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DNBSEQ Fast FS Library Prep Set User Manual

- Cat. No.: 940-001511-00 (16 RXN)
  940-001509-00 (B, 96 RXN)
  940-001870-00 (C, 96 RXN)
  940-001512-00 (192 RXN)
- Set Version: V2.0

For Research Use Only

About the user manual

Manufacturer information

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Revision history

Manual Rev	Set version	Date	Description
2.0	V2.0	Jul. 2024	Update the content of “3.1 Workflow”
1.0	V2.0	Mar. 2024	Initial release

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

Contents

1 Product overview

1.1 Introduction

The DNBSEQ Fast FS Library Prep Set is designed to prepare WGS libraries for DNBSEQ high-throughput sequencing platforms. This library prep set is optimized to convert 1 ng - 1000 ng genomic DNA (gDNA) into a customized library using high-quality fast fragmentase to simplify the operation process, thereby significantly shortening the duration of DNA library preparation. All reagents provided in this set have passed strict quality control and functional verification procedures, ensuring stability and reproducibility.

The DNBSEQ Fast FS Library Prep Set uses dual barcode for library preparation. It is recommended that you prepare 4 or more samples with this set. For fewer than 4 samples, contact Technical Support. The constructed libraries (PCR dsDNA) can be used with the DNBSEQ Dual Barcode Circularization Kit (not included in this set. Cat. No.: 940-001310-00) to create single-strand circular (ssCir) DNA libraries for subsequent DNB preparation. After DNB preparation, it can be directly sequenced on DNBSEQ high-throughput sequencing platforms.

1.2 Intended use

This library prep set is applicable to samples from human (including but not limited to blood, saliva, and oral swabs), animals (including but not limited to rat, and mouse), plants (including but not limited to A. thaliana, and O. sativa), bacteria (including but not limited to E. coli), fungi, and other microbial species.

1.3 Applicable sequencing platforms

DNBSEQ sequencing platform series.

1.4 Components

This library prep set comes in four specifications: 16 RXN, 96 RXN (set B), 96 RXN (set C), and 192 RXN. For component details, refer to the following table.

Table 1 DNBSEQ Fast FS Library Prep Set (16 RXN) (Cat. No.: 940-001511-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
DNBSEQ Fast FS Library Prep Module V2.0 Cat. No.: 940-001505-00	Fast FS Buffer II	Green	215 µL/tube × 1
	Fast FS Enzyme II	Green	105 µL/tube × 1
	Fast Ligation Buffer	Red	450 µL/tube × 1
	Ad Ligase	Red	100 µL/tube × 1
	Ligation Enhancer	Brown	55 µL/tube × 1
	20x Elute Enhancer	Black	7 µL/tube × 1
	PCR Enzyme Mix	Blue	460 µL/tube × 1
DNBSEQ UDB Primers Adapter Kit Cat. No.: 940-001516-00	UDB Adapter (10 µM)	White	80 µL/tube × 1
	UDB PCR Primer Mix 57-64, 89-96 (20 µM)	Blue	10 µL/tube × 16
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 Fast FS Library Prep Set (96 RXN) (Cat. No.: 940-001509-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
DNBSEQ Fast FS Library Prep Module V2.0 Cat. No.: 940-001504-00	Fast FS Buffer II	Green	1440 µL/tube × 1
	Fast FS Enzyme II	Green	660 µL/tube × 1
	Fast Ligation Buffer	Red	1440 µL/tube × 3
	Ad Ligase	Red	600 µL/tube × 1
	Ligation Enhancer	Brown	360 µL/tube × 1
	20x Elute Enhancer	Black	25 µL/tube × 1
	PCR Enzyme Mix	Blue	1400 µL/tube × 2
DNBSEQ UDB Primers Adapter Kit B Cat. No.: 940-001517-00	UDB Adapter (10 µM)	White	480 µL/tue × 1
	UDB PCR Primer Mix 97-192 (20 µM)	/	10 µL/well × 96
DNBSEQ DNA Clean Beads Cat. No.: 940-001283-00	DNA Clean Beads	White	15 mL/tube × 1
	TE Buffer	White	17 mL/tube × 1

Table 3 DNBSEQ Fast FS Library Prep Set C (96 RXN) (Cat. No.: 940-001870-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
DNBSEQ Fast FS Library Prep Module V2.0 Cat. No.: 940-001504-00	Fast FS Buffer II	Green	1440 µL/tube × 1
	Fast FS Enzyme II	Green	660 µL/tube × 1
	Fast Ligation Buffer	Red	1440 µL/tube × 3
	Ad Ligase	Red	600 µL/tube × 1
	Ligation Enhancer	Brown	360 µL/tube × 1
	20x Elute Enhancer	Black	25 µL/tube × 1
	PCR Enzyme Mix	Blue	1400 µL/tube × 2

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
DNBSEQ UDB Primers Adapter Kit C Cat. No.: 940-001862-00	UDB Adapter (10 μM)	White	480 μL/tube × 1
	UDB PCR Primer Mix 193-288 (20 μM)	/	10 μL/well × 96
DNBSEQ DNA Clean Beads Cat. No.: 940-001283-00	DNA Clean Beads	White	15 mL/tube × 1
	TE Buffer	White	17 mL/tube × 1

Table 4 DNBSEQ Fast FS Library Prep Set (192 RXN) (Cat. No.: 940-001512-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
DNBSEQ Fast FS Library Prep Module V2.0 x 2 kits Cat. No.: 940-001504-00	Fast FS Buffer II	Green	1440 μL/tube × 1
	Fast FS Enzyme II	Green	660 μL/tube × 1
	Fast Ligation Buffer	Red	1440 μL/tube × 3
	Ad Ligase	Red	600 μL/tube × 1
	Ligation Enhancer	Brown	360 μL/tube × 1
	20x Elute Enhancer	Black	25 μL/tube × 1
	PCR Enzyme Mix	Blue	1400 μL/tube × 2
DNBSEQ UDB Primers Adapter Kit A Cat. No.: 940-001520-00	UDB Adapter (10 μM)	White	480 μL/tube × 1
	UDB PCR Primer Mix 01-08, 100009-100016, 17-96 (20 μM)	/	10 μL/well × 96
DNBSEQ UDB Primers Adapter Kit B Cat. No.: 940-001517-00	UDB Adapter (10 μM)	White	480 μL/tube × 1
	UDB PCR Primer Mix 97-192 (20 μM)	/	10 μL/well × 96
DNBSEQ DNA Clean Beads x 2 kits Cat. No.: 940-001283-00	DNA Clean Beads	White	15 mL/tube × 1
	TE Buffer	White	17 mL/tube × 1

1.5 Storage and transportation

Table 5 Kit storage and transportation

Modules	Storage temperature	Transport temperature
DNBSEQ Fast FS Library Prep Module	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
DNBSEQ UDB Primers Adapter Kit		
DNBSEQ UDB Primers Adapter Kit A		
DNBSEQ UDB Primers Adapter Kit B		
DNBSEQ UDB Primers Adapter Kit C		
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 lives.
- In DNBSEQ Fast FS Library Prep Module V2.0, 20× Elute Enhancer and Ligation Enhancer should be stored at room temperature. Avoid repeated freeze-thawing. The Ligation Enhancer should be stored away from light.

