Part No.:H-940-001195-00-03

Operation Guide

Version:1.0

MGIEasy Fast FS Exome Library Prep

Leading Life Science Innovation

Research Use Only

About the user manual

Manufacturer information

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Revision history

Manual version	Date	Description
1.0	May. 2024	Initial release

Tips Please download the latest version of the manual and use it with the corresponding kit. Search for the manual by Cat. No. or product name from the following website.

https://en.mgi-tech.com/download/files.html

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1 Product overview

1.1 Introduction

The MGIEasy Fast FS Exome Library Prep Operation Guide is designed for WES libraries for MGI high throughput sequencing platforms. The workflow is optimized to convert 200 ng or 500 ng genomic DNA (gDNA) into a customized library and uses high-quality fast fragmentase to simplify the operation process and significantly shorten the duration of DNA library preparation. All reagents provided have passed strict quality control and functional verification procedures, ensuring stability and reproducibility.

1. MGIEasy Fast FS Library Prep Set (Cat. No.: 940-001193-00 / 940-001194-00 / 940-001196-00 / 940-001831-00)
2. MGIEasy Exome Capture V5 Probe Set (Cat. No.: 940-000187-00)
3. MGIEasy Dual Barcode Exome Cpature Accessory Kit (Cat. No.: 1000018647)
4. MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570)

1.2 Intended use

This guide is applicable to human exome hybridization capture.

1.3 Applicable sequencing platforms

Select the appropriate DNB prep kit, sequencing platform, and sequencing type based on application requirements.

Sequencing platform and sequencing type recommendation :

- MGISEQ-2000RS (PE100/PE150)
- DNBSEQ-T7RS (PE100/PE150)

1.4 Reagent required

Reagents to order for this guide include:

1. MGIEasy Fast FS Library Prep Set (Cat. No.: 940-001193-00 / 940-001194-00 / 940-001196-00 / 940-001831-00)
2. MGIEasy Exome Capture V5 Probe Set (Cat. No.: 940-000187-00)
3. MGIEasy Dual Barcode Exome Cpature Accessory Kit (Cat. No.: 1000018647)
4. MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570)

For component details, refer to the following table.

Table 1 MGIEasy Fast FS Library Prep Set V2.0 (16 RXN) (Cat. No.: 940-001193-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Fast FS Library Prep Module V2.0 Cat. No.: 940-001197-00	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
MGIEasy UDB Primers Adapter Kit Cat. No.: 1000022800	UDB Adapter	White	80 μL/tube × 1
	UDB PCR Primer Mix-57-64, 89-96	Blue	12 μL/tube × 16
MGIEasy DNA Clean Beads Cat. No.: 940-001176-00	DNA Clean Beads	White	3.2 mL/tube × 1
	TE Buffer	White	3.2 mL/tube × 1

Table 2 MGIEasy Fast FS Library Prep Set V2.0 (96 RXN) (Cat. No.: 940-001194-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Fast FS Library Prep Module V2.0 Cat. No.: 940-001195-00	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
MGIEasy UDB Primers Adapter Kit B Cat. No.: 1000022802	UDB Adapter	White	480 μL/tue × 1
	UDB PCR Primer Mix-97-192	/	12 μL/well × 96
MGIEasy DNA Clean Beads Cat. No.: 940-001174-00	DNA Clean Beads	White	15 mL/tube × 1
	TE Buffer	White	17 mL/tube × 1

Table 3 MGIEasy Fast FS Library Prep Set C V2.0 (96 RXN) (Cat. No.: 940-001831-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Fast FS Library Prep Module V2.0 Cat. No.: 940-001195-00	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
MGIEasy UDB Primers Adapter Kit C Cat. No.: 940-001748-00	UDB Adapter	White	480 μL/tue × 1
	UDB PCR Primer Mix-193-288	/	10 μL/well × 96
MGIEasy DNA Clean Beads Cat. No.: 940-001174-00	DNA Clean Beads	White	15 mL/tube × 1
	TE Buffer	White	17 mL/tube × 1

Table 4 MGIEasy Fast FS Library Prep Set V2.0 (192 RXN) (Cat. No.: 940-001196-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Fast FS Library Prep Module V2.0 x 2 Cat. No.: 940-001195-00	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
MGIEasy UDB Primers Adapter Kit A Cat. No.: 1000022801	UDB Adapter	White	480 µL/tube × 1
	UDB PCR Primer Mix-01-96	/	12 µL/well × 96
MGIEasy UDB Primers Adapter Kit B Cat. No.: 1000022802	UDB Adapter	White	480 µL/tube × 1
	UDB PCR Primer Mix-97-192	/	12 µL/well × 96
MGIEasy DNA Clean Beads x 2 Cat. No.: 940-001174-00	DNA Clean Beads	White	15 mL/tube × 1
	TE Buffer	White	17 mL/tube × 1

