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DNA Library Prep with MGIEasy FS DNA Library Prep Set

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We use this protocol and it's working

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Abstract

The MGIEasy FS DNA Library Prep Set(1000006987,MGI Tech) is specifically designed for WGS libraries construction for MGI high-throughput sequencing platform series. This library prep set is optimized to convert 5-400 ng genomic DNA into a customized library. This set uses advanced Adapter Ligation technology and High-fidelity PCR Enzymes to significantly increase library yield and conversion rate. This library prep set is applicable for samples from all common animals, plants, fungus, bacteria, etc., including human, mice, rice, Arabidopsis, yeast, E. coli, Metagenomics.

Materials

Materials
MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570)
Qubit ssDNA Assay Kit
MGIEasy FS DNA Library Prep Set

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

Fragmentation

1 Preparation

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

	Reagent	Requirements
	Dilution Buffer	User-supplied:place at room temperature (RT).
	Frag Buffer II	Thaw at RT, mix well, centrifuge briefly, and place on ice.
	Frag Enzyme II	Keep on ice.

preparing the reagents

2 Fragmentation

2.1 Add 45 µL of genomic DNA to a new 0.2mL PCR tube. Add dilution buffer to make a total volume of 45 µL if the fragmentation gDNA volume not enough. Place the tube(s) On ice .

	Reagent	Volume
	gDNA	X µL
	Dilution buffer	45 - X µL
	Total	45 µL

Normalization of gDNA

2.2 Set and run program as table below. The thermocycle will perform the first step reaction described in table below and be kept at 4 °C .

	Temperature	Time
	70 °C Heated lid	On

	Temperature	Time
	4℃	Hold
	30℃	8 min
	65℃	15 min
	4℃	Hold

Fragmentation reaction conditions

2.3 Mix the Frag Enzyme II by inverting 10 times and flicking the bottom gently, ensure that no residual reagent is left at the bottom. Centrifuge briefly and place On ice .

2.4 Acorrding to the desired reaction number, prepare the fragmentation mixture in a 0.2mL PCR tube On ice . Mix it well by votexing 3 times, centrifuge briefly, and place On ice .

	Reagent	Volume per reaction
	Frag Buffer II	10μL
	Frag Enzyme II	5 μL
	Total	15 μL

Fragmentation mixture

2.5 Add 15 μL of fragmentation mixture to each sample tube. Mix by votexing 3 times, centrifuge briefly, and place On ice .

2.6 Make sure the thermocycler has cooled to 4 °C . Place the PCR tube(s) into the thermocycler, and skip the 4℃ Hold step to start the reaction at 30 °C .

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

3 Double size selection.
The following sample use a 36 μL 1st bead selection and a amount 12 μL 2nd bead selection to target a 330bp size fragement from fragmented DNA(60 μL).

3.1 Transfer all fragmented DNA to a new 1.5mL centrifuge tube. Add TE buffer for a final volume of 60 µL .

3.2 Add 36 µL of DNA Clean Beads to each sample tube. Mix with a vortexer until all beads are suspended.

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

3.4 Centrifuge the tube(s) briefly and place on the magnetic rack for 00:05:00 until the liquid is clear. Then, carefully transfer the supernatant to a new 1.5mL centrifuge tube. Retain the supernatant and dicard the beads. 5m

3.5 Add 12 µL of DNA Clean Beads to the centrifuge tube with 94 µL supernatant. Mix with a vortexer until all beads are suspended.

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

3.7 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. 5m

3.8 While keeping the centrifuge tube 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.

3.9 Repeat step 3.8 and try to remove all of the liquid from the tube.

3.10 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.

3.11 Remove the tube(s) from the magnetic rack and add 43 µL of TE to elute the DNA. Mix with a vortexer until all beads are suspended.

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

3.13 Centrifuge the tube(s) briefly and place on the magnetic rack for 00:05:00 until the liquid is clear. Carefully transfer 41 µL of supernatant to a new 1.5mL centrifuge tube. 5m

3.14 Quantify the purified size selection products with Qubit dsDNA Assay Kit.

End repair

4 End repair

4.1 Transfer 100 ng DNA sample to a new 0.2mL PCR tube and add TE buffer for a total volume of 40 µL .

4.2 Prepare the End repair Mixture On ice .

	Reagent	Volume per reaction
	ERAT Buffer	7.1 µL
	ERAT Enzyme Mix	2.9 µL
	Total	10 µL

End repair Mixture

4.3 Transfer 10 µL of the mixture to the 0.2mL PCR tube from step 3. Votex 3 times and centrifuge briefly.

4.4 Place the 0.2mL PCR tube into the thermocycler and run the program.

	Temperature	Time
	70 °C Heated lid	On
	37 °C	30 min
	65°C	15 min
	4°C	Hold

End repair program

4.5 When the program completed, brifly centrifuge to collect the solution at the bottom of the tube.

Adapter ligation

5 Adapter ligation

5.1 Add 5 µL of adapter to the corresponding sample tube. Vortex it 3 times (3 sec each), centrifuge briefly, and place On ice.

