MGI

MGICare

BRCA1/2 Sequencing Library Preparation Kit User Manual

Cat No.：1000009522（96 RXN）
Kit Version：V1.0
Manual Version：A2

Revision History

Manual Version	Kit Version	Date	Description
A2	V1.0	Jan. 2021	◆ Update contact information.
A1	V1.0	Sept. 2019	◆ Add DNBSEQ series sequencing platform
A0	V1.0	Apr. 2019	◆ Initial release.

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

Search manual by Cat. No. or product name from website:

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

Contents

Chapter 1 Product Description

1.1 Introduction

The MGICare BRCA1/2 Sequencing Library Preparation Kit is designed for the genetic risk assessment of breast and ovarian cancer using MGI sequencing platforms. It can be used to detect germline mutations in the coding regions of BRCA1/2, which are breast/ovarian cancer susceptibility genes. This kit is based on an optimized multiplex PCR technology, which requires a minimum gDNA input of 20ng. All reagents provided within this kit have passed stringent quality control and functional verification procedures, ensuring performance stability and reproducibility.

1.2 Applications

This kit is applicable for gDNA samples from human peripheral blood, saliva and cervical exfoliated cells. Based on optimized multiplex PCR, the kit generates single-stranded circular DNA libraries that are compatible with the MGI sequencing platforms. Germline mutations will be analyzed in all coding regions of BRCA1/2 after high-throughput sequencing.

1.3 Sequencing Platform Compatibility

MGICare BRCA1/2 Sequencing Library Preparation Kit generates libraries that are compatible with:

BGISEQ-500RS(PE100)

MGISEQ-2000RS(PE100)、DNBSEQ-G400RS(PE100)

MGISEQ-200RS(PE100)、DNBSEQ-G50RS(PE100)

1.4 Contents

The MGICare BRCA1/2 sequencing library preparation kit is available in 96 Reactions. Each kit contains three separate boxes. Kit information including Cat. No., Components and Specifications are listed below in Table 1.

Table 1 MGICare BRCA1/2 Sequencing Library Preparation Kit （96 RXN）(Cat. No: 1000009522)

Modules and Cat. No	Component	Color-Coded Screw Cap	Spec & Quantity
MGICare BRCA1/2 Sequencing Library Preparation Kit (Box 1)	Multi-PCR Mix	Colorless	3600μL/tube× 2 tubes
	Multi-PCR Primer Pool 1	Blue	96 μL/tube× 1tube
	Multi-PCR Primer Pool 2	Blue	96 μL/tube× 1tube
	Splint Buffer	Purple	47 μL/tube× 1tube
	DNA Rapid Ligase	Purple	2 μL/tube× 1tube
	Digestion Buffer	White	6 μL/tube× 1tube
	Digestion Enzyme	White	11 μL/tube× 1tube
MGICare BRCA1/2 Sequencing Library Preparation Kit (Box 2)	Barcode Primer (33-128)	Colorless	8 μL/well× 96wells
MGICare BRCA1/2 Sequencing Library Preparation Kit (Box 3)	PCR Enhancer	Blue	173 μL/tube× 1tube
	Digestion Stop Buffer	White	30 μL/tube× 1tube
	TE Buffer	White	3847 μL/tube× 2tubes
	DNA Clean Beads	White	6096 μL/tube× 2tubes

1.5 Storage Conditions and Shelf Life

MGICare BRCA1/2 Sequencing Library Preparation Kit (Box 1)

- Storage temperature: -25°C to -18°C
- Expiration Date: refer to kit label
- Transportation Conditions: transported on dry ice

MGICare BRCA1/2 Sequencing Library Preparation Kit (Box 2)

- Storage temperature：-25°C to -18°C
- Expiration Date: refer to kit label
- Transportation Conditions: transported on dry ice

MGICare BRCA1/2 Sequencing Library Preparation Kit (Box 3)

- Storage temperature: 2°C to 8°C
- Expiration Date: refer to kit label

- Transportation Conditions: transported with ice bags

* Please ensure that an abundance of dry ice remains after transportation.

