Application scope

Cat. No.	Model	Name	Specification
940-000830-00	FCL SE50	DNBSEQ-G400RS High-throughput Sequencing Set	70 Cycles/Set
940-000826-00	FCL SE100	DNBSEQ-G400RS High-throughput Sequencing Set	120 Cycles/Set
940-000828-00	FCL SE400	DNBSEQ-G400RS High-throughput Sequencing Set	420 Cycles/Set
940-000812-00	FCL PE100	DNBSEQ-G400RS High-throughput Sequencing Set	220 Cycles/Set
940-000810-00	FCL PE150	DNBSEQ-G400RS High-throughput Sequencing Set	320 Cycles/Set
940-000814-00	FCL PE200	DNBSEQ-G400RS High-throughput Sequencing Set	420 Cycles/Set
940-000831-00	Small RNA FCL SE50	DNBSEQ-G400RS High-throughput Sequencing Set	70 Cycles/Set
940-000824-00	FCS SE100	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	120 Cycles/Set
940-000820-00	FCS PE100	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	220 Cycles/Set
940-000818-00	FCS PE150	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	320 Cycles/Set
940-000816-00	FCS PE300	DNBSEQ-G400RS High-throughput Rapid Sequencing Set	620 Cycles/Set
940-000822-00	stLFR FCL PE100	DNBSEQ-G400RS High-throughput Sequencing Set	252 Cycles/Set
940-000916-00	/	DNBSEQ-G400RS High-throughput Sequencing Primer Kit (App-D) (Single-End)	1 Rxn/Kit
940-000917-00	/	DNBSEQ-G400RS High-throughput Sequencing Primer Kit (App-D) (Paired-End)	1 Rxn/Kit
940-001749-00	/	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode)	4 Rxn/Kit
940-001750-00	/	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode)	4 Rxn/Kit
940-001648-00	/	DNBSEQ OneStep Library Conversion Kit (Third party)	4 Rxn/Kit
940-002192-00	/	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode)	20 Rxn/Kit
940-002193-00	/	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode)	20 Rxn/Kit
940-002195-00	/	DNBSEQ OneStep Library Conversion Kit (Third party)	20 Rxn/Kit

- DNBSEQ High-throughput Sequencing Primer Kit (App-D) (Single-End) is suitable for SE single barcode and SE dual barcode sequencing of CG libraries and third-party libraries with TruSeq and Nextera adapters.
- DNBSEQ High-throughput Sequencing Primer Kit (App-D) (Paired-End) is suitable for PE single barcode and PE dual barcode sequencing of CG libraries and third-party libraries with TruSeq and Nextera adapters.
- DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) requires the single barcode dsDNA libraries with CG adapters.
- DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) requires the dual barcode dsDNA libraries with CG adapters.
- DNBSEQ OneStep Library Conversion Kit (Third party) requires dsDNA libraries that include either TruSeq or Nextera adapters.

Introduction

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

⚠ WARNING

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

Preparing the sequencer

1. Connect the device to the main power supply.
2. Turn the power switch to the | position.
3. Log in to the computer with the account *Zebra* and password *123*.
4. Log in to the control software with the username *user* and password *Password123*.
5. Check the remaining storage drive space and the waste container.
6. Before each sequencing run, perform a pre-run wash. For details, refer to *Performing a sequencer wash on Page 16*.

Preparing the Sequencing Reagent Cartridge-Part 1

1. Remove the Sequencing Reagent Cartridge from storage.
2. Thaw in a water bath at room temperature until completely thawed or thaw in a 2 °C to 8 °C refrigerator 1 to 2 days in advance. The approximate time to thaw is listed in the following table. Store in a 2 °C to 8 °C refrigerator until use.

Approximate thawing time for various sequencing kits

Model	Method		
	Water bath at room temperature (h)	Refrigerate at 2°C to 8°C overnight, then water bath at room temperature (h)	Refrigerate at 2°C to 8°C (h)
FCL SE50	2.0	0.5	24.0
FCL SE100	2.0	0.5	24.0
FCL SE400	8.0	3.0	48.0
FCL PE100	3.0	1.5	36.0
FCL PE150	5.0	2.0	48.0
FCL PE200	6.0	3.5	48.0
FCS SE100	1.0	0.5	24.0
FCS PE100	2.0	0.5	36.0
FCS PE150	3.0	1.5	36.0
FCS PE300	6.0	3.5	48.0
stLFR FCL PE100	3.0	1.5	36.0

Preparing the flow cell

1. Remove the box containing the flow cell from storage and take out the flow cell.
2. Place the flow cell at room temperature for 30 min to 24 h.
3. Unwrap the outer plastic packaging before use.
4. Take the flow cell out of the inner packaging and inspect it to ensure that it is intact and clean, without scratches.

Preparing DNBs

- App Make DNB Buffer can be used to make DNBs for both CG and third-party libraries.
- Mixed use of reagent components from different batches is not recommended.
- For transferring or mixing DNBs, use the wide-bore pipette tips.
- For other reagents, use a proper pipette tip according to the actual situation. It is recommended that you use the pipette tips from recommended brands and catalog numbers.

