Application scope

Catalog number	Model	Name
940-000838-00	FCL PE100	DNBSEQ-T7RS High-throughput Sequencing Set
940-000836-00	FCL PE150	DNBSEQ-T7RS High-throughput Sequencing Set

Introduction

This quick operation guide provides concise instructions for operating the DNBSEQ-T7RS system.

WARNING

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

Getting started

Preparing the flow cell - Part 1

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

Do not open the outer plastic package yet.

2. Place the flow cell at room temperature for 0.5 h to 24 h.

Preparing the Sequencing Reagent Cartridge - Part 1

1. Remove the Sequencing Reagent Cartridge from the DNBSEQ-T7RS High-throughput Sequencing Kit.
2. According to the table below, select the appropriate thawing method based on the model:

Approximate thaw time for Sequencing Reagent Cartridge		
Model	Method	
	Water bath at room temperature (h)	Refrigerator at 2 °C to 8 °C overnight* then water bath at room temperature (h)
FCL PE100	2.5	2
FCL PE150	4.0	2.0

Overnight refers to 16 h.

3. After the Sequencing Reagent Cartridge is thoroughly thawed, place it at 2 °C to 8 °C until use.

Preparing Post Load Plate

1. Remove the Post Load Plate (T7 FCL PE100 or T7 FCL PE150) from the DNBSEQ-T7RS DNB Load Reagent Kit (FCL PE100 or FCL PE150). Thaw Post Load Plate at 2 °C to 8 °C (at least 16 h in advance) or in a water bath at room temperature (2 h) until it is completely thawed before use.
2. After Post Load Plate (T7 FCL PE100 or T7 FCL PE150) is thoroughly thawed, place it at 2 °C to 8 °C until use.

Preparing DNB Load Buffer

1. Remove the DNB Load Buffer II (FCL PE100) or DNB Load Buffer IV (FCL PE150) from the DNBSEQ-T7RS DNB Load Reagent Kit.
2. Thaw the reagent in a water bath or on ice for approximately 0.5 h.
3. After thawing, mix the reagent by using a vortex mixer for 5 s. Centrifuge briefly and place on ice until use.

   If crystal precipitation is found in DNB Load Buffer II, vigorously mix the reagent with 1 to 2 minutes of continuous vortex to re-dissolve the precipitate before use.

Preparing the 0.1 M NaOH reagent

Prepare 0.1 M NaOH according to the procedure described in Wash preparations on Page 09. Each Post Load Plate requires at least 4 mL of 0.1 M NaOH.

Preparing DNBs

Input circular ssDNA library requirement

DNB preparation starts from a circular ssDNA library with a recommended insert size for different sequencing models with different applications. Recommendations are listed in the following table:

Recommended library insert size and applications		
Model	Recommended library insert distribution (bp)	Applications
FCL PE100	200 to 400	WGS, WES, RNAseq
FCL PE150	300 to 500	WGS, WES, RNAseq

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

Circular ssDNA library concentration requirement	
Library type	Minimum concentration
General libraries	3 fmol/μL
PCR-free libraries	3.75 fmol/μL

Library pooling

The sequencer can simultaneously perform sequencing for up to 4 flow cells. The number of samples that can be pooled per flow cell depends on factors such as the required data output, read length, and barcodes required for specific applications. It is recommended to quantify DNBs and then pool

DNBs after making DNBs is completed.

Examples of various sample pooling			
Model	Minimum data for each sample (Gb)	Pooling sample number	Theoretical data output range for each sample (Gb)
FCL PE100	50	20	52 to 63
	100	10	104 to 127
FCL PE150	50	23 RNAseq	51 to 62
	100	4 WGS	102 to 122

Verifying the base balance for barcode

- The minimum base composition of A, C, G, T at each position in the barcode should not be lower than 12.5%.
- Two or more samples with identical barcodes should not be pooled together, as it will prevent correct assignment of the reads.

Making DNBs

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

Volume of ssDNA libraries (μL)			
Library type	V for a 100 μL DNB reaction	V for a 50 μL DNB reaction	V for a 90 μL DNB reaction
General libraries	60 to 90 fmol/C	30 to 45 fmol/C	60 to 90 fmol/C
PCR-free libraries	75 to 150 fmol/C	37.5 to 75 fmol/C	75 to 150 fmol/C

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.

