Part No.:H-1000004155-01

Leading Life Science Innovation

User Manual

Version:2.0

MGIEasy Universal Library Conversion Kit (App-A)

Cat. No. : 1000004155 (16 RXN)
Kit Version: V1.0

Research Use Only

About the user manual

This user manual and the information contained within are proprietary to MGI and are intended solely for the contractual use of its customers in connection with the use of the product described herein and for no other purpose. Any person or organization cannot entirely or partially reprint, copy, revise, distribute, or disclose to others the user manual without the prior written consent of MGI. Any unauthorized person should not use this user manual.

MGI does not make any promise of this user manual, including (but not limited to) any special commercial purpose and any reasonable implied guarantee. MGI has taken measures to guarantee the correctness of this user manual. However, MGI is not responsible for any missing parts in the manual and reserves the right to revise the manual and the reagent to improve the reliability, performance, or design.

All the pictures in this user manual are schematic diagrams and are for reference only. The content of the pictures may be slightly different from the actual product or the actual layout.

DNBSEQ™, MGISEQ™, Agilent®, Agilent Technologies™, ALPAQUA®, Ambion®, Axygen®, Advanced Analytical®, Beckman Coulter®, Invitrogen®, PerkinElmer®, Qubit®, Thermo Fisher™, or any other company, product names, and trademarks are the property of their respective owners.

Manufacturer information

Company	Shenzhen MGI Biological Electronic Technology Co., Ltd.
Address	2/F, Building 11, Beishan Industrial Zone, No.146, Beishan Road, Yantian Street, Yantian District, Shenzhen, 518083, P.R. China
Service hotline	(+86) 4000-688-114
Email	MGI-service@mgi-tech.com
Website	http://en.mgi-tech.com

Revision history

Manual version	Kit version	Date	Description
2.0	V1.0	Mar. 2024	Update the manufacture information
1.0	V1.0	Sep. 2023	• Update the service hotline. Update the manual style • Modify the section of Cleanup of PCR product • Add 3.4 Pooling(option) • Delete appendix Beads and cleanup • Update Specifications in Table 1 • Change the manual code
A4	V1.0	Mar. 2022	Update Manufacturer LOGO
A3	V1.0	Jan. 2021	Update contact information
A2	V1.0	Sep. 2019	• Add DNBSEQ series sequencing platform and its corresponding sequencing reagents • Delete chapter split barcode
A1	V1.0	May. 2019	• Revise kit name and reagent name • Add the sample require of different sample input • Add different PCR cycles with different sample input • Add MGISEQ-200RS sequencing platform • Add chapter 4 sequencing, add chapter 5 split barcode, add appendix sample barcode pooling strategies
A0	V1.0	Sep. 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
1.1 Introduction 1
1.2 Intended use 1
1.3 Applicable sequencing platforms 1
1.4 Components 2
1.5 Storage and transportation 2
1.6 User-supplied materials 2
1.7 Precautions and warnings 3
1.8 Workflow 5
2 Sample preparation 6
2.1 Sample requirements 6
2.2 Sample preparation 6
3 Adapter conversion protocol 7
3.1 Adapter conversion PCR amplification 7
3.2 Cleanup of PCR product 8
3.3 QC of PCR product 10
3.4 Pooling (option) 10
4 Circularization and digestion 14
4.1 Denaturation, single strand circularization 14
4.2 Digestion 15
4.3 Cleanup of digestion product 16
4.4 QC of digestion product 18
5 Appendix 19
5.1 Sample barcode pooling strategies 19

1 Product overview

1.1 Introduction

The MGIEasy Universal Library Conversion Kit (App-A) is designed for linear dsDNA library conversion and is optimized for MGI sequencing platforms. The linear DNA library can be converted to a single stranded circular (ssCir) DNA library and sequenced on MGI sequencing platforms, including BGISEQ-500RS, MGISEQ-200RS, DNBSEQ-G50RS, MGISEQ-2000RS and DNBSEQ-G400RS with the High-Throughput Sequencing Set (App-A). All reagents provided in this set have passed strict quality control and functional verification procedures, ensuring stability and reproducibility.

1.2 Intended use

The MGIEasy Universal Library Conversion Kit (App-A) is used to convert dsDNA linear libraries prepared with Next Generation Sequencing Library Preparation Kits other than MGI to libraries suitable for MGI sequencing platforms.

