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DNBSEQ OneStep DNB Make Reagent Kit V4.0

Instructions for Use

For Research Use Only.
Complete Genomics, Inc.

Part No.: H-020-000848-00

About the Instructions for Use

CG intends to provide this product solely for research use.

This Instructions for Use is applicable to DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode), DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) and DNBSEQ OneStep Library Conversion Kit (Third party). The Instructions for Use version is 3.0.

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Revision history

	Date	Version
Initial release	November 20, 2023	1.0
Upgraded method for preparing DNB	May 10, 2024	2.0
Update applicable platforms	October 20, 2024	3.0

Contents

01

Kit overview

This chapter describes the intended use, working principle, sample requirements, kit information, and basic safety information about the kit. Carefully read and understand the information before use to ensure correct operations, best performance, and personnel safety. Keep this Instructions for Use at hand for reference at any time.

Intended use

WARNING **This kit is intended only for scientific research and should not be used for clinical diagnosis.**

This kit is used for generating DNA nanoball (DNB) through Rolling Circle Amplification (RCA), which is necessary for sample processing prior to sequencing on CG high-throughput platforms. This kit has been optimized for human whole genome sequencing (WGS) libraries. If you are using libraries from other applications, the quantity of input DNA into this kit may need optimization.

Sample requirements

This kit is applicable to CG dsDNA library and third party dsDNA library that include either TruSeq or Nextera adapters.

Working principle

This kit utilizes the principle of RCA to obtain DNA nanoballs from libraries in a one-reaction system.

Available kit list

- DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) requires single barcode dsDNA library with CG adapters.
- DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) requires dual barcode dsDNA library with CG adapters.
- DNBSEQ OneStep Library Conversion Kit (Third party) requires dsDNA library with either TruSeq or Nextera adapters.

Table 1 Available kit list

Catalog No.	Product name	Spec	Version
940-001749-00	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode)	4 Rxn/Kit	V4.0
940-001750-00	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode)	4 Rxn/Kit	V4.0

Catalog No.	Product name	Spec	Version
940-001648-00	DNBSEQ OneStep Library Conversion Kit (Third party)	4 Rxn/Kit	V2.0
940-002192-00	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode)	20 Rxn/Kit	V4.0
940-002193-00	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode)	20 Rxn/Kit	V4.0
940-002195-00	DNBSEQ OneStep Library Conversion Kit (Third party)	20 Rxn/Kit	V1.0

Supported platforms and read lengths

Table 2 Supported platforms and read lengths

Supported platforms	Supported read lengths
Gene Sequencer (DNBSEQ-G400RS)	FCL PE150 / FCL PE100 / FCL PE50 / FCL SE100 / FCL SE50 / FCS PE150 / FCS PE100 / FCS SE100
Genetic Sequencer (DNBSEQ-T7RS)	FCL PE150 / FCL PE100
Genetic Sequencer (DNBSEQ-G99RS)	FCL PE150 / FCL PE50 / FCL SE100

Biological safety

- Reagents and waste chemicals may cause personal injury through skin, eye, or mucosal contact. Follow the safety standards of your laboratory and wear protective equipment (such as a laboratory coat, protective glasses, a mask, gloves, and shoe covers) when using the kit.
- If you accidentally splash reagents or waste liquids on your skin or into your eyes, immediately flush the affected area with large amounts of water and seek medical aid immediately.
- When disposing of expired reagents, waste liquids, waste samples, and consumables, comply with local regulations.
- Use and store the reagents according to the instructions for use. Failure to do so may negatively impact performance.

- Check the expiration date of all reagents before use. Using expired reagents may cause inaccurate results.

User-supplied equipment and consumables

Before using the kit, prepare the following equipment:

Table 3 User-supplied equipment list

Equipment	Recommended brand
Freezer, -25 °C to -15 °C	General lab supplier
Refrigerator, 2 °C to 8 °C	General lab supplier
Ice bucket	General lab supplier
Pipette, 10 µL	Eppendorf or equivalent
Pipette, 20 µL	Eppendorf or equivalent
Pipette, 200 µL	Eppendorf or equivalent
Electronic pipette	Intergra or equivalent
Vortex mixer	General lab supplier
Qubit Fluorometer	Thermo Fisher
Thermal cycler	Bio-Rad or equivalent
Mini spinner	General lab supplier

It is recommended that you use the following reagents / consumables:

Tips are disposable consumables. Do not reuse them.

Table 4 Recommended reagent / consumable list

Reagent / Consumable	Recommended brand	Purpose
Sterile pipette tip (various types)	General lab supplier	Pipetting for diluting and loading wash and loading reagents
Sterile 200 µL wide-bore, non-filtered pipette tip	Axygen, Cat. No.: T-205-WB-C	Mixing DNBs
Qubit ssDNA Assay Kit	General lab supplier	Library and DNB QC
Qubit dsDNA Assay Kit	General lab supplier	Library QC
Qubit Assay Tubes	Thermo Fisher	Library and DNB QC
Sterile PCR 8-strip tube, 0.2 mL	Thermo Fisher	Making DNB Reaction Mixture
Sterile microcentrifuge tube, 1.5 mL	VWR, Cat. No.: 20170-038, or equivalent	Combining volumes when diluting library
Disposable gloves, powder-free	General lab supplier	General purpose

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02

Making DNBs

This chapter describes the recommended library insert size, DNA library amount, making DNBs and quantifying DNBs procedures. Read and follow the instructions to ensure correct operations.

Recommended library insert size

These kits are compatible with dsDNA libraries with either CG, TruSeq, or Nextera adapters. For optimal performance, it is recommended that the distribution of insert size lies within the range of 50 base pairs to 700 base pairs.

DNA library concentration and amount requirement

- The concentration of dsDNA library requirements:

Table 5 dsDNA library concentration requirements of dsDNA for DNBSEQ-G400RS/ DNBSEQ-G99RS

Type	Library type	dsDNA concentration requirements for DNBSEQ-G400RS (fmol/μL)	dsDNA concentration requirements for DNBSEQ-G99RS (fmol/μL)
OS-Single barcode / OS-Dual barcode	CG PCR	≥6.0	≥3.0
	CG PCR-free	≥3.5	≥1.75
Third party	PCR library without 5'-Phosphorylation	≥10.0	≥5.0
	PCR-free library without 5'-Phosphorylation	≥7.5	≥3.75
	PCR library with 5'-Phosphorylation	≥7.5	≥3.75
	PCR-free library with 5'-Phosphorylation	≥6.0	≥3.0

Table 6 dsDNA library concentration requirements of dsDNA for DNBSEQ-T7RS

Read lengths	Library type	dsDNA concentration requirements (fmol/μL)
FCL PE100	CG PCR	≥6.5
	CG PCR-free	≥4.5
	PCR library without 5-Phosphorylation	≥10.0
	PCR-free library without 5-Phosphorylation	≥7.5
	PCR library with 5-Phosphorylation	≥7.5
	PCR-free library with 5-Phosphorylation	≥5.0
FCL PE150	CG PCR	≥10.0
	CG PCR-free	≥6.5
	PCR library without 5-Phosphorylation	≥17.5
	PCR-free library without 5-Phosphorylation	≥12.5
	PCR library with5-Phosphorylation	≥12.5
	PCR-free library with 5-Phosphorylation	≥10.0

- The DNA library concentration (ng/μL) should be quantified using the dsDNA HS Assay Kit with the Qubit Fluorometer. The concentration will need to be converted from ng/μL to fmol/μL using the following formula:

$$C(\text{fmol}/\mu\text{L}) = \frac{3030 \times c(\text{ng}/\mu\text{L})}{N \times 2}$$

- N represents the average number of nucleotides within the DNA fragments (the length of which includes associated adapter sequences).
- c (ng/μL) represents the DNA library concentration.

- Example: If the necessary input of dsDNA is 100 fmol, the library input volume for each DNB making reaction is determined using the following formula:

$$V\ (\mu\text{L}) = \frac{100\ \text{fmol}}{C(\text{fmol}/\mu\text{L})}$$

Making DNBs

- For transferring or mixing DNBs, use the wide-bore pipette tips.
- For operating other reagents, use a proper pipette tip according to the actual situation. It is recommended that you use the pipette tips with recommended brands and catalog numbers.

Select the appropriate DNB making protocol according to the sequencing model. *Table 7* lists the sections that describe each protocol.

