MGI

MGICare

Detection Set for Single Cell Chromosome Copy Number Variation Test User Manual

Cat. No.:1000014630(48 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	Sep. 2019	• Add DNBSEQ series sequencing platform and its corresponding sequencing reagents
A0	V1.0	Aug. 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

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Chapter 1 Product Description

1.1 Introduction

MGICare Detection Set for Single Cell Chromosome Copy Number Variation Test is designed to construct libraries for detecting single cell chromosome copy number variations These libraries are compatible with MGI high-throughput sequencing platforms This set is applicable for human single cell 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 DNA generation for detection of single cell chromosome copy number variations. All reagents provided within this set have passed stringent quality control and functional verification procedures, ensuring performance stability and reproducibility.

1.2 Application

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

1.3 Platform Compatibility

Constructed libraries are compatible with

BGISEQ-500RS(SE50),

MGISEQ-2000RS(SE50), DNBSEQ-G400RS(SE50);

MGISEQ-200RS(SE50), DNBSEQ-G500RS(SE50)

1.4 Contents

MGICare Detection Set for Single Cell Chromosome Copy Number Variation Test consists of 2 kits (4 boxes). Detailed information on components and specifications are listed in Table 1.

Table 1 MGICare Detection Set for Single Cell Chromosome Copy Number Variation Test (48 RXN)

(Cat. No: 1000014630)

Kit Information	Components	Cap Color	Spec & Quantity
MGICare Detection Kit for Single Cell Chromosome Copy Number Variation Test (Box1) Cat No. 1000005291	Cell Lyase	Yellow	11 μL/tube×1 tube
	Cell Lysis Buffer	Purple	280 μL/tube×1 tube
	Pre-amplification Enzyme	White	11 μL/tube×1 tube
	Pre-amplification Buffer	Red	280 μL/tube×1 tube
	Post-amplification Enzyme	Blue	40 μL/tube×1 tube
	Post-amplification Buffer	Orange	1400 μL/tube×1 tube
	Nuclease-Free Water	Clear	1700 μL/tube×1 tube
	Positive Control	Black	30 μL/tube×1 tube
	Negative Control	Black	30 μL/tube×1 tube
MGICare Detection Kit for Single Cell Chromosome Copy Number Variation Test (Box2) Cat No. 1000005291	Fragmentation Enzyme	Pink	70 μL/tube×3 tubes
	Fragmentation Buffer	Pink	70 μL/tube×3 tubes
	ERAT Buffer Mix	Orange	200 μL/ tube × 3 tubes
	ERAT Enzyme Mix	Orange	15 μL/ tube × 3 tubes
	Ligase Buffer Mix	Red	450 μL/ tube × 3 tubes
	DNA Ligase	Red	30 μL/ tube × 3 tubes
	Barcode Adapter Mix (01-48)	Clear	15 μL/ well × 48 wells
	PCR Enzyme Mix	Blue	475 μL/ tube ×3 tubes
	PCR Primer Mix	Blue	80 μL/ tube × 3 tubes
MGICare Detection Kit for Single Cell Chromosome Copy Number Variation Test (Box3) Cat No. 1000005291	TE Buffer	White	5500 μL/ tube × 2 tubes
	DNA Clean Beads	White	1600 μL/ tube × 6 tubes
MGIEasy Rapid Circularization Module Cat No. 1000005258	Splint Buffer	Purple	186 μL/ tube × 1 tube
	DNA Rapid Ligase	Purple	8 μL/ tube × 1 tube

1.5 Storage Conditions and Shelf Life

MGICare Detection Kit for Single Cell Chromosome Copy Number Variation Test **Box1**

- Storage Temperature: -25°C to -15°C.
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: Dry Ice.

MGICare Detection Kit for Single Cell Chromosome Copy Number Variation Test Box2

- Storage Temperature: -25°C to -15°C.
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: Dry Ice.

MGICare Detection Kit for Single Cell Chromosome Copy Number Variation Test Box3

- Storage Temperature: 2°C to 8°C.
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: Ice Packs.

MGIEasy Rapid Circularization Module

- Storage Temperature: -25°C to -15°C.
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: Dry Ice.

