Part No.:H-940-000188-00-01

User Manual

Version:4.0

MGICare Detection Set for Single Cell Chromosome Copy Number Variation Test

Cat. No.: 940-000188-00 (48 RXN)
Set Version: V1.0

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About the user manual

Manufacturer information

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Revision history

Manual version	Set version	Date	Description
4.0	V1.0	Mar. 2024	Update the manufacturer information
3.0	V1.0	Sep. 2023	• Update the components specification • Update the manual style • Add appendix About sample pooling • Delete appendix Magnetic beads and cleanup
2.0	V1.0	Dec. 2022	Update the service hotline
1.0	V1.0	Mar. 2022	Update Cat. No.

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

Contents

1 Product overview

1.1 Introduction

MGICare Detection Set for Single Cell Chromosome Copy Number Variation Test compatible with MGI high-throughput sequencing platform is designed to construct libraries for detecting single cell chromosome copy number variations, which is applicable for human single cells or traces of cells (biopsied in vitro fertilization embryos, human peripheral blood lymphocytes, and so on). This user manual describes the procedures for whole genome amplification (WGA), library preparation of WGA products, and single strand circular (ssCir) DNA generation for detection of single cell chromosome copy number variations. All reagents provided in this set have passed strict quality control and functional verification procedures, ensuring stability and reproducibility.

1.2 Intended use

This set is applicable for human single cell or multiple cells. It is not applicable for other species. It is used in the field of single cell research (PGS, CTC, and so on).

1.3 Applicable sequencing platforms

The prepared libraries are applicable to the following sequencing platforms.

- BGISEQ-500RS (SE50)
- MGISEQ-200RS (SE50), DNBSEQ-G50RS(SE50)
- MGISEQ-2000RS (SE50), DNBSEQ-G400RS (SE50)

1.4 Components

This library prep set comes in one specification: 48 RXN. Four separate boxes are included for each specification. For component details, refer to the following table.

Each set contains an information card. Relevant manuals and SDS files can be downloaded from the MGI website provided on the information card.

Table 1 MGICare Detection Set for Single Cell Chromosome Copy Number Variation Test (48 RXN) (Cat. No.: 940-000188-00)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGICare Detection Kit for Single Cell Chromosome Copy Number Variation Test (Box 1) Cat. No.: 940-000-176-00	Cell Lyase	Yellow	10 μL/tube × 1
	Cell Lysis Buffer	Purple	235 μL/tube × 1
	Pre-amplification Enzyme	White	10 μL/tube × 1
	Pre-amplification Buffer	Red	235 μL/tube × 1
	Post-amplification Enzyme	Blue	40 μL/tube × 1
	Post-amplification Buffer	Orange	1200 μL/tube × 1
	Nuclease-Free Water	Colorless	1650 μL/tube × 1
	Positive Control	Black	24 μL/tube × 1
	Negative Control	Black	24 μL/tube × 1
MGICare Detection Kit for Single Cell Chromosome Copy Number Variation Test (Box 2) Cat. No.: 940-000-177-00	Fragmentation Enzyme	Pink	48 μL/tube × 3
	Fragmentation Buffer	Pink	48 μL/tube × 3
	ERAT Buffer Mix	Orange	150 μL/tube × 3
	ERAT Enzyme Mix	Orange	10 μL/tube × 3
	Ligation Buffer Mix	Red	384 μL/tube × 3
	DNA Ligase	Red	16 μL/tube × 3
	Barcode Adapter Mix (01-48)	Colorless	10 μL/tube × 48
	PCR Enzyme Mix	Blue	400 μL/tube × 3
	PCR Primer Mix	Blue	64 μL/tube × 3
MGICare Detection Kit for Single Cell Chromosome Copy Number Variation Test (Box 3) Cat. No.: 940-000-178-00	TE Buffer	White	5000 μL/tube × 2
	DNA Clean Beads	White	1320 μL/tube × 6

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy Rapid Circularization Module Cat. No.: 1000005258	Splint Buffer	Purple	186 µL/tube × 1
	DNA Rapid Ligase	Purple	8 µL/tube × 1

1.5 Storage and transportation

Table 2 Kit storage and transportation temperatures

Item	Storage temperature	Transportation temperature
MGICare Detection Kit for Single Cell Chromosome Copy Number Variation Test (Box 1)	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
MGICare Detection Kit for Single Cell Chromosome Copy Number Variation Test (Box 2)		
MGIEasy Rapid Circularization Module		
MGICare Detection Kit for Single Cell Chromosome Copy Number Variation Test (Box 3)	2 ℃ to 8 ℃	2 ℃ to 8 ℃

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 life.