Table 5 MGIEasy Exome Capture V5 Probe Set (16 RXN) (Cat. No.: 940-000187-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Exome Capture V5 Probe kit Cat. No.: 1000007741	MGI Exome V5 Probe	Black	80 μL/tube × 1
MGIEasy Exome Capture Hybridization and Wash Kit (Box 1) Cat. No.: 940-000168-00	Block 1	yellow	40 μL/tube × 1
	Block 2	yellow	40 μL/tube × 1
	Block 5	yellow	8 μL/tube × 1
	Hyb Buffer 3	Green	64 μL/tube × 1
MGIEasy Exome Capture Hybridization and Wash Kit (Box 2) Cat. No.: 940-000169-00	Hyb Buffer 1	Green	160 μL/tube × 1
	Hyb Buffer 2	Green	7 μL/tube × 1
	Hyb Buffer 4	Green	90 μL/tube × 1
	Binding Buffer	White	12800 μL/bottle× 1
	Wash Buffer I	White	8000 μL/bottle × 1
	Wash Buffer II	White	24000 μL/bottle × 1

Table 6 MGIEasy Dual Barcode Exome Cpature Accessory Kit (16 RXN) (Cat. No.: 1000018647)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Dual Barcode Exome Cpature Accessory Kit Cat. No.: 1000018647	UDB Block 3	yellow	16 μL/tube × 1
	UDB Block 4	yellow	16 μL/tube × 1
	Post-PCR Enzyme Mix	Blue	800 μL/tube × 1
	Dual Barcode PCR Primer Mix	Blue	96 μL/tube × 1

Table 7 MGIEasy Dual Barcode Circularization Kit (16 RXN) (Cat. No.: 1000020570)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Dual Barcode Circularization Module Cat. No.: 1000018649	Dual Barcode Splint Buffer	Purple	186 μL/tube × 1
	DNA Rapid Ligase	Purple	8 μL/tube × 1
	Digestion Buffer	White	23 μL/tube × 1
	Digestion Enzyme	White	42 μL/tube × 1
	Digestion Stop Buffer	White	120 μL/tube × 1
MGIEasy DNAClean Beads Cat. No.: 1000007325	DNA Clean Beads	White	1600 μL/tube × 2
	TE Buffer	White	1600 μL/tube × 1

1.5 Storage and transportation

Table 8 Kit storage and transportation

Modules	Cat. No.	Storage temperature	Transport temperature
MGIEasy Exome Capture V5 Probe kit	1000007741	-80 ℃	-80 ℃
MGIEasy Fast FS Library Prep Module V2.0	940-001197-00	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
MGIEasy Fast FS Library Prep Module V2.0	940-001195-00		
MGIEasy UDB Primers Adapter Kit	1000022800		
MGIEasy UDB Primers Adapter Kit A	1000022801		
MGIEasy UDB Primers Adapter Kit B	1000022802		
MGIEasy UDB Primers Adapter Kit C	940-001748-00		
MGIEasy Dual Barcode Exome Cpature Accessory Kit	1000018647		
MGIEasy Exome Capture Hybridization and Wash Kit (Box 1)	940-000168-00		
MGIEasy Dual Barcode Circularization Module	1000018649		
MGIEasy DNA Clean Beads	940-001176-00	2 ℃ to 8 ℃	2 ℃ to 8 ℃
MGIEasy DNA Clean Beads	940-001174-00		
MGIEasy DNA Clean Beads	1000007325		
MGIEasy Exome Capture Hybridization and Wash Kit (Box 2)	940-000169-00	18 ℃ to 25 ℃	18 ℃ to 25 ℃

Tips

- 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 MGIEasy Fast FS Library Prep Module V2.0, 20x 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 9 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	/
Desktop centrifuge	/
Pipettes	/
Thermocycler	/
Magnetic rack DynaMag-2	Thermo Fisher (Cat. No.: 12321D) , or equivalent
96M Magnum plate	ALPAQUA (Cat. No.: A000400, Recommended)
Qubit Fluorometer	Thermo Fisher (Cat. No.: Q33216)
Agilent 2100 Bioanalyzer, or equivalent	Agilent Technologies (Cat. No.: G2939AA)
Eppendorf Concentrator	Eppendorf (Cat. No.: 5305000398)