For information on suitable library types, refer to the *App Series Products Ordering Guide* or contact your local sales representative.

If barcodes need to be sequenced, it is strongly recommended to read Appendix carefully before barcodes ligation, and select barcodes with balanced base compositions at each barcode position before library construction.

1.3 Applicable sequencing platforms

The prepared libraries are applicable to the following sequencing platforms.

- BGISEQ-500RS
- MGISEQ-200RS, DNBSEQ-G50RS
- MGISEQ-2000RS, DNBSEQ-G400RS

1.4 Components

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 Universal Library Conversion Kit (App-A) (16 RXN) (Cat. No.: 1000004155)

Item & Cat. No.	Component	Cap color	Spec & Quantity
MGIEasy Universal Library Conversion Kit (App-A) Cat. No.: 1000004155	AC-PCR Primer	🔵 Blue	48 μL/tube × 1
	AC-PCR Amplification Master Mix	🔵 Blue	400 μL/tube × 1
	App-A Splint Buffer	🔴 Red	186 μL/tube × 1
	Ligation Enzyme	🔴 Red	8 μL/tube × 1
	Digestion Buffer	○ White	23 μL/tube × 1
	Digestion Enzyme	○ White	42 μL/tube × 1
	Digestion Stop Buffer	○ White	120 μL/tube × 1

1.5 Storage and transportation

MGIEasy Universal Library Conversion Kit (App-A)

- 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
1000005279	50 mL	MGIEasy DNA Clean Beads

Table 3 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	/
Mini centrifuge	/
Pipettes	/
Thermocycler	/
Magnetic rack DynaMag -2 or equivalent	Thermo Fisher Scientific, Cat. No. 12321D
Qubit Fluorometer or equivalent	Thermo Fisher, Cat. No. Q33216
Agilent 2100 Bioanalyzer or equivalent	Agilent Technologies , Cat. No. G2939AA

Table 4 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)	/
AMPure XP beads	Beckman
Qubit ssDNA Assay Kit	Invitrogen, Cat. No. Q10212, or equivalent
Qubit dsDNA HS Assay Kit	Invitrogen, Cat. No. Q32854) or equivalent
Agilent High Sensitivity DNA Kit	Agilent, Cat. No. 5067-4626, or equivalent
Agilent DNA 1000 Kit	Agilent, Cat. No. 5067-1504, or equivalent
Pipette 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 in vitro diagnostics. Please read this manual carefully before use.
- Familiarize yourself with the precautions and operation 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 for PCR reaction preparation, PCR reaction, and PCR product cleanup. 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 or eyes, rinse immediately with plenty of water or seek medical advice.
- Conform to the law and regulations when disposing of all samples and reagents.
- If you have questions, contact Technical Support: **MGI-service@mgi-tech.com**.

1.8 Workflow

Section	Workflow	Total time	Hands-on time
3.1	Adapter conversion PCR amplification ⏸	40 - 45 min	10 min
3.2	Cleanup of PCR product ⏸	40 min	10 - 15 min
3.3	QC of PCR product ⏸	15 - 60 min	10 - 20 min
4.1	Denaturation, single strand circularization	45 - 50 min	15 min
4.2	Digestion	35 - 40 min	10 min
4.3	Cleanup of digestion product ⏸	50 min	10 - 15 min
4.4	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 Sample requirements

Sample types: Linear dsDNA library.

Linear dsDNA library size distribution: Between 100 to 500 bp, and the peak size should be at approximately 200 bp (center of the distribution) ±100 bp on either side.

2.2 Sample preparation

- Quantitate the linear dsDNA library with a dsDNA Fluorescence Assay Kit such as Qubit dsDNA HS Assay Kit or Quant-iT PicoGreen dsDNA Assay Kit.
- The input amount of linear dsDNA library is based on the available linear dsDNA library (see the table below). For example, if the amount of the linear dsDNA library available is 20 ng, the linear dsDNA library input should be 10 ng, and the linear dsDNA library concentration should be at least 0.5 ng/μL.

Table 5 The relationship between linear dsDNA library input and total amount of dsDNA library

Library input (ng)	Library amount (ng)	Library concentration (ng/μL)
10	Amount ≤ 25	≥ 0.5
25	25 < Amount ≤ 50	≥ 1.2
50	Amount > 50	≥ 2.3

Tips Because the PCR cycles affect downstream data analysis (e.g. InDel analysis), it is preferred that the linear dsDNA library input be increased and the number of PCR cycles corresponds to library input. Please refer to Table 9 for details.

