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DNA Library Prep optimized for low DNA inputs with MGIEasy Fast FS DNA Library Prep Set

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We use this protocol and it's working

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Abstract

The MGIEasy Fast FS Library Prep Set(1000006987, MGI Tech) is designed to convert genomic DNA (gDNA) into a special library for DNBSEQ^{TM} sequencers and combines the fragmentation, end-repair and add A into one step, to simplify the preparation process and significantly shorten the duration of DNA library preparation, reducing the library prep time in 2 hours.
This library prep set provides to be optimized for low DNA inputs, suitable for microbial sequencing, metagenomics sequencing, WGS.

Materials

Materials
MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570)
Qubit ssDNA Assay Kit
MGIEasy Fast FS Library Prep Set(1000006987, MGI Tech)

Supplies:
- Pipette tips (assorted volumes)
- 5ml round-bottom polystyrene tubes, PCR tube, centrifuge tube

Equipment:
- Qubit Fluorometer
- Vortex
- Micropipettes, various volumes
- Microcentrifuge
- Thermocycler
- Magnetic rack

Troubleshooting

Preparation

1 Reagent preparation

1.1 Prepare the 1x Elute Enhancer according to the following table. Mix it by vortexing, and centrifuge briefly. Store at Room temperature before using. The shelf life of the 1x Elute Enhancer is 7 days.

	Reagent	Volume
	20x Elute Enhancer	1 µL
	Nuclease-Free Water	19 µL
	Total	20 µL

1x Elute Enhancer

1.2 Prepare the En-TE according to the following table. Mix it by vortexing, and centrifuge briefly. Store at 4 °C before using. The shelf life of the En-TE is 60 days.

	Reagent	Volume
	1x Elute Enhancer	3 µL
	TE Buffer	1497 µL
	Total	1500 µL

En-TE

1.3 Prepare the En-Beads according to the following table. Mix it by vortexing, and centrifuge briefly. Store at 4 °C before using. The shelf life of the En-Beads is 60 days.

	Reagent	Volume
	1x Elute Enhancer	10 µL
	DNA Clean Beads	990 µL

	Reagent	Volume
	Total	1000 µL

En-Beads

2 Samples Preparation

2.1 Fragmentation
The extent of fragmentation (size distribution of DNA fragments) is controlled by time and temperature. Therefore, ensure the accuracy of time and temperature during the reaction. Mix the reagents before use and store the remaining reagents **immediately** after use.

2.2 Normalize gDNA.
Refer to the following table. Based on the sample concentration, transfer the appropriate gDNA (recommended 1 ng - 1000 ng) to a new 0.2 mL PCR tube. Add TE Buffer (pH 8.0) to make a total volume of 45 µL . Place the normalized gDNA On ice .

	Components	Volume
	TE Buffer (pH 8.0)	45 - X µL
	gDNA (1 ng - 1000 ng)	X µL
	Total	45 µL

Normalization of gDNA dissolved in TE (pH 8.0)

It is recommended that the normalization buffer should be the same as DNA elution buffer.

3 Enzyme Preparation

3.1 Mix the Fast FS Enzyme II by inverting 10 times and flicking the bottom of the tube(s) gently. Ensure that no residual reagent is left at the bottom each time. Centrifuge briefly, and place it On ice until use.
DO NOT vortex the Fast FS Enzyme II. Insufficient mixing will affect the fragmentation process.

3.2 According to the desired reaction number, prepare the fragmentation mixture in a 1.5 mL centrifuge tube On ice . Vortex it 3 times (00:00:03 each), centrifuge briefly, and place On ice . 3s

3.3 Add 15 µL of fragmentation mixture to each sample tube from step 2.2(45 µL). Vortex it 3 times (00:00:03 each), centrifuge briefly, and place On ice . 3s

3.4 Place the tube(s) into the thermocycler. Skip the first step (4 ℃ Hold) to start the reaction.

	Temperature	Time
	70 ℃ Heated lid	On
	4 ℃	Hold
	30 ℃	10 ng for 18 min; <1ng for 22 min
	65 ℃	15 min
	4 ℃	Hold

Fragmentation reaction conditions (Volume: 60 µL)

3.5 After the reaction, centrifuge the tube(s) briefly and immediately proceed to the next step.
DO NOT STOP AT THIS STEP.

Cleanup of fragmentation product

4 Single size selection,the peak size of the single size product is approximately 500 bp - 750 bp.

4.1 Check the volume of the fragmentation product. If the volume is less than 60 µL, add En-TE to make a total volume of 60 µL .

4.2 Mix the En-Beads thoroughly. Add 48 µL of En-Beads to each sample tube. Mix with a vortexer until all beads are suspended.

