Part No.:SOP-013-B02-134

User Manual

Version:6.0

MGIEasy
Circularization Kit

Cat. No.: 1000005259 (16 RXN)
1000017573 (96 RXN)

Kit Version: V2.0

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Manufacturer information

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Revision history

Manual version	Kit version	Date	Description
6.0	V2.0	Jul. 2024	Added 96 RXN of MGIEasy Circularization Module
5.0	V2.0	May. 2024	• Update the manufacturer information • Update the manual style
4.0	V2.0	Mar. 2022	Update Manufacturer LOGO
3.0	V2.0	Jan. 2022	Update the manual style
A1	V2.0	Jan. 2021	Update contact information
A0	V2.0	Jun. 2018	Initial release

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

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 in this set have passed strict quality control and functional verification procedures, ensuring stability and reproducibility.

1.2 Intended use

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

1.3 Applicable sequencing platforms

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 Components

MGIEasy Circularization Module comes in two specifications: 16 RXN and 96 RXN.

- For 16 RXN, two separate boxes are included in MGIEasy Circularization Kit.
- For 96 RXN, only the MGIEasy Circularization Module is included.

For component details, refer to the following table.

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

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

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy Circularization Module Cat. No.: 1000005260	Splint Buffer	Purple	186 μL/tube x 1
	DNA Rapid Ligase	Purple	8 μL/tube x 1
	Digestion Buffer	White	23 μL/tube x 1
	Digestion Enzyme	White	42 μL/tube x 1
	Digestion Stop Buffer	White	120 μL/tube x 1
MGIEasy DNA Clean Beads Cat. No.: 1000007325	DNA Clean Beads	White	1600 μL/tube x 2
	TE Buffer	White	1600 μL/tube x 1

Table 2 MGIEasy Circularization Module (96 RXN) (Cat. No.: 1000017573)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy Circularization Module Cat. No.: 1000017573	Splint Buffer	Purple	1114 μL/tube x 1
	DNA Rapid Ligase	Purple	48 μL/tube x 1
	Digestion Buffer	White	135 μL/tube x 1
	Digestion Enzyme	White	250 μL/tube x 1
	Digestion Stop Buffer	White	720 μL/tube x 1

Tips When using 96 RXN, it is recommended to select a combination of different specifications of MGIEasy DNA Clean Beads according to the actual experimental sample requirements. The catalog number of the clean beads can be referred to "Table 4 Order information for MGI products" on page 3.

1.5 Storage and transportation

Table 3 Kit storage and transportation temperatures

Item	Storage temperature	Transportation temperature
MGIEasy Circularization Module	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
MGIEasy DNA Clean Beads	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 4 Order information for MGI products

Catalog number	Name	Component	Spec & Quantity
1000005279	MGIEasy DNA Clean Beads	DNA Clean Beads	50 mL/tube × 1
		TE Buffer	25 mL/tube × 1
940-001174-00		DNA Clean Beads	15 mL/tube × 1
		TE Buffer	17 mL/tube × 1

Tips
- The required quantity and specifications are calculated based on the actual number of samples (N) and the DNA clean beads volume used in the 3.3.2 step 1.
- The recommended number of samples used to calculate the amount of DNA clean beads is (N+2).

Table 5 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

Table 6 Recommended reagent/consumable list

Reagent/consumable	Recommended brand
Nuclease Free (NF) water	Ambion, Cat. No. AM9937, or equivalent
TE Buffer, pH 8.0	Ambion, Cat. No. AM9858, or equivalent
100% Ethanol (Analytical Grade)	/
Qubit ssDNA Assay Kit	Invitrogen, Cat. No. Q10212, or equivalent
Qubit dsDNA HS Assay Kit	Invitrogen, Cat. No. Q32854, or equivalent
Pipette tips	/
1.5 mL tube	/

Reagent/consumable	Recommended brand
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 use 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. 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, please contact Technical Support: **MGI-service@mgi-tech.com**.

