## Application scope

| Catalog number | Model | Name |
|---|---|---|
| 940-001250-00 | CM App-D FCL PE150 | DNBSEQ-G800RS CoolMPS High-throughput Sequencing Reagent Set |
| 940-001733-00 | CM App-D FCL SE600 | DNBSEQ-G800RS CoolMPS High-throughput Sequencing Reagent Set |
| 940-001750-00 | / | DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) |
| 940-001648-00 | / | DNBSEQ OneStep Library Conversion Kit (Third Party) |

## Introduction

This quick operation guide provides concise instructions for operating the DNBSEQ-G800RS system.

WARNING

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

## Preparing DNBs

### DNA library concentration and amount requirement

DNB preparation starts from either a circular ssDNA library or dsDNA library with a recommended insert size between 50 bp and 1 K bp.

CAUTION

For SE600 sequencing, DNB preparation starts solely from a circular ssDNA library.

One flow cell contains four lanes. DNBs can be loaded either by sequencer or DL-200H:

- Loading DNBs with sequencer: Four different DNBs can be automatically loaded into four different lanes in a flow cell. Each lane requires 50 µL of DNB making reaction mixture. Loading one flow cell requires four 50 µL of DNB making reaction mixtures.
- Loading DNBs with DL-200H:
  - Four different DNBs can be manually loaded into four different lanes in a flow cell. Each lane requires 25 µL of DNB making reaction mixture. Loading one flow cell requires two 50 µL of DNB making reaction mixtures.

- When 30 µL of DNB making reaction is to be loaded, only the DL-200H can be used for loading, and it is only sufficient for loading one lane.

Typical library requirements are listed in the following tables:

| Circular ssDNA concentration requirement and amount needed for each lane | | | | | |
|---|---|---|---|---|---|
| Library type | Minimum concentration | 50* µL reaction | | 30** µL reaction | |
| | | fmol | V (µL) | fmol | V (µL) |
| PCR libraries | 2.5 fmol/µL | 20 | 20/C*** | 12 | 12/C |
| PCR-free libraries | 3.75 fmol/µL | 30 | 30/C | 18 | 18/C |

5'-phosphorylated or 5'-non phosphorylated dsDNA can be made into DNB using CG DNBSEQ OneStep DNB Make Reagent Kit V4.0.

| Circular dsDNA concentration requirement and amount needed for each lane | | | | | | |
|---|---|---|---|---|---|---|
| Library type | Product name | Minimum dsDNA concentration | 50* µL reaction | | 30** µL reaction | |
| | | | fmol | V (µL) | fmol | V (µL) |
| CG | DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) | 4 fmol/µL | 50 | 50/C | 30 | 30/C |
| Third-Party | DNBSEQ OneStep DNB Make Reagent Kit (Third Party****) | 4 fmol/µL | 90 | 90/C | 54 | 54/C |

- * 50 µL of DNB reaction for loading with sequencer or DL-200H.
- ** 30 µL of DNB reaction only for loading with DL-200H. If the library amount is less than 20 fmol but no less than 12 fmol, use 30 µL of Make DNB reaction mixture. It must be noted that 30 µL of Make DNB reaction mixture may cause data loss and lower sequencing quality than expected.
- *** C in the tables above represents the concentration of libraries (fmol/µL).
- **** DNBSEQ OneStep Library Conversion Kit (Third Party) is compatible with third-party dsDNA libraries that include either TruSeq™ or Nextera™ adapters.

## Making DNBs from ssDNA

Mixing reagent components from different lots is not recommended.

1. Place the libraries on ice until use.
2. Remove the reagents from storage according to your model:
   - For PE150, remove Low TE Buffer, App Make DNB Buffer, Make DNB High-efficiency Enzyme Mix I, and Stop DNB Reaction Buffer from storage and thaw the reagents for approximately 30 min.
   - For SE600, remove Low TE Buffer, App Make DNB Buffer II, Make DNB High-efficiency Enzyme Mix III and Stop DNB Reaction Buffer from storage. Thaw the reagents for approximately 30 min.
3. Vortex the reagents by using a vortex mixer for 5 s. Centrifuge briefly and place them on ice until use.

