MGIEasy

RAD Library Prep Kit User Manual

Cat. No.: 1000005242 (64 RXN)

Kit Version: V1.0

Manual Version: 5.0

Revision History

Manual Version	Kit Version	Date	Description
5.0	V1.0	Mar. 2024	◆ Update Manufacturer information
4.0	V1.0	Mar. 2022	◆ Update Manufacturer LOGO.
A2	V1.0	Jan. 2021	◆ Update contact information.
A1	V1.0	Oct. 2019	◆ The equipment of associated with convaris was removed ◆ Changed the description of 3.5.3 ◆ Add a table 11, and the number of other tables increases in turn. ◆ Add DNBSEQ series sequencing platform
A0	V1.0	May. 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

Chinese Name: MGIEasy 简化基因组文库制备试剂盒

English Name: MGIEasy RAD Library Prep Kit

1.2 Application

MGIEasy RAD Library Prep Kit is specifically designed for library preparation for MGI high-throughput sequencing platforms. This kit is optimized to convert animal and plant genomic DNA (gDNA) into a single strand circularized DNA library. All reagents provided within this set have passed stringent quality control and functional verification procedures to ensure performance, stability, and reproducibility.

1.3 Platform Compatibility

Constructed libraries are compatible with:

BGISEQ-500RS (PE100/PE150)

MGISEQ-2000RS (PE100/PE150), DNBSEQ-G400RS (PE100/PE150)

1.4 Contents

Information on the components of MGIEasy RAD Library Prep Kit is listed in Table 1:

Table 1 MGIEasy RAD Library Prep Kit (64 RXN)

Name	Components	Caps Color	Spec & Quantity
MGIEasy RAD Library Prep Kit Cat. No.: 1000005242	Fragmentation Enzyme I	Colorless	77 μL / tube × 1 tube
	Fragmentation Enzyme II	Colorless	116 μL/ tube × 1 tube
	Fragmentation Buffer	Colorless	384 μL/ tube × 2 tubes
	Ligase Enzyme	Red	125 μL/ tube ×1 tube
	PCR Enzyme Mix	Blue	240 μL/ tube × 2 tubes
	PCR Primer Mix	Blue	20 μL/ tube × 1 tube
	RAD Adapter Mix (64 barcode)	Colorless	10 μL/ well × 64 wells
	Splint Buffer	Purple	112 μL/ tube × 1 tube
	Digestion Buffer	White	14 μL/ tube × 1 tube
	Digestion Enzyme	White	25 μL/ tube × 1 tube
	Digestion Stop Buffer	White	72 μL/ tube × 1 tube

⚠ **Note: Do not mix components of different batch numbers.**

1.5 Storage Conditions and Shelf Life

- Storage Temperature: -25℃ to -18℃
- Shelf Life: 9 months
- Production Date: refer to label
- Expiration Date: refer to label

1.6 Equipment and Materials Required but not Provided

Table 2 Equipment and Materials Required but not Provided

Equipment	Vortex Mixer Desktop Centrifuge Pipets Thermocycler Magnetic rack DynaMagTM-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)
Reagents	Nuclease free water (NF water) (Ambion, Cat. No. AM9937) 100% Ethanol (Analytical Grade) Qubit® ssDNA Assay Kit (Invitrogen, Cat. No. Q10212) AMPure XP Magnetic Beads (Agencourt, Cat. No. A63882) 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)
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 general use only, and may require optimization for specific applications. We recommend adjusting according to the experimental design, sample types, sequencing application, and other equipment.
- Remove the reagents from storage beforehand, and prepare them for use: For enzymes, centrifuge briefly and place on ice until further use. For other reagents, first thaw at room temperature and invert several times to mix properly, then centrifuge briefly and place on ice until 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 thermocyclers with heated lids for reactions. Preheat 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 cleanup, respectively. Use designated equipment for each area and cleaning regularly 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 Preparation

2.1 Sample Requirement

- Minimum DNA input quantity: 1 μg of gDNA
- DNA concentration: ≥50 ng/μL
- DNA integrity: OD_{260}/OD_{280}=1.8 to 2.0
- DNA quality: intact or slightly degraded gDNA from animal or plant

Note:

1.We recommend using 1 μg of intact gDNA (Intact gDNA can be viewed on 1% gel as a band >23 kb with either minimal or absent smear). The library can be prepared from slightly degraded gDNA but with a risk of failure of library construction. (Note: High quality gDNA input yields higher library construction success rate and more reliable sequencing data.)