1.6 User-supplied materials

Table 3 Order information for MGI products

Catalog number	Model	Name
1000002072	SE50	BGISEQ-500RS High-throughput Sequencing Set
1000004635	SE50	MGISEQ-200RS High-throughput Sequencing Set
1000016959	FCL SE50	MGISEQ-G50RS High-throughput Sequencing Set
1000012551	SE50	MGISEQ-2000RS High-throughput Sequencing Set
1000016941	FCL SE50	DNBSEQ-G400RS High-throughput Sequencing Set

Table 4 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	/
Desktop centrifuge	/
Pipettes	/
Thermocycler	/
Magnetic rack DynaMag -2, or equivalent	Thermo Fisher Scientific, Cat. No. 12321D
Qubit Fluorometer, or equivalent	Thermo Fisher, Cat. No. Q33216
Agilent 2100 Bioanalyzer, or equivalent	Agilent Technologies , Cat. No. G2939AA
Sterile containment cabinet (vertical laminar flow cabinet)	/

Table 5 Recommended reagent/consumable list

Reagent/consumable	Recommended brand
Nuclease Free (NF) water or Molecular Grade Water	Ambion, Cat. No. AM9937, or equivalent
TE Buffer, pH 8.0	Ambion, Cat. No. AM9858, or equivalent
100% Ethanol (Analytical Grade)	/
Qubit dsDNA HS Assay Kit/Quant-iT dsDNA HS Assay Kit	Invitrogen, Cat. No. Q32854, or equivalent
High Sensitivity DNA Kits	Agilent TechnologiesTM, Cat. No. 5067-4626
Agilent DNA 1000 Kit	Agilent, Cat. No. 5067-1504
Pipette tips	/
1.5 mL tube	/
0.2 mL PCR tube or 96-well plate	/
Qubit Assay Tubes or 0.5mL Thin Wall PCR Tubes	Invitrogen or Axygen, or equivalent

1.7 Precautions and warnings

- This product is for research use only, not for in vitro diagnosis. Please 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 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 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**.

1.8 Workflow

Section 3.1 to 3.5 describes the process of single-cell whole-genome amplification, including cell lysis, pre-amplification, and post-amplification. The library construction protocol is outlined in sections 4.1 to 4.9, which includes a rapid circularization step. Through these steps, ssCir DNA libraries were finally obtained.

Section	Workflow	Total time	Hands-on time
3.1	Environment and reagent preparation	35 min	5 min
3.2	Sample preparation	5 min	5 min
3.3	Cell lysis	20 min	3 - 5 min
3.4	Pre-amplification	50 - 55 min	5 min
3.5	Post-amplification ⏸	40 - 45 min	5 min
4.1	Fragmention	25 min	5 min
4.2	Cleanup of fragmention product	30 - 40 min	20 - 30 min
4.3	End repair	35 - 40 min	5 - 10 min
4.4	Adapter ligation	30 min	5 - 10 min
4.5	Cleanup of adapter-ligated ⏸	30 - 40 min	20 - 30 min
4.6	PCR	25 - 30 min	5 - 8 min
4.7	Cleanup of PCR product ⏸	30 - 40 min	20 - 30 min
4.8	QC of PCR product ⏸	15 - 20 min	5 - 10 min
4.9	Denaturation, single strand circularization	45 - 50 min	15 min

Tips

- 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.
- ⏸ : The stop point.

2 Sample requirements

- This kit is applicable for samples of human single cell or multiple cells. It is not applicable for other species. Validate cell sample storage and shipment conditions before use.
- **Sample collection and process**: Cells must be suspended in 4 µL 1x PBS (Phosphate Buffered Saline) with a maximum concentration of 0.5% PVP (Polyvinylpyrrolidone) in a 0.2 mL PCR tube.
- **Storage**: The collected cell samples can be stored in PBS below -70 ℃ for less than one month. Keep the sample tube upright. Do not invert the sample tube. The frozen samples should be melted at 2 ℃ to 8 ℃ before whole genome amplification.
- **Transportation**: Ensure that the transportation time is no more than seven days and enough dry ice remains when the sample arrives. The liquid part of the sample tube should be kept frozen.