4. Take out a 0.2 mL 8-strip tube or PCR tubes. Prepare Make DNB reaction mixture 1 according to the following table:

| Make DNB reaction mixture 1 | | | | |
|---|---|---|---|---|
| Model | FCL PE150 | | FCL SE600 | |
| Component | Volume for 50 µL DNB reaction (µL) | Volume for 30 µL DNB reaction (µL) | Volume for 50 µL DNB reaction (µL) | Volume for 30 µL DNB reaction (µL) |
| Low TE buffer | 10-V | 6-V | 8-V | 4.8-V |
| App Make DNB buffer | 10 | 6 | / | / |
| App Make DNB buffer II | / | / | 12 | 7.2 |
| ssDNA library | V | V | V | V |
| Total volume | 20 | 12 | 20 | 12 |

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

| Primer hybridization reaction conditions | |
|---|---|
| Temperature | Time |
| Heated lid (65 °C) | On |
| 60 °C | 2 min |
| 40 °C | 1 min |
| 4 °C | Hold |

7. Remove Make DNB Enzyme Mix II (HF+LC) from storage and place it on ice. Centrifuge briefly for 5 s and place back on ice.

- Do not keep Make DNB Enzyme Mix II (HF+LC) at room temperature.
- Hold tubes at the top to avoid enzyme inactivation caused by high temperature.

8. Remove the PCR tube from the thermal cycler when the temperature has reached 4 °C. Centrifuge briefly for 5 s, place the tube on ice, and prepare Make DNB reaction mixture 2 according to the following table:

| Make DNB reaction mixture 2 | | | | |
|---|---|---|---|---|
| Model | FCL PE150 | | FCL SE600 | |
| Component | Volume for 50 µL DNB reaction (µL) | Volume for 30 µL DNB reaction (µL) | Volume for 50 µL DNB reaction (µL) | Volume for 30 µL DNB reaction (µL) |
| Make DNB Enzyme Mix I | 20 | 12 | / | / |
| Make DNB Enzyme Mix III | / | / | 22.5 | 13.5 |
| Make DNB Enzyme Mix II (HF+LC) | 2 | 1.2 | 2 | 1.2 |

9. Add 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.
10. Place the tube into the thermal cycler for the next reaction. The conditions are shown in the following table:

| RCA (Rolling Circle Amplification) conditions | | |
|---|---|---|
| Model | FCL PE150 | FCL SE600 |
| Temperature | Time | Time |
| Heated lid (35 °C) | On | On |
| 30 °C | 25 min | 100 min |
| 4 °C | Hold | Hold |

11. Immediately remove the PCR tube from the thermal cycler when the temperature has reached 4 °C.
12. Immediately add Stop DNB Reaction Buffer into the tube according to the volume shown in the following table when the temperature reaches 4 °C. Mix the reagent in the tube gently by pipetting 8 times by using a wide-bore, non-filtered pipette tip.

| Volume of Stop DNB Reaction Buffer | | | | |
|---|---|---|---|---|
| Model | FCL PE150 | | FCL SE600 | |
| Component | Volume for 50 µL DNB reaction (µL) | Volume for 30 µL DNB reaction (µL) | Volume for 50 µL DNB reaction (µL) | Volume for 30 µL DNB reaction (µL) |
| Stop DNB Reaction Buffer | 10 | 6 | 8 | 4.8 |
| Final volume | 52 | 31.2 | 52.5 | 31.5 |

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

## Making DNBs from dsDNA

If input sample is dsDNA, you can use DNBSEQ OneStep DNB Make Reagent Kit V4.0 to make DNBs without separate steps for phosphorylation and circularization. Use the corresponding OneStep kit according to the following table:

| Kit name | Brand | Catalog number | Applicable library |
|---|---|---|---|
| DNBSEQ OneStep DNB Make Reagent Kit V4.0 (Dual Barcode) | CG | 940-001750-00 | Dual-barcode PCR or PCR-free libraries with CG adapter |
| DNBSEQ OneStep Library Conversion Kit (Third Party) | CG | 940-001648-00 | Dual-barcode PCR or PCR-free libraries with TruSeq or Nextera adapter |

For details on the one-step DNB making method, refer to the *DNBSEQ OneStep DNB Make Reagent Kit V4.0 User Manual*. Notably, the volume of Make DNB mixture for this platform is 50 µL, so the library input and volumes of other reagents for Make DNB mixture should be reduced by half.

