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· Release date: December 2022

Applicable set/kit

Name	Cat.No.
DNBSEQ-T7RS High-throughput Sequencing Set V3.0 (FCL PE100)	940-000269-00
DNBSEQ-T7RS High-throughput Sequencing Set V3.0 (FCL PE150)	940-000268-00
DNBSEQ-T7RS High-throughput Sequencing Set V1.0 (stLFR FCL PE100)	1000019251
DNBSEQ-T7RS High-throughput Sequencing Set V3.0 (App-A FCL PE100)	940-000298-00
DNBSEQ-T7RS High-throughput Sequencing Set V3.0 (App-A FCL PE150)	940-000300-00

Tips

This table only lists a part of applicable sets or kits. For more details, refer to *DNBSEQ-T7RS High-throughput Sequencing Set User Manual V7.0*.

Making DNBs

Preparing libraries

Tips

- For general purpose, the library refers to single-stranded circular DNA (ssDNA).
- For the stLFR library prepared with MGIEasy stLFR Library Prep Kit, the library is dsDNA.

1. Ensure that the insert size of the ssDNA library ranges between 50 bp and 500 bp, and that of the stLFR library ranges between 200 bp and 1500 bp, with main band centered within ±100 bp. Special requirements from the library preparation kit should prevail if any.
2. Use the Qubit® ssDNA Assay Kit and Qubit® Fluorometer to quantify the library.

The conversion formula between ng and fmol is as follows:

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

N represents the number of nucleotides (total library insert size including the adapter). C represents the concentration.

Library type	Concentration requirements
Regular library	≥3 fmol/μL
PCR-free library	≥3.75 fmol/μL
stLFR library	≥1.9 fmol/μL

3. Calculate the maximum number of pooled samples for each flow cell according to the following formula:

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

Tips

The maximum number of pooled samples depends on the required data output, read length, and specific application.

4. Calculate the required amount of library.

Kit model	Required DNB volume	Volume of Make DNB mixture	The number of Make DNB mixture	required amount of library
FCL SE35/SE50/ SE100/PE100, APP-A FCL PE100	Required data output/ Theoretical data output×270	100 μL	Round (V/100)+1	The number of Make DNB mixture×60 fmol
stFLR FCL PE100		80 μL	Round (V/80)+1	The number of Make DNB mixture×30 ng
FCL PE150/App-D/ APP-A FCL PE150	Required data output/ Theoretical data output×300	90 μL	Round (V/90)+1	The number of Make DNB mixture×60 fmol

5. Place the library on ice until use.

Making DNBs

WARNING

Do not use filtered pipette tips for making DNBs.

1. Take out Make DNB Buffer or App-A Make DNB Buffer for App-A sequencing or App-D Make DNB Buffer for App-D sequencing, Low TE Buffer, and Stop DNB Reaction Buffer and thaw them at room temperature.
2. Take out Make DNB Enzyme Mix I or Make DNB Rapid Enzyme Mix II for FCL PE150 sequencing or Make DNB Enzyme Mix III for stLFR PE100 sequencing, and thaw it on ice for approximately 30 minutes.
3. After thawing, mix these reagents by using a vortex mixer for 5 seconds, centrifuge briefly and place them on ice until use.

Tips

It is not recommended to use reagent components from different batches mixedly.

4. Take out a 0.2 mL PCR tube and prepare the reaction mixture 1 on ice according to the following table:

Kit model	Volume (μL)	Low TE Buffer	Make DNB Buffer	APP-A Make DNB Buffer	APP-D Make DNB Buffer	stLFR Make DNB Buffer	ssDNA library	dsDNA library
FCL SE35/SE50/SE100/ PE100	100 μL DNB reaction	20-V1	20	/	/	/	V1	/
	50 μL DNB reaction	10-V2	10				V2	
FCL PE150	90 μL DNB reaction	20-V1	20				V1	
App-A FCL PE100	100 μL DNB reaction	20-V1	/	20			V1	
	50 μL DNB reaction	10-V2		10			V2	
App-A FCL PE150	90 μL DNB reaction	20-V1		20			V1	
App-D FCL PE150	90 μL DNB reaction	20-V1		/	20		V1	
stLFR FCL PE100	80 μL DNB reaction	16-V3			/	16	/	V3

Tips

- For regular library, *V1* and *V2* equal to 60 fmol/C and 30 fmol/C respectively. For PCR-free library, *V1* and *V2* equal to 75 fmol/C and 37.5 fmol/C respectively. For stLFR library, *V3* equals to 30 ng/C.
- Do not throw away remaining Low TE Buffer that will be used in DNB loading.