4.3 Incubate at Room temperature for 00:05:00 . 5m

4.4 Centrifuge the tube(s) briefly and place on the magnetic rack for 00:05:00 until the liquid is clear. Carefully remove and discard all the supernatant. If liquid remains on the tube wall, centrifuge the tube(s) briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette. 5m

4.5 Remove the tube(s) from the magnetic rack and add 45 µL of En-TE to elute the DNA. Mix with a vortexer until all beads are suspended and centrifuge briefly.
DO NOT STOP AT THIS STEP.

Adapter ligation

5 Adapter ligation

5.1 The UDB Adapter is a universal adapter sequence and does not contain Barcode sequences.
Mix the reagents before using and store the remaining reagents immediately after use.

5.2 Adapter ligation
Dilute the UDB Adapter with TE Buffer (pH 8.0) based on gDNA input.

	gDNA input (N ng)	Dilution of UDB Adapter	Volume after dilution
	10	5 x	5 µL
	<1	50 x	5 µL

Recommended adapter usage and dilutions for different amounts of gDNA input

5.3 Add 5 µL of UDB Adapter to the corresponding sample tube . Vortex it 3 times (00:00:03 each), centrifuge briefly, and place On ice . 3s

5.4 According to the desired reaction number, prepare the adapter ligation mixture in a 1.5 mL centrifuge tube On ice . Vortex it 6 times (00:00:03 each), centrifuge briefly, and place On ice . 30m 3s

	Reagent	Volume per reaction
	Fast Ligation Buffer	23 µL
	Ad Ligase	5 µL
	Ligation Enhancer	2 µL
	Total	30 µL

Adapter ligation mixture

It is recommended to prepare the adapter ligation mixture while waiting for cleanup of fragmentation product. Place it On ice after preparation, and use it within 00:30:00 .

5.5 Slowly pipette 30 µL adapter ligation mixture to each sample tube. Vortex it 6 times (00:00:03 each), centrifuge briefly, and place On ice . 3s

The adapter ligation mixture is highly viscous. Pipette slowly and carefully.

5.6 Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

	Temperature	Time
	30 ℃ Heated lid	On
	25 ℃	10 min
	4 ℃	Hold

Adapter ligation reaction conditions (Volume: 80 µL)

5.7 When the program is completed, centrifuge the PCR tube(s) briefly and place On ice .

DO NOT STOP AT THIS STEP.

Cleanup of adapter-ligated product

6 Cleanup of adapter-ligated product

6.1 Add 22 µL of En-TE to each sample tube.

6.2 Mix the En-Beads thoroughly. Add 20 µL of En-Beads to each sample tube. Mix with a vortexer until all beads are suspended.

6.3 Incubate the sample tube(s) at Room temperature for 00:05:00 . 5m

6.4 00:05:00 00:05:00 Centrifuge the sample tube(s) briefly and place on the magnetic rack for 00:05:00 until the liquid is clear. Carefully remove and discard all the supernatant. 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. 15m

6.5 While keeping the PCR tube(s) on the magnetic rack, add 160 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 00:00:30 . Carefully remove and discard the supernatant. 30s

6.6 Repeat step 6.5. 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.

6.7 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.

6.8 Remove the tube(s) from the magnetic rack and add 20 µL of En-TE to elute the DNA. Mix with a vortexer until all beads are suspended.

6.9 Incubate the tube(s) at Room temperature for 00:05:00 . 5m

6.10 Centrifuge the tube(s) briefly and place on the magnetic rack for 00:05:00 until the liquid is clear. Carefully transfer 19 µL of supernatant to a new 0.2 mL PCR tube. 5m

7 After cleanup, the adapter-ligated product(s) can be stored at -20 °C .

PCR

8 PCR Preparation

8.1 Mix the reagents before using and store the remaining reagents immediately after use. Barcodes are in the UDB PCR Primer Mix.

	Reagent	Requirement
	PCR Enzyme Mix	Thaw at RT: mix by vortexing; centrifuge briefly; place on ice
	UDB PCR Primer Mix	Thaw at RT; mix by vortexing; centrifuge briefly; place at RT

Preparing the reagents

8.2 Add 25 µL PCR Enzyme Mix to each sample tube.

8.3 Add 6 µL of the corresponding UDB PCR Primer Mix . Vortex 3 times (00:00:03 each) and centrifuge briefly to collect the solution at the bottom of the tube. 3s

	Reagent	Volume per reaction
	Adapter-ligated product	19 µL
	PCR Enzyme Mix	25 µL
	Corresponding UDB PCR Primer Mix	6 µL
	Total	50 µL

PCR mixture

9 PCR running

9.1 Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

	Temperature	Time	Cycles
	105 °C Heated lid	On	-
	95 °C	3 min	1
	98 °C	20 sec	10 ng for 8cycles: 1ng for 11cycles; 0.1ng for 13cycles
	60 °C	15 sec	
	72 °C	30 sec	
	72 °C	10 min	1
	4 °C	Hold	-

PCR cycles required to yield 300 ng of libraries

The number of PCR cycles should be strictly controlled.

