DNBSEQ Complete WGS Set (96 samples) User Manual

- Cat. No.: 940-001514-00 (96 samples)
- Kit version: 1.0

For Research Use Only.
Not for use in diagnostic procedures.

Complete Genomics, Inc.

About the user manual

Manufacturer information

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Revision history

Manual Rev	Kit version	Date	Description
1.0	V1.0	June, 2025	Initial release

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

Contents

1 Product overview

1.1 Introduction

DNBSEQ Complete WGS Set (96 samples) is a genomic DNA library preparation kit compatible with DNBSEQ high-throughput sequencing platforms. This kit is used to label long DNA fragments (length greater than 20 kb) from each sample with unique barcodes. After library preparation and sequencing, reads derived from the same initial long DNA molecule can be connected using the barcode. This long fragment read technology enables identification of haplotypes and structural variations as well as de novo assembly. All reagents provided in this set have passed strict quality control and functional verification procedures, ensuring stability and reproducibility.

1.2 Intended use

The library preparation kit is applicable for human genome resequencing and can identify haplotype and structural variants. This kit is also applicable to genome de novo assembly of humans, simple plants, and animals (such as dogs, moths, fishes, rice, and lettuce), achieving high quality assembly performance.

1.3 Applicable sequencing platform

The prepared libraries are applicable to the following sequencing platforms:

DNBSEQ-T7RS series sequencer with Complete WGS high-throughput sequencing kits.

Sequencing strategy is PE150+10+42.

i The length of the sample barcode is 10 bp. The length of the bead barcode is 42 bp.

1.4 Components

This library preparation set is designed for up to 96 samples. Three separate boxes are included. For component details, refer to the following table. Each library prep kit contains an information card. Relevant manuals and MSDS files can be downloaded from the CG website provided on the information card.

Table 1 DNBSEQ Complete WGS Set (96 samples, 940-001514-00)

Item & Cat. No.	Component	Cap color	Spec & Quantity
DNBSEQ CompleteWGS 48-Barcode Kit Cat. No. 940-002746-00	CompleteWGS_Barcodes: 1, 3, 4, 5, 7, 8, 17, 22	Colorless	4 µL/tube × 8 (in strip tube)
	CompleteWGS_Barcodes: 9, 10, 11, 12, 13, 15, 16, 20	Colorless	4 µL/tube × 8 (in strip tubes)
	CompleteWGS_Barcodes: 48, 49, 50, 51, 53, 54, 56, 116	Colorless	4 µL/tube × 8 (in strip tubes)
	CompleteWGS_Barcodes: 43, 57, 58, 59, 60, 61, 63, 64	Colorless	4 µL/tube × 8 (in strip tubes)
	CompleteWGS_Barcodes: 23, 65, 66, 67, 69, 70, 71, 72	Colorless	4 µL/tube × 8 (in strip tubes)
	CompleteWGS_Barcodes: 47, 73, 74, 75, 76, 77, 78, 79	Colorless	4 µL/tube × 8 (in strip tubes)

Item & Cat. No.	Component	Cap color	Spec & Quantity
DNBSEQ Complete WGS Library Prep Kit Cat. No. 940-002745-00	TI Buffer	Green	960 µL/tube × 2
	DNA Ligase	Red	224 µL/tube × 1
	Ligation buffer I	Red	416 µL/tube × 1
	Digestion Enzyme	Purple	80 µL/tube × 1
	Digestion Buffer I	Purple	1520 µL/tube × 1
	Pre Ligation Enzyme	Black	64 µL/tube × 1
	Pre Ligation Buffer	Black	320 µL/tube × 1
	Ligation Buffer II	Orange	768 µL/tube × 1
	Adapter	Orange	288 µL/tube × 1
	PCR Enzyme Mix	Blue	1200 µL/tube × 1
	PCR Primer Mix	Blue	120 µL/tube × 1
	Capture Buffer	Yellow	800 µL/tube × 1
DNBSEQ Complete WGS Reagent Kit Cat. No. 940-002744-00	Capture Beads	Yellow	480 µL/tube × 1
	DNA Clean Beads	White	1760 µL/tube × 1
	Wash Buffer I	White	800 µL/tube × 1
	Wash Buffer II	White	5067 µL/tube × 3
	TIS Buffer	White	176 µL/tube × 1
	TE Buffer	White	2080 µL/tube × 1
	Molecular Grade Water	White	1720 µL/tube × 1

1.5 Storage and transportation

Table 2 Kit storage and transportation temperatures

Item	Storage temperature	Transportation temperature
DNBSEQ CompleteWGS 48-Barcode Kit	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ Complete WGS Library Prep Kit		
DNBSEQ Complete WGS Reagent Kit	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 stated shelf life.