DNB library concentration and amount requirement

DNB preparation starts from either a circular ssDNA library or dsDNA library with a recommended insert size of between 20 bp and 800 bp. For stLFR libraries, the recommended insert size range is between 200 bp and 1500 bp. If the library concentration is not known, you can use the Qubit ssDNA Assay Kit/Qubit dsDNA HS Assay Kit and Qubit Fluorometer to measure it. Typical library requirements are listed in the following tables:

Circular ssDNA library concentration and amount requirement

Library type	Library adapter	Minimum concentration	100 μL reaction		50 μL reaction	
			fmol	V (μL)	fmol	V (μL)
PCR libraries	CG	2 fmol/μL	40	40/C*	20	20/C
PCR-free libraries		3.75 fmol/μL	75	75/C	37.5	37.5/C
Small RNA libraries		3 fmol/μL	60	60/C	30	30/C
Third-party PCR libraries	TruSeq, Nextera	3 fmol/μL	60	60/C	30	30/C
Third-party PCR-free libraries		3.75 fmol/μL	75	75/C	37.5	37.5/C

Circular ssDNA library concentration and amount requirement (FCS PE300)

Library type	Library adapter	Minimum concentration	90 μL reaction		45 μL reaction	
			fmol	V (μL)	fmol	V (μL)
PCR libraries	CG	2 fmol/μL	40	40/C	20	20/C
PCR-free libraries		3.75 fmol/μL	75	75/C	37.5	37.5/C
Third-party PCR libraries	TruSeq, Nextera	3 fmol/μL	60	60/C	30	30/C
Third-party PCR-free libraries		3.75 fmol/μL	75	75/C	37.5	37.5/C

dsDNA library concentration and amount requirement

Library type	Library adapter	Minimum concentration	100 μL reaction	
			fmol	V (μL)
PCR libraries	CG	6 fmol/μL	120	120/C
PCR-free libraries		3.5 fmol/μL	70	70/C
Third-party PCR libraries without 5'-Phosphorylation	TruSeq, Nextera	10.0 fmol/μL	200	200/C
Third-party PCR-free libraries without 5'-Phosphorylation		7.5 fmol/μL	150	150/C
Third-party PCR libraries with 5'-Phosphorylation		7.5 fmol/μL	150	150/C
Third-party PCR-free libraries with 5'-Phosphorylation		6.0 fmol/μL	120	120/C

dsDNA library concentration and amount requirement (stLFR FCL PE100)

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

* C in the tables above represents the concentration of libraries (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.
- Loading DNBs with DL-200H: Different DNBs can be loaded into different lanes of the flow cell.

Making DNBs for ssDNA libraries

Making DNBs (FCL SE50/FCL SE100/FCL SE400/FCL PE100/FCL PE150/FCL PE200/Small RNA FCL SE50/FCS SE100/FCS PE100/FCS PE150)

FCL PE150/FCL PE100/FCL SE100/FCL SE50/FCS PE150/FCS PE100/FCS SE100 are compatible with DNBSEQ High-throughput Sequencing Primer Kit (App-D).

1. Place the libraries on ice until use.
2. Remove Low TE Buffer, Make DNB Buffer, and Stop DNB Reaction Buffer from storage, and thaw the reagents at room temperature.

   For sequencing of third-party libraries, remove App Make DNB Buffer from the DNBSEQ High-throughput Sequencing Primer Kit (App-D) packaging.
3. Remove Make DNB Enzyme Mix I from storage, and thaw the reagent for approximately 30 min on ice .
4. Mix the reagents by using a vortex mixer for 5 s. Centrifuge briefly and place on ice until use.
5. Take out a 0.2 mL 8-strip tube or PCR tubes. Prepare Make DNB reaction mixture 1 according to different ssDNA libraries.

Make DNB reaction mixture 1 for CG libraries			
Component	Cap color	Volume of 100 μL DNB reaction (μL)	Volume of 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

Make DNB reaction mixture 1 for third-party libraries			
Component	Cap color	Volume of 100 μL DNB reaction (μL)	Volume of 50 μL DNB reaction (μL)
Low TE Buffer		20-V	10-V
App Make DNB Buffer		20	10
ssDNA libraries	/	V	V
Total Volume		40	20

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

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

8. Remove Make DNB Enzyme Mix II (LC) from storage and place it on ice. Centrifuge briefly for 5 s and hold on ice.

   - Do not keep Make DNB Enzyme Mix II (LC) at room temperature.
   - Avoid holding the tube for a prolonged time.
9. Remove the PCR tube from the thermal cycler when the temperature has reached 4 °C.
10. Centrifuge briefly for 5 s, place the tube on ice, and prepare Make DNB reaction mixture 2 according to the table below:

Make DNB reaction mixture 2			
Component	Cap color	Volume of 100 μL DNB reaction (μL)	Volume of 50 μL DNB reaction (μL)
Make DNB Enzyme Mix I		40	20
Make DNB Enzyme Mix II (LC)		4	2
Total Volume		44	22

11. Add all of Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer, and centrifuge for 5 s by using a mini spinner.
12. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

RCA (Rolling Circle Amplification) conditions	
Temperature	Time
Heated lid (35 °C)	On
30 °C	25 min
4 °C	Hold

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

Volume of Stop DNB Reaction Buffer			
Component	Cap color	Volume of 100 μL DNB reaction (μL)	Volume of 50 μL DNB reaction (μL)
Stop DNB Reaction Buffer		20	10

- It is very important to mix DNBs gently by using a wide-bore pipette tip. Do not centrifuge, vortex, or shake the tube.
- Store DNBs at 2 °C to 8 °C, and perform sequencing within 48 h.