* Performance of products are guaranteed until the expiration date under appropriate transport, storage and usage conditions.

1.6 Equipment and Materials Required but not Provided

Table 2 Equipment and Materials Required but not Provided

Equipment	Thermo Cycler
	Microfuge
	Magnetic stand (Thermo Fisher, Cat. No. 12321D)
	Vortexer
	Gel electrophoresis apparatus
	Qubit® 3.0 Fluorometer (Invitrogen, Cat. No. Q33216)
	Pipettes
Reagents	Nuclease-free water (Ambion, Cat. No. AM9937)
	Ethanol (Analytical purity)
	Qubit® ssDNA Assay Kit (Invitrogen, Cat. No. Q10212)
	Qubit® dsDNA HS Assay Kit (Invitrogen, Cat. No. Q32854)
Consumables	Qubit® Assay Tubes（Invitrogen，Cat. No. Q32856）or 0.5mL Transparent thin wall tube（Axygen, Cat. No. PCR-05-C）
	Pipette Tips
	1.5 mL microcentrifuge tubes（Axygen, Cat. No. MCT-150-C）
	0.2 mL PCR Tube (Axygen, Cat. No. PCR-02-C) or 96-well PCR Plates (Axygen, Cat. No. PCR-96M2-HS-C)

1.7 Precautions and Warnings

- Instructions provided in this manual are intended for general use only, and further adjustment might be required for optimal performance.
- Remove the reagents from storage beforehand and prepare them for use. The Multi-PCR Mix containing enzymes should be thawed on ice. Repeated freezing and thawing should be avoided to retain full activity. Vortex and centrifuge the DNA Rapid Ligase and Digestion Enzyme, then place on ice before use. Thaw other components at room temperature, then mix thoroughly by pipetting up and down several times and centrifuge briefly before use.
- When preparing mixtures and working solutions, we recommend pipetting at least 10 times to mix thoroughly. Note that vigorous shaking may decrease the yield of the prepared library.
- To prevent cross contamination, we recommend filtered pipette tips. Use a new tip each time for pipetting different solutions.
- Pre-warm the DNA clean beads at room temperature for 30 min before use.
- Prepare 80% ethanol fresh for every experiment.
- Improper handling of samples and reagents may contribute to aerosol contamination of PCR products and may decrease experimental accuracy. Therefore, we recommend two physically separate working areas for PCR reaction preparation and PCR product cleanup in the laboratory. Both areas should have designated equipment and be cleaned regularly to ensure a sterile working environment (0.5% Sodium Hypochlorite or 10% Bleach can be used to clean working area).
- If you have other questions, please contact MGI technical support MGI-service@mgi-tech.com

Chapter 2 Sample Requirement

This kit is applicable for library preparation of genomic DNA extracted from samples of human peripheral blood, saliva, and cervical exfoliated cells. It is recommended to use intact or minimally degraded gDNA with A_{260}/A_{280}=1.8 - 2.3. The gDNA concentration should be greater than 1 ng/ul and the total DNA input is 20 ng.

Chapter 3 Library Construction Protocol

3.1 Phase I PCR

3.1.1 Take out Multi-PCR Mix, Multi-PCR Primer Pool 1 and Multi-PCR Primer Pool 2 from the kit stored at -20°C. Thaw on ice and mix thoroughly.

3.1.2 For phase I PCR, at least 10ng gDNA is recommended for each primer pool (Multi-PCR Primer Pool 1 and Multi-PCR Primer Pool 2).

3.1.3 The PCR reactions are performed in 0.2 mL PCR tubes. Each sample will undergo Phase I PCR in two separate tubes with Multi-PCR Primer Pool 1 in one tube and Multi-PCR Primer Pool 2 in the other tube.