1.6 User-supplied materials

Table 3 Ordering information

Catalog number	Model	Name
940-002496-00	DNBSEQ Complete WGS FCL PE150	DNBSEQ-T7RS High-throughput Sequencing Reagent Set

Table 4 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
Agilent 2100 Bioanalyzer, or equivalent	Agilent Technologies, Cat. No. G2939AA

Table 5 Recommended reagent/consumable list

Reagent/consumable	Recommended brand
Nuclease Free (NF) water	Ambion, Cat. No. AM9937, or equivalent
Molecular Grade Water	Ambion, Cat. No. AM9937, or equivalent
100% Ethanol (Analytical Grade)	/
MGIEasy Magnetic Beads Genomic DNA Extraction Kit	MGI, Cat No. 1000010524
Qubit ssDNA Assay Kit	Invitrogen, Cat. No. Q10212, or equivalent
Qubit dsDNA HS Assay Kit	Invitrogen, Cat. No. Q32854, or equivalent
PicoGreen dsDNA Assay Kit	Invitrogen, Cat. No. P7589, or equivalent
Agilent High Sensitivity DNA Kit	Agilent, Cat. No. 5067-4626, or equivalent
Agilent DNA 1000 Kit	Agilent, Cat. No. 5067-1504, 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 diagnosis. 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.
- For animal and plant samples with different GC content, the usage of Barcodes should be appropriately adjusted according to GC content and the adjustment principle is shown in *"5.3 The CompleteWGS_Barcodes usage for samples with different GC content" on page 28*.
- Before step *"3.5 Termination reaction" on page 17*, all liquid containing target DNA fragments should be treated as gently as possible. Wide-bore tips are highly recommended during these steps and vigorous vortexing should be avoided. For mixing purposes, flicking the bottom of the tube or inverting the tube at very low speed is sufficient.
- During the reaction steps, the room temperature range should be 20 °C to 25 °C.

- Always keep the Capture Beads wet and do not let them dry completely at any time. Add the reaction mix immediately after disposing of bead wash buffer.
- 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.
- Avoid touching the Capture Beads and their solution when pipetting the reaction mix into the sample tube.
- Check the lid of the sample tube after every reaction and make sure there are no residual beads. If there are residual beads, use a small volume of the Wash Buffer II to wash the beads off the lid and collect them into the sample tube.
- It is recommended that you use the thermocyclers with heated lids for reactions. Preheat the thermocyclers to operating temperature before use. The reaction volume of the thermocycler should not be less than 100 µL when doing the PCR step.
- Aerosol contamination may cause inaccurate results. It is recommended that you prepare separate working areas in the laboratory for PCR reaction preparation, PCR reaction, and PCR product cleanup. 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).
- All libraries should be sequenced using the high-throughput sequencing kit designed for Complete WGS.
- Avoid skin and eyes contact with samples and reagents. Do not eat or drink the samples and reagents. In case of contact with skin and eyes, rinse immediately with plenty of water and seek medical advice.
- Conform to the laws 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	Transposon insertion	20 - 25 min	10 - 15 min
3.2	Capture	1 hr 15 min	10 - 15 min
3.3	1st Ligation reaction	1 hr 10 min	10 min
3.4	Digestion reaction	15 min	5 min
3.5	Termination reaction ⏸ Stop point	20 - 25 min	10 -15 min
3.6	Pre-2nd Ligation reaction	35 - 40 min	5 - 10 min
3.7	2nd Ligation reaction ⏸ Stop point	2 hr 10 min	10 min
3.8	PCR ⏸ Stop point	50 - 55 min	10 - 15 min
3.9	Cleanup of PCR product ⏸ Stop point	45 min	10 - 15 min
3.10	QC of PCR product ⏸ Stop point	15 - 60 min	10 - 20 min
	Total expected time	~8 hrs	~1.5 hrs

- 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.

2 Sample preparation

2.1 Sample requirements

- For DNA isolation/purification, we recommend using the MGIEasy Magnetic Beads Genomics DNA Extraction Kit (Cat. No. 1000010524).
- The following criteria are recommended for best performance after sample extraction: sample concentration should be more than 1 ng/ μL, the A260/280 value of the DNA sample should be 1.6 to 2.2, and the mean length of the initial DNA fragments should be longer than 20 Kb. DNA fragments with longer length will produce better results (see Figure 1 for expected performance based on different DNA fragment).
- Store isolated DNA samples at 4 ℃. Samples can also be stored at -20 ℃, but frequently freezing and thawing the DNA should be avoided. DNA samples can be stored at 4 ℃ for 6 months or -20 ℃ for 1 year (samples must be stored separately and removed no more than two times. Avoid physical fragmentation). Use nuclease-free reagents and consumables.
- Protein contamination and/or high concentration of salts and other contaminants may lead to failure of the library construction process.
- All samples must meet the conditions listed above for library construction. If the sample cannot meet the requirements, this may result in failure to prepare a library and may cause unsatisfactory data analysis results.

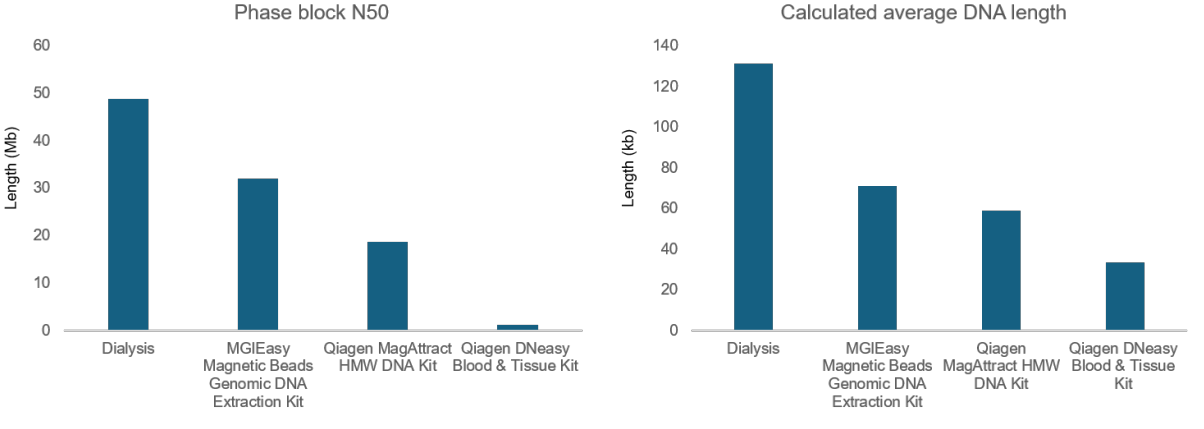

Figure 1 DNA length and phasing performance using different isolation methods.