1.6 User-supplied materials

Table 6 Order information for CG products

Catalog number	Model	Name
940-001310-00	16 RXN	DNBSEQ Dual Barcode Circularization Kit
940-001309-00	96 RXN	DNBSEQ Dual Barcode Circularization Module (customization)
940-001281-00	50 mL	DNBSEQ DNA Clean Beads

Prepare reagent kits based on application requirements.

Table 7 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	General lab supplier
Desktop centrifuge	General lab supplier

Equipment	Recommended brand
Pipettes	General lab supplier
Thermocycler	General lab supplier
96M Magnum Plate	ALPAQUA, Part A000400 (Recommended)
Qubit® Fluorometer 2.0 or higher	Thermo Fisher (Cat. No.: Q33216), or equivalent
Agilent 2100 Bioanalyzer or Tape station	Agilent Technologies (Cat. No.: G2939AA), Tape station, or equivalent

Table 8 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)	Fisher BioReagents™ (Cat. No.: BP2818500), or equivalent
Qubit ssDNA Assay Kit	Invitrogen (Cat. No.: Q10212), or equivalent
Qubit dsDNA HS Assay Kit/Quant-iT	Invitrogen (Cat. No.: Q32854), or equivalent
Agilent High Sensitivity DNA Kit or Tape station High sensitive DNA kit	Agilent (Cat. No.: 5067-4626), or equivalent
Pipette tips	Axygen, or equivalent
1.5 mL tube	Axygen, or equivalent
0.2 mL PCR tube or 96-well plate	Axygen (Cat. No.: PCR-02-C or PCR-96M2-HS-C), or equivalent
Qubit Assay Tubes or 0.5 mL Thin Wall PCR Tubes	Invitrogen (Cat. No.: Q32856) or Axygen (Cat. No.: PCR-05-C), or equivalent

1.7 Precautions

1.7.1 Using the UDB Primers Adapter

This kit is designed for the construction of dual barcode libraries only, and the barcode sequences are designed to be located on the UDB PCR Primer sequences. When multiple samples are mixed together for sequencing, barcodes can be used to accurately attribute the sequencing results to the appropriate samples. To meet the requirements for batch processing

of library construction and multiplex sequencing, the best primer combinations were selected based on the principle of balanced base composition.

Based on the principles of balanced base composition, primers must be used in specific groups. Follow the instructions below to use the primers in the proper combinations. There are four specifications of UDB Primers Adapter Reagent Kits, depending on the number of reactions.

Table 9 The DNBSEQ UDB Primers Adapter of the DNBSEQ Fast FS Library Prep Set

Model	Reagent Kits	Note
16 RXN	DNBSEQ UDB Primers Adapter Kit	2 groups with 8 barcodes each.
96 RXN	DNBSEQ UDB Primers Adapter Kit B	12 groups with 8 barcodes each.
96 RXN	DNBSEQ UDB Primers Adapter Kit C	13 groups: 2 groups with 4 barcodes each; 11 groups with 8 barcodes each.
192 RXN	DNBSEQ UDB Primers Adapter Kit A	12 groups with 8 barcodes each.
	DNBSEQ UDB Primers Adapter Kit B	12 groups with 8 barcodes each.

The specific Barcode coding information refer to "UDB Primers Kit's barcode number and sequence information" on page 33.

1.7.1.1 Note for UDB Adapter and UDB PCR Primer Mix

- In kits with different specifications, the UDB Adapter is the same, while the UDB PCR Primer Mix is different. Each UDB PCR Primer Mix contains two primers, corresponding to 288 unique dual-barcode combinations.
- UDB Adapter is double-stranded. To prevent structure changes that might affect performance, such as denaturation, do not place the adapters in an area that exceeds 30 ℃.
- The UDB Adapter and UDB PCR Primer Mix must be mixed and centrifuged before being used to collect any liquid at the bottom of the tube or plate.
- For tubes, gently remove the cap to prevent liquid from spilling and cross-contamination. Cover the tube immediately after use.
- For 96-well plates, spray 75% alcohol and wipe the surface of the aluminum film of the plate with absorbent wipes. The aluminum film is penetrable. Do not touch the surface of the aluminum film with sharp objects. Pierce the aluminum film to pipette solutions for first-time use. After use, separately transfer the remaining reagents to 1.5 mL centrifuge tube(s) or 0.2 mL PCR tube(s). Label the tubes clearly, and store them at -20 ℃.
- To prevent cross contamination, change tips when pipetting different solutions.

1.7.1.2 UDB PCR Primer Mix pooling guide

It is recommended to optimize the base balance by planning UDB PCR Primer Mix with diverse sequences when pooling libraries across DNBSEQ systems. Pooling combines at least four libraries to sequence in one lane.

The following three application scenarios are predefined for the recommended method of selecting UDB PCR Primer Mix.

1. The sequencing data output requirement is the same for all samples in one lane. Choose the UDB PCR Primer Mix combinations in the table below.

   - Add only one UDB PCR Primer Mix to each sample.
   - Here X means positive integer. For example: 8X = 8 multiplied by X , which means there are 8X samples.

Table 10 UDB PCR Primer Mix pooling guide

Sample/lane	Instruction
4	Only UDB PCR Primer Mix 193-196 or UDB PCR Primer Mix 197-200 can be used.
5	Use the method for (4 samples/lane) above + 1 random UDB PCR Primer Mix.
6	Use the method for (4 samples/lane) above + 2 random UDB PCR Primer Mixes.
7	Use the method for (4 samples/lane) above + 3 random UDB PCR Primer Mixes.
8X	From X set of 8 UDB PCR Primer Mixes (X column total).
8X+1	Add X set of 8 UDB PCR Primer Mixes + 1 random UDB PCR Primer Mix.
8X+2	Add X set of 8 UDB PCR Primer Mixes + 2 random UDB PCR Primer Mixes.
8X+3	Add X set of 8 UDB PCR Primer Mixes + 3 random UDB PCR Primer Mixes.
8X+4	Add X set of 8 UDB PCR Primer Mixes + 4 random UDB PCR Primer Mixes.
8X+5	Add X set of 8 UDB PCR Primer Mixes + 5 random UDB PCR Primer Mixes.
8X+6	Add X set of 8 UDB PCR Primer Mixes + 6 random UDB PCR Primer Mixes.
8X+7	Add X set of 8 UDB PCR Primer Mixes + 7 random UDB PCR Primer Mixes.