14. Quantify the DNBs. For details, refer to *Quantifying DNBs on Page 10.*

Making DNBs (FCS PE300)

FCS PE300 is compatible with DNBSEQ High-throughput Sequencing Primer Kit (App-D).

1. Place the libraries on ice until use.
2. Remove Low TE Buffer, Make DNB Buffer, and Stop DNB Reaction Buffer from storage, and thaw the reagents at room temperature.

   For sequencing of third-party libraries, remove App Make DNB Buffer from the DNBSEQ High-throughput Sequencing Primer Kit (App-D) packaging.
3. Remove Make DNB rapid Enzyme Mix II from storage, and thaw the reagent for approximately 30 min on ice.
4. Mix the reagents by using a vortex mixer for 5 s. Centrifuge briefly and place on ice until use.
5. Take out a 0.2 mL 8-strip tube or PCR tubes. Prepare Make DNB reaction mixture 1 according to different ssDNA libraries.

Make DNB reaction mixture 1 for CG libraries of FCS PE300			
Component	Cap color	Volume of 90 μL DNB reaction (μL)	Volume of 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

Make DNB reaction mixture 1 for third-party libraries of FCS PE300			
Component	Cap color	Volume of 90 μL DNB reaction (μL)	Volume of 45 μL DNB reaction (μL)
Low TE Buffer		20-V	10-V
App Make DNB Buffer		20	10
ssDNA libraries	/	V	V
Total Volume		40	20

Do not discard the Low TE Buffer after you finish this step. The Low TE Buffer will be used in DNB dilution operations.

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

Primer hybridization reaction conditions for FCS PE300	
Temperature	Time
Heated lid (105 °C)	On
95 °C	1 min
65 °C	1 min
40 °C	1 min
4 °C	Hold

8. Remove Make DNB Enzyme Mix II (LC) from storage and place it on ice. Centrifuge briefly for 5 s by a mini spinner, and hold on ice.
9. Remove the PCR tube from the thermal cycler when the temperature has reached 4 °C.
10. Centrifuge briefly for 5 s by using a mini spinner, place the tube on ice, and prepare Make DNB reaction mixture 2 according to the table below.

Make DNB reaction mixture 2 for FCS PE300			
Component	Cap color	Volume of 90 μL DNB reaction (μL)	Volume of 45 μL DNB reaction (μL)
Make DNB rapid Enzyme Mix II		40	20
Make DNB Enzyme Mix II (LC)		1.6	0.8
Total Volume		41.6	20.8

11. Add all Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer, centrifuge for 5 s by using a mini spinner, and place it on ice until use.
12. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

RCA conditions for FCS PE300	
Temperature	Time
Heated lid (35 °C)	On
30 °C	15 min
4 °C	Hold

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

Volume of Stop DNB Reaction Buffer for FCS PE300			
Component	Cap color	Volume of 90 μL DNB reaction (μL)	Volume of 45 μL DNB reaction (μL)
Stop DNB Reaction Buffer		10	5

- Keep DNBs on ice during the entire operation to prevent DNBs from performing secondary replication.
- It is very important to mix DNBs gently by using a wide-bore pipette tip. Do not centrifuge, vortex, or shake the tube.
- This is not a STOP point and immediately go to the next step: *Quantifying DNBs on Page 10.*
- To ensure sequencing quality, it is recommended that you pool and load DNBs for FCS PE300 as soon as possible.

Making DNBs for dsDNA libraries

Making DNBs (FCL SE50/FCL SE100/FCL PE100/FCL PE150/FCS SE100/FCS PE100/FCS PE150)

1. Place the libraries on ice until use.
2. Remove reagents according to the following conditions:

- For CG single barcode libraries, remove Low TE Buffer, Make DNB Buffer (OS-SB), and Stop DNB Reaction Buffer from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) packaging, and thaw the reagents at room temperature.
- For CG dual barcode libraries, remove Low TE Buffer, Make DNB Buffer (OS-DB), and Stop DNB Reaction Buffer from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) packaging, and thaw the reagents at room temperature.
- For third-party libraries, remove Low TE Buffer, App Make DNB Buffer, and Stop DNB Reaction Buffer from the DNBSEQ OneStep Library Conversion Kit (Third party) packaging, and thaw the reagents at room temperature.

3. Remove Make DNB Enzyme Mix I (OS) from storage and thaw the reagent for approximately 30 min on ice.
4. Mix the reagents by using a vortex mixer for 5 s. Centrifuge briefly and place on ice until use.
5. Take out a 0.2 mL 8-strip tube or PCR tubes. Prepare Make DNB reaction mixture 1 according to different dsDNA libraries.

To make DNBs for third-party libraries, remove Conversion Enzyme from DNBSEQ OneStep Library Conversion Kit (Third party). Centrifuge briefly for 5 s, and place on ice until use.