3 Single cell whole genome amplification

3.1 Environment and reagent preparation

3.1.1 Equipment

In this chapter, all steps (cell lysis, pre-amplification and post-amplification) should be done in a dedicated sterile containment cabinet (vertical laminar flow cabinet) and thermo cycler. First switch on the draught fan of the cabinet. And then scrub the materials in the cabinet with 75% ethanol. Finally switch off the draught fan and sterilize the cabinet with ultraviolet for at least 30 min.

3.1.2 Reagent

Take out the reagents from storage beforehand, and prepare them for use: For enzymes, centrifuge briefly and place on ice for further use. For other modules, thaw at 2℃~8℃ and invert several times to mix properly, centrifuge briefly, and place on ice for further use.

CAUTION

- The experimenter should wear a mask and powderless latex gloves during the experiment. If your gloves are in contact with areas outside the cabinet, rub the gloves with 75% ethanol.
- To prevent cross contamination, we recommend using filtered pipette tips. Use a new tip each time for pipetting different solutions.

3.2 Sample preparation

3.2.1 Preparation

Table 6 Preparing the reagents

Reagent	Requirement
NF Water	User-supplied; place at room temperature (RT).
Positive Control	Thaw at RT, mix well, centrifuge briefly, and place on ice.
Negative Control	

3.2.2 Sample preparation

1. Dilute the Negative Control (1 ng/μL) in this kit to 15 pg/μL with NF Water. (Transfer 131 μL of NF Water to a PCR tube and add 2 of Negative Control to the tube. Mix it well and centrifuge briefly.) Pipette 4 μL of diluted Negative Control (15 pg/μL) to a new PCR tube.
2. Prepare the diluted Positive Control (15 pg/μL) in the same way and add 4 μL to a new PCR tube.

   CAUTION Use a clean tip for each tube and cap each tube immediately after transfer. The diluted Positive/Negative Control (15 pg/μL) can't be stored for a long time. We recommend using freshly diluted Positive/Negative Control for every experiment.

3. Thaw the frozen samples on ice before cell lysis process. Centrifuge the sample tubes briefly to collect the liquid to the bottom. Line up the sample tubes in a 96-well rack on ice for later use.

   CAUTION In section Cell lysis and pre-amplification, in order to prevent cell samples adhering to the pipette tip or to the tube wall and cap, it is recommended that you slowly add reagents along the tube wall without inserting the tip into the sample. When mixing the sample, gently flick the bottom or middle of the PCR tube 5-6 times and avoid splashing the liquid. DO not mix the sample by vortexing, inverting, or pipetting.

3.3 Cell lysis

3.3.1 Preparation

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

Table 7 Preparing the reagents

Reagent	Requirement
Cell Lysis Buffer	Thaw at RT, mix well, centrifuge briefly, and place on ice.
Cell Lyase	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.

3.3.2 Cell lysis

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

Table 8 Cell lysis mixture

Reagent	Volume per reaction
Cell Lysis Buffer	4.8 μL
Cell Lyase	0.2 μL
Total	5 μL

2. Add **5 μL of cell lysis mixture** to each sample tube (from step 1/2 in section 3.2.2). **Gently flick the tube bottom** 5 to 6 times to mix it well, centrifuge briefly, and place on ice.

Tips When adding the cell lysis mixture, hold the tips of pipette above the liquid surface.

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

Table 9 Cell lysis reaction conditions (Volume: 9 μL)

Temperature	Time
105 ℃ Heated lid	On
75 ℃	10 min
95 ℃	4 min
4 ℃	Hold

4. After the reaction, centrifuge the PCR tubes briefly and line up in a 96-well rack with ice.

WARNING Do not stop at this step. Proceed to the next reaction.

3.4 Pre-amplification

3.4.1 Preparation

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

Table 10 Preparing the reagents

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

3.4.2 Pre-amplification

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

Table 11 Pre-amplification mixture

Reagent	Volume per reaction
Pre-amplification Buffer	4.8 μL
Pre-amplification Enzyme	0.2 μL
Total	5 μL

2. Add **5 μL of pre-amplification mixture** to each sample tube (from step 4 in section 3.3.2). **Gently flick the tube bottom** 5 to 6 times to mix it well, centrifuge briefly, and place on ice.

   Tips When adding the pre-amplification mixture, hold the tips of pipette above the liquid surface.