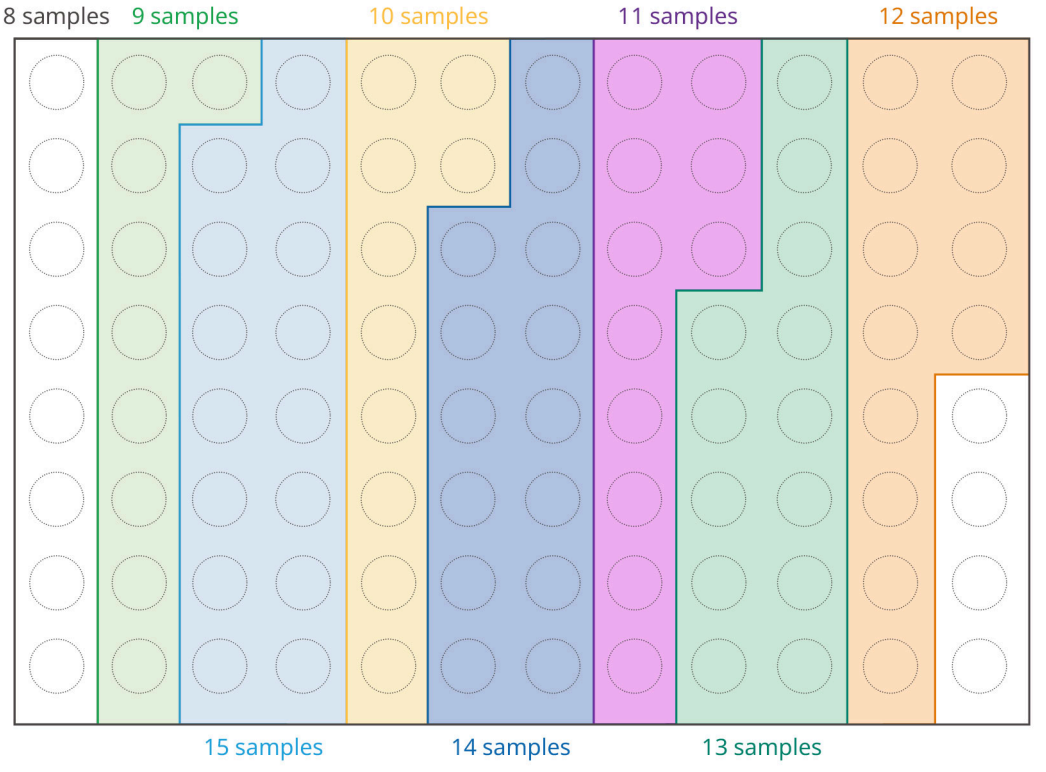

Figure 1 Dual barcode 8 to 15 samples pooling example

2. Under exceptional circumstances (for example, insufficient reagents for a well), when it cannot meet the requirement of at least one balanced UDB Adapter combination for standard pooling or if the required data amount of each library pooled is not equal, make sure to determine the pooling strategy by calculating the content of each base in each sequencing cycle. It is necessary to ensure that each base content **is not less than 12.5% and is not greater than 62.5%** in single sequencing position in the same lane.

Table 11 Balanced 8 UDB Adapter Pooling strategy (8 UDB Adapter from one entire column)

	Position of base in adapter sequence									
	Base 1	Base 2	Base 3	Base 4	Base 5	Base 6	Base 7	Base 8	Base 9	Base 10
Adapter 1	A	G	G	A	C	G	T	A	G	A
Adapter 2	C	T	G	A	A	C	C	G	A	A
Adapter 3	G	A	A	C	G	T	G	T	C	G
Adapter 4	T	C	C	G	T	G	A	C	T	C
Adapter 5	A	A	T	T	C	A	C	T	G	T
Adapter 6	C	C	T	G	A	A	G	G	A	T
Adapter 7	T	T	C	C	T	T	A	C	T	G
Adapter 8	G	G	A	T	G	C	T	A	C	C
Signal % per base	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0

Table 12 Unbalanced 9 UDB Adapter Pooling strategy (UDB PCR Primer Mix from different columns)

	Position of base in adapter sequence									
	Base 1	Base 2	Base 3	Base 4	Base 5	Base 6	Base 7	Base 8	Base 9	Base 10
Adapter 1	A	G	G	A	C	G	T	A	G	T
Adapter 2	A	C	G	A	A	G	G	T	C	C
Adapter 3	G	A	A	C	G	T	G	T	C	G
Adapter 4	T	C	C	G	T	G	A	C	T	C
Adapter 5	A	A	T	T	C	A	C	T	G	T
Adapter 6	G	C	T	G	A	A	G	G	A	T
Adapter 7	T	G	C	C	T	T	A	C	T	G
Adapter 8	G	G	A	T	G	A	T	A	C	C
Adapter 9	G	A	C	G	G	T	C	G	A	G
A signal %	33.3	33.3	22.2	22.2	22.2	33.3	22.2	22.2	22.2	0
T signal %	22.2	0	22.2	22.2	22.2	33.3	22.2	33.3	22.2	33.3
C signal %	0	33.3	33.3	22.2	22.2	0	22.2	22.2	33.3	33.3
G signal %	44.4	33.3	22.2	33.3	33.3	33.3	33.3	22.2	22.2	33.3

1.7.2 General precautions and warnings

- This product is for research use only, not for clinical 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.
- It is recommended that you use pipette tips with filters to prevent cross-contamination. Use a new tip each time you pipette 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.
- To prevent yield loss, try to avoid transferring reaction product to a new tube for bead purification.
- 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, contact Technical Support: **US-TechSupport@CompleteGenomics.com**.

2 Sample preparation

2.1 Sample type

This library prep set is applicable to samples from human (including but not limited to blood, saliva, and oral swabs), animals (including but not limited to rat ,and mouse), plants (including but not limited to A. thaliana, and O. sativa), bacteria (including but not limited to E. coli), fungi, and other microbial species. Different types of samples should be performed in a fragmentation demo test before library preparation to achieve the best results.