Make DNB reaction mixture 1 for CG single barcode libraries		
Component	Cap color	Volume of 100 μL DNB reaction (μL)
Low TE Buffer		20-V
Make DNB Buffer (OS-SB)		20
dsDNA libraries	/	V
Total Volume		40

Make DNB reaction mixture 1 for CG dual barcode libraries		
Component	Cap color	Volume of 100 μL DNB reaction (μL)
Low TE Buffer		20-V
Make DNB Buffer (OS-DB)		20
dsDNA libraries	/	V
Total Volume		40

Make DNB reaction mixture 1 for third-party libraries		
Component	Cap color	Volume of 100 μL DNB reaction (μL)
Low TE Buffer		20-V
Make DNB Buffer (OS-App)		20
dsDNA libraries	/	V
Conversion Enzyme		0.5
Total Volume		40.5

6. Mix the reaction mixture thoroughly by using a vortex mixer. Centrifuge for 5 s by using a mini spinner, and place it on ice until use.
7. Place the mixture into a thermal cycler and start the primer hybridization reaction. The thermal cycler settings are shown in the tables below:

Primer hybridization reaction conditions for CG libraries	
Temperature	Time
Heated lid (105 °C)	On
95 °C	3 min
40 °C	3 min
4 °C	Hold

Primer hybridization reaction conditions for third-party libraries	
Temperature	Time
Heated lid (105 °C)	On
37 °C	5 min
95 °C	3 min
40 °C	3 min
4 °C	Hold

8. Remove Make DNB Enzyme Mix II (OS) from storage and place on ice. Centrifuge briefly for 5 s and place on ice.
9. Remove the PCR tube from the thermal cycler when the temperature has reached 4 °C.
10. Centrifuge briefly for 5 s, place the tube on ice, and prepare Make DNB reaction mixture 2 according to the table below:

Make DNB reaction mixture 2		
Component	Cap color	Volume of 100 μL DNB Reaction (μL)
Make DNB Enzyme Mix I (OS)		40
Make DNB Enzyme Mix II (OS)		2
Total Volume		42

11. Add all Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer, centrifuge for 5 s by using a mini spinner, and place it on ice until use.
12. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

RCA conditions	
Temperature	Time
Heated lid (35 °C)	On
30 °C	30 min
4 °C	Hold

13. Immediately add 20 µL of Stop DNB Reaction Buffer when the temperature reaches 4 °C. Mix gently by pipetting 8 times by using a wide-bore pipette tip.

Volume of Stop DNB Reaction Buffer		
Component	**Cap color**	**Volume of 100 µL DNB reaction (µL)**
Stop DNB Reaction Buffer		20

- It is very important to mix DNBs gently by using a wide-bore pipette tip. Do not centrifuge, vortex, or shake the tube.
- Store DNBs at 2 °C to 8 °C, and perform sequencing within 48 h.

14. Quantify the DNBs. For details, refer to *Quantifying DNBs on Page 10*.

Making DNBs (stLFR FCL PE100)

1. Place the libraries on ice until use.
2. Remove Low TE Buffer, stLFR Make DNB Buffer, and Stop DNB Reaction Buffer from storage, and thaw the reagents at room temperature.
3. Remove Make DNB Enzyme Mix III from storage, and thaw it on ice for approximately 30 min.
4. Mix all the reagents by using a vortex mixer for 5 s. Centrifuge briefly by using a mini spinner, and place it on ice until use.
5. Take out a 0.2 mL 8-strip tube or PCR tubes. Prepare Make DNB reaction mixture 1 according to the table below:

Make DNB reaction mixture 1 for stLFR FCL PE100		
Component	**Cap color**	**Volume of 80 µL DNB reaction (µL)**
Low TE Buffer		16-V
stLFR Make DNB Buffer		16
dsDNA libraries	/	V
Total Volume		32

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

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

8. Remove Make DNB Enzyme Mix IV from storage and place it on ice. Centrifuge briefly for 5 s and hold on ice.
9. Remove the PCR tube from the thermal cycler when the temperature has reached 4 °C.
10. Centrifuge briefly for 5 s, place the tube on ice, and prepare Make DNB reaction mixture 2 according to the table below:

Make DNB reaction mixture 2		
Component	**Cap color**	**Volume of 100 µL DNB Reaction (µL)**
Make DNB Enzyme Mix III		32.0
Make DNB Enzyme Mix IV		3.2
Total Volume		35.2

11. Add all of Make DNB reaction mixture 2 into Make DNB reaction mixture 1. Mix the reaction mixture thoroughly by using a vortex mixer, centrifuge for 5 s by using a mini spinner, and place it on ice until use.
12. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the table below:

RCA conditions	
Temperature	**Time**
Heated lid (35 °C)	On
30 °C	30 min
4 °C	Hold

13. Immediately add 16 µL of Stop DNB Reaction Buffer to the tube when the temperature reaches 4 °C. Mix gently by pipetting 8 times by using a wide-bore pipette tip.

Volume of Stop DNB Reaction Buffer		
Component	**Cap color**	**Volume of 80 µL DNB reaction (µL)**
Stop DNB Reaction Buffer		16

- It is very important to mix DNBs gently by using a wide-bore pipette tip. Do not centrifuge, vortex, or shake the tube.
- Store DNBs at 2 °C to 8 °C, and perform sequencing within 48 h.

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 less than 8 ng/µL, re-make the DNBs.
- For FCS PE300, if the concentration is less than 8 ng/µL, re-make the DNBs.
- For stLFR FCL PE100, if the concentration is less 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.

- For FCS PE300, use Low TE Buffer to dilute the DNBs.
- For DNBSEQ OneStep DNB Make Reagent Kit V4.0/DNBSEQ OneStep Library Conversion Kit (Third party), DNB dilution is not mandatory.