DNA from the cell line GM24385 was isolated using various methods. DNBSEQ Complete WGS libraries were made for each sample and sequenced to a depth of approximately 30X on a DNBseq platform. DNA length was calculated based on the distance between reads mapped to the GRCh38 human reference genome and sharing the same barcode. Each library was phased and haplotypes were constructed using Hapcut2[1,2]. N50 phase block lengths are calculated by the Hapcut2 program. Longer phase blocks are the result of more variants being connected together into haplotypes.

1. Edge P, Bafna V, Bansal V. 2017. Hapcut2: Robust and accurate haplotype assembly for diverse sequencing technologies. Genome Res. 27(5):801-812.

2. Bansal V. 2023. Hapcut2: A method for phasing genomes using experimental sequence data. Methods Mol Biol. 2590:139-147.

2.2 Sample quantification

- Use the Qubit dsDNA HS Assay Kit for quantitation.
- Gently and slowly invert the extracted tube to mix the gDNA. Take 3 µL each of the gDNA sample from top, middle, and bottom part of the tube, and quantitate them with Qubit dsDNA HS Assay Kit. If the replicates differ by more than 10%, repeat the gentle mixing operation and the quantification step until the replicates differ by less than 10% and the concentration range is 1 to 3 ng/µL.
- If the concentration of gDNA is >3 ng/µL, dilute the DNA sample to 1 to 3 ng/ µL in TE Buffer.

  The long-fragment DNA and its dilutions should be stored at 4 °C to prevent freeze-and-thaw cycles. DNA mixing should be performed slowly and gently using wide-bore tips or gentle flicking to avoid physical fragmentation.
- 10 ng of DNA are needed for *"3.1.2 Transposon insertion" on page 10*.
- 15% of transposon-inserted product (~1.5 ng) are required for *"3.2.2 Capture" on page 12*.
- Up to 8 samples can be combined into a single multiplex reaction after the transposon insertion step.

3 Library preparation protocol

3.1 Transposon insertion

- If the GC content of the sample is different from human sample (the GC content of the human sample is about 42%), adjust the usage of CompleteWGS_Barcodes according to *"5.3 The CompleteWGS_Barcodes usage for samples with different GC content" on page 28*. If no adjustment is made the resulting insert size of the library may be suboptimal resulting in potentially lower yields and lower quality results after sequencing.

3.1.1 Preparation

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

Table 6 Preparing the reagents

Reagent	Requirement
CompleteWGS_Barcodes	Thaw on ice, mix thoroughly, and centrifuge briefly.
TI Buffer	Thaw at room temperature (RT), mix by vortexing, centrifuge briefly, and place on ice.
TE Buffer	Place at RT.
Molecular Grade Water	Place at RT.

3.1.2 Transposon insertion

CAUTION

- Mix the gDNA by slowly and gently pipetting with **wide-bore tips 6-8 times** to avoid physical fragmentation.
- Slowly pipette the long fragment DNA with **normal tips** each time when collecting and dispensing.

1. Gently transfer 10 ng long-fragment gDNA into a 0.2 mL PCR tube. Add Molecular Grade Water to make a total volume of 36.8 µL without mixing and place on ice.

2. Dilute the CompleteWGS_Barcodes with TE Buffer.
   1) Prepare the 4 × dilution CompleteWGS_Barcodes: Add 6 µL TE Buffer into a new 0.2 mL PCR tube and transfer 2 µL CompleteWGS_Barcodes to the tube. Vortex intermittently for 4 times (2 sec each) to mix well. Label it as "4 × dilution CompleteWGS_Barcodes".
   2) Prepare the CompleteWGS_Barcodes (Working Mix): For barcode #22, add 12 µL of TE Buffer into a new 0.2 mL PCR tube and transfer 12 µL of 4 × dilution into the tube. For barcode #49, add 14 µL of TE Buffer into a new 0.2 mL PCR tube and transfer 10 µL of 4 × dilution into the tube. For barcode #64, add 15.5 µL of TE Buffer into a new 0.2 mL PCR tube and transfer 8.5 µL of 4 × dilution into the tube. For barcode #73, add 15 µL of TE Buffer into a new 0.2 mL PCR tube and transfer 9 µL of 4 × dilution into the tube. For all remaining barcodes, add 18 µL of TE Buffer into a new 0.2 mL PCR tube and transfer 6 µL of 4 × dilution into the tube. Vortex intermittently for 4 times (2 sec each) to mix the reagent. Label it as "CompleteWGS_Barcodes (Working Mix)". This CompleteWGS_Barcodes (Working Mix) can be used for 8 reactions.

   - Keep all materials on ice. The CompleteWGS_Barcodes dilution must be freshly prepared.
   - Start reaction within 5 min after all reagents were added.