2.Recommend using gDNA sample with high integrity for library construction. Contaminants such as proteins and salts can reduce the performance of enzymes.

2.2 Reagent Preparation

- Remove the reagents from storage beforehand and prepare them for use. For enzymes, centrifuge briefly and place on ice for further use. For other reagents, thaw at room temperature, vortex to mix, then centrifuge briefly and place on ice for use. Warm NF water and TE buffer to room temperature for further use.
- Prepare reaction mixtures on ice.
- The buffers provided in this kit may precipitate when thawed. Precipitation does not affect the performance of the reagents. Vortex to mix thoroughly until the precipitate disappears.

Chapter 3 Library Construction Protocol

3.1 Enzymatic Fragmentation

3.1.1 Based on the quantitation result of the DNA sample, transfer 1 μg of gDNA to a 0.2 mL PCR tube and add TE buffer to bring the total volume to 20 μL.

3.1.2 Prepare the enzymatic fragmentation mixture using the PCR tube from step 3.1.1 (see Table 3):

Table 3 Enzymatic Fragmentation Mixture using animal or plant gDNA

Components	Reaction Mixture	
	Animal gDNA	Plant gDNA
DNA	20 μL	20 μL
Fragmentation Buffer	3 μL	3 μL
Fragmentation Enzyme I	1 μL	1 μL
Fragmentation Enzyme II	1 μL	-
NF water	5 μL	6 μL
Total	30 μL	30 μL

3.1.3 Completely mix the solution then centrifuge briefly.

3.1.4 Place the PCR tube form step 3.1.3 into the thermocycler and run the program in Table 4:

Table 4 The Reaction Conditions of Enzymatic Fragmentation of animal or plant gDNA

Temperature	Time	
	Animal gDNA	Plant gDNA
Heated lid	On	On
37°C	20 min	0 min
65°C	20 min	20 min
4°C	Hold	Hold

3.1.5 After enzymatic fragmentation, set aside 2 μL of enzymatic fragmentation product for quality control of library using agarose electrophoresis analysis. If the library construction fails, the result of quality assessment can help identify the problem. The rest of product can be used in library construction. Proceed to the library construction steps. See quality control of library for detailed instructions.

3.2 Adapter Ligation

3.2.1 Add 5 μL RAD Adapter (referring to Sample Pooling Strategies of 64 barcode in table 5) to the enzymatic fragmentation product and pipette several times to mix thoroughly.

Note: The barcodes are required to be used in groups of 8 consecutive numbers (01-08, 09-16, 17-24, 41-48, 57-64, 65-72, 81-88, 89-96). This ensures that after Adapter Ligation, barcodes are pooled in a normalized set. The Barcode combinations are shown in Table 5:

Table 5 RAD Library Prep Kit Sample Pooling Strategies

Barcode No.	Sample Pooling Solutions		Barcode No.	Sample Pooling Solutions		Barcode No.	Sample Pooling Solutions		Barcode No.	Sample Pooling Solutions	
001			017			057			081		
002			018			058			082		
003			019			059			083		
004	8		020	8		060	8		084	8	
005	samples		021	samples		061	samples		085	samples	
006			022			062			086		
007			023			063			087		
008		16	024		16	064		16	088		16
009		samples	041		samples	065		samples	089		samples
010			042			066			090		
011			043			067			091		
012	8		044	8		068	8		092	8	
013	samples		045	samples		069	samples		093	samples	
014			046			070			094		
015			047			071			095		
016			048			072			096		