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

* Performance of products is 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	Vortex Mixer
	Centrifuge
	Pipets
	Thermo cycler
	Magnetic rack DynaMag™-2 (Thermo Fisher Scientific™, Cat. No. 12321D) or equivalent
	Qubit™ 3 Fluorometer (Thermo Fisher Scientific, Cat. No. Q33216)
	Agilent 2100 Bioanalyzer (Agilent Technologies, Cat. No. G2939AA) / LabChip® GX, GXII, GX Touch (PerkinElmer), or Fragment Analyzer (Advanced Analytical)
	Sterile containment cabinet (vertical laminar flow cabinet)
Reagents	Nuclease free water (NF water) (Ambion, Cat. No. AM9937)
	Absolute ethanol (Analytical Grade)
	PBS pH7.4 (Thermo Fisher Scientific, Cat. No. 10010031)
	Qubit® dsDNA HS Assay Kit (Invitrogen, Cat. No. Q32854)/ Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Cat. No. P7589)
	High Sensitivity DNA Kits (Agilent Technologies™, Cat. No. 5067-4626)
	Agilent DNA 1000 Kit (Agilent, Cat. No. 5067-1504)
	MGISEQ or DNBSEQ or BGISEQ High-throughput Sequencing Set （SE50）
Consumables	Pipette Tips
	1.5 mL centrifuge tubes (Axygen, Cat. No. MCT-150-C)
	0.2 mL PCR tubes (Axygen, Cat. No. PCR-02-C) or 96-well plate (Axygen, Cat. No. PCR-96M2-HS-C)
	Qubit® Assay Tubes (Invitrogen, Cat. No. Q32856) or 0.5 mL Thin Wall PCR Tubes (Axygen, Cat. No. PCR-05-C)

1.7 Precautions and Warnings

- Instructions provided in this manual are intended for research use only, and it may require optimization for specific applications. We recommend customers to make adjustment according to experimental design, sample types, sequencing application, and other equipment.
- Cell sample processing and reagent preparation should be carried out in a sterile containment cabinet (vertical laminar flow cabinet).
- 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 components, first thaw at room temperature and invert several times to mix properly, then centrifuge briefly and place on ice for further use.
- To prevent cross-contamination, we recommend using filtered pipette tips. Use a new tip each time for pipetting different solutions.
- We recommend using thermo cyclers with heated lids for reactions. Preheat the lid to reaction temperature before use.
- Improper handling of samples and reagents may contribute to aerosol contamination of PCR products and may decrease the accuracy of results. Therefore, we recommend physically separating two working areas in the laboratory for PCR reaction preparation and PCR product purification, respectively. Use designated equipment for each area and perform regular cleaning to ensure a sterile working environment. (Use 0.5% Sodium Hypochlorite or 10% Bleach to clean working environment)
- If you have other questions, please contact MGI technical support: MGI-service@mgi-tech.com

Chapter 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 °C for less than one month. Keep the sample tube upright. Do not invert the sample tube. The frozen samples should be melted at 2°C to 8°C 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

Chapter 3 Single Cell Whole Genome Amplification Protocol

This chapter describes the process of single cell whole genome amplification including cell lysis, pre-amplification and post-amplification. Whole genome amplification products are generated through these steps.

3.1 Environment and Reagent Preparation

3.1.1 Equipment Preparation

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 30min.

3.1.2 Reagent Preparation

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, first thaw at 2℃-8℃ and invert several times to mix properly, then centrifuge briefly and place on ice for further use.

⚠ **Note 1: The experimenter should wear a mask and powderless latex gloves during the experiment. If their gloves are in contact with areas outside the cabinet, they should rub the gloves with 75% ethanol and then continue with the experiment;**

⚠ **Note 2. 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 of Positive/Negative Control

Dilute the Positive/Negative Control (1 ng/μL) in this kit to 15 pg/μL separately with Nuclease-Free Water. (gradient dilution: first dilute to 100 pg/μL, and then dilute to 15 pg/μL). Pipette 4 μL diluted Positive/Negative Control (15 pg/μL) to a PCR tube separately.

⚠ **Note: 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.**

3.2.2 Preparation of cell samples.

Thaw the frozen samples on ice before cell lysis process. And then centrifuge the sample tubes briefly to collect the liquid to the bottom. Finally line up the sample tubes in a 96-well rack on ice for later use.

3.3 Cell Lysis

3.3.1 Prepare the cell lysis mix in a 1.5 mL tube on ice following the specifications in Table 3. Mix well by vortex and then briefly centrifuge the tube to collect all liquid to the bottom of the tube.

