length including the adapters).

- If there are any special requirements or specifications for the library preparation kit, then the requirements of the kit should be followed.

Number of samples that can be pooled together

The sequencer can simultaneously perform sequencing of 4 flow cells. The number of samples that can be pooled together for each flow cell depends on the required data output, read length, and specific application.

As a guide, do not pool more samples with their total data output larger than 90% of the theoretical data output, due to variation in pooling and the fact that not all barcodes will generate the same amount of the data output from the same amount of DNB.

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· Version: 1.0
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If pooling variation is within±10%, use the following equation to calculate the maximum number of samples that can be pooled together:

$$\text{Maximum number of samples pooled} = \frac{\text{Total data output of one flow cell} \times 90\%}{\text{Required data per sample}}$$

Tips

For those with special sequencing depth requirements, the number of samples in the pooling can be appropriately increased or decreased.

Examples of various sample pooling				
No.	Read length	Minimum data for each sample (Gb)	Pooling sample number	Theoretical data output range for each sample (Gb)
1	PE100	50	20	52 to 63
2	PE100	100	10	104 to 127
3	PE150	50	23 RNAseq	51 to 62
4	PE150	100	4 WGS	102 to 122

Verifying the base balance for barcode

- A balanced base composition in each sequencing cycle is very important for high sequencing quality. It is strongly recommended that the minimum base composition of A, C, G, T for each position in the barcode is not lower than 12.5%. For a given pooling of samples, if the minimum base composition of A, C, G, T within the barcode is between 5% and 12.5%, the barcode split rate may be compromised. If the minimum base composition of A, C, G, T in any position of the barcode is less than 5%, it is strongly suggested to re-design the pooling strategy to have a more balanced base composition in the barcode.
- It is also important to note that two or more samples with an identical barcode should not be pooled together, otherwise, it is impossible to assign the read correctly.

Making DNBs

1. Calculating the required amount of ssDNA libraries according to the table below:

Library type	V for a 100 μL DNB reaction		V for a 50 μL DNB reaction		V for a 90 μL DNB reaction	
	Required library amount (fmol)	ssDNA library V (μL)	Required library amount (fmol)	ssDNA library V (μL)	Required library amount (fmol)	ssDNA library V (μL)
General libraries (WGS, WES, RNASeq...)	60	60/C	30	30/C	60	60/C
PCR free libraries	75	75/C	37.5	37.5/C	75	75/C

Tips

C is the library concentration in fmol/μL. The volume of the DNB making reaction system depends on the amount of data required for sequencing per sample and the types of DNA libraries.

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

Component	Cap color	Step 1)	Step 2)	Step 3)
Libraries	/	/	/	
Make DNB Enzyme Mix I (FCL SE35/SE50/SE100/PE100) Make DNB Rapid Enzyme Mix II (FCL PE150)		Thaw the reagent on ice for approximately 30 min on ice	Mix the reagents by using a vortex mixer for 5 s, centrifuge briefly	Place on ice until use.
Low TE Buffer		Thaw reagents at room temperature for approximately 30 min		
Make DNB Buffer				
Stop DNB Reaction Buffer				

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

Component	Cap color	FCL SE35/SE50/SE100/PE100		FCL PE150
		Volume for 100μL DNB reaction (μL)	Volume for 50μL DNB reaction (μL)	Volume for 90μL DNB reaction (μL)
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

4. Mix Make DNB reaction mixture 1 thoroughly by using a vortex mixer, centrifuge it for 5 s, and place it on ice until use.
5. Place the mixture into a thermal cycler and start 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 ℃)	95 ℃	65 ℃	40 ℃	4 ℃
Time	On	1 min	1 min	1 min	Hold

6. Remove Make DNB Enzyme Mix II (LC)  from storage and place on ice. Centrifuge briefly for 5 s and place on ice.
7. Remove the PCR tube from the thermal cycler when the temperature reaches 4 ℃. Centrifuge briefly for 5 s, place the tube on ice, and prepare the Make DNB reaction mixture 2 according to the table below:

Component	Cap color	FCL PE100		FCL PE150
		Volume for 100 μL DNB reaction (μL)	Volume for 50 μL DNB reaction (μL)	Volume for 90 μL DNB reaction (μL)
Make DNB Enzyme Mix I		40	20	/
Make DNB Rapid Enzyme Mix II		/	/	40
Make DNB Enzyme Mix II (LC)		4	2	1.6

8. Add the entire volume of the Make DNB reaction mixture 2 into the Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer and centrifuge it for 5 s.

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· Version: 1.0
· Release date: June 2024
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9. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

	Temperature	Heated lid (35 °C)	30 °C	4 °C
FCL SE35/SE50/ SE100/PE100	Time	On	25 min	Hold
FCL PE150	Time	On	10 min	Hold

10. Immediately add the Stop DNB Reaction Buffer when the temperature reaches 4 °C . Mix gently by pipetting 8 times by using a wide-bore, non-filtered pipette tip.

Component	Cap color	FCL PE100		FCL PE150
		Volume for 100 µL DNB reaction (µL)	Volume for 50 µL DNB reaction (µL)	Volume for 90 µL DNB reaction (µL)
Stop DNB Reaction Buffer		20	10	10
Final volume		104	52	91.6

11. Store DNBs at 4 °C and perform sequencing within 48 h.

    For FCL PE150: Immediately go to the next step.

Tips

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

Quantifying DNBs

Tips

- If the concentration is unqualified, please make a new DNB.
- If there are too many samples in a single test, it is recommended to quantify in batches to avoid inaccurate DNB quantification due to fluorescence quenching.
- If the concentration exceeds 40 ng/µL, the DNBs should be diluted 20 ng/µL before loading.

Model	Required DNB concentration	
	Regular libraries	Other libraries
FCL SE35/SE50/SE100/PE100	≥15 ng/µL	≥8 ng/µL
FCL PE150	≥8 ng/µL	≥5 ng/µL

DNB dilution				
Model	Dilution reagent	Cap color	DNB storage temperature	Maximum DNB storage time (h)
FCL SE35/SE50/ SE100/PE100	DNB Load Buffer I		4 °C	≤48
FCL PE150	Low TE Buffer		4 °C	≤8

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.