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

Table 12 Pre-amplification reaction conditions (Volume: 14 μL)

Temperature	Time	Cycles
105 ℃ Heated lid	On	-
95 ℃	2 min	1
95 ℃	15 sec	12
15 ℃	50 sec	
25 ℃	40 sec	
35 ℃	30 sec	
65 ℃	40 sec	
75 ℃	40 sec	
4 ℃	Hold	-

4. After the reaction, centrifuge the PCR tubes briefly and line up in a 96-well rack with ice.

3.5 Post-amplification

3.5.1 Preparation

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

Table 13 Preparing the reagents

Reagent	Requirement
Nuclease-Free Water	Thaw at RT, mix by vortexing, centrifuge briefly, and place at RT.
Post-amplification Buffer	
Post-amplification Enzyme	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.

3.5.2 Post-amplification

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

Table 14 Post-amplification mixture

Reagent	Volume per reaction
Nuclease-Free Water	34.2 μL
Post-amplification Buffer	25 μL
Post-amplification Enzyme	0.8 μL
Total	60 μL

2. Add **60 μL of post-amplification mixture** to each sample tube (from step 4 in section 3.4.2). 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 15 Post-amplification reaction conditions (Volume: 74 μL)

Temperature	Time	Cycles
105 ℃ Heated lid	On	-
95 ℃	2 min	1
95 ℃	15 sec	14
65 ℃	1 min	
75 ℃	1 min	
4 ℃	Hold	-

4. After the reaction, centrifuge the PCR tubes briefly and line up in a 96-well rack with ice.

Stop point Products can be stored at -20 ℃ for a long time.

4 Library preparation protocol

4.1 Fragmentation

4.1.1 Preparation

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

Table 16 Preparing the reagents

Reagent	Requirement
Fragmentation Buffer	Thaw at RT, mix well, centrifuge briefly, and place on ice.
Fragmentation Enzyme	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.

4.1.2 Fragmentation

1. Transfer **24 μL** of sample (from step 4 in section 3.5.2) into a new 0.2 mL PCR tube.
2. According to the desired reaction number, prepare the fragmentation mixture in a 0.2 mL PCR tube on ice. Mix by vortexing 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 17 Fragmentation mixtuer

Reagent	Volume per reaction
Fragmentation Buffer	3 μL
Fragmentation Enzyme	3 μL
Total	6 μL

3. Add **6 μL of fragmentation mixture** to each sample tube (from step 1). Mix by vortexing 3 times (3 sec each), centrifuge briefly, and place on ice.
4. Set up the program in advance. When the thermocycler reaches the reaction temperature, place the PCR tubes into the thermocycler and run the program.

Table 18 Fragmentation reaction conditions (Volume: 30 μL)

Temperature	Time
105 ℃ Heated lid	On
37 ℃	5 min
75 ℃	15 min
4 ℃	Hold

5. When the program is completed, centrifuge the PCR tubes briefly and place on ice.

CAUTION To prevent over-fragmentation, proceed immediately to the next step within 30 min.

4.2 Cleanup of fragmentation 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.

4.2.1 Preparation

Table 19 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.

4.2.2 Cleanup of fragmentation product

Tips Transfer all liquid to a new 1.5 mL centrifuge tube (one tube per reaction) in advance if using a 1.5 mL centrifuge tube and a corresponding magnetic rack for purification.

1. Add **20 μL of TE Buffer** to each fragmentation product (from step 5 in section 4.1.2) for a total volume of 50 μL.
2. Mix the DNA Clean Beads thoroughly. Add **75 μL of DNA Clean Beads** to each sample tube. 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.
3. Incubate the sample(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 the supernatant.

5. While keeping the tube(s) on the magnetic rack, add **150 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 **43 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.
9. Incubate the sample(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 **40 µL** of supernatant to a new 0.2 mL PCR tube.

    Stop point After cleanup, product(s) can be stored at -20 ℃ for no more than 7 days.

4.3 End repair

4.3.1 Preparation

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

Table 20 Preparing the reagents

Reagent	Requirement
ERAT Buffer Mix	Thaw at RT, mix well, centrifuge briefly, and place on ice.
ERAT Enzyme Mix	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.