2.2 Sample purity

It is strongly recommended to use high-quality genomic DNA ($1.8 \leq OD_{260}/OD_{280} \leq 2.0$, $OD_{260}/OD_{230} \geq 1.7$) for fragmentation. If the sample purity does not satisfy the recommended standards, or if enzyme inhibitors are present, there is a risk of low library yield. Because Fast FS Enzyme II is sensitive to pH and components of DNA storage buffer, it is recommended to use TE Buffer (pH 8.0) for DNA dissolution.

- If DNA is dissolved in other buffers, such as 10 mM Tris (pH 6.8-8.0), AE Buffer (pH 8.5), 0.1x TE (pH 8.0) or other special buffers, perform a demo fragmentation test by adjusting the incubation time of 30 ℃ in Table 21 on page 15.
- If the sample contains many impurities and inhibitors, it is recommended to re-purify the sample DNA with 1.8x magnetic beads and elute it with TE Buffer (pH 8.0). After repurification, perform a demo fragmentation test by adjusting the incubation time to 30 ℃ in Table 21 on page 15.

2.3 Sample input

1 ng - 1000 ng gDNA can be used for library preparation. If the amount of genomic DNA is sufficient, it is recommended to use a high input of genomic DNA for library preparation. Qubit or BMG Microplate reader is recommended for the quantification of sample concentrations.

3 Library preparation protocol

3.1 Workflow

Table 13 Workflow

Workflow	Hands-on time	Total processing time
Reagents preparation	3 min	3 min
Fragmentation	2 min	30 - 40 min
Cleanup of fragmentation product	1 - 2 min	7 - 13 min
Adapter ligation	2 min	12 min
Cleanup of adapter-ligated product ⏸	5 min	18 min
PCR	2 min	22 - 40 min
Cleanup of PCR product ⏸	5 min	18 min
quantification of PCR product ⏸	2 min	4 min
Total	~ 20 min	~ 120 min

- Time estimates are based on preparing one reaction with basic molecular biology experience. Experiment duration differs with operator and numbers of samples processed concurrently.
- ⏸ **Stop point.**

3.2 Reagent preparation

3.2.1 Preparation

Table 14 Preparing the reagents

Reagent	Requirement
Nuclease-Free Water	User-supplied; place at room temperature (RT); mix thoroughly.
TE Buffer	
20x Elute Enhancer	Place at RT; mix thoroughly.
DNA Clean Beads	

3.2.2 Procedure

CAUTION The preparation volume of reagents listed below is enough for 8 samples. Increase the preparation reagent volumes in proportion if there are more samples.

1. Prepare the 1x Elute Enhancer according to the following table. Mix it by vortexing, and centrifuge briefly. Store at room temperature before using. The shelf life of the 1x Elute Enhancer is 7 days.

Table 15 1x Elute Enhancer

Reagent	Volume
20x Elute Enhancer	1 µL
Nuclease-Free Water	19 µL
Total	20 µL

2. Prepare the En-TE according to the following table. Mix it by vortexing, and centrifuge briefly. Store at 4 ℃ before using. The shelf life of the En-TE is 60 days.

Table 16 En-TE

Reagent	Volume
1x Elute Enhancer	3 µL
TE Buffer	1497 µL
Total	1500 µL

3. Prepare the En-Beads according to the following table. Mix it by vortexing, and centrifuge briefly. Store at 4 ℃ before using. The shelf life of the En-Beads is 60 days.

Table 17 En-Beads

Reagent	Volume
1x Elute Enhancer	10 μL
DNA Clean Beads	990 μL
Total	1000 μL

3.3 Fragmentation

The extent of fragmentation (size distribution of DNA fragments) is controlled by time and temperature. Therefore, ensure the accuracy of time and temperature during the reaction. Samples and enzyme mix should always be kept on ice.

3.3.1 Preparation

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

Table 18 Preparing the reagents

Reagent	Requirement
TE Buffer (pH 8.0)	User-supplied; place at RT.
Fast FS Buffer II	Thaw at RT; vortex; centrifuge briefly; place on ice.
Fast FS Enzyme II	Keep on ice.
80% ethanol	User-supplied; freshly prepared.
En-TE	Refer to Table 16; place at RT.
En-Beads	Refer to Table 17; allow 30 min to equilibrate to RT before use; mix thoroughly by vortexing before each use.

3.3.2 Fragmentation

1. Normalize gDNA. Refer to the following table. Based on the sample concentration, transfer the appropriate gDNA (recommended 1 ng - 1000 ng) to a new 0.2 mL PCR tube. Add TE Buffer (pH 8.0) to make a total volume of 45 μL. Place the normalized gDNA on ice.

Table 19 Normalization of gDNA dissolved in TE (pH 8.0)

Components	Volume
TE Buffer (pH 8.0)	45-X μL
gDNA (1 ng - 1000 ng)	X μL
Total	45 μL

It is recommended that the normalization buffer should be the same as DNA elution buffer.

2. Set the thermal cycler program according to Table 21 on page 15. Run the program to allow the reaction block to cool to 4 ℃. Hold the program at this step until the fragmentation mixture has been prepared and added to the sample.
3. Mix the Fast FS Enzyme II by inverting 10 times and flicking the bottom of the tube(s) gently. Ensure that no residual reagent is left at the bottom each time. Centrifuge briefly, and place it on ice until use.

CAUTION DO NOT vortex the Fast FS Enzyme II. Strictly follow the manual instructions. Insufficient mixing will affect the fragmentation process.

4. According to the desired reaction number, prepare the fragmentation mixture in a 1.5 mL centrifuge tube on ice. Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 20 Fragmentation mixture

Reagent	Volume per reaction
Fast FS Buffer II	10 μL
Fast FS Enzyme II	5 μL
Total	15 μL

5. Add 15 μL of fragmentation mixture to each sample tube from step 1 (volume: 45 μL). Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.
6. Place the tube(s) into the thermocycler. Skip the first step (4 ℃ Hold) to start the reaction.

Table 21 Fragmentation reaction conditions (Volume: 60 μL)

Temperature	Time
70 ℃ Heated lid	On
4 ℃	Hold
30 ℃	Refer to Table 22
65 ℃	15 min
4 ℃	Hold

The appropriate incubation time at 30 ℃ is shown in the following table.

Table 22 The incubation time for different gDNA input

gDNA input	Incubation time	Size selection method
1000 ng	12 min	Double size selection
500 ng	12 min	Double size selection
200 ng	12 min	Single size selection
100 ng	13 min	Single size selection
50 ng	16 min	Single size selection
25 ng	16 min	Single size selection
10 ng	18 min	Single size selection
5 ng	20 min	Single size selection
1 ng	22 min	Single size selection

7. After the reaction, centrifuge the tube(s) briefly and immediately proceed to the next step.

CAUTION DO NOT STOP AT THIS STEP.