Loading DNBs

If you load DNBs by the sequencer, prepare the sequencing cartridge first according to *Preparing the Sequencing Reagent Cartridge-Part 2 on Page 13*, then proceed to the loading process.

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 30 min.

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. Mix the reagents by using a vortex mixer for 5 s, centrifuge briefly by using a mini spinner, and place it on ice until use.

3. Add the following reagents to a 0.5 mL microcentrifuge tube according to different sequencing read lengths:

DNB loading mixture 1			
Model	Component	Volume (μL)	
		FCL	FCS
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	/

For FCL PE150/FCL PE100/FCL SE100/FCL SE50/FCS PE150/FCS PE100/FCS SE100, if the DNBSEQ OneStep DNB Make Reagent Kit is used for making DNB, prepare DNB loading mixture 1 according to the table below:

DNB loading mixture 1			
Model	Component	Volume (μL)	
		FCL	FCS
FCL SE50, FCL SE100, FCL PE100, FCL PE150, FCS SE100, FCS PE100, FCS PE150	DNB Load Buffer II	64	32
	Make DNB Enzyme Mix II (OS)	2	1
	DNBs	200	100
	Total Volume	266	133

4. Combine the components and mix by gently pipetting 8 times using a wide-bore pipette tip. Store the mixture at 2 °C to 8 °C until use.

- Do not centrifuge, vortex, or shake the DNB tube.
- Prepare a fresh DNB loading mixture 1 immediately before the sequencing run.
- Each FCL requires 266 μL of DNB loading mixture 1, and each FCS requires 133 μL of DNB loading mixture 1.

5. Open the reagent compartment door.
6. Load the DNB loading mixture tube to the DNB loading position.
7. Close the reagent compartment door.
8. Select the **DNB loading** box in the DNB ID entry interface.

Loading DNBs by DL-200H

1. Take out a new 1.5 mL sterile microcentrifuge tube or 0.5 mL sterile microcentrifuge tube and add the reagents by model as shown in the table below:

DNB loading mixture 2			
Model	Component	Volume (μL)	
		FCL	FCS
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	/

For FCL PE150/FCL PE100/FCL SE100/FCL SE50/FCS PE150/FCS PE100/FCS SE100, if the DNBSEQ OneStep DNB Make Reagent Kit is used for making DNB, prepare DNB loading mixture 2 according to the table below:

DNB loading mixture 2			
Model	Component	Volume (μL)	
		FCL	FCS
FCL SE50, FCL SE100, FCL PE100, FCL PE150, FCS SE100, FCS PE100, FCS PE150	DNB Load Buffer II	8	8
	Make DNB Enzyme Mix II (OS)	0.25	0.25
	DNBs	25	25
	Total Volume	33.25	33.25

2. Combine the components and mix by gently pipetting 8 times using a wide-bore pipette tip. Store the mixture at 2 °C to 8 °C until use.

   - Do not centrifuge, vortex, or shake the DNB tube.
   - Prepare a fresh DNB loading mixture 2 immediately before the sequencing run.
   - Each lane requires at least 30 μL of DNB loading mixture 2.

3. Install the sealing gasket and the 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 DL-200H on the laboratory bench with the back facing up. Aspirate 30 μL of DNB loading mixture 2 with a wide-bore pipette tip, and insert the tip into the fluidics inlet. Eject the tip from the pipette. DNBs will automatically flow into the flow cell.
5. Lift up the DL-200H, but do not tilt it (keep it parallel to the bench), and check whether the DNBs flow through the flow cell.
6. Ensure that all DNBs flow into the flow cell, and then hold the DL-200H and rotate the tip counterclockwise to remove it.

7. Repeat steps 4 through 6 to load the DNBs to the rest of the lanes of the flow cell. Ensure that you load DNBs from Lane No. 1 to Lane No. 4 in ascending order.
8. Place the DL-200H on the bench with the front facing up and wait for the DNB loading according to different sequencing:
   - For FCS PE300, it is recommended that you keep the flow cell in the DL-200H and place at 25 °C ± 2 °C for 60 to 90 min for the DNB loading process.
   - For other sequencing models, wait 30 min for the DNB loading process.
9. Open the cover and take out the flow cell and the sealing gasket.
10. After the DNB loading process has completed, immediately transfer the flow cell to the sequencer for sequencing. For details, refer to *Performing a sequencing run on Page 15*.

Preparing the Sequencing Reagent Cartridge-Part 2

1. Invert the cartridge 3 times to mix before use. Shake the cartridge vigorously clockwise 20 times, and then shake it counterclockwise 20 times. Ensure that all reagents are fully mixed. Wipe any water condensation from the cartridge cover and well surround with a KimWipes tissue.
2. Remove dNTPs Mix and dNTPs Mix II from storage 1 h in advance, and thaw them at room temperature. Store them at 2 °C to 8 °C until use.
3. Remove Sequencing Enzyme Mix from storage and place on ice until use.
4. Remove the reagents from storage according to your model:
   - For Small RNA FCL SE50 sequencing, remove Wash Buffer for Small RNA Sequencing from storage, and thaw it at room temperature. Store at 2 °C to 8 °C until use.
   - For FCL SE400 sequencing, remove Wash Buffer for Sequencing from storage, and thaw it at room temperature. Store at 2 °C to 8 °C until use.
   - For SE sequencing of third-party libraries, remove primers reagents from DNBSEQ High-throughput Sequencing Primer Kit (App-D) (Single-End) and thaw them at room temperature. Store at 2 °C to 8 °C until use.

