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DNA Library Prep for BGISEQ-500/MGISEQ-2000/DNBSEQ-G400/MGISEQ-200/DNBSEQ-G50

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ABSTRACT

This DNA library prep protocol is specifically designed for WGS library construction for MGI highthroughput sequencing platforms. It's applicable for samples from all common animals, plants, fungi bacteria, etc., including human, mice, rice, Arabidopsis, yeast, *E. coli,* and Metagenomics samples.

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1 Fragmentation

1.1 Transfer genomic DNA to a new 0.2 mL PCR tube. The volume should be less than or equal to **45 µl** . If the volume is less than **45 µl** , add dilution buffer to bring the final volume to **45 µl**

Citation: Hongfang Zhang (06/03/2021). DNA Library Prep for BGISEQ-500/MGISEQ-2000/DNBSEQ-G400/MGISEQ-200/DNBSEQ-G50. https://dx.doi.org/10.17504/protocols.io.bve2n3ge

Components	Volume
DNA	X µL
dilution buffer	45-X µL
Total	45 µL

Table 1.1 Input DNA Dilution

1.2 Mix Frag Enzyme II by inverting 10 times then centrifuge briefly and place on ice for use. DO NOT vortex Frag Enzyme II. Vortex Frag Buffer II 3 times (3s each) then centrifuge briefly and place on ice for use.

1.3 Prepare the fragmentation mixture on ice. Pipette up and down at least 10 times to mix thoroughly. (DO NOT vortex)

Components	Volume
Frag Buffer II	10 µL
Frag Enzyme II	5 µL
Total	15 µL

Table 1.2 Fragmentation Mixture

1.4 Transfer **15 µl** of the fragmentation mixture to the 0.2 mL PCR tube from step 1.1. Pipette at least 10 times to mix thoroughly and briefly centrifuge to collect the solution at the bottom of the tube.

1.5 Set and run the following program on the thermocycler (see Table 1.3). Make sure the thermocycler has cooled to **4 °C** .Place the 0.2 mL PCR tube from step 1.4 into the thermocycler and skip the **4 °C** Hold step to start the reaction at **30 °C** .

Temperature	Time
Heated lid	On
4°C	Hold
30°C	8 min
65°C	15 min
4°C	Hold

Table 1.3 Fragmentation Reaction Conditions

Briefly centrifuge to collect the solution at the bottom of the tube.

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1.6

2 Size Selection

2.1 Take DNA Clean Beads out of the refrigerator and allow **00:30:00** for the solution to warm to room temperature. Vortex and mix thoroughly before use. 30m

2.2 Transfer **36 µl** of DNA Clean Beads to the 1.5 mL centrifuge tube containing **60 µl** of fragmentation product from step 1.6. Pipette up and down at least 10 times or vortex to mix thoroughly.

2.3 Incubate at room temperature for **00:05:00** . 5m

2.4 Centrifuge briefly and place the tube onto the Magnetic Separation Rack for **00:05:00** until the liquid becomes clear. Then, carefully transfer the supernatant to a new 1.5 mL centrifuge tube. 5m

2.5 Transfer **12 µl** of DNA Clean Beads to the centrifuge tube with **96 µl** of supernatant. Pipette at least 10 times to mix thoroughly.

2.6 Incubate at room temperature for **00:05:00** . 5m

2.7 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for **00:05:00** until the liquid becomes clear. Carefully remove and discard the supernatant with pipette. 5m

2.8 Keep the centrifuge tube on the Magnetic Separation Rack and add **200 µl** of freshly prepared 80% ethanol to wash the beads and the walls of the tube. Carefully remove and discard the supernatant.

2.9 Repeat step 2.8 once. Remove all of the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom of the centrifuge tube, separate the beads magnetically, and then remove any remaining liquid using a small volume pipette.

2.10 Keep the centrifuge tube on the Magnetic Separation Rack with the lid open, and air-dry beads at room temperature until no wetness (reflectiveness) is observed but before the pellet begins to crack.

