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DNBSEQ Dual Barcode Circularization Kit User Manual

- Cat. No.: 940-001310-00 (16 RXN)
  940-001309-00 (96 RXN)
- Kit Version: V1.0

For Research Use Only

About the user manual

Manufacturer information

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Revision history

Manual Rev	Kit version	Date	Description
2.0	V1.0	Jun. 2024	Added 96 RXN of DNBSEQ Dual Barcode Circularization Module
1.0	V1.0	Aug. 2023	Initial release

Use the latest version of the manual and use it with the corresponding kit.

Contents

1 Product overview

1.1 Introduction

DNBSEQ Dual Barcode Circularization Kit is a module library prep kit designed for DNBSEQ high-throughput sequencing platforms. This kit is used to prepare a single-stranded circular DNA (ssCir) library for DNBSEQ sequencers from PCR products with CG UDB primers adapters or "CG Dual-barcode standard PCR Product" (refer to *DNBSEQ platform adapter documentation*). All reagents provided in this set have passed strict quality control and functional verification procedures, ensuring stability and reproducibility.

1.2 Intended use

DNBSEQ Dual Barcode Circularization Kit is applicable to PCR products with DNBSEQ UDB primers adapters or "DNBSEQ Dual-barcode standard PCR Product" (refer to *DNBSEQ platform adapter documentation*) from all CG library prep kits. It is used to convert a PCR product to a ssCir library that can be sequenced on DNBSEQ high-throughput sequencing platforms.

This kit is not suitable for circularization of single barcode libraries.

1.3 Applicable sequencing platforms

Sequencing instrument compatibility is dependent on specific DNBSEQ library prep kits. Libraries created with this kit can be used on any DNBSEQ sequencing platform.

1.4 Components

DNBSEQ Dual Barcode Circularization Module comes in two specification: 16 RXN and 96 RXN.

- For 16 RXN, two separate boxes are included in DNBSEQ Dual Barcode Circularization Kit .
- For 96 RXN, only the DNBSEQ Dual Barcode Circularization Module is included.

For component details, refer to the following table.

Each kit contains an information card. Relevant manuals and SDS files can be downloaded from the CG website provided on the information card.

Table 1 DNBSEQ Dual Barcode Circularization Kit (16 RXN) (Cat. No.: 940-001310-00)

Item & Cat. No.	Component	Cap color	Spec & Quantity
DNBSEQ Dual Barcode Circularization Module Cat. No.: 940-001313-00 Specification: 16 RXN	Dual Barcode Splint Buffer	Purple	186 µL/tube × 1
	DNA Rapid Ligase	Purple	8 µL/tube × 1
	Digestion Buffer	White	23 µL/tube × 1
	Digestion Enzyme	White	42 µL/tube × 1
	Digestion Stop Buffer	White	120 µL/tube × 1
DNBSEQ DNA Clean Beads Cat. No.: 940-001284-00	DNA Clean Beads	White	3.2 mL/tube × 1
	TE Buffer	White	3.2 mL/tube × 1

Table 2 DNBSEQ Dual Barcode Circularization Module (96 RXN) (Cat. No.: 940-001309-00)

Item & Cat. No.	Component	Cap color	Spec & Quantity
DNBSEQ Dual Barcode Circularization Module Cat. No.: 940-001309-00 Specification: 96 RXN	Dual Barcode Splint Buffer	Purple	1114 µL/tube × 1
	DNA Rapid Ligase	Purple	48 µL/tube × 1
	Digestion Buffer	White	135 µL/tube × 1
	Digestion Enzyme	White	250 µL/tube × 1
	Digestion Stop Buffer	White	720 µL/tube × 1

i When using 96RXN, it is recommended to select a combination of different specifications of DNBSEQ DNA Clean Beads according to the actual experimental sample requirements. The catalog number of the clean beads can be referred to "Table 4 Order information for CG products" on page 3.

1.5 Storage and transportation

Table 3 Kit storage and transportation temperatures

Item	Storage temperature	Transportation temperature
DNBSEQ Dual Barcode Circularization Module	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ DNA Clean Beads	2 ℃ to 8 ℃	2 ℃ to 8 ℃

- Production date and expiration date: refer to the label.
- For ice packs or dry ice shipments, ensure that there is enough ice or dry ice remaining after transportation.
- With proper transport, storage, and use, all components can maintain complete activity within their shelf life.

1.6 User-supplied materials

Table 4 Order information for CG products

Catalog number	Name	Component	Spec & Quantity
940-001281-00	DNBSEQ DNA Clean Beads	DNA Clean Beads	50 mL/tube × 1
		TE Buffer	25 mL/tube × 1
940-001283-00	DNBSEQ DNA Clean Beads	DNA Clean Beads	15 mL/tube × 1
		TE Buffer	17 mL/tube × 1

- The DNA Clean Beads in the table above can be used with DNBSEQ Dual Barcode Circularization Module (96 RXN) (Cat. No.: 940-001309-00).
- The required quantity and specifications are calculated based on the actual number of samples (N) and the DNA clean beads volume used in the 3.3.2 step 1.
- The recommended number of samples used to calculate the amount of DNA clean beads is (N+2).