     i
     - App-D Insert Primer 1 and App-D Barcode Primer 1 are for single barcode libraries.
     - App-D Insert Primer 1, App-D Barcode Primer 1, and App-D Barcode Primer 4 are for dual barcode libraries.
   - For PE sequencing:
     1) Remove MDA Reagent from storage and place on ice until use.
     2) For third-party libraries, remove primers reagents from DNBSEQ High-throughput Sequencing Primer Kit (App-D) (Pair-End) and thaw them at room temperature. Store at 2 °C to 8 °C until use.

        i
        - App-D Insert Primer 1, App-D Insert Primer 2, App-D MDA Primer, and App-D Barcode Primer 2 are for single barcode libraries.
        - App-D Insert Primer 1, App-D Insert Primer 2, App-D MDA Primer, App-D Barcode Primer 2, and App-D Barcode Primer 3 are for dual barcode libraries.
5. Prepare well No. 1 and well No. 2:
   1) Pierce the seals in the center of wells No. 1 and No. 2 to make a hole approximately 2 cm in diameter by using a 1 mL sterile pipette tip.

2) According to the following table, premix dNTPs Mix or dNTPs Mix II and Sequencing Enzyme Mix in a new sterile tube, then add all of 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.

Reagent preparation for well No. 1 and well No. 2				
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
Small RNA FCL SE50	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

3) Press the film around the well with your finger. Ensure that the well is tightly sealed and that there are no air bubbles between the film and cartridge surface. This ensure that the reagents will not flow over the cartridge.

4) 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.

5) Carefully remove the seals from the loading wells after fully mixing.

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

- The FCL SE50/FCL SE100/FCS SE100 Sequencing Reagent Cartridge is now ready for CG libraries sequencing.
- For the next step, refer to *Performing a sequencing run on Page 15*.

6. Perform the following steps according to different models:

- For Small RNA FCL SE50 sequencing:
  1) Mix Wash Buffer for Small RNA Sequencing by using a vortex mixer for 5 s and centrifuge briefly before use.
  2) Pierce the seal of well No. 7 and add 4.50 mL of Wash Buffer for Small RNA Sequencing. When adding the reagent, ensure that there are no bubbles at the bottom of the tube.
- For FCL SE400 sequencing:
  1) Mix Wash Buffer for Sequencing by using a vortex mixer for 5 s and centrifuge briefly before use.
  2) Pierce the seal of well No. 7 and add 2.70 mL of Wash Buffer for Sequencing. When adding the reagent, ensure that there are no bubbles at the bottom of the tube.
- For sequencing of third-party libraries (FCL SE50/FCL SE100/FCS SE100) :
  1) Pierce the foil seals with clean pipettes (Well No. 3 and No. 5 for single barcode sequencing; No. 3, No. 4, and No. 5 for dual barcode sequencing).
  2) Discard the reagents in each tube by using a pipette.
  3) Add the primers using appropriate pipettes according to the table below:

Reagent name	Well	Volume (mL)
App-D Insert Primer 1	No. 3	2.20
App-D Barcode Primer 4	No. 4	2.90
App-D Barcode Primer 1	No. 5	2.90

- For sequencing of third-party libraries (FCL PE100/FCL PE150/FCS PE100/FCS PE150/FCS PE300) :
  1) Pierce the foil seals with clean pipettes (Well No. 3, No. 6, No. 7, and No. 8 for single barcode sequencing; No. 3, No. 4, No. 6, No. 7, and No. 8 for dual barcode sequencing).
  2) Discard the reagents in each tube by using a pipette.
  3) Add the primers using appropriate pipettes according to the table below:

Reagent name	Well	Volume (mL)
App-D Insert Primer 1	No. 3	2.20
App-D Barcode Primer 3	No. 4	2.90
App-D Barcode Primer 2	No. 6	2.90
App-D MDA Primer	No. 7	3.10
App-D Insert Primer 2	No. 8	3.30

7. Perform the following steps for PE sequencing:
   1) Pierce the seal of well No. 15 by using a 1 mL sterile pipette tip.
   2) Add 0.5 mL of MDA Enzyme Mix II (FCL PE100/FCL PE150/stLFR FCL PE100/FCS PE100/FCS PE150) or MDA Enzyme Mix (FCL PE200/FCS PE300) to the MDA Reagent tube with a 1 mL pipette.
   3) Invert the tube 6 times to mix the reagents. Add the mixture to well No. 15. When adding the mixture, ensure that there are no bubbles at the bottom of the tube.

   i The FCL PE100/FCL PE150/stLFR FCL PE100/FCL PE200/FCS PE100/FCS PE150/FCS PE300 Sequencing Reagent Cartridge is now ready for use.

Performing a sequencing run

Selecting sequencing parameters

1. In the main interface, select **Sequence** to open the DNB ID entry interface.
2. Select the **DNB ID** box, and enter the DNB ID manually by using the on-screen keyboard.
3. Select a barcode range of different lanes from the list next to the **DNB ID** box.
4. Select an appropriate recipe from the **Recipe** list. One-click sequencing runs and user-customized run (Customize) are available. For Dual Barcode sequencing, select **Customize** from the **Recipe** list.