3. Add the transposon insertion reaction reagents to the sample tube (from step 1) on ice as shown in the table below. Gently pipette 10 times with wide-bore tip. Centrifuge (~1 sec) several times at low speed and place on ice.

Table 7 Transposon insertion reaction system

Reagents	Volume per reaction
gDNA with water (step 1 in section 3.1.2)	36.8 µL
TI Buffer	10 µL
CompleteWGS_Barcodes (Working Mix)	3.2 µL
Total	50 µL

4. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 8 Transposon insertion reaction conditions (Volume: 50 µL)

Temperature	Time
60 ℃ Heated lid	On
55 ℃	10 min
4 ℃	Hold

5. After the reaction, briefly centrifuge (1 sec) the tube(s) at low speed and place on ice.

   Only 15% of transposon-inserted product from step 6 is used for Capture in section 3.2.

6. Remove and transfer 6 µL of each transposon-inserted product, up to a total of 8 different samples, to a new 0.2 mL tube. Bring the total volume to 50 µL with TE. DO NOT use more than 6 µL of a single sample (i.e., if you are not multiplexing-samples, then only use 6 µL transposon-inserted product and add 44 µL of TE). Gently invert the tube to mix. Centrifuge(~1 sec) several times to collect the liquid to the bottom of the tube and place on ice.

3.2 Capture

3.2.1 Preparation

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

Table 9 Preparing the reagents

Reagent	Requirement
Capture Beads	Mix by vortexing and place at RT.
Wash Buffer I	Mix by vortexing, centrifuge briefly, and place at RT.
Capture Buffer	Thaw at RT, mix well, centrifuge briefly, and place on ice.

The Capture Buffer is highly viscous. Mix it well by vortexing 6 times (3 sec each) and centrifuge briefly. When pipetting the Capture Buffer, slowly aspirate to ensure that the volume is accurate.

3.2.2 Capture

1. According to the number of reactions (up to 8 samples can be multiplexed into a single reaction), resuspend the Capture Beads. Vortex the Capture Beads for 1 min. Pipette 30 µL Capture Beads per reaction to the tube*.

   - * If there are multiple reactions, pipette n × 1.1 × 30 µL Capture Beads to the same tube, where n = number of reactions.
   - If n ≤ 3, use 0.2 mL PCR tube. If n > 3, use 1.5 mL tube.

2. Centrifuge the tube briefly and place on a magnetic rack for 2 min until the liquid is clear. Carefully remove and discard the supernatant.
3. Keeping the tube on the magnetic rack, add 50 µL Wash Buffer I per reaction into the 0.2 mL PCR tube or 1.5 mL tube. Ensure that Wash Buffer I can cover all of the Capture Beads*.

   * If there are multiple reactions, pipette n × 50 µL Wash Buffer I to the tube, where n = number of reactions.

4. Rotate the tube 180° while on the magnetic rack to let the beads move through the Wash Buffer I. Repeat the tube rotation. Carefully remove and discard the supernatant when the liquid is clear (~1 min).
5. Remove the tube from the magnetic rack. Add 50 µL Capture Buffer per reaction to resuspend the Capture Beads. Mix with a vortexer.

   - If there are multiple reactions, pipette n × 1.1 × 50 µL Capture Buffer to resuspend the Capture Beads, where n = number of reactions.
   - The Capture Buffer is highly viscous.

6. Transfer 50 µL resuspended Capture Beads to each reaction tube (from step 7 in section 3.1.2). Mix thoroughly by gently inverting at least 10 times, and centrifuge briefly.

   - Due to the high sedimentation rate of the Capture Beads, mix the resuspended Capture Beads once every 30 sec when pipetting it to the reaction tube(s).
   - DO NOT VORTEX the reaction tube at Step 6.
   - To ensure a proper capture reaction, mix all components in the tube by gently inverting several times, followed by brief centrifugation (1 sec) several times. Ensure that Capture Beads are homogeneously resuspended (Figure 2a). Incomplete resuspension of Capture Beads (Figure 2b) may cause poor library performance.

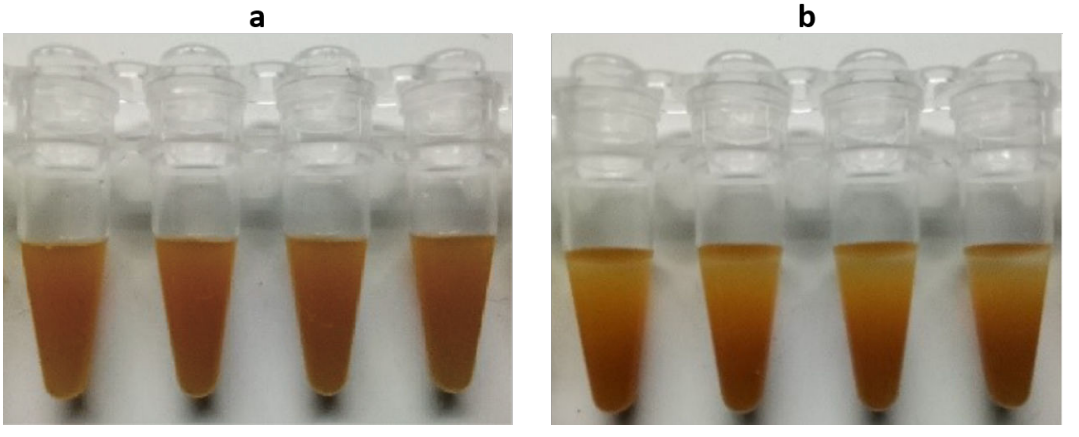

Figure 2 a) Homogenous suspension of capture beads; b) Incomplete suspension of capture beads

7. Centrifuge the tube(s) for 1 sec at low speed. Immediately place the tube(s) into the thermocycler and run the program with the following conditions.