3.4 Cleanup of fragmentation product

Select the appropriate fragment screening method according to "Table 22 The incubation time for different gDNA input" on page 17. Choose single size selection or double size selection of beads according to different gDNA inputs. The PCR products from this library construction process range from 300 bp - 2000 bp.

- When single size selection is selected, the peak size of the single size product is approximately 500 bp - 750 bp.

- When double size selection is selected, the peak size of the double size product is approximately 450 bp - 550 bp.

3.4.1 Single size selection (option 1)

3.4.1.1 Preparation

Table 23 Preparing the reagents

Reagent	Requirement
En-TE	Refer to Table 16; place at RT.
En-Beads	Refer to Table 17; allow 30 min to equilibrate to RT before use; mix thoroughly by vortexing before each use.

3.4.1.2 Size selection

1. Check the volume of the fragmentation product (from step 7 in section 3.3.2). If the volume is less than 60 µL, add En-TE to make a total volume of 60 µL.
2. Mix the En-Beads thoroughly. Add 48 µL of En-Beads to each sample tube. Mix with a vortexer until all beads are suspended.
3. Incubate at room temperature for 5 min.
4. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard all the supernatant. If liquid remains on the tube wall, centrifuge the tube(s) briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
5. Remove the tube(s) from the magnetic rack and add 45 µL of En-TE to elute the DNA. Mix with a vortexer until all beads are suspended and centrifuge briefly.

CAUTION DO NOT STOP AT THIS STEP. Proceed to section 3.5.

3.4.2 Double size selection (option 2)

3.4.2.1 Preparation

Table 24 Preparing the reagents

Reagent	Requirement
En-TE	Refer to Table 16; place at RT.
En-Beads	Refer to Table 17; allow 30 min to equilibrate to RT before use; mix thoroughly by vortexing before each use.

3.4.2.2 Size selection

1. Check the volume of the fragmentation product (from step 7 in section 3.3.2). If the volume is less than 60 µL, add En-TE to make total volume of 60 µL.
2. Mix the En-Beads thoroughly. Add 36 µL of En-Beads to each sample tube. Mix with a vortexer until all beads are suspended.
3. Incubate at room temperature for 5 min.
4. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 96 µL of supernatant to a new 0.2 mL PCR tube.

   In this step, keep the supernatant and discard the beads.

5. Add 12 µL of En-Beads to each sample tube (from step 4, volume: 96 µL). Mix with a vortexer until all beads are suspended.
6. Incubate at room temperature for 5 min. Centrifuge the tube(s) briefly.
7. Place the tube(s) on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant. If some liquid remains on the tube wall, centrifuge the tube(s) briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
8. Remove the tube(s) from the magnetic rack and add 45 µL of En-TE to elute the DNA. Mix with a vortexer until all beads are suspended and centrifuge briefly.

CAUTION DO NOT STOP AT THIS STEP. Proceed to section 3.5.

3.5 Adapter ligation

The UDB Adapter is a universal adapter sequence and does not contain Barcode sequences.

3.5.1 Preparation

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

Table 25 Preparing the reagents

Reagent	Requirement
UDB Primers Adapter Kit	Thaw at RT; mix thoroughly; centrifuge briefly; place on ice.
Fast Ligation Buffer	
Ad Ligase	Flick and/or invert the tube gently; centrifuge briefly; place on ice.
Ligation Enhancer	Mix thoroughly; centrifuge briefly; place at RT.

- Mix the adapter thoroughly before use. Adapters should not be mixed directly with the adapter ligation mixture.
- The Fast Ligation Buffer is highly viscous. Mix it thoroughly by vortexing 6 times (3 sec each) and centrifuge briefly.
- Mix Ad Ligase by inverting the tube 10 times and flicking the bottom gently. Ensure that no residual reagent is left at the bottom. Centrifuge briefly and place them on ice until use.
- After Ligation Enhancer is used for the first time, store it at 10 ℃ - 30 ℃ away from light.

3.5.2 Adapter ligation

1. Dilute the UDB Adapter with TE Buffer (pH 8.0) based on gDNA input.

Table 26 Recommended adapter usage and dilutions for different amounts of gDNA input

gDNA input (*N* ng)	Dilution of UDB Adapter	Volume after dilution
50< N ≤ 1000	No Dilution	5 μL
25	2 ×	5 μL
10	5 ×	5 μL
5	10 ×	5 μL
1	50 ×	5 μL

2. Add 5 µL of UDB Adapter to the corresponding sample tube (from step 5 in section 3.4.1.2 or step 8 in section 3.4.2.2, volume: 45 µL). Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.
3. According to the desired reaction number, prepare the adapter ligation mixture in a 1.5 mL centrifuge tube on ice. Vortex it 6 times (3 sec each), centrifuge briefly, and place on ice.

Table 27 Adapter ligation mixture

Reagent	Volume per reaction
Fast Ligation Buffer	23 µL
Ad Ligase	5 µL
Ligation Enhancer	2 µL
Total	30 µL

It is recommended to prepare the adapter ligation mixture while waiting for cleanup of fragmentation product. Place it on ice after preparation, and use it within 30 min.

4. Slowly pipette 30 µL of adapter ligation mixture to each sample tube. Vortex it 6 times (3 sec each), centrifuge briefly, and place on ice.

The adapter ligation mixture is highly viscous. Pipette slowly and carefully.

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

Table 28 Adapter ligation reaction conditions (Volume: 80 µL)

Temperature	Time
30 ℃ Heated lid	On
25 ℃	10 min
4 ℃	Hold

6. When the program is completed, centrifuge the PCR tube(s) briefly and place on ice.

CAUTION DO NOT STOP AT THIS STEP. Proceed to section 3.6.

3.6 Cleanup of adapter-ligated product

3.6.1 Preparation

Table 29 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
En-TE	Refer to Table 16; place at RT.
En-Beads	Refer to Table 17; allow 30 min to equilibrate to RT before use; mix thoroughly by vortexing before each use.

3.6.2 Cleanup of adapter-ligated product

1. Add 22 µL of En-TE to each sample tube (from step 6 in section 3.5.2, volume: 80 µL).
2. Mix the En-Beads thoroughly. Add 20 µL of En-Beads to each sample tube. Mix with a vortexer until all beads are suspended.
3. Incubate the sample tube(s) at room temperature for 5 min.
4. 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 all the supernatant. 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.
5. While keeping the PCR tube(s) on the magnetic rack, add 160 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
6. Repeat step 5. 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.
7. 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.

   i Over-drying the beads will result in reduced yield.

8. Remove the tube(s) from the magnetic rack and add 20 µL of En-TE to elute the DNA. Mix with a vortexer until all beads are suspended.
9. Incubate the tube(s) at room temperature for 5 min.

10. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 19 µL of supernatant to a new 0.2 mL PCR tube.

Stop point After cleanup, the adapter-ligated product(s) can be stored at -20 ℃.

3.7 PCR

Barcodes are in the UDB PCR Primer Mix. Before operation, carefully read "Precautions" on page 6.

3.7.1 Preparation

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

Table 30 Preparing the reagents

Reagent	Requirement
PCR Enzyme Mix	Thaw at RT; mix by vortexing; centrifuge briefly; place on ice.
UDB PCR Primer Mix	Thaw at RT; mix by vortexing; centrifuge briefly; place at RT.

3.7.2 PCR

1. Add 25 µL PCR Enzyme Mix to each sample tube (from step 13 in section 3.6.2).
2. Add 6 µL of the corresponding UDB PCR Primer Mix according to the pooling guide. Vortex 3 times (3 sec each) and centrifuge briefly to collect the solution at the bottom of the tube.

Table 31 PCR mixture

Reagent	Volume per reaction
Adapter-ligated product (from step 13 in section 3.6.2)	19 µL
PCR Enzyme Mix	25 µL
Corresponding UDB PCR Primer Mix	6 µL
Total	50 µL

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

Table 32 PCR reaction conditions (Volume: 50 μL)

Temperature	Time	Cycles
105 ℃ Heated lid	On	-
95 ℃	3 min	1
98 ℃	20 sec	Refer to Table 33
60 ℃	15 sec	
72 ℃	30 sec	
72 ℃	10 min	1
4 ℃	Hold	-

The number of PCR cycles is shown in the following table.

Table 33 PCR cycles required to yield 300 ng, 600 ng, and 1000 ng of libraries

gDNA input	PCR cycles required for corresponding yield		
	300 ng	600 ng	1000 ng
1000 ng	/	3	4 ~ 5
500 ng	3	4 ~ 5	5 ~ 6
200 ng	3	4 ~ 5	5 ~ 6
100 ng	4 ~ 5	5 ~ 6	6 ~ 7
50 ng	5 ~ 6	6 ~ 7	7 ~ 8
25 ng	6 ~ 7	7 ~ 8	8 ~ 9
10 ng	7 ~ 8	8 ~ 9	9 ~ 10
5 ng	8 ~ 10	9 ~ 11	/
1 ng	11 ~ 12	12 ~ 14	/

The number of PCR cycles should be strictly controlled.

- Insufficient cycles may lead to a reduced library yield.
- Excessive cycles may also lead to adverse effects such as over amplification, an increase in bias, PCR duplicates, chimeric sequences, and accumulated mutations.

The indicated table shows the number of PCR cycles required to yield 300 ng, 600 ng, and 1000 ng of libraries from 1-1000 ng of high-quality gDNA sample. For lower quality, longer DNA fragments, or if the recommended cycle number fails to achieve the ideal yield, PCR cycles should be increased appropriately to generate sufficient yield.

4. When the program is completed, centrifuge the tube(s) briefly.

3.8 Cleanup of PCR product

3.8.1 Preparation

Table 34 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
En-TE	Refer to Table 16; place at RT.
En-Beads	Refer to Table 17; allow 30 min to equilibrate to RT before use; mix thoroughly by vortexing before each use.

3.8.2 Cleanup of PCR product

1. Mix the En-Beads thoroughly. Add 38 µL of En-Beads to each sample tube. Mix with a vortexer until all beads are suspended.
2. Incubate the sample tube(s) at room temperature for 5 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 all the supernatant. 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.
4. While keeping the PCR tube(s) on the magnetic rack, add 160 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.

   i Over-drying the beads will result in reduced yield.

7. Remove the tube(s) from the magnetic rack and add 32 µL of En-TE to elute the DNA. Mix with a vortexer until all beads are suspended.
8. Incubate the tube(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 30 µL of supernatant to a new 0.2 mL PCR tube.

   Stop point After cleanup, the PCR product can be stored at -20 ℃.

3.9 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 35 Different QC methods and standards for library

Method	Equipment/Reagent
dsDNA fluorescence quantification method	Qubit dsDNA HS Assay Kit, Quant-iT PicoGreen dsDNA Assay Kit, or equivalent
Electrophoresis method	Tapestation (Agilent Technologies), Bioanalyzer, LabChip GX, GXII, GX Touch (PerkinElmer), Fragment Analyzer (Advanced Analytical), or equivalent

- If the DNBSEQ Dual Barcode Circularization Kit is used, the required yield for PCR products is ≥ 300 ng. For pooled sequencing, follow the instructions provided by DNBSEQ UDB Primers Adapter Kit. After quantification, different PCR products were mixed. The total yield after pooling should be ≥ 300 ng, with a concentration of ≥ 6.25 ng/µL.
- The Agilent 2100 Bioanalyzer can be used to analyze the fragment distribution of PCR-purified products. The PCR products from this library construction process range from 300 to 2000 bp, with single size selection for peaks of 500 to 750 bp, and double size selection for peaks of 450 to 550 bp.

  i The full length of the dual barcode adapter (includes primer) is 132 bp.

- The figures below show the Agilent 2100 Bioanalyzer detection results of purified PCR products.

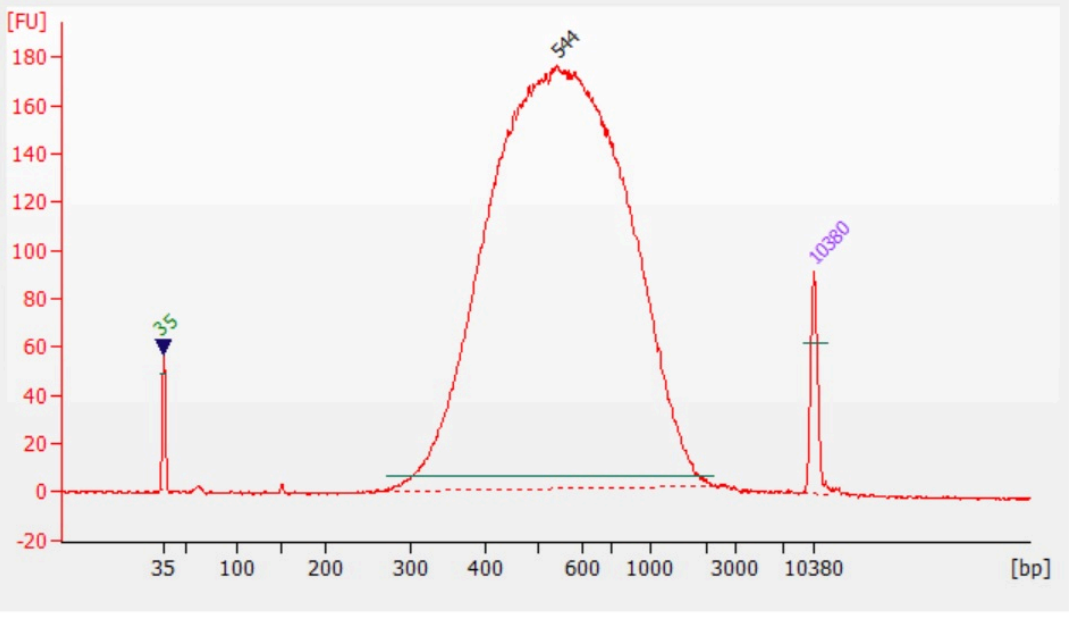

Figure 2 Agilent 2100 Bioanalyzer Image of PCR Products with 200 ng of genome input