3.2.2 Prepare the following Adapter Ligation mixture using the PCR tube from step 3.2.1(see Table 6):

Table 6 Adapter Ligation Mixture

Components	Volume	
	Animal gDNA	Plant gDNA
Fragmentation Buffer	2 μL	2 μL
Fragmentation Enzyme II	0.5 μL	-
Ligation Enzyme	1.6 μL	1.6 μL
NF water	10.9 μL	11.4 μL
Total	15 μL	15 μL

3.2.3 Place the PCR tube from step 3.2.2 into the thermocycler and run the program in Table 7:

Table 7 The Reaction Conditions of Adapter Ligation

Temperature	Time
Heated lid	on
23°C	60 min
65°C	15 min
4°C	Hold

3.3 Pooling of Adapter Ligation Product

- Remove the PCR tube once the reaction is complete. Follow Table 5 to mix 16 Adapter Ligation samples with equal volumes to complete one set. Prepare two sets of Adapter Ligation samples (two sets of 16 barcodes should be used with the correct combination). The volume of the solution should be 200 μL. Add TE buffer until the total volume is 320 μL, then purify the pooled Adapter Ligation samples.
- The following example uses a pool of 32 Adapter Ligation samples consisting of four groups of 8-barcode combinations. Use 8 barcodes with consecutive serial numbers as one group and add a barcode group to one set of 8 samples (e.g. add barcode 01-08, 09-16, 17-24, 41-48 to one of the four sets of samples respectively). Choose two Adapter Ligation samples from the four sets of samples (e.g. 01-08, 09-16), then take 12.5 μL from each of the Adapter Ligation samples in the two sets and mix together. The total volume should be 200 μL. Add 120 μL of TE buffer and mix. Pool the other two sets using the same method. All 16 samples should be pooled as two sets of Adapter Ligation product with a total volume of 320 μL per set. Purify these two sets of products in the following cleanup step.

3.4 Cleanup of Adapter Ligation Product

3.4.1 Take AMPure XP Beads out of refrigerator and allow 30 minutes for the solution to come to room temperature. Vortex and mix thoroughly before use

3.4.2 Transfer 224 µL AMPure XP Beads to the 320 µL Adapter Ligation product from step 3.3 Pipette up and down at least 10 times to mix thoroughly. Ensure that all liquid and beads are fully dispensed from the pipette tip into the centrifuge tube before proceeding.

3.4.3 Incubate at room temperature for 5 minutes.

3.4.4 Centrifuge briefly and place the tube onto a magnetic separation rack for 2-5 minutes until the liquid becomes clear. Then, carefully transfer the supernatant to a new 1.5 mL centrifuge tube.

⚠ **Note: Retain the Supernatant and discard the Beads.**

3.4.5 Transfer 32 µL AMPure XP Beads to the centrifuge tube with 544 µL of supernatant from step 3.4.4. Pipette at least 10 times to mix thoroughly.

3.4.6 Incubate at room temperature for 5 minutes.

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

3.4.8 Keep the centrifuge tube on the magnetic separation rack and add 500 µL of freshly prepared 80% ethanol to wash the beads and the walls of the tube. Carefully remove and discard the supernatant.

3.4.9 Repeat step 3.4.8 once and try to remove all of liquid from the tube.

3.4.10 Keep the centrifuge tube on the magnetic separation rack with the lid open to air-dry the beads until they no longer appear shiny but before the bead pellet starts to crack.

3.4.11 Remove the centrifuge tube from the magnetic separation rack and add 50 µL of TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.4.12 Incubate at room temperature for 5 minutes.

3.4.13 Centrifuge briefly and place the tube onto a magnetic separation rack for 2-5 minutes until the liquid becomes clear. Carefully transfer 47 µL of supernatant to a new 0.2 mL PCR tube.