Table 3 Cell Lysis Mix

Components	Volume (per sample)
Cell Lysis Buffer	4.8 μL
Cell Lyase	0.2 μL
Total	5 μL

3.3.2 For every cell sample and control sample, add 5 μL of freshly prepared Cell Lysis Mix in 3.3.1. Briefly centrifuge sample tubes to get all liquid to the bottom of the tube. Incubate all the samples in the PCR thermal cycler following Table 4.

⚠ **Note: When adding the Cell Lysis Mix, hold the tips of pipette above the liquid surface.**

Table 4 Cell Lysis Procedure

Temperature	Time	Cycles
Heat Lid (105°C)	On	
75°C	10 min	1 cycle
95°C	4 min	1 cycle
4°C	Hold	

3.3.3 When the Cell Lysis process ends, centrifuge the PCR tubes briefly and line up the tubes in a 96-well rack with ice.

3.4 Pre-amplification

3.4.1 Prepare the Pre-amplification Mix in a 1.5 mL tube as in Table 5 Mix well by vortex and then briefly centrifuge the tube to collect all liquid to the bottom of the tube

Table 5 Pre-amplification Mix

Components	Volume (per sample)
Pre-amplification Buffer	4.8 μL
Pre-amplification Enzyme	0.2 μL
Total	5 μL

3.4.2 Add 5 μL of Pre-amplification Mix to the cell lysis product in 3.3.3. Briefly centrifuge sample tubes to collect all liquid to the bottom of the tube. Incubate all the samples in PCR thermal cycler following Table 6.

⚠ **Note: When adding the Pre-amplification Mix, hold the tips of pipette above the liquid surface**

Table 6 Pre-amplification Procedures

Temperature	Time	Cycles
Heat Lid (105°C)	on	
95°C	2 min	1 cycle
95°C	15 s	12 cycles
15°C	50 s	
25°C	40 s	
35°C	30 s	
65°C	40 s	
75°C	40 s	
4°C	Hold	

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

3.5 Post-amplification

3.5.1 Prepare the Post-amplification Mix in a 1.5 mL tube as in Table 7. Mix well by vortex and then briefly centrifuge the tube to collect all liquid to the bottom of the tube.

Table 7 Post-amplification Mix

Components	Volume (per sample)
Post-amplification Buffer	25 μL
Post-amplification Enzyme	0.8 μL
Nuclease-Free Water	34.2 μL
Total	60 μL

3.5.2 Add 60 μL of the freshly prepared Post-amplification Mix to the pre-amplification reaction product in 3.4.3, cap the tube, and invert to mix. Briefly centrifuge sample tubes to get all liquid to the bottom of the tube. Incubate all the samples in PCR thermal cycler following Table 8.

Table 8 Post-amplification Procedures

Temperature	Time	Cycles
95°C	2 min	1 cycle
95°C	15 s	
65°C	1 min	14 cycles
75°C	1 min	
4°C	Hold	

3.5.3 After the Post-amplification reaction ends, centrifuge the PCR tubes briefly and line up the tubes in a 96-well rack for next step reaction.

✔ **Stop Point: Whole genome amplification DNA products can be stored at -25°C to -15°C for a long time.**

Chapter 4 Library Construction Protocol

Library Construction Protocol contains the following steps: DNA fragmentation, purification of fragmentation products, end repair and A-tailing, adapter ligation, purification of adapter-ligated DNA, PCR, purification of PCR product, quantification and pooling, and single strand DNA circularization. Finally, we get the library to be sequenced.

4.1 DNA Fragmentation

4.1.1 Label new PCR tubes according to sample ID. Add 24 μL of whole genome amplification products into the corresponding PCR tubes.

4.1.2 Prepare the Fragmentation Mix in Table 9. Mix well by vortex and then briefly centrifuge the tubes to collect all liquid to the bottom of the tubes.

Table 9 Fragmentation Mix

Components	Volume (per sample)
Fragmentation Enzyme	3 μL
Fragmentation Buffer	3 μL
Total	6 μL

4.1.3 Add 6μL Fragmentation Mix to the 24 μL whole genome amplification product in 4.1.1. Mix well and briefly centrifuge to get all liquid to the bottom of the tubes. Incubate all the samples in PCR thermal cycler as in Table 10.