5.2 Prepare the adapter ligation mixture. Vortex it 6 times (3 sec each), centrifuge briefly, and place On ice.

	Reagent	Volume per reaction
	Ligation Buffer	23.4 µL
	DNA Ligase	1.6 µL
	Total	25 µL

adapter ligation mixture

5.3 Slowly pipette 25 µL of adapter ligation mixture to each sample tube. Vortex it 6 times (3 sec each), centrifuge briefly, and place On ice.

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

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

	Temperature	Time
	30 ℃ Heated lid	On
	23 ℃	30 min
	4℃	Hold

adapter ligation program

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

5.6 Add 20 µL TE buffer, for a total volume of 100 µL and transfer to a new 1.5mL centrifuge tube.

Cleanup of Adapter-ligated DNA

6 Cleanup of Adapter-ligated DNA

6.1 Add 50 µL of DNA Clean Beads to each sample tube. Mix with a vortexer until all beads are suspended.

6.2 Incubate the sample tube at Room temperature for 00:05:00 . 5m

6.3 Centrifuge the sample tube briefly and place on the magnetic rack for 00:05:00 until the liquid is clear. Carefully remove and discard all the supernatant. 5m

6.4 Keep the tube 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.

6.5 Repeat step 6.4 once. 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.

6.6 Keep the tube 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.

6.7 Remove the tube from the magnetic rack and add 21 µL of TE buffer to elute the DNA. Mix with a vortexer until all beads are suspended.

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

6.9 Centrifuge the tube 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

PCR Amplification

7 PCR Amplification

7.1 Prepare the PCR mixture in a 0.2 mL PCR tube On ice . Vortex it 3 times (3 sec each), centrifuge briefly, and place On ice .

	Reagent	Volume per reaction
	PCR Enzyme Mix	25 µL
	PCR Primer Mix	6 µL
	Total	31 µL

PCR mixture

7.2 Add 31 µL of PCR mixture to each sample tube. Vortex 3 times (3 sec each) and centrifuge briefly to collect the solution at the bottom of the tube.

7.3 Place the PCR tube into the thermocycler. Run the program with the following conditions.

	Temperature	Time	Cycles
	Temperature	Time	Cycles
	105 °C Heated lid	on	
	95 °C	3min	1
	98 °C	20s	8
	60 °C	15s	
	72 °C	30s	
	72 °C	10min	1
	4 °C	Hold	1

PCR program

7.4 When the program is completed, centrifuge the tube(s) briefly and transfer to a new 1.5mL centrifuge tube.

Cleanup of PCR Product

8 Cleanup of PCR Product

8.1 Add 50 µL of DNA Clean Beads to each sample tube from 7.4. Mix with a vortexer until all beads are suspended.

8.2 Incubate the sample tube at Room temperature for 00:05:00 . 5m

8.3 Centrifuge the sample tube briefly and place on the magnetic rack for 00:05:00 until the liquid is clear. Carefully remove and discard all the supernatant. 5m

8.4 Keep the tube on the magnetic rack, add 200 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

8.5 Repeat step 8.4 once. 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.

8.6 Keep the tube 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.

8.7 Remove the tube from the magnetic rack and add 32 µL of TE buffer to elute the DNA. Mix with a vortexer until all beads are suspended.

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

8.9 Centrifuge the tube 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 1.5mL centrifuge tube. 5m

8.10 Quality control of PCR product
Quantify the dsDNA with Qubit dsDNA-HS Assay Kit. Yeild for PCR products:>1 pmol.
Assess the size range of purified PCR products with Agilent 2100 Bioanalyzer.

Denaturation and single-stranded circularization 20m 30s

9 Denaturation

9.1 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 48 µL.

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

	Temperature	Time
	100 ℃ Heated lid	On
	95 ℃	3 min
	4 ℃	10 min

Denaturation reaction conditions (Volume: 48 µL)

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

10 Single-stranded circularization

10.1 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 (3 sec each), centrifuge briefly, and place On ice.

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

Circularization reaction mixture

10.2 Add 12.1 µL of circularization reaction mixture to each sample tube . Vortex it 3 times (3 sec each), centrifuge briefly, and place On ice .

10.3 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)

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

11 Digestion

11.1 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 .

11.2 Add 4 µL of digestion mixture to each sample tube. Vortex it 3 times (3 sec each), centrifuge briefly, and then place On ice .

11.3 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)

11.4 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 (3 sec each), centrifuge briefly, and place On ice .

12 Cleanup of digestion product

12.1 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.

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

12.3 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

12.4 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

12.5 Repeat step 12.4. 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.

12.6 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.

12.7 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.

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

12.9 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

13 QC of digestion product

13.1 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%.