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5. Mix the mixture 1 thoroughly by vortexing and centrifuge it for 5 seconds by using a mini centrifuge. Place the tube into a thermal cycler and start the primer hybridization reaction according to the following condition:

Kit model	FCL SE35/SE50/SE100/PE100/PE150, App-A FCL PE100, App-A FCL PE150, App-D FCL PE150	stLFR FCL PE100
Temperature	Time	
105 ℃ (heated lid)	On	On
95 ℃	1 min	3 min
65 ℃	1 min	/
40 ℃	1 min	3 min
4 ℃	Hold	Hold

Tips

It is recommended to preheat the heated lid to the required temperature before DNB reaction, because it might take some time for the lid temperature adjustment.

6. Take out Make DNB Enzyme Mix II (LC) or Make DNB Enzyme Mix IV for stLFR FCL PE100 sequencing and place it on ice. Centrifuge it briefly for 5 seconds and place it on ice until use.
7. Prepare the reaction mixture 2 according to the following table:

<table>
<tr><th>Kit model</th><th>Volume (μL)</th><th>Make DNB Enzyme Mix I</th><th>Make DNB Enzyme Mix III</th><th>Make DNB Rapid Enzyme Mix II</th><th>Make DNB Enzyme Mix II (LC)</th><th>Make DNB Enzyme Mix IV</th></tr>
<tr><td rowspan="2">FCL SE35/SE50/SE100/ PE100</td><td>100 μL DNB reaction</td><td>40 μL</td><td rowspan="6">/</td><td rowspan="2">/</td><td>4 μL</td><td rowspan="6">/</td></tr>
<tr><td>50 μL DNB reaction</td><td>20 μL</td><td>2 μL</td></tr>
<tr><td>FCL PE150</td><td>90 μL DNB reaction</td><td>/</td><td>40 μL</td><td>1.6 μL</td></tr>
<tr><td rowspan="2">App-A FCL PE100</td><td>100 μL DNB reaction</td><td>40 μL</td><td rowspan="2">/</td><td>4 μL</td></tr>
<tr><td>50 μL DNB reaction</td><td>20 μL</td><td>2 μL</td></tr>
<tr><td>App-A FCL PE150</td><td>90 μL DNB reaction</td><td rowspan="3">/</td><td>40 μL</td><td>1.6 μL</td></tr>
<tr><td>App-D FCL PE150</td><td>90 μL DNB reaction</td><td>20 μL</td><td>40 μL</td><td>1.6 μL</td></tr>
<tr><td>stLFR FCL PE100</td><td>80 μL DNB reaction</td><td>32 μL</td><td>/</td><td>/</td><td>3.2 μL</td></tr>
</table>

8. Take the PCR tube out of the thermal cycler when the temperature reaches 4 ℃ and centrifuge it briefly for 5 seconds. Place the tube on ice and add the mixture 2 to the tube.

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9. Mix the tube thoroughly by vortexing, centrifuge it for 5 seconds and place it into a thermal cycler for the next reaction according to the following condition:

Kit model	FCL SE35, FCL SE50, FCL SE100, FCL PE100, App-A FCL PE100	FCL PE150, App-A FCL PE150, App-D FCL PE150	stLFR FCL PE100
Temperature	Time		
35 °C (Heated lid)	On	On	On
30 °C	25 min	10 min	30 min
4 °C	Hold	Hold	Hold

Tips

It is recommended to set the temperature of the heated lid to 35 °C or as close as possible to 35 °C .

10. Add 20 µL of Stop DNB Reaction Buffer for 100 µL DNB reaction, 16 µL of Stop DNB Reaction Buffer for 80 µL DNB reaction and 10 µL of Stop DNB Reaction Buffer for other volumes of DNB reaction to the tube immediately when the temperature reaches 4 °C. Mix the tube gently by using a wide-bore tip to pipette for 5 to 8 times.

Tips

- Do not centrifuge, vortex, or pipette vigorously.
- Store DNBs at 4 °C and use them within 8 hours.