Table 10 DNA Fragmentation Procedures

Temperature	Time	Cycles
Lid (105°C)	On	
37°C	5 min	1 cycle
75°C	15 min	1 cycle
4°C	Hold	

4.1.4 After the Fragmentation reaction ends, centrifuge the PCR tubes briefly and line up the tubes in a 96-well rack for next step reaction

⚠ **Note: To prevent over fragmentation, proceed immediately to the next step.**

4.2 Purification of Fragmentation Products

Purification should be done in 30 min. Take out the DNA Clean Beads from refrigerator in advance Vortex

and mix thoroughly. Put the DNA Clean Beads at room temperature for at least 30 min before use

⚠ **Note: Please read Appendix A carefully before you begin.**

4.2.1 Add 20 μL TE Buffer to each fragmentation product to make the total volume reach 50 μL. Mix well and centrifuge briefly.

4.2.2 Add 75 μL DNA Clean Beads to each fragmentation product in 4.2.1. Pipette up and down at least 10 times to mix thoroughly. Ensure that all liquid and beads are expelled from the pipette tip into the tube

4.2.3 Incubate at room temperature for 5 min. Centrifuge briefly.

4.2.4 Place on a Magnetic Separation Rack and wait until the liquid is clear (~3 min).

4.2.5 Carefully remove and discard the supernatant with a pipette.

4.2.6 Prepare 80% EtOH (the volume ratio of absolute ethanol and Nuclease free water —3:1).

4.2.7 Keep the tubes on the Magnetic Separation Rack. Add 200 μL freshly prepared 80% EtOH to each sample

⚠ **Note. Do not resuspend the beads.**

4.2.8 Incubate on the Magnetic Separation Rack for 1 min. Remove and discard all supernatant from each sample tube.

4.2.9 Repeat 4.2.7-4.2.8 once.

4.2.10 Remove residual EtOH from each tube. Air-dry on the Magnetic Separation Rack for 15 min or until beads are completely dry.

⚠ **Note: Do not over dry the beads. Beads that are over dried appear cracked.**

4.2.11 Remove the sample tubes from the Magnetic Separation Rack.

4.2.12 Add 43 μL TE Buffer to each tube. Mix well and incubate at room temperature for 5 min.

4.2.13 Centrifuge briefly. Place the tubes on a Magnetic Separation Rack and wait until the liquid is clear (~3 min).

4.2.14 Transfer 40 μL supernatant from each sample to a new PCR tube.

✔ **Stop Point: Purified fragmentation products can be stored at -25°C to -15°C for a maximum of 7 days.**

4.3 End Repair and A-tailing

4.3.1 Prepare the End Repair and A-tailing Mix in Table 11. Mix well by vortex and then briefly centrifuge the tube to collect all liquid to the bottom of the tube.

Table 11 End Repair and A-tailing Mix

Components	Volume (per sample)
ERAT Buffer Mix	9.4 μL
ERAT Enzyme Mix	0.6 μL
Total	10 μL

4.3.2 Add 10 μL End Repair and A-tailing Mix to the purified fragmentation products in 4.2.14. Mix well and briefly centrifuge to get all liquid to the bottom of the tube. Incubate all the samples in PCR thermal cycler as in Table 12

Table 12 End Repair and A-tailing Procedure

Temperature	Time	Cycles
Lid (42°C)	On	
37°C	10 min	1 cycle
65°C	15 min	1 cycle
4°C	Hold	

4.3.3 After the reaction, briefly centrifuge to collect all liquid to the bottom of the tube.

⚠ **Warning: DO NOT STOP AT THIS STEP. Please continue to step 4.4.**

4.4 Adapter Ligation

⚠ **Note: Please read Appendix B carefully before you begin.**

4.4.1 Add 5 μL of Barcode Adapter Mix to the sample tubes in step 4.3.3. Vortex 3 times (3 s each time) and centrifuge to collect all liquid to the bottom of the tube.

4.4.2 Prepare the Ligation Mix as in Table 13. Mix well by vortex and then briefly centrifuge the tube to collect all liquid to the bottom of the tube.