Table 5 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	/
Desktop centrifuge	/
Pipettes	/
Thermocycler	/
Magnetic rack DynaMag -2, or equivalent	Thermo Fisher Scientific, Cat. No. 12321D
Qubit Fluorometer, or equivalent	Thermo Fisher, Cat. No. Q33216

Table 6 Recommended reagent/consumable list

Reagent/consumable	Recommended brand
Nuclease Free (NF) water	Ambion, Cat. No. AM9937, or equivalent
TE Buffer, pH 8.0	Ambion, Cat. No. AM9858, or equivalent
100% Ethanol (Analytical Grade)	/
Qubit ssDNA Assay Kit	Invitrogen, Cat. No. Q10212, or equivalent
Qubit dsDNA HS Assay Kit	Invitrogen, Cat. No. Q32854, or equivalent
Pipette tips	/
1.5 mL tube	/
0.2 mL PCR tube or 96-well plate	/
Qubit Assay Tubes or 0.5mL Thin Wall PCR Tubes	Invitrogen or Axygen, or equivalent

1.7 Precautions and warnings

- This product is for research use only, not for use in vitro diagnosis. Please read this manual carefully before use.
- Familiarize yourself with the precautions and operation methods of various instruments before performing the experiment.
- This manual aims to provide a standard protocol. Changes can be made for different applications, but changes must be tested prior to starting the protocol.
- It is recommended that you use pipette tips with filters to prevent cross-contamination. Use a new tip each time for pipetting different solutions or samples.

- It is recommended that you use the thermocyclers with heated lids for reactions. Preheat the thermocyclers to reaction temperature before use. If the thermocycler does not allow for lid temperature adjustments, the preset lid temperature of 105 ℃ is sufficient.
- Aerosol contamination may cause inaccurate results. It is recommended that you prepare separate working areas in the laboratory. Use designated equipment for each area and clean the area regularly to ensure a sterile working environment (use 0.5% Sodium Hypochlorite or 10% Bleach to clean the working area).
- Avoid skin and eye contact with samples and reagents. In case of contact with skin and eyes, rinse immediately with plenty of water and seek medical advice.
- Conform to the law and regulations when disposing of all samples and reagents.
- If you have questions, please contact Technical Support:
  US-TechSupport@CompleteGenomics.com

1.8 Workflow

Section	Workflow	Total time	Hands-on time
3.1	Denaturation and single strand circularization	45 - 50 min	15 min
3.2	Digestion	35 - 40 min	10 min
3.3	Cleanup of digestion product ⏸	50 min	10 - 15 min
3.4	QC of purification product ⏸	15 - 20 min	10 - 15 min

- Total time: The theoretical use time of 8 reactions. The time will be extended if the number of reactions increases.
- Hands-on time: The total required hands-on time in the process.
- ⏸ Stop point.

2 Sample preparation

2.1 Sample requirements

2.1.1 Input requirement

- The recommended input DNA amount is 1 pmol. If the PCR product is not enough, the minimum input DNA amount should be no less than 0.5 pmol.
- If there are special requirements regarding the amount of input PCR product from the library prep kit, follow the special requirements.
- Refer to the formula or the table below to calculate the mass in (ng) that corresponds to 1 pmol of dsDNA sample with varying fragment sizes.

Formula 1 Conversion between 1 pmol of dsDNA sample and mass in ng

Mass corresponding to 1 pmol PCR product (ng) = PCR product peak size (bp) × 0.66

Table 7 The corresponding yield in 1 pmol for different PCR product sizes (dsDNA)

Insert size (bp)	PCR product size (bp)	Corresponding yield in 1 pmol (ng)
150	282	187
200	332	220
250	382	253
300	432	286
350	482	319
400	532	352
450	582	385
500	632	418

2.1.2 Sample pooling requirement

- Input DNA can be a single sample or multiplexed samples with different barcodes.
- Multiplexed samples must satisfy specific barcodes combination requirements. Follow the usage rules of UDB PCR Primer Mix in the DNBSEQ UDB Primers Adapter Kit to use barcodes in the correct combination.
- The recommended total amount of multiplexed samples should be 1 pmol. Pool the samples equally if each sample requires the same amount of sequencing data. Calculate the mass of each sample according to Formula 2.

Formula 2 Calculation of each sample mass

$$\text{Each sample mass (ng)} = \frac{\text{The mass (ng) corresponding to 1 pmol PCR products}}{\text{Number of samples}}$$

- The total volume for circularization should be 48 µL. Add TE Buffer to make a total volume of 48 µL if the volume is not enough.

3 Circularization and digestion

3.1 Denaturation and single strand circularization

Calculate the required purified PCR product volume based on the main fragment size of purified PCR product, concentration of sample, and Formula 1 in Chapter 2.