## Quantifying DNBs

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

- For PE150, if the concentration is lower than 8 ng/µL, re-make DNBs. If the concentration exceeds 40 ng/µL, the DNBs should be diluted to 20 ng/µL using DNB Load Buffer I.
- For SE600, if the concentration is lower than 25 ng/µL, re-make DNBs.

# Preparing the flow cell and the sequencer

## 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 before use.
3. Unwrap the outer plastic packaging before use.
   - If the flow cell is not used within 24 h after being placed at room temperature with the outer plastic packaging intact, the flow cell can be returned to -25 °C to -15 °C for storage. The number of freeze-thaw events must not exceed three cycles.
   - If the outer plastic packaging has been opened but the flow cell cannot be used immediately, store the flow cell at room temperature and use it within 24 h. It is not recommended that you use the flow cell after 24 h once its plastic packaging is opened.
4. Take the flow cell out from the inner packaging and inspect it to ensure that the flow cell is intact and clean, without scratches.

## Preparing the sequencer

1. Connect the device to the 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 *research* and password *Admin123*.

# Preparing the Sequencing Reagent Cartridge

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

| Approximate thawing time for various sequencing kits | | | |
|---|---|---|---|
| Model | Method | | |
| | Water bath at room temperature (h) | Refrigerator at 2 °C to 8 °C overnight then water bath at room temperature (h) | Refrigerator at 2 °C to 8 °C (h) |
| CM App-D FCL PE150 | 5.0 | 2.0 | 48.0 |
| CM App-D FCL SE600 | 8.0 | 4.0 | 72.0 |

3. Invert the cartridge 3 times to mix before use.
4. Using a KimWipes tissue, wipe any water condensation on the cartridge cover and wells. Prepare well No. 9 and well No. 10:

Do not vortex Dye Mix I or Dye Mix II.

According to the following table, invert Dye Mix I 6 times and add it into well No. 9 by using a pipette; invert Dye Mix II 6 times and add it into well No. 10 by using a pipette. Seal the holes of well No. 9 and well No. 10 with the provided transparent sealing films.

| Model | Well No. 9 | Well No. 10 |
|---|---|---|
| | Dye Mix I loading volume (mL) | Dye Mix II loading volume (mL) |
| CM App-D FCL PE150 | 5.60 | 4.90 |
| CM App-D FCL SE600 | 16.80 | 14.70 |

5. Prepare well No. 18:

According to the following table, premix dNTPs Mix II and Sequencing Enzyme Mix II in a 50 mL sterile tube, then add all the mixed reagents to well No. 18. Seal the holes of well No. 18 with the provided transparent sealing film.

| Model | Well No. 18 | |
|---|---|---|
| | dNTPs Mix II loading volume (mL) | Sequencing Enzyme Mix II loading volume (mL) |
| CM App-D FCL PE150 | 12.32 | 11.56 |
| CM App-D FCL SE600 | 14.72 | 13.80 |

6. For SE600 sequencing, prepare well No. 1:

   According to the following table, premix dNTPs Mix II and Sequencing Enzyme Mix II in a 50 mL sterile tube, then add all the mixed reagents to well No. 1. Seal the holes of well No. 1 with the provided transparent sealing film.

| Model | Well No. 1 | |
|---|---|---|
| | dNTPs Mix II loading volume (mL) | Sequencing Enzyme Mix II loading volume (mL) |
| CM App-D FCL SE600 | 7.04 | 6.6 |

7. Press the film around the well with your finger, ensuring that the well is tightly sealed and that no air bubbles exist between the film and cartridge surface.
8. Hold both sides of the cartridge and lift it vertically. Shake it clockwise 20 times, and then counterclockwise 20 times until the color of the reagent in the upper level is the same as that in the bottom level in wells No. 9 and No. 10. Ensure that reagents are fully mixed.
9. Carefully remove the seals from the loading wells after fully mixing.