Table 7 DNB making protocol

Platform and read lengths	Reference process
DNBSEQ-G400RS	*Making DNBs for DNBSEQ-G400RS on Page 10*
DNBSEQ-T7RS FCL PE100	*Making DNBs for DNBSEQ-T7RS FCL PE100 on Page 18*
DNBSEQ-T7RS FCL PE150	*Making DNBs for DNBSEQ-T7RS FCL PE150 on Page 26*
DNBSEQ-G99RS	*Making DNBs for DNBSEQ-G99RS on Page 34*

Making DNBs for DNBSEQ-G400RS

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. For CG single barcode libraries, remove Make DNB Buffer (OS-SB) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) and thaw the reagent at room temperature.
3. For CG dual barcode libraries, remove Make DNB Buffer (OS-DB) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) and thaw the reagent at room temperature.
4. For third party libraries, remove Make DNB Buffer (OS-App) from DNBSEQ OneStep Library Conversion Kit (Third party) and thaw the reagent at room temperature.
5. Remove Low TE Buffer and Stop DNB Reaction Buffer from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) or DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) or DNBSEQ OneStep Library Conversion Kit (Third party) and thaw the reagents at room temperature.

6. Remove Make DNB Enzyme Mix I (OS) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) or DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) or DNBSEQ OneStep Library Conversion Kit (Third party) and thaw the reagent for approximately 30 min on ice.
7. Mix thoroughly using a vortex mixer for 5 sec. Centrifuge briefly and place on ice until use.

Calculating the number of DNB reactions

Each DNBSEQ-G400RS sequencing flow cell contains 4 lanes and each DNBSEQ-G400RS rapid sequencing flow cell contains 2 lanes. DNBs can be loaded into the flow cell using the sequencer or DL-200H.

- Using the sequencer to load DNBs:

  All lanes in the flow cell must be loaded with the same DNBs. Each lane requires 50 μL DNB.

- Using DL-200H to load DNBs:

  Different DNBs can be manually loaded into different lanes. Each lane requires 25 μL DNB.

Table 8 The required number of make DNB reactions for each DNBSEQ-G400RS flow cell

Flow cell type	Loading system	DNB volume (μL) / Lane	Make DNB reaction (μL)	The required number of make DNB reaction / Flow cell
FCL	Sequencer	50	100	2
	DL-200H	25	100	1 to 4
FCS	Sequencer	50	100	1
	DL-200H	25	100	1 to 2

Calculating the required amount of dsDNA libraries

The required volume of dsDNA libraries is determined by the required library amount (fmol) and library concentration quantified in *DNA library concentration and amount requirement on Page 8*.

C in the *Table 9 Volume of libraries on Page 12* represents the concentration of libraries (fmol / μL).

Table 9 Volume of libraries

Model	Library type	Required dsDNA volume of 100 μL DNB reaction (μL)
OS-Single Barcode / OS-Dual Barcode	CG PCR libraries	V = 120 fmol / C
	CG PCR-free ibraries	V=70 fmol / C
Third party	PCR library without 5-Phosphorylation	V=200 fmol / C
	PCR-free library without 5-Phosphorylation	V=150 fmol / C
	PCR library with 5-Phosphorylation	V=150 fmol / C
	PCR-free library with 5-Phosphorylation	V=120 fmol / C

Making DNBs for CG single barcode libraries

Perform the following steps:

1. Take out a 0.2 mL PCR tube. Prepare Make DNB Reaction Mixture 1 according to *Table 10*:

   The following table only illustrates the volume used for one Make DNB reaction. The required number of Make DNB reactions is determined by the actual application as described in *Calculating the number of DNB reactions on Page 11.*

   Table 10 Make DNB Reaction Mixture 1 for CG single barcode libraries of DNBSEQ-G400RS

Component	Volume of 100 μL DNB reaction (μL)
Low TE Buffer	20 - V
Make DNB Buffer (OS-SB)	20
dsDNA libraries	V
Total volume	40

2. Mix Make DNB Reaction Mixture 1 thoroughly by vortexing, and centrifuge for 5 sec.

3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Follow the primer hybridization program described below:

Table 11 Primer hybridization reaction conditions for CG single barcode libraries of DNBSEQ-G400RS

Temperature	Time
105 °C (heated lid)	On
95 °C	3 min
40 °C	3 min
4 °C	Hold

4. Remove Make DNB Enzyme Mix II (OS) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) and place it on ice. Centrifuge briefly for 5 sec, and place on ice until use.

- Keep Make DNB Enzyme Mix II (OS) on ice at all times.
- Avoid holding the tube for a prolonged period of time.

5. Remove the hybridization reaction tube from the thermal cycler when the temperature reaches 4 °C.
6. Centrifuge briefly for 5 sec, place the tube on ice. Prepare the Make DNB Reaction Mixture 2 according to *Table 12*:

Table 12 Make DNB Reaction Mixture 2 for CG single barcode libraries of DNBSEQ-G400RS

Component	Volume of 100 µL DNB reaction (µL)
Make DNB Enzyme Mix I (OS)	40
Make DNB Enzyme Mix II (OS)	2
Total volume	42

7. Add all of Make DNB Reaction Mixture 2 into the Make DNB Reaction Mixture 1. Mix thoroughly by vortexing, and centrifuge for 5 sec.
8. Place the tubes into the thermal cycler for the RCA reaction. The conditions are shown in the *Table 13*:

- When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
- It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 13 RCA conditions for CG single barcode libraries of DNBSEQ-G400RS

Temperature	Time
35 °C (heated lid)	On
30 °C	30 min
4 °C	Hold

9. When the temperature reaches 4 °C, immediately add 20 µL of Stop DNB Reaction Buffer into the RCA reaction tube. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

   - It is very important to use a wide-bore, non-filtered pipette tip.
   - Do not centrifuge, vortex, or shake the tube.
   - Store the DNBs at 4 °C and perform sequencing within 48 hr.

Making DNBs for CG dual barcode libraries

Perform the following steps:

1. Take out a 0.2 mL PCR tube. Prepare Make DNB Reaction Mixture 1 according to *Table 14*:

   The following table only illustrates the volume used for one Make DNB reaction. The required number of Make DNB reactions is determined by the actual application as described in *Calculating the number of DNB reactions on Page 11.*

Table 14 Make DNB Reaction Mixture 1 for CG dual barcode libraries of DNBSEQ-G400RS

Component	Volume of 100 µL DNB reaction (µL)
Low TE Buffer	20 - V
Make DNB Buffer (OS-DB)	20
dsDNA libraries	V
Total volume	40

2. Mix Make DNB Reaction Mixture 1 thoroughly by vortexing, and centrifuge for 5 sec.

3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Follow the primer hybridization program described below:

Table 15 Primer hybridization reaction conditions for CG dual barcode libraries of DNBSEQ-G400RS

Temperature	Time
105 °C (heated lid)	On
95 °C	3 min
40 °C	3 min
4 °C	Hold

4. Remove Make DNB Enzyme Mix II (OS) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) and place it on ice. Centrifuge briefly for 5 sec, and place on ice until use.

- Keep Make DNB Enzyme Mix II (OS) on ice at all times.
- Avoid holding the tube for a prolonged period of time.

5. Remove the hybridization reaction tube from the thermal cycler when the temperature reaches 4 °C.
6. Centrifuge briefly for 5 sec, place the tube on ice. Prepare the Make DNB Reaction Mixture 2 according to *Table 16*:

Table 16 Make DNB Reaction Mixture 2 for CG dual barcode libraries of DNBSEQ-G400RS

Component	Volume of 100 μL DNB reaction (μL)
Make DNB Enzyme Mix I (OS)	40
Make DNB Enzyme Mix II (OS)	2
Total volume	42

7. Add all of Make DNB Reaction Mixture 2 into the Make DNB Reaction Mixture 1. Mix thoroughly by vortexing, and centrifuge for 5 sec.
8. Place the tubes into the thermal cycler for the RCA reaction. The conditions are shown in the *Table 17*:

- When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
- It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 17 RCA conditions for CG dual barcode libraries of DNBSEQ-G400RS

Temperature	Time
35 °C (heated lid)	On
30 °C	30 min
4 °C	Hold

9. When the temperature reaches 4 °C, immediately add 20 µL of Stop DNB Reaction Buffer into the RCA reaction tube. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

   - It is very important to use a wide-bore, non-filtered pipette tip.
   - Do not centrifuge, vortex, or shake the tube.
   - Store the DNBs at 4 °C and perform sequencing within 48 hr.