2.11 Remove the centrifuge tube from the Magnetic Separation Rack and add **43 µl** of TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

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2.12 Incubate at room temperature for **00:05:00** . 5m

2.13 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for **00:05:00** until the liquid becomes clear. Carefully transfer **41 µl** of supernatant to a new 0.2 mL PCR tube. 5m

2.14 Quantify the Size selection products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit.

3 End Repair and A-tailing

3.1 Transfer ≤100 ng of fragmentation product to a new 0.2 mL PCR tube. Add TE Buffer for a total volume of **40 µl** .

3.2 Prepare the end repair and A-tailing mixture on ice (see Table 3.1).

Components	Volume
ERAT Buffer	7.1 µL
ERAT Enzyme Mix	2.9 µL
Total	10 µL

Table 3.1 End Repair and A-tailing Mixture

3.3 Transfer **10 µl** of the end repair and A-tailing mixture to the 0.2 mL PCR tube from step 3.1. Vortex 3 times (3s each) and briefly centrifuge to collect the solution at the bottom of the tube.

3.4 Place the 0.2 mL PCR tube from step 3.3 into the thermocycler and run the program in Table 3.2.

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

Table 3.2 End Repair and A-tailing Reaction Conditions

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3.5 Briefly centrifuge to collect the solution at the bottom of the tube.

4 Adapter Ligation

4.1 Add 5 µL of MGIEasy DNA Adapters (diluent) to the PCR tube from step 3.5. Vortex 3 times (3s each) and briefly centrifuge to collect the solution at the bottom of the tube.

4.2 Prepare the Adapter ligation mixture on ice.

Components	Volume
Ligation Buffer	23.4 µL
DNA Ligase	1.6 µL
Total	25 µL

Table 4.1 Adapter Ligation Mixture

4.3 Pipette slowly and transfer **25 µl** of Adapter ligation mixture to the 0.2 mL PCR tube from step 4.1. Vortex 6 times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube.

4.4 Place the PCR tube from step 4.3 into the thermocycler and run the program.

Temperature	Time
Heated lid	On
23°C	30 min
4°C	Hold

Table 4.2 Adapter Ligation Reaction Conditions

4.5 Centrifuge briefly to collect the solution at the bottom of the tube.

4.6 Add **20 µl** of TE Buffer for a total volume of **100 µl** and transfer all of the solution to a new 1.5 mL centrifuge tube.

5 Adapter-Ligated DNA Cleanup

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5.1 Take DNA Clean Beads out of the refrigerator and allow **00:30:00** for the solution to warm to room temperature. Vortex and mix thoroughly before use. 30m

5.2 Transfer **50 µl** of DNA Clean Beads to the centrifuge tube from step 4.6. Pipette up and down at least 10 times or vortex to mix thoroughly.

5.3 Incubate at room temperature for **00:05:00** . 5m

5.4 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for **00:05:00** until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette. 5m

5.5 Keep the tube on the Magnetic Separation Rack and add **200 µl** freshly prepared 80% ethanol to wash the beads and the walls of the tube. Incubate for 30 seconds, then carefully remove and discard the supernatant.

5.6 Repeat step 5.5 once. Remove all of the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom of the centrifuge tube, separate the beads magnetically and then remove any remaining liquid using a small volume pipette.

5.7 Keep the centrifuge tube on the Magnetic Separation Rack with the lid open and air-dry beads at room temperature until no wetness (reflectiveness) is observed, but before the pellet begins to crack.

5.8 Remove the centrifuge tube from the Magnetic Separation Rack and add **21 µl** of TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

5.9 Incubate at room temperature for **00:05:00** . 5m

5.10 Centrifuge briefly and place the centrifuge tube back onto the Magnetic Separation Rack for **00:05:00** until the liquid becomes clear. Transfer **19 µl** of supernatant to a new 0.2 mL PCR tube. 5m

6 PCR Amplification

6.1 Prepare the PCR amplification mixture on ice.

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6.2 Transfer **31 µl** of PCR amplification mixture to the PCR tube from step 5.10. Vortex 3 times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube.