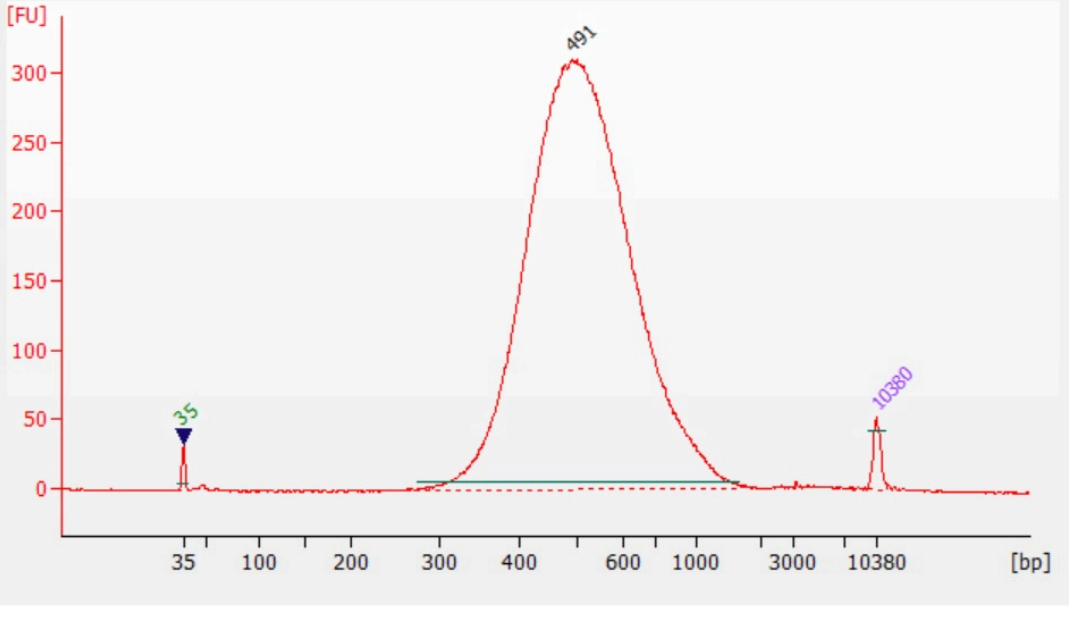

Figure 3 Agilent 2100 Bioanalyzer Image of PCR Products with 500 ng of genome input

4 Circularization and DNB preparation

DNBSEQ Dual Barcode Circularization Kit/ Module (user-supplied, 940-001309-00, 940-001310-00) and DNBSEQ DNA Clean Beads (user-supplied, 940-001281-00) are required for ssCir preparation.

CAUTION Check the name and Cat. No. of the kit carefully before use.

4.1 Denaturation and single-stranded circularization

4.1.1 Preparation

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

Table 36 Preparing the reagents

Reagent	Requirement
Dual Barcode Splint Buffer	Thaw at RT; mix thoroughly; centrifuge briefly; place on ice.
DNA Rapid Ligase	Flick and/or invert the tube gently; centrifuge briefly; place on ice.
TE Buffer, pH 8.0	User-supplied; place at RT.

4.1.2 Denaturation

1. Based on the PCR products concentration, add 300 ng of PCR products (from step 9 in section 3.8.2) into a new 0.2 mL PCR tube. If the volume is less than 48 μL, 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 37 Denaturation reaction conditions (Volume: 48 μL)

Temperature	Time
100 ℃ Heated lid	On
95 ℃	3 min
4 ℃	10 min

3. After the reaction, centrifuge the tube briefly and place on ice.

4.1.3 Single-stranded circularization

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

Table 38 Circularization reaction 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 circularization reaction mixture to each sample tube (from step 3 in section 4.1.2). Vortex it 3 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 39 Single-stranded DNA circularization reaction conditions (Volume: 60 μL)

Temperature	Time
42 ℃ Heated lid	On
37 ℃	10 min
4 ℃	Hold

i Prepare the "Table 41 Digestion mixture" on page 30 in advance of this step.

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

4.2 Digestion

4.2.1 Preparation

Table 40 Preparing the reagents

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

4.2.2 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 41 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 4.1.3, volume: 60 μL). Vortex it 3 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 42 Digestion reaction conditions (Volume: 64 μL)

Temperature	Time
42 ℃ Heated lid	On
37 ℃	10 min
4 ℃	Hold

4. When the program is completed, centrifuge the tube briefly and immediately **add 7.5 μL of Digestion Stop Buffer** to each sample tube. Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.

i DO NOT STOP AT THIS STEP. Proceed to next reaction.

4.3 Cleanup of digestion 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.

4.3.1 Preparation

Table 43 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
TE Buffer, pH 8.0	User-supplied; place at RT.
DNA Clean Beads	User-supplied; allow 30 min to equilibrate to RT before use; mix thoroughly by vortexing before each use.

4.3.2 Cleanup of digestion product

1. Mix the DNA Clean Beads thoroughly. Add 130 µL of DNA Clean Beads to each sample tube (from step 4 in section 4.2.2, volume: 71.5 µL). Mix with a vortexer until all beads are suspended.
2. Incubate at room temperature for 5 min.
3. Centrifuge the 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 160 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 25 µL of TE Buffer to elute the DNA. Mix with a vortexer until all beads are suspended.
8. Incubate at room temperature for 5 min.

9. Centrifuge the tube briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 24 µL of supernatant to a new 1.5 mL centrifuge tube.

Stop point After cleanup, the digestion product(s) (ssCir) can be stored at -20 ℃ for 30 days.

4.4 QC of digestion product

Quantify the ssCir with Qubit ssDNA Assay Kit. The final Enzymatic Digestion products (ssDNA, ng) / input products of PCR (dsDNA, 300 ng) should be ≥ 7%.