Table 13 Ligation Mix

Components	Volume (per sample)
Ligase Buffer Mix	24 μL
DNA Ligase	1 μL
Total	25 μL

4.4.3 Add 25 μL Ligation Mix to each sample tube in step 4.4.1. Mix well and centrifuge briefly.

4.4.4 Incubate all the samples in PCR thermal cycler as in Table 14

Table 14 Ligation Procedure

Temperature	Time	Cycles
Heat Lid(42°C)	Off	
23℃	20 min	1
4℃	Hold	

4.4.5 After the reaction, briefly centrifuge to collect all liquid to the bottom of the tube.

✔ **Stop Point: Adapter-ligated DNA can be stored at -25°C to -15°C for a maximum of 16 hours.**

4.5 Purification of Adapter-Ligated DNA

⚠ **Note: Please read Appendix A carefully before you begin.**

4.5.1 Take out the DNA Clean Beads from refrigerator in advance Vortex and mix thoroughly. Put the DNA Clean Beads at room temperature for at least 30 min before use.

4.5.2 Add 40 μL DNA Clean Beads to each adapter-ligated DNA product in 4.4.5. Pipette up and down at least 10 times to mix thoroughly. Ensure that all liquid and beads are expelled from the pipette tip into the tube.

4.5.3 Incubate at room temperature for 5 min. Centrifuge briefly.

4.5.4 Place on a Magnetic Separation Rack and wait until the liquid is clear (~3 min).

4.5.5 Carefully remove and discard the supernatant with a pipette.

4.5.6 Keep the tubes on the Magnetic Separation Rack. Add 200 μL freshly prepared 80% EtOH to each sample

⚠ **Note. Do not resuspend the beads.**

4.5.7 Incubate on the Magnetic Separation Rack for 1 min. Remove and discard all supernatant from

each sample tube.

4.5.8 Repeat 4.5.6-4.5.7 once.

4.5.9 Remove residual EtOH from each tube. Air-dry on the Magnetic Separation Rack for 15 min or until beads are completely dry.

Note: Do not over dry the beads. Beads that are over dried appear cracked.

4.5.10 Remove the sample tubes from the Magnetic Separation Rack.

4.5.11 Add 23 µL TE Buffer to each tube. Mix well and incubate at room temperature for 5 min.

4.5.12 Centrifuge briefly. Place the tubes on a Magnetic Separation Rack and wait until the liquid is clear (~3 min).

4.5.13 Transfer 21 µL supernatant from each sample tube to a new PCR tube.

Stopping Point: Purified Adapter-ligated DNA can be stored at -25 to -15°C for a maximum of 7 days.

4.6 PCR

4.6.1 Prepare the PCR Mix in Table 15. Mix well by vortex and then briefly centrifuge the tube to collect all liquid to the bottom of the tube.

Table 15 PCR Mix

Components	Volume (per sample)
PCR Enzyme Mix	25 µL
PCR Primer Mix	4 µL
Total	29 µL

4.6.2 Add 29 µL PCR Mix to each PCR tube in step 4.5.13. Vortex 3 times (3 s each time) and centrifuge briefly to collect all the liquid to the bottom of the tube.

4.6.3 Incubate all the samples in PCR thermal cycler as in Table 16.

Table 16 PCR Reaction Procedures

Temperature	Time	Cycles
98°C	2 min	1 cycle
98°C	15 s	
56°C	15 s	12 cycles
72°C	30 s	
72°C	5 min	1 cycle
4°C	Hold	

4.6.4 After the reaction, briefly centrifuge to collect all liquid to the bottom of the tube.

✔ **Stopping Point: PCR products can be stored at -25°C to -15°C for a maximum of 16 hours.**

4.7 Purification of PCR Product

4.7.1 Take out the DNA Clean Beads from refrigerator in advance Vortex and mix thoroughly. Put the DNA Clean Beads at room temperature for at least 30 min before use.

4.7.2 Add 50 μL DNA Clean Beads to each PCR products from 4.6.4. Pipette up and down at least 10 times to mix thoroughly. Ensure that all liquid and beads are expelled from the pipette tip into the tube.

