MGIEasy DNA Fast Library Prep Set (for CycloneSEQ)

Instructions of Use

- Cat. No.: 940-002654-00
- Version: 1.0

For Research Use Only.
Not for use in diagnostic procedures.

MGI Tech Co., Ltd.

About the instructions for use

MGI intends to provide this product solely for research use.

This instructions of use is applicable to MGIEasy DNA Fast Library Prep Set (for CycloneSEQ). The instructions for use version is 1.0. The kit version is V1.

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Revision history

	Date	Version	Revision Description
Initial release	March 19, 2025	1.0	Initial release

Tips

- Please download the latest user manual, and use it with the corresponding version of the preparation set.
- Download the user manual by searching the catalog number or product name from the following website: *https://en.mgi-tech.com/download/files/*

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1 Product information

1.1 Product information

MGIEasy DNA Fast Library Prep Set (for CycloneSEQ) is a fast barcode library preparation set for DNA samples, specifically designed for the Cyclone sequencer. The product uses end repair and a one-step method to connect ligation barcode, barcode sequencing adapter and sample, so as to rapidly prepare the library and efficiently obtain sample data through Cyclone sequencing. The barcode adapters in the set can distinguish different samples, and they are applicable for sequencing multiple samples simultaneously on the same platform. All reagents included in the set have undergone strict quality control and function verification, to ensure the stability and repeatability of the library preparation.

1.2 Intended use

The reagent set is applicable to multiple double-stranded DNA samples, including extracted gDNA, DNA fragments, and amplicons.

The library is used to perform barcode sequencing on CycloneSEQ-WT02 Genetic Sequencer.

1.3 Component

The specification of the library preparation set is 24 RXN. The kit and component information are as follows:

Table 1 MGIEasy DNA Fast Library Prep Set (for CycloneSEQ) (24 RXN) (Cat. No.: 940-002654-00)

Reagent kit and catalog number	Component	Cap color	Specification and quantity
MGIEasy DNA Universal End Repair and Ligation Reagent Module Cat. No.: 950-000142-00	End Repair Buffer		168 μL/tube × 1
	End Repair Enzyme		72 μL/tube × 1
	Rapid Ligase Master Mix		840 μL/tube × 1

Reagent kit and catalog number	Component	Cap color	Specification and quantity
CycloneSEQ 24 Barcode Sequencing Adaptor Module-A Cat. No.: H940-000038	Ligation Barcodes (LB01-LB24)		20 µL/tube × 24
	Barcode Sequencing Adapter		20 µL/tube × 1
CycloneSEQ Barcode Sequencing Adaptor Purification Module-A Cat. No.: H940-000039	DNA Clean Beads		9600 µL/tube × 1
	WB for Short Fragment		14400 µL/tube × 1
	WB for Long Fragment		14400 µL/tube × 1
	Elution Buffer		1632 µL/tube × 1

1.4 Storage and transportation

Table 2 Storage and transportation conditions of kits

Kit	Storage temperature	Transportation temperature
MGIEasy DNA Universal End Repair and Ligation Reagent Module	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃
CycloneSEQ 24 Barcode Sequencing Adaptor Module-A		
CycloneSEQ Barcode Sequencing Adaptor Purification Module-A	2 ℃ to 8 ℃	2 ℃ to 8 ℃

Tips
- For validity period, refer to the label on the box.
- When the product is transported, stored, and used appropriately, all of the components retain full activity within the validity period.