Quantifying DNBs

1. Use the Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify the DNBs.
2. Ensure that the concentration of DNBs meets the following requirements.

Read length	Application library
FCL PE100 and below	≥8 ng/µL
FCL PE150	≥5 ng/µL

- If the concentration is lower than 5 ng/µL for FCL PE150 and lower than 8 ng/µL for FCL PE100 and below, make DNBs again.
- If it is higher than 40 ng/µL, use Low TE Buffer for FCL PE150 and DNB Load Buffer I for FCL PE100 and below to dilute the concentration to 20 ng/µL before loading.

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Pooling DNBs

Calculate the DNB pooling volume for each sample according to the required data output and DNB concentration.

Take 8 samples as an example. Perform the following steps:

1. Calculate the relative amount for each sample.

   The relative amount of sample A (A1) = required data output for sample A/DNB concentration for sample A.

   ……

   The relative amount of sample H (H1) = required data output for sample H/DNB concentration for sample H.
2. Calculate the total relative amount (V) for all samples.

   V=A1+B1+…+H1
3. Calculate the required pooling volume for each sample.
   - For FCL SE35/SE50/SE100/PE100, App-A FCL PE100 and stLFR FCL PE100 kit, pooling volume for sample A=270×A1/V.
   - For FCL PE150, App-A FCL PE150 and App-D FCL PE150 kit, pooling volume for sample A=300×A1/V.

Loading DNBs

Preparing the flow cell

1. Take out the flow cell without unwrapping the outer plastic package until use and place the flow cell at room temperature for at least 30 minutes, but not longer than 24 hours.
2. Before DNB loading, take out the flow cell from the inner package and check whether the flow cell is intact.
3. Use the dust remover to clean the back of the flow cell.

Preparing the post load plate

1. Take out different post load plates according to the kit model.

Kit model	Plate name
FCL SE35/SE50/SE100/PE100 , App-A FCL PE100	Post Load Plate
stLFR PE100	Post Load Plate (stLFR)
FCL PE150, App-A FCL PE150, App-D FCL PE150	Rapid Post Load Plate

2. Thaw it in a water bath at room temperature for 2 hours and place it in a refrigerator at 2 ℃ to 8 ℃ until use, or place it in a refrigerator at 2 ℃ to 8 ℃ overnight.
3. Before use, gently invert the post load plate for 5 times and then centrifuge it for 1 minute.

Preparing DNB loading mixture

⚠ WARNING

Do not use filtered pipette tips for loading DNBs.

1. Take out different reagents according to the kit model.

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Kit model	DNB Load Buffer II	DNB Load Buffer IV	App-A Insert primer 1	App-D Insert primer 1
FCL SE35/SE50/SE100/ PE100, stLFR FCL PE100	√	×	×	×
App-A FCL PE100	√	×	√	×
FCL PE150	×	√	×	×
App-A FCL PE150	×	√	√	×
App-D FCL PE150	×	√	×	√

2. Thaw the reagent(s) in a water bath at room temperature for approximately 0.5 hours.
3. Mix it by using a vortex mixer for 5 seconds, centrifuge it briefly and place it on ice until use.

Tips

If crystal precipitation is found in DNB Load Buffer II, vigorously mix the buffer for 1 to 2 minutes to re-dissolve the precipitation and centrifuge it briefly before use.

4. Take out a new 0.5 mL microfuge tube and add reagents according to the following table:

Kit model	DNB	DNB Load Buffer II	Make DNB Enzyme Mix II (LC)	DNB Load Buffer IV
FCL SE35/SE50/SE100/PE100, stLFR FCL PE100, App-A FCL PE100	270 µL	90 µL	1 µL	/
FCL PE150, App-A FCL PE150, App-D FCL PE150	300 µL	/	/	150 µL

5. Gently pipette the DNB loading mixture for 5 to 8 times by using a wide-bore tip.

Tips

- Do not centrifuge, vortex, or pipette vigorously.
- The DNB loading mixture must be freshly prepared and used within 30 minutes.

Loading DNBs

1. Close the compartment doors and start MGIDL-T7RS.
2. Double click the icon of MGIDL-T7RS to start the program.
3. Enter the user name *user* and password *123*, and tap **Log in**.
4. Tap **A** or **B**.
5. Tap **Loading**.
6. Open the compartment door.
7. Tap the **DNB ID** box, and enter DNB ID.
8. Place the 0.5 mL micro tube containing DNB loading mixture into the DNB tube hole, and wait for the prompt that the DNB tube is loaded.
9. Remove the seal of the post load plate and add 4 mL of 0.1 M NaOH into well No.11.