   - Do not reuse the waste seals.
   - To avoid cross contamination, ensure that the surface around wells No. 1, No. 9, No. 10, and No. 18 is clean.

10. For PE150 sequencing, prepare well No. 15:

    Add 230 µL of MDA Enzyme Mix II to the Inactive MDA Reagent tube with a 1 mL pipette. Invert the tube 6 times to mix the reagents and add the mixture to well No. 15.

    When transferring the mixture, operate carefully to prevent the mixture from spilling out of the reagent tube, and ensure that no bubbles exist at the bottom of the tube.

## Preparing the washing cartridge

CAUTION

- The washing cartridge is required for sequencing and must be used in pairs with the Sequencing Reagent Cartridge. Ensure that the washing cartridge used matches the corresponding Sequencing Reagent Cartridge.
- The washing cartridge and reagents used for sequencing can be subsequently used as the washing cartridge for a single maintenance wash without changing reagents.

Add 200 mL of Laboratory-grade water into well No. 2, and add 100 mL of 0.1 M NaOH into well No. 3.

After the sequencing run, perform a maintenance wash manually using the wash manifold within 24 h.

CAUTION

- Ensure the wash manifold is loaded and used only after the self-check is complete and the objective lens is stationary.
- Sequencing is not allowed during the wash. When using the wash manifold for a wash in stage A or stage B, the other stage cannot be used for sequencing.

# Loading DNBs

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

DNB can be loaded into the flow cell by either the sequencer or DL-200H.

- If 30 µL of DNB making reaction is to be loaded, please use DL-200H.
- If 50 µL of DNB making reaction is to be loaded, please use the sequencer or DL-200H.

## Loading DNBs by using the sequencer

1. Remove DNB Load Buffer II from storage and thaw on ice for approximately 30 min.
2. Mix the reagent by using a vortex mixer for 5 s, centrifuge briefly, and place it on ice until use.

   If crystal precipitation is visible in DNB Load Buffer II, vigorously mix the reagent for 1 to 2 min by using a vortex mixer to re-dissolve the precipitation before use.
3. Take out four 0.5 mL Micro Tubes from the sequencing kit. Label the tubes and add the following reagents into each tube.

| DNB loading mixture 1 | |
|---|---|
| Component | Volume (µL)/lane |
| DNB Load Buffer II | 25 |
| Make DNB Enzyme Mix II (HF+LC) | 0.5 |
| DNBs | 50 |
| Total Volume | 75.5 |

4. Gently pipette 8 times to mix DNB loading mixture 1 using a wide-bore, nonfiltered pipette tip. Place the mixture at 2 °C to 8 °C until use.

## Loading DNBs by using DL-200H

1. Take out 4 new 0.5 mL Micro Tubes and add the reagents shown in the following table:

| DNB loading mixture 2 | |
|---|---|
| Component | Volume (µL)/lane |
| DNB Load Buffer II | 12.5 |
| Make DNB Enzyme Mix II (HF+LC) | 0.25 |
| DNBs | 25 |
| Total Volume | 37.75 |

2. Gently pipette 8 times to mix DNB loading mixture 2 by using a wide-bore, non-filtered pipette tip. Place the mixture at 2 °C to 8 °C until use.
3. Install the sealing gasket and flow cell. Ensure that the label of the flow cell is facing up and in the same position as the sealing gasket.
4. Place the device on the laboratory bench with the back facing up.
5. Aspirate 30 µL of DNB loading mixture 2 with a non-filtered, wide-bore pipette tip, and insert the tip into the fluidics inlet. Eject the tip from the pipette. DNBs automatically flow into the flow cell.

   Do not touch or move the tip when ejecting. Doing so may bring bubbles into the flow cell.
6. Ensure that all DNBs flow into the flow cell. Hold the device and rotate the tip counterclockwise to remove it.
7. Repeat steps 4 to 6 to load the DNBs onto the rest of the lanes of the flow cell. Load the DNBs sequentially, starting with Lane No. 1 and ending with Lane No. 4.
8. Place the DL-200H on the bench with the front facing up. Wait for 10 min for the DNB loading process to complete.