The linear dsDNA library volume can be calculated according to the following formula.

Formula 1 Calculation of linear dsDNA library volume

$$\text{Volume of linear dsDNA library (μL)} = \frac{\text{Linear dsDNA library input (ng)}}{\text{Linear dsDNA library concentration (ng/μL)}}$$

3 Adapter conversion protocol

3.1 Adapter conversion PCR amplification

3.1.1 Preparation

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

Table 6 Preparing the reagents

Reagent	Requirement
Nuclease Free (NF) Water	User-supplied; place at room temperature (RT).
AC-PCR Amplification Master Mix	Mix by vortexing, centrifuge briefly, and place on ice.
AC-PCR Primer	

3.1.2 Adapter conversion PCR

1. Transfer the linear dsDNA library into a new 0.2 mL PCR tube and add NF Water to make a total volume of 22 μL.
2. According to the desired reaction number, prepare the AC-PCR amplification 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 7 AC-PCR amplification mixture

Reagent	Volume per reaction
AC-PCR Amplification Master Mix	25 μL
AC-PCR Primer	3 μL
Total	28 μL

3. Add 28 μL of AC-PCR amplification mixture to each sample tube (from step 1). Vortex 3 times (3 sec each) and centrifuge briefly.

4. Place the PCR tube(s) into the thermocycler and run the program with the following conditions. The PCR cycles for different library input amounts are listed in table 9.

Table 8 AC-PCR amplification reaction conditions (Volume: 50 μL)

Temperature	Time	Cycles
105 ℃ Heated Lid	On	/
98 ℃	3 min	1 cycle
98 ℃	30 sec	N cycles (see table 9)
62 ℃	15 sec	
72 ℃	30 sec	
72 ℃	5 min	1 cycle
4 ℃	Hold	/

Table 9 The PCR cycles for different linear dsDNA library input

Linear dsDNA library input (ng)	PCR cycles
10	10
25	8
50	5

5. When the program is completed, centrifuge the tube(s) briefly.

   Stop point AC-PCR product can be stored at -20 ℃.

3.2 Cleanup of PCR product

Tips

- For use with MGIEasy DNA Clean Beads. If you use the magnetic beads from other brands, 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.

3.2.1 Preparation

Table 10 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied. Freshly prepared.
TE Buffer	User-supplied. Place at RT.
DNA Clean Beads	User-supplied. Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

3.2.2 Cleanup of PCR product

Tips Transfer all liquid to a new 1.5 mL centrifuge tube (one tube per reaction) in advance if using a 1.5 mL centrifuge tube and a corresponding magnetic rack for purification.

1. Mix the DNA Clean Beads thoroughly. Add 60 μL of DNA Clean Beads to each sample tube (from step 5 in 3.1.2). 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.
2. Incubate the sample(s) at room temperature for 5 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 200 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 32 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 5 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 30 μL of supernatant to a new 1.5 mL centrifuge tube or PCR tube.

   Stop point After cleanup, AC-PCR product(s) can be stored at -20 °C.

3.3 QC of PCR product

- **dsDNA fluorescence quantification method**: Quantify the purified PCR products with dsDNA fluorescence assay kits and instructions.
- **Electrophoresis method**: Assess the size range of purified PCR products with electrophoresis based equipment and instructions.

Table 11 Different QC methods and standards for library

Method	Equipment/Reagent	Standard
dsDNA fluorescence quantification method	Qubit dsDNA HS Assay Kit, Quant-iT PicoGreen dsDNA Assay Kit	Yield for PCR products: ≥ 1 pmol
Electrophoresis method	Tapestation (Agilent Technologies), Bioanalyzer, LabChip GX, GXII, GX Touch (PerkinElmer), Fragment Analyzer (Advanced Analytical)	/

Refer to Formula 2 in "Pooling (option)" on page 10 or the table below to calculate the mass (in ng) that corresponds to 1 pmol of dsDNA sample with different fragment sizes.