Making DNBs for third party libraries

Perform the following steps:

1. Remove the Conversion Enzyme from DNBSEQ OneStep Library Conversion Kit (Third party). Centrifuge briefly for 5 sec, and place on ice until use.
2. Take out a 0.2 mL PCR tube. Prepare Make DNB Reaction Mixture 1 according to *Table 18*:

   The following table only illustrates the volume used for one Make DNB reaction. The required number of Make DNB reactions is determined by the actual application as described in *Calculating the number of DNB reactions on Page 11.*

Table 18 Make DNB Reaction Mixture 1 for third party libraries of DNBSEQ-G400RS

Component	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

3. Mix Make DNB Reaction Mixture 1 thoroughly by vortexing, and centrifuge for 5 sec.

4. Place the mixture into a thermal cycler and start the primer hybridization reaction. Follow the primer hybridization program described below:

Table 19 Primer hybridization reaction conditions for third party libraries of DNBSEQ-G400RS

Temperature	Time
105 °C (heated lid)	On
37 °C	5 min
95 °C	3 min
40 °C	3 min
4 °C	Hold

5. Remove Make DNB Enzyme Mix II (OS) from DNBSEQ OneStep Library Conversion Kit (Third party) and place it on ice. Centrifuge briefly for 5 sec, and place on ice until use.

   - Keep Make DNB Enzyme Mix II (OS) on ice at all times.
   - Avoid holding the tube for a prolonged period of time.

6. Remove the hybridization reaction tube from the thermal cycler when the temperature reaches 4 °C.
7. Centrifuge briefly for 5 sec, place the tube on ice. Prepare the Make DNB Reaction Mixture 2 according to *Table 20*:

Table 20 Make DNB Reaction Mixture 2 for third party libraries of DNBSEQ-G400RS

Component	Volume of 100 μL DNB reaction (μL)
Make DNB Enzyme Mix I (OS)	40
Make DNB Enzyme Mix II (OS)	2
Total volume	42

8. Add all of Make DNB Reaction Mixture 2 into the Make DNB Reaction Mixture 1. Mix thoroughly by vortexing, and centrifuge for 5 sec.
9. Place the tubes into the thermal cycler for the RCA reaction. The conditions are shown in the *Table 21*:

   - When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
   - It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 21 RCA conditions for third party libraries of DNBSEQ-G400RS

Temperature	Time
35 °C (heated lid)	On
30 °C	30 min
4 °C	Hold

10. When the temperature reaches 4 °C, immediately add 20 µL of Stop DNB Reaction Buffer into the RCA reaction tube. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

    - It is very important to use a wide-bore, non-filtered pipette tip.
    - Do not centrifuge, vortex, or shake the tube.
    - Store the DNBs at 4 °C and perform sequencing within 48 hr.

Making DNBs for DNBSEQ-T7RS FCL PE100

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. For CG single barcode libraries, remove Make DNB Buffer (OS-SB) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) and thaw the reagent at room temperature.
3. For CG dual barcode libraries, remove Make DNB Buffer (OS-DB) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) and thaw the reagent at room temperature.
4. For third party libraries, remove Make DNB Buffer (OS-App) from DNBSEQ OneStep Library Conversion Kit (Third party) and thaw the reagent at room temperature.
5. Remove Low TE Buffer and Stop DNB Reaction Buffer from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) or DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) or DNBSEQ OneStep Library Conversion Kit (Third party) and thaw the reagents at room temperature.
6. Remove Make DNB Enzyme Mix I (OS) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) or DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) or DNBSEQ OneStep Library Conversion Kit (Third party) and thaw the reagent for approximately 30 min on ice.
7. Mix thoroughly using a vortex mixer for 5 sec. Centrifuge briefly and place on ice until use.

Calculating the number of DNB reactions and amount of dsDNA libraries

- The required volume of dsDNA libraries is determined by the required library amount (fmol) and library concentration quantified in *DNA library concentration and amount requirement on Page 8*.
- 270 µL of DNBs are required to load one flow cell.
- The required library volume to make 100 µL of DNBs (one DNB reaction) is shown in the *Table 22*.

C in the following table represents the concentration of libraries (fmol/µL).

Table 22 Volume of libraries

Model	Library type	Required dsDNA volume of 100 µL DNB reaction (µL)
OS-Single Barcode / OS-Dual Barcode	CG PCR libraries	V = 130 fmol / C
	CG PCR-free ibraries	V=90 fmol / C
Third party	PCR library without 5-Phosphorylation	V=200 fmol / C
	PCR-free library without 5-Phosphorylation	V=150 fmol / C
	PCR library with 5-Phosphorylation	V=150 fmol / C
	PCR-free library with 5-Phosphorylation	V=100 fmol / C

- For a given sample A, if it requires “a” million base data output and the total theoretical expected data output for this flow cell is “b” million bases, then the required DNB volume (V) in the pooling for sample A is as follows:

$$V = a / b \times 270\ (\mu L)$$

- The number of the 100 µL Make DNB reactions is equal to (V / 100)+1 rounded down to the nearest whole number.

Making DNBs for CG single barcode libraries

Perform the following steps:

1. Take out a 0.2 mL PCR tube. Prepare Make DNB Reaction Mixture 1 according to *Table 23*:

   The following table only illustrates the volume used for one Make DNB reaction. The required number of Make DNB reactions is determined by the actual application as described in *Calculating the number of DNB reactions and amount of dsDNA libraries on Page 19*.

Table 23 Make DNB Reaction Mixture 1 for CG single barcode libraries of DNBSEQ-T7RS FCL PE100

Component	Volume of 100 μL DNB reaction (μL)
Low TE Buffer	20 - V
Make DNB Buffer (OS-SB)	20
dsDNA libraries	V
Total volume	40

2. Mix Make DNB Reaction Mixture 1 thoroughly by vortexing, and centrifuge for 5 sec.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Follow the primer hybridization program described below:

Table 24 Primer hybridization reaction conditions for CG single barcode libraries of DNBSEQ-T7RS FCL PE100

Temperature	Time
105 °C (heated lid)	On
95 °C	3 min
40 °C	3 min
4 °C	Hold

4. Remove Make DNB Enzyme Mix II (OS) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) and place it on ice. Centrifuge briefly for 5 sec, and place on ice until use.

   - Keep Make DNB Enzyme Mix II (OS) on ice at all times.
   - Avoid holding the tube for a prolonged period of time.

5. Remove the hybridization reaction tube from the thermal cycler when the temperature reaches 4 °C.

6. Centrifuge briefly for 5 sec, place the tube on ice. Prepare the Make DNB Reaction Mixture 2 according to *Table 25*:

Table 25 Make DNB Reaction Mixture 2 for CG single barcode libraries of DNBSEQ-T7RS FCL PE100

Component	Volume of 100 µL DNB reaction (µL)
Make DNB Enzyme Mix I (OS)	40
Make DNB Enzyme Mix II (OS)	2
Total volume	42

7. Add all of Make DNB Reaction Mixture 2 into the Make DNB Reaction Mixture 1. Mix thoroughly by vortexing, and centrifuge for 5 sec.
8. Place the tubes into the thermal cycler for the RCA reaction. The conditions are shown in the *Table 26*:

- When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
- It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 26 RCA conditions for CG single barcode libraries of DNBSEQ-T7RS FCL PE100

Temperature	Time
35 °C (heated lid)	On
30 °C	30 min
4 °C	Hold

9. When the temperature reaches 4 °C, immediately add 20 µL of Stop DNB Reaction Buffer into the RCA reaction tube. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

- It is very important to use a wide-bore, non-filtered pipette tip.
- Do not centrifuge, vortex, or shake the tube.
- Store the DNBs at 4 °C and perform sequencing within 48 hr.

Making DNBs for CG dual barcode libraries

Perform the following steps:

1. Take out a 0.2 mL PCR tube. Prepare Make DNB Reaction Mixture 1 according to *Table 27*:

   The following table only illustrates the volume used for one Make DNB reaction. The required number of Make DNB reactions is determined by the actual application as described in *Calculating the number of DNB reactions and amount of dsDNA libraries on Page 19*.

Table 27 Make DNB Reaction Mixture 1 for CG dual barcode libraries of DNBSEQ-T7RS FCL PE100

Component	Volume of 100 μL DNB reaction (μL)
Low TE Buffer	20 - V
Make DNB Buffer (OS-DB)	20
dsDNA libraries	V
Total volume	40

2. Mix Make DNB Reaction Mixture 1 thoroughly by vortexing, and centrifuge for 5 sec.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Follow the primer hybridization program described below:

Table 28 Primer hybridization reaction conditions for CG dual barcode libraries of DNBSEQ-T7RS FCL PE100

Temperature	Time
105 °C (heated lid)	On
95 °C	3 min
40 °C	3 min
4 °C	Hold

4. Remove Make DNB Enzyme Mix II (OS) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) and place it on ice. Centrifuge briefly for 5 sec, and place on ice until use.