Table 10 Recommended reagent/consumable list

Reagent/consumable	Recommended brand
DynabeadsTM M-280 Streptavidin	Invitrogen (Cat. No.: 112.06D)
Or Dynabeads MyOne Streptavidin T1	Invitrogen (Cat. No.: 65601)
Nuclease-Free (NF) Water	Ambion (Cat. No.: AM9937), or equivalent
TE Buffer, pH 8.0	Ambion (Cat. No.: AM9858), or equivalent
100% Ethanol (Analytical Grade)	/
Qubit ssDNA Assay Kit	Invitrogen (Cat. No.: Q10212), or equivalent
Qubit dsDNA HS Assay Kit/Quant-iT	Invitrogen (Cat. No.: Q32854), or equivalent
Agilent High Sensitivity DNA Kit	Agilent (Cat. No.: 5067-4626), or equivalent
Pipette tips	/
1.5 mL tube	/
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 0.5mL 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 11 The DNBSEQ UDB Primers Adapter of the DNBSEQ Fast FS Library Prep Set

Model	Reagent Kits	Note
16 RXN	MGIEasy UDB Primers Adapter Kit	2 groups with 8 barcodes each.
96 RXN	MGIEasy UDB Primers Adapter Kit B	12 groups with 8 barcodes each.
96 RXN	MGIEasy UDB Primers Adapter Kit C	13 groups: 2 groups with 4 barcodes each; 11 groups with 8 barcodes each.
192 RXN	MGIEasy UDB Primers Adapter Kit A	12 groups with 8 barcodes each.
	MGIEasy 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 42.

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% ethanol 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 Barcode pooling guide

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

The following 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.

   Tips
   - 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 12 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.

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 13 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 14 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 °C 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: **MGI-service@mgi-tech.com**.

2 Sample preparation

2.1 Sample type

Human genomic DNA samples.

2.2 Sample purity

It is strongly recommended to use high-quality genomic DNA (1.8 ≤ OD_{260}/OD_{280} ≤ 2.0, OD_{260}/OD_{230} ≥ 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.

Tips
- 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 22 Fragmentation reaction conditions (Volume: 60 µL)" on page 19 .
- 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 22 Fragmentation reaction conditions (Volume: 60 µL)" on page 19.

2.3 Sample input

200 ng or 500 ng human genomic DNA can be used for library preparation. Qubit or BMG FLUOstar Omega is recommended for the quantification of sample concentrations.

3 Library preparation protocol

3.1 Workflow

Section	Workflow	Total time	Hands-on time
1	Fragmentation	31 min	2 min
2	Cleanup of fragmentation product	7 - 13 min	1 - 2 min
3	Adapter ligation	12 min	2 min
4	Cleanup of adapter-ligated product ⏸	18 min	5 min
5	PCR	30 min	2 min
6	Cleanup of PCR product ⏸	18 min	5 min
7	QC of PCR product ⏸	4 min	2 min
8	Pre-hybridization preparation	30 - 70 min	5 - 10 min
9	Hybridization and capture	25 hr - 28 hr	20 - 30 min
10	Post-capture PCR	40 - 50 min	10 min
11	Cleanup of post-capture PCR product ⏸	30 - 40 min	20 - 30 min
12	QC of post-capture PCR product ⏸	15 - 60 min	10 - 20 min
13	Denaturation and single strand circularization	45 - 50 min	15 min
14	Digestion	35 - 40 min	10 min
15	Cleanup of digestion product ⏸	50 min	10 - 15 min
16	QC of digestion product ⏸	15 - 20 min	10 - 15 min

Tips
- Total time: The theoretical use time of one reaction when sample input is more than 200 ng. The time will be extended if the number of reactions increases.

- Hands-on time: The total required hands-on time in the process.
- : The stop point.

3.2 Reagent preparation

3.2.1 Preparation

Table 15 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 Operation

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 16 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 17 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 18 En-Beads

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

3.3 Fragmentation

Tips 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 19 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 17 En-TE” on page 16; place at RT.
En-Beads	Refer to “Table 18 En-Beads” on page 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 200 ng or 500 ng, refer to "Table 20 Normalization of gDNA dissolved in TE (pH 8.0)" on page 18) 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 20 Normalization of gDNA dissolved in TE (pH 8.0)

Components	Volume
TE Buffer (pH 8.0)	45-X µL
gDNA (200 ng or 500 ng)	X µL
Total	45 µL

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

2. Set the thermal cycler program according to "Table 22 Fragmentation reaction conditions (Volume: 60 µL)" on page 19. 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 21 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 22 Fragmentation reaction conditions (Volume: 60 μL)

Temperature	Time
70 ℃ Heated lid	On
4 ℃	Hold
30 ℃	16 min
65 ℃	15 min
4 ℃	Hold

Table 23 The incubation time for different gDNA input

gDNA input	Incubation time	Size selection method
500 ng	16 min	Double size selection
200 ng	16 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

Tips Select the appropriate fragment screening method according to "Table 23 The incubation time for different gDNA input" on page 19. Choose single size selection or double size selection of beads according to different gDNA inputs.