Table 12 The corresponding yield in 1 pmol for PCR products with different fragment sizes

AC-PCR product peak size (bp)	Corresponding yield in 1 pmol (ng)	AC-PCR product peak size (bp)	Corresponding yield in 1 pmol (ng)
150	99	350	231
200	132	400	264
250	165	450	297
300	198	500	330

- For 1 sample sequencing in 1 lane, proceed to "Circularization and digestion" on page 14.
- For multiple samples pooled sequencing, refer to "Pooling (option)" on page 10.

3.4 Pooling (option)

⚠ CAUTION Do not pool AC-PCR products with different insert size distributions in the same lane.

There are three stages for multiple samples pooling: purified PCR products (dsDNA libraries) pooling, ssCir libraries (ssDNA, purified digestion products) pooling, and DNB pooling. Unless otherwise required, you can choose one of the three stages for samples pooling based on your needs.

The following methods are suitable for the purified PCR products pooling. For pooling methods in other stages, please refer to the instructions of the library preparation kits, High-throughput sequencing kits, or DNBSEQ sequencing platforms.

- Purified PCR products pooling

CAUTION Before pooling, carefully read Appendix 1 on page 18.

Quantify the purified PCR products before pooling. The total yield after pooling should be 1 pmol, with a total volume ≤ 48 μL.

Calculate the percentage of the required amount of sequencing data for samples on the same lane. Refer to Formula 2 and 3 to calculate the required mass of each sample. Formula 4 shows the calculation of sample volume.

Formula 2 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

Formula 3 Calculation of each sample mass before pooling

Sample mass (ng)= Mass corresponding to 1 pmol PCR product (ng) × Ratio of sample data (%)

Formula 4 Calculation of sample volume

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

For example: For 4 samples (belong to 300 bp insert size libraries) pooled sequencing. The PCR products should have a total mass of 198 ng and be equal to a total yield of 1 pmol.

1. Calculate the mass for each sample.

- The expected amount of sequencingdata for each sample is the same. The ratio of each sample sequencing data is 25%. Referring to Formula 3, the required mass of each PCR sample is 198 ng × 25% = 49.5 ng.
- The expected amount of sequencing data for each sample is different. The ratios of sequencing data for samples 1-4 are 20%, 20%, 30%, and 30%. Referring to Formula 3, the required mass of sample 1 is 39.6 ng. Calculate the mass of samples 2 to 4 in the same way.

2. The concentration of sample 1 is 10 ng/μL. Refer to Formula 4 and the required volume is "A μL". Calculate the volume of samples 2 to 4 in the same way.
3. Transfer A μL of sample 1 into a new 0.2 mL PCR tube.
4. Add other samples into the same PCR tube.
5. Add TE Buffer to make a total volume of 48 μL.

Table 13 Multiple samples pooling (each sample volume should be at least 1 μL)

Name	Volume
Sample 1	A μL
Sample 2	B μL
Sample 3	C μL
Sample 4	D μL
TE Buffer	48 - (A+B+C+D) μL
Total	48 μL

Tips The volumes of A, B, C, and D should each be ≥ 1 μL.

Follow one of the two methods below to pool the samples when the required volume of a sample is less than 1 μL. Method 1 is recommended.

Method 1: Increase the volume of all samples by Z (Z > 1) times. After mixing the samples, take 1/Z of the total volume W μL. Add TE Buffer to make a total volume of 48 μL.

Table 14 Samples mixture: All samples volume increases by Z times

Name	Volume
Sample 1	A × Z μL
Sample 2	B × Z μL
Sample 3	C × Z μL
Sample 4	D × Z μL
Total	W μL

Table 15 Method 1: Multiple samples pooling

Name	Volume
Samples mixture	(W ÷ Z) μL
TE Buffer	48 - (W ÷ Z) μL
Total	48 μL

Tips If necessary, quantify the samples mixture and calculate a new volume X μL (1 pmol). Replace "(W ÷ Z) μL" with "X μL".

Method 2: Dilutes a high concentration sample by Y (Y > 1) times if the required volume is less than 1 μL. Quantify the diluted sample and calculate a new volume. Pool the diluted sample with other samples.

For example: The required volume of sample 3 is < 1 μL. It needs to be diluted by Y times.

Table 16 Diluted sample: Dilute the high concentration sample by Y times

Name	Volume
Sample 3	5 μL*
TE Buffer	5Y - 5 μL
Total	5Y μL

Tips *: The volume of high concentration sample is recommended to be more than 5 μL.