4.3.2 End repair

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

Table 21 End repair mixture

Reagent	Volume per reaction
ERAT Buffer Mix	9.4 µL
ERAT Enzyme Mix	0.6 µL
Total	10 µL

2. Add **10 μL of end repair mixture** to each sample tube (from step 9 in section 4.2.2). Vortex the tube(s) 3 times (3 sec each), entrifuge briefly, and place on ice.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 22 End repair reaction conditions (Volume: 50 μL)

Temperature	Time
70 ℃ Heated lid	On
37 ℃	10 min
65 ℃	15 min
4 ℃	Hold

4. When the program is completed, centrifuge the PCR tube(s) briefly to collect the liquid to the bottom of the tube.

WARNING
- Do not stop at this step. Proceed to the next reaction.
- If the operation stops here, store the end repair product(s) at −20 ℃ overnight with a risk of 20% decrease in yield.

4.4 Adapter ligation

Tips Before operation, carefully read “Using adapters” on page 26.

4.4.1 Preparation

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

Table 23 Preparing the reagents

Reagent	Requirement
Ligation Buffer Mix	Thaw at RT, mix well, centrifuge briefly, and place on ice.
DNA Ligase	Flick or invert the tube gently, centrifuge briefly, and place on ice.
Adapters Mix (Barcode 01-48)	Mix thoroughly, centrifuge briefly, and place on ice.

Tips
- Mix the adapter(s) well before using. Adapters should not be mixed directly with the adapter ligation mixture.
- The Ligation Buffer is highly viscous. Mix it by vortexing 6 times (3 sec each) and centrifuge briefly. When pipetting the Ligation Buffer, slowly aspirate to ensure that the volume is accurate.

4.4.2 Adapter ligation

1. **Add 5 μL of Adapters Mix (Barcode 01-48)** to the corresponding sample tube (from step 4 in section 4.3.2). Vortex the tube(s) 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 0.2 mL PCR tube on ice. Mix it well by vortexing 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 24 Adapter ligation mixture

Reagent	Volume per reaction
Ligation Buffer Mix	24 μL
DNA Ligase	1 μL
Total	25 μL

3. Slowly pipette **25 μL of adapter ligation mixture** to each sample tube and vortex 6 times (3 sec each). Centrifuge briefly to collect the liquid to the bottom of the tube and place on ice.

Tips The adapter ligation mixture is highly viscous. Slowly aspirate to ensure the volume is accurate.

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

Table 25 Adapter ligation reaction conditions (Volume: 80 μL)

Temperature	Time
30 ℃ Heated lid	On
23 ℃	20 min
4 ℃	Hold

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

WARNING Do not stop at this step. Please proceed to next reaction. Otherwise, the yield may decrease.

4.5 Cleanup of adapter-ligated 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.

4.5.1 Preparation

Table 26 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.

4.5.2 Cleanup of adapter-ligated product

Tips Transfer all liquid to a new 1.5 mL centrifuge tube (one tube per reaction) in advance if using a 1.5 mL centrifuge tube and a corresponding magnetic rack for purification.

1. Mix the DNA Clean Beads thoroughly. Add **40 μL of DNA Clean Beads** to each sample tube (from step 5 in section 4.4.2), and 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**150 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 **23 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 **21 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 ℃ for no more than 7 days.

4.6 PCR

4.6.1 Preparation

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

Table 27 Preparing the reagents

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

4.6.2 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 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 28 PCR mixture

Reagent	Volume per reaction
PCR Enzyme Mix	25 μL
PCR Primer Mix	4 μL
Total	29 μL

2. Add **29 μL of PCR mixture** to each sample tube (from step 9 in section 4.5.2). Vortex the tube(s) 3 times (3 sec each) and centrifuge briefly to collect the liquid to the bottom of the tube.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 29 PCR reaction conditions (Volume: 50 μL)

Temperature	Time	Cycles
105 ℃ Heated lid	On	-
98 ℃	2 min	1
98 ℃	15 sec	12
56 ℃	15 sec	
72 ℃	30 sec	
72 ℃	5 min	1
4 ℃	Hold	-

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

Stop point PCR product(s) can be stored at - 20 ℃.

4.7 Cleanup of 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.

4.7.1 Preparation

Table 30 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.

4.7.2 Cleanup of PCR product

Tips Transfer all liquid to a new 1.5 mL centrifuge tube (one tube per reaction) in advance if using a 1.5 mL centrifuge tube and a corresponding magnetic rack for purification.

1. Mix the beads thoroughly. Add **50 μL of DNA Clean Beads** to each sample tube (from step 4 in section 4.6.2). 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**150 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 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, the PCR product(s) can be stored at -20 ℃.