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· Version: 1.0
· Release date: June 2024
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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)

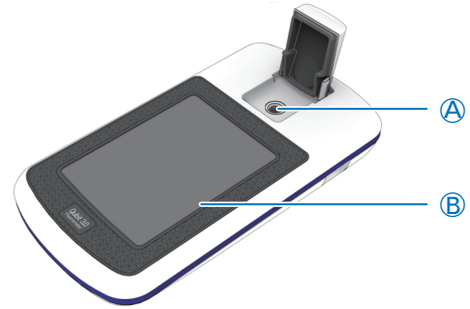
3. Mix the working solution thoroughly with a vortex mixer for 5 s, and centrifuge briefly. Add 190 μL of working solution into 2 standard test tubes, and add 198 μL of working solution into the DNB test tube.
4. Add 10 μL of Qubit ssDNA Standard #1 and Qubit ssDNA Standard #2 to 2 standard test tubes 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 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.

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Pooling DNBs

Tips

Use normal pipette tips to aspirate the required DNB volume of each library, and use wide -bore tips to mix.

1. Calculate the relative amount (V_n) for each sample.

$$V_n = \frac{\text{Data output required for the sample}}{\text{Concentration of DNBs for the sample}}$$

n refers to the sample ID.

2. Calculate the total relative amount (V_2) for all samples.

$$V_2 = V_A + V_B + \ldots + V_H$$

3. Calculate the DNB volume needed for each sample

$$V_{DNB}(\mu L) = \frac{300 \times V_n(\mu L)}{V_2(\mu L)}$$

4. Pool the DNBs of the libraries based on the calculation results.

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· Version: 1.0
· Release date: June 2024
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Loading DNBs

WARNING

Do not use filtered pipette tips in the DNB loading process.

Preparing DNB Load Plate (2)

1. Remove DNB Load Plate from storage.
2. Gently invert the DNB Load Plate 5 times to mix it, and then centrifuge it for 1 min before use.

Preparing DNB loading mixture

1. Take out Micro Tube 0.5 mL (Empty) from DNBSEQ-T7RS DNB Load Reagent Kit and add the following components in order:

No.	Component	Cap color	FCL SE35/SE50/SE100/ PE100	FCL PE150
			Volume (μL)	Volume (μL)
1	DNB*	/	270	300
2	DNB Load Buffer II		90	/
	DNB Load Buffer IV		/	150
3	Make DNB Enzyme Mix II (LC)		1	/

Tips

*DNB in the table refers to the pooled DNBs.

2. 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

- The DNB loading mixture must be prepared fresh on ice and used within 30 min.
- Do not centrifuge, vortex, or shake the tube.

Preparing the flow cell (2)

1. Unwrap the outer package before use. Remove the flow cell from the inner package and inspect it to ensure that no scratches exist on glass panel of the flow cell.

Tips

When using the canned air duster to remove the dust on the back of the flow cell, avoid blowing air into the inlet holes.

2. Clean the back of the flow cell by using a canned air duster.

Tips

- If the flow cell cannot be used within 24 hours after being placed at room temperate and the outer plastics package is intact, the flow cell can be placed back in 2℃ to 8 ℃ for storage. But the switch between room temperature and 2 ℃ to 8 ℃ must not exceed 3 times.
- If the outer plastic package 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 that you use the flow cell.

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· Part No.: H-020-001026-00
· Version: 1.0
· Release date: June 2024
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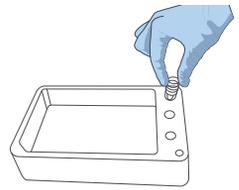
Performing DNB loading

1. Ensure that the compartment doors of MGIDL-T7RS are closed and start the device.
2. Enter the user name *user* and password *Password123* to open the main interface.
3. Select **A** or **B** to continue the operation. Select **Loading** to open the information input interface.
4. Open the loading compartment door.
5. Enter the DNB information into the **DNB ID** text box. Place the Micro Tube 0.5 mL containing DNB loading mixture into the DNB tube hole.



Tips

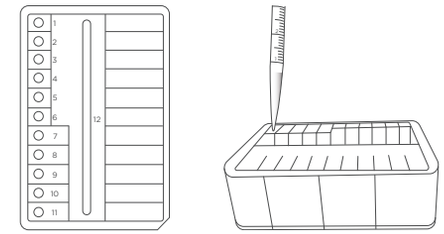
Use only numbers or letters or a combination of numbers and letters for DNB ID.

6. Align the DNB Load Plate to the RFID scanning area and the ID information will appear in the text box.

Tips

- If scanning fails, input the plate ID with the on-screen keyboard.
- Ensure that the ID format is correct when you input ID manually. Otherwise, you will be prompted that the ID is incorrect and the procedure cannot continue.
- The plate ID consists of the catalog number (REF on the label) and serial number (SN on the label). When inputting the ID manually, input the special characters in the catalog number as well.

7. Remove the seal of the DNB Load Plate and add 4 mL of 0.1 M NaOH into well No. 11.



8. Place the prepared DNB Load Plate on the plate tray of MGIDL-T7RS.

9. Enter the flow cell ID in the **Flow cell ID** text box through RFID or manually.
10. Load the flow cell.
    ① Ensure that all the four rubber sealing rings are on the four corners of the flow cell.

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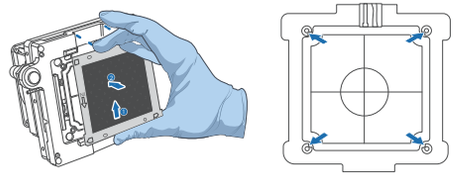
② Orient the flow cell upwards by holding the sides of the flow cell.

③ Align the locating bulge on the flow cell to the locating groove on the flow cell stage.



④ Gently press down the edges of the flow cell.