Table 10 Capture incubation conditions (Volume: 100 µL)

Temperature	Time
65 °C Heated lid	On
60 °C	10 min
45 °C	50 min

8. After the reaction, centrifuge the PCR tube(s) for 1 sec and place at room temperature for 2 minutes to let it cool down. The reactions are now ready to move onto the ligation step. It is not recommend that you stop at this step.

   - Capture Beads may sediment after the reaction (Figure 4), which is a normal phenomenon.
   - Do not stop here. Proceed to the next step.

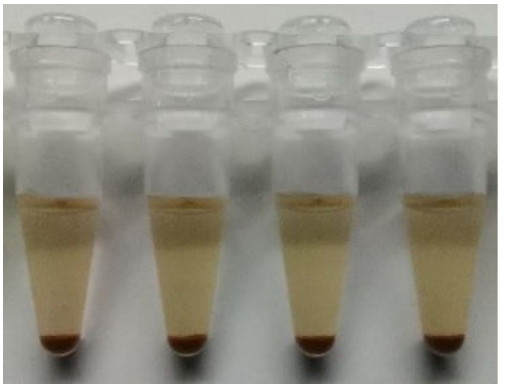

Figure 3 Sedimentation, seen in the tube, may occur after the reaction

3.3 1st ligation reaction

3.3.1 Preparation

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

Table 11 Preparing the reagents

Reagent	Requirement
DNA Ligase	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
Ligation Buffer I	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.
Wash Buffer II	Mix well, place at RT.

CAUTION

- DNA Ligase will be inactivated at high temperatures. Stored it at -20 ℃ immediately after using. Making sure the sample has cooled to room temperature when adding DNA Ligase.
- The Ligation Buffer I is highly viscous. Mix it by vortexing 6 times (3 sec each) and centrifuge briefly. When pipetting the Ligation Buffer I, slowly aspirate to ensure that the volume is accurate.

3.3.2 1st ligation reaction

1. According to the desired reaction number, prepare the 1st Ligation Reaction Mix in a 0.2 mL PCR tube on ice. Vortex it 3 times (3s each), centrifuge briefly, and place on ice. The mix below is for 1 reaction, multiply by 1.1 × n for n reactions.

Table 12 1st ligation reaction mix

Reagents	Volume per reaction
Ligation Buffer I	26 µL
DNA Ligase	4 µL
Total	30 µL

2. Add 30 µL 1st ligation reaction mix to each reaction tube (from step 8 in section 3.2.2). Gently invert the tube(s) at least 10 times and briefly centrifuge (1 sec) at low speed.

- Ensure that the sample has cooled to room temperature. DO NOT VORTEX the sample.
- Mix all components by gently inverting several times followed by brief centrifugation. Ensure that Capture Beads are homogeneously resuspended (Figure 2).

3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 13 1st ligation reaction conditions (Volume: 130 µL)

Temperature	Time
Heated lid	Off
25 °C	60 min

A small amount of sedimentation of Capture Beads is normal after the reaction (see Figure 4).

4. After the incubation, centrifuge the tube(s) briefly and place on a magnetic rack for 1 to 2 min until the liquid is clear. Carefully remove and discard the supernatant.
5. Keeping the tube on the magnetic rack, add 180 µL of Wash Buffer II to each reaction tube. Rotate the tube 180° while on the magnetic rack to let the beads move through the Wash Buffer II. Repeat the tube rotation. Carefully remove and discard the supernatant when the liquid is clear.
6. Digestion Reaction MUST be carried out immediately after discarding Wash Buffer II.

CAUTION

- Do not let the Capture Beads dry.
- Capture Beads can be stored in Wash Buffer II for up to 5 min until Digestion Reaction Mix is prepared.

3.4 Digestion reaction

Keep the reaction tube at room temperature after Digestion Reaction. Add TIS Buffer to reactions at room temperature within 1 min. Prepare the required reagents for Termination Reaction in advance.

3.4.1 Preparation

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

Table 14 Preparing the reagents

Reagent	Requirement
Digestion Enzyme	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
Digestion Buffer I	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.
TIS Buffer	Place at RT until the crystals dissolve (approximately 5 to 10 min). Mix by vortexing, centrifuge briefly, and place at RT. Use for section 3.5.

3.4.2 Digestion reaction

1. According to the desired reaction number, prepare the Digestion Reaction Mix 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. The table below is for a single reaction, multiply by 1.1 × n for n reactions.

Table 15 Digestion reaction mix

Reagents	Volume per reaction
Digestion Buffer I	95 µL
Digestion Enzyme	5 µL
Total	100 µL

2. Add 100 µL digestion reaction mix to each reaction tube (from step 6 in section 3.3.2) on ice. Gently invert the tube at least 10 times to mix and centrifuge briefly (1 sec) at low speed. Place the tube(s) on ice.

   - **DO NOT VORTEX** the sample at Step 2.
   - Mix all components in the tube by gently inverting several times followed by instantaneous centrifugation. Ensure that Capture Beads are homogeneously resuspended (Figure 2).
   - Strictly perform the reaction conditions. Do not incubate at 4 ℃ after the 37 ℃ incubation step.
   - Keep the sample tube on ice before the Digestion Reaction. Strictly control the reaction time of the Digestion Reaction conditions to avoid excessive digestion.