4.8 QC of PCR product

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

Table 31 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	concentration for PCR products: ≥ 2 ng /μL

Refer to Formula 1 in "About samples pooling" on page 30 to calculate the mass (in ng) that corresponds to 1 pmol of dsDNA sample with varying fragment sizes.

- For 1 sample sequencing in 1 lane, please proceed to section 4.9.
- For multiple samples pooled sequencing, refer to "Using adapters" on page 26 and "About samples pooling" on page 30. The mass of PCR products is recommended to be 168 ng with a total volume ≤ 48 μL.

4.9 Denaturation and single strand circularization

Tips Calculate the required purified PCR product volume based on the main fragment size of the purified PCR product, concentration of the sample, and Formula 1 and 2 in "About samples pooling" on page 30.

4.9.1 Preparation

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

Table 32 Preparing the reagents

Reagent	Requirement
Elution Buffer	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.

4.9.2 Denaturation

1. Add **168 ng of PCR product(s)** into a new 0.2 mL PCR tube and add **Elution Buffer** to make a total volume of 48 µL. Mix it well and centrifuge briefly.
2. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 33 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.

4.9.3 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. Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 34 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 section 3.7.2). 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 35 Single strand circularization reaction conditions (Volume: 60.1 μL)

Temperature	Time
45 ℃ Heated lid	On
37 ℃	30 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.

Stop point ssCir DNA can be stored at -20 ℃.

5 Sequencing

About 20 μL of circularized ssDNA product are used to prepare DNA nanoballs (DNBs) and sequencing.

Please follow the protocol described in "BGISEQ/MGISEQ/DNBSEQ High-throughput Sequencing Set Instruction Manual" for DNB making and sequencing. The available sequencing kits including:

- BGISEQ-500RS sequencing platform
  BGISEQ-500RS High-throughput Sequencing Set (SE50)
- MGISEQ-2000RS\ DNBSEQ-G400RS sequencing platform
  MGISEQ-2000RS High-throughput Sequencing Set (SE50)
  DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE50)
- MGISEQ-200RS\ DNBSEQ-G50RS sequencing platform
  MGISEQ-200RS High-throughput Sequencing Set (SE50)
  MGISEQ-G50RS High-throughput Sequencing Set (FCL SE50)

6 Appendix

6.1 Using adapters

MGI currently offers 48 DNA Barcode Adapters to meet requirements for batch processing of library construction and multiplex sequencing. We selected the best adapter combination based on the principle of balanced base composition. However, the numbers of barcode adapter are not continuous. For optimal performance, read the instructions of Adapter Reagent Kits carefully before use.

- Adapters contain overlapping barcodes and cannot be sequenced in the same lane.
- All adapters are double stranded. To prevent structural changes, such as denaturation, which might affect performance, do not place the adapters above 30 ℃.
- Before use, mix the adapter(s) well and centrifuge to collect the liquid at the bottom of tubes or plates.
- Change tips when pipetting different adapters to prevent cross-contamination.
- For tube reagent, carefully open the tube cap to prevent spills or to prevent cross-contamination. Close the cap immediately after use.
- For plate reagent, spray 75% alcohol and wipe the surface of the aluminum film of the plate with absorbent paper. The aluminum film is penetrable and 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 ℃.
- Adapters from other MGI Library Prep Kits are designed differently and cannot be mixed with the adapters described here. Otherwise, errors will occur during barcode demultiplexing procedures on DNBSEQ platforms.

6.1.1 Sample barcode pooling strategies

- For pooled sequencing, sample barcode pooling should follow the principle of base balance.
- Using an 8 bp barcode as an example, the ratio of ATGC at 1-8 bp bases should be 25%, as shown in the table below.

Table 36 Example of sample barcode pooling strategies

Barcode	Sequence	1	2	3	4	5	6	7	8
Example 1	TAGGTCCG	T	A	G	G	T	C	C	G
Example 2	GGACGGAA	G	G	A	C	G	G	A	A
Example 3	CTTACTGC	C	T	T	A	C	T	G	C
Example 4	ACCTAATT	A	C	C	T	A	A	T	T
Barcode 1-8 bp A%		25%	25%	25%	25%	25%	25%	25%	25%
Barcode 1-8 bp T%		25%	25%	25%	25%	25%	25%	25%	25%
Barcode 1-8 bp G%		25%	25%	25%	25%	25%	25%	25%	25%
Barcode 1-8 bp C%		25%	25%	25%	25%	25%	25%	25%	25%

- If the proportion cannot reach 25%, then ATGC should appear in each cycle. The minimum base proportion should not be less than 12.5% and the maximum base proportion should not be greater than 62.5%.
- If the proportion is not between 12.5% and 62.5%, then sequencing quality could be reduced. In this case, it is possible that the sample barcodes might not be properly split.