⑤ Press the flow cell attachment button on the flow cell stage to ensure that the flow cell is securely seated and held on the stage.

Tips

- Remove the dust on both sides of the flow cell with a canned air duster.
- Do not press or touch the glass cover of the flow cell to avoid leaving fingerprints or impurities on the glass surface, and possibly damaging the flow cell.
- Do not move the flow cell after installing the flow cell onto the stage, or it may cause the sealing gaskets to misalign with holes of the fluidics line.
- If flow cell attachment fails, gently wipe the back of the flow cell and flow cell stage with a clean low-lint cloth moistened with 75% ethanol. Dust the flow cell with a canned air duster.

11. Close the loading compartment door.
12. Select **Start** and select **Yes** when prompted to start loading. The process takes around 2 h.
13. When flow cell loading is completed, press the flow cell attachment button and remove the loaded flow cell from the stage. The flow cell is now ready for sequencing.

Tips

- If sequencing cannot be performed immediately, put the loaded flow cell in a clean zip bag and store it at 2 ℃ to 8 ℃ until use.
- The maximum storage time for loaded flow cell is 48 hours.

14. Perform an automatic post-wash:
    ① Install a washing flow cell onto the flow cell stage and press the flow cell attachment button. Close the flow cell compartment door. Select **Confirm**.
    ② Select **Post-wash** and select **Yes** when prompted to start MGIDL-T7RS wash, which will take approximately 20 min.
    ③ Remove the washing flow cell and store it at room temperature.
    ④ Empty any remaining washing solution in the DNB Load Plate into an appropriate waste container.
    ⑤ Dispose of the waste and DNB tube.
    ⑥ Clean the DNB Load Plate 5 times with laboratory-grade water and let it air dry. The cleaned plate can be used as washing plate. Store the plate at room temperature.

    Replace the washing plate every month or after it has been used 10 times.

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· Part No.: H-020-001026-00
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Preparing cartridges and pure water container

Preparing the Sequencing Reagent Cartridge - Part 2

1. Remove the Sequencing Reagent Cartridge from storage.
2. Invert the cartridge 3 times to mix before use. Shake the cartridge 20 times clockwise and counterclockwise respectively. Ensure that all reagents are fully mixed. Wipe any water condensation from the cartridge cover and well surround with a KimWipes tissue.

Tips

It is a normal phenomenon that dark green crystal appears in well No.1, which is crystallization of raw materials of the reagent in this well. When the cartridge is thawed, mix the reagents in the cartridge well and the crystals will dissolve. Sequencing quality will not be affected.

3. Prepare well No. 9 and well No. 10:

① Prepare reagents according to the table below:

Component	Cap color	Operation
dNTPs Mix IV		Remove the reagents from storage 1 hour in advance and thaw them at room temperature. Invert the reagents 6 times. Gently tap the tube on the bench to bring the liquid to the bottom. Place them on ice until use.
dNTPs Mix V		
dNTPs Mix II		
Sequencing Enzyme Mix	/	Invert the reagent 6 times and place it on ice until use

② Take the reagent out of from the kit according to the sequencing type. After thawing at room temperature, mix the reagent thoroughly by using a vortex mixer for 5 s. Centrifuge it briefly and place it on ice until use.

Sequencing type	Kit name	Reagent
Dual barcode SE sequencing	CPAS Barcode Primer 4 Reagent Kit.	1 μM AD153 Barcode Primer 4
Dual barcode PE sequencing	CPAS Barcode Primer 3 Reagent Kit	1 μM AD153 Barcode Primer 3

③ Open the kit cover and wipe any water condensation with lint-free paper. Spray 75% ethanol on the surface of the cartridge seal and clean the seal with a KimWipes tissue. Pierce the seal in the center of well No.9 and No.10 to make a hole around 2 cm in diameter by using a 1 mL sterile tip.

④ Add reagents according to the following table and seal the wells with transparent sealing film.

Model	Well No. 9			Well No. 10	
	dNTPs Mix IV (mL)	dNTPs Mix V (mL)	Sequencing Enzyme Mix (mL)	dNTPs Mix II (mL)	Sequencing Enzyme Mix (mL)
FCL SE35	1.7	/	1.7	4.5	1.5
FCL SE50	2.0	/	2.0	5.4	1.8
FCL SE100	3.0	/	3.0	8.1	2.7
FCL PE100	/	2.76	2.76	8.28	2.76
FCL PE100		3.74	3.74	11.22	3.74

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· Part No.: H-020-001026-00
· Version: 1.0
· Release date: June 2024
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⑤ Press the sealing film with your fingers around the well. Ensure that the well is tightly sealed and that no air bubbles exist between the film and cartridge surface, and that the reagents will not flow over the cartridge.

⑥ Lift the cartridge horizontally, hold both sides of the cartridge with both hands. Shake the cartridge 20 times clockwise and counterclockwise. Ensure that the reagents are fully mixed.

⑦ Carefully remove the seals from the loading wells after fully mixing.

Tips

- Avoid reusing the used sealing film.
- Avoid cross-contamination of the reagents in well No. 9 and No. 10.

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

Tips

The cartridge for FCL SE35/SE50/SE100 is ready for use.

5. Prepare other reagents according to the table below. Ensure that no bubbles exist at the bottom of the wells. Close the sequencing cartridge cover.

Tips

When using the MDA Enzyme Mix, do not touch the wall of the tube. The heat from your hands may affect enzyme activity.

Sequencing type	Operation
Dual barcode SE sequencing	1. Pierce the seal of well No.3 by using a 1 mL sterile tip. 2. Add 3.5 mL of 1 μM AD153 Barcode Primer 4 into well No.3 with a 1 mL pipette.
Single barcode PE sequencing	1. Pierce the seal of well No.8 by using a 1 mL sterile tip. 2. Add 600 μL of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette. 3. Invert the tube 4 to 6 times to mix the reagents. Add the mixture to well No.8.
Dual barcode PE sequencing	On the basis of single barcode PE sequencing operation, the following operations are required: 1. Pierce the seal of well No.3 by using a 1 mL sterile tip. 2. Add 3.5 mL of 1 μM AD153 Barcode Primer 3 into well No.3 with a 1 mL pipette.