⚠ **Note：Two PCR reactions are required for each sample in Phase I PCR, with each tube using different primer pools.**

3.1.4 Prepare phase I PCR mixture in 0.2 mL PCR tubes on ice (see Table 3).

Table 3 Phase I PCR Mixture

Component	volume
gDNA (10ng/ reaction)	X μL
TE Buffer	23.4-X μL
Multi-PCR Mix	25 μL
Multi-PCR Primer Pool *	1 μL
PCR Enhancer	0.6 μL
Total	50 μL

* Multi-PCR Primer Pool 1 or Multi-PCR Primer Pool 2

3.1.5 Vortex the reaction mixture from Step 3.1.4 three times (3s each) then centrifuge briefly.

3.1.6 Load the PCR tubes/plate in Step 3.1.5 into the thermocycler and run the program in Table 4.

Table 4 The thermocycling program of Phase I PCR

Temperature	Time	Cycles
Heated Lid	On	
96°C	10 min	1 Cycle
96°C	1 min	
60°C	15 min	5 Cycles
72°C	1 min	
68°C	10 min	1 Cycle
4°C	Hold	

3.1.7 When the PCR program has completed, centrifuge briefly to spin down solution to the bottom of the tube.

3.1.8 Mix the PCR product of the two amplicon primer pools of each sample into a new 1.5 mL centrifuge tube. Mix thoroughly, then centrifuge briefly.

3.2 Cleanup of Phase I PCR Product

3.2.1 Remove DNA Clean Beads from refrigerator and bring to room temperature for 30 min beforehand. Vortex and mix thoroughly before use.

3.2.2 Pipette 80 μL of DNA Clean Beads into the 100 μL Phase I PCR product from Step 3.1.8. Gently pipette at least 10 times to mix thoroughly. Ensure that all solution and beads are expelled from the tip into the tube.

3.2.3 Incubate the mixture at room temperature for 5 min.

3.2.4 Centrifuge briefly and place the tube on the Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.2.5 Keep the tubes on the magnetic rack and add 200μL freshly prepared 80% ethanol. Incubate the mixture for 30 s. Carefully remove and discard the supernatant without disturbing the beads.

3.2.6 Repeat step 3.2.5 once and remove all supernatant from the tube without disrupting the beads. Briefly centrifuge the tube and return it to the Magnetic Separation Rack. Carefully remove and discard the remaining supernatant using a small volume pipette.

⚠ **Note: Do not disturb the beads.**

3.2.7 Keep the tube on the Magnetic Separation Rack with the lid open to air-dry the beads until no wetness (reflectiveness) is observed (3-5 min). Avoid over-drying beads (cracks can be observed on pellet).

3.2.8 Remove the tube from the Magnetic Separation Rack and add 17 μL of TE Buffer for DNA elution. Gently pipette mixture at least 10 times until the beads are fully resuspended.

3.2.9 Incubate the mixture at room temperature for 5 min.

3.2.10 Centrifuge briefly and place the tubes on the Magnetic Separation Rack for 2-5 min until the solution is clear. Carefully transfer 16.4 μL supernatant to a new 0.2 ml PCR tube without disturbing the beads.

✔ **Stop point: The purified product can be stored at -20°C.**

3.3 Phase II PCR

3.3.1 Remove the Multi-PCR Mix and Barcode Primer (barcode 33-128) from the kit stored at -20°C, thaw on ice, and mix thoroughly.

3.3.2 Add 8 μL Barcode Primer to the PCR tube in Step 3.2.10. Vortex three times (3s each) and centrifuge briefly.

⚠ **Note: Please read Appendix A carefully before starting.**

3.3.3 Prepare phase II PCR mixture on ice (see Table 5).

Table 5 Phase II PCR Mixture

Component	volume
Multi-PCR Mix	25 μL
PCR Enhancer	0.6 μL
Total	25.6 μL

3.3.4 Pipette 25.6 μL Phase II PCR mixture into the PCR tube containing Barcode Primer from Step 3.3.2. Vortex three times (3s each) and centrifuge briefly.