6.1.2 Instructions for Adapters Mix (Barcode 01-48)

Based on the principles of balanced base composition, adapters must be used in specific groups. Please follow the instructions below to use the adapters in the proper combinations.

- 4 sets of 4 adapters: 01-04, 05-08, 09-12, 13-16.
- 4 sets of 8 adapters: 17-24, 25-32, 33-40, 41-48.

If the sequencing data output requirement is the same for all samples in one lane, choose the barcode adapter combinations in the table below.

CAUTION The number of the adapter should not be repeated between samples in one lane.

Table 37 Instructions for Adapters Mix (Barcode 01-48)

Sample/lane	Instruction (Example)
1	Requires at least 1 set of adapters: • For a set of 4 adapters, add 4 adapters to each sample. For example: 01-04. Mix 4 adapters with equal volume and add the mixture to the sample. • Or, for a set of 8 adapters, add 8 adapters to each sample. For example: 41-48. Mix 8 adapters with equal volume and add the mixture to the sample.

Sample/lane	Instruction (Example)
2	Requires at least 1 set of adapters: • For a set of 4 adapters, add 2 adapters to each sample. For example: 01-04. Mix 01 and 02 with equal volume and add the mixture to sample 1; Mix 03 and 04 with equal volume and add the mixture to sample 2. • Or, for a set of 8 adapters, add 4 adapters to each sample. For example: 41-48. Mix 41-44 with equal volume and add the mixture to sample 1; Mix 45-48 with equal volume and add the mixture to sample 2.
3	Requires at least 2 sets of Adapters: 1. For samples 1 and 2, use the method for (2 samples/lane) above. 2. For sample 3, use the method for (1 sample/lane) above. Tips Use different adapter sets for samples 1, 2, and 3.
4	Requires at least 1 set of adapters: • For a set of 4 adapters, add 1 adapter to each sample. For example: 01-04. Add adapter 01, 02, 03, 04 to sample 1, 2, 3, 4, respectively. • Or, for a set of 8 adapters, add 2 adapters to each sample. For example: 41-48. Mix 41-42, 43-44, 45-46, and 47-48 with equal volume. Add the mixture to sample 1, 2, 3, 4, respectively.
5	Requires at least 2 sets of Adapters: • For samples 1-4, use the method for (4 samples/lane) above. • For sample 5, use the method for (1 sample/lane) above. Tips Use different adapter sets for samples 1-4 and for sample 5.
6	Requires at least 2 sets of adapters: 1. For samples 1-4, use the method for (4 samples/lane) above. 2. For samples 5-6, use the method for (2 sample/lane) above. Tips Use different adapter sets for samples 1-4 and for samples 5-6.

Sample/lane	Instruction (Example)
7	Requires all 3 Adapter sets and follow 3 steps: 1. For samples 1-4, use the method for (4 samples/lane) above (use the first adapter set). 2. For samples 5-6, use the method for (2 samples/lane) above (use the second adapter set). 3. For sample 7, use the method for (1 sample/lane) above (use the third adapter set). ▪ Add a single adapter within the adapter set. ▪ Or, add the adapter mix which is mixed from all adapters within the adapter set with an equal volume. Tips Use different adapter sets for samples 1-4, for samples 5-6 and for sample 7.
8	Requires at least 1 set of adapters: • For a set of 8 adapters, add 1 adapter to each sample. For example: 41-48. Add adapters 41-48 to samples 1-8, respectively. • Or, for 2 sets of 4 adapters, add 1 adapter to each sample. For example: 01-04 and 5-8. Add 1 adapter to each sample.
8n+x (x=1-8, Total 9-48)	Perform the following 3 steps: 1. For samples 1 to 8n, use the method for (8 samples/lane) above. 2. For samples X, according to the value of X, use the methods above for 1-8 sample/lane accordingly. Tips Use different adapter sets for steps 1 and 2.

For situations in which sequencing data output requirements are different among samples, any sample with a data output of more than 20% for each lane should use a separate set of adapters.