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

Cleanup of PCR product

10 Cleanup of PCR product

10.1 Mix the En-Beads thoroughly. Add 38 µL of En-Beads to each sample tube. Mix with a vortexer until all beads are suspended.

10.2 Incubate the sample tube(s) at Room temperature for 00:05:00 . 5m

10.3 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 all the supernatant. 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.

10.4 While keeping the PCR tube(s) on the magnetic rack, add 160 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 00:00:30 . Carefully remove and discard the supernatant. 30s

10.5 Repeat step 10.4. 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.

10.6 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.

10.7 Remove the tube(s) from the magnetic rack and add 32 µL of En-TE to elute the DNA. Mix with a vortexer until all beads are suspended.

10.8 Incubate the tube(s) at Room temperature for 00:05:00 . 5m

10.9 Centrifuge the tube(s) briefly and place on the magnetic rack for 00:05:00 until the liquid is clear. Carefully transfer 30 µL of supernatant to a new 0.2 mL PCR tube. 5m

11 After cleanup, the adapter-ligated product(s) can be stored at -20 °C .

Denaturation and single-stranded circularization 20m 30s

12 Combined with MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570) for ssCir preparation and further for DNB preparation.
Denaturation

12.1 Based on the PCR products concentration, add 300 ng of PCR products into a new 0.2 mL PCR tube. If the volume is less than 48 µL , add TE Buffer to make a total volume.

12.2 Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

	Temperature	Time
	100 °C Heated lid	On
	95 °C	3 min
	4 °C	10 min

Denaturation reaction conditions (Volume: 48 µL)

12.3 After the reaction, centrifuge the tube briefly and place On ice .

13 Single-stranded circularization

13.1 According to the desired reaction number, prepare the circularization reaction mixture in a new 0.2 mL PCR tube On ice . Vortex it 3 times (00:00:03 each), centrifuge briefly, and place On ice . 3s

	Reagent	Volume per reaction
	Dual Barcode Splint Buffer	11.6 µL
	DNA Rapid Ligase	0.5 µL
	Total	12.1 µL

Circularization reaction mixture

13.2 Add 12.1 µL of circularization reaction mixture to each sample tube . Vortex it 3 times (00:00:03 each), centrifuge briefly, and place On ice . 3s

13.3 Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

	Temperature	Time
	42 °C Heated lid	On
	37 °C	10 min
	4 °C	Hold

Single-stranded DNA circularization reaction conditions (Volume: 60 µL)

13.4 When the program is completed, place the PCR tube(s) On ice , centrifuge briefly, and immediately proceed to the next step.

14 Digestion

14.1 According to the desired reaction number, prepare the digestion mixture in a 0.2 mL PCR tube On ice . Vortex it 3 times (00:00:03 each), centrifuge briefly, and place On ice . 3s

14.2 Add 4 µL of digestion mixture to each sample tube. Vortex it 3 times (00:00:03 each), centrifuge briefly, and then place On ice . 3s

14.3 Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

	Temperature	Time
	42 °C Heated lid	On
	37 °C	10 min
	4 °C	Hold

Digestion reaction conditions (Volume: 64 µL)

14.4 When the program is completed, centrifuge the tube briefly and immediately add 7.5 µL of Digestion Stop Buffer to each sample tube. Vortex it 3 times (00:00:03 each), centrifuge briefly, and place On ice . 3s

15 Cleanup of digestion product

15.1 Mix the DNA Clean Beads thoroughly. Add 130 µL of DNA Clean Beads to each sample tube. Mix with a vortexer until all beads are suspended.

15.2 Incubate at Room temperature for 00:05:00 . 5m

15.3 Centrifuge the tube(s) briefly and place on the magnetic rack for 00:05:00 until the liquid is clear. Carefully remove and discard the supernatant. 5m

15.4 While keeping the tube(s) on the magnetic rack, add 160 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 00:00:30. Carefully remove and discard the supernatant. 30s

15.5 Repeat step 15.4. 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.

15.6 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.

15.7 Remove the tube(s) from the magnetic rack and add 25 µL of TE Buffer to elute the DNA. Mix with a vortexer until all beads are suspended.

15.8 Incubate at Room temperature for 00:05:00. 5m

15.9 Centrifuge the tube briefly and place on the magnetic rack for 00:05:00 until the liquid is clear. Carefully transfer 24 µL of supernatant to a new 1.5 mL centrifuge tube. 5m

16 QC of digestion product

16.1 Quantify the ssCir with Qubit ssDNA Assay Kit. The final Enzymatic Digestion products (ssDNA,
ng) / input products of PCR (dsDNA, 300 ng) should be ≥ 7%.