3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 16 Digestion reaction conditions (Volume: 100 µL)

Temperature	Time
42 ℃ Heated lid	On
37 ℃	10 min

Do not put the sample on ice after reaction.

4. After the reaction, centrifuge the tube(s) briefly (1 sec) and place at room temperature. Add TIS Buffer within 1 min.

3.5 Termination reaction

- Before adding TIS Buffer, ensure that the samples have cooled to room temperature.
- After adding TIS Buffer, all subsequent steps can be mixed by vortexing as the long fragment DNA has been completely fragmented.
- After adding TIS buffer, slight ivory discoloration of the solution is normal since all enzymes are denatured at this step.
- Residual TIS Buffer will inhibit the following steps. For this reason it is important to wash 3 times with Wash Buffer II to completely remove the TIS Buffer. After adding Wash Buffer II, mix by vortexing at high speed for several seconds to fully rinse all parts of the tube.

3.5.1 Preparation

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

Table 17 Preparing the reagents

Reagent	Requirement
Wash Buffer II	Mix well and place at RT.

3.5.2 Termination reaction

1. Add 11 µL of TIS Buffer to each reaction tube (from step 4 in section 3.4.2).
2. Ensure the tube cap is sealed tightly. Mix the tube(s) by vortexing at medium speed for 3 sec to 5 sec to make sure the beads are fully resuspended. Centrifuge the tube(s) briefly and incubate at room temperature for 10 min.

Table 18 Incubation conditions of the termination reaction (Volume: 111 µL)

Temperature	Time
Room Temperature (20 ℃ to 25 ℃)	10 min

3. After the reaction, centrifuge the tube(s) briefly and place on a magnetic rack for 2 min until the liquid is clear. Carefully remove and discard the supernatant.
4. Keep the tube(s) on the magnetic rack and pipette 150 µL Wash Buffer II into each reaction tube. Mix by vortexing for 10 sec and centrifuge for 3 sec. Place the tube(s) on the magnetic rack for 2 min until the liquid is clear. Carefully remove and discard the supernatant.
5. Repeat step 4 two more times. Try to remove all liquid from the tube. If using the 8 tube-separated PCR strip tubes, replace the PCR tube cap.

CAUTION You may keep the beads in Wash Buffer II for up to 5 min until Pre-2nd Ligation Reaction Mix (step 2 in section 3.6.2) is ready. Pre-2nd Ligation Reaction MUST be carried out immediately after discarding Wash Buffer II.

Stop point During the final washing, you can keep the beads in Wash Buffer II and store them at 4℃ for no more than 24 hr.

3.6 Pre-2nd Ligation reaction

3.6.1 Preparation

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

Table 19 Preparing the reagents

Reagent	Requirement
Pre Ligation Enzyme	Flick and/or invert the tube gently, centrifuge briefly, and place on ice. Return to -20 ℃ immediately after using.
Pre Ligation Buffer	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.

3.6.2 Pre-2nd Ligation reaction

1. According to the desired reaction number, prepare the Pre-2nd Ligation Reaction Mix in a 0.2 mL PCR tube on ice. Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice. The table below is for 1 reaction, multiply by 1.1 x n for n reactions.

Table 20 Pre-2nd ligation reaction mix

Reagents	Volume per reaction
Pre Ligation Buffer	20 μL
Pre Ligation Enzyme	4 μL
Total	24 μL

2. Add 24 μL of Pre-2nd ligation reaction mix to each reaction tube (from step 5 in section 3.5.2). Vortex it 3 times (3 sec each) and centrifuge briefly. Immediately place the PCR tube(s) into the thermocycler and run the program with the following conditions.

   Mix all components in the tube by vortexing several times. Ensure that Capture Beads are homogeneously resuspended (Figure 2).

Table 21 Incubation conditions of Pre-2nd ligation reaction (Volume: 24 μL)

Temperature	Time
42 ℃ Heated lid	On
37 ℃	30 min

3. When the program is completed, immediately take out the tube(s) and centrifuge briefly. Keep it at room temperature and proceed to the next step.

3.7 2nd Ligation reaction

3.7.1 Preparation

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

Table 22 Preparing the reagents

Reagent	Requirement
DNA Ligase	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
Ligation Buffer II	Mix by vortexing, centrifuge briefly, and place on ice.
Adapter	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.
Wash Buffer II	Mix well and place at RT.

- DNA Ligase will be inactivated at high temperatures. Store it at -20 ℃ immediately after using. Be sure the sample has cooled to room temperature when adding DNA Ligase.
- The Ligation Buffer II is highly viscous. Vortex 6 times (3 sec each) and centrifuge briefly. When pipetting the Ligation Buffer II, slowly aspirate to ensure that the volume is accurate.

3.7.2 2nd Ligation reaction

1. According to the desired reaction number, prepare the 2nd Ligation reaction mix in a 0.2 mL PCR tube on ice. Vortex it 6 times (3 sec each), centrifuge briefly, and place on ice. The mix below is for 1 reaction, multiply by 1.1 × n for n reactions.

Table 23 2nd Ligation reaction mix

Reagents	Volume per reaction
Ligation Buffer II	48 µL
Adapter	18 µL
DNA Ligase	10 µL
Total	76 µL

2. Slowly pipette 76 µL of 2nd ligation reaction mix to each reaction tube (from step 3 in section 3.6.2). Mix it well by inverting and vortexing until no pellet residue remaining, centrifuge briefly, and place on ice.