1.8 Workflow

Section	Workflow	Total time	Hands-on time
3.1	Denaturation and single strand circularization	45 - 50 min	15 min
3.2	Digestion	35 - 40 min	10 min
3.3	Cleanup of digestion product ⏸	50 min	10 - 15 min
3.4	QC of purification product ⏸	15 - 20 min	10 - 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.
- ⏸ Stop point.

2 Sample preparation

2.1 Sample 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.
- Refer to the formula 1 or table below to calculate the mass in (ng) that corresponds to 1 pmol of dsDNA sample with varying fragment sizes.

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

Table 7 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	155
200	284	188
250	334	221
300	384	254
350	434	287
400	484	320
450	534	353
500	584	386

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. Refer to the instructions of MGIEasy library prep kits to use barcodes in proper combination.
- 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

$$\text{Each sample mass (ng)} = \frac{\text{The mass (ng) corresponding to 1 pmol PCR products}}{\text{Number of samples}}$$

Formula 3 Calculation of sample volume

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

- The total volume for circularization should be 48 μL. Add TE Buffer to make a total volume of 48 μL if the volume is not enough.

3 Circularization and digestion

3.1 Denaturation and single strand circularization

Tips Calculate the required volume of PCR product, based on the main fragment size of the purified PCR product and concentration of the sample, using Formula 1 in Chapter 2.

3.1.1 Preparation

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

Table 8 Preparing the reagents

Reagent	Requirement
TE Buffer	User-supplied; place at room temperature (RT).
Splint Buffer	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.
DNA Rapid Ligase	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.

3.1.2 Denaturation

1. Add 1 pmol of PCR product into a new 0.2 mL PCR tube. Add TE Buffer to make a total volume of 48 µL.
2. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 9 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.

3.1.3 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. Mix it well by vortexing 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 10 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 3.1.2). Vortex 3 to 6 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 11 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.

3.2 Digestion

3.2.1 Preparation

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

Table 12 Preparing the reagents

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

3.2.2 Digestion

1. According to the desired reaction number, prepare the digestion mixture in a 0.2 mL PCR tube on ice. Vortex it 3 times (3 sec each), centrifuge briefly, and place on ice.

Table 13 Digestion mixture

Reagent	Volume per reaction
Digestion Buffer	1.4 μL
Digestion Enzyme	2.6 μL
Total	4.0 μL

2. Add 4 μL of digestion mixture to each sample tube (from step 4 in section 3.1.3). Vortex 3 to 6 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 14 Digestion reaction conditions (Volume: 64.1 μL)

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

4. When the program is completed, centrifuge the tube(s) briefly. Immediately add **7.5 μL of Digestion Stop Buffer** to each sample tube.
5. Vortex the tube(s) 3 times (3 sec each) and centrifuge briefly. Transfer all liquid to a new 1.5 mL centrifuge tube (one tube per reaction).

3.3 Cleanup of purification product

Tips
- Use the DNA Clean Beads included in this kit (refer to Table 1 and Table 4). If the magnetic beads from other kits or brands are used, optimize the cleanup conditions before getting started.
- 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.

3.3.1 Preparation

Table 15 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.

3.3.2 Cleanup of digestion product

1. Mix the DNA Clean Beads thoroughly. Add 170 µL of DNA Clean Beads to each sample tube (from step 5 in section 3.2.2). 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.

   Tips If other DNA clean beads volumes per sample are required, refer to the corresponding library construction instructions or determine through testing in advance.

2. Incubate the sample(s) at room temperature for 10 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 500 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 22 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 10 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 20 µL of supernatant to a new 1.5 mL centrifuge tube.

   Stop point After cleanup, the purification product(s) can be stored at -20 ℃ for one month.

3.4 QC of digestion product

Quantify the purified digestion product by following the instructions of the Qubit ssDNA Assay Kit.

- The yield of digestion product should be not less than 80 fmol.

Refer to the formula or the table below to calculate the mass of 80 fmol ssCir.

Formula 4 Circular ssDNA fmol and ng conversion

Mass corresponding to 80 fmol circular ssDNA (ng) = 0.08 × PCR product peak size (bp) × 0.33

Table 16 The corresponding yield in 80 fmol for different PCR product sizes (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