1.5 User-supplied equipment, reagents, and consumables

Table 3 User-supplied equipment, reagents, and consumables

	Name	Remarks
Device	Vortex mixer	/
	Mini centrifuge	/
	Pipette	/
	Thermal cycler	/
	Magnetic rack for 96-well plates	ALPAQUA, Part#A00400
	Magnetic rack for 1.5 mL tubes	Thermo Fisher, Cat. No. 12321D
	Qubit® 3.0 Fluorometer	Thermo Fisher, Cat. No. Q33216
Reagent	Nuclease free water (NF water)	Ambion, Cat. No. AM9937
	1× TE buffer, pH 8.0	Ambion, Cat. No. AM9858
	Qubit dsDNA HS Assay Kit	Invitrogen, Cat. No. Q32854
Consumable	Filtered pipette tips	/
	1.5 mL centrifuge tube	/
	0.2 mL PCR tubes or 96-well plates	Axygen, Cat. No. PCR-02-C) or 96-well plate (Axygen, Cat. No. PCR-96M2-HS-C
	Qubit Assay Tubes or 0.5 mL Thin Wall PCR Tubes	Invitrogen, Cat. No. Q32856/Axygen, Cat. No. PCR-05-C

1.6 Precautions and warnings

- This product is for research use only. Please read the instructions for use of the product carefully before use.
- Before the experiment, please be familiar with the operation methods and precautions of various instruments to be used.
- Take out all components of the reagent kit in advance before use. Invert the Enzyme to mix thoroughly. Briefly centrifuge and place it on ice for later use. Thaw other buffer components at room temperature, mix them thoroughly by vortexing, briefly centrifuge, and then place them on ice for later use.
- To prevent cross contamination, it is recommended to use filtered pipette tips during preparation of PCR reaction mixtures and PCR product purification. Use new tips each time when pipetting different samples.
- Regularly clean the laboratory areas using 0.5% Sodium Hypochlorite or 10% Bleach to ensure a clean experimental environment.
- It is recommended to use thermocyclers with heated lids for reactions. Preheat to reaction temperature before use.

- Direct contact with skin and eyes should be avoided for all samples and reagents. Do not swallow. If accidental ingestion occurs, please get medical attention immediately. If skin exposure occurs, rinse with large amounts of water and get medical attention if irritation persists.
- All samples and wastes should be disposed of according to local regulations.
- If you have any other questions, contact the technical support at *mgi-service@mgi-tech.com* .

2 Sample requirements, storage and transportation

2.1 Sample requirements

The reagent set is applicable to multiple double-stranded DNA samples, including extracted gDNA, DNA fragments, and amplicons. For example, the gDNA extracted from bacteria or fungi, amplicon product libraries, and other samples.

- Purity requirement: $OD_{260/280}$ should be greater than or equal to 1.8 and less than or equal to 2.0. $OD_{260/230}$ should be greater than or equal to 2.0 and less than or equal to 2.2.
- Concentration requirement: ≥10 ng/μL
- For gDNA, it is recommended to use samples with good integrity and fragment sizes greater than 10 kb, and to input 500 ng for library preparation.
- For DNA fragment or amplicons, it is recommended to input 200 ng for library preparation.

⚠ WARNING You can also use samples that do not meet the purity requirements to prepare library and perform sequencing. But this may decrease the number of effective sequencing wells, such as abnormal membrane rupture, and other phenomena during sequencing.

2.2 Sample storage and transportation

The sample should be stored and transported under frozen conditions, and repeated freeze-thaw cycles should be avoided during transportation to prevent sample degradation.

3 Library preparation workflow

3.1 End repair

⚠ WARNING
- For genomic DNA, it is recommended to prepare more than 3 libraries to ensure that the input amount of a chip for sequencing reaches 500 ng.
- For fragmented DNA and amplicons, it is recommended to prepare no less than 2 libraries to ensure that the input amount of a chip for sequencing reaches 100 ng.
- For samples with long DNA fragments, use wide-bore tips and gently pipette, or gently flip the tube wall during operation.

1. Take out End Repair Buffer in advance, thaw it at room temperature, and mix it by vortexing.
2. Invert End Repair Buffer, and flip the bottom of the tube 10 times to ensure that no reagent residuals left at the bottom of the tube (do not vortex the tube). Briefly centrifuge the reagent and place it on ice for later use.
3. Based on the number of reactions, prepare the end repair reaction mixture on ice according to the table below.