Preparing the Washing Cartridge

1. Shake the cartridge 10 times clockwise and counterclockwise respectively. Ensure that the reagents are fully mixed.
2. Spray 75% ethanol on the surface of the cartridge seal and clean the seal with lint-free paper. Pierce either side of well No.2 by using a 1 mL sterile tip.
3. Add 45 mL of 0.1 M NaOH into well No. 2 by using an electronic pipette.

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· Version: 1.0
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Filling the pure water container

Tips

- Check to make sure the volume level of the water in the pure water container is sufficient. If the volume is insufficient, sequencing will fail. Replenish the pure water before starting the run. Make sure the air vent opening is unobstructed.
- The pure water will be used in sequencing so it must be kept clean. Change the pure water in the pure water container on a weekly basis.
- Before refilling the pure water container, empty the container and spray 75% ethanol on the inner surface of the container lid and the surface of the pure water tube. Wipe and clean the surfaces with new KimWipes tissues. Rinse the container with fresh pure water 3 times by using 2 L of pure water each time.
- After refilling the pure water container, Insert the pure water tube into the pure water container through the aligned holes until the tube reaches the bottom of the container.
- Refer to the relevant user manual for the preparation of the water container.

Pure water consumption (L)				
Model	1 flow cell	2 flow cells	3 flow cells	4 flow cells
FCL SE35	1.0	2.0	3.0	4.0
FCL SE50	1.0	2.0	3.0	4.0
FCL SE100	1.5	3.0	4.5	6.0
FCL PE100	3.0	6.0	9.0	12.0
FCL PE150	4.5	9.0	13.5	18.0

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Performing a sequencing run

1. Load the cartridges, including the Sequencing Reagent Cartridge and the Washing Cartridge.
   ① Open the reagent compartment door and clean the inner walls with a microfiber clean wiper or lint-free paper moistened with laboratory-grade water. Keep the compartment clean and dry.

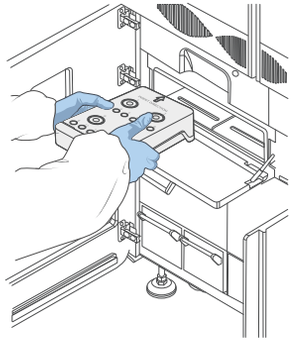
   Tips

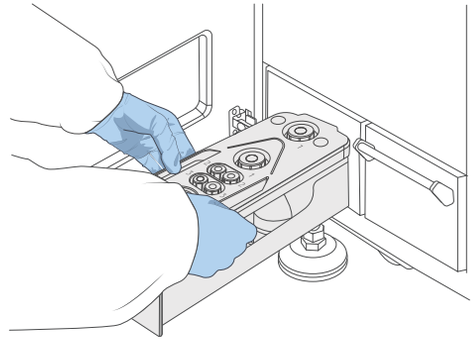
   Be cautious of sharp objects, such as the sampling needles, inside the reagent compartment when cleaning.

   ② Place the sequencing cartridge into the sequencing cartridge compartment and place the washing cartridge into the washing cartridge compartment.

   Tips

   When perform sequencing for only one flow cell, ensure that the cartridges and flow cell are installed on the same side.





2. Select , enter the user name *user* and password *Password123*, and select **Log in** to open the main interface.
3. Load the flow cell:
   ① Select A /B/C/D respectively according to sequencing demand. Select **Sequence** and select **New run**.
   ② Clean the loaded flow cell with a canned air duster to ensure that there is no visible dust on the surface and back of the flow cell. Put the flow cell on the flow cell drive and touch the flow cell drive control button to load the flow cell into the device.
4. Set sequencing parameters:
   ① Align the Sequencing Reagent Cartridge, Washing Cartridge, and flow cell respectively to the RFID scanning area, the ID information will automatically display in the corresponding text box.

   Tips

   If scanning fails, input the cartridge ID with the on-screen keyboard.

   ② Select the first ▼ next to Recipe. Select an appropriate sequencing recipe from the list.

   Tips

   If a customized recipe is required, select **Customize a recipe** from the **Recipe** list

   ③ Select the second ▼ next to **Recipe** and select the corresponding barcode sequence.
   ④ Select the **Split barcode** check box.

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⑤ Open the **Advanced settings** interface to indicate whether primers are custom and whether an auto wash is to be performed.

5. Select **Next**.
6. Review parameters. Ensure that all information is correct. Select **Start** and select **Yes** when prompted to start sequencing.

   During sequencing, you can select to view the sequencing information or change **Auto wash** settings.

Tips

- Do not bump or move the device during sequencing as it may cause inaccurate results.
- 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 DualBarcode sequencing, Read1 length is 100, Read2 length is 100, Barcode read length is 10 and DualBarcode 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 the sequencing is 222.

Automatic post-wash

Auto wash is enabled by default. The system automatically performs a post-wash after each sequencing run.

After the wash is complete, perform the following steps:

1. Select **Finish** to return to the main interface.
2. Open the flow cell retrieval compartment and remove the flow cells.

Tips

- Used sequencing flow cells can be used as washing flow cells.
- Store the flow cells at room temperature.
- Replace the washing flow cell every month or after it has been used 10 times.

3. Open the reagent compartment door and remove the cartridges.
4. Empty the remaining solution in the cartridges into an appropriate waste container.
5. Use pure water to wash the cartridges 3 times and let them airdry.

Tips

- Used sequencing cartridges can be used as washing cartridges.
- Store the washing cartridge at room temperature.
- Replace the washing cartridge every month or after it has been used 10 times.