4.7.3 Incubate at room temperature for 5 min. Centrifuge briefly.

4.7.4 Place on a Magnetic Separation Rack and wait until the liquid is clear (~3 min).

4.7.5 Carefully remove and discard the supernatant with a pipette.

4.7.6 Keep the tubes on the Magnetic Separation Rack. Add 200 μL freshly prepared 80% EtOH to each sample

⚠ **Note. Do not resuspend the beads.**

4.7.7 Incubate on the Magnetic Separation Rack for 1 min. Remove and discard all supernatant from each sample tube.

4.7.8 Repeat 4.7.6-4.7.7 once.

4.7.9 Remove residual EtOH from each tube. Air-dry on the Magnetic Separation Rack until beads are completely dry.

⚠ **Note: Do not over dry the beads. Beads that are over dried appear cracked.**

4.7.10 Remove the sample tubes from the Magnetic Separation Rack.

4.7.11 Add 32 μL TE Buffer to each tube. Mix well and incubate at room temperature for 5 min.

4.7.12 Centrifuge briefly. Place the tubes on a Magnetic Separation Rack for 15 min or wait until the liquid is clear (~3 min).

4.7.13 Transfer 30 μL supernatant from each sample to a new PCR tube.

✔ **Stopping Point: Purified PCR products can be stored at -25°C to -15°C for a maximum of 7 days.**

4.8 Quantification and Pooling of PCR Products

4.8.1 Quantify the purified PCR products with dsDNA Fluorescence Assay Kit such as Qubit® dsDNA HS Assay Kit.

⚠ **Note: Review the manufacturer instruction manuals and safety recommendations before quantifying the samples.**

4.8.2 Pipette 1 μL of the sample for quantification with the Qubit® dsDNA HS Assay Kit. The typical concentration of a successful PCR product should be ≥ 2 ng/μL. If the concentration is < 2 ng/μL, it is recommended to re-prepare the library.

4.8.3 According to the library size, we recommend pooling 168 ng (1 pmol) of all libraries into one PCR tube and each library should be of the same weight. The required weight of each library (ng) =168 ng/N (N=library numbers). Depending on the required weight and concentration of each library, calculate the required volume of each library. The volume of each library= Required weight of each library (ng) /library concentration (ng/μL). Transfer required volume of each library to a PCR tube and add TE Buffer to the pooled libraries to make the total volume to 48uL. Mix well and centrifuge briefly.

4.8.4 We recommend that the volume of each library ≥ 1 μL. After calculating, if the volume of the library is less than 1 μL, we recommend multiplying the pooling amount to 168.X ng(X >1). Then the required weight of each library (ng) =168 X ng/N (N=library numbers). The volume of each library= Required weight of each library(ng) /library concentration (ng/μL). Transfer required volume of each library to a PCR tube and add TE Buffer to the pooled libraries to make the total volume to 48X uL. Mix well and centrifuge briefly. Transfer 48 uL of the mixed libraries to a new PCR tube for further use.

✔ **Stopping Point: After pooling, pooled libraries can be stored at -25°C to -15°C for a maximum of 7 days.**

4.9 Single Strand DNA (ss DNA) Circularization

Circularized ssDNA can be prepared with the "MGI Rapid Circularization Module" (Cat.no.:1000005258) and is compatible with MGI high-throughput sequencing platforms

4.9.1 To prepare circularized ssDNA, incubate the pooled PCR libraries from step 4.8.4 at 95°C for 3 min on a PCR thermocycler and then cool the PCR tubes on the ice for 2 min

4.9.2 Prepare the Circularization Reaction Mix in a new microcentrifuge (see Table 17).

Table 17 Circularization Reaction Mix

Components	Volume
Splint Buffer	11.6μL
DNA Rapid Ligase	0.5 μL
Total	12.1 μL

4.9.3 Add 12.1 μL of the Circularization Reaction Mix into each of the PCR tubes from step 4.9.1. Mix by vortex, centrifuge briefly, then incubate the sample at 37°C for 30 min on the PCR thermocycler.

4.9.4 Centrifuge briefly. The reaction products can be used immediately for DNB making or kept at -20°C.

✔ **Stopping Point: the circularized ssDNA can be stored at -20°C**

4.9.5 About 20 μL of circularized ssDNA product are used to prepare DNA nanoballs (DNBs) and sequencing . Refer to the 'MGISEQ /DNBSEQ /BGISEQ High-throughput Sequencing Set User Manual' for a detailed protocol.

Chapter 5 Sequencing

Select proper sequencer type to sequence according specific requirement. Please follow the protocol described in "BGISEQ/MGISEQ 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) Instruction Manual, PN: 1000002072.