✔ **Stopping Point: Post-bead selection product can be stored at -20°C.**

3.5 PCR Amplification

3.5.1 Prepare the following PCR Amplification mixture in a new 0.2 mL PCR tube on ice (see Table 8).

Table 8 PCR Amplification Mixture

Components	Volume
PCR Enzyme Mix	25 μL
PCR Primer Mix	2 μL
NF water	3 μL
Total	30 μL

3.5.2 Transfer 20 μL adapter ligation product from step 3.4.13 to a new PCR tube. The rest 27 μL of product can be stored at -20°C.

3.5.3 Transfer 30 μL PCR amplification mixture to the PCR tube from step 3.5.2. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.5.4 Place the PCR tube from step 3.5.3 into the thermocycler and run the program in Table 9:

Table 9 PCR Amplification Reaction Conditions

Temperature	Time	Cycles
Heated lid	on	
95°C	3 min	1 cycle
98°C	20 s	
60°C	15 s	9 cycles
72°C	30 s	
72°C	10 min	1 cycle
4°C	Hold	

3.5.5 Centrifuge briefly to collect the solution at the bottom of the tube.

3.5.6 Transfer all of the solution to a new 1.5 mL centrifuge tube.

3.6 Cleanup of PCR Product

3.6.1 Take AMPure XP Beads out of refrigerator and allow 30 minutes for the solution to come to room temperature. Vortex and mix thoroughly before use.

3.6.2 Transfer 30 μL AMPure XP Beads to the centrifuge tube from step 3.5.6. pipette up and down at least 10 times to mix thoroughly. Ensure that all of the liquid and beads are fully dispensed from

the pipette tip into the centrifuge tube before proceeding.

3.6.3 Incubate at room temperature for 5 minutes.

3.6.4 Centrifuge briefly and place the tube onto a magnetic separation rack for 2 minutes until the liquid becomes clear. Then, carefully transfer the supernatant to a new 1.5 mL centrifuge tube.

⚠ **Note: Retain the Supernatant and discard the Beads.**

3.6.5 Transfer 10 μL AMPure XP Beads to the centrifuge tube with 80 μL of supernatant from step 3.6.4. Pipette at least 10 times to mix thoroughly. Incubate at room temperature for 10 minutes.

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

3.6.7 Keep the centrifuge tube on the Magnetic Separation Rack and add 200 μL of freshly prepared 80% ethanol to wash the beads and the walls of the tube. Carefully remove and discard the supernatant.

3.6.8 Repeat step 3.6.7 once and try to remove all of liquid from the tube.

3.6.9 Keep the centrifuge tube on the magnetic separation rack with the lid open to air-dry the beads until they no longer appear shiny but before the bead pellet starts to crack.

⚠ **Note: Do not disturb the beads to ensure cleanup is successful.**

3.6.10 Remove the centrifuge tube from the magnetic separation rack and add 32 μL of TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.6.11 Incubate at room temperature for 5 minutes.

3.6.12 Centrifuge briefly and place the tube onto a magnetic separation rack for 2-5 minutes until the liquid becomes clear. Carefully transfer 30 μL of supernatant to a new 0.2 mL PCR tube.

✔ **Stopping Point: Post-bead selection product can be stored at -20°C.**

3.7 Normalization

3.7.1 Quantitate the purified products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit. Follow the kit user manual. The required yield for PCR products is ≥ 1 pmol. See Table 10 for the corresponding yield for different size fragments of PCR products.

Table 10 The Corresponding Yield in 1 pmol for PCR Products with Different Fragment Sizes

Insert Size (bp)	PCR Product size (bp)	Corresponding Yield in 1 pmol (ng)
250	314	207
280	364	240
300	419	276

3.7.2 Transfer 1 pmol of the sample into a PCR tube and add TE buffer until the final volume is 48.2 µL. If multiple PCR products need to be pooled for Circularization, mix each PCR product with equal amounts such that the total amount of DNA is 1 pmol, with a total volume ≤ 48.2 µL.