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 PE100) Make DNB Rapid Enzyme Mix II (FCL PE150)		Thaw on ice for approximately 30 min	Mix by using a vortex mixer for 5 s, centrifuge briefly	Place on ice until use.
Low TE Buffer		Thaw at room temperature for approximately 30 min		
Make DNB Buffer				
Stop DNB Reaction Buffer				

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

Make DNB reaction mixture 1				
Component	Cap color	FCL 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 the Make DNB reaction mixture 1 thoroughly using a vortex mixer and centrifuge for 5 s.
5. Place Make DNB reaction mixture 1 into a thermal cycler and start primer hybridization reaction. Thermal cycler settings are shown in the table below:

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

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

Make DNB reaction mixture 2				
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

9. Add all of Make DNB reaction mixture 2 into the Make DNB reaction mixture 1. Mix the reaction mixture thoroughly using a vortex mixer and centrifuge for 5 s.
10. Put the PCR tube into the thermal cycler for rolling circle replication reaction. The conditions are shown in the table below:

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

11. Immediately remove the PCR tube from the thermal cycler when the temperature reaches 4 °C and place the tube on ice. Add the Stop DNB Reaction Buffer to the PCR tube according to the table below. Gently mix the product (namely the DNBs) by pipetting 8 times using a wide-bore, non-filtered pipette tip.

Volume of Stop DNB Reaction Buffer				
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

12. Immediately go to the next step: *Quantifying DNBs and pooling*.

Quantifying DNBs and pooling

Quantifying DNBs

Use the Qubit ssDNA Assay Kit and Qubit Fluorometer to measure the concentration of DNBs.

- If the concentration is lower than the minimum DNB concentration, re-make the DNBs.

- If the concentration exceeds 40 ng/μL, the DNBs should be diluted to 20 ng/μL according to the table below:

Model	Minimum DNB concentration	Dilution reagent	Storage conditions	Maximum Storage time (h)
FCL PE100	8 ng/μL	DNB Load Buffer I	2 °C to 8 °C	48
FCL PE150	5 ng/μL	Low TE Buffer	2 °C to 8 °C	8

Pooling DNBs

The amount of DNBs (μL) needed for each sample in the pool depends on the relative amount for each sample and the total amount of DNBs needed for loading one flow cell, which is defined by the specific type of sequencing kit.

Loading DNBs

Preparing DNB loading mixture

1. Take out Micro Tube 0.5 mL (Empty) from the DNBSEQ-T7RS DNB Load Reagent Kit (FCL PE100 or FCL PE150) and add the following components in order:

DNB loading mixture				
No.	Component	Cap color	FCL PE100 Volume (μL)	FCL PE150 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	/

*DNB in the table refers to the pooled DNBs.

2. Combine components and use a wide-bore, non-filtered pipette tip to gently mix the loading mixture 5 to 8 times.

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

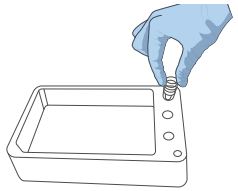
Preparing the flow cell - Part 2

1. Unwrap the outer package before use. Remove the flow cell from the inner package and carefully inspect the glass panel of the flow cell to ensure that there are no scratches.
2. Clean the back of the flow cell using a canned air duster.

- When using the canned air duster to remove the dust on the back of the flow cell, avoid blowing air into the inlet holes.
- If the flow cell cannot be used within 24 h after being placed at room temperature and the outer plastic package remains intact, place it back in 2 °C to 8 °C. However, the temperature transition between room temperature and 2 °C to 8 °C should not exceed 3 times.
- If the flow cell has been taken out of the plastic package and cannot be used immediately, store it at room temperature and use within 24 h. If 24 h is exceeded, it is not recommended to use the flow cell.

Performing DNB loading

1. Ensure that the compartment doors of DL-T7RS are securely closed and start the device.
2. Launch the DL-T7RS control software and log into the main interface
3. Select either **A** or **B** to proceed with the operation. Tap **Loading** to open the information input interface.
4. Open the loading compartment door.
5. Enter the DNB information into the **DNB ID** box. Place the Micro Tube 0.5 mL containing DNB loading mixture into the DNB tube hole. The interface prompts that the DNB tube has been loaded.

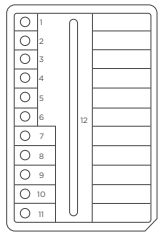
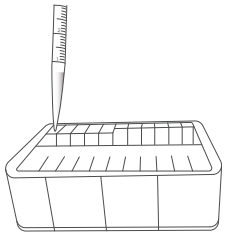


The DNB ID should be only numbers or letters or a combination of numbers and letters.