MGISEQ-2000RS\ DNBSEQ-G400RS sequencing platform

- MGISEQ-2000RS High-throughput Sequencing Set (SE50) Instruction Manual, PN: 1000012551
- DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE50) Instruction Manual, PN: 1000016941

MGISEQ-200RS\ DNBSEQ-G50RS sequencing platform

- MGISEQ-200RS High-throughput Sequencing Set (SE50) Instruction Manual, PN: 1000004635.
- MGISEQ-G50RS High-throughput Sequencing Set (FCL SE50) Instruction Manual, PN: 1000016959.

Appendix

Appendix A DNA Clean Beads and Purification Procedures

Before You Use

To ensure capture efficiency of the Magnetic Beads, take out the beads from 4°C refrigerator, and equilibrate to room temperature for 30 min beforehand. Vortex and mix thoroughly before use.

- Vortex or pipette up and down to ensure that the beads are thoroughly mixed before use.
- The volume of the beads determines the lower limit of fragment size that can be purified.

Operation Notes

- If the sample volume decreases due to evaporation during incubation, add TE buffer to the designated volume before using the beads to purify. This ensures that the correct ratio for the beads is used.
- In the magnetic separation step, please allow the solution to become completely clear before removing the supernatant. This process takes approximately 2-3 min. Consider the different magnetic strength of your specific Separation Plate / Rack, allow enough time for the solution to turn completely clear.
- Avoid contacting the beads with pipette tips when pipetting. 2-3 μL of fluids can be left in the tube to avoid contact. In case of contact between the beads and the pipette tip, expel all the solution and beads to the tube and restart the separation process.
- Use freshly prepared 80% Ethanol (at room temperature) to wash the beads. Keep the centrifuge tube on the Magnetic Separation Rack when washing. Do not shake or disturb the beads in any way.
- After the 2nd wash of beads with Ethanol, try to remove all liquid within the tube. You may centrifuge briefly to collect the remaining liquid to the bottom, separate beads magnetically, and remove the remaining liquid by using a small volume pipette.
- After washing twice with ethanol, air-dry the beads at room temperature. Drying takes approximately 2-5 min depending on your specific lab environment. Observe closely until the pellet appears sufficiently dry with a matt appearance, then continue to the elution step with TE Buffer.

- During the elution step, do not contact the beads with tips when removing the supernatant. Contamination in DNA by the beads may affect subsequent purification. Therefore, the total volume of TE buffer and the beads should be 2μL more than the volume of the supernatant.
- Pay attention when opening/ closing the lids of centrifuge tubes on the Separation Rack. Strong vibrations may cause sample loss because of the spill of liquid or bead. Fix the tubes before opening the lids.

Appendix B Barcode Adapter Mix Instructions

We currently offer 48 DNA Barcode Adapters to meet requirements for batch processing library construction and Multiplex Sequencing. We selected the best adapter combination based on the principle of balancing base composition. For optimum performance, please refer to instructions below. Please note that the identical Barcode Adapters cannot be sequenced in the same lane.

Our adapters are double stranded in nature. Please do not store above room temperature, because structural changes such as denaturation may affect performance.

Before use, centrifuge to collect liquid to the bottom. Gently remove the cap / adhesive film to prevent spillage and cross contamination; Mix the adapters by pipetting up and down before use.

Barcode Adapter Combination (1-48) Instructions

- This set provides 48 Barcode Adapter Mix for high throughput and multiplex library pooling. Please read the following instructions before use to ensure best performance. The Barcode Adapter Mix with the same ID cannot be pooled in one lane, because they have the same barcode sequence.
- Centrifuge briefly to collect the solution to the bottom of the tube, and then open the cap gently to prevent spattering and avoid cross contamination.
- For base balance, please use the Barcode Adapter by set according to the following rules:
- 8 Barcode Adapters a set: 01-08, 09-16, 17-24, 25-32, 33-40, 41-48

  See table 18 below to organize your barcode combinations.

Table 18 Barcode Adapter Combination (1-48) Instructions

8	Requires at least 1 set of Adapter: Take a set of 8 Adapters (eg. 17-24), add 1 Barcode Adapter for each sample of equal volumes.
8n+X (1≤n≤5，X=1-5，total 9-48)	Sample number- 8n+X. (1) 8 samples as a group，use the method for (8 samples/lane) above. (2) For samples not in a group, add a single Barcode Adapter for each sample according to the X.

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