3.4.1 Single size selection (option 1)

3.4.1.1 Preparation

Table 24 Preparing the reagents

Reagent	Requirement
En-TE	Refer to "Table 17 En-TE" on page 16; place at RT.
En-Beads	Refer to "Table 18 En-Beads" on page 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 **54 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 25 Preparing the reagents

Reagent	Requirement
En-TE	Refer to "Table 17 En-TE" on page 16, place at RT.
En-Beads	Refer to "Table 18 En-Beads" on page 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 **42 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 102 μL of supernatant to a new 0.2 mL PCR tube.

   Tips 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: 102 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

Tips 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 26 Preparing the reagents

Reagent	Requirement
UDB Adapter Kit Series	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.

Tips
- 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. 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.
2. 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

Tips 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.

3. 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.

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

4. 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

5. 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 17 En-TE" on page 16; place at RT.
En-Beads	Refer to "Table 18 En-Beads" on page 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 **20 µL of En-TE** to each sample tube (from step 5 in section 3.5.2, volume: 80 µL).
2. Mix the En-Beads thoroughly. Add **28 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.

   Tips 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

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 10 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 10 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	X
60 ℃	15 sec	
72 ℃	30 sec	
72 ℃	10 min	1
4 ℃	Hold	-

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

Table 33 PCR cycles required to yield 1000 ng of libraries

gDNA input	PCR cycles required for corresponding yield
500 ng	5 ~ 6
200 ng	5 ~ 6

Tips 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 1000 ng. 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 17 En-TE" on page 16; place at RT.
En-Beads	Refer to "Table 18 En-Beads" on page 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 **40 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.

   Tips 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

Tips Double barcode PCR adapter length plus primer length is 131 bp.