Table 4 Preparation of the end repair reaction mixture

Component	Volume/reaction
End Repair Buffer	7 μL
End Repair Enzyme	3 μL
Total volume	10 μL

4. Briefly vortex reaction mixture 2 times (3 seconds each) to mix it , briefly centrifuge it, and place it on ice for later use.
5. Add recommended volume of sample into new 0.2 mL PCR tubes, and add NF water in the PCR tubes until the volume reaches 50 μL . Aspirate 10 μL prepared end repair reaction mixture by using a pipette, and dispense the mixture into the PCR tubes. To mix the mixture, gently pipette the mixture 10 times, or flip the tube wall and invert the tube 3 to 5 times. Briefly centrifuge tube and place it on ice.
6. Place the PCR tube into a thermal cycler and start the reaction according to conditions listed in the table below:

Table 5 End repair reaction condition (volume: 60 μL)

Temperature	Time (min)
Heated lid (105 °C)	On

Temperature	Time (min)
30 °C	5
72 °C	10
4 °C	Hold

7. After reaction, briefly centrifuge the tube to collect the reaction mixture at the bottom of the tube.

WARNING Do not stop operation here and proceed to adapter ligation of barcode and motor protein.

3.2 Adapter ligation between Ligation Barcodes, Barcode Sequencing Adapter and samples

1. Take out Ligation Barcodes (LB01-LB24) thaw it, and mix it by shaking. Take out and mix Barcode Sequencing Adapter and Rapid Ligase Master Mix. Briefly centrifuge the three reagents and place them on ice for later use.

CAUTION
- Flip the tube of Barcode Sequencing Adapter to mix it.
- Rapid Ligase Master Mix is thick . It is recommended to mix the mixture 2 minutes by vortex mixer after it is thawed and invert the mixture 3 to 5 times to mix it thoroughly.

2. Add 4 µL of the corresponding Ligation Barcode into the PCR tube prepared in step 5 of *3.1 End repair on page 6*.

WARNING Ligation Barcodes are used to distinguish samples, allowing multiple samples to be sequenced simultaneously on the same platform. One Ligation Barcode is used for library preparation of each sample.

3. Cap the tube, flip the tube wall 3 times, and invert the tube 3 times. Briefly centrifuge the tube to collect the mixture at the bottom of the tube.
4. Based on the number of reactions, prepare the adapter ligation reaction mixture on ice according to the table below:

Table 6 Preparation of the adapter ligation reaction mixture

Component	Volume/reaction
Barcode Sequencing Adapter	0.7 µL
Rapid Ligase Master Mix	35 µL
Total volume	35.7 µL

5. To mix the mixture, pipette the reaction mixture 10 times by using a wide-bore tip, or flip the tube wall more than 10 times and invert the tube 3 to 5 times. Briefly centrifuge the tube to collect the mixture at the bottom of the tube and place it on ice.
6. Slowly aspirate 35.7 µL of the prepared adapter ligation reaction mixture and transfer it into the PCR tube prepared in step 2 by using a pipette. To mix the mixture, pipette the reaction mixture 10 times by using a wide-bore tip, or flip the tube wall more than 10 times and invert the tube 3 to 5 times. Briefly centrifuge the tube to collect the mixture at the bottom of the tube.

Tips The adapter ligation reaction mixture is thick, so please slowly aspirate and pipette the mixture.

7. Place the PCR tube prepared in step 5 into a thermal cycler and start the reaction according to conditions listed in the table below:

Table 7 Adapter ligation reaction conditions (Volume: 100 μL)

Temperature	Time
Heated lid (30 ℃)	On
20 °C	5 min
4 °C	Hold

8. After reaction, briefly centrifuge the tube to collect the reaction mixture at the bottom of the tube.

WARNING It is recommended to proceed to the purification of the adapter-ligated product.