Loading the Sequencing Reagent Cartridge

1. Select the **Sequencing cartridge ID** field, manually enter the cartridge ID according to the serial number (SN) printed on the cartridge label, or use the barcode scanner to scan the cartridge barcode at the lower-right corner of the Sequencing Reagent Cartridge label.
2. Open the reagent compartment door and slowly remove the cleaning cartridge from the compartment.
3. Moisten a KimWipes tissue with laboratory-grade water and use it to wipe the bottom and sides of the compartment to keep it clean and dry.
4. Hold the handle of a new Sequencing Reagent Cartridge with one hand and place the other hand underneath for support. Slide the cartridge into the compartment.
5. Ensure that the cartridge is in the correct position and close the reagent compartment door.

Loading the flow cell

1. Open the flow cell compartment door.
2. Press both sides of the flow cell with one hand, and press the flow cell attachment button with the other hand.
3. After the vacuum is released, remove the washing flow cell from the stage.
4. Use a canned air duster to remove the dust from the flow cell stage and the back of the flow cell.
5. Take out a new flow cell or the loaded flow cell.
6. There are two alignment holes on the left side and one on the right side. The label is on the right. Hold the flow cell by the edges with both hands.
7. Align the holes on the flow cell with the locating pins on the flow cell stage. Gently slide the flow cell at an angle of 45 degrees to the upper-left corner to keep the flow cell aligned with the pin.
8. Press the flow cell attachment button. Press the left and right sides of the flow cell on the stage at the same time to ensure that the flow cell is properly seated on the stage.
9. Ensure that the negative pressure is within the range of -80 kPa to -99 kPa before continuing.
10. Use a canned air duster to remove the dust from the flow cell surface, and then close the flow cell compartment door.
11. Select **Next**. The flow cell ID can be entered using the barcode scanner.

    If automated entry does not work, move the cursor to the **Flow cell ID** box and enter the ID manually.
12. Select **Next**.

Starting sequencing

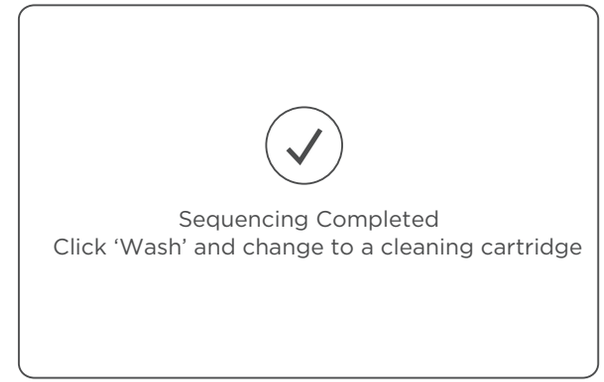
1. Carefully check each item in the Review interface and ensure that all parameters are correct.
2. Select **Start** > **Yes** in the pop-up dialog box to start sequencing.
3. When sequencing has started, immediately open the flow cell compartment door to inspect the flow cells, ensure that the DNBs or reagents are flowing through the flow cell, and close the compartment door.

Maintenance

Performing a sequencer wash

Preparing for a wash

1. When the sequencing run is completed, the device must be washed within 24 h. When the following interface appears, select **Wash** and perform the wash procedures.



2. Select the wash type according to specific situations.

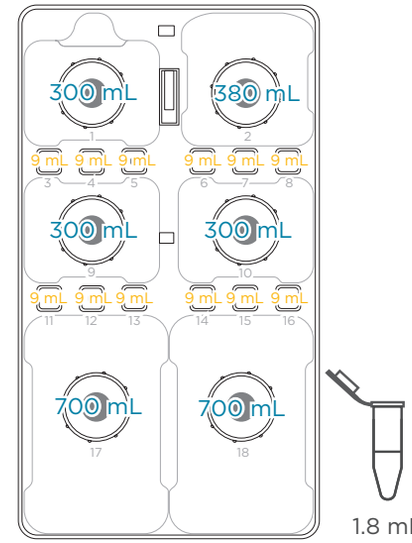
Wash types				
Wash type	**Wash selection**	**Cartridge type**	**DNB loading needle washing tube type**	**Description**
Pre-run wash	Regular (54 min)	Cleaning cartridge 1	DNB loading needle washing tube 1	• Before a sequencing run. • It has been more than 24 h since the last maintenance wash.
Maintenance wash	Maintenance (20 min)	Cleaning cartridge 3	DNB loading needle washing tube 3	• After a sequencing run. • Weekly if the sequencer has been used. • Biweekly if the sequencer is idle or powered off. • When impurities are visible in the image. • After the sequencer maintenance is performed by an engineer. This includes, but not limited to the replacement of pipelines, sample needles, and other accessories exposed to reagents.
	Maintenance (20 min)	Cleaning cartridge 2	DNB loading needle washing tube 2	
	Regular (54 min)	Cleaning cartridge 1	DNB loading needle washing tube 1	
DNBTube wash (Optional)	DNBTube (5 min)	Cleaning cartridge 1	DNB loading needle washing tube 3	If you need an extra wash for the DNB loading tubes after pre-wash and maintenance wash.
	DNBTube (5 min)		DNB loading needle washing tube 2	
	DNBTube (5 min)		DNB loading needle washing tube 1	