   (Optional) For App-A FCL PE100 or App-A FCL PE150 sequencing, use a pipette to completely aspirate all the reagents in well No.1, and add 2 mL of App-A Insert primer 1 to the well.

   (Optional) For App-D FCL PE150 sequencing, use a pipette to completely aspirate all the reagents in well No.1, then add 2 mL of App-D Insert primer to the well.
10. Align the post load plate to the RFID scanning area and the ID will be displayed in the text box.

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11. Place the prepared post load plate on the plate tray and wait for the prompt that the post load plate is loaded.
12. Align the flow cell to the RFID scanning area, and the ID will be displayed in the text box.
13. Hold both sides of the flow cell, upwardly align the locating bulge on the flow cell to the locating groove on the flow cell stage and gently press down the edges of the flow cell.
14. Press the attachment button on the flow cell stage to ensure that the flow cell is securely seated and held on the stage, and wait for the prompt that the flow cell is loaded.

Tips

Ensure that four rubber sealing rings are on the four corners.

15. Close the compartment door.
16. Tap **Start**>**Yes** to start loading the flow cell. The process takes around 2 hours.
17. After loading, press the attachment button and remove the loaded flow cell from the stage.
18. Install the washing flow cell onto the flow cell stage, press the attachment button and tap **Confirm**.
19. Tap **Post-wash**>**Yes** to start a wash. The wash takes around 20 minutes.

    If **Post-wash** is not selected, refer to *Device maintenance* in this operation guide to perform a manual wash.
20. You will be prompted when the wash is completed. Tap **Finish** and perform another flow cell loading process if necessary.

Loading the reagent cartridge and flow cell

Preparing the reagent cartridge

1. Take out the sequencing reagent cartridge.
2. Thaw the cartridge in a water bath at room temperature for 4 to 5 hours and then store it at 2 ℃ to 8 ℃ until use, or thaw it in a refrigerator at 2 ℃ to 8 ℃ for 24 hours in advance.
3. Shake the cartridge vigorously in all directions for 10 to 20 times to mix it thoroughly.
4. Take out dNTPs Mix IV for SE sequencing or dNTPs Mix V for PE sequencing, and dNTPs Mix II, and thaw them at room temperature.
5. After thawing, invert these reagents for 4 to 6 times, centrifuge briefly and place them on ice until use.
6. (Optional) Process the primer according to different situations.
   - For dual barcode PE sequencing, take out the AD153 Barcode Primer 3, and thaw it at room temperature.
   - For dual barcode SE sequencing, take out the AD153 Barcode Primer 4, and thaw it at room temperature.
   - For App-A PE sequencing, take out the App-A Insert Primer 2, App-A MDA primer, and App-A Barcode Primer 2, and thaw them at room temperature.

     (Optional) For dual barcode App-A PE sequencing, take out the App-A Barcode Primer 3 and thaw it at room temperature.
   - For App-D PE sequencing, take out the App-D Insert Primer 2, App-D MDA primer and App-D Barcode Primer 2, and thaw them at room temperature.

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(Optional) For dual barcode App-D PE sequencing, take out the App-D Barcode Primer 3 and thaw it at room temperature.

7. Vortex these components for 5 seconds. Centrifuge briefly and place them on ice until use.
8. Open the cartridge 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 lint-free paper.
9. Use a 1 mL sterile tip to pierce the seal at the edge of well No.9 and No.10 to make a hole around 1 cm in diameter.
10. Take out the Sequencing Enzyme Mix and invert it for 4 to 6 times and place it on ice until use.
11. Add reagents by using a pipette according to the table below:

Model	Well No.9			Well No.10	
	dNTPs Mix IV	dNTPs mix V	Sequencing Enzyme Mix	Sequencing Enzyme Mix	dNTPs Mix II
FCL SE35	1.70 mL	/	1.7 mL	1.5 mL	4.5 mL
FCL SE50	2.0 mL		2.0 mL	1.8 mL	5.4 mL
FCL SE100	3.0 mL		3.0 mL	2.7 mL	8.1 mL
FCL PE100	/	2.76 mL	2.76 mL	2.76 mL	8.28 mL
FCL PE150		3.74 mL	3.74 mL	3.74 mL	11.22 mL
App-A FCL PE100		3.74 mL	3.74 mL	3.74 mL	11.22 mL
App-A FCL PE150		2.76 mL	2.76 mL	2.76 mL	8.28 mL
App-D FCL PE150		3.74 mL	3.74 mL	3.74 mL	11.22 mL
stLFR FCL PE100	5.4 mL	/	5.4 mL	4.9 mL	14.7 mL

12. Seal wells No.9 and No.10 with the transparent sealing film.
13. Place the cartridge horizontally on the bench, and hold both sides of the cartridge with both hands. Shake it clockwise 10 to 20 times, and then counterclockwise 10 to 20 times to mix reagents thoroughly.
14. (Optional) Perform the following steps according to different situations:
    - For dual barcode SE sequencing, pierce the seal of well No.3, and use a 1 mL pipette to add 3.50 mL CPAS AD153 Barcode Primer 4 to well No.3.
    - For PE sequencing, pierce the seal of well No.8, use a 1 mL pipette to add 600 µL MDA Enzyme Mix to the MDA Reagent tube, invert the tube 4 to 6 times to mix the reagents and add the reagents to well No.8 and ensure that no bubbles exist at the bottom of the tube.

Tips

When using MDA Enzyme Mix, do not touch the wall of the tube to prevent influencing the enzyme activity.

- For dual barcode PE sequencing, use a 1 mL sterile tip to pierce the seal of well No.3 and use a 1 mL pipette to add 3.5 mL of AD153 Barcode Primer 3 to well No.3.
- For App-A PE sequencing, use a 1 mL sterile tip to pierce the seals of well No.3, No.4, No.6 and No.13 and use a 1 mL pipette to add reagents to different wells.

Tips

App-A barcode primer 3 is just for dual barcode App-A PE sequencing.

Reagent	Well No.	Volume (mL)
App-A Barcode Primer 2	No.4	3.50
App-A MDA Primer	No.6	4.20
App-A Insert Primer 2	No.13	4.20
App-A Barcode Primer 3	No.3	3.50

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- For App-D PE sequencing, use a 1 mL sterile tip to pierce the seals of well No.3, No.4, No.6 and No.13 and use a 1 mL pipette to add reagents to different wells.

Tips

App-D barcode primer 3 is just for dual barcode App-D PE sequencing.

Reagent	Well No.	Volume (mL)
App-D Barcode Primer 2	No.4	3.50
App-D MDA Primer	No.6	4.20
App-D Insert Primer 2	No.13	4.20
App-D Barcode Primer 3	No.3	3.50

15. Close the sequencing cartridge cover.
16. Gently tap the cartridge on the bench to reduce air bubbles in the reagents.

Tips

After preparation, store the cartridge at 2 °C to 8 °C and load it within 2 hours if it is not loaded immediately.

Preparing the washing cartridge

1. Shake the cartridge clockwise 5 to 10 times, and then counterclockwise 5 to 10 times to 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.
3. Use a 1 mL sterile tip to pierce either of well No.2.
4. Add 45 mL of 0.1 M NaOH into well No.2 by using a pipette.

   For details on how to prepare 0.1 M NaOH, refer to step 2 from *Device maintenance* in this operation guide.

Preparing the pure water container

Fill the pure water container with laboratory-grade water according to the following table:

Kit model	1 flow cell	2 flow cells	3 flow cells	4 flow cells
FCL SE35	1.0 L	2.0 L	3.0 L	4.0 L
FCL SE50	1.0 L	2.0 L	3.0 L	4.0 L
FCL SE100	1.5 L	3.0 L	4.5 L	6.0 L
FCL PE100	3.0 L	6.0 L	9.0 L	12.0 L
FCL PE150	4.5 L	9.0 L	13.5 L	18.0 L
App-A FCL PE100	3.0 L	6.0 L	9.0 L	12.0 L
App-A FCL PE150	4.5 L	9.0 L	13.5 L	18.0 L
App-D FCL PE150	4.5 L	9.0 L	13.5 L	18.0 L
stLFR FCL PE100	3.5 L	7.0 L	10.5 L	14.0 L

Loading the reagent cartridge

1. Open the reagent compartment door, use lint-free paper moistened with laboratory-grade water to clean the inner walls and keep the compartment clean and dry.
2. Place the reagent cartridge into the low-temperature compartment in the upper layer and place the washing cartridge into the room-temperature compartment in the lower layer.
3. Close all compartment doors.

