MGIEasy

Circularization Kit User Manual

Cat. No.: 1000005259

Kit Version: V2.0

Manual Version: 3.0

Revision History

Manual Version	Kit Version	Date	Description
3.0	V3.0	Jan. 2022	◆ Update file template.
A1	V2.0	Jan. 2021	◆ Update contact information.
A0	V2.0	Jun. 2018	◆ 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

MGIEasy Circularization Kit is a modular library prep kit designed for MGI high-throughput sequencing platforms. This kit can be used to prepare a single-stranded circular DNA library for MGI sequencers from PCR products with MGI adapters. All reagents provided within this kit have passed stringent quality control and functional verification procedures to ensure high performance, stability, and reproducibility.

1.2 Applications

This kit is applicable to PCR products with MGI adapters from all MGI library prep kits. It can be used to convert a PCR product to a single-stranded circular DNA library that can be sequenced on MGI high-throughput sequencing platforms.

1.3 Platform Compatibility

Sequencing instrument compatibility is dependent on specific MGI library prep kits. Libraries created with this kit can be used on any MGI sequencer.

1.4 Contents

MGIEasy Circularization Kit can be used for 16 reactions and consists of 2 modules. Further information on Cat. No., Components, and Specifications are listed below:

Table 1 MGIEasy Circularization Kit (16 RXN) (Cat. No.: 1000005259)

Modules and Cat. No.	Components	Color-Coded Screw Caps	Spec & Quantity
MGIEasy Circularization Module Cat. No.: 1000005260	Splint Buffer	Purple	186 μL/tube x 1 tube
	DNA Rapid Ligase	Purple	8 μL/tube x 1 tube
	Digestion Buffer	White	23 μL/tube x 1 tube
	Digestion Enzyme	White	42 μL/tube x 1 tube
	Digestion Stop Buffer	White	120 μL/tube x 1 tube
MGIEasy DNA Clean Beads Cat. No.: 1000007325	DNA Clean Beads	White	1600 μL/ tube× 2 tube
	TE Buffer	White	1600 μL/ tube× 1 tube

1.5 Storage Conditions and Shelf Life

MGIEasy Circularization Module

- Storage temperature: -25℃ to -18℃
- Expiration date: refer to label
- Transport Conditions: transported on dry ice

MGIEasy DNA Clean Beads

- Storage temperature: 2℃ to 8℃
- Expiration date: refer to label
- Transport Conditions: transported on ice packs

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

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

1.6 Equipment and Materials Required but not Provided

Table 2: Equipment and Materials Required but not Provided

Equipment	Vortex mixer Desktop centrifuge Pipette Thermocycler Magnetic Separation Rack (ThermoFisher, Cat. No. 12321D) Qubit® 3.0 Fluorometer (ThermoFisher, Cat. No. Q33216)
Reagents	Nuclease free water (NF water) (Ambion, Cat. No. AM9937) 100% Ethanol (Analytic Grade) Qubit® ssDNA Assay Kit (Invitrogen, Cat. No. Q10212) Qubit® dsDNA HS Assay Kit (Invitrogen, Cat. No. Q32854)
Consumables	Pipette tips and RNase-free tips 1.5 mL tube (Axygen, Cat. No. MCT-150-C) 0.2 mL PCR tube (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 0.5mL 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 adjustments for optimal performance. Adjust the protocol according to your experimental design, sample characteristics, sequencing application, and equipment limitations as necessary.
- 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 and invert several times to mix thoroughly. Centrifuge briefly and place on ice for use.
- When preparing mixtures and working solutions, pipette up and down at least 10 times to mix thoroughly. Vigorous shaking may decrease library yield.
- To prevent cross-contamination, we recommend using filtered pipette tips. Use a new tip each time for pipetting different solutions. Pipette carefully to avoid spillage.
- Use thermocyclers equipped with heated lids for reactions. Preheat the lid to reaction temperature before use. The temperature of Heated lid would be 105℃ if there is no special instruction.
- Avoid contamination of PCR products due to aerosolization of samples and reagents by:
  - Preparing PCR reactions and PCR clean up products in separate working spaces.
  - Using designated equipment for each area.
  - Regularly cleaning working environment with 0.5% Sodium Hypochlorite or 10% Bleach.
- If you have other questions, please contact MGI technical support MGI-service@mgi-tech.com