# Performing a sequencing run

## Entering run information

1. In the main interface, select **Sequence**, and load sequencing materials according to the video guide in the interface.
2. Select **Run Set** to open the Run setting interface and keep the **Resume run** at status. Enter a run name.
3. Place the Sequencing Reagent Cartridge, select on the **Sequencing Cartridge ID** box to scan the ID. If ID is not scanned automatically, use a handheld scanner to scan the 2D Barcode on labels, or manually enter the sequencing cartridge SN by using the on-screen keyboard.

   There are two labels on the sequencing cartridge. Both labels contain the 2D Barcodes to scan with handheld scanner, on top left of the labels. When entering the ID manually, only letters, numbers and a middle bar "-" are allowed. Otherwise, an ID error will occur and the operation cannot continue.
4. Clean the flow cell stage and place the flow cell onto it, ensuring the flow cell is properly seated. Select on the **Flow cell ID** box to scan the ID or manually enter the flow cell ID by using the on-screen keyboard.
5. Place the prepared Washing cartridge into the compartment, select the **Washing cartridge ID** box, and use a handheld scanner to scan the 2D barcode on the prepared washing cartridge, or manually enter the washing cartridge SN by using the on-screen keyboard.

   When entering the flow cell ID or washing cartridge ID manually, only letters, numbers and an underscore "_" are allowed. Otherwise, an ID error will occur and the operation cannot continue.
6. Place the sample tube into the tube rack if you want to load DNBs using the sequencer. If you want to use DL-200H to load DNBs, placing an empty tube into the tube rack is required.
7. Select an appropriate recipe from the **Recipe** list. Select **Customize** to customize a recipe or select a previously saved one, which supports both single barcode and dual barcode sequencing.

   There is no predefined sequencing recipe in the **Recipe** list. For first-timers, select **Customize** from the **Recipe** list to create recipes for use. These recipes can be subsequently selected from the **Recipe** list.
8. For a dual barcode sequencing run, set **Double-index barcode** to the right if Barcode and Dual barcode need to be combined into a dual barcode file.
9. Select **DNB loading** in the process bar if you want to DNBs by using the sequencer. If not, leave it blank.

   Do not select **Auto wash** in the process bar. A manual maintenance wash is required after sequencing.
10. Enter DNB ID(s) and select the barcode range.
11. Close the flow cell compartment door.
12. Select **Review**.

## Starting sequencing

1. Carefully check each item in the Review interface and ensure that each item is correct.
2. Select **Start > Yes** to start sequencing.

## Continuing a stopped sequencing run

If a run is stopped due to some unexpected reasons, you can follow the steps below to resume the sequencing run.

CAUTION

If the run cannot be resumed, it maybe because the run was stopped in some special steps, such as: post-loading, PE synthesis, cleavage, barcode synthesis.

1. In the Run setting interface, select the **Resume run** button to status.
2. Enter the flow cell ID and the sequencing cartridge ID, ensure that those IDs are the same as the IDs of the paused sequencing run.
3. Select **Review** to check each item in the Review interface and ensure that each item is correct.
4. Select **Start**.

## Processing data

For details on processing data, refer to *DNBSEQ-G800RS System Guide*.

After sequencing starts, the sequencing results generated by the control software will be saved to the D drive of the computer.

- The data folder, named after the flow cell ID, mainly contains pictures and data (such as metrics) generated during device operation.
- The results folder, named after the flow cell ID, primarily Bioinfo files, FASTQ files, reports, and *.cal* files.

# Maintenance

For details on wash procedure and maintenance of other accessories, refer to *DNBSEQ-G800RS System Guide*.

## Disposing of the reagent cartridge and flow cell

1. Remove the washing materials from the device.
2. Dispose of the used cartridge, flow cell, and sequencing waste according to local regulations and safety standards of your laboratory.

## Maintaining 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 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.

# Research use only

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