4.5 DNB preparation

Prepare DNBs according to the user manual for the appropriate sequencing platform.

5 Appendix

5.1 UDB Primers Kit's barcode number and sequence information

For detailed sequence information of each barcode, contact Technical Support at: **US-TechSupport@CompleteGenomics.com .**

5.1.1 Instructions for UDB Primers Kit (16 RXN)

Based on the principles of balanced base composition, UDB PCR Primer Mix must be used in specific groups. Follow the instructions to use UDB PCR Primer Mix in the proper combinations:

This kit contains 16 UDB PCR Primer Mix grouped into 2 sets:

- UDB PCR Primer Mix-57 to UDB PCR Primer Mix-64 (inside the blue box in Figure 4).
- UDB PCR Primer Mix-89 to UDB PCR Primer Mix-97 (inside the red box in Figure 4).

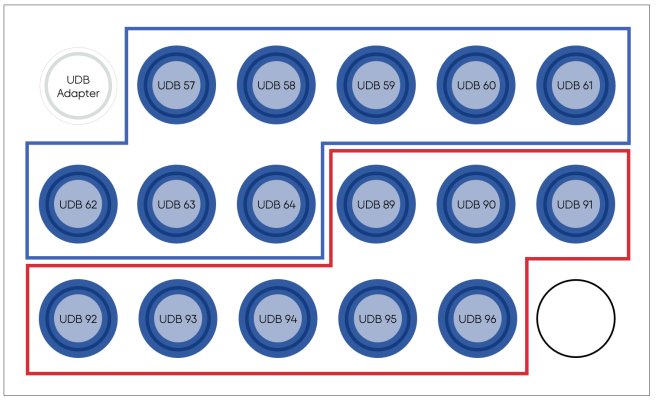

Figure 4 The UDB Adapter and UDB PCR Primer Mix layout

5.1.2 Instructions for UDB Primers Adapter Kit A/B/C (96 RXN)

There is 1 plate of UDB PCR Primer Mix in Set A, Set B, and Set C. Each plate contains 96 UDB PCR Primer Mix, and 8 wells of each column are preset as a balanced dual barcode combination. The detailed layouts are as follows.

The 8- and 12-column Barcode numbers of Set A overlap with those of the 16 RXN kit. The base sequences are the same and cannot be sequenced in the same lane.

Table 44 Set A barcode layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDB1	UDB100009	UDB17	UDB25	UDB33	UDB41	UDB49	**UDB57**	UDB65	UDB73	UDB81	**UDB89**
B	UDB2	UDB100010	UDB18	UDB26	UDB34	UDB42	UDB50	**UDB58**	UDB66	UDB74	UDB82	**UDB90**
C	UDB3	UDB100011	UDB19	UDB27	UDB35	UDB43	UDB51	**UDB59**	UDB67	UDB75	UDB83	**UDB91**
D	UDB4	UDB100012	UDB20	UDB28	UDB36	UDB44	UDB52	**UDB60**	UDB68	UDB76	UDB84	**UDB92**
E	UDB5	UDB100013	UDB21	UDB29	UDB37	UDB45	UDB53	**UDB61**	UDB69	UDB77	UDB85	**UDB93**
F	UDB6	UDB100014	UDB22	UDB30	UDB38	UDB46	UDB54	**UDB62**	UDB70	UDB78	UDB86	**UDB94**
G	UDB7	UDB100015	UDB23	UDB31	UDB39	UDB47	UDB55	**UDB63**	UDB71	UDB79	UDB87	**UDB95**
H	UDB8	UDB100016	UDB24	UDB32	UDB40	UDB48	UDB56	**UDB64**	UDB72	UDB80	UDB88	**UDB96**

Table 45 Set B barcode layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDB97	UDB105	UDB113	UDB121	UDB129	UDB137	UDB145	UDB153	UDB161	UDB169	UDB177	UDB185
B	UDB98	UDB106	UDB114	UDB122	UDB130	UDB138	UDB146	UDB154	UDB162	UDB170	UDB178	UDB186
C	UDB99	UDB107	UDB115	UDB123	UDB131	UDB139	UDB147	UDB155	UDB163	UDB171	UDB179	UDB187
D	UDB100	UDB108	UDB116	UDB124	UDB132	UDB140	UDB148	UDB156	UDB164	UDB172	UDB180	UDB188
E	UDB101	UDB109	UDB117	UDB125	UDB133	UDB141	UDB149	UDB157	UDB165	UDB173	UDB181	UDB189
F	UDB102	UDB110	UDB118	UDB126	UDB134	UDB142	UDB150	UDB158	UDB166	UDB174	UDB182	UDB190
G	UDB103	UDB111	UDB119	UDB127	UDB135	UDB143	UDB151	UDB159	UDB167	UDB175	UDB183	UDB191
H	UDB104	UDB112	UDB120	UDB128	UDB136	UDB144	UDB152	UDB160	UDB168	UDB176	UDB184	UDB192

Table 46 Set C barcode layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDB193	UDB201	UDB209	UDB217	UDB225	UDB233	UDB241	UDB249	UDB257	UDB265	UDB273	UDB281
B	UDB194	UDB202	UDB210	UDB218	UDB226	UDB234	UDB242	UDB250	UDB258	UDB266	UDB274	UDB282
C	UDB195	UDB203	UDB211	UDB219	UDB227	UDB235	UDB243	UDB251	UDB259	UDB267	UDB275	UDB283
D	UDB196	UDB204	UDB212	UDB220	UDB228	UDB236	UDB244	UDB252	UDB260	UDB268	UDB276	UDB284
E	UDB197	UDB205	UDB213	UDB221	UDB229	UDB237	UDB245	UDB253	UDB261	UDB269	UDB277	UDB285
F	UDB198	UDB206	UDB214	UDB222	UDB230	UDB238	UDB246	UDB254	UDB262	UDB270	UDB278	UDB286
G	UDB199	UDB207	UDB215	UDB223	UDB231	UDB239	UDB247	UDB255	UDB263	UDB271	UDB279	UDB287
H	UDB200	UDB208	UDB216	UDB224	UDB232	UDB240	UDB248	UDB256	UDB264	UDB272	UDB280	UDB288

5.2 Acronyms and abbreviations

Table 47 Acronyms and abbreviations

Acronym	Definition
Cat. No.	Catalog Number
DNB	DNA Nanoballs
dsDNA	double-stranded DNA
FS	Fragmentation Shearing
gDNA	genomic DNA
PE	Pair-End
PF	PCR-Free
QC	Quality Control
RT	Room Temperature
RXN	Reaction
ssCir	single-strand Circular

Doc. No.: H-940-001511-00