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· Version: 1.0
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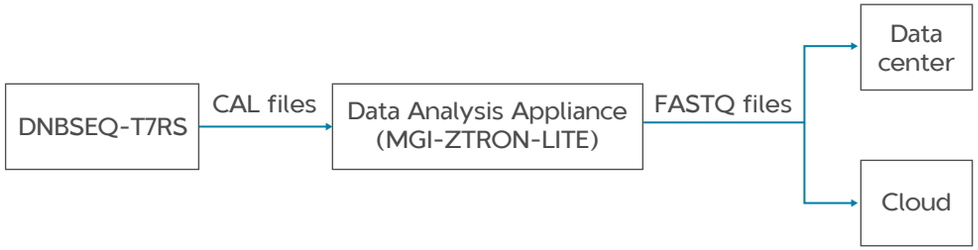
Processing data



If MGI-ZTRON-LITE server is deployed and connected to the sequencer, ZLIMS will monitor the status of the sequencer.

After a sequencing run is complete. the sequencing data will be uploaded to the MGI-ZTRON-LITE server automatically, and ZLIMS can automatically trigger bioinformatics analysis.

For the operation of MGI-ZTRON-LITE, refer to the relevant user manual.

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Wash

Tips

- Automatic wash and manual wash need to be performed on each flow cell stage independently.
- Washing cartridges and washing flow cells are delivered with the device for wash procedures.
- Used sequencing flow cells can be used as washing flow cells.
- Used sequencing cartridges can be used as washing cartridges.
- Used DNB Load Plates can be used as washing plates.
- Before refilled with fresh washing reagents, used washing plates must be cleaned 3 to 5 times with laboratory-grade water.
- Store the washing flow cell, washing cartridge, and washing plate at room temperature. Replace them every month or after they have been used 10 times.

Wash preparations

1. Determine the wash type.

Wash type	Cartridge type	Process time (min)	Description
MGIDL-T7RS Automatic wash	DNB Load Plate	15	When the loading is complete, replace the flow cell with a used flow cell and select **Wash**. The loader will automatically perform the wash without the need to change the DNB Load Plate.
MGIDL-T7RS Manual wash	Washing plate	20	Perform a wash manually under the following conditions: • The device is used for the first time. • The device has not been used for 7 days or longer. • Impurities are found in the device or flow cell. • Tubing, sampling needles or other accessories exposed to the reagents were replaced.
DNBSEQ-T7RS Automatic wash	Sequencing Reagent Cartridge and Washing Cartridge	40	Select **Yes** for **Auto wash**, the system will automatically perform a wash after each sequencing run.
DNBSEQ-T7RS Manual wash	Sequencer Cleaning Cartridge and Cleaning Cartridge	40	Perform a wash manually under the following conditions: • The device is used for the first time. • The device has not been used for 7 days or longer. • Impurities are found in the device or flow cell. • Tubing, sampling needles or other accessories exposed to the reagents were replaced.

2. Prepare washing reagents.
   - Prepare Wash Reagent I (1 M NaCl+0.05% Tween-20):

Reagent	Volume (mL)
5 M NaCl solution	200
100% Tween-20	0.5
Laboratory-grade water	799.5

   - Prepare Wash Reagent II (0.1 M NaOH):

Reagent	Volume (mL)
2 M NaOH solution	50
Laboratory-grade water	950

3. Prepare washing consumables according to the wash type.
   - Prepare the MGIDL-T7RS washing plate

     Take a clean and empty washing plate, add the following reagents:

Well No.	Washing reagent	Volume (mL)
11	0.1 M NaOH	4
10	1 M NaCl+0.05% Tween-20	4
9	Ultrapure water	4

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12	Ultrapure water	20

- Prepare DNBSEQ-T7RS washing cartridges:

Cartridge type	Well No.	Washing reagent	Volume (mL)
Sequencer Cleaning Cartridge	All	NA	NA
Cleaning Cartridge	2	0.1 M NaOH	45
	3	1 M NaCl+0.05% Tween-20	45

- Prepare the washing flow cell:

  Flow cells from previous runs can be used as washing flow cells. Replace the washing flow cell every month or after it has been used 10 times.

Wash procedures

Automatic wash and manual wash need to be performed on each flow cell stage independently.

Performing a manual wash on the loader (about 20 min)

1. Start the loader, enter the password, and then select **Log in** to open the main interface.
2. Select the flow cell stage that needs to be washed. Open the loading compartment door.
3. Place the prepared washing plate into the flow cell stage that needs to be washed. Close the compartment door.
4. Press the flow cell attachment button and wait until the negative pressure is released. Remove the flow cell from the stage.

Tips

Skip this step if no flow cell is on the stage.

5. Place the washing flow cell on the flow cell stage. Press the flow cell attachment button and gently press down on the flow cell to ensure that the flow cell is securely attached to the stage.
6. Return to the main interface. Select **Start** > **Yes** to begin the wash, which takes approximately 20 min.
7. When the wash is complete, take out all the consumables by following the on-screen instructions.
8. Select **Back** to return to the main interface.

Performing a manual wash on the sequencer (about 40 min)

You can perform a wash to remove the remaining reagents from the fluidics lines and flow cell stages to avoid cross-contamination.

When **Auto wash** is enabled, the system automatically performs a wash after each sequencing run. If **Auto wash** is set to **No**, or if the device has not been used for seven days or longer, perform a wash manually.

Perform the following steps:

1. Ensure that the pure water container is filled with at least 4.5 L of laboratory-grade water before performing the wash.
2. Start the sequencer. Enter the user name and password, select **Log in** to open the main interface.
3. Select **Wash**. Touch the flow cell drive control button to install a washing flow cell. Touch the flow cell drive control button again to load the washing flow cell into the device.
4. Place the prepared Sequencer Cleaning Cartridge into the sequencing cartridge compartment on the flow cell stage that needs to be washed. Close the sequencing cartridge compartment door.
5. Place the prepared Cleaning Cartridge into the washing cartridge compartment on the flow cell stage that needs to be washed. Then close the washing cartridge compartment and reagent compartment doors.
6. Select **Start** and select **Yes** when prompted to begin the manual wash, which takes approximately 40 min.
7. When the wash is complete, select **Finish** to return to the main interface.
8. Remove the washing flow cell, Sequencer Cleaning Cartridge, and Cleaning Cartridge.