3.8 Single Strand Circularization

3.8.1 Incubate 48.2 µL Normalized PCR product in the thermocycler at 95°C for 3 minutes. Immediately place the PCR tube on ice and incubate for 2 minutes (see Table 11):

Table 11 Denaturation Reaction Conditions

Temperature	Time
Heated lid	On
95°C	3 min

3.8.2 Prepare the single strand circularization mixture in a new 0.2 mL PCR tube on ice (see Table 12):

Table 12 Single Strand Circularization Mixture

Components	Volume
Splint Buffer	11.6 µL
Ligase Enzyme	0.2 µL
Total	11.8 µL

3.8.3 Transfer 11.8 µL single strand circularization mixture to the 0.2 mL PCR tube from step 3.8.1. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.8.4 Place the PCR tube into the thermocycler and run the program in Table 13:

Table 13 The Reaction Conditions of Circularization

Temperature	Time
Heated lid	On
37°C	30 min
4°C	Hold

3.9 Enzymatic Digestion

3.9.1 Remove the digestion Buffer from storage to thaw and mix thoroughly. Remove the circularization product from the thermocycler in step 3.8.4 and prepare the enzymatic digestion mixture on ice (see Table 14). Mix thoroughly and centrifuge briefly.

Table 14 Enzymatic Digestion Mixture

Components	Volume
Digestion Buffer	1.4 μL
Digestion Enzyme	2.6 μL
Total	4.0 μL

3.9.2 Place the PCR tube from step 3.9.1 into the thermocycler and run the program in Table 15:

Table 15 The Reaction Conditions of Enzymatic Digestion

Temperature	Time
Heated lid	On
37°C	30 min

3.9.3 When the reaction is complete, add 7.5 μL digestion stop buffer to each product and mix to stop the enzymatic digestion reaction.

3.9.4 Transfer all of the solution to a new tube for cleanup.

3.10 Enzymatic Digestion Product Cleanup

3.10.1 Take out AMPure XP Beads out of refrigerator and incubate at room temperature for at least 30 minutes. Vortex and mix thoroughly before use.

3.10.2 Transfer 170 μL AMPure XP Beads to the centrifuge tube from step 3.9.4. pipette up and down at least 10 times to mix thoroughly. Ensure that all of the liquid and beads are fully dispensed from the pipette tip into the centrifuge tube.

3.10.3 Incubate at room temperature for 10 minutes.

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

3.10.5 Keep the non-stick tube on the magnetic separation rack and add 500 μL of freshly prepared 80% ethanol to the tube without disturbing the beads. Incubate for 30 seconds. Carefully remove and discard the supernatant.

3.10.6 Repeat step 3.10.5 once and remove all supernatant from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom, separate the beads magnetically, then remove any remaining liquid using a small volume pipette.

3.10.7 Keep the non-stick tube on the Magnetic Separation Rack with the lid open, and air-dry the beads at room temperature until no wetness (reflectiveness) is observed but before the pellet begins to crack.

3.10.8 Remove the non-stick tube from the magnetic separation rack and add 25 μL of TE Buffer to elute the DNA. Gently pipette up and down at least 10 times to mix thoroughly or until the beads are fully resuspended.

3.10.9 Incubate at room temperature for 5 minutes.

3.10.10 Centrifuge briefly and place the non-stick tube back onto the magnetic separation rack for 2-5 minutes until the liquid is clear. Transfer 23 μL supernatant to a new non-stick tube.

3.10.11 Take 2 μL of purified product for the quantitation with Qubit® ssDNA Assay kit. Store the rest of the product at -20℃ for the make DNB step.

3.11 Quality Control of Enzymatic Digestion Product

3.11.1 Quantify the purified Enzymatic Digestion products with Qubit® ssDNA Assay Kit. The final Enzymatic Digestion products(ssDNA, ng) / input products of PCR (dsDNA, ng) should be ≥ 7%. Please refer to Table 16 or Formula 1 for your calculations.

Table16 The Corresponding Yield in 1 pmol for Different PCR Product Size (Circularized ssDNA)

Insert Size (bp)	PCR Product Size (bp)	Corresponding Yield in 1 pmol (ng)
250	334	≥15.4
280	364	≥16.8
300	384	≥17.5

3.11.2 Formula 1 shows the calculation of the Mass (in ng) that corresponds to 1 pmol of a dsDNA sample with varying fragment sizes. Please refer to Formula 1 to calculate the amount of DNA needed.