Loading the flow cell

1. Enter the user name *user* and password *123*, and tap **Log in** to enter the main interface.

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2. Select A/B/C/D respectively according to sequencing demand. Tap **Sequencing**>**New run**.
3. Use a dust remover to clean the front side of loaded flow cell with and use a dust-free cloth to clean the back side, to ensure that no visible dust exists on the surface and back of the flow cell.
4. Place it on the flow cell drive, and press the button to retract the flow cell drive.

Starting sequencing

1. RFID scans the reagent cartridge ID, washing cartridge ID, and flow cell ID, and the ID will be automatically displayed. Enter the information manually if scanning fails.
2. Tap the first ▼ behind **Recipe** and select required sequencing recipe.
   If customizing a recipe is required, select **Customize** in the drop-down list to enter the interface and fill in required information.
3. Tap the second ▼ behind **Recipe**, select the required barcode and select whether to split the barcode.
4. Tap ⩓ behind **Advanced settings** to select whether the primer is **custom primers** and whether to perform **Auto wash**.
5. Tap **Next** to review the parameters and ensure that all information is correct.
6. Tap **Start**>**Yes**.
7. During sequencing, tap ⧦ to review the information or select whether to cancel auto wash after sequencing. The auto wash takes around 30 minutes.

   If auto wash is canceled, refer to *Device maintenance* in this operation guide to perform a manual wash.

Device maintenance

1. Perform a manual wash for different devices according to specific situations.

Wash method	Description
MGIDL-T7RS/ DNBSEQ-T7RS manual wash	• 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 • After replacing the tubing, sampling needles, or other accessories exposed to the reagents

2. Prepare the following two washing reagents for manual wash.

Washing reagent	Component	Volume (mL)
Washing reagent 1 (1 M NaCl +0.05% Tween-20)	5 M NaCl solution	200
	100% Tween-20	0.5
	Laboratory-grade water	799.5
Washing reagent 2 (0.1 M NaOH)	2 M NaOH solution	50
	Laboratory-grade water	950

3. (Optional) Perform a manual wash for MGIDL-T7RS.
   ① Take out a clean and empty post load plate, and add reagents according to the following table:

Well No.	Reagent	Volume (mL)
Well No.9	Laboratory-grade water	4
Well No.10	1 M NaCl +0.05% Tween-20	4
Well No.11	0.1 M NaOH	4
Well No.12	Laboratory-grade water	20

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② Start the program.

③ Enter the password *123*, and tap **Log in** to enter the main interface.

④ Select the side that needs to wash, open the loading compartment door and place the washing plate with washing reagents into the required side. Close the door.

⑤ Press the attachment button and wait until the negative pressure is released. Remove the flow cell from the stage.

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

⑥ Take out the washing flow cell and place it on the flow cell stage. Press the attachment button and press down the flow cell to ensure that the flow cell is securely attached to the stage.

⑦ Tap **Wash**>**Yes** to start the wash. The wash takes around 20 minutes.

4. (Optional) Perform a manual wash for DNBSEQ-T7RS.

① Prepare an empty washing cartridge labeled as washing cartridge 1.

② Prepare washing cartridge 2 and add reagents according to the following table:

Well No.	Reagent	Volume (mL)
Either of well No.2	0.1 M NaOH	45
Either of Well No.3	1 M NaCl +0.05% Tween-20	45

③ Ensure that the pure water container is filled with at least 4.5 L of laboratory-grade water before performing the wash.

④ Start the program. Enter the user name *user* and password *123*, and tap **Log in** to enter the main interface.

⑤ Tap **Wash**.

⑥ Install a used flow cell from a previous run. Press the flow cell drive control button again to retract the flow cell drive.

⑦ Place the washing cartridge 1 into the low-temperature compartment on the side that needs a wash, and then close the door.

⑧ Place the washing cartridge 2 filled with washing reagents into the room-temperature compartment on the side that needs a wash, and then close the door.

⑨ Close the reagent compartment door, and tap **Start**>**Yes** to start the wash. The wash takes around 40 minutes.

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