Application scope

Catalog number	Model	Name
940-000830-00	FCL SE50	DNBSEQ-G400RS High-throughput Sequencing Set
940-000826-00	FCL SE100	DNBSEQ-G400RS High-throughput Sequencing Set
940-000828-00	FCL SE400	DNBSEQ-G400RS High-throughput Sequencing Set
940-000812-00	FCL PE100	DNBSEQ-G400RS High-throughput Sequencing Set
940-000810-00	FCL PE150	DNBSEQ-G400RS High-throughput Sequencing Set
940-000814-00	FCL PE200	DNBSEQ-G400RS High-throughput Sequencing Set
940-000831-00	Small RNA FCL SE50	DNBSEQ-G400RS High-throughput Sequencing Set
940-000822-00	stLFR FCL PE100	DNBSEQ-G400RS High-throughput Sequencing Set
940-000824-00	FCS SE100	DNBSEQ-G400RS High-throughput Rapid Sequencing Set
940-000820-00	FCS PE100	DNBSEQ-G400RS High-throughput Rapid Sequencing Set
940-000818-00	FCS PE150	DNBSEQ-G400RS High-throughput Rapid Sequencing Set
940-000816-00	FCS PE300	DNBSEQ-G400RS High-throughput Rapid Sequencing Set

Introduction

This quick guide provides concise instructions for operating the DNBSEQ-G400RS.

WARNING

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

Preparing DNBs

Input circular ssDNA library requirement

DNB preparation starts from a circular ssDNA library, for libraries other than stLFR libraries, the recommended insert size ranges between 20 bp and 800 bp. If the library concentration is not known, you can use the Qubit ssDNA Assay Kit and Qubit Fluorometer to measure it. Typical library requirements are listed in the following table.

Library type	Minimum concentration	100* μL reaction		50** μL reaction	
		fmol	V (μL)	fmol	V (μL)
General libraries (WGS, WES, RNASeq...)	2 fmol/μL	40	40/C***	20	20/C
Small RNA libraries	3 fmol/μL	60	60/C	30	30/C
PCR free libraries	3.75 fmol/μL	75	75/C	37.5	37.5/C

For the circular ssDNA library requirement of stLFR FCL PE100, the recommended insert size ranges between 200 bp and 1500 bp. If there are any special requirements or specifications for the library preparation kit, then the requirements of the kit should be followed.

Library type	Minimum concentration	80 μL reaction	
		ng	V (μL)
stLFR libraries	1.5 ng/μL	20	20/C

* 100 μL DNB reaction for loading with sequencer.
** 50 μL DNB reaction for loading with DL-200H.
*** C is the library concentration in fmol/μL.

DNBs can be loaded by either sequencer or DL-200H:

- Loading DNBs with sequencer: All four lanes within a flow cell must be loaded with the same tube of DNBs. One flow cell requires 2 × 100 μL of DNB making reaction.
- Loading DNBs with DL-200H: Different DNBs can be loaded onto different lanes of the flow cell. Each lane requires 50 μL of DNB making reaction.

Making DNBs

You can choose to thaw the flow cell and the sequencing reagent cartridge before making DNBs. For specific thaw times, refer to *Preparing the flow cell* on *Page 3* and *step 1* in *Preparing the sequencing reagent cartridge* on *Page 5*.

Making DNBs for FCL SE50, FCL SE100, FCL SE400, FCL PE100, FCL PE150, FCL PE200, Small RNA FCL SE50, FCS SE100, FCS PE100, and FCS PE150

1. Place the libraries on ice until use.
2. Remove the Low TE Buffer, Make DNB Buffer, and Stop DNB Reaction Buffer from storage and thaw reagents at room temperature.
3. Remove Make DNB Enzyme Mix I from storage and thaw it on ice for approximately 0.5 hours.
4. After thawing, vortex for 5 seconds, centrifuge briefly, and place on ice until use.
5. Use a 0.2 mL 8-strip tube or PCR tubes. Prepare the Make DNB reaction mixture 1 according to the table below:

Component	Volume for 100 μL DNB reaction (μL)	Volume for 50 μL DNB reaction (μL)
Low TE buffer	20-V	10-V
Make DNB buffer	20	10
ssDNA libraries	V	V
Total volume	40	20

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

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

8. Take out the Make DNB Enzyme Mix II (LC) from storage and place on ice. Centrifuge briefly for 5 seconds and place on ice.