Chapter 2 Sample Preparation

2.1 Sample Compatibility and Requirements

2.1.1 Input requirement

- The recommended input DNA amount is 1 pmol. If the PCR product is not enough, the minimum input DNA amount should be no less than 0.5 pmol.
- If there are special requirements regarding the amount of input PCR product from the Library prep kit, please obey the special requirements.
- Formula 1 can be used to calculate the Mass in (ng) that corresponds to 1 pmol of dsDNA sample with varying fragment sizes. Please refer to the Formula 1 to calculate the amount of DNA needed.

Formula 1 dsDNA sample pmol and ng Conversion

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

Table 3 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)
150	234	150
200	284	190
250	334	220
300	384	250
350	434	290
400	484	320
450	534	350
500	584	390

2.1.2 Sample multiplex requirement

- Input DNA can be a single sample or multiplexed samples with different Barcodes.
- Multiplexed samples must satisfy specific Barcodes combination requirements. Please follow the instructions of MGIEasy DNA Adapters Kits in Table 4 to use Barcodes in proper combination.

Table 4 Adapters Kit information

Kit Name	Specifications	Cat. No.
MGIEasy DNA Adapters-16 (Tubes) Kit	16 x 10 μL	1000005284
MGIEasy DNA Adapters-96 (Plate) Kit	96 x 10 μL	1000005282
MGIEasy DNA Adapters-96 (Plate) Kit	96 x 20 μL	1000005283

- The recommended total amount of multiplexed samples should be 1 pmol. If each sample need same sequencing data amount, please multiplex equally and calculate the amount for each sample according to Formula 2.

Formula 2 Calculation of each sample mass for multiplexing

Each sample mass (ng) = The Mass (ng) corresponding to 1 pmol PCR Products / Number of samples

- The total volume for circularization should be 48 μL. Add TE Buffer until the total volume is 48 μL.

2.2 Quality Control of Library

- Quantify the purified circularized ssDNA with Qubit® ssDNA Assay Kit.
- The yield for circularized ssDNA after cleanup must be at least 80 fmol or above for two sequencing runs. Refer to Formula 3 or Table 5 below to calculate the number of mols required.

Formula 3 Circular ssDNA fmol and ng Conversion:

$$\text{The Mass (ng) corresponding to 80 fmol circular ssDNA} = 0.08 \times \frac{\text{DNA Fragment Size (bp)}}{1000\ \text{bp}} \times 330\ \text{ng}$$

Table 5 The Corresponding Yield in 80 fmol for Different PCR Product Size (Circularized ssDNA)

Insert Size (bp)	PCR Product Size (bp)	Corresponding Yield in 80 fmol (ng)
150	234	6.2
200	284	7.5
250	334	8.8
300	384	10.1
350	434	11.5
400	484	12.8
450	534	14.1
500	584	15.4

Chapter 3 Library Construction Protocol

3.1 Denaturation

⚠ **Note: Please read Chapter 2 Sample Preparation carefully before you begin.**

3.1.1 According to the PCR product size and the formula 1 in Chapter 2, transfer 1 pmol of PCR product to a new 0.2 mL PCR Tube. Add TE Buffer to bring the total volume to 48 μL.

3.1.2 Place the PCR tube from step 3.1.1 into the thermocycler and run the program in Table 6:

Table 6 The Reaction Conditions of Denaturation

Temperature	Time
Heated lid	On
95℃	3 min

3.1.3 When the reaction is complete, immediately place the tube on ice for 2 minutes and centrifuge briefly.

3.2 Single Strand Circularization

3.2.1 Prepare the following Single Strand Circularization reaction mixture on ice (see Table 7).

Table 7 Single Strand Circularization Reaction Mixture

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

3.2.2 Transfer 12.1 μL Single Strand Circularization mixture to the PCR Tube from step 3.1.3. Vortex 3 times (3s each) and centrifuge briefly to collect the solution to the bottom of the tube.