Components	Volume
PCR Enzyme Mix	25 µL
PCR Primer Mix	6 µL
Total	31 µL

Table 6.1 PCR Amplification Mixture

6.3 Place the PCR tube from step 6.2 into the thermocycler and run the program in Table 6.2. Please refer to Table 6.2 to adjust the number of cycles with different DNA input.

Temperature	Time	Cycles
Heated lid	on	
95°C	3 min	1 cycle
98°C	20 s	
60°C	15 s	3-12 cycles
72°C	30 s	
72°C	10 min	1 cycle
4°C	Hold	

Table 6.2 PCR Amplification Reaction Conditions

6.4 Centrifuge briefly to collect the solution at the bottom of the tube.

6.5 Transfer all the solution to a new 1.5 mL centrifuge tube.

7 Cleanup of PCR Product

7.1 Take DNA Clean Beads out of the refrigerator and allow **00:30:00** (30m) for the solution to warm to room temperature. Vortex and mix thoroughly before use.

7.2 Transfer **50 µl** of DNA Clean Beads to the centrifuge tube from step 6.5. Pipette up and down at

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least 10 times or vortex to mix thoroughly.

7.3 Incubate at room temperature for **00:05:00** . 5m

7.4 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for **00:05:00** until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette. 5m

7.5 Keep the tube on the Magnetic Separation Rack and add **200 µl** of freshly prepared 80% ethanol to wash the beads and the walls of the tube. Incubate for **00:00:30** and carefully remove and discard the supernatant. 30s

7.6 Repeat step 7.5 once. Remove all of the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom, separate the beads magnetically, and remove any remaining liquid using a small volume pipette.

7.7 Keep the centrifuge tube on the Magnetic Separation Rack with the lid open, and air-dry beads at room temperature until no wetness (reflectiveness) is observed but before the pellet begins to crack.

7.8 Remove the centrifuge tube from the Magnetic Separation Rack and add **32 µl** of TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

7.9 Incubate at room temperature for **00:05:00** . 5m

7.10 Centrifuge briefly, then place the centrifuge tube back onto the Magnetic Separation Rack for **00:05:00** until the liquid becomes clear. Transfer **30 µl** of supernatant to a new 1.5 mL centrifuge tube. 5m

8 Quality Control of PCR Product

8.1 Quantify the purified PCR products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit. The required yield for PCR products is ≥ 1 pmol. Please refer to Formula 1 to calculate the amount of DNA needed. For example, for 384 bp PCR product the yield should reach **250 ng** . For pooled sequencing, please follow instructions provided by MGIEasy DNA Adapters User Manual. Detailed information shows how to plan your sample pooling. Quantify your Adapter-ligated samples before pooling. The total yield after pooling should be 1 pmol, with a total volume ≤ **48 µl** .

Formula 1
Conversion between 1 pmol of dsDNA sample and Mass in ng Mass (ng)
corresponding to 1 pmol PCR Products= DNA Fragment Size (bp) /1000 bp ×660 ng

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8.2 Assess the fragment size distribution of purified PCR products with electrophoresis-based equipment such as Bioanalyzer, Tapestation (Agilent Technologies), LabChip® GX, GXII, GX Touch (PerkinElmer), or Fragment Analyzer (Advanced Analytical).

9 Denaturation

9.1 According to the PCR product size, transfer 1 pmol of PCR product to a new 0.2 mL PCR tube. Add TE Buffer for a total volume of **48 µl** .

9.2 Place the 0.2 mL PCR tube from step 9.1 into the thermocycler and run the program in Table 9.1.

Temperature	Time
Heated lid	On
95°C	3 min

Table 9.1 Denaturation Reaction Conditions

9.3 After the reaction is completed, immediately place the 0.2 mL PCR tube on ice for **00:02:00** , then 2m centrifuge briefly.