For example, 9 samples are pooled into one lane, one sample of which requires 30% of the total data output.

1. 8 samples may use adapter set (41-48).
2. The final sample should use a full adapter set instead of using only a single adapter. (For example: adapter set (01-04) or (13-16)).

6.2 About samples pooling

CAUTION Do not pool samples with different insert size distributions in the same lane.

Tips Before pooling, carefully read Appendix Using adapters.

Purified PCR products pooling

Quantify the purified PCR products before pooling. The total yield after pooling should be 1 pmol, with a total volume ≤ 48 μL.

Calculate the percentage of the required amount of sequencing data for samples on the same lane. Refer to Formula 1 and 2 to calculate the required mass of each sample. Formula 3 shows the calculation of sample volume.

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

Formula 2 Calculation of each sample mass before pooling

Sample mass (ng) = Mass corresponding to 1 pmol PCR product (ng) × Ratio of sample data (%)

Formula 3Calculation of sample volume

$$\text{Sample volume (μL)} = \frac{\text{Sample mass (ng)}}{\text{Sample concentration (ng/μL)}}$$

For example: For 4 samples (belong to 150 to 200 bp insert size libraries, 84 bp adapter) pooled sequencing. The mass of PCR products is recommended to be 168 ng and be equal to a total yield of 1 pmol.

1. Calculate the mass for each sample.
   - The expected amount of sequencingdata for each sample is the same. The ratio of each sample sequencing data is 25%. Referring to Formula 2, the required mass of each PCR sample is 168 ng × 25% = 42 ng.
   - The expected amount of sequencing data for each sample is different. The ratios of sequencing data for samples 1-4 are 20%, 20%, 30%, and 30%. Referring to Formula 2, the required mass of sample 1 is 33.6 ng. Calculate the mass of samples 2 to 4 in the same way.
2. The concentration of sample 1 is 10 ng/μL. Refer to Formula 3 and the required volume is "A μL". Calculate the volume of samples 2 to 4 in the same way.
3. Transfer A μL of sample 1 into a new 0.2 mL PCR tube.
4. Add other samples into the same PCR tube.
5. Add TE Buffer to make a total volume of 48 μL.

Table 38 Multiple samples pooling (each sample volume should be at least 1 μL)

Name	Volume
Sample 1	A μL
Sample 2	B μL

Name	Volume
Sample 3	C μL
Sample 4	D μL
TE Buffer	48 - (A+B+C+D) μL
Total	48 μL

Tips The volumes of A, B, C, and D should each be ≥ 1 μL.

Follow one of the two methods below to pool the samples when the required volume of a sample is less than 1 μL. Method 1 is recommended.

Method 1: Increase the volume of all samples by Z (Z > 1) times. After mixing the samples, take 1/Z of the total volume W μL. Add TE Buffer to make a total volume of 48 μL.

Table 39 Samples mixture: All samples volume increases by Z times

Name	Volume
Sample 1	A × Z μL
Sample 2	B × Z μL
Sample 3	C × Z μL
Sample 4	D × Z μL
Total	W μL

Table 40 Method 1: Multiple samples pooling

Name	Volume
Samples mixture	(W ÷ Z) μL
TE Buffer	48 - (W ÷ Z) μL
Total	48 μL

Tips If necessary, quantify the samples mixture and calculate a new volume X μL (1 pmol). Replace "(W ÷ Z) μL" with "X μL".

Method 2: Dilutes a high concentration sample by Y (Y > 1) times if the required volume is less than 1 μL. Quantify the diluted sample and calculate a new volume. Pool the diluted sample with other samples.

For example: The required volume of sample 3 is < 1 μL. It needs to be diluted by Y times.

Table 41 Diluted sample: Dilute the high concentration sample by Y times

Name	Volume
Sample 3	5 μL*
TE Buffer	5Y - 5 μL
Total	5Y μL

Tips *: The volume of high concentration sample is recommended to be more than 5 μL.

Quantify the diluted sample. Refer to Formula 3 to calculate a new volume "E μL". Pool the diluted sample with other samples. Add TE Buffer to make a total volume of 48 μL.

Table 42 Method 2: Multiple samples pooling

Name	Volume
Sample 1	A μL
Sample 2	B μL
Sample 4	D μL
Diluted sample 3	E μL
TE Buffer	48 - (A+B+D) - E μL
Total	48 μL