   - Keep Make DNB Enzyme Mix II (OS) on ice at all times.
   - Avoid holding the tube for a prolonged period of time.

5. Remove the hybridization reaction tube from the thermal cycler when the temperature reaches 4 °C.

6. Centrifuge briefly for 5 sec, place the tube on ice. Prepare the Make DNB Reaction Mixture 2 according to *Table 29*:

Table 29 Make DNB Reaction Mixture 2 for CG dual barcode libraries of DNBSEQ-T7RS FCL PE100

Component	Volume of 100 µL DNB reaction (µL)
Make DNB Enzyme Mix I (OS)	40
Make DNB Enzyme Mix II (OS)	2
Total volume	42

7. Add all of Make DNB Reaction Mixture 2 into the Make DNB Reaction Mixture 1. Mix thoroughly by vortexing, and centrifuge for 5 sec.
8. Place the tubes into the thermal cycler for the RCA reaction. The conditions are shown in the *Table 30*:

- When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
- It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 30 RCA conditions for CG dual barcode libraries of DNBSEQ-T7RS FCL PE100

Temperature	Time
35 °C (heated lid)	On
30 °C	30 min
4 °C	Hold

9. When the temperature reaches 4 °C, immediately add 20 µL of Stop DNB Reaction Buffer into the RCA reaction tube. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

- It is very important to use a wide-bore, non-filtered pipette tip.
- Do not centrifuge, vortex, or shake the tube.
- Store the DNBs at 4 °C and perform sequencing within 48 hr.

Making DNBs for third party libraries

Perform the following steps:

1. Remove the Conversion Enzyme from DNBSEQ OneStep Library Conversion Kit (Third party). Centrifuge briefly for 5 sec, and place on ice until use.

2. Take out a 0.2 mL PCR tube. Prepare Make DNB Reaction Mixture 1 according to *Table 31*:

   The following table only illustrates the volume used for one Make DNB reaction. The required number of Make DNB reactions is determined by the actual application as described in *Calculating the number of DNB reactions and amount of dsDNA libraries on Page 19*.

Table 31 Make DNB Reaction Mixture 1 for third party libraries of DNBSEQ-T7RS FCL PE100

Component	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

3. Mix Make DNB Reaction Mixture 1 thoroughly by vortexing, and centrifuge for 5 sec.
4. Place the mixture into a thermal cycler and start the primer hybridization reaction. Follow the primer hybridization program described below:

Table 32 Primer hybridization reaction conditions for third party libraries of DNBSEQ-T7RS FCL PE100

Temperature	Time
105 °C (heated lid)	On
37 °C	5 min
95 °C	3 min
40 °C	3 min
4 °C	Hold

5. Remove Make DNB Enzyme Mix II (OS) from DNBSEQ OneStep Library Conversion Kit (Third party) and place it on ice. Centrifuge briefly for 5 sec, and place on ice until use.

   - Keep Make DNB Enzyme Mix II (OS) on ice at all times.
   - Avoid holding the tube for a prolonged period of time.

6. Remove the hybridization reaction tube from the thermal cycler when the temperature reaches 4 °C.
7. Centrifuge briefly for 5 sec, place the tube on ice. Prepare the Make DNB Reaction Mixture 2 according to *Table 33*:

Table 33 Make DNB Reaction Mixture 2 for third party libraries of DNBSEQ-T7RS FCL PE100

Component	Volume of 100 µL DNB reaction (µL)
Make DNB Enzyme Mix I (OS)	40
Make DNB Enzyme Mix II (OS)	2
Total volume	42

8. Add all of Make DNB Reaction Mixture 2 into the Make DNB Reaction Mixture 1. Mix thoroughly by vortexing, and centrifuge for 5 sec.
9. Place the tubes into the thermal cycler for the RCA reaction. The conditions are shown in the *Table 34*:

- When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
- It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 34 RCA conditions for third party libraries of DNBSEQ-T7RS FCL PE100

Temperature	Time
35 °C (heated lid)	On
30 °C	30 min
4 °C	Hold

10. When the temperature reaches 4 °C, immediately add 20 µL of Stop DNB Reaction Buffer into the RCA reaction tube. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

- It is very important to use a wide-bore, non-filtered pipette tip.
- Do not centrifuge, vortex, or shake the tube.
- Store the DNBs at 4 °C and perform sequencing within 48 hr.

Making DNBs for DNBSEQ-T7RS FCL PE150

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. For CG single barcode libraries, remove Make DNB Buffer (OS-SB) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) and thaw the reagent at room temperature.
3. For CG dual barcode libraries, remove Make DNB Buffer (OS-DB) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) and thaw the reagent at room temperature.
4. For third party libraries, remove Make DNB Buffer (OS-App) from DNBSEQ OneStep Library Conversion Kit (Third party) and thaw the reagent at room temperature.
5. Remove Low TE Buffer and Stop DNB Reaction Buffer from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) or DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) or DNBSEQ OneStep Library Conversion Kit (Third party) and thaw the reagents at room temperature.
6. Remove Make DNB Enzyme Mix I (OS) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) or DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) or DNBSEQ OneStep Library Conversion Kit (Third party) and thaw the reagent for approximately 30 min on ice.
7. Mix thoroughly using a vortex mixer for 5 sec. Centrifuge briefly and place on ice until use.

Calculating the number of DNB reactions and the required amount of dsDNA libraries

- 290 µL of DNBs are required to load one flow cell for the T7 FCL PE150. One Make DNB reaction can make 76.6 µL of DNBs. The volume of the Make DNB reaction depends on the amount of data required for sequencing per sample and the types of DNA libraries.
- The required library volume to make 76.6 µL of DNBs (one DNB reaction) is shown in the *Table 35*.

C in the following table represents the concentration of libraries (fmol/µL).

Table 35 Volume of libraries

Model	Library type	Required dsDNA volume of 76.6 μL DNB reaction (μL)
OS-Single Barcode / OS-Dual Barcode	CG PCR libraries	V = 200 fmol / C
	CG PCR-free ibraries	V=130 fmol / C
Third party	PCR library without 5-Phosphorylation	V=350 fmol / C
	PCR-free library without 5-Phosphorylation	V=250 fmol / C
	PCR library with 5-Phosphorylation	V=250 fmol / C
	PCR-free library with 5-Phosphorylation	V=200 fmol / C

- For a given sample A, if it requires "a" million base data output and the total theoretical expected data output for this flow cell is "b" million bases, then the required DNB volume (V) in the pooling for sample A is as follows:

$$V = a / b \times 290\ (\mu L)$$

- The number of the 76.6 μL Make DNB reactions is equal to (V / 76.6)+1 rounded down to the nearest whole number.

Making DNBs for CG single barcode libraries

Perform the following steps:

1. Take out a 0.2 mL PCR tube. Prepare Make DNB Reaction Mixture 1 according to *Table 36*:

   The following table only illustrates the volume used for one Make DNB reaction. The required number of Make DNB reactions is determined by the actual application as described in *Calculating the number of DNB reactions and the required amount of dsDNA libraries on Page 26.*

Table 36 Make DNB Reaction Mixture 1 for CG single barcode libraries of DNBSEQ-T7RS FCL PE150

Component	Volume of 76.6 µL DNB reaction (µL)
Low TE Buffer	20 - V
Make DNB Buffer (OS-SB)	20
dsDNA libraries	V
Total volume	40

2. Mix Make DNB Reaction Mixture 1 thoroughly by vortexing, and centrifuge for 5 sec.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Follow the primer hybridization program described below:

Table 37 Primer hybridization reaction conditions for CG single barcode libraries of DNBSEQ-T7RS FCL PE150

Temperature	Time
105 °C (heated lid)	On
95 °C	3 min
40 °C	3 min
4 °C	Hold

4. Remove Make DNB Enzyme Mix II (OS) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) and place it on ice. Centrifuge briefly for 5 sec, and place on ice until use.

   - Keep Make DNB Enzyme Mix II (OS) on ice at all times.
   - Avoid holding the tube for a prolonged period of time.