Quantify the diluted sample. Refer to Formula 4 to calculate a new volume "E μL". Pool the diluted sample with other samples. Add TE Buffer to make a total volume of 48 μL.

Table 17 Method 2: Multiple samples pooling

Name	Volume
Sample 1	A μL
Sample 2	B μL
Sample 4	D μL
Diluted sample 3	E μL
TE Buffer	48 - (A+B+D) - E μL
Total	48 μL

4 Circularization and digestion

4.1 Denaturation, single strand circularization

Tips Calculate the required purified PCR product volume based on the main fragment size of the purified PCR product ,concentration of the sample, and Formula 2 and 4.

4.1.1 Preparation

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

Table 18 Preparing the reagents

Reagent	Requirement
TE Buffer	User-supplied; place at RT.
APP-A Splint Buffer	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.
Ligation Enzyme	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.

4.1.2 Denaturation

1. Add 1 pmol of PCR product into a new 0.2 mL PCR tube and 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 19 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.

4.1.3 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. Vortex it 3 times (3 s each), centrifuge briefly, and place on ice.

Table 20 Single strand circularization mixture

Reagent	Volume per reaction
APP-A Splint Buffer	11.6 μL
Ligation Enzyme	0.5 μL
Total	12.1 μL

2. Add 12.1 μL of single strand circularization mixture to each sample tube (from 4.1.2 step 3). Vortex the tube(s) 3 times (3 s each), centrifuge briefly, and place on ice.
3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 21 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.

4.2 Digestion

4.2.1 Preparation

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

Table 22 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.

4.2.2 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 23 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 4.1.3). Vortex 3 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 24 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).

4.3 Cleanup of digestion product

Tips
- For use with MGIEasy DNA Clean Beads. If you use the magnetic beads from other brands, 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.

4.3.1 Preparation

Table 25 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied. Freshly prepared.
TE Buffer	User-supplied. Place at RT.
DNA Clean Beads	User-supplied. Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

4.3.2 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 4.2.2). 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.
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 27 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 25 μL of supernatant to a new 1.5 mL centrifuge tube or PCR tube.

   Stop point After cleanup, the digestion product(s) can be stored at -20 ℃.

4.4 QC of digestion product

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

- The final yield should be at least 60 fmol and the cyclization efficiency should be at least 5%.

Refer to the formula or table below to calculate the mass of 60 fmol ssCir.

Formula 5 Conversion between circular ssDNA fmol and mass in ng

$$60 \text{ fmol circular ssDNA (ng)} = 0.06 \times \text{PCR product peak size (bp)} \times 0.33$$

Table 26 The conversion table between ng and PCR product size for 60 fmol of ssDNA

PCR product size (bp)	60 fmol ssDNA (ng)	PCR product size (bp)	60 fmol ssDNA (ng)
150	3	350	7
200	4	400	8
250	5	450	9
300	6	500	10

5 Appendix

5.1 Sample barcode pooling strategies

- For pooled sequencing, sample barcode pooling should follow the principle of base balance.
- Using an 8 bp barcode as an example, the ratio of ATGC at 1-8 bp bases should be 25%, as shown in the table below.

Table 27 Example of sample barcode pooling strategies

Barcode	Sequence	1	2	3	4	5	6	7	8
Example 1	TAGGTCCG	T	A	G	G	T	C	C	G
Example 2	GGACGGAA	G	G	A	C	G	G	A	A
Example 3	CTTACTGC	C	T	T	A	C	T	G	C
Example 4	ACCTAATT	A	C	C	T	A	A	T	T
Barcode 1-8 bp A%		25%	25%	25%	25%	25%	25%	25%	25%
Barcode 1-8 bp T%		25%	25%	25%	25%	25%	25%	25%	25%
Barcode 1-8 bp G%		25%	25%	25%	25%	25%	25%	25%	25%
Barcode 1-8 bp C%		25%	25%	25%	25%	25%	25%	25%	25%

- If the proportion cannot reach 25%, then ATGC should appear in each cycle. The minimum base proportion should not be less than 12.5% and the maximum base proportion should not be greater than 62.5%.
- If the proportion is not between 12.5% and 62.5%, then sequencing quality could be reduced. In this case, it is possible that the sample barcodes might not be properly split.