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DNBSEQ Dual Barcode Exome Capture Accessory Kit User Manual

- Cat. No.: 940-002050-00 (16 RXN)
  940-002051-00 (96 RXN)
- Kit Version: V1.0

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Manufacturer information

Manufacturer	Complete Genomics, Inc.
Address	2904 Orchard Parkway, San Jose, CA 95134
Customer service telephone	+1 (888) 811-9644
Customer service Email	US-CustomerService@CompleteGenomics.com
Technical support Email	US-TechSupport@CompleteGenomics.com
Website	www.CompleteGenomics.com

Revision history

Manual version	Kit version	Date	Description
1.0	V1.0	Aug. 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 Dual Barcode Exome Capture Accessory Kit offers high-quality reagents required to perform hybrid capture experiments using probes. The kit is specifically designed for the DNBSEQ high-throughput sequencing platform series and compatible with various DNA library preparation kits and commercial probes.

- Examples of combining DNBSEQ Dual Barcode Exome Capture Accessory Kit with other products to give a complete library construction process required for hybridization-based target enrichment are listed in table below.
- Choose one of the two categories of probe and hybridization elution kits. Use these in combination with the capture auxiliary kit to perform hybrid capture experiments.

Table 1 The combination of kits for exome capture library construction

DNA library prep kit	Probes and reagents for capture	Accessory kit
DNBSEQ Fast FS Library Prep V2.0 and DNBSEQ Dual Barcode Circularization Kit	RNA probe-related reagents: MGIEasy Exome Capture V5 Probe Set Reagents or kits required by third-party probes for capture	DNBSEQ Dual Barcode Exome Capture Accessory Kit
	DNA probe-related reagents: DNBSEQ Fast Hybridization and Wash Kit and third-party probes for capture	

1.2 Intended use

This kit provides adapter blockers for DNBSEQ platforms and post-capture PCR reagents, collocated with commercial probe products of various vendors, e.g. Nimblegen, IDT, Agilent, CG, and so on.

1.3 Applicable sequencing platforms

The prepared libraries are applicable to the following DNBSEQ sequencing platforms.

- DNBSEQ-G400RS (PE100/PE150)
- DNBSEQ-T7RS (PE100/PE150)
- DNBSEQ-G99RS (PE150)

1.4 Components

This kit comes in two specifications: 16 RXN and 96 RXN. For component details, refer to the following table.

Table 2 DNBSEQ Dual Barcode Exome Capture Accessory Kit (16 RXN) (Cat. No.: 940-002050-00)

Item & Cat. No.	Component	Cap color	Spec & Quantity
DNBSEQ Dual Barcode Exome Capture Accessory Kit Cat. No.: 940-002050-00	Post-PCR Enzyme Mix	Blue	800 µL/tube × 1
	Dual Barcode PCR Primer Mix	Blue	96 µL/tube × 1
	UDB Block 3	Yellow	16 µL/tube × 1
	UDB Block 4	Yellow	16 µL/tube × 1

Table 3 DNBSEQ Dual Barcode Exome Capture Accessory Kit (96 RXN) (Cat. No.: 940-002051-00)

Item & Cat. No.	Component	Cap color	Spec & Quantity
DNBSEQ Dual Barcode Exome Capture Accessory Kit Cat. No.: 940-002051-00	Post-PCR Enzyme Mix	Blue	1200 µL/tube × 4
	Dual Barcode PCR Primer Mix	Blue	576 µL/tube × 1
	UDB Block 3	Yellow	96 µL/tube × 1
	UDB Block 4	Yellow	96 µL/tube × 1

1.5 Storage and transportation

DNBSEQ Dual Barcode Exome Capture Accessory Kit
Storage temperature: -25 ℃ to -15 ℃
Transportation temperature: -80 ℃ to -15 ℃

- Production date and expiration date: refer to the label.
- For dry ice shipments, ensure that there is enough 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

Name	Model	Catalog number
DNBSEQ Fast FS Library Prep Set	16 RXN	940-001511-00
	96 RXN	940-001509-00
	192 RXN	940-001512-00
DNBSEQ Fast Hybridization and Wash Kit	16 RXN	940-001979-00
	96 RXN	940-001980-00
DNBSEQ Dual Barcode Circularization Kit	16 RXN	940-001310-00
DNBSEQ Dual Barcode Circularization Module	96 RXN	940-001309-00
DNBSEQ DNA Clean Beads	50 mL	940-001281-00

Table 5 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	General lab supplier
Mini centrifuge	General lab supplier
Pipettes	General lab supplier
Thermocycler	General lab supplier
Magnetic rack	Thermo Fisher Scientific, Cat. No. 12321D, DynaMag -2, or equivalent
Vacuum concentrator or centrifuge concentrator	Eppendorf, Cat. No. 5305000398, or equivalent