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

Make DNB reaction mixture 2		
Component	**Volume for 100 μL DNB reaction (μL)**	**Volume for 50 μL DNB reaction (μL)**
Make DNB Enzyme Mix I	40	20
Make DNB Enzyme Mix II (LC)	4	2

10. Add all the Make DNB reaction mixture 2 into the Make DNB reaction mixture 1. Mix the reaction mixture thoroughly using a vortex mixer and centrifuge it for 5 seconds.
11. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

Rolling circle replication conditions	
Heated lid (35 °C)	On
30 °C	25 min
4 °C	Hold

12. 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	**Volume for 100 μL DNB reaction (μL)**	**Volume for 50 μL DNB reaction (μL)**
Stop DNB Reaction Buffer	20	10
Final volume	104	52

13. Store DNBs at 4 °C and perform sequencing within 48 hours.

Making DNBs for FCS PE300

1. Place the libraries on ice until use.
2. Remove the Low TE Buffer, Make DNB Buffer, and Stop DNB Reaction Buffer from storage and thaw reagents at room temperature.
3. Remove Make DNB rapid Enzyme Mix II from storage and thaw it on ice for approximately 0.5 hours.
4. After thawing, vortex for 5 seconds, centrifuge briefly, and place on ice until use.
5. Use a 0.2 mL 8-strip tube or PCR tubes. Prepare the Make DNB reaction mixture 1 according to the table below:

Component	**Volume for 90 μL DNB reaction (μL)**	**Volume for 45 μL DNB reaction (μL)**
Low TE buffer	20-V	10-V
Make DNB buffer	20	10
ssDNA libraries	V	V
Total volume	40	20

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

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

8. Take out the Make DNB Enzyme Mix II (LC) from storage and place on ice. Centrifuge briefly for 5 seconds and place on ice.

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

Make DNB reaction mixture 2		
Component	**Volume for 90 μL DNB reaction (μL)**	**Volume for 45 μL DNB reaction (μL)**
Make DNB rapid Enzyme Mix II	40	20
Make DNB Enzyme Mix II (LC)	1.6	0.8

10. Add all the Make DNB reaction mixture 2 into the Make DNB reaction mixture 1. Mix the reaction mixture thoroughly using a vortex mixer and centrifuge it for 5 seconds.
11. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

Rolling circle replication conditions	
Heated lid (35 °C)	On
30 °C	15 min
4 °C	Hold

12. Immediately add the Stop DNB Reaction Buffer when the temperature reaches 2 °C to 8 °C. Mix gently by pipetting 8 times by using a wide-bore, non-filtered pipette tip.

Component	**Volume for 90 μL DNB reaction (μL)**	**Volume for 45 μL DNB reaction (μL)**
Stop DNB Reaction Buffer	10	5
Final volume	91.6	45.8

13. Perform the next step: *Quantifying DNBs* immediately.

Making DNBs for stLFR FCL PE100

1. Place the libraries on ice until use.
2. Remove the Low TE Buffer, stLFR Make DNB Buffer, and Stop DNB Reaction Buffer from storage and thaw reagents at room temperature.
3. Remove Make DNB Enzyme Mix III from storage and thaw it on ice for approximately 0.5 hours.
4. After thawing, vortex for 5 seconds, centrifuge briefly, and place on ice until use.
5. Use a 0.2 mL 8-strip tube or PCR tubes. Prepare the Make DNB reaction mixture 1 according to the table below:

Component	**Volume (μL)**
Low TE buffer	16-V
stLFR Make DNB Buffer	16
dsDNA libraries	V
Total volume	32

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

Primer hybridization reaction conditions	
Heated lid (105 °C)	On
95 °C	3 min
40 °C	3 min
4 °C	Hold

8. Take out the Make DNB Enzyme Mix IV from storage and place on ice. Centrifuge briefly for 5 seconds and place on ice.
9. Remove the PCR tube from the thermal cycler when the temperature reaches 4 °C. Centrifuge briefly for 5 seconds, place the tube on ice, and prepare the Make DNB reaction mixture 2 according to the table below.