5. Remove the hybridization reaction tube from the thermal cycler when the temperature reaches 4 °C.
6. Centrifuge briefly for 5 sec, place the tube on ice. Prepare the Make DNB Reaction Mixture 2 according to *Table 38*:

Table 38 Make DNB Reaction Mixture 2 for CG single barcode libraries of DNBSEQ-T7RS FCL PE150

Component	Volume of 76.6 µL DNB reaction (µL)
Make DNB Enzyme Mix I (OS)	30
Make DNB Enzyme Mix II (OS)	1.6
Total volume	31.6

7. Add all of Make DNB Reaction Mixture 2 into the Make DNB Reaction Mixture 1. Mix thoroughly by vortexing, and centrifuge for 5 sec.
8. Place the tubes into the thermal cycler for the RCA reaction. The conditions are shown in the *Table 39*:

   - When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
   - It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 39 RCA conditions for CG single barcode libraries of DNBSEQ-T7RS FCL PE150

Temperature	Time
35 °C (heated lid)	On
30 °C	12 min
4 °C	Hold

9. When the temperature reaches 4 °C, immediately add 5 µL of Stop DNB Reaction Buffer into the RCA reaction tube. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

   - It is very important to use a wide-bore, non-filtered pipette tip.
   - Do not centrifuge, vortex, or shake the tube.
   - DNBs must be used immediately and cannot be stored.

Making DNBs for CG dual barcode libraries

Perform the following steps:

1. Take out a 0.2 mL PCR tube. Prepare Make DNB Reaction Mixture 1 according to *Table 40*:

   The following table only illustrates the volume used for one Make DNB reaction. The required number of Make DNB reactions is determined by the actual application as described in *Calculating the number of DNB reactions and the required amount of dsDNA libraries on Page 26*.

Table 40 Make DNB Reaction Mixture 1 for CG dual barcode libraries of DNBSEQ-T7RS FCL PE150

Component	Volume of 76.6 µL DNB reaction (µL)
Low TE Buffer	20 - V
Make DNB Buffer (OS-DB)	20
dsDNA libraries	V
Total volume	40

2. Mix Make DNB Reaction Mixture 1 thoroughly by vortexing, and centrifuge for 5 sec.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Follow the primer hybridization program described below:

Table 41 Primer hybridization reaction conditions for CG dual barcode libraries of DNBSEQ-T7RS FCL PE150

Temperature	Time
105 °C (heated lid)	On
95 °C	3 min
40 °C	3 min
4 °C	Hold

4. Remove Make DNB Enzyme Mix II (OS) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) and place it on ice. Centrifuge briefly for 5 sec, and place on ice until use.

   - Keep Make DNB Enzyme Mix II (OS) on ice at all times.
   - Avoid holding the tube for a prolonged period of time.

5. Remove the hybridization reaction tube from the thermal cycler when the temperature reaches 4 °C.
6. Centrifuge briefly for 5 sec, place the tube on ice. Prepare the Make DNB Reaction Mixture 2 according to *Table 42*:

Table 42 Make DNB Reaction Mixture 2 for CG dual barcode libraries of DNBSEQ-T7RS FCL PE150

Component	Volume of 76.6 µL DNB reaction (µL)
Make DNB Enzyme Mix I (OS)	30
Make DNB Enzyme Mix II (OS)	1.6
Total volume	31.6

7. Add all of Make DNB Reaction Mixture 2 into the Make DNB Reaction Mixture 1. Mix thoroughly by vortexing, and centrifuge for 5 sec.
8. Place the tubes into the thermal cycler for the RCA reaction. The conditions are shown in the *Table 43*:

   - When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
   - It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 43 RCA conditions for CG dual barcode libraries of DNBSEQ-T7RS FCL PE150

Temperature	Time
35 °C (heated lid)	On
30 °C	12 min
4 °C	Hold

9. When the temperature reaches 4 °C, immediately add 5 µL of Stop DNB Reaction Buffer into the RCA reaction tube. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

   - It is very important to use a wide-bore, non-filtered pipette tip.
   - Do not centrifuge, vortex, or shake the tube.
   - DNBs must be used immediately and cannot be stored.

Making DNBs for third party libraries

Perform the following steps:

1. Remove the Conversion Enzyme from DNBSEQ OneStep Library Conversion Kit (Third party). Centrifuge briefly for 5 sec, and place on ice until use.
2. Take out a 0.2 mL PCR tube. Prepare Make DNB Reaction Mixture 1 according to *Table 45*:

   The following table only illustrates the volume used for one Make DNB reaction. The required number of Make DNB reactions is determined by the actual application as described in *Calculating the number of DNB reactions and the required amount of dsDNA libraries on Page 26.*

Table 44 Make DNB Reaction Mixture 1 for third party libraries of DNBSEQ-T7RS FCL PE150

Component	Volume of 76.6 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

3. Mix Make DNB Reaction Mixture 1 thoroughly by vortexing, and centrifuge for 5 sec.
4. Place the mixture into a thermal cycler and start the primer hybridization reaction. Follow the primer hybridization program described below:

Table 45 Primer hybridization reaction conditions for third party libraries of DNBSEQ-T7RS FCL PE150

Temperature	Time
105 °C (heated lid)	On
37 °C	5 min
95 °C	3 min
40 °C	3 min
4 °C	Hold

5. Remove Make DNB Enzyme Mix II (OS) from DNBSEQ OneStep Library Conversion Kit (Third party) and place it on ice. Centrifuge briefly for 5 sec, and place on ice until use.

   - Keep Make DNB Enzyme Mix II (OS) on ice at all times.
   - Avoid holding the tube for a prolonged period of time.

6. Remove the hybridization reaction tube from the thermal cycler when the temperature reaches 4 °C.

7. Centrifuge briefly for 5 sec, place the tube on ice. Prepare the Make DNB Reaction Mixture 2 according to *Table 46*:

Table 46 Make DNB Reaction Mixture 2 for third party libraries of DNBSEQ-T7RS FCL PE150

Component	Volume of 76.6 µL DNB reaction (µL)
Make DNB Enzyme Mix I (OS)	30
Make DNB Enzyme Mix II (OS)	1.6
Total volume	31.6

8. Add all of Make DNB Reaction Mixture 2 into the Make DNB Reaction Mixture 1. Mix thoroughly by vortexing, and centrifuge for 5 sec.
9. Place the tubes into the thermal cycler for the RCA reaction. The conditions are shown in the *Table 47*:

- When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
- It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 47 RCA conditions for third party libraries of DNBSEQ-T7RS FCL PE150

Temperature	Time
35 °C (heated lid)	On
30 °C	12 min
4 °C	Hold

10. When the temperature reaches 4 °C, immediately add 5 µL of Stop DNB Reaction Buffer into the RCA reaction tube. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

- It is very important to use a wide-bore, non-filtered pipette tip.
- Do not centrifuge, vortex, or shake the tube.
- DNBs must be used immediately and cannot be stored.

Making DNBs for DNBSEQ-G99RS

Preparing reagents for making DNBs

Perform the following steps:

1. Place the libraries on ice until use.
2. For CG single barcode libraries, remove Make DNB Buffer (OS-SB) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) and thaw the reagent at room temperature.
3. For CG dual barcode libraries, remove Make DNB Buffer (OS-DB) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) and thaw the reagent at room temperature.
4. For third party libraries, remove Make DNB Buffer (OS-App) from DNBSEQ OneStep Library Conversion Kit (Third party) and thaw the reagent at room temperature.
5. Remove Low TE Buffer and Stop DNB Reaction Buffer from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) or DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) or DNBSEQ OneStep Library Conversion Kit (Third party) and thaw the reagents at room temperature.
6. Remove Make DNB Enzyme Mix I (OS) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) or DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) or DNBSEQ OneStep Library Conversion Kit (Third party) and thaw the reagent for approximately 30 min on ice.
7. Mix thoroughly using a vortex mixer for 5 sec. Centrifuge briefly and place on ice until use.

Calculating the number of DNB reactions

Each flow cell contains 1 lane. DNBs can be loaded into the flow cell using the DL-G99.

Table 48 Required number of Make DNB reactions for each flow cell

Loading system	DNB loading volume (µL) / lane	Make DNB reaction (µL)	Required number of Make DNB reactions / flow cell
DL-G99	10	50	1

i The loading volume can vary within the specified range depending on the pipette used.

Calculating the required amount of dsDNA libraries

The required volume of dsDNA libraries is determined by the required library amount (fmol) and library concentration quantified in *DNA library concentration and amount requirement on Page 8*.