3.3.5 Load the PCR tubes/plate from Step 3.3.4 into the thermocycler. Run the thermocycling program as shown in Table 6.

Table 6 The thermocycling program of Phase II PCR

Temperature	Time	Cycles
Heated Lid	On	
96°C	10 min	1 Cycle
96°C	1 min	
62°C	1 min	20 Cycles
72°C	1 min	
68°C	10 min	1 Cycle
4°C	Hold	

3.3.6 Centrifuge briefly to spin down solution to the bottom of the tube.

3.3.7 Transfer all of the reaction mixture into a new 1.5 mL centrifuge tube.

3.4 Cleanup of Phase II PCR Product

3.4.1 Remove DNA Clean Beads from the refrigerator and incubate the beads at RT for 30 min before use. Vortex and mix thoroughly before use.

3.4.2 Pipette 40 μL of DNA Clean Beads into the 50 μL Phase II PCR product from Step 3.3.8. Pipette mixture at least 10 times until thoroughly mixed. Ensure that all liquid is expelled from the pipette tip into the tube before proceeding.

3.4.3 Incubate the mixture at room temperature for 5 min.

3.4.4 Centrifuge briefly and place the tube on the magnetic separation rack for 2-5 min until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.4.5 Keep the tubes on the magnetic separation rack and add 200μL freshly prepared 80% ethanol. Incubate the mixture for 30 s. Carefully remove and discard the supernatant without disturbing the beads.

3.4.6 Repeat Step 3.4.5 once and remove all supernatant from the tube without disrupting the beads. Briefly centrifuge the tube and return it to the Magnetic Separation Rack. Carefully remove and discard the remaining supernatant using a small volume pipette.

⚠ **Note: Do not disturb the beads.**

3.4.7 Keep the tube on the Magnetic Separation Rack with the lid open to air-dry the beads until no wetness (reflectiveness) is observed (3-5 min). Avoid over-drying beads (cracks can be observed on pellet).

3.4.8 Remove the tube from the Magnetic Separation Rack and add 21 μL of TE Buffer for DNA elution.

Gently pipette mixture at least 10 times until the beads are fully resuspended.

3.4.9 Incubate the mixture at room temperature for 5 min.

3.4.10 Place the tubes on the Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Carefully transfer 20 μL supernatant from each well to a new 1.5ml centrifuge tube without disturbing the beads.

✔ **Stop point: The purified product can be stored at -20 °C.**

3.5 Quality control of libraries and pooling for circularization

3.5.1 Prepared libraries (purified product of Phase II PCR) can be quantified using Qubit® dsDNA HS Assay Kit or the Quant-iT™ PicoGreen® dsDNA Assay Kit. DNA concentration must be greater than or equal to 1 ng /μL.

3.5.2 Assess the fragment size distribution of the PCR products using electrophoresis-based equipment such as: Bioanalyzer, Tapestation (Agilent Technologies), LabChip® GX, GXII, GX Touch (PerkinElmer), or Fragment Analyzer™ (Advanced Analytical). The main band of the libraries should be between 200-300 bp. The peak located around 350-500 bp is a PCR product-derived peak.

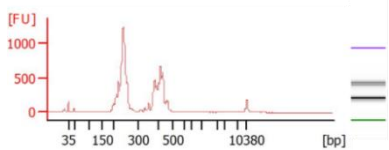

Figure 1 The size of the prepared libraries as analyzed by Agilent 2100 Bioanalyzer.

⚠ **Note: If the library will be delivered to a service lab for sequencing, please stop here.**

Note: If the library will be sequenced in your lab, please go to step 3.5.3.

3.5.3 Depending on the quantitative results of the prepared DNA libraries, pool an equal DNA mass of each sample with different barcode numbers into a new 0.2 mL PCR tube. The total amount of DNA required is 168 ng. Add RNase-free water such that the total volume is 48 μL. Mix thoroughly and centrifuge briefly.