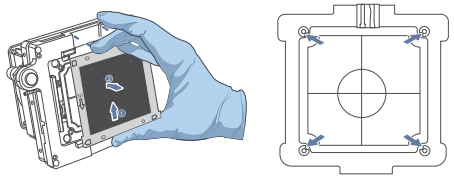
6. Remove Post Load Plate from storage. Align the Post Load Plate to the RFID scanning area and the ID will appear in the **Post-loading plate ID** box.

- If scanning fails, manually enter the plate ID using the on-screen keyboard.
- Ensure that the ID correctly formatted when entering it manually. If the format is incorrect, you will be prompted that the ID is invalid, and the procedure cannot continue.
- The plate ID includes the catalog number (REF) and serial number (SN), as indicated on the label. When entering the ID manually, be sure to include any special characters in the catalog number.

7. Gently invert the Post Load Plate (T7 FCL PE100 or T7 FCL PE150) 5 times, and then centrifuge it for 1 min or gently tap the sealing film and let it sit for 2 to 3 min.
8. Remove the seal of the Post Load Plate and add 4 mL of 0.1 M NaOH into well No. 11. Place the prepared Post Load Plate on the plate tray of DL-T7RS. The interface prompts that the Post Load Plate has been loaded.


9. Align the flow cell to the RFID scanning area and the ID will appear in the **Flow cell ID** box.
10. Load the flow cell.
    1) Ensure that all the four rubber sealing rings are on the four corners of the flow cell.
    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. The flow cell attachment button turns green, and the interface prompts that the flow cell has been loaded.

- Remove dust from both sides of the flow cell using a canned air duster.
- Avoid touching or pressing on the glass cover of the flow cell to avoid leaving fingerprints or impurities, which could damage the glass surface.
- Do not move the flow cell after it is installed onto the stage, as this may misalign the sealing gaskets with the fluidics line holes.
- 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. Then, use a canned air duster to remove any remaining dust.

11. Close the loading compartment door. Select **Start** and select **Yes** when prompted to start loading. The process takes around 2 h.
12. After the DNB 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.

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

Preparing cartridges and pure water container

Preparing the Sequencing Reagent Cartridge - Part 2

1. Ensure that the reagents in the Sequencing Reagent Cartridge are fully thawed.
2. Invert the cartridge 10 times to mix before use. Shake the cartridge 20 times clockwise and counterclockwise. Ensure that all reagents are fully mixed.
3. Prepare well No. 9 and well No. 10:
   1) Prepare reagents according to the table below:

Component	Cap color	Operation
dNTPs Mix V		Remove the reagents from storage 1 h 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 II		
Sequencing Enzyme Mix	/	Invert the reagent 6 times and place it on ice until use

   2) Wipe any water condensation from the cartridge cover and well surround with a Kimwipes tissue. Spray 75% ethanol on the surface of the cartridge seal and clean the seal with Kimwipes tissue. Pierce the seal in the center of well No. 9 and No. 10 respectively by using a 1 mL sterile tip to make a hole around 2 cm in diameter.
   3) According to the following table, add dNTPs Mix V, dNTPs Mix II, and Sequencing Enzyme Mix to well No. 9 or well No. 10.

Model	Well No. 9		Well No. 10	
	dNTPs Mix V (mL)	Sequencing Enzyme Mix (mL)	dNTPs Mix II (mL)	Sequencing Enzyme Mix (mL)
FCL PE100	2.760	2.760	8.280	2.760
FCL PE150	3.740	3.740	11.220	3.740

   4) Seal the loading wells of well No. 9 and well No. 10 with transparent sealing film. 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.
   5) Lift the cartridge horizontally, holding both sides of the cartridge with both hands. Shake the cartridge 20 times clockwise and counterclockwise. Ensure that the reagents are fully mixed. Carefully remove the seals from the wells after fully mixing.

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

4. Prepare well No. 8:
   1) Pierce the seal of well No. 8 using a 1 mL sterile tip.
   2) Add 600 µL of MDA Enzyme Mix to the MDA Reagent tube. Invert the tube 6 times to mix the reagent.
   3) Add all the MDA mixture to well No. 8 and ensure that there is no bubble at the bottom of the tube.

4) Gently tap the Sequencing Reagent Cartridge on the bench to reduce air bubbles in the reagents.