C in the following table represents the concentration of libraries (fmol/μL).

Table 49 Volume of libraries

Model	Library type	Required dsDNA volume of 50 μL DNB reaction (μL)
OS-Single Barcode / OS-Dual Barcode	CG PCR libraries	V = 60 fmol / C
	CG PCR-free ibraries	V=35 fmol / C
Third party	PCR library without 5-Phosphorylation	V=100 fmol / C
	PCR-free library without 5-Phosphorylation	V=75 fmol / C
	PCR library with 5-Phosphorylation	V=75 fmol / C
	PCR-free library with 5-Phosphorylation	V=60 fmol / C

Making DNBs for CG single barcode libraries

Perform the following steps:

1. Take out a 0.2 mL PCR tube. Prepare Make DNB Reaction Mixture 1 according to *Table 50*:

   The following table only illustrates the volume used for one Make DNB reaction. The required number of Make DNB reactions is determined by the actual application as described in *Calculating the number of DNB reactions on Page 34*.

Table 50 Make DNB Reaction Mixture 1 for CG single barcode libraries of DNBSEQ-G99RS

Component	Volume of 50 μL DNB reaction (μL)
Low TE Buffer	10 - V
Make DNB Buffer (OS-SB)	10
dsDNA libraries	V
Total volume	20

2. Mix Make DNB Reaction Mixture 1 thoroughly by vortexing, and centrifuge for 5 sec.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Follow the primer hybridization program described below:

Table 51 Primer hybridization reaction conditions for CG single barcode libraries of DNBSEQ-G99RS

Temperature	Time
105 °C (heated lid)	On
95 °C	3 min
40 °C	3 min
4 °C	Hold

4. Remove Make DNB Enzyme Mix II (OS) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode) and place it on ice. Centrifuge briefly for 5 sec, and place on ice until use.

   - Keep Make DNB Enzyme Mix II (OS) on ice at all times.
   - Avoid holding the tube for a prolonged period of time.

5. Remove the hybridization reaction tube from the thermal cycler when the temperature reaches 4 °C.
6. Centrifuge briefly for 5 sec, place the tube on ice. Prepare the Make DNB Reaction Mixture 2 according to *Table 52*:

Table 52 Make DNB Reaction Mixture 2 for CG single barcode libraries of DNBSEQ-G99RS

Component	Volume of 50 µL DNB reaction (µL)
Make DNB Enzyme Mix I (OS)	20
Make DNB Enzyme Mix II (OS)	1
Total volume	21

7. Add all of Make DNB Reaction Mixture 2 into the Make DNB Reaction Mixture 1. Mix thoroughly by vortexing, and centrifuge for 5 sec.

8. Place the tubes into the thermal cycler for the RCA reaction. The conditions are shown in the *Table 53*:

   - When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
   - It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 53 RCA conditions for CG single barcode libraries of DNBSEQ-G99RS

Temperature	Time
35 °C (heated lid)	On
30 °C	25 min
4 °C	Hold

9. When the temperature reaches 4 °C, immediately add 10 µL of Stop DNB Reaction Buffer into the RCA reaction tube. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

   - It is very important to use a wide-bore, non-filtered pipette tip.
   - Do not centrifuge, vortex, or shake the tube.
   - Store the DNBs at 4 °C and perform sequencing within 48 hr.

Making DNBs for CG dual barcode libraries

Perform the following steps:

1. Take out a 0.2 mL PCR tube. Prepare Make DNB Reaction Mixture 1 according to *Table 54*:

   The following table only illustrates the volume used for one Make DNB reaction. The required number of Make DNB reactions is determined by the actual application as described in *Calculating the number of DNB reactions on Page 34*.

Table 54 Make DNB Reaction Mixture 1 for CG dual barcode libraries of DNBSEQ-G99RS

Component	Volume of 50 µL DNB reaction (µL)
Low TE Buffer	10 - V
Make DNB Buffer (OS-DB)	10
dsDNA libraries	V
Total volume	20

2. Mix Make DNB Reaction Mixture 1 thoroughly by vortexing, and centrifuge for 5 sec.
3. Place the mixture into a thermal cycler and start the primer hybridization reaction. Follow the primer hybridization program described below:

Table 55 Primer hybridization reaction conditions for CG dual barcode libraries of DNBSEQ-G99RS

Temperature	Time
105 °C (heated lid)	On
95 °C	3 min
40 °C	3 min
4 °C	Hold

4. Remove Make DNB Enzyme Mix II (OS) from the DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) and place it on ice. Centrifuge briefly for 5 sec, and place on ice until use.

   - Keep Make DNB Enzyme Mix II (OS) on ice at all times.
   - Avoid holding the tube for a prolonged period of time.

5. Remove the hybridization reaction tube from the thermal cycler when the temperature reaches 4 °C.
6. Centrifuge briefly for 5 sec, place the tube on ice. Prepare the Make DNB Reaction Mixture 2 according to *Table 56*:

Table 56 Make DNB Reaction Mixture 2 for CG dual barcode libraries of DNBSEQ-G99RS

Component	Volume of 50 µL DNB reaction (µL)
Make DNB Enzyme Mix I (OS)	20
Make DNB Enzyme Mix II (OS)	1
Total volume	21

7. Add all of Make DNB Reaction Mixture 2 into the Make DNB Reaction Mixture 1. Mix thoroughly by vortexing, and centrifuge for 5 sec.

8. Place the tubes into the thermal cycler for the RCA reaction. The conditions are shown in the *Table 57*:

   - When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
   - It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

   Table 57 RCA conditions for CG dual barcode libraries of DNBSEQ-G99RS

Temperature	Time
35 °C (heated lid)	On
30 °C	25 min
4 °C	Hold

9. When the temperature reaches 4 °C, immediately add 10 µL of Stop DNB Reaction Buffer into the RCA reaction tube. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

   - It is very important to use a wide-bore, non-filtered pipette tip.
   - Do not centrifuge, vortex, or shake the tube.
   - Store the DNBs at 4 °C and perform sequencing within 48 hr.

Making DNBs for third party libraries

Perform the following steps:

1. Remove the Conversion Enzyme from DNBSEQ OneStep Library Conversion Kit (Third party). Centrifuge briefly for 5 sec, and place on ice until use.
2. Take out a 0.2 mL PCR tube. Prepare Make DNB Reaction Mixture 1 according to *Table 58*:

   The following table only illustrates the volume used for one Make DNB reaction. The required number of Make DNB reactions is determined by the actual application as described in *Calculating the number of DNB reactions on Page 34*.

Table 58 Make DNB Reaction Mixture 1 for third party libraries of DNBSEQ-G99RS

Component	Volume of 50 µL DNB reaction (µL)
Low TE Buffer	10 – V
Make DNB Buffer (OS-App)	10
dsDNA libraries	V
Conversion Enzyme	0.5
Total volume	20.5

3. Mix Make DNB Reaction Mixture 1 thoroughly by vortexing, and centrifuge for 5 sec.
4. Place the mixture into a thermal cycler and start the primer hybridization reaction. Follow the primer hybridization program described below:

Table 59 Primer hybridization reaction conditions for third party libraries of DNBSEQ-G99RS

Temperature	Time
105 °C (heated lid)	On
37 °C	5 min
95 °C	3 min
40 °C	3 min
4 °C	Hold

5. Remove Make DNB Enzyme Mix II (OS) from DNBSEQ OneStep Library Conversion Kit (Third party) and place it on ice. Centrifuge briefly for 5 sec, and place on ice until use.

   - Keep Make DNB Enzyme Mix II (OS) on ice at all times.
   - Avoid holding the tube for a prolonged period of time.

6. Remove the hybridization reaction tube from the thermal cycler when the temperature reaches 4 °C.

7. Centrifuge briefly for 5 sec, place the tube on ice. Prepare the Make DNB Reaction Mixture 2 according to *Table 60*:

Table 60 Make DNB Reaction Mixture 2 for third party libraries of DNBSEQ-G99RS

Component	Volume of 50 µL DNB reaction (µL)
Make DNB Enzyme Mix I (OS)	20
Make DNB Enzyme Mix II (OS)	1
Total volume	21

8. Add all of Make DNB Reaction Mixture 2 into the Make DNB Reaction Mixture 1. Mix thoroughly by vortexing, and centrifuge for 5 sec.
9. Place the tubes into the thermal cycler for the RCA reaction. The conditions are shown in the *Table 61*:

- When a reaction protocol is run, some sample blocks of thermal cyclers may remain at ambient temperatures while the lid is being heated or cooled to operating temperature. For these types of thermal cyclers, pre-heating of the heated lid is required to ensure that the heated lid is at operating temperature during the DNB reactions.
- It is recommended that you set the temperature of the heated lid to 35 °C or as close as possible to 35 °C.