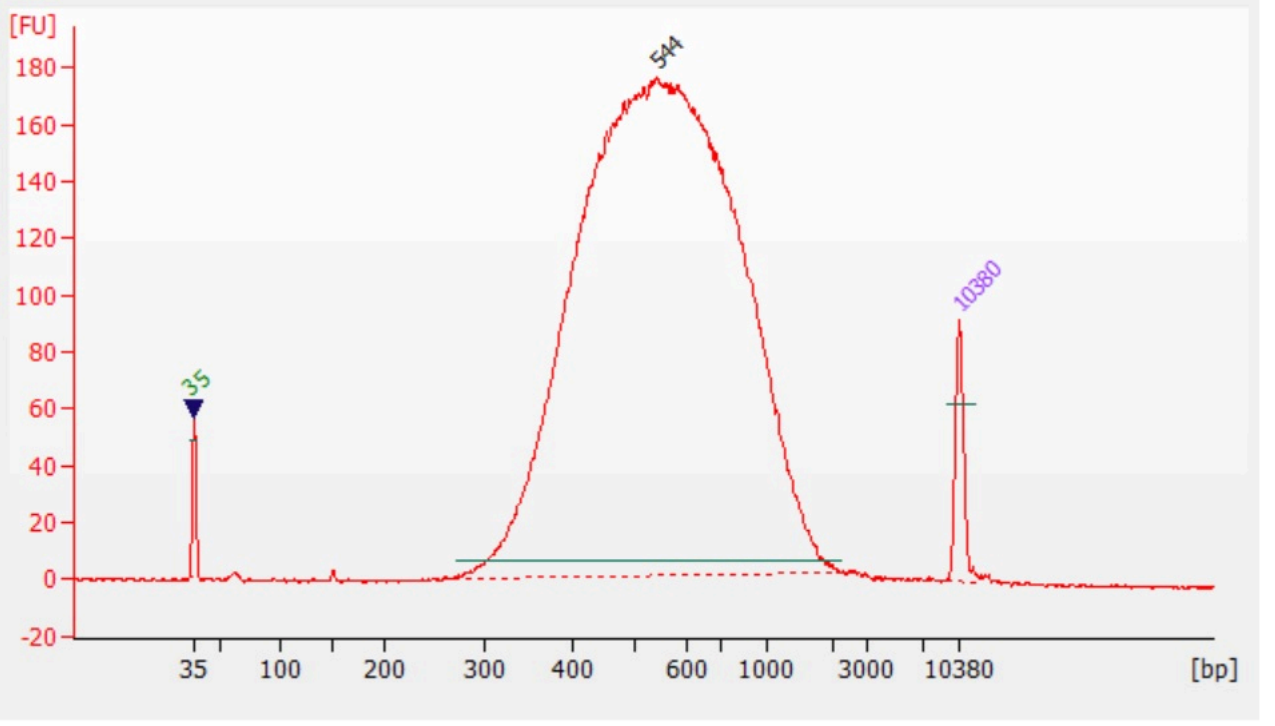

Figure 1 Agilent 2100 Bioanalyzer Image of PCR Products with 200 ng of genome input (single size selection)

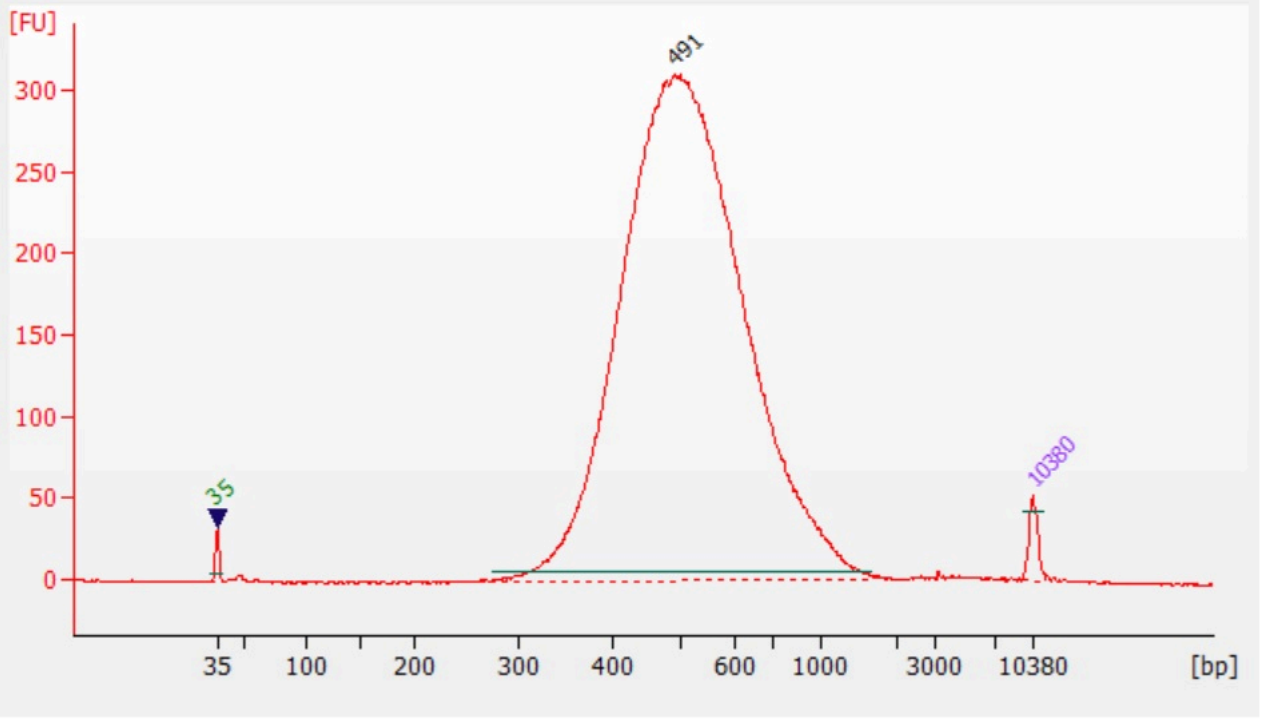

Figure 2 Agilent 2100 Bioanalyzer Image of PCR Products with 500 ng of genome input

3.10 Pre-Hybridization preparation

- The MGIEasy Exome Capture V5 Probe kit is recommended as a priority.
- UDB Block 3 and UDB Block 4 are reagents from the MGIEasy Dual Barcode Exome Cpature Accessory Kit, which are designed exclusively for the MGISEQ/DNBSEQ platform. When preparing samples for a different platform, use reagents applicable for that platform's adaptor sequences.