3. Prepare washing reagent according to the table and figures below:

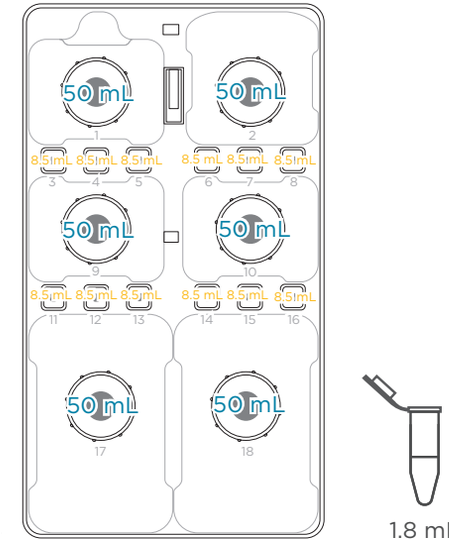
DNB loading needle wash tube type	Washing reagent	Cartridge type	Washing reagent
DNB loading needle washing tube 3	0.05% Tween-20	Cleaning cartridge 3	0.05% Tween-20; 1 M NaCl + 0.05% Tween-20
DNB loading needle washing tube 2	0.1 M NaOH	Cleaning cartridge 2	0.1 M NaOH
DNB loading needle washing tube 1	Laboratory-grade water	Cleaning cartridge 1	Laboratory-grade water

■ Cleaning cartridge 1 and 2

Cleaning cartridge 1 (Laboratory-grade water)

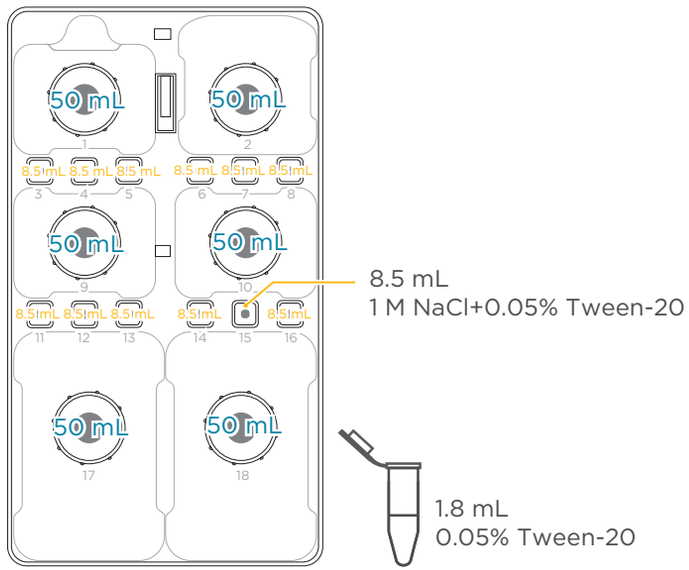


Cleaning cartridge 2 (0.1 M NaOH)



- Cleaning cartridge 3

Cleaning cartridge 3
(0.05% Tween-20/0.05% Tween-20+1 M NaCl)



2.0 mL sterile microcentrifuge tubes are used for DNB loading needle washing.

4. Prepare the washing flow cell.

- A used flow cell without physical damage can be used as a washing flow cell.
- Each washing flow cell, stored at room temperature or in a 2 °C to 8 °C refrigerator, can be reused 20 times.

Performing a wash

When the sequencing run is completed, the device must to be washed within 24 h.

1. Take out the prepared cleaning cartridge, DNB loading needle washing tube, and washing flow cell according to the selected wash type.
2. Slowly insert the cleaning cartridge into the reagent compartment, put DNB loading needle washing tube into the DNB tube rack, and close the compartment door.
3. Load the washing flow cell.
4. Select **Wash** when prompted after the sequencing is completed.
5. Select a wash type from the **Wash type** list, and select **Yes** to start the wash.

Maintaining the DL-200H and sealing gasket

WARNING

- Do not immerse the DL-200H into the liquid for cleaning. Doing so may damage the device.
- Do not use other disinfectants such as dichloroethane ($C_2H_4Cl_2$), trichloroethylene (C_2HCl_3), chloroform ($CHCl_3$), and toluene (C_7H_8) to clean the DL-200H. Doing so may damage the device.
- It is recommended that you replace the DL-200H (Cat. No.: 900-000218-00) with a new one after using it for one year.
- If you have questions about the compatibility of disinfectants, contact CG Technical Support.

After each DNB loading, perform the following steps to maintain the DL-200H and sealing gasket:

1. Wipe all sides of the device with a low-lint cloth moistened with 75% ethanol and a low-lint cloth moistened with ultrapure water.
2. Wipe the device with a low-lint cloth and let it air-dry.
3. Collect the used sealing gasket into a 200 mL beaker.
4. Fill the beaker with ultrapure water and wash the sealing gasket in the beaker, and then empty the beaker. Repeat the wash twice, for a total of 3 times.
5. Fill the ultrasonic cleaner tank with ultrapure water, and wash the sealing gasket in the ultrasonic cleaner tank for about 15 min.
6. Repeat step 4, place the cleaned sealing gasket into a clean container, and let it air-dry.
7. Replace with a new sealing gasket (Cat. No.: 510-003139-00) if any of the following occurs:
   - The sealing gasket has been cleaned 20 times.
   - The sealing gasket has been used for 3 months.
   - The pipette tip loosens during loading DNBs.

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.

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 questions, please contact Complete Genomics at +1 (888) 811-9644.

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