Table 61 RCA conditions for third party libraries of DNBSEQ-G99RS

Temperature	Time
35 °C (heated lid)	On
30 °C	25 min
4 °C	Hold

10. When the temperature reaches 4 °C, immediately add 10 µL of Stop DNB Reaction Buffer into the RCA reaction tube. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip.

- It is very important to use a wide-bore, non-filtered pipette tip.
- Do not centrifuge, vortex, or shake the tube.
- Store the DNBs at 4 °C and perform sequencing within 48 hr.

Quantifying DNBs

When DNB making is completed, take 2 µL of DNBs and use Qubit ssDNA Assay Kit and Qubit Fluorometer to quantify the DNBs. For details, refer to *Using Qubit to quantify the DNBs on Page 49.*

If the DNB concentration is lower than the standard, the DNBs must be prepared again.

Table 62 DNBs concentration standard

Platform and read lengths	DNBs concentration（ng/µL）
DNBSEQ-G400RS	≥ 8
DNBSEQ-T7RS FCL PE100	≥ 8
DNBSEQ-T7RS FCL PE150	≥ 5
DNBSEQ-G99RS	≥ 8

03

Loading DNBs

This chapter describes the loading DNBs procedure. Read and follow the instructions to ensure correct operations.

- Mixed use of reagent components from different batches is not recommended.
- For transferring or mixing DNBs, use the wide-bore, non-filtered pipette tips.

Select the appropriate DNB loading protocol according to sequencing model. *Table 63* lists the sections that describe each protocol.

Table 63 DNB loading protocols

Platform and read lengths	Reference process
DNBSEQ-G400RS	*Loading DNBs for DNBSEQ-G400RS on Page 44*
DNBSEQ-T7RS FCL PE100	*Loading DNBs for DNBSEQ-T7RS FCL PE100 on Page 46*
DNBSEQ-T7RS FCL PE150	*Loading DNBs for DNBSEQ-T7RS FCL PE150 on Page 47*
DNBSEQ-G99RS	*Loading DNBs for DNBSEQ-G99RS on Page 48*

Loading DNBs for DNBSEQ-G400RS

Loading DNBs in sequencer

Perform the following steps:

1. Remove DNB Load Buffer II from the sequencing kit and thaw on ice for approximately 30 min.
2. Mix thoroughly using a vortex mixer for 5 sec. Centrifuge briefly and place on ice until use.

   If crystal precipitation is visible in DNB Load Buffer II, vigorously mix the reagent for 1 to 2 min using a vortex mixer to re-dissolve the precipitation before use.
3. Take out the 0.5 mL Micro Tube and add the reagents listed in the *Table 64*:

Table 64 Making DNB reaction mix 1

Component	FCL volume (µL)	FCS volume (µL)
DNB Load Buffer II	64	32
Make DNB Enzyme Mix II (OS-V4.0)	2	1
DNBs	200	100
Total volume	266	133

4. Mix the components gently by pipetting 5 to 8 times using a wide-bore pipette tip. Place the mixture at 4 °C until use.

   - Do not centrifuge, vortex, or shake the tube.
   - Prepare a fresh DNB loading mix immediately before the sequencing run.
   - Each sequencing flow cell (FCL) requires 266 µL DNB loading mix and each rapid sequencing flow cell (FCS) requires 133 µL DNB loading mix.

Loading DNBs in DL-200H

For wash before the DNBs loading and loading operation, refer to *DL-200H Quick Start Guide*.

Perform the following steps:

1. Remove DNB Load Buffer II from the sequencing kit and thaw on ice for approximately 30 min.
2. Mix thoroughly using a vortex mixer for 5 sec. Centrifuge briefly and place on ice until use.

   If crystal precipitation is visible in DNB Load Buffer II, vigorously mix the reagent for 1 to 2 min using a vortex mixer to re-dissolve the precipitation before use.

3. Take a new PCR 8-strip tube and add the reagents according to *Table 65:*

Table 65 DNB loading mix 2

Component	FCL volume (µL)	FCS volume (µL)
DNB Load Buffer II	8	8
Make DNB Enzyme Mix II (OS-V4.0)	0.25	0.25
DNBs	25	25
Total volume	33.25	33.25

4. Mix the components gently by pipetting 5 to 8 times using a wide-bore pipette tip. Place the mixture at 4 °C until use.

   - Each lane requires 30 µL of DNB loading mix.
   - For detailed flow cell loading instructions, refer to *Chapter 04 of DNBSEQ-G400RS System Guide.*

5. Install the sealing gasket and flow cell.
6. Aspirate 30ul of DNB loading mix 2 with a wide-bore pipette tip and insert the tip into the fluidics inlet.

   - Do not press the control button of the pipette after inserting the tip into the fluidics inlet.
   - Do not move the flow cell during loading.

7. When the tip is ejected from the pipette, the DNB loading mix will automatically flow into the flow cell.
8. After DNBs loading is complete, rotate the tips counterclockwise to remove them.
9. Place the mini-loader on the bench, turn it upside down and leave it that way for 30 min before use.

Loading DNBs for DNBSEQ-T7RS FCL PE100

Preparing DNBs loading mixture

Perform the following steps:

1. Remove DNB Load Buffer II from the sequencing kit and thaw on ice for approximately 30 min.
2. Mix thoroughly using a vortex mixer for 5 sec. Centrifuge briefly and place on ice until use.

   If crystal precipitation is visible in DNB Load Buffer II, vigorously mix the reagent for 1 to 2 min using a vortex mixer to re-dissolve the precipitation before use.

3. Take out a new 0.5 mL Micro Tube, and add the following components.

Table 66 DNB loading mixture for DNBSEQ-T7RS FCL PE100

Adding order	Component	Volume (μL)
1	DNBs	270
2	DNB Load Buffer II	90
3	Make DNB Enzyme Mix II (OS-V4.0)	1

4. Gently pipette the DNB loading mix 5 to 8 times using a wide-bore pipette tip.

   Do not centrifuge, vortex, vigorously pipette, or shake the tube.

DNBs loading

For detailed flow cell loading instructions, refer to *DNBSEQ-T7RS System Guide*.

Loading DNBs for DNBSEQ-T7RS FCL PE150

Preparing DNBs loading mixture

Perform the following steps:

1. Remove DNB Load Buffer IV from the sequencing kit and thaw on ice for approximately 30 min.
2. Mix thoroughly using a vortex mixer for 5 sec. Centrifuge briefly and place on ice until use.
3. Take a new 0.5 mL Micro Tube and add the reagents on ice as described in the *Table 67:*

Table 67 DNB loading mixture for DNBSEQ-T7RS FCL PE150

Adding order	Component	Volume (μL)
1	Low TE Buffer	10
2	DNBs	290
3	DNB Load Buffer IV	150

4. Gently pipette the DNB loading mix 5 to 8 times using a wide-bore pipette tip.

- Do not centrifuge, vortex, vigorously pipette, or shake the tube.
- DNB loading mixture must be prepared fresh and used within 10 min.

DNBs loading

For detailed flow cell loading instructions, refer to *DNBSEQ-T7RS System Guide*.

Loading DNBs for DNBSEQ-G99RS

Preparing DNBs loading mixture

Perform the following steps:

1. Remove DNB Load Buffer II from the sequencing kit and thaw on ice for approximately 30 min.
2. Mix thoroughly using a vortex mixer for 5 sec. Centrifuge briefly and place on ice until use.

   If crystal precipitation is visible in DNB Load Buffer II, vigorously mix the reagent for 1 to 2 min using a vortex mixer to re-dissolve the precipitation before use.
3. Take out the 0.5 mL Micro Tube from the sequencing set and add the following reagents:

Table 68 DNB loading mixture for DNBSEQ-G99RS

Component	Volume (µL)
DNB Load Buffer II	7
Make DNB Enzyme Mix II (OS-V4.0)	1
DNBs	21
Total volume	29

4. Combine the components and mix by gently pipetting 8 times using a wide-bore, non-filtered pipette tip. Place the mixture at 4 °C until use.

   - Do not centrifuge, vortex, vigorously pipette, or shake the tube.
   - Prepare a fresh DNB loading mixture immediately before the sequencing run.
   - Each FCL requires 10 µL of DNB loading mixture.