Table 36 Recommended usages of UDB Block3 and UDB Block4 for different commercial probes

commercial probes	Block 3 usage (volume)	Block 4 usage (volume)	Reagents that need to be replaced in the kits
MGI Exome V5 Probe	1	1	N/A
xGen Exome Research Panel	1	1	xGen Universal Blocking Oligo (1) xGen Universal Blocking Oligo (2) xGen Universal Blocking Oligo (3)
BOKE Core Exome Panel v3.0	1	1	Universal Blocker

Table 37 Post-capture PCR cycles for different commercial probes

commercial probes	Post-capture PCR Cycles
MGI Exome V5 Probe	11 ~ 13
xGen Exome Research Panel	8 ~ 12
BOKE Core Exome Panel v3.0	11 ~ 13

Taking MGI Exome V5 Probe acquisition process as an example, the standard process of experimental operation is as follows:

3.10.1 Sample preparation for hybridization

If only one sample per hybridization reaction, transfer around 1000 ng of PCR products according to the concentrations. If multi-plex in hybridization is needed, ≥250 ng of each sample is required, and the total amount of PCR products should be between 1000ng and 4000 ng. An 8-plex is the maximum pooling possible with this kit.

Please refer to "Barcode pooling guide" on page 11 for the barcode combination strategies.

3.10.2 Prepare the Block Mixture

Prepare the MGIEasy Exome Capture Hybridization and Wash Kit (Box 1) and MGIEasy Dual Barcode Exome Cpature Accessory Kit, melt on ice, mix well for use, and store in the refrigerator at -20℃ after use.

Table 38 Block Mixture

Components	Volume
Block 1	2.5 μL
Block 2	2.5 μL
UDB Block 3	1 μL
UDB Block 4	1 μL
Total	7 μL

3.10.3 Pre-Hybridization Mixture

1. Transfer 7 μL of Block mixture to each of the PCR products from step 3.10.1 for preparation of the Pre-hybridization Mixture. Uncap and place the tubes containing the mixture on the concentrator, set the temperature at 65℃ for spin vacuum around 30 min until the final concentrated volume is 9 μL. If the final volume is less than 9 μL, add NF water to reach a final volume of 9 μL.
2. Place the 9 μL of Pre-hybridization Mixture into a thermocycler and run the program in the table below.

Table 39 Pre-Hybridization Reaction Conditions

Temperature	Time
Heated lid	on
95 ℃	5 min
65 ℃	Hold

3.11 Hybridization and capture

3.11.1 Preparation

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

Table 40 Preparing the reagents

Reagent	Requirement
MGIEasy Exome Capture V5 Probe kit	The MGI Exome V5 Probe must be thawed on ice and added last to the mixture.
MGIEasy Exome Capture Hybridization and Wash Kit (Box 1)	Melt on ice, mix well for use, and store in the refrigerator at -20 ℃ after use.
MGIEasy Exome Capture Hybridization and Wash Kit (Box 2)	Make sure there is no precipitate in the buffer before use. If a precipitate forms, warm the buffer at 65 ℃ for 5 minutes until the precipitation disappear, then mix thoroughly for use.
MGIEasy Dual Barcode Exome Cpature Accessory Kit	Melt on ice, mix well for use, and store in the refrigerator at -20 ℃ after use.
NF water	self-supply
DynabeadsTM M-280 Streptavidin or Dynabeads MyOne Streptavidin T1	Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

3.11.2 Hybridization Mixture

1. Prepare the Hybridization Mixture in a new 0.2 mL PCR tube.

Table 41 Hybridization Mixture

Components	Volume
Hyb Buffer 1	10 μL
Hyb Buffer 2	0.4 μL
Hyb Buffer 3	4 μL
Hyb Buffer 4	5.6 μL
Total	20 μL

2. Incubate the Hybridization Mixture in a thermocycler at 65 ℃ for at least 5 min.

⚠ WARNING Before use, ensure there is no precipitation in the mixture.

3.11.3 Probe Mixture

1. Prepare the Hybridization Mixture in a new 0.2 mL PCR tube.

Table 42 Probe Mixture

Components	Volume
NF water	1.5 μL
Block 5	0.5 μL
MGI Exome V5 Probe	5 μL
Total	7 μL

⚠ WARNING The MGI Exome V5 Probe must be thawed on ice and added last to the mixture. Finger-flick the mixture, centrifuge briefly, and place on ice.

2. Place the PCR tube into the thermocycler. Run the program with the following conditions.

Table 43 Probe Mixture Incubation

Temperature	Time
105 ℃ Heated lid	on
65 ℃	2 min
65 ℃	Hold

3.11.4 Hybridization and capture

1. Keeping all the mixtures above (pre-hybridization mixture, hybridization mixture, and probe mixture) at 65 ℃, quickly transfer 13 μL of the Hybridization Mixture from step 2 in section 3.11.2 into 9 μL Pre-hybridization Mixture from step 2 in section 3.10.3 at 65 ℃. Pipette up and down to mix thoroughly.