⚠ **Note: For the pooling of multiple samples, the DNA input of each library (ng) = 168 ng/N, wherein N is the number of samples to be sequenced. The volume of each library (μL) = the DNA input of each library (ng) / concentration of the library (ng / μL).**

3.6 Denaturation

3.6.1 Load the PCR tubes from step 3.5.3 into a thermocycler and run the program in Table 7.

Table 7 The Reaction Conditions of Denaturation

Temperature	Time
Heated Lid	On
95°C	3 min

3.6.2 Once the program is complete, place the PCR tubes on ice immediately for 2 min, then centrifuge briefly.

3.7 Single Strand Circularization

3.7.1 Prepare the reaction mixture for single strand circularization in a new 0.2 mL PCR tube on ice (see Table 8).

Table 8 The Single Strand Circularization Mixture

Temperature	Time
Splint Buffer	11.6 μL
DNA Rapid Ligase	0.5 μL
Total	12.1 μL

3.7.2 Pipette 12.1 μL of the single strand circularization mixture into the PCR tube from Step 3.6.2, vortex three times (3s each), and centrifuge briefly to collect the liquid.

3.7.3 Load the PCR tubes into a thermocycler and run the program in Table 9.

Table 9 The thermocycling program of single strand DNA circularization

Temperature	Time
Heated Lid	On
37°C	60 min
4°C	Hold

3.7.4 Once the program is complete, immediately place the tube on ice.

3.8 Enzymatic Digestion

3.8.1 Prepare the enzymatic digestion mixture (Table 10) in a new 0.2 mL PCR tube on ice.

Table 10 The Enzymatic Digestion Mixture

Temperature	Time
Digestion Buffer	1.4 μL
Digestion Enzyme	2.6 μL
Total	4.0 μL

3.8.2 Transfer 4 μL enzymatic digestion mixture into the PCR tube from Step 3.7.4, vortex three times (3s each). Centrifuge briefly to collect the liquid.

3.8.3 Load the PCR tube into a thermocycler and run the program in Table 11.

Table 11 The thermocycling program of enzymatic digestion

Temperature	Time
Heated Lid	On
37°C	30 min

3.8.4 Once the program is complete, immediately place the tube on ice.

3.8.5 Add 7.5 μL Digestion Stop Buffer to the tube from Step 3.8.4, vortex three times (3s each). Centrifuge briefly to collect the liquid. Transfer all of the liquid to a new 1.5 mL centrifuge tube.

3.9 Cleanup of the Enzymatic Digestion Product

3.9.1 Remove DNA Clean Beads from refrigerator and bring to room temperature for 30 min beforehand. Vortex and mix thoroughly before use.

3.9.2 Pipette 170 μL of DNA Clean Beads into the digestion product from Step 3.8.5. Gently pipette at least 10 times to mix thoroughly. Ensure that all solution and beads are expelled from the tip into the tube.

3.9.3 Incubate the mixture at room temperature for 5 min.

3.9.4 Centrifuge briefly and place the tube on the Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.9.5 Keep the tubes on the magnetic rack and add 500μL freshly prepared 80% ethanol. Incubate the mixture for 30 s. Carefully remove and discard the supernatant without disturbing the beads.

3.9.6 Repeat Step 3.9.5. once and remove all supernatant from the tube without disrupting the beads. Briefly centrifuge the tube and return it to the Magnetic Separation Rack. Carefully remove and

discard the remaining supernatant using a small volume pipette.

⚠ **Note: Do not disturb the beads.**

3.9.7 Keep the tube on the Magnetic Separation Rack with the lid open to air-dry the beads until no wetness (reflectiveness) is observed (5-10 min). Avoid over-drying beads (cracks can be observed on pellet).

3.9.8 Remove the tube from the Magnetic Separation Rack and add 21 μL of TE Buffer for DNA elution. Gently pipette the mixture at least 10 times until the beads are fully resuspended.