   - Transfer the 2nd ligation reaction mix after the products cool to room temperature.
   - To ensure proper 2nd Ligation reaction, mix all components in the tube by vortexing several times followed by brief centrifugation (1s). Ensure that Capture Beads are homogeneously resuspended (Figure 2).

3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 24 2nd Ligation reaction conditions (Volume: 100 µL)

Temperature	Time
Heated lid	off
25 °C	120 min

Sedimentation of Capture Beads is normal after the reaction (Figure 4).

4. After incubation, centrifuge the sample and add 80 µL of Wash Buffer II to each reaction tube. Place the tube(s) on the magnetic rack for 2 min until the liquid is clear. Carefully remove and discard the supernatant.
5. Keep the tube(s) on the magnetic rack and add 180 µL of Wash Buffer II to each reaction tube. Rotate the tube 180° while on the magnetic rack to let the beads move through the Wash Buffer II. Repeat the tube rotation.
6. Carefully remove and discard the supernatant when the solution is clear. Be sure the Wash Buffer II is completely removed.

CAUTION Keep the beads in Wash Buffer II for up to 5 min until the PCR Reaction Mix (step 1 in section 3.8) is ready. PCR mixture MUST be carried out immediately after discarding Wash Buffer II.

Stop point The washed beads can be stored in Wash Buffer II at 4 °C for no more than 24 h.

3.8 PCR

3.8.1 Preparation

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

Table 25 Preparing the reagents

Reagent	Requirement
PCR Enzyme Mix	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.
PCR Primer Mix	Thaw at RT, mix by vortexing, centrifuge briefly, and place at RT.
Molecular Grade Water	Place at RT.

3.8.2 PCR

1. According to the desired reaction number, prepare the PCR mixture in a 0.2 mL PCR tube on ice. Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice. The table below is for 1 reaction, multiply by 1.1 × n for n reactions.

Table 26 PCR mixture

Reagent	Volume per reaction
Molecular Grade Water	67.5 µL
PCR Enzyme Mix	75 µL
PCR Primer Mix	7.5 µL
Total	150 µL

2. Pipette 150 µL of PCR mixture to each reaction tube (from step 6 in section 3.7.2). Pipette up and down to mix the beads until fully resuspended. Transfer 150 µL of solution into 2 tubes, 75 µL per tube.

- Samples, reagents, and Capture Beads must be thoroughly mixed to ensure complete capture of the Capture Beads. After mixing, place the reaction tube in a low-speed centrifuge to ensure that no liquid remains on the tube cap and the mix is fully suspended.
- Place the tubes when the lid of thermocycler heats up to 105 ℃. Due to the high sedimentation rate of Capture Beads, mix the sample tube again and centrifuge at low speed to make sure that Capture Beads are homogeneously resuspended (Figure 2).

3. Place all the PCR tubes (2 tubes from the same reaction) into the thermocycler and run the program with the following conditions.

Table 27 PCR reaction conditions (Volume: 75 µL)

Temperature	Time	Cycles
105 °C Heated lid	On	-
98 °C	3 min	1
95 °C	30 sec	9
58 °C	30 sec	
72 °C	2 min	
72 °C	10 min	1
4 °C	Hold	-

4. When the program is completed, centrifuge the tube(s) briefly and place on the magnetic rack for 2 min until the liquid is clear.
5. Transfer all the supernatant of the 2 PCR tubes from the same reaction into a new 1.5 mL centrifuge tube. Mix it well and centrifuge briefly.

Stop point The PCR product(s) can be stored at -20 °C for no more than 24 h.

3.9 Cleanup of PCR product

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.

3.9.1 Preparation

Table 28 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.

3.9.2 Cleanup of PCR product

1. Mix the DNA Clean Beads thoroughly. Add 0.7× of DNA Clean Beads to each reaction tube (from step 5 in section 3.8.2). Vortex for 5 to 10 sec to mix well.
2. Incubate the reaction(s) at room temperature for 10 min.
3. Centrifuge the reaction 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 pipettor.
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 (approximately 3 to 5 min). There should be no visible cracking on the surface of the beads.

   i Over-drying the beads will result in reduced yield.
7. Remove the tube(s) from the magnetic rack and add 33 µL of TE Buffer to elute the DNA. Vortex for 3 sec to resuspend the beads and centrifuge briefly.
8. Incubate the reaction(s) at room temperature for 5 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 31 µL of supernatant to a new 1.5 mL centrifuge tube.

   Stop point After cleanup, the PCR product(s) can be stored at 4 ℃ for up to 72 hr or at -20 ℃ for up to 6 months.

3.10 QC of PCR product

- **dsDNA fluorescence quantification method**: Quantify the purified PCR products with dsDNA fluorescence assay kits and instructions.
- **Electrophoresis method**: Assess the size range of purified PCR products with electrophoresis based equipment and instructions.

Table 29 Different QC methods and standards for library

Method	Equipment/Reagent	Standard
dsDNA fluorescence quantification method	Qubit dsDNA HS Assay Kit, Quant-iT PicoGreen dsDNA Assay Kit, or equivalent	Concentration of PCR products: ≥ 2.6 ng/µL

Method	Equipment/Reagent	Standard
Electrophoresis method	Tapestation (Agilent Technologies), Bioanalyzer, LabChip GX, GXII, GX Touch (PerkinElmer), Fragment Analyzer (Advanced Analytical), or 1% TBE agarose gel	Size range: 200 - 2000 bp

The following figure shows the Agilent 2100 Bioanalyzer detection results of purified PCR products.