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· Version: 1.0
· Release date: June 2024
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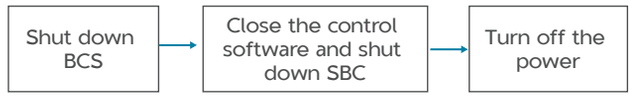
Powering DNBSEQ-T7RS on and off

Powering DNBSEQ-T7RS on

1. Wait for at least 5 min after the previous shutdown, turn the power switch to the **ON** position to power the device on. The BCS is started automatically.
2. Log in to the SBC, the control software is started automatically.

Powering DNBSEQ-T7RS off (FPGA configuration)

- Method 1 (Recommended):



① Return to the desktop of the computer (hereinafter referred to as SBC). For details, contact the technical support.

② Shut down the Basecall server (hereinafter referred to as BCS).

a. Connect the BCS through remote connection to access the BCS desktop.

b. Open the BCS Task Manager, select the Services tab, and stop the Litecall and BIS services. Close Task Manager.

c. Select > > in the task bar of the BCS.

③ Close the remote disconnection window and return to the SBC desktop.

④ Close the control software and SBC:

a. Recover the display of the control software in the task bar.

b. Select >**Shut down** > **Shut down**.

⑤ Wait for 3 min, switch the power switch on the side panel to the **OFF** position to disconnect it from the main power supply.

- Method 2:

① Return to the SBC desktop. For details, contact the technical support.

② Close the control software: Open the SBC Task Manager, select the Services tab, and stop the *ISW.ZebraT7Seq.Service* service. Close Task Manager.

③ Shut down the BCS. For details, refer to step 2 of method 1.

④ Close the remote disconnection window and return to the SBC desktop.

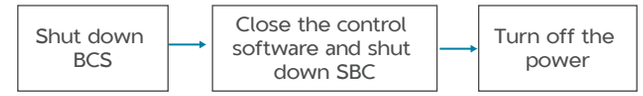
⑤ Select either of the following methods to shut down the SBC:

- Select > > .
- Press the power button in the keyboard tray area.

⑥ Wait for 3 min, turn the power switch on the side panel to the **OFF** position to disconnect it from the main power supply.

Powering DNBSEQ-T7RS off (GPU configuration)

- Method 1 (Recommended):



① Return to the desktop of the SBC. For details, contact the technical support.

② Shut down the BCS:

a. Double-click to open the VNC Viewer.

b. Double-click to open zebracall in the VNC Viewer.

c. Click in the upper right corner to open the shutdown window.

d. Click **Power off**.

③ Close the VNC Viewer and return to the SBC desktop.

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④ Close the control software and SBC:

a. Recover the display of the control software in the task bar.

b. Select >**Shut down** > **Shut down**.

⑤ Wait for 3 min, switch the power switch on the side panel to the **OFF** position to disconnect it from the main power supply.

- Method 2:

① Return to the SBC desktop. For details, contact the technical support.

② Close the control software: Open the SBC Task Manager, select the Services tab, and shutdown the *ISW.ZebraT7Seq.Service*. Close Task Manager.

③ Shut down the BCS. For details, refer to step 2 of method 1.

④ Close the VNC Viewer and return to the SBC desktop.

⑤ Select either of the following methods to shut down the SBC:

- Select > > .
- Press the power button in the keyboard tray area.

⑥ Wait for 3 min, turn the power switch on the side panel to the **OFF** position to disconnect it from the main power supply.

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· Version: 1.0
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FAQs

Sequencer FAQs

Q: What should I do if the device does not power on after turning the power switch to the ON position?

Powering issues arise when the main power supply is in an abnormal condition, not connected to the main power supply/UPS, or if the UPS has run out of power.

Perform the following steps:

1. Check whether the main power supply and UPS is normal.
2. Ensure that the device is connected to the main power supply or UPS.

Q: What should I do if error messages appear when the control software is running?

Errors messages may appear when parameters are not set properly or if an error occurs in software-hardware communication.

Perform the following steps:

1. Perform a self-test in the maintenance interface. Check the record of the hardware that fails the self-test.
2. Check error messages in the log, and troubleshoot the problem according to on-screen instructions.
3. Restart the device.

Q: What should I do if abnormal negative pressure appears during flow cell attachment?

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

1. Dust the stage with a canned air duster. Ensure that no dust is present on the flow cell stage.
2. Dust the back of the flow cell with a canned air duster to ensure no dust is present.

⚠ WARNING

When using the canned air duster to remove the dust on the back of the flow cell, avoid blowing air into the inlet holes.

3. If the problem persists, contact the technical support.

Q: What should I do if temperature error messages and warnings related to the sequencing cartridge compartment appear in the sequencing interface?

If the sequencer has been turned off after a long period of time, the sequencing cartridge compartment will be at room temperature. The sensor may detect that the sequencing cartridge compartment is exceeding the preset temperature. Issues may also occur when there is an error with the temperature control board.

Perform the following steps:

1. Let the sequencer run and let the sequencing cartridge compartment cool. The error message should disappear when the sequencing cartridge compartment is at operating temperature.
2. Restart the sequencer.

Q: What should I do if temperature error messages and warnings related to the LT (Laser Temperature) board appear in the sequencing interface?

Error messages may appear when the temperature of the LT board exceeds the preset limits and/or if there is an error with the temperature sensor error. It is recommended to record the warnings and the related logs of the sequencing run and contact the technical support.

Q: What should I do if the waste level sensor alarm is activated?

The waste level sensor alarm may activate if the waste level exceeds the preset limit, the level sensor is not installed properly, and/or the level sensor is damaged. It is recommended to record the warning and the related logs of the sequencing run and contact the technical support.

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DNB loader FAQs

Q: What should I do if a message, indicating that the compartment door is opened, is displayed in the interface?

This message is displayed when the compartment door is open. The resolve this issue, ensure that the compartment door is closed.