Make DNB reaction mixture 2	
Component	**Volume (μL)**
Make DNB Enzyme Mix III	32.0
Make DNB Enzyme Mix IV	3.2

10. Add all the Make DNB reaction mixture 2 into the Make DNB reaction mixture 1. Mix the reaction mixture thoroughly using a vortex mixer and centrifuge it for 5 seconds.
11. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

Rolling circle replication conditions	
Heated lid (35 °C)	On
30 °C	30 min
4 °C	Hold

12. Immediately add 16 µL of 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	Volume (µL)
Stop DNB Reaction Buffer	16
Final volume	83.2

13. Store DNBs at 2 °C to 8 °C and perform sequencing within 48 hours.

Quantifying DNBs

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

- For FCL SE50, FCL SE100, FCL SE400, FCL PE100, FCL PE150, FCL PE200, Small RNA FCL SE50, FCS SE100, FCS PE100, and FCS PE150, if the concentration is lower than 12 ng/µL, re-make the DNBs.
- For FCS PE300, if the concentration is lower than 8 ng/µL, re-make the DNBs.
- For stLFR FCL PE100, if the concentration is lower than 6 ng/µL, re-make the DNBs.
- If the concentration exceeds 40 ng/µL, the DNBs should be diluted to 20 ng/µL using DNB Load Buffer I.

i For FCS PE300, use Low TE Buffer to dilute the DNBs.

Preparing the flow cell and the sequencer

Preparing the flow cell

Take out the flow cell from the package, and place at room temperature for 1 hour to 24 hours. Before DNB loading, remove the flow cell from the inner package.

Preparing the sequencer

1. Power on the device.
2. Log into the control software.
3. Perform a pre-run wash.

Loading DNBs

i Do not centrifuge, vortex, or shake the DNB tube.

If 4 lanes within a flow cell require different samples, select DL-200H to load DNBs. Otherwise, choose the sequencer.

Loading DNBs by the sequencer

1. Depending upon the sequencing read length, remove the following reagents from storage, and thaw the reagents on ice for approximately 0.5 hours.

Model	Component
FCL SE50, FCL SE100, FCL SE400, FCL PE100, FCL PE150, FCL PE200, Small RNA FCL SE50, FCS SE100, FCS PE100, FCS PE150	DNB Load Buffer II
FCS PE300	DNB Load Buffer IV
stLFR FCL PE100	DNB Load Buffer II

2. After thawing, mix the reagents by using a vortex mixer for 5 seconds, centrifuge briefly, and place on ice until use.
3. Add the following reagents to a Micro Tube 0.5 mL (Empty) according to different sequencing read length:

Model	Component	Volume (μL)	
		FCL (whole flow cell)	FCS (whole flow cell)
FCL SE50, FCL SE100, FCL SE400, FCL PE100, FCL PE150, FCL PE200, Small RNA FCL SE50, FCS SE100, FCS PE100, FCS PE150	DNB Load Buffer II	64	32
	Make DNB Enzyme Mix II (LC)	2	1
	DNBs	200	100
	Total volume	266	133
FCS PE300	DNB Load Buffer IV	/	45
	DNBs	/	90
	Total volume	/	135
stLFR FCL PE100	DNB Load Buffer II	64.0	/
	Make DNB Enzyme Mix IV	2.5	/
	DNBs	200.0	/
	Total volume	266.5	/

4. Combine the components and mix by gently pipetting 8 times using a wide-bore, non-filtered pipette tip. Store the mixture at 2 °C to 8 °C until use.
5. Load the DNB loading mixture tube to the DNB loading position.
6. Select the DNB ID and choose **DNBs loading** from the user interface.
7. Select the required sequencing recipe from the drop-down list and perform the next step: *Preparing the sequencing reagent cartridge*.