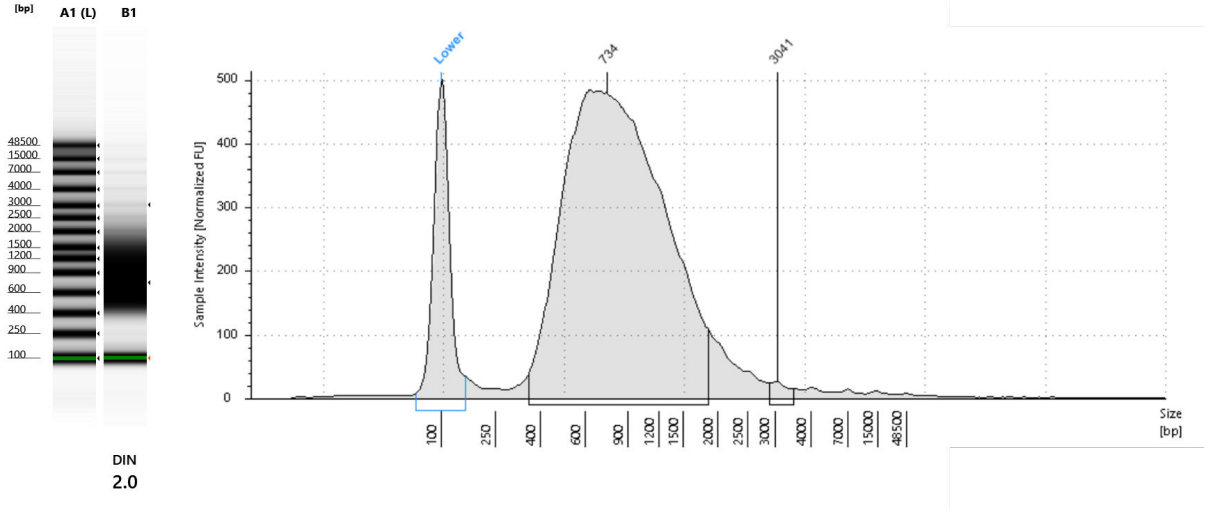

Figure 4 Agilent 2100 Bioanalyzer Image of PCR Products

4 Sequencing and analysis

4.1 Sequencing

The sequencing is optimized for the DNBSEQ-T7RS sequencing platforms with the following reagent kits: DNBSEQ-T7RS High-throughput Sequencing Reagent Set (DNBSEQ cWGS FCL PE150), PN: 940-002496-00.

4.2 Analysis

For human genome sequencing data analysis, and de novo assembly for animal and plant genomes, use the DNBSEQ Complete WGS Analysis Software. The software is available on the website using the following link: http://www.completegenomics.com/methods/completewgs/

5 Appendix

5.1 About CompleteWGS_Barcodes usage

- According to the requirements of high throughput library construction and sample pooling sequencing, to balance the diversity of the sample barcode libraries, the selected CompleteWGS_Barcodes with sample barcode were selected after repeated experimental testing, and the optimal mix mode was tested at the same time. The Barcode number is discontinuous. For best results, read the instructions of CompleteWGS_Barcodes. Samples with the same CompleteWGS_Barcodes number should not be sequenced in the same lane.
- CompleteWGS_Barcodes contains an enzyme. Store them at -20 ℃ and keep them on ice when in use.
- Before use, the liquid must be collected at the bottom of the tube by centrifugation. Carefully remove the tube cap to prevent liquid from splashing and to prevent cross contamination. Cover the tube cap after use.

5.2 Instructions for CompleteWGS_Barcodes

The CompleteWGS_Barcodes contained in the kit can be grouped according to the following rules:

1	9	48	43	23	47
3	10	49	57	65	73
4	11	50	58	66	74
5	12	51	59	67	75
7	13	53	60	69	76
8	15	54	61	70	77
17	16	56	63	71	78
22	20	116	64	72	79

Figure 5 CompleteWGS_Barcodes plate layout

- 6 sets of 8 CompleteWGS_Barcodes: Columns 1-6 (see the red box in the figure above).

- For the preferred case (multiplexing of 8 samples) use entire column as directed.
- For barcoding strategies beyond multiples of 8, please contact Complete Genomics technical support.

5.3 The CompleteWGS_Barcodes usage for samples with different GC content

Refer to the following table to adjust the usage of CompleteWGS_Barcodes according to the different GC content of the genome. Animal and plant samples can be vastly different from human samples, and differences also exist among individual types of organism samples. GC contents differences can result in increased or decreased rates of transposon insertion. The following table provides some suggestion of potential adjustments that can be made to the transposon concentration to generate the proper insert size.

Table 30 Usage reference of CompleteWGS_Barcodes in animal and plant samples with different GC

GC content	CompleteWGS_Barcodes usage reference (pmol/10ng DNA)	CompleteWGS_Barcodes (working mix) volume (μL)	DNA sample normalization volume (μL)
<30%	0.3	1.2	38.8
30%-35%	0.4	1.6	38.4
36%-39%	0.6	2.4	37.6
40%-60%	0.8	3.2	36.8
>60%	0.8	3.2	38.8

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