3.9.9 Incubate the mixture at room temperature for 5 min.

3.9.10 Place the tubes on a magnetic rack for 2-5 min until the solution becomes clear. Carefully transfer 20 μL supernatant from each well to a new 1.5mL centrifuge tube without disturbing the beads.

✔ **Stop point: The purified product can be stored at -20°C.**

3.10 Quality Control of Enzymatic Digestion Product

1 μL of the product is used for quantification using the Qubit® ssDNA Assay kit. The total amount of product must be greater than or equal to 6ng. The product can be stored at -20°C before the DNB preparation.

Appendix

Appendix A. The Combination Barcode Adaptors Strategies

- This kit provides 96 Barcode Primers (barcodes 33-128). The kit was designed for library preparation and sequencing with large sample sizes. For the best results, please refer to Appendix A-1 for the use of Barcode Primers.
- In other BGISEQ library preparation kits, barcodes are generally designed within the adapters. If the adapter numbers in other sequencing libraries are the same as the Barcode Primer number in this kit, then the barcode sequences are the same. These libraries cannot be loaded in the same lane when sequencing.
- Centrifuge the Barcode Primers briefly before use. Remove the silicone film carefully and prevent liquid splashing to avoid cross-contamination. Pipette the solution up and down to mix. Seal the plate with a new adhesive film immediately after use.
- Libraries prepared with this kit are not compatible with libraries prepared with other MGI kits that use adaptors 501-596. DO NOT mix libraries from other kits if they are prepared using adaptors 501-596.

A-1 MGICare BRCA1/2 Sequencing library preparation（Box 2）(plate) kit user instructions

Barcode-Primers in MGI kits are base balanced for optimal sequencing accuracy and should be used in groups. The Barcode Primers provided in this kit has the following grouping rules:

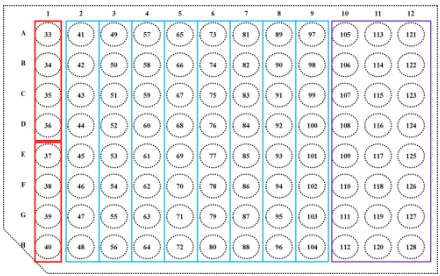

Figure 2 Barcode Primer (33-128) (Plate) Barcode Map and Grouping Rules

4 Barcode Primers per group: 33-36，37-40，41-44，45-48，…，121-124，125-128，a total of 24 groups

(as shown in the red boxes in Figure 3)

8 Barcode Primers per group：33-40，41-48，49-56，57-62，…，121-128，a total of 12 groups (as shown in the blue boxes in Figure 3)

24 Barcode Primer per group：33-56，57-80，81-104，105-128，a total of 4 groups (as shown in the purple box in Figure 3).

The data required for each sample is 2M (reads). It is recommended to use a differently numbered barcode for library construction of each sample. In order to ensure high sequencing quality of barcodes, always use an entire group of barcodes (4, 8, or 24).

If the data required for each sample is the same, use the barcode combination strategy recommended in Table 12:

Table 12 MGICare Barcode Primer（33-128）(plate) kit user instructions

Samples/lane	Instructions (Example)
Case-1: Number of samples (N) equals 4A (A =1-24)	1. Add a set of 4 Barcode Primers (such as 33-36, or 37-40...) Or 2. Add a set of 8 Barcode Primer (such as 41-48, or 49-56...) Or 3. Add a set of 24 Barcode Primer (such as 57-80, or 81-104...) Or 4. Add combination of 4, 8, 24 Barcode Primer set.
Case-2: Number of samples (N) is not equal to 4A (A =1-24) and N = 4B + C (B is integer, C = 1, or 2, or 3)	1. Use 4,8,24 Barcode Primer set combination to assign the barcodes to sample 1 to 4B. 2. The remaining samples (C) can use any other Barcodes.

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Doc.#: B02-138