Loading DNBs with DL-200H

1. Add the following reagents to a new PCR 8-strip tube:

Model	Component	Volume (μL)	
		FCL (whole flow cell)	FCS (whole flow cell)
FCL SE50, FCL SE100, FCL SE400, FCL PE100, FCL PE150, FCL PE200, Small RNA FCL SE50, FCS SE100, FCS PE100, FCS PE150	DNB Load Buffer II	8	8
	Make DNB Enzyme Mix II (LC)	0.25	0.25
	DNBs	25	25
	Total volume	33.25	33.25
FCS PE300	DNB Load Buffer IV	/	11.5
	DNBs	/	22.5
	Total volume	/	34
stLFR FCL PE100	DNB Load Buffer II	8.0	/
	Make DNB Enzyme Mix IV	0.31	/
	DNBs	25.00	/
	Total volume	33.31	/

2. Combine the components and mix by gently pipetting 8 times using a wide-bore, non-filtered pipette tip. Store the mixture at 4 °C until use.
3. Install the sealing gasket and flow cell. Ensure that the label of the flow cell is facing up and in the same position as the sealing gasket.
4. Place the device on the laboratory bench with the back up.

5. Aspirate 30 µL of DNB loading mixture 2 with a wide-bore, non-filtered pipette tip.
6. Insert the tip into the fluidics inlet. Eject the tip from the pipette. The DNBs automatically flow into the flow cell.
7. Ensure that all DNBs flow into the flow cell. While holding the device, rotate the pipette tip counterclockwise and then remove it.

   i

   - To load the DNBs to the rest of the lanes of the flow cell, refer to step 4 through 7.
   - Ensure that you load DNBs to Lane No. 1 to Lane No. 4 of the flow cell in ascending order.
8. Place DL-200H on the bench with the front up. Wait for 30 minutes in room temperature after the DNBs has finished loading. During the waiting time, you can perform the next step: *Preparing the sequencing reagent cartridge*.

Preparing the sequencing reagent cartridge

1. Remove the Sequencing Reagent Cartridge from storage, and thaw it in a 2 °C to 8 °C refrigerator before use or in a water bath at room temperature until completely thawed. The approximate time to thaw is listed in the table below:

Approximate thaw times for various sequencing kits			
Model	Method		
	Water bath at room temperature (hours)	Refrigerator at 2 °C to 8 °C overnight then water bath at room temperature (hours)	Refrigerator at 2 °C to 8 °C (hours)
FCL SE50	2.0	0.5	24.0
FCL SE100	2.0	0.5	24.0
FCL SE400	8.0	3.0	48.0
FCL PE100	3.0	1.5	36.0
FCL PE150	5.0	2.0	48.0
FCL PE200	6.0	3.5	48.0
FCS SE100	1.0	0.5	24.0
FCS PE100	2.0	0.5	36.0
FCS PE150	3.0	1.5	36.0
FCS PE300	6.0	3.5	48.0
stLFR FCL PE100	3.0	1.5	36.0

2. Invert the cartridge 3 times to mix before use. Shake the cartridge vigorously 20 times in a clockwise and counterclockwise direction. Ensure that all reagents are fully mixed. Wipe any water condensation from the cartridge cover and well surround with a Kimwipes tissue.
3. Prepare well No. 1 and well No. 2:
   1) Remove the dNTPs Mix and dNTPs Mix II from storage and thaw them at room temperature. Store at 2 °C to 8 °C refrigerator until use. Remove the Sequencing Enzyme Mix from storage and place on ice until use.According to the following table, premix dNTPs Mix or dNTPs Mix II and Sequencing Enzyme Mix in a new appropriate tube, then add all the mixed reagents to well No. 1 or well No. 2. Seal the loading wells of well No. 1 and well No. 2 with the transparent sealing film provided in the kit.