Preparing the Washing Cartridge

1. Shake the cartridge 10 times clockwise and counterclockwise. Ensure that the reagents are fully mixed.
2. Spray 75% ethanol on the surface of the cartridge seal and clean the seal with Kimwipes tissue. Pierce the seal in the center of well No. 2 by using a 1 mL sterile tip. Add 45 mL of 0.1 M NaOH into well No. 2 through the pierce using an electronic pipette.

You can use laboratory-grade water such as 18 Megohm (MΩ) water, Milli-Q water, Super-Q water, or similar molecular biology-grade water.

Reagent name	Volume (mL)	Final concentration
2 M NaOH	50	0.1 M
Laboratory-grade water	950	/
Total volume	1000	
Shelf life	1 month at 2 °C to 8 °C	

Filling the pure water container

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

Pure water consumption (L)				
Model	**1 flow cell**	**2 flow cells**	**3 flow cells**	**4 flow cells**
FCL PE100	3.0	6.0	9.0	12.0
FCL PE150	4.5	9.0	13.5	18.0

Performing a sequencing run

1. Load the Sequencing Reagent Cartridge and the Washing Cartridge.
2. Select , enter the user name and password, and select **Log in** to enter the main interface.
3. Load the flow cell:
   1) Select the flow cell stage. Ensure the lane selected corresponds to the placement of the Sequencing Reagent Cartridge and Washing Cartridge. 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.
   3) 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) The sequencer will automatically identify the ID information of Sequencing Reagent Cartridge, Washing Cartridge, and flow cell, and display ID in the corresponding text box. If not, enter the ID information manually.
   2) Select Recipe: Select the sequencing recipe from the **Recipe** list. One-click sequencing runs (such as **PE150**, etc.) and user-customized run (**Customize**) options are available.
   3) Select the corresponding barcode sequence.
   4) Select the **Split barcode** check box.

5) Enter the **Advanced settings** interface to indicate whether primers are custom and whether an auto wash is to be performed.
6) Select **Next**.

5. Review parameters. Ensure that all information is correct.
6. 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.

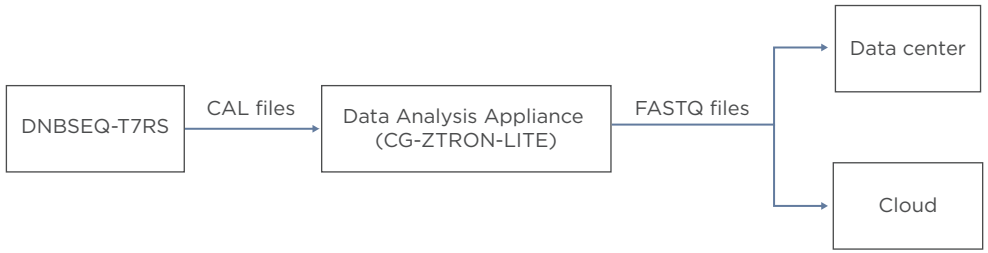
Automatic post-wash

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

Processing data

During the sequencing run, the control software automatically operates the basecalling analysis software and delivers raw sequencing data outputs for secondary analysis.

The data processing workflow is as follows:



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

For deployment of CG-ZTRON-LITE, contact CG Technical Support.

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

For detailed instructions on operating the CG-ZTRON-LITE, refer to the relevant user manual.

Maintenance

Maintaining the devices

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

- DL-T7RS post-wash:
  1) When DNB loading is complete, install a washing flow cell onto the flow cell stage and press the flow cell attachment button.
  2) Close the flow cell compartment door.
  3) Select **Confirm**. Select **Post-wash** and select **Yes** when prompted to start DL-T7RS wash, which will take approximately 20 min.
  4) Remove the washing flow cell and store it at room temperature.
- Manual wash on DL-T7RS and the sequencer: For details, refer to *DNBSEQ-T7RS System Guide (CSS-00165).*

Disposal of waste reagents and flow cells

1. Remove the used DNB Load Plate from the loader.
2. Remove the used cartridges and flow cells from the sequencer.
3. Discard the loading waste, sequencing waste, waste cartridges, DNB tube, and flow cells according to the SDS.

Research use only

Complete Genomics has labeled the product solely for research use only and specified “RS” in the model name which means that it should not be used for clinical diagnosis. Please refer to FDA Guidance, *Distribution of In Vitro Diagnostic Products Labeled for Research Use Only or Investigational Use Only* (Nov. 2013) (available at: *https://www.fda.gov/media/87374/download*). If you have any question, please contact Complete Genomics at +1 (888) 811-9644.