Part No.:SOP-013-B02-181

User Manual

Version:4.0

MGIEasy Rapid Circularization Module

Cat. No.: 1000005258 (16 RXN)
Kit Version: V1.0

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Research Use Only

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Manufacturer information

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Revision history

Manual version	Kit version	Date	Description
4.0	V1.0	May. 2024	• Update the manufacturer information • Update the manual style
3.0	V1.0	Mar. 2022	Update Manufacturer LOGO
A1	V1.0	Jan. 2021	Update contact information
A0	V1.0	Oct. 2019	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 Rapid Circularization Module is specifically designed for MGI high-throughput sequencing platforms. This kit can be used to convert PCR products with MGI adapters into single-stranded circularized DNA (ssCir DNA). 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 that recommend using the MGIEasy Rapid Circularization Module.

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

This kit comes in one specification: 16 RXN. 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 Rapid Circularization Module (16 RXN) (Cat. No.: 1000005258)

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

1.5 Storage and transportation

MGIEasy Rapid Circularization Module

Storage temperature: -25 ℃ to -15 ℃

Transportation temperature: -80 ℃ to -15 ℃

Tips
- Production date and expiration date: refer to the label.
- For dry ice shipments, ensure that there is enough 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 2 Order information for MGI products

Catalog number	Model	Name
1000005284	16 RXN	MGIEasy DNA Adapters-16 (Tubes) Kit
1000005282	96 RXN	MGIEasy DNA Adapters-96 (Plate) Kit

Table 3 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	/
Desktop centrifuge	/
Pipettes	/
Thermocycler	/
Qubit Fluorometer or equivalent	Thermo Fisher, Cat. No. Q33216

Table 4 Recommended reagent/consumable list

Reagent/consumable	Recommended brand
TE Buffer, pH 8.0	Ambion, Cat. No. AM9858 or equivalent
Qubit ssDNA Assay Kit	Invitrogen, Cat. No. Q10212, or equivalent

Reagent/consumable	Recommended brand
Pipette tips and RNase-free 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 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	QC of digestion 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.
- ⏸ : The stop point.

2 Sample preparation

2.1 Input requirement

- The recommended input DNA amount is 1 pmol.
- If there are special requirements regarding the amount of input PCR product from the Library prep kit, 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 1Conversion 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 5 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

2.2 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 Protocol

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 6 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 7 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 8 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 9 Single strand circularization reaction conditions (Volume: 60.1 μL)

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

4. After the reaction, centrifuge the tube(s) briefly. The reaction products can be used immediately for DNB making or kept at -20 ℃.

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

3.2 Quality control

Transfer 20 μL of circularized ssDNA product to the PCR tube to prepare DNA nanoballs (DNBs). Follow the protocol described in BGISEQ/MGISEQ/DNBSEQ High-throughput Sequencing Set Instruction Manual for DNB making.

Quantify the DNB with Qubit ssDNA Assay Kit. The final concentration should be≥ 8 ng/μL. If the concentration of DNB is ≥ 8 ng/μL, the DNB can be directly loaded by the sequencer.