3.2.3 Place the PCR tube into the thermocycler and run the program in Table 8.

Table 8 The Reaction Conditions of Single Strand Circularization

Temperature	Time
Heated lid	On
37℃	30 min
4℃	Hold

3.2.4 When the reaction is complete, immediately place the tube on ice and prepare for the next step.

3.3 Enzymatic Digestion

3.3.1 Prepare the Enzymatic Digestion mixture (see Table 9) on ice during the reaction in step 3.2.3.

Table 9 Enzymatic Digestion Mixture

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

3.3.2 Transfer 4 μL Enzymatic Digestion mixture into the PCR tube from step 3.2.4. Vortex 3 times (3s each) and centrifuge briefly to collect the solution to the bottom of the tube.

3.3.3 Place the PCR tube from step 3.3.2 into the thermocycler and run the program in Table 10.

Table 10 The Reaction Conditions of Enzymatic Digestion

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

3.3.4 Centrifuge briefly to collect the solution to the bottom of the tube.

3.3.5 Add 7.5 μL of Digestion Stop Buffer to the PCR tube. Vortex 3 times (3s each) and centrifuge briefly to collect the solution to the bottom of the tube. Transfer all of the solution to a new 1.5 mL tube.

3.4 Cleanup of Enzymatic Digestion Product

⚠ **Note: Please use the DNA Clean Beads included in this kit. If other Clean beads are used, then purification conditions will need to be optimized.**

3.4.1 Remove DNA Clean Beads from refrigerator and bring to room temperature for at least 30 minutes beforehand. Vortex and mix thoroughly before use.

3.4.2 Transfer 170 μL of DNA Clean Beads to the Enzymatic Digestion product in step 3.3.5. Gently pipette at least 10 times to mix thoroughly. Ensure that all solution and beads are expelled from the tip into the tube.

3.4.3 Incubate at room temperature for 10 minutes.

3.4.4 Centrifuge briefly and place the 1.5 mL tube on the Magnetic Separation Rack for 2-5 minutes until liquid becomes clear. Carefully remove and discard the supernatant using a pipette.

3.4.5 Keep the 1.5 mL tube on the Magnetic Separation Rack and add 500 µL freshly prepared 80% Ethanol to the tube without disturbing the beads. Incubate for 30 seconds. Carefully remove and discard the supernatant.

3.4.6 Repeat step 3.4.5 once. Remove all liquid from the tube without disrupting the beads. Centrifuge briefly to collect any remaining liquid to the bottom, separate beads on the magnetic separation rack, then remove any remaining liquid using a small volume pipette.

3.4.7 Keep the 1.5 mL tube on the Magnetic Separation Rack with the lid open to air-dry the beads until no wetness (reflectiveness) is observed. Avoid over-drying beads (cracks can be observed on pellet).

3.4.8 Remove the 1.5 mL tube from the Magnetic Separation Rack and add 22 µL of TE Buffer to elute DNA. Gently pipette the entire volume up and down 10 times to mix or until the beads are fully resuspended.

3.4.9 Incubate at room temperature for 10 minutes.

3.4.10 Centrifuge briefly. Place the 1.5 mL tube on the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 20 µL supernatant to a new 1.5 mL tube. Take care not to disturb the beads.

✔ **Stopping Point: Purified Enzymatic Digestion products can be stored at -20°C for up to one month.**

3.5 Quality Control of Enzymatic Digestion Product

Quantify the purified Enzymatic Digestion product with Qubit® ssDNA Assay Kit. The final yield should be ≥ 80 fmol (enough for two sequencing runs). Please refer to Table 5 or formula 3 in Chapter 2 for calculations.

Contact Us
Company: MGI Tech Co., Ltd
Address: 2/F, Building 11, Beishan Industrial Zone, Yantian District, Shenzhen, CHINA, 518083
Website: http://en.mgi-tech.com
Email: MGI-service@mgi-tech.com
Service Hotline: (+86) 4000-966-988

MGI Website

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