Q: What should I do if abnormal negative pressure appears during flow cell attachment?

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

1. Gently wipe the stage surface with a damp KimWipes tissue and dust the stage with a canned air duster. Ensure that no dust is present on the flow cell stage.
2. Dust the back of the flow cell with a canned air duster to ensure no dust is present.

⚠ WARNING

When using the canned air duster to remove the dust on the back of the flow cell, avoid blowing air into the inlet holes.

3. If the problem persists, contact the technical support.

Q: What should I do if the sampling needle bumps into the DNB Load Plate and bends during operation?

The sample needle may make contact with the DNB Load Plate if the position settings are incorrect. If this occurs, contact the technical support for assistance.

Q: What should I do if bubbles are present in the fluidics lines of the flow cell?

Bubbles may be present in the fluidics lines of the flow cell when aspirating reagents.

To resolve the issue, perform the following steps:

1. After loading, press the flow cell attachment button to release the flow cell.
2. Check whether the sealing rings installed evenly and properly. If not, re-install the sealing ring.

Q: Why is the flow cell not attaching to the flow cell stage?

If the flow cell is not attaching to the flow cell stage on the loader, it may be due to the flow cell attachment button not being pressed. Any dust, debris, or damage that may be present on the flow cell stage and/or the flow cell can keep the flow cell from attaching.

To resolve the issue, perform the following steps:

1. Check whether the flow cell attachment button is pressed.
2. Check the flow cell stage for dust, debris, or damage. Clean the flow cell stage.

Q: Why is liquid not passing through the fluidics lines of the flow cell?

When foreign particles are present on the sealing ring, or the sealing ring is damaged, liquid may not be able to pass through the fluidics lines. Foreign particles on the rear of the flow cell and blockages in fluidics line may also be present if liquid is unable to pass through the lines.

To resolve the issue, perform the following steps:

1. Check whether the sealing ring on the flow cell stage is intact or if any foreign particles are blocking the holes of the sealing ring.
2. Check whether there are any foreign particles on the rear of the flow cell or the surface of the flow cell stage. If particles are present, clean the flow cell stage.

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Q: What should I do if the flow cell stage is leaking?

The flow cell stage may leak if:

- Sealing rings are not installed.
- Sealing rings are not correctly installed.
- There are foreign particles on the back side of the flow cell.
- The fluidics lines are blocked.

To resolve the issue, perform the following steps:

1. Check whether the sealing rings are installed.
2. Check whether the sealing rings on the flow cell stage are intact or if any foreign particles are blocking the holes of the sealing ring.
3. Check whether there are any foreign particles on the back side of the flow cell or the surface of the flow cell stage. If particles are present, clean the flow cell stage.

Remote server FAQs

Q: What should I do if ZLIMS is disconnected?

Errors messages may appear when the sequencer is disconnected from MGI-ZTRON-LITE or when ZLIMS cannot be opened on MGI-ZTRON-LITE, perform the following steps:

1. Check whether the network cable is firmed attached.
2. If the problem persists, contact the technical support.

Q: What should I do if error messages appear when there is insufficient disk space on the remote server?

Tips

Storage capacity for PE150 sequencing of a single flow cell is around 5 TB and that for PE100 sequencing is around 3.5 TB.

- FPGA configuration
  ① Return to the SBC desktop. For details, contact the technical support.
  ② Access the BIS desktop through remote connection, and open **My computer**.
  ③ Check the available space of the mounted directory, If the space is insufficient, transfer the data into a storage server or portable storage device, and then delete the data in the mounted directory to release space.
- GPU configuration
  ① Return to the SBC desktop. For details, contact the technical support.
  ② Access the BCS desktop through the VNC Viewer.
  a. Double-click to open the VNC Viewer.
  b. Double-click to open zebracall in the VNC Viewer.
  ③ Check the available space of the mounted directory, If the space is insufficient, transfer the data into a storage server or portable storage device, and then delete the data in the mounted directory to release space. If you have any problems, contact the technical support.

Reagent FAQs

Q: What should I do if DNB concentration is low?

When DNB concentration is lower than the specified minimum DNB concentration, perform the following steps:

1. Check whether the DNB preparation kit has expired.
2. Check whether the library meets the requirements.
3. Make DNBs again. If the DNB concentration still does not meet the requirements after a new sample preparation, contact the technical support.

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Q: What should I do if I forgot to add reagent into well No. 8 for PE sequencing run?

MDA Enzyme II is required to make the second-strand template for PE sequencing. When preparing the Sequencing Reagent Cartridge, the appropriate amounts of MDA Enzyme Mix II and MDA Reagent must be added to well No. 8. If MDA mixture was not added into well No. 8 before starting the sequencing run, this can be resolved by performing the following steps, as long as the sequencing run is in the sequencing phase of Read1:

1. Pause the run: Select the pause button ⏸ in the sequencing interface and select **Yes** when prompted.
2. Lift the needle:
   ① Select the stop button ■ and select **Yes** when prompted.
   ② Select **Finish**.
3. Fill well No. 8 of the Sequencing Reagent Cartridge:
   ① Open the reagent compartment door and take out the Sequencing Reagent Cartridge.
   ② Prepare the MDA mixture by adding the appropriate amount of MDA Enzyme Mix II into the MDA Reagent tube.
   ③ Mix thoroughly and transfer all solution into well No. 8. as described in *Preparing the Sequencing Reagent Cartridge - Part 2 on Page 12*.
   ④ Insert the filled sequencing cartridge back into the sequencer.
4. Resume the run:
   ① Select **Sequence** > **Resume run** on the main interface.
   ② Clean the loaded flow cell with a canned air duster to ensure that no visible dust exists on the surface and back of the flow cell. Place the flow cell on the flow cell drive, and touch the flow cell drive control button to load the flow cell into the device.

⚠ WARNING

When using the canned air duster to remove the dust on the back of the flow cell, avoid blowing air into the inlet holes.

