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DNA Library Prep with MGIEasy UDB Universal Library Prep Set

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We use this protocol and it's working

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Abstract

The MGIEasy UDB Universal Library Prep Set(1000022803,MGI Tech) is specifically designed for creating WGS libraries for the MGI high-throughput sequencing platform series. This library prep set is optimized to convert 1-1000ng of fragmented DNA into a customized library. Unique Dual barcode supported double checked barcode split method ensuring sequencing Homogeneity and accuracy.

Materials

Materials
MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570)
Qubit ssDNA Assay Kit

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

Sample Preparation

20m

1 Fragmentation

1.1 Use Covaris series models to fragment 55 µL gDNA sample into size between 300-500bp.

2 Double size selection
The following sample use a 60 µL 1st bead selection and a 20 µL 2nd bead selection to target a 280bp size fragement from fragmented DNA(100 µL).

2.1 Transfer all fragmented DNA to a new 1.5mL centrifuge tube. Add TE buffer for a final volume of 100 µL .

2.2 Add 60 µL of DNA Clean Beads to each sample tube. Mix with a vortexer until all beads are suspended.

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

2.4 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

2.5 Add 20 µL of DNA Clean Beads to the centrifuge tube with 144 µL supernatant. Mix with a vortexer until all beads are suspended.

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

2.7 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

2.8 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 00:00:30 . Carefully remove and discard the supernatant. 30s

2.9 Repeat step 2.8 and try to remove all of the liquid from the tube.

2.10 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.

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

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

2.13 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 1.5mL centrifuge tube.

3 DNA Quantification

3.1 Quantify the purified fragment products with Qubit dsDNA Assay Kit.

End repair and A-tailing

4 End repair and A-tailing

4.1 Transfer DNA sample to a new 0.2mL PCR tube and add TE buffer for a total volume of 39.5 µL .

4.2 Prepare the End repair and A-tailing Mixture On ice .

	Components	Volume
	ERAT Buffer	6 µL
	ERAT Enzyme Mix	4.5 µL
	Total	10.5 µL

End repair and A-tailing Mixture

4.3 Transfer 10.5 µL of the mixture to the 0.2mL PCR tube from step 2.1. Votex 3 times and centrifuge briefly.

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

	Temperature	Time
	Heated lid(105°C)	On
	20°C	30 min
	65°C	15 min
	4°C	Hold

End repair and A-tailing program

4.5 Brifly centrifuge to collect the solution at the bottom of the tube.

Adapter ligation

5 Adapter ligation

5.1 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

5.2 Prepare the adapter ligation mixture. Vortex it 6 times (00:00:03 each), centrifuge briefly, and place On ice . 3s

	Components	Volume
	DNA Ligase	4 µL
	Ligation Buffer	21 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 (00:00:03 each), centrifuge briefly, and place On ice . 3s

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
	Heated lid(50°C)	On
	23°C	30 min
	4°C	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 5m

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 2 to 5 min until the liquid is clear. Carefully remove and discard all the supernatant.

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 00:00:30 . Carefully remove and discard the supernatant. 30s

6.5 Repeat step 4.4 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 40 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 2 to 5 min until the liquid is clear. Carefully transfer 38 µL of supernatant to a new 0.2 mL PCR tube.

PCR Amplification

7 PCR Amplification

7.1 Add 50 µL PCR Enzyme Mix to each sample tube.

7.2 Add 12 µL of UDB PCR Primer Mix to each sample tube . Vortex 3 times (00:00:03 each) and centrifuge briefly to collect the solution at the bottom of the tube. 3s

	Components	Volume
	Ligation product	38 µL
	PCR Enzyme Mix	50 µL

	Components	Volume
	UDB PCR Primer mix	12 µL
	Total	100 µL

PCR Mixture

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

	Temperature	Time	Cycles
	105 °C Heated lid	on	
	95 °C	3min	1
	98 °C	20s	
	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 100 µL of DNA Clean Beads to each sample tube from 5.4. 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 2 to 5 min until the liquid is clear. Carefully remove and discard all the supernatant.

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 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.

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 2 to 5 min until the liquid is clear. Carefully transfer 30 µL of supernatant to a new 1.5mL centrifuge tube.

8.10 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 Combined with MGIEasy Dual Barcode Circularization Kit (Cat. No.: 1000020570) for ssCir preparation and further for DNB preparation.
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 °C Heated lid	On
	95 °C	3 min
	4 °C	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 (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

10.2 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

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 (00:00:03 each), centrifuge briefly, and place On ice. 3s

11.2 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

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 (00:00:03 each), centrifuge briefly, and place On ice. 3s

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%.