Model	Well No. 1		Well No. 2	
	dNTPs Mix (mL)	Sequencing Enzyme Mix (mL)	dNTPs Mix II (mL)	Sequencing Enzyme Mix (mL)
FCL SE50	0.700	0.700	0.600	0.600
FCL SE100	1.100	1.100	0.900	0.900
FCL SE400	4.000	4.000	12.000	4.000
FCL PE100	1.800	1.800	1.500	1.500
FCL PE150	2.400	2.400	2.100	2.100
FCL PE200	3.800	3.800	5.700	3.800
FCL SE50 (small RNA)	0.700	0.700	0.600	0.600
FCS SE100	0.800	0.800	1.600	0.800
FCS PE100	1.400	1.400	2.800	1.400
FCS PE150	1.900	1.900	3.800	1.900
FCS PE300	3.800	3.800	5.700	3.800
stLFR FCL PE100	2.000	2.000	1.700	1.700

2) Use your fingers to press the sealing film onto the well No. 1 and well No. 2. Ensure that there are no air bubbles between the sealing film and the surface of the cartridge to prevent reagents from flowing through the cartridge.
3) Lift the cartridge horizontally, and hold both sides of the cartridge with both hands. Shake the cartridge 20 times in a clockwise and counterclockwise direction. Ensure that the reagents are fully mixed.
4) Carefully remove the seals from the loading wells after thorough mixing.

4. Prepare well No. 7 and well No. 15:
   - For PE sequencing, prepare well No. 15: Add 500 µL of MDA Enzyme Mix to the MDA Reagent tube. Mix the reagent by inverting the tube 6 times. Transfer all the mixture to well No. 15. Ensure that no bubbles exist at the bottom of the tube.
   - For small RNA sequencing, prepare well No. 7: Thaw and vortex the Wash Buffer For Small RNA Sequencing thoroughly, and add 4.50 mL to well No. 7.
   - For SE400 sequencing, prepare well No. 7: Thaw and vortex the Wash Buffer For Sequencing thoroughly, and add 2.70 mL to well No. 7.

Performing a sequencing run

1. Select DNB ID from the user interface.
2. Select Recipe: Select the sequencing recipe from the **Recipe** list. One-click sequencing runs (such as SE50, etc.) and user-customized run (Customize) options are available. For Dual Barcode sequencing, select **Customize** from the **Recipe** list.
3. Place the reagent cartridge into the compartment. Use the barcode reader to scan the reagent cartridge ID, or manually enter the reagent cartridge ID. Close the reagent compartment door.
4. Clean the flow cell stage and place the flow cell onto the stage. Ensure that the flow cell is properly seated on the flow cell stage. The flow cell ID can be entered automatically with the barcode scanner, or it can be entered manually.
5. Close the flow cell compartment door.

Starting sequencing

1. Carefully check each item in the review interface and ensure that all parameters are correct.
2. Click **Start** > **Yes** to start sequencing.

Processing data

After sequencing starts, the sequencing results generated by the control software will appear on the **D** drive, including a **Data** folder and a **Result** folder.

Maintenance

Maintaining the devices

Wash the device within 24 hours after the sequencing run.

1. Perform sequencer maintenance with a regular wash.
2. DL-200H maintenance: Wipe all sides of the device with a low- lint cloth moistened with 75% ethanol, then wipe with a low-lint cloth moistened with ultra-pure water. Dry the device with a dry, low-lint cloth or let it air-dry.
3. Sealing gasket maintenance:
   1) Collect the used sealing gasket into a 200 mL beaker.
   2) Fill the beaker with ultra-pure water and wash the sealing gasket in the beaker, and empty the beaker after wash. Repeat the wash for 2 times.
   3) Fill the ultrasonic cleaner tank with ultra-pure water, and wash the sealing gasket in the ultrasonic cleaner tank for approximately 15 minutes.
   4) Repeat step 2), place the cleaned sealing gasket into a clean container and let it air-dry.
   5) (Optional) Replace with a new sealing gasket (Cat. No.: 510-003139-00) after cleaning for 20 times or every 3 months, depending on the sequencing frequency.

Disposing of the sequencing reagent cartridge and flow cell

1. After the sequencing run, open the reagent compartment door, and remove the used cartridge and flow cell from the device.
2. Discard the sequencing waste, tube, flow cell, and sequencing reagent cartridge in accordance with 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 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.

---This page is intentionally left blank.---