⚠ CAUTION 100 μL Filter Tips are recommended for this step.

2. Transfer 22 μL of the combined pre-hybridization and hybridization mixtures from step 1 in section 3.11.4 into 7 μL of the Probe Mixture from step 2 in section 3.11.3. Pipette up and down to mix thoroughly.

⚠ CAUTION 100 μL Filter Tips are recommended for this step.

While handling large number of samples, in order to reduce the vaporization of the hybridization solution, it is recommended to handle the samples in sets of 1 to 2 reactions and close the heated lid of the PCR thermal cycler for 15 seconds before continuing with the next set of reactions.

Process: Hybridization Mix -> Pre-Hybridization Mix -> Probe Mix.

3. Keep the tube(s) at 65 ℃ (set the heated lid of thermocycler to 105 ℃) for hybridization for 24 hours according to the program in table below.

Table 44 Hybridization Reaction Conditions

Temperature	Time
105 ℃ Heated lid	on
65 ℃	Hold

3.11.5 Pre-Elution Preparation

⚠ CAUTION Please make sure no precipitation in buffers before use. If a precipitate forms, warm the buffer at 65 ℃ for 5 minutes until the precipitation disappear, then mix thoroughly for use.

1. Turn on a Thermomixer and set at 65 ℃ at least 30 min before beginning the elution. For each hybridization reaction, add 1.8 mL of Wash Buffer II in a new 2.0 mL Microcentrifuge Tube and place in the Thermomixer for preheating.
2. Vigorously vortex Dynabeads until evenly mixed. For each hybridization reaction, transfer 50 μL of Dynabeads to a new 2.0 mL Microcentrifuge Tube.
3. Add 200 μL of Binding Buffer to each tube containing Dynabeads and vigorously vortex for 5 s to re-suspend the magnet beads.
4. Centrifuge briefly and place the tube(s) onto a Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.
5. Repeat step 3 to step 4 twice.
6. Add 200 μL of Binding Buffer to each tube containing Dynabeads to re-suspend the beads.

3.11.6 Elution

1. Keep the mixtures on the thermocycler following the 24 hours incubation. Quickly use a pipette to estimate the remaining hybridization solutions one by one and transfer each mixture into a separate tube from step 6 in section 3.11.5 containing prepared Dynabeads.

⚠ CAUTION If the volume of remaining hybridization solution is less than 19 μL, the yield may be low. While handling large number of samples, in order to reduce the vaporization of the hybridization solution, it is recommended to handle the samples in sets of 1 to 2 reactions and close the heated lid of the PCR thermal cycler for 15 seconds before continuing with the next set of reactions.

2. Fix the tubes from step 1 in section 3.11.6 on a Nutator or other similar mixer for mixing by 360° rotation and incubate at room temperature for 30 min.
3. Take the tubes off the mixer. Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

4. Add 500 μL of Wash Buffer I, turn tubes upside down several times to re-suspend the bead mixture, then incubate at room temperature for 15 min (Upside down every 5 minutes).
5. Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.
6. Add 500 μL of pre-heated Wash Buffer II from step 1 in section 3.11.5 . Put the tubes in the Thermomixer and set speed to 1000 rpm. Press 'short' for 10 s to make sure all the beads are re-suspended, then set speed to 0 rpm. Incubate at 65 ℃ in the Thermomixer for 10 min.
7. Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 10s until the liquid becomes clear. Carefully remove and discard the supernatant immediately with a pipette.
8. Repeat step 6 to 7 twice.
9. Add 100 μL NF water to each tube to re-suspend the bead mixtures, then transfer all of the solution (including beads) to a new 1.5 mL Microcentrifuge Tube and centrifuge briefly.
10. Place the centrifuge tube from step 9 onto a Magnetic Separation Rack for 2 min until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.
11. Add 44 μL of NF water to each tube to re-suspend the bead mixtures, then transfer all of the solution (including beads) to a new 0.2 mL PCR tube.

3.12 Post-Capture PCR

3.12.1 Preparation

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

Table 45 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.

3.12.2 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 46 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 47 Post-capture PCR reaction conditions

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

Tips If only one sample per hybridization reaction, 13 cycles are suggested.

If multi-plex in hybridization reaction and the input for hybridization > 1000 ng, it is suggested to reduce the cycles to 12.

If 8-plex in hybridization reaction and the input for hybridization = 4000 ng (500ng of each sample) , it is suggested to reduce the cycles to 11.

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).

3.13 Cleanup of post-capture PCR product

Tips 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.