Equipment	Recommended brand
Thermomixer or water bath equipment	General lab supplier
Nutator or other nutating mixer/shaker	General lab supplier

Table 6 Recommended reagent/consumable list

Reagent/consumable	Recommended brand
MGIEasy Exome Capture V5 Probe Set	MGI, Cat. No. 940-000186-00
Nuclease Free (NF) water	Ambion, Cat. No. AM9937, or equivalent
100% Ethanol (Analytical Grade)	General lab supplier
Reagents or kits required by third-party probes for capture	MGI, Nimblegen, IDT, Agilent, and so on.
Pipette tips	General lab supplier
1.5 mL tube	General lab supplier
0.2 mL PCR tube or 96-well plate	General lab supplier
2.0 mL centrifuge tubes	General lab supplier
8 Strip Domed Caps Fit 0.2 mL PCR Tube Strips	Axygen, Cat. No. PCR-02CP-C, or equivalent
Filter Tips	Axygen, Cat. No. TF-100, or equivalent
Clear Adhesive Film	ABI, Cat. No. 4306311
Blade or knife	General lab supplier
Consumables required by commercial probes for capture	General lab supplier

1.7 Precautions and warnings

- This product is for research use only, not for in vitro diagnosis. Please read this manual carefully before use.
- Familiarize yourself with the precautions and operation 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 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).
- Avoid skin and eyes contact with samples and reagents. Do not eat or drink the samples and reagents. In case of contact with skin or eyes, rinse immediately with plenty of water or 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.**

1.8 Workflow

Section	Workflow	Total time	Hands-on time
3.1	Sample preparation before capture	2 hr - 6 hr	/
3.2	Hybridization and capture	4 hr - 28 hr	/
3.3	Post-capture PCR	50 min	10 min
3.4	Cleanup of PCR product ⏸	30 - 40 min	20 - 30 min
3.5	QC of PCR product ⏸	15 - 60 min	10 - 20 min
Total		5.6 hr - 36.5 hr	> 1 hr

- 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

The sample refers to the dual barcode library (PCR purified product) prepared using the DNBSEQ series library preparation kits or the DNBSEQ platform-compatible library preparation kits before hybridization.

2.2 Sample quantitation and quality control

The quantitation and fragment size distribution of purified PCR products can be assessed according to the *QC of PCR Products* steps in the user manual provided with your library preparation kit

3 Protocol

3.1 Sample preparation before capture

- If you are using the MGI Exome V5 Probe, you need to use the corresponding reagents from MGIEasy Exome Capture V5 Probe Set and conduct the hybridization and capture according to the user manual provided by the set.
- If you are using third-party probes for hybridization, you need to perform the hybridization and capture according to their instruction and replace their sequence adapter reagents with the UDB Block 3 and UDB Block 4 from DNBSEQ Dual Barcode Exome Capture Accessory Kit.

For example, if the user works on the NimbleGen SeqCap EZ, follow sections 3.1 - 3.5.

1. If the user prepares the dual barcode PCR library product according to DNBSEQ series library preparation kits or the DNBSEQ platform-compatible library preparation kits, follow the sections from gDNA to PCR library purification.
2. For 1-plex capture, prepare hybridization product separately for each PCR product. For multiplex capture, refer to the instructions of UDB PCR Primer Mix for detailed information about samples pooling.
3. Mix PCR products to meet the input requirements of the SeqCap EZ Library SR User's Guide.

3.2 Hybridization and capture

3.2.1 Preparation

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

Table 7 Preparing the reagents

Reagent	Requirement
MGI Exome V5 Probe, RSS_SeqCap_EZ, or third-party probes	User-supplied.

Reagent	Requirement
UDB Block 3	Thaw at RT (room temperature), mix by vortexing, centrifuge briefly, and place on ice.
UDB Block 4	

3.2.2 Block replacement notes

Following Chapter 5 Step 3 in the SeqCap EZ Library SR User's Guide v5.1, replace SeqCap HE Universal Oligo to UDB Block 3, and replace SeqCap HE Index 2/4/6/8 to UDB Block 4 in step 4. Refer to the table below for the usage information of UDB Block 3 and UDB Block 4.

- If the usage volume of UDB Block 3 and UDB Block 4 is larger than the volume of the reagents to be replaced in the commercial probe, it is required/strongly recommended to add these two reagents before sample concentration step.
- For example, 'SeqCap EZ Library SR User's Guide' requires performing the concentration step to reduce the mixture volume after adding the Multiplex Hybridization Enhancing Oligo Pool to the sample.