Formula 1 Conversion between 1 pmol of dsDNA sample and Mass in ng

$$\text{Mass (ng) corresponding to 1 pmol PCR Products} = \frac{\text{DNA Fragement Size (bp)}}{1000\ \text{bp}} \times 660\ \text{ng}$$

3.12 Quality Control of Library

Quality assessment helps identify any problems that could contribute to failure of Library Construction. Assess the main size Fragmentation product with 2% agarose gel electrophoresis. Run the electrophoresis at 100 V for 60 minutes. Dye the gel for 15 minutes and perform gel imaging. The electrophoresis gel result shows that the size of enzymatic fragmentation product should be in a dispersion between 200 and 1000 bp, if the size of enzymatic fragmentation product is greater than 1000bp the enzymatic fragmentation step is considered abnormal. See Figure 1:

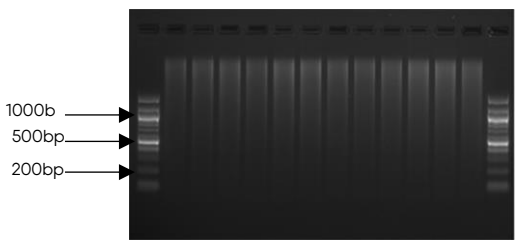

Figure 1 The electrophoresis result of enzymatic fragmentation sample (NEB 100 bp marker)

If the main band is not within the range, then an issue may have occurred during the enzyme fragmentation reaction.

3.12.1 Quantitate purified PCR products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-i PicoGreen® dsDNA Assay Kit following the kit user manual. The total amount of purified product should be ≥200 ng.

3.12.2 Assess the fragment size distribution of purified PCR products with Agilent 2100 Bioanalyzer or other electrophoresis-based equipment. The main DNA fragment size should be around 400 bp without dimers or other contaminants. The Agilent 2100 Bioanalyzer result is shown as Figure 2.

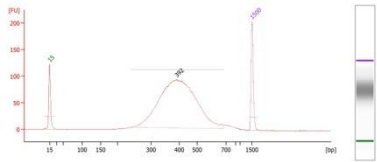

Figure 2 The Agilent 2100 Result of RAD Library PCR Product

3.13 Sequencing Instructions

3.13.1 MGI sequencers require 3 dark reactions to sequence RAD Libraries prepared using the MGIEasy RAD Library Prep Kit. Please remember that this a required step and follow the instructions below.

3.13.2 Follow the MGI sequencer User Manual to turn on the sequencer and to start the sequencing software. In the parameter settings interface, select "dark reaction" and enter the number "3" in the blank called "dark reaction read length." See Figure 3.

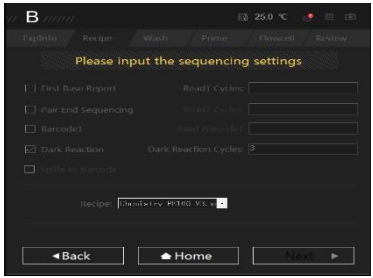

Figure 3 Dark reaction setting instruction（BGISEQ-500RS）

Note: In RAD Library Preparation, the restriction enzyme used inserts a fixed sequence to both ends of the fragments. This fixed sequence results in an unbalanced base composition which causes the sequencer to stop the run. Therefore, sequencing requires dark reactions for the first

three bases.

3.14 Precautions and Warnings

3.14.1 This kit is for scientific research only. It is not intended for use in clinical diagnostics. Please read this manual carefully before use.

3.14.2 Understand all operating procedures, precautions, and warnings for the instruments before use.

3.14.3 Avoid skin and eye contact with all samples and reagents. Do not swallow. If skin or eye contact occurs, immediately flush skin or eyes with plenty of water. Seek medical attention immediately.

3.14.4 Dispose of all samples and waste in accordance with federal, state, and local requirements.

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Doc.#: B02-140