3.13.1 Preparation

Table 48 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 every use.

3.13.2 Cleanup of 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 3.12.2). 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 centrifuge 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 centrifuge 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.

   Tips 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 0.2 mL PCR tube.

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

3.14 QC of post-capture PCR product

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

Table 49 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 1 Conversion between 1 pmol of dsDNA sample and mass in ng

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

- For multiple samples pooling in one land for sequencing, refer to "Barcode pooling guide" on page 11. Detailed information shows how to plan samples pooling.
- Quantify the purified post-captured PCR products before pooling. The total yield after pooling should be 1 pmol, with a total volume ≤ 48 μL.

CAUTION If the library will be delivered to a service lab for sequencing, please stop here. If the library will be sequenced in your lab, proceed to chapter circularization and digestion.

4 Circularization and digestion

4.1 Denaturation and single strand circularization

Tips Calculate the required purified post-capture PCR product volume based on the main fragment size of purified PCR product, concentration of sample, and Formula 1.

4.1.1 Preparation

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

Table 50 Preparing the reagents

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

4.1.2 Denaturation

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

Table 51 Denaturation reaction conditions (Volume: 48 µL)

Temperature	Time
105 ℃ Heated lid	On
95 ℃	3 min

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

4.1.3 Single strand circularization

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

Table 52 Single strand circularization mixture

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

2. Add 12.1 μL of single strand circularization mixture to each sample tube (from step 3 in 4.1.2). Vortex the tube(s) 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 53 Single strand circularization reaction conditions (Volume: 60.1 μL)

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

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

4.2 Digestion

4.2.1 Preparation

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

Table 54 Preparing the reagents

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

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 55 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). Vortex 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 56 Digestion reaction conditions (Volume: 64.1 μL)

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

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

4.3 Cleanup of digestion product

Tips 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 57 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied. Freshly prepared.
TE Buffer	Place at RT.

Reagent	Requirement
DNA Clean Beads	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 170 µL of DNA Clean Beads to each sample tube (from step 5 in section 4.2.2). Gently pipette at least 10 times until all beads are suspended. Ensure that all of the solution and beads in the tip are transferred into the tube after mixing. Or, mix with a vortexer.
2. Incubate the sample(s) at room temperature for 10 min.
3. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant.
4. While keeping the centrifuge tube(s) on the magnetic rack, add 500 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
5. Repeat step 4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
6. Keep the centrifuge 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.

   Tips 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. Gently pipette the liquid at least 10 times until all beads are suspended. Or, mix with a vortexer.
8. Incubate the sample(s) at room temperature for 10 min.
9. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 23 µL of supernatant to a new 1.5 mL centrifuge tube.

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

4.4 QC of digestion product

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

- The yield of digestion product (ssDNA) / post-capture PCR input (dsDNA) should be not less than 7%.

5 Appendix

5.1 UDB Primers Kit's barcode number and sequence information

Tips For detailed sequence information of each barcode, contact Technical Support at: MGI-service@mgi-tech.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).

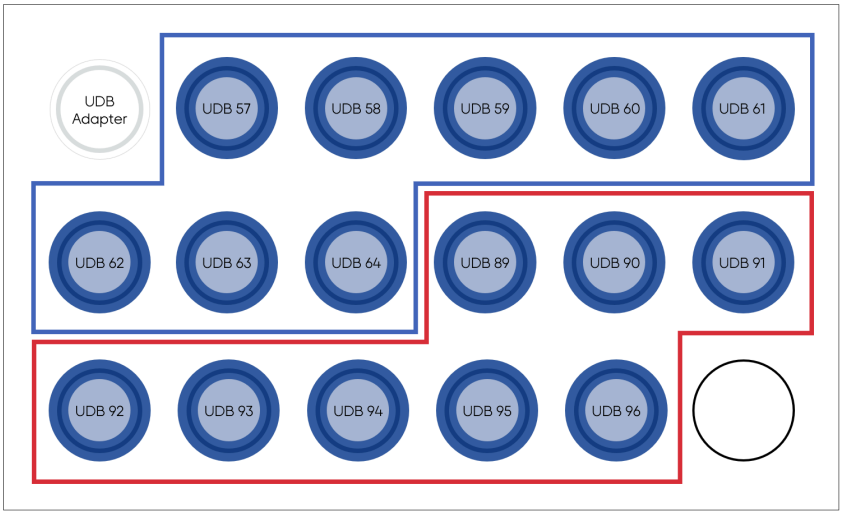

Figure 3 The UDB Adapter and UDB PCR Primer Mix layout

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.

Tips 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 58 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 59 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 60 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