3.3 Adapter-ligated DNA purification

WARNING
- Please read Appendix A before the experiment.
- Select the volume of DNA Clean Beads and washing reagent according to the fragment size in the following table:

Fragment size	Volume of DNA Clean Beads	Washing reagent
< 3 K	60 μL (0.6×)	WB for Short Fragment
≥ 3 K	40 μL (0.4×)	WB for Long Fragment

1. Take out DNA Clean Beads, WB for Short Fragment, or WB for Long Fragment 30 minutes in advance, place them at room temperature, and mix them thoroughly before use.
2. Transfer 60 μL or 40 μL of DNA Clean Beads into the adapter ligation product prepared in step 7 of *3.2 Adapter ligation between Ligation Barcodes, Barcode Sequencing Adapter and samples on page 7* by using a pipette, gently pipette the mixture several times or flip the tube wall until all beads suspend, and ensure that all the liquid and beads in the tip have been added to the PCR tube.
3. Incubate the tube at room temperature for 10 minutes.
4. Briefly centrifuge the PCR tube and place it on the magnetic rack for 2 to 5 minutes. Carefully aspirate and discard the supernatant with a pipette after the liquid in the tube is clear.
5. Remove the PCR tube from the magnetic rack, add 150 μL of WB for Short Fragment or 150 μL of WB for Long Fragment , and gently pipette the mixture several times or flip the tube wall until the beads are mixed. Briefly centrifuge the mixture, put the PCR tube with the mixture on the magnetic rack, and let it stand for 2 to 5 minutes until the mixture becomes clear. Carefully aspirate the supernatant.
6. Repeat step 5. Aspirate the liquid as much as possible. If a small amount of residue remains on the wall, briefly centrifuge the PCR tube and wait until the supernatant and beads separate. Use low-volume pipette to remove all the liquids. Do not dry the beads.

7. Remove the centrifuge tube from the magnetic rack, add 30 µL of Elution Buffer for DNA elution, and gently pipette the mixture several times or flip the tube wall until all beads suspend.
8. Incubate the tube at room temperature for 10 minutes.
9. Centrifuge the PCR tube briefly and place it on the magnetic rack for 2 to 5 minutes. Transfer 27 µL of supernatant into a new 1.5 mL EP tube by using a pipette after the liquid in the PCR tube is clear.
10. Aspirate 1 µL of supernatant for quantification. Use Qubit dsDNA HS Assay Kit, Quant-iT™ PicoGreen® dsDNA Assay Kit or other double-stranded DNA Quantitative PCR (qPCR) Reagent Kit to quantify the second PCR product according to the instructions of the quantification kit. The total amount of recovered sample library should be greater than or equal to 100 ng.

   Stop point The purified product can be stored in a refrigerator at 4 °C for no more than 72 hours.

4 Sequencing

The library preparation set is used with CycloneSEQ WT Sequencing Kit (6 T) to perform sequencing on CycloneSEQ-WT02 Nanopore Gene Sequencer. For details, refer to *CycloneSEQ-WT02 Nanopore Gene Sequencer checklist* .

View the data volume in real-time through the Total Output in the Analysis module of the Cyclone Master software. You can stop or continue sequencing based on the required data volume.

The volume of libraries for sequencing:

- For the sample libraries with fragment sizes exceeding 10 kb, it is recommended to input 500 ng of the libraries for sequencing runs.
- For the DNA fragment or amplicon libraries, it is recommended to input 100 ng of the libraries for sequencing runs.
- If you perform sequencing with multiple samples, pool the libraries according to the required data volume and input 500 ng or 100 ng pooled libraries for sequencing runs.

For the single bacterial genome assembly applications, to ensure the completeness and accuracy of the genome assembly, the sequencing depth for each sample should be greater than or equal to 50×. To achieve an average sequencing depth of 50× per sample, theoretically, the data volume per flow cell (Passed Bases) should be greater than or equal to 8 Gb.