10 Single Strand Circularization

10.1 Prepare the single strand circularization mixture on ice (see Table 10.1).

Components	Volume
Splint Buffer	11.6 µL
DNA Rapid Ligase	0.5 µL
Total	12.1 µL

Table 10.1 Single Strand Circularization Mixture

10.2 Transfer 12.1 µL single strand circularization mixture to the 0.2 mL PCR tube from step 9.3. Vortex 3 times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube.

10.3 Place the PCR tube into the thermocycler and run the program in Table 10.2.

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

Table 10.2 Single Strand DNA Circularization Reaction Conditions

10.4 After the reaction is complete, immediately place the tube on ice for the next reaction.

11 Enzymatic Digestion

11.1 Prepare the following enzymatic digestion mixture (see Table 11.1) on ice during the reaction in step 10.3.

Components	Volume
Digestion Buffer	1.4 µL
Digestion Enzyme	2.6 µL
Total	4 µL

Table 11.1 Enzymatic Digestion Mixture

11.2 Transfer **4 µl** of enzymatic digestion mixture into the PCR tube from step 10.4. Vortex 3 times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube.

11.3 Place the PCR tube from step 11.2 into the thermocycler and run the program in Table 11.2.

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

Table 11.2 Enzymatic Digestion Reaction Conditions

11.4 Centrifuge briefly to collect the solution at the bottom of the tube.

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11.5 Add **7.5 µl** Digestion Stop Buffer to the 0.2 mL PCR tube. Vortex 3 times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube. Transfer all of the solution into a new 1.5 mL centrifuge tube.

12 Enzymatic Digestion Product Cleanup

12.1 Take DNA Clean Beads out of the refrigerator and allow **00:30:00** for the solution to warm to room temperature. Vortex and mix thoroughly before use. 30m

12.2 Transfer **170 µl** of DNA Clean Beads to the Enzymatic Digestion product from step 11.5. Gently pipette at least 10 times or vortex to mix thoroughly.

12.3 Incubate at room temperature for **00:10:00** . 10m

12.4 Centrifuge briefly and place the 1.5 mL tube on the Magnetic Separation Rack for **00:05:00** until the liquid becomes clear. Carefully remove and discard the supernatant using a pipette. 5m

12.5 With the 1.5 mL tube on the Magnetic Separation Rack, add **500 µl** of freshly prepared 80% ethanol to the tube without disturbing the beads. Incubate for **00:00:30** . Carefully remove and discard the supernatant. 30s

12.6 Repeat step 12.5 once. Remove all of the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom, separate the beads magnetically, then remove any remaining liquid using a small volume pipette.

12.7 Keep the 1.5 mL centrifuge tube on the Magnetic Separation Rack with the lid open, and air-dry beads at room temperature until no wetness (reflectiveness) is observed but before the pellet begins to crack.

12.8 Remove the 1.5 mL centrifuge tube from the Magnetic Separation Rack and add **32 µl** of TE Buffer to elute the DNA. Gently pipette up and down at least 10 times to mix thoroughly or until the beads are fully resuspended.

12.9 Incubate at room temperature for **00:10:00** . 10m

12.10 Centrifuge briefly and place the centrifuge tube back onto the Magnetic Separation Rack for **00:05:00** until the liquid becomes clear. Transfer **30 µl** of supernatant to a new 1.5 mL 5m

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centrifuge tube.

13 **Quality Control of Enzymatic Digestion Product**

Quantitate the purified Enzymatic Digestion product with Qubit® ssDNA Assay Kit. The final Enzymatic Digestion products (ssDNA, ng) / input products of PCR (dsDNA, ng) should be ≥ 7%. For example, for 384 bp PCR product the final Enzymatic Digestion products should reach 17.5 ng.

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