   ③ Select **Next** to review the parameters and ensure that all parameters are correct.
   ④ Select **Start** > **Continue**.

Q: What should I do if I want to resume a stopped sequencing run?

The sequencing run might be stopped due to some unexpected errors during the run, such as mechanical gripper operation failure, flow cell transfer failure, fluidics failure, and photographing failure. This stopped run may be continued after resolving the issues causing the run to stop.

Perform the following steps:

1. Select **Finish** in the sequencing interface to end the stopped run.
2. After resolving the issues that caused the run to stop, select **Sequence** > **Resume run** in the main interface.

⚠ WARNING

If the Sequencing Reagent Cartridge or Washing Cartridge is taken out for processing, ensure that the processed Sequencing Reagent Cartridge or Washing Cartridge is placed back in the corresponding compartment before resuming the sequencing run.

3. Re-load the flow cell:
   ① Dust the loaded flow cell of the interrupted sequencing run with a canned air duster. Ensure that no visible dust is present on the surface and back of the flow cell.

⚠ WARNING

When using the canned air duster to remove the dust on the back of the flow cell, avoid blowing air into the inlet holes.

   ② Place the flow cell on the flow cell drive, and touch the flow cell drive control button to load the flow cell into the device.
4. Select **Next** to review the parameters and ensure that all information is correct.
5. Select **Start** > **Continue** to resume the sequencing run.

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Q: What rules should I follow if I need to store a reagent kit temporarily?

- If a kit has been thawed (including the dNTPs) but cannot be used within 24 h, it can be frozen and thawed at most one time.
- If a kit has been thawed (including the dNTPs) but cannot be used immediately, store it at 2 ℃ to 8 ℃ . It is strongly recommended that you use it within 24 h. A thawed kit that is stored at 2 ℃ to 8 ℃ may still be used within seven days, although it may compromise sequencing quality. It is not recommended that you use a kit that has been thawed and stored at 2 ℃ to 8 ℃ for more than seven days.
- If the 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 covered with foil or plastic wrap. Store the kit at 2 ℃ to 8 ℃ and use it within 24 h. 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.

Q: What should I do if bubbles appear in the flow cell?

- MGIDL-T7RS
  - ① Check the sealing gasket to ensure that it is in the right position.
  - ② Check the DNB Load Plate to ensure that enough reagent is in each well.
  - ③ Replace the used flow cell and inspect the pump.
  - ④ If the problem persists, contact the technical support.
- DNBSEQ-T7RS
  - ① Check the water container to ensure that the water volume is sufficient.
  - ② Ensure that the pure water tube goes through the handle.
  - ③ Check the reagent needles to ensure that they can immerse fully into the cartridges. Otherwise, restart the sequencing software.
  - ④ If the problem persists after a restart, contact the technical support.

Q: What should I do if pumping failure occurs during DNB loading and sequencing?

1. Check if the pure water volume is sufficient.
2. When error occurs on MGIDL-T7RS and DNBSEQ-T7RS:
   - Remove the flow cell and check for is dust on the sealing gasket. Remove any dust with a canned air duster.
   - Place the flow cell by following the instructions and start the pump again.
3. Check if the sampling needles are moving properly. If the sampling needles are not moving properly, restart the control software of the sequencer.
4. If the problem persists, contact the technical support.

Q: What should I do if impurities appear in the original sequencing image?

If impurities appear, perform the following steps:

1. Perform a manual wash on MGIDL-T7RS and DNBSEQ-T7RS.
2. If there is still no improvement after manual wash, prepare washing reagents again according to *Wash preparations on Page 19*, and perform a manual wash again on MGIDL-T7RS and DNBSEQ-T7RS.
3. If the problem persists, contact the technical support.

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Customizing a recipe

The rules for filling in the **Customize a recipe** interface are as follows:

- When naming a sequencing recipe, use only letters, numbers, "+", "_" and "–".
- Because a previously named recipe will be saved in the recipe drop-down menu, duplicate name checking will be performed to ensure that each sequencing recipe name is unique (i.e., a new recipe name must not be the same as an existing recipe name).
- Enter numbers in the read length boxes of Read1, Read2, Barcode and DualBarcode.
- Multiple ranges of dark reaction cycles can be set in the Read1 and Read2 entry for "Dark reaction cycles". Use "," to separate the ranges. The dark reaction cycles of the ranges are presented in the format of "number" and "number-number".

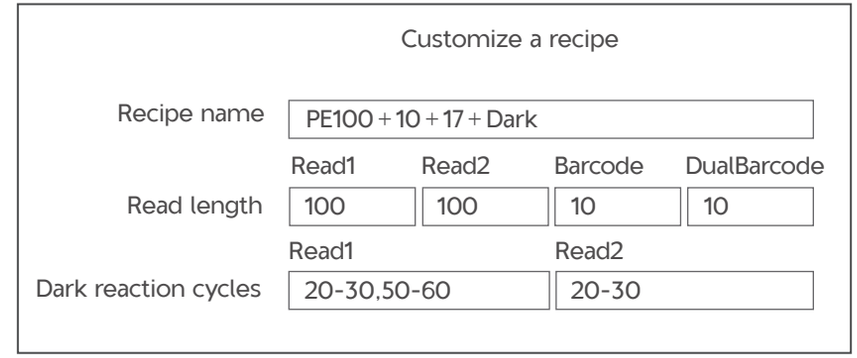
Tips

Dark reaction cycle: A sequencing cycle in which the chemical reaction is performed, but with no imaging.

Assumptions are as below:

- Sequencing run: PE100+10+10.
- Length of Read1: 100.
- Length of Read2: 100.
- Length of barcode: 10.
- Length of DualBarcode: 10.
- Dark cycles: From cycle-20 to cycle-30 and cycle-50 to cycle-60 in Read1 and cycle-16 to cycle-20 in Read2.
- Name the recipe as "PE100+10+10+Dark".

The **Customize a recipe** interface is set as follows:



Research use only

MGI has labeled the product solely for research use only and specified "RS" in the model name which means 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.

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