- For bacterial or fungal samples with a genome size of less than 10 Mb, it is recommended that the number of pooled samples should not exceed 16.
- For bacterial or fungal samples with a genome size between 10 Mb and 20 Mb, it is recommended that the number of pooled samples should not exceed 8.
- For bacterial or fungal samples with a genome size between 20 Mb and 40 Mb, it is recommended that the number of pooled samples should not exceed 4.

⚠ CAUTION Before preparing barcode libraries for multiple samples, it is recommended to estimate the minimum sequencing data volume per sample based on the target genome size, in order to develop a pooled sequencing strategy for different barcode libraries. When performing sequencing by using sample libraries in batches, you may lose a certain proportion of data due to barcode splitting. It is recommended to take the loss into account when estimating the required data volume.

Appendix 1 Magnetic Beads and purification procedures

It is recommended to use DNA Clean Beads included in the library preparation kit for beads purification.

Before you use

- To ensure the capture efficiency of DNA Clean Beads, take out DNA Clean Beads from 4°C refrigerator storage 30 minutes in advance and equilibrate them o room temperature before use. Vortex and mix it thoroughly before use.
- Vortex or pipette up and down DNA Clean Beads to ensure that the beads are thoroughly mixed before each use.
- The beads volume directly affects the lower limit of fragment size. The higher the amount of beads used, the smaller the lower limit of the fragment size becomes.

Operation notes

- It is recommended to use the magnetic rack for 96-well plates or other magnetic rack for 0.2 mL PCR tubes for bead purification. If you use the magnetic rack for 1.5 mL PCR tubes, transfer all reaction samples from 0.2 mL PCR tubes into 1.5 mL PCR tubes before purification. The transfer may cause loss of purification, especially in enzymatic digestion product purification, where it may result in approximately 20% purification loss.
- When separating magnetic beads , wait until the mixture is completely clear before removing the supernatant. This process takes approximately 2 to 3 minutes. The magnetic strength of the magnetic rack might vary, extend the separation as needed until the mixture is completely clear.
- Avoid touching the beads with pipette tips when pipetting. Leave 2 µL to 3 µL of liquid in the tube to prevent aspiration of the beads. If beads are accidentally aspirated, aspirate all of the mixture and beads back into the tube and aspirate the supernatant after the mixture becomes clear.
- Use WB for Short Fragment or WB for Long Fragment that has been equilibrated to room temperature to wash the DNA Clean Beads. Keep the centrifuge tube on the magnetic rack when washing. Keep the pipette tip far away the tube wall when operating. Do not shake or disturb the beads in any way.
- Aspirate the mixture as much as possible during the second wash. If a small volume of residue remains on the wall, briefly centrifuge the PCR tube and wait until the supernatant and beads separate. Use a low-volume pipette to remove all the mixture.
- Do not dry the beads at room temperature and add the elution buffer directly.
- During the elution step, do not touch the beads with the pipette tips when removing the supernatant to prevent contamination of DNA by the beads. The contamination may affect subsequent purification. Therefore, the total volume of elution buffer and the beads should be 2 µL greater than the volume of the supernatant.

- Pay attention when opening and closing the lids of centrifuge tubes on the magnetic rack for 1.5 mL tubes to avoid spilling liquid or beads from the tubes due to strong vibrations. It is recommended to hold the middle-to-lower part of the 1.5 mL tubes before opening or closing the lids.

Appendix 2 About the adapter ligation

- The ligation reaction mixture is quite thick because it contains a high concentration of PEG. Aspirate and dispense it slowly to ensure accurate liquid volume.
- Due to PEG, you can reduce the purification bead multiplier for the ligation product. It is recommended to use the magnetic beads specified in this instructions for use for purification. If the volume of magnetic beads is increased, you may recover some adapter dimers.

Appendix 3 Manufacturer information

Manufacturer	MGI Tech Co., Ltd.
Address	2/F, Building 11, Beishan Industrial Zone, No. 146, Beishan Road, Yantian Street, Yantian District, Shenzhen, 518083, P.R. China
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