3.1.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 8 Preparing the reagents

Reagent	Requirement
TE Buffer	User-supplied; place at room temperature (RT).
Splint Buffer	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.
DNA Rapid Ligase	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.

3.1.2 Denaturation

1. Add 1 pmol of PCR product into a new 0.2 mL PCR tube. Add TE Buffer to make a total volume of 48 µL.
2. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 9 Denaturation reaction conditions (Volume: 48 µL)

Temperature	Time
105 ℃ Heated lid	On
95 ℃	3 min

3. When the program is completed, immediately place the PCR tube(s) on ice for 2 min. Centrifuge briefly and place on ice.

3.1.3 Single strand circularization

1. According to the desired reaction number, prepare the single strand circularization mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 10 Single strand circularization mixture

Reagent	Volume per reaction
Dual Barcode Splint Buffer	11.6 μL
DNA Rapid Ligase	0.5 μL
Total	12.1 μL

2. Add 12.1 μL of single strand circularization mixture to each sample tube (from step 3 in section 3.1.2). Vortex 3 to 6 times (3 sec each), centrifuge briefly, and place on ice.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 11 Single strand circularization reaction conditions (Volume: 60.1 μL)

Temperature	Time
45 ℃ Heated lid	On
37 ℃	30 min
4 ℃	Hold

4. When the program is completed, place the PCR tube(s) on ice, centrifuge briefly, and immediately proceed to the next step.

3.2 Digestion

3.2.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 12 Preparing the reagents

Reagent	Requirement
Digestion Buffer	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.
Digestion Enzyme	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
Digestion Stop Buffer	Thaw at RT, mix by vortexing, centrifuge briefly, and place at RT.

3.2.2 Digestion

1. According to the desired reaction number, prepare the digestion mixture in a 0.2 mL PCR tube on ice. Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 13 Digestion mixture

Reagent	Volume per reaction
Digestion Buffer	1.4 μL
Digestion Enzyme	2.6 μL
Total	4.0 μL

2. Add 4 μL of digestion mixture to each sample tube (from step 4 in section 3.1.3). Vortex 3 to 6 times (3 sec each), centrifuge briefly, and place on ice.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 14 Digestion reaction conditions (Volume: 64.1 μL)

Temperature	Time
45 ℃ Heated lid	On
37 ℃	30 min
4 ℃	Hold

4. When the program is completed, centrifuge the tube(s) briefly. Immediately add **7.5 μL of Digestion Stop Buffer** to each sample tube.
5. Vortex the tube(s) 3 times (3 sec each) and centrifuge briefly. Transfer all liquid to a new 1.5 mL centrifuge tube (one tube per reaction).

3.3 Cleanup of purification product

- Use the DNA Clean Beads included in this kit (refer to Table 1 and Table 4). If the magnetic beads from other kits or brands are used, optimize the cleanup conditions before getting started.
- Do not disturb or pipette the beads when adding reagents or transferring supernatant. If you accidentally disturb or pipette the beads, pipette the solution and beads back into the tube and restart the separation process.

3.3.1 Preparation

Table 15 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied. Freshly prepared.
TE Buffer	Place at RT.
DNA Clean Beads	Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

3.3.2 Cleanup of digestion product

1. Mix the DNA Clean Beads thoroughly. Add 170 µL of DNA Clean Beads to each sample tube (from step 5 in section 3.2.2). Gently pipette at least 10 times until all beads are suspended. Ensure that all of the solution and beads in the tip are transferred into the tube after mixing. Or, mix with a vortexer.

   If other DNA clean beads volumes per sample are required, refer to the corresponding library construction instructions or determine through testing in advance.

2. Incubate the sample(s) at room temperature for 10 min.
3. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant.
4. While keeping the tube(s) on the magnetic rack, add 500 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
5. Repeat step 4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
6. Keep the tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

   Over-drying the beads will result in reduced yield.

7. Remove the tube(s) from the magnetic rack and add 22 µL of TE Buffer to elute the DNA. Gently pipette the liquid at least 10 times until all beads are suspended. Or, mix with a vortexer.
8. Incubate the sample(s) at room temperature for 10 min.
9. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 20 µL of supernatant to a new 1.5 mL centrifuge tube.

   Stop point After cleanup, the purification product(s) can be stored at -20 ℃ for one month.

3.4 Quality control of library

Quantify the purification product by following the instructions of the Qubit ssDNA Assay Kit.

- The yield of purification product should be not less than 80 fmol.

Refer to the formula or the table below to calculate the mass of 80 fmol ssCir.

Formula 3 Circular ssDNA fmol and ng conversion

Mass corresponding to 80 fmol circular ssDNA (ng) = 0.08 × PCR product peak size (bp) × 0.33

Table 16 The corresponding yield in 80 fmol for different PCR product sizes (circularized ssDNA)

Insert size (bp)	PCR product size (bp)	Corresponding yield in 80 fmol (ng)
150	282	7.5
200	332	8.8
250	382	10.1
300	432	11.4
350	482	12.8
400	532	14.1
450	582	15.4
500	632	16.7

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