DNBs loading

For detailed flow cell loading instructions, refer to *DNBSEQ-G99RS&DNBSEQ-G99ARS System Guide.*

Using Qubit to quantify the DNBs

- Working solution should be used within 0.5 hr after preparation.
- It is recommended that the number of quantified samples is no more than 8 per batch to avoid inaccurate DNB quantification due to fluorescence quenching.
- Avoid touching the wall of tapered detection tubes.
- Avoid introducing bubbles in detection tubes.

Perform the following steps:

1. Prepare the Qubit working solution by diluting the Qubit ssDNA Reagent 1:200 in Qubit ssDNA Buffer. Use a clean plastic tube each time you prepare Qubit working solution. Do not mix the working solution in a glass container.

   The final volume in each tube must be 200 µL. Each standards tube requires 190 µL of Qubit working solution, and each sample tube requires 180–199 µL.

   Prepare sufficient Qubit working solution to accommodate all standards and samples.

   For example, for 8 samples, prepare enough working solution for the samples and 2 standards: 200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit reagent plus 1990 µL of Qubit Buffer).
2. Add 190 µL of Qubit working solution to each used for standards.
3. Add 10 µL of each Qubit standard to the appropriate tube, and then mix by vortexing 3–5 sec. Be careful not to introduce bubbles.
4. Set up the required number of 0.5 mL tubes for standards and samples. The Qubit ssDNA Assay requires 2 standards.

   - Use only thin-wall, clear, 0.5 mL PCR tubes. Acceptable tubes include Qubit assay tubes (Cat. No.: Q32856) or Axygen PCR-05-C tubes (Cat. No.: 10011-830).
   - To determine the number of Qubit test tubes needed, add 2 standards tubes to the number of samples. For example, if you have 3 samples, you will need 5 tubes.
5. Label the tube lids. Do not label the side of the tube.

6. Prepare the solutions used for standards and sample tests according to the example provided in the table below:

Table 69 Working solution

	S1 (μL)	S2 (μL)	D1 (μL)	D2 (μL)	D3 (μL)
Working solution	190	190	198	198	198
S1（0 ng/μL）	10	/	/	/	/
S2（20 ng/μL）	/	10	/	/	/
Sample（μL）	/	/	2	2	2
Total volume	200	200	200	200	200

7. Mix well using a vortex mixer, centrifuge briefly for 5 secs, and incubate at room temperature for 2 mins.
8. Refer to the Qubit user manual for instructions on reading standards and samples. Follow the appropriate procedure for your instrument.

List of kit components

Table 70 Components of DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode)
Cat. No.: 940-001749-00 Spec: 4 Rxn/Kit

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Validity period
Low TE Buffer Cat. No.: 530-003851-00		300 µL / tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
Make DNB Buffer (OS-SB) Cat. No.: 530-003894-00		80 µL / tube×1 tube			
Make DNB Enzyme Mix I (OS) Cat. No.: 530-003852-00		160 µL / tube×1 tube			
Make DNB Enzyme Mix II (OS) Cat. No.: 530-003854-00		8 µL / tube×1 tube			
Stop DNB Reaction Buffer Cat. No.: 530-003849-00		100 µL / tube×1 tube			

Table 71 Components of DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode)
Cat. No.: 940-001750-00 Spec: 4 Rxn/Kit

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Validity period
Low TE Buffer Cat. No.: 530-003851-00		300 µL / tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
Make DNB Buffer (OS-DB) Cat. No.: 530-003893-00		80 µL / tube×1 tube			
Make DNB Enzyme Mix I (OS) Cat. No.: 530-003852-00		160 µL / tube×1 tube			
Make DNB Enzyme Mix II (OS) Cat. No.: 530-003854-00		8 µL / tube×1 tube			
Stop DNB Reaction Buffer Cat. No.: 530-003849-00		100 µL / tube×1 tube			

Table 72 Components of DNBSEQ OneStep Library Conversion Kit (Third party)
Cat. No.: 940-001648-00 Spec: 4 Rxn/Kit

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Validity period
Low TE Buffer Cat. No.: 530-003851-00		300 µL / tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
Make DNB Buffer (Third party) Cat. No.: 530-003853-00		80 µL / tube×1 tube			
Conversion Enzyme Cat. No.: 530-003848-00		5 µL / tube×1 tube			
Make DNB Enzyme Mix I (OS) Cat. No.: 530-003852-00		160 µL / tube×1 tube			
Make DNB Enzyme Mix II (OS) Cat. No.: 530-003854-00		8 µL / tube×1 tube			
Stop DNB Reaction Buffer Cat. No.: 530-003849-00		100 µL / tube×1 tube			

Table 73 Components of DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode)
Cat. No.: 940-002192-00 Spec: 20 Rxn/Kit

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Validity period
Low TE Buffer Cat. No.:530-002087-00		960 µL / tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
Make DNB Buffer (OS-SB) Cat. No.: 530-005335-00		400 µL / tube×1 tube			
Make DNB Enzyme Mix I (OS) Cat. No.: 530-005333-00		800 µL / tube×1 tube			
Make DNB Enzyme Mix II (OS) Cat. No.: 530-005332-00		60 µL / tube×1 tube			
Stop DNB Reaction Buffer Cat. No.: 530-002091-00		400 µL / tube×1 tube			

Table 74 Components of DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode)
Cat. No.: 940-002193-00 Spec: 20 Rxn/Kit

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Validity period
Low TE Buffer Cat. No.: 530-002087-00		960 µL / tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
Make DNB Buffer (OS-DB) Cat. No.: 530-005331-00		400 µL / tube×1 tube			
Make DNB Enzyme Mix I (OS) Cat. No.: 530-005333-00		800 µL / tube×1 tube			
Make DNB Enzyme Mix II (OS) Cat. No.: 530-005332-00		60 µL / tube×1 tube			
Stop DNB Reaction Buffer Cat. No.: 530-002091-00		400 µL / tube×1 tube			

Table 75 Components of DNBSEQ OneStep Library Conversion Kit (Third party)
Cat. No.: 940-002195-00 Spec: 20 Rxn/Kit

Component	Cap color	Spec & quantity	Storage temperature	Transportation temperature	Validity period
Low TE Buffer Cat. No.: 530-002087-00		960 µL / tube×1 tube	-25 °C to -15 °C	-80 °C to -15 °C	12 months
Make DNB Buffer (OS-App) Cat. No.: 530-005326-00		400 µL / tube×1 tube			
Conversion Enzyme Cat. No.: 530-005334-00		25 µL / tube×1 tube			
Make DNB Enzyme Mix I (OS) Cat. No.: 530-005333-00		800 µL / tube×1 tube			
Make DNB Enzyme Mix II (OS) Cat. No.: 530-005332-00		60 µL / tube×1 tube			
Stop DNB Reaction Buffer Cat. No.: 530-002091-00		400 µL / tube×1 tube			

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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Order information

Catalog number	Name	Spec	Version
940-001749-00	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode)	4 Rxn/Kit	V4.0
940-001750-00	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode)	4 Rxn/Kit	V4.0
940-001648-00	DNBSEQ OneStep Library Conversion Kit (Third party)	4 Rxn/Kit	V2.0
940-002192-00	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Single Barcode)	20 Rxn/Kit	V4.0
940-002193-00	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode)	20 Rxn/Kit	V40
940-002195-00	DNBSEQ OneStep Library Conversion Kit (Third party)	20 Rxn/Kit	V1.0

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Acronyms and abbreviations

Item	Description
OS	OneStep
SB	Single Barcode
DB	Dual Barcode
DNA	Deoxyribonucleic Acid
dsDNA	Double-stranded DNA
ssDNA	Single-stranded DNA
DNB	DNA Nanoball
PCR	Polymerase Chain Reaction
bp	Base-pair
RCA	Rolling Circle Amplification
DL-200H	Portable DNB Loader (For DNBSEQ-G400)
SE	Single-end sequencing
PE	Pair-end sequencing
FCL	Flow Cell Large, 4 lanes per flow cell in DNBSEQ-G400RS Sequencing FCL Flow Cell, 1 lane per flow cell in DNBSEQ-T7RS Sequencing FCL Flow Cell, 1 lane per flow cell in DNBSEQ-G99RS Sequencing FCL Flow Cell
FCS	Rapid Flow Cell, Flow Cell Small, 2 lanes per flow cell in DNBSEQ-G400 Sequencing FCS Flow Cell.
QC	Quality Control

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