Table 8 Recommended usage of Block3 and Block4 for different commercial probes

Commercial probes	UDB Block 3 usage (volume)	UDB Block 4 usage (volume)	Reagents that need to be replaced in the kits
MGI Exome V5 Probe	1 μL	1 μL	N/A
Kits with SureSelect series probes (SureSelect Human All Exon V6 etc.)	1 μL	1 μL	SureSelect Indexing Block #3
SeqCap EZ Human Exome Probes v3.0	1 μL	1 μL	SeqCap HE Universal Oligo
			SeqCap HE Index 2 Oligo
			SeqCap HE Index 4 Oligo
			SeqCap HE Index 6 Oligo
			SeqCap HE Index 8 Oligo
xGen Exome Research Panel	1 μL	1 μL	xGen Universal Blocking Oligo (1)
			xGen Universal Blocking Oligo (2)
			xGen Universal Blocking Oligo (3)

3.2.3 Hybridization and capture

Conduct the Hybridization capture and elution following Chapter 5-6 of the SeqCap EZ Library SR User's Guide v5.1. Any reagents that are not mentioned here should be used as required in the probe user manual.

After elution, the total volume of the sample solution (including beads) should be 44 μL before the next post-capture PCR step.

- If the volume is less than 44 μL in third-party probe after elution, you need to add NF water to make a final volume of 44 μL.
- If the volume is larger than 44 μL in third-party probe after elution, you need to reduce the volume of the elution buffer.

3.3 Post-Capture PCR

3.3.1 Preparation

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

Table 9 Preparing the reagents

Reagent	Requirement
Post-PCR Enzyme Mix	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
Dual Barcode PCR Primer Mix	Thaw at RT, mix by vortexing, centrifuge briefly, and place at RT.

3.3.2 Post-Capture PCR

1. According to the desired reaction number, prepare the PCR mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing, centrifuge briefly, and place on ice.

Table 10 Post-capture PCR mixture

Reagent	Volume per reaction
Post-PCR Enzyme Mix	50 μL
Dual Barcode PCR Primer Mix	6 μL
Total	**56 μL**

2. Add 56 µL of the post-capture PCR mixture into each sample tube (44 µL sample with beads) . Mix well and centrifuge briefly to collect the solution at the bottom of the tube.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 11 Post-capture PCR reaction conditions

Temperature	Time	Cycles
105 ℃ Heated lid	On	-
95 ℃	3 min	1
98 ℃	20 sec	see table 12
60 ℃	15 sec	
72 ℃	30 sec	
72 ℃	10 min	1
4 ℃	Hold	-

The number of Post-PCR cycles is recommended in the table below, in this condition as an example, the 'X' should be 12.

Table 12 Post-capture PCR cycles for different commercial probes

Commercial probe	PCR cycles
MGI Exome V5 Probe	12
SeqCap EZ Human Exome Probes v3.0	12
xGen Exome Research Panel	6 (12 pool)-10 (1 pool)
SureSelect series probes(SureSelect Human All Exon V6 etc.)	12

4. When the program is completed, centrifuge the tube(s) briefly.
5. Place the tube(s) on a magnetic rack for 2 to 5 min until the liquid becomes clear. Transfer **100 µL** of supernatant to a new 1.5 mL centrifuge tube (one tube per reaction).

3.4 Cleanup of post-capture PCR product

- For use with DNBSEQ DNA Clean Beads (User-supplied). If you use the magnetic beads from other brands, 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.

3.4.1 Preparation

Table 13 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.

3.4.2 Cleanup of post-capture PCR product

1. Mix the DNA Clean Beads thoroughly. Add 100 µL of DNA Clean Beads to each sample tube (from step 5 in section 3.3.2). 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.
2. Incubate the sample(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 the supernatant.
4. While keeping the tube(s) on the magnetic rack, add 200 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 32 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 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 1.5 mL centrifuge tube.

Stop point After cleanup, products can be stored at -20 ℃.

3.5 QC of post-capture PCR product

- **dsDNA fluorescence quantification method**: Quantify the purified PCR products with dsDNA fluorescence assay kits and instructions.

Table 14 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	Yield for PCR products: ≥ 1 pmol

Refer to the formula below to calculate the mass (in ng) that corresponds to 1 pmol of dsDNA sample with varying fragment sizes.

Formula 1Conversion 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

For example, the desired yield for the fragmented DNA with a insert size of 300 bp (post-capture PCR products with a peak size of 432 bp; the length of UDB Primers Mix is 132 bp) should be ≥ 286 ng.

Table 15 The corresponding yield in 1 pmol for PCR products with different fragment sizes

Insert size (bp)	PCR product size /post-capture 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

- If the library will be sequenced on DNBSEQ platform, refer to the instructions of DNBSEQ Dual Barcode Circularization Kit to prepare the ssDNA library.

- For pooled sequencing, please follow instructions provided by DNBSEQ UDB Primers Adapter Kit. Detailed information shows how to plan your sample pooling. Quantify your post-capture PCR products before pooling. The total yield after pooling should be 1 pmol, with a total volume ≤ 48 μL.

Doc. No.: H-940-002050-00